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# ADMA metabolism and clearance

Tom Teerlink

**Abstract:** The plasma concentration of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase, is the resultant of many processes at cellular and organ levels. Post-translational methylation of arginine residues of proteins plays a crucial role in the regulation of their functions, which include processes such as transcription, translation and RNA splicing. Because protein methylation is irreversible, the methylation signal can be turned off only by proteolysis of the entire protein. Consequently, most methylated proteins have high turnover rates. Free ADMA, which is formed during proteolysis, is actively degraded by the intracellular enzyme dimethylarginine dimethylaminohydrolase (DDAH). Some ADMA escapes degradation and leaves the cell via cationic amino acid transporters. These transporters also mediate uptake of ADMA by neighboring cells or distant organs, thereby facilitating active interorgan transport. Clearance of ADMA from the plasma occurs in small part by urinary excretion, but the bulk of ADMA is degraded by intracellular DDAH, after uptake from the circulation. This review discusses the various processes involved in ADMA metabolism: protein methylation, proteolysis of methylated proteins, metabolism by DDAH, and interorgan transport. In addition, the role of the kidney and the liver in the clearance of ADMA is highlighted.

**Key words:** asymmetric dimethylarginine; cationic amino acid transporters; dimethylarginine dimethylaminohydrolase; methylation; nitric oxide; proteolysis

## Introduction

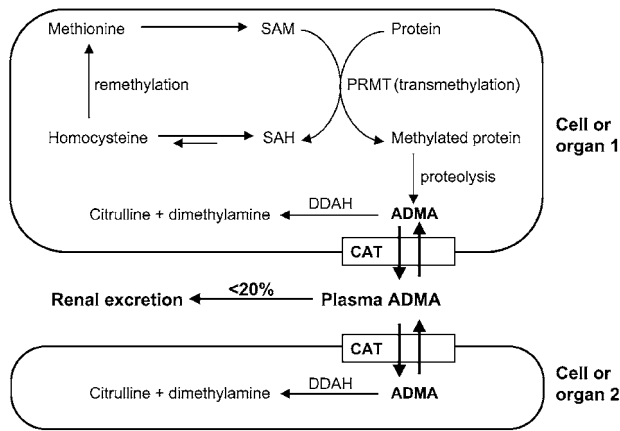
Asymmetric dimethylarginine (ADMA) is a post-translationally modified form of arginine that is generated in all cells during the process of protein turnover. ADMA has been found to be an independent predictor of cardiovascular morbidity and mortality<sup>1,2</sup> and its status as a cardiovascular risk factor is gradually gaining acceptance. Competitive inhibition of nitric oxide synthase (NOS) is the most likely mechanism for the effects of ADMA on the cardiovascular system,<sup>3</sup> although nitric oxide (NO)-independent mechanisms cannot be excluded.<sup>4</sup> Only free ADMA, and not its protein-bound form, is capable of inhibiting NOS. Because NOS is an intracellular enzyme, the intracellular concentration of free ADMA, rather than its concentration in plasma, is relevant with respect to NOS inhibition. This review focuses on the processes that determine the intracellular concentration of free ADMA (Figure 1). There are no indications for the

existence of biosynthetic routes for the direct synthesis of free ADMA by methylation of free arginine. Free ADMA is formed exclusively by the sequence of methylation of arginine residues of proteins, followed by proteolysis of these proteins. There are two pathways for the clearance of ADMA from the cell. In the first, ADMA is enzymatically degraded by dimethylarginine dimethylaminohydrolase (DDAH), which cleaves ADMA into citrulline and dimethylamine. The second pathway consists of export of ADMA from the cell to the plasma via cationic amino acid transporters (CAT) in the plasma membrane. Likewise, clearance from the plasma compartment also occurs by two routes: renal excretion and uptake by cells via CAT. The activity of CAT is thus involved in both cellular release and uptake of ADMA, and as such plays an important role in intercellular and interorgan transport of ADMA. Both the kidney and the liver seem to be important organs in the clearance of ADMA from the plasma. The interorgan transport of ADMA may have serious consequences in diseases and clinical conditions that are associated with altered ADMA metabolism. If, in a particular organ, large amounts of ADMA are formed and subsequently exported to the plasma, this may lead to uptake of ADMA by other organs, which will affect their function.

The processes of protein methylation, proteolysis, degradation of ADMA by DDAH, intracellular traffic

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**Figure 1** Schematic overview of the metabolism and clearance of asymmetric dimethylarginine (ADMA). Arginine residues of proteins are methylated by protein arginine methyltransferases (PRMT). In this transmethylation process, *S*-adenosylmethionine (SAM), which serves as donor of the methyl group, is converted into *S*-adenosylhomocysteine (SAH). SAH is hydrolyzed to homocysteine, which can be remethylated to methionine. Free ADMA is formed on proteolysis of methylated proteins. Most ADMA is hydrolyzed by intracellular dimethylarginine dimethylaminohydrolase (DDAH) into citrulline and dimethylamine. Some ADMA is exported from the cell by cationic amino acid transporters (CAT). A small fraction of circulating ADMA is excreted by the kidneys, but most ADMA is taken up by other cells or organs and degraded by DDAH.

via amino acid transporters, and metabolism of ADMA at organ level are discussed in the following sections, with special emphasis on the regulation of these processes.

### Protein methylation

Methylation of the amino acids lysine, histidine and arginine in proteins is a specific form of post-translational modification used by cells to expand the functional repertoire of their proteome. The terminal guanidino group of arginine can be dimethylated by protein arginine methyltransferases (PRMTs), of which two classes exist.<sup>5</sup> Both classes of PRMT catalyze the monomethylation of arginine but, when a second methyl group is attached to monomethylarginine, the reaction product is PRMT dependent. Type 1 PRMTs catalyze the formation of ADMA, whereas type 2 PRMTs lead to the formation of symmetric dimethylarginine (SDMA). Both types of PRMT preferentially methylate arginine located in arginine-glycine-rich sequences of proteins.<sup>5</sup> A recent proteomic analysis has identified more than 200 proteins that are putatively arginine methylated.<sup>6</sup> Many of these have strong interactions with nucleic acids and are involved in processes such as splicing of pre-mRNA, transcription, protein translation, and signal

transduction.<sup>5-8</sup> Arginine methylation is also involved in targeting proteins for nuclear import.<sup>9</sup>

### Regulation of methylation by *S*-adenosylmethionine and *S*-adenosylhomocysteine

All PRMTs use *S*-adenosylmethionine (SAM), which is synthesized from methionine and ATP, as the methyl group donor. After transfer of its methyl group, SAM is converted into *S*-adenosylhomocysteine (SAH). SAH is enzymatically converted into homocysteine, which is either metabolized in the trans-sulfuration route or remethylated to methionine. Because synthesis of ADMA requires the transfer of two methyl groups, two equivalents of homocysteine are formed as a byproduct. Several groups have investigated the link between the metabolic pathways of ADMA and homocysteine. A positive association between plasma levels of homocysteine and ADMA has been reported,<sup>10</sup> but this was not found in other studies.<sup>11,12</sup> Oral methionine loading can be used to induce acute hyperhomocysteinemia. It is interesting that we and other investigators have observed a significant increase in plasma ADMA concentration after a methionine-loading test.<sup>13-15</sup>

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.<sup>16</sup> In accordance with this mechanism, DNA hypomethylation has been observed in hyperhomocysteinemic patients with end-stage renal disease.<sup>17</sup> It has also been shown that the production of ADMA by endothelial cells is inhibited by incubation with SAH.<sup>18</sup>

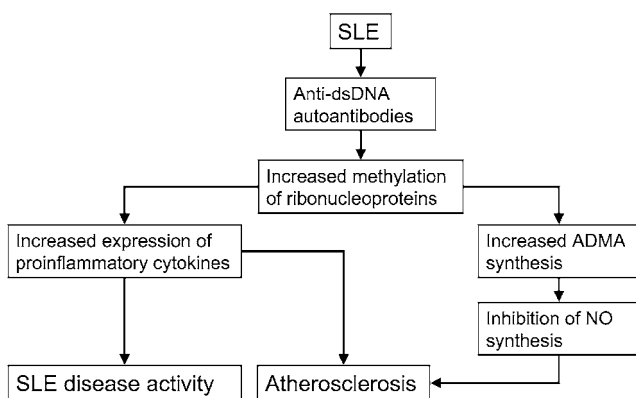
Whether endogenous levels of SAM and SAH play an important role in the regulation of synthesis of ADMA is not clear at present, but this certainly deserves further investigation.

### Stimulation of protein methylation by autoantibodies

Recent reports suggest a link between the occurrence of autoantibodies and protein methylation. Systemic lupus erythematosus (SLE) is a systemic inflammatory disease characterized by the presence of a host of autoantibodies, many of which recognize antigens from the cell nucleus. These autoantibodies play an important role in the pathogenesis and activity of SLE. Common antigenic targets include double-stranded DNA (dsDNA) and several ribonucleoproteins, including the spliceosomal complex. The autoantibodies specifically recognize the basic arginine-glycine-rich domains of the proteins involved.<sup>19</sup> Notably, these domains are also preferred sites for the methylation of arginine residues by PRMTs.<sup>5,20</sup> Indeed, several

spliceosomal proteins have been shown to contain both ADMA and SDMA residues in their sequence.<sup>8,20</sup> It was recently shown that autoantibodies to dsDNA cross-react with the arginine-glycine-rich domain of heterogeneous nuclear ribonucleoprotein A2 (hnRNP A2).<sup>21</sup> In the presence of anti-dsDNA, methylation of hnRNP A2 by PRMT1 was increased in a dose-dependent manner to 3.5 times the control level.<sup>21</sup> Anti-dsDNA antibodies may therefore cause increased ADMA production by upregulating the dimethylation of arginine residues by PRMT1.

This mechanism of excess ADMA production possibly plays a role in the pathology of rheumatoid diseases such as SLE. Cardiovascular disease is an important cause of morbidity and mortality in patients with SLE,<sup>22</sup> but traditional Framingham risk factors fail fully to account for this excess of cardiovascular events.<sup>23</sup> In addition, the endothelial dysfunction associated with SLE cannot be explained by classic risk factors.<sup>24</sup> We have recently assessed the association between ADMA and cardiovascular disease in a cross-sectional study in 107 patients with SLE.<sup>25</sup> The prevalence of cardiovascular disease increased across tertiles of plasma ADMA concentrations ( $p = 0.023$ ). Most importantly, we observed a significant positive association between plasma ADMA concentrations and titres of anti-dsDNA autoantibodies ( $r = 0.43$ ;  $p < 0.001$ ), corroborating the *in vitro* data described above. Anti-dsDNA autoantibodies have also been shown to stimulate the expression of pro-inflammatory cytokines by mononuclear cells<sup>26</sup> and we hypothesize that these antibodies play a dual role in the pathology of SLE by augmenting the inflammatory reaction and stimulating ADMA production (Figure 2).



**Figure 2** Scheme depicting the dual role of autoantibodies against double-stranded DNA (dsDNA) in systemic lupus erythematosus (SLE). These autoantibodies increase the production of asymmetric dimethylarginine (ADMA) by stimulating the asymmetric dimethylation of arginine residues in arginine-glycine-rich domains of ribonucleoproteins. Elevated ADMA levels accelerate atherosclerosis by inhibiting nitric oxide (NO) synthesis. In addition, methylation of ribonucleoproteins leads to enhanced expression of inflammatory cytokines, thereby exacerbating SLE disease activity and atherogenesis.

## Reversibility of arginine methylation

The methylation of proteins is generally considered to be irreversible because demethylating enzymes in eukaryotic cells have been elusive. Recently, this paradigm has been challenged by the identification of an enzyme that can demethylate monomethylated arginine.<sup>27</sup> Two research groups have reported that peptidylarginine deiminase 4 (PAD4), which has a nuclear localization, is able to convert the monomethylated arginine present in histone proteins to citrulline by hydrolytic cleavage of the monomethylamine group.<sup>28,29</sup> It should be noted that PAD4 does not seem to be able to deiminate ADMA<sup>29</sup> but, as monomethylarginine is an intermediate product in the synthesis of ADMA, its deimination by PAD4 interferes with the synthesis of ADMA. In a strict sense, this deimination reaction is not a reversal of arginine methylation because it produces citrulline instead of arginine.

The reaction described above may be considered an exception to the rule. In general, protein methylation is a one-way process and methylated arginine residues remain an integral part of the protein until it is degraded by proteolysis.

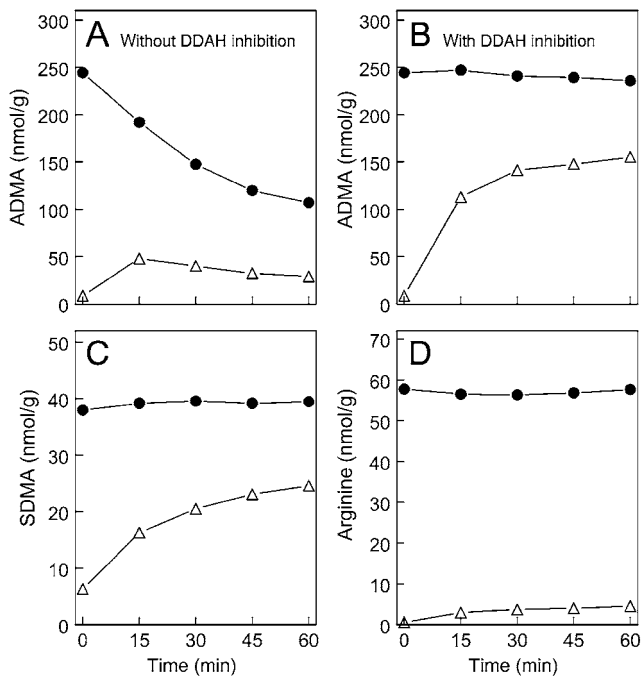
## Proteolysis of methylated proteins

Proteolysis of methylated proteins is the process that leads directly to the liberation of free ADMA, which, in contrast to protein-bound ADMA, serves as a competitive inhibitor of NOS. Protein synthesis and proteolysis are the anabolic and catabolic counterparts of protein turnover, respectively. Under physiological conditions, where no net protein synthesis or degradation occurs, both aspects are in balance. Proteolysis ultimately results in the complete degradation of proteins down to the level of free amino acids, which are re-utilized for *de novo* synthesis of proteins. Amino acids that have been modified by post-translational processes cannot be re-utilized for protein synthesis and are either excreted or metabolized. Whole-body protein turnover has been estimated at 300 g/day.<sup>30</sup> Individual proteins are destroyed at widely differing rates, with half-life times ranging from several minutes to many years.<sup>31</sup> It is very likely that the turnover of methylated proteins occurs at a high rate. In contrast to other post-translational protein modifications that are reversible, such as phosphorylation, arginine methylation is virtually irreversible. This implies that, if arginine methylation is considered as a switch that turns on a particular function of a protein, the only means to switch off that function is by degrading the methylated protein and replacing it by a newly synthesized unmethylated copy.

## Rapid *in vitro* proteolysis of methylated proteins

We have obtained experimental *in vitro* evidence that proteins containing methylated arginine are degraded

at a very high rate. Incubation of a homogenate of rat kidney at 37°C resulted in a very rapid release of free ADMA, which was subsequently degraded by DDAH (Figure 3A). If ADMA degradation was prevented by blocking the activity of DDAH, free ADMA accumulated to a much higher concentration. Comparison with total ADMA (i.e. the sum of free and protein-bound ADMA, measured after acid hydrolysis) revealed that 66% of the protein-bound ADMA was released after 60 minutes (Figure 3B). The amount of protein-bound SDMA was approximately six-fold lower in comparison with protein-bound ADMA, but, in agreement with the data on ADMA, proteolysis resulted in the liberation of more than 60% of SDMA after 60 minutes (Figure 3C). In contrast, the release of protein-bound arginine occurred at a much lower rate, and was less than 8% at the end of the incubation period (Figure 3D). Although the conditions used in this experiment were unphysiological, it shows convincingly that the proteolytic machinery of the cell has the capacity to degrade methylated proteins at a very high rate.



**Figure 3** Rapid in vitro proteolysis of methylated proteins. Rat kidney was homogenized in saline and incubated at 37°C. At specified times, an aliquot was analyzed for free asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA) and arginine (open triangles). The total amount of ADMA, SDMA, and arginine (i.e. the sum of free and protein-bound amino acids) was determined after acid hydrolysis (closed circles). Analysis of ADMA was performed after incubation in the absence (panel A) and presence (panel B) of a large excess monomethylarginine, which inhibits degradation of ADMA by dimethylarginine dimethylaminohydrolase (DDAH).

### Potential role of the proteasome

We can only speculate on the mechanisms involved in the accelerated proteolysis of proteins containing methylated arginine residues. Most intracellular proteins are degraded by the proteasome, which serves as a quality-control system by rapidly degrading misfolded and damaged proteins, whose accumulation would otherwise interfere with normal cellular function.<sup>31,32</sup> The 26S proteasome is a large multiprotein complex, consisting of a barrel-shaped core with combined tryptic, chymotryptic and caspase-like protease activities (the 20S proteasome), which is capped at the ends by 19S regulatory complexes. In contrast to simple proteolytic enzymes, which cut their substrate at a single spot in a scissor-like fashion, the proteasome acts more like a shredder that in a single pass cuts a protein molecule into a large number of peptides. Proteins destined for degradation by the proteasome are conjugated to ubiquitin, which serves as a recognition signal for the regulatory subunits of the 26S proteasome. However, there are strong indications that proteins damaged by oxidative processes do not require ubiquitination, but are directly degraded by the 20S proteasome.<sup>33,34</sup> Oxidation of proteins leads to destabilization of their tertiary structure, resulting in exposure of hydrophobic patches that are normally buried in the interior of the protein. It has been suggested that exposure of these hydrophobic sequences may trigger proteasomal degradation.<sup>35,36</sup> It is tempting to speculate that methylation of arginine residues, which also increases the hydrophobicity of the protein surface, functions as a similar signal. The proteasomal machinery is very effective in cleaving proteins into peptides of approximately 3–22 amino acids in length, with a mean length of 6–9 residues.<sup>37</sup> Final degradation of these peptides into individual amino acids thus requires the adjunctive action of intracellular endo- and exopeptidases. Whether peptidases with specificity for methylated arginine residues exist remains to be established.

### Degradation of ADMA by DDAH

Intracellular ADMA is metabolized to citrulline and dimethylamine, a reaction catalyzed by DDAH.<sup>38,39</sup> Two isoforms of DDAH have been identified that are widely expressed in rat and human tissues.<sup>40–42</sup> A small part of ADMA is cleared by urinary excretion, but it has been estimated that more than 80% is metabolized by DDAH.<sup>30</sup> The very high  $K_m$  value (Michaelis–Menten constant) of DDAH and its sensitivity to oxidative stress are two peculiar features of this enzyme that will be discussed in the following sections.

### Kinetic properties of DDAH

There is abundant evidence that the activity of DDAH plays a critical role in regulating intracellular ADMA

levels. On the one hand, impairment of DDAH activity, with subsequent elevated ADMA levels and reduced production of nitric oxide, can accelerate atherosclerosis.<sup>43</sup> On the other, ADMA acts as an endogenous inhibitor of angiogenesis,<sup>44</sup> and it has been shown that overexpression of DDAH may promote expression of vascular endothelial growth factor and induce tumor neovascularization.<sup>45,46</sup> Apparently, because neither very high nor very low ADMA levels are desirable, ADMA concentrations must be kept between certain limits. The  $K_m$  of DDAH of approximately 180  $\mu\text{mol/l}$ ,<sup>38</sup> much higher than normal intracellular concentrations, may endow the enzyme with the properties to accomplish this task. It ensures that under normal conditions the enzyme works in the linear part of the substrate-velocity curve, which means that the rate of ADMA degradation is roughly proportional to its concentration. This is illustrated in Figure 3A, which shows that at low ADMA concentrations DDAH activity is also very low, leading to a rise in ADMA levels during rapid proteolysis. As the concentration of ADMA increases, DDAH activity also goes up, thereby preventing the accumulation of ADMA to very high levels. The high  $K_m$  value of DDAH may thus provide buffering of ADMA concentrations, ensuring the presence of sufficient amounts of ADMA to allow its function as a regulator of NOS activity, while at the same time serving as a safety valve by rapidly degrading ADMA under conditions of excess supply.

### DDAH and oxidative stress

DDAH is very sensitive to oxidative stress because the active site of the enzyme contains a critical sulfhydryl group that is required for its catalytic activity.<sup>47</sup> Consequently, pathologic stimuli that induce oxidative stress in endothelial cells, such as inflammatory cytokines, hyperhomocysteinemia, hyperglycemia, infectious agents and oxidized low-density lipoprotein (LDL), may reduce DDAH activity and allow ADMA to accumulate.<sup>48–52</sup> It is interesting to note that inhibition of endothelial NOS by elevated ADMA levels may lead to uncoupling of NOS, resulting in a shift from NO production to superoxide production.<sup>53,54</sup> The fact that ADMA itself may induce oxidative stress suggests that under some pathologic conditions the DDAH-ADMA-NOS system may be precipitated into a vicious circle.

The effect of therapy with anti-oxidant vitamins on ADMA levels has not been studied thoroughly, but the results of two small studies suggest that vitamins C and E have no effect.<sup>55,56</sup> In contrast, estrogens, which also have anti-oxidant properties, seem promising. Several *in vitro* and *in vivo* studies on the effect of estrogen on ADMA metabolism have been conducted. Exposure of human and murine endothelial cells to estradiol resulted in an increase in the activity of DDAH and was accompanied by a reduced release of

ADMA.<sup>57</sup> Injection of rats with LDL increased serum levels of ADMA and reduced the endothelium-dependent relaxation of aortic rings. Both effects were attenuated by pretreatment with estradiol.<sup>58</sup> In addition, a negative association between ADMA and endogenous estrogen levels in women with coronary heart disease has been described.<sup>59</sup> We have studied the ADMA lowering effect of estrogens in the setting of hormone replacement therapy in postmenopausal women.<sup>60,61</sup> Both conjugated estrogen and estradiol reduced plasma ADMA concentrations to a similar extent, but the effect of estradiol was found to be strongly enhanced if administered in combination with the synthetic progestogen trimegestone.<sup>60,61</sup> It is striking that this combination of hormones lowered not only ADMA but also arginine levels. It is tempting to speculate that arginine levels were reduced by an increased consumption of arginine by NOS, resulting from diminished inhibition of NOS by ADMA.

### Role of amino acid transporters

Both the generation of free ADMA by proteolysis of methylated proteins and its degradation by DDAH are intracellular processes. Most of the ADMA is probably rapidly degraded in the cytosol of the same cell where it was made. However, a small part may escape degradation and leave the cell to enter the bloodstream. Transport of basic amino acids such as arginine, lysine and ornithine across the cell membrane is facilitated by functionally distinct transport systems, principally systems  $y^+$  and  $y^+L$ . System  $y^+$  belongs to a family of CAT that exclusively transports cationic amino acids, whereas system  $y^+L$  transports both cationic and neutral amino acids.<sup>62</sup> These transporters mediate the bidirectional transport of cationic amino acids, thus supporting important metabolic functions such as protein synthesis, NO production, polyamine biosynthesis, and interorgan amino acid flow. Arginine transport through both  $y^+$  and  $y^+L$  systems is involved in NO synthesis in endothelial cells,<sup>63</sup> whereas arginine uptake by platelets is exclusively mediated by system  $y^+L$ .<sup>64</sup> Altered expression of CAT has been implicated in several clinical conditions, including congestive heart failure, chronic and acute renal failure, and septic shock.<sup>65–67</sup>

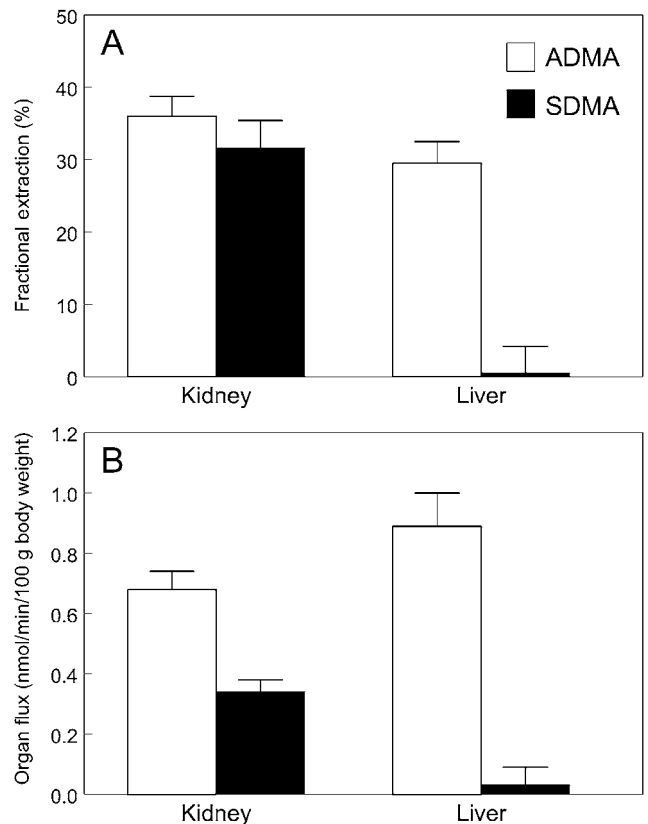
It has been shown that system  $y^+$  is capable of transporting both ADMA and SDMA across the cell membrane.<sup>68</sup> Changes in the expression of CAT may therefore not only influence the interorgan transport and cellular uptake of arginine, but also of ADMA and SDMA. In addition, both methylated arginines may compete with arginine for cellular uptake. It is interesting that both endothelial NOS and system  $y^+$  are localized to caveolae in the plasma membrane.<sup>69</sup> This colocalization ensures an efficient supply of plasma

arginine to NOS, but it may also provide plasma ADMA with direct access to NOS.

### Metabolism of ADMA at organ level

Several studies have shown that ADMA accumulates in renal failure and is associated with cardiovascular mortality in end-stage renal disease.<sup>2,70</sup> The human kidney is responsible for urinary excretion of both ADMA and SDMA but, in addition, the kidneys clear a substantial amount of ADMA by metabolic degradation, probably by DDAH.<sup>71</sup> Apparently, the kidney plays an important role in the metabolism of ADMA, but less information on the role of other organs in the clearance of ADMA is available. In recent years our group has studied the metabolism of both ADMA and SDMA at organ level in the rat.<sup>72-74</sup> In the experimental design, arteriovenous concentration differences and blood flow through the organ were measured, allowing the calculation of fractional extraction and net organ fluxes. Arteriovenous concentration differences were determined by a high-performance liquid chromatography method with high precision and sensitivity<sup>75</sup> and blood flow through individual organs was measured using radioactive microspheres. Data on renal and hepatic clearance of ADMA and SDMA in the rat are summarized in Figure 4. Fractional extraction of ADMA (i.e. the percentage that is cleared from the plasma) is slightly higher in the kidney than in the liver (Figure 4A). However, because total plasma flow through the liver is higher than through the kidney, the liver clears more ADMA from the circulation than the kidney, as evidenced by the higher organ flux of the liver (Figure 4B). Comparable data for SDMA show that SDMA is cleared by the kidney, whereas extraction by the liver is negligible. It can be concluded that the liver plays an important role in the metabolism of ADMA by taking up large amounts from the systemic circulation. Endotoxemia, induced by the administration of lipopolysaccharide, resulted in a lower systemic ADMA concentration, in combination with a reduced uptake of ADMA by the kidney and an increased uptake by the liver.<sup>73,74</sup> Mittermayer et al have studied the effect of endotoxemia on plasma ADMA levels in humans. No effect on ADMA or SDMA levels was observed, but a decreased arginine level and arginine/ADMA ratio were found.<sup>76</sup>

The potential role of the liver in the metabolism of ADMA was already recognized in 1977 by Carnegie et al, who described increased urinary excretion of ADMA in patients with liver disease.<sup>77</sup> We have performed several clinical studies that confirm the essential role of the liver in the elimination of ADMA in humans. In patients undergoing major liver resection, ADMA levels were significantly elevated in a subgroup with prolonged postoperative hepatic injury.<sup>78</sup> In a prospective study in patients undergoing liver



**Figure 4** Renal and hepatic clearance of asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) in the rat. Blood flow through the organs was measured by injection of radiolabeled microspheres and concentrations of ADMA and SDMA were determined in the renal, hepatic and portal veins, and in the aorta. From these measurements the fractional extraction (panel A) and organ flux (panel B) for ADMA (open bars) and SDMA (filled bars) were calculated. Error bars indicate standard error of the mean.

transplantation, preoperative ADMA concentrations were elevated, especially in those with chronic hepatic failure.<sup>79</sup> After transplantation, ADMA concentration decreased very rapidly but, in patients who experienced acute rejection, ADMA concentrations were higher during the first postoperative month compared with nonrejectors. These results are in line with reports by other investigators on elevated plasma levels of ADMA in patients with end-stage liver disease and alcoholic cirrhosis.<sup>80,81</sup>

In a population of critically ill patients with clinical evidence of dysfunction of two or more organs, plasma ADMA concentrations were elevated and significantly related to hepatic function.<sup>82</sup> Moreover, plasma ADMA concentration emerged as a strong and independent risk factor for mortality in these patients. Based on these findings we hypothesize that the accumulation of ADMA is a causative factor in the development of multiple organ failure by interfering with important physiological functions of NO production.<sup>83</sup>

## Conclusions

The sequence of events leading to the generation of free ADMA (i.e. methylation of arginine residues by PRMTs and proteolysis) is not well characterized. Often, the formation of ADMA is regarded as an inevitable byproduct of protein turnover. This seems an oversimplification because both methylation and the breakdown of proteins are highly regulated processes. The crucial role of DDAH in the degradation of ADMA and its extreme sensitivity to oxidative stress are well documented. The bulk of ADMA is metabolized by DDAH, but this degradation may occur in a different cell or organ from the one in which ADMA is formed. CAT play an important role in trafficking of ADMA between cells and organs. The kidney and the liver play a significant role as sinks for excess ADMA. The interorgan transport of ADMA may have serious consequences in disease states. Overproduction or defective clearance of ADMA in a single organ may lead to its accumulation in other organs, thereby impairing their function.

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## References

- Valkonen VP, Päivä H, Salonen JT et al. Risk of acute coronary events and serum concentration of asymmetrical dimethylarginine. *Lancet* 2001; **358**: 2127–28.
- Zoccali C, Bode-Böger SM, Mallamaci F et al. Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: a prospective study. *Lancet* 2001; **358**: 2113–17.
- Vallance P, Leone A, Calver A, Collier J, Moncada S. Endogenous dimethylarginine as an inhibitor of nitric oxide synthesis. *J Cardiovasc Pharmacol* 1992; **20**(suppl 12): S60–62.
- Suda O, Tsutsui M, Morishita T et al. Asymmetric dimethylarginine causes arteriosclerotic lesions in endothelial nitric oxide synthase-deficient mice. Involvement of renin-angiotensin system and oxidative stress. *Arterioscler Thromb Vasc Biol* 2004; **24**: 1682–88.
- Gary JD, Clarke S. RNA and protein interactions modulated by protein arginine methylation. *Prog Nucleic Acid Res Mol Biol* 1998; **61**: 65–131.
- Boisvert FM, Côté J, Boulanger MC, Richard S. A proteomic analysis of arginine-methylated protein complexes. *Mol Cell Proteomics* 2003; **2**: 1319–30.
- Bachand F, Silver PA. PRMT3 is a ribosomal protein methyltransferase that affects the cellular levels of ribosomal subunits. *EMBO J* 2004; **23**: 2641–50.
- Miranda TB, Khusial P, Cook JR et al. Spliceosome Sm proteins D1, D3, and B/B' are asymmetrically dimethylated at arginine residues in the nucleus. *Biochem Biophys Res Commun* 2004; **323**: 382–87.
- Smith WA, Schurter BT, Wong-Staal F, David M. Arginine methylation of RNA helicase A determines its subcellular localization. *J Biol Chem* 2004; **279**: 22795–98.
- Holven KB, Haugstad TS, Holm T, Aukrust P, Ose L, Nenseter MS. Folic acid treatment reduces elevated plasma levels of asymmetric dimethylarginine in hyperhomocysteinaemic subjects. *Br J Nutr* 2003; **89**: 359–63.
- Dierkes J, Westphal S, Martens-Lobenhoffer J, Luley C, Bode-Böger SM. Fenofibrate increases the L-arginine:ADMA ratio by increase of L-arginine concentration but has no effect on ADMA concentration. *Atherosclerosis* 2004; **173**: 239–44.
- Jonasson TF, Hedner T, Hultberg B, Öhlin H. Hyperhomocysteinaemia is not associated with increased levels of asymmetric dimethylarginine in patients with ischaemic heart disease. *Eur J Clin Invest* 2003; **33**: 543–49.
- Böger RH, Lentz SR, Bode-Böger SM, Knapp HR, Haynes WG. Elevation of asymmetrical dimethylarginine may mediate endothelial dysfunction during experimental hyperhomocyst(e)inaemia in humans. *Clin Sci* 2001; **100**: 161–67.
- Stühlinger MC, Oka RK, Graf EE et al. Endothelial dysfunction induced by hyperhomocyst(e)inemia. Role of asymmetric dimethylarginine. *Circulation* 2003; **108**: 933–38.
- Wanby P, Brattström L, Brudin L, Hultberg B, Teerlink T. Asymmetric dimethylarginine and total homocysteine in plasma after oral methionine loading. *Scand J Clin Lab Invest* 2003; **63**: 347–53.
- Yi P, Melnyk S, Pogribna M, Pogribny IP, Hine RJ, James SJ. Increase in plasma homocysteine associated with parallel increases in plasma S-adenosylhomocysteine and lymphocyte DNA hypomethylation. *J Biol Chem* 2000; **275**: 29318–23.
- Ingrosso D, Cimmino A, Perna AF et al. Folate treatment and unbalanced methylation and changes of allelic expression induced by hyperhomocysteinaemia in patients with uraemia. *Lancet* 2003; **361**: 1693–99.
- Böger RH, Sydow K, Borlak J et al. LDL cholesterol upregulates synthesis of asymmetrical dimethylarginine in human endothelial cells: involvement of S-adenosylmethionine-dependent methyltransferases. *Circ Res* 2000; **87**: 99–105.
- McClain MT, Ramsland PA, Kaufman KM, James JA. Anti-Sm autoantibodies in systemic lupus target highly basic surface structures of complexed spliceosomal autoantigens. *J Immunol* 2002; **168**: 2054–62.
- Zieve GW, Khusial PR. The anti-Sm immune response in autoimmunity and cell biology. *Autoimmun Rev* 2003; **2**: 235–40.
- Sun KH, Tang SJ, Wang YS, Lin WJ, You RI. Autoantibodies to dsDNA cross-react with the arginine-glycine-rich domain of heterogeneous nuclear ribonucleoprotein A2 (hnRNP A2) and promote methylation of hnRNP A2. *Rheumatology* 2003; **42**: 154–61.
- Manzi S, Meilahn EN, Rairie JE et al. Age-specific incidence rates of myocardial infarction and angina in women with systemic lupus erythematosus: comparison with the Framingham Study. *Am J Epidemiol* 1997; **145**: 408–15.
- Esdaile JM, Abrahamowicz M, Grodzicky T et al. Traditional Framingham risk factors fail to fully account for accelerated atherosclerosis in systemic lupus erythematosus. *Arthritis Rheum* 2001; **44**: 2331–37.



- 24 El-Magadmi M, Bodill H, Ahmad Y et al. Systemic lupus erythematosus: an independent risk factor for endothelial dysfunction in women. *Circulation* 2004; **110**: 399–404.
- 25 Bultink IEM, Teerlink T, Heijst JA, Dijkmans BAC, Voskuyl AE. Elevated plasma levels of asymmetric dimethylarginine are associated with cardiovascular events, disease activity and organ damage in patients with systemic lupus erythematosus. *Ann Rheum Dis* 2005 (in press).
- 26 Sun KH, Yu CL, Tang SJ, Sun GH. Monoclonal anti-double-stranded DNA autoantibody stimulates the expression and release of IL-1beta, IL-6, IL-8, IL-10 and TNF-alpha from normal human mononuclear cells involving in the lupus pathogenesis. *Immunology* 2000; **99**: 352–60.
- 27 Zhang Y. Molecular biology: no exception to reversibility. *Nature* 2004; **431**: 637–39.
- 28 Wang Y, Wysocka J, Sayegh J et al. Human PAD4 regulates histone arginine methylation levels via demethylination. *Science* 2004; **306**: 279–83.
- 29 Cuthbert GL, Daujat S, Snowden AW et al. Histone demethylation antagonizes arginine methylation. *Cell* 2004; **118**: 545–53.
- 30 Achan V, Broadhead M, Malaki M et al. Asymmetric dimethylarginine causes hypertension and cardiac dysfunction in humans and is actively metabolized by dimethylarginine dimethylaminohydrolase. *Arterioscler Thromb Vasc Biol* 2003; **23**: 1455–59.
- 31 Glickman MH, Ciechanover A. The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. *Physiol Rev* 2002; **82**: 373–428.
- 32 Goldberg AL. Protein degradation and protection against misfolded or damaged proteins. *Nature* 2003; **426**: 895–99.
- 33 Shringarpure R, Grune T, Mehlhase J, Davies KJ. Ubiquitin conjugation is not required for the degradation of oxidized proteins by proteasome. *J Biol Chem* 2003; **278**: 311–18.
- 34 Orłowski M, Wilk S. Ubiquitin-independent proteolytic functions of the proteasome. *Arch Biochem Biophys* 2003; **415**: 1–5.
- 35 Giulivi C, Pacifici RE, Davies KJ. Exposure of hydrophobic moieties promotes the selective degradation of hydrogen peroxide-modified hemoglobin by the multicatalytic proteinase complex, proteasome. *Arch Biochem Biophys* 1994; **311**: 329–41.
- 36 Mehlhase J, Grune T. Proteolytic response to oxidative stress in mammalian cells. *Biol Chem* 2002; **383**: 559–67.
- 37 Kisselev AF, Akopian TN, Woo KM, Goldberg AL. The sizes of peptides generated from protein by mammalian 26 and 20 S proteasomes. Implications for understanding the degradative mechanism and antigen presentation. *J Biol Chem* 1999; **274**: 3363–71.
- 38 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**: 10205–209.
- 39 MacAllister RJ, Parry H, Kimoto M et al. Regulation of nitric oxide synthesis by dimethylarginine dimethylaminohydrolase. *Br J Pharmacol* 1996; **119**: 1533–40.
- 40 Kimoto M, Tsuji H, Ogawa T, Sasaoka K. Detection of NG,NG-dimethylarginine dimethylaminohydrolase in the nitric oxide-generating systems of rats using monoclonal antibody. *Arch Biochem Biophys* 1993; **300**: 657–62.
- 41 Tran CT, Fox MF, Vallance P, Leiper JM. Chromosomal localization, gene structure, and expression pattern of DDAH1: comparison with DDAH2 and implications for evolutionary origins. *Genomics* 2000; **68**: 101–105.
- 42 Leiper JM, Santa Maria J, Chubb A et al. Identification of two human dimethylarginine dimethylaminohydrolases with distinct tissue distributions and homology with microbial arginine deiminases. *Biochem J* 1999; **343**: 209–14.
- 43 Dayoub H, Achan V, Adimoolam S et al. Dimethylarginine dimethylaminohydrolase regulates nitric oxide synthesis. Genetic and physiological evidence. *Circulation* 2003; **108**: 3042–47.
- 44 Cooke JP. NO and angiogenesis. *Atheroscler Suppl* 2003; **4**: 53–60.
- 45 Smith CL, Birdsey GM, Anthony S, Arrigoni FI, Leiper JM, Vallance P. Dimethylarginine dimethylaminohydrolase activity modulates ADMA levels, VEGF expression, and cell phenotype. *Biochem Biophys Res Commun* 2003; **308**: 984–89.
- 46 Kostourou V, Robinson SP, Whitley GS, Griffiths JR. Effects of overexpression of dimethylarginine dimethylaminohydrolase on tumor angiogenesis assessed by susceptibility magnetic resonance imaging. *Cancer Res* 2003; **63**: 4960–66.
- 47 Murray-Rust J, Leiper J, McAlister M et al. Structural insights into the hydrolysis of cellular nitric oxide synthase inhibitors by dimethylarginine dimethylaminohydrolase. *Nat Struct Biol* 2001; **8**: 679–83.
- 48 Ito A, Tsao PS, Adimoolam S, Kimoto M, Ogawa T, Cooke JP. Novel mechanism for endothelial dysfunction: dysregulation of dimethylarginine dimethylaminohydrolase. *Circulation* 1999; **99**: 3092–95.
- 49 Leiper J, Murray-Rust J, McDonald N, Vallance P. S-nitrosylation of dimethylarginine dimethylaminohydrolase regulates enzyme activity: further interactions between nitric oxide synthase and dimethylarginine dimethylaminohydrolase. *Proc Natl Acad Sci U S A* 2002; **99**: 13527–32.
- 50 Lin KY, Ito A, Asagami T et al. Impaired nitric oxide synthase pathway in diabetes mellitus: role of asymmetric dimethylarginine and dimethylarginine dimethylaminohydrolase. *Circulation* 2002; **106**: 987–92.
- 51 Stühlinger MC, Tsao PS, Her JH, Kimoto M, Balint RF, Cooke JP. Homocysteine impairs the nitric oxide synthase pathway: role of asymmetric dimethylarginine. *Circulation* 2001; **104**: 2569–75.
- 52 Cooke JP. Asymmetrical dimethylarginine: the über marker? *Circulation* 2004; **109**: 1813–18.
- 53 Böger RH, Bode-Böger SM, Tsao PS, Lin PS, Chan JR, Cooke JP. An endogenous inhibitor of nitric oxide synthase regulates endothelial adhesiveness for monocytes. *J Am Coll Cardiol* 2000; **36**: 2287–95.
- 54 Sydow K, Münzel T. ADMA and oxidative stress. *Atheroscler Suppl* 2003; **4**: 41–51.
- 55 Engler MM, Engler MB, Malloy MJ et al. Antioxidant vitamins C and E improve endothelial function in children with hyperlipidemia. Endothelial Assessment of Risk from Lipids in Youth (EARLY) trial. *Circulation* 2003; **108**: 1059–63.
- 56 Saran R, Novak JE, Desai A et al. Impact of vitamin E on plasma asymmetric dimethylarginine (ADMA) in chronic kidney disease (CKD): a pilot study. *Nephrol Dial Transplant* 2003; **18**: 2415–20.
- 57 Holden DP, Cartwright JE, Nussey SS, Whitley GS. Estrogen stimulates dimethylarginine dimethylaminohydrolase activity and the metabolism of asymmetric dimethylarginine. *Circulation* 2003; **108**: 1575–80.
- 58 Dai Z, Zhu HQ, Jiang DJ, Jiang JL, Deng HW, Li YJ. 17beta-Estradiol preserves endothelial function by reduction of the endogenous nitric oxide synthase inhibitor level. *Int J Cardiol* 2004; **96**: 223–27.
- 59 Li XP, Zhou Y, Zhao SP, Gao M, Zhou Q, Li YS. Effect of endogenous estrogen on endothelial function in women with coronary heart disease and its mechanism. *Clin Chim Acta* 2004; **339**: 183–88.

- 60 Post MS, Verhoeven MO, van der Mooren MJ, Kenemans P, Stehouwer CD, 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**: 4221–26.
- 61 Teerlink T, Neele SJ, de Jong S, Netelenbos JC, Stehouwer CD. Oestrogen replacement therapy lowers plasma levels of asymmetrical dimethylarginine in healthy postmenopausal women. *Clin Sci* 2003; **105**: 67–71.
- 62 Mann GE, Yudilevich DL, Sobrevia L. Regulation of amino acid and glucose transporters in endothelial and smooth muscle cells. *Physiol Rev* 2003; **83**: 183–252.
- 63 Arancibia-Garavilla Y, Toledo F, Casanello P, Sobrevia L. Nitric oxide synthesis requires activity of the cationic and neutral amino acid transport system y + L in human umbilical vein endothelium. *Exp Physiol* 2003; **88**: 699–710.
- 64 Mendes Ribeiro AC, Brunini TM, Yaqoob M, Aronson JK, Mann GE, Ellory JC. Identification of system y + L as the high-affinity transporter for L-arginine in human platelets: up-regulation of L-arginine influx in uraemia. *Pflugers Arch* 1999; **438**: 573–75.
- 65 Reade MC, Clark MF, Young JD, Boyd CA. Increased cationic amino acid flux through a newly expressed transporter in cells overproducing nitric oxide from patients with septic shock. *Clin Sci* 2002; **102**: 645–50.
- 66 Mendes Ribeiro AC, Brunini TM, Ellory JC, Mann GE. Abnormalities in L-arginine transport and nitric oxide biosynthesis in chronic renal and heart failure. *Cardiovasc Res* 2001; **49**: 697–712.
- 67 Kaye DM, Ahlers BA, Autelitano DJ, Chin-Dusting JP. In vivo and in vitro evidence for impaired arginine transport in human heart failure. *Circulation* 2000; **102**: 2707–12.
- 68 Closs EI, Basha FZ, Habermeyer A, Förstermann U. Interference of L-arginine analogues with L-arginine transport mediated by the y+ carrier hCAT-2B. *Nitric Oxide* 1997; **1**: 65–73.
- 69 McDonald KK, Zharikov S, Block ER, Kilberg MS. 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**: 31213–16.
- 70 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**: 572–75.
- 71 Nijveldt RJ, van Leeuwen PA, van Guldener C, Stehouwer CD, Rauwerda JA, Teerlink T. Net renal extraction of asymmetrical (ADMA) and symmetrical (SDMA) dimethylarginine in fasting humans. *Nephrol Dial Transplant* 2002; **17**: 1999–2002.
- 72 Nijveldt RJ, Teerlink T, Siroen MP, van Lambalgen AA, Rauwerda JA, van Leeuwen PA. The liver is an important organ in the metabolism of asymmetrical dimethylarginine (ADMA). *Clin Nutr* 2003; **22**: 17–22.
- 73 Nijveldt RJ, Teerlink T, van Guldener C et al. Handling of asymmetrical dimethylarginine and symmetrical dimethylarginine by the rat kidney under basal conditions and during endotoxaemia. *Nephrol Dial Transplant* 2003; **18**: 2542–50.
- 74 Nijveldt RJ, Siroen MP, Teerlink T, van Lambalgen AA, Rauwerda JA, van Leeuwen PA. Gut and liver handling of asymmetric and symmetric dimethylarginine in the rat under basal conditions and during endotoxemia. *Liver Int* 2004; **24**: 510–18.
- 75 Teerlink T, Nijveldt RJ, de Jong S, van Leeuwen PA. Determination of arginine, asymmetric dimethylarginine, and symmetric dimethylarginine in human plasma and other biological samples by high-performance liquid chromatography. *Anal Biochem* 2002; **303**: 131–37.
- 76 Mittermayer F, Namiranian K, Pleiner J, Schaller G, Wolzt M. Acute *Escherichia coli* endotoxaemia decreases the plasma L-arginine/asymmetrical dimethylarginine ratio in humans. *Clin Sci* 2004; **106**: 577–81.
- 77 Carnegie PR, Fellows FC, Symington GR. Urinary excretion of methylarginine in human disease. *Metabolism* 1977; **26**: 531–37.
- 78 Nijveldt RJ, Teerlink T, Siroen MP et al. Elevation of asymmetric dimethylarginine (ADMA) in patients developing hepatic failure after major hepatectomy. *JPEN J Parenter Enteral Nutr* 2004; **28**: 382–87.
- 79 Siroen MP, Warlé MC, Teerlink T et al. The transplanted liver graft is capable of clearing asymmetric dimethylarginine. *Liver Transpl* 2004; **10**: 1524–30.
- 80 Tsikas D, Rode I, Becker T, Nashan B, Klempnauer J, Frölich JC. Elevated plasma and urine levels of ADMA and 15(S)-8-iso-PGF<sub>2</sub>alpha in end-stage liver disease. *Hepatology* 2003; **38**: 1063–64.
- 81 Lluch P, Torondel B, Medina P et al. Plasma concentrations of nitric oxide and asymmetric dimethylarginine in human alcoholic cirrhosis. *J Hepatol* 2004; **41**: 55–59.
- 82 Nijveldt RJ, Teerlink T, van der Hoven B et al. Asymmetrical dimethylarginine (ADMA) in critically ill patients: high plasma ADMA concentration is an independent risk factor of ICU mortality. *Clin Nutr* 2003; **22**: 23–30.
- 83 Nijveldt RJ, Teerlink T, van Leeuwen PA. The asymmetrical dimethylarginine (ADMA)-multiple organ failure hypothesis. *Clin Nutr* 2003; **22**: 99–104.