The role of endothelin A receptors in peripheral vascular control at rest and during exercise in patients with hypertension

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Abstract

Patients with hypertension can exhibit impaired muscle blood flow and exaggerated increases in blood pressure during exercise. While endothelin (ET)-1 plays a role in regulating blood flow and pressure during exercise in health, little is known about the role of ET-1 in the cardiovascular response to exercise in hypertension. Therefore, eight volunteers diagnosed with hypertension were studied during exercise with either saline or BQ-123 (ET_A receptor antagonist) infusion following a 2-week withdrawal of anti-hypertensive medications. The common femoral artery and vein were catheterized for drug infusion, blood collection, and blood pressure measurements and leg blood flow was measured by Doppler ultrasound. Patients exercised at both absolute (0, 5, 10, 15 W) and relative (40, 60, 80 % peak power) intensities. BQ-123 increased blood flow at rest (79±87 ml/min; p=0.03) and augmented the exercise-induced hyperemia at most intensities (80% Saline: 3818±1222 vs BQ-123: 4812±1469 ml/min; p=0.001). BQ-123 reduced leg MAP at rest (−8±4 mmHg; p<0.001) and lower intensities (0-10 W; p<0.05). Systemic diastolic blood pressure was reduced (0 W-40 %; p<0.05), but systemic MAP was defended by an increased cardiac output. The exercise pressor response (MAP) did not differ between conditions (80% saline: 25±10, BQ-123: 30±7 mmHg; p=0.17). Thus, ET-1, acting through the ET_A receptors, contributes to the control of blood pressure at rest and lower intensity exercise in these patients. Furthermore, the

finding that ET-1 constrains the blood flow response to exercise suggests that ET_A receptor antagonism could be a therapeutic approach to improve blood flow during exercise in hypertension.

Keywords

ET_A receptor; exercise hyperemia; exercise pressor response

Introduction

Hypertension is characterized by an increased risk of developing cardiovascular disease and diminished exercise tolerance (Lim et al., 1996). An abnormal cardiovascular response to exercise may contribute to this increased risk. In healthy individuals, the blood flow response to exercise is accompanied by a commensurate increase in blood pressure. Together these responses increase proportionally with metabolic demand in a tightly-regulated manner across a wide range of exercise intensities to ensure adequate oxygen delivery to the skeletal muscle (Andersen & Saltin, 1985; Richardson et al., 1993). Regulation of this response is governed by the complex interactions of vasodilators (local) and vasoconstrictors (local- and neurally-mediated) to balance the prodigious capacity for blood flow to the active skeletal muscle with the need to maintain systemic blood pressure in order to adequately perfuse vital organs (Joyner & Casey, 2015). However, individuals with hypertension can exhibit an impaired blood flow response (McEniery et al., 2002; Rondon et al., 2006; Nyberg et al., 2012) or an exaggerated increase in blood pressure (i.e. exercise pressor response) (Delaney et al., 2010; Greaney et al., 2014; Barbosa et al., 2016; Chant et al., 2018) for a given level of exercise compared to healthy controls. This dissociation of the normal blood flow and pressure response likely increases the risk for adverse cardiovascular events during physical activity and diminishes exercise tolerance.

Recently, our laboratory evaluated the role of endothelin-1 (ET-1) in the regulation of blood flow and pressure in both young and older healthy individuals during knee extensor exercise. In these studies, selective antagonism of ET_A receptors, using the drug BQ-123, in the exercising leg augmented leg blood flow progressively with increasing exercise intensity and reduced blood pressure, with the largest changes observed at higher workloads (Barrett-O'Keefe et al., 2013, 2015). Importantly, the ET_A receptor antagonism mediated increases in resting and lower-intensity exercising blood flow were only evident in the older individuals and not the young. This suggests that ET-1 might differentially affect populations and play a more substantial role in the age-related alterations in vascular control mechanisms (Barrett-O'Keefe et al., 2015), as has been previously reported, at rest, in older men and mice (Van Guilder et al., 2007; Donato et al., 2009). Since the augmented exercise pressor response in hypertension has been linked to an amplified metaboreflex (Smith et al., 2006; Delaney et al., 2010; Barbosa et al., 2016), ET_A antagonism-induced increases in blood flow may be a viable therapeutic treatment to reduce the exercise pressor response in hypertensive individuals. Early studies on resting participants reported that hypertensive individuals vasoconstrict more following ET-1 infusion and vasodilate to a greater extent following ET_A receptor antagonism compared to healthy controls (Cardillo et al., 1999; Taddei et al., 1999),

supporting either a greater ET receptor expression or an increased sensitivity to ET-1, which contributes to the altered vascular control and tone in hypertension. A subsequent investigation revealed that ET_A antagonism increased blood flow during moderate-intensity handgrip exercise in hypertensive individuals (McEniery et al., 2002), however, this study did not report the blood pressure response.

There is growing evidence that ET-1 plays a critical role in the regulation of both blood flow and blood pressure in healthy young and older individuals. Since the cardiovascular response to exercise in hypertension can manifest as impaired blood flow and augmented blood pressure responses, we sought to determine the role of ET-1 in this dysregulation. Specifically, we tested the hypotheses that ET_A receptor antagonism with intra-arterial infusion of BQ-123 would: 1) increase leg blood flow at rest and progressively during incremental exercise and 2) attenuate the increase in arterial blood pressure during single-leg knee extensor exercise in individuals with hypertension.

Methods

Eight patients diagnosed with hypertension (2 female, 6 male) volunteered and were enrolled in this study. Participant characteristics are presented in Table 1. All patients were either drug-naïve or withdrew antihypertensive medications, under physician supervision, for two weeks prior to the study. The protocol was approved and written informed consent was obtained according to the University of Utah and Salt Lake City Veterans Affairs Medical Center Institutional Review Board requirements (IRB #30810). All procedures adhered to the *Declaration of Helsinki*, with the exception of registration in a database. All data collection took place at the Utah Vascular Research Laboratory located at the Veterans Affairs Salt Lake City Geriatric, Research, Education, and Clinical Center.

Protocol.

Prior to the experimental day, patients reported to the Utah Vascular Research Laboratory to perform an incremental exercise test to determine their single-leg knee extension peak power on a custom-built knee extension ergometer. The inertial nature of the flywheel in this ergometer localizes effort to the quadriceps and minimizes the activation of the hamstring muscles. On the experimental day, patients reported to the laboratory in the morning following 24 hours of abstinence from exercise and alcohol, an overnight fast, and having not consumed any caffeine the day of the experiment. Participant characteristics were measured and blood samples were taken for lipid and complete blood count panels (Table 1). After 30 min of supine rest, two catheters (20-gauge central venous catheter; Arrow International, Reading, Pennsylvania, USA) were introduced into the right common femoral artery (CFA) and femoral vein using the Seldinger technique, as previously described (Amann et al., 2011; Barrett-O'Keefe et al., 2013, 2015). Following catheter placement, patients rested quietly for an additional 30 min before being moved to the custom-built knee extension ergometer. All data collection took place in a thermoneutral environment with the participant in an upright, seated position.

Due to the extended duration of the present investigation (~6 hrs), patients were given a standardized meal (½ cup of corn flakes and ½ cup of skim milk) 20 min prior to the

initiation of the control (saline) and ET_A receptor blockade (BQ-123) exercise trials (Figure 1) as is customary for our protocols due to the lack of effect of this sustenance on our outcome measures (Barrett-O'Keefe et al., 2013, 2015). Since the effects of BQ-123 are long lasting (Spratt et al., 2001), it was not possible to randomize the drug infusions in each experimental session and the control bout was always performed first. However, previous work from our laboratory indicated that there are no appreciable effects of time or repeated exercise on the primary measurements in the present investigation (Barrett-O'Keefe et al., 2013, 2015).

Drug infusion.

Thigh volume was determined anthropometrically (Andersen & Saltin, 1985) and used to calculate the BQ-123 dosing. The selective ET_A receptor antagonist (BQ-123, Clinalfa, Bachem Americas, Inc., Torrance, California, USA) was prepared in physiological saline (0.9% NaCl) and administered intra-arterially via the CFA catheter at 10 nmol/min/L of thigh volume (infusion rates of 0.8-1.5 ml/min). This dosing protocol induces a plateau in vasodilation without affecting systemic MAP (Verhaar et al., 1998; Helmy et al., 2003; Thijssen et al., 2007; Barrett-O'Keefe et al., 2013, 2015). During the control trial, physiological saline was administered at the same infusion rate as BQ-123. The total drug loading times preceding exercise were 10 and 45 min for saline and BQ-123, respectively (Figure 1) with infusion continued throughout exercise.

Knee extension exercise.

The exercise was performed for 3 min at both absolute (0, 5, 10, 15 W) and relative (40, 60, 80 % peak power) intensities while maintaining 60 contractions per minute. The order of the exercise intensities was arranged such that the work rate increased sequentially and patients were given a 3 min recovery period following three consecutive bouts. The 80 % peak power bout was always performed last following 3 min of rest. After a total of 105 min of recovery (60 min recovery period plus 45 min resting BQ-123 infusion), the same exercise protocol was performed during BQ-123 infusion (Figure 1).

Measurements.

CFA blood velocity and vessel diameter measurements were performed in the infused leg using a Logiq E9 ultrasound Doppler system (9L-D probe; General Electric Medical Systems, Milwaukee, Wisconsin, EISA) operating in duplex mode with both imaging and pulse wave frequencies optimized. The CFA was insonated 2-3 cm proximal to the bifurcation into the superficial and deep femoral arteries. All blood velocity measurements were obtained with the probe appropriately positioned to maintain an insonation angle of 60°. The sample volume was maximized according to the vessel size and was centered within the vessel. At all measurement points, CFA diameter (cm) and angle-corrected, time-averaged, and intensity-weighted mean blood velocity (V_{mean}, cm/s) values were calculated using commercially available software (Logiq e9). Leg blood flow was calculated during the final minute of each stage (Figure 1) as: blood flow (ml/min) = [V_{mean} x π (vessel diameter/2)^2 x 60].

Arterial and venous blood pressures (MAP and MVP, respectively) were measured continuously via the indwelling catheters with pressure transducers placed at the level of the catheter (Transpac IV, ICU Medical, Inc., San Clemente, California, USA) and saved for offline analysis on the data acquisition device (Biopac, Goleta, California, USA). A 6 s mean pressure was taken during the last 30 s of each exercise intensity when drug infusions were temporarily halted. The leg perfusion pressure (mmHg) was calculated as: MAP – MVP. Leg vascular conductance (ml/min/mmHg) was calculated as: leg blood flow / leg perfusion pressure. Leg vascular resistance (mmHg/100ml/min) was calculated as: (leg perfusion pressure / leg blood flow) x 100. Blood pressure was also measured at the level of the brachial artery using an automated sphygmomanometer (Tango M2, SunTech Medical, Inc., Morrisville, North Carolina, USA) and used to calculate systemic MAP as: diastolic blood pressure + (pulse pressure x 0.33). These systemic MAP values were used to quantify the exercise pressor response to exercise as: exercising MAP – resting MAP. Heart rate was monitored via 3-lead ECG and recorded in duplicate on the data acquisition device and Logiq e9. Stroke volume and cardiac output were estimated with a finometer (Finapres Medical Systems, Amsterdam, The Netherlands). Stroke volume was calculated with the Modelflow method (Tam et al., 2004) and cardiac output was calculated as the product of stroke volume and heart rate. The finometer also measured MAP at the finger in order to calculate systemic vascular resistance (mmHg/L/min) as: MAP / cardiac output. Finometer variables were averaged over the last 30 s of each stage.

During the last 30 s of each stage, following the vascular pressure measurements, femoral arterial and venous blood samples (3-4 ml) were collected from the indwelling catheters. Arterial and venous blood (1 ml) were presented anaerobically to a GEM 4000 blood gas analyzer and cooximeter (Instrumentation Laboratories, Bedford, Massachusetts, USA) to quantify arterial and venous total hemoglobin (tHb), oxyhemoglobin saturation, PO_2, hematocrit, and pH. The remaining blood was processed and stored at $-80°C$ until later analysis. Arterial and venous blood O_2 content (ml/dl) were calculated as: $[1.39 \text{ (tHb) x } (O_2$ saturation/100) + (0.003 x PO_2)]. The direct Fick method was used to calculate leg O_2 consumption (ml/min) as: [(arterial blood O_2 content – venous blood O_2 content) x leg blood flow]. Leg O_2 delivery (ml/min) was calculated as: [(leg blood flow x arterial blood O_2 content) / 100], Plasma ET-1 concentrations were measured by quantitative enzyme immunoassay (R&D Systems, Minneapolis, Minnesota, USA) and net ET-1 release (pg/min) was calculated as: [(venous [ET-1] – arterial [ET-1]) x leg blood flow].

Data and statistical analysis.

BQ-123-induced changes were calculated as the difference between the BQ-123 trial and the saline control trial. The leg blood flow-to-work rate relationship was determined for individual participants using linear regression. All statistical analyses and figure production were performed using a commercially available software package (SigmaPlot 12.5, Systat Software, San Jose, California, USA). The effect of BQ-123 on resting variables and the leg blood flow-to-work rate relationship were compared using paired Student's *t*-tests. The comparison of exercising data was performed using two-way repeated measures ANOVA (drug x intensity) separately for the absolute (i.e., 0, 5, 10, 15 W) and relative (i.e., 40, 60,

80 % peak power) intensities with Tukey's *post hoc* tests, as necessary. Data are presented as mean ± SD unless otherwise noted and significance was accepted at $p < 0.05$.

Results

Resting responses.

The effect of local ET_A receptor antagonism via BQ-123 infusion on select physiological variables at rest are presented in Table 2. ET_A receptor antagonism resulted in a doubling of leg net ET-1 efflux (Saline: 235 ± 68 vs BQ-123: 475 ± 209 pg/min; $p < 0.01$). Following BQ-123 infusion, common femoral artery diameter was increased (Saline: 0.97 ± 0.14 vs BQ-123: 1.02 ± 0.15 cm; $p < 0.01$). Leg perfusion pressure was reduced ($p = 0.001$) as a result of a decreased intravascular MAP measured at the level of the femoral artery ($p < 0.001$) without a concomitant change in MVP ($p = 0.09$). Leg blood flow, leg vascular conductance, and leg O_2 delivery were elevated compared to the saline trial (all $p < 0.05$), despite the lower perfusion pressure. There were no differences between conditions in terms of resting heart rate, CaO_2, CvO_2, leg CaO_2-CvO_2 difference, leg O_2 consumption, or venous pH (all $p > 0.05$). Systemic MAP was not statistically different (Saline: 102 ± 11 vs BQ-123: 96 ± 8 mmHg, $p = 0.08$), however, stroke volume and cardiac output were increased (both, $p < 0.05$) resulting in a lower calculated systemic vascular resistance ($p < 0.05$) following BQ-123 infusion.

Exercising responses.

The impact of BQ-123 on select physiological variables during exercise are presented in Tables 3 and 4. Following ET_A receptor antagonism, the net ET-1 efflux was increased for all absolute work rates (0-15 W) and 80 % peak power (all $p < 0.05$). The BQ-123-induced reduction in leg perfusion pressure observed at rest was sustained during exercise up to 60 % peak power (all $p < 0.05$; Figure 2), but was not different at the highest intensity ($p = 0.32$). This response was driven by intravascular MAP changes as MVP was relatively constant across all work rates in both conditions. Absolute leg blood flow was increased and leg vascular resistance was reduced during BQ-123 infusion at all work rates compared to saline (all $p < 0.05$; Figure 2). Due to the baseline increase in leg blood flow, the change in leg blood flow from rest is also presented (Figure 2 B). Moreover, there was a significant interaction (drug x intensity) for the change in blood flow from rest to exercise with differences between drug conditions at all intensities except for 0 and 5 W ($p = 0.1$ and 0.09, respectively), achieving the greatest difference at 80% (Saline: 3483 ± 1149 vs BQ-123: 4398 ± 1428 ml/min; $p = 0.001$). The leg blood flow-to-work rate relationship was increased following ET_A receptor antagonism (Saline: 65 ± 28 vs BQ-123: 83 ± 24 ml/min/W; $p < 0.01$). The fall in leg vascular resistance from rest was reduced at all exercise intensities following BQ-123 (all $p < 0.03$) including 80% (Saline: −28.4 ± 12.3 vs BQ-123: −20.0 ± 5.7 mmHg/100 ml/min; $p = 0.02$).

Leg O_2 delivery was increased at all exercise intensities as a function of the increased blood flow and unchanged CaO_2-CvO_2 difference (Table 3). However, for the three highest intensities, the CaO_2-CvO_2 difference was reduced during BQ-123 infusion compared to saline. Despite this, the leg oxygen consumption was augmented for all intensities 10 W and

higher. Venous pH exhibited an intensity-dependent decrease in both conditions, however, the venous effluent was typically more basic during BQ-123 infusion (Table 3). The relationships between leg vascular conductance and vascular resistance to blood flow are presented in Figure 3. BQ-123 infusion did not change the relationship for either variable, but instead shifted the data points further along the curve.

Systolic blood pressure was not different at any work rate, but approached a significant increase at 40 and 60 % peak power ($p = 0.052$ and 0.07, respectively) with BQ-123 infusion (Table 4). Diastolic blood pressure was lower at all absolute work rates and 40 % (all, $p < 0.05$), but not the two highest exercise intensities ($p = 0.09$ and 0.21, respectively) (Table 4). Systemic MAP during exercise at 0, 5, and 10 W was not different but approached significance with BQ-123 ($p = 0.09$, 0.08, and 0.06, respectively) (Table 4). Systemic vascular resistance was reduced during BQ-123 infusion in a manner similar to leg vascular resistance; however, the fall in systemic vascular resistance was accompanied by increases in heart rate, stroke volume, and cardiac output, likely in an effort to sustain systemic MAP (Table 4). Thus, the reduction in systemic vascular resistance from rest was significantly less following BQ-123 at 60% (Saline: -4.5 ± 3.3 vs BQ-123: -2.8 ± 4.0 mmHg/L/min; $p = 0.04$) and 80% (Saline: -3.4 ± 3.2 vs BQ-123: -1.3 ± 5.0 mmHg/L/min; $p = 0.02$).

Exercise-induced changes in leg MAP and systemic MAP are presented in Figure 4. Both conditions saw an intensity-dependent increase at 60 and 80% peak power. The change in leg MAP was not different between saline and BQ-123 conditions at any work rate, but approached significance at 80% peak power (Saline: 24.4 ± 7.6 vs BQ-123: 29.3 ± 8.1 mmHg; $p = 0.06$). However, the absolute leg MAP for the 80 % peak power intensity was not different between conditions (saline: 144 ± 21 vs BQ-123: 142 ± 21 mmHg; $p = 0.17$), indicating that the reduced leg MAP at rest with BQ-123 was responsible for this response. There were no differences between saline and BQ-123 for the change in systemic MAP at any work rate including 80 % peak power (Saline: 25.4 ± 10.3 vs BQ-123: 29.9 ± 7.4 mmHg; $p = 0.17$), and both conditions achieved similar absolute values during 80 % peak power exercise (Saline: 127 ± 11 vs BQ-123: 126 ± 13 mmHg; $p = 0.72$).

Discussion

The primary novel findings of the present investigation were that local ET_A receptor antagonism markedly increased leg blood flow, vascular conductance, oxygen delivery, and oxygen consumption during exercise in patients with hypertension. Interestingly, these increases occurred in the presence of reduced leg perfusion pressure at rest and during lower intensity exercise, indicating that the increased blood flow was driven by changes in vascular resistance and a shift along the relationship between resistance and blood flow (displayed in Figure 3). These changes in peripheral vascular resistance likely resulted in the reduction in diastolic blood pressure which was accompanied by a concomitant increase in cardiac output, necessary to sustain systemic MAP. Additionally, the exercise pressor response, derived from the change in MAP from rest to exercise, was not lower following local ET_A receptor antagonism. In fact, ET_A receptor antagonism was associated with a reduction in both leg intravascular and systemic diastolic blood pressures which may have triggered an

attendant increase in cardiac output. Thus, ET-1, acting through the ET_A receptors, contributes greatly to the control of blood pressure and blood flow in these patients.

Role of ET-1 in the control of blood flow.

Hypertension is associated with abnormal vascular control that is mediated, in part, by endothelial dysfunction (Panza et al., 1990; Panza et al., 1995; Taddei et al., 1997). Interestingly, the phenotypical expression of this dysfunction in hypertensive individuals appears to represent an accelerated onset of vascular dysfunction typically observed with healthy aging (Taddei et al., 1997). Indeed, the middle-aged hypertensive individuals in the present investigation (~46 years old), expressed a significant level of ET-1-mediated restraint of resting blood flow that paralleled the constraint expressed in older individuals (~67-76 years old) following ET_A receptor antagonism (Thijssen et al., 2007; Barrett-O'Keefe et al., 2015). This is in stark contrast to the negligible effects of ET_A receptor antagonism on resting blood flow in healthy young (Thijssen et al., 2007; Barrett-O'Keefe et al., 2013) and middle-aged (Cardillo et al., 1999; Taddei et al., 1999; McEniery et al., 2002; Weil et al., 2011) participants, further substantiating the role of ET-1 in the hastened onset of vascular control maladaptations associated with hypertension rather than these differences manifesting due solely to advancing age. The resting blood flow data herein, measured in the leg, are in agreement with previous measurements in the arms of individuals with hypertension (Cardillo et al., 1999; Taddei et al., 1999). However, of note, the present investigation offers an important advance beyond previous findings, as the vascular control and function of the upper and lower limbs exhibits substantial heterogeneity (Newcomer et al., 2004; Wray & Richardson, 2006; Calbet et al., 2007). Hence, the current findings indicate that the effects of ET-1 in hypertension are, indeed, systemic and negatively impact the vasculature supplying the ambulatory muscles of the leg.

Following the onset of muscular contractions, skeletal muscle experiences a large increase in blood flow in order to meet the requirements of the increased metabolic demand. Under normal circumstances, this is accomplished through an intricate balance of local vasodilatory factors acting against local and systemic vasoconstrictor signals and a progressive, appropriate increase in systemic blood pressure (Joyner & Casey, 2015). However, in individuals with hypertension, the exercise-induced increase in blood flow can be attenuated compared to age-matched healthy controls (McEniery et al., 2002; Rondon et al., 2006; Nyberg et al., 2012) and the increase in blood pressure may also be exaggerated (Delaney et al., 2010; Barbosa et al., 2016; Chant et al., 2018). This dissociation between blood flow and pressure, in hypertension, indicates that vascular resistance may attenuate the increase in blood flow, resulting in an inappropriate pressor response, which likely contributes to the increased risk for adverse cardiovascular events in this population. In agreement with our first hypothesis, and previous work (Barrett-O'Keefe et al., 2013, 2015), BQ-123 infusion facilitated a progressive increase in the absolute change in blood flow with increasing exercise intensity, which approached 1000 ml/min at the highest intensity. This increased the blood flow-to-work rate relationship in these individuals from 65 ± 28 to 83 ± 24 ml/min/W, which positioned them back in line with the blood flow-to-work rate relationship previously reported in healthy individuals during knee extension exercise (~80 ml/min/W, (Andersen & Saltin, 1985; Richardson et al., 1993)). These findings suggest that ET-1 activity excessively

constrains blood flow and is a fundamental mechanism contributing to the attenuated blood flow in these patients. Importantly, in this investigation, the increase in blood flow was driven by changes in leg vascular resistance, but the vasodilation induced by antagonizing the ET_A receptors did not alter the relationship between flow and resistance. Rather, this change in resistance simply shifted these individuals with hypertension further along the same curvilinear relationship, as illustrated in Figure 3. The result of this shift, at rest and during lower intensities, when blood flow was relatively modest, reduced the perfusion pressure required for a given blood flow. With more intense exercise, when blood flow was much higher, this same shift along the curve actually facilitated an increased blood flow for the same perfusion pressure.

Vascular resistance and blood pressure regulation.

Interestingly, the reduction in leg vascular resistance due to BQ-123 and its subsequent effects on intravascular blood pressure differed based on the intensity of exercise. Following ET_A receptor antagonism, the diameter of the common femoral artery was increased ~5% and this dilation was sustained throughout exercise. Considering the limited vasoactivity of this artery, even during exercise (Radegran, 1997; Lutjemeier et al., 2005; Wray et al., 2007), these data support that ET-1, acting through ET_A receptors, likely contributes to an increased resting vascular tone in this population. Our group has previously reported that intra-arterial infusion of ET-1 does not cause constriction of the common femoral artery in healthy young participants (Wray et al., 2007); however, BQ-123 infusion appears to impart a powerful age-specific vasodilation, whereby, older individuals exhibited an ET-1-mediated restraint on diameter and young individuals had no response (Trinity et al., 2016). Although not directly compared in age-matched normotensive and hypertensive participants, the present data, along with our previous work (Trinity et al., 2016), support the idea that the hypertension phenotype of vascular control dysregulation is an age-accelerated onset that is mediated, in part, by ET-1.

The reduced leg MAP induced by BQ-123 at rest and during exercise at the lower intensities in the present investigation is a novel observation and unique divergence from healthy young and older participants who only experienced an intravascular pressure drop as exercise intensity increased (Barrett-O'Keefe et al., 2015; Trinity et al., 2016). Previous work implicated that the role of ET-1 in the regulation of blood pressure became more vital as exercise intensity increased, potentially serving as a mechanism to preserve systemic MAP in the face of an accumulating drive for dilation in the peripheral skeletal muscle. Interestingly, in the current study, the opposite was found in hypertensive patients. ET-1 was more important in the regulation of blood pressure at rest and during lower intensity exercise. At this time we are unable to determine the precise mechanism responsible for this unique difference between normotensive individuals and patients with hypertension at rest and during lower intensity exercise, however, it may relate to differences in ET-1 sensitivity or ET_A receptor density between the current patients and the previously studied healthy individuals.

The antagonism of ET_A receptors in the current patients, increased stroke volume, heart rate, and cardiac output, which was not evident in either young or older healthy participants

(Thijssen et al., 2007; Van Guilder et al., 2007; Barrett-O'Keefe et al., 2013, 2015; Trinity et al., 2016). These data may indicate that inhibition of ET-1 activity in these patients led to a greater dilation in the periphery downstream of the femoral artery (i.e., reduced leg vascular resistance). This dilation of a large vascular bed, in this case the skeletal muscle of the upper leg, is likely to initiate the baroreceptor response to maintain blood pressure, when receiving a large proportion (25-45%) of cardiac output (O'Leary, 1991). Indeed, in the current study, systemic blood pressure was defended by the, presumed, baroreceptor-mediated increase in cardiac output, as systemic vascular resistance was markedly reduced. The reduced diastolic blood pressure observed across a majority of the work rates in the present investigation likely contributed to the initiation of this baroreceptor-mediated response. This effect appears to be dependent on the size of the vascular bed, as Cardillo and colleagues (Cardillo et al., 1999) reported no change in heart rate or MAP when BQ-123 was infused into the arms in patients with hypertension. Systemic vascular resistance was reduced at all measurement points in the present investigation, and the normal reduction seen with the onset of exercise was no longer evident following BQ-123 infusion. These data may indicate an underlying inability to increase systemic vascular resistance, particularly within inactive tissues, in order to defend MAP in these patients, potentially due to chronically elevated muscle sympathetic nerve activity (Greenwood et al., 1999; Rondon et al., 2006; Lambert et al., 2007), and that an increase in cardiac output was necessary to ensure systemic perfusion pressure. The exercise intensity-dependent increase in systemic blood pressure was preserved during the inhibition of ET-1 despite the augmented central hemodynamics, however, it should be noted that the single-leg knee extension exercise (small muscle mass), used in the present investigation, was chosen to specifically examine the peripheral effects of ET-1 in this population. It is important to note that muscle sympathetic nerve activity is initially withdrawn with the onset of low-to-moderate intensity cycling (Ichinose et al., 2008) and single-leg exercise (Notarius et al., 2019), which may have augmented the reductions in pressure for these patients following ET_A receptor antagonism. Based on the current reductions in leg MAP, systemic diastolic blood pressure, and concomitant central cardiovascular responses (increase in heart rate, stroke volume, and cardiac output) in the present investigation, we speculate that acutely administered ET_A receptor antagonism in both legs or systemically during a whole body exercise modality, where such central cardiovascular responses are more limited, may deleteriously lower systemic MAP in hypertensive individuals. None of the patients in the present investigation reported light-headedness or exhibited any symptoms of syncope, however, this is an important consideration for future research and treatment with ET_A receptor antagonists, particularly when larger vascular beds are targeted.

The exercise pressor response.

An exaggerated exercise pressor response, defined as an augmented change in systemic blood pressure from rest to exercise, has previously been observed in individuals with hypertension during both small muscle mass (Aoki et al., 1983; Hamada et al., 1987; Delaney et al., 2010; Greaney et al., 2014) and whole body exercise (Barbosa et al., 2016; Chant et al., 2018). Previous work demonstrated that the exercise pressor response is reduced in healthy individuals (Amann et al., 2011) and patients with hypertension (Barbosa et al., 2016) following intrathecal administration of the μ-opioid receptor agonist fentanyl,

indicating that group III/IV skeletal muscle afferents are essential for this response. Furthermore, the augmented exercise pressor response in hypertension has been attributed, in part, to an intensified muscle metaboreflex (Smith et al., 2006; Delaney et al., 2010; Barbosa et al., 2016; Chant et al., 2018), which led to our hypothesis that BQ-123 infusion would reduce the exercise pressor response as a consequence of increased blood flow and O_2 delivery to the working skeletal muscle. Although conduit artery blood flow and O_2 delivery were increased (both ~20-25% for the higher intensities) following BQ-123 infusion, the exercise pressor response was unchanged and potentially augmented. The metabolic consequences of this improved blood flow, O_2 delivery, and increased O_2 consumption are unclear and further investigation is warranted to determine if these alterations impact oxidative and non-oxidative phosphorylation in the muscle during exercise and whether they interact with the exercise pressor response.

It has been reported that the pressor response to arm and leg exercise can be reduced following exercise training (Mostoufi-Moab et al., 1998; Fisher & White, 1999), and that the attenuated exercise pressor response following training was principally due to changes in central command (Fisher & White, 1999). Moreover, the exaggerated response in patients with hypertension was reduced ~30% following treatment with the α_1-blocker prazosin (Hamada et al., 1987), implicating sympathetic nerve activity as a culprit in the hypertensive exercise pressor response. The role of sympathetic activity is further substantiated by the evidence of impaired functional sympatholysis found in hypertensive individuals, which may be mediated, in part, by angiotensin II (Vongpatanasin et al., 2011). Taken together, these previous findings suggest that the improved O_2 delivery documented here may have been tempered by a potential baroreceptor-mediated increase in sympathetic outflow and reduced ability to attenuate sympathetic vasoconstriction. Additionally, the increased influence of other non-adrenergic vasoconstrictors (e.g., angiotensin II and neuropeptide Y) may act to preserve, or even augment, systemic pressure and its change during exercise in the face of ET_A receptor antagonism (Holwerda et al., 2015). Although hypertension results in widespread dysfunction of the cardiovascular system, the baroreflex still serves to protect systemic perfusion pressure (Grassi et al., 1998). Indeed, the current patients achieved similar absolute values for leg and systemic MAP during exercise in both drug conditions, this could suggest that the regulation of the exercise pressor response (i.e. change in blood pressure from rest to exercise) was secondary to the absolute level of blood pressure. It was recently reported that hypertensive patients display an exaggerated exercise pressor response regardless of how well resting blood pressure is controlled (Chant et al., 2018), which was attributable to the metaboreflex and could explain the maintained exercise pressor response in the present study.

Experimental considerations.

We cannot exclude the possibility that some of the observed changes in central and peripheral hemodynamics are due to ET_B receptor activation following the inhibition of ET_A receptors with BQ-123. However, previous work suggests that, in the legs of healthy young and older individuals, the combined inhibition of ET_A and ET_B receptors (Thijssen et al., 2007) does not differ appreciably from the sole inhibition of ET_A receptors (Barrett-O'Keefe et al., 2015). Furthermore, the addition of ET_B receptor antagonism over ET_A receptor

antagonism in the arms of young and older individuals had no measureable effect on blood flow (Van Guilder et al., 2007). A two week washout period may have been insufficient to fully mitigate the effects of the anti-hypertensive medications. However, this is likely not the case herein, as all patients had an elevated blood pressure (i.e. SBP > 120 mmHg), with six patients exceeding the classification for hypertension (Stage 1, n = 1 and Stage 2, n = 5) (Whelton et al., 2018). Additionally, we must acknowledge that the increased central hemodynamics at rest and throughout exercise in these patients were likely accompanied by increased sympathetic nerve activity. This systemic alteration could lead to errant conclusions regarding local blood flow regulation, however, as we did not quantify the sympathetic activity in these patients we are unable to dissect this effect. Finally, since we do not have an age-matched control group of normotensive individuals, we cannot conclude whether the lack of a reduction in the exercise pressor response in these patients is due to their lack of an exaggerated response or if there was an increased effect from other pathways (i.e., increased sympathetic nerve activity or activity of another vasoconstrictor) that lead to similar pressure responses following ET_A receptor antagonism.

Conclusions.

This study reveals that ET-1 clearly constrains leg blood flow in hypertension, however, improvements in blood flow were not associated with a concomitant reduction in the exercise pressor response, as previously reported in healthy individuals. However, these patients had large reductions in both local leg and systemic blood pressure at rest and during the lower intensities of exercise. Thus, ET-1, acting through the ET_A receptors, contributes to the control of blood pressure and constrains the blood flow response to exercise and suggests that ET_A receptor antagonism could be a therapeutic approach to augment blood flow during exercise in hypertensive patients.

Acknowledgments

Author Profile

Jesse C. Craig completed his PhD at Kansas State University in 2018. He is currently a postdoctoral fellow in the Utah Vascular Research Laboratory under the tutelage of Prof. Joel D. Trinity. This investigation was carried out as part of an NIH T32 fellowship through the Divisions of Cardiovascular Medicine and Geriatrics. He is interested in how local factors (nitric oxide, reactive oxygen species, endothelin) influence oxygen delivery and utilization in health and disease. The long-term goal of his research is to gain a greater understanding of the mechanisms contributing to dysfunction at the vascular and skeletal muscle systems interface.

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Key Points

- Exercise in patients with hypertension can be accompanied by an abnormal cardiovascular response that includes attenuated blood flow and an augmented pressor response.

- Endothelin-1, a very potent vasoconstrictor, is a key modulator of blood flow and pressure during in health and has been implicated as a potential cause of the dysfunction in hypertension.

- We assessed the role of endothelin-1, acting through endothelin A (ETa) receptors, in modulating the central and peripheral cardiovascular responses to exercise in patients with hypertension via local antagonism of these receptors during exercise.

- ET_A receptor antagonism markedly increased leg blood flow, vascular conductance, oxygen delivery, and oxygen consumption during exercise; interestingly, these changes occurred in the presence of reduced leg perfusion pressure, indicating that these augmentations were driven by changes in vascular resistance.

- These data indicate that ET_A receptor antagonism could be a viable therapeutic approach to improve blood flow during exercise in hypertension.
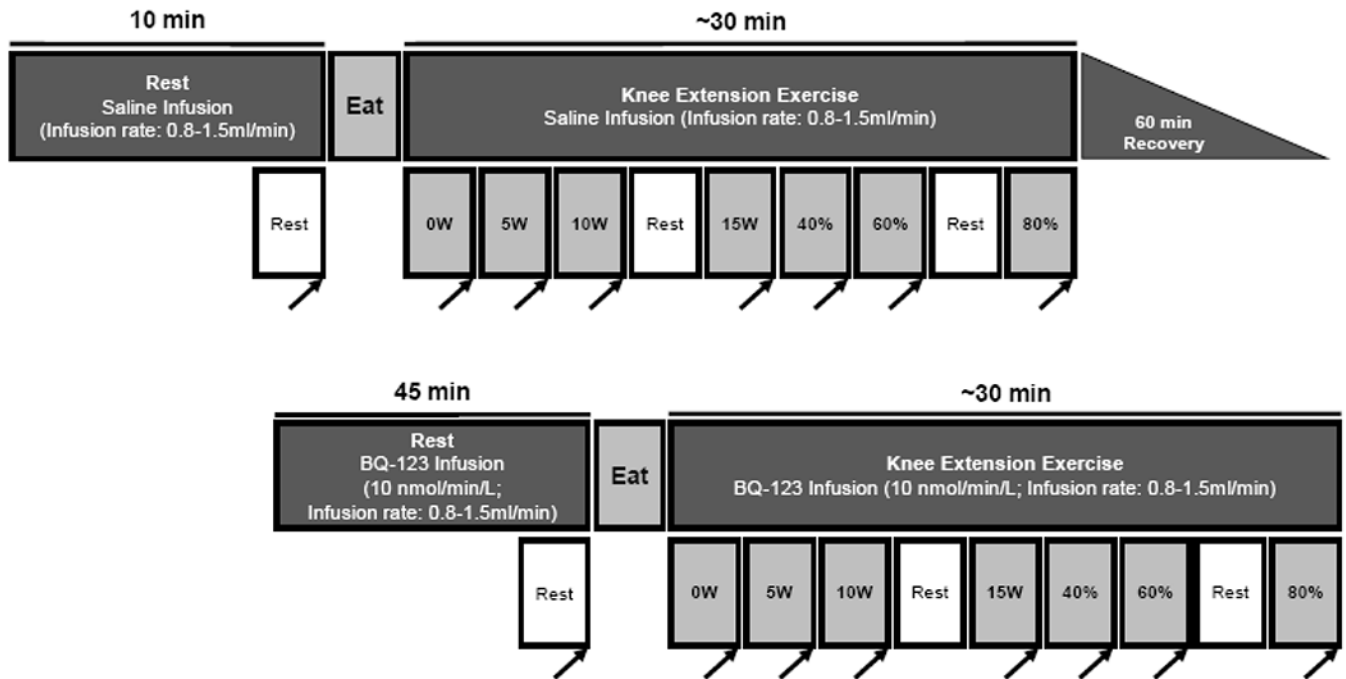
Figure 1.
Schematic representation of the experimental protocol. Arrows indicate points at which primary measurements were made (i.e., common femoral artery blood flow, arterial and venous blood gases, arterial and venous intravascular blood pressures).
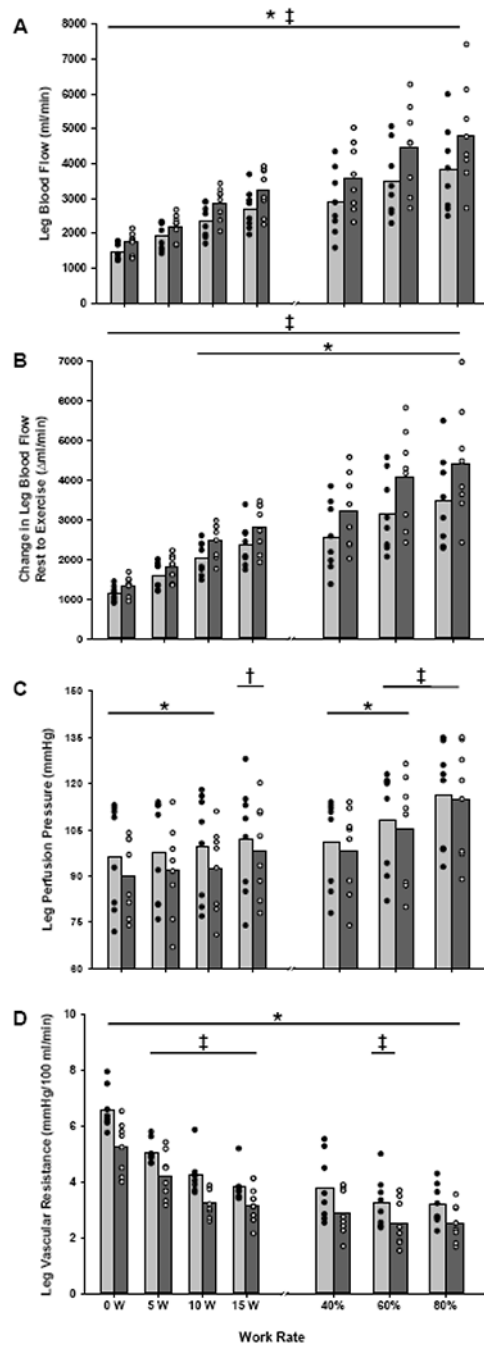
Figure 2.
Individual and group mean leg blood flow (*panel A*), change in leg blood flow from rest (*panel B*), leg perfusion pressure (*panel C*), and leg vascular resistance (*panel D*) during exercise with continued infusion of saline (control) and BQ-123 (ET$_A$ receptor antagonist). Filled circles, saline; Open circles, BQ-123; gray bars, saline mean; black bars, BQ-123 mean (both n = 8).. *Significantly different from saline; ‡Significantly different from the previous work rate for both conditions; †Significantly different from previous work rate for BQ-123 only (all, p < 0.05).
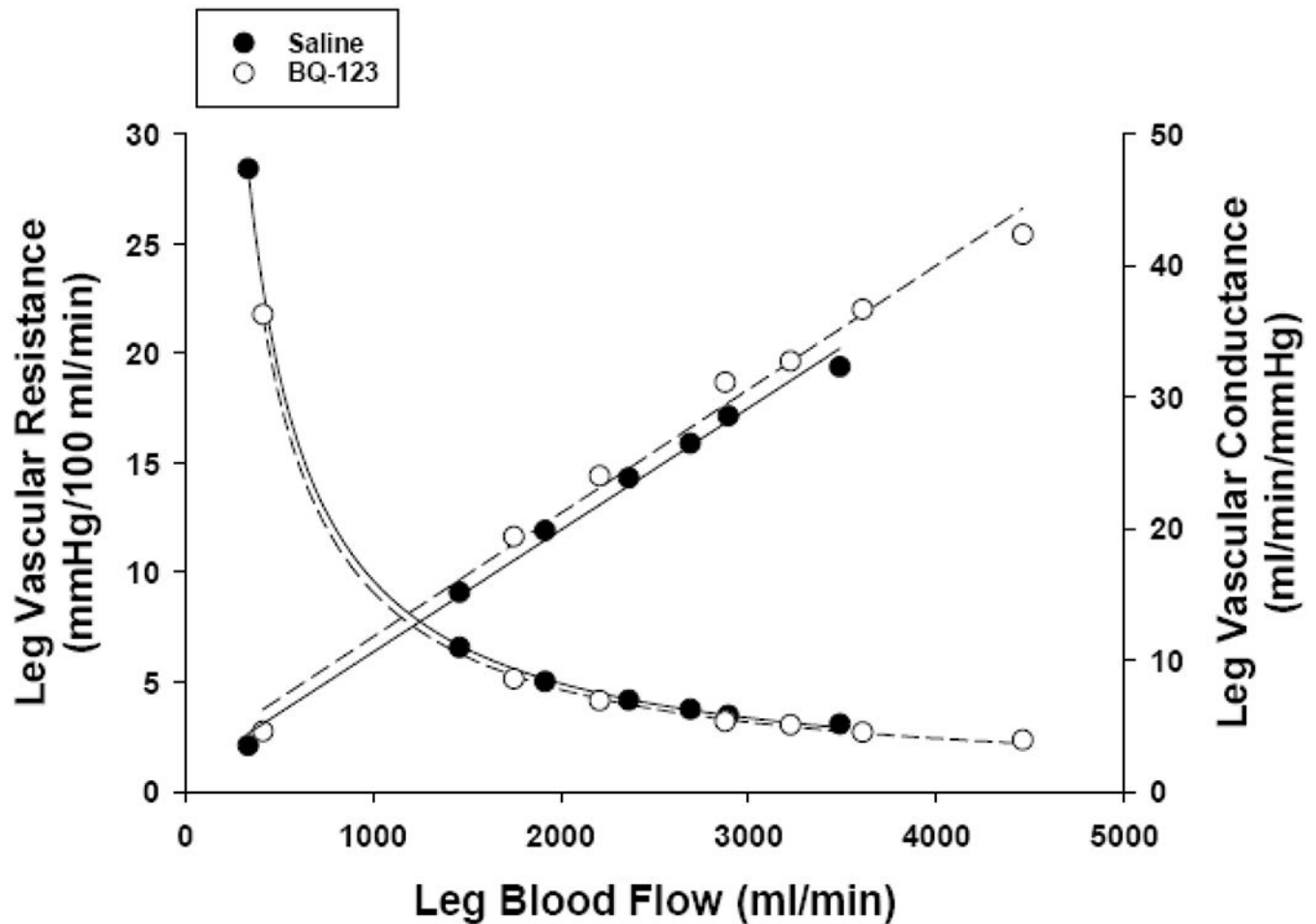
Figure 3.
Relationships between leg vascular resistance (inverse curvilinear response) or leg vascular conductance (direct linear response) and leg blood flow at rest and during exercise with continued infusion of saline (control) and BQ-123 (ET_A receptor antagonist). Filled circles, saline; Open circles, BQ-123 (both n = 8). At rest, blood flow to the skeletal muscle is low, as the vascular bed receives only a small fraction of the resting cardiac output. Vasodilation evoked by BQ-123 at rest is associated with a marked reduction in leg vascular resistance (-9 ± 3 mmHg/100 ml/min); however, the accompanying increase in absolute leg blood flow (79 ± 31 ml/min) and vascular conductance (1.2 ± 0.3 ml/min/mmHg) is relatively small as the skeletal muscle is inactive. This reduction is depicted by the steep slope along the leg vascular resistance curve from the saline to BQ-123 condition at the lower resting leg blood flow. As leg blood flow progressively increases as a result of skeletal muscle activity during knee extension exercise, the relationships between leg vascular resistance or leg conductance and leg blood flow remain constant (i.e. along the same projection) with a notable rightward shift, indicating a relatively greater leg blood flow for a given change of leg resistance or conductance. Therefore, during exercise, when the increase in blood flow is directed towards the active muscle, a high flow condition is created, whereby the same relative vasodilation (~5%) that was observed at rest due to BQ-123 manifests as a much larger absolute increase

in blood flow (994 ± 230 ml/min at 80% peak power). Error bars excluded for clarity (variance available in previous figures).
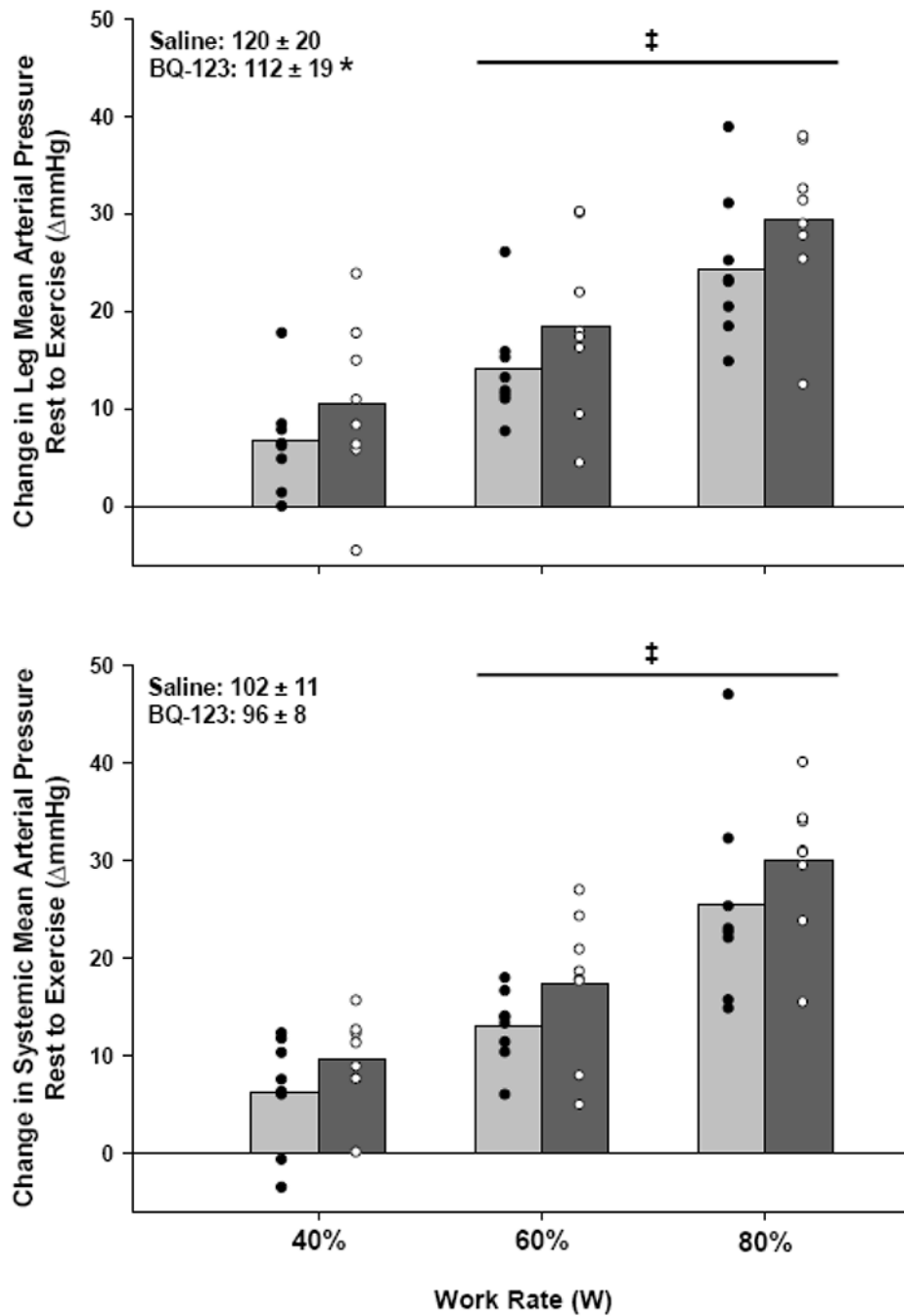
Figure 4.

Individual and group mean exercise-induced changes for leg mean arterial pressure (*panel A*) and systemic mean arterial pressure (*panel B*) during exercise with continued infusion of saline (control) and BQ-123 (ET_A receptor antagonist). Filled circles, saline; Open circles, BQ-123; Gray bars, saline mean; Black bars, BQ-123 mean (both n = 8). Inset values are average resting pressure ± SD. *Significantly different from saline; ‡Significantly different from the previous work rate for both conditions (both, p < 0.05).

Table 1.

Participant Characteristics

Age (years)	46 ± 11
Height (cm)	177 ± 8
Weight (kg)	83 ± 18
Body mass index (kg/m^2)	27 ± 5
Systolic blood pressure (mmHg)	136 ± 9
Diastolic blood pressure (mmHg)	86 ± 15
Quadriceps muscle mass (kg)	2.4 ± 0.5
Peak power (W)	50 ± 20
Glucose (mg/dl)	94 ± 16
Total cholesterol (mg/dl)	181 ± 26
Triglycerides (mg/dl)	115 ± 86
High-density lipoprotein (mg/dl)	54 ± 10
Low-density lipoprotein (mg/dl)	112 ± 25
White blood cells (K/ul)	6.9 ± 1.2
Red blood cells (M/ul)	5.0 ± 0.5
Hemoglobin (g/dl)	15.3 ± 1.5
Platelets (K/ul)	222 ± 46
Drug Status (n, %)	
Drug Naïve	1 (13%)
ACE Inhibitor	3 (38%)
Ca^{2+} Channel Blocker	3 (38%)
Diuretic	4 (50%)

Data are mean $\pm$ SD

Table 2.

Resting physiological variables

	Saline	BQ-123
Arterial [ET-1] (pg/ml)	0.7 ± 0.3	0.6 ± 0.3
Venous [ET-1] (pg/ml)	1.5 ± 0.4	1.8 ± 0.4 [*]
Leg MAP (mmHg)	120 ± 20	112 ± 19 [*]
Leg MVP (mmHg)	24 ± 5	22 ± 4
Leg perfusion pressure (mmHg)	95 ± 17	90 ± 16 [*]
Leg blood flow (ml/min)	334 ± 119	413 ± 79 [*]
Leg vascular resistance (mmHg/100 ml/min)	31.6 ± 12.4	22.6 ± 5.9 [*]
Leg vascular conductance (ml/min/mmHg)	3.5 ± 1.1	4.7 ± 1.4 [*]
Leg O_2 delivery (ml/min)	66 ± 26	80 ± 15 [*]
Leg O_2 consumption (ml/min)	25 ± 11	27 ± 7
Heart rate (beats/min)	65 ± 10	68 ± 11
Stroke volume (ml)	88 ± 25	103 ± 31 [*]
Cardiac output (L/min)	5.8 ± 1.5	6.9 ± 1.4 [*]
Systemic vascular resistance (mmHg/L/min)	19.2 ± 5.7	15.3 ± 4.8 [*]

ET-1, endothelin-1; MAP, mean arterial pressure; MVP; mean venous pressure.

Data are mean ± SD.

[*], $p < 0.05$ vs Saline.

Table 3.

Effect of BQ-123 on select peripheral physiological variables during dynamic single-leg knee extension exercise

Absolute Work Rates	0 Watts		5 Watts		10 Watts		15 Watts	
	Saline	BQ-123	Saline	BQ-123	Saline	BQ-123	Saline	BQ-123
Leg MAP (mmHg)	121 ± 19	$114 \pm 15^{*}$	123 ± 19	$115 \pm 16^{*}$	125 ± 20	$116 \pm 16^{*}$	126 ± 19	$123 \pm 18^{\ddagger}$
Leg MVP (mmHg)	25 ± 5	24 ± 5	25 ± 5	24 ± 4	25 ± 5	24 ± 7	24 ± 4	25 ± 4
Leg Vascular Conductance (ml/min/mm Hg)	15.3 ± 1.6	$19.6 \pm 3.6^{*}$	$19.8 \pm 1.5^{\ddagger}$	$24.5 \pm 4.9^{*\ddagger}$	$24.0 \pm 3.3^{\ddagger}$	$31.4 \pm 4.9^{*\ddagger}$	$26.6 \pm 3.2^{\ddagger}$	$33.1 \pm 6.9^{*}$
Leg O_2 delivery (ml/min)	280 ± 60	$329 \pm 55^{*}$	$370 \pm 88^{\ddagger}$	$414 \pm 73^{*\ddagger}$	$457 \pm 95^{\ddagger}$	$544 \pm 110^{*\ddagger}$	$514 \pm 105^{\ddagger}$	$610 \pm 121^{*\ddagger}$
Leg CaO_2-CvO_2 difference (ml/dl)	11.5 ± 1.6	11.4 ± 1.6	12.2 ± 1.5	$11 5 \pm 2.0$	12.8 ± 1.7	12.1 ± 2.1	13.1 ± 1.7	12.7 ± 2.0
Leg O_2 consumption (ml/min)	168 ± 34	199 ± 40	$233 \pm 56^{\ddagger}$	$253 \pm 59^{\ddagger}$	$302 \pm 68^{\ddagger}$	$346 \pm 85^{*\ddagger}$	$349 \pm 72^{\ddagger}$	$403 \pm 79^{*\ddagger}$
Leg venous PH	7.33 ± 0.02	$7.35 \pm 0.02^{*}$	$7.32 \pm 0.02^{\ddagger}$	$7.33 \pm 0.02^{*\ddagger}$	$7.31 \pm 0.02^{\ddagger}$	$7.31 \pm 0.02^{\ddagger}$	7.30 ± 0.02	$7.31 \pm 0.02^{*}$
Leg net ET-1 efflux (pg/min)	438 ± 160	$704 \pm 250^{*}$	416 ± 363	$738 \pm 335^{*}$	502 ± 283	$950 \pm 479^{*\ddagger}$	424 ± 304	$895 \pm 295^{*}$

Relative Work Rates	40% (20 ± 3 Watts)		60% (30 ± 4 Watts)		80% (40 ± 6 Watts)	
	Saline	BQ-123	Saline	BQ-123	Saline	BQ-123
Leg MAP (mmHg)	126 ± 18	$123 \pm 16^{*}$	$134 \pm 19^{\ddagger}$	$131 \pm 19^{\ddagger}$	$144 \pm 21^{\ddagger}$	$142 \pm 21^{\ddagger}$
Leg MVP (mmHg)	25 ± 6	25 ± 4	25 ± 5	26 ± 4	28 ± 6	27 ± 5
Leg Vascular Conductance (ml/min/mmHg)	28.6 ± 8.3	$37.3 \pm 11.3^{*}$	$32.4 \pm 8.0^{\ddagger}$	$43.3 \pm 13.7^{*\ddagger}$	32.7 ± 7.2	$42.0 \pm 11.2^{*}$
Leg O_2 delivery (ml/min)	564 ± 209	$701 \pm 220^{*}$	$698 \pm 238^{\ddagger}$	$864 \pm 285^{*\ddagger}$	777 ± 280	$938 \pm 342^{*}$
Leg CaO_2-CvO_2 difference (ml/dl)	13.4 ± 1.7	$13.0 \pm 1.9^{*}$	$14 3 \pm 2.1^{\ddagger}$	$13.5 \pm 1.9^{*\ddagger}$	14.6 ± 2.1	$13.7 \pm 2.1^{*}$
Leg O_2 consumption (ml/min)	393 ± 159	$474 \pm 160^{*}$	$504 \pm 192^{\ddagger}$	$606 \pm 205^{*\ddagger}$	$560 \pm 206^{\ddagger}$	$667 \pm 250^{*\ddagger}$
Leg venous pH	7.27 ± 0.02	$7.30 \pm 0.02^{*}$	7.25 ± 0.02	$7.28 \pm 0.03^{*}$	$7.22 \pm 0.03^{\ddagger}$	$7.24 \pm 0.04^{*\ddagger}$
Leg net ET-1 efflux (pg/min)	645 ± 449	726 ± 239	362 ± 475	531 ± 415	729 ± 490	$1022 \pm 480^{*\ddagger}$

MAP, mean arterial pressure; MVP, mean venous pressure; ET-1, endothelin-1.

All data are mean ± SD.

[*], p < 0.05 vs Saline within work rate;

[‡], p < 0.05 vs previous work rate within drag.

Table 4.

BQ-123-induced changes in central hemodynamic variables during dynamic single-leg knee extension exercise

Absolute Work Rates	0 Watts		5 Watts		10 Watts		15 Watts	
	Saline	BQ-123	Saline	BQ-123	Saline	BQ-123	Saline	BQ-123
Heart rate (beats/min)	73 ± 11	$78 \pm 9^{*}$	75 ± 10	$81 \pm 10^{\ddagger}$	$80 \pm 9^{*}$	$85 \pm 10^{*\ddagger}$	84 ± 13	$89 \pm 14^{\ddagger}$
Stroke volume (ml)	88 ± 25	$105 \pm 30^{*}$	93 ± 31	$106 \pm 27^{*}$	95 ± 30	$108 \pm 28^{*}$	94 ± 29	$108 \pm 33^{*}$
Cardiac output (L/min)	6.2 ± 1.6	$8.0 \pm 1.9^{*}$	$6.9 \pm 2.3^{\ddagger}$	$8.4 \pm 2.0^{*}$	$7.6 \pm 2.4^{\ddagger}$	$9.1 \pm 2.2^{*\ddagger}$	7.7 ± 2.3	$9.3 \pm 2.1^{*}$
Systolic blood pressure (mmHg)	146 ± 13	144 ± 15	150 ± 14	148 ± 18	150 ± 16	151 ± 16	150 ± 17	158 ± 19
Diastolic blood pressure (mmHg)	85 ± 13	$78 \pm 15^{*}$	85 ± 13	78 ± 14	$86 \pm 13^{*}$	$76 \pm 14^{*}$	85 ± 14	$78 \pm 12^{*}$
Systemic MAP (mmHg)	105 ± 8	100 ± 8	107 ± 9	101 ± 8	107 ± 9	101 ± 7	107 ± 6	104 ± 4
Systemic Vascular Resistance (mmHg/L/min)	19.0 ± 5.7	$14.0 \pm 3.8^{*}$	$17.5 \pm 6.3^{\ddagger}$	$13.5 \pm 3.9^{*}$	$16.4 \pm 5.3^{\ddagger}$	$12.8 \pm 3.5^{*}$	16.0 ± 5.7	$13.3 \pm 4.5^{*}$

Relative Work Rates	40% (20 ± 3 Watts)		60% (30 ± 4 Watts)		80% (40 ± 6 Watts)	
	Saline	BQ-123	Saline	BQ-123	Saline	BQ-123
Heart rate (beats/min)	87 ± 12	$92 \pm 10^{*}$	$94 \pm 10^{\ddagger}$	$101 \pm 12^{*\ddagger}$	$106 \pm 13^{\ddagger}$	$110 \pm 12^{*\ddagger}$
Stroke volume (ml)	94 ± 25	$109 \pm 30^{*}$	96 ± 27	108 ± 32	93 ± 30	$101 \pm 33^{\ddagger}$
Cardiac output (L/min)	8.1 ± 2.4	$9.9 \pm 2.2^{*}$	$8.9 \pm 2.3^{\ddagger}$	$10.8 \pm 2.7^{*\ddagger}$	$9.6 \pm 2.6^{\ddagger}$	$10.9 \pm 3.0^{*}$
Systolic blood pressure (mmHg)	155 ± 17	162 ± 13	$166 \pm 19^{\ddagger}$	$173 \pm 17^{\ddagger}$	$187 \pm 20^{\ddagger}$	$192 \pm 22^{\ddagger}$
Diastolic blood pressure (mmHg)	85 ± 15	$78 \pm 12^{*}$	89 ± 14	84 ± 16	$97 \pm 19^{\ddagger}$	$93 \pm 18^{\ddagger}$
Systemic MAP (mmHg)	108 ± 8	106 ± 8	$115 \pm 9^{\ddagger}$	$114 \pm 10^{\ddagger}$	$127 \pm 11^{\ddagger}$	$126 \pm 13^{\ddagger}$
Systemic Vascular Resistance (mmHg/L/min)	15.5 ± 4.3	$12.6 \pm 3.2^{*}$	14.7 ± 3.5	$12.5 \pm 3.6^{*}$	15.8 ± 4.6	$14.0 \pm 5.1^{*}$

MAP, mean arterial pressure. All data are mean ± SD.

*, $p < 0.05$ vs Saline within work rate;

‡, $p < 0.05$ vs previous work rate within drag.