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Research Paper

α₁-Adrenergic responsiveness in human skeletal muscle feed arteries: the impact of reducing extracellular pH

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Experimental Physiology

New Findings

- **What is the central question of this study?**
  In human arteries involved in the regulation of muscle blood flow, there is a lack of data about whether acidosis alters vascular sensitivity to vasoactive agents, as well as altering endothelium dependent vasorelaxation. Little is known about the interaction of metabolites and vascular function in human skeletal muscle feed arteries.

- **What is the main finding and its importance?**
  Increasing acidosis attenuated the response and sensitivity of the arteries to phenylephrine; this effect was selective to the receptor over smooth muscle.
  Acidosis did not alter endothelium dependent vasorelaxation. Impaired vasoconstriction coupled with intact vasorelaxation, promotes decreased vascular tone with exposure to acidosis, and may contribute to sympatholysis during exercise.

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. We therefore sought to determine whether pH attenuates α₁-adrenergic receptor sensitivity in human feed arteries. Feed arteries (560 ± 31 μm i.d.) were harvested from 24 humans (55 ± 4 years old) and studied using the isometric tension technique. Vessel function was assessed using KCl, phenylephrine (PE), ACh and sodium nitroprusside (SNP) concentration–response curves to characterize non-receptor-mediated and receptor-mediated vasocontraction, as well as endothelium-dependent and -independent vasorelaxation, respectively. All concentration–response curves were obtained from (originally contiguous) vessel rings in separate baths with a pH of 7.4, 7.1, 6.8 or 6.5. Reduction of the pH, via HCl, reduced maximal PE-induced vasocontraction (pH 7.4 = 85 ± 19, pH 7.1 = 57 ± 16, pH 6.8 = 34 ± 15 and pH 6.5 = $16 \pm 5\%$ KCl$_{max}$), which was partly due to reduced smooth muscle function, as assessed by KCl (pH 7.4 = 88 ± 13, pH 7.1 = 67 ± 8, pH 6.8 = 67 ± 9 and pH 6.5 = $58 \pm 8\%$ KCl$_{max}$). Graded acidosis had no effect on maximal vasorelaxation. In summary, these data reveal that reductions in extracellular pH attenuate α₁-mediated vasocontraction, which is partly explained by reduced smooth muscle function, although vasorelaxation in response to ACh and SNP remained intact. These findings support the concept that local acidosis is likely to contribute to functional

sympatholysis and exercise hyperaemia by opposing sympathetically mediated vasoconstriction while not impacting vasodilatation.

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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 hyperaemia (Daugherty *et al.* 1967*b*; 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 hyperaemia (Andersen & Saltin, 1985). In parallel, exercise is associated with a reduction in vascular responsiveness to both endogenous sympathetic nerve activity (Remensnyder *et al.* 1962) and exogenous sympathomimetics (Wray *et al.* 2004) that is 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 and co-workers (McGillivray-Anderson & Faber, 1990; Tateishi & Faber, 1995) and others (Ryan & Gisolfi, 1995) sought to determine whether the acidosis-induced hyperaemia 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 whether acidosis could attenuate agonist-induced vasoconstriction are equivocal, revealing an increase (Rohra *et al.* 2003*a,b*), a decrease (Medgett *et al.* 1987; McGillivray-Anderson & Faber, 1990; Ryan & Gisolfi, 1995; Peng *et al.* 1998; Rohra *et al.* 2003*b*; 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. noradrenaline or phenylephrine (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.* 2003*b*), vascular location and calibre (Ishizaka *et al.* 1999; Lindauer *et al.* 2003; Hyvelin *et al.* 2004; Heintz *et al.* 2005)

and experimental model (Rohra *et al.* 2003*a*, 2005; Celotto *et al.* 2011).

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 are also likely to 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 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 vasocontraction (Segal, 2005). However, it remains unknown whether acidosis, a consequence of exercise, plays a significant role in modulating vasocontraction of human skeletal muscle feed arteries, which are considered to be a point of blood flow regulation.

Accordingly, using 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 hypotheses that reductions in the pH of the medium surrounding human skeletal muscle feed arteries will attenuate α_1-adrenergic receptor responsiveness, attenuate inherent smooth muscle function and enhance endothelium-dependent vasodilatation. 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 24 subjects (13 men and 11 women, age range 21–86 years) agreed to have their vessels used

Table 1. Subject characteristics (*n* = 24)

Characteristic	Mean ± SEM	Normal range
Age (years)	55 ± 4	–
Men/women (*n*)	13/11	–
Height (cm)	170 ± 2	–
Weight (kg)	83 ± 3	–
Body mass index (kg m^{-2})	29 ± 1	–
Systolic blood pressure (mmHg)	136 ± 4*	–
Diastolic blood pressure (mmHg)	78 ± 3*	–
Mean arterial blood pressure (mmHg)	97 ± 3*	–
Glucose (mg dl^{-1})	90 ± 3	65–110
Blood urea nitrogen (mg dl^{-1})	17 ± 1	6–21
Creatinine (mg dl^{-1})	0.93 ± 0.0	0.52–0.99
Albumin (g dl^{-1})	4.01 ± 0.1	3.3–4.8
Bilirubin (mg dl^{-1})	0.43 ± 0.1	0.2–1.3
Lactate dehydrogenase (U l^{-1})	471 ± 28	313–618
Haemoglobin (g dl^{-1})	14 ± 0.3	12–16
White blood cells ($\times 10^3$ μl^{-1})	6.95 ± 0.3	3.6–10.6
Red blood cells ($\times 10^6$ μl^{-1})	4.73 ± 0.1	4–5.2
Platelets ($\times 10^3$ μl^{-1})	227 ± 9	150–400
Haematocrit (%)	42 ± 1	36–46
Cardiovascular medication (users/*n*)		
Diuretic	5/24	
Ca^{2+} channel blocker	5/24	
β-Blocker	4/24	
Angiotensin blocker	3/24	
Statin	6/24	
Angiotensin-converting enzyme inhibitor	2/24	
Vessel location (*n*/total)		
Axillary	15/24	
Inguinal	9/24	

* Data obtained during preoperative examination.

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, because 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, written informed consent was obtained from all subjects prior to vessel harvesting, and the study conformed with the principles of the Declaration of Helskini.

Vessel harvest

Human skeletal muscle feed arteries from the axillary and inguinal regions were obtained during melanoma-related node dissection surgery at the Huntsman Cancer Hospital, University of Utah. Patients were anaesthetized using a standard protocol that included 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 region (e.g. hip adductors or quadriceps femoris) were identified and classified as feed arteries based on entry into a muscle bed, structure, colouration and pulsatile bleed pattern. The vessels were ligated, excised, and immediately placed in iced physiological saline solution and brought to the laboratory within 15 min of harvesting.

Wire myography

Vessels were dissected under a stereo microscope in cold (~4°C) normal physiological saline solution (mM: 125 NaCl, 4.7 KCl, 1.2 KH$_2$PO$_4$, 1.2 MgSO$_4$, 2.5 CaCl$_2$, 18 NaHCO$_3$, 0.026 Na$_2$EDTA and 11.2 glucose). All normal physiological saline solutions and drugs were prepared fresh daily. Vessel internal diameter was measured using a calibrated micrometer eyepiece and reported in micrometers. Perivascular adipose tissue was dissected from the feed arteries. Normal physiological saline solution was continuously aerated with carbogen gas (95% oxygen and 5% carbon dioxide), and pH was monitored at regular intervals and maintained at 7.35–7.45 by altering the amount of aeration (Orion 3 Star; Thermo Scientific, Waltham, MA, USA).

Vessels were dissected into four rings, each measuring approximately 2 mm in length, and mounted in wire myography baths (700 MO; DMT Systems, Aarhus, Denmark) to be studied using the isometric tension technique as previously used by our group (Ives *et al.* 2011). Once mounted, vessel baths were also aerated with the same carbogen gas mixture, and the medium in the bath was exchanged at 10 min intervals, except during cumulative drug dose responses. Vessel baths were warmed to 37°C over a 30 min 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 100 mM KCl (L_{max}; Symons *et al.* 2002); L_{max} was operationally defined as less than a 10% improvement in developed tension in response to 100 mM 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 (1 N) to the 8 ml vessel bath, an approach that has been used previously (Rohra *et al.* 2005; Celotto *et al.* 2011) and that yields similar results to those obtained with use of acetic acid, 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 (mM: 145 NaCl, 5 KCl, 2 CaCl$_2$, 1 MgCl$_2$, 1 citrate, 10 glucose, 10 Mes and 10 Hepes; Light *et al.* 2008) and, after a similar time to that required to obtain a cumulative concentration–response curve (CRC), the pH was measured and a linear regression between pH and HCl volume constructed. This pH–volume relationship was subsequently tested to determine efficacy, and pH was confirmed *post hoc* in each myograph bath after each protocol. Myograph bath pH values of 7.4 (control), 7.1, 6.8 and 6.5 were chosen because 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). In these conditions, concentration responses were determined, in a balanced manner, to examine the effect of acidosis on vasocontraction (Fig. 1). The following concentration–response curves were determined: KCl (10–100 mM); PE (10^{-9}–10^{-3} log M); ACh (10^{-7}–10^{-3} log M); and sodium nitroprusside (SNP; 10^{-9}–10^{-4} log M) to determine non-receptor-mediated and receptor-mediated vasocontraction, 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 (Fig. 1). In order to normalize vasocontraction 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 percentage of the individual maximal response to 100 mM KCl (% KCl$_{max}$) obtained during the length tension protocol, which typically yields the greatest tension development (S.J. Ives & R.S Richardson, unpublished observations). All vasorelaxation responses are expressed as the percentage relaxation from approximately 60–70%

PE-induced precontraction (Ives *et al.* 2011). All data were acquired at 4 Hz using an analog to digital data acquisition system (Biopac Systems; Goleta, CA, USA) to monitor vessel tensions and allow later offline analyses.

Calculation of receptor-mediated vascular function

Use of both receptor-dependent and receptor-independent agonists allowed comparison of the receptor-mediated response *versus* direct activation of the smooth muscle function for both vasocontraction and vasorelaxation. In order to understand the effects of acidosis on smooth muscle function and the role this may play in the receptor-mediated response, we determined the percentage change in receptor-mediated function associated with acidosis (% ΔpH 7.4–6.5) for both PE and ACh, performing the same calculation for KCl and SNP. We then subtracted the percentage change attributable to vascular smooth muscle for both vasocontraction (% ΔPE % ΔKCl) and vasorelaxation (% ΔACh – % ΔSNP). This approach provides the potential to elucidate the effect of acidosis on receptor-mediated function for both vasocontraction and vasorelaxation, using a common unit of measure (% Δ).

Supplementary study

Employing a repeated-measures approach, not used in the main protocol, to examine the reversibility of the effects of acidosis, we determined a PE CRC in a feed artery from 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 medium in the baths was exchanged several times with fresh normal pH buffer (7.4) and allowed to recover. After recovery, determination of the PE CRC was repeated with all baths at a normal pH. The exposure to acidosis was of a similar duration to that required for determination of the PE CRC in the main protocol.
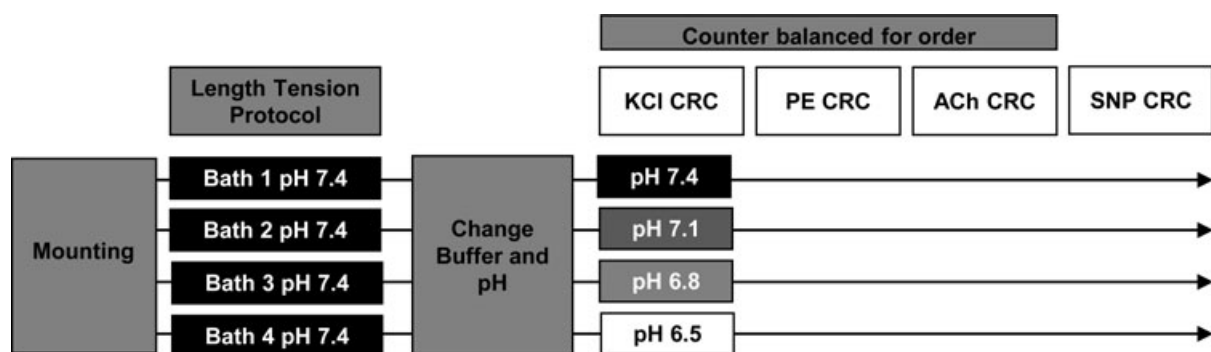
Figure 1. Experimental time line
Total protocol duration was approximately 2.5 h. Abbreviations: CRC, concentration–response curve; PE, phenylephrine; and SNP, sodium nitroprusside.

Statistical analyses

Statistical analyses were performed using commercially available software (SPSS version 16; SPSS Inc., Chicago, IL, USA). Student's unpaired *t* tests were used to determine whether differences in vasocontraction or vasorelaxation responses existed between anatomical locations (axial *versus* inguinal) and sex (males *versus* females). Linear regression was used to determine whether there was a relationship between age and the effect of acidosis. Two-way repeated-measures ANOVAs were used to determine whether an interaction existed between pH (four levels: 7.4, 7.1, 6.8 and 6.5) and concentration in the vasoreactivity to each agonist (PE, KCl, ACh and SNP). Owing to potential individual differences in the concentration eliciting the maximal response, the individual maximal response for each agonist (KCl, PE, ACh and SNP) was analysed using a one-way repeated-measures ANOVA. Additionally, log EC_{50}, an estimate of vascular sensitivity, was calculated individually, for each CRC, in all of the pH conditions using commercially available software (Biodatafit, version 1.02, Chang Bioscience, Castro Valley, CA, USA). A one-way ANOVA was used to determine whether differences existed in EC_{50} across pH for each CRC. Significant differences were assessed using Tukey's least significant difference *post hoc* test to make pairwise comparisons. Student's unpaired *t* test was used to determine whether a difference existed between the effects of acidosis on vasocontraction compared with vasorelaxation. The level of significance was established at $P < 0.05$. All data are reported as means $\pm$ SEM.

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}, vasocontraction or vasorelaxation responses were observed in terms of anatomical location (axial *versus* 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 they measured $1650 \pm 75 \, \mu m$ in length. Values of L_{max} (916 ± 64, 916 ± 64, 900 ± 58 and 916 ± 63 mg) or tension at L_{max} (1085 ± 211, 1414 ± 254, 1040 ± 262 and 1229 ± 262 mg developed tension) were not different across baths. Vessel function protocols revealed robust vasocontraction in response to PE and KCl (83 ± 19 and $87 \pm 13\%$ KCl_{max}, respectively)
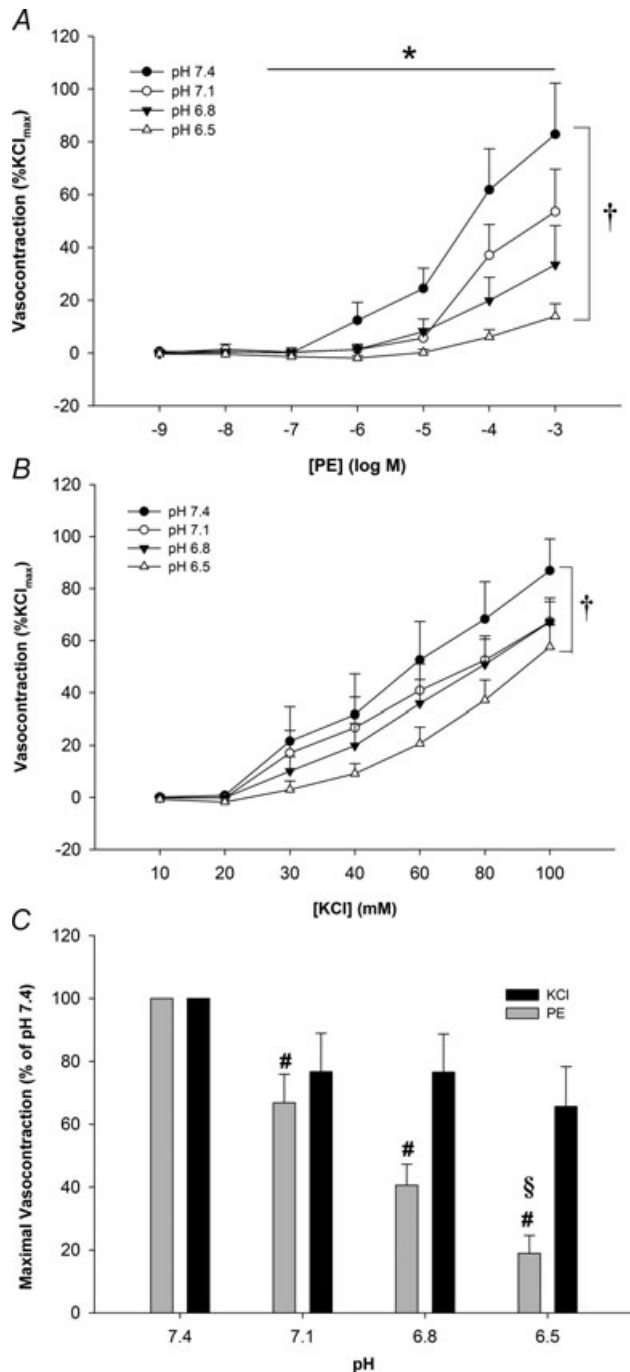
at normal pH (7.4). Vessels were preconstricted to approximately $60 \pm 10\%$ of the maximal PE response prior to determination of ACh and SNP concentration–response curves, and from this point the feed artery segments achieved significant vasorelaxation (102 ± 16 and $78 \pm 8\%$ relaxation, respectively). Taken together, these results indicate that the feed arteries had functional smooth muscle and α_1-adrenergic receptors, as well as an intact endothelium.

pH and vasocontraction

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

pH and vasorelaxation

The ACh (endothelium-dependent) and SNP (endothelium-independent) vasorelaxation responses were obtained with graded reductions in pH to determine whether 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 (Fig. 3*A*) or SNP (Fig. 3*B*) or on the maximal vasorelaxation in response to either ACh or SNP (Fig. 3*C*). Acidosis had no effect on the sensitivity (EC_{50}) to ACh ($P = 0.38$) or SNP ($P = 0.83$; Table 2). In order to determine, *post hoc*, whether the level of precontraction altered the ACh response, the level of precontraction and the maximal ACh-induced vasorelaxation were entered into a simple linear regression equation, and there was no evidence of a relationship ($r^2 = 0.05$, $P > 0.05$). This suggests that the lower level of precontraction associated

Table 2. Concentration–response curve characteristics across pH

Drug	pH	Maximal 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(m M)
	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

with acidosis did not influence the vasorelaxation response.

pH and receptor-mediated vascular function

Calculation of 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 with vasorelaxation, which was relatively unaffected ($6 \pm 2\%$ reduction in receptor function).
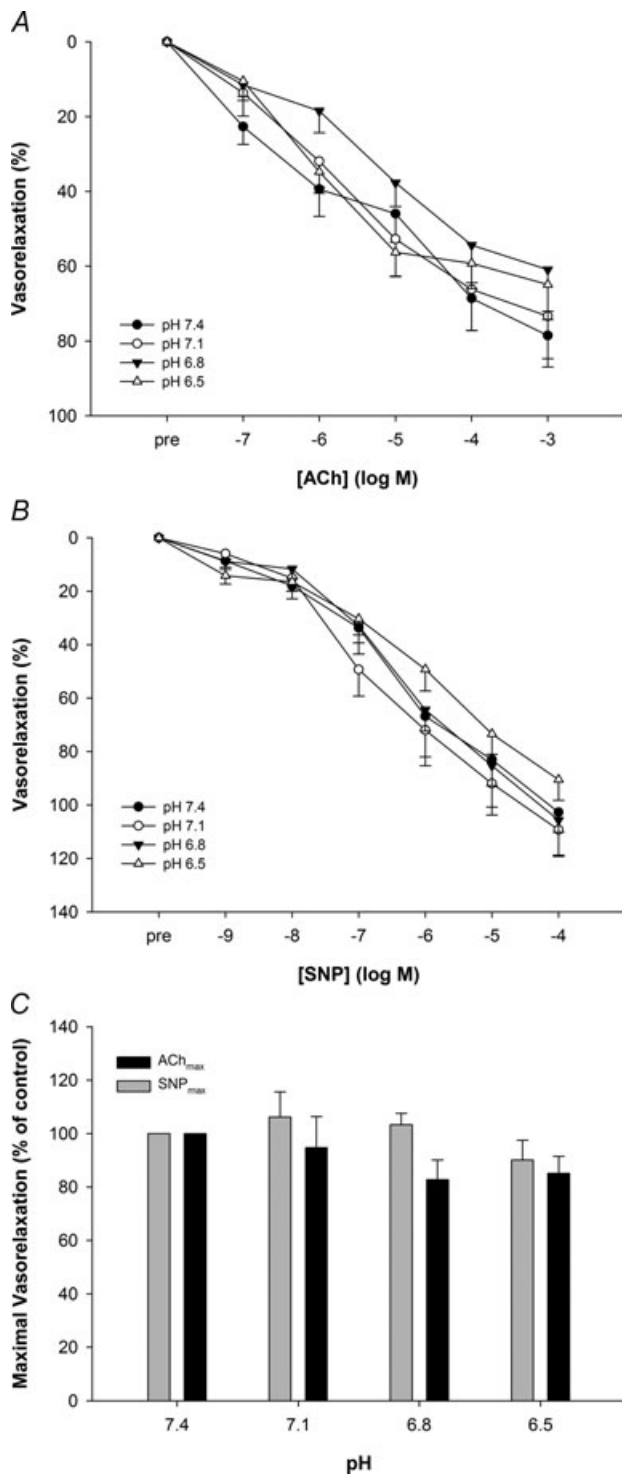
Supplemental study

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 (Fig. 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 (Fig. 4). When returned to normal pH, these vessel rings again exhibited similar responses to the other two rings (Fig. 4). Although 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

Figure 2. Vasocontractile function and acidosis
A, PE concentration responses across varying levels of acidosis. * Significant pH × 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 percentage of control condition (pH 7.4). #*P* < 0.05 *versus* pH 7.4 and §*P* < 0.05 *versus* pH 7.1. Data are presented as means + SEM.

Figure 3. Vasorelaxation function and acidosis
A, ACh concentration–response curves across varying levels of acidosis. *B*, SNP concentration–response curves across varying levels of acidosis. *C*, Maximal ACh and SNP responses expressed as a percentage of control condition (pH 7.4). Data are presented as means ± SEM.

on vasocontraction, there was no significant effect on endothelium-dependent or endothelium-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 that this phenomenon may also be enhanced by unaltered vasodilatory function. These findings therefore 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 an alteration in 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.* (2005) used single doses of the receptor-independent agonist KCl and the 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-induced vasocontraction and, to a much greater extent, PE-induced vasocontraction (Rohra *et al.* 2005). In agreement with Rohra *et al.* (2005), the present findings reveal that α_1-adrenergic receptor function is suppressed in a proportional fashion by reductions in pH (Fig. 2*A*), which persisted across a range of doses, and is, at least in part, mediated by reduced smooth muscle function (Fig. 2*B*). 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 (Fig. 2*C*).

The effect of pH may, or may not, be receptor selective, given that Kluess *et al.* (2005) documented, in the rat femoral artery, that purinergic ($P2X_1$) and

not α_1-adrenergic receptor-mediated vasocontraction was impaired during acidosis. Although the majority of studies indicate that 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.* 2003*b*), vascular location and calibre (Ishizaka *et al.* 1999; Lindauer *et al.* 2003; Hyvelin *et al.* 2004; Heintz *et al.* 2005, 2008) and experimental model (Rohra *et al.* 2003*a*, 2005; Celotto *et al.* 2011). For example, in an *in vitro* rodent study, Rohra *et al.* (2003*b*) determined that rat strain alone created a divergent response, resulting in either a suppressed or an enhanced α_1-mediated vasoconstriction in the face of acidosis. Vessel location appears to be another important determinant of the effect that acidosis may have upon vascular tension development, because 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 is also likely to explain the negative findings of 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, given that the sympatholysis observed by 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, 1991; Anderson & Faber, 1991). While the classic dogma was logical and
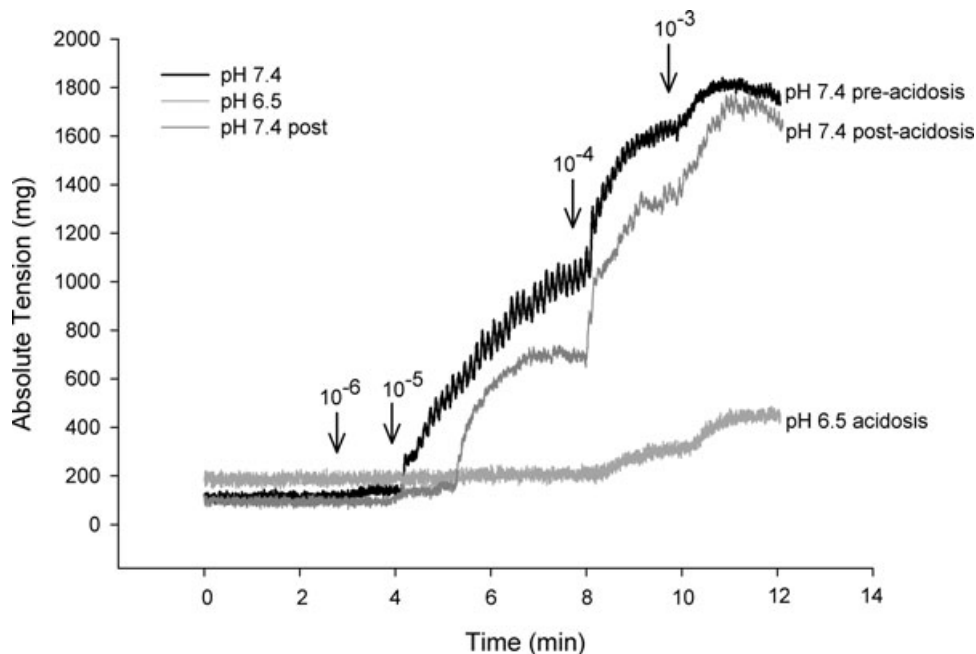
Figure 4. Evidence of the reversibility of the effects of reduced pH
Sample traces from an individual myograph chamber during determination of the phenylephrine concentration–response curve prior to acidosis, during acidosis and after exposure to acidosis, demonstrating the acute effect of reducing pH on α_1-mediated vasocontraction. Note that these data were obtained in a repeated-measures design, in contrast to the data presented in Figs 2 and 3, which were obtained by using each bath for a given pH condition (Fig. 1).

certainly an attractive hypothesis, the work of Moore *et al.* (2010) in essence challenges the applicability of prior findings from the cremaster model, because they found that 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 (2005), much like countercurrent heat exchange, venous drainage of skeletal muscle containing metabolites is likely to be 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 leach from the vein and act on the feed artery itself, yielding a form of blood flow autoregulation. 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 present 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 function. In terms of basic mechanisms, it is tenable to speculate that either or both the transient receptor potential cation channel subfamily V (TRPV) or the 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 *versus* smooth muscle) remain 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 the pH significantly attenuates α_1-mediated vasocontraction.

pH and vasorelaxation

The vasodilatory effects of acidosis have been well described in animals 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, 2008; Celotto *et al.* 2011) and *in vivo* (Deal & Green, 1954; Daugherty *et al.* 1967a,b; Kontos *et al.* 1971), but it is not yet known whether these results translate into humans. Early work in humans indicated that hypercapnia or the infusion of hypercapnic saline elicited a profound

hyperaemia (Kontos *et al.* 1967, 1968a,b). 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 vasodilatation 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 present 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 (Fig. 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 a single study that has investigated the effect of acidosis on agonist-induced vasorelaxation in an either animals or humans; therefore, this study appears to be the first to demonstrate that arterial vasorelaxation, both endothelium 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 in fact be the alteration in the balance between sympathetic vasoconstriction and vasodilatation induced by metabolic byproducts 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, using 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 with 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 vasodilatation. Irrespective of the origin of the acidosis, whether it be 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 hyperaemia during exercise.

Experimental considerations

The present study used 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 was upon functional sympatholysis, the use of HCl instead of lactic acid, which is a 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 CO_2 production (Robergs *et al.* 2004), which is a significant contributor to both local and systemic proton load during exercise ($CO_2 + H_2O \rightarrow HCO_3^- + H^+$). As suggested previously (Hong *et al.* 1997), it is likely that the proton, not lactate *per se*, contributes to physiological responses to acidosis; therefore, the resultant acidosis elicited by HCl would probably yield similar results to lactic acid, because both compounds dissociate to create a proton and a negatively charged molecule (Cl^- in the case of HCl lactate$^-$ in the case of lactic acid). It should be noted, however, that other prior work suggests that the experimental origin of acidosis (HCl *versus* lactic acid) may elicit cardiovascular responses that are specific to the acid used (Shirer *et al.* 1988). Further studies would be necessary to determine whether lactic acid itself has different 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 vasocontraction, ultimately reducing vascular tone. These findings support the concept that local acidosis could be a contributing factor in functional sympatholysis and exercise hyperaemia, both by opposing sympathetically mediated vasoconstriction and by not impacting vasodilatation.

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