Vasodilator prostanoids, but not nitric oxide, may account for skeletal muscle hyperaemia in Type I diabetes mellitus

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ABSTRACT

We and others have previously documented increased resting and exercise-induced skeletal muscle blood flow in young subjects with Type I (insulin-dependent) diabetes mellitus compared with healthy controls. Both NO and prostanoids are important regulators of vascular tone and may therefore contribute to this hyperaemia. The aim of the present study was to determine the contribution of NO and vasodilator prostanoids to this skeletal muscle hyperaemia in diabetes. We assessed the effects of infusion into the intrabrachial artery of the cyclo-oxygenase inhibitor acetylsalicylic acid (ASA; aspirin) and of the l-arginine analogue N\textsubscript{G}-monomethyl-l-arginine (L-NMMA) on skeletal muscle blood flow in subjects with Type I diabetes mellitus (DM subjects) and control subjects. Blood flow was measured by venous occlusion plethysmography. Isotonic forearm exercise involved 2 min of wrist flexion and extension. Resting flow (forearm blood flow; FBF) was augmented in DM subjects, as was peak exercise-related blood flow (PFBF) and the volume repaid to the forearm 5 min after exercise (AUC 5, where AUC is area under the flow–time curve) ($P < 0.05$), even when accounting for differences in basal flow. Infusion of L-NMMA reduced resting flow by 48% in controls ($P < 0.005$) and by 12% in DM subjects (not significant). L-NMMA reduced PFBF and AUC 5 by 29% ($P < 0.05$) and 39% ($P < 0.0005$) respectively in controls, but had no significant effect on these parameters in DM subjects. Infusion of ASA reduced FBF, PFBF and AUC 5 in both DM ($P < 0.05$) and control ($P < 0.05$) subjects, but the magnitude of this reduction was greater in DM than in control subjects (ANOVA, $P < 0.05$), even when differences in resting FBF were accounted for. Indeed, ASA eliminated the differences in FBF, PFBF and AUC 5 between DM and control subjects. Thus increased release of vasodilator prostanoids, rather than of NO, appears to account for skeletal muscle hyperaemia in Type I diabetes.

INTRODUCTION

The haemodynamic hypothesis of diabetic microangiopathy details the pathophysiological events in the development of microvascular disease [1,2]. A cornerstone of this is the early development of microcirculatory hyperaemia, which has been demonstrated in the retinal [3], renal [4], cutaneous [5] and coronary [6] circulations. More recently we have demonstrated augmented resting and exercise-induced skeletal muscle blood flow in subjects with Type I (insulin-dependent) diabetes mellitus, which appears to be a function of insulin levels [7]. The control of skeletal muscle vascular tone at rest and

Key words: artery, blood flow, diabetes mellitus, nitric oxide, prostaglandins.

Abbreviations: ACh, acetylcholine; ASA, acetylsalicylic acid (aspirin); AUC 5, area under the flow–time decay curve at 5 min (representing the hyperaemic volume repaid to the forearm); ΔAUC 5, absolute hyperaemic volume repaid to the forearm 5 min after exercise; DM subjects, subjects with Type I diabetes mellitus; FBF, forearm blood flow; FVR, forearm vascular resistance; MAP, mean arterial pressure; l-NMMA, N\textsubscript{G}-monomethyl-l-arginine; PFBF, peak (exercise-induced) FBF; ΔPFBF, absolute increase in hyperaemic flow following exercise; SNP, sodium nitroprusside.

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in response to exercise and to reactive hyperaemia is dependent not only on systemic influences, such as the autonomic nervous system, but also on local vasoactive metabolites [8] and endothelium-dependent paracrine factors, such as nitric oxide (NO) [9–11], and the vasoactive prostanoids, principally prostacyclin (prosta-
glandin I₂) [12–14]. We and others have demonstrated an impaired NO-mediated vasodilator response to various stimuli in subjects with Type I diabetes [7,15–18]. These findings raise the possibility that, in conditions associated with endothelial vasodilator dysfunction, such as Type I diabetes, abnormal NO-mediated regulation of blood flow might be expected.

In the setting of impaired NO-mediated vasodilation, increased levels of prostacyclin could potentially be responsible for the hyperaemia that is observed in the skeletal muscle circulation of subjects with Type I diabetes. Conflicting reports suggest that levels of this prostanoid are increased [19–21], normal or reduced [22–24] in diabetes. Acetylsalicylic acid (ASA; aspirin), which rapidly inhibits prostaglandin formation by inactivating cyclo-oxygenase, can be used to assess the contribution of prostacyclin to this skeletal muscle hyperaemia [13,25]. In turn, the contribution of NO can be assessed following the infusion of N⁶-monomethyl-L-arginine (L-NMMA), an L-arginine analogue which is used to inhibit the synthesis of NO [26]. We hypothesized that augmented production of prostacyclin, but not of NO, might be responsible for skeletal muscle hyperaemia in subjects with Type I diabetes mellitus.

These data were presented in part at the 70th Scientific Sessions of the American Heart Association in November 1997, and have been published in Abstract form [26a].

METHODS

Subjects

Subjects with Type I diabetes (DM subjects) and healthy control subjects were recruited by advertisement to participate in this study. Subjects who had participated in the first study were subsequently recruited for the second part of the investigation (13 DM subjects and 20 controls received ASA, while 9 DM subjects and 11 controls received L-NMMA). The study was approved by the Human Research Ethics Committee of Monash Medical Centre, and all subjects were fully informed and provided written informed consent. The investigation conforms with the principles outlined in the Declaration of Helsinki.

Participants were clinically well, and were screened for cardiovascular risk factors and disease with a 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 and two 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 between the groups. In addition, there were no differences in fasting total cholesterol, triacylglycerols, low-density lipoprotein (LDL)-cholesterol or high-density lipoprotein (HDL)-cholesterol between the two groups.

DM subjects were all controlled with insulin. The average daily total insulin dose was 71 ± 18 units (range 42–174 units). The duration of diabetes was 113 ± 50 months, with at least fair metabolic control, as represented by levels of glycosylated haemoglobin (Hb A₁c) (8.6 ± 0.3%). Timed overnight urine collections were performed in DM subjects to assess albumin excretion rates, which were within normal limits (14.1 ± 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 (22–23 °C) with dimmed lighting. Subjects attended the laboratory fasted, having refrained from ASA (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. Blood glucose levels were documented in DM subjects at the time of measurement of basal flow and exercise-related blood flow. It was the intention of this

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>DM</th>
<th>Control</th>
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<tr>
<td>Age (years)</td>
<td>23 ± 5*</td>
<td>23 ± 5*</td>
</tr>
<tr>
<td>Gender (male:female)</td>
<td>6:7</td>
<td>10:10</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22.2 ± 0.6</td>
<td>22.7 ± 0.5</td>
</tr>
<tr>
<td>Diabetic complications</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>Duration of diabetes (months)</td>
<td>113 ± 50*</td>
<td>NA</td>
</tr>
<tr>
<td>Hb A₁c (%)</td>
<td>8.6 ± 0.3</td>
<td>NA</td>
</tr>
<tr>
<td>Total daily insulin dose (units)</td>
<td>71 ± 18*</td>
<td>NA</td>
</tr>
<tr>
<td>Fasting cholesterol (mmol/l)</td>
<td>5.2 ± 0.2</td>
<td>4.9 ± 0.1</td>
</tr>
<tr>
<td>Fasting LDL (mmol/l)</td>
<td>3.0 ± 0.2</td>
<td>2.8 ± 0.1</td>
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<tr>
<td>Fasting HDL (mmol/l)</td>
<td>1.5 ± 0.07</td>
<td>1.6 ± 0.08</td>
</tr>
<tr>
<td>Fasting TG (mmol/l)</td>
<td>1.0 ± 0.1</td>
<td>1.1 ± 0.1</td>
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Peak functional hyperaemia

DM
Control
Absolute volume repaid to forearm

Volume repaid (ml/100 ml forearm)

Basal volume

60 300 Time (s)

Figure 1  Time course of blood flow decay following 2 min of isotonic exercise in a typical DM subject (upper trace) and a typical control subject (lower trace)

Immediately on cessation of exercise, peak functional hyperaemic blood flow can be measured. The area under the flow–time curve represents the volume repaid to the forearm at 5 min (AUC 5). By subtracting basal volume from AUC 5, the absolute volume repaid to the forearm can be calculated (\(\Delta AUC\) 5).

To 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 with the subject in the supine position. A 20-gauge, 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 intra-arterial 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. Forearm blood flow (FBF; in units of 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 [13]. In brief, measurement of blood flow was achieved by the well validated technique of venous occlusion plethysmography [27] using a calibrated mercury-in-silastic strain gauge (D. E. Hokanson, Bellevue, WA, U.S.A.). 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. Furthermore, 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 again achieved. This was achieved on average in 13 min. Mean arterial pressure (MAP) was measured online. 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. Acetylcholine (ACh) chloride (Miochol; Iolab Pharmaceuticals, Sydney, Australia), an endothelium-dependent NO-mediated vasodilator, was infused into the brachial artery for 3 min at doses of 2.7, 9 and 27 \(\mu\)g/min. Sodium nitroprusside (SNP) (Faulding, Melbourne, Australia), an endothelium-independent vascular smooth muscle vasodilator, was infused into the brachial artery for 3 min at a dose of 9 \(\mu\)g/min. ASA (ASPISOL; Bayer) was infused into the brachial artery at a dose of 10 mg/min, as previously described [13,25], to achieve a local plasma concentration (assuming an FBF of 2.5 ml min\(^{-1}\) 100 ml\(^{-1}\) forearm) of 500 \(\mu\)g/ml. L-NMMA (Clinalfa AG, Laufelfingen, Switzerland) was infused at a dose of 2 mg/min. 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).
Protocol

Figure 2 Outline of the study protocol
Blood flow was assessed initially during infusion of normal saline at rest (FBF), and subsequently following isotonic exercise (functional hyperaemic blood flow; FHB), in response to ACh and following the infusion of SNP. This order was then repeated during l-NMMA or ASA infusion.

Functional hyperaemia
Functional hyperaemic blood flow was measured in response to 2 min of isotonic wrist flexion and extension exercise against no load, paced by a metronome at 45 cycles/min. This stimulus has been shown to be reproducible in our laboratory [25]. Peak functional hyperaemic FBF (PFBF) was measured immediately on cessation of exercise, and flow measurement was repeated for 5 min thereafter, similar to the procedure described previously for reactive hyperaemia [11]. Flow was measured every 7 s during the first 2 min post-exercise, and 5 times per min thereafter. This enabled the construction of a post-exercise flow decay curve for each individual patient, as shown in Figure 1. The absolute increase in hyperaemic flow following exercise (ΔPFBF) was calculated by subtracting basal FBF immediately prior to exercise from PFBF. Furthermore, the area under the flow–time decay curve (representing the hyperaemic volume repaid to the forearm) was calculated at the arbitrary time interval of 5 min (AUC 5). The absolute hyperaemic volume repaid to the forearm 5 min after exercise (AAUC 5) was also calculated by subtracting the basal volume (basal flow × time) from the total hyperaemic volume at 5 min.

In order to ensure acceptable reproducibility of this technique during the study, we examined forearm exercise on three separate occasions within one morning in a group of nine healthy subjects. The within-day reproducibility of the technique was found to be acceptable, with a coefficient of variation of 4.2%, 6.3% and 7.2% for resting FBF, PFBF and AAUC 5 respectively.

Study protocol
Stable resting FBF was measured during the infusion of physiological saline solution, as shown in Figure 2.

Subsequently, the response to 2 min of isotonic exercise was measured, followed by a dose–response curve to ACh and the response to SNP. ASA or l-NMMA infusion was subsequently carried out for 10 min to record the effect of inhibition of cyclo-oxygenase or NO synthase respectively on basal flow. This infusion was then continued while investigating the effects of ASA or l-NMMA on functional hyperaemia and the responses to ACh and SNP. After each stimulus, a stable resting FBF value, similar to that achieved at the beginning of the study, was obtained before proceeding.

In each individual subject, the two studies (involving infusion of ASA in one and l-NMMA in the other) were completed within 1 week. The average duration of each study was 222 min (range 198–242 min).

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). Assessment of the relative effect of ASA or l-NMMA in the two study groups was performed using two-way repeated-measures ANOVA. Statistical significance was accepted where P < 0.05.

RESULTS

Effect of l-NMMA infusion on resting FBF
Intra-arterial infusion of l-NMMA produced a 48% reduction in resting FBF in the control group (2.1 ± 0.2 to 1.1 ± 0.1 ml min⁻¹ 100 ml⁻¹ forearm; P < 0.0005), with reciprocal changes in FVR (42.7 ± 4.1 to 70.4 ± 7.2 units; P < 0.0005). In DM subjects, infusion of l-NMMA resulted in a non-significant 12% reduction in resting FBF (3.4 ± 0.5 to 3.0 ± 0.5 ml min⁻¹ 100 ml⁻¹ forearm). The magnitude of the decrease in FBF was greater in the control subjects (ANOVA; P < 0.05). Following the infusion of l-NMMA there was still a significant difference in resting FBF between the groups (DM subjects, 3.0 ± 0.5 ml min⁻¹ 100 ml⁻¹ forearm; controls, 1.1 ± 0.1 ml min⁻¹ 100 ml⁻¹ forearm; P < 0.005) (Figure 3a).

There was no significant change in MAP following infusion of l-NMMA.

Effect of ASA infusion on resting FBF
Intra-arterial infusion of ASA produced a 33% decrease in resting FBF in DM subjects (from 3.9 ± 0.4 to 2.6 ± 0.3 ml min⁻¹ 100 ml⁻¹ forearm; P < 0.005) (Figure 3b). There was a decrease of 21% in FBF in the control group (2.8 ± 0.2 to 2.2 ± 0.2 ml min⁻¹ 100 ml⁻¹ forearm; P < 0.05). The magnitude of the reduction was, however, greater in DM than in control subjects (P < 0.005).
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Figure 3 FBF at rest in DM subjects and control subjects (C)
(a) Following the intra-arterial infusion of L-NMMA (black bars), FBF was reduced in the control group by 48% \( (P < 0.005) \), but not significantly in the diabetic group.
(b) ASA (black bars) reduced FBF in both DM \( (P < 0.005) \) and control \( (P < 0.05) \) subjects. However, the magnitude of the decrease in FBF following ASA was greater in DM subjects than in controls ANOVA, \( P < 0.005 \). ASA eliminated the difference in FBF between the two groups (DM subjects, \( 2.6 \pm 0.3 \text{ ml} \cdot \text{min}^{-1} \cdot \text{100 ml}^{-1} \text{ forearm} \); controls, \( 2.2 \pm 0.2 \text{ ml} \cdot \text{min}^{-1} \cdot \text{100 ml}^{-1} \text{ forearm} \); \( P < 0.05 \)).

Figure 4 Absolute peak functional hyperaemic blood flow (\( \Delta PFBF \)) in DM subjects and control subjects (C)
(a) Following the intra-arterial infusion of L-NMMA (black bars), \( \Delta PFBF \) was reduced in the control group \( (P < 0.05) \), but not in the diabetic group. (b) ASA (black bars) reduced \( \Delta PFBF \) in both DM \( (P < 0.05) \) and control \( (P < 0.05) \) subjects. However, the magnitude of the reduction in \( \Delta PFBF \) following ASA was greater in DM subjects than in controls ANOVA, \( P < 0.005 \). ASA eliminated the difference in \( \Delta PFBF \) between the two groups.

The infusion of ASA did not produce any changes in MAP in either the DM subjects (pre-ASA, \( 87 \pm 3 \text{ mmHg} \); post-ASA, \( 85 \pm 2 \text{ mmHg} \)) or the controls (pre-ASA, \( 84 \pm 2 \text{ mmHg} \); post-ASA, \( 85 \pm 3 \text{ mmHg} \)), and there was no significant difference in MAP between the two groups either before or after ASA infusion.

Effect of L-NMMA infusion on PFBF following exercise
L-NMMA reduced PFBF by 29% in control subjects (from \( 11.5 \pm 1.4 \text{ to } 8.1 \pm 0.9 \text{ ml} \cdot \text{min}^{-1} \cdot \text{100 ml}^{-1} \text{ forearm} \);
There was a 39% reduction in volume repaid to the forearm (AUC 5) in response to L-NMMA in the control group (21.4 ± 2.4 to 13.0 ± 1.7 ml/100 ml forearm; *P < 0.0005), whereas there was no significant change in AUC 5 in DM subjects (31.1 ± 5.1 to 27.6 ± 4.1 ml/100 ml forearm). The magnitude of the reduction in AUC 5 following ASA was greater in DM subjects than in controls (ANOVA, *P < 0.005). ASA eliminated the difference in ∆AUC 5 between the two groups.

Effect of ASA infusion on volume repaid following exercise

ASA infusion reduced AUC 5 to a greater extent in DM subjects than in controls (ANOVA, *P < 0.05). In DM subjects this reduction was 33% (from 33.6 ± 4.0 to 22.5 ± 2.3 ml·min⁻¹·100 ml⁻¹ forearm; *P < 0.0005), while the reduction in controls was 20% (from 23.9 ± 1.2 to 19.6 ± 1.4 ml·min⁻¹·100 ml⁻¹ forearm). The infusion of ASA eliminated the difference between the DM and control groups in AUC 5 values.

Similarly, ASA eliminated the difference in ∆AUC 5 values between the two groups, as a consequence of a greater reduction in blood flow in the DM compared with the control subjects (Figure 5b).
Figure 6 Absolute increase in FBF in DM subjects and control subjects in response to the graded intra-arterial infusion of ACh

(a) The change in FBF (ΔFBF) in response to this muscarinic agonist was attenuated in DM subjects (○) compared with controls (□) (ANOVA, P < 0.05). (b) There was little change in the vasodilator response to ACh after the infusion of l-NMMA in DM subjects (●) (ANOVA, not significant). (c) There was a significant reduction in the response to l-NMMA in the control group (■) (ANOVA, P < 0.05). (d) Following the infusion of l-NMMA, there was no difference in response to ACh between the two groups (ANOVA, not significant).

Effect of l-NMMA infusion on response to ACh
In view of the differences in basal FBF, we calculated the absolute changes in FBF and FVR in response to ACh. The vasodilator response to graded intra-arterial infusion of ACh was impaired in DM subjects compared with controls (ANOVA, P < 0.05), with reciprocal changes in FVR (ANOVA, P < 0.05) (Figure 6a). At the highest dose of ACh (27 μg/min), the absolute change in FBF was reduced in DM subjects (8.0 ± 1.2 ml·min⁻¹·100 ml⁻¹ forearm) compared with controls (13.0 ± 1.6 ml·min⁻¹·100 ml⁻¹ forearm; P < 0.05). The slope of the dose–response curve was also reduced in DM subjects (0.25 ± 0.04) compared with controls (0.42 ± 0.06; P < 0.05).

Following the infusion of l-NMMA there was significant blunting of the vasodilator response to ACh in the control group (ANOVA, P < 0.05), but no significant change in DM subjects (Figures 6b and 6c). In the control group absolute FBF at the highest dose of ACh was reduced from 13.0 ± 1.6 to 5.1 ± 1.8 ml·min⁻¹·100 ml⁻¹ forearm following the infusion of l-NMMA (P < 0.005). In DM subjects the decrease in absolute FBF produced by l-NMMA at the highest dose of ACh was from 8.0 ± 1.2 to 6.9 ± 0.8 ml·min⁻¹·100 ml⁻¹ forearm (not significant). After the infusion of l-NMMA there was no significant difference in the vasodilator response to the graded intra-arterial infusion of ACh in DM compared with control subjects (ANOVA) (Figure 6d).

Effect of l-NMMA infusion on response to SNP
The response to SNP was not significantly different in DM subjects compared with controls (4.7 ± 0.5 and 3.9 ± 0.5 ml·min⁻¹·100 ml⁻¹ forearm respectively). The infusion of l-NMMA did not produce any change in the response to the endothelium-independent agonist (DM
DISCUSSION

The results of the present study confirm our previous findings that resting FBF and FHBF are increased in young subjects with Type I diabetes mellitus [7]. L-NMMA, at a dose sufficient to reduce resting FBF in control subjects by 48% and also to reduce FHBF, had no significant effect on the forearm hyperaemia observed in subjects with Type I diabetes. However, ASA reduced blood flow to a greater extent in DM subjects than in controls, and indeed eliminated the relative resting and exercise-induced skeletal muscle hyperaemia observed in the group with diabetes. These results suggest that vasodilator prostanoids may account for skeletal muscle hyperaemia in patients with Type I diabetes.

Endothelium-dependent paracrine factors are important in the control of vascular tone. NO is an important determinant of both resting [9,10,13,28] and exercise-induced [9,10,25,28] blood flow in healthy subjects. However, in Type I diabetes, numerous [7,15–18,29,30], but not all [31–33], studies have demonstrated impaired shear-stress-mediated or agonist-induced NO-dependent vasodilation in the conduit and resistance circulations. It is therefore not surprising that in the present study, as in others [31,34], a reduced contribution of NO to resting blood flow was observed in subjects with Type I diabetes.

Previous studies have not, however, assessed the contribution of NO to exercise-induced vasodilation in diabetes. We have shown previously that NO contributes approximately 30% to metabolic vasodilation during exercise in healthy individuals [38]. In the present study we observed a reduced response to L-NMMA in the diabetic group with respect to all parameters of isotonic exercise. These findings suggest that NO-dependent vasodilation during submaximal exercise is impaired in the forearm circulation of patients with Type I diabetes.

Vasodilator prostanooids also make an important contribution to both resting and exercise-induced hyperaemia in health [12–14,25]. Data from our laboratory have demonstrated that infusion of the cyclo-oxygenase inhibitor ASA will produce a dose-dependent reduction in FBF [13,25] which correlates with diminished production of 6-oxo-prostaglandin F$_1$$_2$, the stable metabolite of prostacyclin. In addition, this effect of ASA does not appear to be related to non-specific actions of ASA, such as alterations in plasma pH or modulation of local noradrenaline release or uptake [13]. In healthy subjects prostacyclin accounts for approx. 20% of resting and exercise-related blood flow (AUC 5), whereas the contribution is approx. 33% in subjects with Type I diabetes. To account for this disparity it is possible that, in conditions of impaired bioavailability of NO (such as Type I diabetes), up-regulation of other endothelium-dependent factors, such as vasodilator prostanooids, may result. As a consequence these substances would play a more prominent role in the control of vascular tone. In keeping with this hypothesis, altered prostanooid production by renal glomeruli (involving increases in vasodilatory prostanooid and decreases in vasoconstrictor prostanooid levels) has been suggested to mediate the hyperfiltration that occurs during the early stages of diabetic nephropathy [36,37].

Our findings are consistent with two previous studies demonstrating elevated levels of prostacyclin in diabetic patients [20,21]. However, interpretation of studies examining prostacyclin production in diabetes are complicated not only by age [22,23,38], variation in metabolic parameters [19,39] and duration of disease [40], but also by the presence of micro- and macro-vascular complications [39,41].

Augmented production of vasoactive prostanoids in diabetes could be related to alterations in aldose reductase activity [42], changes in shear stress [43] or changes in circulating glucose and insulin levels [44]. Insulin is a potent vasodilator that is thought to act at least in part, through an endothelium-dependent NO-mediated mechanism [45,46], but also through other pathways such as prostacyclin [47]. Peripheral insulin levels are known to be elevated in Type I diabetes [48], and these levels are directly related to both resting and exercise-induced hyperaemia [7]. It is thus possible that elevated insulin levels produce relative hyperaemia in subjects with Type I diabetes via a prostacyclin-mediated mechanism, which is susceptible to the effects of ASA.

The results of the present study may have important implications with respect to other regional circulations, particularly those of the retina and kidney, where hyperaemia is thought to lead to microvascular complications [1,2]. If it can be demonstrated that vasodilator prostanoids are also responsible for this phenomenon, ASA may be a therapeutic agent with which to intervene early in the pathogenesis of diabetic retinopathy and nephropathy. However, it should be emphasized that the doses of ASA used in the present study are within the anti-inflammatory range, and the relevance of standard oral doses of ASA (75–325 mg) to the findings presented here are not clear. Notably, 325 mg of ASA appeared to have no effect on FBF responses in Type II (non-insulin-dependent) diabetes [49], suggesting that high-dose ASA is required to adequately block synthesis of vasodilator prostanoids.

Although a reduced response to L-NMMA is widely interpreted as evidence of reduced release of NO, it is possible, particularly in the presence of augmented blood flow, that there is in fact increased NO synthesis. Consequently this would require more L-NMMA to overcome the associated dilation. However, the impaired
vasodilator response to ACh, together with the effect of 1-  
NMMA on skeletal muscle haemodynamics in the  
diabetic patients in the present study, suggests that  
reduced NO bioavailability is the more likely inter-  
pretation. Impaired endothelial uptake or metabolism of 1-  
NMMA is a further possibility that may account for an  
impaired response to this NO synthase inhibitor. Until  
we are able to directly and accurately measure basal  
and stimulated NO production, conclusive proof of reduced  
bioavailability of NO will be lacking.

Measurement of alterations in the levels of 6-oxo-  
prostaglandin F\textsubscript{1\alpha} (the stable metabolite of prostacyclin)  
in response to ASA would have provided corroborating  
evidence regarding our proposed mechanism underlying  
forearm hyperaemia in Type I diabetes. This was not  
performed in the present study although, as discussed  
above, we have shown previously in healthy controls a  
direct correlation between FBF and 6-keto-prostaglandin  
F\textsubscript{1\alpha} levels following ASA infusion [13]. Moreover, non-  
specific effects of ASA do not account for the reduction in  
blood flow seen following ASA infusion [13]. Further-  
more, the fact that ASA reduced, rather than increased, blood flow provides evidence that vasocon-  
strictor prostanooids do not appear to be important in the  
control of blood flow in diabetic subjects.

In conclusion, we have evidence from the present  
study of a reduced contribution of NO to resting and  
exercise-induced hyperaemia in subjects with Type I  
diabetes. The fact that the infusion of ASA eliminates the  
relative hyperaemia at rest and associated with exercise  
provides evidence that this phenomenon may be related to the  
increased release of vasodilator prostanooids. This finding  
may have important implications for the treat-  
ment of augmented microvascular blood flow in subjects  
with Type I diabetes.

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