Basal and exercise-induced skeletal muscle blood flow is augmented in type I diabetes mellitus

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A B S T R A C T

Hyperaemia occurs early in the renal and retinal microcirculation of patients with type I (insulin-dependent) diabetes mellitus, and may be critical in the development of nephropathy and retinopathy. We therefore sought to determine whether resting and exercise-induced hyperaemia was also apparent in the skeletal muscle circulation of young subjects with type I diabetes. Blood flow was assessed by venous occlusion plethysmography in 18 diabetic (DM) subjects and 20 matched controls. Exercise entailed 2 min of isotonic exercise against no load. Endothelium-dependent and -independent vasodilator function was assessed following intra-arterial infusion of acetylcholine and sodium nitroprusside respectively. Forearm blood flow (FBF) was higher in DM subjects than in controls (3.3 ± 0.3 and 2.2 ± 0.2 ml·min⁻¹·100 ml⁻¹ forearm respectively; P < 0.005). This was not due to differences in forearm or body size, blood pressure, heart rate, lipid status or glycaemic control. Peripheral insulin levels were higher in DM subjects than in controls (48.5 ± 8 and 15.5 ± 1.5 ì-units/ml respectively; P < 0.005). Resting FBF was closely correlated with insulin levels (r² = 0.4; P < 0.005). Parameters of exercise-induced hyperaemia [including peak flow (16.4 ± 1.4 and 12.0 ± 0.7 ml·min⁻¹·100 ml⁻¹ forearm in DM and control subjects respectively; P < 0.01) and the volume repaid to the forearm at 5 min post-exercise (32.1 ± 3.1 and 23.1 ± 1.4 ml·100 ml⁻¹ forearm respectively; P < 0.05)] were also significantly greater in DM subjects, even when differences in resting FBF were taken into account. Peak hyperaemic blood flow and the volume repaid at 5 min were also related to insulin levels (r² = 0.16; P < 0.05 and r² = 0.27; P < 0.005 respectively). The vasodilator response to acetylcholine was reduced in DM subjects (P < 0.05; analysis of variance), and the slope of this dose–flow relationship was inversely related to insulin levels (r² = 0.2; P < 0.05). These data show that both resting and exercise-induced skeletal muscle blood flow are augmented in young patients with type I diabetes, possibly due to the vasodilatory effect of increased insulin levels. Diminished vasodilator responses to acetylcholine may also, in part, be a consequence of insulin-augmented resting muscle blood flow.

INTRODUCTION

The regulation of resting forearm blood flow (FBF) and exercise-induced hyperaemia is dependent on the interplay between local influences, such as endothelial paracrine factors (including nitric oxide [1,2], prostaglandins [3,4] and adenosine [5]) and local vasoactive metabolites (potassium, lactate, acid–base regulation) [6],

Key words: endothelium, hyperaemia, insulin, metabolic vasodilation, vasodilation.
Abbreviations: ACh, acetylcholine; AUC, area under the curve; DM subjects, subjects with type I diabetes; FBF, forearm blood flow; FVR, forearm vascular resistance; Hb A₁c, glycosylated haemoglobin; MAP, mean arterial pressure; ΔPeak FBF, absolute increase in peak functional hyperaemic blood flow following exercise; SNP, sodium nitroprusside.
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and systemic counter-regulatory influences such as the autonomic nervous system. Numerous studies have demonstrated the importance of endothelium-derived nitric oxide (NO) in the control of resting tone, as well as in ischaemic and functional hyperaemia [1,2,7,8]. It is now clear that atherosclerosis and its associated risk factors, such as diabetes mellitus, are associated with endothelial vasodilator dysfunction that is due in part to reduced resting and stimulated bioavailability of NO [9,10]. This dysfunction is apparent in both the conduit [11] and resistance [9] circulation. Diabetes and other risk factors may therefore influence the endothelium-dependent control of resting vascular tone and the skeletal muscle blood flow response to both ischaemia and exercise.

Diabetes mellitus is associated with both macro- and micro-vascular diseases, which are a major cause of morbidity and mortality. The microvascular complications of nephropathy and retinopathy are particularly important in type 1 (insulin-dependent) diabetes. The haemodynamic hypothesis of microangiopathy details the pathophysiological events in the development of microvascular disease [12,13]. A cornerstone of this is the early development of microcirculatory hyperaemia, which has been demonstrated in the retinal [14], renal [15], cutaneous [16] and coronary [17] circulations. There is some evidence that resting hyperaemia also exists in the skeletal muscle circulation of patients with type 1 diabetes [10,18], suggesting abnormal control of resting skeletal muscle vascular tone. Whether this is causally or consequentially related to microvascular disease, and whether it affects the physiological response to exercise, are unknown. Moreover, little is known about the factors that precipitate or maintain this inappropriate hyperaemia.

We therefore sought to investigate whether resting and exercise-induced skeletal muscle FBF is elevated in subjects with type 1 diabetes early on in the course of their condition, i.e. before the development of overt vascular disease. In order to gain some insight into the mechanisms underlying any impairment of blood flow regulation in type 1 diabetes, we investigated endothelial function and other factors that might influence blood flow in this group of diabetic subjects.

This work was presented in part at the 70th Scientific Sessions of the American Heart Association in November 1997 [18a].

METHODS

Subjects
A total of 18 young subjects with type I diabetes (DM subjects) and 20 healthy control subjects were recruited by advertisement and participated in this study. The study was carried out in accordance with the Declaration of Helsinki (1989) of the World Medical Association, and was approved by the Human Research Ethics Committee of Monash Medical Centre. All subjects were fully informed and provided written informed consent. Participants were clinically well and were screened for cardiovascular risk factors and disease by medical history, physical examination, full fasting lipid profile, fasting glucose level, urea, creatinine and liver function tests. Subjects were excluded if there were any signs of cardiovascular disease or significant non-cardiovascular disease such as renal impairment or abnormality in liver function. No subjects were taking any vasoactive medication apart from the oral contraceptive pill (three control subjects and four DM subjects).

Baseline characteristics are shown in Table 1. There were no differences in age, gender distribution, body mass index or conventional cardiovascular risk factors, or in fasting total cholesterol, triacylglycerols, low-density lipoprotein cholesterol or high-density lipoprotein cholesterol, between the two groups.

DM subjects were all controlled with insulin. The average daily total insulin dose was 76±32 units (range 42–174 units). The duration of diabetes was 120±50 months, with at least fair metabolic control as represented by levels of glycosylated haemoglobin (Hb A1c) (8.5±0.3%). Timed overnight urine collections were performed in DM subjects to assess albumin excretion rates, which were within normal limits (5.6±3.4 µg/min). None of the DM subjects had clinical evidence of retinopathy or neuropathy.

General procedures
All studies were performed in the morning in a dedicated quiet climate-controlled laboratory (temperature 22–23 °C) with dimmed lighting. Subjects attended the laboratory having fasted (8–12 h), and having refrained from aspirin and non-steroidal anti-inflammatory drugs for at least 5 days before the study and from caffeine-containing beverages for 12 h. Each subject was given a standardized light breakfast, before which DM subjects received their usual insulin dose. In DM subjects, blood glucose levels were documented at the time of measurement of basal flow and exercise-related blood flow. It was the intention of the study to investigate diabetic patients in their usual state but, in order to avoid the potentially confounding haemodynamic effects of marked hyperglycaemia or hypoglycaemia, a prospective decision was made to exclude any individual whose blood glucose deviated from the range 5–20 mmol/l during the study. No active treatment was given for control of blood glucose during the study, and no subjects required exclusion.

Studies were performed in the supine position. A 20 G, 5 cm polyethylene catheter (Cook, Brisbane, Australia)
was introduced into the brachial artery of the non-dominant forearm under local anaesthesia utilizing aseptic conditions. The arterial line was used for on-line measurement of blood pressure and for direct intrararterial drug infusions. The catheter was connected via a minimum-dead-space saline-filled line to a pressure transducer (Biosensors International, Singapore). Physiological saline was infused at a rate of 0.4 ml/min through the catheter into the brachial artery to maintain patency. FBF (ml·min⁻¹·100 ml⁻¹ forearm tissue) was measured at rest and in response to both endothelium-dependent and -independent vasodilators infused directly into the brachial artery, as described previously [8,19]. In brief, blood flow measurement was achieved by the well validated technique of venous occlusion plethysmography [20] using a calibrated mercury-in-silastic strain gauge (D. E. Hokanson, Bellevue, Western Australia). During the recording of FBF, the hands were excluded from the circulation by inflation of a wrist cuff to suprasystolic pressure. Venous occlusion pressure was 40 mmHg. Measurement of resting FBF was carried out at least 30 min after insertion of the brachial artery line and at least 2 h after insulin injection in DM subjects. Measurement of resting FBF was repeated until a stable recording was obtained.

FBF responses were measured continuously for 2 min following infusion of each dose of drug. An average FBF was calculated from at least five venous occlusion cycles. Baseline flow was re-measured at least 10 min after the completion of each drug sequence, and this was repeated until a stable recording similar to the original basal FBF value was achieved. Mean arterial pressure (MAP) was measured on-line. Forearm vascular resistance (FVR) was calculated by dividing MAP by FBF, and was expressed in arbitrary units.

### Drug infusion protocol

The volume of the non-dominant forearm was measured in each subject by water displacement. Doses of drugs were then calculated to obtain a predicted target forearm concentration. Acetylcholine (ACh) chloride (Miochol; Iolab Pharmaceuticals, Sydney, Australia), an endothelium-dependent NO-mediated vasodilator, was infused into the brachial artery for 3 min at target forearm concentrations of 2.7, 9 and 27 μg·min⁻¹·100 ml⁻¹ forearm. Sodium nitroprusside (SNP) (Faulding, Melbourne, Australia), an endothelium-independent vascular smooth muscle vasodilator, was infused into the brachial artery for 3 min at a target forearm concentration of 9 μg·min⁻¹·100 ml⁻¹ forearm. After dilution in normal saline, all drugs were infused at a rate of 0.4 ml/min using a syringe pump (Terumo Corp., Tokyo, Japan).

### Functional hyperaemia

Functional hyperaemic blood flow was measured in response to 2 min of isometric wrist flexion and extension exercise against no load paced by a metronome at 45 cycles per min. This stimulus has been shown to be reproducible in our laboratory [7]. Peak functional hyperaemic blood flow (peak FBF) was measured immediately on cessation of exercise, and flow measurement was repeated for 5 min thereafter, similar to that described previously for reactive hyperaemia [8]. Flow was measured every 7 s during the first 2 min post-exercise and five times per min thereafter. This enabled the construction of a post-exercise flow decay curve for each individual patient, as demonstrated in Figure 1. The absolute increase in peak functional hyperaemic blood flow following exercise (ΔPeak FBF) was calculated by subtracting basal flow immediately before exercise from peak FBF. Furthermore, the area under the flow–time

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>DM subjects (n = 18)</th>
<th>Controls (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>22 ± 4 (mean ± S.D.)</td>
<td>23 ± 5 (mean ± S.D.)</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>10/8</td>
<td>10/10</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.7 ± 0.4</td>
<td>23.7 ± 0.6</td>
</tr>
<tr>
<td>Cardiovascular risk factors</td>
<td>One smoker</td>
<td>0</td>
</tr>
<tr>
<td>Diabetic complications</td>
<td>None</td>
<td>NA</td>
</tr>
<tr>
<td>Duration of diabetes (months)</td>
<td>120 ± 50 (mean ± S.D.)</td>
<td>NA</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>12 ± 1.5*</td>
<td>4.5 ± 0.1</td>
</tr>
<tr>
<td>Hb A₁C (%)</td>
<td>8.5 ± 0.3</td>
<td>NA</td>
</tr>
<tr>
<td>Total daily insulin dose (units)</td>
<td>76 ± 32 (mean ± S.D.)</td>
<td>NA</td>
</tr>
<tr>
<td>Fasting cholesterol (mmol/l)</td>
<td>5.2 ± 0.3</td>
<td>4.8 ± 0.1</td>
</tr>
<tr>
<td>Fasting LDL cholesterol (mmol/l)</td>
<td>3.1 ± 0.2</td>
<td>2.8 ± 0.1</td>
</tr>
<tr>
<td>Fasting HDL cholesterol (mmol/l)</td>
<td>1.5 ± 0.1</td>
<td>1.5 ± 0.07</td>
</tr>
<tr>
<td>Fasting TG (mmol/l)</td>
<td>1.2 ± 0.2</td>
<td>1.1 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. unless indicated. Significance of difference compared with controls: * P < 0.001. Abbreviations: LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triacylglycerol; NA, not applicable.
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...aemic volume at 1 and 5 min. respectively) was also calculated, by subtracting the basal volume from AUC 1 and AUC 5 respectively. By subtracting basal volume from AUC 1 and AUC 5, the absolute volume repaid to the forearm can be calculated (ΔAUC 1 and ΔAUC 5 respectively).

Immediately on cessation of exercise, peak functional hyperaemic blood flow was measured. The area under the flow–time curve (hatched area) represents the volume repaid to the forearm at arbitrary time intervals of 1 and 5 min (AUC 1 and AUC 5 respectively). The absolute hyperaemic volume repaid to the forearm at 1 and 5 min after exercise (ΔAUC 1 and ΔAUC 5 respectively) was also calculated, by subtracting the basal volume (basal flow by time) from the total hyperaemic volume at 1 and 5 min.

Study protocol

Stable resting FBF was measured during the infusion of physiological saline solution (0.9% NaCl). A dose–response curve to ACh was then obtained using three incremental concentrations of ACh, as detailed above. The blood flow response to a single dose of SNP was compared between the two groups. Finally, the response to 2 min of isotonic exercise was measured. Between stimuli, a value for stable resting FBF similar to that achieved at the beginning of the study was obtained before proceeding.

Data

Analogue data were acquired directly on-line using an eight-channel analogue-to-digital computerized chart recorder (Maclab/8s System; AD Instruments, Castle Hill, NSW, Australia) and were analysed subsequently off-line (Chart 3.5; AD Instruments), as described previously [19].

Statistical analysis

Clinical characteristics are expressed as means ± S.D. Data are expressed as means ± S.E.M. Student’s t-test was utilized in the comparison of paired data (baseline characteristics, basal flow, exercise parameters and response to SNP). Differences between groups in the vasodilator responses to ACh were evaluated using two-way repeated-measures analysis of variance. Simple and multiple linear regression analyses were performed to determine the best individual and combination of predictor variables. Statistical significance was accepted where P < 0.05.

RESULTS

Resting FBF

Resting FBF was 50% higher in DM subjects compared with control subjects (3.3 ± 0.3 and 2.2 ± 0.2 ml·min⁻¹·100 ml⁻¹ forearm respectively; P < 0.005), as demonstrated in Figure 2. This was associated with a difference in FVR (29.0 ± 2.6 and 42.5 ± 3.7 arbitrary units respectively; P = 0.005), but no significant difference between groups with respect to MAP (83 ± 1.6 mmHg and 81.0 ± 1.3 mmHg respectively). These observations were not accounted for by differences in systemic haemodynamics, or in morphometric variables such as body mass index, waist/hip ratio, forearm circumference (22.7 ± 0.5 cm and 22.5 ± 0.4 cm respectively) or forearm volume (946 ± 44 ml and 900 ± 40 ml respectively). There were no differences in the fasting lipid profile between the two groups.

Ambient plasma glucose and insulin levels were higher in DM subjects than in controls at the time of resting FBF measurement (glucose, 9.0 ± 1.0 and 5.4 ± 0.4 mmol/l respectively; insulin, 48.5 ± 8 and 15.5 ± 1.5 μ-units/ml respectively; P < 0.0005). Although resting FBF was a function of the presence or absence of diabetes (r² = 0.46; P < 0.005), it was not related to fasting or ambient glucose levels. Within the diabetic group there was no relationship between resting FBF and duration of diabetes or glycaemic control, as measured by Hb A₁c levels. Resting FBF was, however, directly related to plasma insulin levels both within the study cohort as a whole and in the diabetic group (FBF = 0.029[insulin] + 1.7; r² = 0.4, P < 0.005 in both cases; see Figure 3). Resting FBF was also a function of the total prescribed daily insulin dose in the DM subjects (r² = 0.22; P < 0.05).

Exercise-induced hyperaemic FBF

Peak FBF following 2 min of isotonic exercise was 37% higher in DM subjects compared with control subjects (16.4 ± 1.4 and 12.0 ± 0.7 ml·min⁻¹·100 ml⁻¹ forearm respectively; P < 0.05; Figure 4). This increase in peak FBF persisted even when taking into account differences in basal flow between groups (∆Peak FBF). ∆Peak FBF was 41% higher in DM subjects than in controls (12.8 ± 1.4 and 9.1 ± 0.8 ml·min⁻¹·100 ml⁻¹ forearm respectively;
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Figure 2  FBF (left), FVR (middle) and MAP (right) at rest in control subjects (C) and DM subjects

Resting FBF is 50% greater and FVR is 32% lower in the DM subjects compared with the control group (P < 0.005), with no difference in MAP. Abbreviation: AU, arbitrary units.

Figure 3  Correlation of resting FBF with ambient insulin and glucose levels in DM subjects (○) and control subjects (●)

There is a direct relationship between resting FBF and insulin levels in the study cohort (top left) and in DM subjects alone (top right), but no correlation between resting FBF and glucose levels (bottom panels). Abbreviations: μU, μ-units; NS, not significant.

P < 0.05). Peak FBF in the diabetic group was directly related to plasma insulin levels ($r^2 = 0.16; P < 0.05$), although ΔPeak FBF was not.

As shown in Table 2, there was a trend towards a difference in the volume repaid to the forearm at 1 min post exercise (AUC 1) (DM subjects, 9.6 ± 0.7 ml·100 ml⁻¹·min⁻¹ forearm; controls, 7.7 ± 0.4 ml·100 ml⁻¹·min⁻¹ forearm; P = 0.06). This trend was maintained when differences in basal flow were taken into account (DM subjects, 6.7 ± 0.7 ml·100 ml⁻¹·min⁻¹ forearm; controls, 5.0 ± 0.3 ml·100 ml⁻¹·min⁻¹ forearm, P = 0.07) (Table 2). The volume repaid to the forearm at 5 min post exercise (AUC 5) was increased by 39% in DM subjects compared with controls (32.1 ± 3.1 and 23.1 ± 1.4 ml·100 ml⁻¹· forearm respectively; P < 0.01) (Figure 5). This difference also persisted when differences in basal flow were taken into account (DM subjects, 15.1 ± 2.4 ml·100 ml⁻¹· forearm; controls, 9.8 ± 0.9 ml·100 ml⁻¹· forearm; P < 0.05). Both within the study cohort and within DM subjects alone, AUC 5 was correlated with plasma insulin levels ($r^2 = 0.27; P < 0.005$ and $r^2 = 0.25; P < 0.05$ respectively), although ΔAUC 5 was not. MAP did not
change from baseline during exercise in either group (see Table 2). Blood flow in the contralateral arm did not change following exercise.

Table 2 Mean data relating to basal flow and functional hyperaemia of the DM and control groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DM</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting FBF (ml min−1 100 ml−1 forearm)</td>
<td>3.3 ± 0.3</td>
<td>2.2 ± 0.2</td>
<td>0.005</td>
</tr>
<tr>
<td>Resting FVR (arbitrary units)</td>
<td>29.0 ± 2.6</td>
<td>42.5 ± 3.7</td>
<td>0.005</td>
</tr>
<tr>
<td>Resting MAP (mmHg)</td>
<td>83.1 ± 1.6</td>
<td>81.0 ± 1.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Peak FBF (ml min−1 100 ml−1 forearm)</td>
<td>16.4 ± 1.4</td>
<td>12.0 ± 0.7</td>
<td>0.01</td>
</tr>
<tr>
<td>ΔPeak FBF (ml min−1 100 ml−1 forearm)</td>
<td>12.0 ± 1.4</td>
<td>9.1 ± 0.8</td>
<td>0.02</td>
</tr>
<tr>
<td>ΔPeak AUC (ml 100 ml−1 forearm)</td>
<td>9.6 ± 0.7</td>
<td>7.7 ± 0.4</td>
<td>0.06</td>
</tr>
<tr>
<td>ΔAUC 1 (ml 100 ml−1 forearm)</td>
<td>6.7 ± 0.7</td>
<td>5.0 ± 0.3</td>
<td>0.07</td>
</tr>
<tr>
<td>AUC 1 (ml 100 ml−1 forearm)</td>
<td>32.1 ± 3.1</td>
<td>23.1 ± 1.4</td>
<td>0.01</td>
</tr>
<tr>
<td>Exercise MAP (mmHg)</td>
<td>85.2 ± 2.0</td>
<td>84.9 ± 1.9</td>
<td>0.2</td>
</tr>
</tbody>
</table>

FBF response to ACh

Intra-arterial infusion of ACh produced a dose-dependent increase in FBF in both DM subjects and controls (P < 0.005). The vasodilator response in DM subjects was, however, impaired compared with that in controls, with a downward and rightward shift in the dose–flow relationship (slope of dose–response curve: DM subjects, 0.29 ± 0.05; controls, 0.45 ± 0.04; P < 0.05) (Figure 6). At the highest concentration of ACh, FBF was 31% lower in DM subjects than in controls (13.2 ± 1.5 and 17.7 ± 1.4 ml min−1 100 ml−1 forearm respectively). In view of the differences in resting FBF between the two groups, the absolute changes in FBF and FVR from baseline (ΔFBF and ΔFVR respectively) were calculated for each subject at each incremental concentration of ACh. At the highest dose of ACh, ΔFBF was 30% lower in DM subjects than in control subjects (10.2 ± 1.2 and 15.1 ± 1.1 ml min−1 100 ml−1 forearm respectively).

The slope of the ACh dose–flow curve was inversely related to resting FBF (r² = 0.15; P < 0.005 and r² = 0.4; P < 0.005, for the whole-study cohort and the DM group respectively) and to plasma insulin levels (r² = 0.3; P < 0.005 and r² = 0.3; P < 0.005 respectively).

FBF response to SNP

SNP increased blood flow in both groups. In view of the differences in resting FBF between groups, the absolute

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change in flow from baseline was again calculated. There was no difference in the magnitude of this response between DM subjects and controls (DM subjects, 4.4 ± 0.3 ml min⁻¹ 100 ml⁻¹ forearm; controls, 4.4 ± 0.3 ml min⁻¹ 100 ml⁻¹ forearm). Similarly, there was no difference in FVR or MAP between the two groups following the infusion of SNP, and there was no change in blood flow in the contralateral forearm.

**DISCUSSION**

In this study we assessed resting and exercise-induced skeletal muscle FBF in a group of young, otherwise healthy, patients with type I diabetes, and compared these findings with data obtained from healthy age-matched control subjects. Resting FBF, exercise-related peak FBF and the volume repaid at 5 min after exercise (AUC 5) were higher in DM subjects than in the control group. These findings were not accounted for by differences in systemic haemodynamics, such as MAP or heart rate.

Close scrutiny of a few [10,18,21,22], but not all [9,23–27], previous studies using venous occlusion plethysmography suggests that resting skeletal muscle blood flow is increased in subjects with type I diabetes. This has been seen largely as a methodological inconvenience rather than as an important finding in its own right [10,18]. Only one study has stressed the importance of this observation [22]. As far as we are aware, there has been no systematic analysis of the possible contributory factors to this phenomenon in subjects with type I diabetes, and no previous study has related this hyperaemia to elevated peripheral insulin levels. Furthermore, the observation that increased muscle blood flow is maintained even during changes in metabolic demand caused by exercise has not been reported previously.

Augmented blood flow has also been demonstrated in other vascular beds in diabetic subjects, such as the coronary [17], renal [15] and retinal [14] beds. The physiological significance of this hyperaemia is not known, but it precedes the onset of microvascular disease, as detailed in the haemodynamic hypothesis of diabetic microangiopathy [12,13]. Elevated blood flow in the skeletal muscle circulation may thus also be an important initiating factor in the development of conduit [11] and resistance [9,23] vessel dysfunction.

A number of factors, including peripheral insulin levels, could potentially account for the increased resting and exercise-induced muscle blood flow in subjects with type I diabetes. Peripheral insulin levels were higher in subjects with type I diabetes than in our control group. This is consistent with previous observations [28], and is a reflection of subcutaneous administration rather than the central physiological release of insulin into the portal circulation. Importantly, resting FBF, peak exercise-induced FBF and the volume repaid to the forearm at 1 and 5 min after exercise were linearly related to both peripheral insulin levels and the total insulin dose prescribed. Although not proof of a causal relationship, these data suggest that peripheral insulin levels influence regulation of FBF. This view is supported by observations on the relationship between insulin and control of skeletal muscle blood flow in subjects with heart failure [29].

The vasodilator action of insulin is well recognized [30–32] and is thought to be partly mediated through an endothelium-dependent NO-related mechanism [33,34]. Other mechanisms involving adenosine, ATP-sensitive potassium channels and prostacyclin have also been proposed [35–37]. In fact, under conditions of reduced NO bioavailability, such as is thought to occur in diabetes, these alternative factors may play a more prominent role [38].

Interestingly, resting FBF is not increased in type II (non-insulin-dependent) diabetes, which is characterized by high circulating insulin levels and insulin resistance. This may be due to the effects of other factors that are commonly associated with type II diabetes, such as hypertension and dyslipidaemia, which are known to impair vascular function in their own right. Alternatively, it may be due to the occurrence of blunted or impaired insulin-mediated vasodilation in this syndrome [39].

Controversy exists as to whether the insulin resistance of type II diabetes is related to abnormal substrate delivery to skeletal muscle (i.e. impaired blood flow) or to abnormal insulin receptor function. The former possibility is important; if correct, it might be expected that insulin resistance, which may also accompany type I diabetes, would act to reduce FBF. It would therefore have been interesting to correlate the magnitude of hyperaemia in young subjects with type I diabetes with the degree of insulin resistance (if we had obtained an appropriate measure of insulin resistance in this group).

Although the issue is unresolved, glycaemic control is thought to affect the regulation of FBF. In healthy subjects hyperglycaemia has been shown to increase [40], decrease [41] or have no effect on [42] resting vascular tone. However, most evidence indicates that hyperglycaemia reduces NO bioavailability [40], possibly via a glutathione-sensitive free-radical-mediated pathway [43]. In the present study of diabetic patients with fair metabolic control (Hb A1C 8.5%), we found no relationship between fasting glucose, ambient glucose or Hb A1C levels and resting FBF. Peak exercise-induced FBF was, however, correlated with parameters of glycaemic control, suggesting that blood flow during exercise may be more dependent on glucose levels.

Vascular tone is controlled in part by endothelium-dependent mechanisms, including NO [2,7,44] and vasodilator prostanoids [3,4,7]. Thus endothelial vasodilator dysfunction might be expected to result in abnormal
regulation of exercise-related blood flow. In agreement with some [9,23,45], but not all [10,18,27], previous studies, we observed differences between DM subjects and control subjects in the dose–response curves to an endothelium-dependent muscarinic agonist. The vasodilator response to ACh is inextricably related to basal flow [46]. Although the dose–response curves to ACh in DM subjects and controls appeared to be different, we only examined three points on the dose–response curve. It is entirely possible that we are observing different phases of the same relationship. We cannot state for certain that the data points we have gathered in DM subjects and controls are from two different dose–flow curves or from different phases of the same dose–flow curve. Discrepancies between studies with regard to responses to muscarinic agonists may thus be related to differences in basal flow, as a result of differences in insulin levels or other parameters of the diabetic condition. Notably, the vasodilator response to ACh in the present study was inversely correlated with insulin levels. This has been observed previously [9].

The finding of an impaired vasodilator response to ACh in the present study may be consistent with reduced NO bioavailability in the diabetic subjects. This is at first hard to reconcile if one accepts that NO plays a significant role in both resting and exercise-induced blood flow regulation [7,47,48], both of which we have shown to be elevated in DM subjects. This apparent paradox could be explained in several ways. ACh-mediated vasodilation may not be impaired, but simply blunted, in the presence of resting hyperaemia. Alternatively, muscarinic receptor function may be abnormal [49], despite a normal post-receptor NO pathway. Another explanation might be that up-regulation of other endothelium-dependent factors, such as prostacyclin or adenosine, which contribute to resting and exercise-related hyperaemia [4,5,19] may have occurred.

Potential limitations
There are a number of potential limitations to this study. We have measured blood flow only in the forearm, which may not be representative of all muscle groups. Moreover, blood flow varies according to the fibre composition of muscle [50], which could potentially be different between the two groups [51] and thus could contribute to our findings.

Furthermore, although we have demonstrated augmented blood flow in DM subjects, we have not been able to show whether this is nutritive or non-nutritive flow (i.e. whether there is increased blood flow through arteriovenous shunts or if additional capillary recruitment occurs to provide useful extra nutrient delivery to skeletal muscle). If insulin is indeed responsible for the elevated blood flow in DM subjects, we may expect increased capillary recruitment [52]. This will require further investigation.

Examination of a dose–response curve with SNP, instead of the response to a single dose, may have allowed us to draw a better conclusion regarding the presence of endothelial dysfunction. However, the interpretation of these data would also have been complicated by increased resting blood flow, although the finding of a normal response to SNP is consistent with previous studies [9,23,24].

Conclusion
Both resting and exercise-induced FBF is increased in young diabetic subjects, and this increase in flow appears to be more closely related to circulating insulin levels than to plasma glucose. Flow responses to ACh – the conventional yardstick of the integrity of endothelial vasodilator function in vivo – were impaired in this group of young diabetic subjects, but this may be a function of basal flow and its relationship to insulin levels. Whether augmented muscle blood flow in young diabetic subjects is simply an epiphenomenon of the diabetic condition or plays a significant role in the genesis of vascular disease in the peripheral circulation is unclear from the present study. If the latter is true, our observations have important implications for the delivery of insulin in young patients with type I diabetes mellitus.

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