*Original Article*

# Endothelin Limits Insulin Action in Obese/Insulin-Resistant Humans

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**The normal action of insulin to vasodilate and redistribute blood flow in support of skeletal muscle metabolism is impaired in insulin-resistant states. Increased endogenous endothelin contributes to endothelial dysfunction in obesity and diabetes. Here, we test the hypothesis that increased endogenous endothelin action also contributes to skeletal muscle insulin resistance via impairments in insulin-stimulated vasodilation. We studied nine lean and seven obese humans, measuring the metabolic and hemodynamic effects of insulin (300 mU · m$^{-2}$ · min$^{-1}$) alone and during femoral artery infusion of BQ123 (an antagonist of type A endothelin receptors, 1 μmol/min). Endothelin antagonism augmented skeletal muscle responses to insulin in obese subjects through changes in both leg blood flow (LBF) and glucose extraction. Insulin-stimulated LBF was significantly increased in obese subjects only. These changes, combined with differential effects on glucose extraction, resulted in augmented insulin-stimulated leg glucose uptake in obese subjects (54.7 ± 5.7 vs. 107.4 ± 18.9 mg/min with BQ123), with no change in lean subjects (103.7 ± 11.4 vs. 88.9 ± 16.3, $P = 0.04$ comparing BQ123 across groups). BQ123 allowed augmented leg glucose extraction in obese subjects even in the face of NOS antagonism. These findings suggest that increased endogenous endothelin action contributes to insulin resistance in skeletal muscle of obese humans, likely through both vascular and tissue effects. *Diabetes* 56:728–734, 2007**

Increased circulating levels of endothelin have been described in a number of insulin-resistant states, including type 2 diabetes (1,2), relatives of subjects with type 2 diabetes (1), obesity (3–5), and polycystic ovarian syndrome (6). Recent work from our laboratory and others has shown that increased endogenous endothelin activity in subjects with obesity and diabetes contributes to the impaired endothelium-dependent vasodilation that characterizes these states (7,8).

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Received for publication 5 October 2006 and accepted in revised form 24 November 2006.

AV, arteriovenous; ET-1, endothelin 1; IRS, insulin receptor substrate; LBF, leg blood flow; L-NMMA, $N^G$-monomethyl-L-arginine; LVC, leg vascular conductance; MAP, mean arterial pressure; NOx, total serum nitrate; $PIP_2$, phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C.

DOI: 10.2337/db06-1406



We know that a portion of the net metabolic actions of insulin depends on flow redistribution (9–11), which insulin regulates by stimulating production of nitric oxide (NO) in the vasculature (12,13). On this basis alone, one could postulate that endothelin acts to impair insulin action by antagonizing this vascular component of insulin action. Many lines of evidence suggest that endothelin may contribute to insulin resistance and vascular dysfunction. In vitro, sustained exposure of insulin-responsive cells to endothelin 1 (ET-1) induces insulin resistance (14–16). Molecular studies have shown that this effect is mediated by effects of endothelin on a number of components of the insulin signaling cascade, including heterologous desensitization of insulin signaling by a $G_{\alpha q/11}$-mediated pathway (14), impaired stimulation of phosphoinositol-3 kinase by insulin receptor substrate (IRS)-1 and IRS-2 (14,17), and impairments in downstream plasma membrane phosphatidylinositol 4,5-bisphosphate ($PIP_2$) and actin cytoskeleton interactions (18).

Endothelin induces insulin resistance in isolated rat soleus muscle strips (16), and studies of whole organisms have found similar effects of exogenous endothelin. In normal conscious rats, bolus intraperitoneal injection of ET-1 raises endogenous glucose and insulin levels and reduces whole-body responses to infused insulin (19). Sustained exposure to ET-1 (delivered continuously by osmotic minipump for 5 days) induces whole-body insulin resistance and was associated with reduced skeletal muscle glucose uptake with evidence for impaired insulin signaling in muscle (16). In humans, ET-1 infusion induces insulin resistance as measured by hyperinsulinemic-euglycemic clamp (20,21).

The actions of exogenous endothelin on metabolism have not been exclusive to skeletal muscle beds. Effects of ET-1 on splanchnic glucose production independent of glucagon or insulin appear to be a component of the effects of endothelin on insulin sensitivity (22), and using the minimal model approach, one group found that ET-1 infusion produced impairment in the acute pancreatic response to glucose rather than inducing peripheral insulin resistance (23). How these observations relate to the circumstance of endogenous states of insulin resistance is unknown.

The metabolic effects of endothelin antagonists have begun to be evaluated in animal models of insulin resistance. In the Goto-Kakizaki rat, a model of type 2 diabetes, antagonism of the ET(A) receptor improved glucose metabolism sufficiently to significantly reduce hyperglycemia (24). Bosentan, a mixed antagonist of type A and B endothelin receptors, was found to improve insulin action in mouse models of physiological stress and insulin deficiency (25). Atrastentan, an ET(A) receptor selective

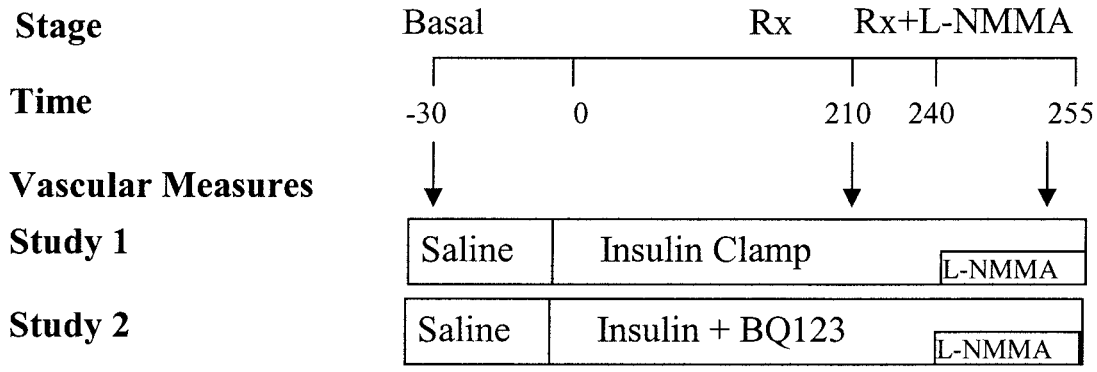


**FIG. 1. Study schema. Subjects were studied on two occasions in random sequence, receiving a systemic hyperinsulinemic-euglycemic clamp (300 mU · m$^{-2}$ · min$^{-1}$) alone or in combination with intrafemoral arterial ET(A) antagonism (BQ123, 1 μmol/min). The NO synthase antagonist L-NMMA was infused at the end of each study (16 mg/min). Arrows indicate stages where vascular and hemodynamic measurements were taken.**

agent, improved fasting and meal-stimulated insulin levels in insulin-resistant Zucker fatty rats (26). No studies have been published of effects of endothelin antagonists on insulin sensitivity in human subjects with endogenous insulin resistance. We have therefore undertaken studies of the effects of endothelin antagonism on insulin-stimulated whole-body and skeletal muscle glucose uptake in lean and obese/insulin-resistant humans.

## RESEARCH DESIGN AND METHODS

Subjects were recruited through newspaper advertisement and classified as either lean or obese according to BMI cut points of ≥26 kg/m$^2$ for men or ≥28 kg/m$^2$ for women. Exclusion criteria included hypertension (systolic blood pressure >140 mmHg diastolic blood pressure >90 mmHg) 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, and significant underlying medical conditions. The use of a peroxisome proliferator–activated receptor γ agonist within the previous 6 months, prior evidence of retinopathy, neuropathy, and nephropathy, was exclusion criteria. 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. 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. Invasively determined MAP was recorded with every other LBF determination.

**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.

ET-1 blockade was achieved with BQ123 (Clinalfa, Basel, Switzerland), a high-affinity competitive inhibitor of ET-1 type A receptors. We have previously demonstrated increased endogenous endothelin activity in obese and diabetic subjects using an intrafemoral arterial infusion rate of 0.6 mg/min (1 μmol/min) (7), and this rate was used in the current study protocol. $N^G$-monomethyl-L-arginine (L-NMMA) (Clinalfa, Basel, Switzerland), a competitive antagonist of L-arginine, was infused at 16 mg/min into the femoral artery to block NO generation by NO synthase. This is the standard infusion rate used in our laboratory (8,27), chosen on the basis that it provides near-maximal effect across all populations. Hyperinsulinemic-euglycemic insulin clamps were performed using a high insulin exposure (300 mU · m$^{-2}$ · min$^{-1}$ administered systemically via an antecubital vein) to provide an unequivocal stimulus to vascular and tissue insulin actions in both lean and obese/insulin-resistant subjects.

The protocol is presented diagrammatically in Fig. 1. Subjects were studied in random sequence on two occasions, receiving insulin alone on one occasion and insulin with concurrent BQ123 on the other occasion. On each study day, basal vascular responses were measured before the initiation of the hyperinsulinemic-euglycemic clamp. Steady state was defined as the stage when glucose infusion rate changes of ≤5% were required to maintain euglycemia and was at least 210 min from the initiation of the clamp procedure. Further vascular measurements were made at this stage, followed by measurements during the co-infusion of L-NMMA.

**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 radioimmune 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 (NO analyzer; Sievers, Denver, CO) after a Greiss reaction to oxidize NO and $NO_2$ to $NO_3$. NOx flux was calculated as venous NOx times LBF and used as an index of net NO production. Endothelin levels were determined by ELISA (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. Statistical significance was accepted at a level of $P < 0.05$. Population descriptive statistics are presented as means ± SD; otherwise, results are presented as means ± SE.

## RESULTS

The subject characteristics are presented in Table 1. We studied nine lean and seven obese subjects. As expected, obese subjects had higher BMI, waist circumference, and insulin levels. Obese subjects had marginally higher glucose and triglyceride levels, not statistically different from lean subjects. One obese subject was found to be diabetic on oral glucose tolerance testing (fasting glucose 5.0 mmol/l, 2-h glucose value of 12.8 mmol/l). The results presented below include this subject; analyses excluding this subject were not materially different. No differences across groups in the lipid profiles were seen.

**Whole-body glucose uptake.** The whole-body response of the obese subjects to insulin was reduced compared

TABLE 1
Subject characteristics

| | Lean | Obese |
|---|---|---|
| *n* | 9 | 7 |
| Age (years) | 37 ± 7 | 33 ± 7 |
| Race (African American/Caucasian) | 5/4 | 6/1 |
| BMI (kg/m$^2$) | 21.8 ± 2.8 | 34.3 ± 4.8* |
| Fat (%) | 16.7 ± 5.6 | 35.7 ± 5.5* |
| Waist (cm) | 72.5 ± 2.9 | 92.7 ± 8.3* |
| Glucose (mmol/l) | 5.2 ± 1.4 | 6.0 ± 2.0 |
| Insulin (pmol/l) | 48.2 ± 32.6 | 199.8 ± 238.9* |
| ET-1 (pg/ml) | 1.05 ± 0.50 | 1.01 ± 0.39 |
| Systolic blood pressure (mmHg) | 93 ± 6 | 107 ± 35 |
| Diastolic blood pressure (mmHg) | 72 ± 15 | 67 ± 7 |
| Cholesterol (mmol/l) | 3.4 ± 0.7 | 3.5 ± 1.0 |
| LDL (mmol/l) | 2.0 ± 0.5 | 2.5 ± 0.7 |
| HDL (mmol/l) | 0.9 ± 0.1 | 1.0 ± 0.3 |
| Triglycerides (mmol/l) | 1.1 ± 0.5 | 1.6 ± 1.0 |

Data are means ± SD. *$P < 0.05$ across groups.

with the lean subjects, as expected (Fig. 2; $P < 0.001$). Concurrent exposure to BQ123 produced a nonsignificant increase in whole-body glucose uptake in obese subjects, with no such increase seen in lean subjects (Fig. 2; means ± SE, 11 ± 13% change in whole-body glucose uptake in obese subjects and −4 ± 9% change in lean subjects). This response was not significantly different across treatment groups. Expressing whole-body glucose uptake per kilogram lean body mass did not materially change this observation (lean, 10.2 ± 0.67 mg · kg$^{-1}$ fat free mass · min$^{-1}$ without BQ123, 10.6 ± 0.9 with BQ123; obese, 5.3 ± 0.4 without BQ123, 5.8 ± 0.2 with BQ123), and again no effect of BQ123 to modulate this response was evident in either group.

**Leg glucose uptake.** Leg glucose uptake was calculated using the Fick equation as LBF multiplied by the arteriovenous (AV) glucose difference (glucose extraction). Changes in these inter-related parameters are presented in Figs. 3–5. Increases in LBF in response to insulin were seen in both subject groups, but as expected, this response was reduced among obese subjects (Fig. 3, $P < 0.001$ comparing groups). Concurrent exposure to BQ123 augmented the leg vascular response to insulin in obese subjects, correcting the deficit in LBF response (Fig. 3, *left*, $P = 0.03$ comparing change in LBF insulin vs. insulin plus BQ123); this effect of BQ123 was not statistically greater in obese than lean subjects ($P = 0.07$ by repeated-measures ANOVA). As expected, given that both insulin and BQ123 are vasodilators, blood pressure dropped in response to both treatments, with a greater effect seen with combined insulin and BQ123 (change in MAP: insulin, −5.9 ± 1.6 mmHg lean and −7.5 ± 2.3 obese; insulin plus BQ123, −9.1 ± 1.6 lean and −14.1 ± 6.9 obese; $P = 0.01$ comparing insulin vs. insulin plus BQ123, NS comparing the effect across groups). Expressing the vascular response as leg vascular conductance (LVC) to adjust for these blood pressure effects again revealed significant

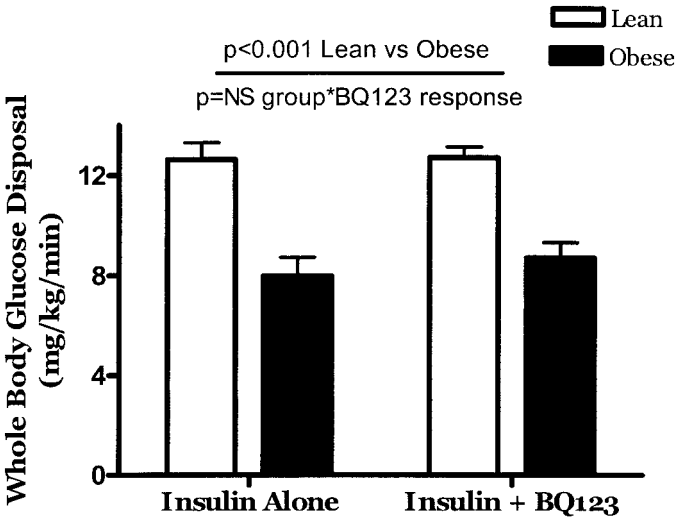


**FIG. 2. Whole-body metabolic response to endothelin antagonism. Changes within each group did not reach significance, and there was no difference across groups in the response to BQ123.**

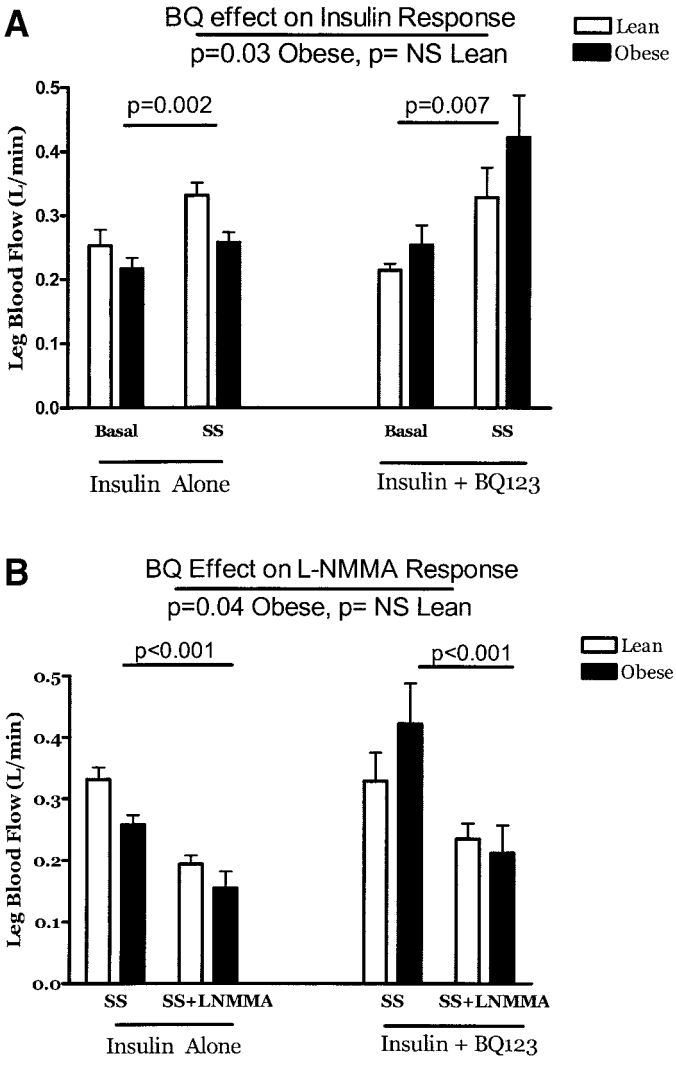


**FIG. 3. Modulation of LBF responses to insulin by endothelin antagonism. Insulin-stimulated increases in LBF were seen in both groups (*A*), with significant reduction in this response after the addition of L-NMMA (*B*). BQ123 augmented the vasodilator response to insulin in obese but not lean subjects ($P = 0.03$ for BQ effect by subject group, repeated-measures ANOVA). The reduction in LBF with L-NMMA was also augmented in obese but not lean subjects. For comparisons within each study, the response did not differ across subject groups except under insulin-stimulated conditions with BQ123, where the response to L-NMMA was greater in obese than lean subjects ($P = 0.015$).**

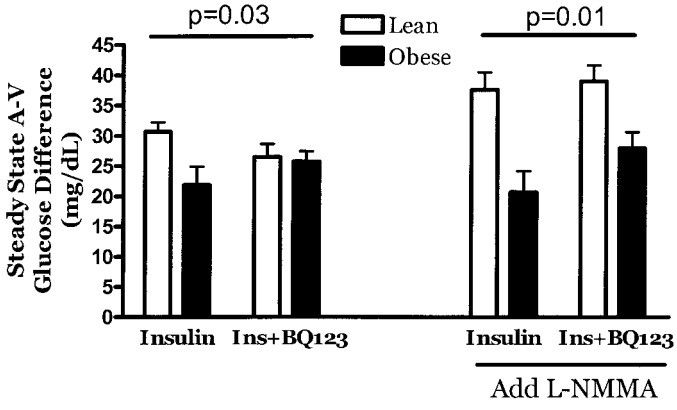


**FIG. 4. Modulation of AV glucose difference under insulin stimulation by endothelin antagonism. Statistics in the figure represent the interaction term for a difference in the observed response to BQ123 across subject groups by repeated-measures ANOVA. L-NMMA significantly changed this parameter compared with the preceding study stages (*left* vs. *right panels*, $P < 0.001$ by repeated-measures ANOVA), driven by a significant increase in AV difference in lean subjects ($P = 0.002$), with a significant difference in this response across subject groups ($P = 0.017$).**

differences in the vascular response with BQ123 (change in LVC: insulin, 1.1 ± 0.3 units lean and 0.5 ± 0.2 obese; insulin plus BQ123, 1.6 ± 0.5 lean and 2.1 ± 0.5 obese; $P = 0.03$ for BQ123 effect, NS across groups).

Glucose extraction, reflected in the AV glucose difference, was modestly increased in obese subjects during concurrent BQ123 exposure compared with insulin alone, although this change did not reach statistical significance (Fig. 4; $P = 0.13$). In lean subjects, a modest, nonsignificant reduction in extraction was seen. By repeated-measures ANOVA, this differential response across groups was statistically significant ($P = 0.03$).

Combined, these effects of BQ123 produced a marked increase in insulin-stimulated leg glucose uptake in obese but not lean subjects (Fig. 5; $P = 0.04$ comparing BQ123 effect across groups). This amounted to a 112 ± 41% increase in leg glucose uptake in obese subjects. This increase was achieved through effects of BQ123 to augment both insulin-stimulated LBF and insulin-stimulated glucose uptake.

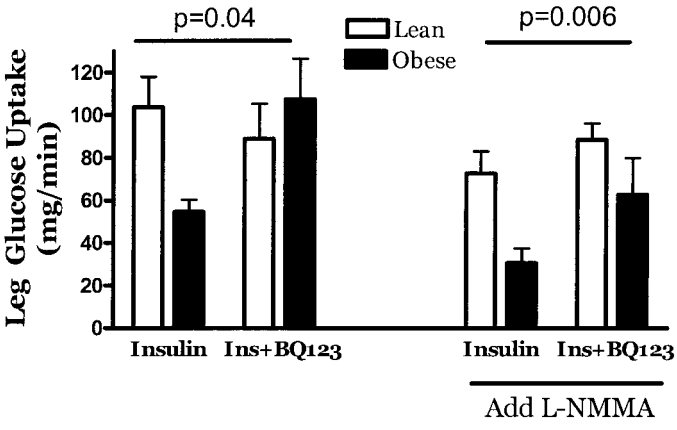


**FIG. 5. Modulation of insulin-stimulated leg glucose uptake by endothelin antagonism. Statistics in the figure represent the interaction term for a difference in the observed response to BQ123 across subject groups by repeated-measures ANOVA. The effect of BQ123 on leg glucose uptake was different across groups (*left*), with a significant increase seen in obese subjects but no significant change seen in lean subjects. The addition of L-NMMA resulted in a reduction in leg glucose uptake in both groups ($P = 0.007$ comparing *left* vs. *right panels*), but overall, obese subjects experienced an augmented post–L-NMMA leg glucose uptake under BQ123-treated conditions ($P < 0.001$).**

**Effects on endothelin.** There were no evident baseline differences in circulating levels of ET-1 between the groups (Table 1). We did not see an effect of insulin to augment systemic levels of ET-1 (steady-state levels 0.89 ± 0.38 and 0.89 ± 0.48 pg/ml in lean and obese subjects, respectively; NS vs. pretreatment levels and NS comparing groups). There was also no evident effect of insulin to augment ET-1 levels specifically in the femoral venous circulation, with or without concurrent BQ123 (not shown).

**Effects on NO.** This protocol included two measures of NO responses. First, the change in leg vascular resistance after exposure to L-NMMA (a competitive antagonist of NO synthase) provides a measure of bioavailable NO (Fig. 3, *bottom*). Second, NOx flux was measured, providing an independent measure of total nitrate production by the leg. Under conditions of insulin stimulation alone, LBF fell significantly in both groups ($P < 0.001$), without a difference in this effect across groups. NOx flux similarly fell significantly (lean 5.3 ± 1.2 μmol/min steady state vs. 2.8 ± 0.6 L-NMMA; obese 5.6 ± 1.4 steady state vs. 3.0 ± 0.9 L-NMMA, $P < 0.001$), without a significant difference across groups in this response. Reductions in LBF with L-NMMA were also seen under conditions of insulin plus BQ123 (Fig. 3, *bottom right*). Under these conditions, the response among lean subjects was not different from insulin alone, but among obese subjects, we observed augmented vasoconstrictor responses to L-NMMA ($P = 0.015$ comparing effect of BQ123 on L-NMMA response across groups). The NOx responses were parallel, although less statistically powerful (lean 5.6 ± 1.4 steady state vs. 3.4 ± 0.6 L-NMMA; obese 8.9 ± 4.3 steady state vs. 5.0 ± 2.4 L-NMMA, $P = 0.02$ for L-NMMA effect, NS comparing effect across groups).

**Metabolic response to L-NMMA.** The reductions in LBF with L-NMMA resulted in increased glucose extraction (i.e., an increased AV glucose difference), evident particularly among lean subjects (Fig. 4, $P < 0.001$ for all subjects, $P = 0.02$ comparing subject groups under conditions with and without L-NMMA). The addition of BQ123 produced a differential response across subject groups ($P = 0.01$), allowing an augmentation of extraction among obese subjects.

Combined with changes in LBF, these changes in glucose extraction produced markedly different leg glucose uptake responses to L-NMMA depending on the prior exposure to BQ123 (Fig. 5, *right*). Overall, insulin-stimulated leg glucose uptake was reduced by L-NMMA ($P < 0.001$, ~33% reduction in lean subjects and ~39% reduction in obese subjects). The prior addition of BQ123 resulted in higher steady-state leg glucose uptake in obese subjects, as noted above. From this shifted starting point, there was a reduction after the addition of L-NMMA, with a significant difference across groups ($P < 0.001$ for steady state vs. L-NMMA, $P = 0.006$ comparing this effect across groups). Nonetheless, the residual insulin-stimulated leg glucose uptake after L-NMMA treatment was augmented by BQ123 in obese subjects ($P < 0.001$ comparing *left* vs. *right panels*).

## DISCUSSION

Using BQ123, an antagonist of the type A endothelin receptor, we have found a significant contribution of

endogenous endothelin to insulin action in obese humans. Through effects to augment insulin-stimulated LBF and insulin-stimulated glucose extraction, a marked increase in leg glucose uptake in response to insulin was observed with endothelin antagonism. No such effect was seen in lean control subjects. Much smaller effects were seen at the level of whole-body glucose metabolism, not reaching statistical significance comparing across or within groups. Overall, these findings suggest that endogenous endothelin contributes to insulin resistance in human obesity/insulin resistance through combined vascular and nonvascular effects in skeletal muscle beds.

This observation places endothelin in a very select group of modulators of vascular tone which are able to also affect tissue metabolism. Vasoconstriction with inhibitors of NO synthase reliably reduces both flow and tissue glucose uptake as previously reported (27,28) and recapitulated here. Catecholamines fall into two camps, either stimulating or impairing metabolism (conceptually, activating either "nutritive" or "non-nutritive" patterns of tissue perfusion) (29,30). By corollary, nonspecific vasodilation is not sufficient in and of itself to augment metabolism (31,32). Even endothelium-dependent vasodilation alone is not sufficient to exert this effect. Rather, this appears to reflect agonist-specific metabolic responses, and until this report, only cholinergic vasodilator agonists and the vasoconstrictors noted above had been observed to enhance the effects of insulin through vascular actions (33,34). Therefore, our observations imply that the actions of endothelin in skeletal muscle vasculature are via mechanisms which support the actions of insulin to redistribute perfusion into nutritive blood flow patterns.

**Endothelin and insulin resistance.** The first suggestion that endothelin interacted with insulin resistance came from observations that states of insulin resistance were associated with increased circulating levels of ET-1 polycystic ovarian syndrome (1–6). Also, improvements in insulin resistance through physiological or pharmacological means are associated with reductions in circulating endothelin (5,6,35,36).

Subsequent experimental studies have shown that the application of exogenous endothelin to intact skeletal muscle (16), intact rats (16,19), and humans can induce impairments in insulin-stimulated glucose uptake (20,21) through mechanisms that involve the type A endothelin receptor (21). However, effects at multiple levels beyond skeletal muscle have been described, including effects on splanchnic glucose production independent of glucagon or insulin (22), effects on hepatic and renal blood flow (37), and effects on islet function (38). These observations highlight the importance of the current observations, which clearly show a significant and relevant effect of endothelin action in skeletal muscle on insulin action.

The current results provide the first data demonstrating a contribution of endothelin to endogenous insulin resistance in humans. This observation is concordant with prior work demonstrating such an effect in the Goto-Kakizaki rat, a model of type 2 diabetes (24), fatty Zucker rats (26), and insulin-deficient rats (25).

It is not obvious that systemic infusions of endothelin appropriately mimic the increased endogenous endothelin activity that is seen in obesity and diabetes. The major source of endothelin is believed to be the endothelial cells lining the vessel wall, but this is a largely paracrine function with the majority of secreted endothelin acting in the underlying tissue rather than being released into the circulation. Therefore, although circulating levels are increased and likely exert actions at a distance, these actions a priori would be expected to represent a minority of the overall effects. To date, little has been done to clarify the balance of these actions in states of endogenous insulin resistance. The contribution of the current study to this discussion lies in the demonstration of a clear and important effect, including a significant vascular effect, within skeletal muscle. Definitive studies regarding the contributions of endothelin to the overall balance of metabolism, including in particular effects on splanchnic glucose metabolism, will be needed to better delineate this issue.

**Vascular versus cellular effects of endothelin.** The current understanding of the mechanisms of action of insulin and cellular interactions of insulin with endothelin supports an expectation for both vascular and nonvascular effects of endothelin antagonism on insulin action. The discovery of endothelin as a vasoconstrictor agent produced by the endothelium prompted initial explorations of its role as a modulator of vascular function (39). The net tissue actions of insulin in skeletal muscle depend in part on the capacity of the vasculature to vasodilate in response to insulin (12,40,41), with this vasodilation actively redistributing blood flow in support of the increased metabolism (13). This vascular action of insulin is impaired in states of insulin resistance (9,42,43), and we and others have previously shown that increased endogenous endothelin contributes to vascular dysfunction in obesity and diabetes (7,8). Based on these observations, we had an a priori expectation that endothelin antagonism would improve insulin-stimulated vasodilation and therefore improve net insulin action in skeletal muscle. This was in fact seen in the current study.

A number of molecular studies have described interactions of endothelin and insulin at the level of insulin signaling. After ET-1 receptor binding, the ET(A) G protein-coupled receptor activates phospholipase Cβ. This, in turn, increases the formation of inositol triphosphate and diacylglycerol, leading to an increase in cytosolic $Ca^{2+}$ and activation of protein kinase C (PKC) (44,45). Acute exposure of insulin-responsive cells to this endothelin stimulates GLUT4 translocation and glucose transport in the absence of insulin (46). Sustained exposure of insulin-responsive cells to ET-1, on the other hand, induces insulin resistance (14,16,17). In vascular smooth muscle cells and adipocytes, ET-1 causes insulin resistance by a PKC-dependent mechanism (14,17). Endothelin has also been found to impair insulin action through interactions with downstream signaling events at the plasma membrane involving $PIP_2$ and actin cytoskeleton components of the insulin response (18), suggesting that ET-1 negatively impacts cytoskeletal structure important for insulin function. From this perspective, the question of effects of endothelin antagonism on glucose extraction as distinct from effects on blood flow is of interest. We observed an increase in insulin-stimulated glucose extraction with BQ123 in obese subjects. Given that impairing insulin-stimulated vasodilation with L-NMMA induced a 30–40% reduction in leg glucose uptake through effects on both blood flow and glucose extraction, concordant with prior reports of this effect (27,47), we cannot attribute the observed increase in glucose extraction to correction of the vascular response alone. Therefore we surmise that this observation reflects actions of BQ123 on the tissue (i.e., cellular) response to insulin over and above the

vascular effects. Together, these effects provided an ~100% increase in leg glucose uptake, not all of which was lost on blocking the NO-mediated vasodilation of insulin. Overall, we interpret these observations as evidence in support of both vascular and nonvascular effects of BQ123 to improve insulin action in human obesity/insulin resistance.

Endothelin and NO are mutually antagonistic at multiple subcellular levels (48,49). This system appears to function with NO and ET-1 as mutual antagonists (50). Under conditions of health, this can produce a tightly regulated balance between these major determinants of vascular tone (8,51,52), and we have recently reported that endothelin antagonism can augment bioavailable NO in obesity (8). In the present study, we performed two unrelated measures of the contribution of NO to the vascular response observed under insulin stimulation with and without endothelin antagonism. The vasoconstriction seen in response to NO synthase antagonism provides a measure of bioactive NO, and the direct measurement of NO flux provides a measure of NO production. Both parameters were increased under insulin stimulation, although the NOx values in particular were highly variable after exposure to BQ123. The changes in LBF are concordant with our prior observations that endothelin antagonism can allow augmented expression of bioavailable NO in obese subjects (8). Reductions in blood flow with L-NMMA were associated with augmented glucose extraction, more prominent among lean than obese subjects, with an effect of BQ123 to restore this effect in obese subjects (Fig. 4). Overall, the net reduction in glucose uptake after L-NMMA was of a similar magnitude to insulin-only conditions but in the setting of an increased starting point, such that the post–L-NMMA levels were significantly higher than without BQ123. These findings suggest that despite changes in NO bioavailability, an independent effect of endothelin antagonism to support or restore glucose metabolism is evident in obese subjects.

**Limitations.** A high dose of insulin used was chosen to ensure that both insulin-mediated vasodilation and augmentation of tissue glucose uptake were evident in both subject groups. However, with this exposure, the responses seen in lean subjects are expected to be near maximal. This limits any possible additional effect of endothelin antagonism to augment the actions of insulin in the lean subjects. Therefore, although the current results provide convincing evidence for an enhancement of these actions of insulin in obese/insulin-resistant subjects, the lack of such an effect in the lean subjects does not exclude contributions of endothelin to insulin action in normal physiology as well.

The current studies use direct intrafemoral arterial exposure of the endothelin antagonist and provide clear effects within the directly perfused skeletal muscle bed. The observed systemic effects therefore reflect changes in whole-body metabolism due to these changes in the directly exposed muscle bed, plus other possible effects in other tissue beds that experience secondary systemic exposure. This balance of effects is not the same as that which would be achieved, for example, with an orally available endothelin antagonist. Therefore questions of insulin-sensitizing effects of systemically or orally delivered endothelin antagonists will need to be specifically investigated.

**ACKNOWLEDGMENTS**

K.J.M. was supported in part by an American Diabetes Association Junior Faculty Award. This work was supported by the Indiana University General Clinical Research Center (National Institutes of Health Grant M01-RR-00750) and directly by the National Institutes of Health (DK-42469).

The expert technical assistance and hyperinsulinemic clamp expertise of Paula Robinett, RN, is gratefully acknowledged.

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