

Interactions Between Endothelin and Nitric Oxide in the Regulation of Vascular Tone in Obesity and Diabetes

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Endothelial dysfunction reflects an imbalance of vasodilators and vasoconstrictors. Endogenous endothelin activity seems to be increased in human obesity and type 2 diabetes, and cellular studies suggest that this factor may itself reduce bioavailable nitric oxide (NO). We studied 20 lean, 20 obese, and 14 type 2 diabetic individuals under three protocols, measuring leg vascular responses to intra-arterial infusions of *N*^G-monomethyl-L-arginine (L-NMMA; an inhibitor of NO synthase) alone or in combination with BQ123 (an antagonist of type A endothelin receptors) or phentolamine (used as a control vasodilator). NO synthase inhibition alone (study 1) produced an ~40% increase in leg vascular resistance (LVR) in all three participant groups, which was not statistically different across groups (increase in LVR: lean, 135 ± 28; obese, 140 ± 32; type 2 diabetic, 184 ± 51 units; NS). By design, BQ123 at the infused rate of 3 μmol/min produced equivalent ~35% reductions in LVR across groups. The subsequent addition of L-NMMA produced a greater increase in LVR among obese participants than lean or type 2 diabetic participants (study 2: lean, 182 ± 48; obese, 311 ± 66; type 2 diabetic, 186 ± 40; *P* = 0.07). Compared with study 1, the effect of L-NMMA was magnified by BQ123 in obese participants but not in lean or type 2 diabetic participants (*P* = 0.005, study 1 vs. 2; *P* = 0.03 for group effect). Phentolamine (75 mg/min) produced vasodilation in obese participants comparable to that seen with BQ123 but failed to augment the L-NMMA response. Endothelin antagonism unmasks or augments NO synthesis capacity in obese but not type 2 diabetic participants. This suggests that impaired NO bioavailability as a result of endogenous endothelin may contribute to endothelial dysfunction in obesity, in addition to direct vasoconstrictor effects of endothelin. In contrast, endothelin antagonism alone is insufficient to restore impaired NO bioavailability in diabetes. *Diabetes* 53: 2060–2066, 2004

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ET, endothelin; LBF, leg blood flow; L-NMMA, *N*^G-monomethyl-L-arginine; LVR, leg vascular resistance; NOS, nitric oxide synthase.

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Vascular tone is regulated through the actions of locally produced agents (1). Among the vasoconstrictors, the most potent agent is endothelin, which exerts its vasoconstrictor actions principally through type A endothelin (ET)_A receptors (2). Of the vasodilators, nitric oxide (NO) seems to be the most important contributor to the acute regulation of vascular tone (1). Vascular tone in health reflects the balance of these opposing factors (3,4).

Endothelial dysfunction in obesity and diabetes is evident clinically as a failure to vasodilate adequately after application of an endothelium-dependent vasodilator, but this may reflect not only impaired NO bioavailability (5–7) but also excess vasoconstrictor tone. Findings from both our laboratory (8) and others (9) suggest that endogenous ET-mediated vasoconstrictor tone is augmented in these insulin-resistant states. It is interesting that ET-1 and NO function in negative feedback loops for each other, each acting to limit the action of the other. These effects are exerted through actions at multiple levels, including reduced transcription (10–13) and modulation of enzyme activity (14). It therefore is possible that ET contributes to endothelial dysfunction both directly, through its vasoconstrictor effects, and indirectly, through inhibitory effects on NO production.

This suggests the hypothesis that the excess ET action in the insulin-resistant states of obesity and type 2 diabetes affects vascular tone in part through impairment of NO generation by the vascular endothelium. To explore this hypothesis, we undertook studies with *N*^G-monomethyl-L-arginine (L-NMMA), an antagonist of NO synthase (NOS), and BQ123, an antagonist of ET_A receptors, in diabetic, nondiabetic obese, and lean individuals.

RESEARCH DESIGN AND METHODS

Nondiabetic individuals were recruited through newspaper advertisement and classified as either lean or obese according to BMI cut points of ≥26 kg/m² for men or ≥28 for women. Individuals 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 were 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 individuals who had taken a peroxisome proliferator-activated receptor-γ agonist within the previous 6 months were excluded. Diabetic individuals with previous evidence of retinopathy, neuropathy, or nephropa-

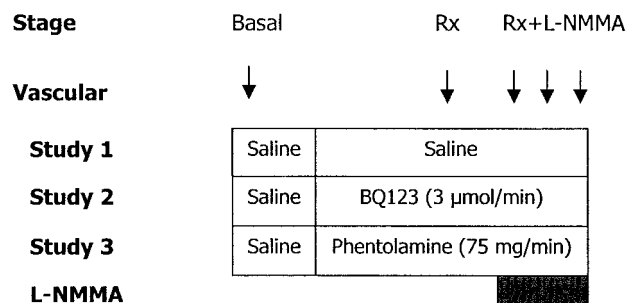


FIG. 1. Schema of the investigation protocols. The arrows represent a set of hemodynamic and LBF measurements. All agents were infused directly into the femoral artery. L-NMMA was infused at 16 mg/min, with continuous measurements during the infusion. For studies 2 and 3, the vasodilator infusions continued during the application of L-NMMA.

thy were excluded. All participants 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. Diabetic participants withheld oral hypoglycemic agents for a minimum of 14 days before the study. This study was approved by the local institutional review board, and all participants gave written informed consent. All procedures were performed in accordance with institutional guidelines.

Technique. All studies were performed after an overnight fast. A 6-F sheath (Cordis, Miami, FL) was placed into the right femoral vein to allow the insertion of a custom-designed 5-F double-lumen thermodilution catheter (Baxter Scientific, Edwards Division, Irvine, CA) to measure leg blood flow (LBF). The right femoral artery was cannulated with a 5.5-F 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 participants in the supine position in a quiet, temperature-controlled room. Baseline LBF and mean arterial pressure 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). At baseline, 24 LBF measurements were obtained at ~ 30 -s intervals. At each subsequent step, the mean of 10 measurements was taken. Invasively determined mean arterial pressure was recorded with every other LBF determination.

Approach. 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 (Clnalfa, Basel, Switzerland), a high-affinity competitive inhibitor of ET-1 type A receptors. On the basis of preliminary studies performed in our laboratory (data not shown), we infused BQ123 into the femoral artery at a rate of 3 μ mol/min, a dose that provided a near-maximal vasodilating effect in all participant groups. L-NMMA (Clnalfa), a competitive antagonist of L-arginine, was infused at 16 mg/min into the femoral artery to block NO generation by NOS. This is the standard infusion rate used in our laboratory and again was chosen on the basis that it provides near-maximal effect across all populations. Control studies were performed with the α -adrenergic receptor antagonist phentolamine (Regitine; Bedford Labs, Bedford, OH), infused at 75 mg/min into the femoral artery, a rate previously shown in our laboratory to induce a 30–40% vasodilation in obese individuals. Study drugs were infused at comparable and low rates (1–2 ml/min), which are without confounding effects on the vasomotor responses to the agents themselves.

Protocols. The protocols are presented diagrammatically in Fig. 1. Although not part of the current analysis, all protocols included measurements of methacholine-stimulated dose responses at each stage, together with a minimum 20-min washout period (confirmed by return of LBF rates to baseline values) before proceeding with subsequent interventions and measurements.

Study 1. Lean, obese, and type 2 diabetic participants were studied under this protocol. Untreated basal LBF and hemodynamic measurements were performed, followed by a washout period. We then applied an intrafemoral arterial infusion of L-NMMA for 15 min, with continuous LBF and hemodynamic measurements during the infusion. The maximal responses to L-NMMA, averaged over 5-min intervals and usually evident during the final 5 min of this infusion, were used in subsequent statistical analyses.

Study 2. Lean, obese, and type 2 diabetic participants were studied under this

protocol. Untreated basal measurements were performed as above. This was followed by the institution of an intrafemoral arterial infusion of BQ123 in a two-stage protocol, including measurements at 1- and 3- μ mol/min infusion rates. The current analysis includes measurements taken at the higher infusion rate, followed by measurements during the coinfusion of L-NMMA with BQ123. As above, this coinfusion lasted 15 min, LBF and hemodynamic measurements were taken continuously during this interval, and maximal responses were used in subsequent analyses.

Study 3. Only obese participants were studied under this protocol. To address the concern that any alterations in NO tone seen during infusion of BQ123 reflected a nonspecific response to vasodilation rather than a specific effect of ET receptor antagonism, we undertook a control study with phentolamine. Untreated basal measurements were performed as above. A direct intra-arterial infusion of phentolamine was then initiated. After 60 min of this infusion (allowing a full and stable effect of phentolamine), LBF and systemic hemodynamic measurements were made. L-NMMA was then coinfused into the femoral artery with phentolamine, as above.

Laboratory. Serum glucose determinations were performed at the bedside using the glucose oxidase method (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 radioimmuno assay, 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 2.6%, respectively. Total serum nitrate (NOx) was determined by chemiluminescence (Sievers NO analyzer, Denver, CO) following a Greiss reaction to oxidize NO and NO₂ to NO₃. NOx flux was calculated as venous NOx times LBF and used as an index of net NO production. Standard methods for cholesterol and triglyceride determinations were used for assays performed through our local hospital's clinical laboratory.

Statistical 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.

In previous studies, we have noted a significant effect of BQ123 on blood pressure as well as LBF; therefore, we planned our analysis using leg vascular resistance (LVR) as the primary end point. Within studies, the groups had comparable initial LVR values, allowing comparisons using both absolute and relative changes in LVR. In comparing L-NMMA effects across studies, with markedly different pre-L-NMMA LVR values, only absolute changes in LVR were used for statistical analysis.

RESULTS

The demographic and clinical characteristics of the individuals who participated in the three studies are presented in Table 1. Within each study, groups differed in expected ways, including higher BMI and blood pressure among obese and type 2 diabetic participants than the lean control participants. Also expected was the elevated glucose and homeostasis model assessment index of insulin resistance among obese and type 2 diabetic participants.

As a whole, the participants in study 1 had higher LDL cholesterol levels than participants in study 2, although no significant differences existed within each group between the studies (Table 1). Also, the obese and type 2 diabetic participants of study 2 had higher mean blood pressures than their counterparts from study 1. There were no differences either within or between studies when effects of sex and race were examined.

Effects of L-NMMA (study 1). Basal NO tone was assessed in studies under protocol 1, using intrafemoral arterial L-NMMA alone at a dose previously demonstrated in our laboratory to provide maximal effectiveness. At baseline, the LVR was not different across the three groups (Fig. 2). NOS antagonism increased blood pressure in all groups ($P < 0.0001$) and reduced LBF in all groups ($P < 0.0001$), producing a net increase in LVR ($P < 0.0001$) that did not differ across groups (LVR increment: lean, $135 \pm$

TABLE 1
Patient characteristics

Measure	Study 1			Study 2			P	Study 3		
	Lean	Obese	Diabetic	Lean	Obese	Diabetic		Obese	Study 1 vs. 2	Study 3 vs. others
Age (years)	32.3 ± 1.9	32.5 ± 2.9	39.9 ± 3.0	36.5 ± 2.2	30.3 ± 3.5	43.0 ± 3.7	0.04	33.6 ± 3.4	NS	NS
Sex (M/F)	10/2	2/6	5/2	7/1	2/4	4/3	NS	3/3	NS	NS
Race (black/white)	4/8	3/4	4/3	2/6	4/2	4/3	NS	4/2	NS	NS
BMI (kg/m ²)	23.8 ± 0.5	34.1 ± 1.4	33.9 ± 2.8	22.9 ± 0.7	36.4 ± 1.0	34.4 ± 3.2	<0.0001	33.5 ± 0.8	NS	NS
% fat	24.7 ± 2.6	43.6 ± 43.8	38.4 ± 5.6	22.4 ± 2.1	43.8 ± 4.3	38.7 ± 6.5	0.0094	37.8 ± 2.6	NS	NS
HbA _{1c} (%)	—	—	9.9 ± 1.2	—	—	11.2 ± 2.8	<0.0001	—	NS	NS
Glucose (mmol/l)	5.0 ± 0.1	5.1 ± 0.1	12.6 ± 1.6	4.9 ± 0.1	5.2 ± 0.1	9.2 ± 1.2	0.0005	5.0 ± 0.2	NS	NS
Insulin (pmol/l)	56.4 ± 13.2	81.6 ± 18.6	102.6 ± 32.4	47.4 ± 6.0	60.6 ± 18.0	90.0 ± 38.4	NS	84.9 ± 15.5	NS	NS
HOMA-IR	2.1 ± 0.5	3.0 ± 0.7	10.6 ± 2.4	1.8 ± 0.2	2.7 ± 0.6	5.5 ± 2.2	0.0007	3.2 ± 0.6	NS	NS
MAP (mmHg)	85.5 ± 2.2	95.4 ± 3.3	94.4 ± 3.9	88.0 ± 2.3	103.6 ± 5.5	99.4 ± 2.1	0.01	96.8 ± 6.3	NS	NS
Cholesterol (mmol/l)	3.6 ± 0.1	3.7 ± 0.2	3.7 ± 0.6	3.1 ± 0.2	3.4 ± 0.2	3.6 ± 0.2	NS	2.1 ± 0.5	NS	0.01
LDL (mmol/l)	2.6 ± 0.3	2.2 ± 0.2	2.3 ± 0.2	1.9 ± 0.2	2.0 ± 0.1	2.2 ± 0.2	NS	2.3 ± 0.4	NS	NS
HDL (mmol/l)	0.8 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	NS	0.8 ± 0.1	NS	NS
Triglycerides (mmol/l)	1.4 ± 0.4	1.5 ± 0.3	2.1 ± 0.4	1.1 ± 0.1	1.2 ± 0.2	1.6 ± 0.2	NS	1.7 ± 0.2	NS	NS

Values are means ± SE for each group. Measurements were made the morning of the study protocol, except for blood lipid levels, which were measured within 4 weeks of the study. P values represent the ANOVA comparing all three groups. HOMA-IR, homeostasis model assessment of insulin resistance; MAP, mean arterial pressure.

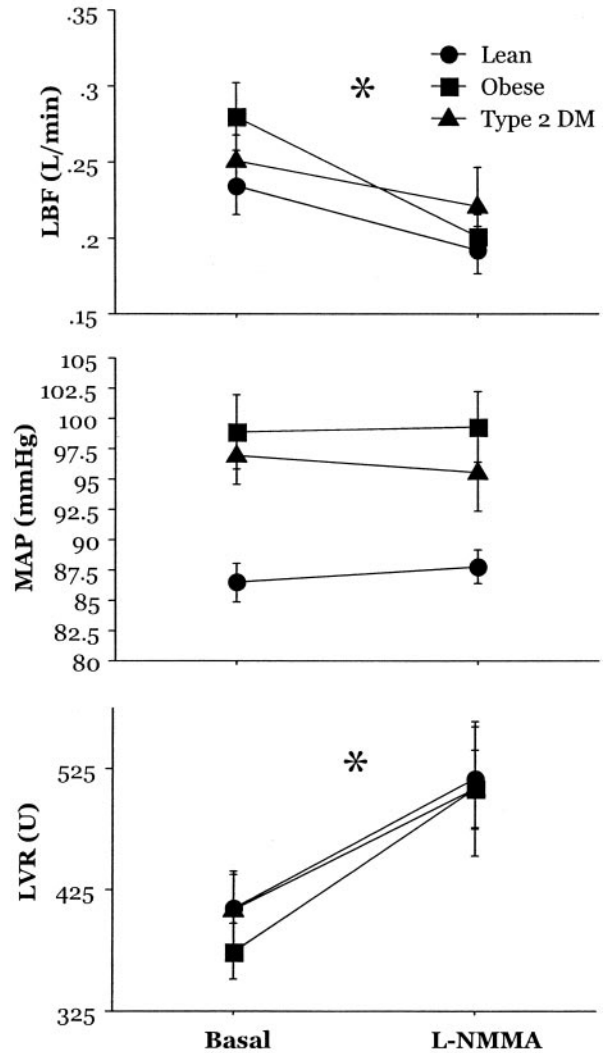


FIG. 2. Study 1 results. MAP, mean arterial pressure. *P < 0.001 for L-NMMA effect; NS for group effect.

28; obese, 140 ± 32; type 2 diabetic, 184 ± 51 units; Fig. 1). There was also no statistical difference across the three groups in the relative increase in LVR after L-NMMA (lean, 35 ± 7%; obese, 46 ± 11%; type 2 diabetic, 47 ± 16%).

Effects of BQ123 and L-NMMA (study 2). The participants who were studied under this protocol again differed in the expected ways, with higher blood pressures among obese and type 2 diabetic participants and elevated glucose, insulin, and triglyceride levels seen in type 2 diabetic participants (Table 1). Also, the baseline circulating ET-1 levels were statistically higher among the type 2 diabetic participants (lean, 8.5 ± 1.3; obese, 7.9 ± 2.6; type 2 diabetic, 15.8 ± 2.4 pg/ml; P = 0.02). BQ123 was without effect on circulating insulin, glucose, or free fatty acid levels (data not shown) and also did not change femoral venous ET-1 levels (lean, 10.7 ± 2.3; obese, 12.9 ± 3.5; type 2 diabetic, 17.5 ± 4.1 pg/ml; NS vs. baseline levels).

The intrafemoral arterial infusion rate of BQ123 was chosen to provide a near-maximal and equivalent reduction in LVR across the study groups (P < 0.0001 for BQ123 effect, NS for ANOVA comparing groups; Fig. 3). This change resulted from concurrent changes in LBF and blood pressure that were more pronounced in the obese

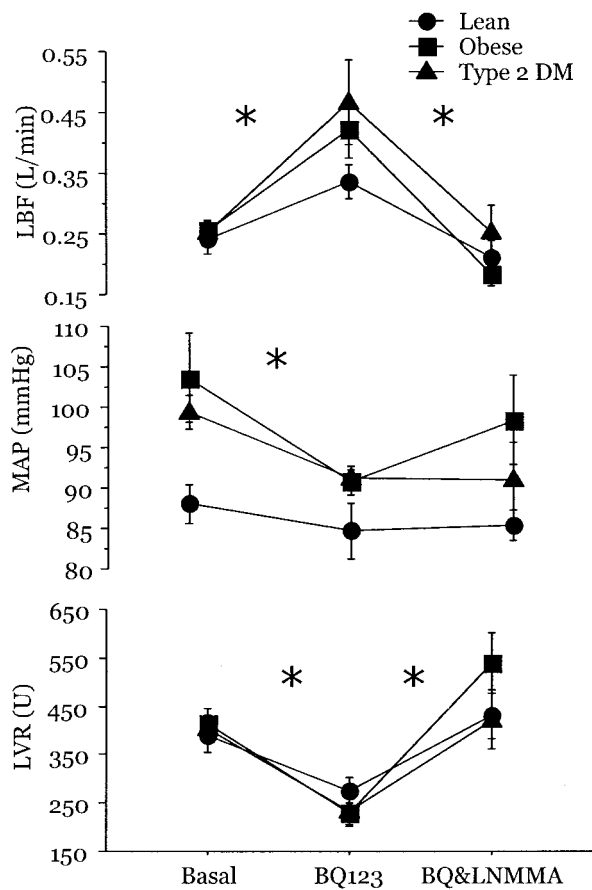


FIG. 3. Study 2 results. * $P < 0.001$ for all groups; NS for group effect. For LVR, $P = 0.07$ for L-NMMA obese vs. others. MAP, mean arterial pressure.

and diabetic participants than in the lean control participants (Fig. 3), although not reaching statistical significance individually ($P = 0.08$). The subsequent addition of intrafemoral arterial L-NMMA produced increases in LVR in all groups ($P < 0.0001$), principally reflecting reductions

in LBF (Fig. 3). Neither the final LVR (lean, 456 ± 61 ; obese, 539 ± 62 ; type 2 diabetic, 419 ± 56 ; $P = 0.3$) nor the absolute increment in LVR (lean, 182 ± 48 ; obese, 311 ± 66 ; type 2 diabetic, 186 ± 40 ; $P = 0.07$) was statistically different across the three groups. In contrast, whereas the relative changes in LVR in response to L-NMMA (percentage increase in LVR: lean, $68 \pm 18\%$; obese, $146 \pm 37\%$; type 2 diabetic, $85 \pm 19\%$) increased in all groups, this increase was greatest among obese participants ($P = 0.04$).

We have previously reported an effect of BQ123 to augment endothelium-independent vasodilation in obese and diabetic individuals using a lower dose of BQ123 (8). This effect was also evident under the current conditions: baseline impairments in methacholine-stimulated vasodilation were present (nadir LVR: lean, 120 ± 16 ; obese, 215 ± 30 ; type 2 diabetic, 203 ± 28 units; $P = 0.01$ including baseline LVR as a covariate), but BQ123 improved the response to methacholine such that the three groups exhibited comparable maximal vasodilation (nadir LVR with BQ123: lean, 100 ± 10 ; obese, 123 ± 13 ; type 2 diabetic, 125 ± 19 units; NS). Of note, no difference between obese and type 2 diabetic participants was apparent in this regard.

Combined ET_A and NOS antagonism produced a small net increase in LVR compared with the basal resting state ($P = 0.04$; Fig. 3). This effect was most evident in the obese participants and reflected small reductions in both mean arterial pressure and LBF in these participants compared with their baseline values.

L-NMMA with and without ET antagonism. The effect of L-NMMA on LVR under the conditions of study 1 and study 2 differed significantly ($P = 0.005$; Fig. 4). Furthermore, this effect differed by participant group ($P = 0.03$), with a pronounced difference between studies seen in the obese participants (Fig. 4) but not in the other two groups. In other words, we observed an effect of ET antagonism to augment the response to L-NMMA in obese but not lean or type 2 diabetic participants.

Total NOx flux. Concordant with the changes in LVR,

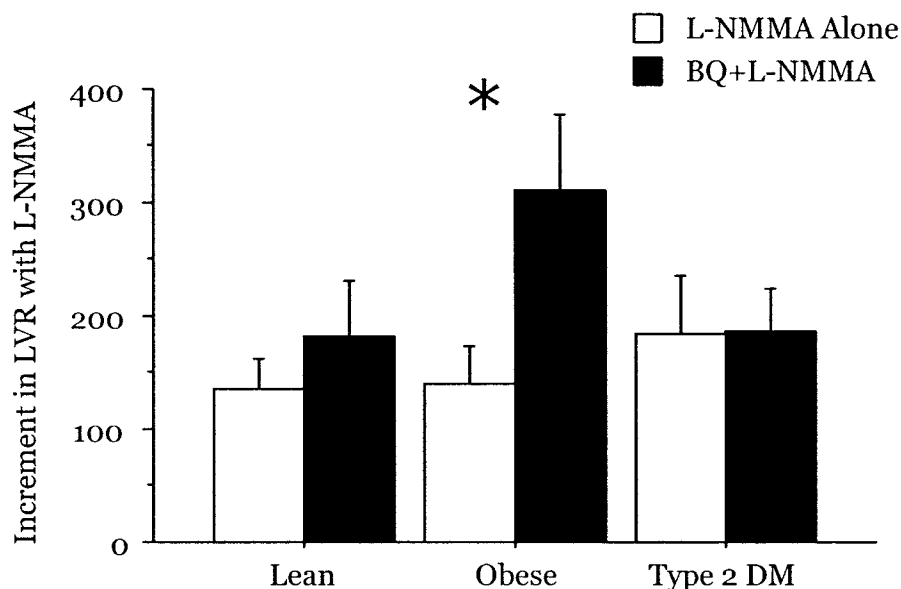


FIG. 4. Comparison of the L-NMMA effect with and without previous application of BQ123. Values are expressed as absolute increment in LVR (see text for discussion). $P = 0.005$ for overall comparison; * $P = 0.04$ for group effect.

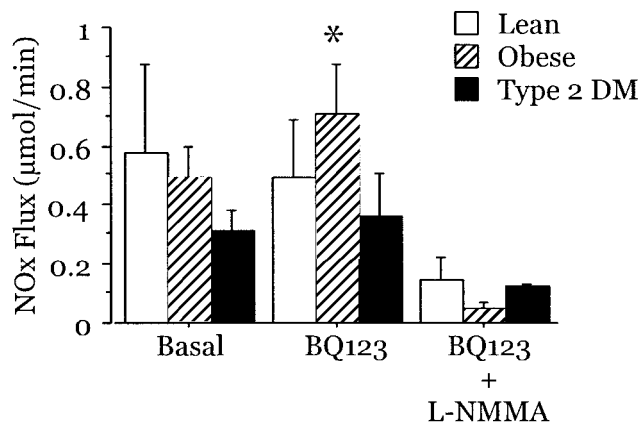


FIG. 5. Effects of BQ123 and L-NMMA on total NOx flux. * $P = 0.03$ for effect of BQ123 to augment NOx flux, significant in obese participants only.

total NOx flux was augmented during the infusion of BQ123 in obese participants ($n = 10$) but not statistically changed in either the lean ($n = 5$) or the diabetic participants ($n = 6$; Fig. 5). L-NMMA markedly reduced NOx flux in all groups, and the final NOx flux rates did not differ across the groups (Fig. 5). Despite a greater mean reduction in NOx flux with L-NMMA in obese participants, as a result of variability in these measurements, the reduction in NOx flux after the addition of L-NMMA to BQ123 did not differ statistically across the three groups.

Effects of phentolamine and L-NMMA (study 3). Control studies with phentolamine were undertaken in obese participants only. These participants were comparable to the obese participants of protocol 2 in clinical and laboratory characteristics (Table 1).

By design, the vasodilation achieved with phentolamine was comparable to that seen with BQ123 under protocol 2 in the obese participants (nadir LVR: BQ123, 229 ± 22 ; phentolamine, 271 ± 47 units; NS). Although subsequently adding L-NMMA clearly produced an increase in LVR ($P < 0.001$), both the absolute and the relative increases in LVR (100 ± 28 units and $47 \pm 14\%$, respectively) were smaller than those seen after the administration of BQ123 ($P = 0.01$; Fig. 6). Of note, these increases in LVR after the application of L-NMMA were comparable to those of study

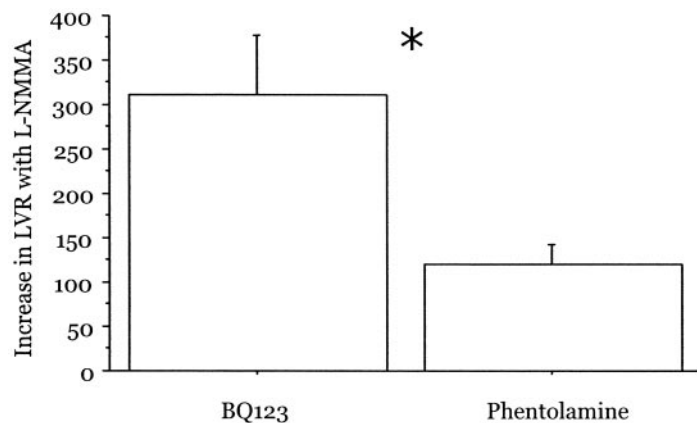


FIG. 6. Study 3 results. Comparison of the L-NMMA effect in obese participants after comparable vasodilation with BQ123 or phentolamine. * $P = 0.006$.

1 (obese participants: absolute increase, 142 ± 32 units; relative increase, $46 \pm 11\%$; NS vs. phentolamine). Therefore, in contrast to the effect seen with ET antagonism, nonspecific vasodilation with α -adrenergic antagonism failed to augment the effect of L-NMMA in obese participants.

DISCUSSION

These studies were undertaken to explore the hypothesis that impaired NO bioavailability in obesity and type 2 diabetes reflects an effect of ET to restrain NO production. We performed leg vascular studies using L-NMMA, a competitive antagonist of NOS, alone or in combination with the ET_A antagonist BQ123 to explore these interactions. L-NMMA alone produced comparable increases in LVR in all groups of participants, a perhaps unexpected result.

The main finding of note is that the effect of L-NMMA was amplified in the obese participants by the previous application of BQ123, which suggests that obese individuals have a capacity for increased NO production that is inhibited by ET's action through ET_A receptors. This difference was not evident in the diabetic participants, in whom the net effect of L-NMMA was unchanged after BQ123.

Resting NO tone in obese and type 2 diabetic participants. Under resting conditions, the relative contribution of NO to resting tone was not different across the groups (Fig. 2). In particular, the effect of L-NMMA alone on vascular tone was statistically equivalent between obese and type 2 diabetic participants, suggesting equal relative dependencies on NO synthesis in these two conditions. This is a point of some debate. Although these two groups exhibit seemingly comparable reductions in bioactive NO (i.e., comparable impairments in endothelium-dependent vasodilator responses [5,8,15]), this does not necessarily result from identical underlying impairments in NO production or increased NO destruction. The literature is divided on the question of whether NO production is decreased in obesity and type 2 diabetes (16–19). Most recently, Avogaro et al. (20) applied a novel approach using labeled arginine and found evidence for reduced conversion of arginine to NO in diabetic individuals.

Combination ET_A and NOS antagonism. The infusion rate for BQ123 was chosen to produce a near-maximal effect, equivalent across the three groups, to allow a comparison of the L-NMMA response. By design, therefore, there was no net difference in nadir LVR with BQ123 across the study groups. Under combined ET_A and NOS antagonism, LVR essentially returned to the level seen in the untreated state. This suggests that, overall, these dominant regulators of vascular tone are in balance, as has been previously shown in lean individuals (4). Although of only moderate statistical significance ($P = 0.04$), with this combined antagonism, we observed a small net increase in vascular tone compared with baseline, most apparent among the obese participants (Fig. 3). This may reflect the action of an unspecified vasoconstrictor, the action of which is normally not evident in the presence of the more dominant vasoactive regulators ET-1 and NO. The present protocols did not allow a more detailed exploration of the nature of this possible third agent.

Perhaps the most interesting finding of the present study

is the unmasking of bioavailable NO in obese participants by ET_A antagonism (Fig. 4). In this analysis, the effect of NOS antagonism under conditions with and without ET antagonism was compared using absolute change in LVR, in view of the markedly different starting points (which would artifactually magnify the apparent relative effects). This allows an unbiased comparison of the effect of L-NMMA between the two studies. This approach reveals an augmented effect of NOS antagonism after ET_A blockade among obese participants only. The specificity of these effects to ET antagonism, rather than being due to a nonspecific effect of the associated vasodilation, is supported by the phentolamine studies presented here (study 3). These findings were also evident with the measurement of NO_x flux, which was increased in obese participants only by the application of BQ123 (Fig. 5). Again, by this measure, ET antagonism alone was not sufficient to exert a similar effect in the diabetic participants.

This augmented NO bioactivity is interesting in that it suggests that obese individuals have an increased underlying capacity (and perhaps stimulus) for the production of NO. However, under normal conditions, NO production seems to be restrained by ET's actions at ET_A receptors in obese individuals. To our knowledge, the only comparable observation in any in vivo model found increased endothelial NOS transcript and activity levels in hypercholesterolemic pigs that were treated chronically with ET receptor antagonists (21). Molecular interactions between the NO and ET systems have been described at multiple levels in vitro. This includes acute regulation of protein function and signal transduction (14), as well as modulation of receptor and converting enzyme levels through effects on transcription and translation (13). This system seems to function with NO and ET-1 as mutual antagonists (22). Under conditions of health, this can produce a tightly regulated balance between these major determinants of vascular tone (3,4,23,24). If, however, the levels or actions of these agents are changed as a result of effects outside this control loop, then this system can be expected to amplify the disparity. With this interpretation, then, we presume that the increased action of ET seen in obesity is due to some feature of obesity itself, but through this feedback system, it serves to directly restrain NO production. Previous results from our laboratory suggested that ET antagonism corrected impaired NO-dependent vasodilation in obese individuals (8), and the present results may explain this earlier finding.

Clearly, one possibility to explain this augmented NO availability is increased production through ET-1 action at ET_B receptors, which have been shown to mediate ET-induced vasodilation in healthy individuals (3,4). The current protocols did not include additional agents to antagonize ET_B receptors; therefore, we cannot make direct comments regarding this possibility. However, even if increased ET_B action accounts in part for this observation, it is difficult to explain the current observation without an augmented capacity for NO production and/or action in obese individuals.

The implicit assumption in assuming increased ET_B action is that tissue ET-1, blocked from acting via ET_A receptors, exerts increased action via ET_B receptors. If so, then we might have expected such actions in all groups, in

particular in the lean control participants whose capacity for NO production is assumed to be normal. Notably, no such augmentation of the L-NMMA response was seen in the lean control participants after BQ123 exposure. Similar results in healthy individuals have been previously reported by other investigators (3,4,23,24). Therefore, although ET_B-mediated NO production is undoubtedly present, augmented action through this system is unlikely to be sufficient to explain the observed increase in NO tone after ET_A antagonism in obese participants. Nevertheless, further studies specifically assessing this possible mechanism will be needed to more fully explore the present observation.

Despite comparable obesity and parallel changes in some metabolic features, no augmentation or improvement in NO bioactivity was seen among the diabetic participants after ET_A antagonism. We have previously observed essentially identical effects of intermediate-dose ET antagonism on vascular responses between these groups (8), suggesting equal ET action between the groups. We interpret this observation as evidence that the removal of ET's restraining effects on NO bioavailability is not sufficient to improve the impairment in NO bioavailability in diabetes. This could reflect additional abnormalities in other influences on NO production, including regulators of NOS activity, such as tetrahydrobiopterin (25,26) or asymmetric dimethylarginine (27), or supervening effects of diabetes to accelerate the destruction of NO before it can act. This last effect is generally attributed to oxidant stress, likely related to hyperglycemia (28,29) and advanced glycation end products (29,30), but possibly also to consumption by glycosylated proteins directly (31). These are only the most prominent of a number of factors that have been found to influence NO production and action and that are abnormal in diabetes. The current experimental design does not allow us to distinguish or comment on these possibilities, but we are confident in the strength of this initial observation of differential responses between obese and diabetic individuals. Specific protocols testing these and other possible factors will be required to better understand the mechanisms underlying this observation.

In summary, in the present work, we present evidence for an effect of BQ123 to augment bioavailable NO, apparent as an increased response to L-NMMA, in obese but not diabetic individuals. Because no such effect was evident in lean control individuals, it is unlikely that this effect is simply due to increased ET_B-mediated NO production. Therefore, it seems that ET_A-mediated actions of ET-1 restrain NO production in obese individuals. The lack of such an effect in the type 2 diabetic individuals suggests that reversal of this effect alone is insufficient to restore NO bioavailability in the setting of the additional abnormalities seen with diabetes. These findings provide evidence that ET contributes to ET dysfunction in obesity through indirect effects on NO bioavailability in addition to the previously demonstrated direct vasoconstrictor effects.

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