

Endothelin Contributes to Basal Vascular Tone and Endothelial Dysfunction in Human Obesity and Type 2 Diabetes

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Endothelium-dependent vasodilation is impaired in clinical states of insulin resistance such as obesity and type 2 diabetes. Individuals who have hyperinsulinemic insulin resistance have relatively elevated circulating levels of endothelin (ET)-1, suggesting that ET-1 may be important in the endothelial dysfunction and alterations of vascular tone in these conditions. In 8 lean subjects, 12 nondiabetic obese subjects, and 8 subjects with type 2 diabetes, we measured basal and methacholine-stimulated rates of leg blood flow (LBF) and total serum nitrates (NOx) before and after the intrafemoral arterial administration of BQ123, a specific blocker of ET_A receptors. BQ123 produced significant vasodilation in the obese and type 2 diabetic subjects (leg vascular resistance = mean arterial pressure/LBF fell by 34 and 36%; $P < 0.005$) but not in the lean subjects (13%; $P = NS$, $P = 0.018$ comparing all groups). ET_A blockade did not change basal NOx flux (NOx*LBF). This suggests increased basal ET-1 constrictor tone among obese and type 2 diabetic subjects. BQ123 corrected the baseline defect in endothelium-dependent vasodilation seen in obese and type 2 diabetic subjects, suggesting an important contribution of ET-1 to endothelial dysfunction in these subjects. In contrast to basal conditions, stimulated NOx flux was augmented by BQ123 in obese and type 2 diabetic subjects but not in L subjects ($P = 0.04$), suggesting a combined effect of ET_A blockade to reduce constrictor tone and augment dilator tone. Endothelin seems to contribute to endothelial dysfunction and the regulation of vascular tone in human obesity and type 2 diabetes. *Diabetes* 51:3517–3523, 2002

The endothelial dysfunction observed in the insulin-resistant states of obesity and type 2 diabetes has been attributed to decreased nitric oxide (NO) availability (1,2), likely related to both impaired production and increased consumption (3,4). However, paracrine control of vascular tone de-

pends on the balance of both vasodilating and vasoconstricting factors (5). Endothelin (ET)-1, produced directly by vascular endothelial cells, is the most important locally produced vasoconstrictor, acting principally through type A (ET_A) receptors on vascular smooth muscle cells (6). ET-1 and NO function as mutual antagonists in the determination of vascular tone but also have important direct interactions at the level of transcription and expression (7) and signal transduction (8), with each acting to limit the net production of the other. This mutual antagonism can serve to amplify any imbalances resulting from other factors, for example insulin resistance/hyperinsulinemia. Furthermore, both ET-1 and NO have effects beyond their contributions to the regulation of vascular tone, which act in net as antiatherosclerotic (NO) or proatherosclerotic (ET-1) factors through modulation of platelet activity, lipid oxidation, leukocyte chemotaxis, and local production of thrombotic factors as well as the growth and proliferation of vascular smooth muscle cells and mural fibroblasts (6,9,10). Imbalances in the actions of NO and ET-1 are therefore of potential importance beyond the acute regulation of vascular tone.

Although data from animal models of insulin resistance and diabetes suggest an important role for ET-1 in the associated vascular dysfunction (11–13), little is known about the contributions of ET-1 to vascular dysfunction in human obesity and type 2 diabetes. Insulin engenders the production and release of ET-1 in vitro (14,15). This effect may also be present in vivo in humans, suggested by observations that circulating ET-1 levels are elevated in hyperinsulinemic states (16–18) and proportional to the hyperinsulinemia (19), that the acute imposition of hyperinsulinemia can result in a concurrent increase in circulating ET-1 levels (20), and that reduction in hyperinsulinemia concurrently reduces circulating insulin and ET-1 levels (21). However, it is unclear how well circulating concentrations of ET-1 reflect vessel biology, because of the preferential abluminal secretion of ET-1 (22) and its paracrine effect in the vessel wall (6). For directly addressing this question, in vivo studies using endothelin receptor antagonists are required.

We applied this approach to investigate the role of ET-1 in vascular dysfunction in insulin-resistant subjects with and without diabetes, by studying the effects of BQ123, a specific blocker of ET_A receptors (which mediate the vasoconstrictor effects of ET-1), on basal tone and endothelium-dependent vascular function in patients with type

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ET, endothelin; HOMA-IR, homeostasis model assessment–insulin resistance; LBF, leg blood flow; LVR, leg vascular resistance; MAP mean arterial pressure; NO nitric oxide; NOx, total nitrate.

2 diabetes, subjects with obesity/insulin resistance, and lean normal control subjects.

RESEARCH DESIGN AND METHODS

Study population. Nondiabetic subjects were recruited through newspaper advertisement and classified as either lean or obese according to BMI cutpoints of ≥ 26 for men or ≥ 28 for women. Subjects with type 2 diabetes were included on the basis of either a historical diagnosis of type 2 diabetes confirmed on screening or a new diagnosis made on screening (according to American Diabetes Association criteria of a fasting glucose >7.0 mmol/l and/or any reading >11.1 mmol/l on 75-g oral glucose tolerance testing). Exclusion criteria included hypertension (systolic blood pressure >140 /diastolic blood pressure >90) or antihypertensive therapy, elevated serum lipids (total cholesterol >5.2 mmol/l, LDL >2.3 mmol/l, or triglycerides >2.0 mmol/l), biochemical evidence of renal or hepatic dysfunction, or significant underlying medical conditions. Diabetic subjects who had taken a peroxisome proliferator-activated receptor- γ agonist within the previous 6 months were excluded. Diabetic subjects with evidence of previous retinopathy, neuropathy, or nephropathy were excluded. All subjects underwent a standard 75-g oral glucose tolerance test to screen for diabetes and had body composition assessed by dual energy X-ray absorptiometry measurement. No insulin-treated diabetic subjects volunteered. Oral hypoglycemic agents were withheld for a minimum of 14 days before the study. This study was approved by the local Institutional Review Board, and all subjects gave written informed consent. All procedures were performed in accordance with institutional guidelines.

Technique. All studies were performed after an overnight fast. A 6F sheath (Cordis, Miami, FL) was placed into the right femoral vein to allow the insertion of a custom-designed 5F double-lumen thermodilution catheter (Baxter Scientific, Edwards Division, Irvine, CA) to measure leg blood flow (LBF). The right femoral artery was cannulated with a 5.5F double-lumen catheter to allow simultaneous infusion of substances and invasive blood pressure monitoring via a vital signs monitor (Spacelabs, Redmond, WA). All hemodynamic measurements were obtained with the subjects in the supine position in a quiet temperature-controlled room. Basal LBF and mean arterial pressure (MAP) measurements were obtained after ≥ 30 min of rest after the insertion of the catheters. Femoral vein thermodilution curves were used to measure rates of LBF, calculated by integration of the area under the curve, using a cardiac output computer (model 9520A; American Edwards Laboratories). Initially, 24 basal LBF measurements were obtained at ~ 30 -s intervals. During subsequent drug infusions, the mean of 10 measurements was taken for basal and methacholine (MCh)-stimulated flows. LBF measurements were begun 2 min after the onset of each incremental dose of MCh. Invasively determined MAP was recorded with every other LBF determination. Graded intrafemoral artery infusions of MCh (Roche Laboratories) were administered at sequential doses of 5.0, 10.0, and 15.0 $\mu\text{g}/\text{min}$ (0.2–0.6 ml/min) to assess endothelium-dependent vasodilation.

Protocol. Vascular responses were measured as changes in LBF as above, with vasoactive agents administered via the ipsilateral femoral artery. Continuous bedside monitoring of heart rate and intra-arterial blood pressure allowed full hemodynamic assessment of the responses.

ET-1 blockade was achieved with BQ123 (Cinalfa, Basel, Switzerland), a high-affinity competitive inhibitor of ET-1_A receptors. In previous studies, achieving local concentrations of BQ123 an order of magnitude higher than the pK_a of the ET_A receptor required BQ123 to be administered at a rate of ~ 100 nmol/min into the forearm circulation, with rates of blood flow on the order of 25–40 ml/min. Typical blood flow rates into the much larger muscle bed of the leg are on the order of 10-fold higher. Therefore, accounting for the diluting effect of this increased blood flow rate, a 10-fold higher dose of BQ123 was chosen.

The protocol is presented in Fig. 1. Basal unstimulated and endothelium-dependent (MCh-stimulated) vascular responses were measured at baseline. BQ123 was then administered intra-arterially at 0.6 mg/min (1 $\mu\text{mol}/\text{min}$) for 90 min, at which point the effect of this dose on basal and MCh-stimulated LBF was determined.

Laboratory. Blood for serum glucose determinations was put in untreated polypropylene tubes and centrifuged using an Eppendorf microcentrifuge (Brinkman, Westbury, NY). The glucose concentration of the supernatant was then measured by the glucose oxidase method using a glucose analyzer (Model 2300 Yellow Springs Instruments, Yellow Springs, OH). Blood for determination of plasma insulin was collected in heparinized tubes, processed immediately, and frozen at -20°C . Insulin determinations were made using a dual-site radioimmunoassay specific for human insulin and with cross-reactivity with proinsulin $<0.2\%$. The lower detection limit is 0.56 pmol/l, and in our laboratory the inter- and intra-assay coefficients of variation are 4.1 and

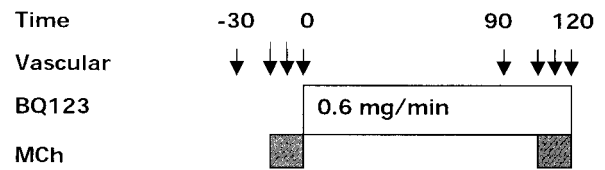


FIG. 1. The investigation protocol. The period before the BQ123 infusion is designated as the saline infusion period. With saline and with BQ123, basal vascular measurements (single arrows) were performed before the MCh dose-response measurements (triple arrows).

2.6%, respectively. Total serum nitrate (NO_x) was determined by chemiluminescence (Sievers NO analyzer, Denver, CO) after a Greiss reaction to oxidize NO and NO₂ to NO₃. NO_x flux was calculated as venous NO_x times LBF and used as an index of net NO production. Endothelin levels were determined by radioimmunoassay (R&D Systems, Minneapolis MN). Standard methodologies for cholesterol and triglyceride determinations were performed through our local hospital's clinical laboratory.

Statistical analysis. Data that were not normally distributed were normalized through logarithmic transformations before analysis. Comparisons between and within groups were performed by *t* tests, ANOVA, and repeated measures ANOVA as appropriate. When significant differences were found by ANOVA, this was followed by post hoc pairwise testing with the Student-Newman-Keuls test. Statistical significance was accepted at a level of $P < 0.05$. All results are presented as the mean \pm SE.

RESULTS

The subject characteristics are presented in Table 1. We studied 8 lean subjects, 12 obese subjects, and 8 subjects with type 2 diabetes. In a simultaneous comparison of all three groups, the diabetic subjects were older and hyperglycemic and had higher homeostasis model assessment–insulin resistance (HOMA-IR) indexes of insulin resistance. The type 2 diabetic subjects had a higher BMI than the obese subjects but equivalent body fat composition. Blood pressure was higher among obese and type-2 diabetic subjects, reaching statistical significance only for the comparison of type-2 diabetic and lean subjects. Lipid values did not differ significantly across the three groups, although fasting triglycerides in particular were lower among the lean subjects. Of note, the type 2 diabetic and obese subjects were comparable with regard to body fat, insulin concentration, blood pressure, and lipids but differed in age, glucose concentration, and HOMA-IR. Endothelin levels were not statistically different across groups.

The MAP fell significantly with the application of BQ123 ($P = 0.002$), from 89 ± 3 to 85 ± 2 mmHg in the lean subjects, from 97 ± 2 to 94 ± 2 mmHg in the obese subjects, and from 104 ± 4 to 96 ± 5 mmHg in the type 2 diabetic subjects (NS comparing the change across groups). No associated changes in heart rate were seen in any group (NS, not shown). In view of these systemic changes, vascular function results were expressed as leg vascular resistance (LVR; calculated as MAP/LBF). No changes in MAP were seen with MCh infusions at baseline or with BQ123 (not shown).

Effect of ET_A blockade on basal vascular tone. BQ123 produced differential vasodilation across the three groups. LBF rose from 0.239 ± 0.022 to 0.253 ± 0.027 l/min in lean subjects, from 0.262 ± 0.028 to 0.0341 ± 0.037 in obese subjects, and from 0.255 ± 0.017 to 0.361 ± 0.016 in type 2 diabetic subjects ($P < 0.001$ for BQ123 effect, $P = 0.02$ for comparison across groups; Fig. 2). In lean subjects, this corresponded to a statistically nonsignificant 13% reduction in basal LVR from 406 ± 55 to 351 ± 59 units (Figs. 2

TABLE 1
Patient characteristics

Measure	Lean subjects	Obese subjects	Type 2 diabetic subjects	P
Age (years)	33.1 ± 2.5	33.7 ± 2.6	44.5 ± 3.5	0.021*
Sex (M/F)	6/2	8/4	5/3	
BMI (kg/m ²)	22.1 ± 1.6	34.2 ± 3.2	38.1 ± 2.7	<0.0001†
Percentage of fat	22.3 ± 8.6	37.1 ± 9.9	37.7 ± 3.9	0.005*
HbA _{1c} (%)	—	—	10.0 ± 1.3	
Glucose (mmol/l)	5.0 ± 0.1	5.4 ± 0.2	9.6 ± 1.1	<0.001*
Insulin (pmol/l)	53.3 ± 5.4	86.7 ± 13.3	101.1 ± 34.2	0.18
HOMA-IR	1.9 ± 0.2	3.6 ± 0.7	6.7 ± 1.9	0.014*
ET-1 (pg/ml)	11.1 ± 1.7	13.7 ± 1.5	14.6 ± 2.7	0.15
MAP (mmHg)	89 ± 3	96 ± 2	103 ± 4	0.009†
Cholesterol (mmol/l)	4.3 ± 0.1	4.0 ± 0.2	4.5 ± 0.2	0.12
LDL (mmol/l)	2.6 ± 0.2	2.7 ± 0.1	2.7 ± 0.2	0.81
HDL (mmol/l)	0.9 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	0.61
Triglycerides (mmol/l)	1.3 ± 0.1	2.2 ± 0.4	2.4 ± 0.3	0.07

Data are means ± SE. P values represent the ANOVA comparing all three groups. Symbols indicate which post hoc pairwise comparisons were significant: *L differs from others; †all three groups differ.

and 3). In contrast, obese subjects experienced a 34% reduction (from 452 ± 47 to 300 ± 27 units) in unstimulated LVR ($P = 0.004$; Figs. 2 and 3). Type 2 diabetic subjects also experienced a 36% reduction in tone, from 418 ± 27 to 267 ± 14 units ($P = 0.0003$). These reductions in LVR differed across groups ($P = 0.018$ by ANOVA; Fig. 3), and by pairwise comparisons, the obese and type 2

diabetic groups were equivalent to each other and both exhibited greater vasodilation to BQ123 than the lean subjects. There was no apparent effect of sex on these responses.

Univariate regression analyses suggested significant relationships of this effect with measures of obesity (BMI and percentage of body fat) but not with serum glucose, insulin, HOMA-IR, blood pressure, or lipid parameters. Stepwise multivariate analysis including all of these variables revealed both BMI and total serum cholesterol as significant independent determinants of this differential sensitivity ($r^2 = 0.474$, $P = 0.0009$).

Under basal conditions, NO_x flux was lower in the diabetic patients but did not reach statistical significance (data available in 7 lean, 10 obese, and 6 type 2 diabetic subjects; $P = 0.13$; Fig. 4). The application of BQ123 did not have a measurable effect on basal NO_x flux in any group (Fig. 4).

ET_A blockade and endothelium-dependent vasodilation. Among lean control subjects, peak responses to MCh were not appreciably altered by the coinfusion of BQ123

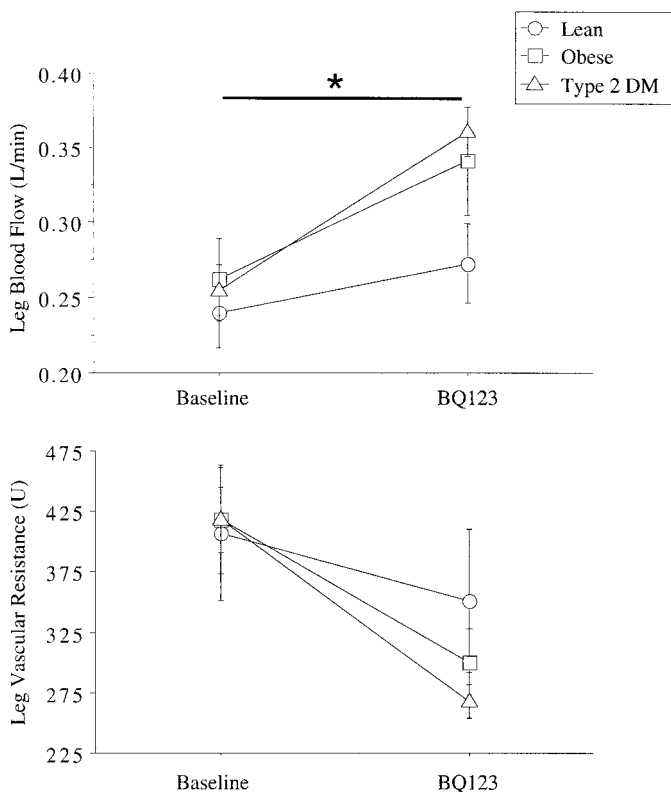


FIG. 2. Effect of BQ123 on basal vascular tone. *Top*: LBF responses. *Bottom*: LVR. BQ123 caused a significant vasodilation, with a rise in blood flow ($P < 0.001$) and a reduction in vascular resistance ($P < 0.001$). This effect was statistically different across the three subject groups for LBF (* $P = 0.02$ by repeated measures ANOVA) with a similar trend for LVR ($P = 0.08$). By pairwise comparisons, the responses in obese and type 2 diabetic subjects were not different.

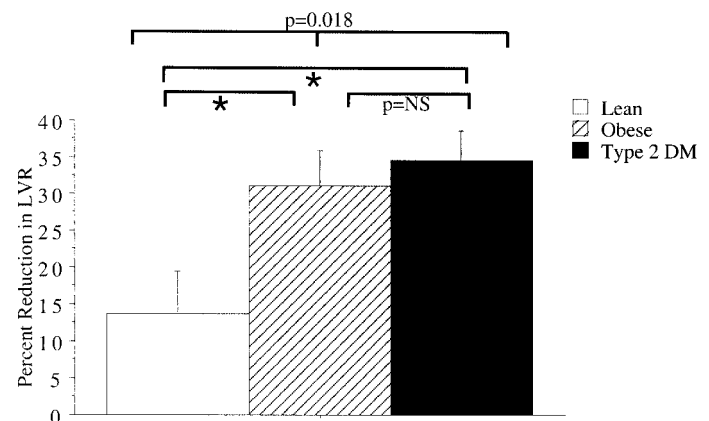


FIG. 3. Mean percentage reduction in LVR with BQ123. The subject groups were different by ANOVA ($P = 0.018$), and by pairwise comparisons (Student-Newman-Keuls, * $P < 0.05$), the lean subjects differed from the other groups. BQ123 had a greater effect in the obese and type 2 diabetic subjects than in lean subjects.

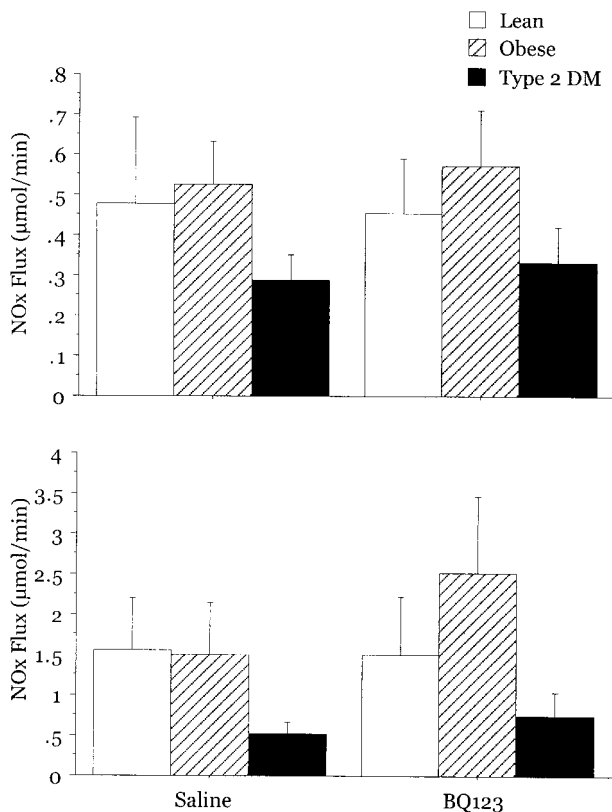


FIG. 4. Effect of BQ123 on total NOx flux. *Top*: basal conditions. *Bottom*: MCh-stimulated conditions (15 µg/min). Stimulated NOx flux was augmented in obese and type 2 diabetic subjects (* $P = 0.04$ by two-way repeated measures ANOVA).

(Fig. 5), suggesting a minimal role of ET-1 to limit endothelium-dependent vasodilation in these subjects. As expected, the MCh dose-response was impaired in the obese subjects compared with the lean control subjects at baseline (Fig. 5, left), evident as a blunted increase in LBF (top) or a blunted reduction in LVR (bottom) across the dose range ($P = 0.003$). An equivalent dysfunction was seen in the type 2 diabetic subjects ($P = 0.005$ vs. lean subjects and $P = \text{NS}$ vs. obese subjects; Fig. 5, left). This baseline defect in endothelium-dependent vasodilation was corrected by the application of ET_A blockade in both obese and type 2 diabetic subjects (Fig. 5, right). This effect of BQ123 to equalize MCh-stimulated vascular responses across groups was significant for both LBF ($P = 0.01$) and LVR ($P = 0.02$) by two-way repeated measures ANOVA. Again, no effect of sex on these responses was evident.

As noted above, in the presence of BQ123 basal (pre-MCh) LVR was lower in obese and type 2 diabetic subjects relative to lean subjects. This difference in basal vascular tone potentially confounds interpreting the effect on MCh-induced endothelium-dependent vasodilation when considering LVR alone. As is usually seen in our laboratory, femoral arterial infusion of MCh did not affect blood pressure, allowing direct comparison of LBF responses to MCh. Increments in LBF with peak MCh were augmented by concurrent BQ123 versus saline in obese subjects ($\Delta\text{LBF } 0.327 \pm 0.103$ to 0.518 ± 0.145 l/min) and in type 2 diabetic subjects (0.298 ± 0.054 to 0.451 ± 0.054 l/min; $P = 0.003$ for both comparing baseline to BQ123). LBF increases with peak MCh did not change in lean subjects

(0.575 ± 0.093 to 0.531 ± 0.08 l/min; $P = \text{NS}$). Similar to the results with absolute LBF values, under BQ123 the increase in LBF above baseline with MCh was equal across groups (NS). The corresponding reductions in LVR were of borderline statistical significance ($P = 0.056$) for the obese and type 2 diabetic groups (comparing peak ΔLVR at baseline with that with BQ123), despite that with unchanged blood pressure this simply represents the mathematical inversion of blood flow. Again, however, the ΔLVR with peak MCh was unchanged by BQ123 in lean subjects, and the difference between groups at baseline was corrected by BQ123. Therefore, ET_A blockade results in an augmented vasodilator response to MCh in obese and type 2 diabetic subjects that cannot be attributed merely to changes in basal tone.

Furthermore, NOx flux under MCh stimulation was unchanged by BQ123 in lean subjects but increased in both obese and type 2 diabetic subjects ($P = 0.04$ by repeated measures ANOVA; Fig. 4). It is interesting that the relative effect of BQ123 to increase NOx flux was matched between obese and type 2 diabetic (percentage increase 69.3 ± 0.3 and 64.0 ± 0.2 for obese and type 2 diabetic subjects, respectively; $P = 0.02$ vs. lean subjects). This improvement did not, however, normalize NOx flux in type 2 diabetic subjects (Fig. 4).

DISCUSSION

Using a specific blocker of ET_A receptors, we found important and differential effects on basal and MCh-stimulated vascular tone in lean, obese, and type 2 diabetic subjects. The novel findings are as follows: 1) the obese and type 2 diabetic subjects demonstrated greater effects of ET_A blockade on basal vascular tone, suggesting a greater contribution of ET-1 to vascular tone in these subjects than in normal lean controls; 2) endothelium-dependent vasodilation among obese and type 2 diabetic subjects was significantly ameliorated during ET_A blockade, suggesting a role for ET-1 in the associated endothelial dysfunction; and 3) in both the measures of basal vascular tone and endothelium-dependent vasodilation, there was no difference in the effect of ET_A blockade between obese and type 2 diabetic subjects, although markedly different NOx flux measures suggest that the underlying balance of dilators and constrictors is not necessarily equivalent in these two groups. These findings suggest that ET-1-mediated vasoconstriction is increased in the insulin-resistant states of obesity and type 2 diabetes and that this accounts in part for the endothelial dysfunction that typifies these states.

ET_A and vascular tone in insulin resistance and diabetes. The observed difference in the contribution of ET_A-mediated vasoconstriction to net vascular tone across groups is perhaps counterintuitive, given the lack of absolute differences in basal LBF or LVR across groups. Indeed, augmented ET-1 constrictor tone in the face of "normal" basal LVR suggests an altered balance of the determinants of vascular tone in obese and type 2 diabetic subjects. Specifically, for this observed increase in ET-1-mediated vasoconstriction, some combination of an increase in vasodilators and/or a reduction in other vasoconstrictors must also be present. One possibility is concurrent increases in both constrictor and dilator tone

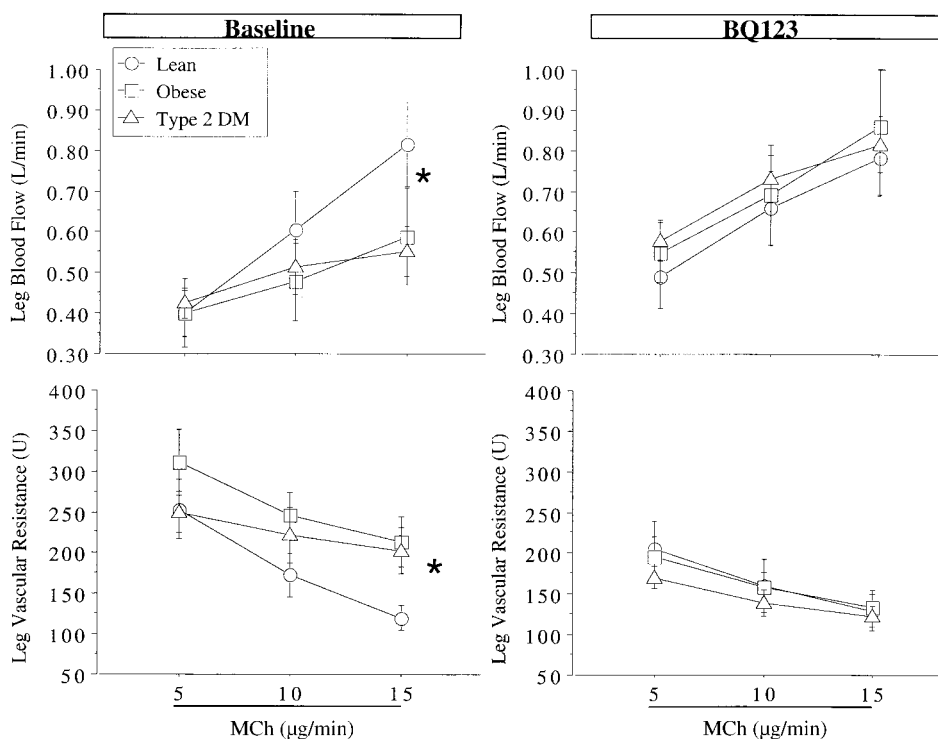


FIG. 5. Effect of BQ123 on endothelium-dependent vasodilation. *Top:* LBF responses. *Bottom:* LVR. *Left:* baseline. *Right:* BQ123 0.6 mg/min. MCh was administered at 5, 10, and 15 $\mu\text{g}/\text{min}$. At baseline, endothelial dysfunction was present in obese and type 2 diabetic subjects ($*P = 0.01$). The effect of BQ123 to correct impaired endothelium-dependent vascular responses in obese and type 2 diabetic subjects was significant for both LBF ($P = 0.01$) and LVR ($P = 0.02$).

exerted by ET-1 itself, through simultaneous actions at ET_A and ET_B receptors (which can engender the production of NO). Regardless of the precise nature of this altered balance of determinants of vascular tone in obese and type 2 diabetic subjects, the present findings suggest that increased ET-1 effect reflects an overall reduction in vasodilator reserve in these subjects and that net vasodilator function can be improved by ET_A blockade.

Although a considerable body of experimental evidence suggests an important role of endothelin in the complications of insulin-deficient diabetes (23), only a few studies have specifically addressed insulin-resistant states and no data exist in animal models of type 2 diabetes. Fructose-induced insulin resistance is associated with augmented vascular ET-1 sensitivity (12) and with beneficial effects of endothelin receptor blockade on vascular function (24). In galactose-fed rats, augmented extracellular matrix production was partially prevented by endothelin receptor blockade (25). Finally, in mesenteric artery and thoracic aorta from spontaneously insulin-resistant obese Zucker rats, increased endothelin receptor mRNA expression and receptor levels were noted (13). These findings are consistent with the current results in that they suggest an association between insulin resistance/hyperinsulinemia and endothelin-mediated vascular dysfunction.

Our observation of increased response to ET_A blockade in type 2 diabetes is in contrast to observations made using forearm plethysmography and the same ET_A antagonist as the present study, at the standard forearm dose (26). In a population of subjects with poorly controlled type 2 diabetes both with and without microvascular disease, there was no overall effect of BQ123 on basal vascular

responses. It is surprising that the subjects who had diabetes without microvascular disease exhibited a trend to vasoconstriction during BQ123 infusion. The authors concluded that this finding reflects impaired responsiveness to endogenous ET-1 among the subjects with diabetes and speculated that this is on the basis of either reduced receptor number or impaired postreceptor responses. These data are difficult to reconcile with the present findings and may relate to differences in sensitivity of the forearm versus leg measurements of vascular responses (27) or to inherent differences in the biology of the two vascular beds. More studies exploring these issues in vivo in humans are needed to be able to explain these contrasting findings.

ET_A and endothelial dysfunction in insulin resistance and diabetes. In absolute terms, the vasodilation achieved with MCh was impaired in obese and type 2 diabetic subjects compared with normal lean control subjects at baseline and similar between groups with BQ123 treatment. This effect of BQ123 to improve net vasodilation with MCh was statistically significant for both LBF and LVR (Fig. 5). An important caveat is that the starting point for this vasodilation, the pre-MCh basal tone, was itself differentially affected by BQ123. The possibility arises, therefore, that the vasodilation afforded by MCh is not in fact improved but rather is simply being added to a greater background vasodilation in obese and type 2 diabetic subjects. In addressing this question, we considered the augmentation in vasodilation seen with MCh stimulation as reflected in both the change in LBF and the change in LVR. MCh did not itself change blood pressures, and so for this evaluation both measures are equally valid. This

evaluation revealed that the net increase in vasodilation reflected an augmentation in MCh-induced vasodilation in addition to the change in underlying tone. Furthermore, the index of MCh-stimulated NO production afforded by NOx flux was augmented by BQ123 in obese and type 2 diabetic but not in lean subjects. Therefore, we conclude that ET_A blockade acts to correct endothelial dysfunction in these subjects.

Determinants of endothelin biology. The lack of a difference in effects of endothelin blockade between obese and type 2 diabetic subjects is a novel finding and raises important questions regarding the endogenous stimuli to endothelin production. The two groups were equivalently hyperinsulinemic but were not otherwise metabolically equivalent and in particular differed in their insulin sensitivity and in the existence of frank hyperglycemia.

It is possible that despite the overall metabolic mismatch between these groups, they were in fact equivalent in the factor(s) most directly relevant to the endothelin system. It is also possible that the equivalence between groups reflects a functional maximum in stimulated endothelin activity reflecting metabolic abnormalities present in obesity alone, such that additional relevant metabolic factors present with frank diabetes are incapable of further augmenting this activity. Alternately, the excess ET-1 tone may reflect a dysfunctional endothelium independent of any direct interactions of insulin and endothelin.

The available literature addresses only the first of these possibilities. A relationship between insulinemia and ET-1 biology is suggested by the increased circulating levels of ET-1 in type 2 diabetes reported in most (16,17,19,21) but not all studies (26,28). In vitro and in vivo evidence suggests that insulin regulates endothelin by acutely stimulating its production (14,15,20,21). In health, the competing effects of insulin as a stimulus to both endothelium-dependent vasodilation and ET-1 production are approximately balanced, at least in the forearm circulation (29). Parallel studies in clinical states of insulin resistance have not been reported. The present finding of a lack of dependence of the differential sensitivity to ET_A blockade on serum insulin concentration or HOMA-IR argues against a prominent direct interaction. However, this is not definitive particularly because no intervention was undertaken to alter insulinemia or insulin resistance, which may be required to reveal such a relationship. Additional experiments under controlled conditions of hyperinsulinemia will be needed to address this question directly.

Under ET_A blockade, the effects of unimpeded endothelin action on vascular smooth muscle cells (rather than endothelial cells) through ET_B receptors have been of concern (30). However, we found no evidence for a net detrimental effect of isolated blockade of the ET_A receptor among our obese subjects with endothelial dysfunction and in fact were able to demonstrate a correction of endothelial function despite the presumed continued access of ET-1 to the vascular wall ET_B receptors. Whether this is in fact beneficial through contributions to the net vasodilator tone is an important unanswered question.

By multivariate analysis, both BMI and total serum cholesterol were predictors of the differential response to BQ123. However, of these, only BMI was a predictor in the initial univariate analysis. It therefore seems appropriate

to limit applying this result to the generation of additional hypotheses.

Limitations. The principal weakness of this study relates to the concurrent changes in systemic hemodynamics seen during infusion of BQ123. Reductions in MAP of 4–5 mmHg were seen, without compensatory increases in heart rate. No such changes have been described in forearm studies with the lower rates of BQ123 infusion that technique allows. Importantly, in the present study, this systemic effect did not differ across groups. We cannot rule out that the intrinsic reflex responses to these changes differed across groups, but the lack of change in heart rate in any group argues against a prominent effect. Furthermore, any potential confounding of our results by this systemic response (i.e., a superimposed reflex vasoconstriction) would be anticipated to reduce the magnitude of the observed vasodilator responses.

CONCLUSIONS

Previous work began to define the normal counterbalance of endothelin and NO in health. The present findings explore for the first time the contributions of ET-1 to vascular tone and endothelial dysfunction in humans with obesity and type 2 diabetes. ET_A blockade revealed increased contributions of ET-1 to vasoconstrictor tone in obese and diabetic subjects and improved endothelial function in these subjects. These findings suggest an important role for excess endothelin tone in the vascular dysfunction in obesity and type 2 diabetes, which may present a novel approach to therapies that target the vasculature in these conditions.

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