

Insulin Therapy Improves Insulin-Stimulated Endothelial Function in Patients With Type 2 Diabetes and Ischemic Heart Disease

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Blunted insulin-stimulated endothelial function may be a mechanism for the development of atherothrombotic disease in type 2 diabetes, but it is unknown whether hypoglycemic drug therapy can modulate this abnormality. We studied patients with type 2 diabetes and stable ischemic heart disease ($n = 28$) and lean, healthy control subjects ($n = 31$). Forearm blood flow was measured by venous occlusion plethysmography during dose-response studies of acetylcholine (ACh) and sodium nitroprusside (SNP) infused into the brachial artery. In the patients and 10 healthy control subjects, ACh was repeated after intrabrachial infusion of insulin. Patients were restudied after 2 months of insulin therapy with four daily subcutaneous injections (treatment group, $n = 19$) or without hypoglycemic drug therapy (time control group, $n = 9$). Insulin infusion raised venous serum insulin in the forearm to high physiological levels (133 ± 14.6 mU/l in patients) with a minor increase in systemic venous serum insulin. This increased the ACh response by 149 ± 47 , 110 ± 33 , 100 ± 45 , and $106 \pm 44\%$ during the four ACh doses in healthy control subjects ($P < 0.0001$) but had no effect in patients ($P = 0.3$). After 2 months, HbA_{1c} in the treatment group had decreased from 10.0 ± 0.4 to $7.5 \pm 0.2\%$. Although neither the ACh response ($P = 0.09$) nor the SNP response ($P = 0.4$) had changed significantly, insulin stimulation had a significant effect, as the ACh response increased by 58 ± 25 , 84 ± 66 , 120 ± 93 , and $69 \pm 36\%$ ($P = 0.0002$). In the time control group, insulin stimulation remained without effect after 8 weeks ($P = 0.7$). In conclusion, insulin therapy partly restores insulin-stimulated endothelial function in patients with type 2 diabetes and ischemic heart disease. *Diabetes* 50:2611–2618, 2001

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ACh, acetylcholine; DIGAMI, Diabetes Mellitus, Insulin Glucose Infusion in Acute Myocardial Infarction; eNOS, endothelial NO synthase; IRK, insulin receptor kinase; IRS, insulin receptor substrate; L-NMMA, N^G-monomethyl-L-arginine acetate; NO, nitric oxide; PI3K, phosphatidylinositol-3 kinase; PKC, protein kinase C; SNP, sodium nitroprusside; TNF- α , tumor necrosis factor- α ; UKPDS, U.K. Prospective Diabetes Study.

Diabetes is an independent risk factor for cardiovascular disease (1). Hyperglycemia (2) and insulin resistance (3) are the characteristics of type 2 diabetes most often attributed a causal relation with atherosclerosis. Their possible influence on vascular endothelial function may explain the particular risk of diabetic vascular disease, because endothelial dysfunction is one of the earliest events identified in the pathogenesis of atherosclerosis and thrombosis (4). Impaired endothelium-dependent vasodilation is associated with insulin resistance (5), and this association may be represented in the vasculature by abnormalities in insulin-stimulated endothelial function. Thus, insulin induces vasorelaxation mediated by endothelium-derived nitric oxide (NO) in isolated rat skeletal muscle arterioles (6), and in healthy humans, insulin has a stimulating effect on NO-dependent basal blood flow (7,8) and on agonist-stimulated endothelium-dependent vasodilation (9,10). However, these effects are blunted in patients with obesity-associated insulin resistance or type 2 diabetes (10–12).

The most important trials of the effect of hypoglycemic drug therapy on clinical cardiovascular end points in type 2 diabetes are the U.K. Prospective Diabetes Study (UKPDS) (13), which included patients with newly diagnosed type 2 diabetes, and the Diabetes Mellitus, Insulin Glucose Infusion in Acute Myocardial Infarction (DIGAMI) trial (14), which included patients with diabetes (>80% with type 2 diabetes) and myocardial infarction. The UKPDS showed a nonsignificant 16% reduction of relative risk of myocardial infarction in patients managed with an intensive treatment policy of blood glucose control during a mean follow-up time of 8.4 years, whereas the DIGAMI trial showed a significant 28% reduction of relative risk of 1-year total mortality in patients who received an insulin-glucose infusion in the acute phase, followed by subcutaneous insulin injections during at least 3 months after discharge. The DIGAMI trial, as opposed to the UKPDS, may have been able to show a statistically significant benefit because it included high-risk patients.

We hypothesized that the beneficial effect of insulin therapy in the DIGAMI trial was due to an improvement of vascular endothelial function. As insulin therapy and other hypoglycemic drug therapy improves metabolic insulin resistance (15), we reasoned that insulin therapy may modulate endothelial insulin resistance. With the results of

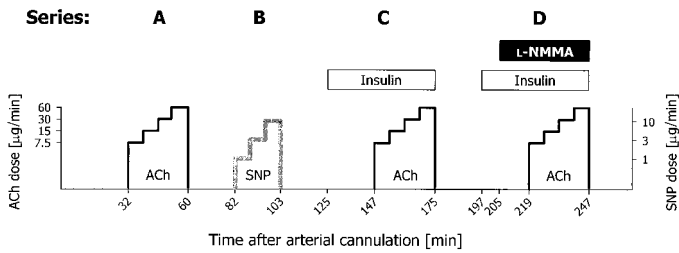


FIG. 1. Infusion protocol for the initial examination. The narrow black graphs represent ACh dose-response studies (doses at left ordinate), and the bold gray graph represents a SNP dose-response study (doses at right ordinate, which is logarithmic like the left ordinate). ACh and SNP were infused for 5 min, followed by measurement of blood flow for 2 min; thus, each dose was given for 7 min. Insulin and L-NMMA were infused in doses of 0.05 mU/kg/min and 3.3 mg/min, respectively. Wash-out periods between dose-response studies were at least 20 min (as shown), but were extended, if necessary, until blood flow had returned to basal levels. In all patients, infusion series A, B, and C were given. In the last 13 consecutively included patients, these three series were followed by series D. In 10 consecutively included healthy control subjects, series A, B, C, and D were given. In the remainder of healthy control subjects, only series A was given.

the DIGAMI trial in mind, we selected patients with type 2 diabetes complicated with manifest and advanced vascular disease. Thus, we examined the effects of 2 months of insulin therapy on endothelial function and insulin-stimulated endothelial function in patients with type 2 diabetes and stable ischemic heart disease.

RESEARCH DESIGN AND METHODS

Patients. A total of 28 patients with type 2 diabetes and ischemic heart disease were included. The sex distribution (26 men and 2 women) reflected that recruitment took place among patients with documented prior myocardial infarction or coronary atherosclerosis demonstrated by angiography and that women declined participation more often than men. All had poor glycemic control (HbA_{1c} >8.0%) and no history of insulin therapy. Patients with systolic dysfunction (left ventricular ejection fraction <50%) or decreased renal function (plasma creatinine >130 μmol/l) were excluded. Microalbuminuria, measured as the urine albumin/creatinine ratio, was present in eight patients. Four patients were cigarette smokers.

Healthy control subjects. A total of 31 healthy control subjects (25 men and 6 women) were recruited after newspaper advertisements and were eligible if they received no medication, were nonsmokers, and did not take vitamin or other dietary supplements. They were lean (BMI 23.1 ± 0.3 kg/m²), had normal plasma cholesterol (4.9 ± 0.1 mmol/l), had a normal electrocardiogram, and had glucose concentrations in venous hemolysed full blood <5.6 mmol/l on two separate occasions. They were slightly younger (58 ± 2 years) than patients (63 ± 2 years). The study was approved by the local ethics committee and by the Danish Medicinal Agency. All subjects gave written informed consent.

Examination preparations. In patients, oral hypoglycemic drugs were withdrawn 2 weeks before the first examination. All medicine was interrupted for 24 h before any of the examinations, and the four patients who were smokers abstained from smoking for 8 h before each examination. Both patients and healthy control subjects arrived at the laboratory at 8:00 A.M. after 8 h of fasting, which was continued during the examination. An arterial cannula, with an external diameter of 1 mm, was inserted into the brachial artery of the nondominant arm. During the whole experiment, isotonic saline was infused through the cannula with intermittent co-infusion of solutions of vasoactive drugs, keeping the total infused volume constant at a rate of 1 ml/min. A venous cannula was placed in the medial cubital vein of both arms (retrogradely in the infused arm) for aspiration of blood samples.

Acetylcholine and sodium nitroprusside dose-response studies. The study subjects were examined in the supine position with both forearms at horizontal level with the right atrium. Forearm blood flow was measured by strain gauge venous occlusion plethysmography (D.E. Hokanson, Bellevue, WA) (16). Measurements were made simultaneously in both arms immediately before the start of any drug infusion and after completion of the infusion periods mentioned below. Each blood-flow measurement was calculated as the mean of at least three stable recordings on the plethysmograph. The vascular studies described below were performed in subgroups of patients, as illustrated in Figs. 1 and 2. Endothelium-dependent vasodilation was exam-

ined by an accumulated dose-response study with acetylcholine chloride (ACh) (Clnalfa, Läufelfingen, Switzerland) 7.5, 15, 30, and 60 μg/min for 5 min at each dose. Endothelium-independent vasodilation was examined by sodium nitroprusside (SNP) dihydrate (Roche, Basel) 1, 3, and 10 μg/min, also for 5 min at each dose. ACh is a receptor-dependent agonist of endothelial NO synthase (eNOS), whereas SNP acts by donating NO in the vascular wall.

Between dose-response studies, saline alone was infused for 20 min or until blood flow in the infused arm had returned to baseline values.

Insulin-stimulated ACh response. To examine the effect of insulin on basal blood flow, insulin (Actrapid; Novo Nordisk Scandinavia, Malmö, Sweden) 0.05 mU · kg⁻¹ · min⁻¹ was infused for 20 min. Immediately after this period, the acute effect of insulin on endothelium-dependent vasodilation was examined by maintaining the insulin infusion during a repeated ACh dose-response study. The NO-dependent proportion of the insulin-stimulated ACh response was assessed by repeating the combined insulin and ACh study during co-infusion of N^G-monomethyl-L-arginine acetate (L-NMMA) (Clnalfa), a competitive inhibitor of NO synthase. L-NMMA 1.6 and 3.3 mg/min was infused for 5 min at each dose preceding ACh infusion and maintained at 3.3 mg/min during ACh infusion.

Effect of short- and long-term insulin therapy on vasodilator responses. A flow diagram of the intervention design is shown in Fig. 2. The first 18 patients consecutively included were randomized 1:1 to a treatment group and a time control group. To obtain a more precise estimate of the changes in insulin-stimulated endothelial function, 10 additional patients were allocated to the treatment group. Clinical characteristics of the two groups are summarized in Table 1. Patients in the treatment group started insulin therapy in the evening of the day of the initial examination, taking fast-acting insulin (Actrapid Pen; Novo Nordisk) three times daily at meals and intermediate-acting insulin (Insulatard Pen; Novo Nordisk) at bedtime. The patients in the time control group continued without hypoglycemic drug therapy and without further advice regarding glycemic control while monitoring and reporting their own blood glucose as a safety measure. In the first nine consecutively included patients in the treatment group, ACh and SNP responses were re-examined after 3 days (early repeat examination). In all patients, the ACh and SNP responses were re-examined after 2 months (late repeat examination). At the early and late repeat examination, patients in the treatment group took no insulin; the last insulin dose was the intermediate-acting insulin at bedtime the night before the study. Medical therapy in addition to hypoglycemic drug therapy was unchanged during the study, except in one patient in the time control group who discontinued atorvastatin due to side effects. One patient in the time control group was removed from the study before the late repeat examination because of symptomatic hyperglycemia, but data from the initial examination were included in analyses.

Reproducibility study. An ACh reproducibility study was performed at the early repeat examination in nine patients and at the late repeat examination in four patients. The infusion series on these examination days were ACh, followed by SNP and ACh.

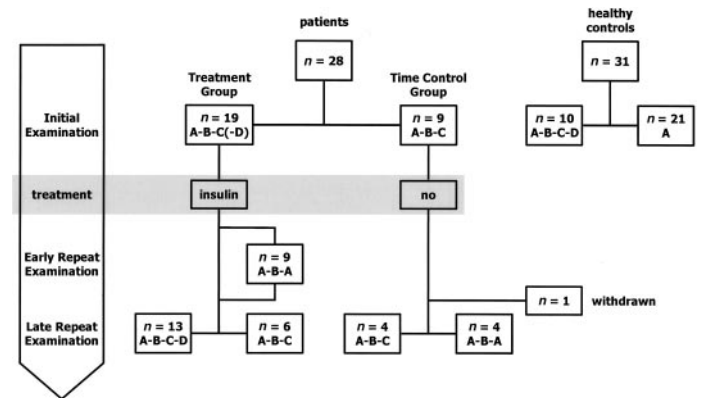


FIG. 2. Flow diagram for clinical interventions and examinations of vascular function. The sequences of infusion series, described by the letters A–D (as defined in Fig. 1), are indicated. The patients in the treatment group who received the sequence A–B–C–D at the initial examination were the same as those who received this sequence at the late repeat examination, except that series D was missing in one patient at the initial examination and in another patient at the late repeat examination for technical reasons. Thirteen patients (nine at the early repeat examination and four at the late repeat examination) received the sequence A–B–A as a reproducibility study with regard to the ACh response. One patient was withdrawn from the time control group before the late repeat examination because of symptomatic hyperglycemia.

TABLE 1
Clinical characteristics of patients in the two treatment groups

	Treatment	Time control
<i>n</i>	19	9
Age (years)	63 ± 2	63 ± 3
Sex (M/F)	17/2	9/0
BMI (kg/m ²)	31.4 ± 1.2	33.4 ± 2.2
Smokers	3 (11)	1 (11)
Microalbuminuria	4/19 (21)	4/9 (44)
Oral hypoglycemic drug therapy	16/19 (84)	5/9 (56)
Aspirin therapy	17/19 (89)	9/9 (100)
Statin therapy	15/19 (79)	9/9 (100)
ACEI therapy	4/19 (21)	1/9 (11)

Data are *n*, means ± SE or *n* (%). ACEI, angiotensin-converting enzyme inhibitor.

Biochemical analyses and systemic circulatory response. Blood samples were drawn immediately before measurements of blood flow. Blood glucose was analyzed in full blood by enzymatic colometry, and insulin was analyzed in serum by double-antibody radioimmunoassay (Insulin RIA 100; Pharmacia & Upjohn Diagnostics, Uppsala, Sweden). Blood pressure and heart rate were determined from recordings of the intra-arterial pressure and an electrocardiogram, respectively, which were obtained immediately after each measurement of blood flow.

Statistical analysis. Comparisons of two continuous variables, including basal blood flow, were performed by paired or unpaired Student's *t* test as appropriate. Blood-flow responses during dose-response studies were compared by mixed models after logarithmic transformation using the PROC MIXED procedure in the Statistical Analysis Software, version 8.0 (SAS Institute, Cary, NC). The dose-response studies in question entered the model as fixed effects, as did the interaction between dose-response study and dose of vasodilator (ACh or SNP). Study subject and the interaction between study subject and dose of vasodilator entered the model as random effects. Statistical significance was *P* < 0.05 (two-sided). Values (including geometric symbols with error bars in the figures) are means ± SE.

RESULTS

Serum insulin and blood glucose during insulin stimulation. In the total patient group at the initial examination, 20 min of insulin infusion raised serum insulin in the infused arm ("local serum insulin") from 18.4 ± 5.6 to 133 ± 14.6 mU/l. This increase is comparable with the increase of the systemic serum insulin level 2 h after a glucose load during an oral glucose tolerance test in a previously studied group of patients with type 2 diabetes and ischemic heart disease (17). Local serum insulin was not statistically different in healthy control subjects (105 ± 16 mU/l, *P* = 0.3) or during insulin stimulation with or without L-NMMA in the different groups of subjects or on different examination days in the two groups of patients (*P* > 0.2). Systemic insulin was raised by 1.3 ± 0.6 mU/l during insulin infusion in healthy control subjects and by ≤3.7 mU/l in patients in either group at any examination

day. Local and systemic blood glucose decreased by ≤0.8 mmol/l at any examination day in any group (Table 2).

Metabolic effects of insulin therapy. Plasma cholesterol was not different in the total patient group (4.6 ± 0.2 mmol/l) compared with the total group of healthy control subjects (4.9 ± 0.1 mmol/l, *P* = 0.1), but LDL cholesterol was lower (*P* = 0.002), whereas plasma triglycerides were higher (*P* < 0.0001). In patients, fasting blood glucose, HbA_{1c}, blood pressure, total plasma cholesterol, and plasma HDL cholesterol were not different in the time control group compared with the treatment group (*P* > 0.2) (Table 3).

Fasting serum insulin was more than twice as high in patients compared with healthy control subjects, reflecting insulin resistance (18) (Table 3). In the treatment group, fasting systemic serum insulin increased significantly from the initial examination to the late repeat examination (*P* = 0.01), which was probably a result of insulin administration. In the time control group, the insulin levels were unchanged (*P* = 0.9).

In the treatment group, fasting blood glucose decreased after 2 months of insulin therapy to about half the value at the initial examination (Table 3), and HbA_{1c} decreased from 10.0 ± 0.4 to 7.5 ± 0.2%. In the time control group, fasting blood glucose was unchanged from the initial to the late repeat examination (Table 3), and although HbA_{1c} deteriorated slightly, this was not statistically significant (*P* = 0.07) (Table 3). Plasma triglyceride decreased in the treatment group (*P* = 0.0004) (Table 2) but was unchanged in the time control group. Total plasma cholesterol, HDL cholesterol, and blood pressure did not change in the treatment group or the time control group (*P* > 0.5) (Table 2).

ACh and SNP responses. Basal blood flow was not different in any group of subjects at any examination. In healthy control subjects (*n* = 31), basal blood flow was 2.1 ± 0.2, and in patients in the treatment group (*n* = 19), it was 1.8 ± 0.2 and 2.1 ± 0.2 ml/(100 ml)/min at the initial and late repeat examinations, respectively (*P* > 0.3 for any comparison). The ACh response was lower in the total patient group at the initial examination compared with that of healthy control subjects (*P* = 0.03) (Fig. 3). In the treatment group, the ACh response had not changed significantly after 2 months of insulin therapy (*P* = 0.09) (Fig. 4). In the time control group, the ACh response was also unchanged [2.4 ± 0.5, 4.1 ± 1.9, 5.3 ± 2.5, and 8.9 ± 3.6 ml/(100 ml)/min at the initial examination; 4.1 ± 1.7, 5.2 ± 1.9, 7.5 ± 2.8, and 8.0 ± 2.9 ml/(100 ml)/min at the late repeat examination; *n* = 9, *P* = 0.09]. The SNP response was lower in all patients compared with all healthy control subjects (3.5 ± 0.3, 8.3 ± 0.6, and 13.4 ±

TABLE 2
Serum insulin and blood glucose during insulin stimulation in healthy control subjects and in patients at the initial examination

	Treatment group		Time control group		Healthy control subjects	
	Before	During	Before	During	Before	During
Local insulin (mU/l)	13.3 ± 1.4	121.2 ± 19.5	13.9 ± 2.3	154.2 ± 20.4	5.4 ± 0.5	105 ± 16
Systemic insulin (mU/l)	13.2 ± 1.44	16.2 ± 1.4	14.0 ± 2.4	17.7 ± 2.3	5.5 ± 0.4	6.8 ± 0.5
Local glucose (mmol/l)	13.2 ± 0.9	12.7 ± 0.8	11.5 ± 0.8	10.7 ± 0.8	4.9 ± 0.1	4.4 ± 0.1
Systemic glucose (mmol/l)	12.8 ± 0.8	12.5 ± 0.8	11.4 ± 0.8	10.9 ± 0.8	5.1 ± 0.2	4.6 ± 0.2

Data are means ± SE. "Local" denotes the arm where insulin is infused. "Before" is concentrations just before insulin infusion. "During" is concentrations 20 min after the start of insulin infusion.

TABLE 3
Clinical parameters for patients according to treatment group and for healthy control subjects

	Treatment group			Time control group		Healthy control subjects
	Primary	Early repeat	Late repeat	Primary	Late repeat	
<i>n</i>	19	9	19	9	8	31
Body mass (kg)	94 ± 4	—	98 ± 5	104 ± 8	104 ± 8	74 ± 2
Fasting blood glucose (mmol/l)	14.7 ± 0.9	8.3 ± 0.6	7.5 ± 0.2	13.1 ± 0.9	13.0 ± 1.0	5.1 ± 0.1
HbA _{1c} (%)	10.0 ± 0.4	—	7.5 ± 0.2	9.5 ± 0.5	10.0 ± 0.6	—
Fasting serum insulin (mU/l)	15.4 ± 1.4	17.1 ± 3.1	19.4 ± 2.4	16.2 ± 2.9	14.7 ± 3.3	5.5 ± 0.3
Systolic blood pressure (mmHg)	142 ± 5	138 ± 6	148 ± 6	143 ± 11	145 ± 10	117 ± 5
Diastolic blood pressure (mmHg)	71 ± 2	64 ± 3	76 ± 3	71 ± 3	75 ± 3	63 ± 1
Plasma cholesterol (mmol/l)	4.6 ± 0.2	—	4.5 ± 0.2	4.6 ± 0.3	4.8 ± 0.4	4.9 ± 0.1
Plasma LDL cholesterol (mmol/l)	2.6 ± 0.2	—	2.6 ± 0.2	2.5 ± 0.2	2.4 ± 0.3	3.1 ± 0.1
Plasma HDL cholesterol (mmol/l)	1.1 ± 0.1	—	1.3 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.3 ± 0.1
Plasma triglyceride (mmol/l)	2.0 ± 0.3	—	1.4 ± 0.2	2.4 ± 0.5	1.6 ± 0.5	0.8 ± 0.1

Data are means ± SE.

0.8 ml/(100 ml)/min and 5.9 ± 0.9, 9.8 ± 1.4, and 15.6 ± 2.1 ml/(100 ml)/min, respectively; *P* = 0.0008). At the late repeat examination, the SNP response had changed in neither the treatment group (*P* = 0.4) (Fig. 5) nor the time control group [3.0 ± 0.4, 8.0 ± 1.0, and 13.7 ± 1.2 ml/(100 ml)/min at the initial examination; 4.0 ± 0.7, 6.8 ± 1.2, and 11.6 ± 2.0 ml/(100 ml)/min at the late repeat examination; *n* = 9, *P* = 0.6].

Surprisingly, at the early repeat examination in the patients in the treatment group, both the ACh and SNP response had decreased [ACh response: 4.2 ± 1.3, 5.8 ± 1.9, 8.7 ± 2.2, and 10.6 ± 2.5 ml/(100 ml)/min at the initial examination; 2.2 ± 0.3, 3.4 ± 0.5, 6.9 ± 1.4, and 9.7 ± 2.2 ml/(100 ml)/min at the early repeat examination; *P* = 0.007. SNP response: 3.8 ± 0.7, 9.1 ± 1.2, and 13.3 ± 1.7 ml/(100 ml)/min at the initial examination; 2.9 ± 0.3, 6.6 ± 0.6, and 11.3 ± 1.2 ml/(100 ml)/min at the early repeat examination; *n* = 9, *P* = 0.009]. At this time, after 3 days of insulin therapy, a considerable decrease of fasting blood glucose was already achieved (Table 2), with a daily insulin dose of 51 ± 12 units (compared with 71 ± 9 units at the late repeat examination).

In nine patients at the early repeat examination and in four patients at the late repeat examination, the ACh response was reproducible when repeated after the SNP infusion, as the responses during the two ACh infusions were not different [2.3 ± 0.2, 3.6 ± 0.5, 6.2 ± 1.1, and 9.3 ± 1.7 ml/(100 ml)/min during the first dose-response study; 2.8 ± 0.4, 4.9 ± 0.8, 7.6 ± 1.4, and 10.6 ± 1.8 ml/(100 ml)/min during the repeated study; *n* = 13, *P* = 0.2].

Insulin-stimulated ACh response. At the initial examination, insulin had no effect on basal forearm blood flow in the total patient group [2.1 ± 0.2 ml/(100 ml)/min without change; *n* = 28], in patients in the treatment group (before insulin stimulation: 2.5 ± 0.5 ml/(100 ml)/min; during insulin stimulation: 2.6 ± 0.6 ml/(100 ml)/min; *n* = 19, *P* = 0.4), or in healthy control subjects [before insulin stimulation: 2.5 ± 0.5 ml/(100 ml)/min; during insulin stimulation: 2.9 ± 0.6 ml/(100 ml)/min; *n* = 10, *P* = 0.2]. However, insulin had a large stimulatory effect on the ACh response in healthy control subjects (149 ± 47, 110 ± 33, 100 ± 45, and 106 ± 44% increase of blood flow during the four doses of ACh; *n* = 10, *P* < 0.0001) (Fig. 6). In contrast, this effect was absent in the total patient group at the initial

examination (*P* = 0.3) (Fig. 3). After 2 months of insulin therapy, insulin stimulation had a significant effect in patients in the treatment group (58 ± 25, 84 ± 66, 120 ± 93, and 69 ± 36% increase of blood flow during the four doses of ACh; *P* = 0.0002) (Fig. 5). In the time control group, insulin stimulation had no effect on the ACh response at the initial or late repeat examination [blood flow increments during insulin infusion of 1.8 ± 1.0, 0.2 ± 1.3, 3.7 ± 2.1, and 0.8 ± 2.9 ml/(100 ml)/min at the early repeat examination and -0.4 ± 0.8, 0.0 ± 0.9, 1.3 ± 1.6, and 2.4 ± 2.4 ml/(100 ml)/min at the late repeat examination; *n* = 9 and *n* = 4, respectively, *P* = 0.7].

In healthy control subjects, L-NMMA decreased blood flow in response to co-infusion of insulin and ACh by 46 ± 9, 43 ± 9, 30 ± 15, and 21 ± 14% with administration of the four doses of ACh [blood flow decrement of 2.7 ± 0.8, 2.6 ± 0.6, 3.7 ± 1.7, and 3.1 ± 2.3 ml/(100 ml)/min; *n* = 10, *P* < 0.0001] (Fig. 6). In patients in the treatment group, the L-NMMA inhibition of the insulin-stimulated ACh response did not change significantly after 2 months [blood flow

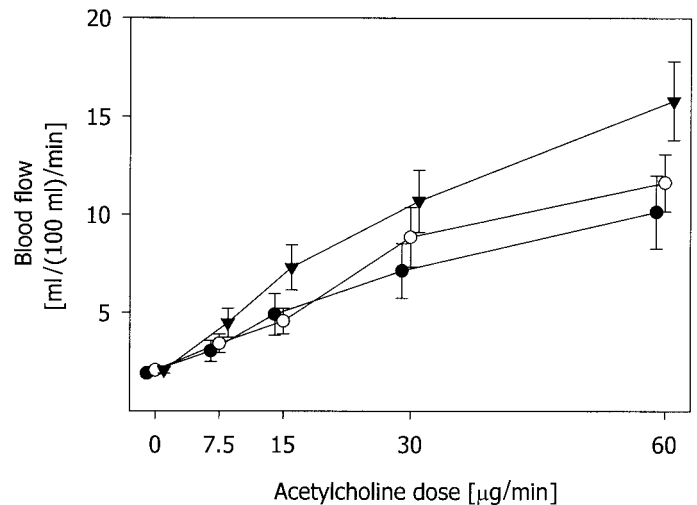


FIG. 3. Forearm blood-flow response to ACh in 31 lean, healthy control subjects (▼) and ACh response (●) and insulin-stimulated ACh response (○) in 28 patients with type 2 diabetes and ischemic heart disease. The ACh response was lower in patients than in healthy control subjects (*P* = 0.03), and insulin stimulation had no effect in patients (*P* = 0.3).

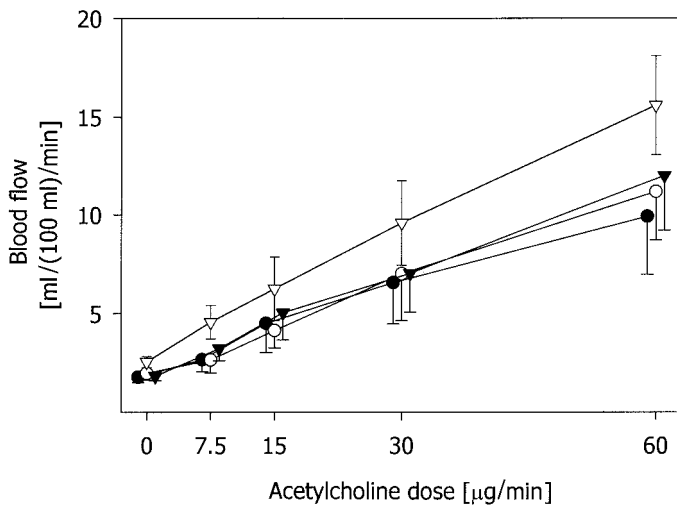


FIG. 4. Forearm blood-flow response to ACh (●) and insulin-stimulated ACh response (○) in 13 patients with type 2 diabetes and ischemic heart disease before insulin therapy as well as ACh response (▼) and insulin-stimulated ACh response (▽) in the same patients after 2 months of insulin therapy. Insulin had no stimulating effect on the ACh response before treatment ($P = 0.3$). After 2 months the ACh response was unchanged ($P = 0.09$) but insulin now had a stimulating effect ($P = 0.0002$).

decrement of 1.5 ± 0.6 , 2.4 ± 1.0 , 4.6 ± 2.7 , and 5.7 ± 2.2 ml/(100 ml)/min at the initial examination; 1.7 ± 0.8 , 2.2 ± 1.5 , 1.6 ± 1.2 , and 5.3 ± 2.1 ml/(100 ml)/min at the late repeat examination; $n = 13$, $P = 0.9$].

Heart rate, blood pressure, and blood flow in the arm contralateral to the infused arm were unchanged throughout the studies (results not shown).

DISCUSSION

This study shows that in patients with type 2 diabetes and ischemic heart disease, long-term insulin therapy improves insulin-stimulated endothelium-dependent vasodilation. To our knowledge, our results are the first to demonstrate that abnormal insulin-stimulated endothelial function improves from hypoglycemic drug therapy. This

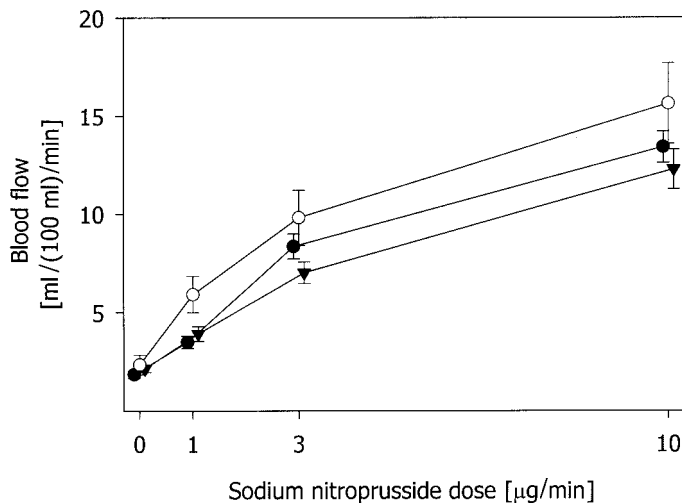


FIG. 5. Forearm blood-flow response to SNP in 10 lean, healthy control subjects (○) and in 19 patients with type 2 diabetes and ischemic heart disease before (●) and after (▼) 2 months of insulin therapy. The SNP response was lower in patients than in healthy control subjects ($P = 0.0008$), and it was not changed by insulin therapy in patients ($P = 0.4$).

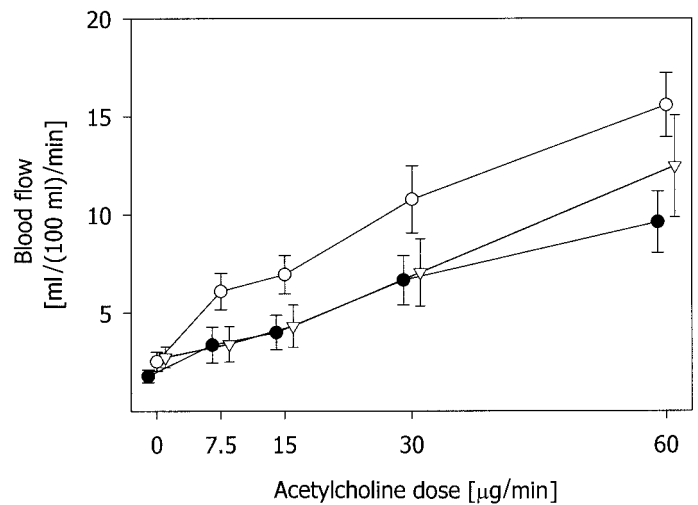


FIG. 6. Forearm blood-flow response to ACh (●), insulin-stimulated ACh response (○), and L-NMMA-inhibited insulin-stimulated ACh response (▼) in 10 lean, healthy control subjects. Insulin had a stimulating effect on the ACh response ($P < 0.0001$), which was inhibited by L-NMMA ($P < 0.0001$).

represents a physiological parallel to the well-established knowledge that insulin treatment and other hypoglycemic drug therapies improve insulin-stimulated glucose uptake and other metabolic functions (15). The results constitute an argument for the concept that endothelial insulin resistance is an aspect of insulin resistance (10), which has traditionally been thought to be primarily confined to skeletal muscle, liver, and fat. Because of the central role of metabolic insulin resistance in the pathogenesis of type 2 diabetes, endothelial insulin resistance may be a mechanism that explains the particular risk for vascular disease in type 2 diabetes. It is also a possible physiological explanation for the fact that insulin resistance is an independent risk factor for atherosclerotic disease and its complications.

The ACh response was lower in patients than in healthy control subjects, an almost uniform finding in studies of forearm ACh responses and other measures of endothelium-dependent vasodilation in patients with type 2 diabetes. The SNP response was also lower in patients than in healthy control subjects, suggesting decreased vascular smooth muscle sensitivity to the vasodilating effects of NO. Previous studies have found decreased vasodilator responses to infusion of nitroglycerin or SNP, both of which donate NO in the vascular wall, in patients with type 2 diabetes compared with healthy individuals (19–21). This may represent decreased NO signaling distal to NO production, e.g., through NO degradation by reactive oxygen species (see below). Thus, verapamil-stimulated vasodilation, which is independent of NO signaling, was found to be unchanged (20). However, many studies have found normal endothelium-independent vasodilation in such patients, as previously reviewed (22), perhaps because they did not have the statistical power to detect differences more subtle than those in endothelium-dependent vasodilation.

High glucose concentrations may directly affect endothelial function, as shown in vitro (23) and in human studies (24). This may be caused by an increased production of endothelium-derived vasoconstricting prosta-

glandins (25). Alternatively, glucose-induced endothelial dysfunction may be mediated by increased production of reactive oxygen species because it is prevented by antioxidants (23,26). Sources of reactive oxygen species may be superoxide production by a vascular NAD(P)H oxidase (27) or by eNOS itself (28). Superoxide production (27,28) and impaired NO-mediated vasodilation (28,29) may be mediated by activation of protein kinase C (PKC).

Our study confirms the previous findings that insulin, given as an intrabrachial infusion for 20 min, is a relatively potent stimulus for ACh-stimulated vasodilation even though it does not have any effect when infused alone at the chosen dose and duration (9). It is also in agreement with the results that metacholine-stimulated leg blood flow is potentiated by an euglycemic-hyperinsulinemic clamp in healthy control subjects but not in people with obesity-associated insulin resistance or type 2 diabetes (10). It is important to note that local insulin stimulation, as in our study, may have advantages compared with systemic hyperinsulinemia, as used in many previous studies (7,8,10–12,30), when the aim is to study the direct effect of insulin on vascular function. Thus, in subjects with a history of regional sympathectomy, vasodilation to systemic insulin infusion is delayed in the innervated calf compared with the denervated forearm (31). This may be explained by the ability of systemic hyperinsulinemia to elicit central activation of the sympathetic nervous system (31). However, vasoconstrictor effects of insulin-stimulated sympathetic activation may not be comparable in insulin-sensitive and insulin-resistant individuals, as sympathetic responses to insulin are altered in obese subjects (32).

The response to ACh alone is only partly mediated by NO, and one study (33), representative of several others, has shown that L-NMMA inhibits 39% of vasodilation to ACh (at an ACh dose of 30 $\mu\text{g}/\text{min}$). In healthy control subjects, we observed a similar relative L-NMMA inhibition of the far greater combined insulin and ACh response. Therefore, the insulin potentiation of the ACh response is most likely mediated by NO to a substantial extent.

Insulin stimulation of glucose uptake and other metabolic actions of insulin are mediated by insulin receptor kinase (IRK), insulin receptor substrate (IRS)-1 and -2, phosphatidylinositol-3 kinase (PI3K), and Akt (protein kinase B) (34). Impairment of the activity of the mentioned protein kinases may constitute a molecular basis of insulin resistance (34). It appears that insulin stimulation of endothelial NO production is mediated by the same protein kinases, i.e., IRK (35–37), IRS-2 (36), PI3K (35–37), and Akt (36), resulting in phosphorylation and activation of eNOS (37). Thus, insulin stimulates endothelial NO production by a mechanism similar to the response to fluid shear stress—considered the most important physiological stimulus for NO production—because shear stress-induced eNOS activation (38) and NO-mediated vasodilation (39) are also dependent on Akt. During the experimental conditions of our study, insulin may have augmented the ACh vasodilator response because Ca^{2+} -dependent activation of eNOS by ACh may be potentiated by Ca^{2+} -independent eNOS phosphorylation, through increased electron flux through the reductase domain of the enzyme at any concentration of intracellular Ca^{2+} (40).

In animal models of obesity and insulin resistance,

insulin-stimulation in vitro or in vivo show decreased protein expression of IRS and impaired activity of IRK, IRS-2, and PI3K in vascular tissue (36). The mechanisms leading to such changes are not yet described, but in metabolic insulin resistance, insulin signal transduction may be inhibited by increased expression of the pro-inflammatory cytokine tumor necrosis factor- α (TNF- α) (34) and by elevated glucose concentrations acting through PKC activation (34). In endothelial cells, TNF- α inhibits NO production through inhibition of insulin-stimulated activation of IRS-1, PI3K, Akt, and eNOS (41), and TNF- α inhibits insulin-stimulated vasodilation (42). Furthermore, PKC activation may inhibit endothelial insulin-stimulated PI3K activity (43).

In our study, the improvement in glycemic control after 2 months in the treatment group was considerable, as shown by the reduction of fasting blood glucose to about half the initial level and by a reduction of HbA_{1c} of 2.5%. Insulin therapy did not improve plasma cholesterol or blood pressure (Table 2). As the pharmacological therapy of the patients was also unchanged, changes in vascular function were likely directly or indirectly mediated by exogenous insulin administration, the decrease of blood glucose, or both. Insulin therapy may directly improve endothelial function because insulin induces eNOS (43) as well as an essential cofactor for eNOS, tetrahydrobiopterin (44). Improved glycemic control may increase the activity of the PI3K pathway of insulin-stimulated NO production in endothelial cells, as it improves a similar pathway leading to glucose uptake in skeletal muscle (45). Differential improvement of the PI3K pathway is a potential explanation for our finding that the insulin-stimulated ACh response was improved in patients even though the ACh response remained unchanged. Speculatively, the mechanism responsible for such improvement could involve a reversal of the hyperglycemia-induced activation of PKC. Such a mechanism would be expected to operate during any approach to improve glycemic control, rather than being a result of insulin therapy per se.

Two months of insulin therapy did not significantly change the ACh or SNP response. In previous studies, long-term treatment with the insulin-sensitizer troglitazone improved brachial artery flow-mediated vasodilation in patients with impaired glucose tolerance (46) but did not change forearm ACh responses or vasodilation to systemic hyperinsulinemia in patients with obesity-associated insulin resistance (30). In these studies, glycemic control was unchanged by troglitazone therapy. The only previous human study of the relation between glycemic control and endothelium-dependent vasodilation showed that the forearm ACh response increased after bedtime long-acting insulin was added to established therapy with metformin in patients with type 2 diabetes without manifest cardiovascular disease (47).

In the present study, after just 3 days of insulin therapy, much of the final insulin dose was attained and the majority of the ensuing long-term reduction of blood glucose was already manifest. At this time, both the ACh and SNP responses had significantly decreased in a subgroup of patients. The reason for this decrement in vasodilator function is not clear. Glucose uptake may be linked to insulin-stimulated vasodilation (48,49). In general, if NO-

mediated vasodilation is dependent on glucose uptake, it is possible that subacute lowering of long-standing hyperglycemia may decrease basal glucose uptake and impair endothelium-dependent and -independent vasodilation in the short term.

After 2 months of insulin therapy in our patients, insulin-stimulation had an effect on the ACh response that was previously absent. If the increase of the combined response to insulin and ACh was mediated by NO, the inhibitory effect of L-NMMA would be expected to increase. However, the vasoconstriction to L-NMMA during co-infusion of insulin and ACh was not different before and after insulin therapy. The most straightforward interpretation of this finding is that insulin therapy resulted in an increased production of insulin-stimulated endothelium-derived vasodilators other than NO or in a decreased production of endothelium-derived vasoconstrictors. It is unknown whether insulin stimulates the production of other vasodilators, e.g., prostacyclin or endothelium-derived hyperpolarizing factor, but there is evidence that release of the endothelium-derived vasoconstrictor endothelin-1 is stimulated by insulin (50). An alternative interpretation is that our study may not have had the statistical power to detect an increase in vasoconstriction to L-NMMA, which inherently contains the variability of both the insulin and ACh response and of the combined L-NMMA, insulin, and ACh response. Furthermore, the second insulin stimulation may have been more potent than the first because insulin induces eNOS. Thus, protein expression and activity of eNOS is already apparent after 1 h in cell culture experiments (43). Accordingly, the serial dose-response studies with insulin may have underestimated the L-NMMA response on each day and thus confounded comparisons of the proportion of insulin stimulation mediated by NO.

We do not have data to exclude that the increase in the combined insulin and ACh response was endothelium-independent. However, a general increase in vascular smooth muscle sensitivity to NO after insulin therapy is unlikely because the SNP response was unchanged by insulin therapy. Unfortunately, with a design of already rather intensive medical intervention and monitoring of vascular function, we did not find it feasible to plan additional examinations of these patients. Nonetheless, it would have been interesting to examine the effect of insulin therapy on the insulin-stimulated SNP response or vasodilation during pharmacological inhibition of prostaglandin synthesis or endothelin-1 action.

In conclusion, this study showed that patients who are individually prone to the vascular complications of type 2 diabetes, revealed by manifest ischemic heart disease, had impaired insulin-stimulated endothelium-dependent vasodilation in addition to impaired endothelium-dependent and -independent vasodilation. Insulin treatment for 2 months did not improve endothelium-dependent vasodilation itself, but partly restored insulin-stimulated endothelium-dependent vasodilation. These results support recent evidence that insulin-stimulated glucose uptake and insulin-stimulated endothelial function are regulated by similar signaling pathways. Furthermore, restoration of insulin-stimulated endothelial function may be a mechanism for the improved survival observed with insulin therapy of patients with diabetes and acute myocardial infarction.

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