Vascular $K_{\text{ATP}}$ channel blockade by glibenclamide, but not by acarbose, in patients with Type II diabetes

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ABSTRACT

Glibenclamide inhibits the opening of vascular ATP-sensitive potassium ($K_{\text{ATP}}$) channels, which represents a protective mechanism during ischaemia. This effect may imply harmful cardiovascular effects of glibenclamide when used under conditions of ischaemia in patients with Type II diabetes. Acarbose is not associated with effects on the cardiovascular system, because the drug is not absorbed from the bowel. Therefore we hypothesized that treatment of Type II diabetes patients with glibenclamide will impair the vasodilator function of $K_{\text{ATP}}$ opening, unlike treatment with acarbose. A double-blind randomized cross-over study in 12 patients with Type II diabetes was performed to compare the effects of glibenclamide with those of acarbose on the vasodilator responses to $K_{\text{ATP}}$ channel opening in the forearm vascular bed. The study consisted of two periods: 8 weeks of treatment with orally administered glibenclamide ($10 \text{ mg} \cdot \text{day}^{-1}$) followed by 8 weeks of treatment with acarbose ($300 \text{ mg} \cdot \text{day}^{-1}$), or vice versa. At the end of each treatment period, forearm blood flow (venous occlusion plethysmography) in response to intra-arterially administered diazoxide, acetylcholine and dipyridamole and to forearm ischaemia was measured. The diazoxide-mediated increase in the forearm blood flow ratio (infused/control arm) was significantly less pronounced after glibenclamide than after acarbose ($290 \pm 58\%$ and $561 \pm 101\%$ respectively; $P < 0.0005$). Forearm blood flow responses to acetylcholine, dipyridamole and forearm ischaemia were similar during glibenclamide and acarbose treatment. Thus, in patients with Type II diabetes mellitus, treatment with glibenclamide is associated with an attenuated response to $K_{\text{ATP}}$ opening as compared with treatment with acarbose. This implies that glibenclamide may affect defensive mechanisms under conditions of $K_{\text{ATP}}$ channel activation.

INTRODUCTION

Type II diabetes mellitus accounts for approximately 85% of all cases of diabetes mellitus, and is an important risk factor for cardiovascular morbidity and mortality [1–4]. Sulphonylurea derivatives have formed the backbone of treatment of Type II diabetes mellitus for several decades [5]. These drugs exert their glucose-lowering effects by closing ATP-sensitive potassium ($K_{\text{ATP}}$) channels in the $\beta$ cells of the pancreas. This closure promotes an influx of calcium, with subsequent stimulation of insulin release [6]. Recent investigations have shown that the vascular system also has functional $K_{\text{ATP}}$ channels [7,8]. Under physiological conditions these channels are

Key words: acarbose, acetylcholine, diazoxide, dipyridamole, glibenclamide, $K_{\text{ATP}}$ channel, reactive hyperaemia, Type II diabetes mellitus, vascular.

Abbreviations: FBF, forearm blood flow; HbA$_1c$, glycated haemoglobin.

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closed or inactive [9]. During hypoxia and/or ischaemia, the intracellular concentration of ATP falls, which results in opening of the $K_{\text{ATP}}$ channels. The subsequent efflux of potassium and hyperpolarization of the cell membrane induces a shortening of the action potential in the myocardium and a relaxation of vascular smooth muscle cells [10,11]. Animal experiments have demonstrated that these mechanisms play a role in protection of the myocardium against ischaemia/reperfusion damage [12].

Glibenclamide inhibits the vasodilator as well as the cardioprotective responses to $K_{\text{ATP}}$ channel opening [12,13]. Moreover, in dogs, sulphonylurea derivatives attenuated the vasodilator response to an ischaemic stimulus [14] and abolished the protective effects of myocardial ischaemic preconditioning [15]. In theory, these observations may imply harmful cardiovascular effects of sulphonylurea derivatives when used under conditions of ischaemia in patients with Type II diabetes mellitus.

It has been shown in healthy volunteers that glibenclamide, at local therapeutic concentrations similar to those that occur in patients with Type II diabetes, can significantly inhibit forearm vasodilation mediated by the pharmacological opening of $K_{\text{ATP}}$ channels with diazoxide [16] or mediated by ischaemia [17]. These properties suggest potentially hazardous cardiovascular effects of glibenclamide. Treatment of Type II diabetes mellitus with the $\alpha$-glucosidase inhibitor acarbose is not associated with effects on the cardiovascular system, because the drug is not absorbed from the bowel. Acarbose therefore will lack harmful cardiovascular effects.

These previous studies [16,17] investigated the acute effects of sulphonylurea derivatives in healthy volunteers. In order to show relevance for daily practice, it is important to confirm these observations during chronic treatment of patients with Type II diabetes mellitus. Therefore a double-blind randomized cross-over study in patients with Type II diabetes was performed to compare the effects of chronic treatment with glibenclamide and acarbose on vasodilator responses to $K_{\text{ATP}}$ channel opening induced by diazoxide in the forearm vascular bed. We hypothesized that glibenclamide impairs vasodilator function in vivo, whereas chronic treatment of Type II diabetes patients with acarbose does not. In addition, differences between glibenclamide and acarbose with respect to forearm vasodilator responses to the endothelium-dependent vasodilator acetylcholine, to the adenosine uptake inhibitor dipryridamole and to an ischaemic stimulus were also studied.

**METHODS**

**Study population**

The study population consisted of 12 patients with Type II diabetes, selected from general practitioner patients and by advertisement. All met the inclusion criteria: age between 18 and 75 years, body mass index between 18 and 35 kg m$^{-2}$ and normal haematological, hepatic and renal laboratory values. For at least 3 months before entering the study, the patients were treated with oral glucose-lowering drugs at a dose equivalent to the doses of the study medication. The study was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association, and all subjects gave their informed consent. The study was approved by the hospital ethics committee.

**Protocol**

The study was designed as a single-centre, double-blind, controlled, cross-over investigation. The protocol consisted of two periods: 8 weeks of treatment with orally administered glibenclamide (10 mg once daily plus placebo three times a day) were followed by 8 weeks of treatment of orally administered acarbose (placebo once daily plus 100 mg of acarbose three times a day), or vice versa. The dosages of glibenclamide and acarbose were expected to be equipotent. Glycaemic control during glibenclamide and acarbose treatment was assessed by measuring glycated haemoglobin ($\text{HbA}_{1c}$) at the end of each 8-week treatment period.

During each treatment period, visits to the outpatient clinic were held after 4 and 8 weeks of treatment. At each visit fasting plasma glucose concentration was determined, weight and blood pressure were measured and compliance was assessed by tablet counts. In addition, at the end of each treatment period, fasting venous blood samples were taken for lipid profiles, and arterial blood samples were taken for insulin, C-peptide and sulphonylurea concentrations.

Participants were instructed to continue with their normal habits and eating patterns during the experimental treatment. Concomitant drug treatment was kept to a minimum during the study period. Participants were instructed to report side effects and hypoglycaemic episodes during the study.

**Experimental procedures**

At the end of each treatment period, forearm vasodilator responses were measured using the perfused forearm model [18], which was also used in previous studies with healthy volunteers [16,17]. The patients were instructed to abstain from caffeine-containing beverages and alcohol for at least 24 h before the experiments. The experiments were performed in the morning after an overnight (10 h) fast, with the subjects supine in a quiet temperature-controlled room (23–24 °C). Blood samples were taken, after which a light standardized breakfast [1255 kJ (300 kcal); 40% carbohydrate, 35% fat and 25% protein] was served. At this time point the subjects also ingested their morning dose of the study medication. After the
breakfast, the participants abstained from further food intake until the end of the experiment. The time between breakfast and the onset of studies was similar in all experiments (45–60 min).

First, the vasodilator responses to diazoxide, acetylcholine and dipyridamole were investigated. Before the experiment, forearm volume was measured by the water-displacement method. Subsequently, a cannula (Angiocath, 20 gauge; Deseret Medical Inc., Becton Dickinson Co., Sandy, UT, U.S.A.) was inserted under local anaesthesia (xylocaine 2%) into the brachial artery for blood pressure measurement (Hewlett Packard monitor type 78353B; Hewlett Packard GmbH, Boeblingen, Germany), and for intra-arterial drug administration by an automated syringe infusion pump (type STC-521; Terumo Corp., Tokyo, Japan). Drugs were infused at a fixed rate of 100 µl min⁻¹ dl⁻¹ forearm volume. Bilateral forearm blood flow (FBF) was measured by venous-occlusion mercury-in-silastic strain-gauge plethysmography (Hokanson EC4; D.E. Hokanson, Inc., Issaquah, WA, U.S.A.) [19]. During all recordings of FBF, the hand circulation was completely occluded by a wrist cuff inflated to 100 mmHg above systolic blood pressure, to ensure that measurements only included the forearm skeletal muscle vascular bed [20].

A schedule of the study protocol is shown in Figure 1. After complete instrumentation, at least 30 min of rest was included in order to obtain a steady state before baseline measurements of blood pressure, heart rate and bilateral FBF were recorded. Then vasodilator responses to the K<sub>ATP</sub> channel opener diazoxide were measured (three intra-arterial dosages: 0.25, 0.75 and 2.25 mg min⁻¹ dl⁻¹; 5 min per dose). After a subsequent equilibration period of 45 min to allow parameters to return to baseline levels, baseline values were again recorded. Then vasodilator responses to the endothelium-dependent vasodilator acetylcholine were measured (three intra-arterial dosages: 0.5, 2.0 and 8.0 µg min⁻¹ dl⁻¹; 5 min per dose). Acetylcholine was investigated because the endothelium-derived vasodilator nitric oxide (NO) has been reported to induce vasodilation via hyperpolarization of vascular smooth muscle by activating K<sub>ATP</sub> channels in an animal experiment [21]. Thus stimulation of the production of NO by acetylcholine could lead to the opening of K<sub>ATP</sub> channels. After a further equilibration period of 45 min, vasodilator responses to the adenosine uptake inhibitor dipyridamole were measured (three intra-arterial dosages: 10, 30 and 100 µg min⁻¹ dl⁻¹; 5 min per dose). Dipyridamole was investigated because a specific adenosine receptor subtype, the A<sub>1</sub>-receptor, has been reported to be coupled to K<sub>ATP</sub> channels [22,23]. Stimulation of the A<sub>1</sub>-receptor leads to the opening of K<sub>ATP</sub> channels, followed by vasodilation. Hence inhibition of adenosine uptake by dipyridamole could lead to more pronounced stimulation of adenosine receptors, and thus to increased opening of K<sub>ATP</sub> channels. Blood samples were taken for measurement of the plasma glucose concentration at the beginning of the experiment, and after the diazoxide, acetylcholine and dipyridamole infusions.

Finally, the vasodilator response to forearm ischaemia was investigated. Occlusion of the brachial artery of the experimental arm was obtained by inflating an extra cuff around the upper arm to 100 mmHg above systolic blood pressure. Then the FBF in response to 2, 5 and 13 min of forearm ischaemia was recorded over a 5 min post-occlusive reperfusion period. During the final 1 min of the 13-min occlusion period, subjects performed forearm exercise, inducing maximal forearm vasodilation during reperfusion [24].

**Drugs**

Diazoxide, acetylcholine and dipyridamole were dissolved in 0.9% NaCl at the start of the study. Diazoxide (Hyperstat<sup>®</sup>) was purchased from Schering-Plough B.V. (Amstelveen, The Netherlands); acetylcholine (acetylcholinii chloridum) was from Dispersa A.G. (Winterthur, Germany); and dipyridamole (Persantin<sup>®</sup>) was from Boehringer Ingelheim B.V. (Alkmaar, The Netherlands). Glibenclamide and acarbose verum and placebo tablets were manufactured by Bayer B.V. (Mijdrecht, The Netherlands).
Analytical methods
Plasma glucose was determined using a commercially available glucose oxidation method. HbA1c was measured using an HPLC technique (Hi-AUTO A1c analyser HA 8140; Menarini), with reference values of 4.2–6.3%. Total plasma cholesterol and triacylglycerol concentrations were determined using commercially available enzymic reagents (reference values 4.7–6.5 and 0.8–2.0 mol·l⁻¹ respectively). Plasma insulin was assessed by means of RIA using ¹²⁵I-labelled human insulin and anti-(human insulin) antiserum; the latter was raised in guinea pigs against a human-insulin–BSA conjugate. Sheep anti-(guinea pig) antiserum was used for separation of bound and free tracer. Human insulin (Novo Biolabs, Copenhagen, Denmark) was used as a standard. The between-assay coefficient of variation was 10.3%.

Calculations and statistics
For each dose of diazoxide, acetylcholine and dipyridamole, FBF registrations during the last 2 min of infusion were averaged. The FBF ratio (ratio of experimental to non-experimental FBF) was calculated to correct for systemic changes due to time or arousal in order to consider only changes induced by local infusions [26]. For comparison of the results of FBF measurements between glibenclamide and acarbose, the percentage change in the FBF ratio was used as the most important parameter, in accordance with recommendations that expressing data as the percentage change in the FBF ratio is the best way to compare changes in flow [27,28]. Results were analysed by repeated-measures ANOVA over all vasodilator dosages for each drug. In a separate analysis, the effect of treatment (glibenclamide versus acarbose) was evaluated by a two-factor repeated-measures ANOVA. During reperfusion after the ischaemic stimuli, maximal FBF was determined. This parameter was also analysed using a repeated-measures ANOVA model. Laboratory values after glibenclamide treatment were compared with those after acarbose treatment using a paired-samples t-test. The SPSS PC + 9.0.1 program (Statistical Package for Social Sciences) was used, and P < 0.05 was considered statistically significant.

RESULTS
Baseline characteristics
The study population consisted of 12 patients with Type II diabetes mellitus (seven male and five female). The mean age of the participants was 60.8 ± 2.5 years, their body mass index was 28.9 ± 0.9 kg·m⁻² (body weight 82.5 ± 3.8 kg) and their HbA₁c level before entering the study was 7.5 ± 0.4%. Mean cholesterol at entry was 5.6 ± 0.2 mmol·l⁻¹, non-fasting triacylglycerols 2.6 ± 0.3 mmol·l⁻¹, systolic blood pressure 150 ± 7 mmHg and diastolic blood pressure 88 ± 2 mmHg (blood pressures measured sphygmomanometrically). The mean duration of diabetes was 4.8 ± 0.8 years. The medication of the diabetic patients consisted of: ACE (angiotensin-converting enzyme) inhibitor (4), calcium antagonist (2), angiotensin II antagonist (1), β blocker (1), thiazide diuretic (1), metformin (5), fibrate (1), hormone replacement therapy (1), theophylline (1), mucolyticum (1), inhalation parasympaticolyticum (1), inhalation sympathicomimeticum (1) and inhalation corticosteroid (1).

Metabolic and clinical parameters (Table 1)
After 8 weeks of treatment with glibenclamide, fasting glucose and HbA₁c values were significantly lower than after treatment with acarbose. The plasma insulin concentration, but not that of C-peptide, was significantly higher during glibenclamide than during acarbose treatment. In 11 of the 12 patients glibenclamide could be demonstrated in plasma after glibenclamide treatment (range 0.01–0.49 µg·ml⁻¹), whereas the sulphonylurea drug was undetectable in all patients after acarbose treatment. Plasma cholesterol was significantly lower after glibenclamide treatment than after acarbose treatment. Plasma triacylglycerols tended to be lower during glibenclamide treatment. Baseline intra-arterial systolic and diastolic blood pressures were similar after glibenclamide and acarbose treatment. The body weight of the participants was significantly higher after glibenclamide as compared with acarbose treatment.

Vasodilator responses
Diazoxide, acetylcholine and dipyridamole all induced significant vasodilation in the infused forearm, with no

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Glibenclamide</th>
<th>Acarbose</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose (mmol·l⁻¹)</td>
<td>8.5 ± 0.7</td>
<td>12.3 ± 1.4</td>
<td>0.01</td>
</tr>
<tr>
<td>HbA₁c (%)</td>
<td>7.3 ± 0.4</td>
<td>8.0 ± 0.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Insulin (m-units·ml⁻¹)</td>
<td>28 ± 4</td>
<td>17 ± 3</td>
<td>0.01</td>
</tr>
<tr>
<td>C-peptide (nmol·l⁻¹)</td>
<td>1.12 ± 0.10</td>
<td>1.00 ± 0.11</td>
<td>0.11</td>
</tr>
<tr>
<td>Sulphonylurea (µg·ml⁻¹)</td>
<td>0.13 ± 0.04</td>
<td>n.d.</td>
<td>0.01</td>
</tr>
<tr>
<td>Cholesterol (mmol·l⁻¹)</td>
<td>5.2 ± 0.3</td>
<td>5.5 ± 0.3</td>
<td>0.01</td>
</tr>
<tr>
<td>Triacylglycerols (mmol·l⁻¹)</td>
<td>1.8 ± 0.3</td>
<td>2.4 ± 0.3</td>
<td>0.06</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>149 ± 4</td>
<td>150 ± 5</td>
<td>0.88</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>70 ± 2</td>
<td>70 ± 1</td>
<td>0.64</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>83.2 ± 4.1</td>
<td>81.1 ± 4.1</td>
<td>&lt; 0.0005</td>
</tr>
</tbody>
</table>
change in FBF in the control arm, after both oral treatment strategies (Table 2). Furthermore, in the ischaemia studies, the longer the arterial occlusion lasted, the higher the maximal FBF that was achieved after release of the cuff around the upper arm.

The three baseline measurements after glibenclamide treatment as well as those after acarbose treatment showed a significant change over time ($P < 0.0005$), but this change was similar in both study arms. As such, there was no difference in baseline recordings between glibenclamide and acarbose treatment at any time point ($P = 0.21$; Table 2). Therefore we can reliably compare the results of the experiments after the two treatment strategies.

Diazoxide induced a significantly larger increase in FBF after acarbose treatment than after glibenclamide treatment ($P = 0.001$; Table 2). These increases in FBF corresponded to increases in the FBF ratio of 561 ± 101% after acarbose treatment and 290 ± 58% after glibenclamide treatment ($P < 0.0005$; Figure 2). There were no statistically significant differences in FBF values in response to acetylcholine and dipyridamole after 8 weeks of treatment with glibenclamide compared with 8 weeks of treatment with acarbose (acetylcholine, $P = 0.97$; dipyridamole, $P = 0.32$; Table 2). The increases in FBF ratio values in response to acetylcholine and dipyridamole were also not significantly different (Figure 2).

The maximal FBF in response to the three occlusion periods was also not significantly different after 8 weeks of treatment with glibenclamide as compared with acarbose (Figure 2).

### Compliance

The compliance in each treatment group was very high. During the acarbose treatment period, altogether six out of 2016 tablets were forgotten. During the glibenclamide treatment period, two out of 672 capsules were forgotten. This corresponds to a compliance of 99.7% for both drugs.

### Side effects and hypoglycaemic symptoms

During acarbose treatment, all participants reported flatulence, whereas none reported this symptom during glibenclamide treatment. Other side effects during acarbose treatment included diarrhoea (2), constipation (1), itching (2) and nausea (2); those reported during glibenclamide treatment were diarrhoea (1) and nausea (1). Three participants reported hypoglycaemic symptoms (hunger, dizziness, perspiration) during glibenclamide treatment, whereas only one participant experienced these symptoms during acarbose treatment. For none of the study subjects were the side effects a reason to discontinue participation in the study.

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Table 2: FBF in the experimental (Exp.) and non-experimental (Non-exp.) arms before and during infusion of three increasing doses of diazoxide, acetylcholine and dipyridamole after 8 weeks of glibenclamide treatment and 8 weeks of acarbose treatment

<table>
<thead>
<tr>
<th>Dose</th>
<th>Glibenclamide</th>
<th>Acarbose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline 1</td>
<td>3.0 ± 0.3</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td>Diazoxide 0.25</td>
<td>6.3 ± 0.5</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td>Diazoxide 0.75</td>
<td>9.8 ± 1.1</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td>Diazoxide 2.25</td>
<td>13.2 ± 1.6</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td>Baseline 2</td>
<td>5.5 ± 0.6</td>
<td>3.6 ± 0.4</td>
</tr>
<tr>
<td>Acetylcholine 0.5</td>
<td>7.9 ± 1.4</td>
<td>3.3 ± 0.4</td>
</tr>
<tr>
<td>Acetylcholine 2.0</td>
<td>10.6 ± 1.7</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td>Acetylcholine 8.0</td>
<td>16.1 ± 2.1*</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>Baseline 3</td>
<td>3.9 ± 0.4‡</td>
<td>3.4 ± 0.3</td>
</tr>
<tr>
<td>Dipyridamole 10</td>
<td>4.4 ± 0.4</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td>Dipyridamole 30</td>
<td>5.4 ± 0.4</td>
<td>3.3 ± 0.4</td>
</tr>
<tr>
<td>Dipyridamole 100</td>
<td>6.5 ± 0.6*</td>
<td>3.5 ± 0.4</td>
</tr>
</tbody>
</table>

The units for infusion of diazoxide were mg·min⁻¹·dl⁻¹, and those for the infusion of acetylcholine and dipyridamole were µg·min⁻¹·dl⁻¹. Significance of differences: *$P < 0.0005$ for repeated-measures ANOVA over all doses; †$P = 0.001$ for two-factor repeated-measures ANOVA compared with glibenclamide-induced vasodilation after glibenclamide treatment; ‡$P < 0.0005$ for repeated-measures ANOVA over the three baseline measurements; §$P = 0.21$ (not significant) for two-factor repeated-measures ANOVA of the three baseline measurements compared with baseline measurements after glibenclamide treatment.
DISCUSSION

The major finding of the present study is that chronic treatment of Type II diabetes patients with glibenclamide attenuates the vasodilator response to K$_{ATP}$ channel opening, as compared with chronic treatment with acarbose. This finding is based on the observation that the vasodilation induced by the K$_{ATP}$ channel opener diazoxide was significantly attenuated after 8 weeks of treatment with glibenclamide as compared with 8 weeks of treatment with acarbose in patients with Type II diabetes.

This finding is in accordance with a previously published study in healthy volunteers indicating that acute exposure to glibenclamide attenuates diazoxide-induced vasodilation [16]. In the present study we investigated the effects of chronic treatment of patients with Type II diabetes with glibenclamide. Thus the effects of chronic treatment of Type II diabetes patients with glibenclamide on vascular K$_{ATP}$ channels appear to be qualitatively similar to the effects of acute glibenclamide administration in healthy volunteers. Our present observations are in contrast with a study indicating that acute orally administered glibenclamide potentiates the vasodilator response to diazoxide in healthy volunteers [29]. However, acute oral administration of glibenclamide induces a short-term increase in insulin concentration throughout the experiment. As insulin itself induces vasodilation, the more pronounced vasodilation during diazoxide infusion after oral glibenclamide could be a result of the vasodilation induced by insulin. Another study showed that diazoxide-induced vasodilation was similar after 1 month of glibenclamide treatment as compared with 1 month of metformin treatment in patients with Type II diabetes [30]. The duration of the two treatment strategies in that study was relatively short, so that carryover effects cannot be excluded. Furthermore, acarbose is a better comparison for the vascular effects of glibenclamide, because it is not absorbed in the bowel. Metformin itself has been reported to affect vascular function [31,32] and is therefore less suitable as a comparison drug in a study on the vascular effects of glibenclamide.

Treatment of Type II diabetes mellitus with acarbose has never been associated with pharmacological effects on the cardiovascular system. Moreover, a cardiovascular effect of acarbose is unlikely, since there is hardly any uptake of this drug from the bowel into the circulation.

In contrast with the difference in diazoxide-induced vasodilation, there were no differences between glibenclamide and acarbose treatments in vascular responses to the endothelium-dependent vasodilator acetylcholine, to the adenosine uptake inhibitor dipyridamole or to an ischaemic stimulus. We have shown that glibenclamide inhibits vasodilation induced by ischaemia in humans [17], but that study was performed in healthy volunteers during acute parenteral administration of glibenclamide. The present study indicates that chronic administration
of glibenclamide in patients with Type II diabetes does inhibit the vasodilation induced by $K_{\text{ATP}}$ channel opening, but not endothelium-dependent, adenosine-mediated or ischaemia-induced vasodilation. Thus glibenclamide affects vascular $K_{\text{ATP}}$ channels when administered chronically in patients with Type II diabetes. Apparently, however, $K_{\text{ATP}}$ channel opening does not contribute significantly to the vasodilator responses to acetylcholine, dipyridamole and ischaemia in this setting.

Three potential confounders of the vasodilator response to acetylcholine can be identified in the present study. First, fasting glucose and HbA$_1c$ values were significantly higher after acarbose treatment than after glibenclamide treatment. This indicates that the dosages used were not completely equipotent in reducing the glucose concentration. Endothelium-dependent vasodilation has been shown to be less pronounced in subjects with increased fasting glucose [33], as well as during glucose infusion in healthy volunteers if combined with octreotide [34]. Therefore, if the higher glucose concentrations during acarbose treatment affected the vasodilator response to acetylcholine, vasodilation would have been reduced. Secondly, the insulin concentration after glibenclamide treatment was higher than after acarbose, probably due to the mechanism of action of glibenclamide. Acute hyperinsulinaemia promotes acetylcholine-induced vasodilation in healthy volunteers [35]. Provided that this is also true during chronic hyperinsulinaemia in patients with Type II diabetes, acetylcholine-induced vasodilation might therefore be more pronounced after glibenclamide. Thirdly, the cholesterol concentration was slightly, but significantly, higher after acarbose treatment. Endothelium-dependent vasodilation is attenuated in hypercholesterolaemic subjects [36,37], so acetylcholine-induced vasodilation might be less pronounced after acarbose. Based on the last two potential confounders (insulin and cholesterol concentrations), acetylcholine-induced vasodilation might be expected to be more pronounced after glibenclamide as compared with acarbose treatment. These observations strengthen the conclusion that acetylcholine-induced vasodilation is not attenuated after glibenclamide as compared with acarbose treatment, because both favour the occurrence of acetylcholine-induced vasodilation after glibenclamide as compared with acarbose treatment.

We do not know whether the observed differences in glucose, insulin and cholesterol concentrations may have affected the vasodilator responses to diazoxide, dipyridamole and ischaemia, as these issues have never been investigated in the literature.

Baseline intra-arterial systolic and diastolic blood pressures were similar after glibenclamide as compared with acarbose treatment. Body weight was higher after glibenclamide than after acarbose. A possible explanation for this difference is the higher glucose concentration during acarbose treatment, which could lead to more pronounced fluid loss in the urine. Additionally, a loss of appetite due to the gastrointestinal side effects of acarbose could explain the difference in body weight.

All patients on acarbose complained of flatulence. This was probably due to the fact that they were treated with the highest permitted dosage straight away. These side effects would have been less pronounced if we had followed the dose titration recommended in the package leaflet. However, the time schedule of the study did not allow this titration approach.

In summary, after glibenclamide treatment, diazoxide-induced vasodilation was significantly less pronounced than after acarbose treatment. The vasodilatory responses induced by acetylcholine, dipyridamole or an ischaemic stimulus were similar after glibenclamide and acarbose treatments. This indicates that chronic treatment of patients with Type II diabetes with glibenclamide is associated with an attenuated vasodilator response to $K_{\text{ATP}}$ activation. This finding implies that glibenclamide may affect defensive mechanisms under conditions of $K_{\text{ATP}}$ channel activation.

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25 Reference deleted.