Type 2 Diabetic Individuals Have Impaired Leg Blood Flow Responses to Exercise

Role of endothelium-dependent vasodilation

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OBJECTIVE — Diabetic individuals have impaired endothelium-dependent forearm vasodilatory responses to ischemia, acetylcholine, and other endothelium-dependent agonists. The functional significance of impaired endothelium-dependent dilation in diabetic individuals is uncertain but is most likely to be manifest during leg muscle exercise and may have relevance to peripheral vascular disease and leg ischemia, which is prevalent in diabetic individuals. The current study aimed to determine the relationship between leg blood flow (LBF) responses to endothelium-dependent vasodilation and dynamic large muscle exercise.

RESEARCH DESIGN AND METHODS — LBF responses (thermodilution) to intrafemoral arterial infusions of an endothelium-dependent (acetylcholine) and endothelium-independent (sodium nitroprusside) vasodilator and a standardized 25-min cycling bout at 60% $V_{O_{2peak}}$ were compared in nine male type 2 diabetic subjects and nine age-, sex-, $V_{O_{2peak}}$-, and weight-matched control subjects.

RESULTS — LBF responses to acetylcholine and exercise but not sodium nitroprusside were significantly ($P < 0.05$) attenuated in patients with diabetes compared with healthy control subjects. The percentage increase in LBF in response to exercise and acetylcholine were significantly correlated ($r = 0.54$, $P = 0.02$). Furthermore, resting plasma glucose was significantly related to the LBF response to exercise ($r = -0.66$, $P = 0.003$) independently of insulin, HbA_{1c}, lipids, BMI, and blood pressure.

CONCLUSIONS — The increase in LBF during exercise is substantially attenuated in type 2 diabetic compared with matched control subjects. Impaired endothelium-dependent vasodilation secondary to elevated plasma glucose may underlie this observation. This mechanism may be of importance in determining the leg ischemic threshold in diabetic individuals with peripheral vascular disease.

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T ype 2 diabetic individuals have impaired dilatory responses to endothelium-dependent agonists such as acetylcholine (1) and methacholine (2,3). Furthermore, forearm flow–mediated dilation is reduced in type 2 diabetic individuals, especially those with peripheral vascular disease (4). There are many possible mediators of endothelial dysfunction in patients with type 2 diabetes. In established disease, it is likely that damage associated with advanced glycosylation end products is most important. In individuals with milder diabetes, dyslipidemia, hypertension, hyperinsulinemia, and hyperglycemia may all contribute. The clinical relevance of impaired endothelium-dependent vasodilation is as an early marker of cardiovascular risk, which may contribute to the atherosclerotic disease process (5). Endothelial-dependent vasodilation may also have relevance to blood flow control during exercise. Specifically, endothelium-derived vasodilator prostanoids and nitric oxide (NO) have been proposed to contribute to exercise hyperemia (6–10). Thus, endothelial dysfunction may also have clinical relevance to leg ischemic threshold in patients with peripheral vascular disease. However, the importance of impaired endothelium-dependent vasodilation with respect to blood flow control during exercise in the early development of diabetes is not known.

The current study examined the relationship between leg blood flow (LBF) responses to endothelium-dependent vasodilation and dynamic large muscle exercise. The leg was studied as a vascular bed relevant to the pathogenesis of atherosclerosis and peripheral vascular disease. Specifically, LBF responses to an endothelium-dependent (acetylcholine) and endothelium-independent (sodium nitroprusside) vasodilator and a standardized exercise bout were compared in type 2 diabetic individuals and age-, sex-, $V_{O_{2peak}}$-, and weight-matched healthy control subjects. The hypothesis was that type 2 diabetic individuals would have impaired LBF responses to both exercise and acetylcholine, and that these responses would be related.

RESEARCH DESIGN AND METHODS

Subjects

After providing written informed consent, nine type 2 non–insulin-dependent

diabetic men aged 48 ± 4 years (mean ± SD) and nine control subjects (46 ± 5 years) participated in the study, which was approved by the Alfred Hospital ethics committee and conducted in accordance with the Declaration of Helsinki of the World Medical Association. All subjects were nonsmokers, free of overt coronary disease (stress electrocardiogram), and with a BMI of <35 kg/m². Control subjects did not take any medication and had fasting and post–2-h 75-g oral glucose load plasma glucose levels of <6.1 mmol/l, whereas type 2 diabetic individuals had fasting plasma glucose >7 mmol/l and/or post–75-g oral glucose load plasma glucose levels of >11.1 mmol/l (Table 1) (11,12). Of the diabetic subjects, seven were controlled by diet and two were medicated with metformin. Of those medicated, one was also taking gliclazide. The two medicated diabetic subjects did not take their medication the night before or the morning of the screening or experimental day, and thus they had a 24-h drug-free period before all measurements. Because hypoglycemic agents, in particular metformin, are known to improve endothelial function, the inclusion of medicated patients is unlikely to account for the findings (13). All were normally active but were not specifically exercise trained.

Subject preparation

Peak pulmonary oxygen uptake ($V_{O_{2peak}}$) was determined during continuous incremental upright cycling to volitional exhaustion on an electronically braked ergometer (900 ergometer, D-7474; Ergo-line, Bitz, West Germany). Expired air was analyzed for volume, O_2, and CO_2 using calibrated analyzers (Quark b²; Cosmed, Rome, Italy). During a subsequent visit, subjects were familiarized with supine cycling during a 30-min bout on an electronically braked ergometer (380B ergometry system; Siemens-Elema, Sweden) at a workload eliciting 60% of the upright $V_{O_{2peak}}$.

Protocol

LBF was measured at rest and at 3, 4, and 5 min during an intrafemoral artery infusion of acetylcholine (6.4 $\mu g \cdot kg^{-1} \cdot min^{-1}$). After a 30-min rest period to allow LBF to return to resting levels, similar measurements were made in response to sodium nitroprusside (0.2 $\mu g \cdot kg^{-1} \cdot min^{-1}$). Both drugs were delivered at a

rate of 2 ml/min. The dose of sodium nitroprusside was calculated so as to elicit LBF responses similar to those observed for acetylcholine in the diabetic patients. LBF was again allowed to return to resting levels over a 30-min period. All subjects performed a 25-min supine cycling bout at 60 ± 2% $V_{O_{2peak}}$ in the supine position. During this trial, saline was infused at a rate of 2 ml/min between 10 and 25 min of exercise. LBF was measured at rest before exercise and at 10, 15, 20, and 25 min during exercise as part of a previous study (14).

Experimental procedures

Subjects were requested to refrain from exercise, alcohol, and caffeine for 24 h before the experimental trial. After an overnight fast, subjects attended the Alfred Hospital at 0800 h. The right femoral artery and vein were cannulated as described previously (14,15) and used for simultaneous arterial and venous blood sampling, arterial blood pressure measurement, arterial drug infusions, and venous blood flow measurement. Blood pressure, blood temperature, and infusate temperature were digitized, and electronic calipers were used to average these signals over appropriate time intervals.

Preparation of drug infusions

Acetylcholine (Clinalfa; Calbiochem-Novabiochem, Läufalfingen, Switzerland)

and sodium nitroprusside (Roche, Basel) were diluted in 0.9% NaCl.

LBF

Right femoral venous blood flow was measured by constant-rate infusion of cold saline according to the thermodilution principle (16), as described previously by our group (14,15). Briefly, cold saline was drawn from a reservoir and then immediately infused through the femoral venous catheter (5–8°C) using a Leibel-Flarsheim Angiomat 3000 Injector (Sybron, Cincinnati, OH). At rest, LBF was measured at an infusion rate of 0.7 ml/s for 20 s. During exercise, LBF was measured using a constant infusion rate between 1.5 and 2.4 ml/s for 15 s, titrated to produce an ~0.9–1.2°C decrease in blood temperature. The coefficient of variation in blood flow measurement during exercise was 6.7%.

Blood sampling and analysis

Triglycerides, glucose concentration, and plasma total, LDL, and HDL cholesterol were measured using enzymatic spectrophotometric techniques with a Cobas-BIO centrifugal analyzer (Roche Diagnostic Systems). Arterial plasma insulin concentration was measured in duplicate by radioimmunoassay (Linco Research, St. Charles, MO). HbA_{1c} was measured using affinity high-performance liquid chromatography (CLC385

Table 1—Subject characteristics

	Control subjects	Type 2 diabetic subjects	P
Age (years)	46 ± 2	48 ± 1	0.23
Weight (kg)	86.8 ± 4.1	88.2 ± 4.8	0.82
BMI (kg/m²)	25.9 ± 1.0	28.1 ± 1.4	0.25
Systolic blood pressure (mmHg)	120 ± 2	128 ± 6	0.27
Diastolic blood pressure (mmHg)	74 ± 2	80 ± 3	0.05*
Fasting plasma glucose (mmol/l)	5.21 ± 0.15	9.07 ± 0.87	<0.001*
Fasting plasma insulin (pmol/l)	61 ± 4	87 ± 18	0.20
HbA_{1c} (%)	4.97 ± 0.08	6.23 ± 0.38	0.005*
Total cholesterol (mmol/l)	5.08 ± 0.22	4.75 ± 0.38	0.46
HDL cholesterol (mmol/l)	1.39 ± 0.09	1.02 ± 0.08	0.01*
LDL cholesterol (mmol/l)	3.25 ± 0.20	2.89 ± 0.33	0.37
Triglycerides (mmol/l)	0.98 ± 0.14	1.82 ± 0.35	0.04*
$V_{O_{2peak}}$ (ml · kg^{-1} · min^{-1})	27.3 ± 2.0	28.8 ± 1.4	0.54
V_{O_2} during exercise (% $V_{O_{2peak}}$)	60.6 ± 1.9	60.0 ± 2.0	0.84
Workload during exercise (W)	91 ± 8	97 ± 7	0.55
Mean heart rate during exercise (beats/min)	112 ± 5	119 ± 4	0.30

Values are expressed as means ± SE. Blood pressures (brachial sphygmomanometry) and fasting plasma glucose and insulin were measured at the initial screening visit. Exercise was at 60% $V_{O_{2peak}}$. *$P < 0.05$ compared with control subjects.

liquid chromatograph with a 234 Autosampler; Primus, Kansas City, MO). This methodology is compatible with that used in the Diabetes Control and Complications Trial.

Statistics

All results are expressed as means ± SE. Group characteristics were compared using unpaired *t* tests. Serial measurements made during drug infusions and exercise were analyzed using repeated-measures ANOVA to examine differences between groups. A Pearson correlation coefficient was used for univariate analysis. Multiple regression was performed by stepped backward and forward entry. Variables were entered if the respective *F* probability was <0.05 and were removed if the *F* probability was >0.1. The null hypothesis was rejected at $P \leq 0.05$.

RESULTS — Control and type 2 diabetic subjects were not different in age, weight, or BMI (Table 1). Fasting plasma glucose and triglycerides were significantly higher and HDL cholesterol significantly lower in diabetic subjects (Table 1). HbA_{1c} was higher in diabetic subjects, attesting to their diabetic status, but the elevation was only mild, indicating that they were well controlled. Furthermore, whereas fasting insulin tended to be higher in diabetic subjects, it was not significantly different from control subjects. There was no difference between groups in total or LDL cholesterol (Table 1). All blood pressures were within the normotensive range; however, resting brachial diastolic blood pressure was 6 mmHg higher in patients with diabetes, whereas systolic blood pressure was not different from control subjects (Table 1).

Leg vascular reactivity

Resting LBF was not significantly different between groups (Fig. 1). In healthy control subjects, LBF increased from 0.370 ± 0.034 l/min at rest to an average (mean 3–5 min) of 1.76 ± 0.30 l/min during acetylcholine infusion (an increase of 394 ± 81%) (Fig. 1*A*). In contrast, the increase in diabetic subjects was significantly less, from 0.279 ± 0.026 to 0.813 ± 0.133 l/min (an increase of 190 ± 36%, $P < 0.05$ for both absolute LBF and percentage change) (Fig. 1*A*). There was no significant difference in the LBF response to sodium nitroprusside between the two groups (Fig. 1*B*). Blood
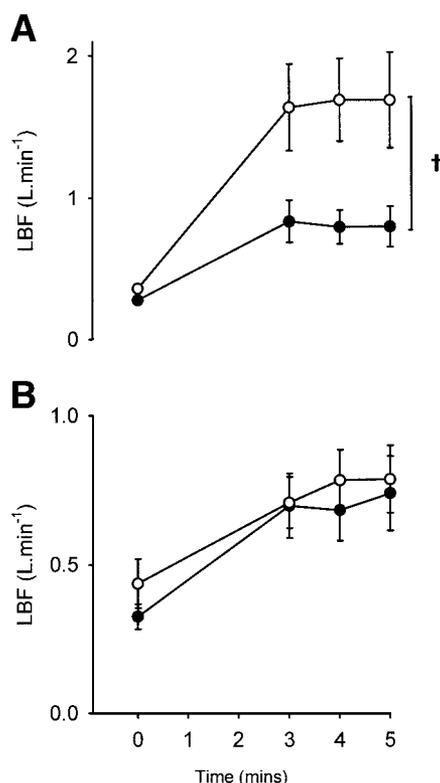
Figure 1—*LBF response to intrafemoral infusion of acetylcholine* (A) *and sodium nitroprusside* (B). ●, *Diabetic subjects;* ○ *control subjects.* †*Significant difference between control subjects and diabetic subjects,* P < 0.05.

pressure and heart rate were unaffected by drug infusion in both groups.

Exercise hemodynamics

VO_{2peak}, exercise workload, and exercise VO_2 as a percentage of VO_{2peak} were not different between groups (Table 1). Furthermore, throughout exercise there was no difference between groups in either leg oxygen consumption, whole-body respiratory exchange ratio, or leg respiratory quotient (14) (Table 1). LBF was not different between control and diabetic subjects before exercise but was significantly lower in diabetic subjects during exercise (mean 10–25 min, control subjects 3.73 ± 0.38 l/min, diabetic subjects 2.78 ± 0.19 l/min, $P = 0.04$) (Fig. 2*A*). At rest, there was no difference in heart rate or intrafemoral artery blood pressure between the diabetic and control groups. Heart rate was not different between groups during exercise (Table 1). Both systolic and diastolic blood pressure were higher in diabetic subjects throughout exercise (mean 10–25 min, control subjects 131/68 ± 3/3 mmHg; diabetic subjects

155/79 ± 7/2 mmHg; $P < 0.01$) (Fig. 2*B*). As a consequence of both lower LBF and higher blood pressure, leg vascular resistance was higher in diabetic subjects (mean 10–25 min, control subjects 27.9 ± 3.2 mmHg · min^{-1} · l^{-1}; diabetic subjects 42.5 ± 3.1 mmHg · min^{-1} · l^{-1}; $P = 0.005$) (Fig. 2*C*).

The percentage changes in LBF in response to exercise and acetylcholine were significantly correlated ($r = 0.54$, $P = 0.02$) (Fig. 3*A*). To determine potential influences on the impaired LBF response to both acetylcholine and exercise in diabetic subjects, univariate analysis was used to examine the relationship of these parameters with resting glucose, insulin, HbA_{1c}, HDL cholesterol, triglycerides, diastolic blood pressure, and BMI. There was a significant negative correlation between the LBF response to exercise and fasting plasma glucose ($r = -0.66$, $P = 0.003$) (Fig. 3*B*) and to a lesser extent fasting insulin ($r = -0.47$, $P = 0.05$) and BMI ($r = -0.49$, $P = 0.04$). There were no significant relationships for the other variables examined. In multiple regression incorporating the significant univariate correlates of exercise LBF, fasting plasma glucose was the only significant independent variable. Relationships with LBF response to acetylcholine were not significant, but there was a trend for a negative relationship with fasting plasma glucose ($r = -0.41$, $P = 0.09$).

CONCLUSIONS — Impaired endothelium-dependent dilatory responses are thought to be an antecedent to atherosclerosis (17) and peripheral vascular disease (4). The current data attribute functional significance to impaired endothelium-dependent vasodilation, demonstrating that LBF is reduced in type 2 diabetic subjects during exercise and that this response is correlated with impaired responsiveness to acetylcholine. Furthermore, the relationship between LBF response to exercise and fasting plasma glucose suggests that the underlying mechanism relates, at least in part, to hyperglycemia.

Hemodynamics during exercise

This is the first demonstration of a reduced LBF response during exercise in diabetic subjects, and it occurred despite higher perfusion pressure. Peripheral vascular resistance was therefore elevated during exercise in the patients with dia-

A

B

C
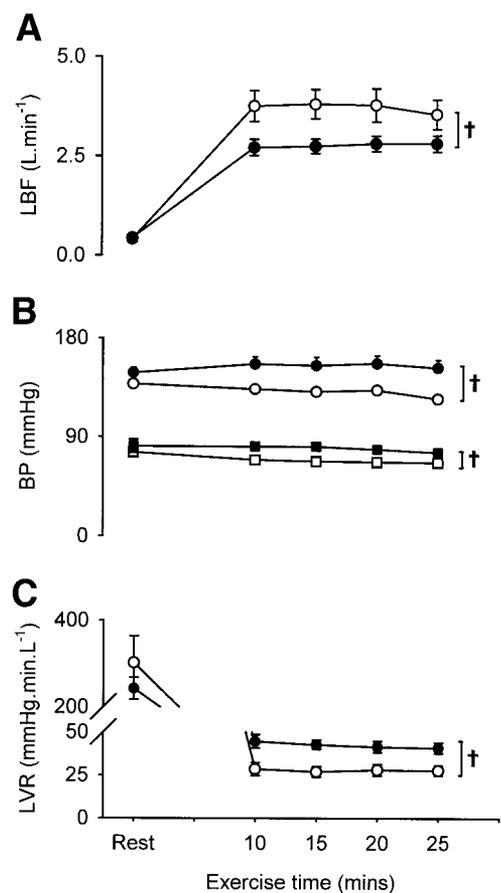
Figure 2— *LBF (A), blood pressure (B), and leg vascular resistance (C) response to 25 min of exercise at 60% VO_{2peak}. BP, blood pressure; LVR, leg vascular resistance. Circles represent systolic blood pressure; squares, diastolic blood pressure; closed symbols, diabetic subjects; open symbols, control subjects. †Significant difference between control subjects and diabetic subjects, P < 0.05.*

betes, suggesting impairment of normal vasodilatory responses. In the only other similar study of which we are aware, Martin et al. (18) found no difference in exercise LBF between type 2 diabetic and control subjects, probably because of recruitment of a fitter cohort.

Endothelial dysfunction in type 2 diabetes
The reduced LBF response to exercise in the type 2 diabetic subjects was moderately well correlated with the LBF response to acetylcholine. Acetylcholine mediates vasodilation through interaction with the endothelial M_3 muscarinic receptor (19) to release NO and vasodilator prostanoids, which diffuse to the underlying smooth muscle and initiate vasorelaxation (20,21). Thus, either the endothelial mechanisms for acetylcholine-stimulated release of NO or prostanoids or arterial smooth muscle reactivity to these substances is reduced in type 2 diabetic subjects. This study implicates these mechanisms in the impaired LBF response to exercise.

Although previous studies have shown impaired endothelium-independent smooth muscle responsiveness to NO in type 2 diabetic subjects (1,2), in the current and previous studies (3), responses to the direct-acting smooth muscle relaxant sodium nitroprusside were normal. This disparity most probably relates to disease severity, which was relatively mild in the current study. The relationship between LBF responses to acetylcholine and exercise therefore appears to be of endothelial origin, at least with respect to NO.

In the current study, there were trends for relationships between lipid parameters and LBF response to exercise; however, significant relationships existed for insulin, glucose, and BMI. In multiple regression analysis, plasma glucose was the only independent predictor of the LBF response to exercise. These data suggest that hyperglycemia may contribute to the impaired LBF response to exercise in type 2 diabetic subjects. Several previous studies have reported impaired forearm blood flow responses to both an ischemic stim-

ulus (22–24) and methacholine (25) during acute hyperglycemia in healthy subjects. Furthermore, acute hyperglycemia has been shown to increase blood pressure and reduce LBF, with such effects being reversible by intravenous L-arginine infusion (26). These previous findings, together with the strength of the correlation between plasma glucose and the percentage change in LBF in response to exercise in the current study, suggest that plasma glucose mediates the impaired LBF response to exercise in diabetic subjects. However, it should be noted that there was only a trend ($P = 0.09$) for a correlation between plasma glucose and LBF response to acetylcholine, suggesting that other mechanisms also contribute.

Mechanisms of glucose-induced endothelial dysfunction
Hyperglycemia may contribute to impaired endothelium-mediated vasodilation through multiple mechanisms. These include increased formation of oxygen-derived free radicals, activation of protein kinase C, and formation of advanced glycosylation end products (25). Advanced glycosylation end products did not appear to be important in the current study because there was no significant relationship between LBF responses to acetylcholine or exercise and HbA_{1c}. Plasma insulin concentration at rest showed a marginally significant correlation ($P = 0.05$) with exercise LBF; however, this was not independent of fasting plasma glucose.

Mechanisms controlling blood flow during exercise
Despite extensive study, the precise mechanisms controlling exercise hyperemia are still unclear (rev. in 27–29). Endothelium-derived vasodilator prostanoids and NO, possibly released in response to shear stress elevation, have been proposed to contribute (6–8); however, their role is controversial (9).

In vascular disease states, impairment of NO and vasodilator prostanoid dilatory mechanisms may have important consequences for exercise hyperemia (30). In support of this contention, an association has been made between impaired endothelium-mediated vasodilation and impaired blood flow to active muscle during exercise in hypercholesterolemic apolipoprotein-E–deficient mice (31). Al-
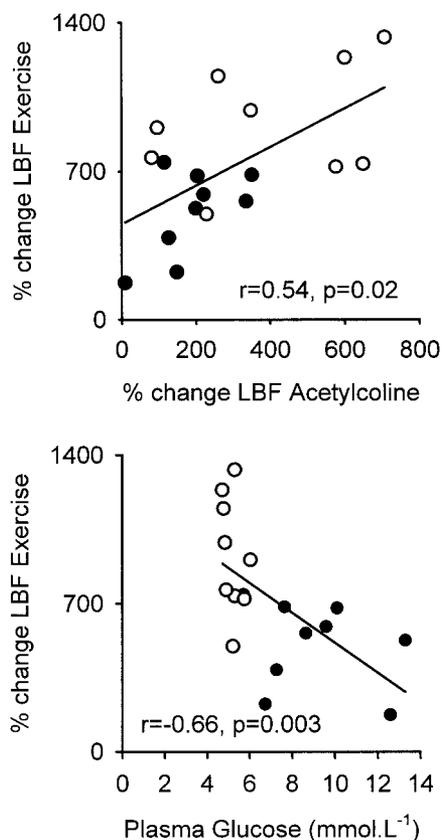
Figure 3— *Relationship between percentage change in LBF in response to acetylcholine and exercise at 60% V_{O_2} peak (A) and relationship between plasma glucose and percentage change in LBF in response to exercise at 60% V_{O_2} peak (B). Closed circles represent diabetic subjects; open circles, control subjects.*

though there are no comparable human studies with relevance to diabetes, it is interesting to note that lipid-lowering therapy associated with improvement in forearm-reactive hyperemia also increased ischemic threshold during treadmill testing in patients with stable angina pectoris (32). Chronic impairment in endothelium-dependent vasodilation would therefore appear to be associated with impaired exercise blood flow responses in both hypercholesterolemic mice and coronary disease patients. It is likely that impairment of NO and the vasodilator prostanoid dilatory mechanism contribute to this attenuation of exercise hyperemia, but other mechanisms may also be important. The current data provides evidence that impaired endothelium-dependent dilatory responses in relatively well-controlled patients with type 2 diabetes limits blood flow during exercise.

Clinical implications

The impaired LBF response to exercise in diabetic subjects and its relationship to acetylcholine reactivity suggests that there are important functional implications for endothelial dysfunction. Thus, although impaired endothelium-dependent dilation may be an antecedent to atherosclerosis (4) and peripheral vascular disease (4), it is also detrimental to blood flow regulation and would be expected to further exacerbate the effects of existing vascular disease in this respect. Indeed, impaired LBF response to exercise may limit exercise capacity in advanced type 2 diabetic subjects (33–35). Furthermore, this may be an important mechanism contributing to reduction in leg ischemic threshold in diabetic subjects with peripheral vascular disease.

Conclusions

The LBF response during exercise is attenuated in type 2 diabetic subjects compared with matched control subjects. Impaired endothelium-dependent vasodilation secondary to elevated plasma glucose may contribute to this observation.

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