

Obesity/Insulin Resistance Is Associated with Endothelial Dysfunction

Implications for the Syndrome of Insulin Resistance

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Abstract

To test the hypothesis that obesity/insulin resistance impairs both endothelium-dependent vasodilation and insulin-mediated augmentation of endothelium-dependent vasodilation, we studied leg blood flow (LBF) responses to graded intrafemoral artery infusions of methacholine chloride (MCh) or sodium nitroprusside (SNP) during saline infusion and euglycemic hyperinsulinemia in lean insulin-sensitive controls (C), in obese insulin-resistant subjects (OB), and in subjects with non-insulin-dependent diabetes mellitus (NIDDM). MCh induced increments in LBF were ~40% and 55% lower in OB and NIDDM, respectively, as compared with C ($P < 0.05$). Euglycemic hyperinsulinemia augmented the LBF response to MCh by ~50% in C ($P < 0.05$ vs saline) but not in OB and NIDDM. SNP caused comparable increments in LBF in all groups. Regression analysis revealed a significant inverse correlation between the maximal LBF change in response to MCh and body fat content. Thus, obesity/insulin resistance is associated with (a) blunted endothelium-dependent, but normal endothelium-independent vasodilation and (b) failure of euglycemic hyperinsulinemia to augment endothelium-dependent vasodilation. Therefore, obese/insulin-resistant subjects are characterized by endothelial dysfunction and endothelial resistance to insulin's effect on enhancement of endothelium-dependent vasodilation. This endothelial dysfunction could contribute to the increased risk of atherosclerosis in obese insulin-resistant subjects. (*J. Clin. Invest.* 1996. 97: 2601–2610.) Key words: obesity • insulin resistance • endothelium-dependent vasodilation • methacholine chloride • sodium nitroprusside

Introduction

Insulin has a specific and physiological action to vasodilate skeletal muscle vasculature in humans (1–3). This hemodynamic action appears to be important both for the maintenance

of vascular tone (4) and the modulation of substrate uptake (5). We have recently reported (6) that insulin's vasodilating effect is mediated by endothelium-derived nitric oxide (EDNO),¹ a finding which has been confirmed by others (7). Importantly, we demonstrated that insulin causes a shift to the left in the endothelium-dependent vasodilation produced by the muscarinic agonist methacholine chloride. In contrast, insulin had no effect upon the endothelium-independent vasodilation produced by sodium nitroprusside (6). These data suggested the novel concept that the endothelium is not merely a passive site for insulin transit to the tissues, but rather a target tissue for insulin action, at least with respect to the nitric oxide system (and perhaps others).

Insulin resistance states such as hypertension (8) and non-insulin-dependent diabetes mellitus (NIDDM) (9) have been reported to be associated with both defective insulin-mediated and endothelium-dependent vasodilation. These findings suggest the possibility that the endothelium may also exhibit resistance to insulin's action to modulate the EDNO system in these disease states. However, states of secondary hypertension, which are not associated with insulin resistance (vis-à-vis carbohydrate metabolism) (10), and hyperglycemia (11, 12) can also result in defective endothelium-dependent vasodilation. Therefore, whether insulin resistance, independent of confounding variables such as hypertension and hyperglycemia, is associated with abnormalities in endothelial function is not known. This question is of considerable clinical importance because the endothelium is considered to play a central role in the pathogenesis of atherosclerosis, and insulin resistance is often accompanied by a cluster of cardiovascular risk factors (insulin-resistance syndrome [13, 14]) that greatly increase the incidence of both coronary and peripheral vascular disease. Thus, endothelial abnormalities associated with insulin resistance could account, at least in part, for the vascular disease associated with insulin resistance.

Glucose tolerant, normotensive obese humans without family history of diabetes display both impaired insulin-mediated vasodilation and resistance to insulin's action to stimulate glucose disposal. Therefore, we tested the hypothesis that insulin resistance of obesity is associated with defective endothelium-dependent vasodilation. To this end, we studied healthy subjects exhibiting a large range of adiposity. In each subject, endothelium-dependent vasodilation was assessed by graded intrafemoral artery infusions of methacholine. In addition, we

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1. Abbreviations used in this paper: BMI, body mass index; C, lean insulin-sensitive controls; EDNO, endothelium-derived nitric oxide; GDR, glucose disposal rate; LBF, leg blood flow; LVR, leg vascular resistance; MAP, mean arterial blood pressure; MCh, methacholine chloride; NIDDM, non-insulin-dependent diabetes mellitus; OB, obese insulin-resistant subjects; SNP, sodium nitroprusside.

tested the ability of euglycemic hyperinsulinemia to shift the methacholine dose response curve to the left, as observed in lean insulin-sensitive subjects. Finally, to better distinguish the effects of adiposity versus those of diabetes we also studied a group of patients with non-insulin-dependent diabetes mellitus.

Methods

Subjects. Demographic characteristics of the subject groups in each study are given in Table I. Subjects were initially recruited and characterized as obese (OB) if their body mass index (BMI) was ≥ 28 and as lean controls (C) if their BMI was < 28 . Fat content was determined by DXA (dual energy x-ray absorptiometry, system software 1.2; Lunar DPX-L, Madison, WI). The weight limit for accurate determination of body composition with our DXA equipment is 110 kg, therefore subjects weighing over 110 kg had their body fat content measured by underwater weighing (15) ($n = 5$). Body fat content was determined in 41 out of 44 subjects among C and OB and in all subjects with NIDDM. Failure to attend the scheduled body fat measurement or equipment failure were the reasons for not obtaining body fat measurements in one OB and two C subjects.

Nondiabetic subjects (C and OB) had normal 75-g oral glucose tolerance tests (16). NIDDM was defined by either fasting hyperglycemia (serum glucose > 140 mg/dl, $n = 11$) or a diabetic range oral glucose tolerance test ($n = 2$) according to national diabetes data group criteria (16). Diabetic subjects were withdrawn from their oral hypoglycemic drugs for 3 wk ($n = 2$) or their insulin injections for 1 wk ($n = 10$) before study (one subject was receiving both oral hypoglycemic and insulin therapy, and two subjects were treated with diet alone). All study subjects except one were normotensive as determined by cuff pressure and were ingesting no medications other than their oral hypoglycemic agents. Studies were approved by the Indiana University Human Subjects Internal Review Board and all volunteers gave informed consent.

Diet. All subjects were admitted to the Indiana University General Clinical Research Center 2 d before study and were fed a weight-maintaining diet of which the caloric content was distributed as 50% carbohydrate, 30% fat, and 20% protein.

Drugs. All infusates were prepared under sterile conditions on the morning of the study. Regular insulin (Humulin; Eli Lilly and Co., Indianapolis, IN) was diluted in normal saline to the desired concentration with added albumin. Methacholine chloride (MCh) (Roche Laboratories, Division of Hoffman-La Roche Inc., Nutley, NJ) was dissolved in normal saline to a concentration of 25 μ g/ml, and sodium nitroprusside (SNP) (Roche Laboratories) was dissolved in normal saline to a concentration of 7 μ g/ml. Insulin was administered through a catheter in the antecubital vein. MCh or SNP was infused directly into the femoral artery using a Harvard programmable pump (model 44; Harvard Apparatus Inc., South Natick, MA).

Protocol. Separate groups of subjects were studied under two distinct study protocols designed to examine endothelium-dependent (methacholine study) and endothelium-independent vasodilation (nitroprusside study) under basal or insulin-stimulated conditions. Aspects of the protocol that are common to both studies are described below.

At $\sim 7:00$ a.m., after an overnight 14-h fast, a catheter was inserted into the antecubital vein for infusion of substances. Subsequently, the right femoral artery and vein were cannulated. A 5 French sheath (Cordis Corp., Miami, FL) was placed in the right femoral vein to allow the insertion of a custom designed 5 French double lumen thermodilution catheter (Baxter Scientific, Edwards Division, Irvine, CA) to measure leg blood flow as previously described (17). The right femoral artery was cannulated with a 5.5 French double lumen catheter (Arrow International, Reading, PA) to allow simultaneous infusion of substances through the proximal (most caudad) and invasive blood pressure monitoring through the distal port (most cephalad). Heart rate and mean arterial blood pressure (MAP) were monitored continuously via precordial leads and a pressure transducer connected to a vital signs monitor (VSM 1; Physiocontrol, Redmond, WA).

Hemodynamic measurements. All hemodynamic measurements were obtained with the subjects in the supine position in a quiet, temperature controlled room and after the subject had emptied his/her bladder. Baseline measurements of leg blood flow, mean arterial pressure, and heart rate were obtained after allowing at least 30 min of rest after the insertion of the catheters. During graded intrafemoral artery infusion of drugs (MCh or SNP), leg blood flow (LBF) measurements were begun 2 min after the onset of each dose. LBF measurements were performed every ~ 30 s for a total of 10 determinations at each drug dose. Invasively determined MAP and heart rate were recorded with every other LBF determination. Graded intrafemoral artery infusion of drugs were repeated after ~ 200 min of euglycemic hyperinsulinemia when glucose disposal rates and hemodynamic parameters were in a near steady state.

Euglycemic hyperinsulinemic clamps. All euglycemic hyperinsulinemic clamps were performed during a square wave systemic infusion of insulin at rates which varied according to the study protocols described below. The serum glucose concentration was kept at the baseline level in nondiabetic subjects and in the normoglycemic range (~ 90 mg/dl) in diabetic subjects by administering a 20% dextrose solution at a variable rate according to arterial serum glucose measurements obtained at 5-min intervals. K_2HPO_4 (~ 0.001 – 0.0038 meq/kg per min) was infused during the euglycemic hyperinsulinemic clamps to prevent hypokalemia and hypophosphatemia. Serum potassium levels were maintained above 3.5 meq/liter during all study conditions.

Insulin-stimulated glucose uptake. By design we individualized insulin infusion rates to cause an increase glucose uptake without markedly altering basal LBF. In previous studies (5), we have demon-

Table I. Demographic Characteristics of the Study Groups

	Methacholine studies			Nitroprusside studies		
	Control	Obese	NIDDM	Control	Obese	NIDDM
<i>n</i>	13	12	8	13	6	5
Age (yr)	35.2 \pm 1.3	35.4 \pm 1.5	40.4 \pm 2.5	33.1 \pm 1.2	28.5 \pm 2.4	36.2 \pm 5.0
BMI (wt/height ²)	23.2 \pm 0.7*	33.8 \pm 1.6	36.0 \pm 3.4	23.5 \pm 0.8*	32.9 \pm 3.0	34.7 \pm 3.4
Percent body fat	19.2 \pm 2.3*	35.7 \pm 3.5	34.4 \pm 3.1	18.7 \pm 1.3*	33.2 \pm 4.1 [‡]	43.5 \pm 3.7
Basal mean arterial blood pressure (mmHG)	89.2 \pm 3.3	99.5 \pm 2.5 [§]	97.5 \pm 2.4	88.0 \pm 3.8	88.9 \pm 5.6	99.7 \pm 3.3 [§]

* $P < 0.01$ control vs. obese and NIDDM (study 1 and study 2); [‡] $P < 0.05$ obese vs. NIDDM; [§] $P < 0.05$ vs. control (study 1 and study 2); $P < 0.01$ control vs. obese (study 2).

Table II. Lipid, Glucose, and Insulin Levels, Insulin Infusion Rates, and GDR of the Study Groups during Saline (Basal) and during Steady State Euglycemic Hyperinsulinemia (Clamp)

	Methacholine studies			Nitroprusside studies		
	Control	Obese	NIDDM	Control	Obese	NIDDM
<i>n</i>	13	12	8	13	6	5
Basal glucose (mg/dl)	92.2±1.0	94.2±3.4	210±23.7*	92.0±1.0	96.1±2.2	273±41*
Clamp glucose (mg/ml)	91.2±1.0	89.4±1.2	92.7±2.9 [‡]	90.4±1.2	93.0±0.9	90.0±1.1 [‡]
Basal insulin (μU/ml)	5.8±0.8 [§]	9.1±1.7	15.7±5.0	4.9±0.6 [§]	11.4±1.6	20.3±6.1
Clamp insulin (μU/ml)	26.9±5.9	66.3±6.8 [‡]	1023±349* [‡]	27.8±6.7 [‡]	54.4±14.5 [‡]	961±69** [‡]
Insulin infusion rates (mU/m ² per min)	18.6±3.6 [¶]	32.1±4.1	256.7±43.3*	17.1±2.1	32.5±7.0	300±0.0*
GDR (mg/kg per min)	5.14±0.69	3.42±0.45	4.98±1.00	4.7±0.2	4.3±0.9	4.1±0.9
Cholesterol (mg/dl)	168±10	193±10	198±16	183±10	173±15	189±14
HDL-cholesterol (mg/dl)	43.5±2.6	35.6±4.6	30.5±1.0	42.5±3.6	33.8±6.8	34.2±2.6
LDL-cholesterol (mg/dl)	106±9	114±9	132±9	115±11	99±16	124±10
Free fatty acids (μmol)	541±88	553±61	793±95	498±44 [§]	623±110	823±85
Triglyceride (mg/dl)	109±12	173±42	195±37	124±15	163±32	154±20

**P* < 0.01 vs. C and OB; [‡]*P* < 0.001 vs. basal; [§]*P* < 0.05 C vs. NIDDM; ^{||}*P* < 0.01 vs. OB; [¶]*P* < 0.05 vs. OB.

strated that low insulin infusion rates are able to significantly increase glucose uptake rates without significantly altering basal LBF. Moreover, we have previously established that insulin's ability to raise LBF is directly related to the degree of insulin sensitivity and inversely related to obesity (1) and baseline blood pressure (18). Therefore insulin infusion rates were chosen empirically and a priori in both C and OB, based on the subject's estimated insulin sensitivity according to (a) the subjects degree of adiposity and (b) the subject's blood pressure as determined by cuff. Since NIDDM are highly resistant to insulin's vasodilatory effect, we arbitrarily chose a supraphysiologic insulin infusion rate to maximally stimulate glucose uptake. The insulin infusion rates utilized in the various subject groups in the methacholine study and the nitroprusside study are shown in Table II.

Tracer determined rates of glucose disposal. The glucose disposal rate (GDR) was determined in C and OB during the low dose insulin infusion studies (15–40 mU/m² per min) by infusion of D[3-³H] glucose in a primed, continuous manner. With this technique, 45 μCi tracer was injected as a bolus dose, followed by a continuous infusion at the rate of 0.6 μCi/min. The tracer was allowed to equilibrate for 120 min, and glucose specific activity was measured for the subsequent 30-min period at 10-min intervals. Blood samples were obtained at 20-min intervals for determination of both the serum glucose concentration and the specific activity, and GDR was calculated after ~ 200 min while in near steady state. The GDR in the NIDDM was assumed to equal the glucose infusion rate at near steady state conditions after ~ 180 min, since during high dose (300 mU/m² per min) insulin infusion, hepatic glucose production is essentially completely suppressed (19, 20). One NIDDM subject in the MCh study and one subject in the SNP study developed hypotension and bradycardia (vaso-vagal reaction) within 40 min of the insulin infusion, and the study was therefore immediately terminated.

Methacholine study: endothelium-dependent vasodilation (MCh dose response curves). To study the effect of insulin resistance and/or NIDDM on endothelium-dependent vasodilation, we administered graded intrafemoral artery infusions of MCh at sequential doses of 2.5, 5.0, 7.5, 10.0, and 12.5 μg/min at baseline (saline infusion) to three subject groups exhibiting different degrees of insulin sensitivity, C (*n* = 13), OB (*n* = 12), and NIDDM (*n* = 8). Each MCh dose was administered for ~ 8 min. To examine whether insulin's ability to enhance endothelium-dependent vasodilation is impaired in OB or NIDDM, graded intrafemoral artery infusions of MCh were repeated after ~ 200 min of euglycemic hyperinsulinemia in a subset of each

group (7 C, 7 OB, and 6 NIDDM). The characteristics of the subjects, who were examined during both basal and insulin-stimulated conditions were not different from those who were assessed during saline infusion only. Thus, dose response curves for MCh were constructed both in the absence and in the presence of exogenous insulin in C, OB, and NIDDM. The volume of MCh infusate delivered ranged from 0.1 to 0.5 ml/min.

Nitroprusside study: endothelium-independent vasodilation (SNP dose response curves). To study the effect of insulin resistance and/or NIDDM on endothelium-independent vasodilation, graded intrafemoral artery infusions of SNP were administered to three groups of subjects (C, *n* = 13; OB, *n* = 6; NIDDM, *n* = 5) at rates of 1.75, 3.5, and 7.0 μg/min during the infusion of saline. Subgroups of C (*n* = 6), OB (*n* = 6), and NIDDM (*n* = 3) were also studied after ~ 200 min of a euglycemic hyperinsulinemic clamp. The demographic characteristics of the subjects studied during insulin infusion were not different from those studied during saline alone. Each SNP dose was administered for ~ 8 min. In this fashion, dose response curves for SNP were obtained both in the presence and absence of exogenous insulin in C, OB, and NIDDM. The volume of SNP delivered ranged from 0.25 to 1.0 ml/min.

Analytical methods. Blood for determination of plasma insulin concentrations was collected in tubes treated with heparin. The specimens were spun, and the supernatant was removed and stored at -20°C. Insulin levels were measured using the "Coat a Count" kit (Diagnostic Products Co., Los Angeles, CA). Blood for serum glucose determinations was drawn, put in untreated polypropylene tubes, and centrifuged with an Eppendorf microcentrifuge (Brinkman Instruments, Inc., Westbury, NY). The glucose levels in the supernatant were determined by the glucose oxidase method with a glucose analyzer YSI 2300 (Yellow Springs Instrument Co., Yellow Springs, OH). Serum total cholesterol and triglyceride levels were measured on an Ektachem 702 analyzer with an enzymatic method. HDL-cholesterol was measured with the "Magnetic HDL" kit (Reference Diagnostics, Inc., Arlington, MA), and LDL-cholesterol was calculated according to the Friedewald formula (21). FFA were measured according to the method described by Novak (22).

Statistical analysis. Results are shown as the mean±SEM. MAP is expressed in mmHg, LBF is expressed in liters/min, and leg vascular resistance (LVR) was defined as $LVR = MAP/LBF$ and is expressed in arbitrary units (U). Changes in blood flow are expressed as percent change (%Δ) to adjust for differences at baseline. Insulin levels are expressed in μU/ml. GDR is expressed as mg/kg per min.

Three-way ANOVA was used to compare the changes in LBF in response to the graded drug infusions between groups before and during the hyperinsulinemic euglycemic clamp. Two-way ANOVA was used to compare the effect of saline vs. hyperinsulinemic clamp on the changes in LBF response to MCh within groups. When significant differences between groups were found by ANOVA this was followed by post hoc testing with Fisher's PLSD.

Simple linear regression analysis was performed to assess the relationship between the maximal increase in LBF in response to the intrafemoral artery infusions of MCh and (a) indices of obesity/insulin resistance and (b) factors reported to impair endothelium-dependent vasodilation. Subsequently, variables whose correlation with the maximum LBF response to the intrafemoral artery infusions of MCh achieved near statistical significance ($P < 0.1$) were entered into a stepwise regression model to assess the magnitude of their individual effects on the maximum LBF response to the intrafemoral artery infusions of MCh.

Statistical significance was accepted at a level of $P < 0.05$. Statistics were performed on a Macintosh computer with StatView IV (Abacus Concepts, Inc., Berkeley, CA).

Results

Methacholine study: endothelium-dependent vasodilation (MCh dose response curves)

Glucose and insulin levels, and glucose disposal rates (Table II). Glucose levels were significantly elevated in NIDDM in the basal state. During steady state euglycemic hyperinsulinemia, glucose levels did not change from baseline in C and OB but decreased significantly in NIDDM. However, steady state glucose concentrations did not differ between groups during the euglycemic clamp.

Insulin levels differed between groups. Compared to C, basal insulin levels were ~ 60 and 160% higher in OB and NIDDM, respectively. During steady state euglycemic hyperinsulinemia insulin levels increased significantly in all groups. Insulin levels were, as expected, in the physiological range in the C and OB, and supraphysiologic in NIDDM.

GDR was near maximally stimulated in the NIDDM group by the supraphysiologic insulin levels. Nevertheless, GDR was lower in the NIDDM as compared to C, despite insulin levels which were more than 30 times higher. Thus, NIDDM displayed severe insulin resistance. OB also exhibited insulin resistance as evidenced by $\sim 35\%$ lower rates of GDR despite of more than twofold higher insulin concentrations compared to C.

Lipids (Table II). Total cholesterol and LDL-cholesterol concentrations were in the normal range, and although they were somewhat higher in the obese and NIDDM groups compared to controls, these differences did not reach statistical significance. HDL-cholesterol levels were highest in C and lowest in the NIDDM group. HDL-cholesterol did not differ between C and OB, but the difference between C and NIDDM was significant. Fasting triglyceride levels were in the normal range in all groups. While triglyceride levels were $\sim 50\%$ lower in C as compared to OB and NIDDM, this difference was not statistically significant. FFA levels were comparable between C and OB. NIDDM had nearly 50% higher FFA levels as compared to C and OB, but this difference was not statistically significant (ANOVA).

Hemodynamic data. Basal MAP values are shown in Table I. Although MAP in the OB was ~ 10 mmHg higher as compared to C ($P < 0.05$), the OB were not clinically hypertensive.

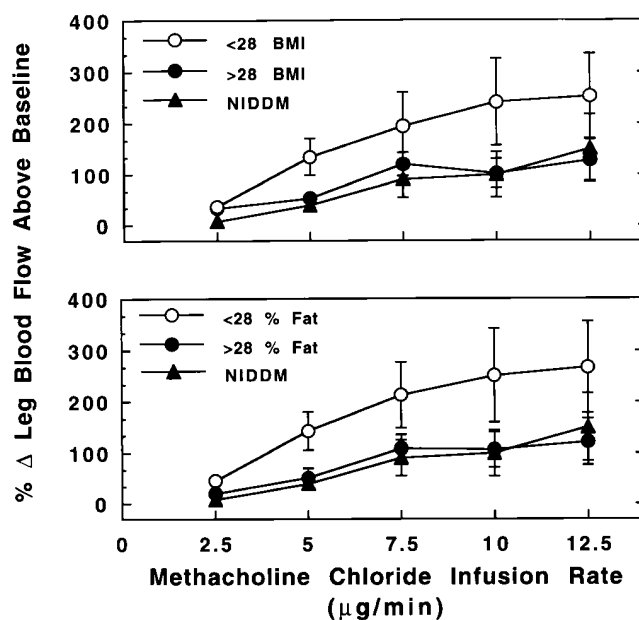


Figure 1. Percent leg blood flow increments ($\% \Delta$) above baseline in response to graded intravenous infusions of the endothelium-dependent vasodilator methacholine chloride under basal (saline) conditions in NIDDM subjects and in control and obese subjects as defined by BMI in A (control: BMI < 28 , obese: BMI ≥ 28) or directly measured percent body fat content in B (control: $< 28\%$, obese: $\geq 28\%$).

MAP did not change in response to euglycemic hyperinsulinemia and was not altered by the intrafemoral artery infusions of MCh.

Basal LBF was 0.22 ± 0.03 , 0.28 ± 0.04 , and 0.29 ± 0.03 liters/min in C, OB, and NIDDM, respectively ($P = \text{NS}$, ANOVA). LBF during euglycemic hyperinsulinemia was unchanged from basal in all groups. In response to the intrafemoral artery infusions of MCh, LBF increased significantly ($P < 0.01$) in a dose-dependent fashion in all groups under both basal and euglycemic hyperinsulinemic conditions.

Compared to controls, LBF increments in response to the intrafemoral artery infusions of MCh were on average ~ 40 and 55% lower (Fig. 1 A) in the OB and NIDDM, respectively ($P < 0.05$ OB vs. C, $P < 0.01$ NIDDM vs. C). When total body fat content (body fat $\geq 28\%$) was used as a criterion for obesity, the adverse effect of obesity on endothelium-dependent vasodilation became even more apparent (Fig. 1 B). OB and NIDDM did not differ in their LBF responses to the intrafemoral artery infusions of MCh. The diminished response to the intrafemoral artery infusions of MCh in both OB and NIDDM groups during saline infusion suggests that endothelium-dependent vasodilation is impaired in both OB and NIDDM under basal conditions.

During steady state euglycemic hyperinsulinemia, the differences in LBF response between C and both OB and NIDDM were more pronounced than during saline infusion. Increments in LBF were, respectively, 55 and 60% lower in the OB and NIDDM compared to C ($P < 0.0001$ C vs. OB and NIDDM). The heightened difference in LBF response between C and both OB and NIDDM groups was due to an effect of euglycemic hyperinsulinemia to markedly augment the

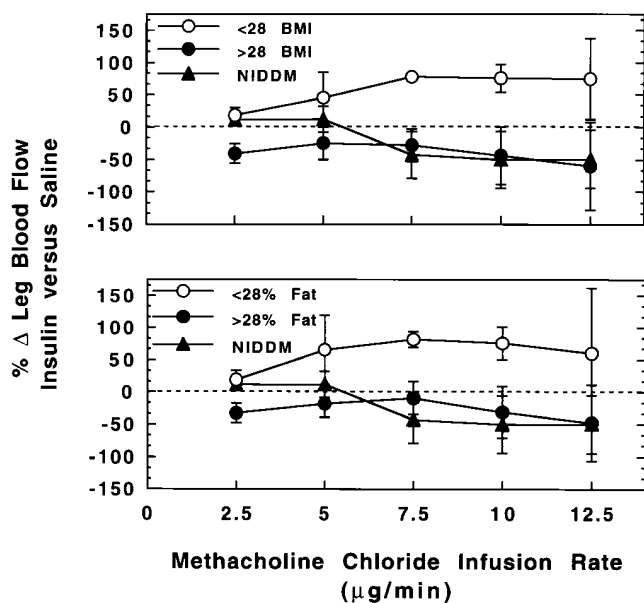


Figure 2. Differences in relative changes (%Δ) of leg blood flow [(%Δ above baseline during insulin) – (%Δ above baseline during saline)] in response to graded intrafemoral artery infusions of methacholine chloride during steady state euglycemic hyperinsulinemia as compared to saline alone in NIDDM subjects and in control and obese subjects as defined by BMI in *A* (control: BMI < 28, obese: BMI ≥ 28) or directly measured percent body fat content in *B* (control: < 28%, obese: ≥ 28%). Note that these data are obtained from a subgroup of the methacholine study group shown in Fig. 1 (C, *n* = 7; OB, *n* = 7; NIDDM, *n* = 6) and represent only subjects who underwent graded intrafemoral artery infusions of methacholine chloride during both saline and subsequent steady state euglycemic hyperinsulinemia.

LBF response to MCh in C, as hyperinsulinemia had no effect on MCh responses in OB and NIDDM which had similar LBF responses. Fig. 2 illustrates the difference in the percent increments in LBF in response to the intrafemoral artery infusions of MCh during steady state euglycemic hyperinsulinemia as compared to saline [(%Δ above baseline during insulin) – (%Δ above baseline during saline)]. Relative to baseline steady state euglycemic hyperinsulinemia enhanced the LBF response to MCh in C by ~ 50% ($P < 0.05$ vs. basal). The results were the same regardless of whether obesity was defined by BMI or percent body fat, suggesting that increased levels of body fat are associated with impaired insulin-mediated augmentation of endothelium-dependent vasodilation. In OB and NIDDM, the increments in LBF were somewhat lower during euglycemic hyperinsulinemia than during saline, but this difference was not significant.

These findings were unchanged when OB were defined as having a BMI ≥ 24 or a percent body fat content ≥ 24% under either basal or insulin-stimulated conditions. Thus, adiposity even when conservatively defined, predicted the MCh response.

Basal LVR in C, OB, and NIDDM, respectively, was 496 ± 65 , 442 ± 57 , and 383 ± 52 U during saline ($P = \text{NS}$) and 364 ± 41 , 363 ± 40 , and 321 ± 46 U during insulin ($P = \text{NS}$). LVR decreased in a dose-dependent manner in all groups, and changes in LVR mirrored the changes in LBF. Changes in LVR in response to the intrafemoral artery infusions of MCh

during both saline and euglycemic hyperinsulinemia were significantly more pronounced in lean as compared to the obese and NIDDM subjects ($P < 0.005$ vs. OB and NIDDM).

Nitroprusside study: endothelium-independent vasodilation (SNP dose response curves)

Glucose and insulin levels, and glucose disposal rates (Table II). Glucose levels were significantly elevated in NIDDM in the basal state. During steady state euglycemic hyperinsulinemia, glucose levels did not change from baseline in C and OB, but decreased significantly in NIDDM. However, steady state glucose concentrations did not differ between groups during the euglycemic clamp.

Insulin levels differed between groups. Compared to C, basal insulin levels were approximately twofold and fourfold higher in OB and NIDDM, respectively. During steady state euglycemic hyperinsulinemia insulin levels increased significantly in all groups. Insulin levels were in the physiological range in the C and OB and supraphysiologic in NIDDM.

GDR was near maximally stimulated in the NIDDM group by the supraphysiologic insulin levels. Nevertheless, GDR was ~ 20% lower in the NIDDM as compared to C, despite of more than 30 times higher insulin levels. Thus, NIDDM displayed severe insulin resistance. OB also exhibited insulin resistance as evidenced by ~ 10% lower rates of GDR despite of in twofold higher insulin concentrations compared to C.

Lipids (Table II). Total cholesterol, LDL-cholesterol, and HDL-cholesterol concentrations were in the normal range. HDL-cholesterol levels were higher in C as compared to OB and NIDDM, but the differences between the groups were not significant. Fasting triglyceride levels were in the normal range and similar in all groups. FFA levels were lowest in C, highest in NIDDM ($P < 0.05$ C vs. NIDDM), and intermediate in OB.

Hemodynamic data. Basal MAP values are shown in Table I. C and OB had comparable blood pressure levels. MAP in the NIDDM group was ~ 10 mmHg higher compared to both C and OB ($P < 0.05$ C vs. NIDDM). During steady state euglycemic hyperinsulinemia, MAP was unchanged from baseline. MAP was not altered by the intrafemoral artery infusions of SNP.

Basal LBF was 0.21 ± 0.02 , 0.27 ± 0.11 , and 0.27 ± 0.02 liters/min in C, OB, and NIDDM, respectively. During euglycemic hyperinsulinemia rates of LBF were unchanged from baseline. LBF increased in a dose-dependent fashion in response to SNP ($P < 0.05$) in all groups during saline infusion and steady state euglycemic hyperinsulinemia.

Fig. 3, *A* and *B* illustrate the relative (percent) increments above baseline in LBF in response to the intrafemoral artery infusions to SNP during saline infusion and during steady state euglycemic hyperinsulinemia, respectively. LBF responses to SNP were similar in C, OB, and NIDDM during both saline and steady state euglycemic hyperinsulinemia. Steady state euglycemic hyperinsulinemia did not augment the vasodilatory effect of SNP in either group. The results remained unchanged when analyzing the data according to body fat content (< 28% vs. ≥ 28%). Thus, obese and NIDDM subjects displayed normal endothelium-independent vasodilation.

Basal LVR in C, OB, and NIDDM, respectively, was 440 ± 31 , 516 ± 106 , and 376 ± 33 U during saline ($P = \text{NS}$) and 355 ± 47 , 513 ± 148 , and 254 ± 32 U during insulin ($P = \text{NS}$). LVR decreased in a dose-dependent manner in all groups, and changes in LVR mirrored the changes in LBF. Changes in LVR in response to the intrafemoral artery infusions of MCh

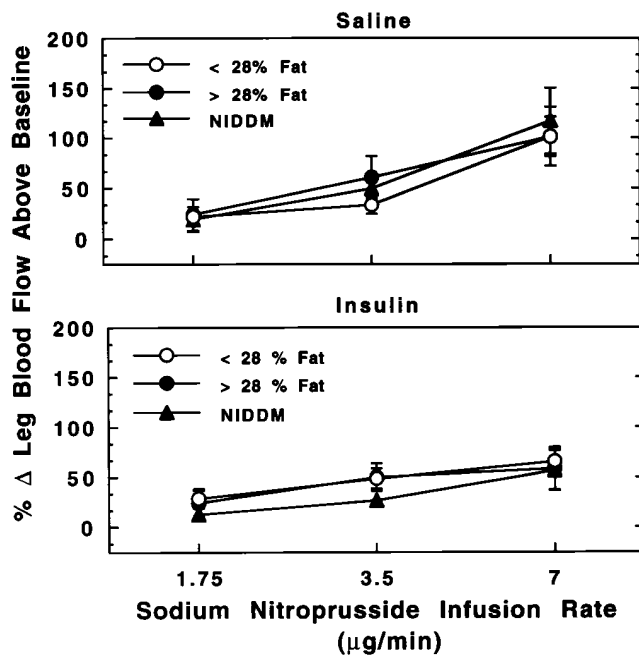


Figure 3. Leg blood flow increments (% Δ) above baseline in response to graded intrafemoral artery infusions of the endothelium-independent vasodilator sodium nitroprusside in NIDDM subjects, and in lean and obese subjects as defined by directly measured percent body fat content (lean: < 28%, obese: \geq 28%) during saline (A) and during steady state euglycemic hyperinsulinemia (B).

during both saline and euglycemic hyperinsulinemia were not different between the groups.

Correlational analyses

The results of our study indicate defective endothelium-dependent vasodilation in OB and NIDDM. To better examine the relationship between obesity/insulin resistance and endothelium-dependent vasodilation, we performed linear regression analyses between the maximum LBF response to MCh and indices of obesity and insulin sensitivity across all subject groups.

Table IV. Stepwise Regression Analysis for the Relationship between BMI or Percent Body Fat Content and LDL-Cholesterol and the Maximum Change in Leg Blood Flow in Response to the Endothelium-dependent Vasodilator Methacholine Chloride (Methacholine Study)

	Saline			Insulin		
	Coefficient	P	R ²	Coefficient	P	R ²
BMI	-11.38 \pm 4.38	0.015	0.20	-9.14 \pm 2.81	0.005	0.34
LDL-cholesterol	-2.45 \pm 1.21	0.053	0.31	-2.12 \pm 0.70	0.008	0.58
Intercept	833 \pm 187	< 0.0001		696 \pm 114	< 0.0001	
	F = 5.92, P = 0.0074			F = 10.9, P = 0.001		
Percent body fat	-7.80 \pm 3.57	0.0389	0.20	-8.27 \pm 0.62	0.0037	0.56
LDL-cholesterol	-2.27 \pm 1.30	0.0943	0.29	-1.41 \pm 0.76	0.0873	0.65
Intercept	702 \pm 170	0.0004		577 \pm 93	< 0.0001	
	F = 4.807, P = 0.0176			F = 11.971, P = 0.0011		

The stepwise regression analysis shows that the maximal leg blood flow response to the intrafemoral artery infusion of MCh is explained most strongly by body fat content. Whether estimated by BMI or directly measured (percent body fat), body fat predicts 20% of the leg blood flow response during saline and up to 56% during euglycemic hyperinsulinemia. The effect of LDL-cholesterol explains an additional 10% of the leg blood flow response during saline and not more than 24% during euglycemic hyperinsulinemia.

Table III. Correlational Analyses between the Maximum Percent Increase in LBF in Response to Graded Intrafemoral Artery Infusions of Methacholine Chloride and Various Relevant Parameters during Saline Infusion (Basal) and During Steady State Euglycemic Hyperinsulinemia

	Basal		Hyperinsulinemia	
	r	P Value	r	P Value
Percent body fat	-0.42	P < 0.05	-0.74	P < 0.01
BMI	-0.44	P < 0.01	-0.58	P < 0.01
Insulin sensitivity index (GDR/insulin level)	ND	ND	-0.6	P < 0.005
Fasting insulin	-0.24	P = 0.19	-0.43	P = 0.056
Cholesterol	-0.29	P = 0.105	-0.48	P < 0.05
LDL-cholesterol	-0.36	P < 0.05	-0.55	P < 0.05
HDL-cholesterol	0.38	P < 0.05	0.41	P = 0.076
Triglyceride	-0.19	P = 0.307	-0.26	P = 0.29
Free fatty acids	0.07	P = 0.7	0.04	P = 0.86
MAP	0.09	P = 0.64	-0.3	P = 0.20

ND, Glucose disposal rate was not determined.

Linear regression analysis was performed between the maximal LBF response to intrafemoral artery infusions of MCh and (a) percent body fat, (b) BMI, (c) fasting insulin levels, and (d) insulin sensitivity index.

The results of the regression analysis are presented in Table III. The results suggest that during saline and euglycemic hyperinsulinemia, increasing degrees of obesity/insulin resistance are associated with increasing impairment of endothelium-dependent vasodilation. The association between fasting insulin levels and the maximal increase in LBF in response to MCh during saline failed to achieve statistical significance, but the insulin sensitivity index (insulin-stimulated glucose uptake divided by prevailing insulin levels) showed a strong and inverse relationship.

Because impaired endothelium-dependent vasodilation has been reported in patients with hypertension (23–27) and hy-

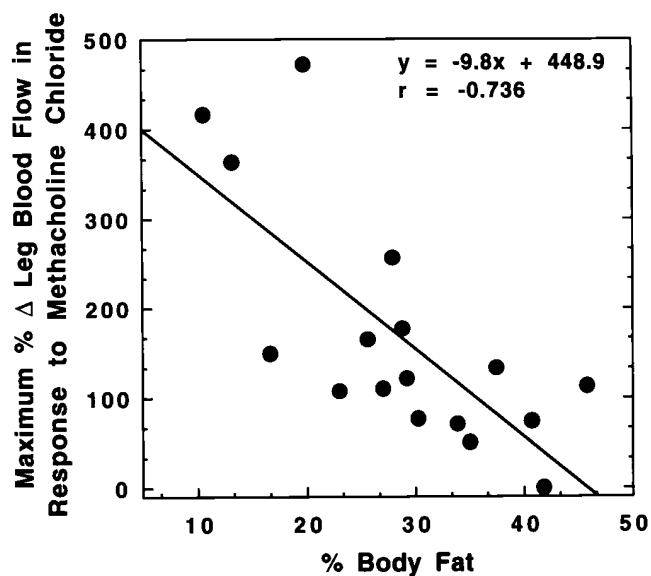


Figure 4. Relationship between the maximum leg blood flow response (% change) to graded intrafemoral artery infusions of methacholine chloride and percent body fat content during steady state euglycemic hyperinsulinemia in C, OB, and NIDDM.

percholesterolemia (28, 29), we also examined the relationship between the maximum LBF response to MCh and MAP and cholesterolemia (Table III). This analysis revealed that LDL-cholesterol was negatively correlated and HDL-cholesterol was positively correlated with the maximal LBF response to MCh.

Stepwise regression analysis (Table IV) revealed (a) that indices of body composition correlated most strongly with the maximum LBF response to MCh during saline and euglycemic hyperinsulinemia, and (b) that LDL-cholesterol had an independent but much weaker effect on the maximum LBF response, which was evident only during euglycemic hyperinsulinemia. BMI predicted 20% ($P < 0.02$) and 34% ($P < 0.01$) of the maximum LBF response to MCh during saline and euglycemic hyperinsulinemia respectively. Percent body fat predicted 20% ($P < 0.05$) and 56% ($P < 0.01$) of the maximum LBF response to MCh during saline and euglycemic hyperinsulinemia, respectively (Fig. 4). In contrast to the strong relationship between body composition and the maximum response to MCh, LDL-cholesterol during euglycemic hyperinsulinemia contributed not more than 11% ($P = 0.087$) to the variance of the maximum LBF response to MCh.

Discussion

Insulin is now well recognized for its physiologic action to vasodilate skeletal muscle vasculature (1–4) in insulin-sensitive but not insulin-resistant humans (1, 4, 18). This vasodilation appears to occur via an increase in the synthesis/release of EDNO (6, 7). EDNO is the most potent endogenous vasodilator in humans and plays a major role in the regulation of vascular tone and blood pressure (30). Moreover, EDNO has marked effects to inhibit vascular smooth muscle proliferation, reduce platelet adhesiveness (31–33), and decrease lipid peroxidation (34–37), actions which have the net effect to reduce the progression of atherosclerosis. Obesity is an insulin-resis-

tant state associated with impaired insulin-mediated vasodilation and is often accompanied by a cluster of potent cardiovascular risk factors (Syndrome X) (13, 14). Therefore, a major goal of this study was to examine the status of the EDNO system in human insulin resistance/obesity. We tested the hypothesis that obesity is associated with (a) impaired endothelium-dependent vasodilation and (b) a defect in insulin's physiological action to enhance EDNO synthesis/release from the endothelium. Finally, we also tested whether NIDDM adds to these abnormalities.

The results of this study indicate that in obese humans with and without NIDDM: (a) endothelium-dependent vasodilation is reduced by 40–50% compared to lean control subjects under basal conditions; (b) insulin's physiologic ability to enhance endothelium-dependent vasodilation is markedly impaired; and (c) endothelium-independent vasodilation is normal. These first time observations suggest the novel idea that obesity is associated with endothelial dysfunction which may be related to insulin resistance.

Endothelium-dependent vasodilation was examined after an overnight fast (basal insulin levels) and during steady state euglycemic hyperinsulinemia by measuring the LBF response to graded intrafemoral artery infusions of MCh. MCh stimulates the release of EDNO, which diffuses through the subendothelium to the vascular smooth muscle and results in vasodilation through the activation of guanylate cyclase (38) and Na^+/K^+ ATPase (39). Therefore, the decreased effect of MCh in obese or NIDDM subjects could be due to either diminished EDNO production/release or reduced EDNO action at the level of the vascular smooth muscle. The latter is unlikely, because the effect of graded intrafemoral artery infusions of the exogenous NO donor SNP did not differ between the control and the obese groups. Thus, it follows, that the diminished vasodilatory response to MCh in the obese and NIDDM is most likely due to impaired production/release of EDNO.

Hyperinsulinemia augmented the LBF response to graded intrafemoral artery infusions of MCh by $\sim 50\%$ in the controls but not in the obese and NIDDM. It is important to emphasize, that while insulin augmented endothelium-dependent vasodilation at low physiologic levels ($\sim 30 \mu\text{U/ml}$) in the controls, much higher insulin levels in the obese ($\sim 65 \mu\text{U/ml}$) and NIDDM ($\sim 1000 \mu\text{U/ml}$) failed to enhance endothelium-dependent vasodilation. Since euglycemic hyperinsulinemia did not change the response to intrafemoral artery infusions of the exogenous NO donor SNP, the enhanced endothelium-dependent vasodilation is most likely to have been secondary to an increase in EDNO production/release. Thus, insulin appears to act at the level of the endothelial cell to modulate EDNO production/release, and this insulin effect is blunted in obese subjects with or without NIDDM.

The mechanism by which insulin regulates EDNO production/release is unknown. Insulin's effect on EDNO could either be coupled to insulin-mediated glucose uptake, or insulin could directly effect the endothelium, independent of its metabolic actions. If insulin's effect on EDNO was coupled to glucose uptake, endothelium-dependent vasodilation should have been augmented proportionally to the increases in glucose disposal in response to hyperinsulinemia. Glucose disposal rates in the obese group were $\sim 35\%$ lower than in controls and did not differ between the controls in the NIDDM. Nevertheless, endothelium-dependent vasodilation was not enhanced in the obese and NIDDM. Thus, the failure to increase endothelium-

dependent vasodilation in response to hyperinsulinemia, despite significantly stimulated glucose uptake in the obese and NIDDM, suggests that (a) insulin's modulating effect on EDNO production/release at the level of the endothelial cell may be independent of its effect on glucose uptake, and (b) in obese and NIDDM the endothelium may be resistant to insulin's modulating effect on EDNO production/release.

Impairment of EDNO-dependent vasodilation has been described in patients with hypercholesterolemia (28, 29), essential hypertension (23–27), and diabetes (9) and in older patients (40–43). Furthermore, free fatty acids which have been reported to be increased in obesity and NIDDM (44) have been shown most recently to increase vasoconstrictor responses in dorsal hand veins (45) and to impair endothelial NO production in vitro (46). Therefore, to ascribe a defect in endothelial function to obesity/insulin resistance it is important to rule out other causal or associated factors.

Total cholesterol and LDL-cholesterol levels in the obese and NIDDM groups were somewhat higher than in the control group but were in the normal range and not statistically different from controls. Moreover there was no relationship between any indices of body mass and cholesterol levels. In previous reports, hypercholesterolemic patients exhibiting abnormalities in endothelium-dependent vasodilation had average total cholesterol and LDL-cholesterol levels in excess of 260 and 180 mg/dl, respectively (28, 29). In our subject groups the mean cholesterol levels did not exceed 200 mg/dl, and the mean LDL-cholesterol levels did not exceed 135 mg/dl. Therefore, it is not likely that hypercholesterolemia was a major cause for the impaired endothelium-dependent vasodilation in these subjects. Nevertheless, it is interesting to note that we found a weak, but independent, inverse correlation of borderline significance between the LDL-cholesterol concentration and the peak vasodilation induced by methacholine. These data suggest that the relationship between LDL-cholesterol level and endothelium-dependent vasodilation may be continuous even within the normal range of cholesterol levels. Thus, LDL-cholesterol concentrations may account for a small portion of the impaired endothelium-dependent vasodilation among normocholesterolemic subjects. It should be noted however, that relative to body fat, cholesterol concentration was a weak predictor of maximal endothelium-dependent vasodilation in a stepwise regression analysis. Finally, because oxidized LDL-cholesterol has been shown to impair nitric oxide production in vitro (47, 48), it is possible that small dense LDL-cholesterol, which is more prevalent in insulin-resistant subjects (49) and more susceptible to oxidation (50), might reveal a stronger association with the impaired endothelial function than LDL-cholesterol.

Differences in FFA concentrations did not account for the marked differences in endothelium-dependent vasodilation between the groups. The FFA levels were the same in control and obese subjects, and the nearly 50% higher levels in the NIDDM did not cause further impairment in the LBF response to MCh as compared to the obese. Finally, there was no relationship between FFA levels and maximum LBF increase in response to the intrafemoral artery infusion of MCh.

With respect to blood pressure, obese and NIDDM subjects exhibited somewhat higher levels of blood pressure than the controls, however they were not clinically hypertensive. Defective endothelium-dependent vasodilation in relation to blood pressure elevation has been reported in patients with es-

tablished essential hypertension (23–27), but this finding has recently been questioned (51). In the current study, blood pressure levels did not correlate with the maximum response to intrafemoral artery infusions of MCh. Therefore, it is not likely that the impaired endothelium-dependent vasodilation observed in obese and NIDDM subjects is due to or associated with the modestly higher (albeit normal) blood pressure levels. In this regard, it is interesting to speculate whether differences in endothelium-dependent vasodilation among various reports examining hypertensive subjects (24–26, 51) may actually reflect differences in body fat content rather than blood pressure alone, as most hypertensives have an increase in body adiposity (52–54).

Interestingly, endothelium-dependent vasodilation was similar in obese and NIDDM groups. Because these groups were equally obese, the data suggest that obesity rather than hyperglycemia has a more potent detrimental effect on endothelium-dependent vasodilation. If hyperglycemia decreases endothelium-dependent vasodilation, one would expect that restoration of normoglycemia should improve endothelial function. Induction of acute euglycemia with the hyperinsulinemic glucose clamp technique did not improve endothelium-dependent vasodilation in the NIDDM, suggesting that decreased EDNO production/release in NIDDM subjects was not the result of acute effects of hyperglycemia. These results do not rule out any deleterious effects of chronic hyperglycemia upon endothelium-dependent vasodilation; nevertheless, they suggest that obesity/insulin resistance may be a more important factor in the etiology of the endothelial dysfunction.

Aging has been reported to be associated with impaired endothelium-dependent vasodilation (40–43). Our study groups were relatively young compared to the elderly subjects previously studied, making it highly unlikely that age was a confounding variable in our analysis. In fact, we were unable to find a relationship between age and peak endothelium-dependent vasodilation ($R^2 = 0.03$, $P = \text{NS}$), which is in contrast to one report where a strong and negative relationship between age and endothelium-dependent vasodilation in the forearm was found (43). Also, body fat content increases with age which may account for some of the impairment of endothelium-dependent vasodilation seen with aging. Furthermore, control and obese groups were the same age, and NIDDM subjects who were an average 5 yr older had nearly identical responses to the intrafemoral artery infusions of MCh when compared to the obese, showing that age was not likely to be a confounding factor in our study.

Finally, an association between obesity/insulin resistance and endothelial dysfunction is perhaps most strongly supported by the fact that endothelium-dependent vasodilation is impaired in proportion to insulin resistance and various indices of adiposity under baseline conditions (Table III). Moreover and importantly, the difference in endothelium-dependent vasodilation between insulin sensitive and insulin resistant groups was accentuated by euglycemic hyperinsulinemia, which enhanced endothelium-dependent vasodilation in the insulin-sensitive subjects but had no effect in the insulin-resistant groups.

Although the accentuated difference in endothelium-dependent vasodilation between insulin-sensitive and insulin-resistant groups during hyperinsulinemia was most likely due to decreased production/release of EDNO, we cannot rule out the possibility that hyperinsulinemia caused the release of vas-

oconstrictor substances in the insulin-resistant groups. The release of a vasoconstrictor substance during hyperinsulinemia could impair endothelium-mediated vasodilation and thus prevent further increases in leg blood flow in response to MCh. Interestingly, it has been shown that the levels of the potent vasoconstrictor endothelin 1 (ET-1) increase in NIDDM subjects in response to hyperinsulinemia (55, 56). Certainly, an increase in ET-1 levels, which were not measured in our study, could explain some of the differences seen in our study. However, if in response to hyperinsulinemia vasoconstrictor levels had increased to sufficiently impair endothelium-dependent vasodilation, one would expect other hemodynamic changes such as increments in systemic blood pressure or decrements in resting leg blood flow.

In summary, we have presented evidence suggesting that obesity/insulin resistance, independent of other risk factors, is associated with endothelial dysfunction. This endothelial dysfunction most likely reflects an abnormality in the generation of EDNO per se or in the release of EDNO in response to endothelium-dependent factors. Given the central role of both EDNO and the endothelium in the maintenance of vascular tone, platelet adhesiveness and smooth muscle cell proliferation, it follows that abnormalities in endothelial function in obese humans could be critically instrumental in conferring an increased risk of macrovascular disease characteristic of obese insulin-resistant subjects.

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