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Preserved vasodilator response to adenosine in insulin-dependent diabetes mellitus

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Abstract. Experimental data derived from animal models suggest that the endogenous nucleoside adenosine has important cardioprotective properties. The potent vasodilator effects of adenosine may contribute to this cardioprotection as ischaemia-induced release of endogenous adenosine has been suggested to adjust local blood flow to the metabolic demands of the tissue. Interestingly, the vascular effects of adenosine appeared to be impaired in animal models for diabetes mellitus. This observation may be of importance with respect to the increased cardiovascular mortality in diabetes. Therefore, the authors investigated the *in vivo* vasodilator effects of adenosine in insulin-dependent diabetic patients. In 12 uncomplicated insulin-dependent male diabetic patients and 12 healthy male age-matched subjects, the brachial artery was cannulated for infusion of adenosine (0.15, 0.5, 1.5, 5, 15 and 50 $\mu\text{g } 100^{-1} \text{ mL min}^{-1}$) and for measurement of mean arterial pressure (MAP). Forearm blood flow (FBF) was measured by venous occlusion mercury-in-silastic strain gauge plethysmography. Maximal vasodilatation was assessed by standardized post occlusive reactive hyperaemia (PORH). Baseline forearm blood flow was 2.7 ± 0.4 and $1.8 \pm 0.2 \text{ mL } 100^{-1} \text{ mL min}^{-1}$ for the diabetic patients and control group respectively. In the diabetic patients, adenosine infusion raised forearm blood flow to 2.4 ± 0.4 , 2.6 ± 0.4 , 4.4 ± 0.7 , 6.3 ± 1.0 , 9.8 ± 1.5 and $14.2 \pm 2.1 \text{ mL } 100^{-1} \text{ mL min}^{-1}$ for the respective dosages. In the control group these values were 1.7 ± 0.2 , 1.9 ± 0.3 , 3.2 ± 0.8 , 6.0 ± 1.2 , 10.9 ± 2.1 and $17.1 \pm 3.4 \text{ mL } 100^{-1} \text{ mL min}^{-1}$ respectively ($P > 0.1$ for between group comparison). Forearm blood flow at the contralateral side was not significantly affected by the placebo and adenosine infusions. Similar results were obtained when results were expressed as changes in forearm vascular resistance or forearm blood flow ratio (FBF infused arm/FBF control arm). Maximal vasodilatation did not differ between the two groups. The authors conclude

that the forearm vasodilator response to adenosine is preserved in uncomplicated insulin-dependent diabetic patients. This observation argues against a primary role of a reduced adenosine responsiveness in the cardiovascular sequelae of diabetes.

Keywords. Adenosine, diabetes mellitus, forearm blood flow, nucleosides, plethysmography, vasodilation.

Introduction

Adenosine has potentially important cardioprotective properties such as inhibition of neutrophil activation with subsequent reduced free radical formation, inhibition of thrombocyte aggregation, vasodilatation, presynaptic inhibition of noradrenaline release and opening of potassium channels [1]. These effects are mediated by adenosine receptors, located on the outer cell membrane. In animals, myocardial infarct size is reduced when adenosine is infused either before ischaemia or during the reperfusion period [2–4]. In addition, adenosine reduces the incidence of ischaemia-induced arrhythmias [5]. Infusion of a selective adenosine receptor antagonist increases infarct size, indicating a role for endogenous adenosine as a cardioprotective autacoid [6]. As the vasodilator action of adenosine is thought to play a role in the local adjustment of oxygen demand to oxygen supply [7,8], this may contribute to the cardioprotective properties of adenosine.

Interestingly, an impaired responsiveness to the vasodilator effect of adenosine has been observed in animal models of diabetes mellitus [9,10]. Several mechanisms may be responsible for this reduced responsiveness to adenosine. Animal and human data have indicated both facilitating as well as inhibiting interactions between adenosine and the sympathetic nervous system [11–16]. Furthermore, human and animal studies show that adenosine-induced vasodilatation is at least partially mediated by the endothelium [17–19]. As insulin alters sympathetic nervous system activity [20,21] and diabetes mellitus has been associated with reduced endothelium-dependent vasodilatation [22–25], both neural and endothelial mechanisms may be involved in the reduced

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reactivity to adenosine in these descriptive animal studies. Additionally, direct actions of adenosine on cardiac and vascular muscle cells may be reduced in patients with diabetes mellitus.

Although vascular reactivity in human diabetes has been studied extensively over the past few years [22–25], no human data are available on responsiveness to the endogenous nucleoside adenosine. As diabetes is an independent risk factor for developing cardiovascular disease [26], and is often associated with concomitant hypercholesterolaemia and hypertension, which further contributes to an increased risk of ischaemic heart disease [27,28], an impairment in adenosine responsiveness may be of clinical interest. Pharmacological compounds are currently being developed to potentiate the action of endogenous adenosine at sites of ischaemia [12]. In this context it is valuable to know whether diabetic patients exhibit decreased responses to adenosine. Furthermore, a reduced vascular responsiveness to adenosine may also be of importance in the metabolic control of patients with diabetes mellitus as adenosine enhances glucose uptake in some animal models [29,30]. This metabolic effect of adenosine may in part be a result of its effect on blood flow [31]. Therefore, we evaluated the vasodilator response to adenosine in patients with uncomplicated insulin-dependent diabetes mellitus and compared these observations with a carefully matched control group.

Patients and methods

Patients

After approval of the local ethics committee, 12 normotensive non-smoking Caucasian male patients with insulin-dependent diabetes mellitus were selected from our outpatient population. Diabetes mellitus was diagnosed at least 5 years before participation in this study. Patients with evidence of macro- or microvascular disease were excluded from the study

because these vascular complications would result in a non-specific impairment of reactivity to any vasodilator substance. Macrovascular disease was assessed by taking patients' history (no coronary artery disease, heart failure, cerebrovascular disease, peripheral vascular disease or foot ulcers), physical examination and a 12-lead electrocardiogram. Microvascular disease was excluded by demonstrating the absence of orthostatic hypotension and peripheral loss of sensibility, by a normal fundoscopy and by an albumin excretion ratio less than $20 \mu\text{g min}^{-1}$. None of the patients used medication other than subcutaneous insulin injection. Only patients with a glycosylated haemoglobin concentration (HbA1c) between 7% and 10% as measured during insulin treatment were included. The control group consisted of 12 male non-smoking healthy Caucasian volunteers. These subjects were carefully matched for age, blood pressure and body weight. They had no history of diabetes mellitus and did not use concomitant medication. Physical examination and 12-lead electrocardiography did not reveal any abnormalities. Demographic data of the study groups are shown in Table 1.

Methods

Before the start of the study, the subjects were asked to abstain from caffeine-containing products for at least 24 h, because caffeine is a potent adenosine receptor antagonist [32]. In all participating subjects, the plasma caffeine concentration was below the limit of detection as measured in a sample that was collected immediately before starting the experiments [reversed-phase high-performance liquid chromatography (HPLC); minimal level of detection: $0.2 \mu\text{g ml}^{-1}$ [33]]. All tests were performed in a temperature-controlled laboratory ($22\text{--}23^\circ\text{C}$), with participants in the supine position, after an overnight fast, starting at 8.00 a.m.

From a methodological point of view, the level of plasma insulin and glucose concentrations throughout

Table 1. Demographic characteristics of the study groups (mean \pm SD)

| | Diabetic subjects | Healthy subjects |
|---|-------------------|------------------|
| <i>n</i> | 12 | 12 |
| Male/female | 12/0 | 12/0 |
| Age (years) | 34.6 ± 5.9 | 34.8 ± 6.0 |
| Body mass index (kg m^{-2}) | 23.6 ± 2.4 | 23.3 ± 2.4 |
| Systolic blood pressure (mmHg)* | 123 ± 10.4 | 118 ± 8.2 |
| Diastolic blood pressure (mmHg)* | 63.2 ± 8.6 | 62.4 ± 4.9 |
| Mean arterial pressure (mmHg)* | 85.2 ± 9.8 | 82.4 ± 6.8 |
| Heart rate (beats min^{-1})† | 67.3 ± 16.8 | 57.4 ± 9.6 |
| HbA1c (%) | 8.5 ± 0.9 | |
| Time after diagnosis (years) | 16.2 ± 8.3 | |
| Glucose (mmol L^{-1})‡ | 13.0 ± 5.1 | |

*Intra-arterially measured during placebo infusion. †Measured by electrocardiographic recordings during placebo infusion. ‡Determined during the experiment; for each subject the six determinations were averaged to one value.

the study is a very important issue. Recent studies have convincingly shown that baseline skeletal muscle flow in humans is not affected by hyperglycaemia [34], whereas hyperinsulinaemia induces an obvious increase in baseline skeletal muscle flow and affects forearm vascular reactivity [35–38]. To avoid confounding of our results by insulin-mediated vasodilatation, we instructed the diabetic subjects to skip their morning dose of insulin and not to have breakfast. This was done in order to achieve low and steady state plasma insulin levels during the experiments. Although even lower insulin levels would have been reached by also skipping the long-acting insulin injection of the evening before the experiment, this would have introduced the risk of the development of ketosis or ketoacidosis, a factor which would certainly have affected the results. As no insulin was administered in the morning hours, we had to accept the varying fasting glucose levels throughout the experiments, as correcting the glucose levels would inevitably have increased insulin levels. As stated above, recent data have convincingly shown that plasma glucose levels up to 15 mmol L affect neither baseline forearm blood flow nor vascular reactivity of the forearm vascular bed [34].

After local anaesthesia (xylocaine, 2%), the left brachial artery was cannulated with a 20-gauge catheter (Angiocath, Deseret Medical, Becton Dickinson, Sandy, UT, USA) for both intra-arterial adenosine infusion (automatic syringe infusion pump, type STC-521, Terumo Corporation, Tokyo, Japan) and blood pressure recording (Hewlett Packard, Böblingen, Germany). Forearm blood flow was registered simultaneously on both forearms by electrocardiography-triggered venous occlusion plethysmography using mercury-in-silastic strain gauges (Hokanson EC4, D.E. Hokanson, Washington, USA). The upper arm collecting cuff was inflated using a rapid cuff inflator (Hokanson E-20, D.E. Hokanson). At least 1 min before the FBF measurements, the circulation of the left hand was occluded by inflation of a wrist cuff to 200 mmHg. Forearm blood flow was recorded three times per minute during the 4-min placebo infusion and during the last 2 min of each adenosine infusion.

The experiment started with the measurement of baseline forearm blood flow during placebo infusion (NaCl, 0.9%). Apart from the course in the forearm blood flow, Fig. 1 shows the schedule of the several drug infusions. The effect of six increasing dosages of adenosine (Sigma, St Louis, MO, USA; 0.15, 0.5, 1.5, 5, 15 and 50 μg 100 mL forearm min^{-1}) were compared with placebo (NaCl 0.9%). Prolonged occlusion of the hand circulation can cause discomfort with subsequent effects on blood pressure and heart rate. Therefore, a 5-min rest period with desufflation of the wrist cuffs was allowed between the placebo infusion and the first adenosine dose and between the third and fourth adenosine doses. During all procedures, total volume infusion was adjusted to

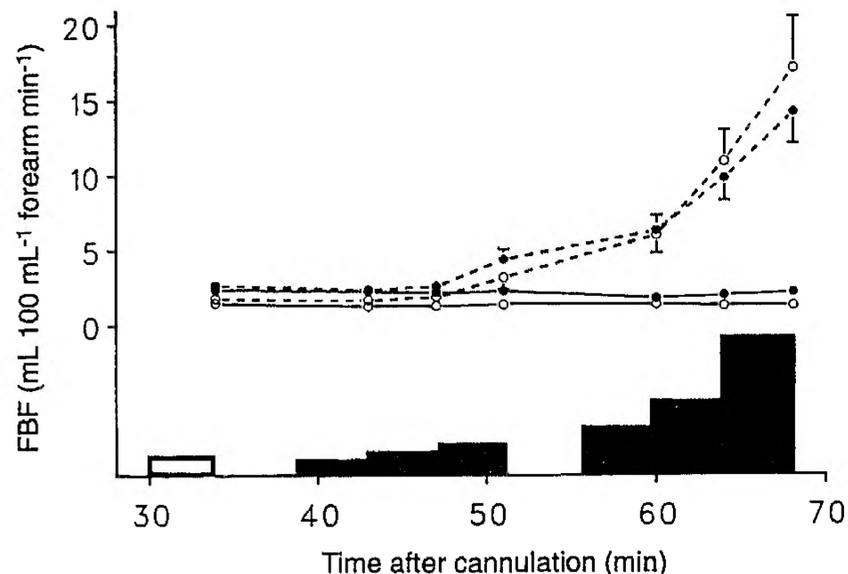


Figure 1. The course in forearm blood flow (FBF) before and during the intra-arterial infusion of adenosine is presented for the diabetic patients and the control group. There was no statistically significant difference between the groups (ANOVA for repeated measurements: $P = 1.0$). Diabetic patients, control arm (\bullet); controls, control arm (\circ); diabetic patients, infused arm (\bullet); controls infused arm (\circ). \square , Placebo to infusion (NaCl 0.9%); \blacksquare , incremental adenosine infusions.

forearm volume as measured by water displacement and kept at a constant rate of 100 μL 100 mL forearm min^{-1} . Placebo and each adenosine dosage were infused over 4 min.

To exclude structural vascular changes in the diabetic patients, maximal vasodilatation was measured during post occlusive reactive hyperaemia (PORH) according to the well-established method of Pedrinelli *et al.* [39,40] 20 min after the end of the final adenosine infusion. A cuff applied to the left upper arm was inflated to 300 mmHg for 13 min. During the last minute of ischaemia the subjects were asked to perform repeated hand contractions. Immediately after desufflation of the upperarm cuff, FBF measurements were started for at least 2 min with occluded hand circulation. The lowest forearm vascular resistance (MAP/FBF) was considered to represent maximal vasodilatation.

In the diabetic group, blood glucose concentrations were determined six times: immediately after arterial cannulation, after infusion of placebo, after the third and after the sixth adenosine dose, just before the test of maximal vasodilatation and just before decannulation (Accutrend, type 1284851, Boehringer, Mannheim, Germany). Before the intra-arterial adenosine infusions, 10 mL of arterial blood was collected with lithium-heparin as coagulant in nine diabetic patients and in four control subjects for the determination of plasma insulin and detection of insulin antibodies. Plasma insulin was measured by radioimmunoassay using a specific antiserum raised in a guinea pig against human insulin. A second antibody was used to separate the antibody-bound and free fractions. Insulin antibodies were detected by incubation of the samples with [^{125}I]-insulin and subsequent precipitation with polyethyleneglycol [41].

Statistics

Mean arterial pressure (MAP) was measured continuously during each recording of forearm blood flow (FBF) and averaged per FBF registration. Forearm vascular resistance (FVR) was calculated from simultaneously measured MAP and FBF (MAP/FBF) and expressed as arbitrary units (AU). Additionally, the ratio of each simultaneously measured FBF (FBF infused arm/FBF control arm) was calculated. Forearm blood flows, the calculated flow ratios and FVRs obtained during each 4 min of placebo infusion or during the last 2 min of each drug infusion were averaged to one value. Adenosine-induced effects were expressed both as absolute and percentage change from preceding placebo infusion. Differences in responses to adenosine between the two study groups were analysed with an ANOVA for repeated measurements with the adenosine dosage as within-subject factor and the presence of diabetes mellitus as between-subject factor. Differences in baseline values were assessed with the unpaired Student *t*-test. Correlations were performed using the Pearson correlation coefficient. Since plasma insulin concentrations did not show a Gaussian distribution, group differences in insulin levels were analysed by the Mann-Whitney *U*-test. All results are expressed as mean \pm SE unless indicated otherwise; $P < 0.05$ (two-sided) was considered to indicate statistical significance.

Results

Baseline FBFs in the infused arm were 2.7 ± 0.4 and 1.8 ± 0.2 mL 100 mL $^{-1}$ forearm min^{-1} for the diabetic

patients and control group, respectively ($P = 0.07$). In the control arm these values were 2.4 ± 0.4 and 1.5 ± 0.1 mL 100 mL $^{-1}$ forearm min^{-1} ($P = 0.05$). During the adenosine infusions, FBF in the infused arm of the diabetic patients amounted to 2.4 ± 0.4 , 2.6 ± 0.4 , 4.4 ± 0.7 , 6.3 ± 1.0 , 9.8 ± 1.5 and 14.2 ± 2.1 mL 100 mL $^{-1}$ forearm min^{-1} for the respective adenosine dosages of 0.15, 0.5, 1.5, 5, 15 and 50 μg adenosine 100 mL $^{-1}$ forearm min^{-1} . In the control group these values were 1.7 ± 0.2 , 1.9 ± 0.3 , 3.2 ± 0.8 , 6.0 ± 1.2 , 10.9 ± 2.1 and 17.1 ± 3.4 mL 100 mL $^{-1}$ forearm min^{-1} , respectively (see Fig. 1). In both groups, FBF in the control arm was not significantly affected during the placebo and adenosine infusions. Overall, repeated measures ANOVA did not reveal a significant difference between the two groups (between subject effect: $P = 1.0$). Similar results were obtained when results were expressed as absolute or relative changes in FBF from baseline.

Baseline FVRs in the infused arm were 39.6 ± 5.2 and 57.5 ± 7.6 AU for the diabetic patients and controls, respectively ($P = 0.07$). In the control arm these values were 45.7 ± 6.3 and 63.7 ± 5.9 AU, respectively ($P < 0.05$). During adenosine infusion, FVRs in the infused arm of the diabetic patients were 44.6 ± 6.6 , 41.9 ± 5.4 , 29.9 ± 7.1 , 20.5 ± 4.6 , 16.6 ± 5.7 and 12.2 ± 5.7 AU for the six increasing adenosine dosages, respectively. In the controls, these values were 57.5 ± 7.6 , 58.5 ± 7.2 , 56.1 ± 7.7 , 45.8 ± 9.0 , 24.6 ± 5.5 , 16.0 ± 4.6 and 13.2 ± 5.2 AU respectively. Overall, the course of FVR in the infused arm did not significantly differ between the two groups (between-subject effect: $P = 0.2$). In both groups, FVR in the control arm was not affected. Similar results were

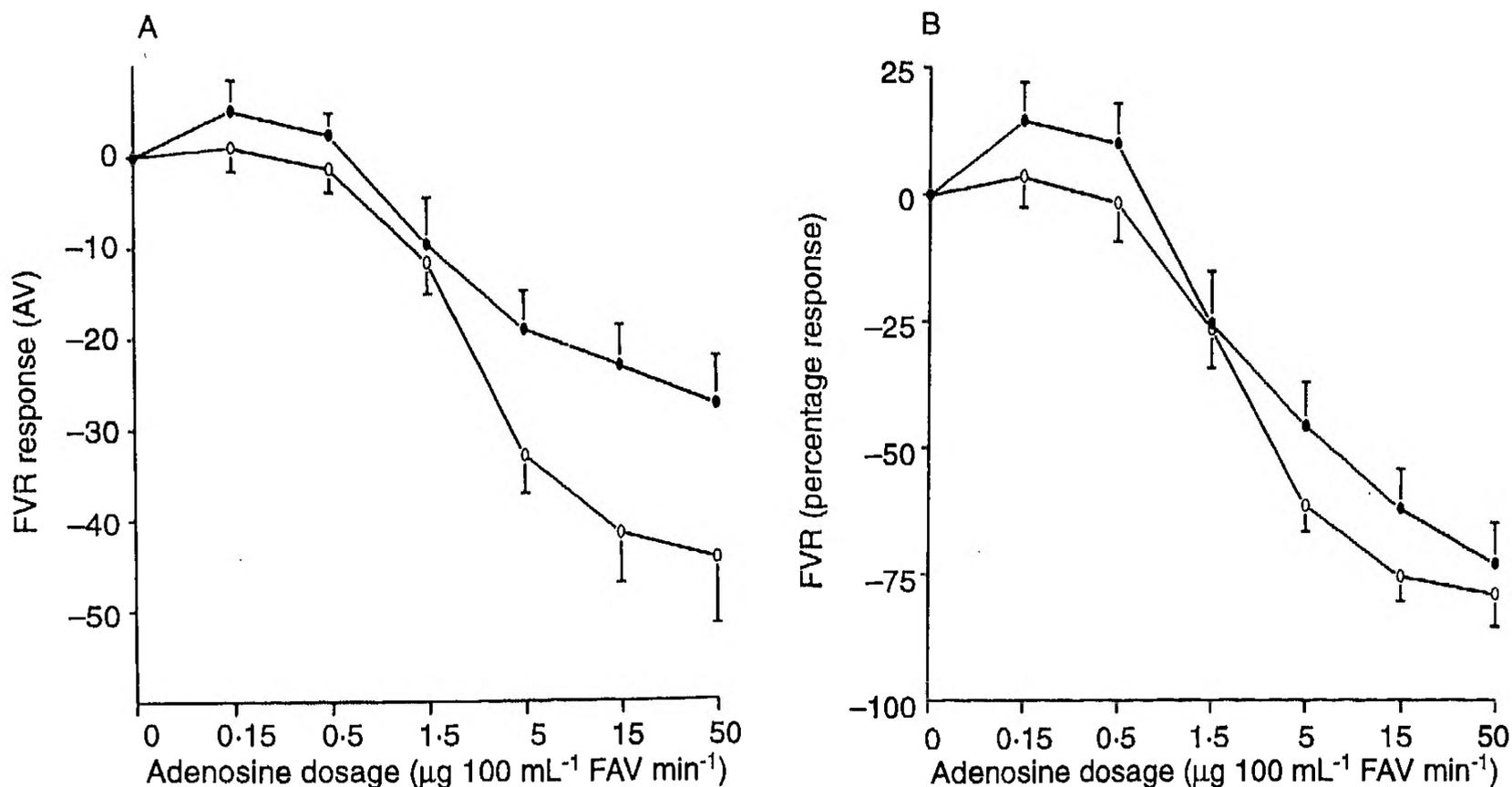


Figure 2. Adenosine-induced forearm vasodilatation in controls (O) and diabetic patients (●) expressed as absolute (A) and percentage change (B) from baseline of the forearm vascular resistance (FVR). *P* values indicate the level of significance for between-group effects (ANOVA for repeated measurements). *P* = not significant.

obtained when adenosine-induced changes in FVR were expressed as absolute or relative changes from baseline (see Fig.2).

Because we observed slight differences in baseline FBF and FVR values between the diabetic patients and the control group, we also analysed the results of the FBF ratio (FBF_{infused arm} divided by FBF_{contralateral arm}). Assessment of the per cent changes in this ratio has been shown to be an appropriate method to analyse dose-response curves [22,42]. During placebo infusion, the FBF ratio was equal in both groups and had a value of numbered 1.2 ± 0.1 .

In the diabetic patients, the FBF ratios increased to 1.0 ± 0.1 , 1.2 ± 0.1 , 2.1 ± 0.4 , 4.4 ± 1.0 , 5.9 ± 1.2 and 9.2 ± 2.5 during the six increasing adenosine dosages respectively. In the controls, these values were 1.3 ± 0.1 , 1.5 ± 0.2 , 2.2 ± 0.4 , 4.2 ± 0.8 , 8.9 ± 1.5 and 14.7 ± 2.1 respectively. Overall, the course of the ratio did not significantly differ between the two groups (between-subject effect: $P = 0.2$). Similar results were obtained when adenosine-induced changes in the ratio were expressed as absolute or relative changes from baseline.

The minimal FVR that occurred during post-occlusive reactive hyperaemia did not differ between diabetic patients and controls: 3.2 ± 0.2 vs. 3.4 ± 0.3 AU ($P = 0.6$). The individual courses of blood glucose concentration are shown in Fig.3. Within each individual, glucose levels remained reasonably stable. However, between patients a high variation in averaged glucose level existed ranging from 3.4 to 20.6 mmol L. There was no correlation between the individual plasma glucose concentration and the vascular responsiveness to adenosine in the diabetic patients ($r = -0.2$, $P = 0.5$). Plasma insulin concentration was 21.8 ± 2.5 m equiv. L⁻¹ ($n = 9$) in the diabetic patients versus 6.5 ± 1.3 m equiv. L ($n = 4$) in the controls ($P < 0.01$). In four diabetic patients, insulin antibodies could be detected. After exclusion of these patients, plasma insulin concentration was still significantly higher in the diabetic patients as

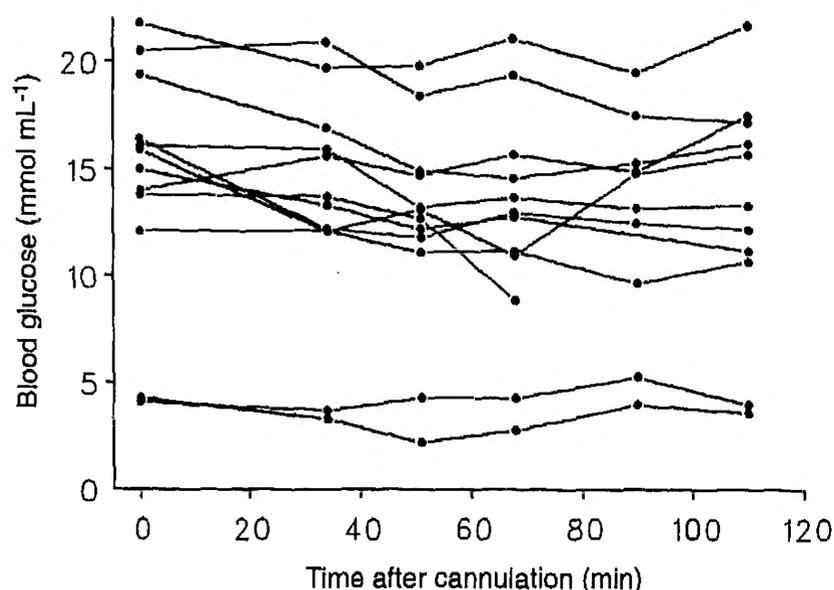


Figure 3. Individual courses of blood glucose concentrations, demonstrating the *large* inter-individual variability as well as the *small* intraindividual variability during the experiments.

compared with the control subjects (20.8 ± 3.8 vs. 6.5 ± 0.9 m equiv. L; $P < 0.05$).

Discussion

This study was performed to investigate whether the forearm vasodilator response to adenosine is affected in patients with insulin-dependent diabetes mellitus. Normotensive non-smoking diabetic patients without evidence of macro- or microvascular complications were selected to prevent possible confounding by structural arteriolar or microvascular changes. Maximal forearm vasodilatation in the diabetic patients appeared to be equal to that of the age-matched control group, confirming the absence of structural abnormalities in the forearm vascular bed of the diabetic subjects. In these carefully selected patients, we observed a preserved vasodilator response to adenosine in the forearm vascular bed. This observation argues against a primary role for reduced adenosine responsiveness in the cardiovascular sequelae of diabetes.

The current results are in contrast with several observations in animal models for diabetes [9,10] that studied the responsiveness of the heart and coronary vasculature to adenosine. We cannot exclude the possibility that diabetes mellitus differentially affects the coronary and forearm vascular bed in humans. However, other possible explanations should be discussed as well.

It has to be emphasized that animal models for diabetes represent a true insulinopenic state. In contrast, as a result of subcutaneous administration of insulin as opposed to the physiological release of insulin into the portal vein, the levels of insulin are elevated in treated patients with insulin-dependent diabetes mellitus. As such, treated patients with insulin-dependent diabetes do not represent an insulinopenic state, not even in the fasting state when plasma insulin concentrations reach their trough level. This may well explain the preserved adenosine responsiveness in our patients, because treatment of diabetic animals with insulin also restored the impaired responses to adenosine [10]. Since we did not study the effects of adenosine in a true insulinopenic state, our data do not exclude an interaction between insulin and adenosine. Nonetheless, our present results do allow the conclusion that the vascular responsiveness to adenosine is preserved in patients with diabetes mellitus who are regularly treated with insulin.

In the diabetic patients, baseline forearm blood flow and forearm vascular resistance were slightly different from that of the control subjects. This interesting phenomenon has been described before [22] and is not only confined to the forearm but has also been shown for the retinal, renal and cutaneous circulation [43–45]. It already exists in the early course of the disease before diabetic complications have developed [22,45]. Although the exact mechanism of this 'hyperdynamic circulation' in diabetic patients is not known,

it may be related to the development of complications such as diabetic nephropathy and diabetic microangiopathy [45]. Especially because of this difference in baseline forearm blood flow, we also included the results on the FBF ratio (see Methods). For the FBF-ratio, the diabetic patients and the control group had exactly the same baseline values. Using this parameter, statistical analysis of the adenosine responses revealed no difference between the two groups.

The main observation of the present study is that adenosine-mediated forearm vasodilatation is not significantly affected in insulin-dependent diabetic patients. The response to the two highest adenosine dosages tended to be slightly reduced in the diabetic patients, but this difference did not reach statistical significance. In contrast to the two highest dosages, the responses to the lower dosages were very similar between the two groups. We regard these lower dosages as being more representative for the local physiological increases in adenosine concentration, which are probably needed for the small adjustments of local flow in order to constantly balance tissue oxygen demand and supply.

The effect of intra-arterially supplied adenosine is determined by the adenosine concentration, adenosine receptor density and receptor function. Adenosine concentrations depend on the rate of cellular adenosine uptake. In theory, diabetes mellitus may impair this cellular uptake of adenosine [46]. Therefore, a reduced adenosine receptor density or function could have been masked by differences in adenosine kinetics between the two groups. However, the clinical significance of a possible receptor dysfunction is limited when the overall vasodilator effect of adenosine is not reduced *in vivo* as shown in the present study.

In conclusion, the vasodilator response to adenosine is preserved in patients with insulin-dependent diabetes mellitus who are regularly treated with insulin. This observation argues against a primary role of impaired adenosine responsiveness in the cardiovascular sequelae of diabetes.

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