Noninvasive Detection of Functional Alterations of the Arterial Wall in IDDM Patients With and Without Microalbuminuria

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OBJECTIVE — To test endothelial function in a group of 10 normoalbuminuric and eight microalbuminuric insulin-dependent diabetes mellitus patients (ages 28 ± 3 [mean ± SE] and 28 ± 1 years, respectively), in comparison with 16 control subjects (age 35 ± 2 years, normal subjects vs. diabetic subjects P = NS), to identify prestructural abnormalities of the arterial wall. An early stage of vascular involvement seems in fact to be characterized by functional alterations of endothelial control on vascular tone and wall interaction with circulating cells. Furthermore, many recent studies suggest the importance of microalbuminuria as an early marker not only of nephropathy but also of retinopathy and macroangiopathy.

RESEARCH DESIGN AND METHODS — Endothelium-mediated flow-dependent vasodilation and endothelium-independent vasodilation (induced by glyceryl trinitrate administration) were evaluated in the right common femoral artery by echo-Doppler ultrasound. Arterial wall distensibility was evaluated at the common femoral artery by an echo-tracking system.

RESULTS — In spite of a comparable increase in flow velocity, endothelium-mediated vasodilation was significantly reduced in diabetic subjects, particularly in microalbuminuric patients. Endothelium-independent vasodilation was also significantly impaired in diabetic subjects, particularly in microalbuminuric subjects; whereas arterial wall distensibility, an index of the viscoelastic properties of the wall, was similar in the three groups.

CONCLUSIONS — These results confirm a reduced vasodilatory capacity in diabetes mellitus, with a more marked alteration in microalbuminuric diabetic subjects. This reliable, noninvasive evaluation of arterial function is particularly useful for early diagnosis of vascular involvement.

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dBP, diastolic blood pressure; EDRF, endothelium-derived relaxing factor; GTN, glyceryl trinitrate; HDL, high-density lipoprotein; IDDM, insulin-dependent diabetes mellitus; LDL, low-density lipoprotein; mBP, mean blood pressure; NIDDM, non-insulin-dependent diabetes mellitus; sBP, systolic blood pressure.

Cardiovascular diseases and end-stage renal failure are the main causes of morbidity and death in patients with insulin-dependent diabetes mellitus (IDDM). Several abnormalities of vascular endothelium have been described in diabetes and play a contributory role in explaining the pathogenesis of its vascular complications (1). Over recent years, increasing evidence has suggested a complex role of the endothelium, not only representing a mechanical barrier but also synthesizing various substances that modulate tone and replicative processes of vascular wall cells. Substances like endothelium-derived relaxing factor (EDRF) and prostacyclin have an important vasodilatory role and an antiaggregatory effect on platelets and granulocytes, whereas endothelin and other products of cyclooxygenase (prostaglandins and thromboxane A_2) have vasoconstrictive and proaggregatory effects. The fine balancing of these substances is fundamental for the prevention of any atherogenous process (2). Recent studies have documented that an early stage of vascular involvement is in fact characterized by functional alterations of the vessel wall, e.g., the impairment of flow-dependent vasodilation, an endothelium-mediated phenomenon (3).

Persistent microalbuminuria is considered a marker of early-stage diabetic nephropathy, preceding the development of overt proteinuria and established nephropathy. It is a functional alteration, depending on the impairment of some vasoregulatory mechanisms. Recent studies have also suggested a relationship between microalbuminuria and the prevalence of cardiovascular complications. Perhaps the subgroup of IDDM patients with proteinuria presents a genetically determined predisposition to the development of renal, cardiovascular, and possibly other vascular complications. Microalbuminuria could therefore be considered a marker of widespread vascular damage, in particular of the early phase of functional involvement, one as-

pect of which is impairment of vasorelaxant capacity (4).

An increase in blood flow velocity, with a resulting increase in shear stress, is a physiological stimulus to induce endothelial production of substances active on the arterial smooth muscle cells (3). The consequent endothelium-dependent vasodilation is not only well-documented in vitro (3) but is also reproducible and quantifiable in humans, even by noninvasive investigation (5). To better characterize vasorelaxant capacity, we also evaluated arterial response to administration of nitro derivatives (endothelium-independent vasodilation). In addition, we studied the viscoelastic properties of the vessel wall in the common femoral artery and common carotid artery. The latter is a good model for investigating the capacity of the arterial system to buffer the pulsatile energy related to the cyclic nature of cardiac contraction (6).

This study evaluated young IDDM patients, with and without persistent microalbuminuria, to consider the relationship between biochemical and hemodynamic aspects of the early vascular alteration, before any morphological damage (7,8).

RESEARCH DESIGN AND METHODS

— We studied 34 subjects, including 10 normoalbuminuric (5 men and 5 women, age 28 ± 3 years [mean ± SE]) and 8 microalbuminuric (2 men and 6 women, age 28 ± 1 years) IDDM patients and 16 control subjects (6 men, 10 women, age 35 ± 2 years). Duration of diabetes was 10 ± 1 years in normoalbuminuric and 11 ± 1 years in microalbuminuric patients. Microalbuminuric patients had an albumin excretion rate between 20 and 200 mg/min in two determinations over a period of 6 months. None of the subjects had hypertension, hyperlipidemia, obesity, or clinical evidence of micro- and macroangiopathy.

Metabolic parameters were evaluated for blood drawn after overnight fasting, on the same day as the hemodynamic evaluations. Serum total cholesterol and triglyceride levels were assayed by an automatic colorimetric method (DAX 96, Bayer Diagnostici, Milan, Italy), high-density lipoprotein (HDL) cholesterol was assayed by precipitation with phosphotungstic acid and magnesium, and low-density lipoprotein (LDL) cholesterol was calculated by the formula of Friedewald. Glycosylated hemoglobin was assayed by high-performance liquid chromatography, and fructosamine was assayed by a colorimetric method.

High-resolution echo-Doppler ultrasound (HP, Sonos 1000), with a 7.5-MHz linear vascular probe with axial resolution of 0.1 mm, was used to measure flow velocity and arterial diameter in the right common femoral artery, at a fixed distance from the femoral bifurcation. This measure was performed basally, during distal postischemic hyperemia, and after administration of glyceryl trinitrate (GTN). Arterial diameter, considered as the distance between the M-lines of the proximal and distal wall (the interface between the tunica media and adventitia, simpler to determine than the surface of the endothelial layer), was measured on a two-dimensional ultrasound image of a longitudinal section, at the end-diastolic phase of the cardiac cycle (identified by an accompanying electrocardiogram), to avoid errors of evaluation due to arterial wall distensibility.

Distal ischemia was obtained by applying a mercury sphygmomanometer cuff to the calf, inflated to suprasystolic pressure for 8 min. During distal hyperemia, femoral artery diameter and flow velocity were measured 30 s and 2, 4, and 8 min after the end of ischemia. An overall evaluation of flow-dependent vasodilation was obtained by calculating the area under the curve of percentage diameter variations as a function of time.

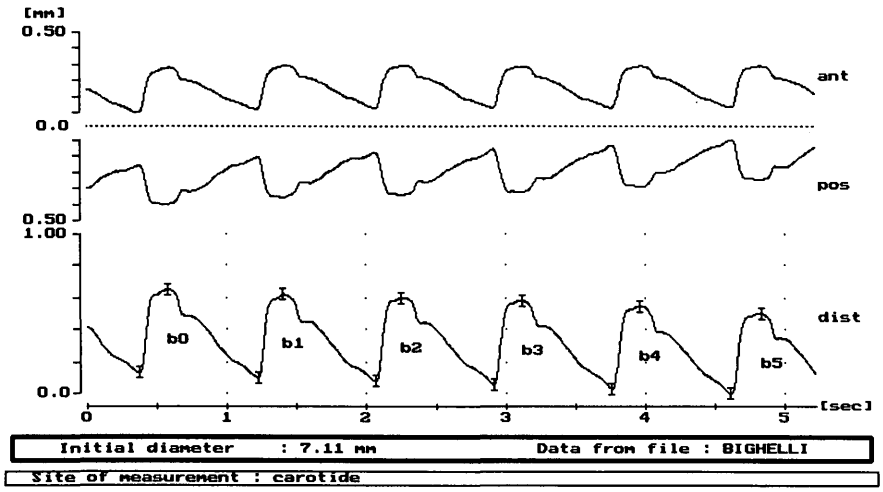
Endothelium-independent vasodilation was evaluated 3.5 and 5 min after administration of GTN (400 mg sublingual). Distensibility and arterial compliance were evaluated in the common femoral and common carotid arteries, using an echo-tracking system (9). This system gives a noninvasive transcutaneous measurement of arterial wall movement throughout the cardiac cycle, allowing evaluation of changes in arterial diameter, as a function of time, in relation to end-diastolic diameter. Data concerning arterial distensibility and compliance were collected before the study of both endothelium-dependent and -independent vasodilation, to avoid the influence of reactive hyperemia and GTN administration on these measurements.

Figure 1 shows a typical curve of movement for the two arterial walls and the corresponding arterial diameter changes during successive cardiac cycles.

The following parameters were computed: internal diastolic diameter (Dd), absolute stroke change in diameter ($Ds - Dd$), and relative stroke change in diameter ($Ds - Dd/Dd$). The stiffness of the vascular wall was estimated using a noninvasive approach to the pressure-diameter curve. The distensibility coefficient (DC) was calculated as $DC = 2(Ds - Dd)/(\text{sBP} - \text{dBP})Dd$, in which sBP is systolic blood pressure and dBP is diastolic blood pressure. DC expresses the tangential strain on the arterial wall for a given pulse pressure and pertains to the mechanical loading of the artery during a cardiac cycle. Cross-sectional compliance (CC) was derived from the dynamic capacitance dV/dP, by rewriting this ratio equation as $CC = \pi (Ds - Dd)Dd/2(\text{sBP} - \text{dBP})$ (10).

All hemodynamic evaluations were conducted by a single observer, blinded to the allocation of the subjects to the three groups.

The reproducibility of these methods was evaluated in five control subjects. The coefficient of variation (SD expressed as a percentage of the mean of several successive measurements) was used for this purpose. Variability was assessed during 12 measurements performed by the observer over a 90-min period in five subjects. Under these conditions, the mean intra-observer coefficient of variation was 1.8 ± 0.8% and



beat	dist (μm)	diam (mm)	dist (%)	dA/A (%)	RR-int (ms)	rise-time (ms)
0	519	6.83	7.60	15.77	846	70
1	522	6.80	7.67	15.94	846	68
2	523	6.78	7.72	16.02	846	66
3	523	6.76	7.74	16.07	841	68
4	519	6.73	7.71	16.01	857	69
5	502	6.70	7.49	15.53	---	66
mean	518	6.77	7.66	15.89	847	68
stdev	8	0.05	0.09	0.20	6	2

Figure 1—*Typical curve for movement in the two arterial walls and the corresponding arterial diameter changes during successive cardiac cycles.*

14 ± 3% for Doppler ultrasound evaluations of diameter and flow velocity, respectively; for the echo-tracking system, it was 1 ± 0.6% for *Dd* and 6 ± 2% for *Ds* − *Dd* and (*Ds* − *Dd*)/*Dd*.

Blood pressure and heart rate were measured automatically, every minute, on the left arm by an oscillometric recorder (Dynamap, model 845, Critikon, Tampa, FL) (11).

Hemodynamic evaluations were performed at a controlled temperature of 22 ± 1°C, after 15 min of rest in a supine position. In diabetic subjects, the evaluations were performed 120–150 min after the last subcutaneous dose of regular insulin.

Statistical analysis

Descriptive data are expressed as means ± SE. The Kruskal-Wallis test was used to compare the three groups.

For diabetic subjects, the relationship between flow-dependent and GTN-induced vasodilation was assessed by linear regression. Statistical significance was taken as $P < 0.05$.

RESULTS — Age, percentage of smokers, body mass index, blood pressure, basal hemodynamic parameters, and plasma lipid concentration were similar in diabetic patients, both normoalbuminuric and microalbuminuric, and in control subjects, whereas heart rate was significantly higher in diabetic patients than in control subjects. Glycemic control was fair in both diabetic groups (Tables 1 and 2).

During the various phases of the study, there were no significant changes in blood pressure and heart rate. During postischemic distal hyperemia, flow velocity in the femoral artery increased significantly and similarly in the three groups (25.4 ± 4.4 and 29.2 ± 6.2 cm/s in normoalbuminuric and microalbuminuric IDDM patients, respectively, and 29.9 ± 3 cm/s in control subjects). Flow-dependent endothelium-mediated vasodilation, calculated from the area under the curve of percentage variations in femoral artery diameter as a function of time, was significantly reduced in diabetic subjects. In particular, microalbuminuric diabetic subjects presented a paradoxical vasoconstrictive response after endothelial stimulation (Fig. 2). Similarly, diabetic patients showed a significant impairment of endothelium-independent vasodilation, induced by sublingual GTN; this was again significantly more evident in the microalbuminuric group (Fig. 3). In all diabetic subjects there was a statistically significant correlation between endothelium-dependent and endothelium-independent responses ($r = 0.597$, $P < 0.01$) (Fig. 4). Common fem-

Table 1—*Hemodynamic details in normoalbuminuric and microalbuminuric IDDM patients and in control subjects*

	Normoalbuminuric	Microalbuminuric	Control
sBP (mmHg)	130 ± 5	123 ± 2	125 ± 3
dBP (mmHg)	72 ± 5	71 ± 3	74 ± 2
mBP (mmHg)	92 ± 4	89 ± 3	90 ± 2
Heart rate (beats/min)	79 ± 7*	76 ± 2*	68 ± 2
Femoral artery diameter (mm)	7.1 ± 0.1	7.5 ± 1	7.4 ± 0.3
Carotid artery diameter (mm)	6.7 ± 0.1	7.6 ± 0.4	6.6 ± 0.3

Data are means ± SE. * $P < 0.05$ compared with control subjects.

Table 2—*Metabolic parameters in normoalbuminuric and microalbuminuric IDDM patients and in control subjects*

	Normoalbuminuric	Microalbuminuric	Control
Total cholesterol (mg/dl)	190 ± 12	184 ± 19	186 ± 8
LDL cholesterol (mg/dl)	129 ± 15	112 ± 11	115 ± 9
HDL cholesterol (mg/dl)	58 ± 4	64 ± 5	59 ± 4
Triglycerides (mg/dl)	73 ± 6	79 ± 6	87 ± 8
Fructosamine (nmol/l)	377 ± 8	369 ± 12	—
HbA_{1c} (%)	7.7 ± 0.2	7.2 ± 0.5	—

Data are means ± SE; NS.

oral artery and common carotid artery distensibility and compliance, indexes of viscoelastic wall properties, showed no significant difference between microalbuminuric, normoalbuminuric, and control subjects (Table 3).

There was no significant relationship between hemodynamic parameters and glycosylated hemoglobin, fructosamine, and duration of the disease.

CONCLUSIONS — Cardiovascular diseases and end-stage renal failure are the main causes of morbidity and mortality in IDDM patients. The prevalence of microalbuminuria among diabetic patients is about 15–20% (12). In recent years, persistent microalbuminuria has come to be considered a marker of generalized vascular alteration (13), and so far a predictive marker not only of nephropathy but also of proliferative retinopathy and of cardiovascular morbidity and mortality, as demonstrated by recent epidemiological studies (14). Against this background, it is important to identify vascular parameters which, together with microalbuminuria, could be useful in clinical practice to detect the onset of arterial involvement.

This study highlighted functional alterations of vascular tone regulation, in the absence of any detectable structural lesion of the vascular wall, in a population of young IDDM patients. The findings of the study contribute to the identification and understanding of the early stages of arterial damage.

Endothelial insult seems to have a crucial role in the beginning of the atherosclerotic process (15). Experimental models have demonstrated early structural lesions of the vessel wall with baring of the subendothelial strata (16). Scope for observing merely functional endothelial damage is of greater potential interest; immunological, chemical, or dietary factors may lead to an alteration of the endothelial phenotype known as "activation" (17). Endothelial production of a series of substances able to regulate vascular tone (18), both vasodilators (like EDRF and prostacyclin) and vasoconstrictors (such as endothelin and thromboxane A_2), becomes "imbalanced" in favor of vasoconstrictors (19, 20), and in turn promotes proliferation of vessel wall smooth muscle cells and platelet aggregation (21).

In recent years, considerable interest has been aroused by the study of the main endothelial functions, such as endothelium-dependent vasodilation. Many authors have suggested that impaired endothelium-dependent vasodilation can be the forerunner of frank atherosclerotic damage (22). Reduced endothelium-dependent vasodilation has been demonstrated in various experimental models of

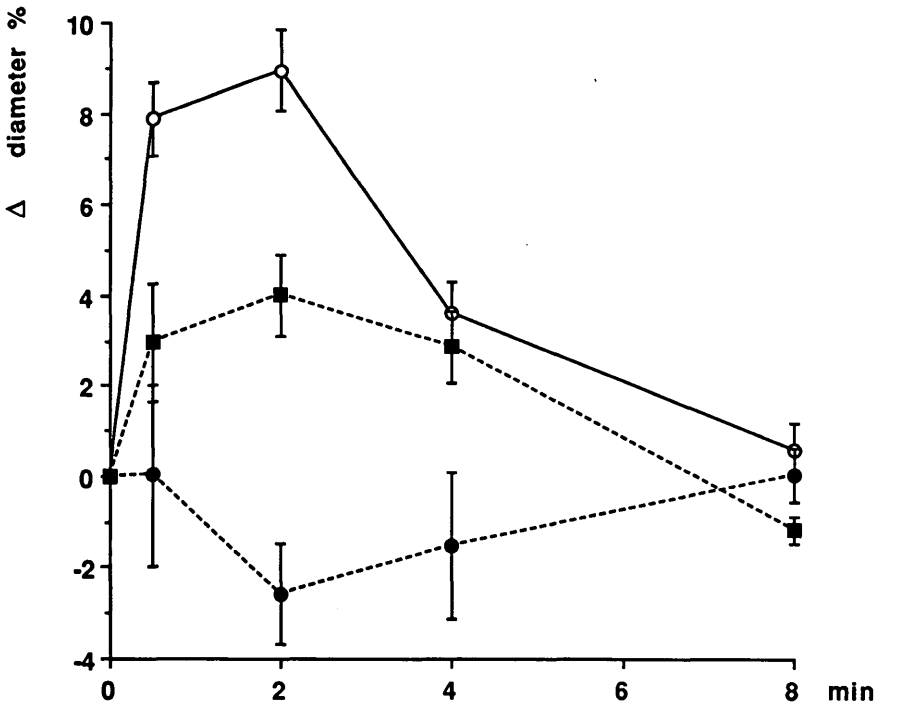


Figure 2—*Time course of flow-dependent changes of common femoral artery diameter in microalbuminuric (●) and normoalbuminuric (■) IDDM patients and in control subjects (○). The time-integrated variations of femoral artery diameter were significantly lower in both groups of patients versus control subjects ($P < 0.01$ and $P < 0.0001$ for normoalbuminuric and microalbuminuric patients, respectively, by the Kruskal-Wallis test) and microalbuminuric versus normoalbuminuric patients ($P < 0.005$ by the Kruskal-Wallis test). Data shown are means ± SE.*

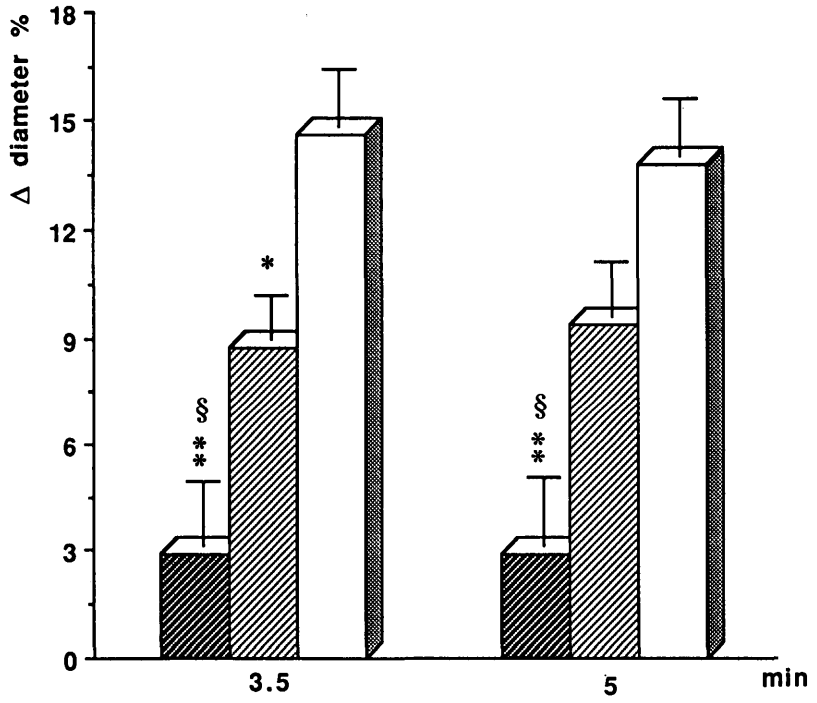


Figure 3—*Common femoral artery diameter changes after GTN administration in microalbuminuric (▨) and normoalbuminuric (▨) IDDM patients and in control subjects (□). Data are means ± SE. *P < 0.05, **P < 0.01 vs. control subjects; §P < 0.05 vs. normoalbuminuric IDDM patients.*

previous human studies (5). This method also seems to be more physiological than the evaluation of endothelial function by acetylcholine infusion, which may be affected by different rates of the molecule metabolism and/or alterations of the specific vessel wall receptors involved (24).

The device used in this study allows morphological evaluation of the artery and the measurements of variations in vessel diameter smaller than those elicited by distal postischemic hyperemia (25).

We studied vasodilatory capacity in 18 IDDM patients, 10 normoalbuminuric and 8 microalbuminuric, with a similar duration of disease, to compare their endothelial function with that of a group of healthy subjects. During postischemic distal hyperemia, the three groups showed a significant, comparable increase in flow velocity, whereas endothelium-mediated vasodilatory response was significantly reduced in normoalbuminuric diabetic subjects by comparison with

diabetes (23). Nevertheless, caution is required in extrapolating these animal findings to humans.

One of the substances most extensively considered in relation to endothelium-dependent vasodilation is EDRF, which has been identified as nitric oxide (NO). It is an important vasodilator as well as an inhibitor of the interaction among leukocytes, platelets, and vessel wall cells. Increased flow velocity within the vessel, leading to increased wall shear stress, is one of the physiological stimuli able to provoke endothelial release of EDRF (3). This phenomenon can be experimentally induced in human arteries by creating distal postischemic hyperemia. Concerning the experimental model used in this study to evaluate endothelium-dependent function, a number of reports confirm endothelial release of NO, with consequent vasodilation, in response to increased flow velocity (3). The suitability and reproducibility of this maneuver has been well-documented in

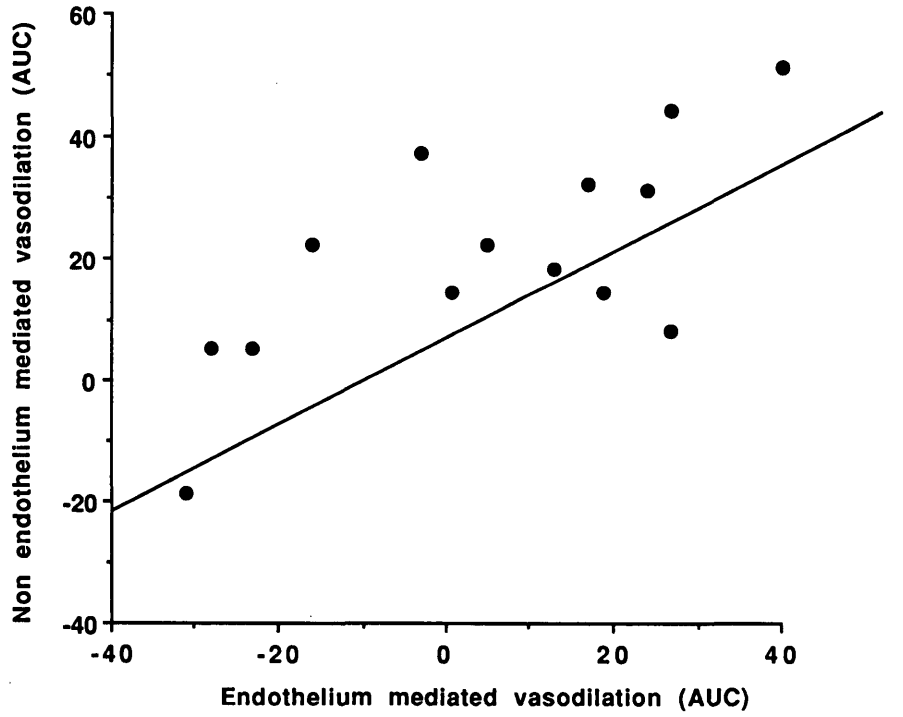


Figure 4—*Correlation between endothelium-mediated and non-endothelium-mediated vasodilation in all IDDM patients. P < 0.01. r = 0.70.*

Table 3—*Artery distensibility parameters in normoalbuminuric and microalbuminuric IDDM patients and in control subjects*

	Normoalbuminuric	Microalbuminuric	Control
Femoral artery			
DC (10^{-3} KPa^{-1})	10.2 ± 1.3	9.1 ± 2	9.9 ± 1.2
CC (10^{-7} m^2 KPa^{-1})	6.3 ± 1.5	3.6 ± 0.6	5.5 ± 1.5
Carotid artery			
DC (10^{-3} KPa^{-1})	12 ± 1.3	16.2 ± 2.1	10.8 ± 1.2
CC (10^{-7} m^2 KPa^{-1})	6.5 ± 0.9	6.2 ± 1.3	4 ± 0.6

Data are means ± SE; NS.

control subjects; in addition, we observed a paradoxical vasoconstrictive response in microalbuminuric patients.

Previous studies have shown impairment of vasodilatory function in vitro, in the presence of high glucose concentrations, or in animals, in hyperglycemic conditions. However, the effects of hyperglycemia on endothelium-mediated vasodilatory capacity are not consistent, either in animals or in humans (26).

Authors reporting no alterations in endothelial vasodilation include Smits and Halkin (27,28), who studied the response to acetylcholine of resistance arterioles in IDDM patients. On the contrary, other studies confirm vascular tone alteration in diabetes mellitus (1). Johnstone et al. (29), for example, found impaired endothelium-dependent vasodilation in forearm resistance vessels in a group of IDDM patients, although they used methacholine chloride for endothelial stimulation, and the response was measured by venous occlusion plethysmography.

Several mechanisms have been suggested to explain impaired endothelial relaxation in diabetes, including a reduction in EDRF production or its early inactivation, e.g., by oxygen free radicals (30) or the accumulation of advanced glycosylation end products, as well as excessive thromboxane A_2 or endothelin production (31), but also a possible functional and/or structural defect involving the muscular layer of the vessel wall (1).

High plasma levels of endothelin have been reported in both non-insulin-dependent diabetes mellitus (NIDDM) diabetic subjects with retinopathy (32) and, of particular interest, in IDDM subjects with microalbuminuria (33). Besides these mechanisms, the impaired endothelium-dependent relaxation in diabetes, particularly the paradoxical vasoconstrictive response in microalbuminuric patients, may be caused by a greater release of endothelium-derived vasoconstrictors (34), i.e., contractile prostanoids. This could counteract the NO-mediated relaxation. Another mechanism of vasoconstrictive response may be F_2-isoprostane production by free radical–catalyzed peroxidation of arachidonic acid (35). These F_2-isoprostanes are formed independently of the cyclooxygenase enzyme, and they are potent vasoconstrictors.

In our diabetic patients, microalbuminuric patients showed a vasoconstrictive response to increased blood flow velocity, compared with a blunted vasodilation in the normoalbuminuric group. This gradient in the vascular functional defect between normoalbuminuric and microalbuminuric diabetic patients was not totally unexpected and may also reflect a gradient with respect to the imbalance between endothelium-derived vasodilating and vasoconstricting substances. In agreement with the recent epidemiological evidence about microalbuminuria as a marker of widespread vascular damage, we consider the gradient in endothelial dysfunction as a continuum, ranging from normoalbuminuric to microalbuminuric diabetic patients.

Looking for a pathogenetic explanation of altered endothelial vasodilation, we also evaluated endothelium-independent vasodilation induced by sublingual GTN. A reduced vasodilatory response was observed in diabetic, particularly microalbuminuric, subjects. This finding has been previously reported in humans only by McVeigh et al. (36), in resistance arterioles of NIDDM diabetic subjects, and in animals by Bucala et al. (37). The suggested mechanisms may be the same as those involved in endothelium-dependent vasodilation, except for a defect in NO production. The correlation that we found between the two altered responses (endothelium- and non-endothelium-mediated responses) is not enough to prove a final common mechanism but suggests a pathological phenomenon that can counteract vasodilatory action of both endogenous and exogenous NO.

Consistent with other reports (4), our hemodynamic results were not correlated with duration of disease and glycemic equilibrium. The observed higher heart rate in our diabetic patients could suggest a sympathetic activation, possibly responsible for increased vascular smooth muscle tone. We exclude higher sympathetic stress during the investigation, considering that both patients and control subjects were extensively informed by the medical staff about the noninvasive nature of the methods. Many authors attribute the hemodynamic effects of insulin to increased levels of vasoconstrictors, such as endothelin (38) or to the stimulatory effect of insulin on the sympathetic nervous system (39–41). Insulin, as a growth factor for the smooth muscle cells (15), and diabetes, like other risk factors for atherosclerosis, can also favor structural damage of the arterial wall. However, in humans, different authors have demonstrated that in the presence of physiological (e.g., postprandial) increases in plasma insulin levels, insulin fails to increase arterial pressure, the sympathetic vasoconstrictor activation being

opposed by vasodilation (42,43). In this study, diabetic patients received four insulin injections per day (three regular and one slow-acting at bedtime); the vascular reactivity tests were all conducted in the postprandial phase, such as 120–150 min after a meal and the accompanying dose of regular insulin.

In addition, in our diabetic patients a role of the sympathetic activation on blunted or abolished vasodilation seems to be excluded by the unchanged blood pressure and arterial wall distensibility values, in comparison to control subjects.

Alterations of the viscoelastic properties of the arterial wall represent one of the first clinically relevant manifestations, leading to early reduction of arterial elasticity. We therefore studied arterial distensibility in the common femoral artery and common carotid artery. The evaluation at the level of the common femoral artery completes the information about vasodilatory capacity obtained in this vessel. The proximity of the common carotid artery to the heart and its prominent elastic component make it a good model for investigating the capacity of the arterial system to buffer the pulsatile energy related to the cyclic nature of cardiac contraction (6). Previous findings in humans have suggested the negative effects of diabetes on arterial distensibility (44).

In our study, normo- and microalbuminuric diabetic subjects did not differ from control subjects in terms of their distensibility coefficient or cross-sectional compliance index. This study allows a direct evaluation of arterial compliance, based on an extremely reliable measurement of arterial wall movement. This is a considerable advance on previous indirect calculations of compliance in humans, based on pulse wave velocity.

In our patients, the integrity of the viscoelastic properties of the arterial wall suggests the absence of any structural alteration. In interpreting reduced endothelium-dependent and endothelium-independent dilation, normal arterial compliance seems to indicate a functional origin for the vascular damage.

In conclusion, IDDM patients with persistent microalbuminuria showed paradoxical vasoconstriction accompanying postischemic enhancement of flow velocity and a reduced vasodilatory response to GTN. These alterations, more evident in microalbuminuric subjects, were also present in normoalbuminuric subjects, allowing an earlier detection of vascular involvement.

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