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 \( \geq 200 \) mg/dL, a fasting glucose concentration \( \geq 126 \) 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 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

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, Predictive 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/m²; p≤0.05), plasma glucose concentration (148±8 vs 105±8 mg/dL; p≤0.001) and HbA1c (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.

Table 1. Clinical characteristics of the study population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Diabetes mellitus</th>
<th>Control</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>[n]</td>
<td>25</td>
<td>25</td>
<td></td>
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<tr>
<td>CAD [n]</td>
<td>[n]</td>
<td>25</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Age [years]</td>
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<td>65 ± 1.4</td>
<td>66 ± 1.6</td>
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<tr>
<td>Male sex [n]</td>
<td>[n]</td>
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<td>21</td>
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<tr>
<td>Body mass index [kg/m²]</td>
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<td>29.7 ± 1.0</td>
<td>26.6 ± 0.7</td>
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<tr>
<td>Systolic blood pressure [mmHg]</td>
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<td>142 ± 3</td>
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<td>86 ± 2</td>
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<td>Mean arterial pressure [mmHg]</td>
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<td>182 ± 9</td>
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<td>107 ± 8</td>
<td>111 ± 7</td>
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<td>HDL cholesterol [mg/dL]</td>
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<td>51 ± 2</td>
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<tr>
<td>Plasma glucose [mg/dL]</td>
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<td>148 ± 8</td>
<td>105 ± 8</td>
<td>p≤0.001</td>
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<tr>
<td>HbA1c [%]</td>
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<td>5.8 ± 0.2</td>
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<td>ASS [%]</td>
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<td>Clopidogrel [%]</td>
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<td>84</td>
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<td>Statin [%]</td>
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<td>Insulin [%]</td>
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<td>44</td>
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</table>

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.
Vascular dysfunction in diabetes mellitus

(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≤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 (0.024±0.002 vs 0.034±0.004 a.u.; p≤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≤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≤0.05) and during reactive hyperemia (10.7±1.0 vs. 15.3±1.4 mL/min per 100mL tissue; p≤0.05).

*Correlation of vascular function and structure with plasma glucose and HbA1c*

Plasma glucose concentration and HbA1c correlated with FMD (glucose: r=-0.321, p≤0.05; HbA1c: r=-0.447, p≤0.01), IMT (glucose: r=0.539, p≤0.001; HbA1c: r=0.479, p≤0.001), and FBF during reactive hyperemia (glucose: r=-0.304, p≤0.05; HbA1c: r=-0.285, p≤0.05). For FDC, the correlations did not reach statistical significance (glucose: r=-0.272, p=0.067; HbA1c: r=-0.239, p=0.110) (Table 2).

*Correlations of vascular function and structure*

FMD correlated with FDC (r=0.429; p≤0.01) and with IMT (r=-0.532; p≤0.001) as simultaneously assessed by high resolution ultrasound of the BA. FMD also correlated with FBF during reactive hyperemia (r=0.307; p≤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-
Vascular 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].

Endothelial dysfunction in diabetes mellitus

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Complex vascular alterations in diabetes mellitus

Endothelial function is understood as an indicati-
Vascular dysfunction in diabetes mellitus

We have shown that FDC and IMT correlated with FMD suggesting that physiomechanical 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 HbA1c, respectively [22]. However, there is only a limited number of studies investigating endothelial function, physiomechanical, 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. HbA1c 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-
Microvascular response to endothelium-dependent and endothelium-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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References


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