ORIGINAL RESEARCH

Non-invasive, Complex Examination of Micro- and Macrovascular System of Patients with Type 1 Diabetes Mellitus with or Without Vascular Complications

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ABSTRACT

Objective: We examined the vascular system, from the microvasculature to the aorta, in diabetes mellitus, using non-invasive methods.
Methods: We enrolled patients with type 1 diabetes: 17 patients without complications (DMW) and 19 patients with clinically manifest complications (DMC). Control group was represented by 34 healthy volunteers (C). We examined microvascular function with laser-Doppler flowmetry, using post-occlusive reactive hyperemia test and local heating. Arterial stiffness was studied by arteriograph, determining augmentation index and pulse wave velocity. We measured serum levels of sE-selectin and sICAM-1, markers of endothelial dysfunction.
Results: Microvascular reactivity was significantly reduced in DMC-, and tendentiously in DMW groups. sE-selectin level was significantly higher in DMC group than in controls. Arterial stiffness was the highest in the DMC group and the lowest in the DMW group. Heart rate was significantly higher in both diabetic groups compared to controls. Time to maximum flow during PORH test tended to be the shortest in DMW group.
Conclusions: Our results confirm impairment of the microvascular system in diabetic patients, even in early, uncomplicated stage of the disease, and might demonstrate diffuse hyperkinesis in the vascular system, resulting from the insulin effect or refering to the "vasodilation phase" of diabetes mellitus.

Keywords: type 1 diabetes mellitus, laser-Doppler flowmetry, arteriograph, microvascular function, arterial stiffness

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INTRODUCTION

Patients suffering from type 1 or type 2 diabetes mellitus are at 2–4 fold risk of cardiovascular events [1]. Vascular function was shown to be impaired at an early stage of the disease, before the onset of microalbuminuria. As a first step, in response to various noxious stimuli, loss or dysregulation of homeostatic endothelial mechanisms — characterized by increased expression of adhesion molecules, increased synthesis of proinflammatory and prothrombotic factors, increased oxidative stress and abnormal modulation of vascular tone — occurs. Thus, endothelial dysfunction is closely associated with subsequent development of diabetic microvascular complica-

tions, both in type 1 and 2 diabetes [2]. Retinopathy, which is the leading cause of blindness in younger patients, nephropathy, which is the leading cause of end-stage renal disease in the Western world, and diabetic neuropathy are the most important clinical manifestations of the microvascular damage [3]. Endothelial dysfunction in diabetic patients can contribute to further increase of arterial stiffness, resulting in macrovascular complications as well. It has been demonstrated previously, that in patients with 5–10 year long history of diabetes despite preserved renal function subtle increase in blood pressure and large-artery stiffness can be observed. Similarly, type 1 diabetes is associated with accelerated stiffening of the elastic arteries. As a result, in terms of cardiovascular (CV) risk, the presence of diabetes is equivalent to 15 years of chronological age in non-diabetics [4].

Recent reports have demonstrated that endothelial dysfunction is an independent predictor of future cardiovascular events in patients with atherosclerotic risk factors, in patients with stable ischemic heart disease, and in patients with acute coronary syndromes [5]. Therefore, the assessment of endothelial integrity and endothelial function has become of critical importance for the understanding of vascular pathophysiology and its clinical implications [6].

According to the clinical practice, assessment of retinopathy, symptoms of diabetic foot and microalbuminuria are performed every year, routinely. Unfortunately, in this case the clinicians detect only definitive microvascular anomalies, not early vascular functional impairment. In the past years, novel, non-invasive techniques have been developed in order to determine micro- and macrovascular damage, already before the onset of symptoms. On the other hand, the relative lack of standardized methods and their implementation in large-scale outcome studies provides only insufficient evidence to support these applications for clinical use at the present time [6]. Evaluation of micro- and macrovascular function represents an extensive area of clinical research.

Among various methods implemented in determination of microvascular function, the non-invasive laser-Doppler flowmetry of the skin has been used frequently. This method is based on the reflection of a beam of laser light, which undergoes changes in wavelength (Doppler shift) when it reflects from moving blood cells [7]. Postocclusive reactive hyperemia (PORH) test is used to estimate microvascular reactivity [8]. Local thermal hyperemia leads to a temperature-dependent increase in the cutaneous flow and achieves a maximal vasodilation between 42–44ºC. This maximal vasodilation corresponds to the maximal vasodilator capacity of the vessels [7]. Endothelial function can be detected also by measuring the plasma levels of endothelium-derived regulatory proteins, such as soluble endothelial-leukocyte adhesion molecule 1 (sE-selectin), soluble intercellular adhesion molecule 1 (sICAM-1), vascular cell adhesion molecule 1 (VCAM-1) and von Willebrand factor (vWF) [2]. Concerning the macrovascular system, augmentation index (AIX) and pulse wave velocity (PWV) are parameters of arterial stiffness of the peripheral resistance vessels and the aorta, respectively. These parameters were shown to be strong indicators of cardiovascular risk [9].

Due to high cardiovascular risk in diabetic patients, the screening of micro- and macrovascular function holds importance, already in the early phase of the disease. Therefore, in the current study, we aimed to broadly examine the vascular system from the microvasculature to the aorta, using non-invasive methods in patients suffering from type 1 diabetes mellitus. Data were compared to healthy volunteers. Patients with or without microvascular complications were separately analyzed in respect to endothelial dysfunction and early signs of arterial stiffness. Based on our results, reduced peripheral microvascular reactivity indicates the systemic endothelial impairment, accompanied by clinically detectable microvascular complications in diabetic patients.

MATERIALS AND METHODS

PATIENTS

Subjects with type 1 diabetes mellitus were recruited from the diabetes outpatient clinic of our hospital. We enrolled 36 patients with diabetes (DM I., 4 male, 32 female), age ranged from 22–47 years. Patients with hypertension were excluded from the study. We divided the DM I. group into two subgroups, based on the presence of the microvascular complications of diabetes. Retinopathy was examined by ophthalmologic evaluation, diagnosis of neuropathy confirmed by neurological examination and patients regularly underwent urine analysis to establish microalbuminuria. Seventeen patients had no diabetic complications (diabetes without complications, DMW group), age ranged from 22–47 years, 3 male, 14 female, diabetes duration was 3–43 years. Nineteen patients had microvascular complications (diabetes with complications, DMC group), age ranged from 25–45 years, 1 male, 18 female, diabetes duration was 13–33 years. As regarding medications aside from insulin, two of the diabetic patients received L-Thyroxin (75 and 100

TABLE 1. Baseline characteristics of the DMW and DMC patients groups

	DMW	DMC	C
N	17	19	34
Mean age ± SE	30 ± 1.56	32.63 ± 1.3	32.21 ± 1.68
Gender (male/female)	3/14	1/18	13/21
Systolic blood pressure (mmHg)	127 ± 3.09	131 ± 4.64	125 ± 2.46
Diastolic blood pressure (mmHg)	72 ± 1.48	77 ± 1.58	73 ± 1.34
Heart rate (beat/s)	77 ± 2.34***	81 ± 2.22***	67 ± 1.5
BMI (kg/m²) ± SE	24.1 ± 0.52	23.55 ± 0.85	23.12 ± 0.56
Current smoker, n (%)	3 (17.65%)	2 (10.53%)	8 (23.53%)
History of tobacco use, n (%)	0	2 (10.53%)	2 (5.88%)
Blood glucose level (mmol/l)	8.37 ± 0.89***	9.14 ± 1.19***	4.62 ± 0.1
Total cholesterol (mmol/l)	4.51 ± 0.24	4.61 ± 0.18	4.89 ± 0.13
Triglycerides (mmol/l)	0.97 ± 0.19	0.93 ± 0.09	1.13 ± 0.11
LDL-cholesterol (mmol/l)	2.28 ± 0.16	2.38 ± 0.18	2.57 ± 0.11
HDL-cholesterol (mmol/l)	1.78 ± 0.12	1.8 ± 0.09	1.78 ± 0.08
C-reactive protein (mg/l)	2.29 ± 0.95	1.61 ± 0.42	1.74 ± 0.49
Diabetes duration (ys)	13.29 ± 2.27###	21.16 ± 1.39	
Insuline consumption (unit/kg)	0.7 ± 0.05	0.68 ± 0.05	
Hb1Ac (%)	7.89 ± 0.22	7.73 ± 0.24	
Fructosamine (µmol/l)	364.41 ± 11.55	360.55 ± 14.02	

Results are expressed as mean ± SE.
***: p<0.001 compared to control (C). ###: p<0.001 compared to DMC

µg, respectively), and one diabetic patient was on benfotiamine therapy for her neuropathy.

Controls were healthy volunteers (C, 13 male, 21 female), age ranged from 20–49 years. Exclusion criteria in the control group were diabetes mellitus, hypertension, cardiovascular disease and taking medications that can interfere with vascular compliance.

All participants gave an informed consent, and the study protocol was approved by the Ethics Committee for Scientific Researches of the Scientific Council for Healthcare Services. Detailed patient demographics are shown in Table 1.

ASSESSMENT OF PERIPHERAL MICROVASCULAR FUNCTION BY LASER DOPPLER FLOWMETRY

Measurements were performed by a single operator after the subjects had been resting supine for 15 minutes in a quiet room. Temperature was adjusted to 22 ± 1 ºC. Patients were asked to avoid consuming caffeine or smoke for at least 30 minutes before the examination. For the examination of the microcirculation we used a Periflux System 5000 Laser-Doppler Perfusion Monitor, with PF 5010 LDPM Unit and PF 5020 Heating Unit and suitable laser-Doppler probes (780 nm wavelengths, Perimed AB, Sweden). Data processing was made by Perisoft for Windows 2.1. Probes were fixed with an adhesive tape to the inner side of the right forearm, at a maximum 5 cm distance from the internal crook of the arm. Basal perfusion was recorded for 2 minutes, then postocclusive reactive hyperemia test was performed. Arterial occlusion was induced by inflating the tourniquet of a sphygmomanometer, positioned on the upper arm, to ≥200 mmHg for 2 minutes. Basal perfusion and the maximal blood flow after the release of the compression were measured in perfusion units (PU), and change of flow (ChF-PORH) has been calculated as the percentage peak/basal flow ratio (%). Time to maximal flow (TM-PORH) was measured in seconds. After skin perfusion had resumed basal levels, and at least 5 minutes after cuff-release, probes were heated to 44ºC for 10 minutes for the local heating test. Basal flow before heating and the maximal blood flow during the local hyperthermia were measured in perfusion units (PU), and change of flow (ChF-LH) has been calculated as the percentage ratio of maximal/basal flow (%). Time to maximum (TM-LH) was measured in minutes. Reproducibility of laser-Doppler method was described elsewhere [7,10,11].

ASSESSMENT OF ARTERIAL STIFFNESS

Arterial stiffness was determined by arteriograph. This method uses an oscillometric technique to determine augmentation index (AIX) and pulse wave velocity (PWV) by analysis of the pressure curves registered on the upper arm. The aortic pulse wave comprises the initial pressure wave from the left ventricle and a later reflected wave. Augmentation index is calculated by the pressure difference between the first and second wave in relation to the pulse pressure (given in %). AIX, corresponding to the stiffness of the peripheral resistance vessels, can have both positive and negative values, the "more negative" the better. Pulse wave velocity is determined by the elapsed time between the onsets of the two waves and the distance from jugulum to symphysis, which refers to the distance the wave travels along (given in m/s). Theoretical basis and clinical validation of the arteriograph was extensively described elsewhere [12,13].

AIX and PWV were measured with TensioMed Arteriograph (TensioMed, Hungary), data processing was performed with TensioClinic 1.9.9.12. software. Cuff was wrapped tightly around the upper arm on the dominant side. Patients were asked to lie still during the procedure. Every measurement was repeated twice, within 15 minutes. Results were calculated as the mean of the two measurements.

ENDOTHELIAL SOLUBLE MARKERS

Venous blood samples for the biochemical analysis were drawn from the cubital vein after the vascular examination. Plasma and serum samples were prepared by centrifugation at 3000 rpm for 10 minutes at room temperature, and stored at -80⁰C until processing in aliquots.

Soluble intercellular adhesion molecule-1 (sICAM-1) was measured by a commercially available ELISA Kit (R&D Systems, Europe Ltd.). Samples were measured in duplicates in 60-fold dilution. The minimum detectable dose of sICAM-1 was 0.35 ng/ml. Soluble sE-selectin levels were determined by the commercial ELISA kit (R&D Systems, Europe Ltd.). Samples were measured in duplicates in 20-fold dilution. The minimum detectable dose of sE-selectin was less than 0.1 ng/ml.
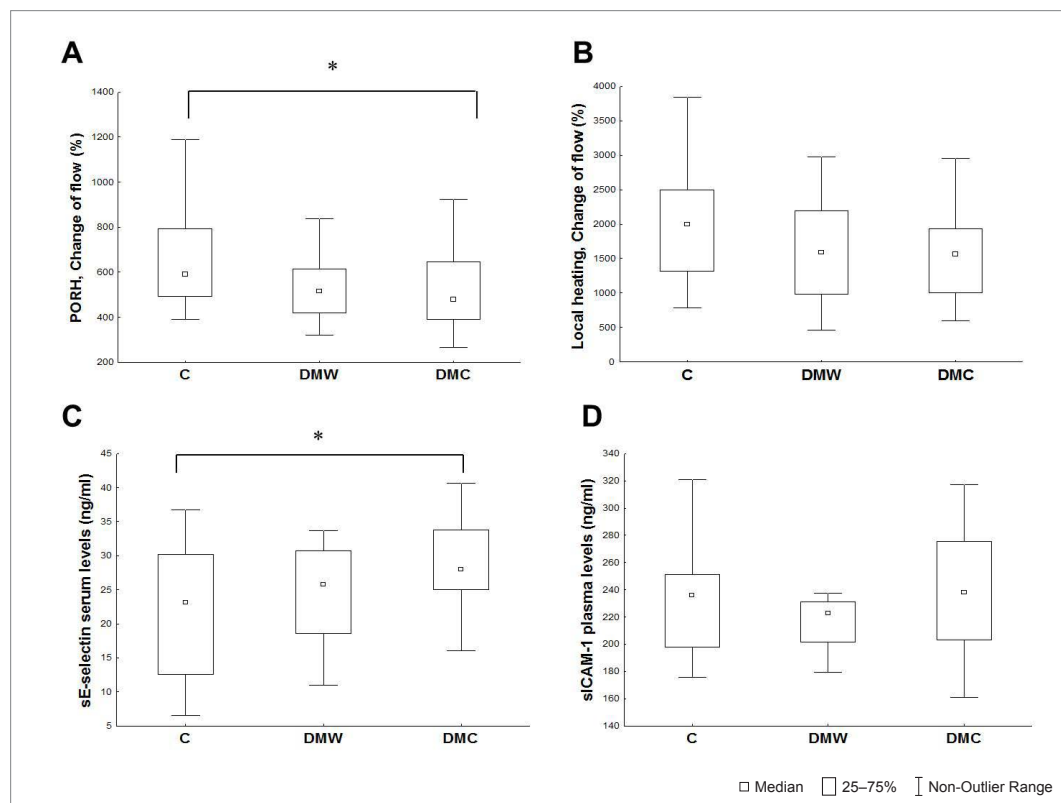
FIGURE 1. Microvascular- and endothelial dysfunction in diabetic (DMW and DMC) and control (C) patient groups. Change of flow during the PORH test (A), change of flow during local heating (B) and serum levels of sE-selectin (C) are expressed as medians and 25–75% IQ range. C: control group, DMW: type 1 diabetes mellitus without complications, DMC: type 1 diabetes mellitus with complications, PORH: Post-occlusive reactive hyperaemia. Non-outlier range is represented by bars. *: p<0.05

STATISTICAL ANALYSIS

Statistica for Windows 8.0 software was used for statistical analysis. Nonparametric Kruskal-Wallis ANOVA and Mann-Whitney U-test were used to determine differences among the study groups. Spearman rank-correlation was used to show relationships between variables. General regression model was used to adjust baseline demographic parameter differences.

Demographical data are listed in mean ± standard error of the mean. Results of the measurements are expressed as median (M) with interquartile range (IQR). Results are considered to be significant at p<0.05.

RESULTS

PATIENT CHARACTERISTICS

Patients' demographic data are shown in Table 1. Mean age, systolic and diastolic blood pressure and BMI were comparable in all groups. Heart rate and blood glucose level were significantly higher in both diabetic groups compared to the control. Regarding the DMW and DMC groups, there were no significant differences in fructoseamine, HbA1c, glucose levels and insulin consumption. Diabetes duration was significantly longer in patients with diabetic complications than in the DMW group.

MICROVASCULAR- AND ENDOTHELIAL DYSFUNCTION

The change of flow during the PORH test was the highest in the control group (C) and the lowest in diabetic patients with complications (DMC) (Figure 1A). The difference was significant between C and DMC groups (C: M: 591.71% (IQR: 493.15–792.21%), DMW: M: 517.66% (IQR: 419.21–612.98%), DMC: M: 477.34% (IQR: 391.67–646.33%), C vs. DMC: p = 0.024). The change of flow during local heating tended to be higher in the control group compared to DMC patients (C: M: 1998.66% (IQR: 1319.61–2499.25%), DMW: M: 1583.35% (IQR: 983.13–2198.63%), DMC: M: 1561.22% (IQR: 1006.22–1932.42%, C vs. DMC: p = 0.093, Figure 1B).

Plasma sE-selectin level, representing increased adhesion and permeability to leukocytes, was the highest in the DMC group and the lowest in control patients, the difference between these two groups was significant (C: M: 23.15 ng/ml (IQR: 12.60–30.20 ng/ml), DMW: M: 25.71 ng/ml (IQR: 18.63–30.68 ng/ml), DMC: M: 28.05 ng/ml (IQR: 25.02–33.82 ng/ml), C vs. DMC: p = 0.019, Figure 1C). In contrast, we found no difference in the level of the common inflammatory marker, sICAM-1 between patient groups (C: M: 235.75 ng/ml (IQR: 197.90–251.10 ng/ml), DMW: M: 223.00 ng/ml (IQR: 201.60–230.95 ng/ml), DMC: M: 237.78 ng/ml (IQR: 203.00–275.60 ng/ml), p = 0.33, Figure 1D).
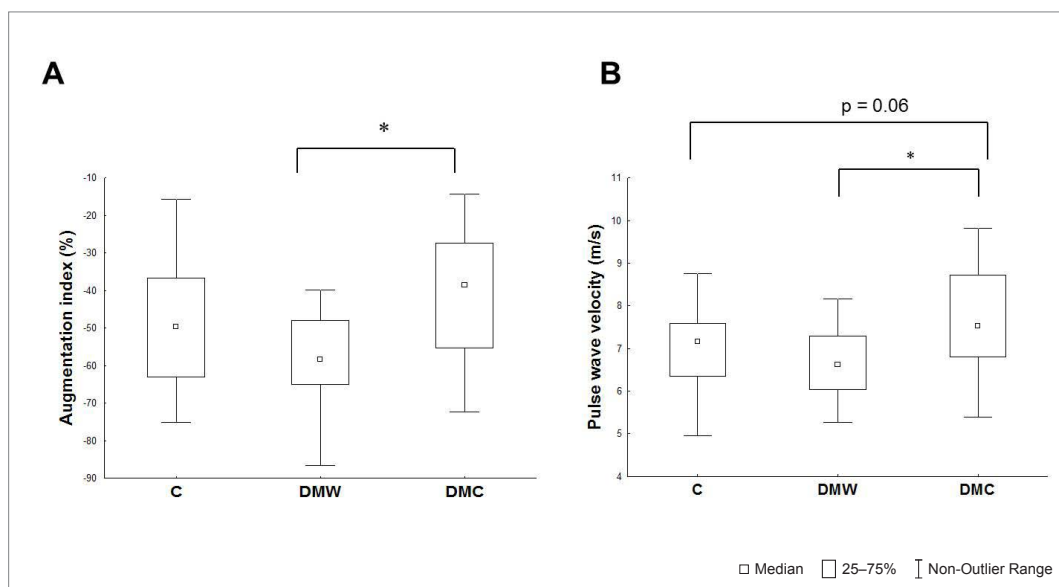
FIGURE 2.   Arterial stiffness and endothelial function in the three patient groups. Augmentation index (AIX) of the brachial artery (A), pulse wave velocity (PWV) in the aorta (B) and plasma levels of sICAM-1 (C) are expressed as medians and 25–75% IQ range. C: control group, DMW: type 1 diabetes mellitus without complications, DMC: type 1 diabetes mellitus with complications, *: p<0.05, $: p = 0.06.
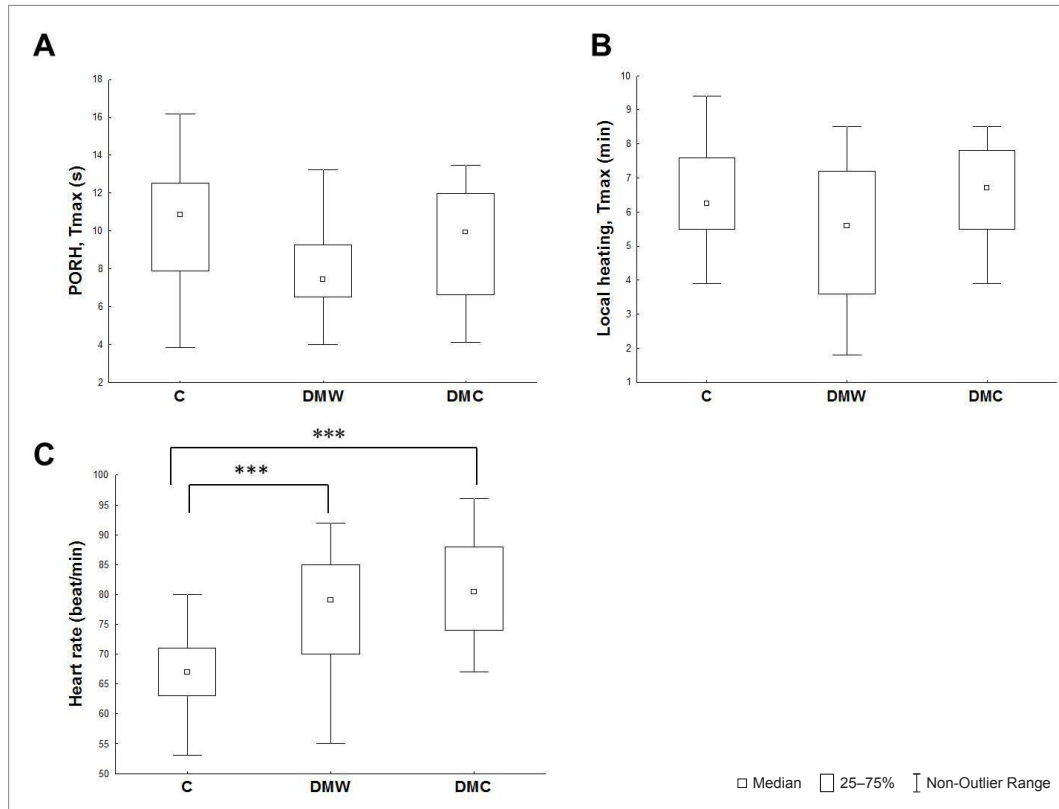
FIGURE 3. Hyperkinesis in the circulation. Time to maximum during PORH test (A), time to maximum during local heating (B) and heart rate (C) are expressed as medians and 25−75% IQ range. PORH: Post-occlusive reactive hyperaemia, C: control group, DMW: type 1 diabetes mellitus without complications, DMC: type 1 diabetes mellitus with complications, ***: p<0.001.

ARTERIAL STIFFNESS

Augmentation index (AIX) was the worse in the DMC group. Interestingly, the DMW patient group had significantly better results of AIX than DMW (C: M (median): -49.56% (IQR: -63.01−-36.6%), DMW: M: -58.17% (IQR: -64.91−-47.85%), DMC: M: -38.52% (IQR: -55.36−-27.3%), DMW vs. DMC: p = 0.016, Figure 2A). Similar pattern was observed when examining pulse wave velocity (PWV). The results of the DMW group were significantly better compared to DMC, although the difference between C and DMC group was only tendentious (C: 7.15 m/s (IQR: 6.35−7.58 m/s), DMW: 6.62 m/s (IQR: 6.03−7.3 m/s), DMC: 7.53 m/s (IQR: 6.8−8.72 m/s), DMW vs. DMC: p = 0.013, C vs. DMC: p = 0.06, Figure 2B).

HYPERKINESIS IN THE SYSTEMIC AND MICROVASCULAR CIRCULATION

Although the patients did not receive any medications influencing heart frequency, heart rate was significantly higher in both diabetic groups than in controls (C: M: 67/min (IQR: 63−71/min), DMW: M: 79/min (IQR: 70−85/min), DMC: M: 80.5/min (IQR: 74−88/min), C vs. DMW: p<0.0001 and C vs. DMC: p<0.00001, Figure 3C).

Interestingly, the TM parameter of the PORH test, representing the time needed to reach the maximum flow, tended to be the shortest in the DMW group (C: M: 10.85 s (IQR: 7.91−12.53 s), DMW: M: 7.46 s (IQR: 6.52−9.25 s), DMC: M: 9.95 s (IQR: 6.64−11.99 s, C vs. DMW: p = 0.029, Figure 3A). Time to maximum flow during local heating was comparable in all study groups (C: M: 6.25 min (IQR: 5.5−7.6 min), DMW: M: 5.6 min (IQR: 3.6−7.2 min), DMC: M: 6.7 min (IQR: 5.5−7.8 min), p = 0.15, Figure 3B).

CORRELATIONS

After adjustment to age, BMI, systolic and diastolic blood pressure, heart rate, gender and smoking, the differences in change of flow during PORH test, pulse wave velocity (PWV) and sE-selectin remained significant in a general regression model.

In diabetic patients, diabetes duration correlated significantly with AIX and PWV (AIX: r = 0.6, PWV: r = 0.43, p<0.05, respectively) (Figure 4A, 4B). Significant inverse
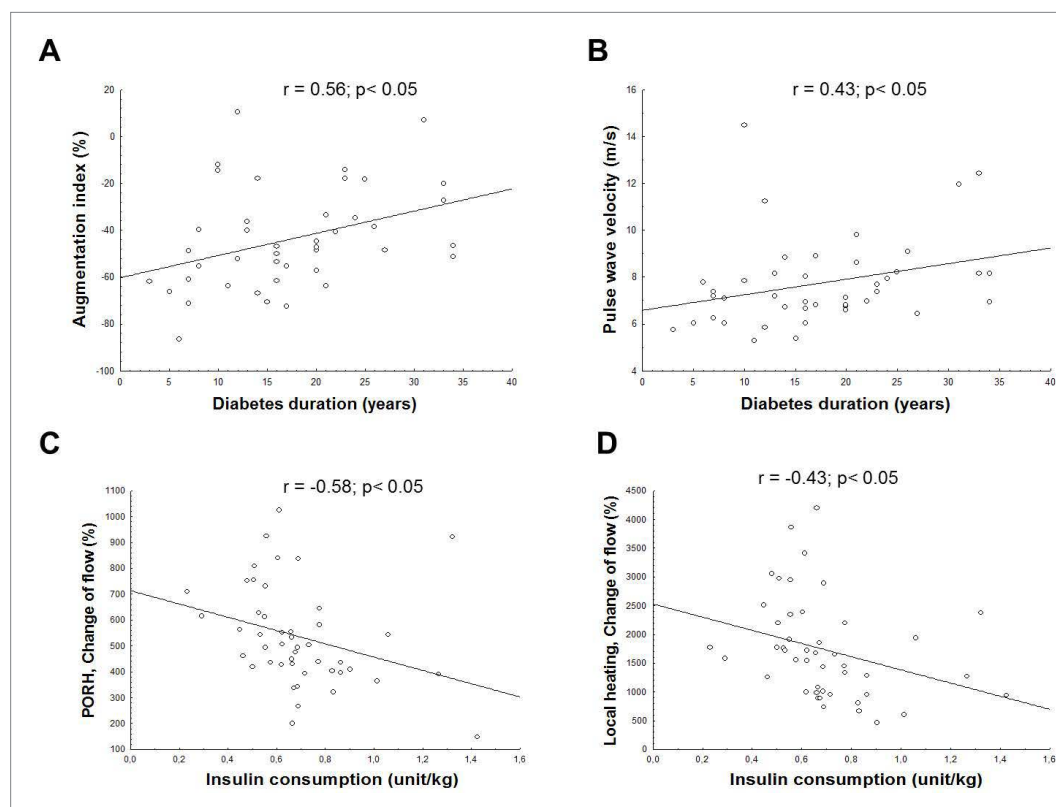
FIGURE 4. Correlations. There were significant correlations between diabetes duration and augmentation index (A, r = 0.6, p<0.05), diabetes duration and pulse wave velocity (B, r = 0.43, p<0.05), insulin consumption and change of flow during PORH test (C, r = −0.58, p<0.05) and insulin consumption and change of flow during local heating (D, r = −0.43, p<0.05) in diabetic patients.

correlation was found between the insulin consumption (total daily subcutaneous insulin dose/patients weight — IU/kg) and change of flow during PORH test (r = −0.58, p<0.05) (Figure 4C) and change of flow during local heating (r = −0.43, p<0.05) (Figure 4D).

As expected, there were significant correlations between change of flow during PORH test and change of flow during local heating (r = 0.71, p<0.05), AIX and PWV (r = 0.61, p<0.05) and sE-selectin and sICAM-1 (r = 0.3, p<0.05).

TABLE 2. Results

	DMW	DMC	C
a) Laser–doppler flowmetry			
PORH test, Change of flow (%)	649.38 ± 37.42	541.02 ± 38.1	523.62 ± 43.14*
PORH test, Time to maximum (s)	10.58 ± 0.65	8.38 ± 0.7	10.36 ± 1.17
Local heating, Change of flow (%)	2094.55 ± 173.27	1623.35 ± 182.55	1592.63 ± 160.28
Local heating, Time to maximum (min)	6.44 ± 0.24	5.48 ± 0.51	6.74 ± 0.3
b) Arteriograph			
Augmentation index (%)	−46.15 ± 3.88	−57.72 ± 3.04	−41.56 ± 4.52
Pulse wave velocity (m/s)	6.92 ± 0.19	6.67 ± 0.21	7.67 ± 0.29#,$
c) Levels of adhesion molecules			
Plasma levels of sICAM–1 (ng/ml)	233.59 ± 7.21	219.16 ± 12.56	238.54 ± 11.38
Serum levels of sE–selectin (ng/ml)	21.81 ± 1.81	25.77 ± 2.62	29.65 ± 2.23*

Data are given in mean ± SE.
*: p<0.05 compared to C. #: p<0.05 compared to DMW. $: p = 0.06 compared to C.

DISCUSSION

Despite the fact that aggressive treatment of conventional cardiovascular (CV) risk factors may lead to marked reduction of CV mortality in diabetic patients, the absolute risk of cardiovascular disease (CVD) remains higher in patients with diabetes compared to those without DM [1]. Diabetic autonomic neuropathy (as a result of microangiopathy), a serious and common complication of diabetes can lead to silent myocardial ischemia, which may impair the recognition of myocardial infarction and may delay revascularization therapy [14]. In type 1 diabetes mellitus vascular changes of large and small arteries occur at an early stage of the disease, even before the manifestation of clinical complications of diabetes [15,16]. These findings suggest that appropriate screening of both micro- and macrovascular complications are very important, mainly in the young population. In our study we examined different parts of the vascular system (capillaries, small and large arteries) and endothelial function with non-invasive reproducible [7,10,11,12] methods, which can also be used in clinical practice.

Postocclusive reactive hyperemia (PORH) and local heating test in type 1 diabetes mellitus

The laser-Doppler method is widely used to assess microvascular function in the skin. Postocclusive reactive hyperemia (PORH) represents a complex microvascular response to an acute period of ischaemia, while maximal thermal vasodilation corresponds to the vasodilator capacity of the vessels [7]. Using the laser-Doppler method we found, that change of flow during the PORH test was slightly reduced in uncomplicated type 1 diabetes mellitus and significantly impaired in type 1 diabetes with confirmed vascular complications. Change of flow during local heating tended to be higher in the control group compared to diabetes with complications. These results point to an impairment of microvascular function of the skin in patients with clinically proven microvascular complications. Importantly, microvascular damage detected in the peripheral microcirculation of the skin observed simultaneously with the well-known clinical complications, suggests systemic microvascular impairment.

In concordance with our findings, previous studies demonstrated endothelial and microvascular dysfunction in early, uncomplicated type 1 diabetes mellitus [11,17,18] as well. Skrha and colleagues reported similar results in case of peak flow of PORH, and maximal flow of thermal hyperemia in type 1 diabetic patients with and without microvascular complications [19]. Tur and colleagues found that in patients with type 1 diabetes peak blood flow during PORH test was significantly lower compared to control subjects, and the ratio of peak flow and time to peak was lower in patients with retinopathy than in patients without microvascular complications [20]. Schalkwijk et al. concluded in their review that endothelial function becomes impaired before the onset of microalbuminuria, at an early stage of the disease [2].

According to our results, microvascular dysfunction occurs already in the early phase of diabetes, before the onset of clinical complications. We assume that laser Doppler flowmetry might be a suitable method to detect the functional abnormalities of the microcirculation. Since the functional impairment of the microvascular system occurs before the onset of the anatomical damage, using the laser-Doppler method an early microvascular defect can be detected before the initiation of the clinically evident microvascular complications.

Interestingly, we found significant inverse correlation between insulin consumption (daily insulin requirement, (unit/kg)) and change of flow during PORH test and local heating, respectively. These results suggest that by increased insulin consumption microvascular reactivity had reduced. The exact mechanism explaining the above results is not elucidated. The need for higher doses of insulin may refer to difficulties in the management of the metabolic status of the patients and may suggest poor glycaemic control. Excessive hyperglycaemia can lead to the up-regulation of alternative glucose metabolic pathways, which can cause more severe damage in the microvascular system.

Endothelial soluble markers in type 1 diabetes mellitus

The microvascular endothelium, with its very large surface area and synthetic capacity, is the most important determinant of plasma levels of endothelium-derived mediators [2], therefore sE-selectin can be considered as a marker of microvascular endothelial dysfunction. In our study we found elevated levels of sE-selectin in diabetic patients. In consistence with our results, soluble sE-selectin levels were also found to be higher in diabetic patients in other studies [19,21]. Results of laser-Doppler flowmetry and significantly higher sE-selectin levels altogether refer to a functional disturbance of the microvascular system of the patients with type 1 diabetes mellitus detected even in early stage.

Arterial stiffness in type 1 diabetes mellitus

Arterial stiffness is a powerful, independent predictor of

cardiovascular disease [22]. Pulse wave velocity (PWV) of the aorta is considered to be a direct marker of arterial stiffness, referring the stiffness of large arteries. PWV is a strong predictor of mortality in diabetes mellitus, too. Augmentation index is an indirect marker of arterial stiffness, represents mainly the peripheral resistance vessels, and is closely correlated with the cardiovascular risk [12]. Systolic blood pressure, pulse pressure, age, heart rate and different medications (i.e. angiotensin-converting enzyme inhibitors, statins) can affect the measured values of arterial stiffness [12,22].

Unexpectedly, in our study, the DMW group had significantly better parameters of arterial stiffness (augmentation index and pulse wave velocity) than patients with complicated type 1 diabetes mellitus. However, there are some other studies available in the literature with corresponding conclusions. Vervoort and colleagues examined the forearm blood flow (FBF) in type 1 diabetic patients, and found that the basal FBF was elevated in diabetic patients compared to controls. The vascular response to norepinephrine was increased in the diabetic group, caused by lower baseline arterial plasma norepinephrine concentrations [23]. On contrary, several other studies found elevated large and small artery stiffness [15,24] and diffuse arterial wall stiffening and thickening [16] in type 1 diabetic patients, even in the absence of any diabetic vascular complications.

We assume that our results can be explained by the insulin effect, or represent the manifestation of the early vasodilative phase of diabetes mellitus. Westerbacka et al. examined the effect of physiological doses of insulin on large artery function by applanation tonometry and pulse wave analysis. They found that insulin caused a significant decrease in both central augmentation and peripheral augmentation index, which means an acute reduction in arterial stiffness [25]. Insulin has also been found to have a long-term beneficial effect on the progression of carotid intima-media thickness, another indicator of atherosclerosis [26]. According to Schalkwijk and colleagues, in reasonably well-controlled type 1 diabetes mellitus, endothelium-dependent and -independent vasodilation of resistance and conduit arteries are neither impaired nor enhanced. In addition, early, uncomplicated type 1 diabetes is accompanied rather by dilation, than constriction of small and large blood vessels [2]. This statement raises the possibility that better results of arterial stiffness measurements in early, uncomplicated type 1 diabetes mellitus do not necessarily refer to better clinical status regarding complications. Therefore, these parameters could not be used in evaluation of the vascular system and establishment of the cardiovascular prognosis by themselves. We

found significant correlation between diabetes duration and arterial stiffness parameters which might demonstrate that arterial stiffness raises in parallel with the duration of diabetes. This finding could resolve the conflict between the results in the literature, mentioned above.

Hyperkinesis in the systemic and microvascular circulation

In our study, the heart rate was significantly higher in both diabetic groups compared to controls. Time to maximum flow during PORH test tended to be shorter in DMW patients. Skrha et al. found the same pattern in case of TM-PORH without significances, as well [19]. In contrary, Gomes and colleges found a longer TM-PORH among diabetic patients [11]. None of these studies used the TM parameters by themselves, but for the calculation of velocity or area under curve. Meyer et al. found a delay of the PORH in diabetic patients. They explained the result as an effect of the redistribution of total skin microcirculation, caused by the insulin-induced increase in the capillary blood flow [27]. The shortening of the time parameters and the elevated heart rate might indicate diffuse hyperkinesis in the whole circulation system, which can be caused by the effect of insulin or can be the manifestation of the hyperkinetic stage of the disease [2]. In summary, we found decreased arterial stiffness and hyperkinesis in the systemic and microvascular circulation in patients with diabetes mellitus without complications. Several mechanisms might explain our results. We assume the common factor can be the hyperkinetic effect of insulin, although this hypothesis needs further investigation. Though both diabetic groups used insulin, in diabetic patients with microvascular complications the hyperkinetic effect of insulin may not occur because of the damage of the capilla-ries. Another possibility is that our findings represent the early, dilative stage of type 1 diabetes mellitus, reported previously [2].

PERSPECTIVES

We demonstrated the impairment of the microcirculation in type 1 diabetic patients, even in the early, uncomplicated phase of the disease. We found impairment in the microcirculation of the skin, corresponding to the clinically proven microvascular complications. On the other hand, we reported an unexpected hyperkinetic circulation which might be caused by the insulin effect or could demonstrate the vasodilation phase of the disease, communicated previously by other studies. According to our results, laser-Doppler flowmetry might represent a useful screening and follow-up method in daily clinical practice. If insulin can

decrease arterial stiffness, high risk patients with type 2 diabetes should be introduced early and intensive insulin therapy as a part of cardiovascular prevention.

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LIST OF ABBREVIATIONS

AIX	augmentation index	**IU**	international unit
BMI	body mass index	**M**	median
C	control	**PORH**	Postocclusive reactive hyperemia
ChF	change of flow	**PU**	perfusion unit
CV	cardiovascular	**PWV**	pulse wave velocity
CVD	cardiovascular disease	**sE-selectin**	soluble endothelial-leukocyte adhesion molecule 1
DM	diabetes mellitus		
DM I.	type 1 diabetes mellitus	**sICAM-1**	soluble intercellular adhesion molecule 1
DMC	diabetes with complications	**TM**	time to maximal flow
DMW	diabetes without complications	**VCAM-1**	vascular cell adhesion molecule 1
FBF	forearm blood flow	**vs**	versus
HbA1c	Hamoglobin A1c	**vWF**	von Willebrand factor
IQR	interquartile range		