Impaired endothelial function in female adolescents with type 1 diabetes measured by peripheral artery tonometry

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Abstract

Background The reactive hyperemia peripheral artery tonometry (RH-PAT) is a newly developed method for non-invasive endothelial function assessment.

Objective The goal of this study is to determine whether a significant difference in RH-PAT score is present between adolescents with type 1 diabetes (T1D) in comparison with controls.

Subjects and methods Thirty-four adolescents with T1D and 25 control subjects (age 12–20 years) underwent RH-PAT endothelial function testing after an overnight fast. Height, weight, body mass index (BMI), blood pressure (BP), fasting lipid profile, Tanner stage, and glucose level were determined in each child.

Results Adolescents with T1D had significantly lower RH-PAT scores compared to healthy controls, and this difference remained significant when overweight cases were not considered ($p < 0.05$). This difference was also observed in the female subgroup ($p = 0.005$). The interindividual variability in RH-PAT observations in T1D cases was not explained by BMI standard deviation score (SDS), BP SDS, age, duration of T1D, hemoglobin A1c, triglycerides, and pubertal stage, respectively.

Conclusions The RH-PAT technique is used as a non-invasive test to assess for early vascular changes in high-risk patient groups. Endothelial dysfunction, measured by RH-PAT, was present in diabetic adolescent, especially in the female subgroup. Although additional longitudinal studies are required, early detection of this reversible process may have therapeutic and prognostic implications.

Keywords Adolescents · Prevention · Type 1 diabetes · Vascular endothelium

Abbreviations

RH-PAT	Reactive hyperemia peripheral artery tonometry
T1D	Type 1 diabetes mellitus
BMI	Body mass index
BP	Blood pressure
SDS	Standard deviation score
HbA1c	Hemoglobin A1c
FMD	Flow-mediated dilatation
NO	Nitric oxide
IMT	Intima-media thickness
LDL	Low-density lipoprotein
HDL	High-density lipoprotein

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Introduction

There is evidence of a rising incidence of pediatric diabetes worldwide [8, 29]. Type 1 diabetes (T1D) is a significant risk factor for early atherosclerotic vascular changes with clinical evidence for manifest coronary disease before 30 years of age [17]. The Diabetes Control and Complications Trial and its longitudinal follow-up study Epidemiology of Diabetes Interventions and Complications have demonstrated that the risk of diabetes-related microvascular and macrovascular

complications relates to long-term glycemic control [25, 33]. However, even with intensive insulin therapy, the majority of children with T1D are unable to maintain near-normal glycaemia. Therefore, measures to identify pediatric patients at risk for the development of premature cardiovascular disease (CVD) are needed. A well-known early surrogate marker of CVD is endothelial function. In adult studies, it has been shown that endothelial dysfunction precedes atherosclerotic plaque formation. Early detection of this process may have therapeutic and prognostic implications [31]. Endothelial dysfunction is described as the inability of the artery to sufficiently dilate in response to an appropriate endothelial stimulus. Several non-invasive methodologies were developed to assess the early stages of atherosclerotic disease, including the flow-mediated dilatation (FMD) technique, the measurement of the carotid or aortic intima-media thickness (cIMT and aIMT, respectively) using B-mode ultrasound scanning and reactive hyperemia techniques by plethysmography or peripheral artery tonometry. The FMD technique was first described in 1992 by Celermajer et al. [2]. The upper arm occlusion results in reactive hyperemia after the release of the cuff. The increased shear stress stimulates an endothelial release of nitric oxide (NO) and results in endothelium-dependent flow-mediated vasodilatation. Brachial artery diameter is measured with high-resolution ultrasound at rest and during reactive hyperemia. The resultant FMD score is a marker of the ability of the vessels to respond to an increased need for local blood supply. For cIMT, the left and right common carotid arteries were imaged in a standardized magnification with images of the posterior wall of the distal 10 mm of the common carotid artery, just proximal to the carotid bulb. The posterior wall of a straight, non-branched 1-cm longitudinal segment of the distal abdominal aorta was imaged just proximal to the aortic bifurcation for aIMT. For each image, the mean and maximum IMT was measured [14, 15]. Increased IMT is a structural marker of early atherosclerosis that correlates with vascular risk factors. These techniques are however operator dependent, more difficult to perform in the pediatric age group, and there are no possibilities to correct for potential measurement-induced changes in the systemic hemodynamics [5].

Alternative devices allow non-invasive measurement of vasoreactivity without the disadvantages of the use of conventional ultrasound techniques. Originally, venous occlusion plethysmography was used as a surrogate non-invasive measure of endothelial function [32]. More recently, the reactive hyperemia peripheral artery tonometry (RH-PAT) has been developed. RH-PAT testing uses fingertip plethysmography probes to measure the changes in pulse wave amplitude observed before and after the period of reactive hyperemia [1]. Endothelium-mediated changes in vascular tone after occlusion of the brachial artery are reflecting a downstream hyperemic response, which is a measure for arterial endothelial function. An unequivocal theory about

the underlying mechanism of the RH-PAT is lacking. It is known that the provoked endothelium-dependent flow-mediated vasodilatation after removing the occlusion is partly (60 %) mediated by endothelial release of NO [27]. The RH-PAT score is calculated as the ratio of the average pulse wave amplitude during the 1-min period starting 60 s after cuff deflation divided by the average pulse wave amplitude of a 210-s pre-occlusion baseline period. Lower RH-PAT scores reflect more pronounced endothelial dysfunction. In adults, the literature describes a significant relation between a low RH-PAT score and several cardiovascular risk factors: tobacco use, hypertension, hyperlipidemia, body mass index (BMI), and diabetes mellitus [13]. In addition, there is a moderate but significant correlation between FMD and RH-PAT as shown in the study of Kuvin et al. ($r=0.55$, $p<0.0001$) and the study of Dhindsa et al. ($r=0.47$, $p<0.01$) [7, 19]. The goal of this study is to determine whether a significant difference in RH-PAT score is found in adolescents with T1D as compared to controls.

Materials and methods

Study population

Thirty-four adolescents with T1D (16 males and 18 females) and 27 healthy age- and sex-matched control subjects (13 males and 14 females) were recruited from our University Diabetes Centre. All patients who participated in this study were Caucasian and aged 12 to 20 years. Only children diagnosed with T1D and treated with insulin for at least 1 year were included. All patients with T1D are using a “multiple daily injection” scheme: fast-acting insulin at meal time and long-acting insulin before going to bed. Controls were age- and sex-matched and were recruited by the diabetic children themselves (friends or family). All subjects were non-smokers. Patients and controls were excluded from the study when a technical problem occurred. Controls were excluded when there was a familial history of premature CVD (having first- or second-degree relatives with premature CVD). Overweight and obesity were no exclusion criteria and were defined by using the age-adjusted BMI cutoff points suggested by Cole et al. in children under the age of 18 [4]. At the age of 18 years, the adult cutoff points were used ($\text{BMI} \geq 25 \text{ kg/m}^2$ for overweight and $\text{BMI} \geq 30 \text{ kg/m}^2$ for obesity).

Methods

Parents and patients received verbal and written information about the study before providing written consent. The study was approved by the Ethical Review Board of the University Hospitals. The study was registered as NCT01267591.

RH-PAT endothelial function was assessed using the EndoPAT device (Itamar Medical Ltd., Caesara, Israel). All measurements were performed in the same noise- and temperature-controlled room, in fasting state. The patient was placed in a comfortable position with the hands at heart level with the fingers hanging freely to avoid muscular activity. They were instructed to remain quiet during the measurements. Two plethysmographic registration probes were placed on both index fingers. After 5 min of baseline measurement, arterial flow to the non-dominant arm was occluded for 5 min using a blood pressure cuff inflated to 40 mmHg above systolic pressure. After the 5-min occlusion, the cuff deflated rapidly to allow for reactive or flow-mediated hyperemia. Pulse wave amplitudes were recorded for at least 5 min after the cuff was deflated. Due to the concurrent signal from the contralateral finger, the method corrects for changes in systemic vascular tone. The probes were connected to a computer for online registration of all recordings. A specialized software program calculated the ratio of the average blood volume before and after occlusion in both fingers to know the RH-PAT score [5]. Following RH-PAT testing, height, weight, blood pressure (BP), and Tanner score were recorded, and blood was obtained in fasting condition for lipid profile, blood glucose, and hemoglobin A1c (HbA1c).

Statistical methods

All data were expressed as median with interquartile range. The primary end point of the study was the RH-PAT score. Other variables were analyzed as secondary end points. The characteristics and RH-PAT values in T1D cases were compared to controls (unpaired analysis, Mann–Whitney *U* test). Correlations between RH-PAT and BMI standard deviation score (SDS), BP SDS, age, duration of T1D, HbA1c, triglycerides, and pubertal stage were studied. A *p* value below 0.05 was considered to be statistically significant. The statistical analysis was performed with the program MedCalc® version 11.4.3 (Mariakerke, Belgium).

Results

Baseline characteristics

We refer to Table 1 for an overview of the baseline characteristics of both T1D and control cases. No difference was observed in variables like age, blood pressure, BMI, or lipid profile (total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglyceride levels). As expected, diabetic patients had higher fasting blood glucose levels ($p<0.0001$) and HbA1c levels ($p<0.0001$). Two children from the control group were excluded from the study: one female had a family history of premature CVD

and there was a technical problem in another male, resulting in 25 control subjects (12 males and 13 females). None of the adolescents with T1D were excluded.

RH-PAT

Children with T1D ($n=34$) had clear endothelial dysfunction as evidenced by lower median RH-PAT scores (1.6 (1.3–2.0) vs. 1.9 (1.7–2.4), $p=0.0154$). As the BMI was higher in the children with T1D, the data were reanalyzed after exclusion of the children with overweight or obesity (five children in the diabetes group: three males and two females). Even after exclusion, the RH-PAT score remained significantly lower in diabetic subjects compared to controls (1.5 (1.3–2.0) vs. 1.9 (1.7–2.4), $p=0.0057$). When subdividing according to sex, it was clear that female diabetic patients had a lower RH-PAT score as compared to controls (1.3 (1.3–1.7) vs. 2.0 (1.7–2.5), $p=0.005$) (Fig. 1b). In contrast, in male adolescents, a non-significant difference in RH-PAT score was observed (1.8 (1.5–2.1) vs. 1.8 (1.5–2.3), $p=0.7277$) (Fig. 1c). This gender-related difference was not due to a difference in HbA1c between both groups. Additionally, we checked if there was a difference in baseline pulse amplitude between our two groups. We found a significantly higher basal pulse amplitude in the group of patients with T1D compared to controls (373.0 (208.3–522.0) vs. 145.3 (52.3–300.2), $p=0.0033$). When subdividing according to sex, this difference was seen in the male subgroup (442.8 (146.7–594.3) vs. 99.0 (48.0–311.1), $p=0.0345$) and the female subgroup (365.1 (295.8–482.3) vs. 154.9 (95.1–318.8), $p=0.0448$).

Finally, individual RH-PAT scores were explored in both T1D and control cases. There was a weak correlation between individual RH-PAT scores and LDL cholesterol levels ($r=0.29$, $p=0.031$). This positive correlation was counterintuitive and might be a type 1 error. No significant correlation was seen between individual RH-PAT scores and BMI SDS, BP SDS, HbA1c, age, duration of T1D, triglycerides, and pubertal stage.

Discussion

The scientific statement from the American Heart Association Expert Panel on Population and Prevention Science identified children with T1D as being in the highest risk tier for early cardiovascular disease [17]. Surrogate markers are needed to assess cardiovascular disease risk in these children. Previous studies using FMD, cIMT, and aIMT have shown that children with T1D have decreased vascular distensibility, increased arterial stiffness, and increased cIMT and aIMT [14, 15]. Studies using venous occlusion plethysmography confirmed a limited vasodilator capacity in adolescents with T1D based on a smaller ischemic response for the diabetic group [24, 26]. In this cross-sectional

Table 1 Demographic and biochemical characteristics of the study patients

Characteristic	Control (n=25)	T1D (n=34)	p value
Male gender (n)	12	16	–
Age (years)	15.5 (13.9 to 16.2)	15.8 (14.4 to 16.6)	0.36
BMI (kg/m ²)	19.9 (19.3 to 20.8)	20.9 (18.9 to 23.4)	0.15
BMI SDS	0 (−0.3 to 0.5)	0.5 (−0.4 to 1.1)	0.14
Tanner	4 (3 to 5)	5 (4 to 5)	0.16
Overweight/n	0/25	5/34	–
Systolic BP (mmHg)	124 (115.0 to 131.3)	123.5 (115.0 to 131.0)	0.78
Systolic BP SDS	0.9 (0.08 to 1.5)	0.85 (0.2 to 1.5)	0.81
Diastolic BP (mmHg)	69.0 (61.8 to 74.5)	67.5 (64.0 to 72.0)	0.85
Diastolic BP SDS	0.1 (−0.4 to 0.5)	0.05 (−0.3 to 0.6)	0.99
Glucose (mg/dL)	92 (85.0 to 98.5)	186.5 (139.0 to 225.0)	<0.0001
HbA1c (%)	5.3 (5.2 to 5.4)	8.4 (7.0 to 9.3)	<0.0001
Total cholesterol (mg/dL)	146 (139.8 to 164.0)	155.5 (139.0 to 168.0)	0.22
LDL (mg/dL)	76 (64.5 to 89.3)	82.5 (69.0 to 89.0)	0.26
HDL (mg/dL)	58 (49.8 to 63.8)	57.5 (52.0 to 65.0)	0.75
Triglycerides (mg/dL)	64 (43.8 to 81.8)	63 (48.0 to 68.0)	0.80

All values were reported as median (P25 to P75)

BMI body mass index, SDS standard deviation score, BP blood pressure, HbA1c hemoglobin A1c, LDL low-density lipoprotein, HDL high-density lipoprotein, T1D type 1 diabetes mellitus

study, we measured endothelial function by RH-PAT. Adolescents with T1D had a significantly lower RH-PAT score as compared to controls. Additionally, we observed a marked difference in baseline pulse amplitude between the two groups, with a significantly higher baseline amplitude in the group of adolescents with T1D. This difference was seen in both sexes. These findings were consistent with the data from the study of Hamburg et al. [13]. They observed that baseline pulse amplitude was directly related to several cardiovascular risk factors, including hypertension, diabetes mellitus, overweight, and smoking. Baseline pulse amplitude was highly dependent on digital blood flow and sympathetic tone. The presence of increased basal blood flow was consistent with the presence of hyperperfusion, which might contribute to microvascular hypertension and microvessel damage. Decreased digital microvessel tone, increased pulse pressure, increased blood flow, and altered microvascular structure might all contribute to higher resting pulse amplitude. The higher baseline perfusion in DM1 adolescents may help explain the smaller RH-PAT index that was observed in female, but not in male adolescents with DM1. Only three other studies have previously used the RH-PAT score to demonstrate alterations in endothelial function in children with diabetes [12, 22, 23]. The first study measured endothelial function in 44 children with T1D and compared these data with the control group of the previous study; they found a significantly lower RH-PAT score in the children with T1D, but no sex difference. The second study described endothelial dysfunction in male adolescents with T1D. The third study measured endothelial function both before and after a high-fat meal and found a worse endothelial function in patients with T1D both before and after this meal. In addition, three

adult studies report a significantly lower RH-PAT score in patients with diabetes mellitus compared to patients without diabetes, possibly due to impaired vasodilator responses in diabetics [9, 11, 13]. One of these studies reported HbA1c as a weak independent predictor of endothelial dysfunction, suggesting a role of glycemic control in the pathogenesis of endothelial dysfunction [11]. In diabetic patients, it seems that the NO-mediated vasodilator response of insulin is blunted, whereas endothelin production is increased [18]. Endothelin-1 is a vasoconstrictor endothelial cell-derived peptide that is of significant importance in the regulation of vascular function. It is involved in the development of endothelial dysfunction including important interactions with nitric oxide.

When analyzing female and male subjects in our study, the female diabetic subgroup showed a significantly lower RH-PAT score compared to controls, while this was not seen in the male subgroup. Our study is the first to demonstrate this gender-related difference in children with diabetes. The small sample size however limits the power of this subanalysis. Gender-related differences in healthy controls have already been described: the Framingham Heart Study reports a lower PAT hyperemic response in healthy adult males compared to females [13]. This might be explained by the fact that estrogens have been shown to stimulate NO synthesis [10, 20]. Diabetes mellitus negates this protective effect of the female sex and it could be one explanation of our noticed results [6, 16, 21]. However, the sex differences may also be influenced by factors beyond biology. The study by Wexler et al. suggested sex disparities in medical management. This prospective cohort study demonstrated that women with diabetes were less likely to be treated until they reached established therapeutic targets than men with diabetes.

Fig. 1 Box plot of RH-PAT score in controls versus subjects with T1D (a), female controls versus female T1D patients (b), and male controls versus male T1D patients (c)

Whether these disparities were related to physician factors or patient factors remained unclear [30]. This factor however cannot

play a role in our study as both female and male patients get the same treatment and follow-up in our diabetic clinic.

The RH-PAT technique, used in our study, has some important advantages. First, it is a non-invasive subclinical marker. Subjects confirmed that the method was easy to tolerate; they described a tingling feeling in the occluded arm rather unpleasant, but not unbearable or painful. In the study of Selamet Tierney et al., the reported median pain score was 1, using the Wong–Baker Faces Pain Scale (ranging 1 to 5), in adolescents with ages 13 to 19 years [28]. Second, in contrast to the other non-invasive methods, the technique is operator independent. Third, the RH-PAT method has excellent reproducibility. In the study of Selamet Tierney et al., the reproducibility and feasibility have been tested prospectively in healthy adolescents [28]. However, the RH-PAT technique also has its limitations. First, there is some uncertainty about the relative importance of endothelium-dependent and endothelium-independent vasodilatation in the RH-PAT response. Second, there is a high cost associated with the finger probes, because each probe can only be used once. Third, the vascular bed is highly responsive to sympathetic tone. Changes in blood volume of the capillary bed can be caused by changes in room temperature or stress. These external influences are minimized by a similar measurement of the control signal at the contralateral finger (each test patient serves as his own control), but a recent study in 248 healthy school children showed that anger, depression, and anxiety are clearly associated with lower RH-PAT scores [3].

We conclude that there is increasing evidence that vascular change occurs early in children with diabetes mellitus, measured by the RH-PAT technique. Our study showed a gender difference, with female sex as a new risk factor. Therefore, attention to the maintenance of near-normal glycemia, control of hypertension, and earlier treatment of dyslipidemia is mandatory in the female adolescents with T1D. However, there certainly is a need for additional scientific evidence validated by longitudinal studies. Further research is also required about the use of the baseline pulse amplitude as a possible marker of endothelial dysfunction.

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Conflict of interest The authors declare no conflict of interest or any financial relationship with the organization that hosted the research.

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