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# Asymmetric Dimethylarginine: Clinical Significance and Novel Therapeutic Approaches

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**Abstract:** Asymmetric dimethylarginine (ADMA) is a competitive endogenous inhibitor of nitric oxide synthase with a key role in the pathophysiology of endothelial dysfunction, in the progression of atherosclerosis and in cardiovascular diseases. Statins, renin-angiotensin-aldosterone system inhibitors, blood glucose lowering agents, insulin sensitizers, beta-blockers, estrogen replacement therapy, antioxidants, complex B vitamins, L-arginine and acetylsalicylic acid have been evaluated for their ability to reduce ADMA levels or inhibit its actions. Despite the major beneficial effects of these agents in cardiovascular disease, research has shown that their favorable actions are only partially mediated by reducing ADMA levels or by bypassing its effect in nitric oxide synthesis. Novel therapeutic approaches targeting selectively ADMA are encouraging, but have only been tested *in vitro* or in animal studies and further research is needed in order to conclude on how therapeutic strategies modulating ADMA actions can affect atherosclerosis progression and cardiovascular diseases.

**Keywords:** Asymmetric dimethylarginine, atherosclerosis, cardiovascular disease, endothelial dysfunction, nitric oxide, therapeutic approaches.

## INTRODUCTION

Vascular endothelium is a monolayer of cells, which lines the interior surface of blood vessels forming an interface between the vessel lumen and the underlying vascular smooth muscle cells. Apart from being a single barrier between blood flow and the intimal wall, endothelium plays a role of crucial importance in the regulation of vascular function and structure via modulating vascular tone, blood flow, platelet function and coagulation [1, 2]. Nitric Oxide (NO) has a central role in vascular homeostasis and is not only a potent vasodilator but acts also as an anti-atherogenic and anti-proliferative molecule [1, 3].

The central role of NO is further highlighted as most of the cardiovascular risk factors, including hypertension, hypercholesterolemia, smoking, diabetes mellitus and hyperhomocysteinemia, have been found to mediate their effects on the vessels through dysfunction of the pathway of endothelial-derived NO synthesis, leading to inactivation or reduced bioavailability of it [4].

Endothelium-derived NO is synthesized from L-arginine by the endothelial isoform of NO synthase (eNOS). Asymmetric dimethylarginine (ADMA), which is formed as a metabolic byproduct of continuous cellular protein turnover, is an endogenous competitive inhibitor of eNOS. Consequently, elevated ADMA levels are found in the presence of cardiovascular risk factors and are associated with atherosclerosis progression and cardiovascular events [5]. In addition, ADMA inhibits NO generation by the two other isoforms of nitric oxide synthase (NOS): neuronal and inducible NOS (nNOS and iNOS). Hence, it affects other organs and tissues, and specifically brain and immune system, as well [6].

In the current review we shortly present the evidence concerning the role of ADMA in endothelial dysfunction, in cardiovascular disease and in systemic pathological conditions and we further focus on established and novel therapeutic approaches aiming to modulate ADMA's function or synthesis. Finally, we discuss the clinical significance and use of currently available treatments.

## BIOSYNTHESIS OF ADMA

Methylation of arginine residues constitutes a mechanism of post-translational modification of proteins in eukaryotic cells influencing various cellular functions [7, 8]. A family of enzymes, termed protein arginine methyltransferases (PRMTs) catalyzes this reaction utilizing a methyl group derived by S-adenosyl-L-methionine and adding it to the guanidino nitrogen atoms of arginine side chains, while producing S-adenosyl-L-homocysteine as a by-product [8, 9]. In a two-step process, PRMTs first catalyze monomethylation of arginine residues and subsequently a second methylation reaction.

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After proteolysis, monomethylarginines, asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) are released into the cytosol. Monoethylarginines are found in the form of NG-monomethyl-L-arginine (L-NMMA), which is formed by arginine residues that escaped second methylation [8-10].

Nine members of PRMTs family have been identified in the mammalian genomes [8]. Based on their substrate, reaction and by-product specificity they are classified as types I, II, III and IV PRMTs. Types I, II and III PRMTs all catalyze the first methylation reaction of arginine residues resulting in formation of L-NMMA. Subsequently, in the second methylation reaction, type I PRMTs (PRMT 1, 2, 3, 4, 6, 8) add a methyl group to the already methylated guanidino nitrogen atom, leading to asymmetric dimethylation (formation of ADMA), whereas type II PRMTs (PRMT 5 and 7) can methylate both atoms leading to symmetric dimethylation (formation of SDMA) [8]. PRMT 7 also acts as a type III enzyme catalyzing monomethylation [11], while PRMT 9, also called FBXO11, is believed to act as a type II enzyme [12], but its function has not yet been determined [8, 12]. While type I, II and III PRMTs catalyze the methylation of terminal guanidino nitrogen atoms, a type IV enzyme, methylating the internal guinidino nitrogen atom of arginine residues has been described in yeast, but never been identified in humans [13]. PRMT 1 is the predominant member of PRMTs family catalyzing protein arginine methylation, since its activity accounts for approximately 85% of arginine methylation reactions [14].

However, it is still under investigation whether protein arginine methylation is a potentially reversible reaction. Two mechanisms have been identified through which protein methylarginine residues may be modified. First, peptidyl-methylarginine residues are deaminated to peptidyl-citrulline by peptidyl-arginine deiminase enzymes and specifically peptidyl-arginine deiminase 4 [15, 16]. However, it has been shown that peptidyl-arginine deiminases are unlikely to remove methyl groups from peptidyl-arginines [17] and therefore it is unknown whether they play a demethylation role *in vivo*. Secondly, Jumonji domain-containing 6 protein, has been reported to exert true demethylation action in histone methylarginine residues [18]. Though, a more recent study did not detect arginine demethylase activity for Jumonji domain-containing 6 protein [19].

## METABOLISM, INTER-ORGAN TRANSPORT AND EXCRETION OF ADMA

ADMA is removed from the body via catabolism and renal excretion. Two catabolic pathways have been identified for methylarginines: the first includes their hydrolysis by NG-dimethylarginine dimethylaminohydrolase (DDAH) enzymes, which is specific for asymmetric methylarginines (L-NMMA and ADMA) [20-22]; the second contributes to the metabolism of asymmetric as well as symmetric methylarginines and includes their transamination by alanine-glyoxylate aminotransferase 2 (AGXT2) [23, 24].

DDAH are mostly cytosolic enzymes that catalyze the hydrolytic degradation of L-NMMA and ADMA to citrulline and monomethylamine or dimethylamine respectively [22]. Two isoforms of DDAH exist: DDAH-1 and DDAH-2 [25]. Heart, endothelium, kidney, lung, pancreas, liver, brain and placenta as well as immune tissue, including macrophages and neutrophils, have been reported to have significant DDAH activity [22]. Based on specific tissue mRNA expression of DDAH isoforms and NOS types, it has been concluded that DDAH-1 is the predominant isoform in tissues with nNOS activity, whereas DDAH-2 tissue expression overlaps in a greater extent with the expression of eNOS and iNOS [22]. Therefore, the catabolism of ADMA in the brain, where only nNOS activity is detected, is believed to be predominantly catalyzed by DDAH-1, while in cardiovascular and immune tissues, where eNOS and iNOS are respectively highly expressed, by DDAH-2 [22]. The primary locations for ADMA metabolism are the kidney and the liver [26, 27]. In the kidney all three isoforms of NOS, namely nNOS, eNOS and iNOS, as well as both DDAH-1 and 2 are highly expressed. Both DDAH and NOS expression within the different sites and cells of the nephron are highly isoform-specific, thus providing discreet cellular localization patterns. This could serve different site-specific regulation of NO generation in different parts inside the nephron [22].

AGXT2 is a pyridoxal phosphate-dependent aminotransferase and one of the two mammalian alanine-glyoxylate aminotransferases, along with AGTX1. AGTX2, but not AGTX1, catalyzes the transamination of ADMA by utilizing it as an amino donor for the formation of α-keto-δ-(*N*,*N*-dimethylguanidino) valeric acid [23, 24, 28]. Although recent *in vivo* evidence suggests that this mechanism applies in mice [29] as well as in humans [29] its contribution to ADMA metabolism has not yet been evaluated.

The synthesized ADMA may remain intracellularly where it exerts its action by inhibiting NOS or is catabolized by the aforementioned mechanisms, but it can also be exported from its site of origin to the extracellular fluid, plasma and subsequently distant tissues. This inter-organ transmembrane transport is an active procedure mediated by cationic amino acid transporters (CATs) of system y+ [30, 31].

Methylarginines that have passed out from the cell to circulation may be eliminated through renal excretion [32]. It is believed that 300 μmol of ADMA are generated in a daily basis, of which approximately 80% is metabolized by DDAH. The rest is removed from the body through urinary excretion [33]. Since SDMA catabolism is not catalyzed by DDAH, renal excretion is believed to be the major eliminatory pathway for SDMA [31, 32].

## ADMA: MECHANISMS OF ACTION

Functional NOS proteins are homodimers that transfer electrons from nicotinamide-adenine-dinucleotide phosphate (NADPH) in the carboxyterminal reductase domain via flavin adenine dinucleotide and flavin mononucleotide to the amino-terminal oxygenase domain. Electrons interact with heme iron and the co-factor (6R)-5,6,7,8-tetrahydrobiopterin (BH4) and are utilized to reduce and activate molecular oxygen and to oxidize L-arginine to L-citrulline and NO [34, 35]. Three different isoforms of NOS have been identified in mammals: nNOS, eNOS and iNOS. NO derived by nNOS participates in synaptic plasticity and central controlling of blood pressure in the central nervous system, while it serves as a neurotransmitter in the peripheral nervous system medi-

ating autonomic functions, like gut peristalsis, penile erection and vasodilation [36]. iNOS activity is induced by inflammatory response and cytokines. It has been reported to be crucial in the elimination of intracellular bacteria, while iNOS-derived NO is believed to mediate the vasodilation observed in inflammation as well as other inflammatory reactions [37]. Lastly, eNOS catalyzes formation of NO in the endothelium. NO is not only a potent vasodilator but also acts as an anti-atherogenic and anti-proliferative molecule and is considered crucial for the maintenance of vascular homeostasis [1]. Protein interacting with NIMA (never in mitosis-A)-1 (Pin1) has been found to bind to eNOS at serine-116, enabling its dephosphorylation which leads also to an increase of NO generation [38-40]. Therefore decrease levels of Pin1 can be used diagnostically in hypertension and inhibition of this factor can act beneficially against Alzheimer's disease [41, 42] as we discuss in the specific section.

Asymmetric methylarginines (L-NMMA and ADMA) are endogenous competitive inhibitors of all three NOS isoforms. Their action is attributed to their ability to bind to the active site of NOS enzymes, thus competing endogenous L-arginine [43, 44]. Plasma concentration of L-NMMA is approximately 10% compared to ADMA and therefore, ADMA is considered to be the predominant endogenous NOS inhibitor. However, since inhibition of NOS is conducted intracellularly, the effect of L-NMMA in some tissues may be of comparable importance [6]. The competitive inhibition of NOS enzymes by ADMA has been reported to be dose-dependent [45].

L-arginine concentrations *in vivo* are much higher than its Michaelis constant (Km) for NOS. However, excess exogenous L-arginine supplementation increases NO bioavailability through a NOS-dependent pathway, a phenomenon called "the L-arginine paradox" [46]. The basic mechanism underlying the L-arginine paradox is believed to be the tissue coexistence of L-arginine and asymmetric methylarginines that activate or inhibit NOS [47, 48]. Indeed, ADMA concentrations in the brain and endothelial cells are also much higher than the respective inhibitor dissociation constant (Ki) of nNOS and eNOS. Therefore, the intracellular L-arginine: ADMA concentration ratio potentially reflects the NOS activation state [31].

In addition, the CAT system has also been implied as a second explanation to the L-arginine paradox. It has been shown that CAT1 and eNOS are both located in membrane caveolae. Therefore, high plasma levels of L-arginine after supplementation could through CATs directly reach eNOS due to their proximity and enhance NO generation [49]. This could possibly explain why serum ADMA levels have been associated with a number of diseases despite the fact that ADMA exerts its action mostly intracellularly. ADMA, as well as the other methylarginines, compete with L-arginine for transmembrane transport in the intracellular levels through CATs [50-53]. Nevertheless, a study by Strobel *et al.*, declared that based on their properties, ADMA in its physiological concentration is unlikely to inhibit transport of L-arginine through CAT1 [54].

In addition to being a competitive inhibitor of NOS, ADMA has also been implicated to play a role in NOS-derived superoxide generation. Previous studies have reported that NOS isoforms generate superoxide instead of NO, under condition of L-arginine or BH4 depletion [55, 56]. Presence of oxidative stress induces this phenomenon which has been referred to as "NOS uncoupling". ADMA serum levels have been associated with the *ex vivo* generation of superoxide, associated with eNOS uncoupling [57]. Evidence suggests that when BH4 is absent, ADMA and L-NMMA increase superoxide production in a dose-dependent manner by uncoupled eNOS [58]. However, the effects observed for the neuronal isoform of NOS were not similar: Cardounel *et al.* demonstrated that ADMA has no impact on superoxide production by nNOS under depletion of BH4, but L-NMMA increased superoxide generation [59].

Although it remains unclear whether ADMA induces NOS uncoupling and subsequent superoxide generation, a relationship between ADMA and oxidative stress is well established [60]. It has been implicated that oxidative stress may lead to reduced DDAH [61] and increased PRMTs activity [62], thus decreasing ADMA degradation and increasing ADMA synthesis. On the other hand, ADMA has also been found *ex vivo* [63] and *in vitro* [64] to increase superoxide radical generation. Therefore, oxidative stress and ADMA may share a relationship of bidirectional causality.

Intriguingly, a feedback regulatory mechanism has been reported for the DDAH-2/ADMA/NOS/NO pathway. Specifically, NO through a cGMP-mediated process increased DDAH-2 gene expression and thus reduced ADMA levels [65].

## ADMA IN SEVERAL PATHOLOGICAL CONDITIONS

Increased ADMA levels have been found in several cardiovascular diseases and in the presence of cardiovascular risk factors**.** To name some of them hypertension [66], chronic heart failure [67], chronic renal failure [45], coronary artery disease [68], stroke [69], diabetes mellitus [70], are conditions in which elevated serum ADMA levels have consistently been measured. However, association of ADMA with other disease state beyond cardiovascular system has also been observed. Table **1** summarizes the literature evidence for the relationship between ADMA and several diseases.

### Atherosclerosis and Cardiovascular Disease

ADMA plays a significant role in endothelial dysfunction and atherogenesis, by suppressing eNOS and consequently NO production. In the endothelium, NO acts as a potent vasodilator, reduces monocytes adhesion, inhibits oxidation of lipoproteins and smooth muscle cells proliferation, suppresses aggregation of platelets and reduces superoxide radical release. Indeed, ADMA has been reported to induce accumulation of oxidized LDL [109], increase adhesiveness of monocytes [110], stimulate expression of chemotactic cytokines [111], facilitates platelet aggregation, induce smooth muscle cell migration [112] and increase vascular resistance [113], leading to atherosclerosis. Intriguingly, long-term infusion of ADMA caused vascular lesions in mice [114], implying that ADMA is an important *in vivo* atherogenic molecule.

**Table 1. Asymmetric dimethylarginine in several pathological conditions.**

| Author/Year | Type of Study | Subjects | Results/Conclusion | Underlined Mechanism |
|---|---|---|---|---|
| *Atherosclerosis and cardiovascular disease* | | | | |
| Bai *et al.* 2013 [71] | Meta-analysis | 6168 subjects (derived from 22 studies) | ADMA levels are positively related with carotid IMT. | ADMA inhibits the generation of NO by eNOS and induces accumulation of oxidized LDL cholesterol, increases adhesiveness of monocytes, stimulates expression of chemotactic cytokines, facilitates platelet aggregation, induces smooth muscle cell migration and increases vascular resistance. These lead to endothelial dysfunction and subsequently atherosclerosis |
| Juonala *et al.* 2007 [72] | Cross-sectional | 2096 adults 24-39 years old | ADMA levels are inversely related to brachial FMD | |
| Willeit *et al*. 2015 [73] | Meta-analysis | 19842 subjects (derived from 22 prospective studies) | Increased ADMA levels are associated with increased risk for CAD and stroke | |
| Lu *et al.* 2003 [68] | Prospective (median follow up 16 months) | 153 subjects with stable CAD undergoing PCI | Higher ADMA levels are independently associated with a higher risk of adverse cardiovascular events after PCI | |
| Yoo *et al.* 2001 [69] | Case control | 52 subjects with stroke and 35 healthy subjects | ADMA is elevated in subjects with stroke | |
| Meinitzer *et al*. 2007 [74] | Prospective (median follow up 5.5 years) | 3148 subjects (2453 with CAD and 695 without CAD) | ADMA concentration predicts all-cause and cardiovascular mortality in individuals with CAD | |
| *Hypertension* | | | | |
| Perticone *et al.* 2010 [66] | Case control | 84 (63 hypertensive/21 healthy) | Hypertensive subjects have higher ADMA levels | By inhibiting formation of NO, ADMA leads to vasoconstriction and increased arterial blood pressure. ADMA also decreases urinary sodium excretion by suppressing the inhibitory effect of NO on tubular sodium re-absorption. |
| Surdacki *et al.* 1999 [75] | Case control | 19 newly diagnosed male hypertensive subjects and 11 normotensive controls | Circulating ADMA levels are increased in hypertensive subjects | |
| Goonasekera *et al.* 1997 [76] | Case control | 38 hypertensive with impaired renal function and 9 healthy control children (median age 7.7 years) | Increased ADMA levels among children with nephrogenic hypertension | |
| Curgunlu *et al.* 2005 [77] | Case control | 102 subjects (34 with white coat hypertension, 34 with hypertension, 34 controls) | ADMA levels are increased among subjects with white coat hypertension and hypertension compared to controls. | |
| *Hypercholesterolemia* | | | | |
| Boger *et al.* 1998 [78] | Case control | 49 hypercholesterolemic and 31 normocholesterolemic subjuects | ADMA levels are more than 2-fold higher in subjects with hypercholesterolemia | LDL cholesterol up-regulates gene expression of PRMTs, thus leading to an increase of ADMA synthesis. This mechanism may mediate part of the atherogenic effects of hypercholesterolemia. |
| Jehlicka *et al.* 2009 [79] | Case control | 32 children with familiar hypercholesterolemia, 30 children with diabetes mellitus type 1, 30 healthy age-matched controls | Baseline ADMA is elevated in children with familiar hypercholesterolemia compared to diabetes mellitus and controls | |
| Chobanyan-Jurgens *et al.* 2012 [80] | Case control | 64 children with hypercholesterolemia type II and 54 healthy controls | Plasma concentration and urinary excretion of ADMA are not different between two groups. Increased DDAH activity is observed. | |

**(Table 1) contd….**

| Author/Year | Type of Study | Subjects | Results/Conclusion | Underlined Mechanism |
|---|---|---|---|---|
| *Hyperhomocysteinemia* | | | | |
| Korandji *et al.* 2007 [81] | Cross-sectional | 138 patients hospitalized for AMI | ADMA is associated with total plasma homocysteine, but the association is attenuated after controlling for eGFR | Homocysteine suppresses activity of DDAHs. |
| Wilcken *et al.* 2006 [82] | Case control | 23 cystathionine beta-synthase deficient subjects and 24 age-matched controls | ADMA levels are increased only in cases with elevated cystatin C but not in those with normal renal function | |
| Jonasson *et al.* 2003 [83] | Cross sectional | 60 patients with ischemic heart disease | ADMA levels are not different among subjects with higher or lower homocysteine levels | |
| *Chronic heart failure* | | | | |
| Saitoh *et al.* 2003 [84] | Case control | 25 subjects with exacerbated chronic heart failure, 23 with compensated chronic heart failure and 26 healthy controls | ADMA levels are increased among subjects with exacerbated heart failure | ADMA via inhibiting eNOS decreases cardiac output and heart rate and increases blood pressure. Oxidative stress observed in heart failure may lead to decreased DDAH and increased PRMT activity, thus elevating ADMA. |
| Usui *et al.* 1998 [67] | Cross sectional | 84 heart failure subjects (NYHA 1 to 4) | ADMA is elevated according to NYHA status | |
| Hsu *et al.* 2012 [85] | Cross sectional | 285 patients with ischemic chronic heart failure | ADMA plasma levels are positively correlated with NYHA functional class and NT-proBNP levels and predict major cardiovascular adverse outcomes and cardiac decompensation | |
| Selijeflot *et al.* 2011 [86] | Cross sectional | 80 patients with chronic heart failure of NYHA II-IIIb on an optimal treatment | ADMA levels are higher in NYHA III than II | |
| *Chronic kidney disease* | | | | |
| Zoccali *et al.* 2001 [87] | Prospective (mean follow-up 33.4 months) | 225 hemodialysis patients | ADMA levels independently predict overall mortality and cardiovascular events | Suppression of DDAHs in chronic kidney disease leads to an increase of ADMA levels. Kidney excretion of ADMA is reduced in renal failure and this may explain the susceptibility of these patients to atherosclerosis. |
| Ravani *et al.* 2005 [88] | Prospective (mean follow-up 27 months) | 131 patients with chronic kidney disease | ADMA levels are inversely related to eGFR and predict progression to end-stage renal disease and death | |
| Filser *et al.* 2005 [89] | Prospective (follow-up up to 7 years) | 227 relatively young patients (mean age 45.7 years) with nondiabetic chronic kidney disease | Baseline ADMA levels are correlated with creatinine levels and predict progression rate of the disease | |
| Sesti *et al.* 2013 [90] | Cross sectional | 2852 white European subjects | Carriers of the C allele with the rs9267551 variant in the DDAH2 gene have significantly lower likelihood of renal dysfunction, possibly due to increased DDAH-2 activity and decreased ADMA | |

**(Table 1) contd….**

| Author/Year | Type of Study | Subjects | Results/Conclusion | Underlined Mechanism |
|---|---|---|---|---|
| *Diabetes and insulin resistance* | | | | |
| Altinova *et al.* 2007 [91] | Case control | 40 patients with type 1 diabetes and 35 controls | ADMA is elevated among diabetic subjects | Hyperglycemia induces oxidative stress and decreases DDAH activity leading to increased ADMA. Increased ADMA may ameliorate insulin resistance. Insulin increases the expression of CATs, thus increasing intracellular transport of L-arginine and ADMA. Hence, hyperinsulinemia counteracts the increase of serum ADMA caused by hyperglycemia. |
| Päivä *et al.* 2003 [70] | Case control | 86 subjects with type 2 diabetes and 65 control | Increased ADMA in diabetic subjects with increased glycosylated hemoglobin | |
| Stühlinger *et al.* 2001 [92] | Cross-sectional | 64 healthy volunteers | Serum ADMA is positively correlated with insulin resistance | |
| Boger *et al.* 2009 [93] | Prospective (mean follow-up 10.7years) | 3320 participants | ADMA is associated with all-cause mortality among non-diabetic but not among diabetic subjects | |
| Lu *et al.* 2011 [94] | Prospective (median follow-up 2.4 years) | 997 individuals referred for coronary angiography | ADMA is increased in subjects with CAD, predicted long-term adverse clinical outcomes only in non-diabetic subjects | |
| Andersohhn *et al.* 2014 [95] | Prospective (follow-up of 4 years) | 783 diabetic subjects | Risk of incident CVD is not associated with ADMA levels | |
| *Pulmonary hypertension* | | | | |
| Zhang *et al.* 2015 [96] | Case control | 35 cases with pulmonary hypertension and 35 healthy controls | ADMA concentration is increased in cases and was positively correlated to mean PAP and PVRI | Decreased DDAH activity in pulmonary hypertension leads to an increase of ADMA. ADMA causes pulmonary vasoconstriction via inhibiting of NO synthesis, but also increases permeability of pulmonary endothelium via inhibition of connexin 43 (gap junctional protein) and acts as a pro-proliferative molecule. |
| Parikh *et al.* 2014 [97] | Cross sectional | 214 HIV- infected subjects | ADMA is positively associated with mean PAP and PASP. Higher values are found in subjects with pulmonary hypertension | |
| Dimitroulas *et al.* 2008 [98] | Case control | 66 patients with systemic sclerosis (24 of whom with pulmonary hypertension) and 30 controls | ADMA is elevated in subjects with pulmonary hypertension | |
| Kielstein *et al.* 2005 [99] | Case control | 57 subjects with idiopathic pulmonary hypertension and 22 controls | Significantly increased serum ADMA levels in cases with idiopathic pulmonary hypertension | |
| *Preeclamspia* | | | | |
| Pettersson *et al.* 1998 [100] | Case control | 12 pregnant women with severe preeclampsia and 12 normotensive pregnant controls | ADMA levels are elevated in the preeclamptic group during the third trimester | Decreased mRNA expression of DDAH1 and DDAH2 in placenta of preeclamptic women explains the accumulation of ADMA. PRMTs are not upregulated. |
| Mao *et al.* 2010 [101] | Case control | 62 preeclamptic women and 30 healthy pregnant controls | Serum ADMA levels are increased in women with preeclampsia | |
| Maas *et al.* 2004 [102] | Case control | 67 women with preeclampsia (49 moderate, 18 severe) and 93 healthy pregnant controls | No significant difference is observed in ADMA levels of preeclamptic and non-preeclamptic pregnant women | |

**(Table 1) contd….**

| Author/Year | Type of Study | Subjects | Results/Conclusion | Underlined Mechanism |
|---|---|---|---|---|
| Bian *et al.* 2015 [103] | Prospective (from 12-16 weeks of gestation up to 6 weeks after delivery) | 740 pregnant women | First trimester ADMA levels are increased among women who later developed preeclampsia | |
| Anderssohnn *et al.* 2012 [104] | Case control | 18 preeclamptic women and 28 controls | Expression and activity of DDAH2 enzyme are undetectable in preeclampsia, but PRMT1 expression is similar among the two groups | |
| *Alzheimer's disease* | | | | |
| Arit *et al.* 2008 [105] | Case control | 80 patients with Alzheimer's disease and 80 age- and gender-matched controls | ADMA levels are increased in plasma, but decreased in CSF. Severity of cognitive impairment is inversely associated with CSF ADMA concentration. | ADMA may ameliorate Aβ-induced toxicity in Alzheimer's disease, but overactivation of the DDAH/ADMA/NOS/NO pathway leads to overproduction of NO, leading to oxidative stress, neurotoxicity and neurodegeneration. |
| Selley 2003 [106] | Case control | 25 subjects with Alzheimer's disease and 25 healthy controls | Plasma ADMA levels are increased in patients | |
| Abe *et al.* 2001 [107] | Case control | 14 Alzheimer's disease patients and 15 controls | ADMA is significantly decreased in CSF of patients | |
| McEvoy *et al.* 2014 [108] | Cross-sectional | 483 community-dwelling subjects aged between 55 and 85 years | Higher ADMA levels are independently associated with subjective memory impairment | |

ADMA: Asymmetric dimethylarginine; IMT: Intima- media thickness; Flow-mediated dilation; CAD: Coronary artery disease; PCI: Percutaneous coronary intervention; NO: Nitric oxide; eNOS: Endothelial nitric oxide synthase; LDL: Low-density lipoprotein; DDAH: dimethylarginine dimethylaminohydrolase; PRMT: protein arginine methyltransferases; eGFR: Estimated glomerular filtration rate; NT-proBNP: N-terminal prohormone of brain natriuretic peptide; NYHA: New York heart association (functional classification); CAT: Cationic amino acid transporter ; CVD: Cardiovascular disease; PAP: Pulmonary artery pressure; PVRI: pulmonary vascular resistance index; PASP: Pulmonary artery systolic pressure; CSF: Cerebrospinal fluid; NOS: Nitric oxide synthase.

In humans, brachial artery flow-mediated dilatation (FMD), a marker of endothelial function has been inversely and independently associated with serum ADMA levels even among healthy young individuals [72], outlining the significance of ADMA in the initiation of endothelial dysfunction which finally progress to atherosclerosis. In addition, ADMA levels have been positively related to carotid intima-media thickness, a proxy of subclinical atherosclerosis in cross-sectional and prospective studies [71]. Besides ADMA, the L-arginine: ADMA serum levels ratio has been associated with intima-media thickness as well [115], indicating the significance of their imbalance.

Prospective studies have further highlighted the prognostic value of serum ADMA regarding its association with adverse cardiovascular outcomes. Specifically, a recent meta-analysis combining 22 prospective studies [73] revealed a statistically significant association between high baseline ADMA serum levels and risk for subsequent cardiovascular disease, coronary artery disease and stroke. Furthermore, there is adequate evidence supporting that elevated ADMA is a significant predictor of cardiovascular mortality in high-, intermediate- and low-risk populations [50].

Lastly, higher plasma ADMA levels in stroke-free individuals have been associated with subsequent magnetic resonance imaging markers of subclinical vascular brain injury (silent brain infarcts and large white-matter hyperintensity volumes), indicating ADMA as a potential new biomarker for risk of stroke [116].

Taken together ADMA can be considered a novel cardiovascular risk factor. Nevertheless, the existing methods for serum ADMA levels quantification do not fulfill the criteria to be characterized as "gold standard", since they do not represent a reliable measurement and we must also further evaluate his additive predictive value in top of classical risk factors before its clinical applicability.

## Chronic Heart Failure

Experimental as well as human data suggest that congestive heart failure is associated with elevated serum ADMA levels [67, 84, 117]. These observations have raised suspicion whether ADMA has any etiological role in congestive heart failure. In the study by Seljeflot *et al*, the association with severity of heart failure was stronger for L-arginine: ADMA serum concentration ratio than for ADMA levels [86]; this may indicate that the competitive inhibition of

eNOS is the main underlying mechanism of the observed association. Studies in humans further imply that ADMA infusion at low doses has the ability to decrease cardiac output and heart rate and increase blood pressure [33, 113]. On the other hand, it could be possible that increased oxidative stress observed in congestive heart failure [118] increases ADMA levels by decreasing DDAH and increasing PRMT 1 activity. Therefore, the mechanism of increased ADMA levels in congestive heart failure as well as the hypothesis that it may be a risk factor for the disease should be further explored.

**Hypertension**

Hypertension is a major risk factor for cardiovascular disease. Human studies have shown that adults as well as children and adolescents with hypertension have higher plasma ADMA levels as compared to controls [66, 75, 76]. Similarly, higher levels of serum ADMA have been observed in subjects with white coat hypertension [77] as well as in children and adolescents with nephrogenic hypertension [76]. ADMA infusion increases also blood vessels resistance and arterial hypertension [33, 113]. Beyond vasoconstriction, due to inhibition of NO synthesis in the endothelial cells, ADMA affect also renal sodium handling. Specifically, ADMA has been shown to decrease urinary sodium excretion by suppressing the inhibitory effect of NO on tubular sodium re-absorption [119]. Moreover, experimental and human studies have suggested that ADMA is involved in the mechanism of salt sensitivity in hypertensive subjects; they showed that ADMA mediates the increase in blood pressure observed after salt intake [120, 121]. Therefore, accumulating evidence indicates that ADMA is involved in the molecular mechanisms of hypertension in humans.

**Diabetes and Insulin Resistance**

It is well established that diabetes mellitus and insulin resistance are conditions associated with endothelial dysfunction and atherosclerotic cardiovascular disease [122]. However, the association of ADMA with diabetes mellitus and insulin resistance is rather complex. Experimental studies have tried to give insight in this complicated relationship. *In vitro* as well as *in vivo* data report that hyperglycemia induces oxidative stress which decreases DDAH activity leading to accumulation of ADMA in cultured endothelial cells and in diabetic rats [61]. Similarly, transgenic mice overexpressing DDAH-1 have been found to have lower levels of ADMA and significantly improved insulin sensitivity compared to mice with normal DDAH-1 activity [123]. On the contrary, based on *in vitro* findings, insulin stimulates mRNA expression of CAT, thus resulting in increased L-arginine intracellular transport and NO production [124]. However, ADMA is also transported through CATs. Therefore, increased ADMA transport intracellularly could be responsible for the lower serum ADMA levels observed in acute hyperinsulinemia [125]. These mechanisms could possibly explain why in patients with insulin resistance, that is characterized by both hyperglycemia and hyperinsulinemia, ADMA levels are not always found elevated; the hyperglycemia-induced elevation of ADMA could be counteracted by the opposing effect of hyperinsulinemia.

More precisely, clinical studies have reported that serum ADMA levels are increased among patients with type 1 diabetes mellitus [91] and are associated with diabetic nephropathy [126]. However, concerning type 2 diabetes mellitus findings are rather equivocal. Some studies have found elevated ADMA levels in diabetic subjects [127, 128], while other reported higher ADMA levels in non-diabetics subjects compared to diabetic individuals or no significant differences between subjects according to the presence of diabetes mellitus type 2 [70, 93, 95]. In insulin resistant patients increased ADMA levels have also been observed [92, 129] but not in women with gestational diabetes [130]. While, in men with coronary artery disease ADMA levels but not insulin resistant was associated with the coronary atherosclerotic burden. Moreover, ADMA and insulin resistance were mutually unrelated [131]. On the other hand, it has been shown that insulin therapy decreases ADMA levels in critically ill humans [132]. An interesting finding was described by Boger *et al*, regarding the association between ADMA and diabetes. In 3320 participants of the Framingham Offspring Study cohort ADMA levels were predictors of mortality only among non-diabetic participants [93]. Similarly, ADMA serum levels were associated with adverse cardiovascular outcomes and all-cause mortality among non-diabetic individuals, but the relationship attenuated in subjects with diabetes [94].

Therefore, clinical data imply that the relationship between diabetes mellitus, insulin resistance, ADMA levels and atherosclerotic burden is not strait forward but rather complicated.

**Hypercholesterolemia**

Hypercholesterolemia and LDL-cholesterol has a central role in the pathogenesis of endothelial dysfunction and the formation of atherosclerotic plaques. Experimental as well as clinical studies have tried to assess whether high levels of cholesterol exert part of their detrimental effects through changes in ADMA levels. In a study in monkeys fed with an atherogenic diet the induced hypercholesterolemia raised ADMA levels [133]. This effect of hypercholesterolemia may be partly regulated by the impact of LDL cholesterol on PRMTs. Specifically, it was found *in vitro* that LDL cholesterol up-regulates the expression of PRMTs, thus leading to increased ADMA synthesis and reduced NO production [134]. Elevation of ADMA in hypercholesterolemia may further lead to impaired angiogenesis, as shown in a study of apolipoprotein E- deficient hypercholesterolemic mice [135].

In view of epidemiological studies, Boger *et al.* were the first to demonstrate elevated serum ADMA levels among hypercholesterolemic patients compared to healthy controls [78] and similar findings were observed in hypercholesterolemic children [136] and children with familiar hypercholesterolemia [79]. However, not all human studies have confirmed the aforementioned observation which may be attributed to age related changes in ADMA concentration and enhance DDAH activity with age [80, 137, 138].

**Hyperhomocysteinemia**

Hyperhomocysteinemia is a condition characterized by increased levels of serum total homocysteine and is caused

by genetic defects in homocysteine or methionine metabolism, nutritional depletion of B complex vitamins, renal failure, hypothyroidism and alcoholism [139]. Moderate hyperhomocysteinemia has been associated with decrease NO bioavailability, endothelial dysfunction [140] and increased cardiovascular risk [141, 142]. Besides generation of oxidative stress, hyperhomocysteinemia has been proposed to cause endothelial dysfunction through ADMA-mediated mechanisms. As shown in Fig. (**1**), methylation of protein arginine residues by PRMTs leads to formation of S-adenosyl-L-homocysteine as a by-product which is further hydrolyzed to L-homocysteine. L-homocysteine is remethylated to L-methionine which is activated to S-adenosyl-L-methionine that could be used as a substrate for PRMTs to catalyze protein arginine methylation [140]. Therefore, a reasonable hypothesis would be that situations associated with increased ADMA synthesis would in parallel induce homocysteine production, while increased bioavailability of homocysteine via its remethylation would provide adequate S-adenosyl-L-methionine as a methyl donor for protein arginine methylation and ADMA formation. In experimental studies homocysteine has been also found to inhibit DDAH activity through an oxidative reaction in its active cysteine residue [143], as well as through methylation in the promoter region of DDAH-2 gene [144]. DDAH-1 and DDAH-2 overexpression has also been reported to ameliorate endothelial dysfunction induced by hyperhomocysteinemia [145, 146]. However, Dayal *et al.*, demonstrated that the observed downregulation of DDAH activity in mice with endothelial dysfunction is tissue-specific, applying mostly in the liver and is not capable of reducing ADMA levels [147] and a more recent study showed that methionine loading cause hyperhomocysteinemia and endothelial dysfunction but not ADMA elevation in a rat model [148]. Interestingly, clinical studies have not revealed a direct relationship between the endothelial dysfunction caused by hyperhomocysteinemia and ADMA [149-151] whereas it has been indicated that the observed association between increased ADMA levels and hyperhomocysteinemia applies only among subjects with renal failure and is secondary to the impaired renal function [81-83]. In conclusion, the role of ADMA-homocysteine interplay in the pathogenesis of endothelial dysfunction is yet unclear, but current data do not support a direct interaction.

**Chronic Kidney Disease**

Since methylarginines are partially eliminated through renal excretion it is not surprising that elevated ADMA levels have been reported among subjects with renal failure [45]. This increase in ADMA may explain the susceptibility of end-stage renal disease patients to cardiovascular disease and atherosclerosis [152]. Indeed, in hemodialysis patients, serum ADMA levels have been found to be a significant predictor of all-cause as well as cardiovascular mortality [87]. Furthermore, ADMA, besides increasing risk for cardiovascular adverse outcomes in renal failure, is a strong predictor of the progression of chronic kidney disease, as shown by clinical studies [88, 89]. This is also supported by experimental data in rats that have revealed an association of higher plasma ADMA levels with peritubular capillary loss, tubulointerstitial fibrosis and proteinuria. Interestingly, overexpression of DDAH-1 ameliorated these effects [153]. In human studies, specific genetic polymorphisms of DDAH-1 and DDAH-2 have been associated with chronic kidney disease progression, an effect possibly exerted due to renal micro-vascular damage caused by ADMA accumulation [90, 153]. However, the respective DDAH-1 polymorphism was unexpectedly related to decrease ADMA levels [153]. ADMA possibly plays a role in the pathophysiology of renal disease via mechanisms other than competitive inhibition of eNOS. In particular, it was revealed that ADMA inhibits eNOS phosphorylation at Serine-1177, via suppression of extracellular signal-related protein kinase (ERK), a major kinase for eNOS phopshorylation, thus leading to decreased eNOS activation [154]. Another molecule, fibroblast growth factor 23, that has been previously linked to renal failure, was reported to interact in the relationship between ADMA and kidney injury progression [155]. In conclusion, current literature supports the role of ADMA in progression and possibly generation of chronic kidney disease as well as in the endothelial dysfunction and atherosclerotic adverse outcomes accompanying it, but further research is required to understand in depth the pathophysiology of these phenomena.

**Preeclampsia**

Despite the fact that the pathogenesis of preeclampsia remains obscure, it is well established that it is a disorder associated with vascular pathology and endothelial dysfunction of the placenta [156]. Human studies have explored whether ADMA plays a role in the pathophysiology of preeclampsia. The majority of the relevant literature demonstrates elevated ADMA levels among women with preeclampsia [100, 101, 157], but there are also references reporting no significant difference between preeclamptic and non-preeclamptic pregnant [102, 158]. Interestingly, elevated ADMA levels during the first and second trimester of pregnancy may have predictive value in recognizing women at higher risk of developing preeclampsia [103, 159, 160]. It is possible that elevated ADMA levels are attributed to its decreased degradation by DDAH enzymes, since DDAH-1 and DDAH-2 mRNA expression is significantly lower in the placental cells of preeclamptic pregnant women [104]. The same study also explored PRMT-1 expression in preeclampsia but no significant difference with healthy pregnant was detected, implying that this pathway does not contribute to ADMA accumulation in this settings [104]. It is anticipated that further research will clarify the clinical significance of circulating ADMA as a biomarker for prediction of preeclampsia and will give insight to its pathophysiological role in the disease.

**Alzheimer's Disease**

Alzheimer's disease is a neurodegenerative disease and the most common type of dementia in the elderly population. As aforementioned, ADMA inhibits nNOS and NO formation in the neurons, which is considered significant for synaptic plasticity. However, over-production of NO may lead to neurodegeneration, implying therefore that its metabolism is critical for balancing its levels [161].

Experimental data show that accumulated ADMA in the neurons stimulates pathogenesis of Alzheimer's disease.

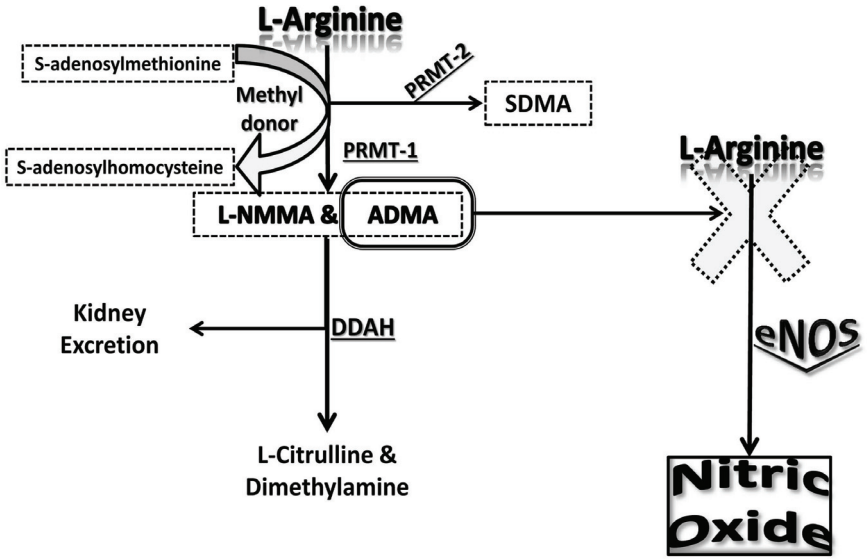


**Fig. (1). Overview of biosynthesis, metabolism, excretion and actions of Asymmetric dimethylarginine.** Arginine residues within proteins are methylated by PRMTs in a two-step reaction leading to the asymmentric and symmetric dimethylation, respectively. PRMTs catalyze these reactions utilizing a methyl group by SAM while producing SAH as a by-product. L-methionine is activated to SAM, while SAH is hydrolyzed to L-homocysteine. L-homocysteine can be further re-methylated to L-methionine. Proteolysis of proteins containing monomethylated arginine residues, which escaped second methylation, results in production of L-NMMA, while proteolysis of proteins containing dimethylated arginine residues leads to the formation of ADMA and SDMA. ADMA may be hydrolyzed by DDAH-1 and -2 to L-citrulline and dimethylamine or transaminated by AGTX2 to DMGV in the mitochondria. ADMA is also transported through cell membrane via CATs to extracellular fluid, circulation and subsequently other tissues. ADMA (and L-NMMA) are competitive inhibitors of all three isoforms of nitric oxide synthase (neuronal, endothelial and inducible), thus blocking the generation of NO. PRMT: Protein arginine methyltransferase; L-NMMA: N-monomethyl-L-arginine; ADMA: Asymmetric dimethylarginine; SDMA: Symmetric dimethylarginine; eNOS: Endothelial Nitric Oxide synthase; nNOS: Neuronal Nitric Oxide synthase; iNOS: Inducible Nitric Oxide synthase; DDAH: dimethylarginine dimethylaminohydrolase; AGXT2: Alanine glyoxylate aminotrasferase; DMGV: dimethyl-guanidino valeric acid; CAT: Cationic amino acid transporter; SAM: S-adenosyl-L-methionine ; SAH: S-adenosyl-L-homocysteine; NO: Nitric oxide; "Me" indicates methyl groups and "(Me)2" asymmetric dimethylation; "A" within proteins indicates arginine residues.

Specifically, a recent study demonstrates that increased ADMA levels in neurons promote beta-amyloid (Aβ) secretion, Aβ-induced oxidative stress and neurotoxicity. In this study, overexpression of DDAH-1, but not knockdown of type I PRMT, attenuated these phenomena [162]. In addition, Pin1 inhibits the production of amyloid-beta besides enhancing eNOS activity which further inhibits accumulation of amyloid-beta, showing that Pin1 may act beneficially against Alzheimer's disease [42]. Furthermore, it was found that increased homocysteine, which shares an established association with risk for Alzheimer's disease, blocks DDAH activity resulting in accumulation of ADMA and decrease of NO production in the brain. It is not clear though, whether this mechanism plays a significant role *in vivo* for the pathogenesis of Alzheimer's disease [163]. Nevertheless, protein tyrosine nitration, that constitutes a post-translational modification associated with neurodegenerative diseases, was found to induce oxidative stress and neurotoxicity through activation of the DDAH/ADMA/NOS/NO pathway leading to accumulation of NO [164].

However, only a few clinical studies of small samples have assessed serum and cerebrospinal fluid levels of ADMA in patients with Alzheimer's disease compared to controls. These studies show equivocal findings. Regarding serum ADMA levels, they have been reported higher among Alzheimer's disease patients in two of the three studies examining this relationship [105, 106], while the third study revealed no significant difference [165]. In another study increased levels of ADMA were associated with subjective memory complaints, a common symptom of dementia [108]. On the other hand cerebrospinal fluid levels of ADMA were decreased among patients with Alzheimer's disease compare to controls in two studies [105, 107], while no association was found in a third study [166]. Despite these findings come from studies with relatively small samples they possibly imply two different mechanisms for participation of ADMA in Alzheimer's disease pathogenesis. First, increased ADMA in the endothelium of cerebral vessels leads to endothelial dysfunction and cerebral angiopathy that is a known risk factor for Alzheimer's disease. Secondly, through decreased ADMA in the cerebral parenchyma, it is possible that nNOS is induced to form NO in levels of toxicity leading to neurodegeneration.

In conclusion, future research is in need in order to clarify the role of ADMA on neurodegeneration. Current evidence suggests that ADMA levels in the brain parenchyma should be kept at middle levels in order to settle a balance between activation and inhibition of NO production and preserve neuronal health.

### Pulmonary Angioproliferative/Fibrotic Disorders

A significant number of studies have associated ADMA levels with idiopathic pulmonary hypertension, and secondary pulmonary hypertension due to HIV and systematic sclerosis [96-99, 167, 168]. In addition, a recent meta-analysis showed that among patients with pulmonary hypertension due to congenital heart disease, serum ADMA levels were significantly higher compared to healthy controls and demonstrated that it has the potential to be a useful biomarker of the disease [169]. Interestingly, Pullamsetti *et al.* showed that ADMA is not only elevated in the serum of patients with pulmonary hypertension, but also in the intracellular level in lung tissue [170]. The same authors reported reduced mRNA and protein expression of DDAH in pulmonary hypertension [170], a finding further supported by experimental research [171-173]. DDAH-1 deficiency or inhibition also leads to increase of right ventricular pressure revealing an association with pathology of pulmonary vasculature [174]. In addition to vasoconstriction of pulmonary vessels, ADMA has also been reported to act via other mechanisms on the pulmonary endothelium. Particularly, ADMA inhibited protein expression and membrane localization of connexin 43, a gap junctional protein in the endothelium, increasing permeability, and decreasing angiogenesis [175]. Lastly, in pulmonary endothelium ADMA may act as a pro-proliferative molecule, since it has been reported to enhance urea production resulting in more viable cells [176].

Idiopathic pulmonary fibrosis is characterized by fibroblast proliferation as well as injury and inflammation of the alveolar epithelium [177]. NO has been reported to increase after bleomycin-induced acute lung injury in response to an increase of eNOS and iNOS expression [178], while it has also been implied to induce fibro-proliferation [179]. Hence, an increase in NO bioavailability may play a significant role in the pathogenesis of idiopathic pulmonary fibrosis. In mice and patients with idiopathic pulmonary fibrosis, an up-regulation of DDAH enzymes was detected in alveolar epithelial type II cells which was accompanied by an increase of the expression of colocalized iNOS through an ADMA-dependent pathway [180]. Administration of a DDAH inhibitor reduced collagen deposition and abnormal epithelial proliferation while enhanced lung function in mice [180]. ADMA may therefore have a beneficial effect against pulmonary fibrosis by decreasing NO generation.

### Neoplasms

There is accumulating evidence that NO production and NOS activity are positively correlated with human neoplasms [181-187]. Indeed, there is evidence that NO promotes mutagenesis, has anti-apoptotic effects, enhances tumor angiogenesis, suppresses immunological response against the neoplasmatic cells and induces neoplasm metastasis. [188]. Therefore, elevation of ADMA levels aiming to knockdown NO formation could possibly represent a promising target for suppressing tumor cell metabolism. Interestingly, over-expression of DDAH-1 has been found to enhance neovascularization as well as the growth rate of the tumor [189-191]. Furthermore, recent experimental data demonstrate that DDAH-1 induces angiogenesis via vascular endothelial growth factor stimulation through an ADMA dependent mechanism, but its effect on tumor growth is independent of ADMA metabolism. Hence, it is possible that unidentified mechanisms other than degradation of ADMA mediate the effects of DDAH-1 on carcinogenesis [192]. The issue is more complicated though, since recent studies suggest that patients with cancer have higher serum ADMA levels than controls [193-195], while ADMA has been reported to have anti-apoptotic effects and contribute to the resistance to chemotherapy of colon cancer cells [195].

## THERAPEUTIC APPROACHES TARGETING ADMA

Since ADMA has a key role in the pathophysiology of endothelial dysfunction and its serum levels have been related to cardiovascular risk and prognosis, it has been hypothesized that established cardiovascular and cardio protective treatments as well as novel therapeutic approaches capable to down regulate its levels would be of clinical benefit. However, results from studies are not consistent as significant inter-study differences exist concerning the study population, the dose and administration way of the therapeutic agents etc. Furthermore, it is difficult to directly connect the decrease in ADMA levels with the clinical benefits observed as most of the studying agents have a broad spectrum of effects and pleiotropic actions. The complex interplay between therapeutic regiments, ADMA levels and clinical effects are summarized in Table **2** and are further discuss in the following section.

### Statins

Statins are considered to exert their beneficial clinical effects via the inhibition of HMG-CoA reductace leading to decrease in cholesterol biosynthesis and consequently reduction of serum LDL-cholesterol levels. However, statins have also been suggested to enhance endothelial function through pleiotropic actions independent of LDL cholesterol reduction. These include up-regulation of eNOS expression, anti-oxidative effects, up-regulation of cyclo-oxygenase-2 and prostacyclin, decrease in endothelin-1 bioavailability and increase in BH4 levels in vascular endothelium [224-227].

The impact of statins on ADMA was firstly documented on experimental models. Rosuvastatin significantly decreased ADMA levels in spontaneously hypertensive rats independently of any lowering in cholesterol levels effect [228]. In another experimental study, rosuvastatin decreased to normal values the elevated ADMA levels in dogs with atrial fibrillation [229]. Atorvastatin has also been reported to modulate DDAH/ADMA pathway in insulin-resistant rats by reversing low DDAH levels, low DDAH-1 aortic expression and high ADMA levels, suggesting that DDAH may be another target of statins through which they exert their anti-atherogenic actions [196].

The effect of statins on ADMA levels has also been examined in a number of studies on human subjects but the results are controversial. In the majority of them no impact was observed on ADMA levels with statins [197], but more recent randomized-control trials reported opposing findings. A significant reduction of ADMA levels after 1 month under simvastatin treatment in patients with hypercholesterolemia was reported. It is possible that simvastatin effects on ADMA levels are dose-dependent since in both studies the

**Table 2. Synopsis of the main treatments capable to modify asymmetric dimethylarginine levels; main findings.**

| Study | Treatment | Subjects | Main Findings |
|---|---|---|---|
| Chen *et al.* [196] | Atorvastatin 30mg/kg/day for 8 weeks | Insulin resistant rats | Atorvastatin inhibits the increase in ADMA levels by almost 50% and enhances the DDAH activity by 18% |
| Young *et al.* [197] | Atorvastatin 40mg/day for 6 weeks | 24 chronic heart failure subjects | No change in ADMA levels |
| Lu *et al.* [198] | Rosuvastatin 10mg/day for 6 weeks | 46 patients with elevated low density lipoprotein cholesterol levels | Rosuvastatin decreases ADMA levels by almost 18% |
| Yang *et al.* [199] | Fonofibrate | Cultured human umbilical vein endothelial cells incubated with oxidized LDL cholesterol and pretreated with fenofibrate (3, 10 or 30 microM) | Pretreatment with fenofibrate inhibited the oxidized-LDL-mediated increase in ADMA |
| Yang *et al.* [200] | Fenofibrate 200mg/day for 8 weeks | 45 subjects with hypertriglyceridemia | Treatment with fenofibrate decreases the levels of ADMA by 15% |
| Dierkes *et al.* [201] | Fenofibrate 200mg/day for 6 weeks | 25 hypertriglyceridemic men | Has no effect on serum ADMA levels but increases the plasma L-arginine/ADMA ratio |
| Westphal *et al.* [202] | Niacin 375mg/day increased to 2000 mg/day over a period of 16 weeks | 26 patients with low HDL cholesterol level | Treatment decreases ADMA levels by almost 10% |
| Fujii *et al.* [203] | renin-angiotensin system inhibitors for 3 months | 23 normotensive patients with chronic glomerulonephritis and normal or mildly impaired renal function | Treatment decrease ADMA levels by almost 10% |
| Delles *et al.* [204] | Enalapril 20mg/day for 1 week | 20 mildly hypertensive young male subjects | Enalapril significantly reduces ADMA levels by 16% |
| Wakino *et al.* [205] | Pioglitazone for 4 weeks | Wister-Kyoto rats and spontaneously hypertensive rats | Treatment decreases ADMA levels in both group by almost 15% |
| Wang *et al.* [206] | Rosiglitazone (3, 10 or 30mg/kg) for 6 weeks | Streptozotocin-induced diabetic rats | Rosiglitazone had no impact in the ADMA levels |
| Wang *et al.* [207] | Rosiglitazone 4mg/day for 8 weeks | 70 non diabetic subjects with metabolic syndrome randomized to either rosiglitazone or placebo | Treatment decreases ADMA by 16% |
| Asagami *et al.* [208] | Metformin at maximal effective dose for 3 months | 31 patients with poorly controlled type 2 diabetes mellitus | Metformin decreases ADMA levels by 27% |
| Cakirca *et al.* [209] | Vildagliptin 100mg/day was added to metformin treatment for 6 months | 68 patients with type 2 diabetes mellitus (33 were assigned to Vildagliptin) | ADMA levels were lower in the Vildagliptin group by 25% |
| Khan *et al.* [210] | Nebivolol for 24 weeks starting with 5mg/day and titrated to 20mg/day | 42 hypertensive African Americans | ADMA levels are decreased by 44% |
| Sen *et al.* [211] | Nebivolol (5mg/day) versus metoprolol (50mg/day) for 12 weeks | 38 patients with cardiac syndrome X were randomized to nebivolol (19 subjects) or metoprolol | Nebivolol reduces ADMA levels by 37% |
| Oguz *et al.* [212] | Nebivolol (5mg/day) or metoprolol (100mg/day) for 12 weeks | 54 patients with type 2 diabetes mellitus were randomized to nebivolol (28 subjects) or metoprolol | In nebivolol group there is no significant changes in serum ADMA levels. |

**(Table 2) contd….**

| Study | Treatment | Subjects | Main Findings |
|---|---|---|---|
| Deng *et al.* [213] | Pretreatment with acetylsalicylic acid (30 or 100 mg/kg/day) for 5 days | Rats with LDL induced vascular endothelial injury | Acetylsalicylic acid inhibits the LDL induced increase in ADMA levels (a relative decrease by 25%) |
| Hetzel *et al.* [214] | Treatment with acetylsalicylic acid range from 81mg/day to 1300 mg/day for 12 weeks | 37 patients with stable coronary artery disease | A mean reduction of ADMA levels by 30% is observed |
| Holden *et al.* [215] | Subcutaneous insertion of a 100-mg ethynylestradiol implant for 2 weeks | 15 postmenopausal women | A decrease in ADMA level by 20% is observed |
| Post *et al.* [216] | Oral 17beta-estradiol 2 mg/day in various combination or placebo for 12 weeks | 60 healthy early postmenopausal women (16 in the control group) | ADMA levels are reduced by 18.7% |
| Wu *et al.* [217] | Folic acid 5mg/day and vitamin 12 500μg/fay for 12 weeks | 120 patients with hypertension | ADMA levels were decreased by 14% |
| Ziegler [218] | A mixture of vitamin-B (50 mg vitamin-B1, 50 mg vitamin-B6, 0.05 mg vit-B12/day) and folic acid (5 mg/day) for 6 weeks | 49 subjects with peripheral arterial disease, stable intermittent claudication and fasting plasma total Homocysteine concentration > 15 μmol/liter | Treatment has no effect on ADMA levels |
| Mittermayer *et al.* [219] | Intravenously administered alpha-lipoic acid 600mg/day for 3 weeks | 30 patients with type 2 diabetes mellitus | Treatment decreases ADMA levels by 9% |
| Thaha *et al.* [220] | N-acetylcysteine intravenously infused during hemodialysis | 40 patients with end stage renal disease | N-acetylcysteine induces a greater decrease in ADMA levels by 30% |
| Nascimento *et al.* [221] | Oral N-acetylcysteine (1200mg/day) for 8 weeks | 22 patients on peritoneal dialysis | Treatment has no effect on ADMA levels |
| Tain *et al.* [222] | Malatonin 0.01% in drinking water for 8 weeks | Spontaneous hypertensive rats | Plasma ADMA levels are decreased by 20% |
| Han *et al.* [223] | Resveratrol 50mg/litter in drinking water (approximately 7-7.5 mg/Kg/day) for 12 weeks | Hypertensive rats | Resveratrol decrease ADMA levels by 50% |

ADMA: Asymmetric dimethylarginine; DDAH: dimethyl-arginine-dimethyl-aminohydrolase LDL: low density lipoprotein; HDL: high density lipoprotein;

dose of 80 mg daily managed to achieve a statistically significant reduction in contrast to 40 mg daily [230]. In another study including patients with hypercholesterolemia rosuvastatin appeared to reduce ADMA levels as well [198]. Interestingly, a recent trial revealed a potential advantage of rosuvastatin versus atorvastatin in decreasing ADMA levels in patients with hyperlipidemia and coronary artery disease, even though both drugs had a significant effect [231]. Finally, in ischemic stroke patients statin treatment is associated with decreased ADMA serum concentration independently of several atherosclerotic risk factors and this was combined with an adequately controlled lipid profile [232].

ADMA may also modulate the therapeutic response to statin treatment regarding endothelium-mediated vasodilatation. As noted above, statins induce vasodilatation via eNOS up-regulation. In a study, ADMA was found to be a significant determinant of enhancement of endothelial function in patients undergoing treatment with simvastatin, since only in those with low ADMA serum levels the endothelial function was improved [233]. This may suggest that even though eNOS is up-regulated after statin therapy, it may be incapable of acting under elevated ADMA concentration.

## Fibrates

Fibrates are widely used for the treatment of dyslipidemia and specifically hypertriglyceridemia. They mainly act through activation of peroxisome proliferator-activated receptor alpha. Moreover, fibrates have anti-oxidant and anti-inflammatory effects and therefore act protectively for the endothelium. Since ADMA is a key molecule for the maintenance of endothelial homeostasis, it has been hypothesized that fibrates may modulate endothelial function by influencing ADMA. It has been found that treatment with fenofibrate reduces ADMA levels in rats with endothelial dysfunction [234] and this effect has been verified in a clinical trial of hypertriglyceridemic patients [200]. A proposed mechanism includes the activation of DDAH possibly by the decrease in

nuclear factor kappa-beta activity due to the activation of PPAR-a, as it was shown in an experimental study of cultured human umbilical vein endothelial cells with LDL-induced endothelial injury [199]. Nevertheless, a previous study had failed to prove that fenofibrate had an impact on ADMA levels, but reported improved endothelial function as a result of increased L-arginine concentration and L-arginine to ADMA ratio [201]. The neutral impact of fenofibrate in the ADMA levels may be explained by the increase in the LDL cholesterol concentration after treatment with fenofibrate, that was observed only in the latest study, as LDL cholesterol tent to increase ADMA plasma concentrations.

### Niacin

Niacin is an agent used for the treatment of dyslipidemia and is the most powerful HDL-raising drug currently available. In addition, it reduces triglycerides, Lipoprotein-a and LDL cholesterol levels. Despite the fact that niacin is used for decades, its exact mechanism of action remains unclear. It is believed that it enhances endothelial function independently of the changes in plasma lipids [235]. The only study examining the association between niacin treatment and ADMA concentration reported a significant dose-dependent decrease in ADMA plasma levels of patients with low HDL cholesterol levels after 6 weeks intervention with niacin [202]. The proposed by the authors' underlying mechanism of this effect is based on the fact that the metabolism of niacin requires a considerable amount of methyl groups. As a result, S-adenosylmethionine, serving as the methyl donor, is depleted and becomes unavailable for the methylation of proteins, including methylarginines. Therefore, niacin may reduce ADMA levels by inhibiting its synthesis.

### Inhibitors of the Renin-Angiotensin-Aldosterone System

Angiotensin Converting Enzyme Inhibitors (ACEIs) and Angiotensin Receptor Blockers (ARBs) are antihypertensive agents. ACEIs block the formation of angiotensin II by inhibiting angiotensin converting enzyme and impede activation of angiotensin I receptors in the adrenal cortex. Therefore, they not only reduce vasoconstriction but also aldosterone release. Angiotensin II receptor blockers (ARBs) displace angiotensin II from the angiotensin I receptor and reduce blood pressure by preventing angiotensin I receptor induced vasoconstriction, aldosterone, catecholamines and arginine-vasopressin release, water intake and hypertrophic responses. However, it has now been established that ACEIs and ARBs act as cardio and renal protective agents and enhance endothelial function, independently of reducing blood pressure levels, through different mechanisms [236].

A number of clinical studies have shown that treatment with ACEIs and ARBs reduces serum ADMA levels. Specifically, ADMA levels have been decreased in patients with chronic kidney disease, hypertension and other cardiovascular risk factors [203, 204].

It has also been shown that ACEIs and ARBs improve endothelial function by increasing NO bioavailability. One proposed mechanism is the decrease in ADMA levels which is possibly mediated by enhancement of DDAH activity. DDAH activity is diminished by ROS [57] and ACEIs and ARBs have been reported to decrease ROS generation by endothelium [237]. This is reinforced by an experimental study in rats with proteinuric nephropathy, in which an increase in DDAH-1 mRNA was observed after treatment with losartan (ARB) and this effect was associated with reduction of reactive oxygen species (ROS) [238]. It has also been suggested that ADMA activates the vascular renin-angiotensin system leading to activation of NADPH-nicotinamide-adenine-dinucleotide phosphate oxidase and generation of oxidative stress [239]. This phenomenon may be mediated by ADMA-induced up-regulation of Angiotensin Converting Enzyme [114]. Therefore, ACEIs and ARBs could impede ADMA as well. Another study on hypertensive rats claims that reduction of ADMA levels is an important pathway through which losartan exerts its cardioprotective effects [240].

Due to ACEIs and ARBs inability to completely block renin-angiotensin-aldosterone system, they do not provide absolute protection against endothelial dysfunction. Aliskirin, a renin inhibitor could theoretically provide a better blockade. In recent experimental studies, aliskirin reduces circulating ADMA levels in hypertensive rats [241].

### Blood Glucose Lowering Medications

ADMA and NO have been found to be significant determinants of insulin resistance, a common feature of diabetes mellitus type 2 [242]. Moreover, ADMA levels have been recorded higher than normal in subjects with diabetes mellitus type 2 [70]. Insulin resistance has been related to endothelial dysfunction and accordingly, drugs increasing insulin sensitivity may improve endothelial function. Thiazolidinedones (or glitazones), which are activators of peroxisome proliferator receptor-γ, and metformin, which exerts its effects via suppressing hepatic gluconeogenesis have been proposed to act independently of these major mechanisms in a beneficial manner for the endothelium.

Since ADMA levels may be associated with insulin resistance and thiazolidinedones enhance insulin sensitivity, it was investigated whether they decrease ADMA levels. Pioglitazone reduces ADMA levels by 20% in both spontaneously hypertensive and normotensive rats [205]. Simultaneously, it increases DDAH-2 renal expression, implying that up-regulation of DDAH-2 in renal tubular cells by pioglitazone may lead to ADMA catabolism and ameliorates endothelial function. On the other hand, rosiglitazone has no impact on serum ADMA levels in diabetic rats, but reverses endothelial dysfunction by inhibiting the increased activity of nuclear factor-kappaB and returned to normal the elevated levels of tumor necrosis factor-alpha and intercellular adhesion molecule-1 [206]. It was found though, that these actions were mediated by the effect of ADMA on the vascular wall. Therefore, rosiglitazone may not reduce its plasma levels, but suppresses its deleterious effects. However, in another study, rosiglitazone significantly decreases ADMA levels in rats with dyslipidemia and this effect was even more profound after combining rosiglitazone with atorvastatin suggesting a possible synergistic role of the two drugs [243].

Data from clinical research studies on the effect of thiazolidinediones on ADMA are controversial as well. Although there is evidence that rosiglitazone after 8 weeks of

treatment reduces plasma ADMA levels in 70 non-diabetic patients with metabolic syndrome [207] and 7 insulin-resistant hypertensive patients [92], in most clinical studies no effect on ADMA levels has been observed after treatment with either pioglitazone or rosiglitazone [244, 245]. Moreover, thiazolidinediones present a high risk of liver toxicity and detrimental effects in the cardiovascular system and therefore the only in use thiazolidinedione at the moment is pioglitazone [246]. These side effects of thiazolidinediones may also explain the aforementioned discrepancies in studies concerning changes in ADMA levels.

Metformin is a glucose lowering drug from the family of biguanides, which is now considered as the first line oral drug for the treatment of diabetes mellitus type 2. Its main mechanism of action is believed to be the inhibition of gluconeogenesis, thus leading to decrease glucose production by the liver [247]. Even though the exact target of metformin remained unknown for years, there is now evidence that it acts in the mitochondria of hepatocytes by inhibiting respiratory-chain complex 1 [248, 249]. In particular, this action leads to a decrease in cellular energy status, which further stimulates the activation of the AMP- activated protein kinase resulting in a switching of the cells from an anabolic to a catabolic state [250]. Metformin has also been reported to reduce ADMA levels, thus acting protectively for the endothelium. Specifically, metformin has been reported to decrease ADMA and blood pressure levels in spontaneously hypertensive rats. This effect was not mediated by either DDAH or PRMT enzymes [251]. In addition, metformin administration for one week has been found to reduce ADMA tissue levels, increase DDAH activity and attenuate histopathological lesions in rat with experimentally induced liver injury [252]. Interestingly, it has been suggested that metformin acts as a competitive antagonist of ADMA [253]. This is based on the fact that the two molecules are chemical structural analogues and they have opposing cardiometabolic effects [253].

There is also evidence that metformin decreases plasma ADMA concentration in humans with polycystic ovaries syndrome [254-256] and in patients with diabetes mellitus type 2 [208]. On the contrary, Lund *et al.*, reported no change in ADMA levels of patients with type 2 diabetes mellitus after administration of metformin for 4 months [257]. Moreover, the ADMA levels were similar among patients with no difference in their glycemic control receiving either metformin either repaglinide [257]. Worth noting, a study among patients with diabetes mellitus type 2 and stable coronary artery disease revealed that patients receiving metformin have higher circulating ADMA levels as compared to subjects not receiving metformin [258]. Therefore, it remains obscure whether metformin actually exerts part of its beneficial action via regulation of ADMA.

Lastly, there are references for vildagliptin and aminoguanidine that they modulate ADMA levels. A recent study found that vildagliptin, an anti-hyperglycemic agent belonging to the dipeptidyl peptidase-4 inhibitors, when combined with metformin cumulatively reduces plasma ADMA in a lower level compared to metformin alone in patients with diabetes mellitus type 2 [209]. Aminoguanidine, as shown in an experimental study in rats, improves endothelial function, increases DDAH activity and prevents elevation of ADMA levels [259].

Generally, high glucose levels promote impairment of endothelial function and this may be mediated in part by ADMA. In a study of 24 patients with diabetes mellitus type 2, it was found that intensive control of glucose levels is more effective in enhancing endothelial function and decreasing ADMA levels than conventional therapy [260].

**Third Generation β-Blockers (Nebivolol, Carvedilol)**

Nebivolol is a selective β1-adrenoreceptor antagonist, but there is evidence that it exerts its cardioprotective activity via various different pathways, including NO-dependent vasodilatation and oxidative stress reduction [261]. ADMA is a potential target for nebivolol, since several clinical studies state that it reduces its circulating levels. Specifically, ADMA levels decrease after treatment with nebivolol in patients with hypertension [210] and in patients with cardiac syndrome X [211], but the drug has no impact on ADMA levels in patients with diabetes mellitus type 2 [212]. However, in this trial of diabetic patients, the group treated with nebivolol did not have an observable change in ADMA levels, whereas in patients treated with metoprolol (a selective β1-blocker) an increase in ADMA levels was observed, implying that nebivolol may inhibit ADMA increase.

It was first proposed that nebivolol attenuated ADMA levels by up-regulating DDAH-2 expression and activity and this hypothesis was verified in a double-blind randomized study which compared 40 essentially hypertensive patients treated with nebivolol or atenolol (a selective β1-blocker) [262]. In this study only patients on nebivolol treatment decreased ADMA levels, increased DDAH-2 expression, enhanced endothelial function (measured by FMD) and increased eNOS activity. Recently an experimental study in spontaneously hypertensive rats found that nebivolol not only augments ADMA hydrolysis by increasing DDAH-2 expression, but also inhibits its generation via down-regulating PRMT-1 expression [263].

Carvedilol, which is a non-selective β-blocker with additional α1-adrenoreceptor antagonist activity, has not yet been in depth investigated about its actions in endothelial function and especially ADMA. In one study that included 22 patients with heart failure, a decrease in ADMA levels was observed after carvedilol treatment but only in patients that recorded treatment response and improvement of heart failure status [264]. Nevertheless, in hypertensive patients undergoing combined carvedilol-lisinopril therapy, no difference was recorded in ADMA levels after seven months [265].

**Acetylsalicylic Acid**

Acetylsalicylic acid is considered to act cardioprotectively mainly through inhibition of cyclooxygenase (COX)-1 leading to decrease synthesis of thromboxane-A2 and attenuation of platelet aggregation. Acetylsalicylic acid can also improve endothelial function. The proposed mechanisms include ADMA regulation. In a study in rats, acetylsalicylic acid at the dose of 30 mg/kg reduced LDL-derived endothelium damage through a decrease in ADMA levels via increasing DDAH activity [213]. Moreover, acetylsalicylic

acid reduces endothelial cell senescence in parallel with elevation of ADMA levels and up-regulation of DDAH activity [266]. Since, ADMA promotes cell senescence it is possible that reduction of endothelial cell senescence by acetylsalicylic acid may be mediated via reduction in ADMA levels. However, acetylsalicylic acid inhibits also endothelial cell senescence via up-regulation of eNOS expression [267]. The aforementioned mechanisms have been further confirmed by clinical studies that have found a decrease in ADMA levels in coronary artery disease patients when treated with aspirin [214].

**Hormone Replacement Therapy**

Estrogen deficiency after menopause may be responsible for the sharp increase in cardiovascular risk in post-menopausal women. The fact that premenopausal women have lower ADMA levels than men of the same age indicates a possible effect of estrogens on ADMA levels [268]. It has been shown that estrogens increase DDAH activation and thus inhibit ADMA accumulation [215]. Estradiol has been found *in vitro* to attenuate the activity of oxidized LDL on endothelium, inhibit DDAH activity and as a result increases ADMA concentration and reduces NO bioavailability [269]. Furthermore, it has been shown that estradiol inhibits DDAH through estrogen receptor alpha (ERα), since its effects were abolished when this receptor was blocked by antagonists [270].

These experimental findings have been verified *in vivo*, since a wide number of clinical studies indicates that hormone replacement therapy regardless of its type, its route of administration, its dose and the progesterone addition or not, decreases significantly ADMA levels [215, 216]. However, progesterone addition [216] seem to cause a greater decline. Lastly, there is evidence that the relationship between estrogen therapy and ADMA levels decline is dose-dependent [215].

**Antioxidants, Folate, Vitamin B12 and Omega-3-Polyunsaturated Fatty Acids**

Several molecules such as vitamins C and E, beta-carotene, ascorbic acid and alpha-lipoic acid have been characterized for their antioxidant capacity. It has been demonstrated that oral administration of vitamin C and/or vitamin E improves endothelial function and these vitamins are considered to have anti-oxidant, anti-thrombotic and anti-inflammatory effects [271]. It is unclear if they have any impact on ADMA levels though. The results from two clinical trials are conflicting. In the first of them that included patients with mild to moderate chronic kidney disease, vitamin E was found to significantly reduce ADMA levels by 4% [272]. When vitamin E was combined with ascorbic acid and beta-carotene and was given to patients with mildly high homocysteine levels, ADMA levels were not altered [273].

Folate and vitamins of complex B are administered as a homocysteine-lowering treatment. They have been studied about their potential to reduce ADMA levels in cardiovascular disease. There is evidence that folate either alone either when combined with methylcobalamine (a form of vitamin B12) decreases ADMA concentration in patients with hyperhomocysteinaemia [274], and essential hypertension [275]. On the contrary, no impact of this treatment on ADMA levels was proven in children with familiar hypercholesterolemia or diabetes mellitus type 1 [79], patients with mild to moderate chronic kidney disease [272] and patients with peripheral artery disease [218]. Nevertheless, folate's circulating metabolite, 5-methyltetrahydrofolate, when infused in patients with chronic heart failure and controls, led to an acute significant decline of ADMA levels [276].

Accumulating evidence suggests that alpha-lipoic acid, a molecule naturally produced in animals, possesses antioxidant properties and acts beneficially against endothelial dysfunction [277, 278]. A number of studies have explored whether this effect is partially mediated by ADMA. In one of them alpha-lipoic acid was found to reverse the increase of ADMA levels observed in high fructose-fed rats [279], while in cultured endothelial cells, alpha-lipoic acid decreased ADMA concentration via activation and promotion of DDAH gene expression [280]. Specifically, this effect was mediated by increasing activity of signal transducer and activator of transcription 3, which is bind to DDAH2 gene promoter and amplified the expression of DDAH2 [280]. Likewise, clinical studies have reported a decrease in ADMA levels after supplementation with alpha-lipoic acid in patients with end-stage renal disease as well as patients with type 2 diabetes mellitus [219, 281]. However, in another study of patients with chronic renal failure under hemodialysis, alpha-lipoic acid was not found to have any effect on ADMA levels [282].

N-acetylcysteine is another molecule with reported direct and indirect antioxidant effects [283]. In human umbilical vein endothelial cells incubated with ADMA, the observed stimulation of senescence was reserved if N-acetylcysteine was pre-incubated [284]. Moreover, N-acetylcysteine attenuates the increase in ADMA levels observed *in vivo* in human renal proximal tubular epithelial cells after Adriamycin treatment [285]. It has also been reported that N-acetylcysteine inhibits the suppression of DDAH2 expression induced by advanced glycation end products, thus blocking accumulation of ADMA in cultured endothelial cells [286]. Similarly, a study of ischemia/reperfusion-injured mice showed that N-acetylcysteine did not allow elevation of ADMA levels by inhibiting the knockdown of DDAH1 enzyme in the kidneys [287]. In spontaneously hypertensive rats, N-acetylcysteine restores DDAH activity and decreases ADMA levels, oxidative stress and blood pressure [288]. Therefore, experimental studies support that N-acetylcysteine inhibits accumulation of ADMA. It is not clear though, whether this is a direct interaction between N-acetylcysteine and molecules that participate in ADMA metabolism or if this effect is mediated by a decrease in oxidative stress production. Studies in humans are contradictory. Thaha *et al.*, reported an approximately 30% decrease in ADMA serum levels of end-stage renal disease patients after a 4-hour hemodialysis session which was accompanied with a 4-hour intravenous infusion of N-acetylcysteine [220]. This decrease was significantly 10% greater than the one observed in the control group of patients who did not receive N-acetylcysteine [220]. On the contrary, oral administration of N-acetylcysteine for 8 weeks did not have any significant effect on ADMA levels of peritoneal dialysis patients [221].

Since omega-3-polyunsaturated fatty acids have a well-recognized clinical benefit, it was explored whether they exert their cardioprotective effect through an ADMA-dependent manner. However, no study that tested the efficacy of supplementary omega-3-polyunsaturated fatty acids verified such a hypothesis [289-292].

### Nitrate/Nitrite

In mammals, NO may be produced via either NOS dependent or NOS independent pathways. The latter includes formation of NO by its oxidation products, nitrate ($NO_3^-$) and nitrite ($NO_2^-$) [293]. Thus, dietary administration of nitrate has revealed a new therapeutic perspective. Indeed, oral intake of nitrate has been found to reduce diastolic and systolic blood pressure in healthy volunteers [294, 295], while nitrite supplementation has been shown to have cytoprotective and anti-apoptotic properties in models of acute myocardial infarction and stroke [296-298]. They also act protectively against renal injury in hypertensive rats [299]. Regarding the effect of NO oxidation products on ADMA levels few studies have explored their actions. In salt-induced hypertensive rats, nitrate supplementation prevents renal and cardiac injury, reduces oxidative stress and normalizes serum and urine levels of ADMA and SDMA [300]. Similarly, dietary sodium nitrate (as well as L-citrulline) was found to decrease ADMA levels in spontaneously hypertensive rats [301]. Due to the paucity of relevant studies, whether oral intake of nitrate and/or nitrite has any direct effect on ADMA needs to be further studied.

### Dietary Intervention

It has been proven that diet plays a crucial role in the development of atherosclerosis and cardiovascular disease. The exact mechanism through which the modification of diet improves endothelial function has not been revealed yet. Some studies indicate that low-calorie dietary intervention, specifically for 12 weeks, decreased the concentration of circulating ADMA in obese and overweight patients [302, 303]. This effect was significant even in obese children after only a 2-week-long low-calorie dietary intervention along with exercise [304].

Mediterranean diet and low-fat dietary patterns have been shown to be efficient in both primary and secondary prevention of cardiovascular events. A clinical trial suggested that such an intervention as secondary prevention in patients who had suffered an acute coronary syndrome, reduced ADMA plasma levels and increased L-arginine/ADMA ratio [305]. Olive oil has a central role in Mediterranean diet. Polyphenols, are considered partly responsible for its beneficial effect. Polyphenol-rich olive oil intake seems to significantly decrease serum ADMA levels after 4 months, implying a potential explanation for its protective action against endothelial dysfunction [306].

Moreover, high salt intake has been correlated with higher ADMA concentration in normotensive patients [307]. Interestingly, it was found that high-salt diet in rats increases ADMA levels via up-regulating DDAH expression and this effect was independent of the blood pressure measurements [308].

Lastly, in patients with chronic kidney disease a 3-year-low-protein dietary intervention improves endothelial function and reduces ADMA levels [309].

### Interferon-a

Treatment with interferon-a in patients with hepatitis C causes depression which is driven by elevated ADMA levels linking depression to increased cardiovascular risk. A potential modification in the expression of interferon-a may also result in alteration in ADMA levels [310]. Nevertheless at the moment there are no such studies.

### Tumor Necrosis Factor Antagonists

It is now well established that inflammatory arthropathies are associated with increased risk for cardiovascular disease, due to impaired endothelial function [311]. Even though the exact mechanisms underlying this phenomenon are still unclear, ADMA levels have been found elevated in patients with rheumatoid arthritis and ankylosing spondylitis [312-316]. Tumor necrosis factor is a molecule of crucial importance in the inflammatory process and antagonists of his action are used as a treatment. Initially reports noted a decrease in ADMA levels after initiation of anti-TNF therapy in patients with rheumatoid arthritis [317] but later studies with higher samples of patients with rheumatoid arthritis and ankylosing spondylitis and longer follow-up failed to verify such an effect [318-321]. However, one of these studies recorded a significant increase in the L-arginine/ADMA ratio after 3 and 12 months of anti-TNF treatment [318]. These results imply that TNF-antagonists may have an additional atheroprotective role mediated by modulation of ADMA/NO cascade, but further research is required.

### Retinoic Acid

Vitamin A derivatives and particularly all-trans-retinoic acid are believed to have important effects on cardiovascular system and due to their beneficial antiatherogenic action they may have therapeutic potential. It has been shown that all-trans-retinoic acid increases NO production by up-regulating DDAH2 expression and reducing ADMA in endothelial cells [322]. This effect should be further studied in clinical trials.

## THERAPEUTIC PERSPECTIVE

Despite the proven ability of current treatments to significantly reduce ADMA levels, as shown in Table **2**, these agents are not exclusively targeting ADMA and there is still no established clinical benefit of this reduction. Previous reports have already discussed the clinical impact of the decrease in ADMA levels caused by classical regimens [6, 323-325]. Therefore, in the next section, novel approaches under investigation, targeting specifically ADMA synthesis and metabolism, as well as other innovative ADMA lowering agents, are discussed.

### Gene Therapy Targeting DDAH

As it has already been mentioned, DDAH metabolizes ADMA and this action is a promising pharmaceutical target to indirectly reduce ADMA levels and increase NO formation. It has been reported that DDAH-1 and 2 genes poly-

morphisms influence ADMA levels in patients with type 2 diabetes mellitus [326] and recent evidence suggests that a specific variant in DDAH-2 gene (polymorphism rs9267551) is associated with insulin sensitivity [327]. Therefore, DDAH-2 gene was transferred to thoracic aortas of hyperlipedemic rabbits and in diabetic rats by recombinant adenoviruses. In both experiments DDAH expression was increased and led to improvement in the endothelial-dependent relaxation by decreasing ADMA levels [328, 329]. Moreover, in another study in human umbilical vein endothelial cells infected with recombinant DDAH-1 and DDAH-2 adenovirus an increase in DDAH expression and a decrease in ADMA levels was observed. Similarly, in the same study, overexpression of DDAH-1 or 2 in DDAH-1(+/-) mice increased NO production and improved acetylocholine induced relaxation was observed in carotid vessels [330]. However, such a therapeutic prospective is still far away from clinical application.

**Modulation of Protein Arginine Methyltransferases Activity**

It has already been noted that PRMT catalyzes the formation of ADMA. There is a number of PRMT isoforms, but PRMT-1 is responsible for asymmetrical dimethylarginines synthesis [10]. Therefore, PRMT-1 inhibitors would be potential a novel therapeutic agents for cardiovascular disease. Despite the fact, that various PRMT inhibitors have been developed, most of them are S- adenosylmethionine analogues, such as sinefungin and *S*–adenosylhomocysteine, and inhibit the activity of a wide variety of S-adenosylmethionine –dependent methyltransferases. As a result, they lack specificity for PRMT-1 [9]. However, more recent agents, like A9 and A36 seem to be more selective against PRMT-1 [331]. In addition, research findings suggest that using small molecules targeting the substrates of PRMT-1 rather than the enzymes themselves provides higher specificity and more effective inhibition of PRMT-1 activity [332]. Development of more effective agents could possibly play a key role in the future in the modulation of PRMT-1/ADMA/NO cascade in order to enhance endothelial function. For the time being, there is no experimental or clinical trial exploring the effect of PRMT-1 inhibitors in cardiovascular disease, though [333].

**Serelaxin**

Relaxin is a small peptide hormone produced mainly during pregnancy which is thought to be a vasodilator agent among its other actions. There is evidence suggesting that relaxin increases NO synthesis both acutely and chronically, possibly after connecting to relaxin/insulin-like family peptide receptor (RXFP1) [334]. Serelaxin, a recombinant form of human relaxin-2, is a novel investigational drug for the treatment of acute heart failure [335]. Whereas it is known that serelaxin exerts part of its beneficial effect via inducing the activation of NOS, there is no well established proposed mechanism for this action. An experimental study reported a decrease in ADMA circulating levels and normalization of oxidative stress products in drug-induced hypertensive rats receiving serelaxin. In addition, serelaxin did not influence PRMT or DDAH activity and the authors suggested that serelaxin reduces ADMA and increases NO bioavailability through its antioxidant action [336].

**Melatonin**

Melatonin is an indoleamine produced form the pineal gland. Its main role is the regulation of circadian rhythms but antioxidant properties have also been attributed to this indoleamine. Experimental evidence support that melatonin exerts part of its beneficial action via increasing DDAH activity and subsequently decreasing ADMA levels [222, 337-342]. A possible explanation for this action has been given by Tain *et al.*, who showed that melatonin suppresses the inhibitory effect of oxidative stress on DDAH, thus not allowing its down-regulation [339]. No clinical studies are available for the effect of exogenously administered melatonin on ADMA levels. In a study of 852 community-dwelling elderly individuals though, a decrease of ADMA levels was found to mediate the night-time blood pressure decrease caused by endogenous melatonin secretion [343]. Further clinical evidence is needed to give insight in the potential beneficial effect of melatonin administration against diseases associated with elevated ADMA levels.

**Resveratrol**

Resveratrol is a phytoalexin naturally produced by several plants and present in many natural products, including red wine [344]. It has been reported to have beneficial effects against diabetes, obesity and cardiovascular disease, while it possesses anti-ageing and neuroprotective properties [345]. Resveratrol was found to act protectively against the high-glucose-induced senescence of endothelial cells via up-regulating DDAH and restoring ADMA levels [346]. It was found that resveratrol up-regulates DDAH possibly through activation of silent information regulator 1 [346]. In line with these evidences, resveratrol was found to increase DDAH activity and reduce ADMA levels in endothelial cells in a dose-dependent manner [347]. Resveratrol was also found to act against ADMA accumulation and restore L-arginine in rats with vascular dysfunction induced by high fructose supplementation [348]. Similar effects were observed in a more recent study, where endothelial cells senescence by high glucose intake was inhibited by resveratrol via up-regulation of DDAH-2 [349]. In this study it was also reported that expression of sirtuin 1 was decreased in endothelial cells pretreated with high glucose as well as in patients with type 2 diabetes mellitus [349]. Since resveratrol activates sirtuin 1, it was hypothesized that the up-regulation of DDAH-2 may be mediated by this phenomenon [349]. Lastly, resveratrol has been reported to decrease ADMA levels in rats with deoxycorticosterone acetate induced hypertension [223]. Besides endothelial dysfunction, resveratrol has been reported to exert a protective effect against gastric mucosal injury in rats via increasing DDAH activity and thus decreasing ADMA concentration and increasing NO bioavailability in the gastric mucosal cells [350]. Studies in humans should further investigate the role of resveratrol in the DDAH/ADMA/NO pathway, as well as the potential mediating effect of sirtuin 1 in this phenomenon.

### L-Arginine

L-arginine is a semi essential amino acid. It is the substrate for eNOS as the precursor molecule for NO synthesis. It has been recently suggested that eNOS inhibition by its endogenous competitive inhibitors, the most known and crucial of which is ADMA, leads to eNOS "uncoupling". As a result, instead of NO, ROS are released and oxidative stress is generated [351]. Therefore, L-arginine acts beneficially for endothelium via competing the harmful results of ADMA on eNOS function. Based on this hypothesis, L-arginine supplementation has been tested as a potential therapeutic approach. It was shown from clinical studies that L-arginine only increases NO synthesis when administered to patients with baseline elevated ADMA levels [352] and that it has no impact on healthy subjects [353]. Moreover, short-term treatment with L-arginine prevents the smoking-induced impairment of endothelial function and vascular elastic properties in healthy smokers [354]. The underlying mechanism was proposed to be the saturation of eNOS with substrate (L-arginine) under physiological conditions when ADMA levels are low. Indeed, in healthy subjects the physiological concentration of L-arginine was found to be much higher than the Michaelis-Menten constant Km of eNOS [47]. In addition to its antagonism with ADMA, L-arginine can further enhance endothelial function by stimulating insulin and growth hormone which in turn up-regulates eNOS expression and decreases ADMA levels [355]. Finally, L-arginine seems to have anti-oxidant and anti-apoptotic abilities [356].

Despite the theoretical protective mechanisms stated above, data from clinical studies are inconsistent with the theory that L-arginine supplementation acts protectively for the heart and the vascular wall in patients suffering from cardiovascular disease. It has been found that exogenous L-arginine is able to restore NO bioavailability, but in patients with hypertension or heart failure an enhancement in endothelial function has not been confirmed in all studies. This may be explained by the decrease in expression of L-arginine intracellular transporter in these diseases [357]. In a randomized control trial (VINTAGE MI Study) it was investigated whether L-arginine should be administered to post-myocardial infarction patients [358]. Interestingly, it was found that L-arginine not only had no effect on ejection fraction and arterial stiffness but it may increase post-infarction mortality when compared to placebo. Therefore they concluded that L-arginine should not be given after myocardial infarction.

### L-Citrulline

L-citrulline is an amino acid that may serve as a precursor of L-arginine. Particularly, L-citrulline is converted to L-argininosuccinate by argininosuccinate synthetase and subsequently to L-arginine by argininosuccinate lyase [359]. Oral administration of L-citrulline has an advantage over L-arginine because it is not subject of pre-systemic elimination, i.e. bacteria of the gastrointestinal tract [359].

The effect of L-citrulline supplementation on ADMA concentration and L-arginine to ADMA concentration ratio has been evaluated in experimental and clinical studies. In a study of offspring rats whose mothers had been exposed to caloric restriction, L-citrulline intake, among others, prevented the increase of plasma ADMA and SDMA and decreased the L-arginine to ADMA ratio, thus increasing NO concentration [360]. Similar findings were observed in spontaneously hypertensive rats when kidney concentrations of L-arginine and ADMA were measured [301]. In a more recent study in porcine coronary arteries ADMA-induced endothelial dysfunction was attenuated by L-citrulline due to restoration of NO bioavailability [361]. Underlying mechanisms include up-regulation of eNOS expression and stimulation of its activation, inhibition of reactive oxygen species formation and enhancement of argininosuccinate synthetase expression [361].

In a study of 20 heathy volunteers, oral supplementation of L-citrulline for 1 week significantly increased L-arginine to ADMA circulating ratio in a dose-dependent manner [362]. Another study in healthy males reported that despite a significant increase of L-arginine to ADMA ratio after L-citrulline supplementation, this effect was not associated with a decrease in ADMA plasma levels [363]. On the contrary, in vasospastic angina patients, plasma ADMA decreased by approximately 15% after daily intake of 800 mg/kg of L-citrulline for 8 weeks [364]. As expected, L-arginine to ADMA ratio was significantly increased [364]. Finally, L-citrulline administration may have beneficial properties during mid-pregnancy, since it was found to increase L-arginine to ADMA ratio, enhance endothelial function and lower blood pressure [365].

### Farnesoid X Receptor Agonists

Farnesoid X receptor (FXR) is a type of nuclear receptor mostly expressed in liver and intestine. Its primary effect is the regulation of gene expression in order to reduce bile acid toxicity in these tissues [366]. FXR is a promising target for pharmacological intervention due to its regulating effect in the homeostasis of cholesterol, bile acid and glucose. *In vivo* administration of GW4064, a FXR agonist in Zucker diabetic fatty rats stimulated a dose-dependent increase in hepatic DDAH-1 gene expression leading to a respective decrease in ADMA levels [367]. Another study demonstrated that the same FXR agonist, in addition to DDAH-1, increased CAT1 gene expression in mouse liver and kidney, thus regulating ADMA uptake in these tissues [368]. GW4086 was also found to protect against ischemia/reperfusion-induced gastric lesions via increasing of ADMA [369]. Another FXR agonist, INT-747, has been reported to inhibit DDAH suppression after high salt intake in rat models with salt-sensitive hypertension and insulin resistance [370]. This effect led to enhanced insulin sensitivity [370]. Lastly, recent evidence suggests that administration of a FXR agonist (obeticholic acid) in cirrhotic rats reduces significantly portal hypertension via up-regulation of DDAH-1 and subsequent decrease of ADMA [371].

## CONSIDERATIONS-DRUG INTERACTIONS WITH ADMA METABOLIC PATHWAYS

Proton pump inhibitors are gastric acid-suppressing agents widely prescribed for the treatment of gastroesophageal reflux disease. Several studies have documented an increase in cardiovascular events in patients treated with this class of agents. Recently, the finding that proton pump in-

hibitors bind to and inhibit DDAH leading to elevated plasma ADMA levels and reduced NO levels and endothelium-dependent vasodilation in a murine model and *ex vivo* human tissues raised concerns about the use of this agents in subjects with atherosclerosis and coronary artery disease [372].

Recombinant human erythropoietin is frequently used in patients suffering from anemia, specifically due to chronic kidney disease. It is known that erythropoietin causes hypertension and it has been suggested that this is the effect of its action on endothelial cells. In particular, one study found that it impairs DDAH activity thus increasing ADMA levels [373]. However, more recent evidences indicate that despite its aforementioned action, erythropoietin does not affect NO synthesis [374]. In accordance to this finding, a prospective study of patients with chronic kidney injury and anemia undergoing treatment with erythropoietin for 6 months demonstrated a protective role of the agent against atherosclerosis and endothelial dysfunction. This study reported a decrease in ADMA levels as well [375].

It is usual for patients with epilepsy to be treated with one or more anti-epileptic drugs for long periods of time, possibly life-long. Consequently, findings linking anti-epileptic agents to atherosclerosis create a dilemma regarding their use. Interestingly, patients under therapy with carbamazepine or valproic acid had higher levels of ADMA compared to measures before initiation of the treatment [376]. A more recent study did not report elevated ADMA levels in patients under oxcarbazepine or valproic acid but increased risk for hyperhomocysteinemia, which the authors implicated as responsible for the higher atherosclerosis risk [377]. It is important that these effects have not been studied for each agent on a separate basis despite their different mechanisms of action. Nevertheless, in future studies it should be determined which antiepileptic drugs have the least cardiovascular risk.

## CONCLUSION

The role of endothelial dysfunction in atherosclerosis and cardiovascular disease is undoubted and ADMA is a key molecule in its pathophysiology. Elevated levels of ADMA have been correlated with a number of cardiovascular risk factors and diseases. Several established cardiovascular treatments such as ACEIs and ARBs, nebivolol, metformin and acetylsalicylic acid are among the classic pharmaceutical agents that their ability to decrease ADMA levels is almost proven, whereas this effect is still controversial for thiazolidinedones and statins. However, reducing pharmaceutically ADMA levels has uncertain clinical benefit and therapeutic regimens are not indicated in the absence of other cardiovascular risk factors. Consequently, further research is needed to give insights in the potential of novel therapeutic approaches targeting specifically ADMA synthesis and metabolism to modify atherosclerosis progression.

## CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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## REFERENCES

[1] Tousoulis, D.; Kampoli, A.M.; Tentolouris, C.; Papageorgiou, N.; Stefanadis, C. The role of nitric oxide on endothelial function. *Curr. Vasc. Pharmacol.,* **2012**, *10*(1), 4-18.

[2] Tousoulis, D.; Psaltopoulou, T.; Androulakis, E.; Papageorgiou, N.; Papaioannou, S.; Oikonomou, E.; Synetos, A.; Stefanadis, C. Oxidative stress and early atherosclerosis: novel antioxidant treatment. *Cardiovasc. Drugs Ther.,* **2014**.

[3] Tousoulis, D.; Simopoulou, C.; Papageorgiou, N.; Oikonomou, E.; Hatzis, G.; Siasos, G.; Tsiamis, E.; Stefanadis, C. Endothelial dysfunction in conduit arteries and in microcirculation. Novel therapeutic approaches. *Pharmacol. Ther.,* **2014**, *144*(3), 253-267.

[4] Celermajer, D.S.; Sorensen, K.E.; Bull, C.; Robinson, J.; Deanfield, J.E. Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. *J. Am. Coll. Cardiol.,* **1994**, *24*(6), 1468-1474.

[5] Tousoulis, D.; Siasos, G.; Oikonomou, E.; Stougianos, P.; Papageorgiou, N.; Papavassiliou, A.G.; Stefanadis, C. Asymmetric dimethylarginine (ADMA): is really a biomarker for cardiovascular prognosis? *Int. J. Cardiol.,* **2011**, *153*(2), 123-125.

[6] Leiper, J.; Nandi, M. The therapeutic potential of targeting endogenous inhibitors of nitric oxide synthesis. *Nat. Rev. Drug Discov.,* **2011**, *10*(4), 277-291.

[7] Paik, W.K.; Kim, S. Enzymatic methylation of protein fractions from calf thymus nuclei. *Biochem. Biophys. Res. Commun.,* **1967**, *29*(1), 14-20.

[8] Bedford, M.T.; Clarke, S.G. Protein arginine methylation in mammals: who, what, and why. *Mol. Cell.,* **2009**, *33*(1), 1-13.

[9] Krause, C.D.; Yang, Z.H.; Kim, Y.S.; Lee, J.H.; Cook, J.R.; Pestka, S. Protein arginine methyltransferases: evolution and assessment of their pharmacological and therapeutic potential. *Pharmacol. Ther.,* **2007**, *113*(1), 50-87.

[10] McBride, A.E.; Silver, P.A. State of the arg: protein methylation at arginine comes of age. *Cell.,* **2001**, *106*(1), 5-8.

[11] Miranda, T.B.; Miranda, M.; Frankel, A.; Clarke, S. PRMT7 is a member of the protein arginine methyltransferase family with a distinct substrate specificity. *J. Biol. Chem.,* **2004**, *279*(22), 22902-22907.

[12] Cook, J.R.; Lee, J.H.; Yang, Z.H.; Krause, C.D.; Herth, N.; Hoffmann, R.; Pestka, S. FBXO11/PRMT9, a new protein arginine methyltransferase, symmetrically dimethylates arginine residues. *Biochem. Biophys. Res. Commun.,* **2006**, *342*(2), 472-481.

[13] Bedford, M.T. Arginine methylation at a glance. *J. Cell. Sci.,* **2007**, *120*(Pt 24), 4243-4246.

[14] Tang, J.; Frankel, A.; Cook, R.J.; Kim, S.; Paik, W.K.; Williams, K.R.; Clarke, S.; Herschman, H.R. PRMT1 is the predominant type I protein arginine methyltransferase in mammalian cells. *J. Biol. Chem.,* **2000**, *275*(11), 7723-7730.

[15] Wang, Y.; Wysocka, J.; Sayegh, J.; Lee, Y.H.; Perlin, J.R.; Leonelli, L.; Sonbuchner, L.S.; McDonald, C.H.; Cook, R.G.; Dou, Y.; Roeder, R.G.; Clarke, S.; Stallcup, M.R.; Allis, C.D.; Coonrod, S.A. Human PAD4 regulates histone arginine methylation levels via demethylimination. *Science,* **2004**, *306*(5694), 279-283.

[16] Cuthbert, G.L.; Daujat, S.; Snowden, A.W.; Erdjument-Bromage, H.; Hagiwara, T.; Yamada, M.; Schneider, R.; Gregory, P.D.; Tempst, P.; Bannister, A.J.; Kouzarides, T. Histone deimination antagonizes arginine methylation. *Cell,* **2004**, *118*(5), 545-553.

[17] Raijmakers, R.; Zendman, A.J.; Egberts, W.V.; Vossenaar, E.R.; Raats, J.; Soede-Huijbregts, C.; Rutjes, F.P.; van Veelen, P.A.; Drijfhout, J.W.; Pruijn, G.J. Methylation of arginine residues interferes with citrullination by peptidylarginine deiminases *in vitro*. *J. Mol. Biol.,* **2007**, *367*(4), 1118-1129.

[18] Chang, B.; Chen, Y.; Zhao, Y.; Bruick, R.K. JMJD6 is a histone arginine demethylase. *Science,* **2007**, *318*(5849), 444-447.

[19] Han, G.; Li, J.; Wang, Y.; Li, X.; Mao, H.; Liu, Y.; Chen, C.D. The hydroxylation activity of Jmjd6 is required for its homo-oligomerization. *J. Cell. Biochem.,* **2012**, *113*(5), 1663-1670.

[20] Ogawa, T.; Kimoto, M.; Sasaoka, K. Purification and properties of a new enzyme, NG,NG-dimethylarginine dimethylaminohydrolase, from rat kidney. *J. Biol. Chem.,* **1989**, *264*(17), 10205-10209.

[21] Ogawa, T.; Kimoto, M.; Sasaoka, K. Occurrence of a new enzyme catalyzing the direct conversion of NG,NG-dimethyl-L-arginine to L-citrulline in rats. *Biochem. Biophys. Res. Commun.,* **1987**, *148*(2), 671-677.

[22] Palm, F.; Onozato, M.L.; Luo, Z.; Wilcox, C.S. Dimethylarginine dimethylaminohydrolase (DDAH): expression, regulation, and function in the cardiovascular and renal systems. *Am. J. Physiol. Heart Circ. Physiol.,* **2007**, *293*(6), H3227-3245.

[23] Rodionov, R.N.; Murry, D.J.; Vaulman, S.F.; Stevens, J.W.; Lentz, S.R. Human alanine-glyoxylate aminotransferase 2 lowers asymmetric dimethylarginine and protects from inhibition of nitric oxide production. *J. Biol. Chem.,* **2010**, *285*(8), 5385-5391.

[24] Ogawa, T.; Kimoto, M.; Sasaoka, K. Dimethylarginine:pyruvate aminotransferase in rats. Purification, properties, and identity with alanine:glyoxylate aminotransferase 2. *J. Biol. Chem.,* **1990**, *265*(34), 20938-20945.

[25] Leiper, J.M.; Santa Maria, J.; Chubb, A.; MacAllister, R.J.; Charles, I.G.; Whitley, G.S.; Vallance, P. Identification of two human dimethylarginine dimethylaminohydrolases with distinct tissue distributions and homology with microbial arginine deiminases. *Biochem. J.,* **1999**, *343. Pt. 1*, 209-214.

[26] Nijveldt, R.J.; Teerlink, T.; van Guldener, C.; Prins, H.A.; van Lambalgen, A.A.; Stehouwer, C.D.; Rauwerda, J.A.; van Leeuwen, P.A. Handling of asymmetrical dimethylarginine and symmetrical dimethylarginine by the rat kidney under basal conditions and during endotoxaemia. *Nephrol. Dial. Transplant.,* **2003**, *18*(12), 2542-2550.

[27] Nijveldt, R.J.; Teerlink, T.; Siroen, M.P.; van Lambalgen, A.A.; Rauwerda, J.A.; van Leeuwen, P.A. The liver is an important organ in the metabolism of asymmetrical dimethylarginine (ADMA). *Clin. Nutr.,* **2003**, *22*(1), 17-22.

[28] Lee, I.S.; Muragaki, Y.; Ideguchi, T.; Hase, T.; Tsuji, M.; Ooshima, A.; Okuno, E.; Kido, R. Molecular cloning and sequencing of a cDNA encoding alanine-glyoxylate aminotransferase 2 from rat kidney. *J. Biochem.,* **1995**, *117*(4), 856-862.

[29] Kittel, A.; Muller, F.; Konig, J.; Mieth, M.; Sticht, H.; Zolk, O.; Kralj, A.; Heinrich, M.R.; Fromm, M.F.; Maas, R. Alanine-glyoxylate aminotransferase 2 (AGXT2) polymorphisms have considerable impact on methylarginine and beta-aminoisobutyrate metabolism in healthy volunteers. *PLoS One,* **2014**, *9*(2), e88544.

[30] Deves, R.; Boyd, C.A. Transporters for cationic amino acids in animal cells: discovery, structure, and function. *Physiol. Rev.,* **1998**, *78*(2), 487-545.

[31] Teerlink, T.; Luo, Z.; Palm, F.; Wilcox, C.S. Cellular ADMA: regulation and action. *Pharmacol. Res.,* **2009**, *60*(6), 448-460.

[32] Nijveldt, R.J.; Van Leeuwen, P.A.; Van Guldener, C.; Stehouwer, C.D.; Rauwerda, J.A.; Teerlink, T. Net renal extraction of asymmetrical (ADMA) and symmetrical (SDMA) dimethylarginine in fasting humans. *Nephrol. Dial. Transplant.,* **2002**, *17*(11), 1999-2002.

[33] Achan, V.; Broadhead, M.; Malaki, M.; Whitley, G.; Leiper, J.; MacAllister, R.; Vallance, P. Asymmetric dimethylarginine causes hypertension and cardiac dysfunction in humans and is actively metabolized by dimethylarginine dimethylaminohydrolase. *Arterioscler. Thromb. Vasc. Biol.,* **2003**, *23*(8), 1455-1459.

[34] Alderton, W.K.; Cooper, C.E.; Knowles, R.G. Nitric oxide synthases: structure, function and inhibition. *Biochem. J.,* **2001**, *357*(Pt 3), 593-615.

[35] Forstermann, U.; Sessa, W.C. Nitric oxide synthases: regulation and function. *Eur. Heart. J.,* **2012**, *33*(7), 829-837, 837a-837d.

[36] Zhou, L.; Zhu, D.Y. Neuronal nitric oxide synthase: structure, subcellular localization, regulation, and clinical implications. *Nitric. Oxide.,* **2009**, *20*(4), 223-230.

[37] Sharma, J.N.; Al-Omran, A.; Parvathy, S.S. Role of nitric oxide in inflammatory diseases. *Inflammopharmacology,* **2007**, *15*(6), 252-259.

[38] Ruan, L.; Torres, C.M.; Qian, J.; Chen, F.; Mintz, J.D.; Stepp, D.W.; Fulton, D.; Venema, R.C. Pin1 prolyl isomerase regulates endothelial nitric oxide synthase. *Arterioscler. Thromb. Vasc. Biol.,* **2011**, *31*(2), 392-398.

[39] Chiasson, V.L.; Munshi, N.; Chatterjee, P.; Young, K.J.; Mitchell, B.M. Pin1 deficiency causes endothelial dysfunction and hypertension. *Hypertension,* **2011**, *58*(3), 431-438.

[40] Lu, K.P.; Zhou, X.Z. The prolyl isomerase PIN1: a pivotal new twist in phosphorylation signalling and disease. *Nat. Rev. Mol. Cell. Biol.,* **2007**, *8*(11), 904-916.

[41] Wang, J.Z.; Li, S.R.; Li, Y.L.; Zhang, Y.Z.; Zhang, T.; Zhao, C.X.; Yao, C.X.; Du, L.F. Could Pin1 help us conquer essential hypertension at an earlier stage? A promising early-diagnostic biomarker and its therapeutic implications for the disease. *Med. Hypotheses,* **2013**, *81*(5), 931-935.

[42] Wang, J.Z.; Zhu, W.D.; Xu, Z.X.; Du, W.T.; Zhang, H.Y.; Sun, X.W.; Wang, X.H. Pin1, endothelial nitric oxide synthase, and amyloid-beta form a feedback signaling loop involved in the pathogenesis of Alzheimer's disease, hypertension, and cerebral amyloid angiopathy. *Med. Hypotheses,* **2014**, *82*(2), 145-150.

[43] Leiper, J.; Vallance, P. Biological significance of endogenous methylarginines that inhibit nitric oxide synthases. *Cardiovasc. Res.,* **1999**, *43*(3), 542-548.

[44] Boger, R.H.; Vallance, P.; Cooke, J.P. Asymmetric dimethylarginine (ADMA): a key regulator of nitric oxide synthase. *Atheroscler. Suppl.,* **2003**, *4*(4), 1-3.

[45] Vallance, P.; Leone, A.; Calver, A.; Collier, J.; Moncada, S. Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet,* **1992**, *339*(8793), 572-575.

[46] Kurz, S.; Harrison, D.G. Insulin and the arginine paradox. *J. Clin. Invest.,* **1997**, *99*(3), 369-370.

[47] Boger, R.H. Asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, explains the "L-arginine paradox" and acts as a novel cardiovascular risk factor. *J. Nutr.,* **2004**, *134*(10 Suppl), 2842S-2847S; discussion 2853S.

[48] Tsikas, D.; Boger, R.H.; Sandmann, J.; Bode-Boger, S.M.; Frolich, J.C. Endogenous nitric oxide synthase inhibitors are responsible for the L-arginine paradox. *FEBS Lett.,* **2000**, *478*(1-2), 1-3.

[49] McDonald, K.K.; Zharikov, S.; Block, E.R.; Kilberg, M.S. A caveolar complex between the cationic amino acid transporter 1 and endothelial nitric-oxide synthase may explain the "arginine paradox". *J. Biol. Chem.,* **1997**, *272*(50), 31213-31216.

[50] Boger, R.H.; Maas, R.; Schulze, F.; Schwedhelm, E. Asymmetric dimethylarginine (ADMA) as a prospective marker of cardiovascular disease and mortality--an update on patient populations with a wide range of cardiovascular risk. *Pharmacol. Res.,* **2009**, *60*(6), 481-487.

[51] Luiking, Y.C.; Deutz, N.E. Biomarkers of arginine and lysine excess. *J. Nutr.,* **2007**, *137*(6 Suppl 2), 1662S-1668S.

[52] Brunini, T.; Moss, M.; Siqueira, M.; Meirelles, L.; Rozentul, A.; Mann, G.; Ellory, J.; Soares de Moura, R.; Mendes-Ribeiro, A. Inhibition of l-arginine transport in platelets by asymmetric dimethylarginine and N-monomethyl-l-arginine: effects of arterial hypertension. *Clin. Exp. Pharmacol. Physiol.,* **2004**, *31*(10), 738-740.

[53] Closs, E.I.; Basha, F.Z.; Habermeier, A.; Forstermann, U. Interference of L-arginine analogues with L-arginine transport mediated by the y+ carrier hCAT-2B. *Nitric. Oxide.,* **1997**, *1*(1), 65-73.

[54] Strobel, J.; Mieth, M.; Endress, B.; Auge, D.; Konig, J.; Fromm, M.F.; Maas, R. Interaction of the cardiovascular risk marker asymmetric dimethylarginine (ADMA) with the human cationic amino acid transporter 1 (CAT1). *J. Mol. Cell. Cardiol.,* **2012**, *53*(3), 392-400.

[55] Xia, Y.; Tsai, A.L.; Berka, V.; Zweier, J.L. Superoxide generation from endothelial nitric-oxide synthase. A Ca2+/calmodulin-dependent and tetrahydrobiopterin regulatory process. *J. Biol. Chem.,* **1998**, *273*(40), 25804-25808.

[56] Klatt, P.; Schmidt, K.; Uray, G.; Mayer, B. Multiple catalytic functions of brain nitric oxide synthase. Biochemical characterization, cofactor-requirement, and the role of N omega-hydroxy-L-arginine as an intermediate. *J. Biol. Chem.,* **1993**, *268*(20), 14781-14787.

[57] Antoniades, C.; Shirodaria, C.; Leeson, P.; Antonopoulos, A.; Warrick, N.; Van-Assche, T.; Cunnington, C.; Tousoulis, D.; Pillai, R.; Ratnatunga, C.; Stefanadis, C.; Channon, K.M. Association of plasma asymmetrical dimethylarginine (ADMA) with elevated vascular superoxide production and endothelial nitric oxide synthase uncoupling: implications for endothelial function in human atherosclerosis. *Eur. Heart J.,* **2009**, *30*(9), 1142-1150.

[58] Druhan, L.J.; Forbes, S.P.; Pope, A.J.; Chen, C.A.; Zweier, J.L.; Cardounel, A.J. Regulation of eNOS-derived superoxide by endogenous methylarginines. *Biochemistry,* **2008**, *47*(27), 7256-7263.

[59] Cardounel, A.J.; Xia, Y.; Zweier, J.L. Endogenous methylarginines modulate superoxide as well as nitric oxide generation from neuronal nitric-oxide synthase: differences in the effects of mono-

methyl- and dimethylarginines in the presence and absence of tetrahydrobiopterin. *J. Biol. Chem.,* **2005**, *280*(9), 7540-7549.

[60] Sydow, K.; Munzel, T. ADMA and oxidative stress. *Atheroscler. Suppl.,* **2003**, *4*(4), 41-51.

[61] Lin, K.Y.; Ito, A.; Asagami, T.; Tsao, P.S.; Adimoolam, S.; Kimoto, M.; Tsuji, H.; Reaven, G.M.; Cooke, J.P. Impaired nitric oxide synthase pathway in diabetes mellitus: role of asymmetric dimethylarginine and dimethylarginine dimethylaminohydrolase. *Circulation,* **2002**, *106*(8), 987-992.

[62] Tran, C.T.; Leiper, J.M.; Vallance, P. The DDAH/ADMA/NOS pathway. *Atheroscler. Suppl.,* **2003**, *4*(4), 33-40.

[63] Scalera, F.; Borlak, J.; Beckmann, B.; Martens-Lobenhoffer, J.; Thum, T.; Tager, M.; Bode-Boger, S.M. Endogenous nitric oxide synthesis inhibitor asymmetric dimethyl L-arginine accelerates endothelial cell senescence. *Arterioscler. Thromb. Vasc. Biol.,* **2004**, *24*(10), 1816-1822.

[64] Toth, J.; Racz, A.; Kaminski, P.M.; Wolin, M.S.; Bagi, Z.; Koller, A. Asymmetrical dimethylarginine inhibits shear stress-induced nitric oxide release and dilation and elicits superoxide-mediated increase in arteriolar tone. *Hypertension,* **2007**, *49*(3), 563-568.

[65] Sakurada, M.; Shichiri, M.; Imamura, M.; Azuma, H.; Hirata, Y. Nitric oxide upregulates dimethylarginine dimethylaminohydrolase-2 via cyclic GMP induction in endothelial cells. *Hypertension,* **2008**, *52*(5), 903-909.

[66] Perticone, F.; Sciacqua, A.; Maio, R.; Perticone, M.; Galiano Leone, G.; Bruni, R.; Di Cello, S.; Pascale, A.; Talarico, G.; Greco, L.; Andreozzi, F.; Sesti, G. Endothelial dysfunction, ADMA and insulin resistance in essential hypertension. *Int. J. Cardiol.,* **2010**, *142*(3), 236-241.

[67] Usui, M.; Matsuoka, H.; Miyazaki, H.; Ueda, S.; Okuda, S.; Imaizumi, T. Increased endogenous nitric oxide synthase inhibitor in patients with congestive heart failure. *Life Sci.,* **1998**, *62*(26), 2425-2430.

[68] Lu, T.M.; Ding, Y.A.; Lin, S.J.; Lee, W.S.; Tai, H.C. Plasma levels of asymmetrical dimethylarginine and adverse cardiovascular events after percutaneous coronary intervention. *Eur. Heart J.,* **2003**, *24*(21), 1912-1919.

[69] Yoo, J.H.; Lee, S.C. Elevated levels of plasma homocyst(e)ine and asymmetric dimethylarginine in elderly patients with stroke. *Atherosclerosis,* **2001**, *158*(2), 425-430.

[70] Paiva, H.; Lehtimaki, T.; Laakso, J.; Ruokonen, I.; Rantalaiho, V.; Wirta, O.; Pasternack, A.; Laaksonen, R. Plasma concentrations of asymmetric-dimethyl-arginine in type 2 diabetes associate with glycemic control and glomerular filtration rate but not with risk factors of vasculopathy. *Metabolism,* **2003**, *52*(3), 303-307.

[71] Bai, Y.; Sun, L.; Du, L.; Zhang, T.; Xin, W.; Lan, X.; Du, G. Association of circulating levels of asymmetric dimethylarginine (ADMA) with carotid intima-media thickness: evidence from 6168 participants. *Ageing Res. Rev.,* **2013**, *12*(2), 699-707.

[72] Juonala, M.; Viikari, J.S.; Alfthan, G.; Marniemi, J.; Kahonen, M.; Taittonen, L.; Laitinen, T.; Raitakari, O.T. Brachial artery flow-mediated dilation and asymmetrical dimethylarginine in the cardiovascular risk in young Finns study. *Circulation,* **2007**, *116*(12), 1367-1373.

[73] Willeit, P.; Freitag, D.F.; Laukkanen, J.A.; Chowdhury, S.; Gobin, R.; Mayr, M.; Di Angelantonio, E.; Chowdhury, R. Asymmetric dimethylarginine and cardiovascular risk: systematic review and meta-analysis of 22 prospective studies. *J. Am. Heart Assoc.,* **2015**, *4*(6).

[74] Meinitzer, A.; Seelhorst, U.; Wellnitz, B.; Halwachs-Baumann, G.; Boehm, B.O.; Winkelmann, B.R.; Marz, W. Asymmetrical dimethylarginine independently predicts total and cardiovascular mortality in individuals with angiographic coronary artery disease (the Ludwigshafen Risk and Cardiovascular Health study). *Clin. Chem.,* **2007**, *53*(2), 273-283.

[75] Surdacki, A.; Nowicki, M.; Sandmann, J.; Tsikas, D.; Boeger, R.H.; Bode-Boeger, S.M.; Kruszelnicka-Kwiatkowska, O.; Kokot, F.; Dubiel, J.S.; Froelich, J.C. Reduced urinary excretion of nitric oxide metabolites and increased plasma levels of asymmetric dimethylarginine in men with essential hypertension. *J. Cardiovasc. Pharmacol.,* **1999**, *33*(4), 652-658.

[76] Goonasekera, C.D.; Rees, D.D.; Woolard, P.; Frend, A.; Shah, V.; Dillon, M.J. Nitric oxide synthase inhibitors and hypertension in children and adolescents. *J. Hypertens.,* **1997**, *15*(8), 901-909.

[77] Curgunlu, A.; Uzun, H.; Bavunoglu, I.; Karter, Y.; Genc, H.; Vehid, S. Increased circulating concentrations of asymmetric dimethylarginine (ADMA) in white coat hypertension. *J. Hum. Hypertens.,* **2005**, *19*(8), 629-633.

[78] Boger, R.H.; Bode-Boger, S.M.; Szuba, A.; Tsao, P.S.; Chan, J.R.; Tangphao, O.; Blaschke, T.F.; Cooke, J.P. Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia. *Circulation,* **1998**, *98*(18), 1842-1847.

[79] Jehlicka, P.; Stozicky, F.; Mayer, O., Jr.; Varvarovska, J.; Racek, J.; Trefil, L.; Siala, K. Asymmetric dimethylarginine and the effect of folate substitution in children with familial hypercholesterolemia and diabetes mellitus type 1. *Physiol. Res.,* **2009**, *58*(2), 179-184.

[80] Chobanyan-Jurgens, K.; Fuchs, A.J.; Tsikas, D.; Kanzelmeyer, N.; Das, A.M.; Illsinger, S.; Vaske, B.; Jordan, J.; Lucke, T. Increased asymmetric dimethylarginine (ADMA) dimethylaminohydrolase (DDAH) activity in childhood hypercholesterolemia type II. *Amino Acids,* **2012**, *43*(2), 805-811.

[81] Korandji, C.; Zeller, M.; Guilland, J.C.; Vergely, C.; Sicard, P.; Duvillard, L.; Gambert, P.; Moreau, D.; Cottin, Y.; Rochette, L. Asymmetric dimethylarginine (ADMA) and hyperhomocysteinemia in patients with acute myocardial infarction. *Clin. Biochem.,* **2007**, *40*(1-2), 66-72.

[82] Wilcken, D.E.; Wang, J.; Sim, A.S.; Green, K.; Wilcken, B. Asymmetric dimethylarginine in homocystinuria due to cystathionine beta-synthase deficiency: relevance of renal function. *J. Inherit. Metab. Dis.,* **2006**, *29*(1), 30-37.

[83] Jonasson, T.F.; Hedner, T.; Hultberg, B.; Ohlin, H. Hyperhomocysteinaemia is not associated with increased levels of asymmetric dimethylarginine in patients with ischaemic heart disease. *Eur. J. Clin. Invest.,* **2003**, *33*(7), 543-549.

[84] Saitoh, M.; Osanai, T.; Kamada, T.; Matsunaga, T.; Ishizaka, H.; Hanada, H.; Okumura, K. High plasma level of asymmetric dimethylarginine in patients with acutely exacerbated congestive heart failure: role in reduction of plasma nitric oxide level. *Heart Vessels.,* **2003**, *18*(4), 177-182.

[85] Hsu, C.P.; Lin, S.J.; Chung, M.Y.; Lu, T.M. Asymmetric dimethylarginine predicts clinical outcomes in ischemic chronic heart failure. *Atherosclerosis,* **2012**, *225*(2), 504-510.

[86] Seljeflot, I.; Nilsson, B.B.; Westheim, A.S.; Bratseth, V.; Arnesen, H. The L-arginine-asymmetric dimethylarginine ratio is strongly related to the severity of chronic heart failure. No effects of exercise training. *J. Card. Fail.,* **2011**, *17*(2), 135-142.

[87] Zoccali, C.; Bode-Boger, S.; Mallamaci, F.; Benedetto, F.; Tripepi, G.; Malatino, L.; Cataliotti, A.; Bellanuova, I.; Fermo, I.; Frolich, J.; Boger, R. Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: a prospective study. *Lancet,* **2001**, *358*(9299), 2113-2117.

[88] Ravani, P.; Tripepi, G.; Malberti, F.; Testa, S.; Mallamaci, F.; Zoccali, C. Asymmetrical dimethylarginine predicts progression to dialysis and death in patients with chronic kidney disease: a competing risks modeling approach. *J. Am. Soc. Nephrol.,* **2005**, *16*(8), 2449-2455.

[89] Fliser, D.; Kronenberg, F.; Kielstein, J.T.; Morath, C.; Bode-Boger, S.M.; Haller, H.; Ritz, E. Asymmetric dimethylarginine and progression of chronic kidney disease: the mild to moderate kidney disease study. *J. Am. Soc. Nephrol.,* **2005**, *16*(8), 2456-2461.

[90] Sesti, G.; Mannino, G.C.; De Lorenzo, C.; Greco, A.; Sciacqua, A.; Marini, M.A.; Andreozzi, F.; Perticone, F. A functional variant of the dimethylarginine dimethylaminohydrolase-2 gene is associated with chronic kidney disease. *Atherosclerosis,* **2013**, *231*(1), 141-144.

[91] Altinova, A.E.; Arslan, M.; Sepici-Dincel, A.; Akturk, M.; Altan, N.; Toruner, F.B. Uncomplicated type 1 diabetes is associated with increased asymmetric dimethylarginine concentrations. *J. Clin. Endocrinol. Metab.,* **2007**, *92*(5), 1881-1885.

[92] Stuhlinger, M.C.; Abbasi, F.; Chu, J.W.; Lamendola, C.; McLaughlin, T.L.; Cooke, J.P.; Reaven, G.M.; Tsao, P.S. Relationship between insulin resistance and an endogenous nitric oxide synthase inhibitor. *JAMA,* **2002**, *287*(11), 1420-1426.

[93] Boger, R.H.; Sullivan, L.M.; Schwedhelm, E.; Wang, T.J.; Maas, R.; Benjamin, E.J.; Schulze, F.; Xanthakis, V.; Benndorf, R.A.; Vasan, R.S. Plasma asymmetric dimethylarginine and incidence of cardiovascular disease and death in the community. *Circulation,* **2009**, *119*(12), 1592-1600.

[94] Lu, T.M.; Chung, M.Y.; Lin, M.W.; Hsu, C.P.; Lin, S.J. Plasma asymmetric dimethylarginine predicts death and major adverse car-

diovascular events in individuals referred for coronary angiography. *Int. J. Cardiol.,* **2011**, *153*(2), 135-140.

[95] Anderssohn, M.; McLachlan, S.; Luneburg, N.; Robertson, C.; Schwedhelm, E.; Williamson, R.M.; Strachan, M.W.; Ajjan, R.; Grant, P.J.; Boger, R.H.; Price, J.F. Genetic and environmental determinants of dimethylarginines and association with cardiovascular disease in patients with type 2 diabetes. *Diabetes Care,* **2014**, *37*(3), 846-854.

[96] Zhang, S.; Yang, T.; Xu, X.; Wang, M.; Zhong, L.; Yang, Y.; Zhai, Z.; Xiao, F.; Wang, C. Oxidative stress and nitric oxide signaling related biomarkers in patients with pulmonary hypertension: a case control study. *BMC Pulm. Med.,* **2015**, *15*(1), 50.

[97] Parikh, R.V.; Scherzer, R.; Nitta, E.M.; Leone, A.; Hur, S.; Mistry, V.; Macgregor, J.S.; Martin, J.N.; Deeks, S.G.; Ganz, P.; Hsue, P.Y. Increased levels of asymmetric dimethylarginine are associated with pulmonary arterial hypertension in HIV infection. *AIDS,* **2014**, *28*(4), 511-519.

[98] Dimitroulas, T.; Giannakoulas, G.; Sfetsios, T.; Karvounis, H.; Dimitroula, H.; Koliakos, G.; Settas, L. Asymmetrical dimethylarginine in systemic sclerosis-related pulmonary arterial hypertension. *Rheumatology (Oxford).,* **2008**, *47*(11), 1682-1685.

[99] Kielstein, J.T.; Bode-Boger, S.M.; Hesse, G.; Martens-Lobenhoffer, J.; Takacs, A.; Fliser, D.; Hoeper, M.M. Asymmetrical dimethylarginine in idiopathic pulmonary arterial hypertension. *Arterioscler. Thromb. Vasc. Biol.,* **2005**, *25*(7), 1414-1418.

[100] Pettersson, A.; Hedner, T.; Milsom, I. Increased circulating concentrations of asymmetric dimethyl arginine (ADMA), an endogenous inhibitor of nitric oxide synthesis, in preeclampsia. *Acta Obstet. Gynecol. Scand.,* **1998**, *77*(8), 808-813.

[101] Mao, D.; Che, J.; Li, K.; Han, S.; Yue, Q.; Zhu, L.; Zhang, W.; Li, L. Association of homocysteine, asymmetric dimethylarginine, and nitric oxide with preeclampsia. *Arch. Gynecol. Obstet.,* **2010**, *282*(4), 371-375.

[102] Maas, R.; Boger, R.H.; Schwedhelm, E.; Casas, J.P.; Lopez-Jaramillo, P.; Serrano, N.; Diaz, L.A. Plasma concentrations of asymmetric dimethylarginine (ADMA) in Colombian women with pre-eclampsia. *JAMA,* **2004**, *291*(7), 823-824.

[103] Bian, Z.; Shixia, C.; Duan, T. First-Trimester Maternal Serum Levels of sFLT1, PGF and ADMA Predict Preeclampsia. *PLoS One,* **2015**, *10*(4), e0124684.

[104] Anderssohn, M.; Maass, L.M.; Diemert, A.; Luneburg, N.; Atzler, D.; Hecher, K.; Boger, R.H. Severely decreased activity of placental dimethylarginine dimethylaminohydrolase in pre-eclampsia. *Eur. J. Obstet. Gynecol. Reprod. Biol.,* **2012**, *161*(2), 152-156.

[105] Arlt, S.; Schulze, F.; Eichenlaub, M.; Maas, R.; Lehmbeck, J.T.; Schwedhelm, E.; Jahn, H.; Boger, R.H. Asymmetrical dimethylarginine is increased in plasma and decreased in cerebrospinal fluid of patients with Alzheimer's disease. *Dement. Geriatr. Cogn. Disord.,* **2008**, *26*(1), 58-64.

[106] Selley, M.L. Increased concentrations of homocysteine and asymmetric dimethylarginine and decreased concentrations of nitric oxide in the plasma of patients with Alzheimer's disease. *Neurobiol. Aging,* **2003**, *24*(7), 903-907.

[107] Abe, T.; Tohgi, H.; Murata, T.; Isobe, C.; Sato, C. Reduction in asymmetrical dimethylarginine, an endogenous nitric oxide synthase inhibitor, in the cerebrospinal fluid during aging and in patients with Alzheimer's disease. *Neurosci. Lett.,* **2001**, *312*(3), 177-179.

[108] McEvoy, M.; Schofield, P.; Smith, W.; Agho, K.; Mangoni, A.A.; Soiza, R.L.; Peel, R.; Attia, J. Memory impairment is associated with serum methylarginines in older adults. *Curr. Alzheimer Res.,* **2014**, *11*(1), 97-106.

[109] Smirnova, I.V.; Kajstura, M.; Sawamura, T.; Goligorsky, M.S. Asymmetric dimethylarginine upregulates LOX-1 in activated macrophages: role in foam cell formation. *Am. J. Physiol. Heart Circ. Physiol.,* **2004**, *287*(2), H782-790.

[110] Chan, J.R.; Boger, R.H.; Bode-Boger, S.M.; Tangphao, O.; Tsao, P.S.; Blaschke, T.F.; Cooke, J.P. Asymmetric dimethylarginine increases mononuclear cell adhesiveness in hypercholesterolemic humans. *Arterioscler. Thromb. Vasc. Biol.,* **2000**, *20*(4), 1040-1046.

[111] Boger, R.H.; Bode-Boger, S.M.; Tsao, P.S.; Lin, P.S.; Chan, J.R.; Cooke, J.P. An endogenous inhibitor of nitric oxide synthase regulates endothelial adhesiveness for monocytes. *J. Am. Coll. Cardiol.,* **2000**, *36*(7), 2287-2295.

[112] Sun, L.; Zhang, T.; Yu, X.; Xin, W.; Lan, X.; Zhang, D.; Huang, C.; Du, G. Asymmetric dimethylarginine confers the communication between endothelial and smooth muscle cells and leads to VSMC migration through p38 and ERK1/2 signaling cascade. *FEBS Lett.,* **2011**, *585*(17), 2727-2734.

[113] Kielstein, J.T.; Impraim, B.; Simmel, S.; Bode-Boger, S.M.; Tsikas, D.; Frolich, J.C.; Hoeper, M.M.; Haller, H.; Fliser, D. Cardiovascular effects of systemic nitric oxide synthase inhibition with asymmetrical dimethylarginine in humans. *Circulation,* **2004**, *109*(2), 172-177.

[114] Suda, O.; Tsutsui, M.; Morishita, T.; Tasaki, H.; Ueno, S.; Nakata, S.; Tsujimoto, T.; Toyohira, Y.; Hayashida, Y.; Sasaguri, Y.; Ueta, Y.; Nakashima, Y.; Yanagihara, N. Asymmetric dimethylarginine produces vascular lesions in endothelial nitric oxide synthase-deficient mice: involvement of renin-angiotensin system and oxidative stress. *Arterioscler. Thromb. Vasc. Biol.,* **2004**, *24*(9), 1682-1688.

[115] Notsu, Y.; Yano, S.; Shibata, H.; Nagai, A.; Nabika, T. Plasma arginine/ADMA ratio as a sensitive risk marker for atherosclerosis: Shimane CoHRE study. *Atherosclerosis,* **2015**, *239*(1), 61-66.

[116] Pikula, A.; Boger, R.H.; Beiser, A.S.; Maas, R.; DeCarli, C.; Schwedhelm, E.; Himali, J.J.; Schulze, F.; Au, R.; Kelly-Hayes, M.; Kase, C.S.; Vasan, R.S.; Wolf, P.A.; Seshadri, S. Association of plasma ADMA levels with MRI markers of vascular brain injury: Framingham offspring study. *Stroke,* **2009**, *40*(9), 2959-2964.

[117] Feng, Q.; Lu, X.; Fortin, A.J.; Pettersson, A.; Hedner, T.; Kline, R.L.; Arnold, J.M. Elevation of an endogenous inhibitor of nitric oxide synthesis in experimental congestive heart failure. *Cardiovasc. Res.,* **1998**, *37*(3), 667-675.

[118] Searles, C.D. The nitric oxide pathway and oxidative stress in heart failure. *Congest. Heart Fail.,* **2002**, *8*(3), 142-147, 155.

[119] Kielstein, J.T.; Simmel, S.; Bode-Boger, S.M.; Roth, H.J.; Schmidt-Gayk, H.; Haller, H.; Fliser, D. Subpressor dose asymmetric dimethylarginine modulates renal function in humans through nitric oxide synthase inhibition. *Kidney Blood Press Res.,* **2004**, *27*(3), 143-147.

[120] Fujiwara, N.; Osanai, T.; Kamada, T.; Katoh, T.; Takahashi, K.; Okumura, K. Study on the relationship between plasma nitrite and nitrate level and salt sensitivity in human hypertension : modulation of nitric oxide synthesis by salt intake. *Circulation,* **2000**, *101*(8), 856-861.

[121] Matsuoka, H.; Itoh, S.; Kimoto, M.; Kohno, K.; Tamai, O.; Wada, Y.; Yasukawa, H.; Iwami, G.; Okuda, S.; Imaizumi, T. Asymmetrical dimethylarginine, an endogenous nitric oxide synthase inhibitor, in experimental hypertension. *Hypertension,* **1997**, *29*(1 Pt 2), 242-247.

[122] Tousoulis, D.; Kampoli, A.M.; Stefanadis, C. Diabetes mellitus and vascular endothelial dysfunction: current perspectives. *Curr. Vasc. Pharmacol.,* **2012**, *10*(1), 19-32.

[123] Sydow, K.; Mondon, C.E.; Schrader, J.; Konishi, H.; Cooke, J.P. Dimethylarginine dimethylaminohydrolase overexpression enhances insulin sensitivity. *Arterioscler. Thromb. Vasc. Biol.,* **2008**, *28*(4), 692-697.

[124] Gonzalez, M.; Flores, C.; Pearson, J.D.; Casanello, P.; Sobrevia, L. Cell signalling-mediating insulin increase of mRNA expression for cationic amino acid transporters-1 and -2 and membrane hyperpolarization in human umbilical vein endothelial cells. *Pflugers Arch.,* **2004**, *448*(4), 383-394.

[125] Eid, H.M.; Reims, H.; Arnesen, H.; Kjeldsen, S.E.; Lyberg, T.; Seljeflot, I. Decreased levels of asymmetric dimethylarginine during acute hyperinsulinemia. *Metabolism,* **2007**, *56*(4), 464-469.

[126] Tarnow, L.; Hovind, P.; Teerlink, T.; Stehouwer, C.D.; Parving, H.H. Elevated plasma asymmetric dimethylarginine as a marker of cardiovascular morbidity in early diabetic nephropathy in type 1 diabetes. *Diabetes Care,* **2004**, *27*(3), 765-769.

[127] Abbasi, F.; Asagmi, T.; Cooke, J.P.; Lamendola, C.; McLaughlin, T.; Reaven, G.M.; Stuehlinger, M.; Tsao, P.S. Plasma concentrations of asymmetric dimethylarginine are increased in patients with type 2 diabetes mellitus. *Am. J. Cardiol.,* **2001**, *88*(10), 1201-1203.

[128] Ito, A.; Egashira, K.; Narishige, T.; Muramatsu, K.; Takeshita, A. Angiotensin-converting enzyme activity is involved in the mechanism of increased endogenous nitric oxide synthase inhibitor in patients with type 2 diabetes mellitus. *Circ. J.,* **2002**, *66*(9), 811-815.

[129] McLaughlin, T.; Stuhlinger, M.; Lamendola, C.; Abbasi, F.; Bialek, J.; Reaven, G.M.; Tsao, P.S. Plasma asymmetric dimethylarginine concentrations are elevated in obese insulin-resistant women and

fall with weight loss. *J. Clin. Endocrinol. Metab.,* **2006**, *91*(5), 1896-1900.

[130] Mittermayer, F.; Mayer, B.X.; Meyer, A.; Winzer, C.; Pacini, G.; Wagner, O.F.; Wolzt, M.; Kautzky-Willer, A. Circulating concentrations of asymmetrical dimethyl-L-arginine are increased in women with previous gestational diabetes. *Diabetologia,* **2002**, *45*(10), 1372-1378.

[131] Kruszelnicka, O.; Surdacki, A.; Golay, A. Differential associations of angiographic extent and severity of coronary artery disease with asymmetric dimethylarginine but not insulin resistance in non-diabetic men with stable angina: a cross-sectional study. *Cardiovasc. Diabetol.,* **2013**, *12*, 145.

[132] Siroen, M.P.; van Leeuwen, P.A.; Nijveldt, R.J.; Teerlink, T.; Wouters, P.J.; Van den Berghe, G. Modulation of asymmetric dimethylarginine in critically ill patients receiving intensive insulin treatment: a possible explanation of reduced morbidity and mortality? *Crit. Care Med.,* **2005**, *33*(3), 504-510.

[133] Boger, R.H.; Bode-Boger, S.M.; Sydow, K.; Heistad, D.D.; Lentz, S.R. Plasma concentration of asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, is elevated in monkeys with hyperhomocyst(e)inemia or hypercholesterolemia. *Arterioscler. Thromb. Vasc. Biol.,* **2000**, *20*(6), 1557-1564.

[134] Boger, R.H.; Sydow, K.; Borlak, J.; Thum, T.; Lenzen, H.; Schubert, B.; Tsikas, D.; Bode-Boger, S.M. LDL cholesterol upregulates synthesis of asymmetrical dimethylarginine in human endothelial cells: involvement of S-adenosylmethionine-dependent methyltransferases. *Circ. Res.,* **2000**, *87*(2), 99-105.

[135] Jang, J.J.; Ho, H.K.; Kwan, H.H.; Fajardo, L.F.; Cooke, J.P. Angiogenesis is impaired by hypercholesterolemia: role of asymmetric dimethylarginine. *Circulation,* **2000**, *102*(12), 1414-1419.

[136] Hasanoglu, A.; Okur, I.; Oren, A.C.; Biberoglu, G.; Oktar, S.; Eminoglu, F.T.; Tumer, L. The levels of asymmetric dimethylarginine, homocysteine and carotid intima-media thickness in hypercholesterolemic children. *Turk. J. Pediatr.,* **2011**, *53*(5), 522-527.

[137] Maas, R.; Schwedhelm, E.; Kahl, L.; Li, H.; Benndorf, R.; Luneburg, N.; Forstermann, U.; Boger, R.H. Simultaneous assessment of endothelial function, nitric oxide synthase activity, nitric oxide-mediated signaling, and oxidative stress in individuals with and without hypercholesterolemia. *Clin. Chem.,* **2008**, *54*(2), 292-300.

[138] Lucke, T.; Kanzelmeyer, N.; Kemper, M.J.; Tsikas, D.; Das, A.M. Developmental changes in the L-arginine/nitric oxide pathway from infancy to adulthood: plasma asymmetric dimethylarginine levels decrease with age. *Clin. Chem. Lab. Med.,* **2007**, *45*(11), 1525-1530.

[139] Lentz, S.R.; Haynes, W.G. Homocysteine: is it a clinically important cardiovascular risk factor? *Cleve. Clin. J. Med.,* **2004**, *71*(9), 729-734.

[140] Dayal, S.; Lentz, S.R. ADMA and hyperhomocysteinemia. *Vasc. Med.,* **2005**, *10. Suppl. 1*, S27-33.

[141] Wald, D.S.; Law, M.; Morris, J.K. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. *BMJ,* **2002**, *325*(7374), 1202.

[142] Homocysteine Studies, C. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. *JAMA,* **2002**, *288*(16), 2015-2022.

[143] Hong, L.; Fast, W. Inhibition of human dimethylarginine dimethylaminohydrolase-1 by S-nitroso-L-homocysteine and hydrogen peroxide. Analysis, quantification, and implications for hyperhomocysteinemia. *J. Biol. Chem.,* **2007**, *282*(48), 34684-34692.

[144] Zhang, J.G.; Liu, J.X.; Li, Z.H.; Wang, L.Z.; Jiang, Y.D.; Wang, S.R. Dysfunction of endothelial NO system originated from homocysteine-induced aberrant methylation pattern in promoter region of DDAH2 gene. *Chin. Med. J. (Engl).,* **2007**, *120*(23), 2132-2137.

[145] Liu, L.H.; Guo, Z.; Feng, M.; Wu, Z.Z.; He, Z.M.; Xiong, Y. Protection of DDAH2 overexpression against homocysteine-induced impairments of DDAH/ADMA/NOS/NO pathway in endothelial cells. *Cell. Physiol. Biochem.,* **2012**, *30*(6), 1413-1422.

[146] Rodionov, R.N.; Dayoub, H.; Lynch, C.M.; Wilson, K.M.; Stevens, J.W.; Murry, D.J.; Kimoto, M.; Arning, E.; Bottiglieri, T.; Cooke, J.P.; Baumbach, G.L.; Faraci, F.M.; Lentz, S.R. Overexpression of dimethylarginine dimethylaminohydrolase protects against cerebral vascular effects of hyperhomocysteinemia. *Circ. Res.,* **2010**, *106*(3), 551-558.

[147] Dayal, S.; Rodionov, R.N.; Arning, E.; Bottiglieri, T.; Kimoto, M.; Murry, D.J.; Cooke, J.P.; Faraci, F.M.; Lentz, S.R. Tissue-specific downregulation of dimethylarginine dimethylaminohydrolase in hyperhomocysteinemia. *Am. J. Physiol. Heart Circ. Physiol.,* **2008**, *295*(2), H816-825.

[148] Magne, J.; Huneau, J.F.; Borderie, D.; Mathe, V.; Bos, C.; Mariotti, F. Plasma asymmetric and symmetric dimethylarginine in a rat model of endothelial dysfunction induced by acute hyperhomocysteinemia. *Amino Acids,* **2015**.

[149] van Guldener, C.; Nanayakkara, P.W.; Stehouwer, C.D. Homocysteine and asymmetric dimethylarginine (ADMA): biochemically linked but differently related to vascular disease in chronic kidney disease. *Clin. Chem. Lab. Med.,* **2007**, *45*(12), 1683-1687.

[150] Antoniades, C.; Tousoulis, D.; Marinou, K.; Vasiliadou, C.; Tentolouris, C.; Bouras, G.; Pitsavos, C.; Stefanadis, C. Asymmetrical dimethylarginine regulates endothelial function in methionine-induced but not in chronic homocystinemia in humans: effect of oxidative stress and proinflammatory cytokines. *Am. J. Clin. Nutr.,* **2006**, *84*(4), 781-788.

[151] Tousoulis, D.; Bouras, G.; Antoniades, C.; Marinou, K.; Papageorgiou, N.; Miliou, A.; Hatzis, G.; Stefanadi, E.; Tsioufis, C.; Stefanadis, C. Methionine-induced homocysteinemia impairs endothelial function in hypertensives: the role of asymmetrical dimethylarginine and antioxidant vitamins. *Am. J. Hypertens,* **2011**, *24*(8), 936-942.

[152] Boger, R.H.; Zoccali, C. ADMA: a novel risk factor that explains excess cardiovascular event rate in patients with end-stage renal disease. *Atheroscler Suppl.,* **2003**, *4*(4), 23-28.

[153] Matsumoto, Y.; Ueda, S.; Yamagishi, S.; Matsuguma, K.; Shibata, R.; Fukami, K.; Matsuoka, H.; Imaizumi, T.; Okuda, S. Dimethylarginine dimethylaminohydrolase prevents progression of renal dysfunction by inhibiting loss of peritubular capillaries and tubulointerstitial fibrosis in a rat model of chronic kidney disease. *J. Am. Soc. Nephrol.,* **2007**, *18*(5), 1525-1533.

[154] Kajimoto, H.; Kai, H.; Aoki, H.; Yasuoka, S.; Anegawa, T.; Aoki, Y.; Ueda, S.; Okuda, S.; Imaizumi, T. Inhibition of eNOS phosphorylation mediates endothelial dysfunction in renal failure: new effect of asymmetric dimethylarginine. *Kidney Int.,* **2012**, *81*(8), 762-768.

[155] Tripepi, G.; Kollerits, B.; Leonardis, D.; Yilmaz, M.I.; Postorino, M.; Fliser, D.; Mallamaci, F.; Kronenberg, F.; Zoccali, C. Competitive interaction between fibroblast growth factor 23 and asymmetric dimethylarginine in patients with CKD. *J. Am. Soc. Nephrol.,* **2015**, *26*(4), 935-944.

[156] Masoura, S.; Kalogiannidis, I.A.; Gitas, G.; Goutsioulis, A.; Koiou, E.; Athanasiadis, A.; Vavatsi, N. Biomarkers in pre-eclampsia: a novel approach to early detection of the disease. *J. Obstet. Gynaecol.,* **2012**, *32*(7), 609-616.

[157] Holden, D.P.; Fickling, S.A.; Whitley, G.S.; Nussey, S.S. Plasma concentrations of asymmetric dimethylarginine, a natural inhibitor of nitric oxide synthase, in normal pregnancy and preeclampsia. *Am. J. Obstet. Gynecol.,* **1998**, *178*(3), 551-556.

[158] Kim, Y.J.; Park, H.S.; Lee, H.Y.; Ha, E.H.; Suh, S.H.; Oh, S.K.; Yoo, H.S. Reduced L-arginine level and decreased placental eNOS activity in preeclampsia. *Placenta,* **2006**, *27*(4-5), 438-444.

[159] Rizos, D.; Eleftheriades, M.; Batakis, E.; Rizou, M.; Haliassos, A.; Hassiakos, D.; Botsis, D. Levels of asymmetric dimethylarginine throughout normal pregnancy and in pregnancies complicated with preeclampsia or had a small for gestational age baby. *J. Matern. Fetal Neonatal. Med.,* **2012**, *25*(8), 1311-1315.

[160] Savvidou, M.D.; Hingorani, A.D.; Tsikas, D.; Frolich, J.C.; Vallance, P.; Nicolaides, K.H. Endothelial dysfunction and raised plasma concentrations of asymmetric dimethylarginine in pregnant women who subsequently develop pre-eclampsia. *Lancet,* **2003**, *361*(9368), 1511-1517.

[161] Moncada, S.; Bolanos, J.P. Nitric oxide, cell bioenergetics and neurodegeneration. *J. Neurochem.,* **2006**, *97*(6), 1676-1689.

[162] Luo, Y.; Yue, W.; Quan, X.; Wang, Y.; Zhao, B.; Lu, Z. Asymmetric dimethylarginine exacerbates Abeta-induced toxicity and oxidative stress in human cell and Caenorhabditis elegans models of Alzheimer disease. *Free Radic. Biol. Med.,* **2015**, *79*, 117-126.

[163] Selley, M.L. Homocysteine increases the production of asymmetric dimethylarginine in cultured neurons. *J. Neurosci. Res.,* **2004**, *77*(1), 90-93.

[164] Zhang, F.; Chen, L.; Liu, C.; Qiu, P.; Wang, A.; Li, L.; Wang, H. Up-regulation of protein tyrosine nitration in methamphetamine-induced neurotoxicity through DDAH/ADMA/NOS pathway. *Neurochem. Int.,* **2013**, *62*(8), 1055-1064.

[165] Richardson, C.; Nilforooshan, R.; Gard, P.R.; Weaving, G.; Tabet, N. Impaired renal function and biomarkers of vascular disease in Alzheimer's disease. *Curr. Alzheimer Res.,* **2014**, *11*(3), 253-258.

[166] Mulder, C.; Wahlund, L.O.; Blomberg, M.; de Jong, S.; van Kamp, G.J.; Scheltens, P.; Teerlink, T. Alzheimer's disease is not associated with altered concentrations of the nitric oxide synthase inhibitor asymmetric dimethylarginine in cerebrospinal fluid. *J. Neural. Transm.,* **2002**, *109*(9), 1203-1208.

[167] Skoro-Sajer, N.; Mittermayer, F.; Panzenboeck, A.; Bonderman, D.; Sadushi, R.; Hitsch, R.; Jakowitsch, J.; Klepetko, W.; Kneussl, M.P.; Wolzt, M.; Lang, I.M. Asymmetric dimethylarginine is increased in chronic thromboembolic pulmonary hypertension. *Am. J. Respir. Crit. Care. Med.,* **2007**, *176*(11), 1154-1160.

[168] Gorenflo, M.; Zheng, C.; Werle, E.; Fiehn, W.; Ulmer, H.E. Plasma levels of asymmetrical dimethyl-L-arginine in patients with congenital heart disease and pulmonary hypertension. *J. Cardiovasc. Pharmacol.,* **2001**, *37*(4), 489-492.

[169] Giannakoulas, G.; Mouratoglou, S.A.; Gatzoulis, M.A.; Karvounis, H. Blood biomarkers and their potential role in pulmonary arterial hypertension associated with congenital heart disease. a systematic review. *Int. J. Cardiol.,* **2014**, *174*(3), 618-623.

[170] Pullamsetti, S.; Kiss, L.; Ghofrani, H.A.; Voswinckel, R.; Haredza, P.; Klepetko, W.; Aigner, C.; Fink, L.; Muyal, J.P.; Weissmann, N.; Grimminger, F.; Seeger, W.; Schermuly, R.T. Increased levels and reduced catabolism of asymmetric and symmetric dimethylarginines in pulmonary hypertension. *FASEB J.,* **2005**, *19*(9), 1175-1177.

[171] Sasaki, A.; Doi, S.; Mizutani, S.; Azuma, H. Roles of accumulated endogenous nitric oxide synthase inhibitors, enhanced arginase activity, and attenuated nitric oxide synthase activity in endothelial cells for pulmonary hypertension in rats. *Am. J. Physiol. Lung. Cell. Mol. Physiol.,* **2007**, *292*(6), L1480-1487.

[172] Millatt, L.J.; Whitley, G.S.; Li, D.; Leiper, J.M.; Siragy, H.M.; Carey, R.M.; Johns, R.A. Evidence for dysregulation of dimethylarginine dimethylaminohydrolase I in chronic hypoxia-induced pulmonary hypertension. *Circulation,* **2003**, *108*(12), 1493-1498.

[173] Arrigoni, F.I.; Vallance, P.; Haworth, S.G.; Leiper, J.M. Metabolism of asymmetric dimethylarginines is regulated in the lung developmentally and with pulmonary hypertension induced by hypobaric hypoxia. *Circulation,* **2003**, *107*(8), 1195-1201.

[174] Leiper, J.; Nandi, M.; Torondel, B.; Murray-Rust, J.; Malaki, M.; O'Hara, B.; Rossiter, S.; Anthony, S.; Madhani, M.; Selwood, D.; Smith, C.; Wojciak-Stothard, B.; Rudiger, A.; Stidwill, R.; McDonald, N.Q.; Vallance, P. Disruption of methylarginine metabolism impairs vascular homeostasis. *Nat. Med.,* **2007**, *13*(2), 198-203.

[175] Tsang, H.; Leiper, J.; Hou Lao, K.; Dowsett, L.; Delahaye, M.W.; Barnes, G.; Wharton, J.; Howard, L.; Iannone, L.; Lang, N.N.; Wilkins, M.R.; Wojciak-Stothard, B. Role of asymmetric methylarginine and connexin 43 in the regulation of pulmonary endothelial function. *Pulm. Circ.,* **2013**, *3*(3), 675-691.

[176] Chen, B.; Strauch, K.; Jin, Y.; Cui, H.; Nelin, L.D.; Chicoine, L.G. Asymmetric dimethylarginine does not inhibit arginase activity and is pro-proliferative in pulmonary endothelial cells. *Clin. Exp. Pharmacol. Physiol.,* **2014**, *41*(7), 469-474.

[177] King, T.E., Jr.; Pardo, A.; Selman, M. Idiopathic pulmonary fibrosis. *Lancet,* **2011**, *378*(9807), 1949-1961.

[178] Jang, A.S.; Lee, J.U.; Choi, I.S.; Park, K.O.; Lee, J.H.; Park, S.W.; Park, C.S. Expression of nitric oxide synthase, aquaporin 1 and aquaporin 5 in rat after bleomycin inhalation. *Intensive Care Med.,* **2004**, *30*(3), 489-495.

[179] Romanska, H.M.; Polak, J.M.; Coleman, R.A.; James, R.S.; Harmer, D.W.; Allen, J.C.; Bishop, A.E. iNOS gene upregulation is associated with the early proliferative response of human lung fibroblasts to cytokine stimulation. *J. Pathol.,* **2002**, *197*(3), 372-379.

[180] Pullamsetti, S.S.; Savai, R.; Dumitrascu, R.; Dahal, B.K.; Wilhelm, J.; Konigshoff, M.; Zakrzewicz, D.; Ghofrani, H.A.; Weissmann, N.; Eickelberg, O.; Guenther, A.; Leiper, J.; Seeger, W.; Grimminger, F.; Schermuly, R.T. The role of dimethylarginine dimethylaminohydrolase in idiopathic pulmonary fibrosis. *Sci. Transl. Med.,* **2011**, *3*(87), 87ra53.

[181] Thomsen, L.L.; Miles, D.W.; Happerfield, L.; Bobrow, L.G.; Knowles, R.G.; Moncada, S. Nitric oxide synthase activity in human breast cancer. *Br. J. Cancer,* **1995**, *72*(1), 41-44.

[182] Cobbs, C.S.; Brenman, J.E.; Aldape, K.D.; Bredt, D.S.; Israel, M.A. Expression of nitric oxide synthase in human central nervous system tumors. *Cancer Res.,* **1995**, *55*(4), 727-730.

[183] Tanriover, N.; Ulu, M.O.; Isler, C.; Durak, H.; Oz, B.; Uzan, M.; Akar, Z. Neuronal nitric oxide synthase expression in glial tumors: correlation with malignancy and tumor proliferation. *Neurol. Res.,* **2008**, *30*(9), 940-944.

[184] Beevi, S.S.; Rasheed, M.H.; Geetha, A. Evidence of oxidative and nitrosative stress in patients with cervical squamous cell carcinoma. *Clin. Chim. Acta,* **2007**, *375*(1-2), 119-123.

[185] Yagihashi, N.; Kasajima, H.; Sugai, S.; Matsumoto, K.; Ebina, Y.; Morita, T.; Murakami, T.; Yagihashi, S. Increased in situ expression of nitric oxide synthase in human colorectal cancer. *Virchows Arch.,* **2000**, *436*(2), 109-114.

[186] Masri, F.A.; Comhair, S.A.; Koeck, T.; Xu, W.; Janocha, A.; Ghosh, S.; Dweik, R.A.; Golish, J.; Kinter, M.; Stuehr, D.J.; Erzurum, S.C.; Aulak, K.S. Abnormalities in nitric oxide and its derivatives in lung cancer. *Am. J. Respir. Crit. Care. Med.,* **2005**, *172*(5), 597-605.

[187] Rasheed, M.H.; Beevi, S.S.; Geetha, A. Enhanced lipid peroxidation and nitric oxide products with deranged antioxidant status in patients with head and neck squamous cell carcinoma. *Oral. Oncol.,* **2007**, *43*(4), 333-338.

[188] Choudhari, S.K.; Chaudhary, M.; Bagde, S.; Gadbail, A.R.; Joshi, V. Nitric oxide and cancer: a review. *World J. Surg. Oncol.,* **2013**, *11*, 118.

[189] Kostourou, V.; Robinson, S.P.; Cartwright, J.E.; Whitley, G.S. Dimethylarginine dimethylaminohydrolase I enhances tumour growth and angiogenesis. *Br. J. Cancer,* **2002**, *87*(6), 673-680.

[190] Kostourou, V.; Robinson, S.P.; Whitley, G.S.; Griffiths, J.R. Effects of overexpression of dimethylarginine dimethylaminohydrolase on tumor angiogenesis assessed by susceptibility magnetic resonance imaging. *Cancer Res.,* **2003**, *63*(16), 4960-4966.

[191] Kostourou, V.; Troy, H.; Murray, J.F.; Cullis, E.R.; Whitley, G.S.; Griffiths, J.R.; Robinson, S.P. Overexpression of dimethylarginine dimethylaminohydrolase enhances tumor hypoxia: an insight into the relationship of hypoxia and angiogenesis *in vivo*. *Neoplasia,* **2004**, *6*(4), 401-411.

[192] Boult, J.K.; Walker-Samuel, S.; Jamin, Y.; Leiper, J.M.; Whitley, G.S.; Robinson, S.P. Active site mutant dimethylarginine dimethylaminohydrolase 1 expression confers an intermediate tumour phenotype in C6 gliomas. *J. Pathol.,* **2011**, *225*(3), 344-352.

[193] Yoshimatsu, M.; Toyokawa, G.; Hayami, S.; Unoki, M.; Tsunoda, T.; Field, H.I.; Kelly, J.D.; Neal, D.E.; Maehara, Y.; Ponder, B.A.; Nakamura, Y.; Hamamoto, R. Dysregulation of PRMT1 and PRMT6, Type I arginine methyltransferases, is involved in various types of human cancers. *Int. J. Cancer,* **2011**, *128*(3), 562-573.

[194] Szuba, A.; Chachaj, A.; Wrobel, T.; Dzietczenia, J.; Mazur, G.; Antonowicz-Juchniewicz, J.; Kuliczkowski, K.; Andrzejak, R. Asymmetric dimethylarginine in hematological malignancies: a preliminary study. *Leuk. Lymphoma.,* **2008**, *49*(12), 2316-2320.

[195] Li, H.; Zhou, Y.; Zhao, A.; Qiu, Y.; Xie, G.; Jiang, Q.; Zheng, X.; Zhong, W.; Sun, X.; Zhou, Z.; Jia, W. Asymmetric dimethylarginine attenuates serum starvation-induced apoptosis via suppression of the Fas (APO-1/CD95)/JNK (SAPK) pathway. *Cell. Death Dis.,* **2013**, *4*, e830.

[196] Chen, P.; Xia, K.; Zhao, Z.; Deng, X.; Yang, T. Atorvastatin modulates the DDAH1/ADMA system in high-fat diet-induced insulin-resistant rats with endothelial dysfunction. *Vasc. Med.,* **2012**, *17*(6), 416-423.

[197] Young, J.M.; Strey, C.H.; George, P.M.; Florkowski, C.M.; Sies, C.W.; Frampton, C.M.; Scott, R.S. Effect of atorvastatin on plasma levels of asymmetric dimethylarginine in patients with non-ischaemic heart failure. *Eur. J. Heart Fail.,* **2008**, *10*(5), 463-466.

[198] Lu, T.M.; Ding, Y.A.; Leu, H.B.; Yin, W.H.; Sheu, W.H.; Chu, K.M. Effect of rosuvastatin on plasma levels of asymmetric dimethylarginine in patients with hypercholesterolemia. *Am. J. Cardiol.,* **2004**, *94*(2), 157-161.

[199] Yang, T.L.; Chen, M.F.; Luo, B.L.; Xie, Q.Y.; Jiang, J.L.; Li, Y.J. Fenofibrate decreases asymmetric dimethylarginine level in cultured endothelial cells by inhibiting NF-kappaB activity. *Naunyn Schmiedebergs Arch Pharmacol.,* **2005**, *371*(5), 401-407.

[200] Yang, T.L.; Chen, M.F.; Xia, X.; Luo, B.L.; Li, Y.J. Effect of fenofibrate on the level of asymmetric dimethylarginine in individuals with hypertriglyceridemia. *Eur. J. Clin. Pharmacol.,* **2006**, *62*(3), 179-184.

[201] Dierkes, J.; Westphal, S.; Martens-Lobenhoffer, J.; Luley, C.; Bode-Boger, S.M. Fenofibrate increases the L-arginine:ADMA ratio by increase of L-arginine concentration but has no effect on ADMA concentration. *Atherosclerosis,* **2004**, *173*(2), 239-244.

[202] Westphal, S.; Borucki, K.; Luley, C.; Martens-Lobenhoffer, J.; Bode-Boger, S.M. Treatment with niacin lowers ADMA. *Atherosclerosis,* **2006**, *184*(2), 448-450.

[203] Fujii, H.; Kono, K.; Nakai, K.; Goto, S.; Kitazawa, R.; Fukagawa, M.; Nishi, S. Renin-Angiotensin system inhibitors reduce serum asymmetric dimethylarginine levels and oxidative stress in normotensive patients with chronic kidney disease. *Nephron Extra,* **2014**, *4*(1), 18-25.

[204] Delles, C.; Schneider, M.P.; John, S.; Gekle, M.; Schmieder, R.E. Angiotensin converting enzyme inhibition and angiotensin II AT1-receptor blockade reduce the levels of asymmetrical N(G), N(G)-dimethylarginine in human essential hypertension. *Am. J. Hypertens.,* **2002**, *15*(7 Pt 1), 590-593.

[205] Wakino, S.; Hayashi, K.; Tatematsu, S.; Hasegawa, K.; Takamatsu, I.; Kanda, T.; Homma, K.; Yoshioka, K.; Sugano, N.; Saruta, T. Pioglitazone lowers systemic asymmetric dimethylarginine by inducing dimethylarginine dimethylaminohydrolase in rats. *Hypertens. Res.,* **2005**, *28*(3), 255-262.

[206] Wang, S.; Jiang, J.L.; Hu, C.P.; Zhang, X.J.; Yang, D.L.; Li, Y.J. Relationship between protective effects of rosiglitazone on endothelium and endogenous nitric oxide synthase inhibitor in streptozotocin-induced diabetic rats and cultured endothelial cells. *Diabetes Metab. Res. Rev.,* **2007**, *23*(2), 157-164.

[207] Wang, T.D.; Chen, W.J.; Cheng, W.C.; Lin, J.W.; Chen, M.F.; Lee, Y.T. Relation of improvement in endothelium-dependent flow-mediated vasodilation after rosiglitazone to changes in asymmetric dimethylarginine, endothelin-1, and C-reactive protein in nondiabetic patients with the metabolic syndrome. *Am. J. Cardiol.,* **2006**, *98*(8), 1057-1062.

[208] Asagami, T.; Abbasi, F.; Stuelinger, M.; Lamendola, C.; McLaughlin, T.; Cooke, J.P.; Reaven, G.M.; Tsao, P.S. Metformin treatment lowers asymmetric dimethylarginine concentrations in patients with type 2 diabetes. *Metabolism,* **2002**, *51*(7), 843-846.

[209] Cakirca, M.; Karatoprak, C.; Zorlu, M.; Kiskac, M.; Kanat, M.; Cikrikcioglu, M.A.; Soysal, P.; Hursitoglu, M.; Camli, A.A.; Erkoc, R.; Abdul-Ghani, M. Effect of vildagliptin add-on treatment to metformin on plasma asymmetric dimethylarginine in type 2 diabetes mellitus patients. *Drug Des. Devel. Ther.,* **2014**, *8*, 239-243.

[210] Khan, B.V.; Rahman, S.T.; Haque, T.; Merchant, N.; Bhaheetharan, S.; Harris, J., 3rd; Umar, K.; Wahi, J.; Ferdinand, K.C. Vascular effects of nebivolol added to hydrochlorothiazide in African Americans with hypertension and echocardiographic evidence of diastolic dysfunction: the NASAA study. *J. Cardiovasc. Pharmacol. Ther.,* **2012**, *17*(3), 291-297.

[211] Sen, N.; Tavil, Y.; Erdamar, H.; Yazici, H.U.; Cakir, E.; Akgul, E.O.; Bilgi, C.; Erbil, M.K.; Poyraz, F.; Okyay, K.; Turfan, M.; Cemri, M. Nebivolol therapy improves endothelial function and increases exercise tolerance in patients with cardiac syndrome X. *Anadolu Kardiyol Derg.,* **2009**, *9*(5), 371-379.

[212] Oguz, A.; Uzunlulu, M.; Yorulmaz, E.; Yalcin, Y.; Hekim, N.; Fici, F. Effect of nebivolol and metoprolol treatments on serum asymmetric dimethylarginine levels in hypertensive patients with type 2 diabetes mellitus. *Anadolu Kardiyol Derg.,* **2007**, *7*(4), 383-387.

[213] Deng, S.; Deng, P.Y.; Jiang, J.L.; Ye, F.; Yu, J.; Yang, T.L.; Deng, H.D.; Li, Y.J. Aspirin protected against endothelial damage induced by LDL: role of endogenous NO synthase inhibitors in rats. *Acta Pharmacol. Sin.,* **2004**, *25*(12), 1633-1639.

[214] Hetzel, S.; DeMets, D.; Schneider, R.; Borzak, S.; Schneider, W.; Serebruany, V.; Schroder, H.; Hennekens, C.H. Aspirin increases nitric oxide formation in chronic stable coronary disease. *J. Cardiovasc. Pharmacol. Ther.,* **2013**, *18*(3), 217-221.

[215] Holden, D.P.; Cartwright, J.E.; Nussey, S.S.; Whitley, G.S. Estrogen stimulates dimethylarginine dimethylaminohydrolase activity and the metabolism of asymmetric dimethylarginine. *Circulation.,* **2003**, *108*(13), 1575-1580.

[216] Post, M.S.; Verhoeven, M.O.; van der Mooren, M.J.; Kenemans, P.; Stehouwer, C.D.; Teerlink, T. Effect of hormone replacement therapy on plasma levels of the cardiovascular risk factor asymmetric dimethylarginine: a randomized, placebo-controlled 12-week study in healthy early postmenopausal women. *J. Clin. Endocrinol. Metab.,* **2003**, *88*(9), 4221-4226.

[217] Wu, C.J.; Wang, L.; Li, X.; Wang, C.X.; Ma, J.P.; Xia, X.S. [Impact of adding folic acid, vitamin B(12) and probucol to standard antihypertensive medication on plasma homocysteine and asymmetric dimethylarginine levels of essential hypertension patients]. *Zhonghua Xin Xue Guan Bing Za Zhi,* **2012**, *40*(12), 1003-1008.

[218] Ziegler, S.; Mittermayer, F.; Plank, C.; Minar, E.; Wolzt, M.; Schernthaner, G.H. Homocyst(e)ine-lowering therapy does not affect plasma asymmetrical dimethylarginine concentrations in patients with peripheral artery disease. *J. Clin. Endocrinol. Metab.,* **2005**, *90*(4), 2175-2178.

[219] Mittermayer, F.; Pleiner, J.; Francesconi, M.; Wolzt, M. Treatment with alpha-lipoic acid reduces asymmetric dimethylarginine in patients with type 2 diabetes mellitus. *Transl. Res.,* **2010**, *155*(1), 6-9.

[220] Thaha, M.; Widodo; Pranawa, W.; Yogiantoro, M.; Tomino, Y. Intravenous N-acetylcysteine during hemodialysis reduces asymmetric dimethylarginine level in end-stage renal disease patients. *Clin. Nephrol.,* **2008**, *69*(1), 24-32.

[221] Nascimento, M.M.; Suliman, M.E.; Silva, M.; Chinaglia, T.; Marchioro, J.; Hayashi, S.Y.; Riella, M.C.; Lindholm, B.; Anderstam, B. Effect of oral N-acetylcysteine treatment on plasma inflammatory and oxidative stress markers in peritoneal dialysis patients: a placebo-controlled study. *Perit. Dial. Int.,* **2010**, *30*(3), 336-342.

[222] Tain, Y.L.; Huang, L.T.; Lin, I.C.; Lau, Y.T.; Lin, C.Y. Melatonin prevents hypertension and increased asymmetric dimethylarginine in young spontaneous hypertensive rats. *J. Pineal. Res.,* **2010**, *49*(4), 390-398.

[223] Han, S.; Uludag, M.O.; Usanmaz, S.E.; Ayaloglu-Butun, F.; Akcali, K.C.; Demirel-Yilmaz, E. Resveratrol affects histone 3 lysine 27 methylation of vessels and blood biomarkers in DOCA salt-induced hypertension. *Mol. Biol. Rep.,* **2015**, *42*(1), 35-42.

[224] Tousoulis, D.; Simopoulou, C.; Papageorgiou, N.; Oikonomou, E.; Hatzis, G.; Siasos, G.; Tsiamis, E.; Stefanadis, C. Endothelial dysfunction in conduit arteries and in microcirculation. Novel therapeutic approaches. *Pharmacol. Ther.,* **2014**.

[225] Tousoulis, D.; Oikonomou, E.; Siasos, G.; Chrysohoou, C.; Zaromitidou, M.; Kioufis, S.; Maniatis, K.; Dilaveris, P.; Miliou, A.; Michalea, S.; Papavassiliou, A.G.; Stefanadis, C. Dose-dependent effects of short term atorvastatin treatment on arterial wall properties and on indices of left ventricular remodeling in ischemic heart failure. *Atherosclerosis,* **2013**, *227*(2), 367-372.

[226] Tousoulis, D.; Antoniades, C.; Vasiliadou, C.; Kourtellaris, P.; Koniari, K.; Marinou, K.; Charakida, M.; Ntarladimas, I.; Siasos, G.; Stefanadis, C. Effects of atorvastatin and vitamin C on forearm hyperaemic blood flow, asymmentrical dimethylarginine levels and the inflammatory process in patients with type 2 diabetes mellitus. *Heart,* **2007**, *93*(2), 244-246.

[227] Tousoulis, D.; Koutsogiannis, M.; Papageorgiou, N.; Siasos, G.; Antoniades, C.; Tsiamis, E.; Stefanadis, C. Endothelial dysfunction: potential clinical implications. *Minerva Med.,* **2010**, *101*(4), 271-284.

[228] Sicard, P.; Delemasure, S.; Korandji, C.; Segueira-Le Grand, A.; Lauzier, B.; Guilland, J.C.; Duvillard, L.; Zeller, M.; Cottin, Y.; Vergely, C.; Rochette, L. Anti-hypertensive effects of Rosuvastatin are associated with decreased inflammation and oxidative stress markers in hypertensive rats. *Free Radic. Res.,* **2008**, *42*(3), 226-236.

[229] Li, J.; Xia, W.; Feng, W.; Qu, X. Effects of rosuvastatin on serum asymmetric dimethylarginine levels and atrial structural remodeling in atrial fibrillation dogs. *Pacing Clin. Electrophysiol.,* **2012**, *35*(4), 456-464.

[230] Vladimirova-Kitova, L.G.; Deneva, T.I. Simvastatin and asymmetric dimethylarginine-homocysteine metabolic pathways in patients with newly detected severe hypercholesterolemia. *Clin. Lab.,* **2010**, *56*(7-8), 291-302.

[231] Kurtoglu, E.; Balta, S.; Sincer, I.; Altas, Y.; Atas, H.; Yilmaz, M.; Korkmaz, H.; Erdem, K.; Akturk, E.; Demirkol, S.; Can, C. Comparision of effects of rosuvastatin versus atorvastatin treatment on plasma levels of asymmetric dimethylarginine in patients with hyperlipidemia having coronary artery disease. *Angiology,* **2014**, *65*(9), 788-793.

[232] Nishiyama, Y.; Ueda, M.; Otsuka, T.; Katsura, K.; Abe, A.; Nagayama, H.; Katayama, Y. Statin treatment decreased serum asymmetric dimethylarginine (ADMA) levels in ischemic stroke patients. *J. Atheroscler. Thromb.,* **2011**, *18*(2), 131-137.

[233] Boger, G.I.; Rudolph, T.K.; Maas, R.; Schwedhelm, E.; Dumbadze, E.; Bierend, A.; Benndorf, R.A.; Boger, R.H. Asymmetric dimethy-

larginine determines the improvement of endothelium-dependent vasodilation by simvastatin: Effect of combination with oral L-arginine. *J. Am. Coll. Cardiol.,* **2007**, *49*(23), 2274-2282.

[234] Yang, T.L.; Chen, M.F.; Luo, B.L.; Yu, J.; Jiang, J.L.; Li, Y.J. Effect of fenofibrate on LDL-induced endothelial dysfunction in rats. *Naunyn Schmiedebergs Arch Pharmacol.,* **2004**, *370*(2), 79-83.

[235] Boden, W.E.; Sidhu, M.S.; Toth, P.P. The therapeutic role of niacin in dyslipidemia management. *J. Cardiovasc. Pharmacol. Ther.,* **2014**, *19*(2), 141-158.

[236] Hornig, B.; Landmesser, U.; Kohler, C.; Ahlersmann, D.; Spiekermann, S.; Christoph, A.; Tatge, H.; Drexler, H. Comparative effect of ace inhibition and angiotensin II type 1 receptor antagonism on bioavailability of nitric oxide in patients with coronary artery disease: role of superoxide dismutase. *Circulation,* **2001**, *103*(6), 799-805.

[237] Fu, Y.F.; Xiong, Y.; Guo, Z. A reduction of endogenous asymmetric dimethylarginine contributes to the effect of captopril on endothelial dysfunction induced by homocysteine in rats. *Eur. J. Pharmacol.,* **2005**, *508*(1-3), 167-175.

[238] Park, S.H.; Hyun, S.H.; Ryu, H.M.; Ahn, J.S.; Oh, S.H.; Oh, E.J.; Yoon, S.H.; Choi, J.Y.; Cho, J.H.; Kim, C.D.; Kim, Y.L. Effects of losartan and pentoxifylline on renal dimethylarginine dimethylaminohydrolase-1 expression in proteinuric nephropathy. *Am. J. Nephrol.,* **2013**, *37*(5), 491-500.

[239] Veresh, Z.; Debreczeni, B.; Hamar, J.; Kaminski, P.M.; Wolin, M.S.; Koller, A. Asymmetric dimethylarginine reduces nitric oxide donor-mediated dilation of arterioles by activating the vascular renin-angiotensin system and reactive oxygen species. *J. Vasc. Res.,* **2012**, *49*(4), 363-372.

[240] Li, D.; Xia, K.; Li, N.S.; Luo, D.; Wang, S.; Jiang, D.J.; Deng, H.W.; Li, Y.J. Reduction of asymmetric dimethylarginine involved in the cardioprotective effect of losartan in spontaneously hypertensive rats. *Can. J. Physiol. Pharmacol.,* **2007**, *85*(8), 783-789.

[241] Tain, Y.L.; Hsu, C.N.; Lin, C.Y.; Huang, L.T.; Lau, Y.T. Aliskiren prevents hypertension and reduces asymmetric dimethylarginine in young spontaneously hypertensive rats. *Eur. J. Pharmacol.,* **2011**, *670*(2-3), 561-565.

[242] Cook, S.; Hugli, O.; Egli, M.; Menard, B.; Thalmann, S.; Sartori, C.; Perrin, C.; Nicod, P.; Thorens, B.; Vollenweider, P.; Scherrer, U.; Burcelin, R. Partial gene deletion of endothelial nitric oxide synthase predisposes to exaggerated high-fat diet-induced insulin resistance and arterial hypertension. *Diabetes,* **2004**, *53*(8), 2067-2072.

[243] Zhang, W.L.; Yan, W.J.; Sun, B.; Zou, Z.P. Synergistic effects of atorvastatin and rosiglitazone on endothelium protection in rats with dyslipidemia. *Lipids. Health. Dis.,* **2014**, *13*(1), 168.

[244] Kelly, A.S.; Thelen, A.M.; Kaiser, D.R.; Gonzalez-Campoy, J.M.; Bank, A.J. Rosiglitazone improves endothelial function and inflammation but not asymmetric dimethylarginine or oxidative stress in patients with type 2 diabetes mellitus. *Vasc. Med.,* **2007**, *12*(4), 311-318.

[245] King, D.E.; Player, M.; Everett, C.J. The impact of pioglitazone on ADMA and oxidative stress markers in patients with type 2 diabetes. *Prim. Care. Diabetes.,* **2012**, *6*(2), 157-161.

[246] Consoli, A.; Formoso, G. Do thiazolidinediones still have a role in treatment of type 2 diabetes mellitus? *Diabetes Obes. Metab.,* **2013**, *15*(11), 967-977.

[247] Viollet, B.; Guigas, B.; Sanz Garcia, N.; Leclerc, J.; Foretz, M.; Andreelli, F. Cellular and molecular mechanisms of metformin: an overview. *Clin. Sci. (Lond).,* **2012**, *122*(6), 253-270.

[248] El-Mir, M.Y.; Nogueira, V.; Fontaine, E.; Averet, N.; Rigoulet, M.; Leverve, X. Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I. *J. Biol. Chem.,* **2000**, *275*(1), 223-228.

[249] Owen, M.R.; Doran, E.; Halestrap, A.P. Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. *Biochem. J.,* **2000**, *348. Pt. 3*, 607-614.

[250] Stephenne, X.; Foretz, M.; Taleux, N.; van der Zon, G.C.; Sokal, E.; Hue, L.; Viollet, B.; Guigas, B. Metformin activates AMP-activated protein kinase in primary human hepatocytes by decreasing cellular energy status. *Diabetologia,* **2011**, *54*(12), 3101-3110.

[251] Tsai, C.M.; Kuo, H.C.; Hsu, C.N.; Huang, L.T.; Tain, Y.L. Metformin reduces asymmetric dimethylarginine and prevents hypertension in spontaneously hypertensive rats. *Transl. Res.,* **2014**, *164*(6), 452-459.

[252] Bal, F.; Bekpinar, S.; Unlucerci, Y.; Kusku-Kiraz, Z.; Onder, S.; Uysal, M.; Gurdol, F. Antidiabetic drug metformin is effective on the metabolism of asymmetric dimethylarginine in experimental liver injury. *Diabetes Res. Clin. Pract.,* **2014**, *106*(2), 295-302.

[253] Bestermann, W.H., Jr. The ADMA-Metformin Hypothesis: Linking the Cardiovascular Consequences of the Metabolic Syndrome and Type 2 Diabetes. *Cardiorenal. Med.,* **2011**, *1*(4), 211-219.

[254] Heutling, D.; Schulz, H.; Nickel, I.; Kleinstein, J.; Kaltwasser, P.; Westphal, S.; Mittermayer, F.; Wolzt, M.; Krzyzanowska, K.; Randeva, H.; Schernthaner, G.; Lehnert, H. Asymmetrical dimethylarginine, inflammatory and metabolic parameters in women with polycystic ovary syndrome before and after metformin treatment. *J. Clin. Endocrinol. Metab.,* **2008**, *93*(1), 82-90.

[255] Ozgurtas, T.; Oktenli, C.; Dede, M.; Tapan, S.; Kenar, L.; Sanisoglu, S.Y.; Yesilova, Z.; Yenen, M.C.; Erbil, M.K.; Baser, I. Metformin and oral contraceptive treatments reduced circulating asymmetric dimethylarginine (ADMA) levels in patients with polycystic ovary syndrome (PCOS). *Atherosclerosis,* **2008**, *200*(2), 336-344.

[256] Kilic, S.; Yilmaz, N.; Zulfikaroglu, E.; Erdogan, G.; Aydin, M.; Batioglu, S. Inflammatory-metabolic parameters in obese and nonobese normoandrogenemic polycystic ovary syndrome during metformin and oral contraceptive treatment. *Gynecol. Endocrinol.,* **2011**, *27*(9), 622-629.

[257] Lund, S.S.; Tarnow, L.; Stehouwer, C.D.; Schalkwijk, C.G.; Teerlink, T.; Gram, J.; Winther, K.; Frandsen, M.; Smidt, U.M.; Pedersen, O.; Parving, H.H.; Vaag, A.A. Impact of metformin versus repaglinide on non-glycaemic cardiovascular risk markers related to inflammation and endothelial dysfunction in non-obese patients with type 2 diabetes. *Eur. J. Endocrinol.,* **2008**, *158*(5), 631-641.

[258] Kruszelnicka, O.; Chyrchel, B.; Golay, A.; Surdacki, A. Differential associations of circulating asymmetric dimethylarginine and cell adhesion molecules with metformin use in patients with type 2 diabetes mellitus and stable coronary artery disease. *Amino Acids,* **2015**.

[259] Yin, Q.F.; Fu, S.H.; He, P.; Xiong, Y. Dimethylarginine dimethylaminohydrolase inhibition and asymmetric dimethylarginine accumulation contribute to endothelial dysfunction in rats exposed to glycosylated protein: effects of aminoguanidine. *Atherosclerosis,* **2007**, *190*(1), 53-61.

[260] Yasuda, S.; Miyazaki, S.; Kanda, M.; Goto, Y.; Suzuki, M.; Harano, Y.; Nonogi, H. Intensive treatment of risk factors in patients with type-2 diabetes mellitus is associated with improvement of endothelial function coupled with a reduction in the levels of plasma asymmetric dimethylarginine and endogenous inhibitor of nitric oxide synthase. *Eur. Heart J.,* **2006**, *27*(10), 1159-1165.

[261] Mason, R.P.; Kalinowski, L.; Jacob, R.F.; Jacoby, A.M.; Malinski, T. Nebivolol reduces nitroxidative stress and restores nitric oxide bioavailability in endothelium of black Americans. *Circulation,* **2005**, *112*(24), 3795-3801.

[262] Pasini, A.F.; Garbin, U.; Stranieri, C.; Boccioletti, V.; Mozzini, C.; Manfro, S.; Pasini, A.; Cominacini, M.; Cominacini, L. Nebivolol treatment reduces serum levels of asymmetric dimethylarginine and improves endothelial dysfunction in essential hypertensive patients. *Am. J. Hypertens.,* **2008**, *21*(11), 1251-1257.

[263] Wang, Y.; Zhang, M.; Liu, Y.; Liu, Y.; Chen, M. The effect of nebivolol on asymmetric dimethylarginine system in spontaneously hypertension rats. *Vascul. Pharmacol.,* **2011**, *54*(1-2), 36-43.

[264] Alfieri, A.B.; Briceno, L.; Fragasso, G.; Spoladore, R.; Palloshi, A.; Bassanelli, G.; Montano, C.; Arioli, F.; Cuko, A.; Ruotolo, G.; Margonato, A. Differential long-term effects of carvedilol on proinflammatory and antiinflammatory cytokines, asymmetric dimethylarginine, and left ventricular function in patients with heart failure. *J. Cardiovasc. Pharmacol.,* **2008**, *52*(1), 49-54.

[265] Kelly, A.S.; Gonzalez-Campoy, J.M.; Rudser, K.D.; Katz, H.; Metzig, A.M.; Thalin, M.; Bank, A.J. Carvedilol-lisinopril combination therapy and endothelial function in obese individuals with hypertension. *J. Clin. Hypertens (Greenwich).,* **2012**, *14*(2), 85-91.

[266] Bode-Boger, S.M.; Martens-Lobenhoffer, J.; Tager, M.; Schroder, H.; Scalera, F. Aspirin reduces endothelial cell senescence. *Biochem. Biophys. Res. Commun.,* **2005**, *334*(4), 1226-1232.

[267] Yi, T.N.; Zhao, H.Y.; Zhang, J.S.; Shan, H.Y.; Meng, X.; Zhang, J. Effect of aspirin on high glucose-induced senescence of endothelial cells. *Chin. Med. J. (Engl).,* **2009**, *122*(24), 3055-3061.

[268] Schulze, F.; Maas, R.; Freese, R.; Schwedhelm, E.; Silberhorn, E.; Boger, R.H. Determination of a reference value for N(G), N(G)-dimethyl-L-arginine in 500 subjects. *Eur. J. Clin. Invest.,* **2005**, *35*(10), 622-626.

[269] Liao, Q.; Li, X.; Zhou, S.; Liu, L.; Zhao, S.; Lian, Y.; Dong, H. Estrogen treatment inhibits vascular endothelial senescence and asymmetrical dimethylarginine in ovariectomized rabbits. *J. Cardiovasc. Pharmacol.,* **2011**, *57*(2), 174-182.

[270] Novella, S.; Laguna-Fernandez, A.; Lazaro-Franco, M.; Sobrino, A.; Bueno-Beti, C.; Tarin, J.J.; Monsalve, E.; Sanchis, J.; Hermenegildo, C. Estradiol, acting through estrogen receptor alpha, restores dimethylarginine dimethylaminohydrolase activity and nitric oxide production in oxLDL-treated human arterial endothelial cells. *Mol. Cell. Endocrinol.,* **2013**, *365*(1), 11-16.

[271] Antoniades, C.; Tousoulis, D.; Tentolouris, C.; Toutouza, M.; Marinou, K.; Goumas, G.; Tsioufis, C.; Toutouzas, P.; Stefanadis, C. Effects of antioxidant vitamins C and E on endothelial function and thrombosis/fibrinolysis system in smokers. *Thromb. Haemost.,* **2003**, *89*(6), 990-995.

[272] Nanayakkara, P.W.; Kiefte-de Jong, J.C.; ter Wee, P.M.; Stehouwer, C.D.; van Ittersum, F.J.; Olthof, M.R.; Teerlink, T.; Twisk, J.W.; van Guldener, C.; Smulders, Y.M. Randomized placebo-controlled trial assessing a treatment strategy consisting of pravastatin, vitamin E, and homocysteine lowering on plasma asymmetric dimethylarginine concentration in mild to moderate CKD. *Am. J. Kidney Dis.,* **2009**, *53*(1), 41-50.

[273] O'Doherty, M.G.; Gilchrist, S.E.; Young, I.S.; McKinley, M.C.; Yarnell, J.W.; Gey, K.F.; Evans, A.; Skidmore, P.M.; Woodside, J.V. Effect of supplementation with B vitamins and antioxidants on levels of asymmetric dimethylarginine (ADMA) and C-reactive protein (CRP): a double-blind, randomised, factorial design, placebo-controlled trial. *Eur. J. Nutr.,* **2010**, *49*(8), 483-492.

[274] Holven, K.B.; Haugstad, T.S.; Holm, T.; Aukrust, P.; Ose, L.; Nenseter, M.S. Folic acid treatment reduces elevated plasma levels of asymmetric dimethylarginine in hyperhomocysteinaemic subjects. *Br. J. Nutr.,* **2003**, *89*(3), 359-363.

[275] Koyama, K.; Ito, A.; Yamamoto, J.; Nishio, T.; Kajikuri, J.; Dohi, Y.; Ohte, N.; Sano, A.; Nakamura, H.; Kumagai, H.; Itoh, T. Randomized controlled trial of the effect of short-term coadministration of methylcobalamin and folate on serum ADMA concentration in patients receiving long-term hemodialysis. *Am. J. Kidney Dis.,* **2010**, *55*(6), 1069-1078.

[276] Paul, B.; Whiting, M.J.; De Pasquale, C.G.; Mangoni, A.A. Acute effects of 5-methyltetrahydrofolate on endothelial function and asymmetric dimethylarginine in patients with chronic heart failure. *Nutr. Metab. Cardiovasc. Dis.,* **2010**, *20*(5), 341-349.

[277] Reed, L.J.; De, B.B.; Gunsalus, I.C.; Hornberger, C.S., Jr. Crystalline alpha-lipoic acid; a catalytic agent associated with pyruvate dehydrogenase. *Science.,* **1951**, *114*(2952), 93-94.

[278] Park, S.; Karunakaran, U.; Jeoung, N.H.; Jeon, J.H.; Lee, I.K. Physiological effect and therapeutic application of alpha lipoic acid. *Curr. Med. Chem.,* **2014**, *21*(32), 3636-3645.

[279] Ozdogan, S.; Kaman, D.; Simsek, B.C. Effects of coenzyme Q10 and alpha-lipoic acid supplementation in fructose fed rats. *J. Clin. Biochem. Nutr.,* **2012**, *50*(2), 145-151.

[280] Lee, W.J.; Kim, S.W.; Kim, G.H.; Han, S.M.; Won, J.C.; Jung, C.H.; Park, H.S.; Choi do, S.; Lee, K.U.; Park, J.Y. Alpha-lipoic acid activates dimethylarginine dimethylaminohydrolase in cultured endothelial cells. *Biochem. Biophys. Res. Commun.,* **2010**, *398*(4), 653-658.

[281] Chang, J.W.; Lee, E.K.; Kim, T.H.; Min, W.K.; Chun, S.; Lee, K.U.; Kim, S.B.; Park, J.S. Effects of alpha-lipoic acid on the plasma levels of asymmetric dimethylarginine in diabetic end-stage renal disease patients on hemodialysis: a pilot study. *Am. J. Nephrol.,* **2007**, *27*(1), 70-74.

[282] El-Nakib, G.A.; Mostafa, T.M.; Abbas, T.M.; El-Shishtawy, M.M.; Mabrouk, M.M.; Sobh, M.A. Role of alpha-lipoic acid in the management of anemia in patients with chronic renal failure undergoing hemodialysis. *Int. J. Nephrol. Renovasc. Dis.,* **2013**, *6*, 161-168.

[283] Samuni, Y.; Goldstein, S.; Dean, O.M.; Berk, M. The chemistry and biological activities of N-acetylcysteine. *Biochim. Biophys. Acta.,* **2013**, *1830*(8), 4117-4129.

[284] Adelibieke, Y.; Shimizu, H.; Muteliefu, G.; Bolati, D.; Niwa, T. Indoxyl sulfate induces endothelial cell senescence by increasing reactive oxygen species production and p53 activity. *J. Ren. Nutr.,* **2012**, *22*(1), 86-89.

[285] Kaida, Y.; Ueda, S.; Yamagishi, S.; Nakayama, Y.; Ando, R.; Iwatani, R.; Fukami, K.; Okuda, S. Proteinuria elevates asymmetric dimethylarginine levels via protein arginine methyltransferase-1 overexpression in a rat model of nephrotic syndrome. *Life Sci.,* **2012**, *91*(9-10), 301-305.

[286] Ando, R.; Ueda, S.; Yamagishi, S.; Miyazaki, H.; Kaida, Y.; Kaifu, K.; Yokoro, M.; Nakayama, Y.; Obara, N.; Fukami, K.; Takeuchi, M.; Okuda, S. Involvement of advanced glycation end product-induced asymmetric dimethylarginine generation in endothelial dysfunction. *Diab. Vasc. Dis. Res.,* **2013**, *10*(5), 436-441.

[287] Nakayama, Y.; Ueda, S.; Yamagishi, S.; Obara, N.; Taguchi, K.; Ando, R.; Kaida, Y.; Iwatani, R.; Kaifu, K.; Yokoro, M.; Toyonaga, M.; Kusumoto, T.; Fukami, K.; Okuda, S. Asymmetric dimethylarginine accumulates in the kidney during ischemia/reperfusion injury. *Kidney Int.,* **2014**, *85*(3), 570-578.

[288] Fan, N.C.; Tsai, C.M.; Hsu, C.N.; Huang, L.T.; Tain, Y.L. N-acetylcysteine prevents hypertension via regulation of the ADMA-DDAH pathway in young spontaneously hypertensive rats. *Biomed. Res. Int.,* **2013**, *2013*, 696317.

[289] Egert, S.; Baxheinrich, A.; Lee-Barkey, Y.H.; Tschoepe, D.; Wahrburg, U.; Stratmann, B. Effects of an energy-restricted diet rich in plant-derived alpha-linolenic acid on systemic inflammation and endothelial function in overweight-to-obese patients with metabolic syndrome traits. *Br. J. Nutr.,* **2014**, *112*(8), 1315-1322.

[290] Ayer, J.G.; Harmer, J.A.; Xuan, W.; Toelle, B.; Webb, K.; Almqvist, C.; Marks, G.B.; Celermajer, D.S. Dietary supplementation with n-3 polyunsaturated fatty acids in early childhood: effects on blood pressure and arterial structure and function at age 8 y. *Am. J. Clin. Nutr.,* **2009**, *90*(2), 438-446.

[291] Eid, H.M.; Arnesen, H.; Hjerkinn, E.M.; Lyberg, T.; Ellingsen, I.; Seljeflot, I. Effect of diet and omega-3 fatty acid intervention on asymmetric dimethylarginine. *Nutr. Metab. (Lond).,* **2006**, *3*, 4.

[292] Zebrowska, A.; Mizia-Stec, K.; Mizia, M.; Gasior, Z.; Poprzecki, S. Omega-3 fatty acids supplementation improves endothelial function and maximal oxygen uptake in endurance-trained athletes. *Eur. J. Sport. Sci.,* **2014**, 1-10.

[293] Lundberg, J.O.; Weitzberg, E.; Gladwin, M.T. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. *Nat. Rev. Drug Discov.,* **2008**, *7*(2), 156-167.

[294] Webb, A.J.; Patel, N.; Loukogeorgakis, S.; Okorie, M.; Aboud, Z.; Misra, S.; Rashid, R.; Miall, P.; Deanfield, J.; Benjamin, N.; MacAllister, R.; Hobbs, A.J.; Ahluwalia, A. Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite. *Hypertension,* **2008**, *51*(3), 784-790.

[295] Larsen, F.J.; Ekblom, B.; Sahlin, K.; Lundberg, J.O.; Weitzberg, E. Effects of dietary nitrate on blood pressure in healthy volunteers. *N. Engl. J. Med.,* **2006**, *355*(26), 2792-2793.

[296] Webb, A.; Bond, R.; McLean, P.; Uppal, R.; Benjamin, N.; Ahluwalia, A. Reduction of nitrite to nitric oxide during ischemia protects against myocardial ischemia-reperfusion damage. *Proc. Natl. Acad. Sci. U. S. A.,* **2004**, *101*(37), 13683-13688.

[297] Gonzalez, F.M.; Shiva, S.; Vincent, P.S.; Ringwood, L.A.; Hsu, L.Y.; Hon, Y.Y.; Aletras, A.H.; Cannon, R.O., 3rd; Gladwin, M.T.; Arai, A.E. Nitrite anion provides potent cytoprotective and anti-apoptotic effects as adjunctive therapy to reperfusion for acute myocardial infarction. *Circulation,* **2008**, *117*(23), 2986-2994.

[298] Jung, K.H.; Chu, K.; Ko, S.Y.; Lee, S.T.; Sinn, D.I.; Park, D.K.; Kim, J.M.; Song, E.C.; Kim, M.; Roh, J.K. Early intravenous infusion of sodium nitrite protects brain against *in. vivo* ischemia-reperfusion injury. *Stroke,* **2006**, *37*(11), 2744-2750.

[299] Kanematsu, Y.; Yamaguchi, K.; Ohnishi, H.; Motobayashi, Y.; Ishizawa, K.; Izawa, Y.; Kawazoe, K.; Kondo, S.; Kagami, S.; Tomita, S.; Tsuchiya, K.; Tamaki, T. Dietary doses of nitrite restore circulating nitric oxide level and improve renal injury in L-NAME-induced hypertensive rats. *Am. J. Physiol. Renal. Physiol.,* **2008**, *295*(5), F1457-1462.

[300] Carlstrom, M.; Persson, A.E.; Larsson, E.; Hezel, M.; Scheffer, P.G.; Teerlink, T.; Weitzberg, E.; Lundberg, J.O. Dietary nitrate attenuates oxidative stress, prevents cardiac and renal injuries, and reduces blood pressure in salt-induced hypertension. *Cardiovasc. Res.,* **2011**, *89*(3), 574-585.

[301] Chien, S.J.; Lin, K.M.; Kuo, H.C.; Huang, C.F.; Lin, Y.J.; Huang, L.T.; Tain, Y.L. Two different approaches to restore renal nitric ox-

ide and prevent hypertension in young spontaneously hypertensive rats: l-citrulline and nitrate. *Transl. Res.,* **2014**, *163*(1), 43-52.

[302] Maeda, S.; Miyaki, A.; Kumagai, H.; Eto, M.; So, R.; Tanaka, K.; Ajisaka, R. Lifestyle modification decreases arterial stiffness and plasma asymmetric dimethylarginine level in overweight and obese men. *Coron. Artery Dis.,* **2013**, *24*(7), 583-588.

[303] Rudofsky, G.; Roeder, E.; Merle, T.; Hildebrand, M.; Nawroth, P.P.; Wolfrum, C. Weight loss improves endothelial function independently of ADMA reduction in severe obesity. *Horm. Metab. Res.,* **2011**, *43*(5), 343-348.

[304] Koncsos, P.; Seres, I.; Harangi, M.; Pall, D.; Jozsa, L.; Bajnok, L.; Nagy, E.V.; Paragh, G. Favorable effect of short-term lifestyle intervention on human paraoxonase-1 activity and adipokine levels in childhood obesity. *J. Am. Coll. Nutr.,* **2011**, *30*(5), 333-339.

[305] Thomazella, M.C.; Goes, M.F.; Andrade, C.R.; Debbas, V.; Barbeiro, D.F.; Correia, R.L.; Marie, S.K.; Cardounel, A.J.; daLuz, P.L.; Laurindo, F.R. Effects of high adherence to mediterranean or low-fat diets in medicated secondary prevention patients. *Am. J. Cardiol.,* **2011**, *108*(11), 1523-1529.

[306] Moreno-Luna, R.; Munoz-Hernandez, R.; Miranda, M.L.; Costa, A.F.; Jimenez-Jimenez, L.; Vallejo-Vaz, A.J.; Muriana, F.J.; Villar, J.; Stiefel, P. Olive oil polyphenols decrease blood pressure and improve endothelial function in young women with mild hypertension. *Am. J. Hypertens.,* **2012**, *25*(12), 1299-1304.

[307] Fang, Y.; Mu, J.J.; He, L.C.; Wang, S.C.; Liu, Z.Q. Salt loading on plasma asymmetrical dimethylarginine and the protective role of potassium supplement in normotensive salt-sensitive asians. *Hypertension.,* **2006**, *48*(4), 724-729.

[308] Cao, Y.; Mu, J.J.; Fang, Y.; Yuan, Z.Y.; Liu, F.Q. Impact of High Salt Independent of Blood Pressure on PRMT/ADMA/DDAH Pathway in the Aorta of Dahl Salt-Sensitive Rats. *Int. J. Mol. Sci.,* **2013**, *14*(4), 8062-8072.

[309] Teplan, V.; Schuck, O.; Racek, J.; Mareckova, O.; Stollova, M.; Hanzal, V.; Maly, J. Reduction of plasma asymmetric dimethylarginine in obese patients with chronic kidney disease after three years of a low-protein diet supplemented with keto-amino acids: a randomized controlled trial. *Wien Klin Wochenschr,* **2008**, *120*(15-16), 478-485.

[310] Baranyi, A.; Meinitzer, A.; Putz-Bankuti, C.; Stauber, R.; Kapfhammer, H.P.; Rothenhausler, H.B. Asymmetric dimethylarginine responses during interferon-alpha-induced depression in patients with chronic hepatitis C infection. *Psychosom. Med.,* **2014**, *76*(3), 197-207.

[311] Vaudo, G.; Marchesi, S.; Gerli, R.; Allegrucci, R.; Giordano, A.; Siepi, D.; Pirro, M.; Shoenfeld, Y.; Schillaci, G.; Mannarino, E. Endothelial dysfunction in young patients with rheumatoid arthritis and low disease activity. *Ann. Rheum. Dis.,* **2004**, *63*(1), 31-35.

[312] Sandoo, A.; Dimitroulas, T.; Veldhuijzen van Zanten, J.J.; Smith, J.P.; Metsios, G.S.; Nightingale, P.; Stavropoulos-Kalinoglou, A.; Kitas, G.D. Lack of association between asymmetric dimethylarginine and *in. vivo* microvascular and macrovascular endothelial function in patients with rheumatoid arthritis. *Clin. Exp. Rheumatol.,* **2012**, *30*(3), 388-396.

[313] Sari, I.; Kebapcilar, L.; Alacacioglu, A.; Bilgir, O.; Yildiz, Y.; Taylan, A.; Yuksel, A.; Kozaci, D.L. Increased levels of asymmetric dimethylarginine (ADMA) in patients with ankylosing spondylitis. *Intern. Med.,* **2009**, *48*(16), 1363-1368.

[314] Turiel, M.; Atzeni, F.; Tomasoni, L.; de Portu, S.; Delfino, L.; Bodini, B.D.; Longhi, M.; Sitia, S.; Bianchi, M.; Ferrario, P.; Doria, A.; De Gennaro Colonna, V.; Sarzi-Puttini, P. Non-invasive assessment of coronary flow reserve and ADMA levels: a case-control study of early rheumatoid arthritis patients. *Rheumatology (Oxford).,* **2009**, *48*(7), 834-839.

[315] Surdacki, A.; Martens-Lobenhoffer, J.; Wloch, A.; Gluszko, P.; Rakowski, T.; Dubiel, J.S.; Bode-Boger, S.M. Plasma asymmetric dimethylarginine is related to anticitrullinated protein antibodies in rheumatoid arthritis of short duration. *Metabolism,* **2009**, *58*(3), 316-318.

[316] Surdacki, A.; Martens-Lobenhoffer, J.; Wloch, A.; Marewicz, E.; Rakowski, T.; Wieczorek-Surdacka, E.; Dubiel, J.S.; Pryjma, J.; Bode-Boger, S.M. Elevated plasma asymmetric dimethyl-L-arginine levels are linked to endothelial progenitor cell depletion and carotid atherosclerosis in rheumatoid arthritis. *Arthritis Rheum.,* **2007**, *56*(3), 809-819.

[317] Spinelli, F.R.; Metere, A.; Barbati, C.; Pierdominici, M.; Iannuccelli, C.; Lucchino, B.; Ciciarello, F.; Agati, L.; Valesini, G.; Di Franco, M. Effect of therapeutic inhibition of TNF on circulating endothelial progenitor cells in patients with rheumatoid arthritis. *Mediat. Inflamm.,* **2013**, *2013*, 537539.

[318] Angel, K.; Provan, S.A.; Mowinckel, P.; Seljeflot, I.; Kvien, T.K.; Atar, D. The L-arginine/asymmetric dimethylarginine ratio is improved by anti-tumor necrosis factor-alpha therapy in inflammatory arthropathies. Associations with aortic stiffness. *Atherosclerosis,* **2012**, *225*(1), 160-165.

[319] Genre, F.; Lopez-Mejias, R.; Miranda-Filloy, J.A.; Carnero-Lopez, B.; Gomez-Acebo, I.; Blanco, R.; Ochoa, R.; Rueda, J.; Gonzalez-Juanatey, C.; Llorca, J.; Gonzalez-Gay, M.A. Asymmetric dimethylarginine serum levels in non-diabetic ankylosing spondylitis patients undergoing TNF-alpha antagonist therapy. *Clin. Exp. Rheumatol.,* **2013**, *31*(5), 749-755.

[320] Korkosz, M.; Gasowski, J.; Surdacki, A.; Leszczynski, P.; Pawlak-Bus, K.; Jeka, S.; Siedlar, M.; Grodzicki, T. Disparate effects of anti-TNF-alpha therapies on measures of disease activity and mediators of endothelial damage in ankylosing spondylitis. *Pharmacol. Rep.,* **2013**, *65*(4), 891-897.

[321] Sandoo, A.; Dimitroulas, T.; Toms, T.E.; Hodson, J.; Veldhuijzen van Zanten, J.J.; Smith, J.P.; Kitas, G.D. Clinical remission following treatment with tumour necrosis factor-alpha antagonists is not accompanied by changes in asymmetric dimethylarginine in patients with rheumatoid arthritis. *Clin. Biochem.,* **2012**, *45*(16-17), 1399-1403.

[322] Achan, V.; Tran, C.T.; Arrigoni, F.; Whitley, G.S.; Leiper, J.M.; Vallance, P. all-trans-Retinoic acid increases nitric oxide synthesis by endothelial cells: a role for the induction of dimethylarginine dimethylaminohydrolase. *Circ. Res.,* **2002**, *90*(7), 764-769.

[323] Arrigoni, F.; Ahmetaj, B.; Leiper, J. The biology and therapeutic potential of the DDAH/ADMA pathway. *Curr. Pharm. Des.,* **2010**, *16*(37), 4089-4102.

[324] Trocha, M.; Szuba, A.; Merwid-Lad, A.; Sozanski, T. Effect of selected drugs on plasma asymmetric dimethylarginine (ADMA) levels. *Pharmazie,* **2010**, *65*(8), 562-571.

[325] Beltowski, J.; Kedra, A. Asymmetric dimethylarginine (ADMA) as a target for pharmacotherapy. *Pharmacol. Rep.,* **2006**, *58*(2), 159-178.

[326] Abhary, S.; Burdon, K.P.; Kuot, A.; Javadiyan, S.; Whiting, M.J.; Kasmeridis, N.; Petrovsky, N.; Craig, J.E. Sequence variation in DDAH1 and DDAH2 genes is strongly and additively associated with serum ADMA concentrations in individuals with type 2 diabetes. *PLoS One,* **2010**, *5*(3), e9462.

[327] Andreozzi, F.; Presta, I.; Mannino, G.C.; Scarpelli, D.; Di Silvestre, S.; Di Pietro, N.; Succurro, E.; Sciacqua, A.; Pandolfi, A.; Consoli, A.; Hribal, M.L.; Perticone, F.; Sesti, G. A functional variant of the dimethylarginine dimethylaminohydrolase-2 gene is associated with insulin sensitivity. *PLoS One,* **2012**, *7*(4), e36224.

[328] Feng, M.; He, Z.M.; Zhu, Y.X.; Liu, L.H.; Lu, C.W.; Xiong, Y. Improvement of endothelial dysfunction in atherosclerotic rabbit aortas by ex vivo gene transferring of dimethylarginine dimethylaminohydrolase-2. *Int. J. Cardiol.,* **2010**, *144*(2), 180-186.

[329] Lu, C.W.; Guo, Z.; Feng, M.; Wu, Z.Z.; He, Z.M.; Xiong, Y. Ex vivo gene transferring of human dimethylarginine dimethylaminohydrolase-2 improved endothelial dysfunction in diabetic rat aortas and high glucose-treated endothelial cells. *Atherosclerosis,* **2010**, *209*(1), 66-73.

[330] Torondel, B.; Nandi, M.; Kelly, P.; Wojciak-Stothard, B.; Fleming, I.; Leiper, J. Adenoviral-mediated overexpression of DDAH improves vascular tone regulation. *Vasc. Med.,* **2010**, *15*(3), 205-213.

[331] Wang, J.; Chen, L.; Sinha, S.H.; Liang, Z.; Chai, H.; Muniyan, S.; Chou, Y.W.; Yang, C.; Yan, L.; Feng, Y.; Li, K.K.; Lin, M.F.; Jiang, H.; Zheng, Y.G.; Luo, C. Pharmacophore-based virtual screening and biological evaluation of small molecule inhibitors for protein arginine methylation. *J. Med. Chem.,* **2012**, *55*(18), 7978-7987.

[332] Feng, Y.; Li, M.; Wang, B.; Zheng, Y.G. Discovery and mechanistic study of a class of protein arginine methylation inhibitors. *J. Med. Chem.,* **2010**, *53*(16), 6028-6039.

[333] Rochette, L.; Lorin, J.; Zeller, M.; Guilland, J.C.; Lorgis, L.; Cottin, Y.; Vergely, C. Nitric oxide synthase inhibition and oxidative stress in cardiovascular diseases: possible therapeutic targets? *Pharmacol. Ther.,* **2013**, *140*(3), 239-257.

[334] Conrad, K.P.; Shroff, S.G. Effects of relaxin on arterial dilation, remodeling, and mechanical properties. *Curr. Hypertens. Rep.,* **2011**, *13*(6), 409-420.

[335] Teerlink, J.R.; Cotter, G.; Davison, B.A.; Felker, G.M.; Filippatos, G.; Greenberg, B.H.; Ponikowski, P.; Unemori, E.; Voors, A.A.; Adams, K.F., Jr.; Dorobantu, M.I.; Grinfeld, L.R.; Jondeau, G.; Marmor, A.; Masip, J.; Pang, P.S.; Werdan, K.; Teichman, S.L.; Trapani, A.; Bush, C.A.; Saini, R.; Schumacher, C.; Severin, T.M.; Metra, M.; Investigators, R.E.i.A.H.F. Serelaxin, recombinant human relaxin-2, for treatment of acute heart failure (RELAX-AHF): a randomised, placebo-controlled trial. *Lancet,* **2013**, *381*(9860), 29-39.

[336] Sasser, J.M.; Cunningham, M.W., Jr.; Baylis, C. Serelaxin reduces Oxidative Stress and Asymmetric Dimethylarginine in Angiotensin II Induced Hypertension. *Am. J. Physiol. Renal. Physiol.,* **2014**, ajprenal 00407 02014.

[337] Ersahin, M.; Sehirli, O.; Toklu, H.Z.; Suleymanoglu, S.; Emekli-Alturfan, E.; Yarat, A.; Tatlidede, E.; Yegen, B.C.; Sener, G. Melatonin improves cardiovascular function and ameliorates renal, cardiac and cerebral damage in rats with renovascular hypertension. *J. Pineal Res.,* **2009**, *47*(1), 97-106.

[338] Tain, Y.L.; Hsieh, C.S.; Chen, C.C.; Sheen, J.M.; Lee, C.T.; Huang, L.T. Melatonin prevents increased asymmetric dimethylarginine in young rats with bile duct ligation. *J. Pineal Res.,* **2010**, *48*(3), 212-221.

[339] Tain, Y.L.; Kao, Y.H.; Hsieh, C.S.; Chen, C.C.; Sheen, J.M.; Lin, I.C.; Huang, L.T. Melatonin blocks oxidative stress-induced increased asymmetric dimethylarginine. *Free Radic. Biol. Med.,* **2010**, *49*(6), 1088-1098.

[340] Cheng, M.C.; Wu, T.H.; Huang, L.T.; Tain, Y.L. Renoprotective effects of melatonin in young spontaneously hypertensive rats with L-NAME. *Pediatr. Neonatol.,* **2014**, *55*(3), 189-195.

[341] Tain, Y.L.; Huang, L.T.; Hsu, C.N.; Lee, C.T. Melatonin therapy prevents programmed hypertension and nitric oxide deficiency in offspring exposed to maternal caloric restriction. *Oxid. Med. Cell. Longev.,* **2014**, *2014*, 283180.

[342] Kantar, S.; Turkozkan, N.; Bircan, F.S.; Pasaoglu, O.T. Beneficial effects of melatonin on serum nitric oxide, homocysteine, and ADMA levels in fructose-fed rats. *Pharm. Biol.,* **2015**, *53*(7), 1035-1041.

[343] Obayashi, K.; Saeki, K.; Kurumatani, N. Asymmetric dimethylarginine attenuates the association of melatonin secretion with night-time blood pressure and dipping in elderly individuals. *Circ. J.,* **2014**, *78*(12), 2908-2914.

[344] Soleas, G.J.; Diamandis, E.P.; Goldberg, D.M. Resveratrol: a molecule whose time has come? And gone? *Clin. Biochem.,* **1997**, *30*(2), 91-113.

[345] Novelle, M.G.; Wahl, D.; Dieguez, C.; Bernier, M.; de Cabo, R. Resveratrol supplementation: Where are we now and where should we go? *Ageing Res. Rev.,* **2015**, *21*, 1-15.

[346] Yuan, Q.; Peng, J.; Liu, S.Y.; Wang, C.J.; Xiang, D.X.; Xiong, X.M.; Hu, C.P.; Li, Y.J. Inhibitory effect of resveratrol derivative BTM-0512 on high glucose-induced cell senescence involves dimethylaminohydrolase/asymmetric dimethylarginine pathway. *Clin. Exp. Pharmacol. Physiol.,* **2010**, *37*(5-6), 630-635.

[347] Frombaum, M.; Therond, P.; Djelidi, R.; Beaudeux, J.L.; Bonnefont-Rousselot, D.; Borderie, D. Piceatannol is more effective than resveratrol in restoring endothelial cell dimethylarginine dimethylaminohydrolase expression and activity after high-glucose oxidative stress. *Free Radic. Res.,* **2011**, *45*(3), 293-302.

[348] Develi-Is, S.; Ozen, G.; Bekpinar, S.; Topal, G.; Unlucerci, Y.; Dogan, B.S.; Uysal, M. Resveratrol improves high-fructose-induced vascular dysfunction in rats. *Can. J. Physiol. Pharmacol.,* **2014**, *92*(12), 1021-1027.

[349] Yuan, Q.; Hu, C.P.; Gong, Z.C.; Bai, Y.P.; Liu, S.Y.; Li, Y.J.; Jiang, J.L. Accelerated onset of senescence of endothelial progenitor cells in patients with type 2 diabetes mellitus: role of dimethylarginine dimethylaminohydrolase 2 and asymmetric dimethylarginine. *Biochem. Biophys. Res. Commun.,* **2015**, *458*(4), 869-876.

[350] Li, L.; Luo, X.J.; Liu, Y.Z.; Zhang, Y.S.; Yuan, Q.; Tan, N.; Xiang, D.X.; Peng, J. The role of the DDAH-ADMA pathway in the protective effect of resveratrol analog BTM-0512 on gastric mucosal injury. *Can. J. Physiol. Pharmacol.,* **2010**, *88*(5), 562-567.

[351] Channon, K.M.; Guzik, T.J. Mechanisms of superoxide production in human blood vessels: relationship to endothelial dysfunction, clinical and genetic risk factors. *J. Physiol. Pharmacol.,* **2002**, *53*(4 Pt 1), 515-524.

[352] Boger, R.H. Asymmetric dimethylarginine (ADMA): a novel risk marker in cardiovascular medicine and beyond. *Ann. Med.,* **2006**, *38*(2), 126-136.

[353] Gates, P.E.; Boucher, M.L.; Silver, A.E.; Monahan, K.D.; Seals, D.R. Impaired flow-mediated dilation with age is not explained by L-arginine bioavailability or endothelial asymmetric dimethylarginine protein expression. *J. Appl. Physiol. (1985),* **2007**, *102*(1), 63-71.

[354] Siasos, G.; Tousoulis, D.; Vlachopoulos, C.; Antoniades, C.; Stefanadi, E.; Ioakeimidis, N.; Andreou, I.; Zisimos, K.; Papavassiliou, A.G.; Stefanadis, C. Short-term treatment with L-arginine prevents the smoking-induced impairment of endothelial function and vascular elastic properties in young individuals. *Int. J. Cardiol.,* **2008**, *126*(3), 394-399.

[355] Heffernan, K.S.; Fahs, C.A.; Ranadive, S.M.; Patvardhan, E.A. L-arginine as a nutritional prophylaxis against vascular endothelial dysfunction with aging. *J. Cardiovasc. Pharmacol. Ther.,* **2010**, *15*(1), 17-23.

[356] Lerman, A.; Burnett, J.C., Jr.; Higano, S.T.; McKinley, L.J.; Holmes, D.R., Jr. Long-term L-arginine supplementation improves small-vessel coronary endothelial function in humans. *Circulation,* **1998**, *97*(21), 2123-2128.

[357] Chin-Dusting, J.P.; Willems, L.; Kaye, D.M. L-arginine transporters in cardiovascular disease: a novel therapeutic target. *Pharmacol. Ther.,* **2007**, *116*(3), 428-436.

[358] Schulman, S.P.; Becker, L.C.; Kass, D.A.; Champion, H.C.; Terrin, M.L.; Forman, S.; Ernst, K.V.; Kelemen, M.D.; Townsend, S.N.; Capriotti, A.; Hare, J.M.; Gerstenblith, G. L-arginine therapy in acute myocardial infarction: the Vascular Interaction With Age in Myocardial Infarction (VINTAGE MI) randomized clinical trial. *JAMA,* **2006**, *295*(1), 58-64.

[359] Curis, E.; Nicolis, I.; Moinard, C.; Osowska, S.; Zerrouk, N.; Benazeth, S.; Cynober, L. Almost all about citrulline in mammals. *Amino Acids.,* **2005**, *29*(3), 177-205.

[360] Tain, Y.L.; Hsieh, C.S.; Lin, I.C.; Chen, C.C.; Sheen, J.M.; Huang, L.T. Effects of maternal L-citrulline supplementation on renal function and blood pressure in offspring exposed to maternal caloric restriction: the impact of nitric oxide pathway. *Nitric Oxide,* **2010**, *23*(1), 34-41.

[361] Xuan, C.; Lun, L.M.; Zhao, J.X.; Wang, H.W.; Wang, J.; Ning, C.P.; Liu, Z.; Zhang, B.B.; He, G.W. L-citrulline for protection of endothelial function from ADMA-induced injury in porcine coronary artery. *Sci. Rep.,* **2015**, *5*, 10987.

[362] Schwedhelm, E.; Maas, R.; Freese, R.; Jung, D.; Lukacs, Z.; Jambrecina, A.; Spickler, W.; Schulze, F.; Boger, R.H. Pharmacokinetic and pharmacodynamic properties of oral L-citrulline and L-arginine: impact on nitric oxide metabolism. *Br. J. Clin. Pharmacol.,* **2008**, *65*(1), 51-59.

[363] Ochiai, M.; Hayashi, T.; Morita, M.; Ina, K.; Maeda, M.; Watanabe, F.; Morishita, K. Short-term effects of L-citrulline supplementation on arterial stiffness in middle-aged men. *Int. J. Cardiol.,* **2012**, *155*(2), 257-261.

[364] Morita, M.; Sakurada, M.; Watanabe, F.; Yamasaki, T.; Doi, H.; Ezaki, H.; Morishita, K.; Miyakex, T. Effects of oral L-citrulline supplementation on lipoprotein oxidation and endothelial dysfunction in humans with vasospastic angina. *Immunol. Endocr. Metab. Agents Med. Chem.,* **2013**, *13*(3), 214-220.

[365] Powers, R.; Weissgerber, T.L.; McGonigal, S.; Myerski, A.; Gallaher, M.; Speer, P.D.; Roberts, J.M.; Jeyabalan, A.; Hubel, C.A. [7-OR]: L-Citrulline administration increases the arginine/ADMA ratio, decreases blood pressure and improves vascular function in obese pregnant women. *Pregnancy Hypertens.,* **2015**, *5*(1), 4.

[366] Rader, D.J. Liver X receptor and farnesoid X receptor as therapeutic targets. *Am. J. Cardiol.,* **2007**, *100*(11 A), n15-19.

[367] Hu, T.; Chouinard, M.; Cox, A.L.; Sipes, P.; Marcelo, M.; Ficorilli, J.; Li, S.; Gao, H.; Ryan, T.P.; Michael, M.D.; Michael, L.F. Farnesoid X receptor agonist reduces serum asymmetric dimethylarginine levels through hepatic dimethylarginine dimethylaminohydrolase-1 gene regulation. *J. Biol. Chem.,* **2006**, *281*(52), 39831-39838.

[368] Li, J.; Wilson, A.; Gao, X.; Kuruba, R.; Liu, Y.; Poloyac, S.; Pitt, B.; Xie, W.; Li, S. Coordinated regulation of dimethylarginine dimethylaminohydrolase-1 and cationic amino acid transporter-1 by farnesoid X receptor in mouse liver and kidney and its implication in the control of blood levels of asymmetric dimethylarginine. *J. Pharmacol. Exp. Ther.,* **2009**, *331*(1), 234-243.

[369] Magierowski, M.; Jasnos, K.; Sliwowski, Z.; Surmiak, M.; Krzysiek-Maczka, G.; Ptak-Belowska, A.; Kwiecien, S.; Brzozowski, T. Exogenous asymmetric dimethylarginine (ADMA) in pathogenesis of ischemia-reperfusion-induced gastric lesions: interaction with protective nitric oxide (NO) and calcitonin gene-related peptide (CGRP). *Int. J. Mol. Sci.,* **2014**, *15*(3), 4946-4964.

[370] Ghebremariam, Y.T.; Yamada, K.; Lee, J.C.; Johnson, C.L.; Atzler, D.; Anderssohn, M.; Agrawal, R.; Higgins, J.P.; Patterson, A.J.; Boger, R.H.; Cooke, J.P. FXR agonist INT-747 upregulates DDAH expression and enhances insulin sensitivity in high-salt fed Dahl rats. *PLoS One,* **2013**, *8*(4), e60653.

[371] Mookerjee, R.P.; Mehta, G.; Balasubramaniyan, V.; Mohamed Fel, Z.; Davies, N.; Sharma, V.; Iwakiri, Y.; Jalan, R. Hepatic dimethylarginine-dimethylaminohydrolase1 is reduced in cirrhosis and is a target for therapy in portal hypertension. *J. Hepatol.,* **2015**, *62*(2), 325-331.

[372] Ghebremariam, Y.T.; LePendu, P.; Lee, J.C.; Erlanson, D.A.; Slaviero, A.; Shah, N.H.; Leiper, J.; Cooke, J.P. Unexpected effect of proton pump inhibitors: elevation of the cardiovascular risk factor asymmetric dimethylarginine. *Circulation,* **2013**, *128*(8), 845-853.

[373] Scalera, F.; Kielstein, J.T.; Martens-Lobenhoffer, J.; Postel, S.C.; Tager, M.; Bode-Boger, S.M. Erythropoietin increases asymmetric dimethylarginine in endothelial cells: role of dimethylarginine dimethylaminohydrolase. *J. Am. Soc. Nephrol.,* **2005**, *16*(4), 892-898.

[374] Desai, A.; Zhao, Y.; Warren, J.S. Human recombinant erythropoietin augments serum asymmetric dimethylarginine concentrations but does not compromise nitric oxide generation in mice. *Nephrol. Dial. Transplant.,* **2008**, *23*(5), 1513-1520.

[375] Fujiwara, N.; Nakamura, T.; Sato, E.; Kawagoe, Y.; Hikichi, Y.; Ueda, Y.; Node, K. Renovascular protective effects of erythropoietin in patients with chronic kidney disease. *Intern. Med.,* **2011**, *50*(18), 1929-1934.

[376] Oz, O.; Gokcil, Z.; Bek, S.; Cakir, E.; Odabasi, Z. Is asymmetric dimethylarginine responsible for the vascular events in patients under antiepileptic drug treatment? *Epilepsy Res.,* **2009**, *87*(1), 54-58.

[377] Emeksiz, H.C.; Serdaroglu, A.; Biberoglu, G.; Gulbahar, O.; Arhan, E.; Cansu, A.; Arga, M.; Hasanoglu, A. Assessment of atherosclerosis risk due to the homocysteine-asymmetric dimethylarginine-nitric oxide cascade in children taking antiepileptic drugs. *Seizure,* **2013**, *22*(2), 124-127.

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