Elevated plasma adiponectin and decreased plasma homocysteine and asymmetric dimethylarginine in children with type 1 diabetes

Kaire Heilman, Mihkel Zilmer, Kersti Zilmer, Pille Kool, Vallo Tillmann

* Department of Paediatrics, University of Tartu, Tartu, Estonia
\nDepartment of Biochemistry, Centre of Molecular and Clinical Medicine, University of Tartu, Tartu, Estonia

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Elevated plasma adiponectin and decreased plasma homocysteine and asymmetric dimethylarginine in children with type 1 diabetes

Kaire Heilman1, Mihkel Zilmer 2, Kersti Zilmer 2, Pille Kool1 and Vallo Tillmann1

1Department of Paediatrics, University of Tartu, Tartu, Estonia; 2Department of Biochemistry, Centre of Molecular and Clinical Medicine, University of Tartu, Tartu, Estonia

Objective. Type 1 diabetes has a bad prognosis concerning the pathogenesis of cardiovascular diseases (CVD). The purpose of this study was to evaluate different possible new risk indices for CVD in children with type 1 diabetes. Material and methods. The present study included 30 children with diabetes (mean HbA1C 9.8%), aged between 4.7 and 18.6 years and with no clinical evidence of vascular complications, and 30 healthy subjects matched by sex, age and body mass index. Blood pressure was measured and blood samples were obtained for lipid profile, creatinine, glucose, high sensitive C-reactive protein (hsCRP), intercellular adhesion molecule-1 (ICAM-1), asymmetric dimethylarginine (ADMA), adiponectin and homocysteine.

Results. Children with diabetes had significantly higher blood pressure, plasma hsCRP, ICAM-1, adiponectin levels and lower homocysteine, ADMA concentrations than their control subjects. In multivariate regression analysis, the best predictors for systolic blood pressure were diabetes group, plasma homocysteine concentration and BMI (Adj $R^2=0.38$, $p<0.0001$), and for diastolic blood pressure diabetes group and triglycerides level (Adj $R^2=0.27$, $p<0.0001$).

Conclusions. Children with diabetes, in view of their higher future risk of CVD, are characterized by a higher concentration of protective adiponectin and paradoxically lower blood concentrations of some other possible risk markers of atherosclerosis, i.e. ADMA and homocysteine compared to healthy children.

Keywords: ADMA; adiponectin; homocysteine; hsCRP; ICAM-1; type 1 diabetes

Introduction

Type 1 diabetes is an important risk factor for cardiovascular diseases (CVD) [1]. The cumulative incidence of CVD increases significantly with the presence of diabetic nephropathy [2] and when conventional cardiovascular risk factors such as hyperlipidemia, smoking and hypertension are present [3]. Over the past decade, several new possible risk factors have been suggested as contributors for CVD. An increase in intercellular cell adhesion molecule-1 (ICAM-1) [4], total homocysteine (tHcy) [5] and asymmetric dimethylarginine (ADMA) levels [6] or a decrease in plasma adiponectin concentrations [7] are some of these new factors.

Endothelial dysfunction is an underlying cause of atherosclerosis and CVD. With type 1 diabetes, hyperglycaemia, increased free fatty acids and insulin resistance provoke molecular mechanisms that contribute to vascular dysfunction: decreased bioavailability of nitric oxide, elevated oxidative stress and prothrombotic activity, disturbances of intercellular signal transduction and activation of receptors for advanced glycosylation end products [8].

Hyperglycaemia is known to modulate expression of cell adhesion molecules and cytokines which, through monocyte-endothelium interaction, leads to the initiation and progression of atherosclerosis. The adipocytokine adiponectin has an anti-inflammatory effect and improves insulin sensitivity [9]. As both insulin resistance and inflammation are important predictors of CVD, adiponectin may be a link between these two causal pathways. Recent studies on experimental animals have indicated that in hyperglycaemia NO production is increased, and when NO reacts with a superoxide anion it forms peroxynitrite, a powerful damaging factor to endothelium [10]. Increased NO levels have also been found in human diabetes [11].

The aim of our study was to evaluate different possible new risk indices for CVD in children with type 1 diabetes without clinical evidence of vascular complications. We measured markers of endothelial activation and inflammation – hsCRP, ICAM-1 and adiponectin – as a marker of anti-atherosclerotic process. As the exact mechanism for increased NO levels in diabetes is not known, we decided to...
measure ADMA, a powerful endogenous inhibitor of NO production [12], as well. As there are conflicting results regarding whether hyperhomocysteinemia is a cause or a result of CVD [13], we also included tHcy in our panel to clarify its relation with early courses of atherosclerosis. A better understanding of the early mechanisms leading to vascular dysfunction may direct progress to new prevention/treatment strategies reducing cardiovascular morbidity and mortality in patients with diabetes.

**Material and methods**

This cross-sectional study included 30 children with type 1 diabetes (mean age 13.6 [range 4.7–18.6] years; 19 boys) attending the Diabetes Clinic at the University of Tartu Children’s Hospital, Estonia, and 30 healthy control subjects, matched by sex, age (±2 years) and BMI (±3 kg/m²). All patients with diabetes were without clinical evidence of vascular complications. The mean duration of diabetes was 5.7 [range 1.0–14.6] years. Inclusion criteria for children with diabetes and control subjects alike were: age between 3 and 20 years and no history of using antihypertensive or lipid-lowering medications. Children with diabetes were included only if at least 1 year had passed from the diagnosis. The diagnosis of type 1 diabetes was based on the American Diabetes Association criteria. Informed consent was obtained from each subject and/or parents participating in the study. The protocol was approved by the local ethics committee. The investigations were carried out in accordance with the principles of the Declaration of Helsinki.

**Clinical and laboratory investigations**

All investigations were performed after overnight fasting between 0800 and 0900 h in the morning in subjects with diabetes, i.e. before insulin administration. Arterial blood pressure was measured three times after at least 5 min rest using a validated oscillometric technique and a cuff of appropriate size on the patient lying in the supine position, and the mean of the triplicate measurements was used in analysis (OMRON M4-I; Omron Healthcare Europe BV®, Hoofddorp, The Netherlands). Height and weight were measured using a wall-mounted stadiometer and digital scale to the nearest 0.1 cm and 0.1 kg, respectively. Stage of puberty was assessed according to Tanner.

Blood was obtained for glucose, HbA1c, creatinine, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, hsCRP, ICAM-1, tHcy, ADMA and adiponectin. Subjects above the upper limit of the normal range of hsCRP (>5 mg/L) were excluded in the comparison of hsCRP between the diabetes and control groups (n=3 in the diabetes group and n=1 in the control group) as having apparently some infection or inflammatory disorder.

ADMA was determined in plasma using a validated ELISA kit (DLD Gesellschaft für Diagnostika und Medizini-sche Geräte mbH; Hamburg), ICAM-1 using human soluble ICAM-1 CD54 immunoassay (R&D Systems Europe, UK), tHcy using an Axis Homocysteine EIA kit (Axis-Shield Diagnostics Ltd, UK), adiponectin using Human Adiponectin/Acrp30 Immunoassay (R&D Systems Europe Ltd, UK) and hsCRP, determined in serum using a chemiluminescence enzyme immunometric assay on an Immulite immunoassay analyser (DPC, USA) in accordance with the manufacturer’s instructions. The intra- and interassay coefficients of variation determined in our laboratory were, respectively, 3.9 % and 5.3 % for ADMA, 7.5 % and 8.5 % for tHcy, 3.4 % and 5.8 % for adiponectin. Glycosylated haemoglobin (HbA1c) was measured by latex immunoagglutination inhibition using a DCA 2000+ Analyser (Bayer Diagnostics Europe Ltd, Ireland). Creatinine, glucose and lipids were analysed by standard laboratory methods using certified assays in the local clinical laboratory. Glomerular filtration rate was calculated using the paediatric Schwartz formula [14].

We obtained information on family history of hypertension and early heart disease in the first- and second-degree relatives of the child. Data on age, diabetes duration, diabetes complications, insulin daily doses and mean HbA1c over the 12 months before the study were obtained from the registry of the Outpatient Diabetes Clinic.

**Statistical analysis**

Continuous variables are presented as mean values with 95% confidence intervals (95% CI), while qualitative variables are presented as absolute and relative frequencies. Comparisons between the groups were made using the Mann-Whitney U test. All data were tested for normality using the Kolmogorov-Smirnov test. However, we used a non-parametric test for all comparisons between the groups because of a “small sample size”. To compare proportions (qualitative variables), Fisher’s exact test for counting data was used. Odds ratios (OR) and 95% CI were used to estimate the relative risk. Bivariate correlations were analysed using the Spearman rank correlation test. Multiple linear regression analysis was used to determine the best set of predictors of blood pressure. Since the distribution of the hsCRP values was skewed to the left, logarithmic transformation and a natural
logarithm were employed to achieve approximate normality. All p-values were two-sided and differences were considered statistically significant if \( p < 0.05 \). All statistical calculations were performed using the SAS Version 8.02 statistical package (SAS Institute Inc., USA).

## Results

The clinical characteristics of the study groups are given in Table I. The two groups did not differ regarding age, gender, BMI, height, weight, pubertal stage, serum creatinine concentration, lipid profile or calculated creatinine clearance. In the diabetes group, there were more cases with a positive family history of arterial hypertension than in the control group, but statistically not significant (15 cases versus 9, OR = 2.3 [95% CI: 0.7–7.7], \( p = 0.19 \)). Children with diabetes had significantly higher systolic and diastolic blood pressure than the controls (Table I). In the multivariate regression analysis diabetes group, BMI and tHcy were the most important determinants of systolic blood pressure, explaining 38% of its variability (\( \text{Adj } R^2 = 0.38; p < 0.0001 \)). Triglycerides and the diabetes group were the most important predictors of diastolic blood pressure, explaining 27% of its variability (\( \text{Adj } R^2 = 0.27; p < 0.0001 \)).

### hsCRP and ICAM-1

The levels of hsCRP and ICAM-1 were significantly higher in the diabetes group than in the control group (Table I). There were no significant associations between ICAM-1 or hsCRP and other measured CVD risk factors.

### Homocysteine

The mean plasma tHcy concentration was significantly lower in the diabetes group compared to the control group (Table I). Plasma tHcy concentration correlated positively with pubertal stage, serum creatinine level and systolic blood pressure and inversely with plasma ICAM-1 concentration in both groups. It also correlated positively with age and BMI in the diabetes group (Table II).

### ADMA

The mean plasma ADMA concentration was significantly lower in the diabetes group compared with the control group (Table I). Plasma ADMA concentration correlated inversely with age (Figure 1), pubertal stage and BMI in both groups and in addition with creatinine level in the diabetes group (Table II).

### Table I. Clinical characteristics of the study groups. The means with 95% confidence intervals are shown.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Type 1 diabetes group (n=30)</th>
<th>Control group (n=30)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>13.1 (11.7–14.4)</td>
<td>13.2 (11.7–14.6)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>19/11</td>
<td>19/11</td>
<td></td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>5.4 (4.1–6.6)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>9.8 (9.4–10.5)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Insulin (IU/day)</td>
<td>0.88 (0.79–0.97)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>52.2 (45.6–58.8)</td>
<td>54.5 (46.9–62.1)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>155.9 (148–163.7)</td>
<td>160 (153–167.3)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>20.2 (19–21.5)</td>
<td>20.4 (18.9–21.9)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>115 (112–118)</td>
<td>108 (105–111)</td>
<td>0.002</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>63 (61–65)</td>
<td>57 (55–59)</td>
<td>0.004</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>60.4 (55.1–65.8)</td>
<td>62.0 (56–68)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Creatinine clearance (mL/min/1.73 m²)</td>
<td>124.8 (117–132.5)</td>
<td>127.7 (114.7–140.7)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>15.6 (13.2–18)</td>
<td>5.2 (5.3–5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.6 (4.2–5.0)</td>
<td>4.2 (3.9–4.5)</td>
<td>n.s.</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/L)</td>
<td>2.8 (2.5–3.2)</td>
<td>2.5 (2.3–2.8)</td>
<td>n.s.</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/L)</td>
<td>1.7 (1.6–1.8)</td>
<td>1.7 (1.5–1.8)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.0 (0.7–1.2)</td>
<td>0.8 (0.6–0.9)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Homocysteine (µmol/L)</td>
<td>5.9 (5.3–6.5)</td>
<td>7.8 (6.9–8.8 )</td>
<td>0.0008</td>
</tr>
<tr>
<td>ADMA (µmol/L)</td>
<td>0.55 (0.5–0.6)</td>
<td>0.67 (0.6–0.8)</td>
<td>0.03</td>
</tr>
<tr>
<td>Adiponectin (µg/mL)</td>
<td>14.45 (11.8–17.1)</td>
<td>10.67 (8.5–12.8)</td>
<td>0.03</td>
</tr>
<tr>
<td>hsCRP (mg/L)</td>
<td>0.9 (0.7–1.2)</td>
<td>0.7 (0.3–1.2)</td>
<td>0.02</td>
</tr>
<tr>
<td>ICAM-1 (ng/mL)</td>
<td>293 (276–310)</td>
<td>251 (227–276)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

n.m. = not measured; n.s. = not significant (\( p > 0.05 \)). ADMA = Asymmetric dimethylarginine; ICAM-1 = intercellular adhesion molecule-1; hsCRP = high sensitivity C-reactive protein.
Adiponectin

The mean plasma adiponectin concentration was significantly higher in the diabetes group than in the control group (Table I). In the diabetes group, adiponectin correlated inversely with BMI \(r=0.36\), but it was statistically not significant \(p=0.06\). In the control group, adiponectin correlated positively with ICAM-1 concentration and negatively with pubertal stage and creatinine level (Table II).

The duration of diabetes, HbA1c and blood glucose level did not demonstrate any significant associations with ADMA, tHcy or adiponectin plasma concentrations.

Discussion

The most important finding of this study is that children with type 1 diabetes, despite their higher future risk of CVD, have a higher concentration of protective adiponectin and paradoxically lower blood concentrations of other possible biochemical risk markers of atherosclerosis, i.e. ADMA and tHcy, compared with healthy children. Traditional risk factors of CVD, such as diabetes itself, higher serum triglycerides level and BMI were significant predictors of higher blood pressure in our study. It should be noted that, as far as we know, this is one of the first studies describing the plasma ADMA levels in children with type 1 diabetes. Although the number of subjects in our study was not very big, every patient with diabetes had a healthy control who was well matched by sex, age and body composition, thus giving the study the statistical power sufficient to find significant differences in the majority of measured biochemical markers between the groups. The two groups did not differ regarding total body fat percentage measured by DXA and physical activity level measured by accelerometer except the diabetic boys who were slightly less active than the control boys (data not shown).

Patients with diabetes are at a high risk of incurring atherosclerotic CVD [1]. Endothelial dysfunction has been demonstrated to occur early in the course of diabetic vascular complications, starting in childhood [15]. Children with diabetes had higher blood pressure and an elevated level of markers of endothelial activation and inflammation, as ICAM-1

<table>
<thead>
<tr>
<th></th>
<th>Type 1 diabetes group (n=30)</th>
<th>Control group (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adiponectin ((P))</td>
<td>tHcy ((P))</td>
</tr>
<tr>
<td>Age</td>
<td>n.s.</td>
<td>0.50 (0.005)</td>
</tr>
<tr>
<td>Pubertal stage</td>
<td>n.s.</td>
<td>0.54 (0.007)</td>
</tr>
<tr>
<td>BMI</td>
<td>n.s.</td>
<td>0.36 (0.05)</td>
</tr>
<tr>
<td>Creatinine</td>
<td>n.s.</td>
<td>0.48 (0.007)</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>n.s.</td>
<td>-0.45 (0.01)</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>n.s.</td>
<td>0.38 (0.04)</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

ADMA=Asymmetric dimethylarginine; tHcy=total Homocysteine; ICAM-1=Intercellular adhesion molecule-1; BP=Blood pressure. n.s.=not significant \(p>0.05\).

Figure 1. Correlation between plasma asymmetric dimethylarginine (ADMA) level and age in patients with diabetes (diamonds, solid line) \(r=-0.59; p=0.001\) and in the control group (crosses, dashed line) \(r=-0.51; p=0.004\).
and hsCRP, compared to the healthy children in our study. Several inflammatory biomarkers, particularly hsCRP, have been identified as likely predictors of the development of CVD [16]. According to a statement by the American Heart Association and the Centers for Disease Control and Prevention (2003), an hsCRP value > 1 mg/L is considered to be a risk factor for CVD in the adult population [17]. Our children with diabetes had a mean hsCRP 0.9 mg/L, which is very close to this limit.

This study evaluated the plasma ADMA levels in children with type 1 diabetes for the first time. We found reduced ADMA concentration in children with diabetes. The decreased ADMA level may be one of the possible explanations why patients with diabetes have a higher level of NO and nitrosative stress. Furthermore, low ADMA is found to be a significant determinant of a high LDL oxidation rate [18]. There may be a number of reasons why children with type 1 diabetes have low plasma ADMA levels; one of the possible explanations is as follows: ADMA is formed during proteolysis of methylated proteins. Protein methylation is a mechanism of post-translational modification of proteins that results in a modification of the tertiary structure and the biofunction of proteins. This process is catalysed by a group of enzymes named S-adenosylmethionine protein N-methyltransferases. Thus, formation of ADMA is a very complicated process and a persistent elevated level of glucose may result in the non-enzymatic glycation (modification) of enzymes/proteins involved in the formation of ADMA. Recently markedly elevated ADMA levels have been demonstrated in adult patients with diabetic nephropathy [19]. One conclusion could be that ADMA level is low in young patients with diabetes, whereas it increases in later stages with renal impairment.

Homocysteine level depends on the kinetic properties of enzymes that participate simultaneously in tHcy and ADMA synthesis. A link between plasma hyperhomocysteinemia and nephropathy in patients with both type 1 and type 2 diabetes has been reported [20,21]. However, reduced tHcy concentrations have been found in children and adults without diabetic nephropathy [22–24]. In our study, children with diabetes had significantly lower tHcy levels than the controls. However, the exact tHcy values remained within the normal reference values published by Vilaseca et al. [25]. Increased glomerular filtration has been proposed as the mechanism explaining the lower than normal tHcy in patients without overt nephropathy [26]. Plasma tHcy level correlated positively with creatinine level in our study, too, but mean serum creatinine concentration and creatinine clearance did not differ between the groups. As we used calculated and not measured glomerular filtration rate, it is possible that in this way we were unable to detect mild changes in renal function. The decreased tHcy concentrations have also been partially explained by higher B12 vitamin and folate status in children with diabetes [24] and by insulin deprivation [27]. We did not measure serum folate and vitamin B12 levels in these subjects and therefore cannot rule out their protective effect on plasma tHcy levels. Blood glucose level, HbA1c or insulin daily dose did not demonstrate any significant correlation with tHcy in our study. Although patients with diabetes had a lower plasma concentration of tHcy, in multivariate analysis tHcy was a significant predictor of higher systolic blood pressure, indicating its value as a risk factor for CVD.

The present study demonstrates that children with type 1 diabetes also have elevated adiponectin levels, concurring with the results of Celi et al. and Galler et al. [28,29]. Adiponectin, a polypeptide synthesized mainly by adipocytes, has a wide range of biological activities, including an anti-inflammatory and antiatherogenic effect [9]. Plasma adiponectin levels are reduced in patients with type 2 diabetes [30], obesity [31] and coronary artery disease [7]. Conversely, increased plasma levels of adiponectin have been observed in patients with type 1 diabetes [32,33]. Patients with microvascular complications have even higher serum adiponectin levels than patients without complications [34]. The mechanism responsible for the elevated adiponectin levels is not clear. Adiponectin levels have also been reported to be higher in dialysis patients [35]. While adiponectin is excreted through the kidneys, it has been suggested that decreased renal function may lead to an elevated adiponectin concentration [36], but this does not explain why children with a normal or increased glomerular filtration rate have elevated adiponectin levels. Previous studies have found an independent positive correlation between plasma adiponectin and markers of endothelial activation/injury in patients with type 1 diabetes [37], chronic renal failure and healthy individuals [38]. The adiponectin expression is shown to be induced by inflammatory cytokines in vivo and in vitro [39]. Therefore, increased adiponectin concentration could represent a beneficial compensatory mechanism to existing vascular damage. The increased plasma adiponectin levels have been found in patients with heart failure [40] and high adiponectin levels were predictive of mortality in patients already afflicted with CVD [41].

Weaknesses of the present study are the wide age distribution with different pubertal stages and inhomogeneity in the range of diabetes onset of this cohort, which makes it difficult to generalize our
findings. It has to be mentioned that our patients had relatively poor glycaemic control and our findings may only apply in the case of diabetic children with poor metabolic control. In our study group, about two-thirds (n=23) of children with diabetes were in puberty. It has been shown that in puberty the average HbA1c is significantly higher than in childhood [42,43]. Poor compliance and decreased insulin sensitivity are part of that. During the study, the government reimbursed only 300 BM strips per year for each child, which definitely had a negative impact on diabetic control. Thus, achieving good metabolic control in patients with type 1 diabetes remains a challenge for paediatric endocrinologists. A limitation of the present study was that the different isoforms of adiponectin were not measured. Adiponectin circulates as various isoforms and polymers, which may differ in receptor affinities as well as metabolic effects. Recent data indicate that it is the plasma fraction of high molecular-weight polymers rather than the total concentration of adiponectin that is associated with its vasculoprotective effects [44].

In summary, we found that children with type 1 diabetes with relatively poor glycaemic control have increased levels of plasma inflammatory markers and endothelial activation. We found lower levels of new possible cardiovascular risk factors such as tHcy and ADMA and higher levels of protective adiponectin. Future prospective studies are needed to clarify the biological role of these changes, but it seems that lower tHcy and ADMA and higher adiponectin plasma concentrations do not reduce the future risk of developing cardiovascular diseases in these patients.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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