Human skeletal muscle feed arteries studied *in vitro*: The effect of temperature on α1-adrenergic responsiveness

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Abstract

Heat and cold exposure can decrease and increase total peripheral resistance, respectively, in humans. With unique access to human skeletal muscle feed arteries, we sought to both characterize these vessels and determine the interaction between temperature and α1-adrenergic receptor responsiveness. We hypothesized that α1-mediated vasocontraction of human feed arteries would be attenuated in response to 39 or 35°C. Skeletal muscle feed arteries were harvested from thirty-two human volunteers and studied using isometric technique. Vessel function was assessed using potassium chloride (KCl), sodium nitroprusside (SNP), phenylephrine (PE), and acetylcholine (ACh) dose response curves (DRCs) to characterize non-receptor and receptor mediated vasocontraction and vasorelaxation. Single doses of PE (1mM) and KCl (100mM) were administered at 37°C and then, in a balanced design, repeated at both 35° and 39°C. The KCl and PE DRCs elicited significant vasocontraction (2009±407; 1974±508 developed tension (mg), respectively), whereas SNP and ACh induced the expected vasorelaxation (102±6; 73±10 %relaxation, respectively). Altering temperature had no effect on inherent smooth muscle function (KCl response), but both a reduction (35°C) and an increase (39°C) in temperature decreased the vasocontractile response to 1mM PE (37°C: 1478 ± 338, 35°C: 546 ± 104, and 39°C: 896 ± 202 mg; $p<0.05$), or across PE dose ($p<0.05$; 35 and 39°C vs. 37°C). Despite clear heterogeneity between both the human volunteers and the feed arteries themselves, this novel approach to the procurement of human vessels revealed a robust "inverted U" response to altered temperature, such that α1-mediated vasocontraction was attenuated with either warming or cooling.

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INTRODUCTION

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 (Williams & Segal, 1993; Segal, 2000). Human skeletal muscle feed arteries, while relatively large compared to the equivalent vessels in rodents are by human standards very small (1–2% of aortic diameter), have a similar anatomical location. 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*. However, not only are the vasocontraction and vasorelaxation characteristics of human feed arteries not well characterized, the impact of temperature upon vasoconstrictor agents important in the regulation of muscle blood flow in response to stress, such as norepinephrine, is unknown.

The specific effect of temperature on vasoconstrictor responsiveness has been extensively studied *in vitro* in vessels from animals. However, potentially, at least in part, due to methodological differences the results have been equivocal, with alterations in temperature leading to an increase (Padilla *et al.*, 1998; Massett *et al.*, 1999), a decrease (Vanhoutte & Lorenz, 1970; Cooke *et al.*, 1984; Kregel & Gisolfi, 1990; Ryan & Gisolfi, 1995; Massett *et al.*, 1998b; Mustafa *et al.*, 2004), or no change (Massett *et al.*, 1998a; Kluess *et al.*, 2005; Siddegowda *et al.*, 2007) in α-adrenergic mediated vasoconstriction. While there are no human *in vitro* studies with which to compare, prior *in vivo* animal and human studies on this topic reveal a general reduction in α-adrenergic vascular reactivity during local heating (Wilson *et al.*, 2002) or systemic increases in core temperature (Kregel *et al.*, 1997; Massett *et al.*, 2000; Wilson *et al.*, 2002; Brothers *et al.*, 2009). However, recent work performed by Keller et al. (Keller *et al.*, 2010) suggested that adrenergic responsiveness is maintained during heat stress localized to the leg. Such *in vivo* measurements represent the summation of the net vascular response and thus with such an approach it is currently not possible to differentiate the contribution of various levels of the arterial tree. An *in vitro* assessment of temperature and adrenergic responsiveness in human vessels recognized to regulate blood flow could provide greater clarity to this issue.

Thus, with the novel approach of harvesting human skeletal muscle feed arteries and an interest in α_1-receptor responsiveness in humans, utilizing an isolated *in vitro* model, we sought to a) assess the vasocontraction and vasorelaxation characteristics of these human feed arteries and b) determine if changes in temperature exert an effect on the α_1-adrenergic receptor responsiveness of these human feed arteries. Specifically, with implications for blood flow regulation in the face of both heating and cooling, we hypothesized that both an increase or a decrease in temperature from 37°C would attenuate the vasocontractile response to the α_1-agonist phenylephrine.

METHODS

Subjects and General Procedures

A heterogeneous group of 32 subjects (20 males and 12 females, 37–93 yrs old) agreed to have their vessels used in this study (Table 1). Although medical conditions and medications were noted, by means of medical records, (Table 1), 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 by all subjects prior to vessel harvesting.

Vessel Harvest

Human skeletal muscle feed arteries from the axillary and inguinal regions were obtained during elective surgeries for melanoma 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, 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 isotonic saline and brought to the laboratory within 15 minutes of harvesting. Vessels were dissected under a stereo microscope at room temperature in normal physiological saline solution (NPSS) (125 NaCl, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 2.5 $CaCl_2$, 18 $NaHCO_3$, 0.026 Na_2EDTA, and 11.2 Glucose mM). All NPSS solutions and drugs were newly prepared on the morning of the experiment. 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 chambers (700 MO, DMT Systems, Aarhus, DK). Once mounted vessel chambers were also aerated with the same carbogen gas mixture, and chamber NPSS was exchanged at 10 minute intervals, except during cumulative drug dose responses. Vessel chambers were warmed to 37°C over a 30 minute equilibration period prior to the protocol.

Vessel Function Protocols

The experimental timeline is illustrated in Figure 1. 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.*, 2000). L_{max} was operationally defined as less than a 10% improvement in developed tension in response to 100mM KCl. The viability of all vessels was characterized using KCl (10–100mM), phenylephrine (PE; $10^{-9} – 10^{-3}$ M), and acetylcholine (ACh; $10^{-7} – 10^{-3}$ M) dose response curves to determine non-receptor and receptor mediated vasocontraction and vasorelaxation at 37°C. All vasorelaxation responses are expressed as percent relaxation (%)

from PE pre-contraction. Temperature changes were achieved by changing myograph chamber and solution temperature utilizing multiple water baths set to the corresponding temperatures. Temperatures of 35, 37, and 39°C were chosen as they can be achieved under physiologic conditions, either during cold exposure, normothermia, or during exercise (Saltin & Hermansen, 1966; Myrer *et al.*, 1998). All feed arteries started experimentation at 37°C to confirm normal vessel function (via KCl, PE, and ACh dose response curves) prior to further experimental procedures (Figure 1). Single doses of PE (10^{-3} M) and KCl (100mM) were administered at 37°C, and were then, in a balanced design, randomized to either 35, or 39°C. All vessel segments were exposed to all temperatures, though to minimize the potential for an ordering effect, the temperature perturbations away from 37°C were balanced with an equal number of vessels starting with either 35 or 39°C, and were then crossed over to the remaining temperature. Each experimental protocol was separated by 30 minutes. To determine endothelium independent vasorelaxation, a sodium nitroprusside (SNP; $10^{-9} – 10^{-4}$ M) dose response curve was performed in all vessels at 37°C, and was conducted last in every case because of its known effect of abolishing vessel reactivity *in vitro*.

In addition to these main vessel function studies, three supplemental studies were performed. In the first, complete dose response curves for PE were performed across each temperature in a longitudinal design, as in the main vessel function studies (Figure 1). In the second, complete dose response curves for PE were again performed at 35°, 37°, and 39°C, but this time in a cross sectional design, where each vessel only experienced a single temperature. The developed tension (current tension – baseline tension) at each PE dose, for each condition, was expressed as a percent of the maximal tension developed at 37°C. Finally a third supplemental study examined stability of the preparation and the potential for tachyphylaxis with repeated contractions. Specifically, time control experiments were performed using repeated single doses of 1 mM PE and 100mM KCl, each separated by at least 30 minutes to replicate the timing employed in the main studies in which each experimental protocol was separated by 30 minutes. All the vasocontraction responses were expressed as percent of initial tension. For each of these supplemental studies subject and vessel characteristics were also assessed.

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. In the majority of cases (including supplemental studies) the data represents the average response of vessel rings in four chambers (average number of baths was 3.2 ±0.4 for each patient; CV across rings = 10–14%).

Statistical Analyses

Statistical analyses were performed using commercially available software (SPSS v. 17, Chicago, IL). A non-linear slope analysis and one-way repeated measures Analysis of Variance (ANOVA) were performed on the PE dose response data to determine the effect of temperature on PE-induced vasocontraction across doses. Two-way Repeated Measures ANOVA were utilized to determine if an interaction existed between order (2 levels; 35 or 39°C) and temperature (3 levels; 37, 39, 35°C) on baseline tension and in the

vasocontraction response to phenylephrine and KCl. One-way repeated measures ANOVA were used to determine if time control data differed over time and if vasocontraction was different between 35 and 39°C when expressed as a percent change from 37°C. Significant differences were further analyzed using Tukeys' Least Significant Difference *post hoc* test to make pair wise comparisons. Intraclass correlation coefficient analyses were performed on the time control experiments to determine the stability of the preparation for both PE and KCl mediated vasocontraction. The level of significance was set to $p < 0.05$. All data are reported as mean +/− standard error (SE).

RESULTS

Subject Characteristics

The subject characteristics, attained by medical records for all studies (main and supplemental), are listed in Table 1. The subjects were varied in terms of demographics (e.g. age), and tended to be overweight and with evidence of systolic hypertension, although it should be noted that these blood pressures were obtained during pre-operative examination. Given the blood chemistry and complete blood count (CBC) results (Table 1), which were on average within normal range except for creatinine, which was slightly elevated, the subjects appeared otherwise to be relatively healthy.

Vessel Characteristics

Thirty two human arteries were successfully harvested (19 inguinal, 13 axial). No statistical differences in L_{max}, vasocontraction or vasorelaxation responses were observed in terms of anatomical location (axial vs. inguinal) or gender (male vs. female). Consequently, responses from all vessels were combined. The average unpressurized internal diameter for these feed arteries was 500 ± 200 μM. Baseline tensions prior to KCl and PE contractile function curves were 105 ± 29 mg and 106 ± 33 mg, respectively. Vessel function protocols revealed robust vasocontraction (peak KCl 2009 ± 407 mg and peak PE 1974 ± 508 mg) (Figure 2, panel A and B). Vessels were pre-contracted to 1460 ± 330 mg of tension prior to Ach and SNP dose response curves, corresponding to approximately 70% of the maximal PE response, and from this point feed artery segments achieved significant vasorelaxation (max SNP 102 ± 6 and max ACh 73 ± 10 % relaxation) (Figure 2, panel C and D). Taken together, these results indicate the feed arteries had functional smooth muscle, α_1-adrenergic receptors, and an intact endothelium. Interestingly, there was no relationship between α_1-adrenergic responsiveness, KCL-induced contraction, or the ratio of these two variables and age.

Temperature and α_1-mediated vasocontraction

In the main protocol, baseline tensions were lower with a temperature of 35°C (111 ± 42 mg) or 39°C (85 ± 39 mg) as compared to 37°C (158 ± 42 mg) ($p < 0.05$). There was a decreased vasocontractile response to a single dose of PE as a consequence of altering temperature from 37°C (1478 ± 338 mg), to either 35°C (546 ± 104 mg) or 39°C (896 ± 202 mg) ($p < 0.05$ vs. 37°C) (Figure 3, panel B). Cooling tended to attenuate vasocontraction more so than heating, however this trend did not achieve statistical difference (PE-induced vasocontraction at 35 vs. 39°C; $p = 0.07$). However, when expressed as percent change from

37°C, the vasocontraction at 35°C was significantly more attenuated than that at 39°C ($p <$ 0.05; 53.5 ± 7.1 vs. 20.6 ± 12.3 %, respectively). The supplemental studies also indicated that altering temperature prior to a phenylephrine dose response curve, significantly reduced α_1-mediated vasocontraction at both 35 and 39°C (Figure 4; $p < 0.05$ 35 and 39°C vs. 37°C) utilizing either a longitudinal (panel A) or cross sectional (panel B) study design. Additionally, standard curve analysis revealed a reduced Hillslope at 35 and 39°C, as compared to 37°C, indicating a reduced α_1-receptor sensitivity with altered temperature.

Smooth Muscle Function

In the main protocol baseline tensions prior to KCl were lower with a temperature of 35°C (127 ± 46mg) or 39°C (107 ± 40mg), as compared to 37°C (207± 35mg) ($p < 0.05$). The effects of temperature were not due to altered inherent smooth muscle function, as measured by KCl mediated vasocontraction, evidenced by a lack of effect ($p = 0.96$) of changing temperature from 37°C (Figure 3, panel A). Additionally, the temperature effects did not appear to be the result of the passage of time, as the time control experiments revealed significant intraclass correlation coefficients for repeated single doses of KCl (0.964, $p <$ 0.001) and PE (0.873, $p < 0.01$) over time (Figure 5), and responses were not different across time ($p > 0.10$). These time intervals were similar to those employed in the protocols that did reveal differences in PE-induced tension at different temperatures. Furthermore, all vessels achieved complete relaxation in response to SNP at the end of the experiment providing additional evidence of intact inherent smooth muscle function after exposure to different temperatures. Taken together these data provide evidence in support of the stability of the preparation, and indicate that the effect of temperature on α_1-mediated vasoconstriction is not the result of altered inherent smooth muscle function.

DISCUSSION

The successful harvesting of human skeletal muscle feed arteries, their characterization, and subsequent use in an *in vitro* isolated vessel study documents the usefulness of this approach to better understand human vascular control. Beyond this novel approach, the main finding of this study was that a change in temperature from the homeostatic set point (37°C), reduced α_1-mediated vasocontraction in human skeletal muscle feed arteries. Of importance, this observation was determined to not be a consequence of altered inherent smooth muscle function. Thus, in combination, these data reveal an "inverted U" response, such that α_1-receptor responsiveness in human skeletal muscle feed arteries is temperature dependent. The potential implications of this finding are discussed.

Hyperthermia and inherent smooth muscle function

Elevated temperature may alter inherent smooth muscle function, as some *in vitro* findings have also revealed an altered depolarization-induced contraction (Padilla *et al.*, 1998; Massett *et al.*, 1999), although not all agree (Vanhoutte & Shepherd, 1970). This clearly has the potential to confound findings that suggest heating results in altered receptor-mediated vasoconstriction. Although, the current study did reveal an altered baseline tension, indicating an altered basal smooth muscle tone, this change was diminutive, particularly in light of such large reductions in α_1-mediated vasocontraction, and stable KCl-mediated

vasocontraction across temperature. In addition, the seminal works of Vanhoutte and Shepard (Vanhoutte & Shepherd, 1970) concluded that temperature does not exert its effect through reduced inherent smooth muscle contractility, which is in accordance with the results of the current study (Figure 3, panel A).

Effects of hyperthermia on vasoconstrictor agents

Hyperthermia has previously been demonstrated to attenuate adrenergic mediated vasoconstriction *in vivo* in humans (Wilson *et al.*, 2002; Brothers *et al.*, 2009) as well as in animal studies utilizing *in vivo* or *in vitro* approaches (Roberts *et al.*, 1989; Kregel *et al.*, 1997; Massett *et al.*, 1998a; Massett *et al.*, 1998b; Massett *et al.*, 1999; Massett *et al.*, 2000). However, not all agree, with recent evidence from Keller and colleagues (Keller *et al.*, 2010) indicating that adrenergic responsiveness may be preserved during isolated heat stress in humans *in vivo*. Indeed, Keller et al. (2010) concluded that passive leg heating increases muscle blood flow and that, despite an apparent intact adrenergic sensitivity, whole limb vascular conductance remained significantly elevated with local hyperthermia in the face of clear sympathomimeticly-induced vasoconstriction in the femoral artery. In contrast, *in vitro*, utilizing the isolated rat femoral artery, Kluess et al. (2005) found that heating had no effect on PE-induced vasocontraction, but reduced purinergic-mediated vasocontraction (Kluess *et al.*, 2005). As already indicated, Keller et al. (Keller *et al.*, 2010) found no such effect, indicating, not surprisingly, that the femoral is not involved in the generation of resistance *in vivo*. In this context, the negative findings of Kluess and colleagues, again studying the femoral, but this time in rats, are also not surprising (Kluess *et al.*, 2005). In fact, the present study appears to be the first to indicate that local heating can reduce α_1-adrenergic responsiveness in human feed arteries, an important site of blood flow regulation (Williams & Segal, 1993; Segal, 2000). As the skin, although likely effected by differing mechanisms, has been extensively studied it is interesting to note that the current data from feed arteries respond in a similar fashion to cutaneous vessels with respect to heating (Wilson *et al.*, 2002).

While not directly assessed in the current study, the effect of elevated temperature on agonist-receptor interaction may, in fact, be non-selective (Kregel & Gisolfi, 1990; Massett *et al.*, 1998b), although not all agree (Kluess *et al.*, 2005). Indeed, Massett and colleagues (Massett *et al.*, 1998b) found, *in vitro*, in rat vessels that increasing temperature not only reduced norepinephrine mediated vasocontraction, but also the vasocontractile response to endothelin-1, and angiotensin II. Therefore, it is likely that heating results in an attenuation of receptor mediated vasoconstriction that may be indiscriminant of receptor type. It appears that attenuated temperature-dependent receptor-mediated vasoconstriction, as demonstrated here in human feed arteries with phenylephrine (Figures 3 and 4), may serve as an autoregulatory mechanism that increases perfusion to metabolically active skeletal muscle.

Temperature, α_1-mediated responsiveness, and functional sympatholysis

The precise factor or combination of factors that contribute to functional sympatholysis has yet to be identified in humans. Indeed, during exercise many variables likely contribute to functional sympatholysis (e.g. K+, nitric oxide, ATP, H$^+$, etc.), however a local elevation in temperature is also a potential candidate (Buckwalter & Clifford, 2001; Kluess *et al.*, 2005)

which has not been thoroughly examined, especially not in human feed arteries. The increase in skeletal muscle metabolism, associated with exercise, releases copious amounts of heat as a by-product. Indeed, as much as 65% of the energy conversion in the metabolic pathways may be lost as heat (Krustrup *et al.*, 2003), elevating intramuscular temperature to ~39°C during moderate intensity exercise (Saltin & Hermansen, 1966; Magnusson *et al.*, 2000). Mechanistically, increased temperature may contribute to functional sympatholysis through the direct or indirect attenuation of α_1-adrenergic vasoconstriction, decreasing vascular resistance and enhancing blood flow to the active skeletal muscle (Buckwalter & Clifford, 2001; Kluess *et al.*, 2005).

Effect of Hypothermia on Vascular Responsiveness

Previous *in vitro* animal studies have revealed that cooling alters receptor mediated vasocontraction in both cutaneous veins and arteries in skeletal muscle (Flavahan *et al.*, 1985; Faber, 1988). Faber (1988), found that cooling appeared to exert a direct effect on basal vasomotor tone such that, upon cooling, vessels exhibited a transient vasorelaxation, revealing a suppressant effect of cooling on vascular tone. In terms of α-adrenergic stimulation, it appears that cooling selectively attenuates α_1-mediated vasocontraction, whereas α_2-induced vasocontraction remains intact or increased perhaps to compensate for reduced α_1 responsiveness (Flavahan *et al.*, 1985; Faber, 1988). More recently, another elegant series of experiments performed by Flavahan and colleagues (Chotani *et al.*, 2000; Chotani *et al.*, 2004; Bailey *et al.*, 2005; Chotani *et al.*, 2005; Eid *et al.*, 2007; Eid *et al.*, 2008) has provided convincing evidence, in cells and cutaneous arteries, that cold exposure induces translocation of previously quiescent α_2c-adrenergic receptors, in part mediated by reactive oxygen species, towards the cell surface which increases vasoconstriction to selective α_2c-adrenergic agonists. However, this effect was not apparent with α_1-adrenergic agonists (Chotani *et al.*, 2000), as the response to PE was unaffected by cold exposure which contrasts somewhat with the current finding of reduced α_1-adrenergic responsiveness with cooling. Although, *in vivo* cold exposure is well known to stimulate a reduction in cutaneous blood flow to maintain core temperature, very little is known regarding the specific effect of a fall in temperature on skeletal muscle blood flow.

Teleologically, and in a not too dissimilar scenario to voluntary exercise and heating, it is possible that cold mediated sympatholysis plays a role in supporting the metabolic demand associated with shivering thermogenesis during cold stress (Colquhoun *et al.*, 1990). Indeed, cold exposure in humans has been documented to increase cutaneous vascular resistance which resulted in a stroke volume-induced increase in cardiac output and prevented any decrement in MAP, despite a significantly reduced total peripheral resistance (Raven *et al.*, 1970). While blood flow to the thermogenically active skeletal muscle was not measured in this study, the current data suggest that during cold exposure, blood flow to deep skeletal muscle may increase to support shivering thermogenesis, ultimately reducing resistance in the skeletal muscle vasculature. However, this observation may only be evident in arteries feeding deep skeletal muscle, such as those feed arteries used in the current study, is likely only to be of significance under prolonged and severe cooling conditions that cannot be overcome by shivering thermogenesis, but these hypotheses need to be confirmed *in vivo*. Potential explanations for the observed reduction in α_1-adrenergic responsiveness in the

current study (Figures 3 and 4) include reduced membrane permeability, decreased diffusional capacity, or a conformational change in the adrenergic receptor as a consequence of cooling (Fick, 1855; Krogh, 1919). Reduced adrenergic responsiveness could be a mechanism by which cold induced vasodilation occurs (Flouris & Cheung, 2009) and may augment pathological hypotension during hypothermia (Vanamoorthy *et al.*, 2010).

Origin of the Vessel

The subjects who took part in this study were certainly heterogeneous in terms of age, gender, and health, but, although exhibiting a tendency to be overweight and some evidence of systolic hypertension (although obtained during pre-operative examination), they were taking minimal medications and had normal blood chemistry and CBC data (Table 1). However, it should also be recognized these subjects were undergoing prophylactic surgical treatment for melanoma and vessels were harvested during sentinel node dissection, although most lymph nodes were found to be negative for melanoma metastasis via Polymerase Chain Reaction analysis. In addition, it is of note the lactate dehydrogenase values (considered to be a crude indicator of metastasis), were within the normal range for all subjects and subjects were not undergoing any chemotherapy.

Despite rather varied origin, the novel approach of harvesting these human arteries during such surgeries yielded the expected receptor mediated and non-receptor mediated vasocontraction and vasorelaxation characteristics (Figure 2), suggestive of normal physiology. With our historical interest in limb specific vascular differences (Wray *et al.*, 2010) and each harvest site (axial and inguinal) being associated with either the upper or lower extremities, it was anticipated that vessel characteristics may vary by limb proximity. However, although the magnitude of within site structural heterogeneity was of interest, this variation was too great to reveal any limb specific information. Therefore, despite a group of heterogeneous subjects, varied vessel harvest location (i.e. axial and inguinal) and potential pathology, the notion that temperature exhibited a common effect speaks to the robust nature of this response as it relates to the regulation of sympathetically mediated vasoconstriction. Specifically, independent of age, gender, or disease status, altering temperature from the homeostatic set-point significantly attenuates α_1-mediated vasocontraction.

Limitations

While the novel approach of harvesting human skeletal muscle feed arteries affords a unique experimental paradigm, it was not achieved without limitation. The human feed arteries used in the current study were inarguably heterogeneous (i.e. harvest location, vessel size, age, gender, medications, health, etc.) and this could be considered a limitation of this study. However, a repeated measures design was employed specifically to reduce the impact of inter-individual variation. Additionally, although relatively small, some tachyphylaxis was observed in the time control studies (Figure 5), and thus may have contributed to the reduction in PE-induced tension development observed during measurements at subsequent temperatures. However, in supplemental studies, when PE dose responses at the different temperatures were performed in a cross sectional design, to avoid any time/tachyphylaxis effects, essentially the same temperature-dependent results were observed (Figure 4, panel B). Additionally, it is also acknowledged that in these supplemental and time control studies

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tension development was, on average, lower than the main protocol. This was attributed to differences in vessel caliber and arterial ring length and an unavoidable consequence of limited human feed artery availability. However, despite these differences, it is important to note that the functional characteristics of the vessels included in these supplemental studies were not different from those utilized in the main protocol. Finally, a reduction in baseline tension over time was also observed, which may have altered the contractile response to PE. However, it should be noted that KCl-induced tension remained stable across temperature, and the magnitude of the baseline shift (~70 mg tension) is highly unlikely to account for the dramatic reduction in tension (~500 mg tension) observed during the temperature perturbations.

Conclusion

Altering temperature, by either cooling or heating, reduces the contractile response of human skeletal muscle feed arteries to the α_1-adrenergic receptor agonist phenylephrine *in vitro*. Importantly, these findings were independent of alterations in inherent smooth muscle function, as measured by KCl-mediated vasocontraction and have implications for human blood flow regulation in both hyperthermic and hypothermic conditions.

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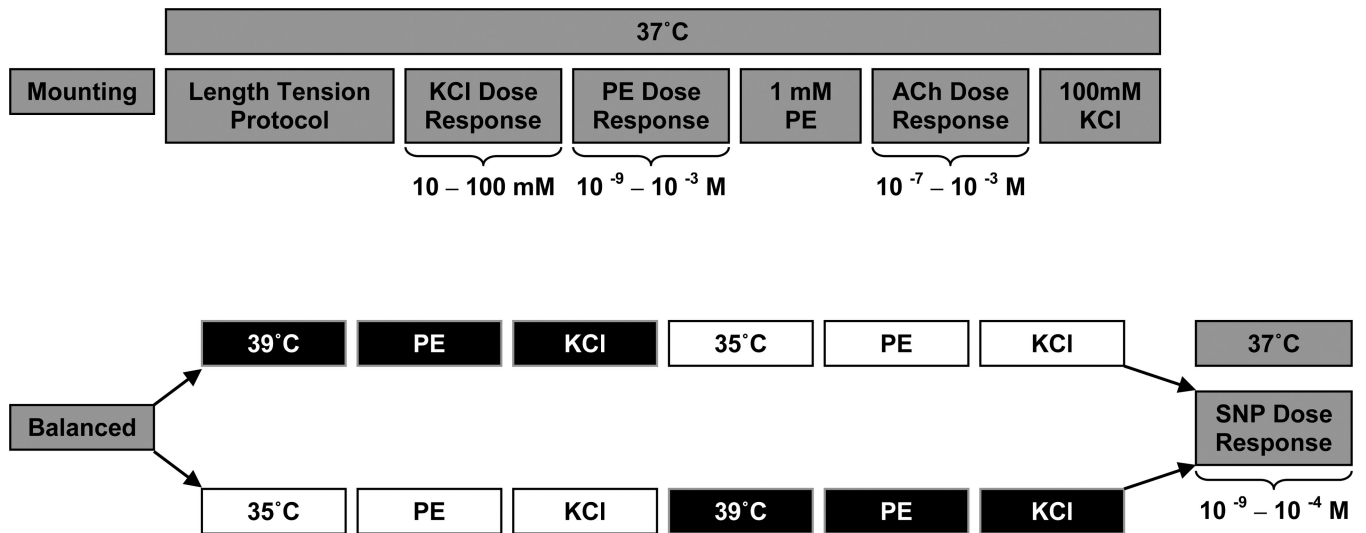
Figure 1. Experimental Timeline

Potassium Chloride (KCl), Phenylephrine (PE), Acetylcholine (ACh) & Sodium Nitroprusside (SNP) concentrations in millimolar (mM) or log molar (10^{-x} M).
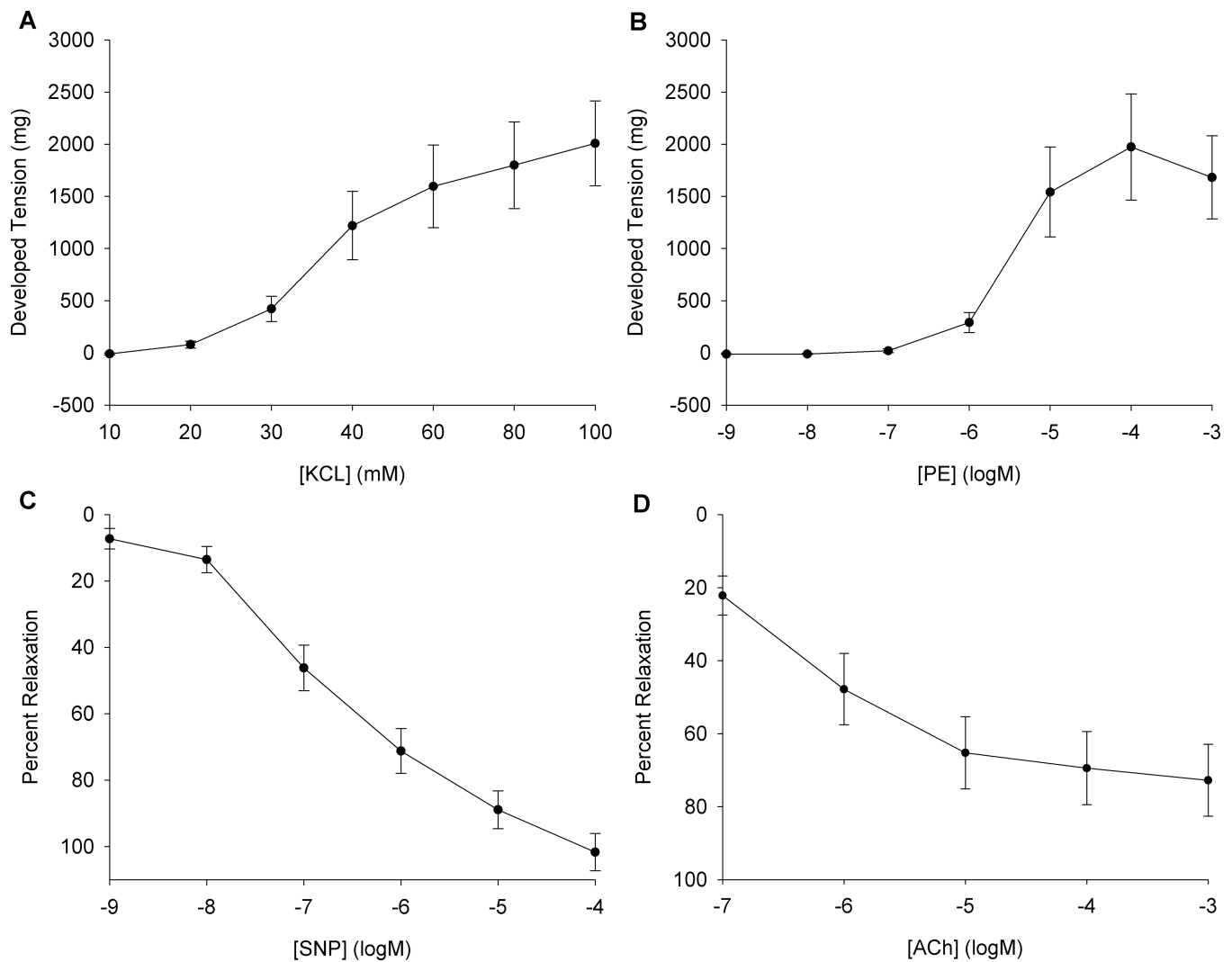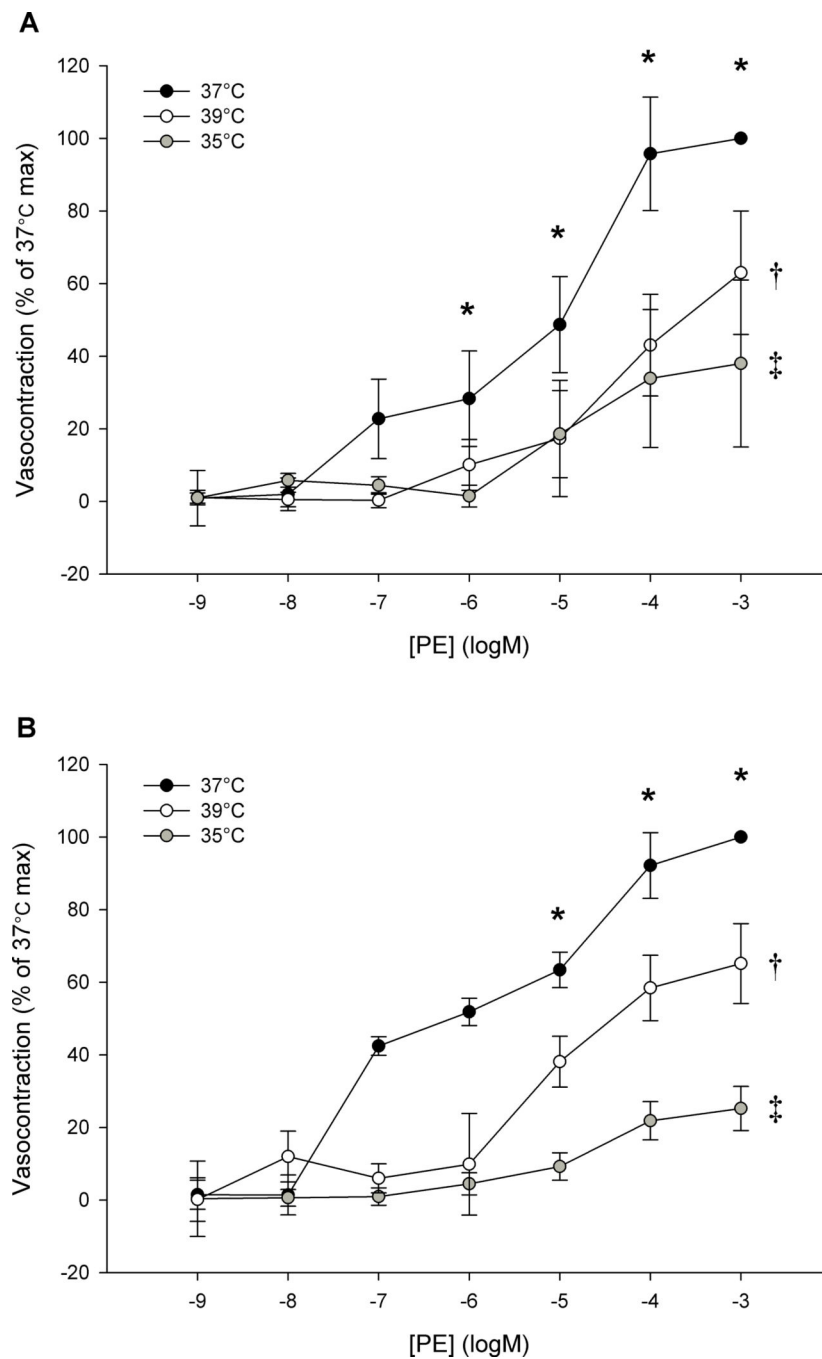
Figure 2. Human skeletal muscle feed artery functional characteristics assessed at normothermia (37°C)

A) Non-receptor mediated vasocontraction dose response for KCl. B) Phenylephrine (PE) dose response for α_1-adrenergic mediated vasocontraction. C) Sodium Nitroprusside (SNP) dose response (% relaxation from PE pre-contraction). D) Acetylcholine (ACh) dose response (% relaxation from PE pre-contraction). Data are presented as mean +/−SE (n=32).

A

B
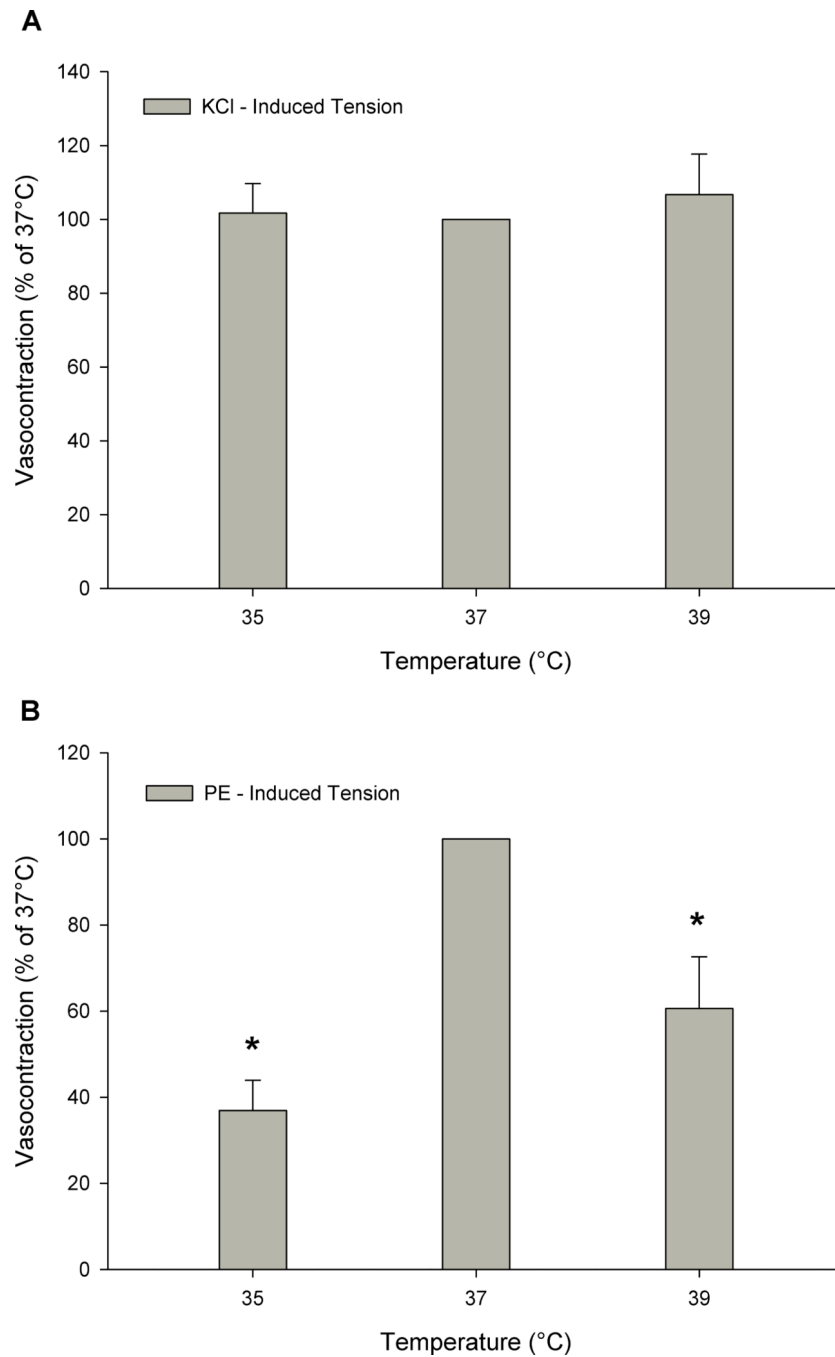
Figure 3. The effect of temperature (35°, 37°, and 39° C) on the contractile response to (A) KCl and (B) a single dose of phenylephrine (PE) (1 mM) in human skeletal muscle feed arteries
* $p < .05$ vs. 37°C. Data are presented as mean +/−SE (n = 14).

Figure 4. Phenylephrine Dose Response Curves at 35°, 37°, and 39°C in human skeletal muscle feed arteries assessed in both a longitudinal (A) and cross sectional design (B)

(Temperature effect: †$p < 0.05$, 39 vs. 37°C; ‡$p < 0.05$, 35 vs. 37°C; * dose effect: $p < 0.05$ vs. baseline). <u>Note</u>: These data represent an additional 6 feed arteries not represented in figures 3, or 5. Data are presented as mean +/−SE (n = 6 per panel).
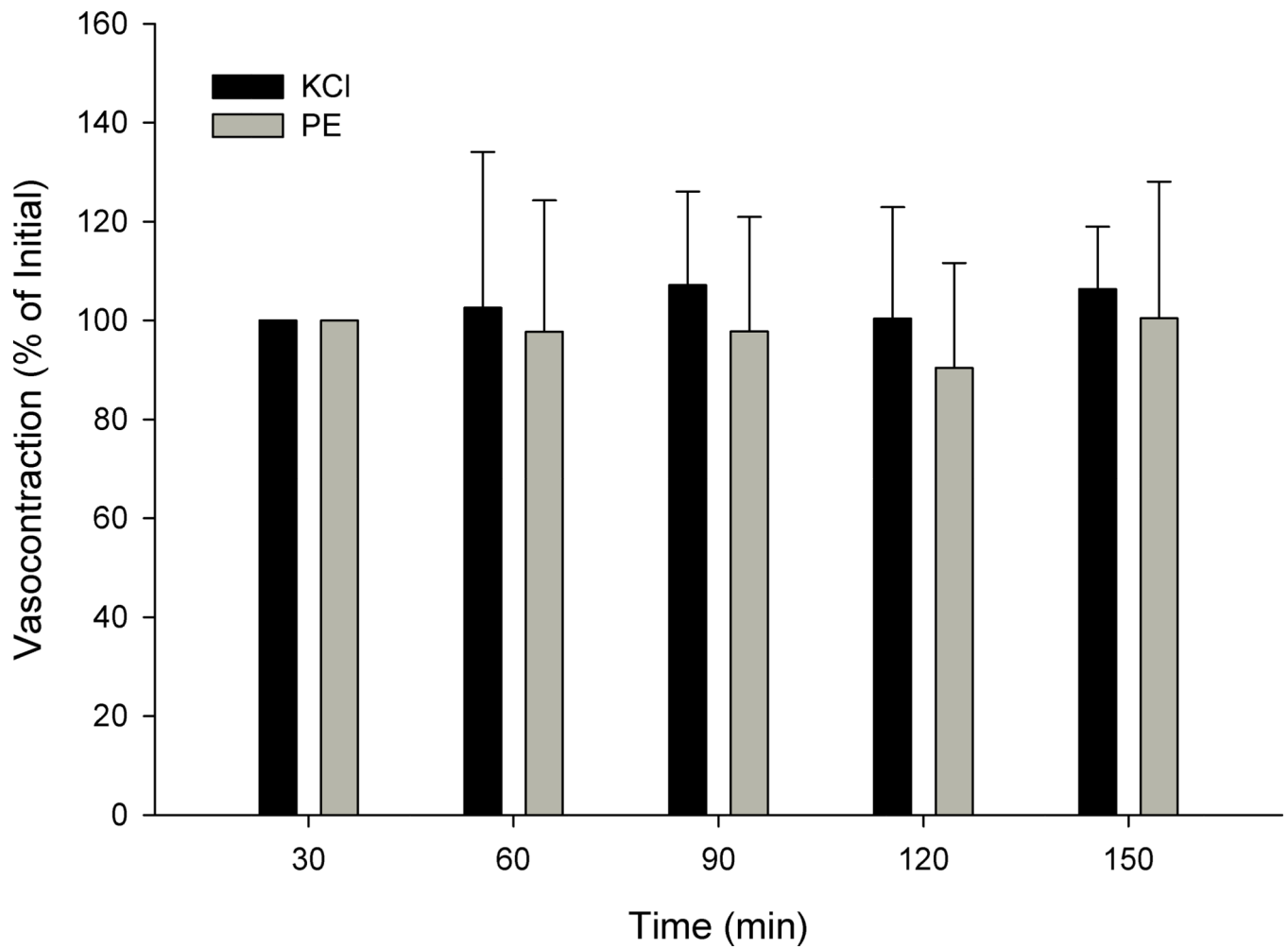
Figure 5. Time control experiments for KCl and PE mediated vasocontraction expressed as developed tension (mg) at 37°C in human skeletal muscle feed arteries

Note: These data represent an additional 6 feed arteries not represented in figures 3, or 4.

Data are presented as mean +/−SE (n = 6).

Table 1

Characteristics of the subject population (n = 32).

	Mean ± SE	Normal Range
Age (yr)	59.00 ± 4.2	--
Height (cm)	174.00 ± 3.5	--
Weight (kg)	89.50 ± 6.1	--
Systolic Blood Pressure	135.60 ± 6.4 [†]	--
Diastolic Blood Pressure	75.10 ± 2.4 [†]	--
Glucose (mg/dl)	109.10 ± 9.3	65 – 110
Blood Urea Nitrogen (mg/dl)	16.39 ± 1.8	6 – 21
Creatinine (mg/dl)	1.03 ± 0.1	0.52 – 0.99 [*]
Albumin (g/dl)	4.17 ± 0.3	3.3 – 4.8
Bilirubin (mg/dl)	0.37 ± 0.2	0.2 – 1.3
Lactate Dehydrogenase (U/L)	494.45 ± 36.3	313 – 618
Hemoglobin (g/dl)	14.40 ± 0.7	12 – 16
WBC (K/uL)	8.12 ± 0.9	3.6 – 10.6
RBC (M/uL)	4.61 ± 0.2	4 – 5.2
Platelets (K/uL)	238.22 ± 20.1	150 – 400
Hematocrit (%)	41.90 ± 1.9	36 – 46
Lymphocyte (%)	30.30 ± 3.9	24 – 44
Monocyte (%)	7.97 ± 1.2	0 – 12
Neutrophil (%)	59.75 ± 3.0	36 – 66
Eosinophil (%)	2.07 ± 0.4	0 – 5
Basophil (%)	0.56 ± 0.1	0 – 5
Medications (users/n)		
Cardiovascular		
Diuretic	2/32	
Ca^{++} Channel Blocker	2/32	
Statin	1/32	
ACE inhibitor	2/32	
Beta Blocker	1/32	
Thyroid		
Levothyroxine	1/32	
Other		
Insulin	1/32	
Antibiotic	1/32	

[*] Average outside of normal range

[†] Data obtained during pre-operative examination