Normal Effect of Insulin to Stimulate Leg Blood Flow in NIDDM

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In patients with non-insulin-dependent diabetes mellitus (NIDDM), a decreased effect of insulin in stimulating leg blood flow (LBF) has been reported. We reinvestigated the effect of insulin on LBF and validated our data by use of other measures. Eight healthy men (control group) and seven men with NIDDM were studied (age 59 ± 1 and 58 ± 3 years, weight 83 ± 3 and 86 ± 6 kg, fat-free mass 66 ± 1 and 64 ± 3 kg, respectively [mean ± SE, all $P > 0.05$]; body mass index 26 ± 1 and 29 ± 1 kg/m^2, fasting plasma insulin 72 ± 7 and 187 ± 22 pmol/l, fasting plasma glucose 5.8 ± 0.2 and 10.2 ± 1.7 mmol/l [all $P < 0.05$]). A three-step hyperinsulinemic glucose clamp (ambient glucose level) was performed, combined with catheterization of an artery and both femoral veins. Expiratory air was collected, LBF was measured by thermodilution, and blood was sampled and analyzed for oxygen content. Insulin concentration was increased to 416 ± 22 and 509 ± 43 (step I), 1,170 ± 79 and 1,299 ± 122 (step II), and 15,936 ± 1,126 and 16,524 ± 1,916 (step III) pmol/l in control and NIDDM subjects, respectively ($P > 0.05$). LBF increased similarly ($P > 0.05$) in the two groups (from 287 ± 23 and 302 ± 12 [basal] to 308 ± 31 and 362 ± 9 [I], 371 ± 29 and 409 ± 17 [II], and 434 ± 32 and 472 ± 29 [III] $ml \cdot min^{-1} \cdot leg^{-1}$ in control and NIDDM subjects, respectively). Leg oxygen uptake always increased in the face of constant venous Po_2 ($P > 0.05$; 4.3 ± 0.2 and 4.5 ± 0.2 [basal], 4.3 ± 0.2 and 4.6 ± 0.2 [I], 4.8 ± 0.2 and 4.6 ± 0.2 [II], and 4.7 ± 0.1 and 4.4 ± 0.2 [III] kPa in control and NIDDM subjects, respectively). Both leg and whole body O_2 uptake increased similarly in the two groups. In conclusion, at ambient glucose levels, the effect of insulin in stimulating LBF is normal in NIDDM. Moreover, insulin-mediated vasodilation is closely linked to muscle metabolic rate. *Diabetes* 44:221–226, 1995

I n healthy subjects, extremity blood flow has been reported to increase in response to increasing plasma insulin concentrations (1–5). The mechanism by which insulin acts as a vasodilator is not fully understood, and both systemic and local mechanisms have been proposed (6). In patients with non-insulin-dependent diabetes mellitus (NIDDM), Laakso et al. (7) have found an impaired

insulin-mediated increase in leg blood flow (LBF). Based on the defective ability of insulin to increase LBF in patients with NIDDM, the authors have noted that insulin-stimulated muscle glucose uptake rates in NIDDM could be improved by up to 40% if the blood flow response to insulin is normalized (7). This issue is therefore important for our understanding of the pathophysiology of the decreased insulin sensitivity in NIDDM, particularly in the skeletal muscle.

In contrast to the findings of Laakso et al. (7), we have now found that insulin-stimulated blood flow in the legs does not differ between healthy subjects and patients with NIDDM. Our blood flow measurements are supported by measurements with other techniques, which, in addition, elucidate the mechanism behind the vasodilator action of insulin.

RESEARCH DESIGN AND METHODS

Eight healthy men (control group; age 59 ± 1 years [mean ± SE]) and seven men with NIDDM (age 58 ± 3 years) gave their informed consent to participate in the study, which was approved by the Ethical Committee of Copenhagen. All subjects in the control group had normal glucose tolerance (assessed by a 75-g oral glucose tolerance test), and none were taking any medication. The duration of diabetes in the NIDDM group was on average 5 years 1 month (range 1.5–12.0 years). Three subjects were treated by diet alone, and four subjects were treated with both diet and oral antidiabetic drugs (subject 1: gliklazide, 3 × 80 mg; subject 3: metformin 3 × 1 g + chlorpropamide 1 × 250 mg; subject 5: glipizide 2 × 3.5 mg + metformin 2 × 500 mg; subject 7: tolbutamide 2 × 500 mg). In none of the groups did the subjects have clinical or laboratory evidence of any other endocrine diseases. In the NIDDM group, one subject (subject 5) was treated for hypertension (diltiazem 2 × 120 mg) as well. Clinical and laboratory characteristics of both groups are shown in Table 1.

On the 3 days before the experimental day, the subjects were fed a diet containing a least 250 g carbohydrate per day. The subjects were studied postabsorptive (10 h) at 8 A.M. and had abstained from unusual physical activity and intake of alcohol the day before. On the day of experiments, no medication was taken. After arrival in the laboratory, the subjects were weighed, their height was measured, and they went to bed. Electrocardiogram and heart rate were monitored by precordial electrodes. A cannula was inserted into a brachial or radial artery and used for blood sampling and measurement of arterial blood pressure. Another cannula was inserted in the medial cubital vein for later infusion of insulin and glucose. After application of local anesthesia, Teflon catheters were inserted in both femoral veins (Seldinger technique) 5–7 cm below the inguinal ligament and advanced so that the tip of the catheter was located ~2 cm distal to the inguinal ligament. The catheters were conical, with the hole at the tip being just wide enough for a thermistor to pass through. Four small (0.3 mm in diameter) side holes were drilled 1.5 cm from the tip, allowing blood drawing and injection of cold saline (see below). A thermistor (Edslab probe 94–030–2.5F, Baxter) was inserted into the catheter and advanced 6–8 cm beyond the catheter tip. All cannulas were kept patent with a slow drip of saline (artery and cubital vein) or Na/KCl (femoral veins). After a 45-min rest, expiratory air was collected in Douglas bags through a respiratory valve. Subjects were accustomed to the respiratory valve for at least 4 min before collection of air (~10 min). Basal blood samples were then drawn simultaneously from the arterial and venous catheters. This was done twice, with an interval of 10 min. Immediately after blood

sampling, blood flow was measured (see below). One minute before and during every blood sampling and blood flow measurement, pneumatic cuffs placed around the ankles were inflated to systolic pressure + 50 mmHg. This was done in order to minimize contributions from the feet, which have relatively little muscle tissue and many arteriovenous shunts.

A three-step (designated I, II, and III) sequential hyperinsulinemic glucose clamp was then carried out. For each subject, a 50-ml insulin infusate had been prepared for each clamp step from insulin (Actrapid, Novo-Nordisk; 100 IU/ml), saline, and 2.5 ml of 20% human serum albumin. At the start of each clamp step, insulin was given as a 2-ml bolus followed by a constant infusion for 120 min, using a Braun precision pump at a rate of 258 μl/min. Insulin infusion rates were 28, 88, and 480 mU · min^{-1} · m^{-2}. Arterial plasma glucose concentration was measured at least every 5 min. Based on the glucose concentration and a modification (8) of the algorithm of DeFronzo et al. (9), a computer-adjusted glucose infusion (20%) was given to maintain plasma glucose concentration at fasting level throughout the clamp. For each subject, the glucose concentration was clamped at the concentration measured at basal. At $t = 75$ min in each clamp step, expiratory air was collected in Douglas bags. At $t = 85$, 100, and 115 min, blood samples and flow measurements were obtained.

Analyses, analytical procedures, and calculations. Blood sampled for analysis of glucose was collected in heparinized tubes and immediately high-speed centrifuged, whereupon plasma glucose concentration was measured by an automated glucose analyzer (YSI 23AM, Yellow Springs Instruments). Blood for determination of catecholamines was collected in iced tubes, stabilized with 5 μmol ethylene glycol-bis-(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) and 4 μmol reduced glutathione in 20 μl 0.6 N sodium hydroxide/ml blood, and stored at −80°C until analysis with a single-isotope radioenzymatic assay (10). Blood for determination of O_2 and CO_2 were drawn anaerobically in heparinized syringes, placed on ice, and within 1 h analyzed for content of O_2 using an automatic blood gas analyzer (ABL4, Radiometer). Concentrations of CO_2 in whole blood were calculated from hematocrit, pH, O_2 saturation in blood, and CO_2 tension in plasma, assuming normal concentrations of 2,3-diphosphoglycerate in erythrocytes (11). Hematocrit was measured by the microhematocrit method. Expiratory air fractions of O_2 and CO_2 were analyzed by paramagnetic (Servomex OA 189) and infrared (Capnograph Godard 146) electronic gas analyzers, respectively. The volume of air expired in Douglas bags was measured in a giant spirometer. Leg volume was estimated by water displacement and calculated as total leg volume minus volume of the foot. Leg weight was calculated assuming a specific gravity of 1. Body fat was estimated by measurement of seven skin-folds (12). Waist and hip circumferences were measured in the standing position at the level of the umbilicus and the level of the anterior superior iliac spine, respectively.

Blood flow was measured by use of the thermodilution technique. The thermistor probe was connected to an amplifier and a recorder and, through an analog-to-digital converter, also to a personal computer. A bolus (5 ml) of cold saline (2.3°C) was injected rapidly in the femoral catheter. Blood temperature was continuously recorded and sampled, and blood flow was calculated according to the formula

$$F = \frac{V_i \cdot (T_b - T_i) \cdot \dfrac{\rho_i \cdot c_i}{\rho_b \cdot c_b} \cdot k_T}{\displaystyle\int_0^\infty \Delta T\, dt}$$

where F = blood flow, V_i = bolus volume, T_b = basal blood temperature, T_i = temperature in bolus, ρ_b = density of blood, ρ_i = density of injected saline, c_b = specific heat of blood, c_i = specific heat of injected saline, and k_T = temperature correction factor.

Blood flow and oxygen extraction did not differ between the two legs, and pooled data are presented accordingly. Furthermore, these data are presented as the means of the triplicate blood flow and blood gas measurements at each clamp step.

Statistical analysis. Results are presented as means ± SE. To detect differences between the groups in responses to increasing insulin concentrations, a two-way analysis of variance (ANOVA) for repeated measures was performed. If ANOVA indicated significant differences, these were located by a pairwise multiple comparison procedure (Student-Newman-Keuls test). To detect differences between the groups in parameters represented by single measurements, nonparametric tests for unpaired data (Mann-Whitney) were used. $P < 0.05$ was considered

TABLE 1
Clinical and laboratory characteristics of the two groups

	Control group	NIDDM group
n	8	7
Body weight (kg)	83.3 ± 2.6	85.9 ± 3.5
Height (cm)	180 ± 2	172 ± 3*
Body mass index (kg/m^2)	25.7 ± 0.8	29.1 ± 0.3*
Body fat (%)	20.8 ± 2.2	25.5 ± 1.3*
Fat-free mass (kg)	65.6 ± 1.1	64.0 ± 2.9
Waist circumference (cm)	99.7 ± 2.2	104.4 ± 1.7
Waist-to-hip ratio	1.00 ± 0.01	1.03 ± 0.02
Fasting plasma glucose (mmol/l)	5.8 ± 0.2	10.2 ± 1.7*
HbA$_{1c}$ (%) ($n = 5$)	ND	7.6 ± 0.8

Data are means ± SE. Waist and hip circumference were measured at the level of the umbilicus and at the level of the anterior superior iliac spine, respectively. Normal range for HbA$_{1c}$ is 4.2–6.3%. *$P < 0.05$ different from control group; ND, not determined.

significant in two-tailed testing. The sensitivity (power) of the test applied on blood flow measurements was ~85% for detection of a difference at a single time point between NIDDM and control subjects of 20%.

RESULTS

Plasma glucose, insulin, and catecholamine concentrations. Fasting plasma glucose and insulin concentrations were higher ($P < 0.05$) in the NIDDM than in the control group (Tables 1 and 2). During insulin infusion, plasma glucose concentrations were clamped with a coefficient of variation of <5.0%, and insulin concentrations were similar in the two groups (Table 2). Norepinephrine concentrations were always higher ($P < 0.05$) in the control group than in the NIDDM group and increased in both groups in response to insulin infusion (Table 2). Epinephrine concentrations were always higher in control subjects compared with NIDDM subjects ($P < 0.05$) and remained unchanged throughout the clamp (Table 2).

Hemodynamic data. Blood flow did not differ between the two groups at any insulin concentration (Fig. 1). In both groups, blood flow increased with increasing insulin concentrations (Fig. 1). At the first clamp step, the increase was significant only in NIDDM subjects (Fig. 1).

At basal, mean arterial blood pressure was higher in NIDDM compared with control subjects, and it increased gradually in response to insulin infusion, the increase being similar in the two groups ($P > 0.05$) (Table 2).

Leg vascular resistance at basal was higher in the NIDDM group than in the control group ($P < 0.05$) and decreased ($P < 0.05$) in both groups with increasing insulin concentration (Table 2). Heart rate did not differ between NIDDM and control subjects and increased in response to insulin infusion ($P < 0.05$) (Table 2).

Oxygen uptake and carbon dioxide release. The venous CO_2 pressures (Pv_{CO_2}) were always similar in NIDDM and control subjects (Fig. 2). A small but significant increase in Pv_{CO_2} was seen with increasing insulin concentrations (Fig. 2). Oxygen partial pressures in the venous blood (Fig. 2) as well as arterio-venous oxygen differences (Fig. 3) were similar in the two groups and did not change with increasing insulin concentrations. Oxygen uptakes in the legs were similar at basal ($P > 0.05$) and increased ($P < 0.05$) in both groups with increasing insulin concentrations (Fig. 4B). Whole-body oxygen uptake also increased similarly in both

TABLE 2
Arterial plasma hormone concentrations and hemodynamic data for control and NIDDM groups

	Clamp step			
	Basal	I	II	III
Insulin (pmol/l)				
Control	72 ± 7	416 ± 22†	1,170 ± 79†	15,936 ± 1,126†
NIDDM	187 ± 22*	509 ± 43†	1,299 ± 122†	16,524 ± 1,916†
Norepinephrine (nmol/l)				
Control	1.47 ± 0.22	1.61 ± 0.24	1.76 ± 0.25†	1.92 ± 0.30†
NIDDM	0.72 ± 0.13*	0.84 ± 0.14*†	1.03 ± 0.15*†	1.11 ± 0.15*†
Epinephrine (nmol/l)				
Control	0.18 ± 0.04	0.19 ± 0.05	0.21 ± 0.04	0.28 ± 0.08
NIDDM	0.09 ± 0.03*	0.09 ± 0.02*	0.12 ± 0.02*	0.12 ± 0.03*
Mean blood pressure (mmHg)				
Control	95 ± 6	102 ± 10	105 ± 9	108 ± 8
NIDDM	114 ± 11*	110 ± 8	117 ± 11	129 ± 13‡*
Leg vascular resistance ($100 \times (mmHg \cdot ml^{-1} \cdot min^{-1})$)				
Control	35 ± 4	35 ± 4	29 ± 2†	26 ± 3‡
NIDDM	38 ± 4*	30 ± 2‡	29 ± 3‡	28 ± 3*‡
Heart rate (beats/min)				
Control	63 ± 2	65 ± 2	68 ± 3	73 ± 3†‡
NIDDM	69 ± 3	73 ± 2	72 ± 3	77 ± 4†‡

Data are means ± SE. Blood pressure was measured intra-arterially. Leg vascular resistance was calculated by dividing mean arterial blood pressure by LBF. * $P < 0.05$ different from control group. † $P < 0.05$ different from preceding clamp step. ‡ $P < 0.05$ different from basal.

groups with increasing insulin concentrations ($P < 0.05$) (Fig. 4A).

DISCUSSION

A major finding of the present study is that LBF in patients with NIDDM increases with increasing insulin concentrations and that the response is not different from insulin-stimulated blood flow in age-, weight-, and lean body mass–matched healthy control subjects (Fig. 1). As mean blood pressure increased, the increase in flow was due to a decrease in vascular resistance, reflecting insulin-mediated vasodilation (Table 2).

During insulin infusion, oxygen tension in the venous blood and arterio-venous oxygen extraction remained unchanged (Figs. 2 and 3), and, accordingly, leg oxygen uptake increased (Fig. 4B). That the increase in leg oxygen uptake did in fact exist is supported by the findings of a simultaneous increase in whole-body oxygen uptake with increasing insulin concentrations (Fig. 4A). Furthermore, assuming that muscle mass of the whole body and of the legs are 40 and 64% (13), respectively, the increase in oxygen uptake in leg muscle from basal to clamp step III can be calculated and extrapolated to whole-body muscle mass. For the NIDDM group, the increase in oxygen uptake in the legs was 1.23 ± 0.23 ml · min^{-1} · kg leg muscle^{-1}, and whole-body muscle mass was 34 ± 1 kg. This extrapolates to an oxygen uptake in the total muscle mass of 43 ± 9 ml/min, which corre-
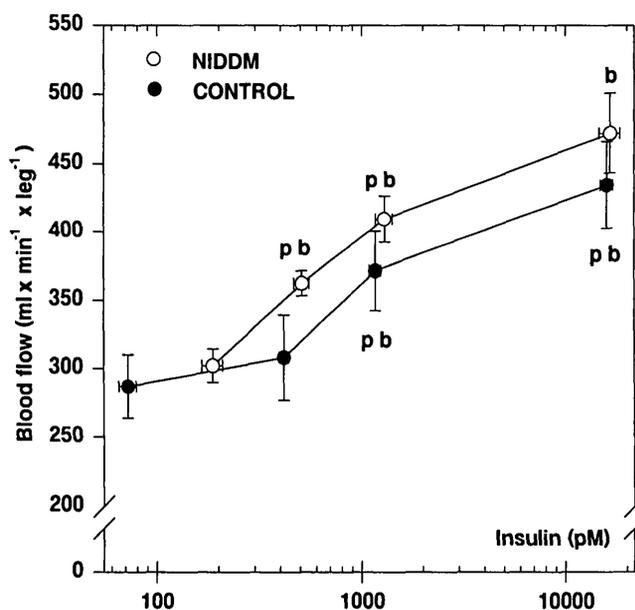

FIG. 1. Blood flow in response to insulin infusion in seven patients with NIDDM and eight healthy age-, weight-, and fat-free mass–matched subjects (control). Blood flow was measured in triplicate before and at the end of each step of a three-step hyperinsulinemic basal glycemia clamp. Data are presented as means ± SE of pooled data from both legs of each subject. b, difference from basal, $P < 0.05$; p, difference from preceding clamp step, $P < 0.05$.
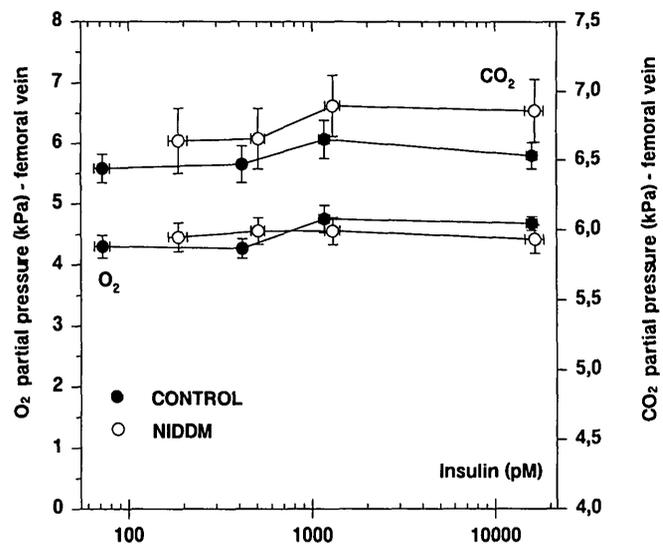

FIG. 2. CO_2 and O_2 tension in femoral venous blood at basal and during a three-step hyperinsulinemic basal glycemia clamp in seven patients with NIDDM and eight healthy age-, weight-, and fat-free mass–matched subjects (control). Blood was sampled before (in duplicate) and at the end (in triplicate) of each clamp step. Data are presented as means ± SE of pooled data from both legs of each subject. Two-way ANOVA for repeated measures detected a significant increase in Pco_2 with increasing insulin concentration ($P = 0.009$).
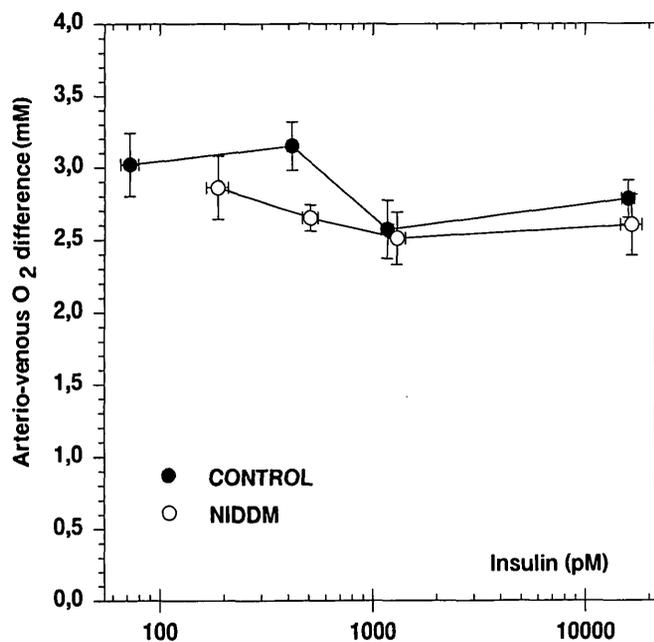
FIG. 3. Arterio-venous oxygen difference in response to insulin infusion in seven patients with NIDDM and eight healthy age-, weight-, and fat-free mass–matched subjects (control). Blood was sampled before (in duplicate) and at the end (in triplicate) of each step of a three-step hyperinsulinemic basal glycemia clamp. Data are presented as means ± SE of pooled data from both legs of each subject. By ANOVA for repeated measures, no change in arterio-venous oxygen difference could be detected in either group.

FIG. 4. Whole-body (A) and leg (B) oxygen uptake in response to insulin infusion in seven patients with NIDDM and eight healthy age-, weight-, and fat-free mass–matched subjects (control). Blood was sampled before (in duplicate) and at the end (in triplicate) of each step of a three-step hyperinsulinemic basal glycemia clamp. Expiratory air was collected in Douglas bags at the end of each clamp step. Data are presented as means ± SE. In each subject, leg oxygen uptake measurements for the two legs were pooled. b, different from basal, $P < 0.05$; p, different from preceding clamp step, $P < 0.05$.

sponds well with the measured increase in whole-body oxygen uptake of 50 ± 6 ml/min (Fig. 4A).

Our present findings disagree with those of Laakso et al. (7), who found impaired LBF in NIDDM patients compared with control subjects. Indeed, even when they increased plasma insulin concentrations to ~82,000 pmol/l (approximately five times the maximal insulin concentration in the present study), blood flow in NIDDM was not statistically different from baseline blood flow (7). At the studied insulin levels, differences in LBF between NIDDM patients and control subjects were at least 20% (7). The risk that we missed such differences was calculated to be minimal. Furthermore, the increased whole-body oxygen uptake during insulin stimulation can only be explained by an increased LBF, considering that arterio-venous oxygen differences remained unchanged throughout the clamp ($P > 0.05$ by ANOVA for repeated measures) (Fig. 3).

The reason for the discrepancy between the findings of Laakso et al. (7) and our present study is not evident. The fact that Laakso et al., in contrast to our present study, found an impaired blood flow response in patients with NIDDM is probably not explained by differences in the duration of the disease, as this was much shorter in the former (1.3 ± 0.3 years) than in the present (5.1 ± 1.3 years) study. However, possible cultural differences in screening for NIDDM makes this parameter quite uncertain. In the present study, four of seven patients were treated with antidiabetic drugs in addition to diet (on the day of experiments, no medication was taken), while the patients studied by Laakso et al. (7) were not on antidiabetic therapy. Residual effects of antidiabetic drugs on the experiment day apparently did not influence the results of the present study, because there was no difference in whole-body oxygen uptake or LBF response to insulin infusion between subjects treated with both diet and antidi-
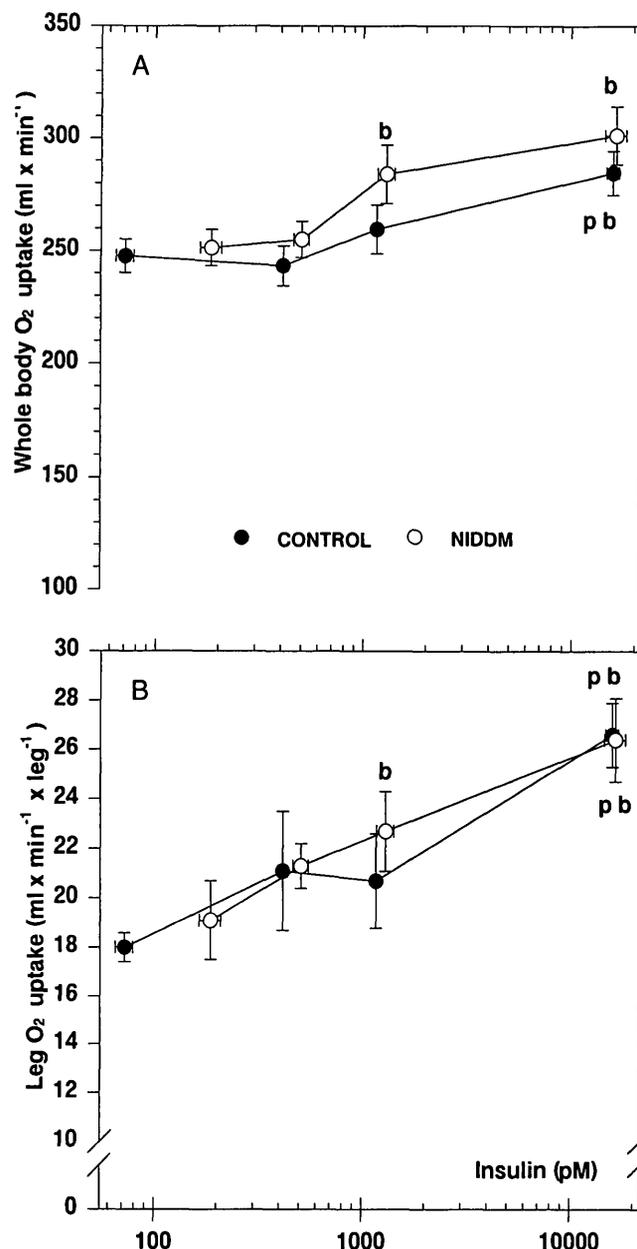
abetic drugs and those treated with diet alone (individual data not shown). Obesity has been shown to impair the LBF response to submaximal insulin concentrations (5), and it cannot be excluded that differences in body composition partly explain the difference in blood flow response between the two studies. Thus, the NIDDM patients studied by Laakso et al. (7) were somewhat more obese (body weight 103 ± 9 kg and body mass index 33 ± 3 kg/m^2) than the patients we studied (Table 1).

In our present study, fasting plasma glucose concentrations were maintained (median 8.8 mmol/l [range 5.8–15.9] and 5.8 mmol/l [range 5.2–6.2], NIDDM and control subjects, respectively) during insulin infusions. In the study by Laakso

et al. (7), however, the clamp was carried out at euglycemia (~4.5 mmol/l). Thus, the impaired blood flow response to insulin in NIDDM subjects was found during euglycemic conditions (7), and it appears from the present study that hyperglycemia may compensate for this defect. Because increases in glucose uptake rates in NIDDM subjects, at comparable increases in insulin concentrations, were higher in the present study (data not shown) compared with the study by Laakso et al. (7), increases in nonoxidative glucose disposal were probably also higher. It might therefore be argued that a higher increase in nonoxidative glucose disposal and in inherent energy costs in NIDDM subjects accounted for the fact that we, in contrast to Laakso et al., found in these subjects an increase in blood flow in response to insulin that was identical to that of healthy control subjects. However, the cost of nonoxidative glucose disposal is probably only a modest fraction of the total insulin-stimulated energy expenditure (14). In accordance with this, we found only a weak correlation between leg glucose uptake rates and leg $\dot{V}o_2$, since leg $\dot{V}o_2$ was similar in control and NIDDM subjects with increasing insulin, while leg glucose uptake rates were significantly lower in the NIDDM group at low insulin concentrations and significantly higher at high insulin concentrations compared with the control group (data not shown).

The patients with NIDDM in the present study were studied in their habitual hyperglycemic state, resembling everyday life conditions. Therefore, in contrast to what has previously been proposed (7), it is probably not a lack of sufficient increase in insulin-stimulated blood flow that ordinarily is responsible for decreased insulin-stimulated glucose clearance rates in NIDDM.

Plasma epinephrine concentrations did not increase with increasing insulin concentrations (Table 2), and skeletal muscle vasodilation was probably due to a vasodilatory action of insulin. This effect of insulin is most likely either secondary to an insulin-mediated increase in local metabolism (5,6) or due to a direct or nerve-mediated relaxing effect on smooth vascular muscle (1,6). Supporting the view that local metabolism is responsible for an increase in blood flow is the fact that insulin enhances the oxidation of carbohydrates at the expense of fat oxidation (15), thus increasing $Pvco_2$, which, in turn, would have a vasodilating effect. A two-way ANOVA for repeated measures did, in fact, reveal that $Pvco_2$ increased slightly with increasing insulin concentration ($P = 0.009$), with no difference between the two groups (Fig. 2). Taken together with the findings of constant venous oxygen tension (Pvo_2) (Fig. 2), this indicates that blood flow in the legs is linked to skeletal muscle metabolism. Nonmetabolic vasodilation would have caused an increase in oxygen and a decrease in CO_2 tension in the venous blood.

In response to hyperinsulinemia, mean blood pressure increased even though vascular resistance in muscle decreased (Table 2). The increase in blood pressure was accompanied by an increase in heart rate (Table 2) and was probably caused by an increase in cardiac output (16). Resistance in nonmuscular tissues may have increased, as suggested by the finding that during hyperinsulinemia systemic vascular resistance decreases less than resistance in muscle (16).

In accordance with previous findings (4,17,18), plasma norepinephrine concentrations increased with increasing insulin concentrations (Table 2). This reflects an insulin-mediated increase in sympathetic nervous activity (2,17,19), which, in turn, could account for increases in cardiac output and extramuscular vascular resistance. The findings of lower basal and insulin-stimulated arterial plasma concentration of norepinephrine in NIDDM compared with control subjects (Table 2) are in line with the previous findings in insulin-resistant compared with control subjects (16) and compatible with findings of lesser insulin-stimulated increase in muscle sympathetic nerve activity in obese compared with lean subjects (20).

In conclusion, the study has demonstrated that in patients with NIDDM studied at their habitual hyperglycemic state, a normal effect of insulin in stimulating LBF is seen. Furthermore, the vasodilating effect of insulin in skeletal muscle is linked to the insulin-mediated increase in local metabolic rate. Finally, basal and insulin-stimulated increases in plasma norepinephrine concentrations are lower in patients with NIDDM compared with control subjects.

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