

Vasomotor Responses to Hypoxia in Type 2 Diabetes

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Type 2 diabetes is associated with vascular dysfunction, accelerated atherosclerotic morbidity, and mortality. Abnormal vasomotor responses to chemoreflex activation may contribute to the acceleration of atherosclerotic diabetes complications, but these responses have not previously been investigated. We measured forearm mean blood flow (MBF) and mean vascular conductance (MVC) responses to isocapnic hypoxia in seven healthy and eight type 2 diabetic subjects during local intra-arterial saline infusion and α -adrenergic blockade (phentolamine). The effects of hypoxia on saline and phentolamine responses significantly differed between groups; relative to normoxia, the % Δ MVC with hypoxia during saline was $-3.3 \pm 11.2\%$ in control and $24.8 \pm 13.3\%$ in diabetic subjects, whereas phentolamine increased hypoxic % Δ MVC to similar levels ($39.4 \pm 9.7\%$ in control subjects and $48.0 \pm 11.8\%$ in diabetic subjects, $P < 0.05$, two-way ANOVA). Absolute normoxic MBF responses during saline infusion were 91.9 ± 21.1 and 77.9 ± 15.3 in control and diabetic subjects, respectively, and phentolamine increased normoxic MBF to similar levels (165.2 ± 40.1 ml/min in control subjects and 175.9 ± 32.0 ml/min in diabetic subjects; both $P < 0.05$). These data indicate that diabetic and control subjects exhibit similar responses to hypoxia in the presence of α -adrenergic blockade despite evidence of exaggerated α -mediated vasoconstriction at rest. *Diabetes* 53:2073–2078, 2004

Type 2 diabetes is associated with accelerated atherosclerotic morbidity and mortality. It is characterized, early in its clinical course, by abnormal vascular function (1), which may ultimately contribute to the clinical manifestations of neuropathy and micro- and macrovascular disease. These abnormal vasomotor responses may be related to insulin resistance (2), hyperinsulinemia (3), hyperglycemia (4), endothelial dysfunction (1,5), dyslipidemia (3), or changes in sensitivity to norepinephrine (6). In particular, diabetes may be associated with abnormal sympathetic nervous

system (SNS)-related vascular control. For example, several studies have demonstrated that hyperinsulinemia increases SNS activity (7,8) and that type 2 diabetic subjects exhibit increased peripheral norepinephrine-mediated α -adrenergic vasoconstriction for their level of SNS activity (6). Conversely, other studies (9) have demonstrated that patients with diabetes have decreased circulating plasma norepinephrine.

Hypoxia is a common physiological stimulus that elicits chemoreflex-mediated changes in vasomotor control. In a recent study (10), we examined peripheral vasomotor response to hypoxia in healthy humans. Using the α -adrenergic receptor blocker phentolamine, we demonstrated that sympathetic vasoconstrictor tone masks underlying hypoxic vasodilatation, which was largely β -adrenoceptor mediated, possibly involving nitric oxide release. Multiple pathways may therefore be implicated in potential abnormalities in efferent vasomotor control in response to hypoxia, and it is possible that several of these may be abnormal in type 2 diabetes. This is supported by findings that patients with obstructive sleep apnea and the metabolic syndrome, common comorbidities in type 2 diabetic subjects, exhibit abnormal sympathetic and paracrine control of vascular function (11,12), including abnormal vasomotor responses to hypoxia (11). To our knowledge, however, diabetic vascular responses to hypoxia have not previously been studied in humans, despite the fact that hypoxia is an important physiological stimulus associated with acute local and reflex vascular adaptations.

RESEARCH DESIGN AND METHODS

All subjects were nonsmokers and did not have current or past evidence of any cardiovascular, respiratory, or neural disorder, including peripheral neuropathy. Subjects were also excluded based on the following: creatinine levels >30 μ g/l; more than mild renal impairment (urinary albumin >15 mg/l); hepatic impairment, gout, or hyperuricemia; more than mild high cholesterol (total cholesterol >7.0 mmol/l), hypertension (arterial pressure $>140/95$ mmHg), thyroid dysfunction (thyroid-stimulating hormone >3.8 mU/l or FT4 >20 pmol/l), or history of asthma; and obstructive sleep apnea. In addition, subjects had no clinical history or evidence of vasculopathy, retinopathy, nephropathy, or neuropathy. The latter was assessed as the absence of any history of abnormal bowel or bladder function, impaired heart rate response to postural change or Valsalva maneuver, orthostatic intolerance, burning or numbness in the feet, gastroparesis, abnormal thermoregulatory control of skin blood flow, or microalbuminuria. None of the subjects had been at altitude ($>1,500$ m) for at least 5 months, and all female subjects were postmenopausal. This study was approved by the Ethics Committee of Royal Perth Hospital, and all of the procedures were performed in accordance with institutional guidelines and the Declaration of Helsinki. Before the study, each subject gave written informed consent to participate.

Eight otherwise healthy subjects with type 2 diabetes (five men and three women, aged 56 ± 2 years [mean \pm SE]) participated in this study (height 1.72 ± 0.1 m, body mass 85.6 ± 9.6 kg, and BMI 29.1 ± 3.8 kg/m). None of the diabetic subjects were taking medications other than those to treat diabetes, and medications remained unchanged on the study day (one was unmedicated; three were on gliclazide; one was on metformin; one was on metformin

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FBG, fasting blood glucose; MBF, mean forearm blood flow; MVC, mean vascular conductance; SNS, sympathetic nervous system.

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and gliclazide; one was on metformin, gliclazide, and insulin; and one was on gliclazide and insulin). On the screening day, fasting blood glucose (FBG) was 9.1 ± 2.8 mmol/l, HbA_{1c} $7.9 \pm 1.7\%$, average Hb 154.6 ± 16.1 g/l, and duration of diabetes 5.1 ± 3.0 years. All other screening measures were normal, including lipid profile (total cholesterol 4.7 ± 0.5 mmol/l).

Seven healthy unmedicated control subjects (six men and one woman) were matched to diabetic subjects according to age and BMI. Control subject characteristics were as follows: age 53 ± 4 years, height 1.72 ± 0.03 m, body mass 80.9 ± 6.8 kg, and BMI 27.2 ± 2.1 kg/m. On the screening day, FBG was 5.4 ± 0.1 mmol/l, HbA_{1c} $5.4 \pm 0.1\%$, Hb 146 ± 3.1 g/l, and total cholesterol 5.2 ± 0.3 mmol/l. Routine resting lung function tests were not different between control (forced vital capacity, 3.83 ± 0.39 l, and forced expiratory volume over 1 s, 3.27 ± 0.34 l) and diabetic (forced vital capacity, 3.44 ± 0.17 l, and forced expiratory volume over 1 s, 2.85 ± 0.11 l) subjects. No significant differences existed between groups in any baseline or screening measures except for FBG and HbA_{1c} ($P < 0.05$).

All subjects fasted for 8 h and abstained from caffeine, alcohol, and exercise for 24 h before the study. Each study began with the subject supine and the nondominant arm, from which blood flow measures were assessed, supported perpendicular to the body at heart level. A 20-gauge, 5-cm arterial catheter (Arrow, Reading, PA) was introduced into the brachial artery of the nondominant arm under local anesthesia (1% lidocaine; Astra Pharmaceuticals, Westborough, MA) for the infusion of drugs and measurement of arterial pressure.

When subject preparation was complete, four consecutive 10-min trials, separated by 15-min rest periods, were undertaken. During each trial, subjects breathed a normoxic then a hypoxic mixture for 5 min each. The first three trials were used to familiarize subjects with breathing on the mouthpiece and with the hypoxic stimulus. All intertrial rest periods involved intrabrachial saline infusion. Five minutes before trial 4, a loading dose of phentolamine (100 μ g/min for 5 min, 500 μ g total) (10-mg vial; Novartis, Castle Hill, Australia) was infused, followed by a continuous dose for the duration of the trial (25 μ g/min for 10 min, 250 μ g total). Previous studies (10,13) have demonstrated that this dose of phentolamine is sufficient for local blockade of α -adrenergic receptors without circulating centrally.

Interventions

Hypoxia. To establish and maintain isocapnic hypoxia, the alveolar ventilation "clamp" method developed by Banzett et al. (14) was adopted. During the experimental procedures, we "clamped" alveolar ventilation such that end-tidal CO₂ (P_{ETCO_2}) was relatively constant despite large changes in minute ventilation (\dot{V}_E). The level of inspired O₂ was modified by blending medical air with a compressed gas mixture (8% O₂/balance N₂) until 85% arterial O₂ saturation (S_{aO_2}), monitored by pulse oximetry of the index finger (Ohmeda, Boulder, CO), was achieved.

Inspired gases were humidified (Model HC325; Fisher & Paykel, Auckland, NZ), and concentrations were monitored at the mouthpiece with O₂ and CO₂ monitors (Models OM-11 and LB2, respectively; Beckman, Fullerton, CA). Respiratory flow rates from the mouth were measured using a heated pneumotachograph (Model 2A; Fleisch, Lausanne, Switzerland) and a differential pressure transducer (Validyne, Northridge, CA). All signals were digitized and stored on a computer at 250Hz, and data were analyzed offline on a breath-by-breath basis (ADInstruments, Colorado Springs, CO).

Measurements. Heart rate was continuously monitored using a 12-lead electrocardiogram (Model 8370; Nihon Kohden, Tokyo, Japan). A three-port connector was placed in series with the arterial cannula so that arterial pressure could be continuously measured, blood samples could be taken, and drugs infused.

Forearm blood flow assessment. Forearm blood flow was calculated from measurements using high-resolution vascular ultrasonography with synchronized Doppler velocity measurement. The brachial artery of the nondominant arm was imaged in the distal third of the upper arm with a 10-MHz multifrequency linear array probe attached to a high-resolution ultrasound machine (Aspen; Acuson/Siemens, Malvern, PA). Ultrasonic parameters were set to optimize longitudinal B-mode images of the lumen and arterial wall interface. Continuous Doppler velocity assessment was recorded using an insonation angle of 60°. Brachial artery diameter was assessed posttest using custom-designed edge detection and wall-tracking software, which is independent of investigator bias, as previously described (15,16).

Doppler/ultrasound images were recorded during the final minute of normoxia and hypoxia in each trial. Mean integral diameter, mean velocity, and mean blood flow (MBF) measurements were averaged across this minute. Mean vascular conductance (MVC) was calculated as $(100 \times \text{MBF})/\text{mean arterial pressure}$ and expressed in arbitrary units.

Blood gas and catecholamine analysis. Brachial artery blood samples were collected in preheparinized blood gas syringes and analyzed using a clinical blood gas analyzer (800 Series; Bayer, Pittsburgh, PA) for P_{O₂}, P_{CO₂}, pH, S_{aO₂},

Hb, and hematocrit. Plasma catecholamine (epinephrine and norepinephrine) levels were determined by high-performance liquid chromatography with electrochemical detection.

Effect of hypoxia on hand blood flow. Blood flow measures were performed in the absence of an occlusive wrist cuff. To determine the significance of the contribution of the hand to limb blood flow measured via brachial Doppler/ultrasound during hypoxia, we studied MBF responses to normoxia and hypoxia with and without a wrist cuff inflated to 200 mmHg in a separate group of healthy subjects ($n = 6$). Two 10-min trials were performed on each subject, consisting of 5 min normoxia followed by 5 min hypoxia, with data from each trial averaged.

Data analysis. All data are reported as means \pm SE for the final minute of each normoxia or hypoxic bout. Two-way ANOVA was performed to compare the effect of each drug administered between the groups (blockade state versus diabetes/control). Other variables were analyzed using two-way ANOVA (blockade state versus normoxia/hypoxia), with differences considered significant when $P < 0.05$. Post hoc *t* test analysis, including Bonferroni correction, was performed to identify differences.

RESULTS

No differences in either normoxic or hypoxic MBF or MVC were evident between the initial three saline infusion trials, which were undertaken to familiarize subjects with breathing through the mouthpiece and breathing hypoxic air. The third trial has therefore been used as the baseline for phentolamine infusion.

Effects of hypoxia on blood gas levels. In control subjects during saline infusion, normoxic S_{aO₂} (by pulse oximetry) was $98.5 \pm 0.2\%$ and decreased to $84.5 \pm 1.0\%$ in response to hypoxia ($P < 0.05$) (Table 1). Similar decreases occurred with phentolamine (98.3 ± 0.2 to $86.1 \pm 1.0\%$; $P < 0.05$). Saturation responses to hypoxia were similar in diabetic subjects: hypoxia decreased S_{aO₂} from 97.4 ± 0.5 to $85.5 \pm 0.4\%$ (saline) and from 97.6 ± 0.4 to $84.1 \pm 0.7\%$ (phentolamine; $P < 0.05$). No differences in normoxic or hypoxic saturations across trials were evident in either group or between groups. Blood P_{O₂} demonstrated similar patterns to the oximetric S_{aO₂} data above.

Hypoxia caused no significant changes in P_{ETCO₂}. Furthermore, no difference existed between phentolamine administration in terms of the effect of either normoxia or hypoxia on P_{ETCO₂} in either control or diabetic subjects. Hypoxia significantly increased pH during saline in diabetic subjects and during phentolamine in control subjects, and the change in pH between normoxia and hypoxia during saline infusion was significantly greater in diabetic than control subjects ($P < 0.05$).

Effects of hypoxia on ventilation and systemic hemodynamics. The effects of hypoxia on \dot{V}_E , heart rate, and arterial pressure are also detailed in Table 1. In response to hypoxia, \dot{V}_E increased in control and diabetic subjects in both trials, but the responses did not reach significance. Furthermore, the magnitude of the hypoxic effect on \dot{V}_E did not differ between saline and phentolamine trials. Heart rate consistently increased in response to hypoxia in both trials in control subjects and during saline in the diabetic group ($P < 0.05$), although the hypoxic effect did not differ between saline and phentolamine administration. During saline infusion, the heart rate response to hypoxia was significantly less in diabetic than in control subjects ($P < 0.05$). Arterial pressure responses to hypoxia were not significantly different compared with normoxic responses in either group under either condition. Arterial pressure increased more in control than in diabetic subjects ($P < 0.05$); however, the magnitude of this increase in control subjects was modest (~ 2 mmHg).

TABLE 1
Effects of hypoxia on blood gases, ventilation, and systemic hemodynamics

Variable/condition	Saline		Phentolamine	
	Control	Diabetes	Control	Diabetes
Hb saturation (%)				
Normoxia	97.1 ± 0.3	97.4 ± 0.3	97.6 ± 0.2	97.0 ± 0.3
Hypoxia	82.1 ± 1.3*	81.9 ± 1.2*	83.4 ± 1.3*	83.6 ± 1.5*
P _{O₂} (mmHg)				
Normoxia	91.9 ± 4.5	91.8 ± 3.3	92.3 ± 3.5	90.9 ± 3.6
Hypoxia	43.7 ± 1.1*	43.9 ± 0.9*	43.7 ± 0.5*	44.9 ± 1.4*
P _{ETCO₂} (mmHg)				
Normoxia	36.3 ± 0.9	35.4 ± 2.9	35.8 ± 1.4	37.0 ± 1.8
Hypoxia	36.2 ± 1.1	34.0 ± 2.9	35.1 ± 1.1	35.7 ± 1.7
pH				
Normoxia	7.42 ± 0.01	7.40 ± 0.01	7.42 ± 0.01	7.41 ± 0.01
Hypoxia	7.43 ± 0.01	7.44 ± 0.01*†	7.44 ± 0.01*	7.45 ± 0.01
Minute ventilation (l/min BTPS)				
Normoxia	6.4 ± 1.2	6.4 ± 1.1	6.8 ± 1.4	9.4 ± 2.1
Hypoxia	17.0 ± 5.0	9.5 ± 2.0†	13.3 ± 5.0	12.5 ± 2.0
Heart rate (bpm)				
Normoxia	65.6 ± 3.9	63.9 ± 2.4	66.4 ± 3.1	64.3 ± 2.5
Hypoxia	83.1 ± 5.0*	75.2 ± 3.3*†	79.5 ± 3.4*	77.2 ± 3.4
Arterial pressure (mmHg)				
Normoxia	93.7 ± 4.0	94.7 ± 2.0	90.7 ± 3.8	92.3 ± 2.1
Hypoxia	104.1 ± 7.4	96.2 ± 2.6	93.9 ± 4.7	92.5 ± 2.6†

Data are means ± SE. **P* significant by Bonferroni-corrected post hoc *t* test, hypoxia versus normoxia; †*P* < 0.05, by two-way ANOVA, control versus diabetic subjects between normoxia and hypoxia within drug trial.

Effects of phentolamine on blood flow responses during hypoxia.

Although no significant within-group changes were evident in arterial pressure across the saline and phentolamine infusion trials during normoxia, we have presented data as absolute flow and as MVC (Table 2), consistent with a previous hypoxia studies (10,17). Since normoxic values of MBF and MVC were altered by phentolamine administration (see absolute MBF and MVC data in Table 2), we assessed hypoxic vasomotor responses to phentolamine as the percentage change from within-trial normoxia (i.e., %ΔMBF, %ΔMVC). This is the preferred way to compare interventions that cause vasodilatation or vasoconstriction under conditions in which marked differences in baseline flow are evident (18–21).

The effects of hypoxia on saline and phentolamine significantly differed between groups: %ΔMVC with hypoxia during saline was $-3.3 \pm 11.2\%$ in control subjects and $24.8 \pm 13.3\%$ in diabetic subjects, whereas during phentolamine hypoxic %ΔMVC was $39.4 \pm 9.7\%$ in control subjects and $48.0 \pm 11.8\%$ in diabetic subjects (*P* < 0.05, two-way ANOVA) (Fig. 1). Similarly, in control subjects the %ΔMBF response to hypoxia in the presence of phen-

tolamine ($44.1 \pm 9.8\%$) was significantly greater than that during saline ($6.0 \pm 11.0\%$; *P* < 0.05), while no such difference in hypoxic responses was evident in diabetic subjects (saline, $26.1 \pm 12.8\%$, and phentolamine, $48.3 \pm 12.0\%$; *P* = NS).

To further investigate the reasons for these differences in %ΔMBF and %ΔMVC, we examined the effects of hypoxia and phentolamine on absolute forearm blood flow and conductance responses (Table 2). During normoxia, absolute MBF was 91.9 ± 21.1 ml/min in control subjects. The corresponding value in diabetic subjects (77.9 ± 15.3 ml/min) was lower but not significantly so. Despite this lower MBF in diabetic subjects during normoxia, hypoxia increased MBF to similar levels in the two groups (100.8 ± 28.2 and 100.2 ± 23.1 ml/min) (Table 2). Conductance responses showed a similar pattern, control and diabetic normoxic levels during saline (102.9 ± 25.8 and 82.1 ± 16.4 units, respectively) increased to 105.8 ± 32.2 and 105.4 ± 24.9 units during hypoxia (Fig. 2).

The difference in normoxic MBF and MVC data evident between the groups during saline infusion was abolished by phentolamine administration (Fig. 2). Phentolamine

TABLE 2
Effects of hypoxia on MBF and forearm MVC

Variable/condition	Saline		Phentolamine	
	Control	Diabetes	Control	Diabetes
MBF (ml/min)				
Normoxia	91.9 ± 21.1	77.9 ± 15.3	165.2 ± 40.1*	175.9 ± 32.0*
Hypoxia	100.8 ± 28.2	100.2 ± 23.1	243.8 ± 66.8†	250.2 ± 41.5†
MVC (units)				
Normoxia	102.9 ± 25.8	82.1 ± 16.4	186.7 ± 47.0*	191.00 ± 35.0*
Hypoxia	105.8 ± 32.2	105.4 ± 24.9	266.0 ± 71.7	273.27 ± 47.5†

Data are means ± SE. **P* is significant by Bonferroni-corrected post hoc *t* test versus saline trial within condition and subject group; †*P* is significant by Bonferroni-corrected post hoc *t* test, hypoxia versus normoxia.

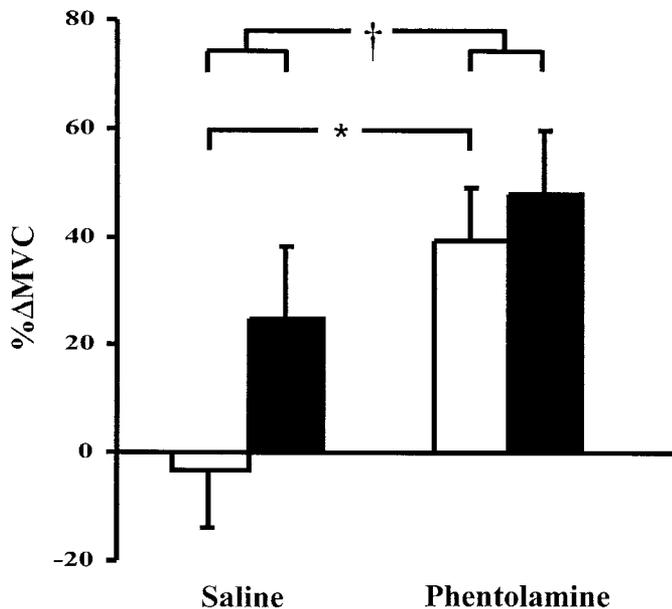


FIG. 1. Change in forearm vascular conductance responses with hypoxia. The percentage change in forearm MVC in control (□) and diabetic (■) subjects. Data represent the percentage change in conductance in response to hypoxia, as a proportion of within-trial normoxic conductance, in the presence of either saline or phentolamine (18–21). * $P = 0.007$, phentolamine versus saline within subject group; † $P < 0.05$, two-way ANOVA, control versus diabetic subjects for the difference in response to hypoxia induced during saline and phentolamine treatments.

significantly increased normoxic MBF in control subjects from 91.9 ± 21.1 ml/min during saline to 165.2 ± 40.1 ml/min ($P < 0.05$) and in diabetic subjects from 77.9 ± 15.3 (saline) to 175.9 ± 32.0 ml/min ($P < 0.05$) (Table 2). Conductance data followed a similar pattern. In control subjects, phentolamine significantly increased normoxic MVC from 102.9 ± 25.8 (saline) to 186.7 ± 47.0 units ($P < 0.05$) and in diabetic subjects from 82.1 ± 16.4 (saline) to 191.0 ± 35.0 units ($P < 0.05$). The abolition of baseline (saline) differences in blood flow by the addition of phentolamine strongly suggests the presence of elevated baseline α -mediated vasoconstrictor tone in the diabetic subjects.

Effects of hypoxia on arterial catecholamines. Plasma norepinephrine levels significantly increased during hypoxia, relative to normoxia, in both groups under both saline and phentolamine conditions ($P < 0.05$) (Fig. 3). However, no differences existed between groups with respect to the magnitude of these hypoxic changes in norepinephrine. Plasma epinephrine levels increased significantly with hypoxia in both groups across all conditions ($P < 0.05$; Fig. 3). Normoxic and hypoxic epinephrine responses were consistently lower in diabetic compared with control subjects during both saline and phentolamine conditions ($P < 0.05$). Neither normoxic nor hypoxic catecholamine levels changed between the two trials.

Contribution of hand blood flow. In the absence of wrist cuff inflation, absolute MBF was increased to 72.1 ± 14.4 ml/min during hypoxia. With cuff inflation, MBF was similarly increased to 72.8 ± 17.8 ml/min during hypoxia. Neither normoxic nor hypoxic MBF was significantly different between cuff inflation or deflation. The % Δ MBF with hypoxia increased by $29.0 \pm 11.9\%$ without cuff inflation and by $21.9 \pm 4.6\%$ with cuff inflation ($P = 0.57$).

DISCUSSION

The aim of the current study was to compare the effects of hypoxia on forearm vasomotor responses in subjects with type 2 diabetes to age-matched healthy control subjects. In a recent publication (10), we established that in young healthy humans α -adrenoceptor-mediated sympathetic vasoconstriction masks local dilator effects of hypoxia on vascular tone. To determine whether this pattern of response to hypoxia is also evident in subjects with type 2 diabetes, we measured forearm blood flow responses to isocapnic hypoxia during saline infusion and α -adrenergic blockade in the present study. The principal findings of this study are that diabetic and control subjects exhibit similar responses to hypoxia in the presence of α -adrenergic blockade despite evidence of exaggerated α -mediated vasoconstriction at rest.

The mechanisms responsible for vascular function changes with hypoxia have not previously been studied in either human or animal models of type 2 diabetes. In response to hypoxia, in the presence of saline alone, diabetic subjects demonstrated an increase in vascular conductance, whereas vascular conductance did not substantially change in response to an identical stimulus in control subjects. We believe the most likely explanation for this difference relates to elevated baseline α -mediated vasoconstrictor tone in the diabetic subjects. That is, despite an apparent difference between baseline normoxic vascular conductance, infusion of phentolamine during normoxia produced similar responses in control and diabetic subjects, suggesting that diabetic subjects possess elevated α -mediated vasoconstrictor tone at rest. The similarity in hypoxic vasodilator responses between groups during saline infusion (Fig. 2) and in the presence of phentolamine (Fig. 1) suggests that, despite elevated α -mediated vasoconstriction at baseline, the diabetic subjects in the present study demonstrate intact responses to hypoxia.

Vascular tone represents a balance between vasodilation and autonomic vasoconstriction. The current data indicate that elevated baseline α -mediated vasoconstriction exists in the diabetic subjects. This could be due to an increase in sympathetic vasoconstrictor tone or, possibly, a consequence of impaired vasodilation in diabetes. In our previous study (10) of healthy subjects, intrabrachial phentolamine administration revealed an underlying vasodilation during hypoxia, which was due to both β -receptor stimulation by circulating adrenaline and a small contribution from local nitric oxide production. The hypoxic vasodilation in the present study under saline conditions was greater in diabetic than in control subjects, whereas arterial pressure and catecholamine concentrations tended to increase less in the diabetic subjects. The sympathetic vasoconstrictor response to hypoxia may therefore have been blunted in diabetic subjects, and, as a consequence, less vasoconstrictor competition to the underlying vasodilation may be evident in response to hypoxia. This would explain the observed increase in hypoxia-induced vasodilation in the diabetic subjects. Indeed, during phentolamine the vasodilation was the same in both groups, suggesting that the dilator responses to hypoxia per se were not blunted in hypoxia. Although this explanation seems logical on the basis of our findings, further studies employing blockers of vasodilator pathways will be re-

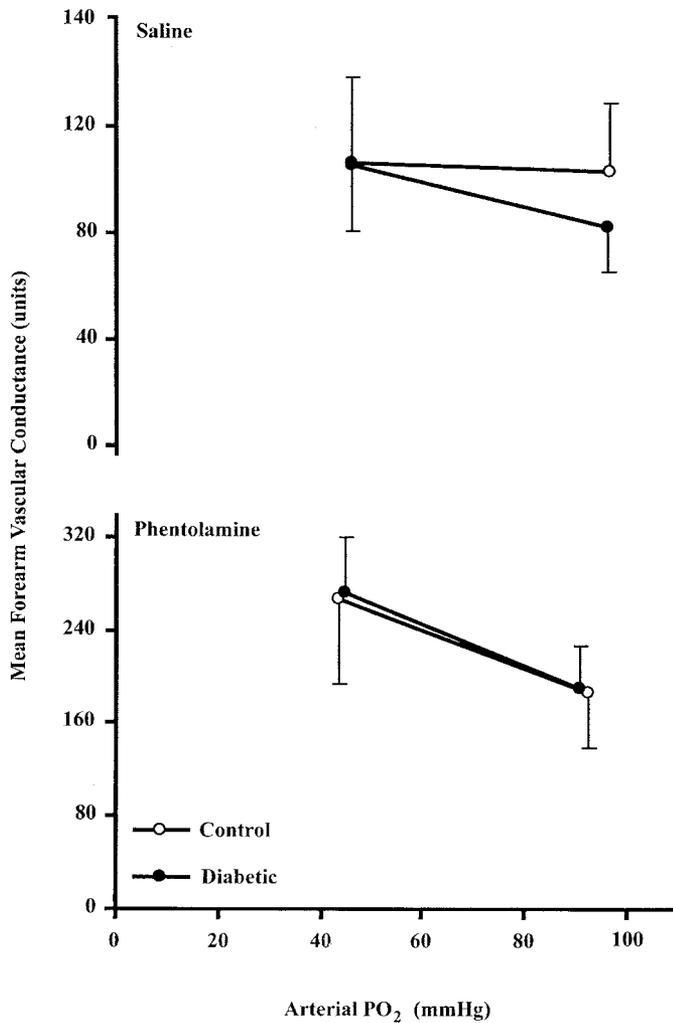


FIG. 2. Absolute forearm vascular conductance responses to hypoxia. MVC responses to arterial normoxia (~ 100 mmHg) and hypoxia (~ 40 mmHg) in control (○) and diabetic (●) subjects in the presence of saline (*upper panel*) and phentolamine (*lower panel*).

quired to investigate the contribution of these mechanisms in diabetic subjects.

Although this is the first study to specifically investigate changes in vascular function in response to hypoxia in type 2 diabetes, several previous studies have examined SNS activity and vasomotor control in these subjects, with divergent results. Studies that have measured plasma norepinephrine concentrations have revealed increased (22), similar (23,24), or decreased (25) levels in diabetic subjects compared with healthy control subjects, whereas those (6,26) that have measured spillover indicate that norepinephrine release is normal in diabetic subjects. Hogikyan et al. (6) demonstrated that type 2 diabetic subjects exhibit augmented α -adrenergic vasomotor tone and an increased vasoconstriction in response to norepinephrine. We observed a similar result in the present study; during normoxia and infusion of phentolamine, MBF increased to a greater degree, relative to saline responses, in diabetic subjects (77.9 ± 15.3 to 175.9 ± 32.0 ml/min) than in control subjects (91.9 ± 21.1 to 165.2 ± 40.1 ml/min; $P < 0.05$) (Table 2). Although our diabetic subjects exhibited better diabetes control and fewer comorbidities and complications, our finding of increased

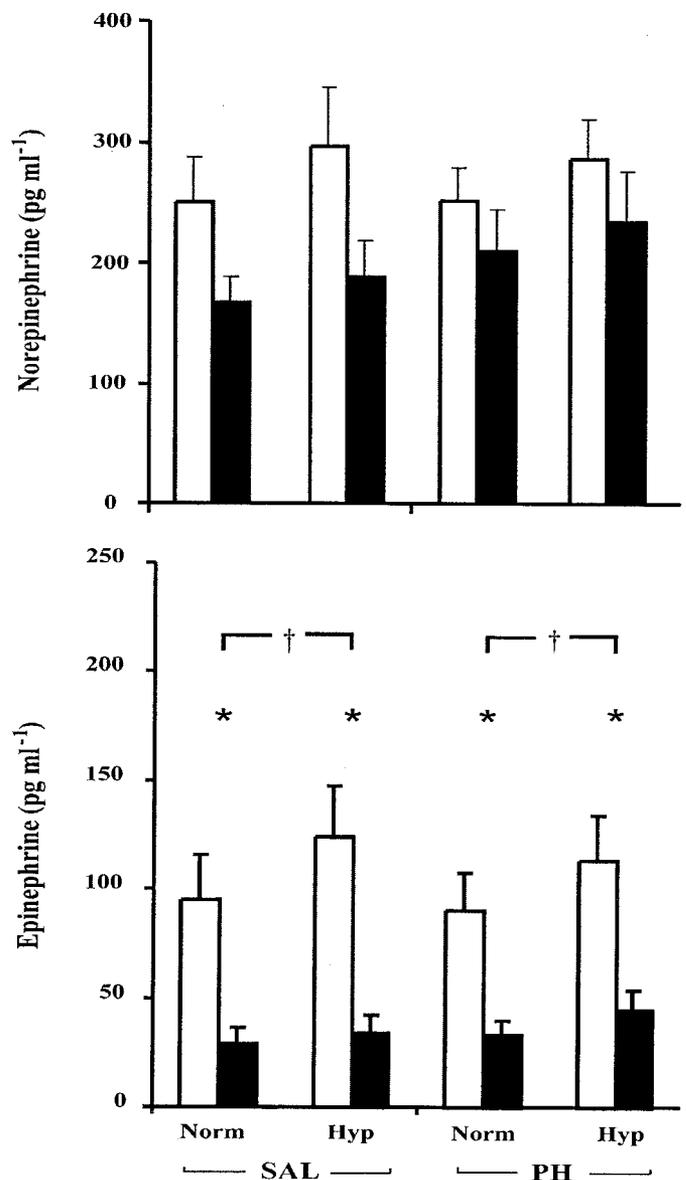


FIG. 3. Arterial catecholamine responses to hypoxia. Plasma norepinephrine (*upper panel*) and epinephrine (*lower panel*) concentrations during normoxia (Norm) and hypoxia (Hyp) in control (□) and diabetic (■) subjects are shown for the saline and phentolamine trials. * $P < 0.05$, control versus diabetic subjects within condition; † $P < 0.05$, control versus diabetic subjects between normoxia and hypoxia.

α -mediated tone at rest in diabetic subjects essentially agrees with that of Hogikyan et al. (6). Taken together, these findings indicate that α -adrenoceptor-mediated control of the vasculature may be exaggerated in diabetic subjects under basal conditions, whereas vasoresponsiveness to hypoxia is preserved.

There are several important limitations of the present study. First, our findings do not exclude the possibility of qualitatively different or exaggerated physiological abnormalities in patients with more advanced disease status of comorbidities, such as obesity and obstructive sleep apnea. In addition, although we excluded patients with clinical signs of peripheral neuropathy, we cannot eliminate the possibility that subclinical autonomic neuropathy may have influenced the results. We measured blood flow responses using high-resolution brachial Doppler/ultra-

sound, an approach we recently validated (16). However, previous studies have reported relative changes in flow using plethysmography, and we cannot exclude the possibility that differences in methodological approaches may have influenced the results. We think it unlikely that inclusion of hand blood flow in the Doppler/ultrasound measures was responsible for any disparity, however, because the magnitude and pattern of responses to hypoxia were not influenced by wrist cuff occlusion. It is important to note, however, that skin microvascular responses are physiologically distinct from those of skeletal muscle resistance vessels, and future studies will be required to characterize the effects of hypoxia on skin blood flow responses in diabetic subjects. Finally, the diabetic subjects studied were treated with a range of different medications including insulin, a known vasodilator (27), in two subjects. However, reanalysis, which excluded these subjects, did not alter the study outcomes ($P = 0.023$ for $n = 6$ vs. $P = 0.012$ for $n = 8$, ANOVA).

In summary, the present study demonstrated that despite exaggerated α -mediated vasoconstriction at rest, type 2 diabetic subjects in the present study possessed intact α -mediated responses during hypoxia.

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REFERENCES

- McVeigh GE, Brennan GM, Johnston GD, McDermott BJ, McGrath LT, Henry WR, Andrews JW, Hayes JR: Impaired endothelium-dependent and independent vasodilation in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 35:771–776, 1992
- Godsland I, Stevenson J: Insulin resistance: syndrome or tendency? *Lancet* 346:100–103, 1995
- Stout R: Insulin and atheroma: 20-year perspective. *Diabetes Care* 13:631–654, 1990
- Williams SB, Goldfine AB, Timimi FK, Ting H, Roddy M, Simonson D, Creager M: Acute hyperglycemia attenuates endothelium-dependent vasodilation in humans in vivo. *Circulation* 97:1695–1701, 1998
- Williams SB, Cusco JA, Roddy MA, Johnstone MT, Creager MA: Impaired nitric oxide-mediated vasodilation in patients with non-insulin-dependent diabetes mellitus. *J Am Coll Cardiol* 27:567–574, 1996
- Hogikyan RV, Galecki AT, Halter JB, Supiano MA: Heightened norepinephrine-mediated vasoconstriction in type 2 diabetes. *Metabolism* 48:1536–1541, 1999
- Anderson E, Hoffman R, Balon T, Sinkey C, Mark A: Hyperinsulinaemia produces both sympathetic neural activation and vasodilation in normal humans. *J Clin Invest* 87:2246–2252, 1991
- Berne C, Fagius J, Pollare T, Hjemdahl P: The sympathetic response to euglycaemic hyperinsulinaemia: evidence from microelectrode nerve recordings in healthy subjects. *Diabetologia* 35:873–879, 1992
- Giacca A, Groenwoud Y, Tsui E, McClean P, Zinman B: Glucose production, utilization, and cycling in response to moderate exercise in obese subjects with type 2 diabetes and mild hyperglycemia. *Diabetes* 47:1763–1770, 1998
- Weisbrod CJ, Minson CT, Joyner MJ, Halliwill JR: Effects of regional phentolamine on hypoxic vasodilatation in healthy humans. *J Physiol* 537:613–621, 2001
- Remsburg S, Launois S, Weiss J: Patients with obstructive sleep apnea have an abnormal peripheral vascular response to hypoxia. *J Appl Physiol* 87:1148–1153, 1999
- Brunner E, Hemingway H, Walker B, Page M, Clarke P, Jeneja M, Shipley M, Kumari M, Andrew R, Seckl J, Papadopoulos A, Checkley S, Rumley A, Lowe G, Stansfield S, Marmot M: Adrenocortical, autonomic, and inflammatory causes of the metabolic syndrome: nested case-control study. *Circulation* 106:2659–2665, 2002
- Eklund B, Kaijser L: Effect of regional alpha- and beta-adrenergic blockade on blood flow in the resting forearm during contralateral isometric handgrip. *J Physiol* 262:39–50, 1976
- Banzett RB, Garcia RT, Moosavi SH: Simple contrivance “clamps” end-tidal PO₂, and PCO₂ despite rapid changes in ventilation. *J Appl Physiol* 88:1597–1600, 2000
- Woodman RJ, Playford DA, Watts GF, Cheatham C, Reed C, Taylor RR, Puddey IB, Beilin LJ, Burke V, Green DJ: Improved analysis of brachial artery ultrasound images using a novel edge-detection software system. *J Appl Physiol* 91:929–937, 2001
- Green DJ, Cheatham C, Reed C, O'Driscoll G: Continuous assessment of brachial artery blood flow across the cardiac cycle: retrograde flows during lower limb exercise. *J Appl Physiol* 93:361–368, 2002
- Dinenno F, Joyner M, Halliwill J: Failure of systemic hypoxia to blunt α -adrenergic vasoconstriction in the human forearm. *J Physiol* 549:985–994, 2003
- Tschakovsky M, Sujirattanawimol K, Ruble S, Valic Z, Joyner MJ: Is sympathetic neural vasoconstriction blunted in the vascular bed of exercising human muscle? *J Physiol* 541:623–635, 2002
- Rosenmeier J, Dinenno F, Fritzlar S, Joyner MJ: alpha1- and alpha2-adrenergic vasoconstriction is blunted in contracting human muscle. *J Physiol* 547:971–976, 2003
- Lautt W: Resistance or conductance for expression of arterial vascular tone. *Microvasc Res* 37:230–236, 1989
- O'Leary DS: Regional vascular resistance vs. conductance: which index for baroreflex responses? *Am J Physiol* 260:H632–H637, 1991
- Christensen N: Plasma norepinephrine and epinephrine in untreated diabetics, during fasting and after insulin administration. *Diabetes* 23:1–8, 1974
- Beretta-Piccoli C, Weidmann P: Exaggerated pressor responsiveness to norepinephrine in nonazotemic diabetes mellitus. *Am J Med* 71:829–835, 1981
- Weidmann P, Beretta-Piccoli C, Trost B: Pressor factors and responsiveness in hypertension accompanying diabetes mellitus. *Hypertension* 7 (Suppl.):II33–II42, 1985
- Eckberg D, Harkins S, Fritsch J, Musgrave G, Gardner D: Baroreflex control of plasma norepinephrine and heart period in healthy subjects and diabetic patients. *J Clin Invest* 78:366–374, 1986
- Tack C, Smits P, Willemsen J, Lenders J, Thien T, Lutterman J: Effects of insulin on vascular tone and sympathetic nervous system in NIDDM. *Diabetes* 45:15–22, 1996
- Vicent D, Ilany J, Kondo T, Naruse K, Fisher S, Kisanuki Y, Bursell S, Yanagisawa M, King G, Kahn C: The role of endothelial insulin signalling in the regulation of vascular tone and insulin resistance. *J Clin Invest* 111:1373–1380, 2003