Amino Acids (2012) 43:805–811
DOI 10.1007/s00726-011-1136-3

ORIGINAL ARTICLE

Increased asymmetric dimethylarginine (ADMA) dimethylaminohydrolase (DDAH) activity in childhood hypercholesterolemia type II

Kristine Chobanyan-Jürgens · Anne-Jule Fuchs · Dimitrios Tsikas · Nele Kanzelmeyer · Anibh M. Das · Sabine Illsinger · Bernhard Vaske · Jens Jordan · Thomas Lücke

Received: 17 June 2011 / Accepted: 24 October 2011 / Published online: 11 November 2011

Abstract Asymmetric dimethylarginine (ADMA) systemic concentrations are elevated in hypercholesterolemic adults and contribute to nitric oxide (NO) dependent endothelial dysfunction. Decreased activity of the key ADMA-hydrolyzing enzyme dimethylarginine dimethylaminohydrolase (DDAH) may be involved. Yet, the ADMA/DDAH/NO pathway has not been investigated in childhood hypercholesterolemia. We studied 64 children with hypercholesterolemia type II (HCh-II) and 54 normocholesterolemic (NCh) children (mean ± SD; age, years: 11.1 ± 3.5 vs. 11.9 ± 4.6). Plasma and urine ADMA was measured by GC–MS/MS. Dimethylamine (DMA), the ADMA metabolite, creatinine, nitrite and nitrate in urine were measured by GC–MS. The DMA/ADMA molar ratio in urine was calculated to estimate whole body DDAH activity. ADMA plasma concentration (mean ± SD; nM: 571 ± 85 vs. 542 ± 110, $P = 0.17$) and ADMA urinary excretion rate (mean ± SD: 7.1 ± 2 versus 7.2 ± 3 μmol/mmol creatinine, $P = 0.6$) were similar in HCh-II and NCh children. Both DMA excretion rate [median (25th–75th percentile): 56.3 (46.4–109.1) vs. 45.2 (22.2–65.5) μmol/mmol creatinine, $P = 0.0004$] and DMA/ADMA molar ratio [median (25th–75th percentile): 9.2 (6.0–16.3) vs. 5.4 (3.8–9.4), $P = 0.0004$] were slightly but statistically significantly increased in HCh-II children compared to NCh children. Plasma and urinary nitrite and nitrate were similar in both groups. In HCh-II whole body DDAH activity is elevated as compared to NCh. HCh-II children treated with drugs for hypercholesterolemia had lower plasma ADMA levels than untreated HCh-II or NCh children, presumably via increased DDAH activity. Differences between treated and untreated HCh-II children were not due to differences in age. In conclusion, HCh-II children do not have elevated ADMA plasma levels, largely due to an apparent increase in DDAH activity. While this would tend to limit development of endothelial dysfunction, it is not clear whether this might be medication-induced or represent a primary change in HCh-II children.

Keywords ADMA · Children · DDAH · DMA · Hypercholesterolemia · Nitric oxide

K. Chobanyan-Jürgens (✉) · N. Kanzelmeyer · A. M. Das · S. Illsinger
Department of Paediatrics, Hannover Medical School, Carl-Neuberg Str. 1, 30625 Hannover, Germany
e-mail: chobanyan.kristine@mh-hannover.de

A.-J. Fuchs
Department of Paediatrics, University of Mayence, Mayence, Germany

D. Tsikas · J. Jordan
Institute of Clinical Pharmacology, Hannover Medical School, Hannover, Germany

B. Vaske
Institute of Biometry, Hannover Medical School, Hannover, Germany

T. Lücke
Department of Neuropaediatrics, Children's Hospital, University of Bochum, Bochum, Germany

Introduction

Asymmetric dimethylarginine (ADMA), the asymmetrically dimethylated analogue of the semi-essential amino acid L-arginine, is an endogenous nitric oxide synthase (NOS) inhibitor and an established cardiovascular risk factor in adults (Leiper and Vallance 2006; Wu 2009; Wu

et al. 2009). Elevated plasma ADMA concentrations have been observed in various conditions associated with NO-dependent endothelial dysfunction (Horowitz and Hereszтyn 2007). Soluble ADMA is released from proteolysis of post-translational asymmetric dimethylation of the guanidine group of L-arginine residues in proteins catalyzed by protein-arginine-methyltransferase (PRMT).

ADMA is excreted unchanged in the urine, but the major metabolic pathway of ADMA (about 90%) involves its hydrolysis by dimethylarginine dimethylaminohydrolase (DDAH) into L-citrulline and dimethylamine (DMA) that is excreted in the urine (Leiper and Vallance 2006). Oxidative stress, inflammatory factors, and elevated LDL-cholesterol, particularly oxidized LDL particles, are thought to reduce DDAH activity, thereby elevating the circulating ADMA concentration (Ito et al. 1999; Palm et al. 2007; Tan et al. 2007). These mechanisms and/or elevated ADMA synthesis appear to contribute to excessive plasma ADMA levels in hypercholesterolemic adults (Böger et al. 1998). Unlike in adults, ADMA metabolism in children has been rarely investigated. Increased ADMA levels have been reported in children with hypertension (Goonasekera et al. 1997), citrullinemia (Lücke et al. 2006), focal-segmental glomerulosclerosis (Lücke et al. 2008), familial hypercholesterolemia (Jehlicka et al. 2009), and chronic kidney disease (Brooks et al. 2009).

ADMA plasma concentrations in young healthy children are much higher than in adults and decrease with advancing age to reach the levels prevailing in adulthood (Lücke et al. 2007). The observation suggests that the L-arginine/NO pathway is differentially regulated in children and in adults. Thus, data on this pathway reported for adults cannot be simply extended to children. In the present study, we tested the hypothesis that in children with hypercholesterolemia type II (HCh-II), ADMA synthesis, metabolism and elimination are altered as compared to children with normal cholesterol levels.

Materials and methods

Subjects

We investigated 64 children with HCh-II (36 males, 28 females) and 54 children (31 males, 23 females) with normal cholesterol levels (Table 1). The study was designed as a cross-sectional pilot-study aimed to investigate the ADMA/DDAH/NO pathway in children with HCh-II referred to our outpatient clinic. The study was approved by the Ethics Committee of the Hannover Medical School and written informed consent was obtained from parents and children. The study was performed in accordance with the guidelines of the Declaration of Helsinki and of Good Clinical Practice (GCP).

Hypercholesterolemia type II was diagnosed according to the criteria of the working group for paediatric metabolic disturbances (APS). We included in the HCh-II group otherwise healthy children with low-density lipoprotein above 130 mg/dL. Exclusion criteria were ongoing infections, chronic kidney, heart, or metabolic disease, malignancies, hypertension, or dystrophy. Children on medication (other than listed below) or food supplements containing vitamin E or L-arginine were also excluded. Sixteen patients received a lipid-lowering therapy with pravastatin, ezetimibe or cholestyramine (either as monotherapy or as combination) with the hypocholesterolemic diet recommendations. Twenty-five patients received only hypocholesterolemic diet. Twenty-three patients with newly diagnosed HCh-II did not receive lipid-lowering medication or hypocholesterolemic diet at the time of our

Table 1 Clinical and biochemical characteristics of hypercholesterolemic and normocholesterolemic children in the study

	Hypercholesterolemia	Normocholesterolemia	*P*
Number of children	64	54	Not applicable
Age (years)	11.1 ± 3.5	11.9 ± 4.6	0.52
Cholesterol, total (mg/dL)	245 ± 40	172 ± 21	0.0001
Arginine in plasma (μM)	71.6 ± 13	72.4 ± 16	0.87
ADMA in plasma (nM)	571 ± 85	542 ± 110	0.17
ADMA in urine (μmol/mmol creatinine)	7.1 ± 2	7.2 ± 3	0.6
DMA in urine (μmol/mmol creatinine)	88.2 ± 72.2; 56.3 (46.4–109.1)	56.3 ± 60.6; 45.2 (22.2–65.5)	0.0004
DMA/ADMA ratio	11.6 ± 8.1; 9.2 (6.0–16.3)	10.8 ± 18.7; 5.4 (3.8–9.4)	0.0004
Nitrate in plasma (μM)	33 ± 12; 29.3 (27–33.6)	34 ± 13; 29.4 (25–38)	0.73
Nitrite in plasma (μM)	2.69 ± 1.43; 2.75 (1.67–3.17)	2.98 ± 1.43; 2.75 (2.42–2.98)	0.68
Nitrate in urine (μmol/mmol creatinine)	140 ± 83; 125 (99–164)	160 ± 145; 118 (78–154)	0.32
Nitrite in urine (μmol/mmol creatinine)	0.36 ± 0.43; 0.19 (0.10–0.48)	0.37 ± 0.37; 0.23 (0.15–0.45)	0.22

Data are presented as mean ± SD and/or median (range 25th–75th percentile)

Table 2 Clinical and biochemical characteristics in three subgroups of the hypercholesterolemic children

	Diet	Medication	Newly diagnosed[a]	P[b]
Number of patients	25	16	23	Not applicable
Age (years)	10.4 ± 3.1	13.2 ± 3.4	10.1 ± 3.7	0.009
BMI (kg/m^2)	19 ± 3; 19 (17–20)	21 ± 4; 21 (19–23)	16 ± 2; 16 (15–18)	0.0003
Cholesterol, total (mg/dL)	234 ± 37	255 ± 39	252 ± 43	0.18
Triglycerides (mg/dL)	81 ± 26; 83 (63–95)	71 ± 44; 62 (47–69)	92 ± 44; 93 (56–112)	0.06
LDL (mg/dL)	170 ± 36; 152 (143–208)	196 ± 42; 184 (163–234)	185 ± 42; 185 (147–224)	0.08
Hemoglobin (g/dL)	13 ± 1	14 ± 1	13 ± 1	0.07
Arginine in plasma (μM)	75 ± 15	82 ± 22	97	0.07
ADMA in plasma (nM)	604 ± 77; 598 (567–640)	514 ± 108; 521 (422–552)	570 ± 62; 577 (531–609)	0.001
ADMA in urine (μmol/mmol creatinine)	7.6 ± 1.9; 7.4 (6.7–8.3)	5.6 ± 1.6; 4.9 (4.6–6.8)	7.6 ± 1.8; 7 (6.4–9.0)	0.001
DMA in urine (μmol/mmol creatinine)	93 ± 69; 58 (49–146)	78 ± 55; 55 (46–107)	90 ± 88; 54 (43–83)	0.81
DMA/ADMA molar ratio	12 ± 8; 8 (6–19)	14 ± 7; 12 (9–19)	13 ± 14; 7 (5–10)	0.16
Nitrite in plasma (μM)	2.91 ± 1.80; 2.82 (1.86–3.24)	2.23 ± 0.97; 2.75 (1.16–3.07)	2.95 ± 1.87; 2.82 (2.03–3.06)	0.55
Nitrate in plasma (μM)	30 ± 8.5; 29 (26–30)	39 ± 20; 30 (27–46)	33 ± 8; 33 (28–35)	0.04
Nitrite in urine (μmol/mmol creatinine)	0.50 ± 0.56; 0.35 (0.14–0.65)	0.12 ± 0.05; 0.11 (0.08–0.17)	0.37 ± 0.35; 0.20 (0.11–0.70)	0.003
Nitrate in urine (μmol/mmol creatinine)	134 ± 100; 112 (92–165)	143 ± 86; 123 (94-152)	143 ± 61; 128 (100–170)	0.49

Data are presented as mean ± SD and/or median (range 25th–75th percentile)

[a] Newly diagnosed: patients with newly diagnosed HCh-II without diet or medication

[b] P value for Kruskal–Wallis ANOVA

investigation (Table 2). HCh-II and normocholesterolemic (NCh) children did not follow a standardized nitrite/nitrate diet in the present study and did not take organic nitrates before sampling. At least 24 h before blood and urine sampling they have not consumed seafood in order to minimize dietary DMA intake (Tsikas et al. 2007).

Analytical methods

Venous blood and urine sampling was performed after overnight fasting. EDTA blood samples were immediately put on ice, centrifuged (4,500×g, 4°C, 10 min), and plasma samples were frozen at −80°C until analysis. Urine samples from spontaneous micturition were 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 measured by GC–MS/MS (Tsikas et al. 2003), and urinary DMA (Tsikas et al. 2007) and creatinine (Tsikas et al. 2010) were measured by GC–MS. Arginine in plasma was determined by GC–MS as described elsewhere (Tsikas et al. 2003). Nitrite and nitrate in plasma and urine were determined simultaneously by GC–MS as described previously (Tsikas 2000).

Statistical analysis

The D'Agostino and Pearson omnibus K2 and Kolmogorov–Smirnov normality tests were used to evaluate data distribution. The non-parametric Mann–Whitney test was used for statistical analysis of variables which were not normally distributed. These data are presented as mean ± SD and median (25th–75th percentile). Spearman's correlation coefficient for non-normally distributed variables was assessed. Normally distributed variables were analyzed by unpaired t test and data are reported as mean ± SD and the Pearson's correlation coefficient was determined. Due to small age span age-matching was not necessary in the present work. Age-dependence of variables was analyzed by ANCOVA. One-way ANOVA (for the non-parametric variables Kruskal–Wallis test) was used for intergroup analysis of patients on diet or on medication or of newly diagnosed patients. Two-tailed P values <0.05 were considered as statistically significant. Pairwise comparisons (subgroup analysis of HCh-II, diet vs. medication vs. newly diagnosed) were made with Bonferroni correction. For each pairwise comparison, $P \leq 0.017$ was considered as statistically significant ($P < 0.05$ divided by 3,

i.e., the number of comparisons). All calculations were performed using GraphPad Prism software (GraphPad Prism Software Inc. San Diego, CA, USA) and the SPSS package (SPSS Inc., Chicago, IL, USA).

Results

The clinical and biochemical characteristics of the HCh-II and NCh children investigated in the study are given in Table 1. Figure 1 shows the relationship between ADMA in plasma and ADMA in urine for both groups. Table 2 summarizes the data of the three subgroups of the HCh-II children, i.e., children on hypocholesterolemic diet, on medication, and newly diagnosed HCh-II children.

Age was similar in the HCh-II and NCh groups. Total cholesterol differed significantly between HCh-II and NCh children ($P = 0.0001$). Plasma ADMA concentration and ADMA excretion rate were not significantly different in HCh-II versus NCh children. DMA excretion rate and DMA/ADMA molar ratio in urine were significantly higher in the HCh-II than in the NCh children. There was no statistically significant difference between plasma arginine, plasma nitrite, plasma nitrate and urinary nitrite and urinary nitrate levels between HCh-II and NCh children, respectively (Table 1).

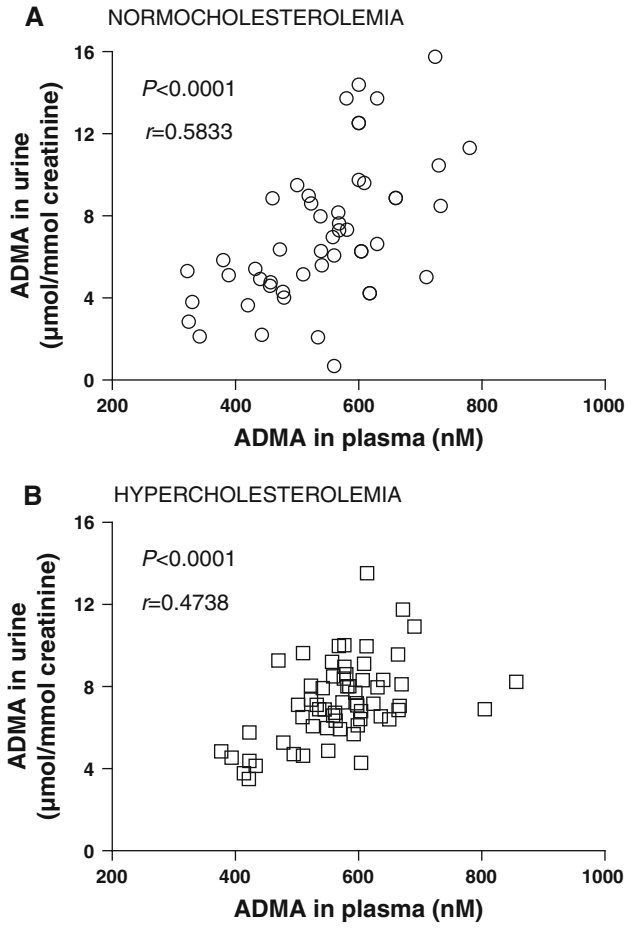

Fig. 1 Spearman's correlation between plasma ADMA and urinary ADMA in normocholesterolemic **a** and hypercholesterolemic **b** children

Both in HCh-II children ($r = 0.47$, $P = 0.0001$; Fig. 1a) and in NCh children ($r = 0.58$, $P < 0.0001$; Fig. 1b) there was a positive correlation between ADMA in plasma and ADMA in urine. In HCh-II children, correlations were found between ADMA in plasma and nitrite in urine ($r = 0.28$, $P = 0.029$), and between ADMA in plasma and blood hemoglobin ($r = -0.29$, $P = 0.024$). In NCh children, weak correlations were found between ADMA in plasma and nitrate in plasma ($r = 0.33$, $P = 0.03$), between ADMA in plasma and nitrite in urine ($r = 0.44$, $P = 0.002$), and between ADMA in plasma and DMA/ADMA ratio in urine ($r = -0.35$, $P = 0.013$).

In HCh-II children, no correlation was found between ADMA in plasma and DMA in urine, total cholesterol, or LDL-cholesterol, and between nitrate and nitrite in plasma. In NCh children, no correlation was found between ADMA in plasma and DMA in urine, between ADMA in plasma and total cholesterol in plasma, between ADMA in plasma and nitrite in plasma, and between ADMA in plasma and blood hemoglobin.

In HCh-II children on lipid-lowering medication, we observed significantly lower plasma ADMA concentrations and lower ADMA excretion rates in urine compared to HCh-II children on hypocholesterolemic diet and to newly diagnosed HCh-II children (Table 2). In order to analyse the possible influence of age on biochemical parameters, we performed an ANCOVA test between the parameters and the age in the HCh-II subgroups.

We did not find any dependence of ADMA in plasma ($P = 0.98$), ADMA in urine ($P = 0.066$), DMA in urine ($P = 0.79$), DMA/ADMA ratio in urine ($P = 0.469$), nitrate and nitrite in plasma and urine (data not shown) on the age. In contrast, there was a dependence of total cholesterol ($P = 0.009$), triglycerides ($P = 0.024$) and arginine ($P = 0.001$) on the children's age. Regarding ADMA plasma concentration, paired multiple comparison with Bonferroni correction revealed differences between diet and medication ($P = 0.0005$) and between medication and newly diagnosed ($P = 0.0092$) groups. On the other hand, there was no difference between the HCh-II children on hypocholesterolemic diet and the newly diagnosed HCh-II children ($P = 0.13$). We found similar results with respect to ADMA excretion rate in the subgroups, i.e., diet versus medication ($P = 0.003$), medication versus newly diagnosed ($P = 0.01$), and diet versus newly diagnosed ($P = 0.57$).

Lower urine nitrite excretion rates were found between diet and medication ($P = 0.0009$), between medication and

newly diagnosed ($P = 0.01$), but not between diet and newly diagnosed ($P = 0.5$). There was a significant difference in plasma nitrate concentrations between diet and newly diagnosed ($P = 0.013$). No differences were found between diet and medication ($P = 0.11$) or between medication and newly diagnosed ($P = 0.96$). No differences were found for plasma arginine concentration, DMA excretion rate and the DMA/ADMA ratio between the subgroups.

Discussion

In previous studies, we showed that the urinary DMA/ADMA molar ratio is a useful estimate of whole body DDAH activity in humans in adulthood in health and disease (Tsikas et al. 2007; Becker et al. 2009; Wolf et al. 2011). Adult patients suffering from end-stage liver disease have a lower DMA/ADMA molar ratio (9 ± 1:1) than healthy adults (11 ± 2:1) (Tsikas et al. 2007). In contrast, adult patients with coronary artery disease have a higher DMA/ADMA molar ratio (17 ± 3:1) compared to healthy adults (Tsikas et al. 2007; Wolf et al. 2011). Considering the ADMA plasma and ADMA urine concentrations in the above mentioned studies, the measured DMA/ADMA molar ratio suggests that ADMA synthesis is elevated both in end-stage liver disease and in coronary artery disease. Because liver and kidney are the major eliminating organs for ADMA, the lower DMA/ADMA molar ratio measured in end-stage liver disease suggests that ADMA elimination is diminished in this disease, presumably due to insufficiently active and/or expressed hepatic DDAH. In previous studies, we also showed that healthy children are not just healthy small adults and that the L-arginine/NO pathway is altered in many diseases (Surdacki et al. 2003; Lücke et al. 2006, 2007, 2008; Kanzelmeyer et al. 2011). In the present study, we investigated the L-arginine/NO pathway in hypercholestolemic children. In adults, circulating and excretory ADMA is an established cardiovascular risk factor (Leiper and Vallance 2006; Wu 2009; Wu et al. 2009; Wolf et al. 2011). We were interested to know whether ADMA may also play a pivotal role in children hypercholesterolemia like in adults (Böger et al. 1998). We, therefore, investigated the L-arginine/NO pathway in 64 HCh-II and 54 NCh children of the same age by measuring the concentration of relevant members of the L-arginine/NO family in plasma and urine samples. The urinary DMA/ADMA molar ratio was used as a measure of the whole body DDAH activity.

The three main findings of our study are: first, HCh-II and NCh children have very similar ADMA concentrations in plasma and urine. Second, the DMA concentration and the DMA/ADMA molar ratio in urine are higher in HCh-II children compared to NCh children. These findings suggest that both ADMA synthesis and whole body DDAH activity are elevated in childhood hypercholesterolemia type II. Finally, the NO synthesis rate is not altered in HCh-II when compared to NCh.

Our HCh-II cohort was heterogeneous due to the involvement of newly diagnosed non-medicated HCh-II children ($n = 23$; referred to as *newly diagnosed group*), of children being treated by drugs ($n = 16$; referred to as *medication group*), and of children having received hypocholesterolemic diet ($n = 25$; referred to as *diet group*). Hence, the impact of the individual subgroups may have contributed differently to the biochemical parameters measured in the entire cohort of the HCH children. For this reason, these subgroups in the HCh-II cohort were analyzed separately (Table 2).

Obvious differences existed regarding age and BMI. Differences between the three subgroups with regard to total cholesterol, triglycerides, LDL and hemoglobin did not reach statistical significance. Interestingly, the ADMA plasma concentration in the medication group was lower than in the other two groups, i.e., by 90 nM compared to the diet group and by 56 nM compared to the newly diagnosed group. In the medication group ($n = 16$), the higher age could have reduced the ADMA plasma concentration by about 45 nM in total. Theoretically, medication with lipid-lowering drugs may also have lowered the ADMA plasma concentration in the HCh-II children of the medication group. For instance, rosuvastatin (Lu et al. 2004) and fluvastatin (Oguz and Uzunlulu 2008) were reported to decrease ADMA plasma concentration in adults. Both factors, i.e., higher age and medication with lipid-lowering drugs in the medication subgroup, are likely to have lowered the ADMA plasma concentration by 90 nM compared to the diet group and by 56 nM compared to the newly diagnosed group. However, because the medication subgroup size accounts for about 25% of the entire HCh-II group, the medication group cannot lower the mean ADMA plasma concentration of the HCH group by more than about 23 nM. Interestingly, the lowest ADMA and DMA excretion rates in the urine were measured in the medication group suggesting that lipid-lowering medication may have decreased ADMA synthesis in the HCh-II children of this subgroup. The borderline higher urinary DMA/ADMA ratio in the medication group may suggest that pharmacological intervention may have increased the DDAH activity in this subgroup.

The idea that lipid-lowering drugs may have influenced ADMA metabolism is supported by in vitro and animal experiments. Incubation of human coronary artery endothelial cells with LDL and oxidized LDL increased ADMA synthesis (Böger et al. 2000). ADMA accumulation in conditioned medium has been reported for ECV 304 cells

incubated with oxidized LDL or TNF-alpha (Ito et al. 1999). Finally, DDAH activity in liver, kidney and aorta is decreased in New Zealand white rabbits fed a high-cholesterol diet compared to control animals on normal chow. However, the DDAH expression has been reported to remain unchanged in that study (Ito et al. 1999).

Children with familial HCh have elevated ADMA plasma concentrations (Jehlicka et al. 2009). In that study, ADMA plasma concentration in the healthy controls was approximately by 200 nM higher compared to NCh children in our study, i.e., 770 ± 140 nM (Jehlicka et al. 2009) versus 571 ± 85 nM (present study). This discrepancy is likely to be related to differences in analytical methodologies, i.e., ELISA (used by Jehlicka et al. 2009) and GC–MS/MS (used in the present study); for a discussion see two recent review articles (Horowitz and Heresztyn 2007; Tsikas 2008a). In most studies using the commercially available ELISA method, ADMA basal values were in average by about 200 nM higher than in studies that used chromatographic or mass spectrometric methods, and the ELISA method seems not to work equally in different investigator groups (Tsikas 2008b).

With regard to the NO metabolites nitrite and nitrate, the greatest differences among the groups were seen for urinary nitrite in the HCh-II subgroups. In average, creatinine-corrected excretion of nitrite was 5 and 4 times higher than in the diet and newly diagnosed groups when compared with the nitrite excretion in the medication group. We have recently shown that nitrite is reabsorbed in the urine in the proximal tubule (Tsikas et al. 2010). Lipid-lowering drugs may have improved the renal nitrite absorption. As nitrite still bears NO bioactivity (Gladwin et al. 2005), enhanced nitrite reabsorption in the kidney may be of particular interest and demands further elucidation.

HCh children in the medication group received pravastatin and/or ezetimibe and/or cholestyramine. Experimental and clinical studies in adults did not show any effects of pravastatin on ADMA plasma concentration and/or DDAH expression and activity (Eid et al. 2003; Nanayakkara et al. 2009). In adult non-diabetic chronic kidney disease patients, ezetimibe lowered ADMA serum concentration in a cholesterol-independent manner (Nakamura et al. 2009). As the HCh children in our study were not randomised, we can only speculate that ezetimibe may have lowered ADMA plasma concentration in the medication subgroup.

Conclusions

In adults, more than 90% of the daily produced ADMA is eliminated through hydrolysis to DMA and L-citrulline mainly by hepatic and renal DDAH. Whole body DDAH activity cannot be estimated solely by measuring ADMA concentration in plasma or serum. Additional measurement of ADMA and DMA in urine and estimation of the urinary DMA/ADMA molar ratio are required. As the L-arginine/NO pathway in children is not well comparable to that in adults, findings from clinical studies on adults cannot be simply extrapolated to children. Our study suggests that both ADMA synthesis and DDAH activity are elevated in childhood HCh-II.

Whether treatment of children with lipid-lowering drugs such as pravastatin and ezetimibe also lowers circulating ADMA concentrations requires further investigation. Given the constantly increasing number of children with cardiovascular risk factors such as dyslipidemia, adiposity and insulin resistance, the importance of ADMA in childhood and the need for its control by pharmacological means remains to be investigated in detail in larger cohorts.

Acknowledgments The authors thank B. Beckmann and A. Mitschke for excellent laboratory assistance and F.-M. Gutzki for performing GC–MS and GC–MS/MS analyses. We are also thankful to Deutsches Stiftungszentrum (die Stiftung Dr. Edith Grünheit; S150/10028/2010) for the travel support given to KCJ for the presentation of the study results at the 5th International Symposium on ADMA, Chicago/Ill, 2010.

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

References

Becker T, Mevius I, de Vries DK, Schaaherder AFM, Meyer zu Vilsendorf A, Klempnauer J, Frölich JC, Tsikas D (2009) The L-arginine/NO pathway in end-stage liver disease and during orthotopic liver and kidney transplantation: biological and analytical ramifications. Nitric Oxide 20:61–67

Böger RH, Bode-Böger SM, Szuba A, Tsao PS, Chan JR, Tangphao O, Blaschke TF, Cooke JP (1998) Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia. Circulation 98:1842–1847

Böger RH, Sydow K, Borlak J, Thum T, Lenzen H, Schubert B, Tsikas D, Bode-Böger SM (2000) LDL cholesterol upregulates synthesis of asymmetrical dimethylarginine in human endothelial cells: involvement of *S*-adenosylmethionine-dependent methyltransferases. Circ Res 87:99–105

Brooks ER, Langman CB, Wang S, Price HE, Hodges AL, Darling L, Yang AZ, Smith FA (2009) Methylated arginine derivatives in children and adolescents with chronic kidney disease. Pediatr Nephrol 24:129–134

Eid HM, Eritsland J, Larsen J, Arnesen H, Seljeflot I (2003) Increased levels of asymmetric dimethylarginine in populations at risk for atherosclerotic disease. Effects of pravastatin. Atherosclerosis 166:279–284

Gladwin MT, Schechter AN, Kim-Shapiro DB, Patel RP, Hogg N, Shiva S, Cannon RO 3rd, Kelm M, Wink DA, Espey MG, Oldfield EH, Pluta RM, Freeman BA, Lancaster JR Jr, Feelisch M, Lundberg JO (2005) The emerging biology of the nitrite anion. Nat Chem Biol 1:308–314

Goonasekera CDA, Rees DD, Woolard P, Frend A, Shah V, Dillon MJ (1997) Nitric oxide synthase inhibitors and hypertension in children and adolescents. J Hypertens 15:901–909

Horowitz JD, Heresztyn T (2007) An overview of plasma concentrations of asymmetric dimethylarginine (ADMA) in health and disease and in clinical studies. J Chromatogr B 851:42–50

Ito A, Tsao PS, Adimoolam S, Kimoto M, Ogawa T, Cooke JP (1999) Novel mechanism for endothelial dysfunction: dysregulation of dimethylarginine dimethylaminohydrolase. Circulation 99:3092–3095

Jehlicka P, Stozicky F, Mayer OJR, Varvarovska J, Racek J, Trefil L, Siala K (2009) Asymmetric dimethylarginine an the effect of folat substitution in children with familial hypercholesterolemia and diabetes mellitus type 1. Physiol Res 58:179–184

Kanzelmeyer N, Tsikas D, Chobanyan-Jürgens K, Beckmann B, Vaske B, Illsinger S, Das AM, Lücke T (2011) Asymmetric dimethylarginine in children with homocystinuria or phenylketonuria. Amino Acids. doi:10.1007/s00726-011-0892

Leiper JM, Vallance P (2006) The synthesis and metabolism of asymmetric dimethylarginine (ADMA). Eur J Clin Pharmacol 62:33–38

Lu TM, Ding YA, Leu HB, Yin WH, Sheu WH, Chu KM (2004) Effect of rosuvastatin on plasma levels of asymmetric dimethylarginine in patients with hypercholesterolemia. Am J Cardiol 94:157–161

Lücke T, Tsikas D, Kanzelmeyer N, Vaske B, Das AM (2006) Elevated plasma concentrations of the endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine 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 AM (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 Transplant 23:734–740

Nakamura T, Sato E, Fujiwara N, Kawagoe Y, Ueda Y, Suzuki T, Ueda S, Adachi H, Okuda S, Yamagishi S (2009) Ezetimibe decreases serum levels of asymmetric dimethylarginine (ADMA) and ameliorates renal injury in non-diabetic chronic kidney disease patients in a cholesterol-independent manner. Pharmacol Res 60:525–528

Nanayakkara PW, Kiefte-de Jong JC, ter Wee PM, Stehouwer CD, van Ittersum FJ, Olthof MR, Teerlink T, Twisk JW, van Guldener C, Smulders YM (2009) Randomized placebo-controlled trial assessing a treatment strategy consisting of pravastatin, vitamin E, and homocysteine lowering on plasma asymmetric dimethylarginine concentration in mild to moderate CKD. Am J Kidney Dis 53:41–50

Oguz A, Uzunlulu M (2008) Short term fluvastatin treatment lowers serum asymmetric dimethylarginine levels in patients with metabolic syndrome. Int Heart J 49:303–311

Palm M, Onozato ML, Luo Z, Wilcox CS (2007) Dimethylarginine dimethylaminohydrolase (DDAH): expression, regulation and function in the cardiovascular and renal systems. Am J Physiol Heart Circ Physiol 293:3227–3245

Surdacki A, Tsikas D, Mayatepek E, Frölich JC (2003) Elevated urinary excretion of nitric oxide metabolites in young infants with Zellweger syndrome. Clin Chim Acta 334:111–115

Tan B, Jiang DJ, Huang H, Jia SJ, Jiang JL, Hu CP, Li YJ (2007) Taurine protects against low-density lipoprotein-induced endothelial dysfunction by the DDAH/ADMA pathway. Vascul Pharmacol 46:338–345

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 (2008a) A critical review and discussion of analytical methods in the L-arginine/nitric oxide (NO) area of basic and clinical research. Anal Biochem 379:139–163

Tsikas D (2008b) Determination of asymmetric dimethylarginine (ADMA) in biological fluids: a paradigm for a successful analytical story. Curr Opin Clin Nutr Metab Care 11:592–600

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

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 (ADMA) in health and disease. 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

Wolf C, Lorenzen JM, Stein S, Tsikas D, Störk S, Weidemann F, Ertl G, Anker S, Bauersachs J, Thum T (2011) Urinary asymmetric dimethylarginine (ADMA) is a predictor of mortality risk in patients with coronary artery disease. Int 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 YL (2009) Arginine metabolism and nutrition in growth, health and disease. Amino Acids 37:153–168