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ORIGINAL ARTICLE

Asymmetric dimethylarginine in children with homocystinuria or phenylketonuria

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Abstract Plasma concentration of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide (NO) synthesis from L-arginine and a cardiovascular risk factor, was found to be elevated in plasma of homocysteinemic adults. Enhanced cardiovascular risk due to homocystinuria and impaired renal function has been found in patients with phenylketonuria (PKU) on protein-restricted diet. However, it is still unknown whether ADMA synthesis is also elevated in children with homocystinuria due to cystathionine beta-synthase deficiency (classical homocystinuria), and whether ADMA may play a role in phenylketonuria in childhood. In the present study, we investigated the status of the L-arginine/NO pathway in six young patients with homocystinuria, in 52 young phenylketonuria patients on natural protein-restricted diet, and in age- and gender-matched healthy children serving as controls. ADMA in plasma and urine was determined by GC–MS/MS. The NO metabolites nitrate and nitrite in plasma and urine, and urinary dimethylamine (DMA), the dimethylarginine dimethylaminohydrolase (DDAH) metabolite of ADMA, were measured by GC–MS. Unlike urine ADMA excretion, plasma ADMA concentration in patients with homocystinuria was significantly higher than in controls (660 ± 158 vs. 475 ± 77 nM, $P = 0.035$). DMA excretion rate was considerably higher in children with homocystinuria as compared to controls (62.2 ± 24.5 vs. 6.5 ± 2.9 μmol/mmol creatinine, $P = 0.068$), indicating enhanced DDAH activity in this disease. In contrast and unexpectedly, phenylketonuria patients had significantly lower ADMA plasma concentrations compared to controls (512 ± 136 vs. 585 ± 125 nM, $P = 0.009$). Phenylketonuria patients and controls had similar L-arginine/ADMA molar ratios in plasma. Urinary nitrite excretion was significantly higher in phenylketonuria as compared to healthy controls (1.7 ± 1.7 vs. 0.7 ± 1.2 μmol/mmol creatinine, $P = 0.003$). Our study shows that the L-arginine/NO pathway is differently altered in children with phenylketonuria and homocystinuria. Analogous to hyperhomocysteinemic adults, elevated ADMA plasma concentrations could be a cardiovascular risk factor in children with homocystinuria. In phenylketonuria, the L-arginine/NO pathway seems not be altered. Delineation of the role of ADMA in childhood phenylketonuria and homocystinuria demands further investigation.

Keywords ADMA · L-Arginine · Dimethylamine · Dimethylarginine dimethylaminohydrolase · Homocysteine · Nitric oxide

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Introduction

Nitric oxide (NO) is generated from L-arginine by nitric oxide synthase (NOS) isoforms virtually in all types of

cells including endothelial cells. NO is a potent vasodilator, has effects on hemostasis, leukocyte adhesion and neurotransmission (Cines et al. 1998), and exerts anti-proliferative and anti-atherosclerotic actions (Cooke and Dzau 1997). Elevated plasma concentrations of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NOS (Achan et al. 2003; Tsikas et al. 2000), have been found in a variety of diseases, most of them being associated with NO-dependent endothelial dysfunction (Böger et al. 1997). Thus, ADMA in plasma and urine is considered to be a novel marker for cardiovascular risk and mortality in adults (Kielstein et al. 2006; Böger and Zoccali 2003; Wolf et al. 2010).

Homocystinuria (MIM 236200), a rare inherited disease with a worldwide incidence of about 1:200,000–1:300,000 due to cystathionine beta-synthase (EC 4.2.1.22) deficiency, is a well-known cardiovascular risk factor in adults (Welch and Loscalzo 1998). In homocystinuria, elevated concentrations of homocysteine in plasma are found and an enhanced cardiovascular risk was described several years ago (Mudd et al. 1985). Interestingly, a positive correlation between ADMA and homocysteine plasma concentrations was found in adults (Korandji et al. 2007). Yet, data on the L-arginine/NO pathway in children with homocystinuria are still lacking.

Enhanced cardiovascular risk has also been proposed for patients with phenylketonuria (PKU, MIM 261600), an inherited defect of phenylalanine hydroxylase (PAH, EC 1.14.16.1) with an incidence of about 1:10,000 (Williams et al. 2008). In phenylketonuria, levels of the cardioprotective HDL cholesterol have been reported to be low (Verduyn et al. 2005). Furthermore, renal function was found to be impaired in phenylketonuria patients (Hennermann et al. 2005). Phenylketonuria patients are on a special diet (restricted to natural proteins and supplemented by special formulae) that could be accompanied by nutritional imbalances and insufficient vitamin supplementation. In fact, in phenylketonuria patients on protein-restricted diet, a moderate homocystinuria was found; the authors assumed that homocystinuria is the cause for an elevated cardiovascular risk in phenylketonuria patients (Schulpis et al. 2002).

In previous studies, we investigated the role of ADMA and other members of the L-arginine/NO pathway in children with renal and metabolic diseases (Lücke et al. 2006, 2008). In the present study, we investigated the status of this pathway in children with homocystinuria and in children suffering from phenylketonuria. The aim of our study was to prove the hypothesis that ADMA synthesis was elevated and consequently NO synthesis was decreased both in homocystinuria and in phenylketonuria. Analogous to adults, ADMA would then represent a cardiovascular risk factor in children suffering from these diseases. It is worth mentioning that healthy children possess clearly higher NO synthesis rates despite higher ADMA synthesis as compared to healthy adults (Lücke et al. 2007), indicating that children are not small adults with respect to the L-arginine/NO pathway. To our knowledge, the present work is the first study to report quantitative data on the L-arginine/NO pathway in children with homocystinuria and in children with phenylketonuria. For a comprehensive description of amino acids metabolism, in general, and of L-arginine metabolism, in particular, in health and disease we refer to recent review articles (Wu 2009; Wu et al. 2009).

Materials and methods

Subjects

In total, 58 patients were investigated in this study, i.e., 6 patients with homocystinuria and 52 patients with phenylketonuria. All patients were under a common therapeutic regimen regarding their underlying disease. Age-matched control groups consisting of 6 healthy children for the homocystinuria group and 46 healthy children for the phenylketonuria group were recruited. Clinical information of the patients is presented in Table 1. Homocysteine and methionine plasma levels of the patients with homocystinuria were in parts greatly elevated as compared to normal levels (Table 2). Protein intake by phenylketonuria patients was not lower compared to healthy controls. The lower percentage of natural protein intake in phenylketonuria patients was compensated by phenylalanine-free amino acid supplementations, which also contained adapted concentrations of minerals and vitamins including vitamin B12. In our phenylketonuria children who were recruited by outpatient clinics, we did not observe megaloblastic anemia or methylmalonic aciduria, a sensitive marker for vitamin B12 deficiency. Also, all phenylketonuria patients included in the study had normal renal function parameters and no arterial hypertension (Table 1). The healthy children were admitted to hospital for minor elective procedures. Subjects with underlying systemic diseases, such as cardiovascular, oncological, renal or metabolic disorders were excluded from the control groups. There was no dietary control of nitrite and nitrate intake by patients and

Table 1 Age, body mass index, blood pressure and serum creatinine of the study patients with homocystinuria and phenylketonuria

Parameter	Homocystinuria	Phenylketonuria
Age (years)	11 ± 7	12 ± 7
Body mass index (percentile)	67 ± 27	63 ± 35
Blood pressure (percentile)	42 ± 48	34 ± 38
Serum creatinine (μM)	45 ± 18	46 ± 15

Table 2 Homocystine, total homocysteine and methionine concentrations in plasma of the six patients with homocystinuria

Patient no.	Homocystine (μM)	Total homocysteine (μM)	Methionine (μM)
1	7.0	91.1	272
2	<1.0	15.5	278
3	<1.0	14.3	20
4	<1.0	23.8	844
5	80	219	611
6	89	225	251

Homocystine, total homocysteine and methionine concentrations in plasma of healthy children are <1 μM, <10 μM and <20 μM, respectively

healthy controls. Also, all children were not fasting. The study was approved by the Ethics Committee of the Hannover Medical School and written consent was obtained from the parents.

Analytical methods

EDTA venous blood was drawn, put immediately on ice and centrifuged (4,500×*g*, 4°C, 10 min). Plasma samples were frozen at −80°C until analysis. Urine samples were obtained from spontaneous micturition and frozen immediately at −20°C until analysis. Urinary ADMA, DMA, nitrate and nitrite excretion was corrected for creatinine excretion.

ADMA in plasma and urine was determined by gas chromatography–tandem mass spectrometry (GC–MS/MS) as described elsewhere (Tsikas et al. 2003). L-Arginine in plasma was measured by GC–MS as reported previously (Tsikas et al. 2003). Nitrate and nitrite in plasma and urine were determined simultaneously by GC–MS as described previously (Tsikas 2000). Urinary creatinine was determined by high-performance liquid chromatography (HPLC) as described (Tsikas et al. 2004). DMA in urine was determined by GC–MS as reported earlier (Tsikas et al. 2007). Methionine and homocystine (i.e., homocysteine disulfide) were determined using a commercially available amino acid analyzer model Biochrom 30Plus (Laborservice Onken GmbH, Gründau, Germany) based on ion-exchange HPLC and post-column derivatization with ninhydrin. Plasma total homocysteine concentrations, which include all homocysteine species present in plasma (Tsikas 2003), were determined by a commercially available standard clinical chemistry fluorescence polarimetry assay (FPA).

Statistical analysis

Data are presented as mean ± SD. Results were compared using the Wilcoxon test (SPSS, version 17). Values of $P < 0.05$ were considered to be significant.

Results

Homocystinuria

Plasma concentrations of ADMA in the hyperhomocysteinemic patients were significantly higher (by 28%) as compared to controls (660 ± 158 vs. 475 ± 77 nM, $P = 0.028$) (Fig. 1a). Creatinine-corrected excretion rate of ADMA did not differ significantly in both groups (8.1 ± 6.6 vs. 5.3 ± 1.7 μmol/mmol creatinine, $P = 0.225$) (Fig. 1b). Creatinine-corrected excretion rate of DMA in urine was almost tenfold higher in the patients as compared to controls (62.2 ± 24.5 vs. 6.5 ± 2.9 μmol/mmol creatinine, $P = 0.068$) (Fig. 1c). Concentrations of L-arginine in plasma were not statistically different between the groups ($P = 0.6$). Also, the L-arginine/ADMA molar ratio did not differ between patients and controls (105 ± 51:1 vs. 104 ± 42:1).

Nitrate and nitrite concentrations measured in plasma and urine are summarized in Table 3. In the patients, plasma nitrate concentration was significantly higher than in controls (77.9 ± 24.2 vs. 29.8 ± 4.2 μM, $P = 0.043$). Nitrite in plasma was not significantly higher in the patients compared to controls (2.7 ± 0.6 vs. 1.9 ± 0.3 μM, $P = 0.08$). Urinary excretion rate of nitrate did not differ between patients and healthy controls (107 ± 51.6 vs. 103.5 ± 31.7 μmol/mmol creatinine, $P = 0.686$). Nitrite excretion in the urine was significantly elevated in patients with homocystinuria compared to healthy controls (0.4 ± 0.3 vs. 0.1 ± 0.04 μmol/mmol creatinine, $P = 0.043$).

Phenylketonuria

Plasma concentrations of ADMA in the phenylketonuria children were significantly lower (by 12%) than those of the controls (512 ± 136 vs. 585 ± 125 nM, $P = 0.002$) (Fig. 2a). In confirmation of a previous study (Lücke et al. 2007), we found a negative correlation between ADMA concentration in plasma and age ($r = -0.597$, $P < 0.01$). Renal excretion of ADMA was not significantly lower in patients with phenylketonuria (6.8 ± 3.8 vs. 10 ± 15.1 μmol/mmol creatinine, $P = 0.456$) (Fig. 2b). The concentration of L-arginine in plasma was slightly, but significantly, lower in children with phenylketonuria as compared to healthy controls (64 ± 23 vs. 72 ± 14 μM, $P = 0.007$) (Fig. 2c). The L-arginine/ADMA molar ratio in plasma did not differ in both groups (133 ± 32:1 vs. 134 ± 41:1, $P = 0.94$, Fig. 2d).

Plasma and urine nitrate and nitrite values of phenylketonuria patients and controls are summarized in Table 3. The concentration of plasma nitrate (46 ± 30 vs. 41 ± 18 μM, $P = 0.922$) and plasma nitrite (2.5 ± 0.8 vs. 3.2 ± 2.1 μM, $P = 0.174$) did not differ between the

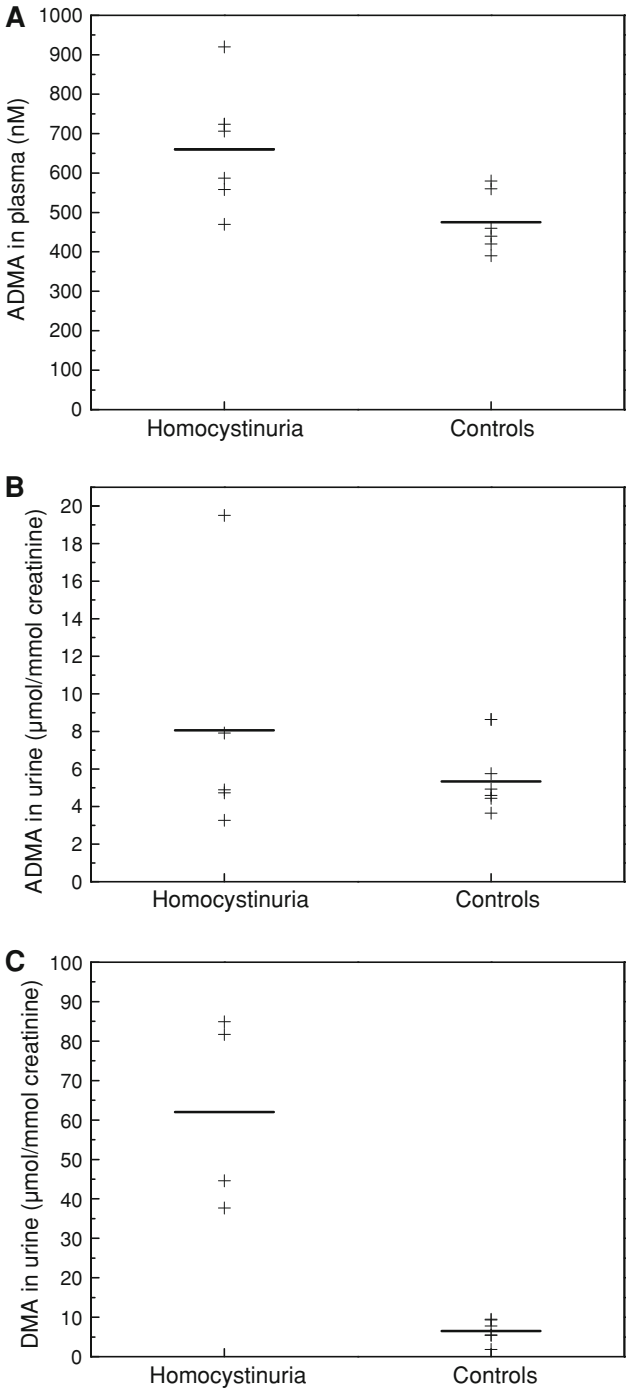

Fig. 1 ADMA in **a** plasma ($P = 0.035$) and **b** urine ($P = 0.416$) and **c** DMA in urine ($P = 0.02$) of patients with homocystinuria and healthy controls

groups. Also, creatinine-corrected excretion of nitrate did not differ between the groups (218 ± 218 vs. 280 ± 439 µmol/mmol creatinine, $P = 0.627$). Nitrite excretion in the urine was significantly higher in phenylketonuria patients than in healthy controls (1.7 ± 1.7 vs. 0.7 ± 1.2 µmol/mmol creatinine, $P = 0.003$).

Discussion

The L-arginine/NO pathway is poorly understood in healthy and ill children. Previously, we found that apparently healthy children had elevated NO synthesis as compared to adults, although the ADMA concentrations in plasma and urine of children (Lücke et al. 2006, 2007, 2008) were considerably higher than those in adults (Tsikas et al. 2006; Tsikas 2008). We also found that ADMA synthesis decreased from infancy to adulthood up to the age of about 16–18 years (Lücke et al. 2007). From a biochemical point of view, ADMA and homocysteine pathways are closely associated to each other, at least in vitro and in vivo in mice (Dayal and Lentz 2005; Dayal et al. 2008), but in vivo in humans, the relationship between homocysteine and ADMA is rather nebulous. The aim of the present study was to define the status of the L-arginine/NO pathway in children with homocystinuria or phenylketonuria, i.e., two diseases with hyperhomocysteinemia as the common feature, albeit not of the same extent. For comparison, we included in our study age-, gender- and number-matched apparently healthy children serving as the control groups. ADMA synthesis, metabolism and elimination via the DDAH pathway were assessed by measuring ADMA and DMA concentrations in plasma and urine (Tsikas et al. 2007). NO synthesis was assessed by measuring nitrite and nitrate concentrations in plasma and urine (Tsikas et al. 2006).

When discussing the results of the present study, it should be kept in mind that all patients examined in the present study were under a common therapeutic regimen. It is known from the literature that folate therapy, an established therapy in homocystinuria, may alter nitrate concentration in plasma (Mansoor et al. 2005). Also, treatment of homocystinuria patients with B vitamins reduced plasma homocysteine, but did not affect plasma ADMA concentration (Spoelstra-de Man et al. 2006). Patients and healthy controls of the present study were not on standardized low nitrite/nitrate diet and were not fasting overnight. Therefore, alimentary contribution to nitrite and nitrate formed from oxidation of endogenously produced NO cannot be excluded (Tsikas 2008). Finally, the small number of the examined homocystinuria children due to the rarity of this disease and of the respective control group renders conclusive interpretation of the findings difficult. In consideration of the above-mentioned limitations, we attempted to roughly delineate the L-arginine/NO pathway status in homocystinuria and phenylketonuria in the childhood (Fig. 3).

NO biosynthesis and bioavailability in homocystinuria and phenylketonuria

NO biosynthesis seems not to be altered in children with homocystinuria and much less in phenylketonuria. Yet,

Table 3 Plasma concentration (μM) and urine excretion rate (μmol/mmol creatinine) of nitrate and nitrite in homocystinuria (HCys) and phenylketonuria (PKU) patients and the respective healthy controls (HC)

Matrix/analyte	HCys	HC for HCys	*P*	PKU	HC for PKU	*P*
Plasma nitrate	77.9 ± 24.2	29.8 ± 4.2	0.043	46 ± 30	41 ± 18	0.06
Urine nitrate	107 ± 51.6	103.5 ± 31.7	0.686	218 ± 218	280 ± 439	0.47
Plasma nitrite	2.7 ± 0.6	1.9 ± 0.3	0.08	2.5 ± 0.8	3.2 ± 2.1	0.05
Urine nitrite	0.4 ± 0.3	0.1 ± 0.1	0.043	1.7 ± 1.7	0.7 ± 1.2	0.003

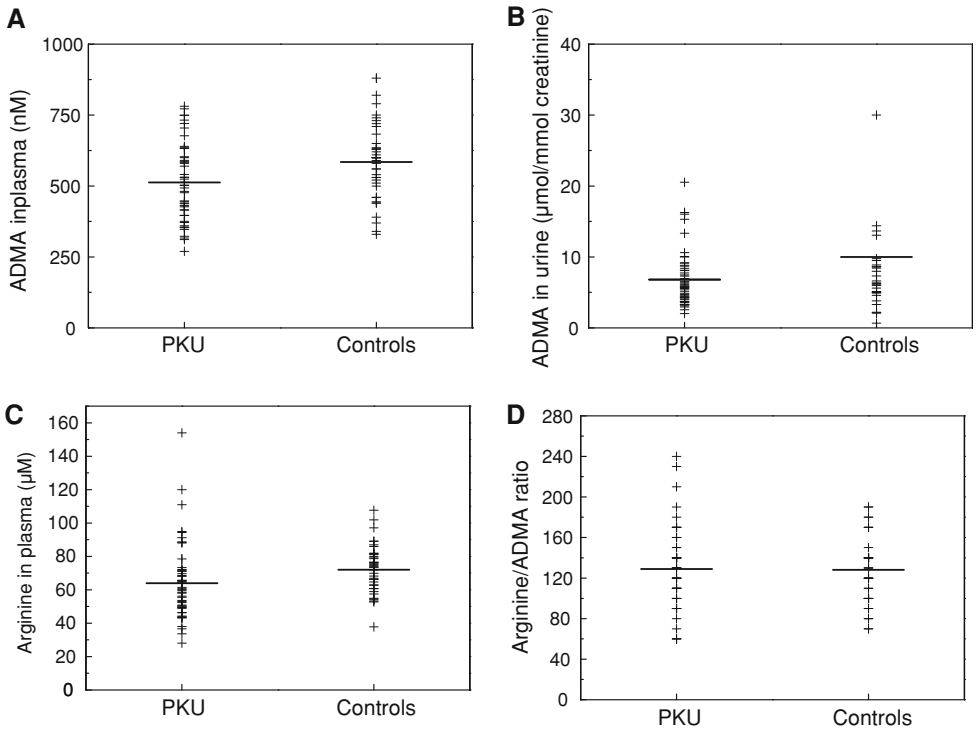

Fig. 2 ADMA in **a** plasma ($P = 0.009$) and **b** urine ($P = 0.013$), **c** L-arginine in plasma ($P = 0.41$), and **d** L-arginine/ADMA molar ratio ($P = 0.94$) in patients with phenylketonuria (*PKU*) and healthy controls

because urinary nitrate, the major NO metabolite, indicates whole body synthesis of NO (Tsikas et al. 2006), and since various NOS isozymes are distinctly inhibited by ADMA, for instance neuronal NOS much stronger ($IC_{50} = 1.5$ μM) than endothelial NOS ($IC_{50} = 12$ μM) (Kielstein et al. 2007), it cannot be excluded that particular NOS isozymes are nevertheless inhibited by ADMA in childhood homocystinuria and phenylketonuria. Furthermore, both nitrate and nitrite are actively reabsorbed in the proximal tubulus of the nephron (Tsikas et al. 2010). It seems that accumulation of nitrate in the blood occurs in homocystinuria rather than in phenylketonuria in childhood (Table 3).

In our study only nitrite excretion in the urine differed statistically significantly in phenylketonuria as compared to healthy controls. Nitrite in urine is often used as an indicator of bacteriuria. Mild bacteriuria may be an explanation for the elevated nitrite excretion rate in the urine of phenylketonuria children. On the other hand, elevated excretion rate of nitrite was found in adult patients with rheumatism and may indicate enhanced myeloperoxidase activity (Pham et al. 2009). Thus, increased nitrite excretion in childhood phenylketonuria may indicate enhanced oxidative stress and myeloperoxidase activity in this disease. Indeed, there is some evidence for the involvement of elevated oxidative stress in phenylketonuria (Sitta et al. 2006), which could diminish NO bioavailability and promote NO-dependent endothelial dysfunction despite unaltered whole body NO synthesis in phenylketonuria. Compromised NO bioavailability in phenylketonuria could result from altered metabolism of tetrahydrobiopterin (BH_4), the common cofactor of NOS and PAH (Ozkor and Quyyumi 2008). Lower BH_4 levels in phenylketonuria may finally lead to endothelial dysfunction (Moens and Kass 2006; Schmidt and Alp 2007).

ADMA synthesis, metabolism and elimination in homocystinuria and phenylketonuria

In consideration of the present knowledge of homocysteine and ADMA biochemistry, we assumed that ADMA biosynthesis would be elevated both in homocystinuria and

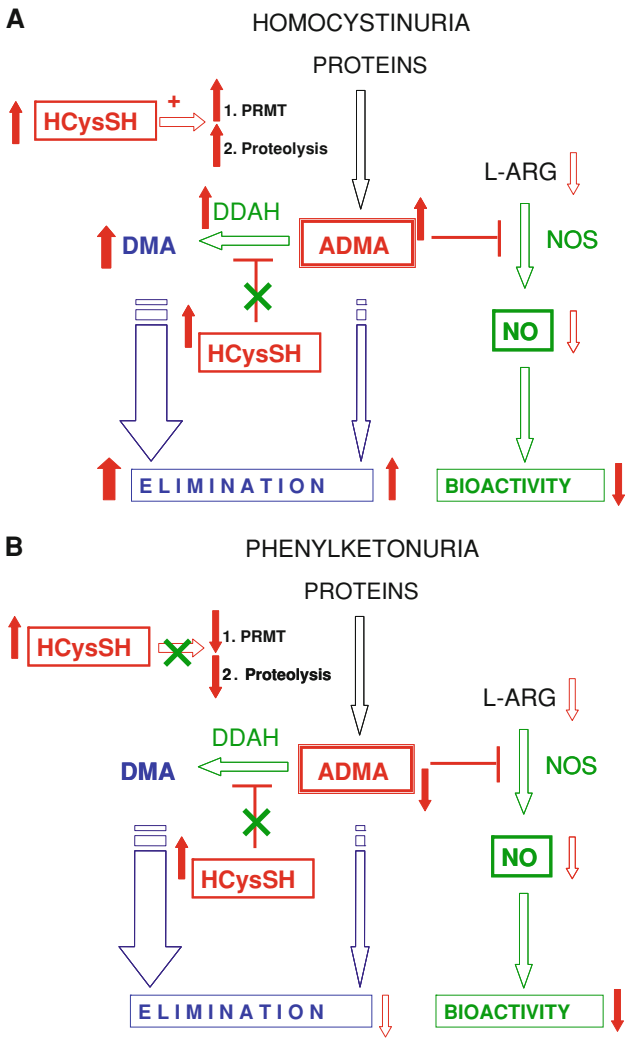

Fig. 3 A schematic proposal of the status of the L-arginine/NO pathway and the role of homocysteine in **a** homocystinuria and **b** phenylketonuria in childhood. In childhood, homocysteine (*HCys*) seems to play distinct roles in phenylketonuria and homocystinuria. At very high concentrations, as they prevail in homocystinuria, HCys could increase the expression and/or activity of a protein arginine methyltransferase (*PRMT*), which methylates asymmetrically the guanidine group of L-arginine residues in proteins to produce ADMA residues in proteins; elevated HCys levels could also induce proteolysis that yields soluble ADMA (Austin et al. 2004). Because of uncompromised DDAH activity, ADMA elimination by renal excretion of DMA is elevated in homocystinuria. ADMA synthesis rate is higher than total ADMA elimination, so that ADMA finally accumulates in the circulation of hyperhomocysteinemic children (**a**). In phenylketonuria (**b**) the effects of HCys on the L-arginine/NO pathway seem to be much less pronounced. *Open small arrows* indicate mild effects

phenylketonuria in childhood. In homocystinuria, we found considerably higher concentrations of ADMA in plasma as compared to healthy controls, comparable excretion rates of ADMA and higher excretion rates of DMA. These findings suggest that ADMA synthesis is indeed elevated in childhood homocystinuria. In contrast, we found that children with phenylketonuria have even significantly lower concentrations of ADMA in plasma and lower ADMA excretion rates in the urine than healthy children of the same age. To our knowledge, this is the first study to report of diminished concentrations of ADMA in plasma in human disease and may have resulted from a lifelong natural protein-restricted diet of phenylketonuria patients. Hydrolysis of ADMA to DMA and L-citrulline by DDAH represents the major elimination pathway of ADMA (Achan et al. 2003). Unfortunately, we have no information about urinary DMA excretion in our phenylketonuria patients. Therefore, elevated DDAH cannot be excluded in phenylketonuria and may have led to the lower concentrations of ADMA in plasma and urine measured in our study. High levels of reduced homocysteine can inhibit DDAH activity in vitro (Dayal and Lentz 2005) and can downregulate DDAH expression in a tissue-specific manner without altering plasma ADMA concentrations in mice with endothelial dysfunction (Dayal et al. 2008). The results of the present study, especially those measured in childhood homocystinuria, indicate increased rather than diminished DDAH activity and argue against an inhibitory effect of reduced homocysteine on DDAH activity in vivo in humans. The particularly high homocysteine levels measured in the homocysteinemic children may have increased the synthesis of ADMA by increasing both protein arginine methyltransferase (PRMT) activity and proteolysis (Austin et al. 2004) (Fig. 3). Supportive of this idea are findings from in vitro studies that demonstrated a coincidental production of homocysteine and ADMA in human peripheral blood mononuclear cells (PBMC) and a release of ADMA from stimulated PBMC (Schroecksnadel et al. 2007). Yet, it is unknown whether PBMC can serve as an additional ADMA source in inherited metabolic disorders. Elucidation of the prevailing mechanism of ADMA elevation in homocystinuria requires further investigations and inclusion of a much larger number of patients.

L-Arginine levels in the phenylketonuria group were significantly lower than those in controls. Since both L-arginine and ADMA concentrations in plasma were lower in the phenylketonuria group, no significant difference in the L-arginine/ADMA molar ratio resulted in comparison with healthy controls.

In conclusion, children with homocystinuria have elevated concentrations of ADMA in plasma, while phenylketonuria children have lower concentrations of ADMA in plasma than healthy controls. The association between ADMA and homocysteine seems to be diametrically opposite in these diseases in the childhood. NO synthesis and metabolism seem to be unaltered in childhood homocystinuria and phenylketonuria. The cardiovascular risk in these diseases could originate from diminished NO bioavailability and/or from other pathways. Elucidation of the underlying mechanisms leading to cardiovascular risks in childhood

homocystinuria and phenylketonuria is an emerging task and may help better understand the biochemistry and pathophysiology in childhood.

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Conflict of interest The authors declare that they have no conflict of interest.

References

Achan V, Broadhead M, Malaki M, Whitley G, Leiper J, MacAlister R, Vallance P (2003) Asymmetric dimethylarginine causes hypertension and cardiac dysfunction in humans and is actively metabolized by dimethylarginine dimethylaminohydrolase. Arterioscler Thromb Vasc Biol 23:1455–1459

Austin RC, Lentz SR, Werstuck GH (2004) Role of hyperhomocystinuria in endothelial dysfunction and atherothrombotic disease. Cell Death Differ 11:56–64

Böger RH, Zoccali C (2003) ADMA: a novel risk factor that explains excess cardiovascular event rate in patients with end-stage renal disease. Atheroscler Suppl 4:23–28

Böger RH, Bode-Böger SM, Thiele W, Junker W, Alexander K, Frölich JC (1997) Biochemical evidence for impaired nitric oxide synthesis in patients with peripheral arterial occlusive disease. Circulation 95:2068–2074

Cines DB, Pollak ES, Buck CA, Loscalzo J, Zimmerman GA, McEver RP, Pober JS, Wick TM, Konkle BA, Schwarzt BS, Barnathan ES, McCrae KR, Hug BA, Schmidt AM, Stern DM (1998) Endothelial cells in physiology and in the pathophysiology of vascular disorders. Blood 91:3527–3561

Cooke JP, Dzau VJ (1997) Nitric oxide synthase: role in the genesis of vascular disease. Annu Rev Med 48:489–509

Dayal S, Lentz SR (2005) ADMA and hyperhomocystinuria. Vasc Med 10(Suppl 1):27–33

Dayal S, Rodionov RN, Arning E, Bottiglieri T, Kimoto M, Murry DJ, Cooke JP, Faraci FM, Lentz SR (2008) Tissue-specific downregulation of dimethylarginine dimethylaminohydrolase in hyperhomocystinuria. Am J Physiol Heart Circ Physiol 295:H816–H825

Hennermann JB, Roloff S, Gellermann J, Vollmer I, Mönch E (2005) Renal function in adolescent and adult patients with phenylketonuria. J Inherit Metab Dis 28(Supp 1):18

Kielstein JT, Tsikas D, Fliser D (2006) Effects of asymmetric dimethylarginine (ADMA) infusion in humans. Eur J Clin Pharmacol 62:39–44

Kielstein A, Tsikas D, Galloway GP, Mendelson JE (2007) Asymmetric dimethylarginine (ADMA): A modulator of nociception in opiate tolerance and addiction? Nitric Oxide 17:55–59

Korandji C, Zeller M, Guilland JC, Vergely C, Sicard P, Duvillard L, Gambert P, Moreau D, Cottin Y, Rochette L (2007) Asymmetric dimethylarginine (ADMA) and hyperhomocystinuria in patients with acute myocardial infarction. Clin Biochem 60:66–72

Lücke T, Tsikas D, Kanzelmeyer N, Vaske B, Das AM (2006) Elevated plasma concentrations of the endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine (ADMA) in citrullinemia. Metabolism 55:1599–1603

Lücke T, Kanzelmeyer N, Kemper MJ, Tsikas D, Das AM (2007) Developmental changes in the L-arginine/nitric oxide pathway from infancy to adulthood: plasma asymmetric dimethylarginine levels decrease with age. Clin Chem Lab Med 45:1525–1530

Lücke T, Kanzelmeyer N, Chobanyan K, Tsikas D, Franke D, Kemper MJ, Ehrich JH, Das A (2008) Elevated asymmetric dimethylarginine (ADMA) and inverse correlation between circulating ADMA and glomerular filtration rate in children with sporadic focal segmental glomerulosclerosis (FSGS). Nephrol Dial Transpl 23:734–740

Mansoor MA, Kristensen O, Hervig T, Stakkestad JA, Berge T, Drablos PA, Rolfsen S, Wentzel-Larsen T (2005) Relationship between serum folate and plasma nitrate concentrations: possible clinical implications. Clin Chem 51:1266–1268

Moens AL, Kass DA (2006) Tetrahydrobiopterin and cardiovascular disease. Arterioscler Thromb Vasc Biol 26:2439–2444

Mudd SH, Skovby F, Levy HL, Pettigrew KD, Wilcken B, Pyeritz RE, Andria G, Boers GHJ, Bromberg IL, Cerone R, Fowler B, Gröbe H, Schmidt H, Schweitzer L (1985) The natural history of homocystinuria due to cystathionine beta-synthase deficiency. Am J Hum Genet 37:1–31

Ozkor MA, Quyyumi AA (2008) Tetrahydrobiopterin. Curr Hypertens Rep 10:58–64

Pham VV, Stichtenoth DO, Tsikas D (2009) Nitrite correlates with 3-nitrotyrosine but not with the F_2-isoprostane 15(*S*)-8-*iso*-$PGF_{2\alpha}$ in urine of rheumatic patients. Nitric Oxide 21:210–215

Schmidt TS, Alp NJ (2007) Mechanisms for the role of tetrahydrobiopterin in endothelial function and vascular disease. Clin Sci 113:47–63

Schroecksnadel K, Weiss G, Stanger O, Teerlink T, Fuchs D (2007) Increased asymmetric dimethylarginine concentrations in stimulated peripheral blood mononuclear cells. Scand J Immunol 65:525–529

Schulpis KH, Karikas GA, Papakonstantinou E (2002) Homocysteine and other vascular risk factors in patients with phenylketonuria on a diet. Acta Paediatr 91:905–909

Sitta A, Barschak AG, Deon M, Terroso T, Pires R, Giugliani R, Dutra-Filho CS, Wajner M, Vargas CR (2006) Investigation of oxidative stress parameters in treated phenylketonuric patients. Metab Brain Dis 21:287–296

Spoelstra-de Man AM, Teerlink T, Brouwer CB, Rauwerda JA, Stehouwer CD, Smuöders YM (2006) No effect of B vitamins an ADMA levels in patients at increased cardiovascular risk. Clin Endocrinol 65:495–501

Tsikas D (2000) Simultaneous derivatization and quantification of the nitric oxide metabolites nitrite and nitrate in biological fluids by gas chromatography–mass spectrometry. Anal Chem 72:4064–4072

Tsikas D (2003) Incorrect and inconsistent use of homocysteine's nomenclature: a potential source of misunderstandings. Eur J Clin Invest 33:1095–1096

Tsikas D (2008) A critical review and discussion of analytical methods in the L-arginine/nitric oxide area of basic and clinical research. Anal Biochem 379:139–163

Tsikas D, Böger RH, Sandmann J, Bode-Böger SM, Frölich JC (2000) Endogenous nitric oxide synthase inhibitors are responsible for the L-arginine paradox. FEBS Lett 478:1–3

Tsikas D, Schubert B, Gutzki FM, Sandmann J, Frölich JC (2003) Quantitative determination of circulating and urinary asymmetric dimethylarginine (ADMA) in humans by gas chromatography–tandem mass spectrometry as methyl ester tri(*N*-pentafluoropropionyl) derivative. J Chromatogr B 798:87–99

Tsikas D, Wolf A, Frölich JC (2004) Simplified HPLC method for urinary and circulating creatinine. Clin Chem 50:201–203

Tsikas D, Gutzki FM, Stichtenoth DO (2006) Circulating and excretory nitrite and nitrate as indicators of nitric oxide synthesis in humans: methods of analysis. Eur J Clin Pharmacol 62:51–59

Tsikas D, Thum T, Becker T, Pham VV, Chobanyan K, Mitschke A, Beckmann B, Gutzki FM, Bauersachs J, Stichtenoth DO (2007) Accurate quantification of dimethylamine (DMA) in human

urine by gas chromatography–mass spectrometry as pentafluorobenzamide derivative: evaluation of the relationship between DMA and its precursor asymmetric dimethylarginine. J Chromatogr B 851:229–239

Tsikas D, Schwarz A, Stichtenoth DO (2010) Simultaneous measurement of [^{15}N]nitrate and [^{15}N]nitrite enrichment and concentration in urine by gas chromatography mass spectrometry as pentafluorobenzyl derivatives. Anal Chem 82:2585–2587

Verduyn I, Bosdet T, Selvage C, Sirrs S, O'Riley M (2005) Reduced HDL levels in adult patients with phenylketonuria (PKU). J Inherit Metab Dis 28(Suppl 1):56–64

Welch GN, Loscalzo J (1998) Homocysteine and atherothrombosis. N Engl J Med 338:1042–1050

Williams RA, Mamotte CDS, Burnett JR (2008) Phenylketonuria: an inborn error of phenylalanine metabolism. Clin Biochem Rev 29:31–41

Wolf C, Lorenzen JM, Stein S, Tsikas D, Störk S, Weidemann F, Ertl G, Anker SD, Bauersachs J, Thum T (2010) Urinary asymmetric dimethylarginine (ADMA) is a predictor of mortality risk in patients with coronary artery disease. Intern J Cardiol. doi: 10.1016/j.ijcard.2010.11.003

Wu G (2009) Amino acids: metabolism, functions, and nutrition. Amino Acids 37:1–17

Wu G, Bazer FW, Davis TA, Kim SW, Li P, Rhoads JM, Satterfield MC, Smith SB, Spencer TE, Yin Y (2009) Arginine metabolism and nutrition in growth, health and disease. Amino Acids 37:153–168