

# Interactions between Insulin and Norepinephrine on Blood Pressure and Insulin Sensitivity

## Studies in Lean and Obese Men

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## Abstract

To explore the interactions between insulin action and norepinephrine (NE) on blood pressure and muscle vascular resistance, we studied seven lean ( $66 \pm 1$  kg) sensitive and seven age-matched obese ( $96 \pm 3$  kg) insulin-resistant men after an overnight fast. Both groups were normotensive; however, the obese exhibited higher basal blood pressure,  $90.8 \pm 2.2$  vs.  $83.4 \pm 1.6$  mmHg,  $P < 0.04$ . Each subject was studied on two separate days during either saline (S) infusion or a euglycemic hyperinsulinemic clamp (I) achieving insulin concentrations of  $\sim 70$   $\mu$ U/ml. After 180 min of either S or I, NE was infused systemically at rates of  $\sim 50$ , 75, and 100 pg/kg per min. Glucose uptake was measured in whole body ( $[3\text{-}^3\text{H}]\text{glucose}$ ) and in leg by the balance technique. The results indicate: (a) the NE/pressor dose-response curve was decreased (shifted to the right) during I in lean but not in obese subjects, (b) I enhanced NE metabolic clearance by 20% in lean but not in obese, (c) NE decreases leg vascular resistance more in lean than in obese, and (d) NE causes a  $\sim 20\%$  increase in insulin-mediated glucose uptake in both groups.

In conclusion, insulin resistance of obesity is associated with an apparent augmented NE pressor sensitivity and decreased NE metabolic clearance. Both of these mechanisms can potentially contribute to the higher incidence of hypertension in obese man. Insulin resistance is likely to be a predisposing but not sufficient factor in the pathogenesis of hypertension. Because the obese group exhibited higher basal blood pressure, it is possible that our results reflect this difference. Further studies will be required to clarify this issue. (*J. Clin. Invest.* 1994; 93:2453–2462.) Key words: glucose uptake • vascular resistance • cardiac output • norepinephrine • metabolic clearance • blood pressure

## Introduction

The interaction between insulin and the sympathetic nervous system (SNS)<sup>1</sup> has been the focus of much investigation and

controversy over the last decade. SNS activation related to increments in circulating insulin concentrations has been documented in humans. Avasthi et al. (1) reported elevations of circulating norepinephrine (NE) levels after mixed meals and Berne et al. (2) reported increased plasma NE concentrations and nerve firing rates by microneurography after glucose but not water ingestion. By means of the euglycemic clamp technique, Rowe and co-workers (3) have documented a rise in NE levels with concomitant elevations in blood pressure during intravenous insulin infusion to achieve supraphysiologic insulinemia. In contradistinction, more recently Anderson et al. (4) found that physiologic hyperinsulinemia achieved during a euglycemic clamp increased muscle SNS activity (measured by microneurography) and was accompanied by a fall in forearm vascular resistance without a change in systemic blood pressure. We have recently reported a dose-dependent effect of intravenous insulin to decrease blood pressure and both systemic and leg vascular resistance in the face of a  $\sim 30\%$  rise in circulating NE concentrations (5), which would have been expected to raise the blood pressure. Further confounding the interaction between insulin and the SNS is the report by Gans et al. (6), suggesting that insulin sensitizes the vasculature to the pressor effects of NE. Their data indicate that the circulating level of NE required to raise the diastolic blood pressure above baseline by 20 mmHg is lower during euglycemic hyperinsulinemia than during a saline infusion. In contrast, in vitro data suggest that insulin causes a shift to the right in the NE and angiotensin II pressor dose-response curves in isolated vascular rings of femoral artery and vein from rabbits (7). Another in vivo report suggests an effect of insulin to decrease vascular reactivity to NE (8).

Recent epidemiological data have indicated an important association between insulin resistance/hyperinsulinemia and hypertension (9–12). Some workers have suggested that hyperinsulinemia may itself play a primary role in the pathogenesis of hypertension through its action to activate the SNS (3, 11, 12) and others have suggested that activation of the SNS causes insulin resistance (13). Therefore, a better understanding of the interactions among the SNS, insulin action, and insulin sensitivity is desirable. To this end, the current study was designed to examine the modulating effects of insulin on the dose response of NE on blood pressure, skeletal muscle blood flow, and vascular resistance in lean insulin-sensitive and obese insulin-resistant humans. In addition, the effect of NE on insulin's action to stimulate glucose uptake was also examined.

## Methods

### Subjects

The clinical characteristics of the study groups are shown in Table I. Groups of seven lean and seven obese volunteers were studied after an overnight  $\sim 14$ -h fast and were required to refrain from smoking at least 12 h before each study. Each subject was admitted to the Indiana

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1. Abbreviations used in this paper: AVGA, arteriovenous glucose difference; CO, cardiac output; FAVA, femoral arteriovenous difference; LBF, leg blood flow; LGU, leg glucose uptake; LVR, leg vascular resistance; MAP, mean arterial pressure; NE, norepinephrine; SNS, sympathetic nervous system; WBGU, whole-body glucose uptake.

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Table I. Subject Characteristics

Characteristic	Lean	Obese
n	7	7
Gender	male	male
Weight (kg)	66.0±1.5	96.1±2.9 <sup>‡</sup>
Percent fat	20.3±0.9	29.7±0.9 <sup>‡</sup>
Body mass index	21.1±0.4	32.3±0.8 <sup>‡</sup>
Fasting insulin (μU/ml)	2.92±0.7	15.6±2.4 <sup>‡</sup>
Fasting glucose (mg/dl)	91.0±0.9	94.0±1.2
Basal MAP (mmHg)	83.4±1.6	90.8±2.2 <sup>*</sup>

\*  $P < 0.04$  vs. lean.

<sup>‡</sup>  $P < 0.01$  vs. lean.

University General Clinical Research Center 2 d before each study and was fed an isocaloric diet with a caloric distribution of 50% carbohydrate, 30% fat, and 20% protein. All subjects were free of any diseases and none were on medication. All had been weight stable for at least 3 mo before the study and none were participating in any regular exercise program. All volunteers had normal glucose tolerance to a 75-g oral glucose load (14). The blood pressure in the obese was higher than in the lean group but was within the range considered clinically normotensive. All subjects underwent under water weighing to determine body composition, by the method of Brozek and Herschel (15). Leg volume was measured by water displacement.

#### Protocol

The study protocol (Fig. 1) was designed to assess in lean and obese individuals mean arterial pressure (MAP) and leg (muscle) blood flow (LBF) and leg vascular resistance (LVR) during a graded infusion of NE in the presence or absence of insulin. For this purpose, each volunteer was studied on two occasions ~ 3 wk apart. The order of the studies was assigned at random.

At ~ 7:00 a.m. a catheter was inserted into a right brachial vein for the purpose of infusion of substances. In a subset of five lean subjects, a 12-in. catheter was also inserted into a left brachial vein for the injection of indocyanine green dye to measure cardiac output (see technique below). After insertion of the brachial venous catheter, a bolus of D-[3-<sup>3</sup>H]glucose was administered, followed by continuous infusion (see method below). At 7:30 a.m. a 5-French pediatric sheath (Cordis

Corp. Miami, FL) was inserted into the femoral vein and a 16-gauge Teflon catheter was placed into the femoral artery ~ 2–3 cm below the inguinal ligament. After a 2-h tracer equilibration period basal bloods were obtained over the subsequent 30 min for the determination of glucose specific activity, femoral arteriovenous glucose differences (AVGΔ), insulin, and NE. In addition, basal MAP and LBF were ascertained to calculate leg vascular resistance (MAP/LBF) and leg glucose uptake (LGU),  $LGU = FAVΔ \cdot LBF$  (16). After basal measurements, subjects received either a square wave infusion of regular insulin (humulin, a kind gift from Eli Lilly & Co., Indianapolis, IN) at a rate of 40 mU/m<sup>2</sup> per min (insulin study) with euglycemia was maintained via the clamp technique (17) or a saline infusion (saline study). During the saline study the saline load was matched to the overall volume of saline received during the insulin study without attempting to replace the free water load obligate as 20% dextrose during the hyperinsulinemic euglycemic clamp studies. Saline infusions and hyperinsulinemic euglycemic clamps were carried out for 180 min. Repeat measurements of rates of whole-body glucose uptake (WBGU) and LGU, MAP, LBF, and LVR were obtained over the last 30 min (150–180 min) of each study.

At ~ 180 min a graded infusion of NE was begun with each infusion rate maintained for 30 min. The goal NE infusion rates were ~ 50, 75, and 100 pg/kg per min. This NE infusion protocol was followed unless the systolic blood pressure reached ≥ 160 mmHg, in which case the infusion rate was lowered to the maximal tolerated dose without exceeding the systolic blood pressure limit. A number of volunteers of both groups (but relatively more obese subjects) were not able to tolerate all NE doses. Moreover, a number of individuals (mostly obese subjects) tolerated pressor doses of NE during hyperinsulinemia, which they could not tolerate during saline. Therefore, only paired data, i.e., data from subjects tolerating a NE dose during both insulin and saline conditions were included in the statistical analysis unless otherwise stated. The actual average NE infusion rates in lean were 50±7 (n = 7), 83±8 (n = 7), and 113±8 (n = 5) pg/kg per min during saline and 61±7 (n = 7), 86±8 (n = 7), and 110±7 (n = 7) pg/kg per min during insulin, and in obese 40±7 (n = 7), 67±6 (n = 3), and 87±13 (n = 2) during saline and 40±7 (n = 7), 70±4 (n = 6), and 88±13 (n = 2) pg/kg per min during insulin. In the last 10 min of each NE plateau measurements of glucose turnover and hemodynamics were repeated.

During the saline studies the prevailing glucose level rose as a result of NE's effect to stimulate hepatic glucose output. Because this rise in serum glucose concentration was modest and unlikely to confound the hemodynamic data, no attempt was made to match the serum glucose

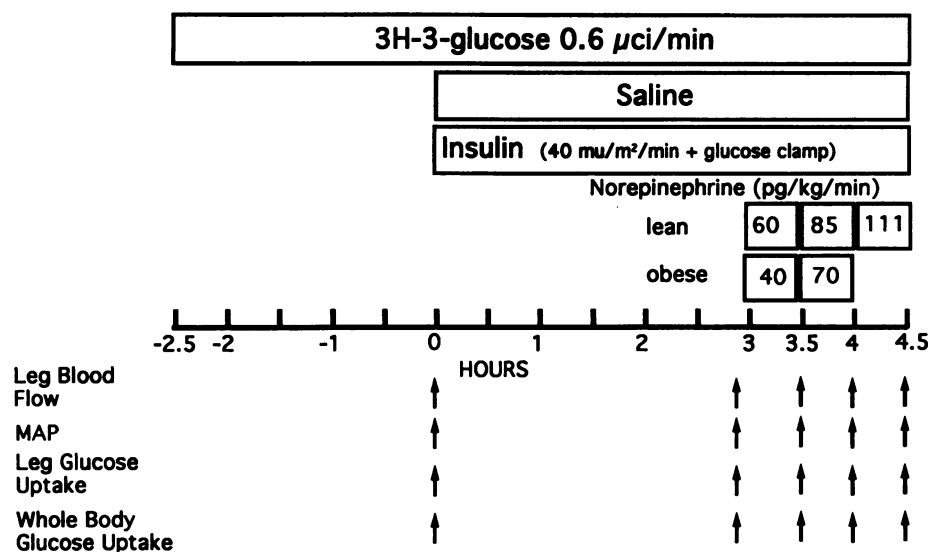


Figure 1. Study protocol. Whole-body and leg glucose uptake, MAP, LBF, and LVR = MAP/LBF were measured at baseline, after 3 h of saline infusion or a euglycemic hyperinsulinemic clamp performed on a separate day and during a superimposed graded venous infusion of norepinephrine in a group of seven lean and seven obese volunteers.

level during the hyperinsulinemic clamp studies. Serum glucose concentrations during hyperinsulinemia were clamped and thus, did not change from baseline.

### Glucose turnover

**Glucose clamp studies.** After basal measurements were obtained, insulin was infused in a square wave fashion and a 20% dextrose solution was infused at a variable rate to keep the serum glucose concentration at the baseline level according to arterial serum glucose determinations performed at ~ 5-min intervals. The clamp studies were carried out for 180 min to achieve near steady-state glucose infusion rates and glucose disposal rates were calculated on the basis of data obtained over the last 40 min of each study. During each clamp  $K_2HPO_4$  (~ 0.0038 meq/kg per min) was infused to prevent hypokalemia and hypophosphatemia. Serum potassium levels were > 3.5 meq/liter at all times during hyperinsulinemic clamps. Serum phosphate was not measured.

**Isotopic studies.** Rates of glucose appearance ( $R_a$ ) and glucose disappearance ( $R_d$ ) during basal conditions and during saline and euglycemic hyperinsulinemic clamp studies were measured isotopically by a primed continuous infusion of high performance liquid chromatography purified D-[3- $^3H$ ]glucose (sp act 16.8 Ci/mmol, New England Nuclear, Boston, MA) as previously described (18). The tritiated glucose infusion was allowed to label the glucose pool for 120 min; subsequently, blood for plasma glucose specific activity was obtained at 10-min intervals for 30 min. Basal  $R_d$  was calculated by dividing the tritiated counts infused by the mean specific activity calculated over the 30-min basal period. During infusion studies blood for plasma glucose specific activity was obtained at 20-min intervals and glucose turnover was calculated assuming non-steady-state kinetics. During NE infusion blood for glucose specific activity was attained at 10-min intervals. Glucose was assumed to distribute a volume of 19% of body weight and the pool fraction was assumed to be 0.65. Although this tracer technique has been criticized for underestimating the  $R_a$  and thus  $R_d$ , this is largely due to modeling error inherent in the assumption of volume of distribution and pool fraction. As glucose turnover enters a near-steady state, the impact of these assumed parameters becomes negligible and underestimates of  $R_a$  are small. In the current studies  $R_a$  was calculated over the last 40 min of each clamp study (140–180 min) when the glucose infusion rate was in a near-steady state. Underestimates of  $R_a$  were never greater than 10% of the glucose infusion rate. Negative rates of endogenous glucose output were assumed to represent 0 glucose output. During NE infusion, conditions deviated greatly from steady state and thus, isotopically determined  $R_d$  is likely to be underestimated to a greater extent.

### Hemodynamic measurements

All hemodynamic measurements were carried out with subjects in the supine position under quiet conditions with the room temperature maintained at ~ 22°C.

**Cardiac output.** Cardiac output (CO) was measured by the dye dilution technique at baseline and during NE infusion in a group of four lean subjects. A bolus of 5 mg of indocyanine green dye (Cardio-green, Becton-Dickinson Microbiology Systems, Cockeysville, MD) was injected into the central venous circulation via a 12-in. catheter (Intracath, Deseret Medical, Sandy, UT) inserted into a left antecubital vein and threaded cephalad to lodge in a thoracic vein. After dye injection, arterial blood was continuously withdrawn via a model SW-367 withdrawal pump through a model DC-410 densitometer cuvette connected to a model CO-10 cardiac output (CO) computer (Waters Instruments, Rochester, MN), which integrates the dye dilution curves. Each dilution curve was recorded on a chart recorder and inspected for integrity. The mean of three CO measurements was taken as the representative value.

**MAP and heart rate.** MAP was continuously monitored via a transducer (Sorenson Transpac, Abbott Critical Care Systems, North Chicago, IL) connected to a vital signs monitor (Physiocontrol VSM 1, Redmond, WA). The mean of five MAP values was taken as the representative MAP.

Heart rate was monitored via precordial leads connected to the vital signs monitor. The representative heart rate was the mean of five values.

**Vascular resistance.** Systemic and leg vascular resistance was calculated by dividing the MAP (mmHg) by the mean CO (liters/min) and LBF (liters/min) and expressed in arbitrary units.

### Analytical methods

Blood for serum glucose determinations was drawn, put in untreated polypropylene tubes, and centrifuged with an Eppendorf microcentrifuge (Brinkmann Instruments Inc., Westbury, NY). The glucose concentration of the supernatant was then measured by the glucose oxidase method with a glucose analyzer (model 23A, Yellow Springs Instruments, Yellow Springs, OH). Blood for determination of serum insulin concentrations was collected in tubes treated with aprotinin (500 kIU/ml) and allowed to clot. The specimens were spun and the supernatant was removed and stored at -20°C. Serum insulin levels were measured by double-antibody radioimmunoassay. Norepinephrine levels were measured in heparinized plasma by the radioenzymatic assay utilizing purified phenylethanolamine *N*-methyl transferase (19). Blood for determination of plasma glucose specific activity was collected in sodium fluoride-treated tubes and immediately placed on ice. The specimens were spun and the supernatant was removed and stored at -20°C. At the time of assay, the serum was thawed and diluted and the proteins were precipitated with 0.6 M perchloric acid. The supernatant was aliquoted and evaporated to dryness, resuspended in 0.5 ml of distilled water to which 10 ml of liquid scintillation counting fluid was added (Ecosinct, Manville, NJ), and counted for 5 min.

### Statistical analysis

Data are expressed as mean±SEM. Comparisons between body type groups at baseline were made with a group *t* test. Comparisons between studies were made using repeated measures analysis of variance. Body type was used as a grouping factor while infusate and time were treated as repeated measures. If the ANOVA had significant interaction terms or main effects, *t* tests were used to define where the differences occurred. During NE infusion, percent changes in the various parameters are expressed relative to the value obtained after either the 3-h saline or 3-h insulin infusion.

## Results

### Glucose and hormone data (Table II)

Fasting arterial plasma glucose concentrations were similar in lean and obese groups. Fasting insulin concentrations were higher in the obese than in lean,  $P < 0.04$ . Baseline recumbent arterial plasma NE levels were comparable in both groups,  $P = NS$ .

Steady-state glucose concentrations were not different from baseline at any time during the euglycemic hyperinsulinemic clamps. In contrast, during saline infusion arterial glucose concentrations rose ~ 20% above baseline in both lean and obese at the second highest NE infusion rate,  $P = 0.01$ .

Serum insulin concentrations during the 3-h saline infusion remained unchanged from baseline in both groups. During combined saline and NE infusion, serum insulin levels were unchanged from baseline despite the significant rise in the prevailing serum glucose concentration, suggesting inhibition of endogenous insulin secretion by NE.

Steady-state insulin levels achieved during the clamp period were not different between obese and lean and tended to fall but not significantly during the NE infusion period in either group.

Steady-state arterial NE concentrations were unchanged from baseline during saline infusion but increased ~ 30% dur-

Table II. Serum Insulin, Arterial Plasma Glucose and Femoral Blood Arteriovenous Glucose Differences (FBAVGΔ) at Baseline, during Saline, or Euglycemic Hyperinsulinemia and Superimposed Graded Norepinephrine Infusion

		Bas	Clamp/Sal	NE-1	NE-2	NE-3
Saline						
Serum insulin ( $\mu\text{U/ml}$ )	Lean	2.3 $\pm$ 0.64	2.1 $\pm$ 0.5	2.3 $\pm$ 0.6	2.2 $\pm$ 0.005	2.2 $\pm$ 0.06
	Obese	15.5 $\pm$ 4.0	13.8 $\pm$ 4.7	11.7 $\pm$ 2.7	7.2 $\pm$ 0.5	7.4 $\pm$ 0.1
Plasma glucose (mg/dl)	Lean	91.9 $\pm$ 1.2	90.8 $\pm$ 1.3	101.8 $\pm$ 1.7	107.4 $\pm$ 2.2	111.9 $\pm$ 3.3
	Obese	93.4 $\pm$ 1.7	90.4 $\pm$ 2.0	103.6 $\pm$ 3.5	112.8 $\pm$ 5.3	114.7 $\pm$ 15.4
FBAVGΔ (mg/dl)	Lean	1.7 $\pm$ 0.3	1.8 $\pm$ 0.4	4.3 $\pm$ 1.0	3.5 $\pm$ 0.9	2.6 $\pm$ 0.9
	Obese	1.6 $\pm$ 0.5	0.5 $\pm$ 0.3	3.5 $\pm$ 0.8	3.0 $\pm$ 0.7	3.4 $\pm$ 0.8
Insulin						
Serum insulin ( $\mu\text{U/ml}$ )	Lean	3.6 $\pm$ 1.2	67.7 $\pm$ 4.5	64.6 $\pm$ 3.9	60.7 $\pm$ 3.8	59.21 $\pm$ 5.0
	Obese	17.6 $\pm$ 4.1	87.3 $\pm$ 13.7	76.7 $\pm$ 14.1	81.7 $\pm$ 15.7	50.13 $\pm$ 16.1
Plasma glucose (mg/dl)	Lean	90.5 $\pm$ 1.4	89.9 $\pm$ 1.7	84.6 $\pm$ 2.1	86.3 $\pm$ 1.7	87.4 $\pm$ 1.7
	Obese	94.0 $\pm$ 1.8	90.9 $\pm$ 2.2	85.7 $\pm$ 2.1	91.1 $\pm$ 2.1	89.5 $\pm$ 3.0
FBAVGΔ (mg/dl)	Lean	2.5 $\pm$ 0.5	21.7 $\pm$ 1.3	26.5 $\pm$ 2.7	26.3 $\pm$ 2.1	26.2 $\pm$ 2.0
	Obese	1.2 $\pm$ 0.3	7.5 $\pm$ 2.1	10.3 $\pm$ 2.3	11.5 $\pm$ 2.0	12.6 $\pm$ 5.6

NE 1-2-3, norepinephrine infusion dose level.

ing euglycemic hyperinsulinemia in both groups,  $P < 0.05$ . During NE infusion (first two doses and paired saline-insulin data only), the NE levels achieved were not significantly different in lean and obese during either study condition despite  $\sim 25\%$  lower NE infusion rates in the obese, suggesting reduced NE metabolic clearance in obese subjects. NE concentrations were  $\sim 16\text{--}20\%$  higher during saline vs. insulin infusion ( $P < 0.04$ ) lean (paired data only), suggesting an effect of insulin to increase NE metabolic clearance. Indeed, the slope of the regression line relating NE infusion rate to NE levels (including all NE doses and nonpaired data) in lean subjects was 25% greater during saline than insulin studies,  $y = 29.2x - 89$  vs.  $y = 23.4x - 103$ ,  $P < 0.05$  (Fig. 2). Because of the small number of obese subjects receiving all doses of NE during saline infusion, the effect of insulin on NE metabolic clearance could not be ascertained in this group. However, analysis of the combined data during insulin infusion from the first and second NE infusion levels (12 data points) reveals that obese subjects exhibit a  $\sim 20\%$  reduction in NE metabolic clearance compared to lean (14 data points),  $P = 0.056$ .

#### Whole-body and leg glucose kinetics (Table III)

Basal rates of glucose uptake at the level of whole body and leg (expressed in mg/100 ml leg volume per min) were unchanged during the 3-h saline infusion. When NE infusion was superimposed upon the saline infusion, rates of LGU and WBGU increased in lean ( $P < 0.001$ ) and obese ( $P < 0.001$ ) over the first two doses of NE. This rise in glucose uptake was likely accounted for by the rise in the prevailing serum glucose concentration and muscle blood flow observed during NE infusion.

During hyperinsulinemia, basal LGU increased markedly in both groups ( $P < 0.001$ ). Steady-state insulin mediated LGU was  $\sim 50\%$  lower in obese vs. lean,  $P < 0.001$ . In lean, when the graded NE infusion was superimposed upon the hy-

perinsulinemic clamp, rates of LGU and WBGU exhibited a maximal rise of  $\sim 24\%$  and  $\sim 17\%$   $P < 0.05$ , respectively. In obese, low dose NE infusion caused a  $\sim 24\%$  and  $\sim 8\%$  rise in LGU and WBGU ( $P < 0.04$ ), respectively.

#### Hemodynamic data

Mean arterial pressure (Fig. 3). Basal MAP was somewhat higher (but not significantly) on the day of insulin than on the day of saline studies  $86.6 \pm 1.6$  vs.  $80.3 \pm 2.4$  mmHg,  $P = \text{NS}$  in

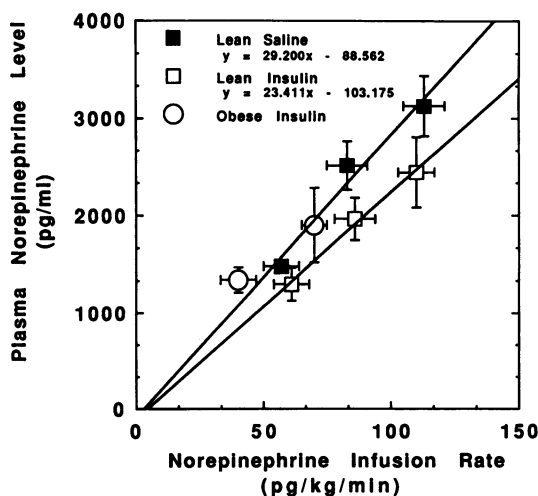


Figure 2. Relationship between three NE infusion rates and the prevailing plasma NE concentrations achieved at each infusion rate in lean subjects during saline or insulin infusion and in obese subjects during insulin infusion only. Data were insufficient in obese subjects infused with NE during saline and at the third and highest NE infusion rate during hyperinsulinemia.

Table III. Rates of Glucose Uptake in Whole-Body and Leg during Saline and Hyperinsulinemic Euglycemic Clamp Studies and Superimposed Norepinephrine Infusion

	Basal	Clamp/Sal	NE-1	NE-2	NE-3
<b>Saline</b>					
Lean					
LGU (mg/100 ml leg volume per min)	0.37±0.00 (7)	0.42±0.09 (7)	1.15±0.26 <sup>‡</sup> (7)	1.19±0.42 <sup>‡</sup> (7)	0.926±0.32 <sup>‡</sup> (7)
WBGU (mg/kg per min)	1.90±0.14 (7)	1.85±0.09 (7)	1.95±0.05 <sup>‡</sup> (7)	2.03±0.12 <sup>‡</sup> (7)	1.77±0.06 (5)
Obese					
LGU (mg/100 ml leg volume per min)	0.50±0.24 (7)	0.19±0.12 (7)	1.46±0.69* (5)	1.26±0.37* (2)	0.96 (2)
WBGU (mg/kg per min)	1.48±0.08 (7)	1.27±0.06 (7)	1.37±0.11* (7)	1.37±0.12* (3)	1.52 (2)
<b>Insulin</b>					
Lean					
LGU (mg/100 ml leg volume per min)	0.56±0.11 (7)	7.75±0.96 (7)	10.14±1.24* (7)	10.31±1.22* (7)	9.38±1.31* (7)
WBGU (mg/kg per min)	1.97±0.15 (7)	8.18±0.41 (7)	8.73±0.39* (7)	8.93±0.31* (7)	9.13±0.44 (7)
Obese					
LGU (mg/100 ml leg volume per min)	0.28±0.09 (7)	3.76±1.28 (7)	4.91±1.62* (6)	4.19±1.87* (5)	6.33* (2)
WBGU (mg/kg per min)	1.54±0.08 (7)	3.66±0.61 (7)	4.02±0.66* (7)	4.17±0.78* (6)	5.23* (2)

\*  $P < 0.05$  vs. steady-state (clamp) hyperinsulinemia or saline infusion.

<sup>‡</sup>  $P < 0.01$  vs. steady-state (clamp) hyperinsulinemia or saline infusion.

Abbreviations: (n), number of subjects studied at each condition; NE 1-2-3, norepinephrine infusion dose level.

lean and not different on either day in obese  $91.8 \pm 3.1$  vs.  $89.8 \pm 3.4$  mmHg,  $P = \text{NS}$ . Although clinically normotensive obese subjects had higher basal MAP compared to lean,  $P < 0.04$ . During saline infusion basal MAP was unchanged in both lean and obese groups. During euglycemic hyperinsulinemia basal MAP fell  $\sim 4.16 \pm 1.59\%$  in lean and by  $\sim 5.33 \pm 1.7\%$  in obese  $P < 0.03$  vs. baseline for both groups so that steady-state MAP was not significantly different between groups.

During insulin studies all seven lean volunteers were able to tolerate all three NE doses ( $\sim 0.06, 0.09, 1.1 \mu\text{g/kg per min}$ ) and maintain a systolic blood pressure  $\leq 160$  mmHg. During saline studies seven lean subjects received the two first doses but only five were able to tolerate the third and highest dose. During insulin studies in the obese group all seven volunteers tolerated an average initial NE dose of  $0.4 \pm 0.01 \mu\text{g/kg per min}$ , six tolerated a second dose of  $0.07 \pm 0.0 \mu\text{g/kg per min}$  and only two tolerated a third and highest dose of  $0.09 \pm 0.01 \mu\text{g/kg per min}$ . During saline studies seven obese volunteers tolerated the initial NE dose, three the second dose and only two received the third dose.

Among lean subjects only five tolerated all NE doses during both saline and insulin conditions. The maximal NE dose lead to an augmentation of MAP from  $77.2 \pm 2.4$  (value after 3-h saline infusion) to  $101.1 \pm 2.3$  mmHg ( $31 \pm 4\%$ ,  $p < 0.004$ ) during saline infusion. In contrast, during hyperinsulinemia MAP only rose  $17.5 \pm 2.8\%$  from  $84.7 \pm 2.3$  (value after 3-h hyperinsulinemic clamp) to  $99.3 \pm 1.5$  mmHg ( $P < 0.004$ ), a level significantly lower than that achieved during saline only,  $P < 0.001$ . Thus, insulin caused a shift to the right in NE/pressor dose response curve in lean subjects (Fig. 3).

Given the high rate of intolerance of obese subjects to the pressor effects of NE (particularly during saline studies),

meaningful statistical analyses could only be carried out for the first and second NE dose (paired data only). In obese the increment in MAP at the first dose was similar during saline or hyperinsulinemia,  $12.5 \pm 1.8$  vs.  $12.5 \pm 1.8$  mmHg,  $P = \text{NS}$ . Similarly, at the second NE dose MAP increased by equivalent amounts ( $14.7 \pm 2.0$  vs.  $16.6 \pm 2.0$ ) to  $105.2 \pm 3.4$  and  $102.7 \pm 1.5$  mmHg with saline and insulin, respectively  $P = \text{NS}$  (Fig. 3). Thus, in obese insulin had no effect to modulate the NE/pressor dose-response relationship.

Because of the small number of obese subjects who tolerated the higher NE infusion rates during saline infusion, a meaningful comparison of the NE pressor effect on lean vs. obese could not be accomplished. However, such comparison was possible during insulin infusion. At similar prevailing NE concentrations ( $1965 \pm 219$  vs.  $1900 \pm 382$ ) the rise in MAP was  $10.95 \pm 2.2\%$  in lean ( $n = 7$ ) and  $17.6 \pm 2.8\%$  ( $n = 6$ ) in obese, respectively,  $P < 0.05$  lean vs. obese (Fig. 4).

In both groups increments in MAP were secondary to roughly equivalent proportional increases in both systolic and diastolic blood pressure (data not shown). As expected, the heart rate decreased consistently in both groups in response to the rise in MAP and the magnitude of the decrease was similar in each group.

*Leg blood flow, vascular resistance, and cardiac output.* Over the 3-h saline infusion, LBF was unchanged from baseline in both lean and obese groups (Fig. 5). After three hours of euglycemic hyperinsulinemia LBF increased by  $57.0 \pm 8.8\%$  in lean ( $P < 0.003$ ) and  $35.3 \pm 19.4\%$  in obese ( $P = \text{NS}$ ).

In lean during NE infusion with saline, LBF increased from a baseline value of  $0.20 \pm 0.03$  liters/min (value after 3-h saline infusion) to  $0.24 \pm 0.03$ ,  $0.27 \pm 0.04$ , and  $0.31 \pm 0.05$  liters/min ( $\sim 55\%$  increase above baseline) over the range of NE doses,  $P < 0.001$  (Fig. 5A). In lean during hyperinsulinemia, superim-

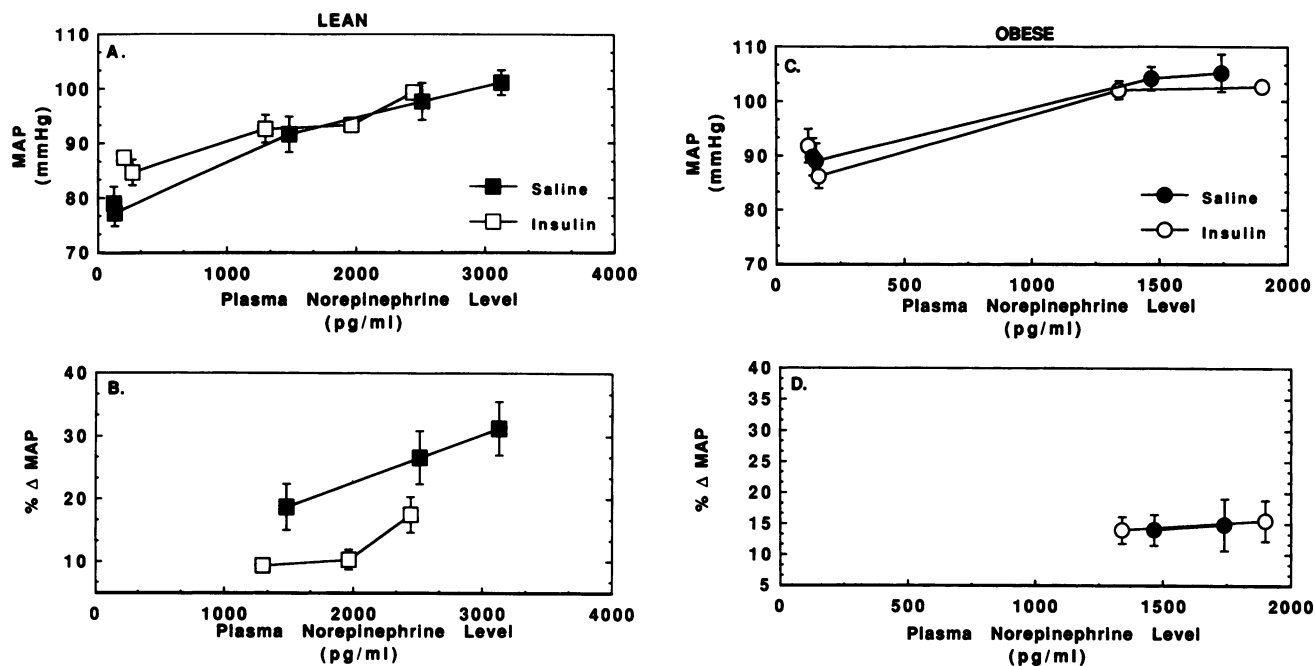


Figure 3. Upper panels: MAP as a function of prevailing plasma NE concentrations at baseline, during 3 h of saline infusion or steady-state euglycemic hyperinsulinemia and during three graded NE infusions in lean (A) and obese (C). Note that during saline infusion, MAP was unchanged and thus, the 3-h data point is superimposed over the basal data point. Lower panels: Percent increment in MAP during three graded NE infusions. Percent changes are calculated relative to the MAP values observed after 3 h of saline or euglycemic hyperinsulinemia in lean (B) and obese (D).

posed NE infusion had no significant effect to decrease LBF below that achieved by insulin alone (Fig. 5). NE infusion in obese led to no significant change in LBF during either hyperinsulinemia or saline infusion (Fig. 5). However, because of the small number of obese receiving the higher NE doses and because in one obese subject leg blood flow could not be measured for technical reasons, there are not enough data to ana-

lyze or examine the effects of higher doses of NE on LBF (or LVR).

LVR (Fig. 6) was unchanged from baseline in both lean and obese after 3 h of saline infusion. With euglycemic hyperinsulinemia LVR fell  $37.8 \pm 3.6\%$  ( $P < 0.001$ ) in lean and  $21.9 \pm 10.0\%$  ( $P = 0.056$ ) in obese,  $P = \text{NS}$  lean vs. obese.

In lean at the highest NE level during saline infusion, LVR fell  $17.5 \pm 5.3\%$  below baseline ( $P < 0.05$ ). With insulin infusion LVR at the highest NE level was somewhat higher but not significantly changed from steady-state hyperinsulinemia. In obese, NE infusion after 3 h of either saline or insulin did not significantly change LVR.

Because of the unexpected effect of NE in lean subjects to increase LBF and reduce LVR in the face of a large rise in MAP, we performed simultaneous measurements of LBF and CO in a subset of four lean subjects. The effect of NE given at two doses ( $0.05$  and  $0.01 \mu\text{g/kg}$  per min) on systemic and leg (muscle) vascular resistance (Fig. 7) was examined. In response to NE infusion systemic vascular resistance (SVR) maximally rose by  $44.6 \pm 23.9\%$  and MAP by  $22.9 \pm 4.1\%$ , respectively,  $P < 0.001$  between baseline and all values. In contrast, leg blood flow rose maximally by  $75.7 \pm 48.0\%$ , and LVR fell by  $25.9 \pm 9.9\%$ ,  $P < 0.001$  between baseline and all values. Thus, systemic NE infusion sufficient to cause a significant rise in MAP and SVR was accompanied by a significant fall in skeletal muscle vascular resistance.

## Discussion

The current study reveals a number of novel observations regarding the physiologic interactions among insulin action, insulin sensitivity, and noradrenergic agonism in humans: (a)

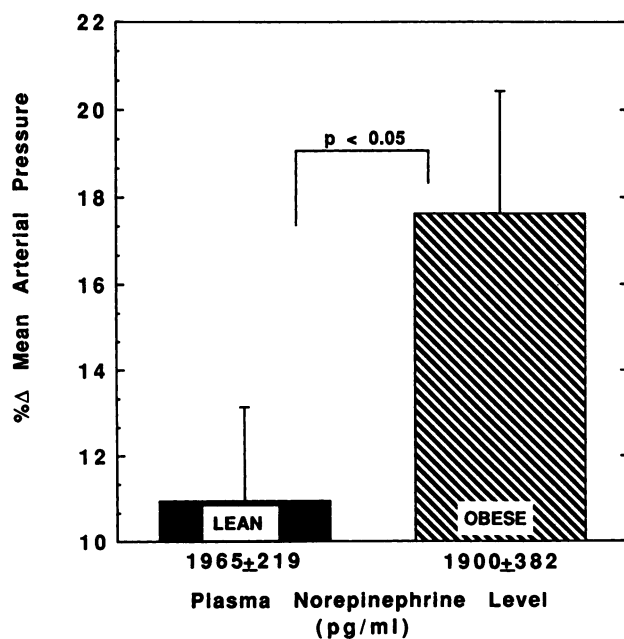


Figure 4. Relative incremental rise in MAP at equivalent prevailing plasma NE concentrations in lean and obese subjects.

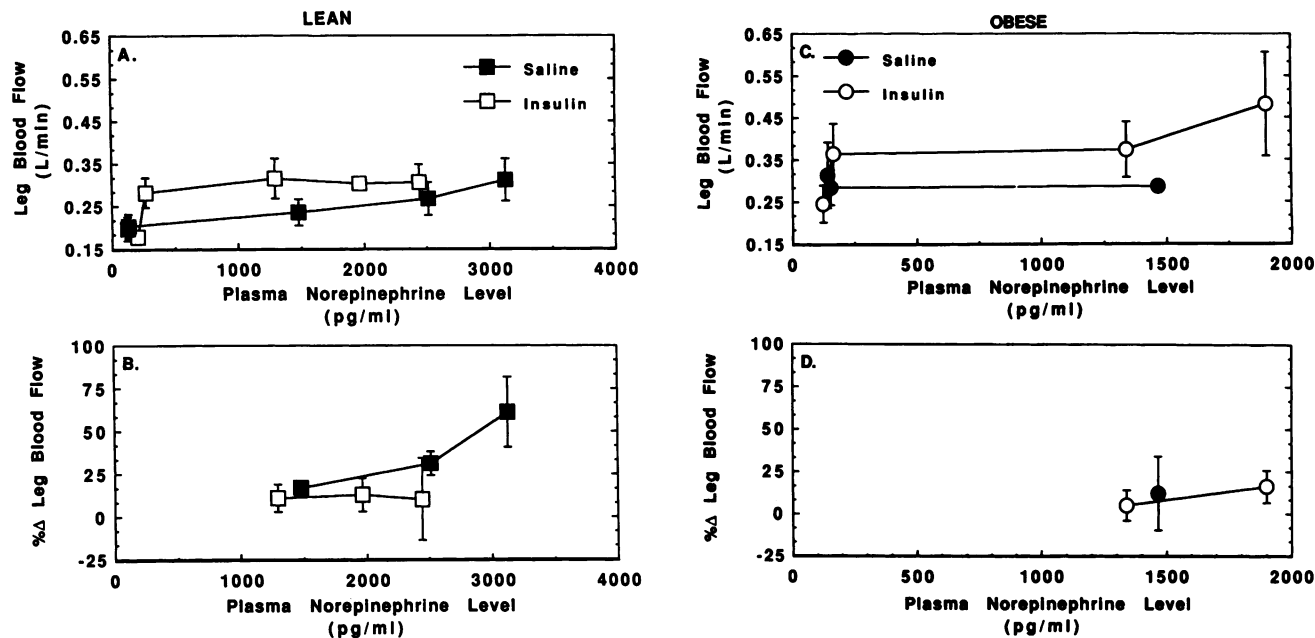


Figure 5. Upper panels: Rates of leg blood flow as a function of prevailing plasma NE concentrations at baseline, after 3 h of saline infusion or hyperinsulinemic euglycemia (steady-state) and three graded NE infusions in lean (A) and obese (C). Lower panels: Percent increment in LBF during three graded NE infusions. Percent changes are calculated relative to the LBF rates observed at steady-state in lean (B) and obese (D).

compared to lean insulin-sensitive subjects, obese insulin-resistant subjects appear more sensitive to NE's pressor action; (b) insulin causes a shift to the right in the NE dose response curve and this effect is more marked in lean than in obese; (c) insulin increases the metabolic clearance rate of NE in lean and this effect may be blunted in obese; (d) systemically infused NE in doses sufficient to cause a rise in MAP and SVR can reduce

LVR (skeletal muscle vascular resistance); and lastly, (e) pressor doses of NE lead to an increase in insulin mediated glucose uptake.

One of the major objectives of this study was to determine if insulin resistance associated with the state of obesity is associated with enhanced pressor responsiveness. In our study design it is very apparent that obese insulin-resistant subjects ex-

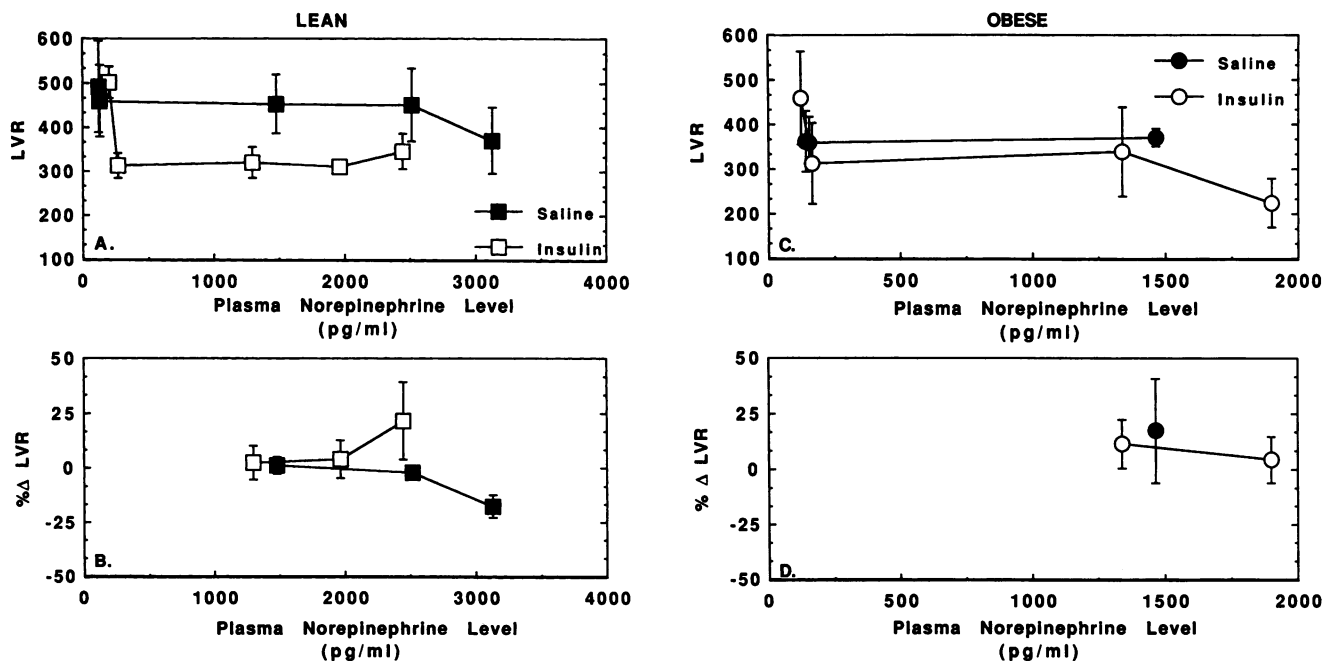


Figure 6. Upper panels: LVR (expressed in arbitrary units) as a function of prevailing plasma NE concentrations at baseline after 3 h of saline infusion or hyperinsulinemic euglycemia (steady-state) and during three graded NE infusions in lean (A) and obese (C). Lower panels: Percent increment in LVR during three graded NE infusions. Percent changes are calculated relative to the LVR rates observed at steady state in lean (B) and obese (D).

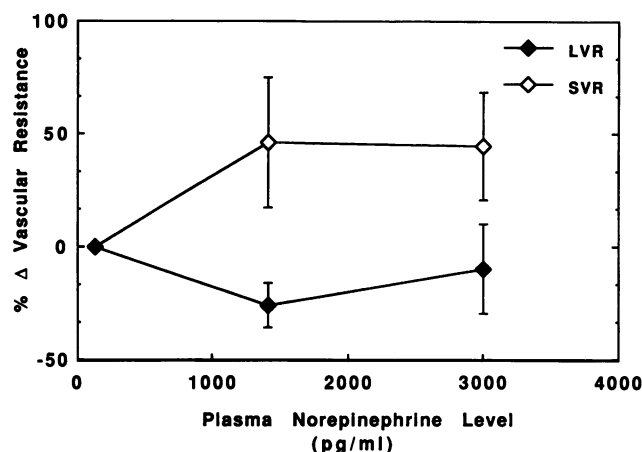


Figure 7. Relative changes from baseline in LVR and systemic vascular resistance (SVR) in response to a two-step NE infusion of 50 and 100 pg/kg per min in a group of four lean volunteers.

hibited greater increments in MAP than lean insulin-sensitive subjects in response to similar prevailing NE concentrations. Moreover, NE metabolic clearance was reduced in obese, leading to higher NE concentrations for a given NE infusion rate. As a result, compared to lean subjects fewer obese were able to tolerate the NE infusions without exceeding a systolic blood pressure of 160 mmHg. It is important to make very clear that because of the large attrition rate of obese subjects undergoing NE infusion it was not possible to directly compare the slopes of the complete NE pressor dose–response curves between lean and obese groups. This is so because with progressively higher NE infusion rates only those obese subjects able to tolerate the NE pressor effect (only two at the highest NE infusion rate) are included in the analysis, thus selecting for those obese subjects least sensitive to the pressor and, therefore, tending to minimize any differences between the groups.

It is also important to note that the resting MAP was ~ 9% higher in the obese group. Therefore, it is possible that the apparent increase in NE sensitivity in obese is related to their relative elevation in baseline MAP. Notwithstanding, the pressor response to NE during euglycemic hyperinsulinemia (when steady-state or clamp MAP was similar in both groups) indicates that for a given level of circulating NE (~ 1,900 pg/ml) MAP rises less in lean than in obese (Fig. 4). Thus, these data demonstrate the greater sensitivity to the pressor effect of NE in obese compared to lean subjects during hyperinsulinemia.

Whether the apparent increase in NE sensitivity in obese during saline infusion is secondary to their relatively higher basal MAP cannot be completely ruled out. However, within the obese group there was no relationship between basal MAP and NE tolerance, i.e., those with the highest basal MAP were not necessarily those who could not receive all three NE doses. Nevertheless, further studies will be necessary to definitively sort out whether greater NE sensitivity is secondary to obesity per se versus higher blood pressure.

Hyperinsulinemia caused a shift to the right in the NE pressor dose–response curve in the lean, but not in the obese (Fig. 3). These data demonstrate the potent mitigating effect of insulin on the NE pressor response in insulin-sensitive individuals and the lack of this depressor effect in obese insulin-resistant individuals. This observation has obvious and important im-

plications with respect to the high prevalence of hypertension in insulin resistant states, such as obesity and non-insulin-dependent diabetes mellitus (20, 21). Indeed, if one considers that vascular tone and blood pressure are in large part set by the balance of pressor and depressor forces (both hormonal and neural), it is apparent that obesity (and probably other insulin-resistant states) is associated with a diminution or even loss of the potent depressor effects of insulin. While it is clear that, in itself, loss of insulin mediated vasodilation is not sufficient to cause hypertension, the collusion of one or more superimposed pressor insults such as an increase in the activity of the SNS or of the renin–angiotensin–aldosterone system might be sufficient for the clinical expression of frank hypertension. Therefore, one can reasonably propose a hypothetical pathogenetic scheme where impairment of insulin-mediated vasodilation associated with insulin resistance of obesity acts as a risk factor for the development of hypertension. It is important to emphasize that in this context, insulin resistance and not hyperinsulinemia per se is putatively instrumental in the development of hypertension. This view is contrary to numerous recently published opinions suggesting a role for hyperinsulinemia in the causation of hypertension via a number of previously cited mechanisms (3, 9, 10, 12, 23).

An effect of insulin to decrease vascular reactivity to pressors has previously been reported in isolated vessels (7, 22), animals (24) and most recently in humans (8). Our study employing systemic NE infusions cannot assess vascular reactivity per se (which can only be assessed by local infusions) but rather addresses for the first time the effects of insulin and insulin sensitivity to modulate pressor action at the systemic level.

The mechanism underlying insulin's effect to reduce NE's pressor action is not known. Although it is possible that insulin modulates the NE signal transduction/effector system mechanism, it is more likely that insulin has independent vasodilating actions which mitigate NE pressor effects.

At this juncture, a brief comment is warranted with respect to the study of obesity as a model of insulin resistance. Obesity is associated with a particular pattern of hemodynamics (21, 25). Therefore, it may not necessarily follow that our findings in obese subjects are generalizable to all insulin-resistant states. Since most insulin-resistant states are associated with an increase in adipose mass, the distinction between insulin resistance per se and obesity is difficult. Thus, it is not possible to ascertain whether hypersensitivity to NE's pressor effect is a property of obesity, insulin resistance, or both. On the other hand, differences in NE action between obese and lean in the context of insulin infusion are more likely the result of differences in insulin sensitivity rather than obesity per se. Previous reports of diminished insulin mediated vasodilation in both insulin-dependent (26) and non-insulin-dependent diabetes (27) strongly suggest that our observations are relevant to other states of insulin resistance but confirmation of this notion will require further study.

It is interesting to note that hyperinsulinemia led to a 25% increase in the metabolic clearance rate of NE in lean. The effect of insulin on NE metabolic clearance in obese was not possible to assess given the small number of subjects who tolerated the infusion protocol. However, the metabolic clearance of NE in obese during insulin infusion was similar to that of lean during saline infusion. These data suggest that the metabolic clearance of NE in obese was either reduced or that the effect of insulin to increase the metabolic clearance rate of NE



was diminished in these subjects (Fig. 2). An effect of insulin to increase NE metabolic clearance has previously been reported (6), however, the apparent lack of this effect in obese insulin resistant man is a novel observation. However, because of the relatively small number of subjects and the borderline significance level ( $P = 0.056$ ), further studies will be required to confirm this finding.

NE is principally a neurotransmitter although it can also act as a classic hormone (28). As a neurotransmitter it is released at the level of the neural synaptic cleft where some escapes or spills over to the circulation. The principal mode of NE metabolic clearance is reuptake at the level of the neuronal synapse (29). Therefore, insulin's effect to cause an apparent increase in NE metabolic clearance is most likely to have occurred via an increase in the reuptake rate of NE as previously suggested (30). Regardless of the mechanism for enhanced NE clearance by insulin, reduced NE metabolic clearance in obese insulin resistant subjects could represent another mechanism for the increase in susceptibility to the development of hypertension in this population. It should be noted, however, that the cardiovascular effects of infused NE may not reflect normal physiology inasmuch as it may not mimic the effects of neuro-nally released NE.

A paradoxical observation of our study is that of increased insulin sensitivity in response to NE infusion, particularly among lean subjects. Indeed, when NE infusion was superimposed after 180 min of euglycemic hyperinsulinemia we observed a significant and apparently dose-dependent increase in whole body and leg glucose uptake, which was greater and more rapid than that expected from merely prolonging the insulin infusion beyond 180 min (31). Recent published reports have indicated that essential hypertension is an insulin resistant state (10, 32), and others (33) have suggested that SNS activation could lead to insulin resistance. Moreover, local NE infusion is classically associated with a rise in limb vascular resistance (decreased blood flow) via  $\alpha$ -adrenergic agonism. Therefore, one would not have expected NE infusion, which caused a marked rise in MAP, to enhance insulin sensitivity. Our data indicate that in the absence of insulin effect, NE infusion caused both a marked rise in SVR (44%), and a significant  $\sim 25\%$  fall in LVR (75% augmentation of LBF). It should be noted that this rise in muscle blood flow observed was only partially responsible for the augmentation of glucose uptake since the femoral  $\Delta$ AVG also increased during the NE infusion (Table II). Similar doses of NE delivered intra-arterially with no significant systemic pressor effect have clearly been shown to cause vasoconstriction (28), therefore, the decrease in LVR was unexpected. The most plausible mechanism for the NE induced increase in blood flow is via a baroreflex suppression of skeletal muscle SNS activity with ensuing vasodilation. It is noteworthy that in response to a similar rise in MAP, obese subjects did not exhibit as great an increase in leg blood flow or fall in LVR. Thus, it appears that obesity (or insulin resistance?) is associated with a diminished baroreflex. On a more speculative note, it is not unreasonable to postulate that an altered baroreflex in obese subjects (and patients with hypertension) could contribute to the insulin resistance observed in these patients via a hemodynamic mechanism (reduced glucose and insulin delivery). In addition, alterations in baroreflex could act as a predisposing factor in the pathogenesis of hypertension. Finally, because muscle (leg) vascular resistance was largely unchanged during NE infusion in the lean, it follows that the

rise in MAP must have been secondary to a rise in resistance in other vascular beds, the most likely of these is the splanchnic circulation.

In conclusion, the current report suggests novel dynamic interactions between NE and insulin action. Specifically, (a) insulin sensitivity plays an important modulating role on NE pressor action and in the physiological regulation of vascular tone; (b) insulin resistance in obese subjects is likely to act as a predisposing but not sufficient factor in the development of hypertension; and (c) elevated but physiological circulating NE concentrations do not cause insulin resistance but actually enhance insulin's action to stimulate glucose uptake.

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