Angiotensin II Receptors Modulate Muscle Microvascular and Metabolic Responses to Insulin In Vivo

Weidong Chai,[1] Wenhui Wang,[1,2] Zhenhua Dong,[1,2] Wenhong Cao,[3] and Zhenqi Liu[1]

OBJECTIVE—Angiotensin (ANG) II interacts with insulin-signaling pathways to regulate insulin sensitivity. The type 1 (AT_1R) and type 2 (AT_2R) receptors reciprocally regulate basal perfusion of muscle microvasculature. Unopposed AT_2R activity increases muscle microvascular blood volume (MBV) and glucose extraction, whereas unopposed AT_1R activity decreases both. The current study examined whether ANG II receptors modulate muscle insulin delivery and sensitivity.

RESEARCH DESIGN AND METHODS—Overnight-fasted rats were studied. In protocol 1, rats received a 2-h infusion of saline, insulin (3 mU/kg/min), insulin plus PD123319 (AT_2R blocker), or insulin plus losartan (AT_1R blocker, intravenously). Muscle MBV, microvascular flow velocity, and microvascular blood flow (MBF) were determined. In protocol 2, rats received ^{125}I-insulin with or without PD123319, and muscle insulin uptake was determined.

RESULTS—Insulin significantly increased muscle MBV and MBF. AT_2R blockade abolished insulin-mediated increases in muscle MBV and MBF and decreased insulin-stimulated glucose disposal by ~30%. In contrast, losartan plus insulin increased muscle MBV by two- to threefold without further increasing insulin-stimulated glucose disposal. Plasma nitric oxide increased by >50% with insulin and insulin plus losartan but not with insulin plus PD123319. PD123319 markedly decreased muscle insulin uptake and insulin-stimulated Akt phosphorylation.

CONCLUSIONS—We conclude that both AT_1Rs and AT_2Rs regulate insulin's microvascular and metabolic action in muscle. Although AT_1R activity restrains muscle metabolic responses to insulin via decreased microvascular recruitment and insulin delivery, AT_2R activity is required for normal microvascular responses to insulin. Thus, pharmacologic manipulation aimed at increasing the AT_2R-to-AT_1R activity ratio may afford the potential to improve muscle insulin sensitivity and glucose metabolism. *Diabetes* 60:2939–2946, 2011

S keletal muscle microvascular perfusion distribution is determined by precapillary terminal arteriolar tone. Dilating these arterioles increases microvascular perfusion and expands the capillary exchange surface area, whereas constriction leads to the opposite (1,2). Microvascular insulin resistance and dysfunction are closely related with metabolic insulin resistance in diabetes (2–4). Insulin-mediated microvascular recruitment precedes insulin-stimulated glucose uptake in skeletal muscle (5), and blockade of insulin's microvascular action with N^{ω}-nitro-L-arginine methyl ester (L-NAME) decreases steady-state insulin-stimulated glucose disposal by ~40% (5,6).

To act on muscle, insulin must first traverse the microvasculature perfusing the muscle and then be transported through the vascular endothelium into muscle interstitium. Recent evidence suggests that altered muscle microvascular perfusion profoundly affects insulin delivery and action in muscle (2). Many physiological factors regulate muscle microvascular perfusion in vivo, including insulin, mixed meals, and muscle contraction (7–12). Increased muscle microvascular recruitment induced by muscle contraction is associated with increased muscle insulin uptake (11).

The renin-angiotensin system (RAS) plays a central role in maintaining hemodynamic stability (13,14), and angiotensin (ANG) II can interact with the insulin-signaling pathways to regulate insulin sensitivity. In cultured cells, ANG II acts via the ANG II type 1 receptor (AT_1R) to impair insulin actions (15–17). On the other hand, acutely raising ANG II systemically improves insulin-stimulated muscle glucose utilization in humans (18–20) and increases muscle microvascular recruitment independent of blood pressure changes in rodents (21). Both the AT_1R and ANG II type 2 receptor (AT_2R) are present on endothelial cells, vascular smooth-muscle cells, and other vessel-associated cells throughout skeletal muscle microcirculation (13,22,23). ANG II stimulates cell proliferation and vasoconstriction via the AT_1Rs and promotes vasodilation through the AT_2R (24,25). We recently have reported that both the AT_1Rs and the AT_2Rs significantly regulate basal microvascular tone and glucose use by muscle (21). Although basal AT_2R activity increases muscle microvascular blood volume (MBV) (an index of microvascular surface area and perfusion) and glucose extraction, basal AT_1R activity decreases both (21).

In the current study, we assessed whether ANG II receptors modulate muscle insulin delivery and sensitivity in vivo. Our results indicate that both AT_1Rs and AT_2Rs regulate insulin's microvascular and metabolic action in muscle. Although AT_1R activity restrains muscle metabolic responses to insulin via decreased microvascular recruitment and insulin delivery, AT_2R activity is required for normal microvascular responses to insulin.

RESEARCH DESIGN AND METHODS

Adult male SD 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-h light-dark cycle and fed standard laboratory diet and water ad libitum prior to the study. After being anesthetized with pentobarbital sodium (50 mg/kg i.p.; Abbott Laboratories, North Chicago, IL), the rats were placed in a supine position on a heating pad to ensure euthermia and intubated to maintain a patent airway. 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- to 45-min baseline period to assure hemodynamic stability

and a stable level of anesthesia, rats were studied under the following two protocols.

Protocol 1. Five groups of rats were studied under this protocol (Fig. 1*A*). Group 1 rats received an intravenous infusion of regular insulin (3 mU/kg/min) for 120 min. Group 2 received systemic infusion of insulin (3 mU/kg/min) and PD123319 (AT_2R blocker, 50 µg/kg/min, started at −5 min) for 120 min. Group 3 received a systemic infusion of insulin (3 mU/kg/min) for 120 min and PD123319 (50 µg/kg/min) 30 min after the initiation of insulin clamp. Group 4 received a bolus intravenous injection of losartan (AT_1R blocker, 0.3 mg/kg) 5 min before the initiation of the insulin clamp. 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 (26,27). Group 5 rats received a saline infusion for 120 min.

Skeletal muscle microvascular blood volume (MBV), microvascular flow velocity (MFV), and microvascular blood flow (MBF) were determined using contrast-enhanced ultrasound, and femoral artery blood flow (FBF) was measured using a flow probe (VB series, 0.5 mm; Transonic Systems), as previously described (5,10,11,21). Plasma nitric oxide (NO) concentration was determined at the beginning and the end of insulin infusion, as described below. Rats then were killed and their gastrocnemius muscles freeze-clamped for later measurement of Akt phosphorylation using Western blotting, as previously described (26,27).

Protocol 2. Two groups of rats were studied under this protocol (Fig. 1*B*). Group 1 received a continuous infusion of saline at 10 µL/min for 120 min. Group 2 received a systemic infusion of PD123319 (50 µg/kg/min) for 120 min. At 115 min, each rat received a bolus intravenous injection of ^{125}I-insulin (1.5 µCi; Perkin Elmer, Boston, MA). Rats were killed at 120 min. Plasma and gastrocnemius were obtained for determination of ^{125}I-insulin uptake.

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 AD Instruments, Colorado Springs, CO). Pentobarbital sodium was infused at a variable rate to maintain steady levels of anesthesia and blood pressure throughout the study. Losartan and PD123319 were obtained from Sigma Chemicals (St. Louis, MO). Losartan was given as a bolus injection because of its longer plasma half-life (1.5–2.5 h for losartan and 6–9 h for its active metabolite), whereas PD123319 was given as continuous infusion because of its much shorter half-life (22 min). At the doses selected, neither losartan nor PD123319 significantly altered systemic blood pressure (21,28) but had a profound effect on basal microvascular perfusion (21). The plasma concentrations of losartan achieved were ~0.25 µg/mL (29) and of PD123319 were ~3×10^{-6} mol/L (30); values remained highly specific for AT_1R and AT_2R, respectively. The investigation conformed to the *Guide for the Care and Use of Laboratory Animals*, published by the National Institutes of Health (publ. no. 85-23, revised 1996). The study protocols were approved by the animal care and use committee of the University of Virginia.

Measurement of plasma NO levels. Plasma NO levels were measured using the 280i Nitric Oxide Analyzer (GE Analytical), according to the manufacturer's instructions. In brief, ice-cold ethanol was added into plasma samples at a ratio

of 2 to 1. The mixture was vortexed, kept at 0°C for 30 min, and then centrifuged at ~14,000 rpm for 5 min. The supernatant then was used for NO analysis.

Muscle ^{125}I-insulin uptake. In protocol 2 studies, each rat received a bolus intravenous injection of 1.5 µCi ^{125}I-insulin 5 min prior to the end of the study. This tracer amount of insulin does not increase systemic insulin concentrations and thus can be used to track the uptake of native insulin. We chose this shorter duration of insulin exposure because of the short circulating half-life (<5 min) for intact insulin (31). At the end of the experiment, a blood sample was collected and each rat then was flushed with 120 mL ice-cold saline (10 mL/min) via the carotid artery catheter. Gastrocnemius muscles were dissected from the right hindlimbs. Protein-bound ^{125}I in blood and muscle samples were precipitated with 30% trichloroacetic acid and was radioactivity measured. Skeletal muscle insulin uptake was calculated using the following formula: muscle insulin uptake = ^{125}I-insulin in muscle (dpm/g dry wt/5 min)/blood ^{125}I-insulin (dpm/mL).

Statistical analysis. All data are presented as means ± SEM. Statistical analyses were performed with SigmaStat 3.1.1 software (Systat Software), using one-way ANOVA or one-way repeated-measures ANOVA with post hoc Holm-Sidak or Dunn analysis, where appropriate. A *P* value <0.05 was considered statistically significant.

RESULTS

We have previously demonstrated that basal AT_1R and AT_2R regulate muscle microvascular volume and glucose use (21). Because the microvasculature plays an important role in insulin delivery and substrate exchange between the plasma and interstitial compartments, we first examined whether changes in AT_1R and AT_2R activities altered insulin-mediated glucose disposal. As shown in Fig. 2, injection of losartan had no effect on insulin-mediated glucose disposal. On the other hand, PD123319 infusion, given either immediately before or 30 min after the initiation of insulin infusion, promptly attenuated insulin-stimulated whole-body glucose disposal, and this effect was maintained during the entire 120 or 90 min of PD123319 infusion ($P < 0.02$, ANOVA). At steady state, AT_2R blockade decreased the whole-body glucose disposal rate by ~30% ($P = 0.02$ for both PD123319 groups). In saline-only rats, the microvascular parameters did not change significantly during the course of the study.

We have shown that basal AT_1R tone restricts skeletal muscle MBV, whereas basal AT_2R activity increases muscle MBV (21). We next examined whether selective stimulation of the ANG II subtype receptors by endogenous ANG II regulate insulin-mediated microvascular recruitment.
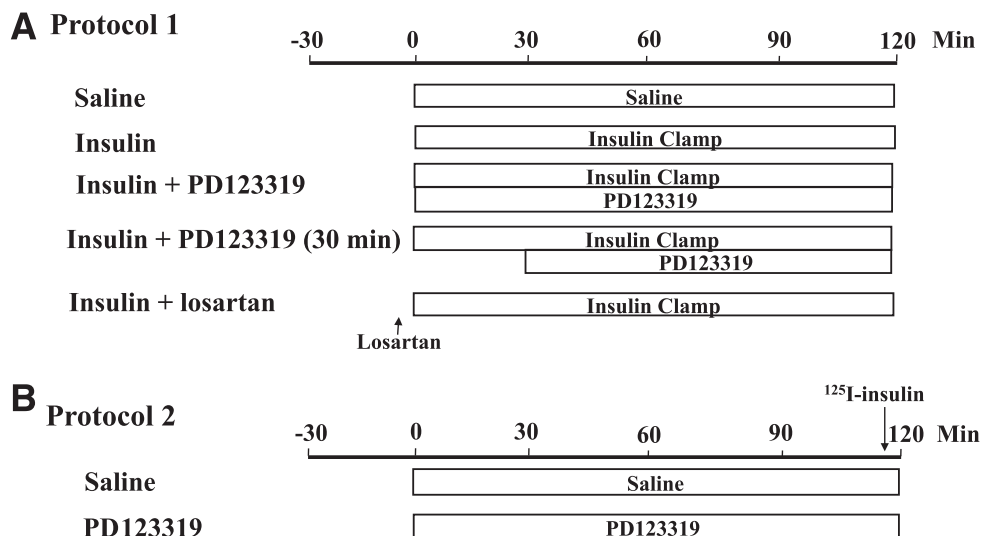

A Protocol 1

| | -30 | 0 | 30 | 60 | 90 | 120 Min |

Saline — Saline
Insulin — Insulin Clamp
Insulin + PD123319 — Insulin Clamp / PD123319
Insulin + PD123319 (30 min) — Insulin Clamp / PD123319
Insulin + losartan — Insulin Clamp

Losartan

B Protocol 2

[125]I-insulin

| | -30 | 0 | 30 | 60 | 90 | 120 Min |

Saline — Saline
PD123319 — PD123319

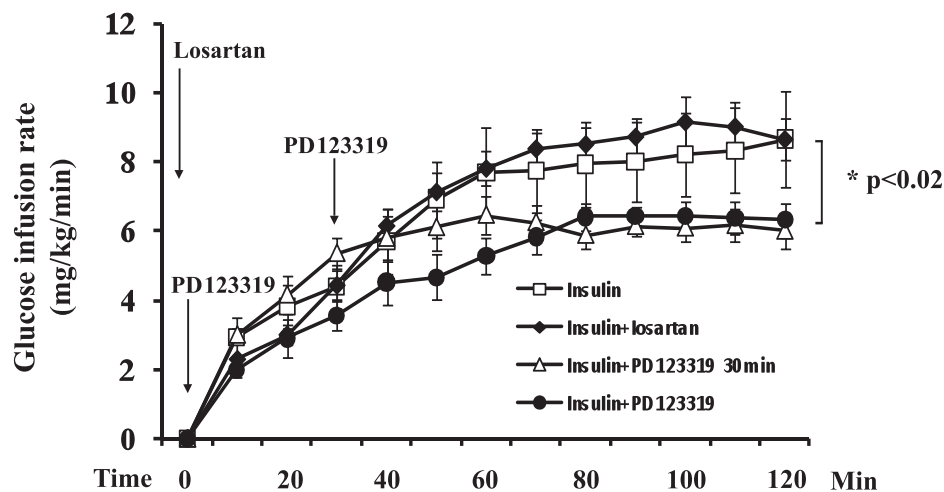**FIG. 1. Experimental protocols.**

FIG. 2. AT$_2$R antagonism attenuates insulin-mediated whole-body glucose disposal. Each rat received 2 h of insulin infusion in the absence or presence of either PD123319 infusion (50 μg/kg/min, started at time −5 or 30 min) or losartan injection (0.3 mg/kg i.v. at time −5 min). n = 8–16. *P < 0.02 (ANOVA).

Figure 3 shows the microvascular responses to insulin infusion. Insulin infusion raised plasma insulin concentrations from 99 ± 15 pmol/L to 685 ± 151 pmol/L (n = 5, P < 0.02). As expected, insulin increased both MBV and MBF without altering MFV (Fig. 3). The addition of PD123319 to insulin infusion promptly inhibited this insulin-mediated increase in MBV and MBF and significantly increased the MFV (Fig. 4). When PD123319 was given before insulin infusion, insulin-induced increases in muscle MBV and MBF were completely prevented. On the other hand, infusion of PD123319 after insulin had already significantly recruited muscle microvasculature (i.e., 30 min after insulin infusion) abrogated the insulin effects. These changes did not seem to be secondary to changes in blood pressure or total blood flow because both MAP and FBF remained stable during the study (Table 1). Injection of losartan 5 min prior to the initiation of insulin promptly increased muscle MBV within 30 min, and the extent of the increase (2.4- to 3.2-fold) was quite similar to what we previously observed using losartan alone (21). The increase in MBV lasted for the entire 120 min. Muscle MFV did not change in the first 90 min but decreased at 120 min. As a result, muscle MBF increased promptly at 30 min, remained elevated at 60 min, and trended down afterward (Fig. 5). Though MAP did not change, FBF decreased by nearly 30% (P < 0.01) (Table 1).

The rapid attenuation of insulin-stimulated whole-body glucose disposal and muscle microvascular recruitment by PD123319 prompted us to further examine the impact of ANG II receptor stimulation by endogenous ANG II on the insulin effects on the vasculature (NO production) and muscle (Akt phosphorylation). Both insulin and losartan increase microvascular recruitment via a NO-dependent pathway (6,21). As shown in Fig. 6A, 2 h of insulin infusion increased plasma NO levels by nearly twofold (P < 0.01). Concurrent PD123319 infusion decreased insulin-mediated increases in plasma NO levels by 37% (P < 0.04) to levels that were not significantly different from the saline controls. On the other hand, losartan injection had no significant impact on insulin-stimulated NO production. Figure 6B shows insulin-stimulated Akt phosphorylation in the skeletal muscle. Similar to the pattern of plasma NO levels, insulin significantly increased muscle Akt phosphorylation, and this effect was suppressed back to the saline control level when PD123319

infusion was superimposed on insulin infusion. Again, losartan had no effect on insulin-stimulated Akt phosphorylation in muscle. Muscle endothelial NO synthase (eNOS) phosphorylation did not differ among all four groups (Fig. 6C).

The above data clearly indicate that AT$_2$R blockade attenuates insulin-mediated muscle microvascular recruitment, glucose disposal, and signaling. To this end, we examined whether modulating the ANG II receptor
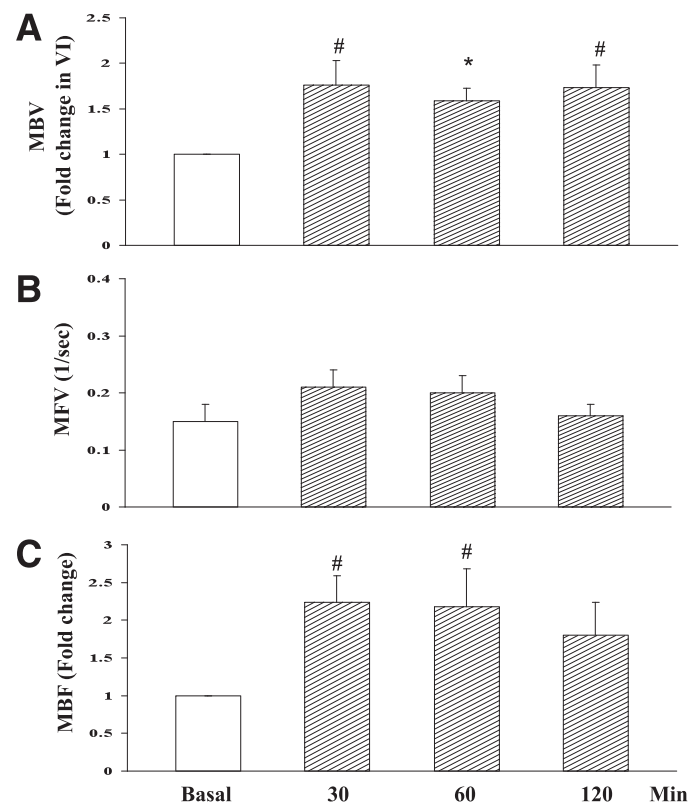

FIG. 3. Insulin increases skeletal-muscle microvascular recruitment in rats. A: Changes in muscle MBV. P < 0.03 (ANOVA). B: Changes in muscle MFV. C: Changes in muscle MBF. P < 0.03 (ANOVA). n = 5–6. Compared with basal level, *P < 0.05 and #P < 0.01.
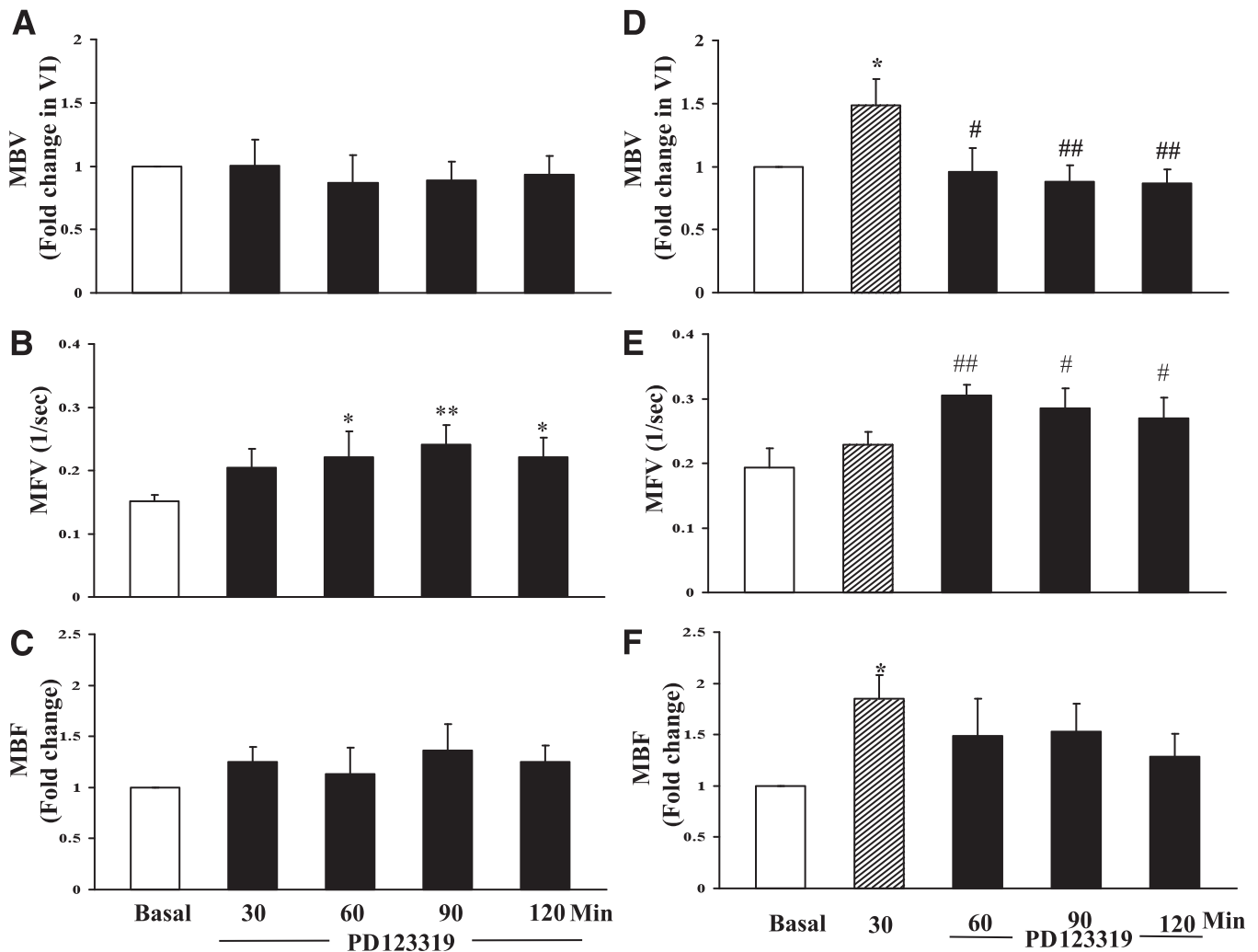
FIG. 4. AT$_2$R blockade attenuates insulin-mediated microvascular recruitment. PD123319 (50 µg/kg/min) was infused systemically either immediately before (*A–C*) or 30 min after (*D–F*) the initiation of insulin clamp. *A* and *D*: Changes in muscle MBV. *A*: $P = 0.86$. *D*: $P = 0.02$ (ANOVA). *B* and *E*: Changes in muscle MFV. *B*: $P < 0.05$. *D*: $P < 0.02$ (ANOVA). *C* and *F*: Changes in muscle MBF. $n = 6–7$ for each. Compared with basal level, *$P < 0.05$ and **$P < 0.01$; compared with 30 min, #$P < 0.05$ and ##$P < 0.01$.

stimulation by endogenous ANG II would alter muscle insulin delivery and uptake (protocol 2). As shown in Fig. 7, infusion of PD123319 did not significantly alter ^{125}I-insulin degradation in the plasma (Fig. 7A). However, it decreased muscle ^{125}I-insulin content by nearly 50% ($P < 0.05$), confirming a decreased muscle insulin uptake in the presence of AT$_2$R blockade.

DISCUSSION
In humans, ANG II infusion acutely enhances insulin-stimulated muscle glucose disposal (18–20). This was suggested to be attributable to enhanced tissue perfusion, although evidence for this was not available. We recently have found that both the AT$_1$R and AT$_2$R exert potent basal tonic effects on microvasculature in skeletal muscle (21).

TABLE 1
Changes in MAP and FBF in protocol 1 experiments

	Basal	30 min	60 min	90 min	120 min
MAP (mmHg)					
Insulin	106 ± 3	107 ± 5	107 ± 2	108 ± 3	108 ± 2
Insulin + PD123319	96 ± 5	92 ± 6	93 ± 4	104 ± 6	96 ± 6
Insulin + losartan	107 ± 3	100 ± 4	97 ± 7	106 ± 5	101 ± 5
FBF (mL/min)					
Insulin	0.78 ± 0.06	0.78 ± 0.05	0.82 ± 0.04	0.84 ± 0.07	$0.94 \pm 0.07*$
Insulin + PD123319	0.75 ± 0.04	0.80 ± 0.04	0.85 ± 0.09	0.73 ± 0.06	0.73 ± 0.07
Insulin + losartan	0.83 ± 0.10	0.75 ± 0.10	0.70 ± 0.09	0.68 ± 0.08	$0.60 \pm 0.07*$

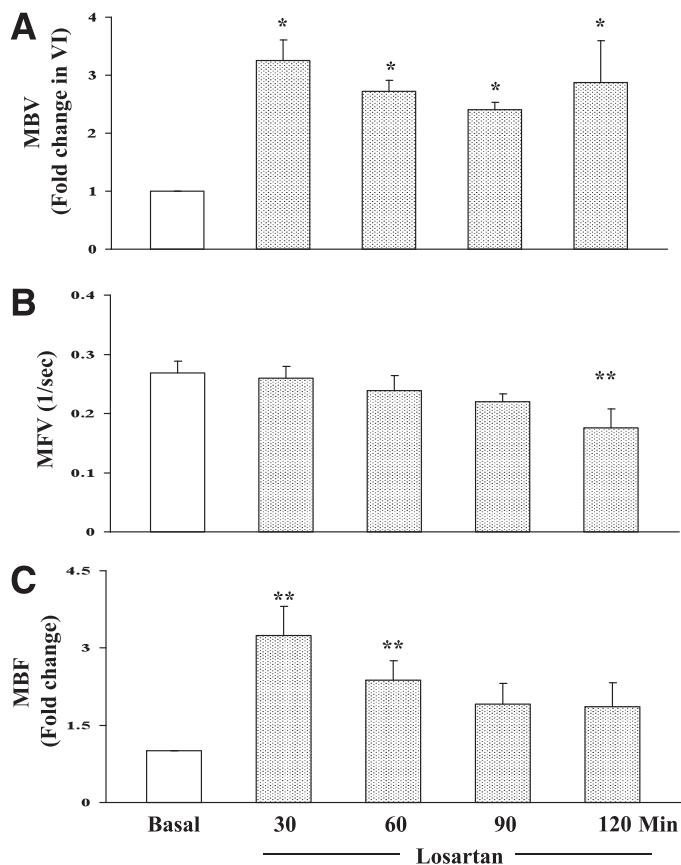Data are means $\pm$ SEM. $n = 5–7$. *Compared with basal (0 min), $P < 0.01$.

FIG. 5. Combined effects of insulin and AT_1R blockade on skeletal-muscle microvascular parameters. Losartan (0.3 mg/kg i.v.) was given 5 min before the initiation of insulin clamp. *A*: Changes in muscle MBV. $P < 0.001$ (ANOVA). *B*: Changes in muscle MFV. $P < 0.01$ (ANOVA). *C*: Changes in muscle MBF. $P < 0.01$ (ANOVA). $n = 7$. Compared with 0 min, $*P < 0.05$ and $**P < 0.01$.

FIG. 6. AT_2R blockade decreases insulin-stimulated NO production and skeletal muscle Akt phosphorylation. *A*: Changes in plasma NO levels. $n = 5–6$. $P = 0.005$ (ANOVA). Compared with saline, $*P < 0.01$; compared with insulin, $\#P < 0.05$. *B*: Changes in muscle Akt phosphorylation. $n = 7–9$. $P < 0.001$ (ANOVA). Compared with saline, $*P < 0.01$; compared with insulin, $\#P < 0.04$. *C*: Changes in muscle eNOS phosphorylation. $n = 4$. $P > 0.05$ (ANOVA). For gel images in *B* and *C*, they were from different parts of the same gels with same exposure.

Here, we show that both AT_1R and AT_2R regulate insulin's microvascular and metabolic actions in muscle. Although the blockade of AT_1R significantly increased muscle microvascular perfusion, it did not further enhance insulin-stimulated whole-body glucose disposal, NO production, and muscle Akt phosphorylation. In contrast, AT_2R antagonism promptly attenuated muscle metabolic responses to insulin, which was associated with decreased muscle insulin uptake and insulin-mediated NO production and microvascular recruitment. Although we previously have demonstrated that dual blockade of both AT_1R and AT_2R had neutral effects on muscle microvascular recruitment and basal glucose extraction, whether dual blockade alters microvascular and muscle insulin sensitivity is unclear. In addition, our observations may represent a combined peripheral and central effect because it is likely that both losartan and PD123319 could exert central effects via either penetrating the blood-brain barrier or acting on the blood vessels in the central nervous system (28). An alternative is to administrate these compounds locally into the microvasculature via the epigastric artery (32). However, this would require abdominal incision, which further stresses the animals, and prolonged infusions would likely also raise systemic concentrations.

Similar to the previous reports (8,12,33–36), insulin potently increased muscle MBV by 58–75%. Injection of losartan prior to the initiation of insulin infusion increase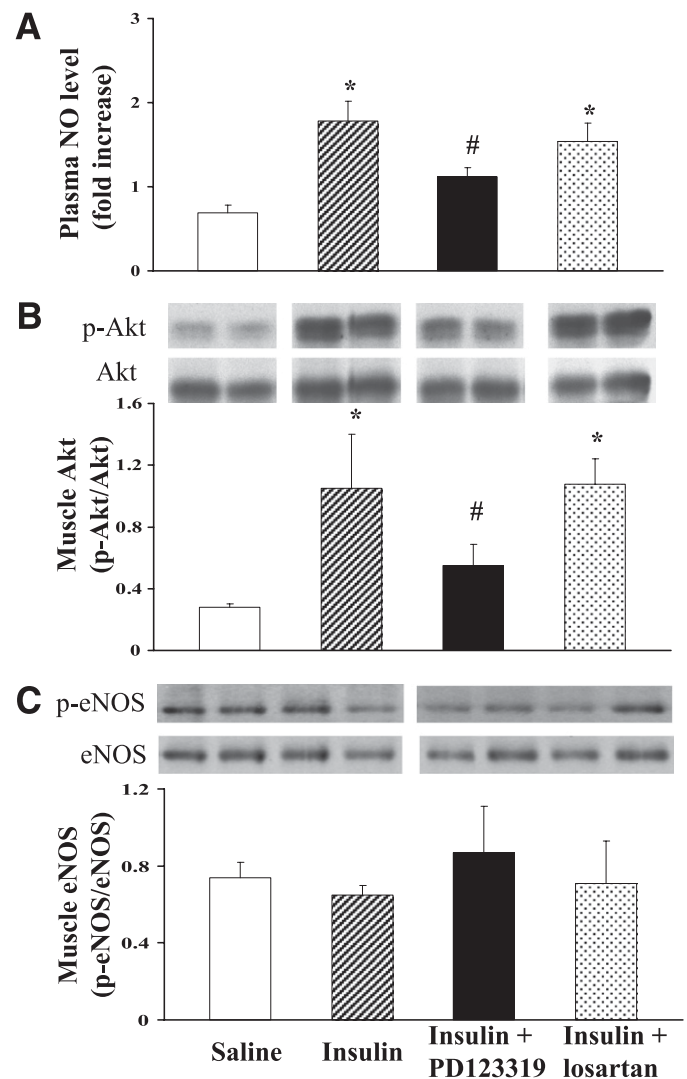d muscle MBV by approximately two- to threefold (Fig. 5), which is similar to what we observed previously with losartan alone (21). Thus, there seems to be no additive effects of losartan and insulin on muscle MBV. It is likely that the large increase in MBV achieved with the losartan injection may have masked the insulin effect. The large increase in muscle MBV at 30 min without an increase in FBF likely reflects a shift in flow distribution from nonnutritive to nutritive microvascular beds (37). Of interest, MBF trended down after 90 min, which was likely secondary to the gradual decline in FBF and a decrease in MFV at 120 min. Whether these findings are caused by either losartan alone or losartan plus insulin remain to be clarified because MFV and MBF were not determined in our previous study using losartan alone (21). Compared with increases induced by insulin alone (Fig. 3), the increases in muscle MBV induced by losartan and insulin combination was only significantly higher at 30 min ($P < 0.03$), and increases in muscle MBF were not statistically different between the two groups. Thus, it is not surprising that the addition of
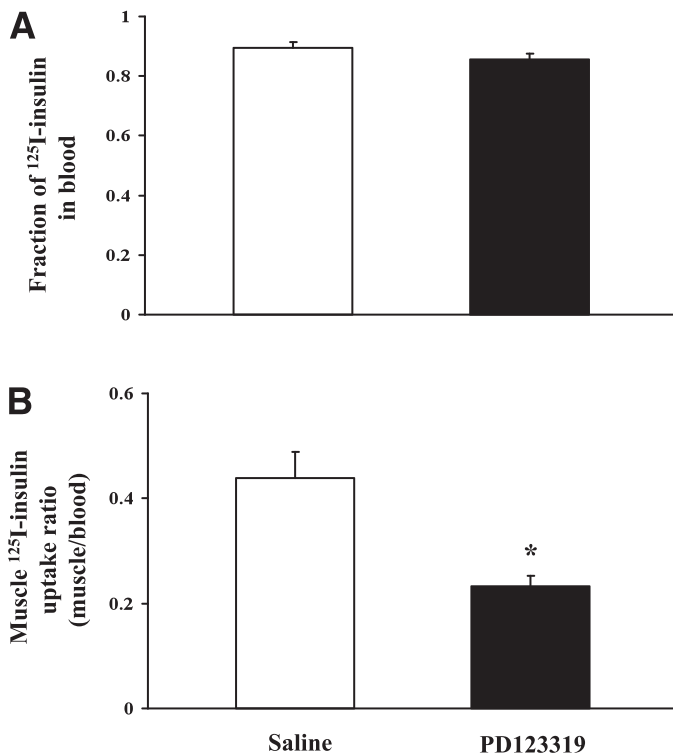
FIG. 7. AT$_2$R blockade decreases skeletal muscle ^{125}I-insulin uptake. Five minutes after bolus injection of ^{125}I-insulin (1.5 μCi i.v.), blood and skeletal muscle samples were collected, and intact ^{125}I-insulin was determined after trichloroacetic acid precipitation. A: Fraction of plasma-intact ^{125}I-insulin. B: Muscle ^{125}I-insulin uptake. n = 4–6. Compared with saline, $*P < 0.05$.

losartan to insulin did not further increase muscle insulin action beyond what we saw with insulin alone because insulin-mediated microvascular recruitment is tightly coupled with insulin's metabolic action.

AT$_2$R has been associated with vasodilation in large capacitance vessels, resistance arterioles, as well as the microvasculature (21,23,38–41), likely via the bradykinin-NO-cGMP signaling cascade because the AT$_2$R antagonist PD123319, the bradykinin receptor 2 antagonist icatibant, and the NO synthase inhibitor L-NAME each independently blocks this action (25,38,42,43). We previously have reported that skeletal muscle precapillary arterioles are a major site of AT$_2$R action in vivo because blockade of the AT$_1$R with losartan causes a threefold increase in muscle MBV, whereas blockade of the AT$_2$R renders an ~80% reduction in muscle MBV (21). Our current results confirmed that AT$_2$R activity also plays an important role in the regulation of insulin's microvascular and metabolic responses in muscle as well. Indeed, PD123319 caused a prompt decrease in insulin-mediated glucose disposal, which was associated with abrogation of insulin-stimulated muscle microvascular recruitment, NO production and muscle Akt phosphorylation, and muscle insulin uptake. Thus, AT$_2$Rs are important regulators of both basal microvascular tone and insulin delivery/action in muscle via its effects on the microvasculature, which is consistent with the current understanding that the regulation of microvascular insulin delivery is a major rate-limiting step in skeletal muscle insulin action (2,44–47).

It is important to distinguish between the acute effects we observed and what might be going on when the RAS already is at a higher level as occurs in obesity and diabetes. Increased muscle microvascular perfusion induced by muscle contraction is clearly associated with increased muscle insulin uptake even in the presence of high plasma free fatty acids (10,11). In humans, simple obesity blunts insulin- and mixed-meal–stimulated muscle microvascular recruitment (9,35). Likewise, in obese Zucker rats, an animal model of metabolic syndrome and insulin resistance, basal skeletal muscle MBV is decreased, which is coupled with impaired insulin-mediated glucose disposal and microvascular recruitment (48). Insulin uptake has not been assessed in this model. However, treatment of the Zucker diabetic fatty rats with the ACE inhibitor quinapril restores insulin's microvascular action and improves insulin-mediated whole-body glucose disposal (49). Because the cardiovascular RAS is upregulated in diabetes, which has been implicated in the development of diabetic cardiovascular complications (7,13,24,50), blockade of the AT$_1$R and/or activation of the AT$_2$R may help to improve insulin delivery and sensitivity and potentially improve the cardiovascular outcomes in patients with diabetes. Although the current study revealed important modulatory roles of ANG II subreceptors on insulin action under normal physiology, whether the current findings can be extrapolated to the insulin-resistant state is unclear and requires additional studies.

We previously have shown in humans that physiologic hyperinsulinemia increases muscle MBF solely by increasing MBV without changing the MFV (8,35,36). Our current study in rodents confirmed this lack of change in MFV after insulin stimulation as well (Figs. 3 and 4). Although the addition of losartan to the insulin infusion caused a decrease in MFV at 120 min, superimposing the PD123319 infusion not only promptly attenuated insulin-stimulated MBV but it also increased muscle MFV. Because there was no change in total FBF, this increase in MFV was likely secondary to the acute decrease in MBV.

Our finding that insulin increased plasma NO levels is consistent with many previous reports demonstrating that insulin causes vasodilation via the phosphatidylinositol 3-kinase/Akt/eNOS pathway (2,4–6,51). We previously have demonstrated that losartan treatment led to microvascular recruitment via the AT$_2$R-NO–dependent mechanism because L-NAME treatment abolished losartan-induced increases in MBV and glucose extraction (21). The increase in plasma NO levels seen with combined losartan and insulin treatment most likely reflects increased release of NO from the endothelium secondary to endogenous ANG II stimulation of the AT$_2$R/B$_2$R/eNOS pathway and insulin stimulation of the phosphatidylinositol 3-kinase/Akt/eNOS pathway. The NO release is less likely from skeletal muscle cells, because they express predominantly AT$_1$R (52), which activates NADPH oxidase, increases reactive oxygen species, and results in decreased NO bioavailability. In the current study, NO production did not further increase with the combined losartan and insulin treatment, which is consistent with the findings that the increases in MBV and MBF were not statistically different between the two groups.

According to the Fick principle, increases in the endothelial exchange surface area between the plasma and the interstitial compartments in muscle could significantly increase the delivery of insulin to the muscle microvasculature and facilitate its transfer to the interstitial space and enhance its metabolic action. Our observation that insulin-stimulated glucose disposal and muscle Akt phosphorylation, two major indices of insulin metabolic actions in muscle, closely parallel the changes in insulin-mediated

microvascular recruitment and NO production, and muscle insulin uptake strongly supports this concept. Indeed, PD123319 superimposing on insulin infusion decreased all these major end points in the current study. The abrogation of insulin-induced microvascular recruitment with PD123319 infusion most likely resulted in a decreased insulin delivery and uptake in the muscle because it is in the microvasculature where insulin uptake takes place and decreased exchange surface area could significantly decrease muscle insulin uptake. The decreases in NO production and Akt phosphorylation with PD123319 infusion are likely secondary to decreased stimulation of the AT_2R by ANG II and insulin receptor by insulin on the endothelium (for NO) and decreased insulin delivery to the muscle (for p-Akt).

In conclusion, both AT_1Rs and AT_2Rs regulate insulin's microvascular and metabolic actions in muscle. Our current findings strongly suggest that ANG receptors affect insulin sensitivity in vivo via modulating microvascular perfusion. This is an important finding because changes in the microvascular endothelial surface area significantly affects insulin delivery to and action in muscle (2). Additional studies are needed to define the underlying molecular mechanisms. Although AT_1R activity restrains muscle metabolic responses to insulin via decreased muscle microvascular recruitment and insulin delivery, AT_2R activity is required for normal muscle microvascular and metabolic responses to insulin. Thus, pharmacologic manipulation aimed at increasing the AT_2R-to-AT_1R activity ratio may afford potential to improve muscle insulin sensitivity and glucose metabolism and to reduce the cardiovascular complications associated with diabetes and insulin resistance.

REFERENCES

1. Segal SS. Regulation of blood flow in the microcirculation. Microcirculation 2005;12:33–45
2. Barrett EJ, Eggleston EM, Inyard AC, et al. The vascular actions of insulin control its delivery to muscle and regulate the rate-limiting step in skeletal muscle insulin action. Diabetologia 2009;52:752–764
3. Kim JA, Koh KK, Quon MJ. The union of vascular and metabolic actions of insulin in sickness and in health. Arterioscler Thromb Vasc Biol 2005;25:889–891
4. Kim JA, Montagnani M, Koh KK, Quon MJ. Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. Circulation 2006;113:1888–1904
5. Vincent MA, Clerk LH, Lindner JR, et al. Microvascular recruitment is an early insulin effect that regulates skeletal muscle glucose uptake in vivo. Diabetes 2004;53:1418–1423
6. Vincent MA, Barrett EJ, Lindner JR, Clark MG, Rattigan S. Inhibiting NOS blocks microvascular recruitment and blunts muscle glucose uptake in response to insulin. Am J Physiol Endocrinol Metab 2003;285:E123–E129
7. Ko S-H, Cao W, Liu Z. Hypertension management and microvascular insulin resistance in diabetes. Curr Hypertens Rep 2010;12:243–251
8. Liu Z, Liu J, Jahn LA, Fowler DE, Barrett EJ. Infusing lipid raises plasma free fatty acids and induces insulin resistance in muscle microvasculature. J Clin Endocrinol Metab 2009;94:3543–3549
9. Keske MA, Clerk LH, Price WJ, Jahn LA, Barrett EJ. Obesity blunts microvascular recruitment in human forearm muscle after a mixed meal. Diabetes Care 2009;32:1672–1677
10. Inyard AC, Chong DG, Klibanov AL, Barrett EJ. Muscle contraction, but not insulin, increases microvascular blood volume in the presence of free fatty acid-induced insulin resistance. Diabetes 2009;58:2457–2463
11. Inyard AC, Clerk LH, Vincent MA, Barrett EJ. Contraction stimulates nitric oxide independent microvascular recruitment and increases muscle insulin uptake. Diabetes 2007;56:2194–2200
12. Coggins M, Lindner J, Rattigan S, et al. Physiologic hyperinsulinemia enhances human skeletal muscle perfusion by capillary recruitment. Diabetes 2001;50:2682–2690
13. Carey RM, Siragy HM. Newly recognized components of the renin-angiotensin system: potential roles in cardiovascular and renal regulation. Endocr Rev 2003;24:261–271
14. Paul M, Poyan Mehr A, Kreutz R. Physiology of local renin-angiotensin systems. Physiol Rev 2006;86:747–803
15. Andreozzi F, Laratta E, Sciacqua A, Perticone F, Sesti G. Angiotensin II impairs the insulin signaling pathway promoting production of nitric oxide by inducing phosphorylation of insulin receptor substrate-1 on Ser312 and Ser616 in human umbilical vein endothelial cells. Circ Res 2004;94:1211–1218
16. Velloso LA, Folli F, Sun XJ, White MF, Saad MJA, Kahn CR. Cross-talk between the insulin and angiotensin signaling systems. Proc Natl Acad Sci USA 1996;93:12490–12495
17. Folli F, Kahn CR, Hansen H, Bouchie JL, Feener EP. Angiotensin II inhibits insulin signaling in aortic smooth muscle cells at multiple levels: a potential role for serine phosphorylation in insulin/angiotensin II crosstalk. J Clin Invest 1997;100:2158–2169
18. Buchanan TA, Thawani H, Kades W, et al. Angiotensin II increases glucose utilization during acute hyperinsulinemia via a hemodynamic mechanism. J Clin Invest 1993;92:720–726
19. Jamerson KA, Nesbitt SD, Amerena JV, Grant E, Julius S. Angiotensin mediates forearm glucose uptake by hemodynamic rather than direct effects. Hypertension 1996;27:854–858
20. Fliser D, Arnold U, Kohl B, Hartung R, Ritz E. Angiotensin II enhances insulin sensitivity in healthy volunteers under euglycemic conditions. J Hypertens 1993;11:983–988
21. Chai W, Wang W, Liu J, et al. Angiotensin II type 1 and type 2 receptors regulate basal skeletal muscle microvascular volume and glucose use. Hypertension 2010;55:523–530
22. Nora EH, Munzenmaier DH, Hansen-Smith FM, Lombard JH, Greene AS. Localization of the ANG II type 2 receptor in the microcirculation of skeletal muscle. Am J Physiol 1998;275:H1395–H1403
23. Carey RM. Cardiovascular and renal regulation by the angiotensin type 2 receptor: the AT2 receptor comes of age. Hypertension 2005;45:840–844
24. Liu Z. The renin-angiotensin system and insulin resistance. Curr Diab Rep 2007;7:34–42
25. Batenburg WW, Garrelds IM, Bernasconi CC, et al. Angiotensin II type 2 receptor-mediated vasodilation in human coronary microarteries. Circulation 2004;109:2296–2301
26. Chai W, Wu Y, Li G, Cao W, Yang Z, Liu Z. Activation of p38 mitogen-activated protein kinase abolishes insulin-mediated myocardial protection against ischemia-reperfusion injury. Am J Physiol Endocrinol Metab 2008;294:E183–E189
27. Wu Y, Barrett EJ, Long W, Liu Z. Glucocorticoids differentially modulate insulin-mediated protein and glycogen synthetic signaling downstream of protein kinase B in rat myocardium. Endocrinology 2004;145:1161–1166
28. Ye S, Zhong H, Duong VN, Campese VM. Losartan reduces central and peripheral sympathetic nerve activity in a rat model of neurogenic hypertension. Hypertension 2002;39:1101–1106
29. Zhuo J, Song K, Abdelrahman A, Mendelsohn FAO. Blockade by intravenous losartan of AT1 angiotensin II receptors in rat brain, kidney and adrenals demonstrated by in vitro autoradiography. Clin Exp Pharmacol Physiol 1994;21:557–567
30. Macari D, Bottari S, Whitebread S, De Gasparo M, Levens N. Renal actions of the selective angiotensin AT2 receptor ligands CGP 42112B and PD 123319 in the sodium-depleted rat. Eur J Pharmacol 1993;249:85–93
31. Zeleznik AJ, Roth J. Demonstration of the insulin receptor in vivo in rabbits and its possible role as a reservoir for the plasma hormone. J Clin Invest 1978;61:1363–1374
32. Ross RM, Wadley GD, Clark MG, Rattigan S, McConell GK. Local nitric oxide synthase inhibition reduces skeletal muscle glucose uptake but not capillary blood flow during in situ muscle contraction in rats. Diabetes 2007;56:2885–2892
33. Rattigan S, Clark MG, Barrett EJ. Hemodynamic actions of insulin in rat skeletal muscle: evidence for capillary recruitment. Diabetes 1997;46:1381–1388

34. Clerk LH, Rattigan S, Clark MG. Lipid infusion impairs physiologic insulin-mediated capillary recruitment and muscle glucose uptake in vivo. Diabetes 2002;51:1138–1145

35. Clerk LH, Vincent MA, Jahn LA, Liu Z, Lindner JR, Barrett EJ. Obesity blunts insulin-mediated microvascular recruitment in human forearm muscle. Diabetes 2006;55:1436–1442

36. Liu Z. Insulin at physiological concentrations increases microvascular perfusion in human myocardium. Am J Physiol Endocrinol Metab 2007; 293:E1250–E1255

37. Clark MG. Impaired microvascular perfusion: a consequence of vascular dysfunction and a potential cause of insulin resistance in muscle. Am J Physiol Endocrinol Metab 2008;295:E732–E750

38. Hannan RE, Davis EA, Widdop RE. Functional role of angiotensin II AT2 receptor in modulation of AT1 receptor-mediated contraction in rat uterine artery: involvement of bradykinin and nitric oxide. Br J Pharmacol 2003; 140:987–995

39. Yayama K, Hiyoshi H, Imazu D, Okamoto H. Angiotensin II stimulates endothelial NO synthase phosphorylation in thoracic aorta of mice with abdominal aortic banding via type 2 receptor. Hypertension 2006;48:958–964

40. Yayama K, Horii M, Hiyoshi H, et al. Up-regulation of angiotensin II type 2 receptor in rat thoracic aorta by pressure-overload. J Pharmacol Exp Ther 2004;308:736–743

41. Zhang C, Hein TW, Wang W, Kuo L. Divergent roles of angiotensin II AT1 and AT2 receptors in modulating coronary microvascular function. Circ Res 2003;92:322–329

42. Katada J, Majima M. AT$_2$ receptor-dependent vasodilation is mediated by activation of vascular kinin generation under flow conditions. Br J Pharmacol 2002;136:484–491

43. Thai H, Wollmuth J, Goldman S, Gaballa M. Angiotensin subtype 1 rReceptor (AT1) blockade improves vasorelaxation in heart failure by up-regulation of endothelial nitric-oxide synthase via activation of the AT2 receptor. J Pharmacol Exp Ther 2003;307:1171–1178

44. Muniyappa R, Montagnani M, Koh KK, Quon MJ. Cardiovascular actions of insulin. Endocr Rev 2007;28:463–491

45. Chiu JD, Richey JM, Harrison LN, et al. Direct administration of insulin into skeletal muscle reveals that the transport of insulin across the capillary endothelium limits the time course of insulin to activate glucose disposal. Diabetes 2008;57:828–835

46. Yang YJ, Hope ID, Ader M, Bergman RN. Insulin transport across capillaries is rate limiting for insulin action in dogs. J Clin Invest 1989; 84:1620–1628

47. Miles PD, Levisetti M, Reichart D, Khoursheed M, Moossa AR, Olefsky JM. Kinetics of insulin action in vivo: identification of rate-limiting steps. Diabetes 1995;44:947–953

48. Wallis MG, Wheatley CM, Rattigan S, Barrett EJ, Clark ADH, Clark MG. Insulin-mediated hemodynamic changes are impaired in muscle of Zucker obese rats. Diabetes 2002;51:3492–3498

49. Clerk LH, Vincent MA, Barrett EJ, Lankford MF, Lindner JR. Skeletal muscle capillary responses to insulin are abnormal in late-stage diabetes and are restored by angiotensin-converting enzyme inhibition. Am J Physiol Endocrinol Metab 2007;293:E1804–E1809

50. Lim HS, MacFadyen RJ, Lip GYH. Diabetes mellitus, the renin-angiotensin-aldosterone system, and the heart. Arch Intern Med 2004;164:1737–1748

51. Baron AD, Steinberg H, Brechtel G, Johnson A. Skeletal muscle blood flow independently modulates insulin-mediated glucose uptake. Am J Physiol 1994;266:E248–E253

52. Qi JS, Minor LK, Smith C, et al. Characterization of functional urotensin II receptors in human skeletal muscle myoblasts: comparison with angiotensin II receptors. Peptides 2005;26:683–690