Low Levels of Asymmetric Dimethylarginine in Children with Diabetes Mellitus Type I Compared with Healthy Children

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Objective Although high levels of asymmetric dimethylarginine (ADMA) are associated with an increased risk for vasculopathy in adults, elevated ADMA concentrations also have been found in healthy young children. Patients with diabetes mellitus type 1 (DM1) are at risk for vasculopathy, and because the function of ADMA in the development of vascular symptoms is incompletely understood, we investigated ADMA concentrations in pediatric patients with DM1 compared with healthy age- and sex-matched individuals.

Study design This cross-sectional study included 85 pediatric patients with DM1 and 89 age- and sex-matched healthy controls.

Results ADMA concentrations were significantly lower in the patients with DM1 and were inversely correlated with hemoglobin A1c concentrations.

Conclusions Besides its vasoprotective function, nitric oxide itself may exert oxidative stress by generating free radicals. In these circumstances, ADMA would protect the system from nitric oxide overproduction and perpetuation of oxidative stress. This theory is supported by the physiologically higher ADMA concentrations in healthy children. Thus, low ADMA concentrations in children with DM1 may be an indicator of impaired protection against oxidative stress. *(J Pediatr 2011;158:602-6)*.

Asymmetric dimethylarginine (ADMA) is synthesized by dimethylation of protein-bound L-arginine residues by arginine methylases.[1] The methionine–homocysteine remethylation pathway serves as main source for the methyl groups.[2] Free ADMA evolves from the degradation of proteins containing dimethylated L-arginine. Free ADMA is catabolized mainly to L-citrulline and dimethylamine by dimethylarginine dimethylaminohydrolase (DDAH)[1,3,4] (**Figure 1**).

Because free ADMA is an analogue of L-arginine, the substrate for nitric oxide synthase (NOS), elevated ADMA causes a reduced L-arginine:ADMA ratio and impairs nitric oxide (NO) synthesis. NO is a potent vasodilator that protects the endothelial integrity and function and inhibits platelet aggregation.[1,4] In adults with or at high risk for cardiovascular disease, ADMA was found to be a predictor of coronary heart disease, with an odds ratio of 2.61 for a 0.1-μmol/L increase in plasma free ADMA concentration.[5] Elevated ADMA levels have been reported in children with chronic renal failure,[6] arterial hypertension,[7,8] and pulmonary hypertension.[9] In addition, high ADMA levels (2.6 $\pm$ 1.9 μmol/L) have been measured in young adults with diabetes mellitus type 1 (DM1) without clinical signs of vasculopathy.[10] Mean ADMA concentration was found to be significantly higher in adults with DM1 (mean age, 42.7 years) with diabetic nephropathy compared with those without nephropathy (0.46 $\pm$ 0.08 μmol/L vs 0.40 $\pm$ 0.06 μmol/L).[11] In contrast, other studies have found lower ADMA levels in children (0.55 μmol/L)[12] and young females (0.58 $\pm$ 0.2 μmol/L)[13] with DM1 compared with healthy controls (0.67 and 0.68 $\pm$ 0.15 μmol/L, respectively).

Because the published data offer arguments for both higher and lower ADMA in young patients with DM1 without manifest vasculopathy, in the present study we tested the undirected hypothesis that ADMA concentration differs significantly in pediatric patients with DM1 and healthy controls. In addition, we investigated whether classical cardiovascular risk factors and disease-specific markers (ie, hemoglobin A1c [HbA1c] and microalbuminuria) had a significant impact on ADMA concentration.

ADMA	Asymmetric dimethylarginine
BP	Blood pressure
BMI	Body mass index
DDAH	Dimethylarginine dimethylaminohydrolase
DM1	Diabetes mellitus type 1
HDL	High-density lipoprotein
HbA1c	Hemoglobin A1c
NO	Nitric oxide
NOS	Nitric oxide synthase
tHcy	Total homocysteine

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Supported by the Federal Government of Vorarlberg, Austria. The authors declare no conflicts of interest.

 10.1016/j.jpeds.2010.09.058

Methods

This cross-sectional study included 85 children and adolescents (age range 2-18 years) with DM1. Data from 89 age- and sex-matched healthy controls collected during a previous study (unpublished data) were used for comparison. The study protocol was approved by the local Ethics Committee (Protocol 2006-3/1). Informed consent/assent were obtained from all participants aged >8 years and their parents/guardians. Blood samples were obtained by venipuncture for independent medical reasons.

The participants with DM1 were recruited from the diabetes outpatient clinics of the participating hospitals. The control group was recruited from healthy children and adolescents scheduled for elective ear, nose, and throat or general surgery or allergy testing.

In all participants, body weight, length, body mass index (BMI), blood pressure (BP), creatinine, blood glucose, ADMA, total homocysteine (tHcy), folate, L-arginine as well as family and individual history of vascular disease were investigated. In addition, HbA1c, microalbuminuria, disease duration and total dose of insulin per kg body weight per day were assessed in the DM1 group.

Body weight was measured with an electronic scale accurate to 0.05 kg, and height was measured with a stadiometer accurate to 0.5 cm. BMI was calculated as weight (in kg)/height (in m)^2. Using a standardized data sheet age, sex, ethnic background, and family and individual history of cardiovascular disease were recorded. BP was measured according to the Riva-Rocci method.

Blood samples (5 mL) were obtained after an overnight fast. L-arginine and ADMA were analyzed using stable isotope dilution techniques and liquid chromatography–tandem mass spectrometry (Waters Micromass Quattro Micro LC-MS/MS, Manchester, United Kingdom). Blood was placed in prechilled heparinized tubes and immediately spun down at 4°C. The plasma was deproteinized using perchloric acid (2:1) and spun down, after which the supernatant was transferred and stored at -80°C until analysis. Separation was done by liquid chromatography on a 150-mm × 3-mm silica column with an isocratic mobile phase consisting of water, acetonitrile, trifluoroacetic acid, and propionic acid (10:90:0.025:1 by volume) with a chromatographic run time of ~7 minutes, as reported previously.[14] Plasma tHcy concentrations were determined by automated fluorescence polarization immunoassay (Abbott IMx Analyzer, Abbott Laboratories, Abbott Park, Illinois),[15] and folate concentrations were measured by microparticle enzyme immunoassay (Abbott Laboratories).[16] Serum creatinine, triglycerides, total cholesterol, high-density lipoprotein (HDL), glucose, and HbA1c were measured at 37°C by standard laboratory assays. Urine albumin was measured by enzyme-linked immunosorbent assay. Microalbuminuria was defined as albumin excretion >30 mg/L in 2 out of 3 urine collections.

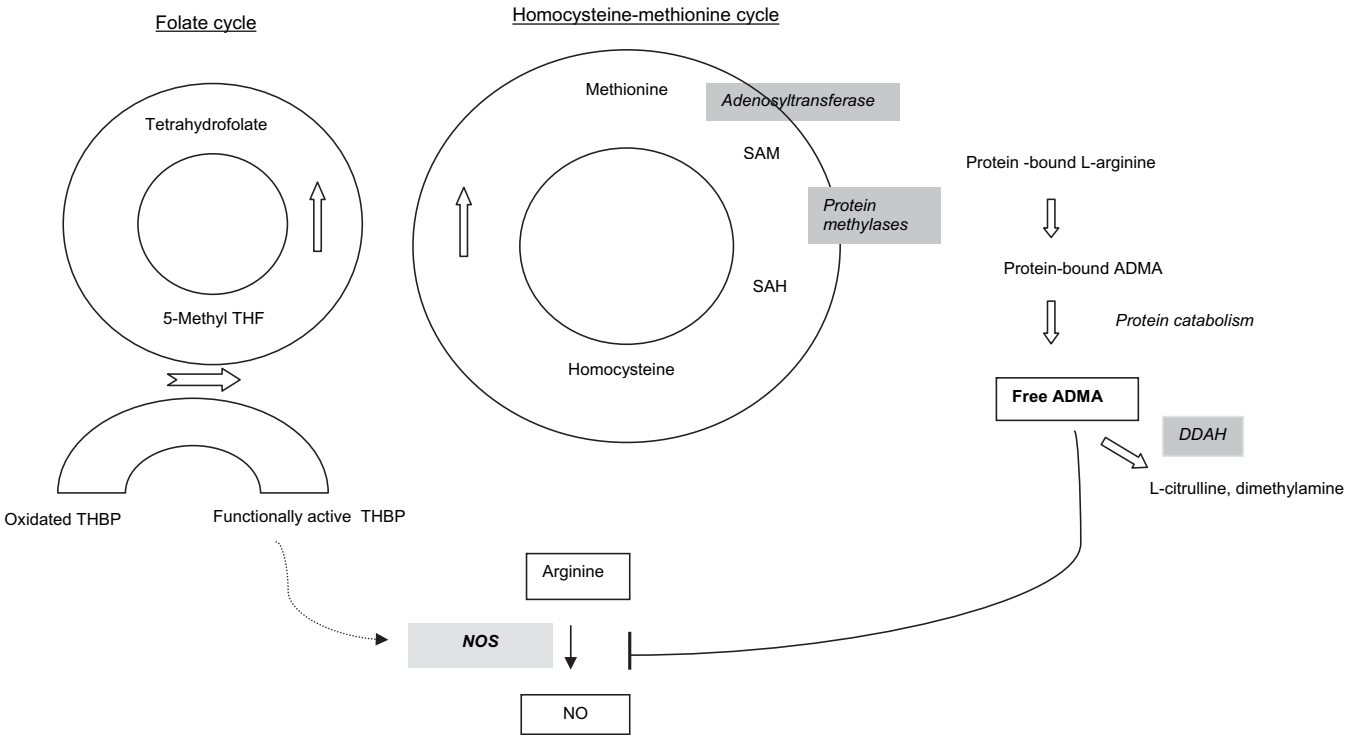

Figure 1. ADMA is synthesized from arginine by protein methylases. The methyl groups for this reaction are derived from the homocysteine–methionine pathway, which is interconnected with the folate cycle. ADMA blocks the synthesis of NO by the enzyme NOS, which requires active tetrahydrobiopterine (THBP) as a cofactor.