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ARTICLE

Microvascular autoregulation in children and adolescents with type 1 diabetes mellitus

O. Schlager · A. Hammer · A. Willfort-Ehringer · M. Fritsch · B. Rami-Merhar · E. Schober · K. Nagl · A. Giurgea · C. Margeta · S. Zehetmayer · G. H. Schernthaner · R. Koppensteiner · M. E. Gschwandtner

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Abstract
Aims/hypothesis Deterioration of microvascular function may have an early onset in individuals with type 1 diabetes mellitus. We hypothesised that microvascular autoregulation is impaired in children with type 1 diabetes and can be detected non-invasively by postocclusive reactive hyperaemia (PORH).
Methods Microvascular autoregulation was assessed in 58 children with type 1 diabetes and 58 age- and sex-matched healthy controls by PORH using laser Doppler fluxmetry. Baseline perfusion, biological zero (defined as a 'no flow' laser Doppler signal during suprasystolic occlusion), peak perfusion following occlusion, time to peak and recovery time (time until baseline perfusion is resumed) were recorded and compared between the groups.
Results Peak perfusion was higher in children with type 1 diabetes than in healthy controls (1.7±0.93 AU [arbitrary units] vs 1.29±0.46 AU; $p=0.004$), and biological zero was lower in children with type 1 diabetes vs controls (0.14±0.04 AU vs 0.19±0.04 AU; $p<0.0001$). No differences were seen between the groups in baseline perfusion, time to peak during PORH and recovery time following PORH.
Conclusions/interpretation PORH reveals impaired microvascular autoregulation in children with type 1 diabetes. The higher peak perfusion might reflect a decline in the vasoconstrictive ability of arteriolar smooth muscle cells upstream of capillary beds in children with type 1 diabetes.

Keywords Autoregulation · Children · Laser Doppler · Microcirculation · Type 1 diabetes mellitus

Abbreviations

AU	Arbitrary units
SDS	Standard deviation score
PORH	Postocclusive reactive hyperaemia

Introduction

Type 1 diabetes mellitus is a major risk factor for the development of atherosclerosis. Epidemiological data on type 1 diabetes have shown an age- and sex-standardised annual incidence rate of 8–15 per 100,000 person-years among children (age below 15 years) in central Europe [1, 2]. In recent years, the incidence of type 1 diabetes in childhood has even increased. Type 1 diabetes in childhood might promote the atherosclerotic process at an early stage

O. Schlager · A. Hammer · A. Willfort-Ehringer · K. Nagl · A. Giurgea · C. Margeta · G. H. Schernthaner · R. Koppensteiner · M. E. Gschwandtner (✉)
Department of Medicine II, Division of Angiology,
Medical University of Vienna,
Währinger Gürtel 18-20,
1090 Vienna, Austria
e-mail: michael.gschwandtner@meduniwien.ac.at

M. Fritsch · B. Rami-Merhar · E. Schober
Department of Pediatrics and Adolescent Medicine,
Medical University of Vienna,
Vienna, Austria

S. Zehetmayer
Center for Medical Statistics, Informatics and Intelligent Systems,
Medical University of Vienna,
Vienna, Austria

[3]. Apart from macrovascular complications, such as ischaemic heart disease, microvascular injury is a matter of concern in patients with type 1 diabetes [4, 5].

Microcirculatory autoregulation can be assessed non-invasively by laser Doppler fluxmetry measuring postocclusive reactive hyperaemia (PORH) [6–9]. Laser Doppler fluxmetry is a non-invasive technique which allows investigation of the microcirculatory network that is situated 1–1.5 mm below the epidermis [10, 11]. Perfusion of this microvascular network of the papillary dermis is mainly regulated by the tone of smooth muscle cells in the vessel wall of afferent arterioles [10].

Previously it has been proposed that, during PORH, vasoconstriction of these smooth muscle cells is provoked by myogenic, neurogenic, metabolic and physical factors [12, 13]. Thereby, precapillary vasoconstriction prevents the terminal microvascular bed from hyperperfusion and subsequently attenuates postocclusive peak perfusion in the microcirculation.

Previous studies have demonstrated that microvascular autoregulation seems to be impaired by metabolic disorders [12, 14]. Importantly, it has been shown that microvascular regulatory dysfunction—when detected by a change in peak perfusion patterns during PORH—is related to an elevated albumin excretion rate as a surrogate marker for end-organ damage and cardiovascular mortality [13, 15]. This may suggest that microcirculatory autoregulation of the skin additionally reflects microvascular function of other vascular beds.

Therefore, we hypothesised that microcirculatory autoregulation of the skin is affected by type 1 diabetes at an early stage and that impairments can easily be detected in children. The aim of the present study was to assess PORH using laser Doppler fluxmetry in children with type 1 diabetes in comparison with age- and sex-matched healthy controls to detect early signs of microvascular dysfunction in type 1 diabetes.

Methods

Study design The study was performed according to the recommendations of the Declaration of Helsinki and the protocol was approved by the local ethics committee (EK 607/2006). Informed assent was obtained from all children and written informed consent was obtained from their parents.

Consecutive children with type 1 diabetes who were admitted to the Department of Pediatrics and Adolescent Medicine at the Medical University of Vienna were eligible for the present study. After inclusion, laboratory tests were obtained at the paediatric outpatient clinic and microvascular function was assessed at the microcirculation laboratory. Type 1 diabetes was diagnosed according to the current recommendations of the American Diabetes Association [16]. Included children with type 1 diabetes were free of clinical manifestations of microvascular damage, i.e. retinal lesions, microalbuminuria and neuropathy were not present in those children.

Healthy children, recruited from Austrian primary and secondary schools within the context of an epidemiological project, served as controls. None had a history of any cardiovascular, metabolic or other chronic disease, and routine laboratory values (including blood glucose and lipids) were within the normal range. BMI was normal in controls according to age- and sex-matched percentiles [17].

Laboratory and clinical data Fasting venous blood samples were drawn from all children with type 1 diabetes. Serum lipids (total cholesterol, triacylglycerol, LDL-cholesterol and HDL-cholesterol) and blood glucose levels were assessed at the institutional laboratory. HbA_{1c} was measured as a percentage of total haemoglobin using high-performance liquid chromatography at the institutional laboratory.

In healthy controls, lipid levels and fasting blood glucose levels were assessed from whole blood obtained by a finger prick using the Cholestech LDX System (Cholestech Corporation, Hayward, CA, USA). This device uses enzymatic solid-phase technology to measure total cholesterol, HDL-cholesterol, triacylglycerol and glucose levels. All blood tests were conducted by the same trained investigator. According to the manufacturer, the intra-day coefficients of variations range between 2.4% and 2.5% for total cholesterol, 3.4% and 4.8% for HDL-cholesterol, 1.6% and 3.6% for triacylglycerol, 3.8% and 4.9% for LDL-cholesterol, and 4.5% and 6.2% for blood glucose. According to the Cholesterol Reference Method Laboratory Network, the Cholestech LDX System meets the National Cholesterol Education Program criteria for accuracy and precision and is comparable to centralised laboratory testing.

To determine the comparability of the Cholestech LDX System with our institutional laboratory we measured total cholesterol, HDL-cholesterol, triacylglycerol and glucose levels in 15 children using both methods on the same day and observed the following percentages of measurement error for the Cholestech LDX System: 6.3% for total cholesterol, 7% for LDL-cholesterol, 4.9% for HDL-cholesterol, 4.9% for triacylglycerol and 5.7% for blood glucose. Comparing both methods we observed the following correlation coefficients: $r=0.97$ for total cholesterol, $r=0.99$ for LDL-cholesterol, $r=0.98$ for HDL-cholesterol, $r=0.97$ for triacylglycerol and $r=0.99$ for blood glucose (all $p<0.001$).

In all participating children blood pressure was measured on both arms with appropriate cuffs following a resting period of 20 min in a supine position. To account for the influence of sex, age and height on blood pressure in children we calculated blood pressure z scores for systolic and diastolic blood pressure.

Laser Doppler A laser Doppler (PIM II with LISCA Opto-Isolation Unit; Perimed, Järfälla, Sweden) was used for continuous fluxmetry to assess total skin perfusion at a predefined single measurement site over time. The first interspace between the thumb and the index finger of the dorsum of the patient´s hand was chosen [14, 18]. The skin of the selected site had to be intact and superficial veins were avoided. Using the duplex mode a laser beam (wavelength 632 nm, power 1 mW, beam diameter 1 mm) was directed to the skin at the selected site [6]. The processor bandwidth was 20–13 kHz with a respective time constant of 0.3–0.4 s (laser classification: class 2 according to EN60825-1, and class II according CFR1040.10 and CFR1040.11); the sampling frequency was 1–100 Hz. Sampling frequency and bandwidth were not changed throughout the course of the study.

Through transmission of superficial tissue layers the laser beam is Doppler-shifted by interfering with moving blood cells. The reflected signal derives from a depth 1–1.5 mm below the epidermis and is processed to form a photocurrent [10]. The photocurrent scales linearly with tissue perfusion, which is defined as the product of average speed and concentration of blood cells. The output signal can be displayed as a continuous time–perfusion diagram illustrating the change of microcirculatory blood flow at the selected site.

All children were investigated under standardised conditions in a quiet and darkened room at a constant room temperature of 23.3±0.6°C. All measurements were performed in the morning. Study participants were requested to refrain from coffee or tea intake ahead of laser Doppler measurements. All study participants were non-smokers. Before measurements the children were kept in a supine position for 20 min and a sphygmomanometer cuff was placed around the upper arm above the antecubital area for determination of PORH. All PORH tests were supervised by the same two experienced investigators (O. Schlanger and M.E. Gschwandtner).

PORH Microvascular function was investigated by PORH to determine the following variables: baseline perfusion, biological zero, time to peak, peak perfusion and recovery time.

Baseline perfusion was recorded under resting conditions without proximal arterial occlusion. Biological zero, defined as a 'no flow' laser Doppler signal, was measured during proximal arterial occlusion. Arterial occlusion was obtained by suprasystolic cuff inflation (10 mmHg above systolic blood pressure). Referring to previously published recommendations, an occlusion time of 3–5 min has been proposed [19]. In the present study we chose an occlusion time of 3 min, since—especially in children—longer occlusion periods might have been associated with substantial discomfort and a subsequent decline in compliance.

Both baseline perfusion and biological zero were determined as mean perfusion values over the predefined time period (3 min) and were given in arbitrary units (AU). Directly following suprasystolic arterial occlusion, cuff deflation led to immediate hyperaemia, which then provoked vasoconstriction of the smooth muscle cells of the precapillary arterioles.

Peak perfusion (AU) and time to peak (s), defined as the time span between cuff deflation and peak perfusion, were determined. Recovery time (s), the time until mean baseline perfusion was attained for the first time, was measured (Fig. 1).

All perfusion values as well as the measured time spans were determined using device-related software (LDPIWin 2 for Windows, www.perimed-instruments.com/software/ldpiwin).

For determination of reproducibility, we investigated 10 healthy children on 3 consecutive days and found the following intra-individual coefficients of variation: 12.9% for baseline perfusion, 11.4% for biological zero, 16% for time to peak, 19.8% for peak perfusion and 18.3% for recovery time. These coefficients of variation are in accordance with those found in previous investigations [20].

Sample size In previous investigations using laser Doppler fluxmetry to measure PORH in microcirculation we observed a mean peak perfusion of 1.26 AU with an SD of 0.5 AU for healthy children [14]. According to these data we assumed that diabetic children had a mean peak perfusion of 1.6 AU and an equal SD of 0.5 AU. A sample size of 45 children per group was calculated to have 80% power for detection of the proposed difference using a two-group *t* test with a 0.05 two-sided significance level. If the SD increased to 0.6 AU, more than 50 children per group would have been needed. Thus, we set the group-wise sample size to 58.

Statistical analysis Absolute and relative frequencies are given for categorical variables; number of observations, mean and SD, are given for continuous variables in the text. Logistic regression analyses of the target variable, type 1 diabetes, were calculated for the variables baseline perfusion, biological zero, time to peak, peak perfusion and recovery time adjusting for age and sex. The probability of a case was modelled and *p* values are reported. Finally, we performed a multiple analysis for each significant microcirculation parameter adjusting for all univariately significant risk factors (BMI, total cholesterol, HDL-cholesterol, blood glucose) as well as the matching variables age and sex. Correlations of influencing variables were calculated using Pearson's method.

Analyses were performed using SAS 9.1 (www.sas.com) and R-2.8 software (www.r-project.org). All p values <0.05 were considered to indicate statistical significance.

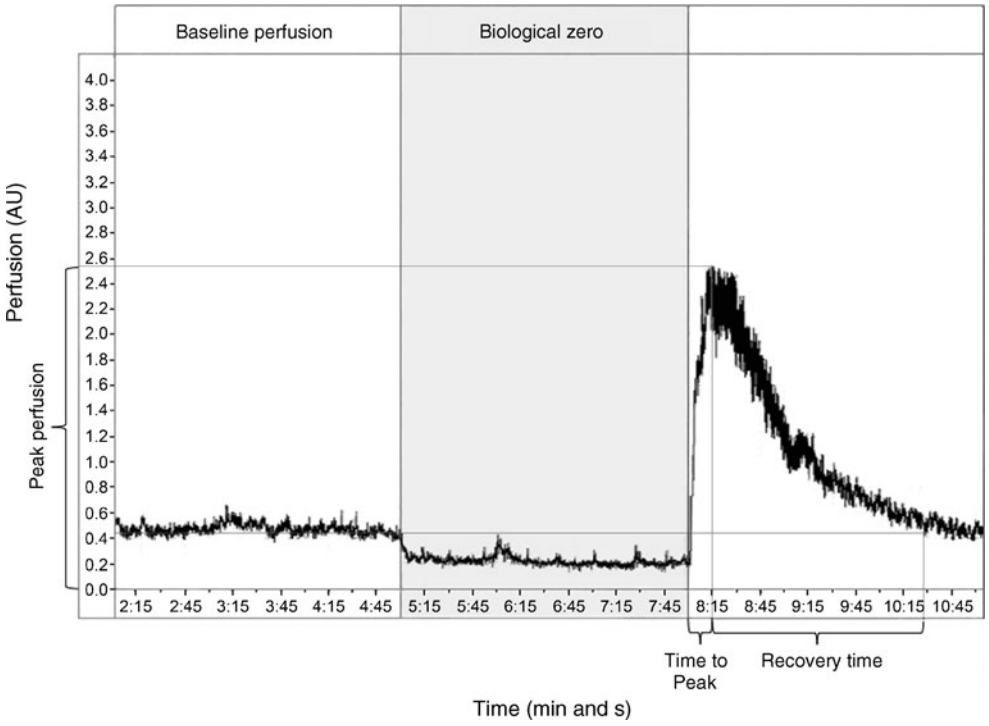

Fig. 1 Time–perfusion graph illustrating total skin perfusion during PORH when measured by laser Doppler fluxmetry

Results

The cohort comprised 58 children with type 1 diabetes (27 males, 31 females; mean age ± SD: 14.1±1.7 years) and 58 age- and sex-matched healthy children (34 males, 24 females; 13.6±2.0 years). Demographic and clinical data are shown in Table 1. Fasting blood glucose (mean ± SD) was higher in children with type 1 diabetes than in healthy controls (8.48±4.7 vs 4.74±0.39 mmol/l). In children with type 1 diabetes the mean (± SD) duration of disease was 7.8±3.3 years and the mean HbA_{1c} was 7.9±1.0% (63.4±11.4 mmol/mol). Body weight, BMI and the corresponding BMI standard deviation score (BMI-SDS) were higher in children with type 1 diabetes than in healthy controls. Further, total cholesterol and HDL-cholesterol levels were higher in the type 1 diabetes group. Absolute diastolic blood pressure was higher in healthy controls than in children with type 1 diabetes, while blood pressure *z* scores did not vary significantly between groups.

Analysing PORH, peak perfusion (mean ± SD) was higher in children with type 1 diabetes than in controls (1.7±0.93 AU vs 1.29±0.46 AU, $p=0.004$; Fig. 2a), whereas biological zero was lower in the patient group (0.14±0.04 AU vs 0.19±0.04 AU, $p<0.0001$; Fig. 2b). Baseline perfusion (mean ± SD) was similar in both groups (type 1 diabetes 0.45±0.26 AU; controls 0.40±0.16 AU; $p=0.15$). In addition, time to peak (type 1 diabetes 14.8±14.1 s; controls 17.6±14.7 s; $p=0.21$) and recovery time following peak perfusion (type 1 diabetes 80.3±43.7 s; controls 87.6±38.0 s; $p=0.39$) did not differ between groups.

We found no correlation of HbA_{1c} and the significant microcirculatory parameters (peak perfusion and biological zero). Regarding the duration of disease we found a correlation with peak perfusion ($r=0.287$, $p=0.03$) but no correlation with biological zero ($r=0.02$, $p=0.99$).

In a multiple analysis for each microcirculatory parameter we adjusted for all univariately significant risk parameters (BMI, total cholesterol, HDL-cholesterol, blood glucose) as well as for age and sex. Blood pressure *z* scores did not enter the model since univariately no significance was observed with respect to this parameter. Thus, after adjusting for univariately significant risk parameters, peak perfusion remained higher in children with type 1 diabetes than in controls ($p=0.017$) and biological zero remained lower in children with type 1 diabetes than in healthy children ($p=0.009$).

Discussion

We observed differences in microvascular reactivity between children with type 1 diabetes and healthy controls. A main finding was that peak perfusion in superficial microcirculatory networks of the dermis was higher in children with type 1 diabetes than in healthy controls. Since, in the past, various techniques have been used to assess vascular alterations, for interpretation of this result it is important to distinguish between these methods: regarding macrocirculation, it is well known that a sudden release of proximal suprasystolic arterial occlusion causes an immediate increase in blood flow velocity in the large arteries. The corresponding

Table 1 Demographic data and clinical characteristics of children with type 1 diabetes and healthy controls

Characteristic	Type 1 diabetes (n=58)	Controls (n=58)	p value
Age (years)	14.1±1.7	13.6±2.0	0.23
Male (n)	27 (46.6%)	34 (58.6%)	0.26
Body weight (kg)	59.5±11.6	48.6±11.1	<0.001
Body height (cm)	163.3±10.1	159.3±10.3	0.04
BMI (kg/m^2)	22.2±3.1	18.9±2.5	<0.001
BMI-SDS	0.79±0.74	−0.16±0.8	<0.001
Systolic blood pressure (mmHg)	117.2±11.1	114.5±10.1	0.18
Systolic blood pressure z score	0.52±1.01	0.22±0.9	0.11
Diastolic blood pressure (mmHg)	61.7±6.9	72.3±10.0	<0.001
Diastolic blood pressure z score	0.07±0.59	0.18±0.83	0.08
Fasting blood glucose (mmol/l)	8.48±4.7	4.74±0.39	<0.001
Total cholesterol (mmol/l)	4.48±0.83	3.96±0.65	<0.001
Triacylglycerol (mmol/l)	1.25±0.69	1.12±0.4	0.22
HDL-cholesterol (mmol/l)	1.75±0.38	1.32±0.39	<0.001
LDL-cholesterol (mmol/l)	2.18±0.7	2.09±0.63	0.49

Data are given as mean±SD or as counts (%)

increase in wall shear stress and the subsequent release of nitric oxide conjointly result in a gain of diameter in the large arteries [21].

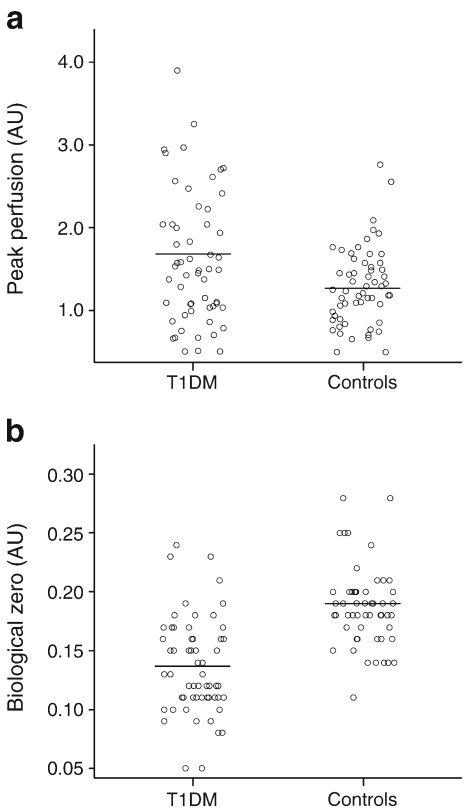

Fig. 2 Peak perfusion **(a)** and biological zero **(b)** in children with type 1 diabetes and controls (T1DM n=58; controls n=58). Dot plots show the distribution of measured values. A jitter function was used to make all observation points visible. The marked lines indicate the means. T1DM, type 1 diabetes

Over the past two decades flow-mediated dilation of the large arteries has been assessed in numerous studies by duplex ultrasound to detect endothelial dysfunction in the macrocirculation [22]. Furthermore, in recent years peripheral arterial tonometry has evolved to identify endothelial dysfunction by measuring volume changes in the digital arteries using fingertip plethysmography [23–25]. Peripheral arterial tonometry thereby reflects reactive vasodilation of the peripheral arteries; however, no conclusions can be drawn with respect to microvascular regulation of perfusion in the terminal microcirculatory beds. Most of the cutaneous microcirculation is situated 1–2 mm below the epidermal surface in the papillary dermis and comprises vessels with a diameter below 100 μm [10, 26]. Laser Doppler fluxmetry gathers signals derived from blood cells circulating through this microcirculatory network [10].

Previous studies have shown that thermally and pharmacologically provoked vasodilation—when measured by laser Doppler fluxmetry—is affected in diabetic individuals [27, 28]. While these effects were related to an impaired release of vasoactive agents and reduced vascular susceptibility to these agents, a distinction needs to be drawn with respect to the PORH-induced microvascular response. Employing PORH, the sudden cuff release provokes a complex microvascular response involving myogenic, neurogenic, metabolic and physical factors conjointly aiming at homeostasis of the terminal microcirculatory beds [13]. Homeostasis of capillary perfusion is regulated by the tone of arteriolar smooth muscle cells upstream of the capillary beds. Especially during the early phase of PORH, the rapid increase in blood flow elicits constriction of smooth muscle cells in the wall of precapillary arterioles, which thereby prevents capillary hyperperfusion [12, 13, 29].

Therefore, the higher peak perfusion, which was observed in the microcirculatory beds of children with type 1 diabetes, could be attributed to an impaired regulatory ability of the precapillary arteriolar units.

Several factors could be linked to an altered microvascular autoregulation in children with type 1 diabetes: diabetes mellitus leads to an increase in oxidative stress as well as elevated serum levels of proinflammatory cytokines, which both affect micro- and macrovascular reactivity [30]. Apart from these functional impairments, which primarily involve the vascular endothelium, previous investigations have shown that diabetes mellitus in childhood causes structural changes, such as clumping of elastic fibres and thickening of vascular basement membranes [27, 31]. A thickened arterial wall structure as well as endothelial dysfunction might conjointly cause a reduced ability of microvascular autoregulation. Furthermore, previous studies have shown that autonomic neuropathy potentially occurs at an early stage in individuals with type 1 diabetes [32]. Therefore, affected skin sympathetic activity as a consequence of diabetic autonomic neuropathy might contribute to the hampered regulatory ability within the microcirculatory networks [13, 33].

According to the complexity of the microvascular response, however, it seems reasonable that several pathophysiological mechanisms are responsible for impaired microvascular autoregulation, which might explain the higher peak perfusion in children with type 1 diabetes.

Interestingly, we observed a correlation of peak perfusion with duration of disease in children with type 1 diabetes, while HbA_{1c} levels had no influence on this microcirculatory parameter. In addition, included children with type 1 diabetes were free of clinical manifestations of end-organ damage. This may suggest that apart from glucose metabolism alone, other factors contribute to impaired microvascular autoregulation in type 1 diabetes.

Apart from peak perfusion following PORH, biological zero was lower in children with type 1 diabetes than in healthy controls. Biological zero is the signal that can be detected by laser Doppler fluxmetry when afferent flow is arrested by suprasystolic cuff occlusion [19]. Regarding the origin of biological zero, several physiological sources of this signal have been discussed: apart from collateral circulation through bones, fluid or cellular movement within the interstitial space, movements within vessels unrelated to flow (e.g. red cell sedimentation) and Brownian motion of interstitial macromolecules, residual microvascular vasomotion might contribute to biological zero [19, 34]. However, since the mechanisms causing biological zero are poorly understood, it can only be speculated that the lower biological zero in children with type 1 diabetes might be caused by impaired spontaneous vasomotion.

Notably, vasomotion can be further assessed by spectral analysis of baseline skin perfusion [35]. In former studies spectral analysis revealed impaired neurogenic activity in patients with diabetes mellitus [36]. However, in more recent investigations wavelet analysis, which particularly focused on the neurogenic spectrum of the recorded signal, was unable to identify specific pathophysiological mechanisms in patients with diabetes by means of spectral analysis [37]. Therefore, further investigations are needed to clarify the extent by which several components of spontaneous vasomotion might be affected by diabetes mellitus.

Regarding total resting skin blood flow we compared baseline perfusion between both groups and found no difference between children with type 1 diabetes and healthy controls. Naturally, baseline perfusion can be regarded as a semiquantitative recording comprising total resting skin blood flow. Therefore, from baseline perfusion alone—without PORH—no conclusion can be drawn with respect to the regulation of microvascular blood flow.

Further, recovery time was similar in both groups. During recovery time microcirculation regenerates to its homeostasis. According to the observed difference in peak perfusion one would have expected a prolonged recovery time in children with type 1 diabetes. The missing difference in recovery times between both groups may be explained by different mechanisms that contribute to microcirculatory homeostasis during the initial and late phase of PORH: while the initial phase of PORH is determined by vasoconstrictive ability of smooth muscle cells of precapillary arterioles, recovery time during the late phase of PORH is mainly triggered by mediators of endothelial function such as nitric oxide, prostanoids and adenosine [13, 38–41]. Therefore, at an early stage type 1 diabetes might influence the vasoconstrictive ability of the precapillary arterioles rather than the release of vasodilatory mediators.

Finally, we observed higher absolute diastolic blood pressure values in healthy controls than in diabetic children. Importantly, blood pressure varies with age, sex and height in childhood. Therefore, blood pressure can be categorised by predefined percentiles and expressed by resulting *z* scores [42]. To account for age, sex and height dependency of blood pressure we compared *z* scores but found no significant difference between the groups.

The following limitations of the present study should be acknowledged: previous studies have found that variations in sex hormone levels during puberty or the menstrual cycle might have an impact on vascular reactivity [43, 44]. However, findings on the impact of hormone levels such as serum oestradiol on PORH in microcirculation are inconsistent [45, 46]. Regarding the proportion of female study participants in both groups, a subgroup analysis addressing the stages of the menstrual cycle would have considerably lowered the statistical power of our study. Further, we did not assess pubertal status, since—especially in healthy controls—the respective examinations might have raised

severe concerns among volunteering children and parents. Aiming to minimise a possible bias by differences in pubertal development, we therefore strictly compared age- and sex-matched groups. Nevertheless, we cannot exclude a residual confounding by variations in sex hormone levels.

In addition, in controls capillary blood tests were used to determine blood lipid levels, while in children with type 1 diabetes venous blood samples were used. These different techniques had to be applied for logistical reasons. However, the Cholestech LDX System has been proven to have satisfactory accuracy and utility for lipid measurements [47, 48].

In summary, we found that autoregulation of microcirculation of the skin is affected by type 1 diabetes in children. The higher peak perfusion in children with type 1 diabetes might be attributed to a decline in the vasoconstrictive ability of arteriolar smooth muscle cells upstream of capillary beds.

Duality of interest The Cholestech LDX System was provided by AstraZeneca Austria. The authors declare that there is no further duality of interest associated with this manuscript.

Contribution statement AWE, GHS and RK were responsible for the conception and design of the study. OS and MEG drafted the manuscript. OS, AH, AWE, MF, BRM, ES, KN, AG, CM, SZ, GHS, RK and MEG analysed and interpreted the data. AH, AWE, MF, BRM, ES, KN, AG, CM, GHS, RK and MEG critically revised the manuscript and contributed to the discussion. SZ was responsible for statistical data analysis and critically revised the manuscript. All authors approved the final version of the manuscript.

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