Original Article
Characterization of macro- and microvascular function and structure in patients with type 2 diabetes mellitus

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Abstract: Objective: Diabetes mellitus (DM) leads to accelerated progression of arteriosclerosis with an increased risk of coronary events in comparison to non-diabetic patients with coronary artery disease (CAD). The precise and early detection of DM-induced vascular alterations is crucial to identify patients with high risk for cardiovascular complications. Thus, we aimed at simultaneously characterizing functional, physicomechanical, and structural vascular alterations in diabetic patients using a non-invasive approach. Research Design and Methods: In CAD patients with and without type 2 diabetes mellitus (n=50), we non-invasively measured flow-mediated dilation (FMD) of the brachial artery as a marker for endothelial function, fractional diameter changes (FDC) as a marker for physicomechanical properties, intima-media thickness (IMT) as a marker for structural properties, and forearm blood flow (FBF) as a marker for microvascular function. Results: DM was associated with reduced FMD (2.5±0.2 vs 4.8±0.4%; p≤0.001) indicating impaired macrovascular endothelial function. In parallel, reduced FDC (0.024±0.002 vs 0.034±0.004; p≤0.05) and increased IMT (0.38±0.01 vs 0.31±0.01mm; p≤0.001) indicated increased stiffness and enhanced structural alterations. Furthermore, reduced forearm blood flow during reactive hyperemia (10.7±1.0 vs. 15.3±1.4mL/min*100mL; p≤0.05) was found indicating microvascular dysfunction. Plasma glucose and HbA_{1c} correlated with FMD (glucose: r=-0.32; HbA_{1c}: r=-0.45), IMT (glucose: r=0.54; HbA_{1c}: r=0.48) and FBF (glucose: r=-0.30) suggesting diabetes-specific effects on vascular properties. Conclusion: In patients with CAD, DM leads to functional and structural vascular alterations of the peripheral vasculature which are determined by the control of the disease underlining the relevance of a strict control of the DM to prevent accelerated atherosclerosis.

Keywords: Diabetes mellitus, coronary artery disease, endothelial function, intima media thickness, microcirculation

Introduction

The number of patients with diabetes mellitus (DM) is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity [1]. It was estimated that there were 171 million people suffering from diabetes mellitus in 2000 and the number is expected to nearly double to 366 million people in North America and Europe by 2030 [1]. The expected rise in the number of diabetic patients will lead to an enormous increase in the socioeconomic burden. Beside the severe microvascular complications, which become clinically evident as diabetic nephropathy, retinopathy or neuropathy [2,3], macrovascular complications including coronary artery disease (CAD) are frequent among diabetic patients. DM leads to premature and accelerated atherosclerosis with an increased risk of cardiovascular events [4,5]. Moreover, myocardial ischemia due to coronary atherosclerosis commonly occurs without symptoms in patients with diabetes [6]. As a result, multivessel atherosclerosis is often present before ischemic symptoms occur and before treatment is instituted. A delayed recognition of various forms of arteriosclerotic complications undoubtedly worsens the prognosis for survival for many diabetic patients [7].

The early and effective detection of macrovascular and microvascular alterations is of highest interest for the primary and secondary prevention of cardiovascular disease and monitoring of optimal medical treatment. Recently, we have introduced a novel non-invasive methodology

simultaneously monitoring functional, physicomechanical, and structural properties of the brachial artery using high resolution ultrasound in a "one stop shop" fashion [8]. In a single session, the flow-mediated dilation (FMD), the fractional diameter change (FDC), and the intima-media thickness (IMT) of the brachial artery can be determined in the same vascular segment simultaneously.

The aforementioned non-invasive methods may help in the early detection of vascular functional and structural alterations and may identify patients with high risk for cardiovascular complications. We aimed to assess diabetes-associated additive vascular functional, physicomechanical and structural alterations in the conduit arteries as well as functional alterations in the microvascular bed in patients with CAD using the "one-stop shop" approach complemented with venous occlusion plethysmography, a standard read-out of microvascular function.

Materials and methods

Study populations

We studied 50 patients with CAD with or without type 2 diabetes mellitus (65 ±1 years). All subjects were screened by clinical history, physical examination, ECG at rest, and routine chemical analysis. Diabetes mellitus was defined by symptoms of hyperglycemia and casual plasma glucose ≥200 mg/dL, a fasting glucose concentration ≥126 mg/dL or medical antidiabetic treatment. Diabetic patients were treated by dietetic control, oral hypoglycemic agents, or insulin therapy. CAD was diagnosed by coronary angiography. Patients with chronic heart failure, chronic renal failure, a malignant disease, an inflammatory disease, vasculitis, or Raynaud's syndrome were excluded. The study was approved by the local ethics committee and written informed consent was obtained from all study subjects prior to enrolment.

Vascular studies

All investigations were performed in the morning between 8:00 and 11:00 a.m. in an air-conditioned room at a temperature of 23±2°C in supine position. Cigarettes, beverages containing caffeine, and alcohol were prohibited for at least 12 hours prior to the investigation. Each subject rested in a supine position quietly for at least 10 minutes before the first scan.

Flow-mediated dilation

Flow-mediated brachial artery dilation was measured as previously described [9,10]. Briefly, the diameter of the brachial artery (BA) was measured from images assessed with a 15 MHz linear array transducer (Vivid i, GE Healthcare) using an automated software analysis (Brachial Analyzer, Medical Imaging Applications, Iowa City, IA, USA). Following measurement of the baseline diameter of the brachial artery, a blood pressure tourniquet located at the proximal forearm was inflated to a pressure of 200 mmHg for 5 minutes. Brachial artery dilation following reactive hyperemia was recorded for 60-90 seconds after release of the cuff. Endothelium-independent dilation of the brachial artery was measured after sublingual nitroglycerin (NTG 400 µg). The time interval between end of hyperemia and NTG test was 20 min to the re-establish baseline conditions. Both FMD and endothelium-independent vasodilation following nitroglycerin were expressed as the percent increase compared to the diameter of the resting scans.

Fractional diameter change

Images for the measurement of FDC of the brachial artery were assessed during the same setting with the FMD as previously described [8]. In order to evaluate FDC, a scan was made in a longitudinal section with regards to a clear differentiation of the intima-media complex of the anterior and posterior wall. FDC was determined during one heart cycle and calculated as the difference between maximum systolic diameter and the minimum diastolic diameter in relation to the minimum diastolic diameter using an automated analysis system (Brachial Analyzer, Medical Imaging Applications, Iowa City, IA, USA).

Intima-media thickness

Images for the measurement of the brachial artery IMT were assessed during the same setting with the FMD as previously described [8]. In order to evaluate IMT, a scan was made in a longitudinal section with regards to a clear differentiation of the intima-media complex of the posterior wall. PC-based measurement of IMT was performed according to the method of

Table 1. Clinical characteristics of the study population

Parameter	Unit	Diabetes mellitus	Control	p value
n		25	25	
CAD	[n]	25	25	
Age	[years]	65 ± 1.4	66 ± 1.6	n.s.
Male sex	[n]	15	21	n.s.
Body mass index	[kg/m^2]	29.7 ± 1.0	26.6 ± 0.7	p≤0.05
Systolic blood pressure	[mmHg]	151 ± 5	142 ± 3	n.s.
Diastolic blood pressure	[mmHg]	87 ± 2	86 ± 2	n.s.
Mean arterial pressure	[mmHg]	108 ± 3	105 ± 2	n.s.
Triglycerides	[mg/dL]	142 ± 12	133 ± 9	n.s.
Total cholesterol	[mg/dL]	174 ± 9	182 ± 9	n.s.
LDL cholesterol	[mg/dL]	107 ± 8	111 ± 7	n.s.
HDL cholesterol	[mg/dL]	51 ± 2	52 ± 3	n.s.
Plasma glucose	[mg/dL]	148 ± 8	105 ± 8	p≤0.001
HbA$_{1c}$	[%]	7.0 ± 0.2	5.8 ± 0.2	p≤0.001
ASS	[%]	88	100	n.s.
Clopidogrel	[%]	20	68	p≤0.001
Beta blocker	[%]	80	84	n.s.
ACE-I or ARB	[%]	92	84	n.s.
Statin	[%]	84	96	n.s.
Oral antidiabetics	[%]	60	0	p≤0.001
Insulin	[%]	44	0	p≤0.001

CAD: coronary artery disease; LDL cholesterol: low density lipoprotein cholesterol; HDL cholesterol: high density lipoprotein cholesterol; ACE-I: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker.

Wendelhag et al. [11] using an automated analysis system (Artery Measurement System, AMS, Wallenberg Laboratory for Cardiovascular Research, Goteborg, Sweden).

Venous occlusion plethysmography

Forearm blood flow (FBF) was measured by mercury-in-rubber strain gauge plethysmography (Periquant 833, Gutman, Eurasburg, Germany) according to standard techniques as previously described [12]. FBF was measured at rest and during reactive hyperemia and expressed as mL•min^{-1}•100 mL^{-1} of tissue.

Statistical analysis

Data are expressed as means ± SEM. Comparisons between groups were analyzed for normal distribution using Shapiro-Wilk test and differences between groups by the unpaired Student t-test. Chi square test was used for comparison of non-continuous data. Univariate correlations were calculated using Pearson's coefficient (r). P-values ≤ 0.05 were accepted as statistically significant. Data processing was performed with the software modules of SPSS® (Statistical package for analysis in social sciences, Predic-

tive analysis software release 18, SPSS Inc., Chicago, USA).

Results

Patient characteristics

The clinical characteristics are presented in **Table 1**. The study groups were well matched for cardiovascular risk factors such as age, sex, blood pressure, or lipid levels. As expected, patients with DM had significantly increased body mass index (29.7±1.0 vs 26.6±0.7 kg/m^2; p≤0.05), plasma glucose concentration (148±8 vs 105±8 mg/dL; p≤0.001) and HbA$_{1c}$ (7.0±0.2 vs 5.8±0.2%; p≤0.001) as compared to non-diabetic patients. The medication was comparable between the study groups except for clopidogrel which was present in a lower percentage of diabetic patients (20 vs 68%; p≤0.001).

Endothelial function

FMD of the BA was significantly reduced (2.5±0.2 vs 4.8±0.4%; p≤0.001; **Figure 1A**) in patients with DM as compared to control subjects. Endothelium-independent dilation of the BA was comparable between the study groups
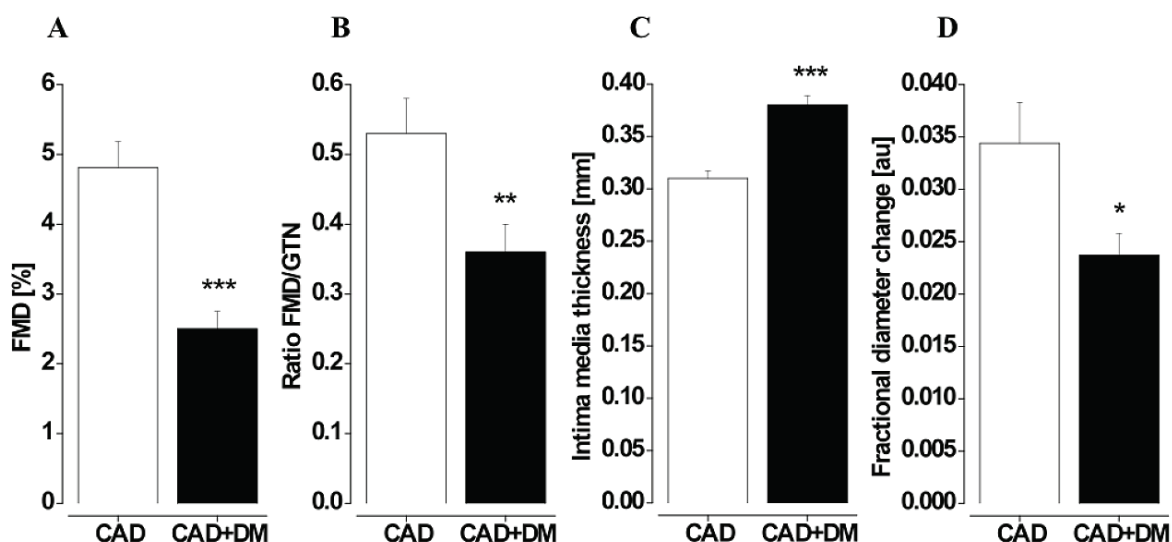
Figure 1. Vascular function and structure in conduit arteries. Flow-mediated dilation (FMD; **A**), ratio FMD/GTN response (**B**); fractional diameter change (**C**) and intima-media thickness (**D**) of the brachial artery were significantly impaired in patients with coronary artery disease and type 2 diabetes mellitus (CAD+DM) in comparison to control patients with coronary artery disease (CAD). DM is presented as white bars, C as black bars. * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

(8.4±0.7 vs 9.8±0.5%; p=n.s.). The ratio of FMD/GTN response was significantly lower in patients with DM (0.34±0.04 vs 0.53±0.05; $p \leq 0.01$; **Figure 1B**) thereby indicating endothelium specific vasodilator dysfunction of conduit arteries.

Physicomechanical properties

FDC of the BA was significantly lower in patients with DM as compared to control patients (0.024±0.002 vs 0.034±0.004 a.u.; $p \leq 0.05$; **Figure 1C**), indicating an increased arterial stiffness of the muscular conduit arteries.

Vascular structure: IMT of the BA was significantly increased (0.38±0.01 vs 0.31±0.01 mm; $p \leq 0.001$; **Figure 1D**) in patients with DM as compared to control patients. Increased IMT reflects accelerated structural alterations of the diabetic BA.

Microvascular function

Microvascular dysfunction in patients with diabetes mellitus was shown by reduced FBF at rest (2.2±0.2 vs 3.1±0.3 mL/min per 100mL tissue; $p \leq 0.05$) and during reactive hyperemia (10.7±1.0 vs. 15.3±1.4 mL/min per 100mL

tissue; $p \leq 0.05$).

Correlation of vascular function and structure with plasma glucose and HbA_{1c}

Plasma glucose concentration and HbA_{1c} correlated with FMD (glucose: r=-0.321, $p \leq 0.05$; HbA_{1c}: r=-0.447, $p \leq 0.01$), IMT (glucose: r=0.539, $p \leq 0.001$; HbA_{1c}: r=0.479, $p \leq 0.001$), and FBF during reactive hyperemia (glucose: r=-0.304, $p \leq 0.05$; HbA_{1c}: r=-0.285, $p \leq 0.05$). For FDC, the correlations did not reach statistical significance (glucose: r=-0.272, p=0.067; HbA_{1c}: r=-0.239, p=0.110) (**Table 2**).

Correlations of vascular function and structure

FMD correlated with FDC (r=0.429; $p \leq 0.01$) and with IMT (r=-0.532; $p \leq 0.001$) as simultaneously assessed by high resolution ultrasound of the BA. FMD also correlated with FBF during reactive hyperemia (r=0.307; $p \leq 0.05$) indicating a potential mutual interaction of macrovascular and microvascular function (**Table 3**).

Discussion

Herein, we demonstrate that in patients with CAD, diabetes mellitus leads to enhanced func-

Table 2. Correlations of vascular function and structure with plasma glucose concentration and HbA_{1c}

		Plasma glucose	HbA_{1c}
FMD	r	-0.321	-0.447
	p value	0.032	0.002
GTN	r	-0.135	-0.252
	p value	0.377	0.095
FDC	r	-0.272	-0.239
	p value	0.067	0.110
IMT	r	0.539	0.479
	p value	<0.001	0.001
FBF_{RH}	r	-0.304	-0.285
	p value	0.045	0.061

FMD: flow-mediated dilation; GTN: glyceroltrinitrate-induced dilation; IMT: intima-media thickness; FDC: fractional diameter change; FBF_{RH}: forearm blood flow during reactive hyperaemia

Table 3. Correlations of vascular function and structure

		FMD	IMT	FDC	FBF_{RH}
FMD	r	1	-0.532	0.429	0.307
	p value		<0.001	0.003	0.038
IMT	r	-0.532	1	-0.523	-0.140
	p value	<0.001		<0.001	0.355
FDC	r	0.429	-0.523	1	0.065
	p value	0.003	<0.000		0.671
FBF_{RH}	r	0.307	-0.140	0.065	1
	p value	0.038	0.355	0.671	

FMD: flow-mediated dilation; IMT: intima-media thickness; FDC: fractional diameter change; FBF_{RH}: forearm blood flow during reactive hyperemia

tional, physicomechanical and structural alterations on the macrovascular level as well as functional alterations on the microvascular level in the peripheral vasculature which are determined by the glucose control.

Endothelial dysfunction in diabetes mellitus

We here show that in patients with CAD diabetes mellitus is associated with deterioration of endothelial function underlining the results from a previous study by Reyes-Soffer et al [13]. Remarkably, diabetes mellitus leads to a further aggravation of endothelial dysfunction in patients with CAD as a manifestation of atherosclerosis thereby indicating the pronounced effects of diabetes mellitus on vascular function. Hyperglycemia is considered as the most relevant cause of endothelial dysfunction and

diabetic complications [14]. The CATHAY study has shown that even in non-diabetic individuals FMD was significantly associated with increasing levels of glycemia. Fasting glucose level was identified as an independent predictor of vascular function. Additionally, other conventional cardiovascular risk factors, including obesity, blood pressure, and an adverse lipid profile, were also related to levels of glycemia, further contributing to impaired vascular function [15]. Other factors promoting endothelial dysfunction in DM also include hyperinsulinemia, insulin resistance, and inflammation as recently reviewed elsewhere [14].

Complex vascular alterations in diabetes mellitus

Endothelial function is understood as an indica-

tor of the integrity of the arterial wall reflecting both functional as well as structural and physicomechanical properties i.e. vessels may not be able respond to endothelial vasodilator mediators due to increased vascular stiffness or increased intima-media thickness. In addition to endothelial function, we measured physicomechanical and structural properties of the brachial artery in one examination at the same site. In parallel with endothelial dysfunction, we found increased arterial stiffness and increased IMT in the brachial artery thereby indicating extensive systemic physicomechanical alterations and structural alterations of the vascular wall in diabetes mellitus explaining part of the observed impaired FMD response. Typically, carotid-femoral pulse wave velocity (PWV) is considered as the gold-standard measurement of arterial stiffness [16]. However, local measurements of arterial stiffness are indicated for mechanistic analyses in pathophysiology, pharmacology, and therapeutics [16]. Henry et al. measured carotid, femoral, and brachial artery distensibility in a population-based cohort including study subjects with type 2 DM. They found an increased arterial stiffness in both elastic and muscular arteries in patients with DM which indicates early physicomechanical alterations in different arterial segments, even in peripheral arteries which are not typically affected by atherosclerosis [17]. In the presence of atherosclerotic disease, DM even leads to a further increase in arterial stiffness as detected in the carotid artery [18] as well as in peripheral arteries. In the above mentioned study by Henry et al., the increase in stiffness indices were explained by decreases in distension, increases in pulse pressure, an increase in carotid IMT, and, for the femoral artery, a decrease in diameter [17]. Typically, IMT is measured in the carotid artery and large studies have shown the predictive value of the carotid IMT for cardiovascular events [19]. It has been shown that diabetic patients without a history of myocardial infarction have a comparable IMT as non-diabetic patients with a history of myocardial infarction [20]. It may be assumed that DM leads to marked systemic structural alterations of the vascular wall affecting elastic as well as muscular arteries.

The presented approach of parallel measurement of FMD, FDC, and IMT in the same vascular segment may give further important insights into the mechanisms of vascular remodelling.

We have shown that FDC and IMT correlated with FMD suggesting that physicomechanical and structural properties determine endothelial function or, more general, the vascular response to an increase blood flow. Soltesz et al. measured FMD of the brachial artery, IMT of the carotid artery and arterial stiffness in patients with systemic autoimmune disease who are at an increased risk of vascular dysfunction and increased cardiovascular mortality due to accelerated atherosclerosis. They showed a reduced FMD, an increased IMT and an increased PWV in patients with systemic autoimmune disease. In this patient group, FMD was inversely correlated with IMT and PWV [21]. Ravikumar and colleagues also measured FMD of the brachial artery, IMT of the carotid artery and arterial stiffness in patients with or without DM. They found a correlation of reduced FMD and augmentation index (AI) with the carotid IMT. FMD and AI correlated with plasma glucose concentration and HbA$_{1c}$, respectively [22]. However, there is only a limited number of studies investigating endothelial function, physicomechanical, and structural properties in the same vascular bed [23]. Bjarnegard et al. showed in female patients with type 1 diabetes mellitus a reduced FMD and GTN response whereby IMT and arterial distensibility were comparable with non-diabetic control patients. HbA$_{1c}$ was an independent predictor of the reduced GTN response in DM [23]. In contrast to our results in patients with type 2 DM, in these patients with type 1 DM the impairment of endothelium-independent response has been seen as the most remarkable vascular alteration suggesting different pathways of vascular remodelling in type 1 and type 2 diabetes. Further longitudinal studies are needed to fully characterize the course of vascular remodelling in the peripheral vasculature in diabetes mellitus.

Interaction of macrocirculation and microcirculation

We found a reduced forearm blood flow at rest and during reactive hyperemia suggesting concomitant microvascular dysfunction in patients with type 2 DM. From clinical studies, it is well known that DM is characterized by its microvascular complications such as diabetic nephropathy, retinopathy or neuropathy. In addition, DM is also associated with homeostatic alterations which are suggested to produce a procoagulant state [24,25]. In previous studies, reduced mi-

crovascular response to endothelium-dependent and endothelial-independent stimuli in resistance arteries and capillaries has been presented by different methods in patients with diabetes mellitus [26,27,28]. Again, forearm blood flow during reactive hyperemia correlated with FMD of the brachial artery indicating an interaction between macrovascular and microvascular function as shown before [12,29]. It may be argued that the reduced forearm blood flow in diabetic patients led to a reduced stimulus of flow during reactive hyperemia which might contribute to the impaired FMD.

Conclusion

In patients with CAD, DM leads to functional and structural vascular alterations which are determined by the glycemic control underlining the relevance of a strict control of the DM to prevent accelerated atherosclerosis. The approach of a single non-invasive one-stop-shop examination allows a frequent and non-invasive monitoring of vascular status in diabetics.

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