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Alpha₁-adrenergic responsiveness in human skeletal muscle feed arteries: The impact of reducing extracellular pH

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Abstract

Graded exercise results not only in the modulation of adrenergic mediated smooth muscle tone and a preferential increase in blood flow to the active skeletal muscle termed “functional sympatholysis”, but is also paralleled by metabolically-induced reductions in pH. Therefore, we sought to determine if pH attenuates α_1 -adrenergic receptor sensitivity in human feed arteries. Feed arteries (560 ± 31 μ m ID) were harvested from 24 humans (55 ± 4 yrs) and studied using the isometric tension technique. Vessel function was assessed using potassium chloride (KCl), phenylephrine (PE), acetylcholine (ACh), and sodium nitroprusside (SNP) concentration response curves (CRCs) to characterize non-receptor and receptor-mediated vasoconstriction as well as endothelium-dependent and independent vasorelaxation, respectively. All CRCs were conducted on, originally contiguous, vessel rings in separate baths with a pH of: 7.4, 7.1, 6.8, or 6.5. Reducing pH, via HCl, reduced maximal PE-induced vasoconstriction (pH 7.4 = 85 ± 19 ; 7.1 = 57 ± 16 ; 6.8 = 34 ± 15 ; 6.5 = 16 ± 5 %KCl_{max}), which was partially due to reduced smooth muscle function, as assessed by KCl, (pH 7.4 = 88 ± 13 ; 7.1 = 67 ± 8 ; 6.8 = 67 ± 9 ; 6.5 = 58 ± 8 %KCl_{max}). Graded acidosis had no effect on maximal vasorelaxation. In summary, these data reveal that reductions in extracellular pH attenuate α_1 -mediated vasoconstriction, which is partially explained by reduced smooth muscle function, although vasorelaxation to ACh and SNP remained intact. These findings support the concept that local acidosis likely contributes to functional sympatholysis and exercise hyperemia by opposing sympathetically-mediated vasoconstriction while not impacting vasodilation.

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INTRODUCTION

In 1880 Gaskell originally proposed that acidosis could exert a suppressant effect on vasculature tone (Gaskell, 1880), and this hypothesis has since been confirmed by other researchers who found, *in vivo*, that hypercapnic acidosis was capable of producing significant hyperemia (Daugherty, 1967a; Kontos *et al.*, 1967). Interestingly, exercise of sufficient intensity in humans results in acidosis not only within the active muscle bed itself, with intramuscular and interstitial compartments reaching a pH of 6.47 and 6.9, respectively (Richardson, 2000; Street *et al.*, 2001), but also in both arterial and venous blood (pH 7.07) (Nielsen *et al.*, 2002; Péronnet *et al.*, 2007) and is correlated with profound hyperemia (Andersen & Saltin, 1985). In parallel, exercise is associated with a reduction in vascular responsiveness to both endogenous sympathetic nerve activity (Remensnyder *et al.*, 1962), or exogenous sympathomimetics (Wray *et al.*, 2004), termed “functional sympatholysis” (Remensnyder *et al.*, 1962). However, the mechanistic link between exercise-induced acidosis and functional sympatholysis in humans is not well understood.

In a series of *in vitro* studies using rodent vessels, Faber (McGillivray-Anderson & Faber, 1990; Tateishi & Faber, 1995) and others (Ryan & Gisolfi, 1995) sought to determine if the acidosis-induced hyperemia previously observed *in vivo*, was the result of altered vascular reactivity to sympathetic neurotransmitters, as in functional sympatholysis during exercise. In the *in vitro* rodent model it was determined that arteriolar α_2 -adrenergic function was disrupted with acidosis (pH 7.1), leaving α_1 -receptor function intact (Medgett *et al.*, 1987; McGillivray-Anderson & Faber, 1990; Thomas *et al.*, 1994; Tateishi & Faber, 1995; Kluess *et al.*, 2005). However, other studies that have used a similar approach to determine if acidosis could attenuate agonist-induced vasoconstriction are equivocal, revealing an increase (Rohra *et al.*, 2003a, b), decrease (Medgett *et al.*, 1987; McGillivray-Anderson & Faber, 1990; Ryan & Gisolfi, 1995; Peng *et al.*, 1998; Rohra *et al.*, 2003b; Hyvelin *et al.*, 2004), or no change (Medgett *et al.*, 1987; McGillivray-Anderson & Faber, 1990; Tateishi & Faber, 1995; Kluess *et al.*, 2005) in the maximal response or sensitivity to an α -agonist (e.g. norepinephrine or PE). Additional studies have determined that the disparate effect of pH upon vasocontraction may depend upon species (Medgett *et al.*, 1987), rodent strain (Rohra *et al.*, 2003b), vascular location and caliber (Ishizaka *et al.*, 1999; Lindauer *et al.*, 2003; Hyvelin *et al.*, 2004; Heintz *et al.*, 2005), and experimental model (Rohra *et al.*, 2003a; Rohra *et al.*, 2005; Celotto *et al.*, 2011).

Again in rodent skeletal muscle, it has been established that a primary control point for regulating total muscle blood flow during exercise is the feed artery (Meininger, 1987; Meininger *et al.*, 1987; Hester & Duling, 1988; Williams & Segal, 1993; Lash, 1994). Therefore human feed arteries also likely contribute significantly to blood flow regulation by varying vascular resistance prior to entry into the muscle bed. Although difficult to obtain, human skeletal muscle feed arteries can, in fact, be harvested during certain surgical

procedures and studied *in vitro* (Ives *et al.*, 2011). While the feed artery is extrinsic to skeletal muscle, the potential for an exercise-induced fall in pH within arterial blood and the close proximity of H⁺ laden veins, which may act on the arterial vessels, provide a reasonable rationale for exercise-induced reductions in pH contributing to functional sympatholysis by modulating α_1 -mediated vasoconstriction (Segal, 2005). However, it remains unknown if acidosis, a consequence of exercise, plays a significant role in modulating vasoconstriction of human skeletal muscle feed arteries, considered to be a point of blood flow regulation.

Accordingly, with the novel approach of harvesting human skeletal muscle feed arteries, the purpose of this study was to determine the effect of pH on vascular reactivity in these vessels. Specifically, we sought to determine the effect of acidosis on α_1 -adrenergic receptor responsiveness and the role of smooth muscle and endothelial function in mediating this process. We tested 3 hypotheses: reductions in the pH of the medium surrounding human skeletal muscle feed arteries will 1) attenuate α_1 -adrenergic receptor responsiveness, 2) attenuate inherent smooth muscle function, and 3) enhance endothelium dependent vasodilation. If confirmed, such findings would provide translational evidence that the acidosis documented to contribute significantly to the local regulation of skeletal muscle blood flow by feed arteries in rodents occurs also in humans. Additionally, this work will offer insight into the potential mechanisms responsible for functional sympatholysis during exercise.

METHODS

Subjects and General Procedures

A group of twenty-four subjects (13 males and 11 females, age range 21–86 yrs) agreed to have their vessels used in this study (Table 1). Although medical conditions and medications were noted there were no exclusions based on this information. All subjects included in this study had not received chemotherapy as this was a contraindication for surgery. All protocols were approved by the Institutional Review Boards of the University of Utah and the Salt Lake City VA Medical Center, and written informed consent was obtained from all subjects prior to vessel harvesting.

Vessel Harvest

Human skeletal muscle feed arteries from the axillary and inguinal regions were obtained during melanoma-related node dissection surgeries at the Huntsman Cancer Hospital, University of Utah. Patients were anaesthetized using a standard protocol including: propofol, fentanyl, benzodiazepines, and succinylcholine. After removal of sentinel lymph nodes or lymph node dissection, skeletal muscle feed arteries in the axillary (e.g. serratus anterior, or latissimus dorsi) or inguinal (e.g. hip adductors, or quadriceps femoris) regions were identified and classified as feed arteries based on entry into a muscle bed, structure, coloration, and pulsatile bleed pattern. The vessels were ligated, excised, and immediately placed in iced physiological saline solution and brought to the laboratory within 15 minutes of harvesting.

Wire Myography

Vessels were dissected under a stereo microscope in cold (~4°C) normal physiological saline solution (NPSS) (125 NaCl, 4.7 KCl, 1.2 KH₂PO₄, 1.2 MgSO₄, 2.5 CaCl₂, 18 NaHCO₃, 0.026 Na₂EDTA, and 11.2 Glucose mM). All NPSS solutions and drugs were prepared fresh daily. Vessel internal diameter was measured using a calibrated micrometer eyepiece and reported in micrometers (µm). Perivascular adipose tissue was dissected from the feed arteries. NPSS was continuously aerated with carbogen gas (95% oxygen, 5% carbon dioxide), and pH was monitored at regular intervals and maintained at pH 7.35 – 7.45 by altering the amount of aeration (Orion 3 Star, Thermo Scientific, Waltham MA).

Vessels were dissected into four rings measuring approximately 2 mm in length, and mounted in wire myography baths (700 MO, DMT Systems, Aarhus, DK) to be studied using the isometric tension technique as previously utilized by our group (Ives *et al.*, 2011). Once mounted, vessel baths were also aerated with the same carbogen gas mixture, and the media in the bath was exchanged at 10 minute intervals, except during cumulative drug dose responses. Vessel baths were warmed to 37°C over a 30 minute equilibration period prior to the start of a protocol.

All vessel segments underwent length tension procedures at 37°C to determine the length at which the vessels produced the greatest tension in response to a single dose of 100mM KCl (L_{max}) (Symons *et al.*, 2002). L_{max} was operationally defined as less than a 10% improvement in developed tension in response to 100mM KCl.

pH and Vascular Reactivity

All pH experiments were conducted using wire myography. Changes in pH were achieved by adding specific volumes (13 – 38 µL) of hydrochloric acid (1N HCl) to the 8 mL vessel bath, an approach that has been utilized previously (Rohra *et al.*, 2005; Celotto *et al.*, 2011), and yields similar results to acetic acid use, indicating that the effects of pH are not specific to HCl (Celotto *et al.*, 2011). Pilot work was performed, without vessels, to determine the appropriate volume of HCl necessary to achieve a target pH. Specifically, 30 different volumes of HCl were added separately to the myograph bath containing a bicarbonate-free medium (in mM: 145 NaCl, 5 KCl, 2 CaCl₂, 1 MgCl₂, 1 citrate, 10 glucose, 10 MES, 10 HEPES) (Light *et al.*, 2008) and, after a similar time to that required to perform a cumulative concentration response curve, the pH was measured and a linear regression between pH and HCl volume was constructed. This pH to volume relationship was subsequently tested to determine efficacy and pH was confirmed *post hoc* in each myograph bath after each protocol. Myograph bath pH of 7.4 (control), 7.1, 6.8, and 6.5 were chosen as they are physiologically relevant and can be achieved in arterial blood (pH 7.07) (Nielsen *et al.*, 2002) and skeletal muscle (pH 6.47) during exercise (Richardson, 2000). Under these conditions, concentration responses were performed, in a balanced manner, to determine the effect of acidosis on vasoconstriction (Figure 1). The following concentrations response curves were performed: KCl (10–100mM), PE (10⁻⁹ – 10⁻³ log M), ACh (10⁻⁷ – 10⁻³ log M), and SNP (10⁻⁹ – 10⁻⁴ log M) to determine non-receptor and receptor mediated vasoconstriction, and vasorelaxation. Baths were replenished with buffer of normal pH (7.4) in between CRCs. It should be noted that each bath contained, originally contiguous, vessel

rings which were simultaneously exposed to the different pH conditions for each CRC. This approach was adopted to minimize the effect of time for a given CRC in these experiments (Figure 1). To normalize vasoconstriction data to the individual maximal response as described elsewhere (Jarajapu *et al.*, 2001; Wareing *et al.*, 2002; Kluess *et al.*, 2005), all vasocontractile responses are expressed as a percent of the individual maximal response to 100mM KCl (% KCl_{max}) obtained during the length tension protocol, which typically yields the greatest tension development (unpublished observations). All vasorelaxation responses are expressed as percent relaxation (%) from approximately 60–70% PE pre-contraction (Ives *et al.*, 2011). All data were acquired at 4Hz using an analog to digital data acquisition system (Biopac Systems, Goleta, CA) to monitor vessel tensions and allow later offline analyses.

Calculation of Receptor-Mediated Vascular Function

By utilizing both receptor-dependent and receptor-independent agonists allowed the comparison of receptor-mediated response versus direct activation of the smooth muscle function for both vasoconstriction and vasorelaxation. To understand the effects acidosis on smooth muscle function and the role this may play in the receptor mediated response, we determined the percent change in receptor-mediated function associated with acidosis (% pH 7.4-6.5) for both PE and ACh, performing the same calculation for KCl and SNP, we then subtracted the percent change attributable to vascular smooth muscle for both vasoconstriction (% PE - % KCl) and vasorelaxation (% ACh - % SNP). This approach provides the potential to elucidate the effect of acidosis on receptor-mediated function for both vasoconstriction and vasorelaxation, using a common unit of measure (%)

Supplementary Study

Employing a repeated measures approach, not used in the main protocol, to examine the reversibility of the effects of acidosis, we performed a PE CRC in a single subject with two vessel rings at normal pH (7.4), and two vessel rings exposed to acidosis (pH 6.5), after which the media in the baths was exchanged several times with fresh normal pH buffer (7.4) and allowed to recover. After recovery the PE CRC was repeated with all baths at a normal pH. The exposure to acidosis was of a similar duration required to perform the PE CRC in the main protocol.

Statistical Analyses

Statistical analyses were performed using commercially available software (SPSS v. 16, Chicago, IL). Independent t-tests were used to determine if differences in vasoconstriction or vasorelaxation responses existed between anatomic location (axial vs. inguinal) and sex (males vs. females). Linear regression was used to determine if there was a relationship between age and the effect of acidosis. Two way Repeated Measures ANOVA were utilized to determine if an interaction existed between pH (4 levels; 7.4, 7.1, 6.8, 6.5) and concentration in the vasoreactivity to each agonist (PE, KCl, ACh, SNP). Due to potential individual differences in the concentration eliciting the maximal response, the individual maximal response for each agonist (KCl, PE, ACh, SNP) was analyzed using a one-way repeated measures ANOVA. Additionally, log EC₅₀, an estimate of vascular sensitivity, was

calculated individually, for each CRC, in all of the pH conditions using commercially available software (Biodatafit, v.1.02). A one-way ANOVA was used to determine if differences existed in EC_{50} across pH for each CRC. Significant differences were assessed using Tukeys' Least Significant Difference *post hoc* test to make pair wise comparisons. An independent t-test was used to determine if a difference existed between the effects of acidosis on vasoconstriction compared to vasorelaxation. The level of significance was established at $p < 0.05$. All data are reported as mean $\pm$ standard error (SE).

RESULTS

Vessel Characteristics

Twenty-four human skeletal muscle feed arteries were successfully harvested (Table 1). Given the blood chemistry and complete blood count results, these individuals, while quite varied, were relatively healthy. No statistical differences in L_{max} , vasoconstriction or vasorelaxation responses were observed in terms of anatomic location (axial vs. inguinal ($p = 0.44$), sex ($p = 0.25$), or the relationship between age and the effect of acidosis ($r^2 = 0.05$). Consequently, responses from all vessels were combined. The average internal diameter with minimal tension for these feed arteries was $560 \pm 31 \mu m$, and measured $1650 \pm 75 \mu m$ in length. L_{max} (916 ± 64 , 916 ± 64 , 900 ± 58 , 916 ± 63 mg), or tension at L_{max} (1085 ± 211 , 1414 ± 254 , 1040 ± 262 , 1229 ± 262 , mg developed tension) were not different across baths. Vessel function protocols revealed robust vasoconstriction in response to PE and KCl (83 ± 19 ; 87 ± 13 %KCl $_{max}$, respectively) at normal pH (7.4). Vessels were pre-constricted to approximately $60 \pm 10\%$ of the maximal PE response prior to ACh and SNP concentration response curves, and from this point feed artery segments achieved significant vasorelaxation (102 ± 16 ; 78 ± 8 %relaxation, respectively). Taken together, these results indicate the feed arteries had functional smooth muscle, α_1 -adrenergic receptors, and an intact endothelium.

pH and Vasoconstriction

Reducing extracellular pH significantly attenuated vascular tension development in response to both the α_1 -adrenergic agonist PE, and KCl (Figure 2A and B). Reducing pH significantly attenuated KCl induced vasoconstriction (pH effect $p < 0.05$; concentration effect $p < 0.05$), but there was no difference in the maximal responses (Figure 2C). The suppressant effect of incremental acidosis was evident in response to cumulative doses of PE (pH $\times$ concentration $p < 0.05$; pH effect $p < 0.05$; concentration effect $p < 0.05$; Figure 2A). When expressed as percent of control (pH 7.4), the effect of pH on the arteries was more pronounced in response to PE, when compared to KCl (Figure 2C). Acidosis had no effect on the sensitivity (EC_{50}) to KCl ($p = 0.78$), in contrast the sensitivity to PE was significantly ($p < 0.05$) reduced (Table 2). These results are not likely due to differences in baseline tension, as altering pH had no significant effect on this variable ($p = 0.69$). Indeed, even the largest difference in baseline tension, approximating 3% of KCl $_{max}$, could not account for the 30 – 80% reduction in maximal α_1 -mediated vasoconstriction observed with acidosis.

pH and Vasorelaxation

The ACh (endothelium dependent) and SNP (endothelium independent) vasorelaxation responses were performed with graded reductions in pH to determine if net relaxation or relaxation kinetics were altered by acidosis. There was no significant effect of graded reductions in pH on the concentration response to ACh (Figure 3A), SNP (Figure 3B), or the maximal vasorelaxation to either ACh or SNP (Figure 3C). Acidosis had no effect on the sensitivity (EC_{50}) to ACh ($p = 0.38$), or SNP ($p = 0.83$) (Table 2). To determine, *post hoc*, if the level of pre-contraction altered the ACh response, the level of pre-contraction and the maximal ACh-induced vasorelaxation were entered into a simple linear regression, and there was no evidence of a relationship ($r^2 = 0.05$, $p > 0.05$). This suggests that the lower level of pre-contraction, associated with acidosis, did not influence the vasorelaxation response.

pH and Receptor-Mediated Vascular Function

Calculating the effect of acidosis on receptor mediated function taking into account any alteration in smooth muscle function as a consequence of acidosis, revealed that vasocontraction was significantly ($p < 0.05$) altered ($47 \pm 10\%$ reduction in receptor function) compared to vasorelaxation which was relatively unaffected ($6 \pm 2\%$ reduction in receptor function).

Supplemental Study

Using a repeated measures experimental approach, in contrast to the cross sectional design of the main study, the effect of acidosis was again clearly evident and determined to be reversible (Figure 4). Specifically, two of four vessel rings were exposed to a pH of 6.5, attenuating vasocontraction in comparison to the other two rings at normal pH (Figure 4A). When returned to normal pH, these vessel rings again exhibited similar responses to the other two rings (Figure 4B). Though not directly related to the main goal of the study, these results highlight the reversibility of the attenuating effect of acidosis on vasoconstrictor function.

DISCUSSION

The main finding of this study was that graded reductions in extracellular pH resulted in significant and progressive decreases in the response to the sympathomimetic PE. This attenuated response to the α_1 -adrenergic agonist PE cannot be fully explained by the much smaller concomitant suppression of smooth muscle function. In stark contrast to the detrimental impact of acidosis on vasocontraction, there was no significant effect on endothelium dependent or independent maximal vasorelaxation. These results reveal, for the first time in human skeletal muscle feed arteries, that not only does acidosis suppress vasocontractile capacity, but this phenomenon may also be enhanced by unaltered vasodilatory function. Therefore, these findings imply that acidosis, associated with skeletal muscle metabolism, could be a contributing factor in the reduction of sympathetically mediated vasoconstriction, or functional sympatholysis, observed during exercise by reducing vasocontractile function while leaving vasorelaxation function intact, ultimately producing significant reductions in vascular resistance.

pH and vasocontraction

As already indicated, *en masse*, the results of studies that have attempted to determine the effect of acidosis on agonist-induced vasoconstriction remain equivocal. Although, early studies focused on the receptor-mediated responses (McGillivray-Anderson & Faber, 1990), later studies acknowledged that any observed reduction in agonist-induced vasocontraction could be, at least in part, mediated by altered inherent smooth muscle function (Tateishi & Faber, 1995). Most studies (Aalkjær & Poston, 1996; Aalkjær & Peng, 1997; Peng *et al.*, 1998; Ishizaka *et al.*, 1999; Austin & Wray, 2000) now agree that acidosis exerts a direct effect on the vascular smooth muscle, via ion channels (ATP sensitive K^+ channels in particular), which may act in conjunction with reduced receptor sensitivity resulting in attenuated maximal receptor-mediated responsiveness. In light of this, Rohra *et al.*, (Rohra *et al.*, 2005) used single doses of the receptor-independent agonist KCl and receptor agonist PE to determine the effects of acidosis on vasocontractile function in the human internal mammary artery. Their findings indicated that acidosis reduced KCl and, to a much greater extent, PE-induced vasocontraction (Rohra *et al.*, 2005). In agreement with Rohra and colleagues (Rohra *et al.*, 2005) the current findings reveal that α_1 -adrenergic receptor function is suppressed in a proportional fashion by reductions in pH (Figure 2A), which persisted across a range of doses, and is, at least in part, mediated by reduced smooth muscle function (Figure 2B). Also in agreement with this prior work in a human artery, it appears that in human feed arteries the receptor mediated vasocontraction, induced by the sympathomimetic PE, is far more susceptible to the effects of acidosis than the non-receptor mediated KCl-induced vasocontraction (Figure 2C).

The effect of pH may, or may not, be receptor selective as Kluess *et al.*, (Kluess *et al.*, 2005) documented, in the rat femoral artery, that purinergic (P_2X_1) and not α_1 -adrenergic receptor-mediated vasocontraction was impaired during acidosis. Although, the majority of studies indicate acidosis is inhibitory despite the agonist employed (Rohra *et al.*, 2005), some of the variation in the effect of acidosis can be explained by differences in species (Medgett *et al.*, 1987), genetic strain (Rohra *et al.*, 2003b), vascular location and caliber (Ishizaka *et al.*, 1999; Lindauer *et al.*, 2003; Hyvelin *et al.*, 2004; Heintz *et al.*, 2005; Heintz *et al.*, 2008), and experimental model (Rohra *et al.*, 2003a; Rohra *et al.*, 2005; Celotto *et al.*, 2011). For example, an *in vitro* rodent study determined that rat strain alone created a divergent response resulting in either a suppressed or enhanced α_1 -mediated vasoconstriction in the face of acidosis (Rohra *et al.*, 2003b). Vessel location appears to be another important determinant of the effect acidosis may have upon vascular tension development as cerebral and coronary vessels seem highly sensitive to acidosis (Medgett *et al.*, 1987; Peng *et al.*, 1998; Heintz *et al.*, 2008; Celotto *et al.*, 2011), whereas the pulmonary artery appears to be less so (Medgett *et al.*, 1987; Hyvelin *et al.*, 2004). Additionally, vessel order appears to alter the vascular sensitivity to acidosis, meaning that it has been documented that smaller arterioles appear to be more sensitive to acidosis than larger arterioles (McGillivray-Anderson & Faber, 1990; Tateishi & Faber, 1995), which has implications for blood flow regulation and redistribution. This, also likely explains the negative findings of Kluess *et al.* (Kluess *et al.*, 2005) where there was no effect of acidosis on PE-induced vasocontraction in the rat femoral artery, an unlikely site of blood flow regulation. Utilizing an artery more likely to be involved in the regulation of skeletal muscle blood flow, our findings indicate

the acidosis does, in fact, reduce α_1 -mediated vasocontraction. Indeed, as the sympatholysis observed by Wray et al. (Wray *et al.*, 2004) could not be wholly explained by changes in femoral artery diameter, acidosis mediated suppression of α_1 -receptor responsiveness in the feed artery may have been one of the blood flow control points acting to prevent PE-induced vasoconstriction during exercise.

It should also be noted that studies which determined that acidosis suppressed α_2 - and not α_1 -adrenergic responsiveness using selective and non-selective adrenergic agonists, employed the rat cremaster muscle model (McGillivray-Anderson & Faber, 1990; Tateishi & Faber, 1995). This model has recently been suggested to be unrepresentative of locomotor muscles (Moore *et al.*, 2010), and challenges the dogma that terminal arterioles are primarily under α_2 -receptor control and more susceptible to “metabolic inhibition”, while proximal arteries are under α_1 -receptor control (McGillivray-Anderson & Faber, 1990; Anderson & Faber, 1991; McGillivray-Anderson & Faber, 1991). While the classic dogma was logical and certainly an attractive hypothesis, the work of Moore and colleagues (Moore *et al.*, 2010) in essence challenges the applicability of prior findings from the cremaster model, as they found terminal arterioles in locomotor muscle were, in fact, primarily under α_1 -receptor control, but also varied in prevalence down the arterial tree. In addition to direct exposure to arterial blood, which can experience a fall in pH (Nielsen *et al.*, 2002; Péronnet *et al.*, 2007), feed arteries that are extrinsic to the muscle itself, may also be exposed to venous arterial feedback. Specifically, as suggested by Segal (Segal, 2005), much like countercurrent heat exchange, venous drainage of skeletal muscle containing metabolites is likely capable of countercurrent ion exchange. Based upon the intimate relationship between the feed artery and the H^+ enriched veins, H^+ ions released from the muscle into the venous system may leech from the vein and act on the feed artery itself, yielding a form of blood flow auto-regulation. In this context, and in light of the potential for the feed artery to be a locus of skeletal muscle blood flow regulation (Meininger, 1987; Meininger *et al.*, 1987; Hester & Duling, 1988; Williams & Segal, 1993; Lash, 1994), the findings from the current study support the suppressant effect of acidosis on α_1 -mediated vasoconstriction, which appears to be due to reduced receptor sensitivity, and inherent smooth muscle. In terms of basic mechanisms, it is tenable to speculate that either or both the transient receptor potential cation channel subfamily V (TRPV) or acid-sensing ion channels (ASICs) receptors are playing a role in the pH-induced attenuation in vasocontraction documented here, but this and the site of their action (endothelial vs. smooth muscle) remains to be investigated.

Finally, in terms of pH and vasocontraction, despite a group of heterogeneous subjects, varied vessel harvest location (i.e. axial and inguinal), and potential underlying pathology, the notion that pH exhibited a common effect speaks to the robust nature of this response as it relates to sympathetically mediated vascular regulation. Specifically, independent of age, sex, vessel location, or disease status, reducing pH significantly attenuates α_1 -mediated vasocontraction.

pH and vasorelaxation

The vasodilatory effects of acidosis have been well described both *in vitro* (Kontos *et al.*, 1977; Ishizaka & Kuo, 1996; Peng *et al.*, 1998; Ishizaka *et al.*, 1999; Lindauer *et al.*, 2003;

Heintz *et al.*, 2005; Heintz *et al.*, 2008; Celotto *et al.*, 2011), and *in vivo* in animals (Deal & Green, 1954; Daugherty, 1967b, a; Kontos *et al.*, 1971), yet the question remains if these results translate into humans. Early work in humans indicated that hypercapnia or the infusion of hypercapnic saline elicited a profound hyperemia (Kontos *et al.*, 1967, 1968b, a). The prior human work, although quite impressive for the era, was not able to determine the mechanism by which acidosis elicited a vasodilatory effect. Subsequent animal work (Lindauer *et al.*, 2003; Celotto *et al.*, 2011), determined that acidosis elicited vasodilation in a dose-dependent manner, and endothelial denudation or pharmacological blockade significantly reduced vessel sensitivity to the vasodilatory effect of acidosis, but not the maximal response. The results of the current study were similar to the findings of these previous studies (Lindauer *et al.*, 2003; Celotto *et al.*, 2011), where the maximal response to ACh or SNP was unchanged (Figure 3). Thus, the effect of acidosis on vasorelaxation does not appear to be significantly impacted by altered smooth muscle cyclic-GMP function. To our knowledge, there has not been single study that has investigated the effect of acidosis on agonist-induced vasorelaxation in either animals or humans. Therefore, this study appears to be the first to demonstrate that arterial vasorelaxation, both endothelial dependent and independent, remains intact when exposed to physiological levels of acidosis.

pH and the balance between vasoconstrictors and vasodilators: Implications for functional sympatholysis

There is a growing recognition that functional sympatholysis may actually be the alteration in the balance between sympathetic vasoconstriction and vasodilation induced by metabolic by-products from active muscle (Hansen *et al.*, 2000). In this context we sought to determine the effect of acidosis on vasoreactivity and the varying contribution of vasorelaxation and vasocontractile function in this response. Additionally, utilizing receptor-dependent and receptor-independent agonists allowed the comparison of receptor versus smooth muscle function for both vasocontraction and vasorelaxation. Specifically, using the subtraction approach, described in the methods, it is apparent that acidosis had a more profound effect upon receptor-mediated vasocontraction compared to vasorelaxation. In the context of exercise and functional sympatholysis, these results provide support for the concept that acidosis can indeed elicit a sympatholytic effect, but that the impact of this effect would be augmented by the concomitant maintenance of endothelium mediated vasodilation. Irrespective of the origin of the acidosis, albeit arterial pH changes, venous countercurrent ion exchange, or from surrounding skeletal muscle, it is likely that, *in vivo*, these contrasting vascular responses to acidosis act in concert to override sympathetic activity and facilitate hyperemia during exercise.

Experimental Considerations

The current study utilized HCl to elicit a non-specific reduction in pH, a common methodological approach used by others (Ishizaka *et al.*, 1999; Kluess *et al.*, 2005; Celotto *et al.*, 2011). As a focus of this work is upon functional sympatholysis, the use of HCL instead of lactic acid, an actual component of the metabolic milieu during exercise, may evoke some concern. However, it is important to note that, although lactic acid is often associated with exercise-induced changes in pH, there are other significant sources of protons during muscular work that result in acidosis. These sources include not only ATP

hydrolysis (Robergs *et al.*, 2004) and mitochondrial H⁺ leak (Monemdjou *et al.*, 2000; Lanza *et al.*, 2010), but also metabolic CO₂ production (Robergs *et al.*, 2004), the latter of which is a significant contributor to both local and systemic proton load during exercise ($\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{HCO}_3^- + \text{H}^+$). As suggested previously (Hong *et al.*, 1997), it is likely the proton, not lactate *per se*, that contributes to physiological responses to acidosis. Therefore, the resultant acidosis elicited by HCl would likely yield similar results to lactic acid, as both compounds dissociate creating a proton and a negatively charged molecule (HCl: Cl⁻; lactic acid: lactate⁻). However, it should be noted that other prior work suggests that the experimental origin of acidosis (HCl vs. lactic acid) may elicit cardiovascular responses that are specific to the acid used (Shirer *et al.*, 1988). Further studies would be necessary to determine if lactic acid, itself, has differing effects from HCL-induced acidosis on α_1 -adrenergic responsiveness in human feed arteries.

Conclusions

This study has demonstrated that local changes in pH significantly reduce α_1 -adrenergic receptor function and this observation is due, at least in part, to reduced smooth muscle function. Interestingly, endothelium-dependent and endothelium-independent vasorelaxation were unchanged which may act to magnify the impact of the attenuated sympathetically mediated vasoconstriction, ultimately reducing vascular tone. These findings support the concept that local acidosis could be a contributing factor in functional sympatholysis and exercise hyperemia by both opposing sympathetically mediated vasoconstriction and not impacting vasodilation.

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Figure 1. Experimental Timeline
CRC, concentration response curves. Total protocol duration was approximately 2.5 hours.

Figure 2. Vasocontractile Function and Acidosis

A) PE concentration responses across varying levels of acidosis. * Significant pH $\times$ concentration interaction, $p < 0.05$, †main effect of pH, $p < 0.05$. B) KCl concentration response curves across varying levels of acidosis. †main effect of pH, $p < 0.05$, C) Maximal PE and KCl responses expressed as a percent of control condition (pH 7.4). # $p < 0.05$ vs. pH 7.4, § $p < 0.05$ vs. pH 7.1. Data are presented as mean $\pm$ SE.

Figure 3. Vasorelaxation Function and Acidosis

A) ACh concentration response curves across varying levels of acidosis. B) SNP Dose response curves across varying levels of acidosis. Data are presented as mean $\pm$ SE.

Figure 4. Evidence of the reversibility of the effects of reduced pH

Sample tracing from an individual myograph chamber during phenylephrine concentration response curve (CRC), prior to acidosis, during acidosis, and after exposure to acidosis demonstrating the acute effect of reducing pH on α_1 -mediated vasocontraction. Note: These data were obtained in a repeated measures design in contrast to the data presented in figures 2 and 3, which were conducted using each bath for a given pH condition (figure 1).

Table 1

Subject Characteristics (n =24).

	Mean $\pm$ SE	Normal Range
Age (yr)	55 $\pm$ 4 (21–86)	
Males/Females (n)	13/11	
Height (cm)	170 $\pm$ 2	
Weight (kg)	83 $\pm$ 3	
Body Mass Index	29 $\pm$ 1	
Systolic Blood Pressure (mmHg)	136 $\pm$ 4 [†]	
Diastolic Blood Pressure (mmHg)	78 $\pm$ 3 [†]	
MAP (mmHg)	97 $\pm$ 3 [†]	
Glucose (mg/dl)	90 $\pm$ 3	65 – 110
Blood Urea Nitrogen (mg/dl)	17 $\pm$ 1	6 – 21
Creatinine (mg/dl)	0.93 $\pm$ 0.0	0.52 – 0.99
Albumin (g/dl)	4.01 $\pm$ 0.1	3.3 – 4.8
Bilirubin (mg/dl)	0.43 $\pm$ 0.1	0.2 – 1.3
Lactate Dehydrogenase (U/L)	471 $\pm$ 28	313 – 618
Hemoglobin (g/dl)	14 $\pm$ 0.3	12 – 16
WBC (K/uL)	6.95 $\pm$ 0.3	3.6 – 10.6
RBC (M/uL)	4.73 $\pm$ 0.1	4 – 5.2
Platelets (K/uL)	227 $\pm$ 9	150 – 400
Hematocrit (%)	42 $\pm$ 1	36 – 46
Medications (users/n)		
Cardiovascular (users/n)		
Diuretic	5/24	
Ca ⁺⁺ Channel Blocker	5/24	
Beta Blocker	4/24	
Angiotensin Blocker	3/24	
Statin	6/24	
ACE inhibitor	2/24	
Beta Blocker	4/24	
Vessel Location (#/total)		
Axillary	15/24	
Inguinal	9/24	

[†]Data obtained during pre-operative examination

Table 2

Concentration Response Curve Characteristics Across pH

Drug	pH	Max Response	EC ₅₀
PE	7.4	85 ± 19 (%KCl _{max})	− 5.0 ± 0.2 (log M)
	7.1	56 ± 16*	− 4.4 ± 0.2*
	6.8	34 ± 15*	− 4.4 ± 0.2*
	6.5	16 ± 5*	− 3.8 ± 0.2*
KCl	7.4	88 ± 13 (%KCl _{max})	62 ± 6.2 (mM)
	7.1	67 ± 8	57 ± 7.5
	6.8	67 ± 9	62 ± 7.0
	6.5	58 ± 8	67 ± 5.5
ACh	7.4	81 ± 8 (% vasorelaxation)	− 5.5 ± 0.2 (log M)
	7.1	77 ± 12	− 5.2 ± 0.2
	6.8	67 ± 7	− 5.0 ± 0.2
	6.5	68 ± 6	− 5.6 ± 0.2
SNP	7.4	103 ± 17(% vasorelaxation)	− 6.4 ± 0.3 (log M)
	7.1	110 ± 9	− 6.3 ± 0.3
	6.8	107 ± 4	− 6.4 ± 0.1
	6.5	93 ± 7	− 6.1 ± 0.2