Impaired Endothelium-Dependent Vasodilation in Patients With Insulin-Dependent Diabetes Mellitus

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Background. Endothelium-dependent vasodilation is abnormal in experimental models of diabetes mellitus. We postulated that abnormalities of endothelial function are also present in patients with insulin-dependent diabetes mellitus and may contribute to the pathogenesis of vascular disease in these individuals.

Methods and Results. Vascular reactivity was measured in the forearm resistance vessels of 15 patients with insulin-dependent diabetes mellitus and 16 age-matched normal subjects. No patient had hypertension or dyslipidemia. Each subject was pretreated with aspirin to inhibit endogenous production of prostanooids. Methacholine chloride (0.3 to 10 μg/min) was administered via the brachial artery to assess endothelium-dependent vasodilation. Sodium nitroprusside (0.3 to 10 μg/min) and verapamil (10 to 300 μg/min) were infused intra-arterially to assess endothelium-independent vasodilation; phenylephrine (0.3 to 3 μg/min) was administered to examine vasoconstrictor responsiveness. Forearm blood flow was determined by venous occlusion plethysmography, and dose-response curves were generated for each drug. Basal forearm blood flow in diabetic and normal subjects was comparable (2.6±0.2 versus 2.1±0.3 mL·100 mL−1·min−1, respectively; P=NS). The forearm vasodilative response to methacholine was less in diabetic than in normal subjects. At the highest dose of methacholine, the forearm blood flow increased 9.5±1.1 mL·100 mL−1·min−1 in diabetic subjects and 15.3±1.4 mL·100 mL−1·min−1 in normal subjects (P<.01). The forearm blood flow responses to nitroprusside and verapamil and the forearm vasoconstrictor responses to phenylephrine were similar in diabetic and healthy subjects. In diabetic subjects, endothelium-dependent vasodilation correlated inversely with serum insulin concentration but not with glucose concentration, glycosylated hemoglobin, or duration of diabetes.

Conclusions. Endothelium-dependent vasodilation is abnormal in forearm resistance vessels of patients with insulin-dependent diabetes mellitus. This abnormality may be relevant to the high prevalence of vascular disease that occurs in these individuals. (Circulation. 1993;88:2510-2516.)

KEY WORDS • relaxing factor, endothelium-derived • flow, regional blood • insulin • diabetes
dependent vasodilation is impaired in patients with insulin-dependent diabetes mellitus. We chose patients with insulin-dependent diabetes mellitus rather than non-insulin-dependent diabetes mellitus since these patients are younger and it would be easier to identify individuals who did not have other conditions that might affect endothelial function such as dyslipidemia or hypertension. Furthermore, we used a technique that enabled us to measure vasomotor reactivity in forearm resistance vessels, thereby precluding the potential confounding effects of atherosclerosis.

Methods

Subjects

The study participants included 15 patients (4 men and 11 women) with insulin-dependent diabetes mellitus. The age of the diabetic subjects ranged from 23 to 39 years and averaged 30±1 years. The duration of diabetes ranged from 6 months to 25 years, averaging 14±2 years. The control population included 16 healthy volunteers (4 men and 12 women), whose ages ranged from 23 to 43 and averaged 31±2 years (P=NS versus diabetic subjects). All healthy subjects and 13 of 15 diabetic subjects were recruited from the Boston area via advertisements in local newspapers. Two diabetic subjects were referred by their physicians. Each subject was evaluated with a careful history, physical examination, ECG, and laboratory analysis that included hematologic and biochemistry profiles. Criteria for exclusion for both diabetic and healthy volunteers included hypertension, elevated low-density lipoprotein (LDL) cholesterol concentration (>75th percentile for age and gender), cardiac or pulmonary disease, serologic evidence of hepatic or renal dysfunction, or use of any antihypertensive, cardiac, or vasoactive medication. No subject had any overt evidence of atherosclerosis as judged by absence of symptoms of angina, claudication, or cerebrovascular ischemia, and each had a normal vascular examination, including normal pulses and no bruises, and a normal ECG. This study was approved by the Human Research Committee of Brigham and Women’s Hospital, and each subject gave written informed consent.

Experimental Protocol

All participants received 325 mg acetylsalicylic acid daily for 3 days, including the day of the study, to exclude potential confounding effects of vasoactive prostanooids.23 Each subject was studied in the morning in a 23°C temperature-controlled room in the postabsorptive state. Alcohol, caffeine, and insulin (in the case of diabetics) were prohibited within 12 hours of study. Under local anesthesia and sterile conditions, a 20-gauge polyethylene catheter was inserted into a brachial artery of each subject for determination of blood pressure and for infusion of drugs. The vascular research laboratory was quiet, and lights were dimmed. All subjects rested at least 30 minutes after catheter placement to establish a stable baseline before data collection.

Measurements of forearm blood flow and blood pressure were repeated every 15 minutes until stable. To determine the maximal vasodilator potential of the resistance vessels, forearm blood flow was measured in the basal state and during reactive hyperemia, after 5 minutes of an ischemic stimulus. Ischemia was induced by inflation of a sphygmomanometric cuff on the upper arm to suprasystolic pressure. Abnormalities in peak reactive hyperemic blood flow often imply structural problems in the resistance vessels preventing maximal vasodilation. To assess endothelium-dependent vasodilation, methacholine chloride (a congener of acetylcholine) was administered via the brachial artery to all participants. Forearm blood flow was measured during infusion of increasing concentrations of methacholine at doses of 0.3, 1, 3, and 10 μg/min. To distinguish abnormalities of endothelial function from abnormalities of vascular smooth muscle, all subjects received an intra-arterial infusion of sodium nitroprusside. This agent, which acts directly on vascular smooth muscle by stimulating soluble guanylate cyclase and inducing hyperpolarization, was given at doses of 0.3, 1, 3, and 10 μg/min. It was considered that abnormalities of vasodilation may be confined to the nitric oxide–guanylate cyclase pathway or represent a generalized impairment of vascular smooth muscle. To examine this possibility, the calcium channel blocker, verapamil was administered intra-arterially at doses of 10, 30, 100, and 300 μg/min to 9 normal subjects and 7 diabetic subjects. Finally, to determine whether vasoconstrictor reactivity was altered, phenylephrine, an α1-adrenergic agonist, was infused intra-arterially in doses of 0.3, 1, and 3 μg/min in 7 normal and 7 diabetic subjects. Vehicle (5% dextrose) and drug infusions were administered at a rate of 0.4 mL/min. Hemodynamic measurements commenced after each drug dose had been delivered for 3 minutes. The doses of each drug were chosen to achieve a change in forearm vascular resistance without causing systemic effects. Basal conditions were reestablished between each intervention.

Hemodynamic Measurements

Bilateral forearm blood flow was determined by venous occlusion strain-gauge plethysmography, using calibrated mercury-in-Silastic plethysmography, and expressed as mL/100 mL of tissue per minute (D.E. Hokanson, Inc, Bellevue, Wash).24 Each arm was supported above the heart level. Venous occlusion pressure averaged 33±1 mm Hg. Circulation to the hand was prevented by inflating a wrist cuff to suprasystolic pressures during each blood flow determination. Forearm blood flow measured during each experimental period comprised at least five separate measurements performed at 10- to 15-second intervals. By measuring blood flow in the infused arm, the direct effect of the drug can be determined. By measuring blood flow in the noninfused arm, confirmation can be made that the drug is not causing systemic effects. Forearm vascular resistance was calculated as the ratio of mean blood pressure to forearm blood flow and expressed as units reflecting mm Hg/mL/100 mL tissue/min.

Blood pressure was measured via an arterial cannula that was attached to a Statham P23 pressure transducer aligned to an amplifier on a Gould physiologic recorder. Heart rate was determined from a simultaneously obtained ECG signal and calculated from the RR interval.
TABLE 1. Characteristics of Study Population

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>31±2</td>
<td>30±1</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>65±3</td>
<td>70±3</td>
</tr>
<tr>
<td>Smoker, no.</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Mean blood pressure, mm Hg</td>
<td>78±2</td>
<td>81±2</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>100±5</td>
<td>254±35↑</td>
</tr>
<tr>
<td>Glycosylated hemoglobin, % total hemoglobin</td>
<td>4.9±0.2</td>
<td>11.9±0.6↑</td>
</tr>
<tr>
<td>Insulin, MU/L</td>
<td>17±2</td>
<td>48±15*</td>
</tr>
<tr>
<td>Blood urea nitrogen, mg/dL</td>
<td>15±1</td>
<td>16±1</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>1.0±0.04</td>
<td>1.0±0.03</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>164±5</td>
<td>175±6</td>
</tr>
<tr>
<td>High-density lipoprotein cholesterol, mg/dL</td>
<td>52±3</td>
<td>52±3</td>
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<tr>
<td>Low-density lipoprotein cholesterol, mg/dL</td>
<td>101±4</td>
<td>109±5</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>78±8</td>
<td>76±12</td>
</tr>
</tbody>
</table>

*P<.05, †P<.01 compared with normal subjects.

Blood Samples

Blood samples for chemistry analysis and lipid profiling were obtained before the study to determine if the subject met the inclusion criteria noted earlier. At the beginning of each study, blood samples were obtained for serum glucose, glycosylated hemoglobin, and insulin levels.

Statistical Analysis

Forearm blood flow, blood pressure, and heart rate are presented as mean±SE. Statistical analysis comprised ANOVA of independent groups for repeated measures for parametric data, followed by Duncan’s test for statistical significance.25 Student’s t test was used to analyze the difference between the means in each group. Linear regression analysis was performed for selected variables. Statistical significance was accepted at the 95% confidence level (P<.05).

Results

The baseline characteristics of the normal and diabetic subjects are provided in Table 1. Age, weight, and blood pressure of each group were similar. Serum glucose, glycosylated hemoglobin, and insulin levels were higher in the diabetic than in the normal subjects. There was no significant difference in renal function (blood urea nitrogen, creatinine) or lipid values (total, high-density lipoprotein, LDL, cholesterol, and triglyceride concentrations).

Basal and Reactive Hyperemic Forearm Blood Flow

The basal forearm blood flow in diabetic and normal subjects averaged 2.6±0.2 and 2.1±0.3 mL/100 mL tissue/min, respectively (P=NS) (Fig 1). Forearm vascular resistance was 34±3 in diabetic subjects and 44±5 in normal subjects (P=NS). Peak reactive hyperemic blood flow was virtually identical in diabetic and normal subjects (21.4±1.2 versus 21.1±1.9 mL/100 mL tissue/min, respectively; P=NS) (Fig 1). The corresponding minimal forearm vascular resistance was 3.9±0.2 U in diabetic participants and 4.2±0.4 U in normal subjects (P=NS).

Endothelium-Dependent Responses to Methacholine Chloride

Intra-arterial infusion of methacholine chloride increased forearm blood flow in both diabetic and normal subjects. However, the vasodilative response to methacholine was significantly attenuated in patients with diabetes (Fig 2). At the two highest doses of methacholine, the changes in forearm blood flow and forearm vascular resistance were less in diabetic than in normal subjects. At the highest dose of methacholine (10 μg/min), the forearm blood flow increased 9.5±1.1 mL/100 mL tissue/min in the diabetic subjects and 15.3±1.4 mL/100 mL tissue/min in the normal volunteers (P<.01). At this dose, forearm vascular resistance decreased 27±3 U in the diabetic participants and 39±4 U in the normal subjects (P<.05).

Methacholine chloride did not affect forearm blood flow or forearm vascular resistance in the contralateral arm. Also, there was no change in blood pressure or heart rate during drug administration.

Fig 1. Bar graph of forearm blood flow under basal conditions and during reactive hyperemia in normal and diabetic subjects. Basal and reactive hyperemic blood flows were not significantly different between the two groups.

Fig 2. Plot of forearm blood flow response to intra-arterial infusion of methacholine chloride in normal and diabetic subjects. Cholinergic vasodilation was less in the diabetic group than in the normal group. The difference between groups was significant at the 3- and 10-μg/min doses.
Endothelium-Independent Response to Nitroprusside

Sodium nitroprusside increased forearm blood flow in diabetic and normal subjects (Fig 3). In contrast to the findings with methacholine, the vasodilative response to nitroprusside was similar in each group. At the maximal dose of nitroprusside (10 μg/min), the increase in forearm blood flow was 8.0±0.8 versus 9.0±1.2 mL/100 mL tissue/min in diabetic and normal subjects, respectively (P=NS). Similarly, the decline in forearm vascular resistance was not significantly different between groups (−25±2 versus −31±4 in diabetic and normal subjects, respectively; P=NS). Forearm blood flow did not increase in the contralateral arm in either group. However, during the 10-μg/min dose, mean blood pressure decreased slightly in both diabetic (−4±1 mm Hg) and normal (−5±1 mm Hg) subjects (each P<.01 versus baseline, P=NS between groups).

Endothelium-Independent Response to Verapamil

Verapamil also increased forearm blood flow and decreased forearm vascular resistance in both diabetic and normal subjects (Fig 4). The vasodilative response to the calcium channel blocker was similar in each group. At the highest dose of verapamil (300 μg/min), forearm blood flow increased 7.4±1.2 mL/100 mL tissue/min in diabetic subjects and 9.1±1.4 mL/100 mL tissue/min in normal subjects (P=NS). The decrease in forearm vascular resistance in diabetic and normal subjects was −30±4 versus −37±4 U, respectively (P=NS). Intra-arterial infusion of verapamil did not significantly change forearm blood flow or vascular resistance in the contralateral arm. During the 300-μg/min dose, blood pressure decreased 5±1 mm Hg in diabetic patients (P<.01 versus baseline) and 2±1 mm Hg in normal subjects (P=NS versus baseline, P=NS between groups).

Response to Phenylephrine

Phenylephrine decreased forearm blood flow and increased forearm vascular resistance comparably in each group (Fig 5). The change in forearm blood flow at the maximal dose (3 μg/min) was −1.2±0.2 versus −1.2±0.2 mm Hg in diabetic and normal subjects, respectively (P=NS). The change in forearm vascular resistance at this dose of phenylephrine did not differ significantly between these two groups (31±6 U in diabetic subjects versus 36±5 U in normal subjects, P=NS). The infusion of phenylephrine did not significantly change the forearm blood flow or vascular resistance in the contralateral arm, nor did it change blood pressure in either group.

Relationship of Endothelium-Dependent Vasodilation to Glucose, Glycosylated Hemoglobin, and Insulin Levels

The degree of attenuation of forearm blood flow response during the methacholine infusion was greater in some diabetic subjects than in others. To determine whether biochemical markers of diabetes, glucose control, or duration of diabetes correlate with endothelial function, the relationship between biochemical markers of diabetes and forearm blood flow response was assessed. The biochemical markers included serum glucose concentration, glycosylated hemoglobin, duration of diabetes, and insulin concentration. The relationship between these markers and forearm blood flow response was evaluated using linear regression analysis. The results are presented in Table 2.

Table 2. Relationship of Biochemical Markers of Diabetes With Maximal Endothelium-Dependent Vasodilation

<table>
<thead>
<tr>
<th>Marker</th>
<th>R</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum glucose concentration</td>
<td>.07</td>
<td>NS</td>
</tr>
<tr>
<td>Glycosylated hemoglobin</td>
<td>−.15</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td>.18</td>
<td>NS</td>
</tr>
<tr>
<td>Insulin concentration</td>
<td>−.60</td>
<td>.02</td>
</tr>
</tbody>
</table>
dysfunction, linear regression analyses were performed relating the forearm blood flow measured at the maximal dose of methacholine to glucose, glycosylated hemoglobin and insulin levels, and duration of diabetes (Table 2). There was an inverse correlation between insulin level and the vasodilative response to methacholine (r = -0.60, P < 0.03). There was no significant correlation between the vasodilative response to methacholine and glucose concentration, glycosylated hemoglobin level, or duration of diabetes mellitus.

Discussion

The important new information derived from these experiments is that endothelium-dependent vasodilation is impaired in forearm resistance vessels of patients with insulin-dependent (type 1) diabetes mellitus. The vasodilator response to methacholine chloride was reduced in diabetic subjects, whereas the responses to sodium nitroprusside, verapamil, and reactive hyperemia were preserved.

Methacholine has several potential mechanisms of action that could reduce vascular resistance, including receptor-stimulated release of EDRF, activation of vasodilator prostanoids, and inhibition of norepinephrine release from sympathetic nerve terminals. In this study, all patients were pretreated with the cyclooxygenase inhibitor, aspirin, making it unlikely that the abnormal response to methacholine occurred as a result of altered prostanoid formation. Previous studies in humans have shown that the α-adrenoceptor antagonist phentolamine does not affect the forearm vasodilator effects of methacholine. Thus, the impaired response to methacholine in insulin-dependent diabetics must be secondary to reduced synthesis, release, or activity of EDRF.

Evidence for Endothelial Dysfunction in Diabetes

Evidence that endothelial function is abnormal in diabetes comes, in large part, from studies that measured endothelial substances that mediate fibrinolysis and coagulation. In both type I and type II diabetic patients, fibrinolytic activity is decreased and plasminogen activator inhibitor activity is increased. Abnormalities of endothelial function have also been implicated by studies in animals and humans with diabetes that reported decreased production of prostacyclin.

Abnormal endothelium-dependent vasodilation has been shown repeatedly, albeit not consistently, in animal models of diabetes. Studies using drugs such as acetylcholine, ADP, and histamine to stimulate release of EDRF have found that endothelium-dependent vasodilation is abnormal in both conduit arteries and resistance vessels of diabetic animals. Also, cGMP levels are low in aortas of diabetic rats, suggesting that basal concentrations of EDRF are reduced in these vessels. Supporting evidence comes from studies that found that impaired acetylcholine-induced vasorelaxation of diabetic rat aortae was unaffected by pretreatment with indomethacin. Other investigators, however, have argued that impaired endothelium-dependent vasodilation in diabetes occurs because the endothelium releases vasoconstrictor prostanoids that counteract the effect of EDRF. Yet, not all studies have confirmed abnormal endothelium-dependent vasodilation in diabetic animals. The reasons for the conflicting results are not clear and may be attributed to the duration of the diabetic state, species or gender of the animal, the nature of the agent used to induce diabetes, or the choice of the artery used in these experiments.

The findings in our study support those reported by Saenz de Tejada et al who found that endothelium-dependent relaxation is impaired in corpora cavernosa excised from diabetic men with impotence. The recent work of Calver and colleagues also complements our findings. They administered the EDRF antagonist Nω-nitro-L-arginine into the forearms of insulin-dependent diabetics and healthy subjects. They found that the vasoconstrictor response to this nitric oxide synthase antagonist is reduced in insulin-dependent diabetic subjects, thus implicating impaired basal release of EDRF. In contrast, Halkin et al were unable to detect any abnormality in endothelium-dependent or -independent vasodilation in insulin-dependent diabetics. McVeigh et al reported that the vasodilator responses to both acetylcholine and nitroglycerin were reduced in patients with non-insulin-dependent diabetes mellitus, implicating abnormalities in the EDRF-nitric oxide pathway. Alternatively, the ability of the vascular smooth muscle to respond to vasodilator stimuli may be impaired in non-insulin-dependent diabetics, who are generally older than insulin-dependent diabetics and often affected by other disorders such as hypertension and dyslipidemia.

Possible Mechanisms for Endothelial Dysfunction

Several mechanisms have been proposed to explain abnormal endothelium-dependent relaxation in diabetics. These include abnormalities in signal transduction, reduced synthesis of EDRF, accelerated inactivation of nitric oxide, and generation and release of competing vasoconstrictor substances. Faulty signal transduction has been variably attributed to decreased expression of inhibitory G proteins, reduced phosphoinositol metabolism, and increased activation of protein kinase C. Abnormalities in the endothelial milieu might hasten the inactivation of nitric oxide. These include high levels of oxygen-derived free radicals, advanced glycosylation end-products, and transport barriers such as thickened basement membranes. Several groups of investigators have reported that endothelial generation and release of vasoconstrictor prostanoids compete with, and thereby attenuate, endothelium-dependent relaxation in diabetes. In these studies, endothelium-dependent relaxation has been restored by the cyclooxygenase inhibitor indomethacin and by a prostaglandin H2 and thromboxane A2 receptor antagonist. In our study, we blocked prostanoid formation with aspirin, making it unlikely that this mechanism accounted for impaired endothelium-dependent relaxation in diabetic forearm resistance vessels.

In addition, there is evidence to suggest that vasoconstrictor responsiveness is enhanced in diabetes, a circumstance that may limit vasodilation. Calcium influx is greater in diabetic than in nondiabetic vessels, possibly accounting for enhanced α-adrenoceptor sensitivity in some experimental models. Increased activity of protein kinase C and decreased activity of the sodium-potassium ATPase pump also may contribute to increased vasoconstrictor responsiveness in diabetes.
We considered this possibility and measured the vasoconstrictive response to phenylephrine in our diabetic and healthy subjects. The dose-response curves to this adrenoceptor agonist were similar in each group. Furthermore, the vasodilator effects of nitroprusside and verapamil were not hindered in the diabetics. Taken together, these observations indicate that competing vasoconstrictor stimuli cannot account for the abnormal endothelium-dependent vasodilation found in patients with insulin-dependent diabetes mellitus.

**Possible Mediators of Endothelial Dysfunction**

The principal biochemical conditions that are likely to mediate abnormal endothelium-dependent relaxation are hyperglycemia and hyperinsulinemia. Tesfamariam et al.\(^5\)\(^5\)\(^7\) have shown that endothelium-dependent relaxation is abnormal in normal rabbit aorta exposed to hyperglycemic conditions. We cannot discount this possibility but were unable to find any correlation between abnormal endothelium-dependent vasodilation and either glucose concentration or glycosylated hemoglobin in our diabetic subjects. We did, however, find a significant inverse correlation between serum insulin levels and endothelium-dependent vasodilation. Insulin had been withheld in all the diabetic subjects for at least 12 hours, but all were on long-acting preparations with residual effects that were still evident on assay. This observation is intriguing but involves a small number of individuals and should not imply causality. Nonetheless, it is noteworthy that three large population-based studies have found a significant correlation between insulin concentration and atherosclerosis.\(^5\)\(^8\)-\(^6\)\(^0\)

**Study Limitations**

We eliminated all patients with overt, clinically significant atherosclerosis on the basis of a careful history and physical examination. We cannot discount the possibility that our diabetic patients had occult atherosclerosis. However, we used a technique that measures resistance in forearm vessels. The upper extremity arteries are less likely to develop atherosclerosis than those of the lower extremities, heart, or aorta. Furthermore, resistance vessels are not subject to atheroma. Structural abnormalities within resistance vessels that might limit vasodilation were further excluded by our finding that peak reactive hyperemic blood flow was not reduced in our diabetic subjects. Finally, patients with additional conditions that affect endothelium-dependent vasodilation, such as hypercholesterolemia and hypertension, were excluded from this study.

**Clinical Significance**

Our findings that abnormalities of endothelium-dependent vasodilation are present in patients with insulin-dependent diabetes mellitus provide some insight into the high prevalence of vascular disease that occurs in these patients. In many respects, nitric oxide is protective against the development of vascular disease. It inhibits platelet aggregation, monocyte adhesion to endothelial cells, and vascular smooth muscle proliferation. We have shown that endothelium-dependent vasodilation is abnormal in patients with insulin-dependent diabetes mellitus and that this abnormality is caused by decreased release or activity of EDRF. These findings are directly applicable only to vasomotor function, but the implications regarding vascular injury and disease cannot be ignored. Further study is required to unravel the mechanisms of endothelial dysfunction in insulin-dependent diabetes and to determine whether these findings are relevant to subjects with non-insulin-dependent diabetes mellitus.

**Acknowledgments**

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