ENOS AND NNOS CONTRIBUTION TO REFLEX CUTANEOUS VASODILATION DURING DYNAMIC EXERCISE IN HUMANS

by

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Abstract

Recent data suggests nNOS mediates the NO-component of reflex cutaneous vasodilation with passive heat stress. Our hypothesis was nNOS, but not eNOS, inhibition would attenuate reflex cutaneous vasodilation during dynamic exercise. Protocol 1: subjects performed a VO_2 peak test on a supine cycle ergometer. Protocol 2: with experimental arm at heart level subjects cycled in supine posture at 60% VO_2 peak to raise core temperature (Tc) 0.8-1.0°C (35-45 min). In protocol 2 subjects were equipped with 4 microdialysis fibers on the forearm and each randomly assigned as: 1) lactated Ringer's (control); 2) 5mM NPLA (nNOS inhibition); 3) 10mM L-NIO (eNOS inhibition); and 4) 20mM L-NAME (non- selective NOS inhibition). At the end of protocol 2 all sites were locally heated to 43°C and infused with SNP to elicit maximal dilation. Mean arterial pressure (MAP), skin blood flow via laser- Doppler flowmetry (LDF), and Tc via ingestible telemetric pill were measured; cutaneous vascular conductance (CVC) was calculated as LDF/MAP and normalized to maximum. In protocol 2 there was no significant difference between control (62±5 %CVCmax) and NPLA (61±6 %CVCmax). L-NIO (38±4 %CVCmax) and L-NAME (41±7 %CVCmax) significantly attenuated CVC compared to control and NPLA ($p<0.001$ all conditions). There was no difference between L-NIO and L-NAME. We conclude eNOS, not nNOS, contributes to reflex cutaneous vasodilation during dynamic exercise.

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Dedication

To my family and parents: You have supported and guided me to my every success.

Chapter 1 - Introduction

In humans, the primary autonomic response to an increase in core temperature is increased blood flow to the skin and sweating. The cutaneous vasodilation and sweat response to heat stress is mediated by two branches of the sympathetic nervous system: the adrenergic vasoconstrictor system and the cholinergic vasodilator system (23). The initial increase in skin blood flow in response to passive heating is achieved by withdrawal of tonic sympathetic vasoconstrictor tone (23), while further cutaneous vasodilation and instigation of sweating is achieved via activation of sympathetic cholinergic nerves (16).

The sympathetic cholinergic active vasodilator nerves are responsible for the majority of vasodilation (28) through co-transmission with acetylcholine of an unknown substance (Figure 1). The co-transmission theory suggests acetylcholine is responsible for the sweat response, while the unknown substance(s) are responsible for the vasodilation. The specific substance(s) and mechanism by which the vasodilation occurs and to what degree each contributes has been the subject of controversy and continuing research. Several vasodilator substances have been proposed in conjunction with the co-transmission theory including: substance P, vasoactive intestinal peptide (20) , H1 histamine receptor activation (55), and prostaglandins (36). In addition, nitric-oxide (NO) has been shown to directly contribute ~30-45% to cutaneous active vasodilation during passive heating (22, 25, 47). The source(s) of NO during passive heating are unclear, but recent data suggests neuronal NO synthase (nNOS) is the primary nitric-oxide synthase (NOS) isoform contributing to NO during passive heating (27, 29).

During dynamic exercise, a large proportion of muscle mass is engaged and the most abundant byproduct of the muscle contraction and increased metabolism is heat. As a result, core

temperature (Tc) increases. As with passive heating, the increase in core temperature during dynamic exercise results in an increase in skin blood flow and sweating; however, these responses are delayed compared to passive heating. This rightward shift in the core temperature threshold for cutaneous vasodilation results from the competition between working skeletal muscle and the skin for cardiac output (15, 30). In addition, the initial skin blood flow response to dynamic exercise is adrenergic sympathetic vasoconstriction. Although several studies have documented an increase in skin blood flow during exercise and have studied modifiers of the skin blood flow response (e.g. changes in skin temperature), there have been no studies investigating mechanisms of cutaneous vasodilation during dynamic exercise. The underlying assumption has been the mechanisms are the same during passive heat stress. The purpose of this study was to investigate the mechanisms directly involved with cutaneous vasodilation due to endogenous heat stress. Specifically, we investigated the contribution of endothelial NO synthase (eNOS) and nNOS to the cutaneous vasodilation during dynamic exercise. We hypothesized nNOS, not eNOS, would contribute to reflex cutaneous vasodilation during dynamic exercise.

Chapter 2 - Literature Review

The mechanisms through which humans vasodilate the cutaneous circulation for thermoregulation during passive whole body heating or dynamic exercise are complicated. In addition, there has been no research concentrating on the mechanisms of cutaneous vasodilation during dynamic exercise. The research surrounding cutaneous vasodilation has focused on the mechanisms responsible during passive heating. Several possible mechanisms have been identified for cutaneous vasodilation and include: Substance P (54), vasoactive intestinal peptide (20) , H1 histamine receptor activation (55), and prostaglandins (36). In addition, NO has been shown to directly contribute ~30-45% to cutaneous active vasodilation during passive heating (22, 25, 47). How and when these mechanisms are activated and the magnitude of the role they play in cutaneous vasodilation is still being studied but the research suggests there is no one substance or mechanism primarily responsible. Instead, the research suggests there is a redundant and complex system surrounding cutaneous vasodilation.

The first section of this review will focus on the mechanisms of vasodilation during passive heating and the role of the sympathetic nervous system. Next, the mechanisms believed to be responsible for the observed response during passive heating will be discussed. The second section of this review will focus on the vasodilation response observed during dynamic exercise. Inasmuch as there have been no mechanistic studies during dynamic exercise, this second section will be brief.

Skin Blood Flow Response to Passive Heating

Theories surrounding the vasodilation process began with observations of vasoconstrictor and vasodilator nerves by Bernard in 1852 (2) and in 1858 (3), as well as Brown-Sequard's work in 1852 (5). Research continues to study the human response to changes in temperature. Early experiments designed to study the effects of heat on blood flow were conducted by submerging a limb in warm water. These methods resulted in an increased blood flow in the limb not placed in warm water (13, 34), suggesting the increase in skin blood flow to limb heating is of reflex origin. When the same methods were used but sympathetic nerves were either blocked or cut, the blood flow response was abolished (1, 16, 17), providing evidence that sympathetic nerves were involved in the reflex increase in skin blood flow. These studies provided the first evidence for the role of the sympathetic nervous system in the response of increased limb blood flow during passive heating. Further studies revealed both the adrenergic vasoconstrictor system and the cholinergic systems have a role in the vasodilation (23). In addition, it was subsequently found that the initial increase in skin blood flow in response to passive heating was achieved by withdrawal of the tonic sympathetic vasoconstrictor tone (23) while further cutaneous vasodilation and the instigation of sweating was achieved via activation of the sympathetic cholinergic nerves (16).

Although evidence was provided for a cutaneous vasodilation mechanism mediated by the two branches of the sympathetic nervous system, the mechanisms involved in this process remained unknown. It remained unclear if acetylcholine was responsible for both the sweat response and increase in cutaneous vasodilation. To investigate the role of acetylcholine a

number of studies used atropine to block acetylcholine receptors. In these studies the sweat response was abolished while atropine delayed but did not affect the magnitude of the vasodilation (9, 11, 43). The co-transmission theory has been proposed to explain these results by suggesting acetylcholine is responsible for the sweat response while an unknown substance is responsible for the vasodilation (28). In order to establish the role of the cholinergic response to passive whole body heating Kellogg administered botulinum treatment to human skin (28). The treatment inhibited presynaptic release of cholinergic vesicles and transmitters and in turn cutaneous vasodilation and sweating responses were abolished. These observations, combined with previous observations showing muscarinic receptor blockade using atropine abolished only the sweat response (9, 11, 43, 44), led Kellogg and colleagues to conclude cutaneous vasodilation mechanisms were mediated by one or more unknown neurotransmitters (30). The specific substance and mechanism by which the vasodilation occurs and to what degree each may contribute has been the subject of controversy and continuing research.

Several mechanisms have been presented in conjunction with the co-transmission theory in regards to the redundant thermoregulatory vasodilation response. These potential mechanisms include substance P, vasoactive intestinal peptide (20) , H1 histamine receptor activation (55), prostaglandins (36), and NO (22, 25, 47) (Figure 1).

Several studies have shown NO directly contributes ~30-45% to cutaneous active vasodilation during passive whole body heating (22, 25, 47). During passive whole body heating in humans, if the cholinergic and adrenergic nerves are blocked, the vasodilation is only partially attenuated (31, 32, 42, 43, 50). The cause of the remaining vasodilation was hypothesized to be due to NO. This hypothesis was tested by Taylor and Bishop in the rabbit ear (51) where they showed the blockade of NO synthase eliminated the rise in blood flow during heating. This result

suggested NO may be responsible for the rise in blood flow seen in humans after the cholinergic and adrenergic systems had been blocked. However, a study by Dietz (8) demonstrated in humans, this is not the case. Dietz demonstrated the NO synthase blocker N5-[imino(methylamino)methyl]-L-ornithine citrate (L-NMMA) did not decrease the rise in forearm blood flow or skin blood flow in humans. In their study, the L-NMMA was administered via an intra-arterial infusion. Dietz did not see a decrease in blood flow after L-NMMA was administered, as seen in the rabbit ear. Therefore, Dietz concluded NO did not play a significant role in increasing cutaneous blood flow during body heating in humans.

Subsequently, Kellogg (24) and Shastry (48) independently presented data exhibiting the need for functional NO synthase in order to achieve full expression of active cutaneous vasodilation. Their studies offered evidence to refute observations of NO synthase blockade failing to reduce blood flow in humans where an active vasodilation is present (8) and independently confirmed the results of Taylor and Bishop's study in the rabbit ear (51).

Skin Blood Flow Response to Dynamic Exercise

During dynamic exercise, a large proportion of muscle mass is engaged and the most abundant byproduct of the muscle contraction and increased metabolism is heat. As a result, core temperature increases. As with passive heating, the increase in core temperature during dynamic exercise results in an increase in skin blood flow and sweating; however, these responses are delayed compared to passive heating. This rightward shift in the core temperature threshold for cutaneous vasodilation results from the competition between working skeletal muscle and the skin for cardiac output (15, 30). In addition, the initial skin blood flow response to dynamic exercise is vasoconstriction from the adrenergic sympathetic nervous system. Although several studies have documented an increase in skin blood flow during exercise (6, 7, 10, 38-41, 45, 49,

52) and have studied modifiers of the skin blood flow response (e.g. changes in skin temperature), there have been no studies investigating mechanisms of cutaneous vasodilation during dynamic exercise. The underlying assumption has been the mechanisms are the same as during passive heat stress. The lack of mechanistic studies investigating the increase in skin blood flow during dynamic exercise served as the basis for this thesis project. Specifically, the contribution of NO to the cutaneous vasodilation during dynamic exercise was investigated.

Chapter 3 - Methods

Ethical Approval

Protocols utilized were approved by the Institutional Review Board at Kansas State University. Subjects were volunteers from the Kansas State University student population. An informed consent was reviewed and signed by each subject prior to participation in the study.

Subjects

There were eight subjects, all men between the ages of 20 and 25 years. Prior to participation, all subjects filled out a health history form to ensure nonsmoking status, the absence of disease and a family history absent of cardiovascular disease. None of the subjects were taking any medications. All subjects were asked to refrain from caffeine, alcohol and strenuous exercise for 12 hours prior to each protocol. Each subject participated in two protocols: The first was a VO_{2peak} test and the second, a prolonged bout of cycling.

Subject Monitoring

Subjects were monitored with an electrocardiogram throughout the second protocol (S/5 Light Monitor; Datex-Ohmeda, GE Healthcare; Madison, WI, USA). Blood pressure was monitored beat-by-beat via photoplethysmography (NexfinHD; BMEYE, Amsterdam, The Netherlands), which was verified via automated brachial auscultation (S/5 Light Monitor; Datex-Ohmeda, GE Healthcare; Madison, WI, USA) every 5 minutes. Skin blood flow measurements were taken from the lateral aspect of the left forearm, while subjects were in the supine position with the experimental arm at heart level for the entire protocol. All experiments were performed in a thermoneutral laboratory.

Near the end of protocol two, local skin temperature was increased from 33°C to 43°C to induce maximal vasodilation utilizing local heating devices (PF5020 local heating units and PeriFlux 5020 Temperature Unit; Perimed; Jarfalla, Sweden). The local heating units used in the second protocol covered a 10mm area of the skin and allowed for temperature increases in 1°C increments.

During protocol two, core temperature was measured via telemetric ingestible pill (CorTemp Data Recorder, CorTemp Temperature Sensor, Wireless Sensing Systems and Design, Palmetto, FL, USA) every minute.

Subject Instrumentation

Microdialysis Technique

In protocol two, all subjects had four microdialysis fibers placed into the dermal layer of the skin of the left ventral forearm. Microdialysis fibers were used to administer drugs to local areas of skin in the forearm. Drug sites were chosen at random. The administration of drugs was accomplished via the semi-permeable membrane found in each fiber, as seen in Figure 2. The membrane allowed for the diffusion of drugs from the fiber into the local area. The membranes of the microdialysis fibers were 10 mm in length with a 55-kDa molecular mass cutoff (CMA 31 Linear Probe; CMA Microdialysis, Sweden).

Fibers were placed approximately 3-5 cm apart on the ventral surface of the left forearm in the absence of anesthetics; however, ice was used to numb the skin prior to placement (18). Fiber placement was accomplished by first threading a 23-gauge needle through the skin at each desired site of microdialysis placement. A microdialysis fiber was then threaded through the lumen of the needle, and the needle removed, leaving the membrane in place. To account for trauma, cutaneous blood flow was monitored via Laser-Doppler Flowmetry as described below

and allowed to return to resting values before the start of the protocol. During this time, all fibers were perfused with lactated Ringer's solution at a rate of 4 μl/min.

Laser-Doppler Flowmetry

Laser-Doppler Flowmetry (LDF) was used as an index of red blood cell (RBC) flux. Laser-Doppler Flowmetry measures the doppler shift of a laser as it is reflected off red blood cells moving through the skin. Laser-Doppler Flowmetry is a non-invasive method for obtaining a continuous index of RBC flow and has been shown to be a reliable method of determining skin blood flow without influence from blood flow in the underlying muscle (21, 46).

The vasodilator response during dynamic exercise and local heating was observed at the site of each of the four microdialysis fibers. Local heating units were placed on the skin centered on each semi-permeable membrane. An integrated laser-Doppler probe, (Probe 413; Perimed; Jarfalla, Sweden) each with seven emitting and receiving probes, was placed at each microdialysis site directly over each semi-permeable membrane in the center of each local heating unit to estimate RBC flow directly over each microdialysis membrane site. The placement and function of the microdialysis fiber and its membrane along with the LDF probe is shown in Figure 2.

Protocols

Protocol One: Determination of VO_{2Peak}

VO_{2peak} was measured with the subjects cycling in the supine position, on a custom built stationary cycle ergometer. After a five minute unloaded warm up at 60 rpm, the workload was

increased 25 watts each minute until the subjects reached exhaustion. Subjects were instructed to maintain 60 rpm and were assisted via audio tone from an electronic metronome. Exhaustion was determined when the subject could no longer maintain 60rpm despite verbal encouragement.

Protocol Two: Dynamic Exercise-Induced Heat Stress/ Prolonged Cycling

Heat stress was accomplished by having the subjects cycle in the supine position, on a stationary bicycle ergometer, at a workload sufficient to elicit and maintain 60% VO_{2peak}. Subjects were instructed to maintain 60rpm and were assisted via audio tone from an electronic metronome. Cycling was stopped when core temperature rose 0.8-1.0°C. It took subjects a range of 35-45 min to reach the desired rise in core temperature.

Drugs Administered

Nitric Oxide Synthase Inhibition

A 20 mM dose of the L-arginine analog *N^G-nitro-L-arginine* methyl ester (L-NAME; Tocris Bioscience; Minneapolis, MN, USA), dissolved in lactated Ringer's solution was used to inhibit NO synthase. This concentration has been shown to inhibit NO synthase in human skin (19, 56). A 5mM dose of N-Propyl-L-Arginine (NPLA; Tocris Bioscience; Minneapolis, MN, USA), dissolved in lactated Ringer's solution was used to inhibit neuronal nitric oxide synthase (nNOS). This concentration has been shown to inhibit nNOS in human skin (29). A 10mM dose of N5-(1-iminoethyl)-L-Ornithine dihydrochloride (L-NIO; Tocris Bioscience; Minneapolis, MN, USA), dissolved in lactated Ringer's solution was used to inhibit endothelial nitric oxide synthase (eNOS). Pilot work in our laboratory determined this concentration effectively inhibits the NO-dependent vasodilation to local heating of the skin. All drugs were administered simultaneously at the start of exercise via microdialysis at a rate of 4µl/min.

Nitric Oxide Donor

Maximal skin blood flow was elicited after cycling at all microdialysis sites, once the subject was at rest, via infusion of 28 mM sodium nitroprusside (SNP) at a rate of 4μl/min and application of local heat via heating units to a skin temperature of 43ºC. This concentration of SNP has been previously shown to elicit maximal vasodilation in human skin (26, 37).

Data Collection and Analysis

Data were digitized and stored at 100 Hz on a personal computer. Data were analyzed offline using signal-processing software (Windaq; Dataq Instruments, Akron, OH, USA). Skin blood flow data were converted to cutaneous vascular conductance (CVC), calculated as the ratio of skin blood flow to mean arterial pressure (RBC flux/mean arterial pressure). Cutaneous vascular conductance data were expressed as a percentage of maximal vasodilation (%CVC_{max}) via SNP infusion and local heating to 43°C.

All data were analyzed using a one way repeated measures ANOVA and Tukey's post hoc analysis was used to determine where significant differences occurred. All statistical analyses were performed using SigmaStat 3.5 (Systat Software; Point Richmond, CA, USA). All values are presented as mean ± SEM, and P-values < 0.05 were considered to be significant.

Chapter 4 - Results

Subject characteristics are described in Table 1. On average, subjects cycled at 53% of their peak power from protocol 1 in order to elicit the desired 60% VO_{2peak} during protocol 2. The group mean hemodynamic data at rest and during exercise at an increase of core temperature of 0.8ºC is summarized in Table 2. Compared to baseline, there was a significant increase in heart rate, cardiac output and core temperature ($p<0.001$) for all subjects. Compared to baseline, there was a significant increase in systolic blood pressure, diastolic blood pressure and systemic arterial pressure ($p<0.05$) for all subjects.

The group mean data for the increase in $\%CVC_{max}$ elicited following a 0.8ºC increase at each treatment site are summarized in Figure 3. There was no significant difference between control (65 ± 1 $\%CVC_{max}$) and NPLA (64 ± 6 $\%CVC_{max}$). L-NIO (44 ± 3 $\%CVC_{max}$) and L-NAME (39 ± 2 $\%CVC_{max}$) significantly attenuated CVC compared to control and NPLA ($p<0.001$ all conditions). There was no difference between L-NIO and L- NAME responses.

The percent contribution of eNOS and nNOS to the increase in $\%CVC_{max}$ is shown in Figure 4. The contribution of eNOS (32 ± 4 $\%CVC_{max}$) was significantly greater than the contribution of nNOS (3 ± 7 $\%CVC_{max}$) ($p<0.05$).

Figure 5 shows the increase in $\%CVC_{max}$ as a function of increasing core temperature from baseline to the end of the exercise at 0.1ºC increments. The increase in $\%CVC_{max}$ occurred with an increase in core temperature of 0.4 ºC at all treatment sites.

Chapter 5 - Discussion

This study is the first to investigate the mechanisms of reflex cutaneous vasodilation during dynamic exercise in humans. The main finding of this study was that eNOS not nNOS contributed to reflex cutaneous vasodilation during dynamic exercise. This finding was inconsistent with our original hypothesis that nNOS but not eNOS inhibition would attenuate reflex cutaneous vasodilation during dynamic exercise. Our finding was substantiated by decreased skin blood flow during dynamic exercise at microdialysis sites where an eNOS and non selective NOS specific antagonist where present. These data suggest eNOS is the primary NOS enzyme responsible for the NO-component of reflex cutaneous vasodilation during dynamic exercise as the control site was not significantly different from the nNOS (NPLA) antagonist site. In as much as the eNOS (L-NIO) site was not significantly different from the non selective NOS antagonist site (L-NAME) these data further suggest a role for eNOS but not nNOS during endogenous heat stress due to dynamic exercise.

The data from this study suggest NO directly contributes ~30% to the increase in skin blood flow during dynamic exercise, which is similar to several studies that have shown NO directly contributes ~30-45% to cutaneous active vasodilation during passive whole body heating (22, 25, 47). Although precise mechanisms underlying the NO-component of cutaneous active vasodilation remains vague, Kellogg et al. (29) recently demonstrated nNOS is the primary NOS isoform activated during passive whole body heating (29). These data from passive heat stress are contrary to our current findings.

Several studies have clearly shown a robust cutaneous vasodilation during exercise (6, 7, 10, 38-41, 49, 52). However, mechanistic data underlying this cutaneous vasodilation is lacking. To date, only one study by Blair et al. in 1961 (4) has provided evidence that the increase in skin

blood flow during exercise may be of reflex origin. It has often been assumed mechanisms are similar during dynamic exercise and passive whole body heating. However, data from our study suggest these mechanisms may differ depending on the means by which hyperthermia is induced.

Our data clearly suggest eNOS is responsible for the NO-component of cutaneous vasodilation during dynamic exercise; however, it is unclear what is responsible for activating eNOS. There are at least three possible mechanisms for this observed response including cutaneous heat load, shear stress, and heat shock proteins (HSP) (Figure 6). The difference in the application of the heat stress resulting in a different cutaneous heat load may be responsible for the difference in nNOS and eNOS activation between passive and active heating protocols. During passive heating, skin temperature is elevated (cutaneous heat load) whereas during exercise, evaporation of sweat cools the skin. Neuronal nitric oxide has been observed to be responsible for the cutaneous vasodilation response during passive heating protocols in which the subject's skin temperature is elevated, which differs from our study where subjects' sweat evaporated and cooled the skin.

Shear stress may also play a role in the response seen in our experiment. Shear stress is thought to be activated by chemical or physical stimuli which lead to a release of vasoactive substances in turn allowing for an increase in flow (14). The increase in flow increases the friction between a fluid and a stable surface, in this case the blood and endothelium respectively, resulting in an elevated wall shear stress. Vasoactive substances have been shown *in vitro* to be released in response to any increase in wall shear stress by way of stimulating the endothelial cells (6, 7, 10, 38-41, 49). As vessel diameter is inversely associated with wall shear stress (14) the potential for the cutaneous vasculature to react to small changes increases substantially.

Heat shock proteins are another possible mechanism responsible for the activation of eNOS during dynamic exercise. There are many HSPs in the human body but HSP90 in particular has been observed to increase eNOS activity (12). In addition, HSP90 has been shown to be activated by fluid shear stress (12). An experiment which exposed endothelial cells to fluid shear stress resulted in an increased association of eNOS and HSP90 (12). A possible cause for the interaction between these proteins may be an enhanced molecular attraction activated by fluid shear stress (12). According to Garcia-Cardena et al. (12) eNOS activation may result from HSP90 inducing a conformational change in eNOS by acting as an allosteric modulator or by stabilizing the dimeric form. To examine the dependence of eNOS activation by HSP90, an HSP90 specific inhibitor was used and the observed decrease in maximal response was similar to NOS inhibitors such as L-NAME, suggesting HSP90 may be necessary for NO release. Data further suggest HSPs may be cell-type specific (33) such that HSP90 may only bind to eNOS and not nNOS in addition to having a specific binding site on eNOS.

Limitations

There are four possible limitations to our study, which should be addressed. First, most human thermoregulation studies have been performed in the forearm and therefore the mechanisms controlling the cutaneous vasculature may vary according to anatomical location. Second, the mechanisms involved with cutaneous active vasodilation during dynamic exercise remain unresolved. In an experiment conducted by Blair and colleagues (4), in which the cutaneous nerves to the forearm were blocked, results suggested the vasodilation response during leg exercise was due to an active vasodilator mechanism; however, there have been no subsequent studies designed to elucidate potential mechanisms. Third, training has been shown

to have an effect on eNOS expression (35, 53) and our subjects were all at least moderately trained individuals. The data from this study show eNOS, not nNOS, contributes to reflex cutaneous vasodilation during dynamic exercise. Fourth, this is the first *in vivo* human study to use L-NIO to specifically inhibit eNOS. Although pilot work in our laboratory demonstrated 10mM L-NIO maximally inhibited the skin blood flow response to local heating, it is possible higher concentrations would attenuate the response further.

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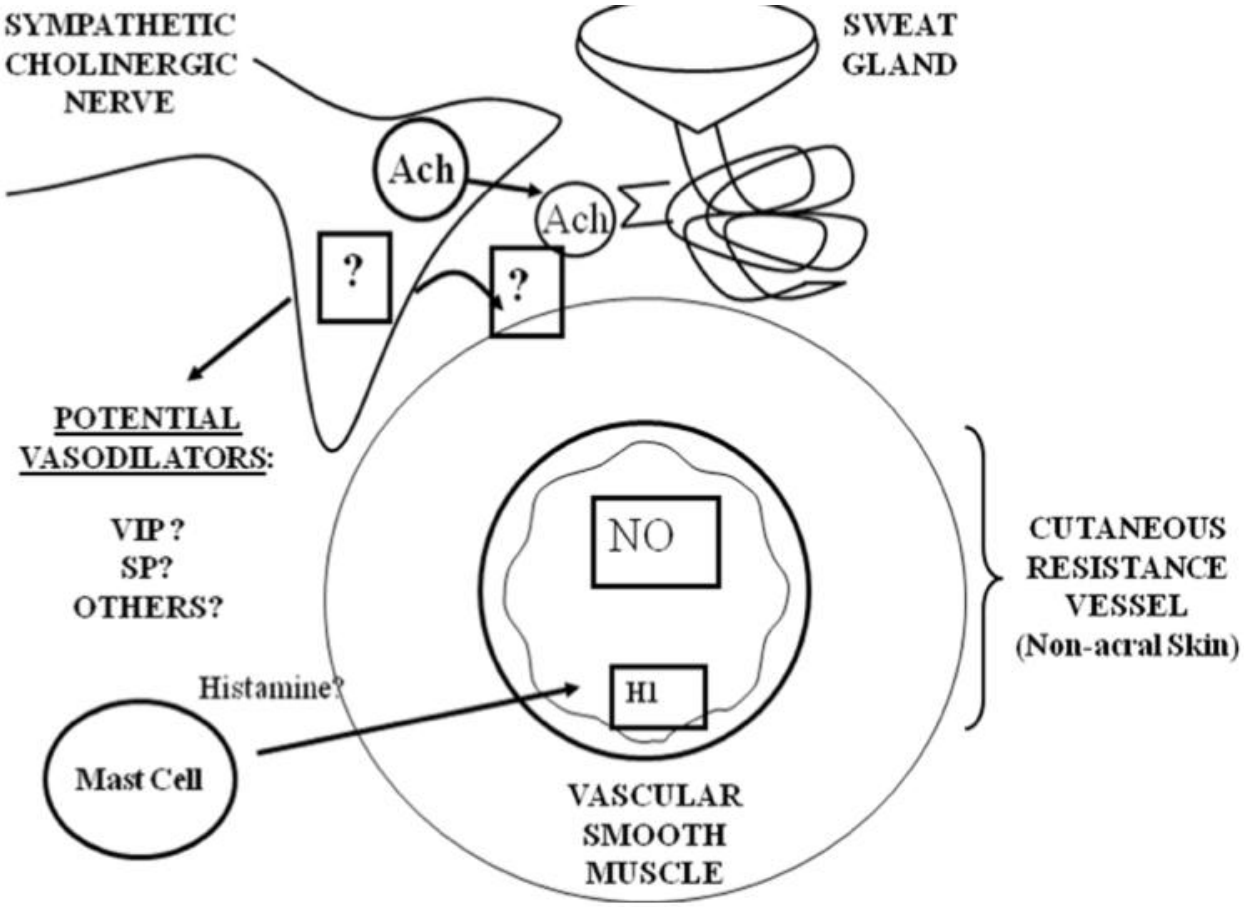

Figure 1: Schematic of the Co-Transmission theory.

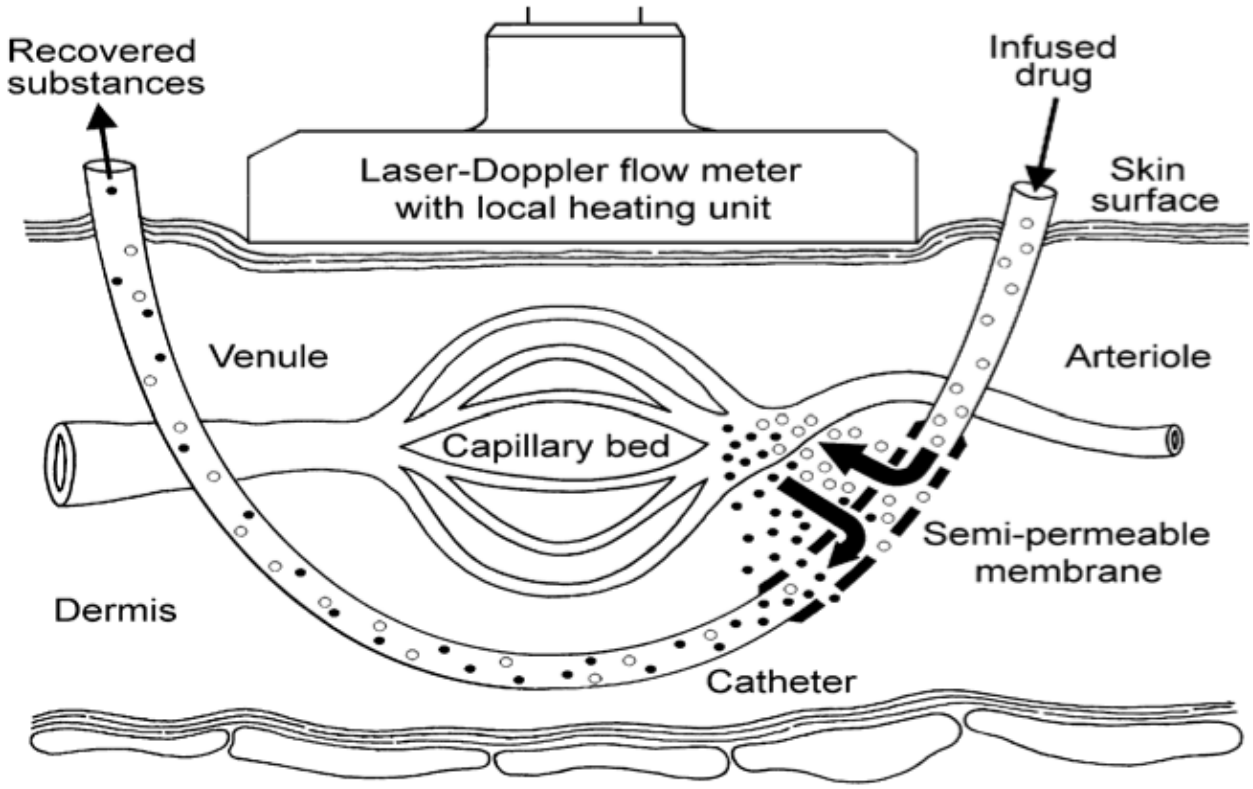

Figure 2: Schematic drawing of the microdialysis technique and placement of a laser-Doppler flow probe.

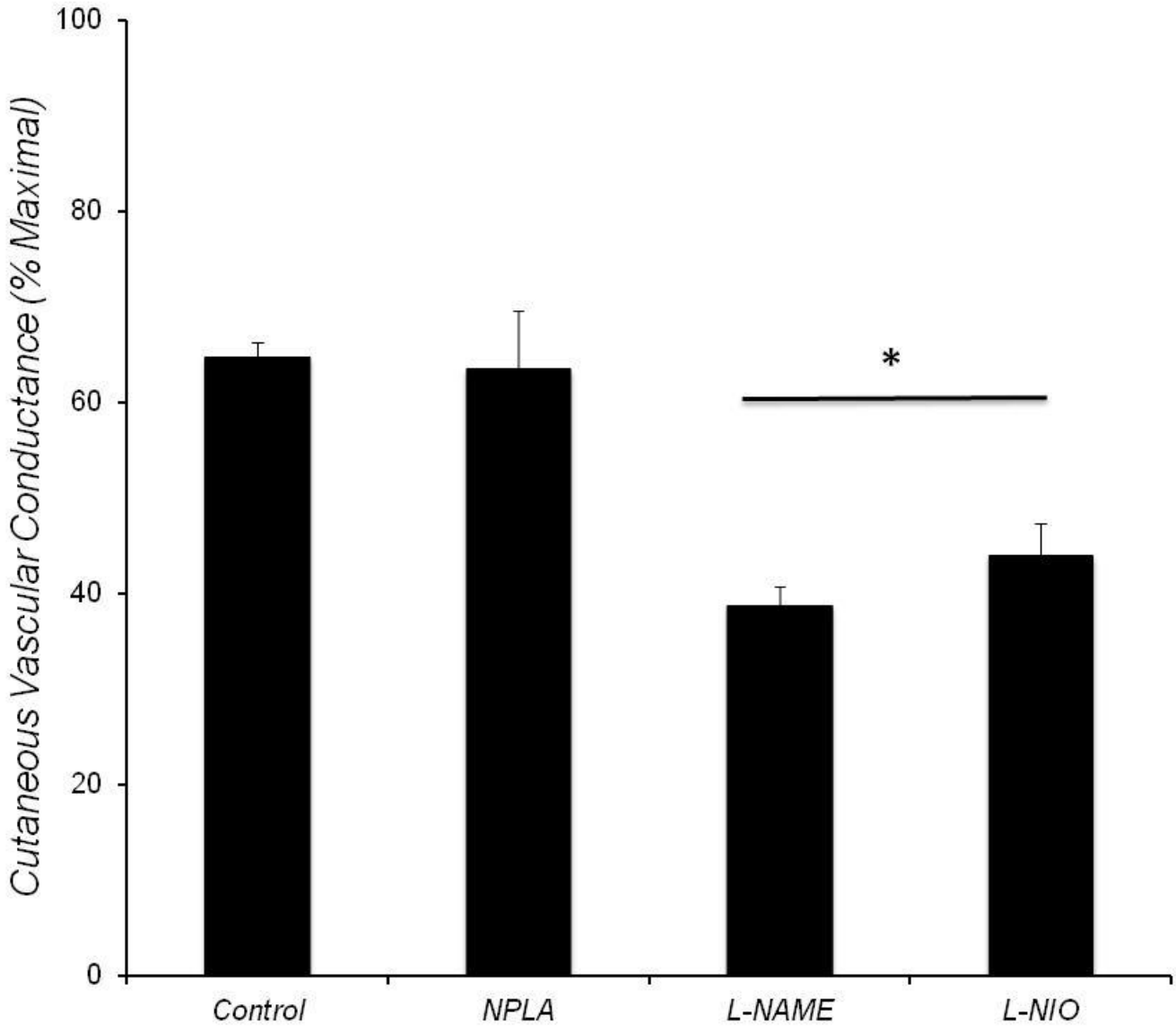

Figure 3: Group mean (± SEM) CVC data from *protocol 2*. Data are from a 0.8ºC increase in core temperature during exercise. NPLA had no significant effect on mean CVC. However, L-NAME and L-NIO significantly reduced CVC compared to control and NPLA. L-NAME and L-NIO were not significantly different from the other.

* $p<0.001$ vs. Control and NPLA.

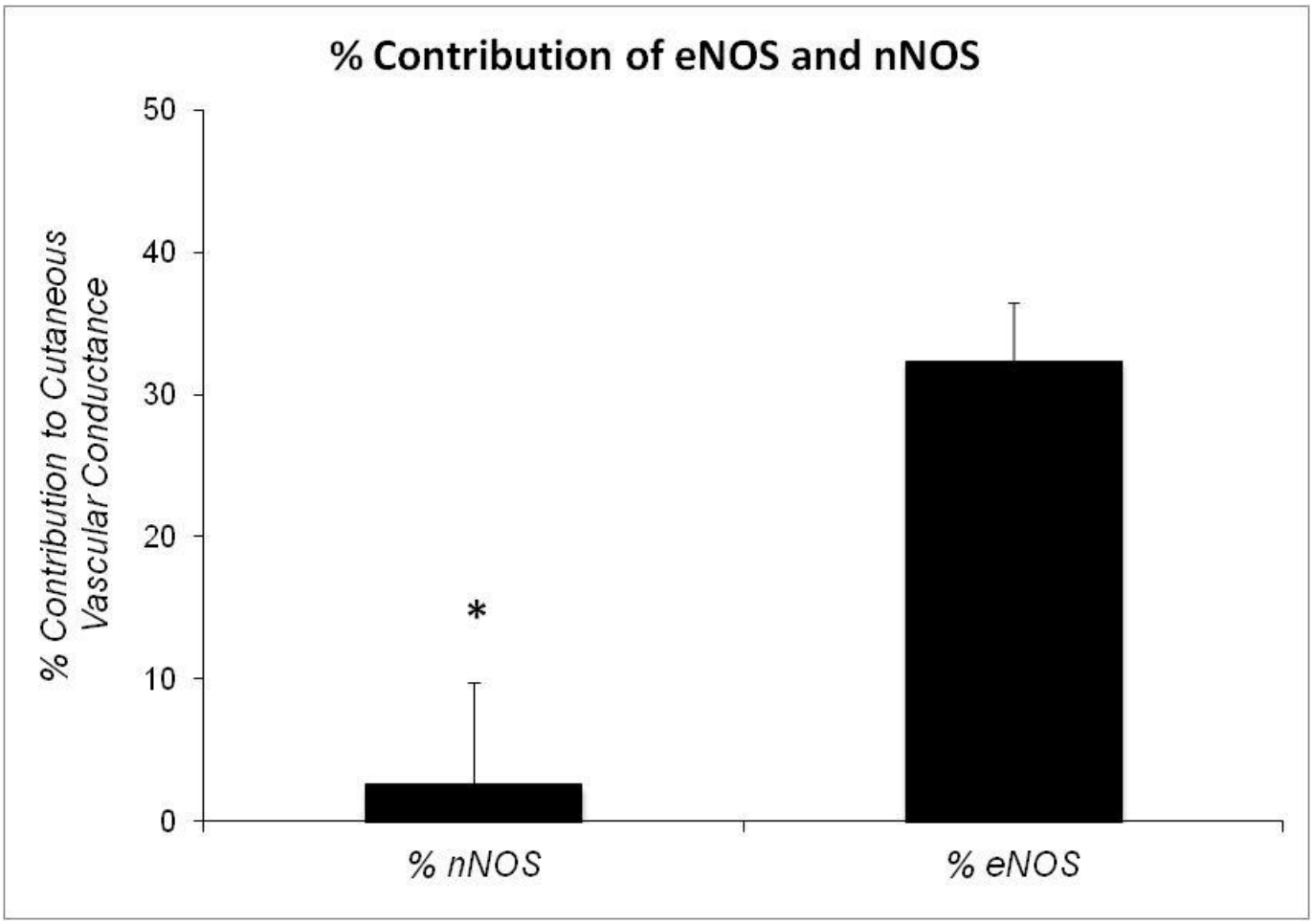

Figure 4: Group mean (± SEM) CVC data from *protocol 2* percent contribution of eNOS and nNOS. The contribution of eNOS to the increase in CVC during exercise was significantly greater than the contribution of nNOS.

* $p<0.01$ vs. eNOS.

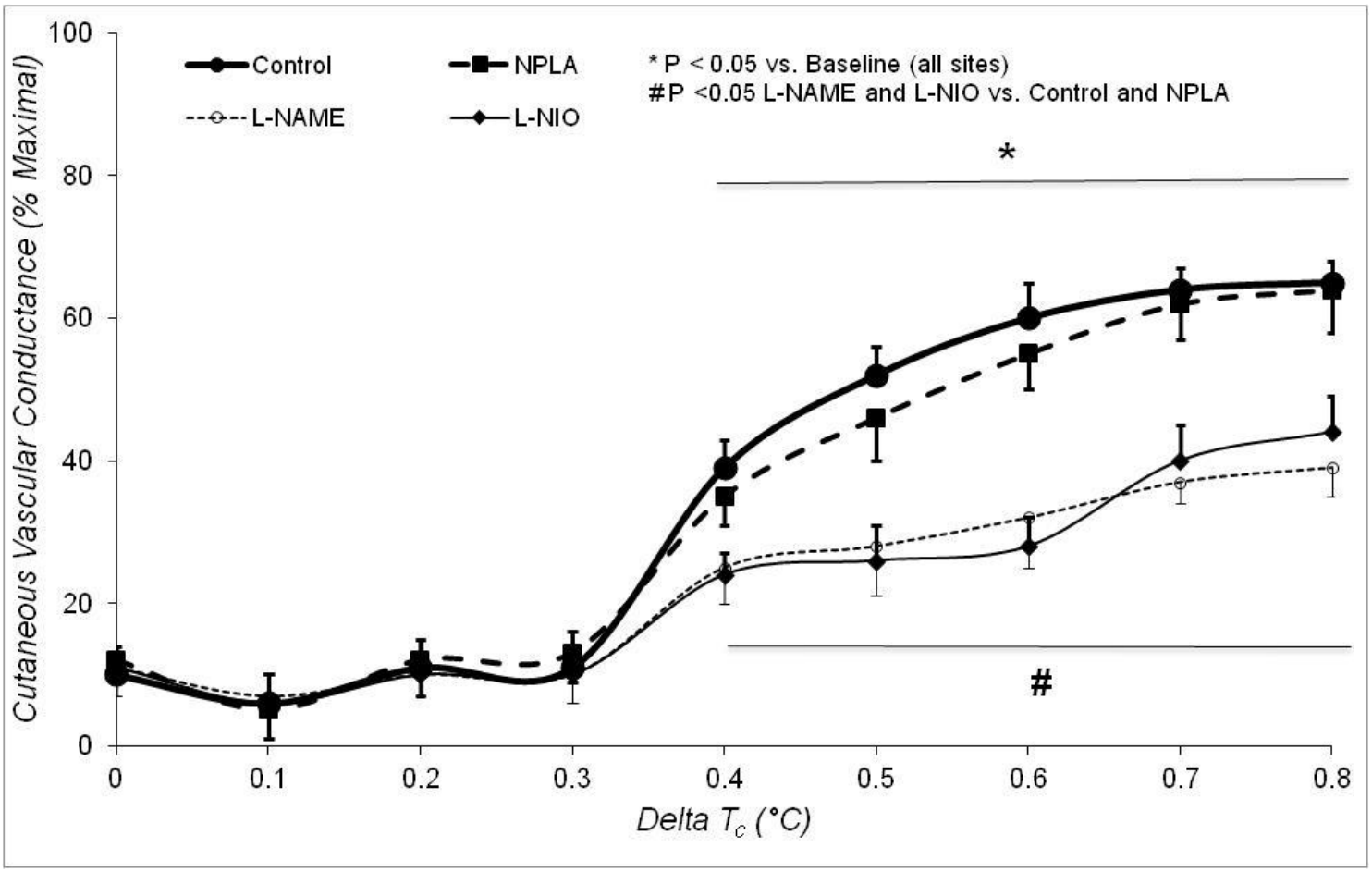

Figure 5: Mean skin blood flow tracing for all treatment sites. The increase in $\%CVC_{max}$ occurred with an increase in core temperature of 0.4 ºC at all treatment sites. The increase in $\%CVC_{max}$ was significantly less for L-NAME and L-NIO sites * $p<0.05$ vs. baseline ($\Delta Tc = 0$ºC) for all sites vs. Control and NPLA sites. Data are means ± SEM.

# $p<0.01$ vs. Control and NPLA.

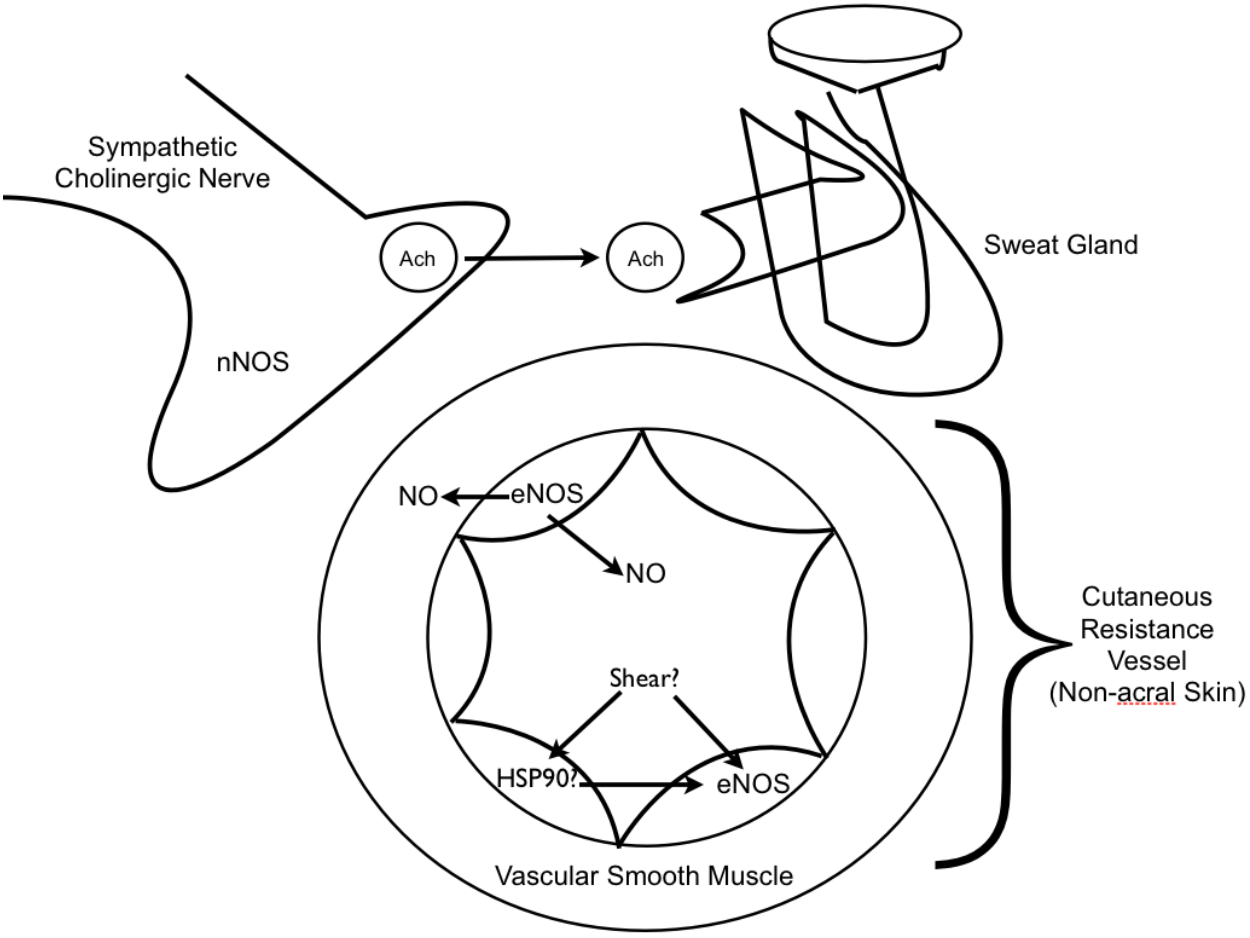

Figure 6: Schematic of proposed mechanisms responsible for CVC observations during dynamic exercise.

Age (Year)	Height (cm)	Weight (kg)	Absolute VO2 (L/min)	Relative VO2 (ml/kg/min)	Peak Power (Watt)	Cycling Power (Watt)
22 ± 1	177 ± 5	78 ± 13	3.51 ± 0.59	44.87 ± 9.52	278 ± 41	147 ± 34

Table 1: Subject characteristics. Values are mean ± SD.

HEMODYNAMIC DATA

	SBP (mmHg)	DBP (mmHg)	SAP (mmHg)	HR (beat/min)	CO (L/min)	Tc (°C)
Baseline	112 ± 2	64 ± 1	80 ± 4	58 ± 4	7 ± 1	36.51 ± 0.3
Exercise	153 ± 11*	70 ± 2*	99 ± 2*	145 ± 6 #	17 ± 0.4 #	37.32 ± 0.4 #

* P < 0.05 vs. Baseline
# P < 0.001 vs. Baseline

Table 2: Group mean hemodynamic data for baseline and exercise. Compared to baseline, there was a significant increase in heart rate (HR), cardiac output (CO) and core temperature (Tc) ($p<0.001$) for all subjects. Compared to baseline, there was a significant increase in systolic blood pressure (SBP), diastolic blood pressure and systemic arterial pressure (SAP) ($p<0.05$) for all subjects.