JACC Vol. 27, No. 3
March 1, 1996:567–74

Impaired Nitric Oxide–Mediated Vasodilation in Patients With Non–Insulin-Dependent Diabetes Mellitus

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Objectives. **This study sought to determine whether nitric oxide–mediated vasodilation is abnormal in patients with non–insulin-dependent diabetes mellitus.**

Background. **Multiple investigations, both in experimental models and in patients with insulin-dependent diabetes mellitus, demonstrate impaired endothelium-dependent vasodilation. Decreased availability of endothelium-derived nitric oxide may contribute to the high prevalence of vascular disease in diabetes.**

Methods. **Vascular reactivity was measured in the forearm resistance vessels of 21 patients with non–insulin-dependent diabetes mellitus and 23 matched healthy control subjects. No patient had hypertension or hypercholesterolemia. Each subject was pretreated with aspirin to inhibit endogenous production of vasoactive prostanoids. Methacholine chloride (0.3 to 10 μg/min) was administered through a brachial artery cannula to assess vasodilation to endothelium-derived nitric oxide. Sodium nitroprusside (0.3 to 10 μg/min) was infused to evaluate vasodilation to an exogenous nitric oxide donor. Verapamil (10 to 300 μg/min) was administered to distinguish impaired nitric oxide–mediated vasodilation from general dysfunction of vascular smooth muscle. Forearm blood flow was determined by venous occlusion plethysmography, and dose-response curves were generated for each agent. To assess the role of vasoconstrictor prostanoids, a subset of eight diabetic subjects were reexamined in the absence of aspirin treatment.**

Results. **Basal forearm blood flow in diabetic and nondiabetic subjects was comparable. The forearm blood flow responses to both methacholine chloride and nitroprusside were significantly attenuated in diabetic compared with nondiabetic subjects ($p < 0.005$ by analysis of variance for both agents). In contrast, the response to verapamil was not significantly different between the groups ($p > 0.50$). The forearm blood flow responses to these agents were not significantly affected by cyclooxygenase inhibition.**

Conclusions. **Nitric oxide–mediated vasodilation is impaired in non–insulin-dependent diabetes mellitus. Vasoconstrictor prostanoids do not contribute significantly to vascular dysfunction. The attenuated response to exogenous as well as endogenous nitric oxide donors suggests that the abnormality is due to increased inactivation of nitric oxide or to decreased reactivity of the vascular smooth muscle to nitric oxide.**

(J Am Coll Cardiol 1996;27:567–74)

Vascular disease is a major cause of morbidity and mortality in patients with diabetes mellitus (1). Atherosclerosis, resulting in myocardial infarction, stroke and ischemic extremities, is much more frequent in diabetic than nondiabetic populations (1). Furthermore, small-vessel disease, resulting in diabetic retinopathy and nephropathy, also contributes importantly to the morbidity associated with this disease (2).

Decreased availability of endothelium-derived nitric oxide may contribute to vascular disease in diabetes. Nitric oxide is a potent vasodilator released by the endothelium, which plays a pivotal role in maintaining vascular homeostasis (3–6). Nitric oxide has been shown to inhibit platelet aggregation (7), proliferation of vascular smooth muscle (8) and leukocyte adhesion to the vascular wall (9). Decreased release or activity of nitric oxide could contribute to abnormalities of vasomotor function as well as to atherogenesis through loss of these protective properties.

There is now substantial evidence that endothelium-dependent vasodilation is abnormal in animal models of diabetes mellitus (10–18). We and others have demonstrated that endothelium-dependent vasodilation is impaired in patients with insulin-dependent diabetes mellitus, probably as a result of the decreased availability of endothelial nitric oxide (19–21). McVeigh et al. (22) reported that patients with non–insulin-dependent diabetes mellitus exhibit attenuated vasodilation to both acetylcholine and glyceryl trinitrate. Interpretation of these data is difficult, however, given the possibility of increased nitrate tolerance to indirect nitric oxide donors (such as glyceryl trinitrate) in patients with diabetes (23). Furthermore, the contribution of vasoconstrictor prostanoids, implicated as a mediator of vascular dysfunction in experimen-

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Manuscript received June 26, 1995; revised manuscript received October 6, 1995, accepted October 17, 1995.

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0735-1097/96/$15.00
0735-1097(95)00522-6

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Table 1. Characteristics of the Study Patients

	Diabetic Subjects (n = 21)	Control Subjects (n = 23)
Age (yr)	44 ± 2	41 ± 1
Female/male	7/14	6/17
Smoker (yes/no)	5/16	4/19
Mean blood pressure (mm Hg)	92 ± 2	87 ± 2
Body mass index (kg/m^2)	29 ± 1	24 ± 1*
Total cholesterol (mg/dl)	189 ± 6	175 ± 6
HDL cholesterol (mg/dl)	42 ± 2	48 ± 3
LDL cholesterol (mg/dl)	122 ± 5	116 ± 4
Triglycerides (mg/dl)	187 ± 25	111 ± 11*
Glucose (mg/dl)	195 ± 14	85 ± 2†
Glycosylated hemoglobin (%)	11 ± 1	5 ± 0.4†
Insulin (mU/ml)	40 ± 8	12 ± 2†
Blood urea nitrogen (mg/dl)	15 ± 1	15 ± 1
Creatinine (mg/dl)	1.1 ± 0.07	1.1 ± 0.03

*$p < 0.05$. †$p < 0.0001$. Data presented are mean value ± SD or number of subjects. HDL = high density lipoprotein; LDL = low density lipoprotein.

tal models of diabetes mellitus (24), has not yet been assessed in diabetic humans. For these reasons, the mechanisms of vascular dysfunction in non–insulin-dependent diabetes remains unclear.

Accordingly, the overall objective of this study was to determine whether vasodilator reactivity is abnormal in patients with non–insulin-dependent diabetes mellitus. Furthermore, we sought to determine whether the mechanisms responsible for vascular dysfunction are confined to abnormalities in the nitric oxide pathway, to increased release of vasoconstrictor prostanoids that counteract nitric oxide–mediated vasodilation or to generalized impairment of vascular smooth muscle.

Methods

Subjects. The study group included 21 subjects with non–insulin-dependent diabetes mellitus and 23 healthy control subjects (Table 1). The two groups were closely matched with respect to age. The average duration of diabetes was 4 years (range 6 months to 12 years). Glycemic control was achieved by diet alone (n = 2), by diet plus sulfonylurea (n = 18) or by diet plus daily insulin injections (n = 1). The patient receiving insulin met criteria for classification as non–insulin-dependent diabetes since he had previously achieved adequate control of hyperglycemia with diet and sulfonylurea and had never had diabetic ketoacidosis. Subjects were recruited from the Boston area by advertisements in local newspapers. All subjects underwent a screening that included medical history, physical examination, electrocardiogram (ECG) and laboratory testing, including complete blood count, serum electrolytes, blood glucose, insulin, glycosylated hemoglobin, serum transaminases, blood urea nitrogen and serum creatinine. The criteria for exclusion of both diabetic and healthy volunteers included hypertension (>145/90 mm Hg), elevated low density lipoprotein (LDL) cholesterol concentration (>75th percentile for age and gender), cardiac or pulmonary disease or use of any antihypertensive, cardiac or vasoactive medication. No subject had overt evidence of atherosclerosis as judged by the absence of symptoms of angina, claudication or cerebrovascular ischemia, and each had a normal vascular examination, including normal pulses and absence of bruits. The protocol was approved by the Committee for the Protection of Human Subjects of the Brigham and Women's Hospital, and each volunteer gave written informed consent.

Experimental protocol 1: assessment of nitric oxide–mediated vasodilation. All participants (21 diabetic subjects, 23 nondiabetic control subjects) received 325 mg of acetylsalicylic acid daily for 3 days (including the day of the study) to eliminate the confounding effects of vasoactive prostanoids in the evaluation of endothelium-dependent vasodilation (25). Each subject was studied the morning after an overnight fast. Alcohol, caffeine, cigarettes and use of any medications (including insulin or oral hypoglycemic agents) were prohibited within 12 hours of the study. Under local anesthesia and sterile conditions, a 20-gauge Teflon catheter was inserted into the brachial artery of each subject for determination of blood pressure and infusion of drugs. Blood samples were obtained for serum glucose, glycosylated hemoglobin, serum insulin and lipid profile. The vascular research laboratory was noise free; the lights were dimmed; and room temperature was controlled at 23°C. All subjects rested for a minimum of 30 min after catheter placement and before data acquisition to establish a stable baseline.

Measurements of basal forearm blood flow and blood pressure were repeated every 10 min until stable. To determine the maximal vasodilator potential of the resistance vessels, forearm blood flow was measured during reactive hyperemia induced by inflation of a sphygmomanometric cuff on the upper arm to suprasystolic pressure for 5 min. Abnormalities in peak reactive hyperemic blood flow are indicative of structural defects in the resistance vessels, preventing maximal vasodilation.

Methacholine chloride (Roche Laboratories) was administered through the brachial artery cannula to all participants to assess vasodilation to endothelium-derived nitric oxide. In preliminary experiments, we found that the nitric oxide synthase inhibitor N^G-monomethyl-L-arginine significantly attenuated the vasodilator response to methacholine chloride, providing direct evidence for methacholine-induced release of endothelium-derived nitric oxide. Forearm blood flow was measured during infusion of increasing concentrations of methacholine chloride at doses of 0.3, 1, 3 and 10 μg/min. Sodium nitroprusside (Elkins-Sinn Inc.) was infused in all subjects to assess the response of the vascular smooth muscle to an exogenous nitric oxide donor. This direct-acting nitric oxide donor, which releases nitric oxide in situ, was administered in doses of 0.3, 1, 3 and 10 μg/min. The calcium channel blocking agent verapamil (American Reagent Laboratory Inc.) was used as an endothelium-independent and nitric oxide–independent vasodilator to examine the possibility that abnormalities of vasodilation are not confined to the nitric oxide–

guanylate cyclase pathway, and instead represent a generalized impairment of vascular smooth muscle. Data in support of the use of verapamil as a nitric oxide–independent agent comes from experiments in our laboratory demonstrating that the forearm blood flow response to verapamil was not significantly attenuated by the concomitant administration of N^G-monomethyl-L-arginine (26). Verapamil was administered at doses of 10, 30, 100 and 300 μg/min in 18 of the 21 diabetic subjects and 20 of the 23 nondiabetic control subjects.

The range of doses for each drug was chosen to achieve a maximal change in forearm blood flow and forearm vascular resistance without concomitant systemic effects (19). Baseline infusions of vehicle (5% dextrose) and of drug (diluted in 5% dextrose) were administered at 0.4 ml/min. Hemodynamic measurements were performed after each drug dose had been delivered for 3 min—the shortest time for steady state effect as determined from previous studies (19). Basal conditions were reestablished between each intervention after waiting a minimum of 30 min for the previous drug to clear.

Experimental protocol 2: assessment of the role of vasoconstrictor prostanoids. To gain insight into the effect of vasoconstrictor prostanoids on the vascular function in non–insulin-dependent diabetes, a subgroup of eight diabetic subjects chosen at random were reexamined in the absence of acetylsalicylic acid. All cyclooxygenase inhibitors were prohibited 1 week before examination. All other aspects of the protocol were identical to that outlined above.

Hemodynamic measurements. Bilateral forearm blood flow was determined by venous occlusion mercury-in-Silastic strain-gauge plethysmography (Hokanson EC4, DE Hokanson) (in ml blood/min per 100 ml tissue) (27). Both arms were suspended above the level of the heart to allow sufficient venous return. Venous occlusion pressure averaged 36 ± 1 mm Hg (mean ± SE). To ensure that blood flow measurements referred predominantly to the vasculature supplying muscular tissue, hand circulation was occluded by inflating a wrist cuff to suprasystolic pressures during each measurement interval (28). The forearm blood flow measurements comprised a minimum of five separate measurements performed at 10- to 15-s intervals. The blood flow measurements in the infused study arm allowed determination of the response to the drugs, whereas measurements in the contralateral arm verified that these agents were not causing systemic effects. Forearm vascular resistance was calculated as the ratio of mean blood pressure to forearm blood flow and is expressed in arbitrary units (AU).

Blood pressure was measured by means of the arterial cannula, which was attached to a Statham P23 pressure transducer aligned to an amplifier on a Gould physiologic recorder (Gould Inc.). Heart rate was determined from a simultaneously obtained ECG and was calculated from the RR interval.

Statistical analyses. Results are presented as mean value ± SE. Comparison of group mean values between diabetic and nondiabetic subjects (Table 1, Fig. 1) was made using unpaired two-tailed *t* tests and chi-square tests, as appropriate. Statistical analyses of the dose-response curves for each drug (methacholine chloride, sodium nitroprusside and verapamil) were conducted for the changes from baseline (to control for baseline variability). Two-way repeated-measures analysis of variance (ANOVA) was performed to compare the dose-response curves between the two groups. Although only the results of the repeated-measures ANOVA are reported, comparisons of the slopes of the dose-response curves between groups gave similar results. The Bonferroni correction for multiple testing was used to test the differing response between diabetic and nondiabetic subjects to each drug dose (29). Univariate linear regression analyses were performed to correlate the vasodilator response of each drug to selected variables. Statistical significance was accepted at the 95% confidence level ($p \leq 0.05$).

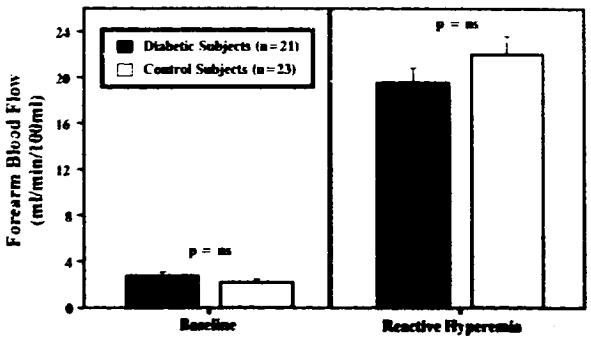

Figure 1. Baseline and reactive hyperemic forearm blood flow in diabetic and nondiabetic subjects. No statistical difference was seen between diabetic and nondiabetic subjects for baseline blood flow ($p > 0.10$) and reactive hyperemic response ($p > 0.20$), as analyzed by unpaired *t* test.

Results

Baseline values of study groups. The baseline characteristics of the diabetic and nondiabetic study groups are provided in Table 1. Age, gender and smoking status were matched between groups. No patient in either group had hypertension, and mean arterial pressure was not statistically different between groups. Diabetic subjects had elevated body mass index relative to control subjects ($p < 0.05$). There was no difference in total, high density lipoprotein (HDL) or LDL cholesterol between diabetic and nondiabetic subjects. However, diabetic subjects did have elevated triglyceride levels ($p < 0.05$). As expected, serum glucose, glycosylated hemoglobin and insulin levels were all significantly higher in diabetic than nondiabetic subjects ($p < 0.001$ for each). Renal function, as assessed by blood urea nitrogen and serum creatinine, was comparable between the two groups.

There was a trend toward a higher baseline forearm blood flow in diabetic subjects (2.8 ± 0.3 ml/min per 100 ml) than nondiabetic subjects (2.3 ± 0.2 ml/min per 100 ml), but the difference was not statistically significant ($p > 0.10$) (Fig. 1). The corresponding baseline forearm vascular resistance was

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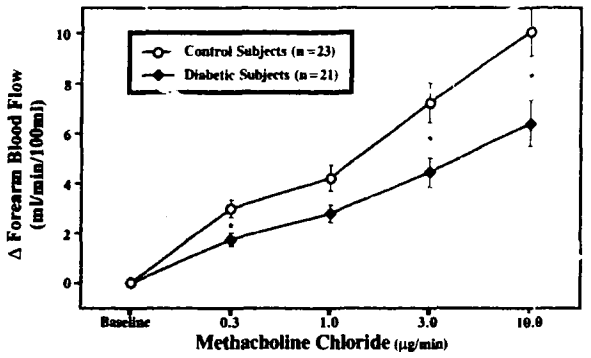


Figure 2. Forearm blood flow increase from baseline during graded intraarterial infusion of methacholine chloride in diabetic and nondiabetic subjects. The response to methacholine chloride was significantly attenuated in diabetic compared with nondiabetic subjects, as analyzed by repeated-measures analysis of variance ($p < 0.005$). The difference between groups was significant at three of the four doses by the Bonferroni correction for multiple comparisons (*$p < 0.05$).

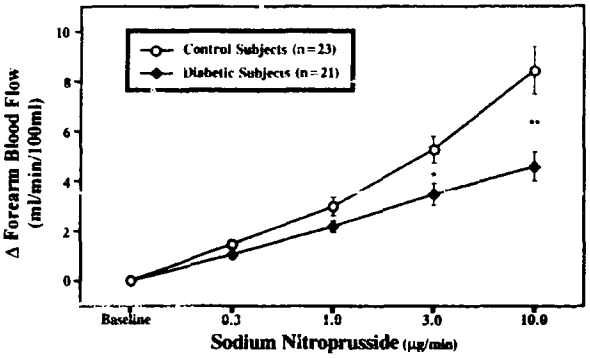


Figure 3. Forearm blood flow increase from baseline during graded intraarterial infusion of sodium nitroprusside in diabetic and nondiabetic subjects. The response to sodium nitroprusside was significantly attenuated in diabetic compared with nondiabetic subjects, as analyzed by repeated-measures analysis of variance ($p < 0.005$). The difference between groups was significant at the 3.0- (*$p < 0.05$) and 10.0-μg/min doses (**$p < 0.005$).

38 ± 3 AU in diabetic and 45 ± 4 AU in control subjects ($p > 0.10$).

Forearm blood flow response to methacholine chloride. Intraarterial infusion of methacholine chloride increased forearm blood flow in both diabetic and nondiabetic subjects (Fig. 2). However, the vasodilator response to methacholine was significantly attenuated in diabetic compared with nondiabetic subjects ($p < 0.005$ by ANOVA). At the highest dose of methacholine (10 μg/min), forearm blood flow increased only 6.4 ± 0.9 ml/min per 100 ml in diabetic compared with 10.1 ± 1 ml/min per 100 ml in nondiabetic subjects ($p < 0.05$, Bonferroni correction for multiple comparisons). Similarly, the corresponding decreases in forearm vascular resistance in response to methacholine infusion were attenuated in diabetic compared with nondiabetic subjects ($p < 0.05$, ANOVA); at the highest dose of methacholine, forearm vascular resistance decreased only 26 ± 3 AU from baseline in diabetic compared with 37 ± 4 AU in control subjects ($p < 0.05$, Bonferroni correction).

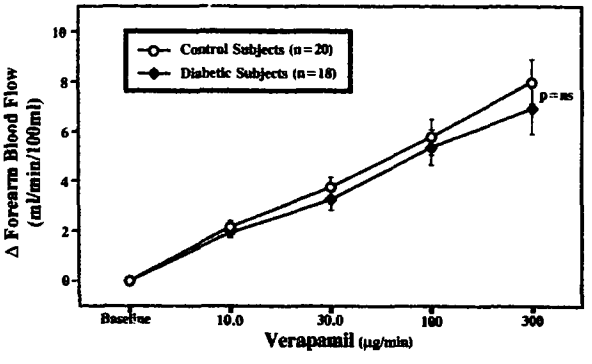
There were no systemic effects of the methacholine infusion in either group; the infusion did not change contralateral forearm blood flow, and there was no change in mean arterial pressure or heart rate during drug administration.

Forearm blood flow response to sodium nitroprusside. Sodium nitroprusside also increased forearm blood flow in both diabetic and nondiabetic subjects (Fig. 3). However, the vasodilator response to nitroprusside was significantly attenuated in diabetic compared with nondiabetic subjects ($p < 0.005$, ANOVA). At the highest dose of nitroprusside (10 μg/min), forearm blood flow increased only 4.6 ± 0.6 ml/min per 100 ml in diabetic compared with 8.4 ± 1 ml/min per 100 ml in control subjects ($p < 0.005$, Bonferroni correction). When expressed as forearm vascular resistance, the data demonstrate an impaired response to nitroprusside in diabetic relative to control subjects ($p < 0.05$, ANOVA); at the highest dose of nitroprusside, forearm vascular resistance decreased only 21 ± 2 AU from baseline in diabetic compared with 32 ± 4 AU in nondiabetic subjects ($p < 0.05$, Bonferroni correction).

Infusion of sodium nitroprusside did not affect forearm blood flow in the contralateral arm in either group, and there was no significant change in blood pressure or heart rate during drug administration.

Forearm blood flow response to verapamil. Verapamil increased forearm blood flow and decreased forearm vascular resistance in both diabetic and nondiabetic subjects (Fig. 4). In contrast to the findings with methacholine and nitroprusside, the vasodilator response to verapamil was not statistically different between the groups ($p > 0.50$, ANOVA). At the maximal dose of verapamil (300 μg/min), the increase in forearm blood flow was similar between the two groups (6.9 ± 1.0 vs. 8.0 ± 0.9 ml/min per 100 ml in diabetic and nondiabetic

Figure 4. Forearm blood flow increase from baseline during graded intraarterial infusion of verapamil in diabetic and nondiabetic subjects. No significant difference was seen between diabetic and nondiabetic subjects in the response to verapamil, as analyzed by repeated-measures analysis of variance ($p > 0.50$).



subjects, respectively). Similarly, the decline in forearm vascular resistance was not significantly different between groups ($p > 0.40$, ANOVA); the decrease in forearm vascular resistance at the highest dose was 26 ± 2 AU in diabetic versus 29 ± 2 AU in nondiabetic subjects.

Forearm blood flow did not increase in the contralateral arm in either group during infusion of verapamil. Also, there was no change in blood pressure or heart rate during drug administration.

Reactive hyperemic forearm blood flow. Reactive hyperemic blood flow is illustrated in Figure 1. The peak reactive hyperemic blood flow was similar in diabetic subjects (19.6 ± 1.2 ml/min per 100 ml) and nondiabetic subjects (22.1 ± 1.5 ml/min per 100 ml, $p > 0.20$). Similarly, minimal vascular resistance (calculated as the ratio of the baseline mean arterial pressure to peak reactive hyperemic blood flow) was not statistically different between the groups (5.1 ± 0.4 in diabetic vs. 4.3 ± 0.3 in nondiabetic subjects, $p > 0.10$).

Relation of vasodilator response to clinical variables. In an attempt to gain insight into the biochemical mechanism or mechanisms by which the diabetic state leads to altered vascular function in non–insulin-dependent diabetes, univariate linear regression analyses were performed on data derived from the diabetic subjects. In this analysis, each of the putative markers of diabetes were correlated with the slope of the dose-response to both methacholine and nitroprusside. There was no significant correlation between the vasodilator response to either methacholine or nitroprusside and the following clinical variables: serum glucose, glycosylated hemoglobin, serum insulin, weight, body mass index, duration of diabetes, total cholesterol, LDL cholesterol, HDL cholesterol or triglyceride concentration.

Subset analyses were performed to determine whether body mass index or triglyceride concentration (which differed between the diabetic and nondiabetic groups) could account for the observed difference in vascular function between the groups. Obese patients with diabetes (>95th percentile body mass index for gender and age, $n = 5$) were excluded, and the vasodilator responses of the remaining nonobese diabetic subjects were compared with those of the nondiabetic subjects. There was no significant difference in either body mass index or weight between these subgroups, yet the findings of the study were not altered ($p < 0.03$ and $p < 0.01$ between groups for methacholine and nitroprusside, respectively, whereas the responses to verapamil were not significantly different, $p > 0.60$). A similar subset analysis was performed by excluding the hypertriglyceridemics (>95th percentile for gender and age, $n = 4$) to evaluate the possibility of hypertriglyceridemia in the diabetic patients as a potential confounder in this study. Excluding hypertriglyceridemic subjects also did not alter the findings of the study ($p < 0.01$ between groups for both methacholine and nitroprusside, whereas the responses to verapamil were not significantly different, $p > 0.60$). Finally, excluding the patient who was receiving exogenous insulin therapy did not alter the findings of the study ($p < 0.01$ between groups for both methacholine and nitroprusside,

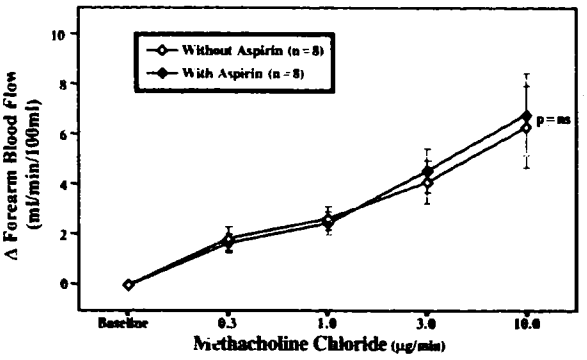


Figure 5. Forearm blood flow increase from baseline in diabetic subjects during graded intraarterial infusion of methacholine chloride in the presence and absence of cyclooxygenase inhibition by acetylsalicylic acid. There was no significant effect of cyclooxygenase inhibition on the responses to methacholine, as analyzed by repeated-measures analysis of variance ($p > 0.80$).

whereas the responses to verapamil were not significantly different between groups, $p > 0.50$).

Assessment of role of vasoconstrictor prostanoids. Baseline blood flow was similar in diabetic subjects in the presence and absence of acetylsalicylic acid (2.8 ± 0.5 vs. 2.5 ± 0.5 ml/min per 100 ml, $p > 0.20$). The reactive hyperemic response also was similar before and during acetylsalicylic acid (17 ± 1 vs. 19 ± 2 ml/min per 100 ml, respectively, $p > 0.40$). The vasodilator response to methacholine was not significantly affected by the presence or absence of acetylsalicylic acid ($p > 0.80$, ANOVA) (Fig. 5). Similarly, the responses to nitroprusside ($p > 0.30$, ANOVA) and verapamil ($p > 0.80$, ANOVA) were unaffected by cyclooxygenase inhibition.

Discussion

The salient information derived from these experiments is that nitric oxide–mediated vasodilation is abnormal in patients with non–insulin-dependent diabetes mellitus. The attenuated response to both methacholine and nitroprusside in non–insulin-dependent diabetic subjects compared with that in nondiabetic subjects indicates impaired vasodilation to both endogenous and direct-acting exogenous nitric oxide donors. The observation that the responses to both verapamil and reactive hyperemia were similar in diabetic and nondiabetic subjects suggests that the functional integrity of the vascular smooth muscle is not compromised in non–insulin-dependent diabetes. Furthermore, the finding that cyclooxygenase inhibition had no effect on the response to any of these vasoactive agents eliminates increased vasoconstrictor prostanoid release as the etiology of diabetic vasodilator dysfunction.

Evidence of endothelial dysfunction in diabetes mellitus. Evidence for endothelial dysfunction in diabetes mellitus comes, in large part, from animal models of diabetes (10–18). Observations in patients with insulin-dependent diabetes mellitus corroborate the findings in animal models. We previously

reported (19) that patients with insulin-dependent diabetes have an attenuated forearm vasodilator response to methacholine compared with matched healthy subjects. In that study, we also demonstrated normal vasodilation to the exogenous nitric oxide donor nitroprusside and to the calcium channel blocker verapamil. The work of Calver et al. (20) and Elliott et al. (21) complements our findings in insulin-dependent diabetes mellitus. They found that the vasoconstrictor response to the nitric oxide synthase antagonist N^G-monomethyl-L-arginine is reduced in insulin-dependent diabetes, implicating impaired basal release of nitric oxide from the endothelium. In contrast, Halkin et al. (30) and Smits et al. (31) did not observe a defect in endothelium-dependent vasodilation in patients with insulin-dependent diabetes. Both of these latter studies included detectable microalbuminuria as an exclusion criterion for their diabetic population, the presence of which correlates with the severity of endothelial dysfunction (21). Despite the inconsistency of the data, however, it is clear that the vasodilator defect in insulin-dependent diabetes, when detected, involves endothelium-derived nitric oxide.

In our study, patients with non–insulin-dependent diabetes exhibited an attenuated response to both endothelium-derived nitric oxide (released in response to methacholine), as well as to the direct-acting exogenous nitric oxide donor nitroprusside. These results are consistent with the report by McVeigh et al. (22), who demonstrated impaired vasodilation to both acetylcholine and glyceryl trinitrate in non–insulin-dependent diabetic subjects relative to control subjects. However, in interpreting the abnormal response to glyceryl trinitrate, McVeigh et al. concluded that this unexpected finding was due to nitroglycerin tolerance in the diabetic patients, and they speculated that the response to a direct-acting nitric oxide donor (such as nitroprusside) would not be attenuated (23). Our data refute this hypothesis, confirm the impaired response to exogenous nitric oxide donors, and thus indicate that the vascular defect in non–insulin-dependent diabetes mellitus cannot be attributed solely to abnormal endothelial production of nitric oxide. Furthermore, the demonstration of normal nitric oxide–independent vasodilation in our protocol (as assessed by verapamil) eliminates the possibility that the vascular abnormality is simply due to generalized impairment of vascular smooth muscle, and instead isolates the impairment to the nitric oxide pathway.

Mechanisms of vascular dysfunction in diabetic patients. Several potential mechanisms have been proposed to explain abnormal endothelium-dependent vasodilation in patients with diabetes. These include 1) decreased synthesis of nitric oxide by the endothelium, 2) increased inactivation of nitric oxide, 3) decreased responsiveness of the nitric oxide–guanylate cyclase pathway at the level of the vascular smooth muscle, and 4) increased release of vasoconstrictor prostanoids that counteract the vasodilation by nitric oxide. The differing vasodilator responses in the two diabetic groups (i.e., an attenuated response to endogenous but not to exogenous nitric oxide donors in insulin-dependent diabetes and an attenuated response to endogenous as well as exogenous nitric oxide donors in non–insulin-dependent diabetes) implicate different mechanisms of vascular dysfunction. Having now completed studies in both insulin-dependent and non–insulin-dependent diabetes with an identical protocol and techniques, we believe that the differing responses in these two groups are, in fact, due to different pathophysiologic processes and are not simply the result of pharmacologic or technical differences between experiments.

Whereas abnormal synthesis or release of nitric oxide by the endothelium is the most likely mechanism that could account for the data in insulin-dependent diabetes, the data from the non–insulin-dependent diabetic studies implicate increased inactivation of nitric oxide or an abnormal response of the vascular smooth muscle to nitric oxide (involving either the receptor for guanylate cyclase or subsequent signal transduction). Of these, evidence derived from experimental models of diabetes suggests that the most likely mechanism is increased inactivation of nitric oxide by either oxygen-derived free radicals (32) or advanced glycosylation end products (18). Oxygen radicals, known to mediate the breakdown of endothelium-derived nitric oxide (33), can be produced in diabetes by a number of reactions, including glucose autooxidation, nonenzymatic protein glycation and cyclooxygenase catalysis (32). Indeed, a number of investigators have reported that scavengers of oxygen radicals improve endothelium-dependent relaxation in diabetic animals both in vitro (34–37) and in vivo (38). Furthermore, the attenuated vasodilator response to acetylcholine, observed in normal vessels exposed to hyperglycemic medium, is restored by oxygen radical scavengers (39, 40). Administration of advanced glycosylation end products to nondiabetic rats inhibits the vasodilator response to both acetylcholine and nitroglycerin in vivo (41), and incubation of rat aortic rings with glycosylated human hemoglobin inhibits endothelium-dependent relaxation in vitro (42).

In our experiments in both insulin-dependent and non–insulin-dependent diabetes, the diabetic and control groups were pretreated with the cyclooxygenase inhibitor aspirin at a sufficient dose and duration to block prostanoid formation (25). Furthermore, we demonstrated in the present protocol that the hemodynamic responses to methacholine, nitroprusside and verapamil in non–insulin-dependent diabetes are similar in the presence and absence of cyclooxygenase inhibition. Therefore, we think it unlikely that vasoconstrictor prostanoids have a significant role in the vascular function in diabetes mellitus.

Potential confounders and study limitations. Although the non–insulin-dependent diabetic and nondiabetic groups were relatively well matched, there was a difference between them with respect to body mass index and triglyceride concentrations, and, as such, these variables have the potential to confound our results. High body mass index is a risk factor for non–insulin-dependent diabetes, and hypertriglyceridemia is a common finding in this disease; thus, these variables may be considered representatives of the diabetic state (as are hyperglycemia and hyperinsulinemia) (1). We decided a priori not to exclude non–insulin-dependent diabetic candidates on the

basis of either criterion. Excluding such diabetic patients would encompass only a subset of the non–insulin-dependent diabetic group and thus would greatly diminish the generalizability of our findings. Nonetheless, subset and univariate regression analyses indicate that neither elevated body mass index nor hypertriglyceridemia were likely to be responsible for the vascular dysfunction that was observed in the subjects with non–insulin-dependent diabetes mellitus.

Sulfonylureas, used as hypoglycemic agents in non–insulin-dependent diabetes, have the potential to attenuate vasodilation by blocking adenosine triphosphate-sensitive potassium channels on vascular smooth muscle, thereby inhibiting hyperpolarization (43). We attempted to minimize the confounding effects of sulfonylurea agents in the design of the study by prohibiting their use within 12 h of the experiment. Furthermore, data from our laboratory indicate that sulfonylureas do not inhibit vasodilation to acetylcholine in normal subjects (44), rendering sulfonylurea use an unlikely confounder for our study.

The vasodilator response to methacholine chloride in the control group, although significantly higher than the response in the diabetic group, was relatively modest compared with groups of healthy control subjects previously reported (19). This difference is the result of the older group required to age match the non–insulin-dependent diabetic group. It is now known that endothelium-dependent vasodilation decreases with age, and the response in the nondiabetic control subjects is appropriate for the average age of that group (45).

Conclusions. We demonstrated an abnormality in the nitric oxide pathway in patients with non–insulin-dependent diabetes mellitus and suggest that this may be secondary to increased inactivation of nitric oxide or an inability of the vascular smooth muscle to respond to nitric oxide. We speculate that loss of the protective properties of nitric oxide may contribute to the increased incidence of vascular disease in these patients. Understanding the mechanisms by which vascular function is impaired in non–insulin-dependent diabetes may lead to novel therapeutic strategies to reduce cardiovascular morbidity and mortality in patients with this disease.

We gratefully acknowledge Nan M. Laird, PhD (Professor and Department Chair, Department of Biostatistics, Harvard School of Public Health, Harvard University) for assistance in statistical analyses. We also thank Joanne Normandin for assistance with manuscript preparation.

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