Losartan Increases Muscle Insulin Delivery and Rescues Insulin’s Metabolic Action during Lipid Infusion via Microvascular Recruitment

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ABSTRACT:

Insulin delivery and transendothelial insulin transport are two discrete steps that limit muscle insulin action. Angiotensin II type 1 receptor (AT₁R) blockade recruits microvasculature and increases glucose use in muscle. Increased muscle microvascular perfusion is associated with increased muscle delivery and action of insulin.

To examine the effect of acute AT₁R blockade on muscle insulin uptake and action, rats were studied after an overnight fast to examine the effects of losartan on muscle insulin uptake (protocol 1), microvascular perfusion (protocol 2), and insulin’s microvascular and metabolic actions in the state of insulin resistance (protocol 3). Endothelial cell insulin uptake was assessed using ¹²⁵I-insulin as tracer. Systemic lipid infusion was used to induce insulin resistance. Losartan significantly increased muscle insulin uptake (~60%, p<0.03), which was associated with 2-3-fold increase in muscle microvascular blood volume (MBV, p=0.002) and flow (MBF, p=0.002). Losartan ± angiotensin II had no effect on insulin internalization in cultured endothelial cells. Lipid infusion abolished insulin-mediated increases in muscle MBV and MBF, and lowered insulin-stimulated whole body glucose disposal (p=0.0001) which were reversed by losartan administration. Inhibition of nitric oxide synthase abolished losartan-induced muscle insulin uptake and reversal of lipid-induced metabolic insulin resistance.

We conclude that AT₁R blockade increases muscle insulin uptake mainly via microvascular recruitment and rescues insulin’s metabolic action in the insulin resistant state. This may contribute to the clinical findings of decreased cardiovascular events and new onset of diabetes in patients receiving AT₁R blockers.
Keywords: Angiotensin receptors; Insulin uptake; Insulin action; Microvascular blood volume;
Muscle
INTRODUCTION

Muscle microvasculature provides endothelial surface area for the exchanges of oxygen, nutrients and hormones between the plasma compartment and muscle interstitium. In the insulin sensitive state, insulin enhances its own delivery to muscle interstitium by relaxing pre-capillary arterioles to recruit microvasculature thus expanding endothelial exchange surface area, and trans-endothelial transport, two discrete steps that limit insulin’s action (1, 2, 12, 45). Insulin’s microvascular action is coupled with its metabolic action as inhibition of insulin-mediated microvascular recruitment with nitric oxide (NO) synthase inhibitor decreases insulin-stimulated glucose disposal by ~40% (39, 40).

Insulin resistance is clearly present in the skeletal muscle microcirculation in patients with or animal models of obesity and/or diabetes and microvascular insulin resistance is closely associated with metabolic insulin resistance in diabetes (1, 23, 24, 37). Both obese Zucker rats (41) and Zucker diabetic fatty rats (14) have decreased basal muscle microvascular blood volume (MBV) and impaired insulin-mediated glucose disposal and microvascular recruitment. In humans with moderate insulin resistance as seen in simple obesity, basal muscle MBV is not decreased but insulin fails to induce muscle microvascular recruitment (15). In experimental insulin resistant states, both inflammatory cytokines and high concentrations of plasma free fatty acids (FFAs) are able to decrease insulin-mediated whole body glucose disposal, along with blunted insulin-mediated microvascular recruitment (13, 21, 49, 50).

We and others have shown that factors that increase muscle microvascular recruitment, such as low intensity muscle contraction (22) and systemic administration of glucagon-like peptide 1 (8), increase muscle insulin uptake. On the other hand, microvascular decruitment induced by PD123319 is associated with decreased muscle insulin delivery and attenuated
insulin action in muscle (9). Thus, modulation of muscle microvascular perfusion could actively alter muscle insulin action and as such pre-capillary arterioles could be a therapeutic target for insulin resistance.

Microvessels express both angiotensin II type 1 (AT₁R) and type 2 (AT₂R) receptors (5, 6, 34). While AT₁Rs mediate vasoconstriction, AT₂R activities promote vasodilation (3, 27, 30). AT₁R blockers have been widely used clinically in patients with various cardiovascular conditions and have been shown to decrease cardiovascular morbidity and mortality and new onset of diabetes in patients on chronic treatment (30, 36). We have recently reported that acute AT₁R blockade potently recruits muscle microvasculature and increases glucose use and AT₂R antagonism yields exactly the opposite (10). While microvascular recruitment associated with AT₂R blockade reduces muscle insulin uptake (9), it remains unknown whether AT₁R blockade-induced microvascular recruitment contributes to increased muscle delivery of insulin hence muscle insulin action.

In the current study, we hypothesized that acute AT₁R blockade increases muscle insulin delivery and improves insulin sensitivity in the insulin resistant state. Our results indicate that administration of losartan increases muscle uptake of insulin via increased microvascular recruitment and prevents lipid-induced metabolic insulin resistance, both via a NO-dependent pathway.

**RESEARCH DESIGN AND METHODS:**

*Animal Preparations and Experimental Protocols:*

Adult male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 220-320 g were studied after an overnight fast. Rats were housed at 22 ± 2°C, on a 12 hr light-dark
cycle, and fed standard lab chow and water ad libitum prior to study. After being anesthetized with pentobarbital sodium (50 mg/kg ip; Abbott Laboratories, North Chicago, IL), rats were placed in a supine position and intubated to maintain a patent airway. A heating pad was used to ensure euthermia. The carotid artery and the jugular vein were cannulated with polyethylene tubing (PE-50, Fisher Scientific, Newark, DE) for arterial blood pressure monitoring, arterial blood sampling, and various infusions. After a 30-45 min baseline period to assure hemodynamic stability and a stable level of anesthesia, rats were studied under the following three protocols (Fig. 1):

**Protocol 1:** Three groups of rats were studied under this protocol (Fig. 1, top panel). Group 1 received a continuous infusion of saline (10 µL/min) for 30 min. Group 2 received a bolus injection of losartan (AT$_1$R blocker, 0.3 mg/kg, i.v.) at time 0 and then saline infusion for 30 min. Group 3 received losartan injection and then systemic infusion of $N^G$-nitro-L-arginine methyl ester (L-NAME, 50 µg/kg/min, Sigma-Aldrich, St. Louis, MO) for 30 min. Blood samples were collected at 25 min for insulin measurement and then each rat received a bolus i.v. injection of $^{125}$I-insulin (1.5 µCi, PerkinElmer Inc, Boston, MA). Rats were sacrificed at 30 min. Blood and gastrocnemius were obtained for determination of muscle $^{125}$I-insulin uptake.

**Protocol 2:** Two groups of rats were studied under this protocol (Fig. 1, middle panel). One group received a continuous infusion of saline (10 µL/min) for 30 min while the other received a bolus injection of losartan (0.3 mg/kg, i.v.) at time 0 and then saline infusion for 30 min. Skeletal muscle MBV, microvascular flow velocity (MFV), and microvascular blood flow (MBF = MBV...
x MFV) were determined using contrast-enhanced ultrasound (CEU) at time 0 and 30 min, as described previously (8, 10, 21, 22, 40).

Protocol 3: Four groups of rats were studied under this protocol (Fig. 1, lower panel). Each rat received either saline (Group 1, 10 µL/min) or Intralipid + heparin (3.3% and 30 U/ml, Groups 2-4) infusion for 3 hrs with a euglycemic hyperinsulinemic clamp (3 mU/kg/min) superimposed in the last 2 hrs (time 0 to 120 min). Groups 3 and 4 received a bolus i.v. injection of losartan (0.3 mg/kg) 5 min before the initiation of insulin clamp. Group 4 rats also received a simultaneous infusion of L-NAME in the last 2 hrs. Arterial blood glucose was determined every 10 min using an Accu-Chek Advantage glucometer (Roche Diagnostics, Indianapolis, IN), and 30% dextrose (30% wt/vol) was infused at a variable rate to maintain blood glucose within 10% of basal (11, 44). Skeletal muscle MBV, MFV and MBF were determined at time 0, 30, 60, and 120 min. Plasma NO concentrations were determined at time 0, 30 and 60 min, as described below. Rats were then sacrificed, gastrocnemius muscle freeze-clamped for later measurement of Akt and Erk1/2 phosphorylation using Western blotting, as described previously (11, 44).

Throughout the study, mean arterial blood pressure (MAP) and heart rate were monitored via a sensor connected to the carotid arterial catheter (Harvard Apparatus, Holliston, MA and ADInstruments, Inc., Colorado Springs, CO). Pentobarbital sodium was infused at a variable rate to maintain steady levels of anesthesia and blood pressure throughout the study. Insulin infusion at 3 mU/kg/min has been shown to potently recruit muscle microvasculature in rats (40). Losartan was obtained from Sigma Chemicals (St. Louis, MO) and at the doses selected does not significantly alter systemic blood pressure (10, 48), but significantly recruit muscle
microvasculature in the postabsorptive state (10). L-NAME at the dose selected (50 µg/kg/min) raises MAP by 20–30 mmHg above baseline without affecting heart rate and completely inhibits insulin-mediated increases in muscle MBV (39).

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (Publication No. 85-23, revised 1996). The study protocols were approved by the Animal Care and Use Committee at the University of Virginia.

Muscle $^{125}$I-insulin uptake:

Muscle insulin uptake was determined in protocol 1 studies using $^{125}$I-insulin as tracer to track the uptake of native insulin as described previously (8, 9, 22). Blood samples were collected for insulin determination and each rat received a bolus i.v. injection of 1.5 µCi $^{125}$I-insulin 5 min prior to the end of the study. Protein-bound $^{125}$I-iodine in blood and muscle samples was precipitated with 30% trichloroacetic acid, and radioactivity was measured. Skeletal muscle insulin uptake was calculated using the following formula: Muscle insulin uptake (fmol/g muscle/5 min) = $^{125}$I-insulin in muscle (DPM/g dry weight/5 min)/blood $^{125}$I-insulin (DPM/ml) x plasma insulin (fmol/ml).

Measurement of Plasma NO levels:

Plasma NO levels were measured using 280i Nitric Oxide Analyzer (GE Analytical Inc.), according to the manufacturer’s instructions. In brief, ice-cold ethanol was added into plasma samples at a ratio of 2:1. The mixture was vortexed, kept at 0°C for 30 min, and then centrifuged at ~ 14,000 RPM for 5 min. The supernatant was then used for NO analysis.
Culture of endothelial cells and determination of insulin uptake:

Endothelial cell insulin uptake was assessed using $^{125}$I-insulin as previously reported (4, 17-19).

In brief, bovine aortic endothelial cells (bAECs) in primary culture were purchased from Lonza (Walkersville, MD). Cells between passages 3 to 6 were cultured in 6-well plates until 80% confluence, serum starved for 18 - 22 hrs and then incubated with pre-warmed N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES)-binding buffer (HBB) (0.1 M HEPES, 0.12 M NaCl, 5 mM KCl, 1.2 mM MgSO$_4$, 8 mM glucose, and 1% bovine serum albumin; pH 7.8) containing 200 pM $^{125}$I-insulin in the presence or absence of unlabelled regular insulin (2 µM), angiotensin II (1 µM), angiotensin II (1 µM) + losartan (10 µM) or losartan (10 µM) at 37°C for 15 min. The reaction was stopped by transferring the culture plates onto ice and washed with ice-cold HBB. Cells were then washed twice with ice-cold acid solution (0.5 M NaCl, 0.2 M acetic acid, pH 3.0) to remove surface bound $^{125}$I-insulin and lysed with 0.5 ml 1 M NaOH on ice for 1 hr. Aliquots of cell lysate were used for protein content determination and radioactivity quantification using a gamma counter. After subtracting the non-specific binding, insulin uptake was calculated and expressed as cpm/µg protein.

Statistical Analysis:

All data are presented as mean ± SEM. Statistical analyses were performed with SigmaStat 3.1.1 software (Systat Software, Inc), using student t-test or ANOVA with post-hoc analysis where appropriate. A p-value of < 0.05 was considered statistically significant.

RESULTS:
Losartan acutely increases muscle uptake via NO-dependent pathway:

We have previously reported that systemic administration of losartan markedly recruits muscle microvasculature (9, 10) and microvascular recruitment is associated with increased muscle delivery of insulin (1, 2). To assess whether losartan indeed increases muscle insulin delivery/uptake, we used $^{125}$I-insulin to trace native insulin movement in vivo. Plasma insulin concentrations did not differ significantly among all three groups at either baseline (83 ± 19 vs. 93 ± 8 vs. 81 ± 4 pM, Saline vs. Losartan vs. Losartan + L-NAME, p=0.687, ANOVA) or at 25 min (86 ± 14 vs. 116 ± 11 vs. 76 ± 6 pM, p=0.06, ANOVA). As shown in Fig. 2, losartan did not alter insulin degradation rates in either blood or in muscle, as reflected by stable fraction of intact $^{125}$I-insulin in each compartment. Muscle clearance of blood insulin trended up, but this increase was not statistically significant (p=0.158, ANOVA). However, losartan injection potently increased muscle insulin uptake (by ~ 60%, p<0.03) and this effect was completely abolished by systemic infusion of L-NAME, suggesting that losartan-mediated muscle uptake of insulin is NO-dependent.

Losartan acutely recruits muscle microvasculature but does not affect endothelial internalization of insulin

To assess whether the increased muscle uptake of insulin was secondary to increased insulin delivery to the microcirculation and/or increased insulin transportation through the vascular endothelium, we first re-confirmed in the current study that losartan injection indeed acutely recruited muscle microvasculature (Fig. 3). Consistent with our prior report, losartan increased muscle MBV ad MBF by 2-3-fold (p=0.002 for both) within 30 min, without affecting muscle MFV. We next determined whether losartan altered insulin internalization by the endothelial
cells, a key step in the process of transendothelial insulin transport. In the presence of excess unlabelled regular insulin, $^{125}$I-insulin internalization decreased by 43% (Fig 4, p=0.0005). On the contrary, losartan, in the presence or absence of angiotensin II, had no effect on $^{125}$I-insulin internalization (Fig 4). This suggests that the increased muscle uptake of insulin induced by losartan was secondary to increased insulin delivery via microvascular recruitment and endothelial uptake of insulin is not the rate-limiting step in this process.

Losartan prevents lipid-induced metabolic insulin resistance via NO-dependent mechanism:

We next examined whether the increase in muscle microvascular recruitment and insulin uptake help prevent lipid-induced metabolic insulin resistance. Systemic lipid infusion potently inhibited insulin-stimulated whole body glucose disposal (by ~ 40%, p<0.0001) but this inhibitory effect was completely reversed with one dose of losartan injection before the initiation of insulin clamp (Fig. 5). In the presence of systemic infusion of L-NAME, this salutary effect of losartan disappeared.

Effect of losartan on insulin-stimulated Akt and Erk1/2 phosphorylation during lipid infusion:

Fig. 6 shows insulin-stimulated phosphorylation of muscle Akt (top panel) and Erk1/2 (lower panel) in the presence or absence of lipid infusion and losartan. There was no statistically significant difference among three groups in insulin-stimulated Akt phosphorylation. Lipid infusion decreased insulin-stimulated Erk1/2 phosphorylation in muscle and this effect was prevented by systemic administration of losartan prior to initiation of insulin infusion (p<0.03).
Lipid infusion inhibits insulin-mediated but not losartan and insulin-mediated microvascular recruitment:

As shown in Fig. 7, insulin infusion potently recruited muscle microvasculature by increasing both muscle MBV (p<0.02) and MBF (p<0.04) without affecting muscle MFV. This effect was completely abolished by systemic lipid infusion. Administration of losartan prior to insulin infusion recruited muscle microvasculature in the presence of lipid infusion, as evidenced by a 2-3-fold increases in both MBV (p<0.02) and MBF (p<0.04). As with insulin alone, MFV did not change. Co-infusion of L-NAME completely abolished losartan-induced increases in both MBV and MBF. Consistent with the changes in MBV and MBF, lipid infusion abolished insulin-mediated increases in plasma NO concentrations which were restored back to insulin alone levels in the presence of losartan (Fig. 8, upper panel). As expected, lipid infusion did not alter MAP values but L-NAME infusion did increase MAP by 20-30 mmHg (Fig. 8, lower panel) which was similar to our prior report (10).

DISCUSSION:

The current study for the first time demonstrated that acute AT$_1$R blockade with losartan potently increased muscle insulin uptake and prevented lipid-induced metabolic insulin resistance in vivo via a NO-dependent mechanism. As in cultured endothelial cells losartan in the presence or absence of AT$_1$R ligand angiotensin II did not alter insulin internalization, a surrogate index of insulin uptake by the endothelial cells, the increased muscle insulin uptake is likely secondary to microvascular recruitment and expanded endothelial surface area. Our findings thus strongly suggest that AT$_1$R blockade improves insulin action via microvascular
insulin delivery which is a major rate-limiting step in skeletal muscle insulin action (1, 12, 32, 33, 45).

While we have previously reported that losartan administration acutely increases muscle microvascular recruitment and glucose use in the postabsorptive state (10), losartan administration did not significantly alter insulin-mediated glucose disposal in the hyperinsulinemic euglycemic state (9). It is very likely that in the insulin sensitive state insulin per se acted to increase its own delivery by recruiting muscle microvasculature and enhancing its own transendothelial transport via activation of insulin signaling pathways (1, 2, 42, 43). As both steps are impaired in the insulin resistant states, our observation that losartan treatment acutely increases muscle microvascular recruitment and restores insulin’s metabolic sensitivity during lipid infusion is of particular significance. Indeed numerous clinical trials have confirmed a salutary effect of AT₁R blockade on decreasing cardiovascular morbidity and mortality and on decreasing the incidence of new onset of diabetes in patients who are chronically on AT₁R blockers (30, 36). Thus, chronic therapy with AT₁R blocker could enhance insulin action via increased muscle delivery of insulin as evidence has strongly suggested that it is the insulin concentrations in the muscle interstitium, not plasma, that correlate with insulin’s metabolic effects (7).

We and others have previously reported that insulin potently increases muscle MBV thus expands microvascular endothelial surface area (13, 15, 16, 29, 31, 35). In the current study, insulin again significantly increased muscle MBV and MBF, and these effects were abolished in the presence of systemic lipid infusion, which is consistent with prior reports in both laboratory animals (13, 21) and humans (28, 31). Administration of losartan prior to the initiation of insulin infusion in the presence of lipid infusion increased muscle MBV by ~ 2-3-fold (Fig. 5), to the
levels seen with losartan alone (10). This was associated with almost a complete restoration in insulin-mediated glucose disposal. While addition of losartan to insulin did not further increase muscle insulin action beyond what we saw with insulin alone in the insulin sensitive state (9), our current results strongly suggest that increased microvascular recruitment must have contributed to this complete restoration in insulin’s metabolic action in the presence of lipid infusion and once again demonstrates a tight coupling between microvascular recruitment and insulin’s metabolic action.

Whether the effect of losartan on insulin uptake and insulin action is secondary to AT1R blockade alone or due to a combination of AT1R blockade and unopposed AT2R activity in vivo needs further investigation. AT2R mediates a vasodilatory effect in large capacitance vessels, resistance arterioles as well as the microvasculature (5, 10, 46, 47) via the bradykinin-NO-cGMP signaling cascade (3, 20, 38). We have previously demonstrated that skeletal muscle pre-capillary arterioles are a major site of AT1R and AT2R action in vivo in insulin sensitive rodents (9, 10). While in the postabsorptive state AT1R blockade with losartan causes a 3-fold increase in muscle MBV and muscle use of glucose, AT2R antagonism with PD123319 renders an ~ 80% reduction in muscle MBV and a reduced muscle use of glucose (10). In the hyperinsulinemic state, unopposed stimulation of AT1R by administration of AT2R antagonist PD123319 markedly recruits microvasculature, reduces muscle insulin uptake and attenuates insulin’s metabolic action (9). Our current results demonstrate that AT1R activity plays important role in the regulation of insulin’s microvascular and metabolic responses in muscle in the insulin resistant state and strongly suggest that pharmacological manipulation of microvascular AT1R and AT2R activity ratio could be an important therapeutic target for the prevention and management of obesity and diabetes.
Despite lipid infusion induced a significant decrease in insulin-mediated glucose disposal, insulin-stimulated Akt phosphorylation was similar among all groups. This is not surprising as previous evidence has suggested that Akt is probably not the critical node in insulin resistance. Indeed, insulin activation of Akt isoforms is normal in muscle of obese nondiabetic and obese diabetic subjects, despite decreases of ~ 50% and 40% in IRS-1- and IRS-2-associated PI3-kinase activity, and ~ 60% decrease in insulin-stimulated glucose disposal in obese diabetic subjects (25). While lipid infusion for a longer period of time than the current study (5.5 vs. 3 hrs) did reduce insulin-mediated Akt1 phosphorylation by 55%, insulin-stimulated Akt2 phosphorylation paradoxically increased by ~40% and Akt3 phosphorylation did not change in rat muscle (26).

In conclusion, acute AT1R blockade with losartan increases muscle insulin uptake mainly via microvascular recruitment and rescues insulin’s metabolic action in the insulin resistant state induced by systemic lipid infusion. This may contribute to the clinical findings of decreased cardiovascular events and new onset of diabetes in patients receiving AT1R blockers. It is likely that AT2R may have also played important role in this process as AT1R blockade leads to unopposed stimulation of AT2R whose antagonism decriuts microvasculature, decreases insulin delivery and attenuates insulin action in muscle.

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FIGURE LEGENDS:

Figure 1. Experimental protocols.

Figure 2. AT_1R blockade increases skeletal muscle ^{125}I-insulin uptake. Five minutes after bolus injection of ^{125}I-insulin (1.5 µCi, i.v.), blood and skeletal muscle samples were collected, and intact ^{125}I-insulin was determined after TCA precipitation. A. Fraction of blood and muscle intact ^{125}I-insulin. B. Muscle clearance of ^{125}I-insulin. C. Muscle ^{125}I-insulin uptake. n = 5 - 9. * p < 0.03 (ANOVA).

Figure 3. AT_1R blockade acutely recruits skeletal muscle microvasculature. CEU measurements were done before and 30 min after bolus injection of losartan (0.3 mg/kg, i.v.). A. MBV. B. MFV. C. MBF. n = 5 - 7. Compared with saline control, * p =0.002.

Figure 4. AT_1R blockade does not affect endothelial internalization of ^{125}I-insulin. bAECs were incubated with ^{125}I-insulin for 15 min in the presence or absence of unlabelled regular insulin (2 µM), angiotensin (Ang) II (1 µM), Ang II (1 µM) + losartan (10 µM), or losartan (10 µM). Compared with control, * p=0.0005.

Figure 5. AT_1R antagonism prevents lipid-induced metabolic insulin resistance. Each rat received 3 hrs of saline or Intralipid + heparin infusion with insulin clamp (1 mU/kg/min) superimposed on the last 2 hrs with or without losartan injection (0.3 mg/kg, i.v.) at time -5 min.
A. Time course of glucose infusion rates (GIR). B. Steady-state GIR. n = 6 - 11. * p<0.001

(ANOVA).

Figure 6. Effect of AT1R blockade on insulin-stimulated skeletal muscle Akt (upper panel) and Erk1/2 (lower panel) phosphorylation. Compared with Lipid + Insulin, * p < 0.03.

Figure 7. AT1R blockade recruits muscle microvasculature during lipid infusion via an NO-dependent mechanism. A. Changes in muscle MBV. * p<0.02 (ANOVA). B. Changes in muscle MFV. C. Changes in muscle MBF. ** p<0.04 (ANOVA). n = 5 - 6.

Figure 8. Changes in plasma NO concentrations (upper panel) and MAP (lower panel). n = 2 - 9. Compared with saline, * p = 0.03.
Figure 1

Protocol 1

0  
25  
30 min

Saline
Losartan
Losartan + L-NAME

Protocol 2

CEU  
CEU

Saline
Losartan

Protocol 3

-60  
0  
120 min

Saline
Intralipid
Intralipid + Losartan @ time 0
Intralipid + Losartan + L-NAME
Figure 2

A. Fraction of intact [125I]-insulin

B. Muscle [125I]-insulin Clearance

C. Muscle [125I]-insulin Uptake

- Saline
- Losartan
- Losartan + L-NAME

Blood
Muscle

NAME

Losartan
Losartan + L-NAME
Figure 3

A. Changes in MBV
B. Changes in MFV
C. Changes in MBF

0 min - 30 min

Losartan
Saline
Figure 4

125I-Insulin Internalization (cpm/µg protein)

Control  Insulin  Ang II  Ang II + Losartan  Losartan

*
Figure 5

A. GIR (mg/kg/min)

- Insulin
- Lipid + Losartan + Insulin
- Lipid + Insulin
- Lipid + Losartan + L-NAME + Insulin

B. Steady-state GIR (mg/kg/min)

- Insulin
- Insulin + Losartan
- Losartan + L-NAME + Insulin
- Lipid Infusion

* Significant difference
Figure 6

Phosphorylated Akt/total

0.8

Lipid + Losartan
Lipid + Insulin

Erk1/2 Phosphorylation

Lipid + Insulin

Insulin

0.8

0.6

0.4

0.2

0

1.6

1.2

0.8

0.4

0

1.6

1.2

0.8

0.4

0

Phosphorylated Akt/total

(p-Akt/total)

(p-Erk/total)
Figure 7

MBV (fold change in VI)

0.0 0.1 0.2 0.3 0.4 1.0

MFV (1/sec)

0.0 0.1 0.2 0.3 0.4

MBF (fold change in VI/sec)

0.0 1.0 2.0 3.0 4.0

0 min 30 min 60 min 120 min

- Insulin
- Lipid+Insulin
- Lipid+Losartan+Insulin
- Lipid+Losartan+L-NAME+Insulin

** *
Figure 8

Plasma NO Concentrations (Fold changes)

MAP (mmHg)

**Legend:**
- Insulin
- Lipid + Insulin
- Lipid + Losartan + Insulin
- Lipid + Losartan + L-NAME + Insulin