Annu. Rev. Nutr. 2006. 26:203–28
doi: 10.1146/annurev.nutr.26.061505.111320

THE CLINICAL SIGNIFICANCE OF ASYMMETRIC DIMETHYLARGININE

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Key Words ADMA, SDMA, arginine, nitric oxide, DDAH

■ **Abstract** In 1992, asymmetrical dimethylarginine (ADMA) was first described as an endogenous inhibitor of the arginine-nitric oxide (NO) pathway. From then, its role in regulating NO production has attracted increasing attention. Nowadays, ADMA is regarded as a novel cardiovascular risk factor. The role of the kidney and the liver in the metabolism of ADMA has been extensively studied and both organs have proven to play a key role in the elimination of ADMA. Although the liver removes ADMA exclusively via degradation by the enzyme dimethylarginine dimethylaminohydrolase (DDAH), the kidney uses both metabolic degradation via DDAH and urinary excretion to eliminate ADMA. Modulating activity and/or expression of DDAH is still under research and may be a potential therapeutic approach to influence ADMA plasma levels. Interestingly, next to its association with cardiovascular disease, ADMA also seems to play a role in other clinical conditions, such as critical illness, hepatic failure, and preeclampsia. To elucidate the clinical significance of ADMA in these conditions, the field of research must be enlarged.

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INTRODUCTION

In humans, the gaseous molecule nitric oxide (NO) is formed by three isoforms of NO synthase (34). NO synthase uses arginine as a substrate to produce NO, which plays a pivotal role in biology by exerting a wide variety of regulatory functions of the pulmonary, cardiovascular, immune, gastrointestinal, and neurological systems. Production of NO by the endothelial-derived NO synthase (eNOS) is important for preservation of organ blood flow by regulating vascular tone, influencing the interaction of white blood cells and platelets with the endothelium, and limiting the development of neointimal hyperplasia by inducing apoptosis of vascular smooth muscle cells (75). In addition, the inducible isoform of NO synthase (iNOS) is able to produce large amounts of NO during inflammation. Since NO is a free radical gas, it plays a crucial role in host defense by acting as a cytotoxic agent (10). NO derived from the neuronal isoform (nNOS) is mainly important in relaxation of smooth muscle cells, but also plays a role in behavioral inhibition (8, 94).

The synthesis of NO is selectively inhibited by guanidino-substituted analogues of arginine, including monomethylarginine (MMA) and asymmetric dimethylarginine (ADMA) (96, 120). Symmetric dimethylarginine (SDMA), the inactive stereoisomer of ADMA, does not directly inhibit the enzyme NO synthase but is able to interfere with NO synthesis by competing with arginine, MMA, and ADMA for cellular transport across cationic amino acid transporters (CAT) of system y^+ (22). Since physiological concentrations of ADMA are approximately tenfold higher than that of MMA, ADMA can be regarded as the predominant endogenous inhibitor of NO biosynthesis (119).

Modification of ADMA concentrations has been shown to change vascular NO production and thereby vascular tone and systemic vascular resistance. Therefore, it has been suggested that elevated ADMA levels may explain the "arginine paradox" (116); the observation that, although the arginine plasma levels fully saturate the enzyme NO synthase (physiologic arginine plasma levels in humans: 90–110 μmol/L, K_m NO synthase: 3 μmol/L), supplementation with exogenous arginine improves NO production and NO-mediated functions. The study by Hornig and coworkers (39) is illustrative for this paradox; supplementation of arginine in patients with chronic heart failure and with increased plasma levels of ADMA

resulted in endothelium-dependent vasodilation, whereas arginine supplementation in patients without chronic heart failure and with normal plasma levels of ADMA did not affect the endothelium-dependent vasodilation. Thus, arginine administration may help to normalize the arginine/ADMA ratio when disturbed. On the other hand, the effect of arginine supplementation may be less pronounced in patients without a disturbed arginine/ADMA ratio.

Since the discovery in 1992 that ADMA plays a regulatory role in the arginine-NO pathway by inhibiting the enzyme NO synthase (120), many researchers have focused on ADMA, especially with respect to cardiovascular disease. ADMA concentrations are increased in a number of clinical conditions, including many cardiovascular disorders (1, 6, 15, 27, 38, 106, 113, 127), critical illness (79, 101), and dysfunction of ADMA-eliminating organs such as the liver (59, 77, 79, 103, 117) and the kidney (31, 47, 119, 132). There have been a growing number of publications concerning ADMA during the past two decades and nowadays ADMA is regarded as a novel cardiovascular risk factor (12). This review article discusses the origin and fate of dimethylarginines and particularly focuses on the organs involved in the elimination of ADMA and on the significance of ADMA in different clinical conditions.

PRODUCTION AND ELIMINATION OF METHYLARGININES

Synthesis of Methylarginines

Methylarginines are synthesized by posttranslational modification, involving addition of methyl groups from the donor methionine to arginine residues in proteins by enzymes called protein arginine methyltransferases (PRMTs). These methylated proteins are predominantly found in the nucleus and play a role in RNA processing and transcriptional control (73). ADMA is formed by PRMT type 1 and SDMA is formed by PRMT type 2. Both methyltransferase types are able to form MMA (68).

Methylarginines are released into the cytosol when these proteins are hydrolyzed, thereby being an obligatory product of protein turnover. Thus, the amount of ADMA generated is dependent on the extent of arginine methylation in proteins and on the rate of protein turnover. It should be noted that plasma ADMA concentrations probably represent cellular spillover and therefore weakly mirror intracellular concentrations.

Eliminatory Pathways of Methylarginines

The kidney plays an important role in the elimination of dimethylarginines from the body by excreting ADMA and SDMA into the urine (19, 129). There is an additional eliminatory pathway for ADMA, namely the conversion of ADMA by dimethylarginine dimethylaminohydrolase (DDAH) into citrulline and dimethylamine (83). The enzyme DDAH is widely distributed in the rat and the human

body but is particularly present in pancreas, spleen, liver, kidney, and endothelium (50, 51). It has been estimated that humans generate approximately 300 μmol of ADMA per day, of which more than 80% is metabolized by DDAH (2). Inhibition of DDAH causes vasoconstriction in vascular segments that is reversed by arginine, indicating that regulation of intracellular ADMA by DDAH affects NO synthase activity (64). In addition, overexpression of human DDAH in transgenic mice results in a reduction of plasma ADMA levels with a concomitant increase in tissue NO synthase activity, providing strong evidence for an important role of endogenous ADMA in regulating NO synthase activity (25).

There are two isoforms of DDAH: type 1, which is found in tissues expressing nNOS, and type 2, which is predominantly located in tissues expressing eNOS (115). The activity of both types 1 and 2 DDAH depends on regulatory mechanisms that are not fully understood. A reduced activity of DDAH after incubation of endothelial cells with tumor necrosis factor-alpha (TNF-α) or oxidized low-density lipoprotein (LDL) has been reported (40). Oxidative stress by *S*-nitrosylation inactivates DDAH (55), which provides probably an important homeostatic mechanism whereby high levels of NO upregulate the levels of ADMA, thereby limiting further NO generation. High glucose levels and homocysteine have also been demonstrated to impair activity of DDAH, thereby causing ADMA accumulation (57, 108).

Interestingly, daviditin A, a xanthone compound with antioxidant properties, has been shown to increase activity of DDAH in endothelial cells damaged by lysophosphatidylcholine (42). In addition, in endothelial cells DDAH expression is increased by retinoic acid, a vitamin A derivate that has some beneficial effects on the cardiovascular system (3). Other recent studies provided evidence for upregulation of DDAH expression by thiazolidinediones (105, 122) and stimulation of DDAH activity by the sulfhydryl antioxidant pyrrolidine dithiocarbamate (100) and estrogens (36). The ADMA-lowering effect of estrogens has been confirmed in placebo-controlled clinical studies on oral hormone replacement therapy in postmenopausal women (93, 114). Metformin and angiotensin-converting enzyme inhibitors have also been shown to reduce ADMA concentrations, but their working mechanisms have not been elucidated (6, 21).

It may be concluded that the regulation of gene expression and activity of DDAH is not yet fully understood, but considering the differential expression of DDAH in different organs, it can be hypothesized that NO synthesis may be differentially regulated in different vascular beds in order to modulate vascular tone during various pathophysiological processes.

RENAL HANDLING OF DIMETHYLARGININES

Studies on the Role of the Kidney in the Metabolism of Dimethylarginines

In the past, several groups have demonstrated that the kidney of both animals and humans is capable of excreting both ADMA and SDMA (4, 69, 129).

McDermott and coworkers (69) revealed that urinary excretion was the main elimination route for SDMA in rabbits, whereas ADMA was partly eliminated by other metabolic pathways. In addition, Al Banchaabouchi and coworkers (4) investigated the relationship between plasma levels and urinary concentrations of ADMA and SDMA. They calculated clearances of dimethylarginines and fractional excretion rates (clearance of dimethylarginines divided by the clearance of creatinine times 100%) in healthy humans. They found similar fractional excretion rates for ADMA and SDMA (68% and 71%, respectively). Moreover, it was calculated that approximately 30% of both dimethylarginines was reabsorbed. The limitation of this study is the fact that no arteriovenous concentration differences were measured and therefore no net renal extraction could be determined, thereby making it impossible to calculate true reabsorption rates. The role of the kidney as an ADMA-eliminating organ has been confirmed in another study with healthy humans (82), where plasma concentrations of dimethylarginines were determined in both arterial and renal venous blood in 20 fasting patients with normal renal function. Renal extraction of ADMA, as a measure of ADMA elimination, was calculated as the arteriovenous concentration difference divided by the arterial concentration times 100%. The main result was a significant net renal extraction for both dimethylarginines. Interestingly, for ADMA, a higher net renal extraction was found when compared with SDMA (16% and 11%, $p = 0.001$, respectively). Arterial SDMA concentration, but not ADMA concentration, was significantly correlated to arterial creatinine concentration ($r = 0.6$, $p = 0.005$). In addition, the presence of a higher renal extraction of ADMA strongly suggested the presence of a catabolic pathway for ADMA in the kidney.

The role of the kidney was further explored in metabolic studies in rats (80). A significant net uptake of both ADMA and SDMA by the rat kidney has been found, with fractional extraction rates of 35% and 31%, respectively. Furthermore, strong evidence was obtained for a differential renal handling of the two dimethylarginines. It was also found that the elimination of ADMA by the rat kidney could not be explained by urinary excretion, because urinary concentration of unchanged ADMA was negligible. This finding points to a high metabolic turnover of ADMA in the kidney, which is fully responsible for the observed net renal uptake of ADMA.

In contrast to the rat kidney, human kidneys are capable of excreting unchanged ADMA (19, 82). Thus, there also seems to be a difference in the handling of dimethylarginines between humans and rats. Ogawa and coworkers (86) investigated the metabolic fate of ADMA and SDMA isotopically in the rat and, interestingly, they found that only 4.6% of injected ADMA was found in the first 12-h urine as unchanged ADMA, compared with 17.8% for SDMA. Furthermore, they showed that both dimethylarginines are metabolized by a pathway forming the corresponding α-ketoacid analogues and the oxidatively decarboxylated products of the α-ketoacids in addition to N-acetyl conjugates, and that these metabolites were mainly found in urine. The potential presence of these metabolites, which would not have been detected by the actual HPLC methods for determination of dimethylarginines, may explain the controversy between the data obtained from

rat and human experiments. Their study provided strong evidence for the existence of an additional pathway for the elimination of ADMA, leading to the formation of citrulline and related amino acids. This pathway seemed to be the main route for ADMA elimination, as most ADMA-derived radioactivity was found in tissues instead of urine. Later this catalytic pathway was recognized both in rats and humans and proven to be degradation by the enzyme DDAH (84, 85).

Theoretically, a reduced activity of DDAH may be responsible for elevated ADMA concentrations (64). DDAH activity is influenced by factors such as oxidative stress and inflammation. In an in vitro model of human umbilical vein endothelial cells, a reduced activity of DDAH was found after exposure to oxidized LDL and TNF-α (40). In vivo, confirmation of the potential role of oxidized LDL was obtained by the occurrence of high ADMA levels in hypercholesterolemia, making ADMA a potential risk factor for atherosclerosis (15, 40). However, no in vivo data were present on the role of TNF-α and inflammation in the metabolism of dimethylarginines. Therefore, the effect of inflammation induced by lipopolysaccharide (LPS), as a natural mediator in the cascade of inflammatory mediators, was studied. In rats treated with endotoxin, a significantly lower ADMA concentration was found, which suggests an increased metabolic turnover of ADMA during severe inflammation (80). Interestingly, the increased metabolic turnover of ADMA was not accompanied by an increased renal elimination of ADMA, as both renal fractional extraction rate and net renal uptake were significantly lower in LPS-treated rats. In contrast to the decreased ADMA levels, SDMA levels were higher in endotoxin-treated rats and the increase of SDMA was accompanied by a reduced renal fractional extraction and a reduced net uptake by the kidney. As creatinine levels were also significantly higher in LPS-treated rats, an impaired renal clearance of SDMA could underlie the rise in SDMA levels. Thus, the kidney does not seem to be responsible for this ADMA-lowering effect of endotoxemia. A potential explanation for the lower plasma concentration of ADMA might be the increased uptake by the y^+ transporter during endotoxemia. Cationic amino acids such as arginine, ornithine, and lysine are also transported into endothelial cells by this y^+ pump. Closs and coworkers (22) investigated transport of dimethylarginines by CAT and found that both ADMA and SDMA were transported across this y^+ carrier. In rats, it has been shown that the expression of CAT was significantly increased in lung, heart, and kidney by LPS injection (35). Clinical conditions associated with severe endotoxemia include sepsis and multiple organ failure. These conditions are characterized by overproduction of NO due to inducible NO synthase activity. One of the biological questions that must be answered is, What is the potential role of ADMA during inflammation and infection? It has been speculated that ADMA could possibly serve as a "brake" on the action of iNOS and inhibit overwhelming NO synthesis (23) (see also Critical Illness section below).

Clinical Studies on Dimethylarginines and Renal Dysfunction

The recognition of ADMA as a potential player in diverse cardiovascular diseases paralleled the discovery of elevated levels of dimethylarginines in patients with end-stage renal disease. A lot of research on the role of the kidney has evolved

since then. First, in 1992, Vallance and coworkers (119) reported elevated levels of ADMA in patients with renal failure. Kielstein and coworkers (47) showed that ADMA was higher in dialysis patients with clinically manifest atherosclerosis than in those without atherosclerotic disease. It was suggested that elevated levels of dimethylarginines may be responsible for the hypertension seen in patients with end-stage renal disease. In patients with end-stage renal disease, ADMA was independently associated with intima-media thickness of the carotid artery and predicted the progression of intimal thickening during one year of follow-up (131). Recently, an independent association between plasma ADMA concentration and carotid intima-media thickness was also described in patients with mild-to-moderate renal failure (74).

Zoccali and coworkers (132) studied the relation between cardiovascular risk factors and plasma ADMA concentration in a cohort of 225 hemodialysis patients, and found that plasma ADMA is a strong and independent risk factor of overall mortality and cardiovascular outcome. In another study in patients with end-stage renal disease, the same investigators revealed that raised plasma concentration of ADMA was associated with left ventricular dysfunction and left ventricular hypertrophy, which are important risk factors for mortality in these patients (133). Furthermore, in a recent study on cardiovascular risk stratification in patients with end-stage renal disease, it was demonstrated that ADMA significantly adds predictive value to all-cause and cardiovascular mortality in dialysis patients (66). Interestingly, a sharp rise of SDMA, the stereoisomer of ADMA, has been reported in patients with chronic renal failure (31). Although SDMA has no direct inhibitory activity toward the enzyme NO synthase, Fleck and coworkers (31) pointed out the potential importance of SDMA and concluded in their study of a large population of renal failure patients that not only ADMA levels, but also SDMA levels, were likely responsible for hypertension, possibly by competition for reabsorption between SDMA and arginine in the kidney.

Recently, it was demonstrated that plasma ADMA is inversely related to glomerular filtration rate in patients with mild-to-advanced chronic kidney disease (95). In these patients, ADMA represented a strong and independent risk marker for progression to end-stage renal disease and mortality. Another recent study showed that ADMA levels above median in patients with nondiabetic kidney diseases and mild-to-moderate renal failure were associated with a faster progression of kidney disease (32). The findings of the above-mentioned studies indicate a potential role for ADMA in diagnostic and therapeutic strategies aimed at detection and treatment of atherosclerotic complications in patients with renal disease.

HEPATIC HANDLING OF DIMETHYLARGININES

Studies on the Role of the Liver in the Metabolism of Dimethylarginines

The first report suggesting a role for the liver in the metabolism of ADMA was published in 1977 by Carnegie and coworkers (19). They studied urinary excretion

of methylarginines in human disease and showed an increased excretion rate of ADMA in patients with liver disease compared with healthy adults. Unfortunately, ADMA plasma concentrations were not measured. Therefore, no definite conclusions on the liver as an ADMA-clearing organ can be drawn from these results. Later, it was revealed that hepatocytes abundantly express CAT (35) and contain large amounts of the ADMA-degrading enzyme DDAH (50, 51). These findings strongly suggested a role for the liver in the metabolism of dimethylarginines. This hypothesis was confirmed by an organ balance study in rats in which net organ fluxes and fractional extraction rates of dimethylarginines across the liver and kidney were determined by measuring arteriovenous concentration differences and blood flow using radiolabeled microspheres (78). The main finding of this study was a high uptake of ADMA by the liver while the concentration of SDMA was barely affected. A comparable animal study investigated dimethylarginine handling of both the liver and the gut under basal conditions and during endotoxemia (76). Results showed that arterial plasma ADMA concentrations were lower in endotoxemic rats and that the hepatic uptake of ADMA was stimulated by LPS administration. In contrast, the gut released ADMA, which increased after infusion of LPS. These findings may indicate that in the early phase of endotoxemia, when an adequate hepatic function is still present, ADMA degradation is enhanced, thereby causing lower systemic ADMA levels and facilitating local organ blood flow. The clinical significance of ADMA in conditions associated with endotoxemia, like sepsis and multiple organ failure, is discussed in the Critical Illness section below.

A recent study performed in patients undergoing hepatic surgery confirmed the uptake of ADMA by the liver (102). Moreover, it was shown that, in contrast to rats, the clearing of SDMA is not only confined to the kidney, but the human liver also takes up small amounts of SDMA from the portosystemic circulation. These basal metabolic studies have proven that the liver is a key organ in regulating dimethylarginine plasma concentrations.

Clinical Studies on ADMA and Hepatic Dysfunction

Theoretically, dysfunction of the liver may disturb dimethylarginine metabolism. Indeed, in critically ill patients, hepatic dysfunction has proven to be the most prominent determinant of ADMA plasma concentration (79). Moreover, in these patients, ADMA ranked as the strongest predictor of intensive care unit (ICU) mortality (see also Critical Illness section below). Furthermore, increased plasma concentrations of ADMA have been measured in the postoperative course in patients undergoing major hepatectomy compared with patients undergoing colorectal surgery (77). Additional research on the clinical significance of the liver as an ADMA-clearing organ has been performed in patients eligible for liver transplantation. Martín-Sanz and coworkers (67) showed that methylated arginine derivates are produced in human livers during the cold ischemia period of the graft and that a longer ischemia time caused significantly greater concentrations of these inhibitors

in the preservation solution. Of particular interest was the significant relationship between the extent of NO synthase inhibition and early liver graft function, which suggests that the concentration of methylarginines in the graft preservation solution may be used as a predictor of early liver graft function. In another study, it has been revealed that plasma concentrations of ADMA and the oxidative stress marker 15(S)-8-*iso*-$PGF_{2\alpha}$ were increased in patients with end-stage liver disease (117). The clinical significance of ADMA was further substantiated in patients undergoing liver transplantation (103). In these patients, preoperative plasma ADMA levels were highly elevated, and plasma ADMA concentrations decreased significantly on the day after the transplantation. Interestingly, in patients suffering from acute hepatic failure, preoperative ADMA levels were significantly higher compared with patients with chronic hepatic failure. Moreover, in patients who experienced acute rejection, ADMA concentrations were higher during the whole first postoperative month compared with nonrejectors. The results of this study confirmed the important role of the human liver in clearing ADMA and also showed that increased concentrations of ADMA during the posttransplantation period reflect impaired hepatic function during rejection.

Dimethylarginines may also be of significance in the pathophysiology of liver cirrhosis, a condition characterized by endothelial dysfunction (i.e., NO deficiency) in the intrahepatic circulation and, paradoxically, overproduction of NO by the splanchnic circulation. Lluch and coworkers (59), who showed elevated plasma concentrations of nitrate and nitrite and ADMA in patients with decompensated alcoholic cirrhosis (Child-Pugh score $\geq$8), suggested that ADMA might oppose the peripheral vasodilation caused by excessive systemic NO production during liver cirrhosis. Furthermore, the development of renal failure in patients with severe liver disease is characterized by renal hypoperfusion. The kidney is highly vulnerable to accumulation of ADMA because renal blood flow and glomerular filtration are both dependent on basal NO synthesis. Therefore, a causal role for ADMA in the development of the hepatorenal syndrome has been suggested (81).

THE ROLE OF ADMA IN CARDIOVASCULAR DISEASE

Hypercholesterolemia

Cholesterol feeding of rabbits increases their plasma ADMA concentration (13, 18, 128). These animals develop atherosclerosis and endothelial dysfunction, and it has been shown that the production of NO is inversely associated with the plasma concentration of ADMA (18). Also, in monkeys with diet-induced hypercholesterolemia, plasma concentrations of ADMA were elevated and inversely associated with endothelial function (14). Dietary arginine can reduce the progression of atherosclerosis and improve endothelium-dependent vasodilation in cholesterol-fed rabbits (13). Overall, these animal experiments provide evidence that inhibition of NO synthase by elevated ADMA concentrations plays a role in cholesterol-induced atherosclerosis.

In humans, a positive association between cholesterol and ADMA has been observed as well. In a group of asymptomatic hypercholesterolemic subjects, the mean plasma ADMA concentration was approximately twofold higher than in age-matched normocholesterolemic subjects, and in the combined groups, ADMA was positively associated with LDL cholesterol (15). Notably, other studies have observed no significant association between ADMA and LDL cholesterol (89, 105), and in most intervention studies, plasma ADMA concentrations were not influenced by aggressive lowering of LDL cholesterol by statin treatment (27, 41, 89, 97). Despite these negative results, it was shown that the effect of pravastatin treatment was modified by ADMA, with low baseline concentrations of ADMA being predictive of a significant improvement in adenosine-induced myocardial blood flow (41).

Oxidation of LDL (ox-LDL) in the vascular wall plays a key role in the process of atherogenesis, and results from in vitro experiments suggest that oxidation of LDL, rather than its cholesterol content per se, may be responsible for the ADMA-increasing effect of LDL. Incubation of endothelial cells with ox-LDL has been shown to lead to increased levels of ADMA in the culture medium, due to a reduction of DDAH activity (40). The lectin-like oxidized LDL receptor-1 (LOX-1) is a major receptor for ox-LDL in endothelial cells, and binding of ox-LDL to LOX-1 increases the intracellular generation of reactive oxygen species. Interestingly, it has been reported that incubation of endothelial cells with ADMA results in upregulation of LOX-1 (104). It is noteworthy that ADMA itself may also increase oxidative stress by uncoupling of endothelial NO synthase, resulting in a shift from NO production to superoxide production (104, 110). From these data, it seems that ADMA and ox-LDL are part of a vicious cycle in which oxidative stress induced by uptake of ox-LDL leads to inhibition of DDAH, and the ensuing increased ADMA concentration further enhances oxidative stress and induces LOX-1 expression, thereby resulting in the augmented uptake of ox-LDL.

Hyperhomocysteinemia

The metabolic pathways of homocysteine and ADMA are strongly intertwined, and emerging evidence suggests that ADMA is a key mediator of the link between hyperhomocysteinemia and endothelial dysfunction (107). During the process of arginine methylation, S-adenosylmethionine (SAM), which serves as methyl group donor, is converted into S-adenosylhomocysteine (SAH), which is subsequently hydrolyzed to homocysteine. Because ADMA contains two methyl groups, its synthesis is accompanied by the generation of two equivalents of homocysteine. It should be noted, however, that synthesis of ADMA is only a minor source of homocysteine.

Homocysteine affects the metabolism of ADMA in several ways. First, homocysteine, by inducing disulfide exchange reactions, can disrupt protein folding (7). This may accelerate protein degradation, resulting in an increased release of free ADMA from methylated proteins. Second, as already mentioned, the enzymatic

activity of DDAH, which is responsible for the breakdown of ADMA, is inhibited by homocysteine (108). Finally, it is important to note that the enzymatic hydrolysis of SAH to homocysteine is reversible, with equilibrium dynamics that strongly favor SAH synthesis rather than hydrolysis. In situations where homocysteine is elevated, intracellular SAH levels may thus increase. Because SAH is a potent inhibitor of transmethylation reactions, its accumulation may lead to hypomethylation of macromolecules (126). That increased SAH levels also inhibit the methylation of arginine residues in proteins is supported by the observation that the production of ADMA by endothelial cells is reduced upon incubation with SAH (17).

Oral methionine loading induces acute hyperhomocysteinemia, which is associated with impairment of vascular endothelial function (9). Several studies have reported a significant increase of plasma ADMA concentrations after a methionine-loading test (16, 106, 123), suggesting that inhibition of NO synthase by increased ADMA concentrations contributes to the acute impairment of endothelial function.

Lowering of elevated homocysteine concentrations is easily accomplished by treatment with B vitamins, of which folic acid is especially effective. There is a single report showing that ADMA concentrations decreased in parallel with homocysteine concentrations during folic acid treatment (38), but several other studies have shown no effect of treatment with combined B vitamins on ADMA concentrations (26, 43, 111, 130).

A significant positive association between basal plasma concentrations of homocysteine and ADMA has been reported in subjects with hyperhomocysteinemia (38) and patients with peripheral arterial disease (106), incipient primary renal disease (46), and stroke (127). This association seems not to be restricted to specific patient groups, because a moderate but highly significant positive association between plasma concentrations of ADMA and log-transformed homocysteine in a large population-based cohort study has been observed recently (113).

Diabetes Mellitus

There is accumulating evidence that ADMA plays a role in insulin resistance (20, 109). In a study by Miyazaki and coworkers (72) of subjects without symptoms of coronary or peripheral artery disease, age, mean arterial blood pressure, and insulin resistance were the main determinants of plasma ADMA. Stühlinger and coworkers (105) also demonstrated a positive relationship between insulin resistance and plasma ADMA concentration in healthy, nondiabetic subjects. In addition, pharmacological agents that improve insulin sensitivity, such as rosiglitazone and metformin, have been shown to lower plasma ADMA concentrations (6, 105, 122).

In rats, streptozotocin-induced type 2 diabetes results in elevated ADMA levels (57, 125). Treatment of these animals with insulin results in normalization of ADMA, a finding that suggests the elevation of ADMA is closely related to glycemic control (124). In diabetic rats, aortic DDAH activity was significantly

reduced and negatively associated with ADMA levels in plasma (57). It has been shown that the reduction of DDAH activity in human endothelial cells exposed to high glucose conditions can be reversed by coincubation with a superoxide-dismutase conjugate (57). Hence, glucose-induced oxidative stress leading to impairment of DDAH seems a likely mechanism for accumulation of ADMA.

Data on plasma levels of ADMA in subjects with diabetes are inconclusive. In patients with poorly controlled type 2 diabetes, increased plasma concentrations of ADMA were found (1, 6). However, Päivä and coworkers (90) reported decreased ADMA concentrations in type 2 diabetes patients, which were ascribed to renal hyperfiltration. Increased concentrations of ADMA have also been reported in women with gestational diabetes (71) and in subjects with type 1 diabetes (27). In a large cohort of patients with long-standing type 1 diabetes, plasma ADMA concentrations were in the normal range. However, ADMA was significantly higher in patients with diabetic nephropathy compared with those with normoalbuminuria (112).

Although there is strong evidence for a relation between insulin sensitivity and ADMA, the association between ADMA and diabetes per se may be confounded by renal function and diabetes-associated vascular pathology. Of particular interest is the reported relationship between ADMA concentrations and the beneficial effects of tight glucose regulation in critically ill patients with insulin resistance (101) (see also Critical Illness section below).

The Effect of ADMA on Cardiac Physiology

Proteome analysis of the canine myocardium has revealed that regulation of NO synthase activity by ADMA plays an important role in local distribution of blood flow within the left ventricular myocardium (54). Specifically, in areas of low flow, expression of the ADMA-degrading enzyme DDAH was more pronounced and ADMA was reduced to only 25% of the ADMA content in high-flow areas. The strong reduction of ADMA in the presence of identical levels of NO synthase strongly suggests enhanced NO formation in low-flow areas. Recent work of Osanai and coworkers (88) demonstrated that the release of ADMA by vascular endothelial cells was increased after shear stress $\leq$15 dyne/cm^2, which is comparable to mechanical forces on the arterial wall under physiological conditions, but was unchanged by shear stress at 25 dyne/cm^2. In addition, the activity of DDAH was enhanced by shear stress at 25 dyne/cm^2, but was not affected by shear stress $\leq$15 dyne/cm^2. Since eNOS is also stimulated by shear stress, ADMA and NO synthase activity might antagonistically regulate production of NO in the systemic circulation. In transgenic mice expressing human DDAH, plasma ADMA was twofold reduced and tissue NO synthase activity was increased (25). Compared with wild-type control animals, systemic vascular resistance and blood pressure were decreased. Interestingly, basal heart rate was increased by 10%, but this was balanced by a 10% reduction in stroke volume, resulting in unaltered cardiac output (25). Conversely, studies in humans have shown that systemic infusion of ADMA in healthy subjects results in an immediate increase of systemic vascular

resistance and blood pressure and a decreased heart rate and cardiac output (2, 48). Resting cardiac output, as well as exercise-induced stimulation of cardiac output, was strongly attenuated after infusion of ADMA, suggesting a possible role for ADMA in the pathophysiology of heart failure and reduced exercise tolerance (2). Indeed, increased plasma levels of ADMA have been observed in animal models of congestive heart failure (29, 87). It has also been reported that plasma concentrations of ADMA are elevated in patients with heart failure (45) and coronary syndrome X (92). Finally, ADMA was significantly associated with left ventricular dysfunction and hypertrophy in hemodialysis patients (133).

Prospective Studies

The results of prospective clinical studies provide the most compelling evidence for a role of ADMA in the development of cardiovascular disease. In patients with end-stage renal disease, ADMA and age were the strongest predictors of cardiovascular events and total mortality, after correction for other traditional and novel risk factors (132). In a Finnish study with a nested case-control design, high concentrations of ADMA were associated with an increased risk of acute coronary events among nonsmoking middle-aged men, especially in men with previous coronary heart disease (118). In a third study, conducted in patients with stable angina pectoris, high plasma concentrations of ADMA predicted adverse cardiovascular events after percutaneous coronary intervention (62). Also, in patients with idiopathic pulmonary arterial hypertension, ADMA was an independent predictor of mortality (44). Furthermore, in a large prospective study, high levels of baseline ADMA independently predicted future cardiovascular events in patients with coronary artery disease (99). Finally, in a prospective study with critically ill patients, ADMA turned out to be the strongest predictor of ICU death, with a 17-fold increased risk in mortality for patients in the highest ADMA quartile (79) (see also Critical Illness section below).

In all of these studies, the association between ADMA and risk was studied by multivariate analysis, including other risk factors and confounding variables, in support of the contention that ADMA is an independent cardiovascular risk factor (11, 24). However, most of these studies were performed in specific patient groups and should therefore be interpreted with some caution. The results of large prospective studies in the general population are urgently needed to establish definitively the position of ADMA in the hierarchy of classic and novel cardiovascular risk factors.

CRITICAL ILLNESS

ADMA Levels in ICU Patients with Multiple Organ Failure

Highly raised ADMA levels are present in critically ill patients with multiple organ dysfunctions (79). This is most likely caused by the unfavorable combination of

increased protein turnover (hypercatabolic state and synthesis of acute-phase proteins) and a decreased elimination of ADMA when hepatic and renal dysfunction are present. In a cross-sectional study of 52 critically ill patients with clinical evidence of dysfunction of more than two organs, plasma ADMA concentration was independently related to the presence of hepatic and renal failure (79). Moreover, in a logistic regression model, plasma ADMA ranked as the first and strongest predictor for outcome, with a 17-fold increased risk for ICU death in patients who were in the highest quartile for ADMA.

The most likely mechanism by which ADMA increases the risk of adverse outcome in critically ill patients is inhibition of the constitutively expressed eNOS. NO produced by eNOS is important for preservation of organ blood flow by regulating vascular tone and influencing the interaction of white blood cells and platelets with the endothelium. In addition, NO is involved in host defense by acting as a cytotoxic agent. During inflammation, iNOS is able to produce large amounts of NO. Overproduction of NO may aggravate tissue damage and may cause systemic vasodilation with therapeutically refractory hypotension and coagulation disorders as seen in septic shock. Assuming that inhibition of these adverse effects has therapeutic potential, the pharmacological NO synthase inhibitor MMA has been given to septic patients. However, the results of this study revealed increased mortality rates in patients receiving MMA (60). In several human and animal studies reporting adverse effects of NO synthase inhibition, the inhibitors were nonselective; inhibiting both eNOS and iNOS. ADMA is also a nonselective inhibitor of NO synthase and is endogenously produced. Therefore, when plasma levels of ADMA increase, interference with physiological functions of NO may be expected.

The Link Between Glucose Regulation and ADMA Concentrations in ICU Patients

Tight glucose regulation by insulin administration has been shown to reduce morbidity and mortality in a large population of critically ill patients (121). In a subgroup of these patients, ADMA levels were measured to determine whether modulation of ADMA concentration by insulin could explain the beneficial effects of intensive insulin therapy (101). The most important finding of this study was the different course in ADMA levels from day 0 to day 2 due to type of insulin treatment: The ADMA plasma concentration significantly increased during the first 2 days after assignment of conventional insulin treatment, whereas the ADMA levels did not change between day 0 and day 2 in patients receiving intensive insulin treatment. Moreover, at the end of the ICU period, ADMA levels were still significantly lower in the intensive treatment group compared with the conventional treatment group. These results, together with the positive association between mean daily insulin dose and ADMA concentration of all patients on the last day, strongly suggest that ADMA is influenced by insulin therapy (101). It seems most likely that this modulation is caused by a combination of factors. Insulin may be able to preserve DDAH activity since high glucose inhibits activity of DDAH (57). In addition,

insulin is an anabolic hormone, thereby reducing protein breakdown and thus causing less ADMA release. Furthermore, insulin affects transport systems and may thereby increase uptake of ADMA in organs that eliminate ADMA (33, 58).

Besides the association between ADMA and insulin, ADMA was also related to several outcome parameters, including duration of ICU stay, duration of ventilatory support, duration of inotropic and vasopressor treatment, number of red cell transfusions, duration of antibiotic treatment, presence of critical illness polyneuropathy, mean Acute Physiology and Chronic Health Evaluation (APACHE II) score (52), and cumulative simplified Therapeutic Intervention Scoring System (TISS-28) score (70). Moreover, ADMA levels in patients who died were significantly higher compared with survivors, and a change in the early and late course of the ADMA concentration proved to be a strong and independent predictor of ICU mortality (101). These relationships strongly suggest that ADMA influences recovery of the critically ill patient but they do not prove causality between ADMA and insulin. Therefore, this study must be regarded as a first step in the field of clinical research on ADMA and glucose/insulin.

PREECLAMPSIA

There is ample evidence that impaired endothelial function (i.e., NO deficiency) is involved in the etiology of preeclampsia. Endothelial-derived NO regulates physiological changes during normal pregnancy, including vasodilation and an increase in circulating blood volume. In the past decennium, several investigators have focused on ADMA as a potential causative factor in the development of preeclampsia. ADMA reduces bioavailability of NO and is thought to impair the physiological adaptation process during pregnancy, thereby causing preeclampsia and the hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome. The first report showing increased plasma concentrations of ADMA in females with preeclampsia in comparison with healthy pregnant women during the third trimester was published in 1993 by Fickling and coworkers (30). They also revealed that in comparison with nonpregnant females, the ADMA levels of pregnant females decreased during normal pregnancy. These findings have been confirmed in other studies (28, 37, 91, 98). Changes in renal function during normal pregnancy and during preeclampsia may explain the difference in ADMA concentration between these patient groups, but the exact cause of elevated ADMA concentrations during preeclampsia has still not been elucidated.

Interestingly, the placenta contains the ADMA-degrading enzyme DDAH (56, 115). Therefore, placental dysfunction has been suggested as an initiator in the development of preeclampsia (98). Maeda and coworkers (65) have reported significantly higher ADMA concentrations in umbilical venous blood compared with maternal concentrations at term, which suggests that the placenta plays an important role in placental transport/metabolism of ADMA. Unfortunately, ADMA concentrations and DDAH activity within the placenta were not determined in this study.

In a recent study, it was shown that placental DDAH activity, placental ADMA content, and fetomaternal ADMA gradient did not differ significantly between preeclamptic patients and healthy pregnant females (100a). In addition, ADMA plasma levels were neither higher in preeclamptic patients in comparison with pregnant controls nor significantly lower in normotensive pregnant females in comparison with nonpregnant women. Although these results are in contrast with the results of above-mentioned studies, the results are in concordance with other recent studies (49, 61, 63).

The contradictory findings about ADMA levels during preeclampsia may be explained by the presence of impaired renal function in the studies reporting elevated ADMA levels in patients suffering from preeclampsia. Some studies did not present results about the severity of renal dysfunction (30, 37, 98), while other studies reported increased creatinine concentrations and a decreased ADMA/SDMA quotient, thereby confirming the presence of renal dysfunction (28, 91). In addition, since the liver also is a major determinant in regulating ADMA plasma concentrations (see Hepatic Handling of Dimethylarginines section above), hepatic dysfunction could also have affected ADMA plasma concentration during preeclampsia. However, most studies did not show results about the function of the liver. Interestingly, elevated liver enzymes and hepatic function parameters indicate progression of preeclampsia to the HELLP syndrome. It has been shown that ADMA plasma levels in patients with the HELLP syndrome are elevated in comparison with preeclamptic patients and normotensive pregnant females, whereas ADMA levels were not elevated in patients with preeclampsia compared with nonpregnant women and normotensive pregnant females (100a). Thus, it can be concluded that when the clinical course of preeclampsia deteriorates, and dysfunction of ADMA-eliminating organs (i.e., liver, kidney, and endothelium) becomes involved, ADMA concentrations increase and may thereby adversely affect organ blood flow.

The presence of a reduced placental perfusion results in a decreased renal plasma flow and glomerular filtration rate (5). Deteriorating renal function results in accumulation of ADMA, thereby probably affecting the integrity of the liver due to prolonged inhibition of NO synthase. In turn, the risk of developing serious complications accompanying the HELLP syndrome further increases. Since placental activity of DDAH was found not to be different between preeclamptic patients and normotensive pregnant women (100a), it can be hypothesized that DDAH activity within the placenta should be upregulated during preeclampsia in order to compensate for the reduced placental perfusion.

SUMMARY AND CONCLUSIONS

Endogenously produced ADMA plays a causative role in cardiovascular diseases. The mechanism by which accumulation of ADMA is detrimental seems to be by nonselective inhibition of NO synthase, thereby interfering with important

physiological functions that are dependent on constitutive NO production. Multiple studies using multivariate regression analyses, thereby taking into account all traditional risk factors, conclude that ADMA is a strong and novel cardiovascular risk factor. Determining ADMA levels in patients who are at risk for developing cardiovascular events may be helpful in the near future, but is still not executable because ADMA levels vary markedly within different clinical populations and between different analytical methods.

Several studies show that ADMA accumulates during renal failure, and ADMA has been held responsible for the cardiovascular complications accompanying end-stage renal disease (32, 47, 66, 95, 132, 133). In addition, the liver has also been proven to be a crucial organ in the handling of ADMA (78, 102), and in patients suffering from hepatic failure, ADMA levels increase (59, 77, 79, 103, 117). ADMA may be of clinical significance in patients who underwent a liver transplantation procedure. In these patients, ADMA levels have been shown to increase during acute liver allograft rejection (103). Future studies investigating the relation between histological (i.e., liver biopsy) and biochemical parameters of hepatic function must reveal whether ADMA may be used as a marker of acute liver graft rejection.

Interestingly, ADMA concentration has been shown to be a strong and independent risk factor for ICU mortality in critically ill patients, and a causal role for ADMA in the development of organ failure has been hypothesized (79, 81). By nonspecific inhibition of NO synthase, ADMA may interfere with important physiological functions, eventually leading to the cascade of organ dysfunction and injury that may be fatal to the critically ill patient. Hypothetical consequences of nonselective inhibition of NO synthase, ultimately leading to organ failure, are reduced perfusion of organs, reduced cardiac output, cardiac ischemia, capillary leakage, thrombocyte aggregation, reduced glomerular filtration rate, pulmonary hypertension, and increased adhesiveness of leucocytes. In our opinion, this appealing role of ADMA as a causative factor in the pathophysiological alterations of multiple organ failure demands further studies in the near future. It is known that metabolic support is essential in critically ill patients. In these patients, intensive insulin therapy improves outcome and reduces morbidity (121). The exact mechanism by which these beneficial effects are brought about remains to be elucidated, but ADMA plasma concentration can be modulated by insulin treatment (101). It seems most likely that this modulation is caused by the combined effect of preserving DDAH activity, reduction of protein breakdown and thus less ADMA release, and increased uptake of ADMA via transport systems in ADMA-eliminating organs. Future studies lowering ADMA levels with, e.g., selective hemodialysis or upregulation of the ADMA-degrading enzyme DDAH, must confirm the finding that ADMA is associated with outcome parameters in critically ill patients.

Considering the fact that ADMA reduces NO production and thereby possibly impairs the physiological adaptation process during pregnancy, it seemed very reasonable to assume that ADMA is involved in the development of preeclampsia. However, results of studies investigating the role of ADMA during preeclampsia

are contradictive. Recently, it became clear that during the HELLP syndrome, i.e., when organ dysfunction is present, ADMA levels were significantly increased compared with healthy pregnant females and preeclamptic women (100a). In order to prove causality between ADMA and the development of preeclampsia, a prospective study investigating the course of ADMA levels in a large group of pregnant patients during pregnancy must be executed.

NO became the molecule of the year in 1992 (53), and it was also in that year that Vallance and coworkers (120) discovered that ADMA plays a regulatory role in the arginine-NO pathway by inhibiting all isoforms of NO synthase. Since this observation, there has been a growing interest in ADMA, finally leading to its recognition as a novel cardiovascular risk factor. Interestingly, many other clinical conditions, such as preeclampsia, critical illness, and hepatic failure, also seem to be linked to ADMA. Therefore, the field of research on ADMA must be widened in order to elucidate the clinical significance of ADMA in other important clinical conditions.

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