Chronic activation of the sympathetic nervous system may be a pathogenetic mechanism by which hyperinsulinemia induces cardiovascular damage in insulin-resistant NIDDM patients. The influence of physiological hyperinsulinemia (< 700 pmol/l) on basal and stimulated sympathetic outflow was studied in 12 lean normotensive subjects with well-controlled NIDDM without complications and in 13 matched control subjects. Forearm blood flow (FBF) was measured with forearm plethysmography; sympathetic nervous system activity was assessed by the [1H]norepinephrine (NE) tracer method. NIDDM patients were insulin resistant (glucose infusion rates 31.8 ± 3.8 vs. 48.7 ± 2.0 pmol · kg⁻¹ · min⁻¹ in control subjects, P < 0.01). After a mixed meal, NIDDM patients showed a hyperinsulinemic response (2-h insulin levels: NIDDM patients 321 ± 31 pmol/l, control subjects 165 ± 19 pmol/l, P < 0.001). Insulin infusion induced a vasodilator response (not significantly different between the groups). Arterial plasma NE levels and total-body NE spillover increased significantly (total spillover in NIDDM patients from 0.77 ± 0.09 to 1.18 ± 0.16 nmol · m⁻² · min⁻¹, in control subjects from 0.98 ± 0.14 to 1.23 ± 0.18 nmol · m⁻² · min⁻¹, P < 0.01 for all, not different between groups). Total-body NE clearance did not change. Sympathetic stimulation (lower-body negative pressure [LBNP] 15 mmHg) induced forearm vasoconstriction and increased arterial and venous plasma NE and total NE spillover. Responses of FBF and NE kinetics to LBNP were not significantly different between groups and were not altered by hyperinsulinemia. Although these nonobese subjects with uncomplicated NIDDM showed postprandial hyperinsulinemia and resistance to the effect of insulin on glucose metabolism, this group was not resistant to the vasodilator and sympathetic stimulant effects of insulin. Responses to sympathetic stimuli (LBNP) were normal and unaffected by physiological hyperinsulinemia. Therefore, because of daily life hyperinsulinemia, chronic sympathetic stimulation could be operative in these patients and may explain the increased incidence of hypertension and/or cardiovascular complications. Diabetes 45:15–22, 1996

As a consequence of insulin resistance, hyperinsulinemia has been related to disorders such as hypertension, obesity, dyslipidemia, and NIDDM (1–3). Furthermore, hyperinsulinemia has been directly associated with atherosclerosis and cardiovascular events (4–6). The cause of this relationship between hyperinsulinemia and cardiovascular morbidity is not clear, but it may be mediated by some cardiovascular effect of insulin. Besides insulin's key role in the regulation of carbohydrate metabolism (7), it has become clear that it also has important effects on the cardiovascular system (8,9). In acute experiments in humans, insulin infusion exerts a vasodilatory effect (10,11). In addition, acute hyperinsulinemia induces systemic sympathetic activation as measured by direct muscle sympathetic nerve activity (MSNA) recordings (11,12). Togote insulin-induced vasodilation and sympathetic stimulation will induce a state of hyperdynamic circulation, characterized by a high heart rate, wide pulse pressure, and increased cardiac output. Recent epidemiological studies, indeed, found a relationship between a hyperdynamic circulation and insulin resistance (13,14).

Continuous sympathetic stimulation has been related to increased cardiovascular morbidity (15). Chronic stimulation of the sympathetic nervous system due to chronic hyperinsulinemia could therefore be an important factor explaining the well-known increased incidence of cardiovascular disease that can be observed in NIDDM patients (16). Therefore, it seems a valid question whether individuals with chronic hyperinsulinemia, which holds true for the initial phase of NIDDM, are exposed to normal insulin-induced sympathetic stimulation. In patients with longstanding established NIDDM, resistance to the vasodilatory effects of insulin has been reported (17,18). Data on the early phase of NIDDM are lacking, while endogenous hyperinsulinemia is most prominent at this early stage (19,20). Moreover, the ability of insulin to stimulate sympathetic neural outflow in NIDDM has not been examined before.

In the present study, we hypothesized that NIDDM patients in the early phase of their disease are still sensitive to the sympathetic stimulant effects of insulin. To address our hypothesis, sympathetic nervous activity was investigated at baseline and during stimulation, both before and during
acute hyperinsulinemia, in patients with early mild uncomplicated NIDDM and well-matched healthy control subjects. To quantify the response of the sympathetic nervous system in detail, the \textsuperscript{3}H]norepinephrine (NE) tracer technique was used (21).

**RESEARCH DESIGN AND METHODS**

**Subjects.** The study group consisted of 13 patients with NIDDM. All met the inclusion criteria: age between 25 and 60 years, non-smoking, without hyperinsulinemia (off-label blood pressure <160/90 mmHg, measured after a 6-min rest in the supine position), absence of macrovascular and microvascular complications, as assessed by physical examination (normal tendon reflexes and normal vibration sense), normal ophthalmoscopic examination, normal renal function and albumin excretion rate (<29 mg/min); body mass index <27 kg/m\(^2\), and good metabolic control (HbA\(_1c\) <7.5%) with diet alone or low doses of the first-generation sulfonylurea derivative tolbutamide (<1,000 mg/day).

Participants used no other medication. Patients were recruited from our outpatient department (of ~700 NIDDM patients, 11 met the inclusion criteria, of whom 6 participated) and through calls for patients treated by general practitioners (20 candidates, of whom 7 were included). Of the 19 patients, 10 who had a family history positive for diabetes. One of these who had NIDDM diagnosed before the age of 25 years, could be classified as having maturity-onset diabetes of the young and was excluded from further analysis because the etiology of this disease is different (22). Mean diabetes duration was 4.5 ± 0.5 years (mean ± SD). A control group consisted of 13 healthy, lean, normotensive, and non-smoking (meeting the above-mentioned criteria) age- and sex-matched subjects. These participants were selected by advertisement and received a payment. All of these subjects had normal glucose tolerance and a family history negative for diabetes and hypertension. All participants gave written informed consent. The experimental protocol was approved by the hospital ethics committee.

**Experimental procedures and protocol.** Before the experiment, all participants underwent a mixed-meal test. After an overnight fast, they ingested a liquid meal containing 25 g proteins, 18 g lipids, and 72 g carbohydrates, partly disaccharides, including gelatin and fibers (total energy content 548 calories). Before and after 30, 60, 120, and 180 min, blood samples were taken for plasma insulin and glucose determinations.

Within 7 days after the mixed-meal test, the main experiment was performed after an overnight fast with the subjects in supine position in a quiet temperature-controlled room (25–26°C). Under local anesthesia (0.3–0.4 mg lidocaine HCl, 20 mg/ml), a 20-gauge catheter (Angiocath, Becton Dickinson, Sandy, Utah) was inserted into the left brachial artery and connected with an arterial pressure monitoring line (Viggo Spec- traflow, 5250-120) to a Hewlett Packard 78505B monitor. In the same arm, a catheter (Vendon, 22 gauge) was inserted into a forearm vein to obtain venous blood samples. On the contralateral side, an identical catheter was inserted into a large forearm vein for the infusion of \(\textsuperscript{3}H\)NE, insulin, and 20% glucose. After complete instrumentation, at least 30 min of rest were included.

Previously prepared aliquots of \(\textsuperscript{3}H\)NE were thawed and diluted with 0.9% saline to an activity of 4 \(\mu\)Ci/ml. The weight of the syringe containing the raditracers was measured just before and just after the infusion to verify the infusion rate. At the end of the experiment, a portion of the radiotracer was frozen and stored at -80°C for analysis at the time the plasma determination was performed. After a priming dose of 16 \(\mu\)C/min, a continuous infusion of 0.35 \(\mu\)Ci • m\(^{-2}\) • min\(^{-1}\) of \(\textsuperscript{3}H\)NE was given during the entire experiment. Again, 30 min were allowed to obtain a steady-state, where baseline hemodynamic and humoral measurements were performed. Then, lower-body negative pressure (LBNP) at -15 mmHg, using a Perspex box, was applied for 15 min to stimulate the sympathetic nervous system (23). Subsequently, 30 min were again taken to allow all parameters to return toward baseline level. The hyperinsulinemic euglycemic clamp was then started and was continued for 90 min. During insulin infusion, hemodynamic data were obtained at 20-min intervals after which the raditracers were obtained at 45 and 90 min. Plasma insulin concentrations were measured at 45 and 90 min. Finally, with the continuation of insulin and glucose infusion, a second application of LBNP at -15 mmHg was performed for 15 min. A schedule of the study protocol is shown in Fig. 1.

**Forearm blood flow (FBF) was measured using mercury-in-Silastic strain gauge venous occlusion plethysmography as previously described (24).** One minute before the start of the measurements, a wrist cuff was inflated to 100 mmHg above systolic blood pressure. The collecting cuff around the upper arm was inflated to a pressure of 30 mmHg during eight heart cycles using a Hokanson E20 rapid cuff inflator. The strain gauges were connected with Hokanson Ec4 plethysmographs.

Insulin (Actrapid, Novo Nordisk, Bagsvaerd, Denmark; 430 pmol • m\(^{-2}\) • min\(^{-1}\) (60 mU • m\(^{-2}\) • min\(^{-1}\)) was diluted in 50 ml of 0.9% NaCl with the addition of 2 ml of the patient's plasma to a concentration of 1 Unit. Plasma glucose levels were kept at euglycemic levels by variable arterial plasma glucose levels measured at 5-min intervals (25). During the last 30 min of the clamp, the glucose infusion rate was calculated in micromoles per kilogram per minute to obtain the whole-body glucose uptake, a measure of insulin sensitivity (26). Healthy subjects were clamped at the fasting level; in diabetic patients, blood glucose was allowed to fall to a level of 5 mmol/l and kept there. In three patients, in whom no steady-state plasma insulin concentration was observed after 45 min, the clamp was continued until 30 min of steady state and lasted for a maximum of 120 min.

All studies were performed with the patients off medication. Of the diabetic patients, five used (low-dose) tolbutamide. This medication was stopped 7 days before the meal test and at least 10 days before the experiment. The chance that glucose hyperglycemia would develop was therefore minimal.

**Analytical methods.** Plasma glucose was measured in duplicate by the glucose oxidase method (Beckman Glucose Analyzer II, Beckman, Fullerton, CA) in arterial blood samples that were immediately centrifuged for 20 s. Plasma insulin was measured with a double-antibody radioimmunoassay (interassay coefficient of variation 4%–6.2%). It may be expected that this conventional insulin assay cross-reacts with proinsulin or insulin (being the most important insulin split products). Plasma C-peptide was measured with a commercially available double-antibody radioimmunoassay (Eugene Science). Plasma NE was measured using a high-performance liquid chromatography (HPLC) technique (Bio-Rad, The Netherlands) with reference values of 0.6–6.2 ng/ml.

Titrated norepinephrine (1-([3-phenylpropyl]amino)pentane, specific activity 30–60 Ci/mmol) was obtained from Du Pont-NEN’s l’Heretogues, L’Hˆtel, The Netherlands). It was sterilized using a micropore filter (0.22 \(\mu\)m) and diluted in 0.9% NaCl containing glacial acetic acid (0.2 mol/l) and ascorbic acid (1 mol/l) and stored at -80°C until use, which was always within 3 months of preparation. Sterilization, dilution, and batch division were carried out under nitrogen.

Blood samples for measurement of plasma catecholamines were collected in precollimated tubes containing glutathione (0.2 mol/l) and EDTA (0.25 mol/l) on melting ice. The tubes were centrifuged at 4°C, and plasma was stored at -30°C. Analyses of plasma samples and urine occurred within 2 days of their collection. Plasma samples were analyzed for concentrations of unlabeled and tritiated-labeled NE by HPLC with fluorometric detection after precolumn derivatization with the selective detection agent 1,2-diphenylethylidenemine. A modification of an earlier described method (27) was performed.

**The instrumentation for the chromatography consisted of a model 470 pump (Systech Instruments, Co., Fullerton, CA), a model 470 scanning fluorometer (16-kDa cut-off), and an integrated sample processor WISP 710B (all from Waters, Milford, MA). The experiments were performed on a silica analytical Nova-Pak C\(_18\) (150 • 3.9 mm) column coupled to a Nova-Pak C\(_18\) guard column.** The \(\textsuperscript{3}H\)NE activity was measured in the eluate collected for 5 min by a Dionex fraction collector (model 201-202) connected to the WISP 710B and programmed to start...
TABLE 1
Baseline characteristics of the two groups

<table>
<thead>
<tr>
<th></th>
<th>Diabetes</th>
<th>Normal</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>12</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td><strong>Sex (F:M)</strong></td>
<td>5:7</td>
<td>5:8</td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>46.5 ± 11.8</td>
<td>45.7 ± 0.0</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Systolic blood pressure (mmHg)</strong></td>
<td>24.2 ± 2.5</td>
<td>24.2 ± 1.7</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Systolic blood pressure (mmHg)</strong></td>
<td>130 ± 10</td>
<td>123 ± 11</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Diastolic blood pressure (mmHg)</strong></td>
<td>67 ± 10</td>
<td>65 ± 7</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Pulse pressure</strong></td>
<td>2.80 ± 0.88</td>
<td>2.19 ± 1.26</td>
<td>NS</td>
</tr>
<tr>
<td><strong>FVR (AU)</strong></td>
<td>35.7 ± 12.1</td>
<td>35.2 ± 17.6</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Fasting glucose (mmol/l)</strong></td>
<td>8.8 ± 2.0</td>
<td>5.0 ± 0.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>IbaA$_1$(%)</strong></td>
<td>6.8 ± 0.8</td>
<td>5.5 ± 0.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Glucose infusion rate, 60-80 min (µmol.kg$^{-1}$.min$^{-1}$)</strong></td>
<td>31.8 ± 13.2</td>
<td>48.7 ± 0.9</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are means ± SD.

at the beginning of the NE peak position in the chromatogram of the standard solution, which was run before the plasma samples and $^{[3]}$HNE infusate in each assay. The $^{[3]}$HNE activity of all 1-ml fractionated samples with 8 ml scintillation solution (Aqualuma) added was counted for 20 min in a Packard Tri-Carb 4600 C.

Calculations and data analysis. The following kinetic variables were calculated according to these formulas (21):

Total clearance ($t$ min$^{-1}$) = Total NE overflow (µmol.min$^{-1}$) / Total clearance ($t$ min$^{-1}$)

Total NE spillover (µmol.min$^{-1}$) = Total clearance ($t$ min$^{-1}$) * $^{[3]}$HNE (µmol/l)

Forearm plasma flow (FPF) (ml.min$^{-1}$) = (1 - hematocrit) * PBF

Fractional extraction (FE) = $^{[3]}$HNE in (µmol/min) / $^{[3]}$HNE in (µmol/min) * $^{[3]}$HNE in (µmol/min)

Forearm spillover (µmol.min$^{-1}$) = PFP * [NE]$^{[3]}$HNE in (µmol/min)

Forearm removal (µmol.min$^{-1}$) = PFP * FE * [NE]$^{[3]}$HNE in (µmol/min)

TABLE 2
Responses of NE kinetic data to LBNP

<table>
<thead>
<tr>
<th></th>
<th>Baseline 1</th>
<th>LBNP 1</th>
<th>Baseline 2</th>
<th>Insulin 45 min</th>
<th>Insulin 90 min</th>
<th>LBNP 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Arterial NE (µmol/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>NIDDM</td>
<td>1.02 ± 0.11</td>
<td>1.48 ± 0.14*</td>
<td>1.01 ± 0.12</td>
<td>1.56 ± 0.16§</td>
<td>1.46 ± 0.16§</td>
<td>1.83 ± 0.21</td>
</tr>
<tr>
<td>Control</td>
<td>1.05 ± 0.12</td>
<td>1.41 ± 0.19*</td>
<td>1.14 ± 0.14</td>
<td>1.60 ± 0.23§</td>
<td>1.54 ± 0.21§</td>
<td>1.91 ± 0.19</td>
</tr>
<tr>
<td><strong>Venous NE (µmol/l)</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIDDM</td>
<td>1.16 ± 0.17</td>
<td>1.65 ± 0.23*</td>
<td>1.27 ± 0.18</td>
<td>1.46 ± 0.20§</td>
<td>1.50 ± 0.22§</td>
<td>1.81 ± 0.27</td>
</tr>
<tr>
<td>Control</td>
<td>1.27 ± 0.13</td>
<td>1.77 ± 0.14*</td>
<td>1.35 ± 0.13</td>
<td>1.63 ± 0.16§</td>
<td>1.44 ± 0.14§</td>
<td>2.13 ± 0.14</td>
</tr>
<tr>
<td><strong>Total NE spillover (µmol.min$^{-1}$)</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>NIDDM</td>
<td>0.97 ± 0.12</td>
<td>1.28 ± 0.22*</td>
<td>0.77 ± 0.09</td>
<td>1.15 ± 0.14§</td>
<td>1.18 ± 0.16§</td>
<td>1.29 ± 0.14*</td>
</tr>
<tr>
<td>Control</td>
<td>0.97 ± 0.13</td>
<td>1.17 ± 0.19*</td>
<td>0.98 ± 0.14</td>
<td>1.19 ± 0.17 §</td>
<td>1.23 ± 0.18 §</td>
<td>1.42 ± 0.16*</td>
</tr>
<tr>
<td><strong>Forearm NE spillover (µmol.min$^{-1}$)</strong></td>
<td></td>
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</tr>
<tr>
<td>NIDDM</td>
<td>1.31 ± 0.28</td>
<td>1.24 ± 0.30</td>
<td>1.55 ± 0.31</td>
<td>1.82 ± 0.30</td>
<td>1.88 ± 0.36</td>
<td>1.98 ± 0.29</td>
</tr>
<tr>
<td>Control</td>
<td>1.42 ± 0.16</td>
<td>1.66 ± 0.22</td>
<td>1.26 ± 0.13</td>
<td>1.52 ± 0.20</td>
<td>1.52 ± 0.32</td>
<td>1.65 ± 0.31</td>
</tr>
<tr>
<td><strong>Total NE clearance (µmol.min$^{-1}$)</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>NIDDM</td>
<td>0.85 ± 0.07</td>
<td>0.85 ± 0.12</td>
<td>0.81 ± 0.09</td>
<td>0.74 ± 0.05</td>
<td>0.81 ± 0.07</td>
<td>0.73 ± 0.07*</td>
</tr>
<tr>
<td>Control</td>
<td>0.85 ± 0.03</td>
<td>0.80 ± 0.05*</td>
<td>0.88 ± 0.06</td>
<td>0.74 ± 0.05</td>
<td>0.81 ± 0.04</td>
<td>0.76 ± 0.05</td>
</tr>
<tr>
<td><strong>Forearm NE removal (µmol.min$^{-1}$)</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>NIDDM</td>
<td>1.13 ± 0.20</td>
<td>1.09 ± 0.16</td>
<td>1.05 ± 0.20</td>
<td>1.52 ± 0.20§</td>
<td>1.84 ± 0.23§</td>
<td>1.92 ± 0.32</td>
</tr>
<tr>
<td>Control</td>
<td>1.17 ± 0.20</td>
<td>1.31 ± 0.25*</td>
<td>1.13 ± 0.14</td>
<td>1.09 ± 0.25§</td>
<td>1.80 ± 0.37§</td>
<td>1.75 ± 0.35</td>
</tr>
</tbody>
</table>

Data are means ± SE. *P < 0.05 (FP < 0.01) LBNP 1 vs. baseline 1, §P < 0.05 and $§P < 0.01$ insulin vs. baseline 2, ||P < 0.05 (FP < 0.01) LBNP 2 vs. insulin 90 min. *P

Note: There were no significant differences between the values of baseline 1 and those of baseline 2.

RESULTS
Baseline measurements. Baseline characteristics of the two groups are given in Table 1. Baseline hemodynamic variables were not different between NIDDM patients and control subjects. NIDDM patients had higher fasting glucose levels and IbaA$_1$ values than control subjects. The fasting
insulin concentration was higher in NIDDM patients (106 ± 17 vs. 63 ± 6 pmol/l in control subjects, \( P = 0.02 \)), as was fasting plasma C-peptide level (0.59 ± 0.07 vs. 0.40 ± 0.02 nmol/l in control subjects, \( P < 0.02 \)). Baseline NE kinetic parameters (arterial and venous plasma concentrations, total-body and forearm spillover, total-body clearance, and forearm removal) were not different between NIDDM patients and control subjects (Table 2).

**Responses to a mixed meal.** In control subjects, the standardized mixed meal induced a slight increase in plasma glucose values, from 5.0 ± 0.1 to maximal 6.0 ± 0.2 mmol/l. NIDDM patients showed a more pronounced and prolonged increase of glucose levels, from 8.8 ± 0.6 to maximal 13.4 ± 1.2 mmol/l after 60 min and to 9.4 ± 1.1 mmol/l after 180 min. The insulin response to a mixed meal was delayed in NIDDM patients, and on every time point it was larger than in control subjects (Fig. 2, lines). When expressed as area under the insulin curve, the responses in NIDDM patients were significantly increased compared with those in control subjects (58.0 ± 7.3 vs. 37.0 ± 3.7 nmol ⋅ l⁻¹ ⋅ min⁻¹, \( P < 0.02 \)). Also, when measured with a more specific insulin assay, NIDDM patients had higher insulin levels than control subjects (baseline: 80 ± 15 [NIDDM] vs. 51 ± 5 pmol/l [control], \( P = 0.08 \); 120 min: 269 ± 33 [NIDDM] vs. 133 ± 19 pmol/l [control], \( P = 0.001 \) (Fig. 2, bars).

**Metabolic response to euglycemic clamp.** Blood glucose values during the last 30 min of the clamp were stable in all individuals (mean glucose concentration 4.9 ± 0.1 [NIDDM] vs. 4.9 ± 0.1 mmol/l [control]; CV 4.8 ± 0.5% [NIDDM] and 4.3 ± 0.6% [control]). After 60 min, plasma insulin concentration was 746 ± 65 pmol/l in NIDDM patients and 645 ± 36 pmol/l in control subjects (\( P = 0.18 \)). After 90 min, it was 810 ± 50 pmol/l in NIDDM patients and 645 ± 50 pmol/l in control subjects (\( P < 0.05 \)). Whole-body glucose uptake was significantly different between NIDDM patients and control subjects (31.8 ± 3.8 vs. 48.7 ± 2.0 pmol ⋅ kg⁻¹ ⋅ min⁻¹, \( P < 0.01 \)).

**Effects of insulin on hemodynamic parameters.** As indicated in Fig. 3, 90 min of hyperinsulinemia induced a forearm vasodilation (FVF: 2.75 ± 0.23 to 3.90 ± 0.40 ml ⋅ min⁻¹ ⋅ dl⁻¹ [NIDDM] vs. 2.98 ± 0.36 to 3.82 ± 0.56 ml ⋅ min⁻¹ ⋅ dl⁻¹ [control], both \( P < 0.05 \) by ANOVA) accompanied by a clear decrease of peripheral resistance (FVR: 36.9 ± 3.1 to 28.9 ± 3.2 [NIDDM] vs. 35.1 ± 4.8 to 27.2 ± 3.1 AU [control], both \( P < 0.01 \)). Systolic blood pressure did not change significantly and diastolic blood pressure decreased significantly in control subjects (from 66.3 ± 2.0 to 65.1 ± 2.1 mmHg, \( P < 0.01 \)), but not in NIDDM patients (from 68.6 ± 2.4 to 66.7 ± 3.0 mmHg, \( P = 0.09 \)). Pulse pressure increased in both groups (64.6 ± 2.9 to 66.6 ± 2.9 [NIDDM] vs. 58.1 ± 1.9 to 63.1 ± 1.8 mmHg [control], both \( P < 0.05 \)). Heart rate tended to increase significantly only in NIDDM patients (63.3 ± 1.8 to 69.0 ± 2.3 bpm, \( P < 0.01 \)) and not in control subjects (60.7 ± 2.4 to 61.5 ± 2.5 bpm, \( P = 0.14 \)). Responses of heart rate, pulse pressure, and FVR to the hyperinsulinemic clamp are depicted in Fig. 3.

There were no significant differences in hemodynamic parameters between NIDDM patients and control subjects at different time points; nor were there significant differences in hemodynamic responses to insulin.

**Effect of insulin on NE kinetics.** Absolute NE kinetic data for the entire experiment are given in Table 2. During insulin infusion, arterial and venous NE concentrations increased (percentage increases after 45 and 90 min: arterial NE in NIDDM patients 62.8 ± 14.0 and 49.8 ± 10.4%, in control subjects 41.3 ± 9.1 and 33.5 ± 6.1%, \( P < 0.01 \) vs. baseline for all; venous NE in NIDDM patients 17.0 ± 4.8 and 22.4 ± 6.7%, in control subjects 26.8 ± 8.3%, all \( P < 0.01 \), and 21.3 ± 7.7%, \( P < 0.05 \) vs. baseline).

Calculated total-body NE spillover increased (percentage changes after 90 min: 51.8 ± 9.4% [NIDDM] vs. 26.5 ± 7.0% [control], both \( P < 0.05 \) vs. baseline).
The major new finding of the present study is that patients who are in the early phase of NIDDM show preserved cardiovascularr and sympathetic responses to physiological hyperinsulinemia. Moreover, on top of this preserved cardiovascular responsiveness to an exogenous sympathetic stimulus, this being in our study the application of

and heart rate (Fig. 3). Arterial and venous NE increased (Table 2 and Figs. 4 and 5), and total-body NE spillover tended to increase, but not significantly. Baseline levels at the start of this LBNP were higher (previous insulin effect), but absolute responses to LBNP on both occasions were not different. Here again, the two study groups responded similarly.

**Control experiments.** In four young healthy volunteers (age 23.8 ± 0.3 years, BMI 20.8 ± 0.5 kg/m²), control experiments were performed. These individuals were studied with an intra-arterial line, but did not undergo any intervention and did not receive any infusion. At the respective time periods, blood samples were taken and blood flow and blood pressure were measured. During the 3-h experiment, blood flow, vascular resistance, and arterial and venous plasma NE concentrations remained at a fairly constant level (baseline 1 to LBNP 1: FVR 48.2 ± 7.3 to 47.5 ± 8.1 AU, arterial NE from 0.82 ± 0.09 to 0.73 ± 0.13 nmol/l; baseline 2 to insulin 90 min: FVR 51.4 ± 7.1 to 61.7 ± 4.9 AU, arterial NE from 0.77 ± 0.14 to 0.91 ± 0.13 nmol/l; insulin 90 min to LBNP 2: FVR 71.7 ± 4.9 to 56.3 ± 3.5 AU, arterial NE from 0.91 ± 0.13 to 0.94 ± 0.16 nmol/l).

**DISCUSSION**

The major new finding of the present study is that patients who are in the early phase of NIDDM show preserved vasoconstrictr and sympathetic responses to physiological hyperinsulinemia. Moreover, on top of this preserved cardiovascular response to insulin, these NIDDM patients exhibit normal cardiovascular responsiveness to an exogenous sympathetic stimulus, this being in our study the application of
LBNP. In contrast, this group was resistant to the effect of insulin on glucose metabolism and was hyperinsulinemic after a regular meal. These observations imply that NIDDM patients at this early stage of their disease might be exposed to insulin-induced vasodilation and sympathetic stimulation because of their hyperinsulinemia throughout the day. We speculate that the combination of increased skeletal muscle blood flow and increments in heart rate and pulse pressure, reflecting a hyperdynamic state, could be important in the development of hypertension and/or cardiovascular complications later in the course of the disease.

**Metabolic effects of insulin.** Most NIDDM patients pass through prediabetic and early diabetic stages (20), characterized by high fasting and postprandial insulin levels (19,29) with concomitant existence of insulin resistance (30). In this study, we tried to select a group with very early NIDDM, making it as homogeneous as possible by using strict selection criteria. This patient group is exceptional from a clinical point of view; in most patients, the diabetic state is more advanced and patients are obese and have complications or comorbidity. Our nonobese NIDDM subjects were indeed insulin resistant, as has been reported before (31). Insulin sensitivity was assessed before the second LBNP and a sufficient time after the first LBNP to allow all parameters to return to baseline; hence, the determination of insulin sensitivity could not be influenced by these sympathetic stimulation procedures.

In NIDDM, proinsulin and proinsulin-split products have been found to be elevated (32,33) and may explain part of the measured hyperinsulinemia (27,34). However, using a specific insulin assay, we were still able to demonstrate that our NIDDM group showed true hyperinsulinemia, as were others (19).

**Vasodilator response to insulin.** Since 1990, a number of groups have demonstrated the vasodilatory effect of systemic hyperinsulinemia (10,11,35). In NIDDM patients, Laakso et al. (17) have reported a decrease in insulin-induced vasodilation, partly reversible by improving metabolic control with insulin therapy (18). The reported studies, however, concern individuals with long-standing established diabetes who are in poor metabolic control and are considerably obese. This may be an important drawback because, as mentioned above, in NIDDM, endogenous hyperinsulinemia can be found especially in the early phase of the disease. Furthermore, insulin-induced vasodilation is attenuated in obesity itself (10,36). Recently it has also been reported that insulin-induced vasodilation was preserved in NIDDM subjects (37), a finding that is compatible with our results.

**Sympathetic stimulant effects of insulin.** The relationship between insulin and the sympathetic nervous system has been studied using variable techniques. Some studies have reported an increase in venous plasma NE concentrations during insulin infusion (8,9,11), with the earliest report being from Gundersen and Christensen (38). The venous NE concentration, however, results from total NE production and clearance on the one hand, local production and removal on the other hand, and blood flow. By measuring not only venous but also arterial blood samples and by using tracer NE infusion (21), a far more detailed estimate of sympathetic neural activity is possible. It is, however, important to realize that the [3H]NE kinetic tracer technique represents only the rate by which NE is entering the plasma compartment, which is not necessarily the same as the rate of NE release from sympathetic nerve endings.

With the use of microneurographic readings (MSNA), the insulin-induced increase in sympathetic nervous system activity has been quite consistently reported (11,12,36,39). The MSNA technique has drawbacks as well as advantages. It is technically demanding and is unsuccessful in a proportion of cases. Moreover, neural outflow at only one location is measured, which does not necessarily reflect total sympathetic activity. Until now, data on sympathetic responses in NIDDM patients have been sparse. As we show in our study, 90 min of physiological hyperinsulinemia induced an increase of total-body NE spillover. Since this spillover is an index of sympathoneural activity, our data indicate that hyperinsulinemia induces sympathoneural stimulation. This sympathoneural stimulation was of a moderate degree, around the same magnitude as stimulation induced by LBNP of $-15$ mmHg. The insulin-induced sympathoneural stimulation was clearly similar in both experimental groups.

Insulin concentrations during the clamps were, nevertheless, slightly higher in the NIDDM group, which is probably caused by a diminished insulin clearance, a feature coupled with insulin resistance (26,41,42). The difference in insulin level between the groups was small, however, furthermore, not only concentration but also duration of hyperinsulinemia seems to be important with respect to sympathoneural stimulation (11). Finally, the relationship between insulin concentration and sympathoneural stimulation is not linear; Vollenweider et al. (36) found comparable increases in sympathetic nerve activity (MSNA) despite threefold differences in insulin concentrations. Probably sympathoneural stimulation is already maximal at low insulin doses. Therefore, it is highly unlikely that sympathoneural responses in diabetic subjects would have been less if insulin concentrations would have been more similar.

**Response to sympathetic stimuli.** The diabetic subjects in our study had normal responses to the non-insulin-sympathetic stimuli LBNP, despite higher prevailing glucose levels (fasting state, diabetes versus control). Also, insulin-resistant obese subjects have been reported (36,40) as having a diminished insulin-induced increase in sympathetic nervous system activity (MSNA) despite threefold differences in insulin concentrations. Probably sympathoneural stimulation is already maximal at low insulin doses. Therefore, it is highly unlikely that sympathoneural responses in diabetic subjects would have been less if insulin concentrations would have been more similar.

The response to the sympathetic stimulus LBNP was not affected by acute hyperinsulinemia; baseline values just before the second test were indeed higher, but absolute changes were similar. These findings are in complete agreement with the aforementioned studies of Vollenweider et al. (36), Grassi et al. (40), and Hoffman et al. (38).

**Mechanisms of sympathetic activation.** While we confirm the vasodilatory and sympathetic stimulant effects of insulin, we are not able to explain the mechanism of these effects. It has been demonstrated previously that activation of the sympathetic nervous system is due to hyperinsulinemia and not to glucose infusion (44,45). Hypoglycemic episodes were avoided, so sympathetic activation cannot be due to hypoglycemia. The observed sympathetic activation could be based on a baroreceptor reflex to a slight insulin-induced decline of
blood pressure. This concept, in which vasodilation and sympathetic stimulation are coupled, is supported by the fact that in autonomous failure, insulin induces hypotension (46-47). From our data, it seems, however, that most of the sympathetic stimulation had already been established after 45 min of insulin infusion, suggesting that the activation of the sympathetic nervous system is merely a predecessor rather than a consequence of the insulin-induced vasodilation.

Pathophysiological implications. Since endogenous hyperinsulinemia is indeed present in the fasting as well as in the postprandial state in our patients, we hypothesize that daily life hyperinsulinemia could induce a chronic stimulation of the sympathetic nervous system. An increased sympathetic drive has been related to an increase in cardiovascular disease (15) and might in particular be relevant to the development of hypertension.

Endogenous insulin levels are indeed linked to cardiovascular risk in NIDDM subjects (48). One might speculate that as the disease proceeds, the chronic endogenous hyperinsulinemia could lead to an adaptive mechanism in which vascular and neurogenic responses are blunted. In support of this hypothesis are recent, epidemiological studies (13,14) in which sympathetic stimulation had already been established after hypertensive compared with normal subjects has been increased sympathetic activation in response to insulin in established hypertension with increased peripheral resistance (15,41)).

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20. In conclusion, we report that nonobese patients in the early phase of NIDDM, who are hyperinsulinemic and resistant to the metabolic effect of insulin, are normally responsive to the effects of insulin on vascular tone and sympathetic activity. Furthermore, the sympathetic nervous system is normally responsive to stimuli. This means that during daily life an excessive sympathetic neural outflow could exist. This fact could be an explanation for the increased incidence of hypertension and/or cardiovascular disease in this group and contribute to a hyperdynamic circulation, probably inducing secondary changes in time. Hyperinsulinemia did not change the absolute responses to sympathetic stimulation but may affect further sympathetic stimulation.

The cardiovascular effects of insulin warrant further investigation.

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