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

Martina Huemer, MD, Burkhard Simma, MD, Dieter Mayr, MD, Adolf Mühl, PhD, Birgit Rami, MD, Edith Schober, MD, Hanno Ulmer, PhD, Ulrike Zanier, MD, and Olaf A. Bodamer, MD

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

From the Department of Pediatrics, Landeskrankenhaus Bregenz, Bregenz, Austria and University Children's Hospital Zürich, Zürich, Switzerland (M.H., D.M.); Department of Pediatrics, Landeskrankenhaus Feldkirch, Feldkirch, Austria (B.S.); Centogene Vienna, Vienna, Austria (A.M.); University Children's Hospital Vienna, Vienna, Austria (B.R., E.S.); Department of Medical Statistics, Informatics and Health Economics, Innsbruck Medical University, Innsbruck, Austria (H.U.); Department of Pediatrics, Krankenhaus Dornbirn, Dornbirn, Austria (U.Z.); and University Children's Hospital and Institute for Inborn Errors of Metabolism, Paracelsus Medical University, Salzburg, Austria (O.B.)

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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.

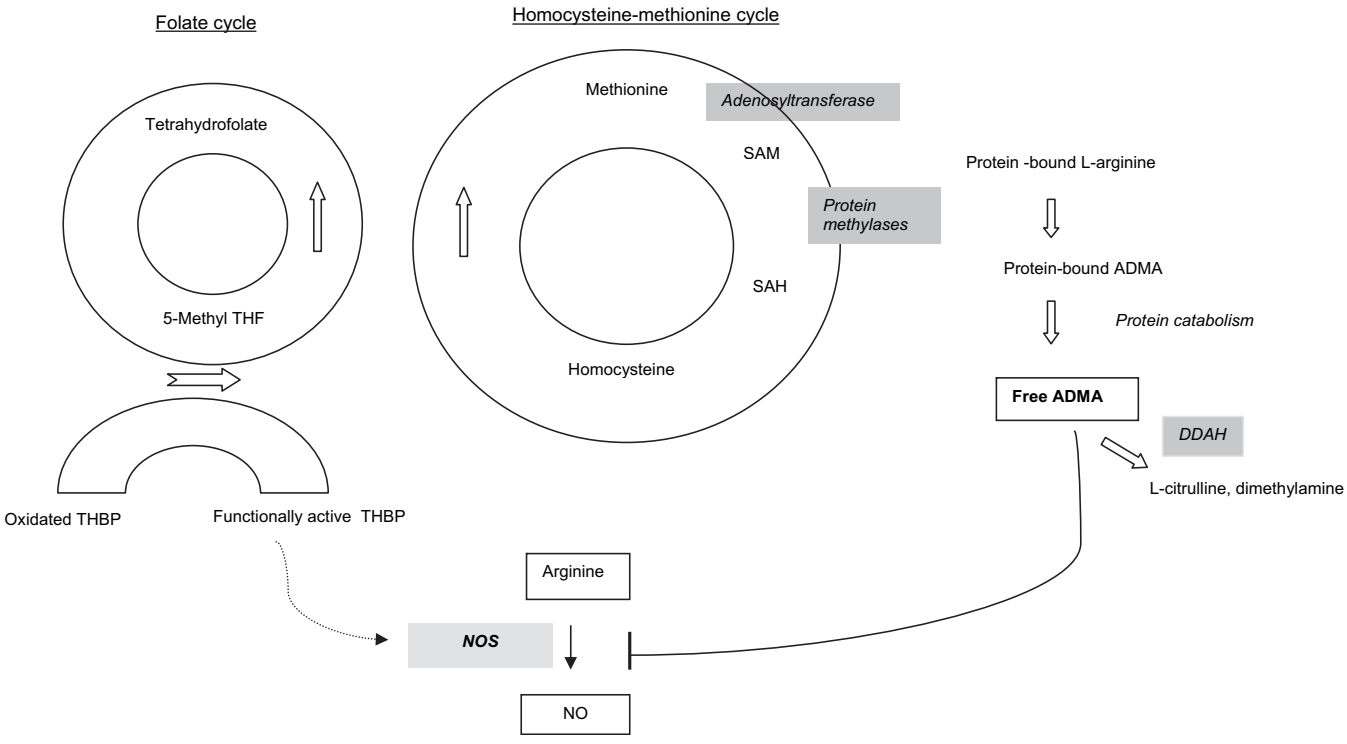

Figure 1. ADMA is synthesized from arginine by protein methylases. The methyl groups for this reaction are derived from the homocysteine–methionine pathway, which is interconnected with the folate cycle. ADMA blocks the synthesis of NO by the enzyme NOS, which requires active tetrahydrobiopterine (THBP)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

A sample size of 64 probands in each group was estimated to have 80% power to detect a difference in mean of 0.1 μmol/L of ADMA between patients with DM1 and healthy controls assuming a common standard deviation of ADMA of 0.2 μmol/L, based on data from Kari et al[6] using a two-sample *t* test with a 0.05 two-sided level of significance.

Between-group differences in secondary or confounding variables were assessed using the *t* test or Mann-Whitney *U* test for continuous variables and the χ^2 test for categorical variables. Bivariate correlation and multivariate regression analyses were performed for the overall study population, as well as separately for the DM1 and control groups.

Results

A total of 174 children and adolescents participated, including 85 with DM1 (mean age, 11.6 ± 3.7 years; 34 females; mean duration of disease, 49 months; range, 1-150 months) and 89 controls (mean age, 12.1 ± 3.1 years; 32 females). There were no significant differences between the groups in terms of sex distribution, age, family history of cardiovascular disease, BMI, systolic BP, and creatinine, triglycerides, tHcy, folic acid, and arginine concentrations. Diastolic BP and total cholesterol and ADMA levels (all $P < .0001$) were higher in controls, and blood glucose and HDL levels (both $P < .0001$) were higher in the DM1 group (**Table** and **Figures 2** and **3**; available at www.jpeds.com).

For the 76 participants in the DM1 with available urine albumin data, only 3 had persistent microalbuminia (ie, 2 out of 3 measurements) and 4 had intermittent microalbuminia (defined as urine albumin >30 mg/L). ADMA concentration did not differ significantly between the participants with and without microalbuminuria (data not shown).

Multiple regression analyses with ADMA as a dependent variable were assessed for all participants and separately for the DM1 and control groups, using the following measures: age, sex, diagnosis (ie, presence or absence of DM1), blood glucose, BP, HDL, triglycerides, total cholesterol, and tHcy. In the model for all participants, the diagnosis of DM1 was the only significant predictive variable for ADMA concentration ($R^2 = 27\%$; $P < .0001$). In the model for the controls, blood glucose level was a significant predictive variable for ADMA concentration ($R^2 = 19\%$; $P = .049$;). In the model for the DM1 group, low HbA1c level significantly predicted higher ADMA concentration ($R^2 = 27\%$; $P = .01$).

Spearman correlation analysis revealed a significant inverse correlation between ADMA concentration and age in the control and DM1 group ($P = .034$ and .01, respectively) in all participants. In the control group, blood glucose and ADMA concentration were significantly correlated ($P = .002$). The DM1 group demonstrated a significant positive correlation between ADMA and HDL concentrations ($P = .004$), and inverse correlations between ADMA concentration and systolic BP ($P = .021$), diastolic BP ($P = .021$), and levels of HbA1c ($P = .028$) and triglycerides ($P = .026$).

Discussion

In our sample, ADMA concentration was not correlated with or predicted by any cardiovascular risk factor, including lipid profile, tHcy, or cardiovascular death/disease in first-degree relatives. ADMA concentration was positively correlated with HDL level and inversely correlated with HbA1c level. ADMA concentration differed significantly between the participants with DM1 and the healthy age- and sex-matched controls, with significantly lower levels in the DM1 group. Elevated ADMA concentrations have been reported in adults with DM1 complicated by retinopathy[11] or vasculopathy.[17,18] In addition, Altinova et al[10] reported a higher mean ADMA concentration in 40 young adult patients (mean age, 28 ± 7 years) with DM1 without clinical evidence of vascular complications compared with healthy controls (2.6 ± 1.9 μmol/L vs 1.7 ± 0.7 μmol/L; $P < .01$). Surprisingly, the ADMA concentrations in both groups in that study are higher than those reported in many different populations.[1,3,4,12,13,17-19]

In contrast, in our study, ADMA concentration was lower in the children and adolescents with DM1 without vasculopathy. Marcovecchio et al[20] reported that a experimentally

Table. Age, BMI, BP, and laboratory test values in patients with DM1 and controls

	DM1 (n = 85; 34 females)		Controls (n = 89; 32 females)		
Variables	Median	Range	Median	Range	*P* value
Age, years	12.3	2.1-18.4	12	2-18.6	NS
BMI, kg/m^2	19.2	13.3-28.4	18.6	13.8-36.6	NS
Systolic BP, mmHg	112	92-152	117	80-157	NS
Diastolic BP, mmHg	65	42-95	78	50-94	<.0001
Total cholesterol, mmol/L	4.37	2.95-8.87	3.72	1.35-5.59	<.0001
HDL, mmol/L	1.68	0.91-3.98	1.35	0.72-2.51	<.0001
Triglycerides, mmol/L	0.66	0.17-4.72	0.69	0.23-3.31	NS
tHcy, μmol/L	10.6	1-33.2	12.4	3.1-43.9	NS
Folic acid, nmol/L	5.9	2.3-15.6	6	2.5-13	NS
ADMA, μmol/L	0.48	0.27-0.72	0.64	0.36-1.61	<.0001
Glucose, mmol/L	9.32	2.5-25.92	4.88	3.16-7.44	<.0001
Insulin dose, IE/kg/day	0.88	0.16-1.5		-	-
HgA1c, %	8.2	5-13		-	-
Creatinine, μmol/L	52.8	26.4-114.4	52.8	26.4-79.2	NS
L-arginine, μmol/L	58	29-118	59	5-186	NS

NS, not significant.

induced (ie, through hyperinsulinemic clamping) high insulin level was correlated with a decrease in ADMA concentration, attributed to impaired protein catabolism. Parallel to the reduction in ADMA, hyperinsulinemia leads to decreased levels of amino acids, including arginine. In our sample, arginine concentrations were similar in the DM1 and control groups, suggesting that the proposed impaired protein catabolism due to high insulin levels seems to be not causative of the lower ADMA concentrations in our DM1 group. We can only speculate on the effect of enhanced renal clearance of ADMA in persons with DM1. An increased filtration fraction after dopamine infusion might even be present before clinical signs of nephropathy and microalbuminuria in patients with DM1;[21] however, filtration fraction was not quantified in our sample.

As in our sample, Heilman et al[12] reported lower ADMA concentrations in 30 children with DM1 without vasculopathy compared with controls. In addition, Pitocco et al[13] found lower ADMA concentrations in 99 young females with DM1 with disease duration <10 years and without clinical evidence for vascular complications compared with 44 controls (0.58 $\pm$ 0.2 vs 0.68 $\pm$ 0.15 μmol/L). In that study, uric acid levels were lower in subjects with DM1 and were inversely correlated with the sum of nitrite and nitrate concentrations, which reflects NO production.[13] Uric acid synthesis is known to be impaired by NO, which down-regulates the enzyme xanthine oxidase. NO plays a role in the development and perpetuation of oxidative stress.[22] Dominguez et al[23] found that parameters of oxidative stress, including malondialdehyde (an end product of polyunsaturated fatty acid peroxidation) level, protein carbonyl group levels, and erythrocyte superoxide dismutase activity, were higher in subjects with DM1 compared with healthy controls, and that erythrocyte glutathione peroxidase and glutathione levels were lower in young subjects with DM1 even at onset and during the early course of the disease.

Various models have been suggested to explain the complex relationship of NO, ADMA, and oxidative stress. It has been shown that ADMA concentrations rise during periods of oxidative stress due to inhibition of the enzyme DDAH by free radicals.[24] ADMA then competes with arginine at the substrate-binding site of NOS, resulting in a relative shortage of arginine. A lack of arginine may cause the NOS to shift from NO to free radical synthesis. Through that step, the NOS perpetuates and aggravates oxidative stress.[24] In contrast, data exist supporting the concept that along with its vasoprotective effects, NO itself may directly exert oxidative stress. NO may interfere with DNA synthesis and the respiratory chain[13] or react with superoxides and form peroxynitrite, a potent agent in oxidative stress. In this model, ADMA would protect the system from NO overproduction and thus discontinue the vicious cycle of oxidative stress[13] (**Figure 4**).

The correlation between ADMA concentration and age in children is also interesting. Younger children have higher ADMA concentrations, and the levels observed in these healthy young individuals are similar to those associated with vascular dysfunction in adults.[25] Under the assumption that higher ADMA concentrations in healthy children are not harmful but rather are a physiological phenomenon, a function of ADMA might be the reduced production of NO to control the development of oxidative stress by excessive NO and peroxynitrite synthesis.[13] Thus, the finding of lower ADMA concentrations in children and adolescents with DM1 might not be interpreted as positive considering vasculopathy, but instead may be the hallmark of impaired protection against oxidative stress in DM1.[13] This assumption is also supported by the correlation between higher HbA1c level

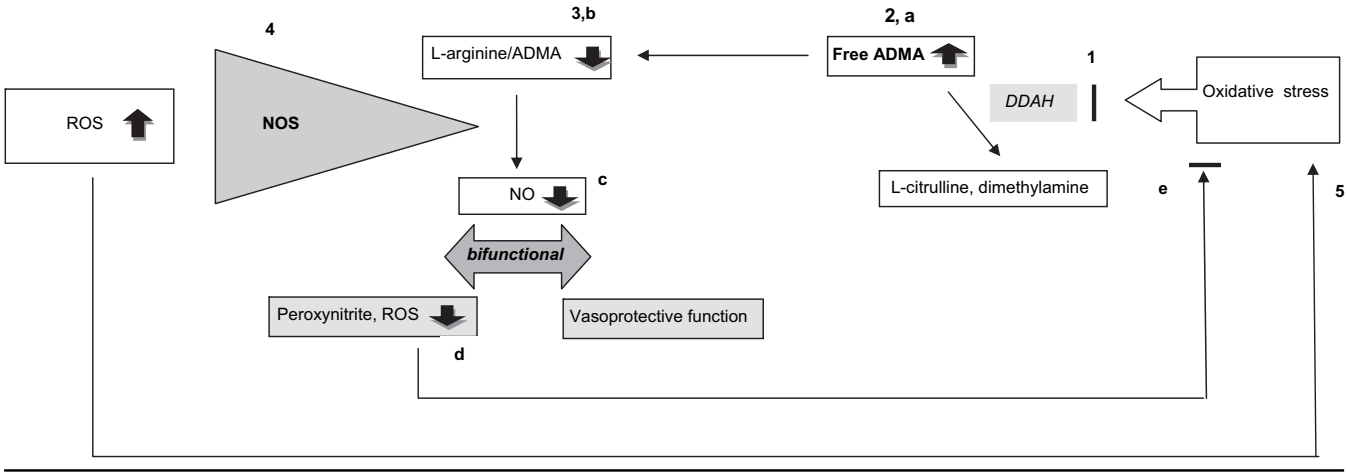

Figure 4. Steps 1-5 summarize the hypothesis that oxidative stress blocks **1,** DDAH, causing the enhancement of **2,** ADMA, and relative **3,** arginine depletion, which induces the shift of **4,** NOS activity from NO synthesis toward generation of reactive oxidative species (ROS) and perpetuates **5,** oxidative stress. Steps a-e illustrate the **a** and **b**, hypothesis that the relative arginine depletion decreases **c,** NO synthesis, and the generation of **d,** peroxynitrite and other ROS, thereby reducing **e,** oxidative stress.

and low ADMA concentration found in our sample, given the association between poor disease control and enhanced oxidative stress.[26]

Long-term studies in children and adolescents are warranted to elucidate the potentially dual roles of both ADMA and NO against the background of oxidative stress in pediatric patients with DM1. ■

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Reprint requests: Martina Huemer, MD, Department of Pediatrics, Landeskrankenhaus Bregenz, Carl Pedenz Str 2, 6900 Bregenz, Austria. E-mail: martina.huemer@lkhb.at

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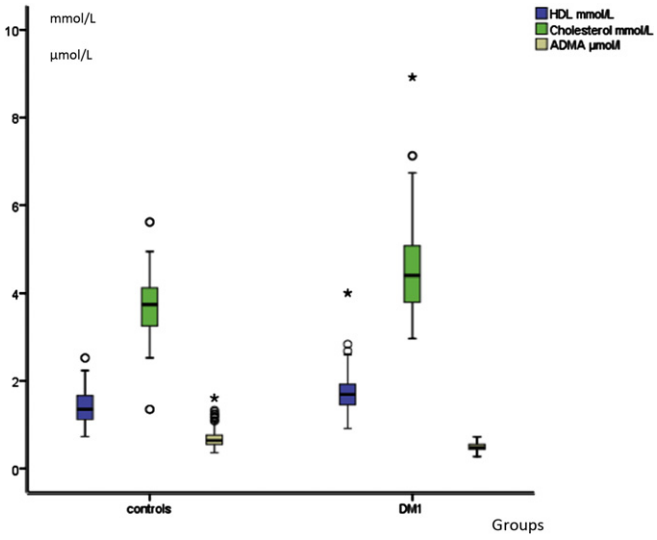

Figure 2. HDL (mmol/L), total cholesterol (mmol/L), and ADMA (μmol/L) concentrations in the DM1 and control groups. Asterisks denote extreme outliers; circles, mild outliers.

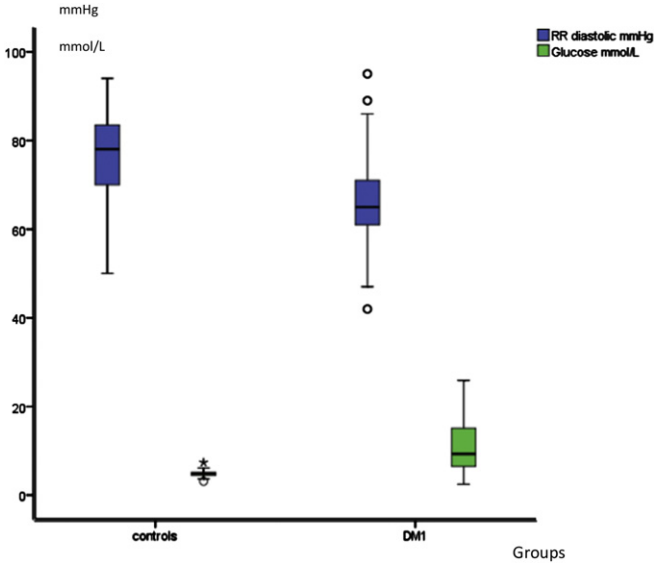

Figure 3. Blood glucose level (mmol/L) and diastolic BP (mm Hg) in the DM1 and control groups. Asterisks denote extreme outliers; circles, mild outliers.