REGIONAL DIFFERENCES IN THE CONTROL OF THE 
CUTANEOUS CIRCULATION IN HUMANS

by

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ABSTRACT

The purpose of this series of studies was to compare regional skin blood flow (SkBF) in adult humans. Forearm and calf skin sites were instrumented with microdialysis fibers, local skin heaters, and laser-Doppler probes in nine healthy volunteers. Baseline cutaneous vascular conductance (CVC) (laser-Doppler flow / blood pressure) was measured at a local skin temperature (Tloc) of 33 °C and 42 °C. All data are expressed as CVC. In study 1, forearm and calf sites were compared to determine if SkBF differed by region. Baseline CVC was higher in the calf than in the forearm (24±2 and 16±1 %max) (P=0.04). At a Tloc of 42 °C, initial peak CVC was higher in the forearms (78±3 %max) than the calf (63±3 %max) (P=0.004). Plateau phase CVC was higher in forearms (90±2 %max) than calves (98±1 %max) (P=0.008). In study 2, the role of nitric oxide synthase (NOS) in the cutaneous vasodilator response in the skin of the forearms and calves using the NOS inhibitor L-NAME was investigated. Baseline CVC between control and L-NAME treated sites was higher (27±2 vs 17±1 %max, respectively; P=0.04) for the calf, but did not differ (P=0.26) for forearms. At a Tloc of 42°C, CVC at forearm and calf L-NAME sites did not differ (P=0.45). In study 3, bretylium tosylate (BT) was used to examine the role of sympathetic nerves on regional SkBF. Baseline BT sites differed between the forearm and calf (P=0.04). Initial peak CVC for forearm sites treated with BT (62±3 %max) were lower than the calf sites (78±2 %max) (P=0.02). CVC achieved at arm and calf BT sites did not differ at plateau (P=0.28). We conclude that differences exist in regional SkBF control at rest (thermoneutral) and in response to local skin warming. Differences observed suggest that the
NOS contribution to basal vascular tone was higher in calves than forearms and accounts for the higher basal SkBF and vasodilator response to a Tloc of 42 °C in the legs. Initial peak CVC in the calf treated with BT increased which may, in part, indicate increased vasoconstrictor tone.
DEDICATION

This dissertation is dedicated to everyone who helped me and guided me through the process of creating this manuscript, in particular, my wife and children as well as my Nana, Mrs. Jacqueline R. Manning who all stood by me and encouraged me through the trials and tribulations of life.
LIST OF ABBREVIATIONS AND SYMBOLS

α  
Alpha is used to denote the area underneath a normal curve in statistics to denote significance.

%  
Percent

% CVCmax  
Percentage of Maximal Cutaneous Vascular Conductance

°  
Degree

μl  
Microliter

μm  
Micrometer

Ach  
Acetylcholine

ANOVA  
Analysis of variance

AU  
Arbitrary Unit

BP  
Blood Pressure

BT  
Bretylium Tosylate

C  
Celsius

cm  
centimeter

CVC  
Cutaneous Vascular Conductance

EC  
Expected Change

EDRF  
Endothelium Derived Relaxing Factor

h  
Hour

HR  
Heart rate
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>kcal</td>
<td>kilocalorie</td>
</tr>
<tr>
<td>kDA</td>
<td>kilodalton</td>
</tr>
<tr>
<td>l</td>
<td>Liter</td>
</tr>
<tr>
<td>LDF</td>
<td>Laser-Doppler flow</td>
</tr>
<tr>
<td>L-NAME</td>
<td>$N^G$-nitro-L-arginine methyl ester</td>
</tr>
<tr>
<td>min</td>
<td>Minute</td>
</tr>
<tr>
<td>mL</td>
<td>Milliliter</td>
</tr>
<tr>
<td>mM</td>
<td>Millimolar</td>
</tr>
<tr>
<td>mm Hg</td>
<td>Millimeter of Mercury</td>
</tr>
<tr>
<td>n</td>
<td>Sample Size</td>
</tr>
<tr>
<td>NE</td>
<td>Norepinephrine</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric Oxide</td>
</tr>
<tr>
<td>NOS</td>
<td>Nitric Oxide Synthase</td>
</tr>
<tr>
<td>NPY</td>
<td>Neuropeptide-Y</td>
</tr>
<tr>
<td>s</td>
<td>Second</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SNP</td>
<td>Sodium Nitroprusside</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for the Social Sciences</td>
</tr>
<tr>
<td>VIP</td>
<td>Vasoactive Intestinal Peptide</td>
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</tbody>
</table>
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I am pleased to have this opportunity to thank the many colleagues, friends, and faculty members who have helped me with this research project. I am most indebted to Dr. Gary J. Hodges, the co-chairman of this dissertation, for his research expertise and experience with this type of research. I would also like to thank Dr. Phillip A. Bishop the other co-chairman for all of his insightful comments and suggestions through the writing process. As well I would like to thank all of my committee members, Dr. Mark T. Richardson, Dr. Jonathan E. Wingo, and Dr. Stephen M. Secor for their input, questions, and support of the dissertation. I would like to thank my wife and children; Joanne M. Del Pozzi, Kylie M. Del Pozzi, Andrew W. Del Pozzi, Jacqueline E. Del Pozzi, and Katherine A. Del Pozzi for their support of my educational goals. They all have sacrificed in the present so that our collective future could be brighter; choosing to live like most won’t for these few years so that we can live like most can’t in our future. I love you all and this could not have been possible without your encouragement and selfless support.

This research would not have been possible without the support of my friends and fellow graduate students, Miss Ann B. Collins and Mr. Stephen J. Carter and of course of my Mom, Mrs. Wilma M. Manning who never stopped asking questions or filling our refrigerator. Of course I could not forget my Nana, Mrs. Jacqueline R. Manning who was always there for us and continued to fill our hearts with fuel as well as our gas tanks. I thank all of the volunteers.

Finally, The University of Alabama Department of Kinesiology and Graduate School for had you not believed in me from the beginning I would not be here today.
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AUTHOR’S NOTE

All studies are written in journal format for the *Journal of Applied Physiology*.

For the body of the dissertation, the following key will be used in reference to my own research.

It is my plan to publish all works in the following order:

**Study 1**: Regional differences in skin blood flow to local skin warming.

**Study 2**: Regional differences in the contribution of nitric oxide synthase to the cutaneous vasodilator response to local warming.

**Study 3**: Effect of sympathetic nerve blockade on regional skin blood flow responses to local skin warming
CHAPTER I
INTRODUCTION

Initial exposure to the thermal environment in all homeotherms starts at the skin surface. This thermal environment is composed of not only air temperature, but also humidity, thermal radiation, barometric pressure, and other factors produced by the weather conditions, as well as the integument and clothing. Ultimately, the ambient environment plus clothing plays a crucial role in modifying heat transfer from the skin surface. At rest, human thermoregulation is comprised of heat production and heat dissipation (4, 6, 10, 11). Exposure to a warm environment leads to cutaneous blood vessel dilation and increased blood flow to the skin, consequently this increases the volume of blood in the cutaneous circulation and enhances surface cooling via conduction (3, 4). By contrast, exposure of bare skin to a cool ambient environment leads to vasoconstriction, reduced heat loss and potentially heat generation through shivering (4).

In a thermoneutral environment, it has been estimated that at rest, skin blood flow for the average individual is on the order of 275 mL·min\(^{-1}\) (8, 10, 11). This amount of blood flow has been estimated to be responsible for approximately 1500 kcal·h\(^{-1}\) of heat dissipation to the environment (4, 9). Heat exposure or exercise increases both skin and core temperature prompting vasodilation of the blood vessels of the skin, which can achieve blood flow of greater than 7 l·min\(^{-1}\) (4, 10, 11). This dramatic increase in blood flow is achieved by increases in cardiac output and a shunting of blood from the splanchnic region (8).
In addition to the thermoregulatory adjustments elicited in response to changes in core temperature, local skin temperature also plays a significant role in the cutaneous vasoconstrictor and vasodilator responses to changes in ambient temperature (10). It has been shown that local skin temperatures in the range of 42 °C can elicit maximal vasodilation regardless of core temperature (1, 21). It is important to note that, unlike other vascular beds, the cutaneous circulation receives dual sympathetic innervation: a noradrenergic system similar to that which is found in almost all blood vessels that release norepinephrine and neuropeptide Y, and a cholinergic branch which releases acetylcholine and other unknown transmitters (6, 13, 19).

The response of the cutaneous vasculature to local skin warming is biphasic (6, 12, 17). The first phase, a rapid, transient initial peak, is mediated by neurotransmitter release from sympathetic and sensory nerves (2, 22, 23). This is followed by a brief nadir and then a secondary phase of a prolonged plateau which is primarily mediated by the activity of nitric oxide synthase (NOS) (5, 7, 14, 17). It has been well established that NOS is responsible for ~70 % of the prolonged plateau phase (14, 17). The majority of the work examining the mechanisms governing cutaneous vascular responses has been performed on the forearm. However, recently, one set of researchers has embarked on examining the cutaneous vascular responses of the calf, and they have reported marked differences in the cutaneous vasodilator responses to local skin warming (15, 16, 20).

The latter findings suggest that there might be regional differences in the mechanisms responsible for skin blood flow control. It has been shown in muscle that those limbs that are chronically placed in a gravitational dependant state have increased amounts of blood flow (18).
It is to this end that the following collection of studies aimed to investigate: 1) regional differences in the cutaneous circulation; 2) the extent to which nitric oxide (NO) contributes to the vasodilator responses when comparing each region; 3) and the role of sympathetic noradrenergic neural control in the cutaneous vasodilator response to local skin heating in relation to the region.
CHAPTER II

REGIONAL DIFFERENCES IN THE SKIN BLOOD FLOW RESPONSE TO LOCAL SKIN WARMING

ABSTRACT

The purpose of this study was to determine whether there are regional differences in the skin blood flow response to local skin warming between the forearm and the calf in healthy, active, young, adult humans. Forearm and calves were instrumented with microdialysis fibers, local skin heaters, and laser-Doppler probes. We compared CVC and CVC as a percentage of maximum CVC (%CVCmax) obtained through pharmacological stimulus (SNP). Baseline initial peak and secondary plateau responses were compared. When CVC was expressed as %CVCmax baseline for the arm (16 ± 1%) differed statistically from the leg (24 ± 2%, P = 0.043). The initial peak values for the arms (78 ± 3 %) differed from legs (63 ± 3 %; P = 0.004). The plateau values for the arms (90 ± 2 %) were statistically different from legs (98 ± 1, P = 0.008). When the data was expressed as absolute CVC there were no differences between the arms and legs at the baseline (P = 0.231), initial peak (P = 0.146), and at the plateau (P = 0.254). Additionally, there was no difference between the absolute CVC values obtained during a pharmacological max (P = 0.075). The current study found apparent differences in CVC between the forearm and the calf. We found that the initial peak response was higher in the forearm compared to the calf; conversely, the baseline and plateau phases were higher in the leg compared to the arm.
These findings to suggest that there might be more tonic constriction in the legs than in the arms, based on the findings that the physiological maximum reached in the legs (CVC at 42 °C) accounts for a greater proportion of the total blood flow achievable (CVC in response to SNP).

**Key Words:** Local Control, Microdialysis, Laser-Doppler, Cutaneous Circulation
INTRODUCTION

In humans, the cutaneous circulation plays a major role in the regulation of internal temperature through manipulation of the level of perfusion. Under conditions of hyperthermia, blood flow through the skin can represent ~60% of cardiac output. In nonglabrous (hairy) skin, the blood flow response to local warming has been shown to be achieved through a combination of sympathetic noradrenergic nerves releasing norepinephrine and neuropeptide Y (9) and a nonadrenergic system previously shown to be dependent on nitric oxide (18).

In 1948, Hertzman and Randall (4) investigated whether differences existed in regional cutaneous blood flow at resting (baseline) and maximal rates. Using photoelectric plethysmography they reported that the human cutaneous circulation could essentially be divided into two distinct types: that which experiences high rates of flow and that which experiences low rates of flow. The circulation of the head, palm of the hand, and the plantar region of the foot, (glabrous skin, not hairy) all were reported to have high rates of blood flow; whereas the circulation of the trunk, forearm, thigh and lower leg (nonglabrous skin, hairy) all had relatively lower rates of blood flow (4). Since 1948, technological advances have been made in the measurement of skin blood flow (SkBF), yet we are still uncertain if differences in SkBF exist within nonglabrous skin by region. Recently, Yamazaki and Yuge (41) compared forearm and calf SkBF to help understand limb-specific differences in response to adrenergic agonists, with the cutaneous circulation of the legs showing a greater sensitivity to the adrenergic agonist norepinephrine (41). They found that baseline and maximum SkBF values did not differ
statistically between regions. Whether these differences occur with local skin warming remains unknown.

During local skin warming in healthy, human adults, the SkBF response is biphasic response (6, 16, 25). There is an initial, transient increase to a peak which is thought to be dependent on sensory and sympathetic nerves (2, 8, 9, 25, 34, 37). The initial peak is then followed by a brief nadir and succeeded by a secondary vasodilation producing a prolonged plateau phase which has been shown to be primarily dependent on nitric oxide (18). While it has been shown that rates of flow can be heterogeneous throughout the human body it remains unclear if there are differences in the cutaneous circulation in the arms and legs during local skin warming. This gap in our understanding of regional differences is clearly demonstrated by discordant findings of Kellogg et al. (18) who examined the SkBF responses in the arm and Stewart et al. (29) who examined SkBF responses in the leg. These two groups have reported differences in the vascular response to local skin warming. However, these data were performed by different groups, using different methods, protocols, and populations; thus direct comparisons of their data are difficult. Therefore, the purpose of this study was to determine whether there are regional differences in the SkBF response to local skin warming between the forearm and the calf.
METHODOLOGY

A power analysis indicated that 9 participants would be required with a P < 0.05 with 80% power, expected required mean differences and standard deviations were taken from our laboratory’s previous work within the field (1, 22-25, 27) (nQuery Advisor v. 3).

Nine healthy, active participants aged 27 ± 4 years (5 men and 4 women). Inclusion criteria were age 19 years or older and not diagnosed with any metabolic or cardiovascular disease. Exclusion criteria included tobacco use or current training for an endurance athletic event as well as being on any medication known to effect exercise or blood pressure. All participants gave verbal and written consent for their participation in the study. All informed consent documentation was approved by the local Institutional Review Board. Subjects were asked to refrain from caffeine and alcohol for 24 hours prior to testing. The subjects were also instructed to report for the testing after a 2-h fast, but were allowed to drink water ad libitum. Female subjects were all using oral contraceptives and in the high hormone phase of their cycle (3). Phase of their cycle was determined by week of use in the oral contraceptives (28).

Instrumentation

Participants laid supine on an adjustable gurney and were evaluated for the placement of microdialysis fibers, laser-Doppler flow probes, and local skin heaters. This evaluation involved finding an area of skin free from scars, superficial veins, inflammation, or any other damage to the skin that would affect SkBF. The subjects were instrumented with 4 custom-built microdialysis fibers. Using a 22-gauge needle, 2 microdialysis fibers were placed in the dorsal aspect of the forearm, and 2 were placed in the lateral aspect of the calf. Following a 90-min
trauma resolution period the subject was outfitted with 4 skin heaters and 4 laser-Doppler probes placed directly over the microdialysis fibers.

Red blood cell flux, measured by laser-Doppler flowmetry (MoorVMS-LDF2, Moor Instruments, Devon, UK), was used to provide an index of SkBF (14, 27). Local skin temperature was monitored and adjusted using an integrated skin heater and temperature monitor (SH02, Moor Instruments, Devon, UK). Laser-Doppler flow (LDF) probes (VP12 probe, Moor Instruments, Devon, UK) were placed inside the local skin heaters (SH02-SHP1, Moor Instruments, Devon, UK). Blood pressure was measured through oscillometry in the contra-lateral arm every 10 minutes throughout the duration of the study.

Experimental procedure

Local skin heaters were set at 33 °C (thermoneutral) 30 min prior to instrumentation and placed on the skin for 30 min prior to data recording. Baseline SkBF measurements were recorded at 33 °C for 10 min, then a local skin warming was performed by increasing skin temperature by 0.5 °C · 10 s⁻¹ until all skin sites had reached 42 °C (2, 8, 9, 11, 31, 34-36). All sites were maintained at 42 °C for 35 minutes, whereupon a plateau was reached and a physiological maximum blood flow was achieved (30). Finally, 58 mM sodium nitroprusside (SNP) was infused via microdialysis for 35 min to achieve a pharmacological maximum SkBF response (2, 19-22, 36) for normalization purposes due to the large heterogeneity of SkBF responses (15). Then, instrumentation was removed.
Data and Statistical Analysis

LDF was recorded at 50 Hz and stored for offline analysis (Biopac MP150, Camino Goleta, CA). LDF was expressed as cutaneous vascular conductance (CVC), LDF (mV)/mean arterial pressure (mm Hg), and normalized to maximal CVC (% CVCmax).

CVC data were averaged over 20 s throughout the data collection period (2, 5, 7-13, 17). Five min of data were used for baseline and plateau measurements and comparisons (2, 8, 9, 23, 24, 38-40). Due to the rapid and transient nature of the initial peak and the nadir responses, a 40-s portion of CVC was used (2, 8, 9, 33, 34, 36). Results are reported as mean ± standard deviation. Data from the respective sites were compared using paired samples t-tests or when appropriate, a repeated measure analysis of variance (ANOVA) (SPSS, inc., Chicago, Illinois, USA).
RESULTS

Figure 1 is a tracing from a single representative participant for the entire protocol. Note that at baseline the legs experienced higher amounts of flow when compared to the arm. Conversely, the initial peak response in the arm was higher when compared to leg. Additionally, the leg had a higher plateau phase response than that of the arm.

During baseline measurements at a local skin temperature of 33 °C, CVC at the arm (16 ± 1 %max) was lower than in the leg (24 ± 2 %max; P = 0.04). Once the local skin warming protocol was started, the initial peak value for the arms was significantly higher than for the legs (78 ± 3 vs. 63 ± 3 %max, respectively; P = 0.004) (Fig. 2). After the local skin temperature had reached 42 °C and was maintained for 35 min, CVC during the plateau values for the arms was statistically different from legs (90 ± 2 and 98 ± 1 %max, respectively; P = 0.008) (Fig. 3).
DISCUSSION

The main finding of the current study was that when CVC was expressed as a percentage of maximum, basal and vasodilator responses to local skin warming differed between arms and legs. The current study indicates that there are likely differences in the regulation and control of the cutaneous circulation between the arm and the legs. The initial peak for the legs was significantly lower than that of the arms. Hodges et al. (8, 9) reported that the initial peak of the vasodilator response was dependent on sympathetic nerve function. While counter-intuitive that sympathetic nerves would be involved in a vasodilator response, this observation was consistent whether the actions of the cutaneous sympathetic nerves were blocked pre-synaptically (8) or post-synaptically (9).

Sensory nerves have also been implicated in the initial peak response to local skin warming (25). Thus, the differences observed in the initial peak responses between the arms and legs suggest that sensory and sympathetic nerve regulation of the arm and leg circulations differ. While it is impossible from the present study to determine which system is working differently between the forearm and calf, we speculate that the leg has a reduced initial response due to gravity-related blood pressure control. For example, if legs responded more quickly, thereby resulting in a rapid increase in blood flow to peripheral circulations, this could cause catastrophic problems in the presence of low total peripheral resistance, venous return, and could ultimately disrupt blood pressure control. Indeed, Yamazaki and Yuge (41) demonstrated a far higher adrenergic responsiveness in the cutaneous circulation of the calf compared to the forearm.
Additionally, the CVC, in response to sustained local warming, was significantly higher during the plateau phase in the legs when compared to the arms. Kellogg et al. (18) showed that NO plays a prominent role in vasodilator response to local skin warming of the forearm, accounting for approximately 70% of the increase in SkBF during the plateau phase. The greater vasodilator response of the legs might be due to greater thermal demands of the local tissue; the volume of the calf is much greater than that of the forearm. The current study examined the vascular response to changes in local temperature, thus it might be that the vasculature is capable of achieving a greater level of perfusion to facilitate the dissipation of increased metabolic heat.

Experimental limitations

Many different measure or indices of SkBF have been proposed and used for studies of that circulation (14). Each method has its particular advantages and limitations, with no single method being universally considered problem-free. Indeed, a major problem with laser-Doppler flowmetry is the lack of a credible unit (e.g. ml · 100 ml⁻¹ · min⁻¹). Also, LDF probes obtain their measures from a relatively small area of measurement, 1 mm³. Consequently, the number of capillaries under the LDF probe could vary sufficiently among studies and regions to cause significant discrepancies (15). Therefore, these factors could have affected the results of the present study.

Finally, the current study was descriptive, i.e. there was no way to determine the mechanisms responsible for the differences in SkBF that were found between the upper and lower body; consequently, we can only speculate as to why differences in the vasodilator response were observed.
CONCLUSION

The current study found differences in CVC between the forearm and the calf. We found that the initial peak response was higher in the forearm compared to the calf; conversely, the baseline and plateau phases were higher in the leg compared to the arm.

The mechanisms responsible for the differences in SkBF between regions still need to be determined. The next step in understanding the differences would be to investigate the roles of NO (plateau phase) as previously determined by Kellogg (9) and the sympathetic noradrenergic systems (initial peak) as determined by Hodges (9, 18).

DISCLOSURES

The authors have no disclosures or conflicts of interest.

ACKNOWLEDGMENTS

We thank the participants for their time and commitment to this study. As well we extend our greatest gratitude to Mr. Stephen J. Carter and Miss. Ann B. Collins for their help with data collection and participant recruitment and screening.
REFERENCES


FIGURE LEGENDS
Figure 1. Data tracing from a single, representative participant. Note the higher baseline values, lower initial peak (A), but the higher plateau phase (B) in the leg (open circles) compared to the arm (closed circles). The three small horizontal bars indicates the section of data used for the statistical comparison. The width of each bar is proportional to the duration of each section of analysis, i.e. 5 min for baseline and plateau and 30 s for the initial peak.

Figure 2. Average initial peak responses in %CVCmax to local skin warming at arm and calf from all 9 participants. Note the lower cutaneous vascular response at the leg sites compared to the arm skin sites. * indicates P < 0.05 compared to arm.

Figure 3. Increase in %CVCmax in response to prolonged local skin warming at arm and leg sites in 9 participants. The leg skin sites had a higher CVC response to 42 °C than the arm skin sites. * indicates P < 0.05 compared to arm.
Figure 1.
Figure 2.
Figure 3.
CHAPTER III
REGIONAL DIFFERENCES IN THE CONTRIBUTION OF NITRIC OXIDE SYNTHASE TO THE CUTANEOUS VASODILATOR RESPONSE TO LOCAL WARMING

ABSTRACT
We investigated the roles of nitric oxide synthase (NOS) on regional cutaneous vasodilator responses to increased local skin temperature (Tloc). Forearm and calf sites were instrumented with microdialysis fibers, local heaters, and laser-Doppler probes. Skin sites were heated from 33 to 42 °C. Each limb had 1 skin site treated with L-NAME to inhibit NOS, and 1 site left untreated to serve as a control. When Tloc was held at 33 °C, CVC averaged 17 ± 1 %max at control sites and 14 ± 1 %max at L-NAME treated sites (P = 0.26). CVC at untreated legs sites was 27 ± 2 %max and 17 ± 1 %max at L-NAME treated sites (P = 0.04). CVC was significantly higher in the legs compared to the arms at control sites (P = 0.03). In contrast, at L-NAME treated sites there was no difference between arms and legs (P = 0.23). During the plateau phase, with Tloc at 42 °C, CVC at the control sites was 93 ± 1 %max for arms and 98 ± 1 %max for the legs (P = 0.02). There were no significant differences between the arm and leg L-NAME treated sites at 42 °C Tloc (P = 0.45). The two main findings of the current study were that the contribution of NO to the vasodilator response to increased Tloc is consistent between the arm and the leg, and second, under thermoneutral conditions (33 °C), NO plays a larger role in the legs than that of the arms.

Key Words: Local Control, Microdialysis, Laser-Doppler, Blood Flow
INTRODUCTION

A constant, normal body temperature is the consequence of a regulated balance between heat production and heat loss. Controlling the level of perfusion of the skin manipulates the rate of heat transfer from the core to the surface of the body. Adjustments in perfusion of the skin are largely driven by changes in core temperature (Tcore) and local skin temperature (Tloc). The dynamic fluctuations in skin perfusion in response to changes in Tloc are achieved primarily through two pathways. One pathway involves sympathetic noradrenergic system, relying heavily on norepinephrine (NE) and neuropeptide Y (NPY), which operates principally at the onset of vasodilation (initial peak response) (2, 8, 9, 26). The other pathway involves a nitric oxide (NO) based system that mediates the persistent vasodilation that occurs in response to prolonged skin warming (plateau phase) (13, 18).

The extant data on the contribution of NO to local warming induced vasodilation is equivocal. Kellogg et al. (13, 15-17) examined arms and Stewart et al. (20, 21) examined legs, found differing contributions of NO to the persistent plateau phase vasodilator response to prolonged increases in Tloc. However, differing methods and protocols make direct comparisons impossible. In a previous study (Study 1) we found that the plateau phase vasodilator responses to increased Tloc were higher in the legs compared to the arms suggesting that there might be a greater role for NO in leg skin blood flow than in the arms. Our finding is, however, discordant with observations from Bussell and Cable (1), who, using acetylcholine and sodium nitroprusside to examine endothelium-dependent and -independent vasodilation, respectively, reported that the contribution of the endothelium (primarily NO) to cutaneous vasodilation did not differ between
forearm and calf skin. It is important to note that this latter finding was achieved via pharmacological stimuli (acetylcholine and sodium nitroprusside via iontophoresis) rather than through physiological stimuli (local skin warming).

Consequently, we sought to directly examine whether the contribution of NO to the vasodilator response to local skin warming differed in the arms and the legs. To that end, we examined the effect of NO synthase (NOS) inhibition with $N_\omega$-Nitro-L-arginine methyl ester hydrochloride (L-NAME) (9, 12, 13, 15, 17) on the vasodilator response to local skin warming in the arms and legs of young healthy humans. Based on the findings in a previous study (Study 1) exhibiting a greater plateau phase vasodilator response (primarily NO-mediated), in the legs compared to arms. We hypothesized that the contribution of NO to vasodilation in response to an increase in Tloc would be greater in the legs than in the arms.
METHODS

A power analysis indicated that 9 participants would be required with a P < 0.05 with 80% power, expected required mean differences and standard deviations were taken from our laboratory’s previous work within the field (1, 22-25, 27) (nQuery Advisor v. 3). Nine healthy, active participants (27 ± 4 years, 5 men and 4 women) volunteered for this study. Only participants who were 19 years or older, and not diagnosed with any metabolic or cardiovascular disease were included. Participants were excluded from the study if they were using tobacco or currently training for an endurance athletic event. Participants were also excluded from participation if they were currently taking any medication that could affect exercise tolerance or manipulate blood pressure. All participants gave verbal informed consent as well as completed a local Institutional Review Board approved consent form. Participants were instructed to discontinue the use of caffeine and alcohol for 24 h prior to testing. Additionally, the participants were instructed to fast for 2 h prior to the testing session and were instructed to drink water ad libitum. Female subjects were all using oral contraceptives and currently in the high hormone phase of their routine verified through self report (3, 19). The current study required each participant to make two visits to the laboratory. The order of testing body regions was counterbalanced.

Instrumentation and experimental procedure

Participants laid supine on an adjustable gurney for the duration of the experiment. Two skin sites on either the dorsal forearm or the lateral calf were prepared for each session. The sites for a test session (in counterbalanced order) were prepared for the placement of
microdialysis fibers by placing ice packs over the skin sites where the fibers were to be placed to act as a temporary anesthetic (6). Participants were instrumented with 2 custom-built microdialysis fibers (2, 6-9, 11, 12). Following a 90-min trauma resolution period, two local skin heaters and laser-Doppler probes were placed directly over the microdialysis fibers (6). Local skin temperature was monitored and adjusted using an integrated skin heater and temperature monitor (model SH02, Moor Instruments Devon, UK). Red blood cell flux measured via laser-Doppler flowmetry (MoorVMS-LDF2, Moor Instruments, Devon, UK) was used to provide an index of skin blood flow. Laser-Doppler flow (LDF) probes (VP12, Moor Instruments, Devon, UK) were placed in a small aperture in the skin heaters to monitor skin blood flow.

During the trauma resolution period, lactated Ringer’s (study vehicle) was perfused at both sites at a rate of 4 µl · min⁻¹. After 30 min of trauma resolution, one site, either forearm or calf depending on the day, was chosen as the experimental skin site, and L-NAME (US Pharmacopeia, Rockville, MD) was administered at a concentration of 20 mM (9, 12, 13, 15, 17). After 30 min of drug infusion, local skin heaters were set at 33 °C (thermoneutral) and placed on the participant to control and monitor Tloc at the site of measurement. Ninety min after microdialysis fiber insertion, baseline measurements were recorded. After 10 min of baseline data recording, a standard local warming protocol was initiated by increasing Tloc by 0.5 °C · 10 s⁻¹ until Tloc had reached 42 °C (2, 9-11, 24-26). Tloc was maintained at 42 °C for 35 min at which time a stable plateau had been reached and which represented a physiological maximum (23). Following the stabilization of the plateau phase, sodium nitroprusside (SNP) was infused at both sites at a concentration of 58 mM for 35 min to pharmacologically induce maximal
cutaneous vasodilation (2, 14-17, 25). Blood pressure was measured through oscillometry in the contra-lateral arm every 10 min throughout the duration of the study protocol.

Data Collection and Analysis

LDF was collected at 50 Hz and digitized for storage on a personal computer for subsequent offline analysis using signal-processing software (Acqknowledge, Biopac MP150, Camino Goleta, CA). Mean arterial pressure (MAP) was calculated as diastolic blood pressure + \( \frac{1}{3} \) pulse pressure from oscillometry measures. Cutaneous vascular conductance (CVC) was calculated as red blood cell flux divided by mean arterial pressure and normalized to maximal vasodilation achieved in response to a local skin temperature of 42 °C and 58 mM of SNP.

Due to the rapid and transient response of the initial peak, 40 s portions of CVC were used. Due to the prolonged status of both the baseline and the plateau phases, stable 5 min periods of CVC data were used.

Text and illustrative results are reported as the mean ± standard deviation. All analysis performed was by paired statistics or when appropriate, a repeated measure analysis of variance (ANOVA). Data were analyzed using SPSS software version 19.0 (SPSS, inc., Chicago, Illinois, USA).
RESULTS

Figure 1 shows responses in CVC from a representative subject to 35 min of local skin warming at two arm and two legs sites. Each limb had a site treated with L-NAME and the other site left untreated. In response to an increase in Tloc at the 10-min mark, CVC increased similarly to that described previously, i.e., an initial peak and prolonged plateau associated with increasing Tloc (13). Note that during L-NAME treatment at Tloc of 33 °C, CVC was only reduced only in the legs. During the plateau phase CVC of both the arm and leg was reduced in response to L-NAME.

When Tloc was held at 33 °C, CVC averaged 17 ± 1 %max at the arm control sites and 14 ± 1 %max at sites perfused with L-NAME (P = 0.26; Fig. 2). CVC at the leg control sites averaged 27 ± 2 %max and at sites perfused with L-NAME 17 ± 1 %max (P = 0.04). CVC was significantly higher in the legs compared to the arms at control sites (P = 0.03), whereas at the L-NAME treated sites there was no difference in CVC between the arm and leg (P = 0.23).

In response to increasing Tloc to 42 °C, CVC increased significantly and achieved stable plateaus at all skin sites (P < 0.001, 33 °C vs. 42 °C for both sites and conditions) (Fig. 3). During the plateau phase, with Tloc maintained at 42 °C, CVC at the control sites averaged 93 ± 1 %max and 98 ± 1 %max in the arms and legs, respectively (P = 0.02). CVC at arm and leg sites treated with L-NAME rose to 70 ± 3 %max and 71 ± 3 %max, respectively. At 42 °C Tloc, CVC was significantly less at the L-NAME treated sites compared to the untreated control sites (P = 0.001 for both arm and leg). There were no significant differences between the arm and leg L-NAME treated sites at 42 °C Tloc (P = 0.45).
DISCUSSION

The goal of the current study was to examine the relative contribution of NO in the vasodilator response to local skin warming in the arms and legs. We found that L-NAME attenuated the CVC increases induced by local skin warming in both arms and legs. However, at a Tloc of 33 °C, L-NAME reduced CVC in the legs only and not in the arms. The main findings of the current study are: 1) the contribution of NO to the vasodilator response to increased Tloc is consistent between the arm and the leg; and 2) at rest, under thermoneutral conditions, NO plays a larger role in basal vascular function in the legs than in the arms. This latter finding is congruent with what we observed in our previous study (Study 1). Moreover, the present study suggests that the higher basal CVC in the legs is due to increased NOS activity.

It is well established that shear-stress is a potent mediator of NOS activity and the synthesis of NO (5). The increased hydrostatic pressure exposure of the legs relative to the arms has been shown to increase both antegrade and retrograde flow, particularly during locomotion (4), which greatly increases shear-stress and NO production. This chronically elevated level of shear stress in the legs compared to the arms might explain why there is a significantly larger contribution of NO to basal vascular tone in the legs compared to the arms. Also, these findings may, in part, explain the discrepancies in the contributions of NO seen by Kellogg et al. (13, 15-17) and Stewart et al. (20-22) who examined arm and leg skin blood flow, respectively.

In a previous study (Study 1) we noticed a paradoxical relationship between the vasodilator responses in the arms and legs, in that when values were expressed as a percentage of maximal vasodilation, there were significant differences between the arms and legs. However,
when data were presented as absolute CVC, not normalized to a percentage of maximum, values did not differ between the arms and legs. We interpreted this as a suggestion that there may be more tonic vasodilation present in the legs than in the arms. The current study’s finding indicates that the contribution of NO to basal vascular tone was much higher in legs than in arms, and appeared to fully explain the differences between these circulations observed in the present study and previous studies.

Experimental Limitations

The data in the present study are limited to a discrete population: young, healthy, active but not trained individuals. It is important to realize that these findings may not be applicable to athletes or individuals with metabolic or cardiovascular disorders. Thus, extrapolation of these data, that skin blood flow is elevated under basal conditions, to such groups to describe phenomena such as post-exercise syncope or other orthostatic intolerance should be performed with caution.

CONCLUSION

The current study found that the contribution of NO to the vasodilator response during local skin warming was similar in arms and legs. However, the contribution of NO to basal vascular tone was higher in legs than in arms and appeared to fully explain the differences between these circulations observed in the present study and previous studies.

DISCLOSURES

The authors have no disclosures or conflicts of interest.
ACKNOWLEDGMENTS

We thank the participants for their time and commitment to this study. As well we extend our greatest gratitude to Miss Ann B. Collins and Mr. Stephen J. Carter for their help with data collection and participant screening and recruitment.
REFERENCES


FIGURE LEGENDS

Figure 1. Responses from a single, representative participant in CVC, expressed as a percentage of max, to local skin warming from 33 to 42 °C. Closed circles indicate control for the arm site. Open circles are for the L-NAME treated arm site. Note the differences at baseline for the leg control (closed triangles) versus the L-NAME treated leg site open triangles). Furthermore, note the absence of a difference during the plateau phase under conditions of L-NAME treatment between arms (open circles) and legs.

Figure 2. Average baseline responses to local skin warming at arm and calf in %CVCmax from all 9 participants. Note the higher cutaneous vascular response at the leg sites compared to the arm skin sites. * indicates P < 0.05 compared to arm † indicates P < 0.05 compared to control.

Figure 3. Increase in %CVCmax in response to prolonged local skin warming at arm and leg sites in 9 participants. The leg skin sites had a higher CVC response to 42 °C than the arm skin sites. * indicates P < 0.05 compared to arm † indicates P < 0.05 compared to control.
**Figure 2**

![Graph showing cutaneous vascular conductance (% Max) for Arm and Leg with Control and L-NAME conditions.](image)

- **Control** represented by solid bars.
- **L-NAME** represented by open bars.

**Legend:**
- Arm
- Leg

**Axes:**
- Y-axis: Cutaneous vascular conductance (% Max)
- X-axis: Arm and Leg

**Annotations:**
- *: Arm Control
- †: Arm L-NAME
- #: Leg Control
- ‡: Leg L-NAME
Figure 3
CHAPTER IV

EFFECT OF SYMPATHETIC NERVE BLOCKADE ON REGIONAL SKIN BLOOD FLOW RESPONSES TO LOCAL SKIN WARMING

ABSTRACT

We investigated the role of sympathetic nerves on regional cutaneous vasodilator response to a localized warming stimulus. Forearm and calf sites were instrumented with microdialysis fibers, local heaters, and laser-Doppler probes. Skin sites were heated from 33 °C to 42 °C. Baseline, initial peak, and secondary plateau values were compared between control and bretylium tosylate (BT) treatment sites. During baseline, CVC at arm control sites (15 ± 1 %max) and at arm sites treated with BT (15 ± 1 % max) were not different ($P = 0.5$). CVC at control leg sites (25 ± 2 %max) and at BT sites (29 ± 4 %max) did not differ ($P = 0.34$). CVC was significantly higher in the leg compared to the arm at control site ($P = 0.03$), and at BT site ($P = 0.04$). The initial peak response in CVC for the arm for BT site (62 ± 3 %max) was lower than for the control site (86 ± 2 %max) ($P = 0.04$). The BT treated leg site during the initial peak (78 ± 2 %max) was significantly higher than the control site (70 ± 3 %max) ($P = 0.03$). For BT site, arm CVC (62 ± 3 %max) was lower than that of the leg site (78 ± 2 %max) ($P = 0.02$).

During the plateau phase, arm CVC at control (89 ± 2 %max) were lower than leg control (99 ± 1 %max) ($P = 0.03$). CVC at arm BT site (89 ± 3 %max) was similar to the leg (93 ± 2 %max) ($P = 0.28$). During the plateau phase, CVC in the legs differed between BT (93 ± 2 %max) and control (99 ± 1 %max) ($P = 0.03$). At plateau BT and control did not differ in the arms ($P = 0.47$), and there were no significant differences between the arm and leg BT treated sites ($P =
0.28). We found that leg CVC was higher at baseline than that of the arm under both the control and BT conditions suggesting no differences in sympathetic regulation at rest between the arms and legs.

**Key Words:** Local Heating, Microdialysis, Norepinephrine, Laser-Doppler, Neuropeptide Y
INTRODUCTION

The skin circulation plays a pivotal role during thermoregulation in humans. Controlling the level of perfusion of the cutaneous vasculature manipulates the rate of heat dissipation from the core of the body to the surrounding environment. The change in skin perfusion is largely brought on by changes in core and local skin temperature (Tloc) through two separate sympathetic pathways. The first is a noradrenergic system, relying on the neurotransmitters neuropeptide Y (NPY) and norepinephrine (NE). The second system is a cholinergic active vasodilator system releasing acetylcholine and other unknown transmitter(s) (4).

The response of the skin blood flow to a local warming stimulus is biphasic. In response to increasing Tloc there is a rapid, transient initial peak (4, 13, 20), which is succeeded by a brief nadir and a prolonged secondary vasodilation, commonly referred to as the plateau phase (4, 13, 20). The initial peak is thought to be mediated by sympathetic and possibly sensory nerves (7, 11, 20).

Previously, our laboratory found that cutaneous vascular conductance (CVC) in the arms and legs was different at rest and in response to local skin warming (Study 1). In particular; CVC during the initial peak at leg skin sites was significantly lower than that achieved in the arms (Study 1). Hodges et al. (6, 7) and Tew et al. (30) reported that the initial peak of the vasodilator response to increased Tloc was dependent on sympathetic nerve function. This loss of vasodilation was observed when the skin sympathetic nerves were blocked pre-synaptically (6, 30) or post-synaptically (7). This leads us to believe that leg sites have a lower sympathetic activity than that of the forearm.
As a result, we sought to determine whether the previously observed difference in the initial peak between the arms and the legs (Study 1) was due to sympathetic nerve involvement. To that end, we examined the effects of a pre-synaptic sympathetic nerve blockade, using bretylium tosylate (BT), on the vasodilator response to local skin warming in the arms and legs in young, healthy humans. Based on the findings in Study 1, we hypothesized that blockade of the sympathetic nerves would reduce the initial peak response to local skin warming more in the arms than in the legs.
METHODS

A power analysis indicated that 99 participants would be required with a \( P < 0.05 \) with 80\% power, expected required mean differences and standard deviations were taken from our laboratory’s previous work on skin blood flow (1, 25-28, 30) (nQuery Advisor v. 3).

Volunteers for this study were five healthy men and four healthy women with an average age of 27 ± 4 years. Only individuals not diagnosed with cardiovascular or any metabolic disease and a minimum 19 years old were included. If during the screening process participants were found to be currently using tobacco products, training for an endurance athletic event, or, taking any medication that could affect exercise tolerance or manipulate blood pressure, they were excluded from the study. All participants gave verbal informed consent as well as completed a local Institutional Review Board approved consent form. Participants were instructed not to use caffeine or alcohol for 24 h prior to testing. Additionally, the participants were instructed to drink water ad libitum; but, to withhold all food for 2 h prior to the testing.

All 4 of the female participants were using prescribed oral contraceptives and currently in the high hormone phase of their birth control routine (2, 23). Each participant made two visits to the Exercise Physiology Laboratory at the University of Alabama separated by 3 to 7 days.

During each testing session, the region of the body tested (i.e. forearm or calf) was counterbalanced per day of testing.

Instrumentation and experimental procedure

Participants laid supine on an adjustable gurney and remained there for the duration of the experiment. Two skin sites were chosen on either the forearm or the calf and were prepared
for the placement of microdialysis fibers. As a temporary analgesic, ice packs were placed over the skin sites where the fibers were to be placed (3). Following ice pack placement, microdialysis fibers were placed using the same techniques as previously described by our laboratory (1). The participant was instrumented with 2 custom built microdialysis fibers (1, 3, 5-7, 9, 10). Depending on the day, the microdialysis fibers were either placed in the dorsal aspect of the forearm, or in the lateral aspect of the calf. An integrated skin heater and temperature monitor (model SH02, Moor Instruments Devon, UK) was used to monitor and adjust local skin temperature. Red blood cell flux was measured via laser-Doppler flowmetry (MoorVMS-LDF2, Moor Instruments, Devon, UK) and was used to provide an index of skin blood flow (12, 22). Laser-Doppler flow (LDF) probes (VP12, Moor Instruments, Devon, UK) were placed within a small opening in the skin heaters to monitor red blood cell flux.

Lactated Ringer’s (study vehicle) was perfused at both sites at a rate of 4 µl · min⁻¹ for the first 30 min of the trauma resolution period. After 30 min of trauma resolution, 1 site, either forearm or calf depending on the day, was chosen as a control, and lactated Ringer’s (study vehicle) continued to be perfused. At the other experimental skin site bretylium tosylate (BT) (US Pharmacopeia, Rockville, MD) was administered at a concentration of 10 mM (1, 11). After 30 min of drug infusion, local skin heaters were set to 33 °C (thermoneutral). Baseline measurements were taken following 90 min of trauma resolution (3). After baseline data were recorded (10 min), the same heating protocol as used in Studies 1 and 2 was performed, increasing the Tloc by 0.5 °C · 10 s⁻¹ until Tloc had reached 42 °C (1, 7-9, 28-30). Tloc was maintained at 42 °C for 35 min at which time a stable plateau had been reached representing a
physiological maximum (24). Following the stabilization of the plateau phase, sodium nitroprusside (SNP) was infused at both sites at a concentration of 58 mM for 35 min to pharmacologically induce maximal cutaneous vasodilation (1, 15-18, 29). Blood pressure was measured in the contra-lateral arm every 10 min throughout the duration of the study protocol via auscultation of the brachial artery.

Data Collection and Analysis

Data were collected at 50 Hz and stored on a personal computer to be analyzed offline using signal-processing software (Acqknowledge, Biopac MP150, Camino Goleta, CA). Mean arterial pressure (MAP) was calculated as diastolic blood pressure + \( \frac{1}{3} \) pulse pressure. LDF data were converted to CVC by dividing LDF (mV) by MAP (mm Hg). All data were normalized to maximal vasodilation and expressed as a percentage of maximum achieved in response to a Tloc of 42 °C and 58 mM of SNP (1, 16, 18, 19, 30). Due to the rapid and transient response of the initial peak, 40 s portions of CVC were used. Stable 5-min periods of CVC data were used for both the baseline and the plateau phases.

Text and illustrative results are reported as the mean ± standard deviation. All analysis performed was by paired \( t \)-statistics or when appropriate, a repeated measure analysis of variance (ANOVA). Statistical significance was set \textit{a priori} at \( P < 0.05 \). All data were analyzed using SPSS software version 19.0 (SPSS, Inc., Chicago, Illinois, USA).
RESULTS

When Tloc was held at 33 °C, CVC averaged 15 ± 1 %max at the arm control site and 15 ± 1 %max at the site perfused with BT (Fig. 1). There was no significant difference between these values at 33 °C Tloc (P = 0.50). CVC at control leg site averaged 25 ± 2 %max and at BT site 29 ± 4 %max (P = 0.34). CVC was significantly higher in the legs compared to the arms at control (P = 0.03) and BT treated skin sites (P = 0.04).

At onset of the warming protocol, the initial peak response in CVC at the arm skin site treated with BT was significantly lower than at the control skin site (62 ± 3 %max and 86 ± 2 %max, respectively; P = 0.04) (Fig. 2). In contrast, CVC responses at the leg skin site treated with BT was higher than at the untreated control skin site (78 ± 2 % max and 70 ± 3 %max, respectively; P = 0.03). When comparing the arm and leg sites treated with BT, there was a significant difference in the initial peak response. Arm site (62 ± 3 %max) was lower than that of the leg site (78 ± 2 %max) (P = 0.02). Additionally, the initial peak of the untreated arm (78 ± 3 %max) was significantly higher than that of the leg (63 ± 3 %max) (P = 0.04) (Fig. 2).

In response to increasing Tloc to 42 °C, CVC increased significantly and achieved stable plateaus at all skin sites (P < 0.001, 33 °C vs. 42 °C for all 4 sites). During the plateau phase, with Tloc maintained at 42 °C, CVC at the control sites averaged 89 ± 2 %max and 99 ± 1 %max in the arms and legs, respectively (P = 0.03) (Fig. 3). CVC at the arm and leg sites treated with BT rose to 89 ± 3 %max and 93 ± 2 %max, respectively. These values did not differ significantly (P = 0.28). At a Tloc of 42 °C, CVC was significantly less at the BT treated when compared to
the untreated control sites for legs ($P = 0.03$) but not for arms ($P = 0.47$). There were no significant differences between the arm and leg BT treated sites at 42 °C Tloc ($P = 0.28$).
DISCUSSION

The aim of this study was to examine the contribution of sympathetic nerves to the skin blood flow response to local skin warming in the arms and the legs. We found that the initial peak response was higher in the legs than in the arms, suggesting that sympathetic nerves evidenced differing roles in arm and leg skin. As we have reported previously, in the skin of the arms, the sympathetic nerves play a role in the vasodilator response to local skin warming (6, 7, 30); this is inferred from the reduced response under conditions of sympathetic nerve block. In contrast, sympathetic nerve block in the skin of the legs increases the initial peak response, suggesting a removal of vasoconstrictor effects. In this study, we found similar results to Study 1 and Study 2 in that leg CVC was higher at baseline than that of the arm under both the control and BT treated conditions (Fig. 3). However, when comparing control site to BT treated site there was no difference for either the arm or leg, suggesting little, if any role for sympathetic nerves in the regulation of basal vascular tone in the skin at thermoneutral (Tloc 33 °C) temperatures.

Similar to previous studies utilizing sympathetic blockade we found a decreased initial peak in the arm (7, 11). In contrast, the initial peak at the leg sites increased under sympathetic blockade. This may in part be due to an increase in basal vasoconstrictor tone in the vasculature of the legs, partially attributed to the higher hydrostatic pressure that legs experience being in a more frequent state of dependency.

Increased CVC in the legs when compared to the arms is consistent with our previous findings (Studies 1 and 2). BT treatment had no effect on the plateau phase in the arms indicating
no sympathetic involvement. This finding differs from previous work examining the role of sympathetic nerves in the vasodilator response to local skin warming (6, 7, 11). However, the rate of skin warming in the present study was much faster (0.5 °C · 10 s⁻¹) than was employed in those studies (0.1 °C · min⁻¹). Indeed, Hodges et al. (6) reported that the faster the rate of warming of the skin, the less sympathetic nerves appeared to be involved in the plateau phase. Carter and Hodges (1) demonstrated that rapid, noxious heating abolished the sympathetic nerve involvement to local skin warming in both the initial peak and plateau phases. Thus, there does appear to be a rate-dependency regarding the involvement of sympathetic nerves in the local warming-induced cutaneous vasodilator response. However, sympathetic nerve blockade did significantly lower the plateau phase in the legs. This supports the suspicions raised in Studies 1 and 2 that the leg cutaneous vasculature experiences an increase in sympathetic tone.

While sympathetic nerves influencing vasodilation is an intriguing notion, the role for NE and NPY in the vasodilation to local skin warming is counterintuitive, as the receptors that preferentially bind these neurotransmitters lead to vasoconstriction in almost every vascular bed, in every species, including human skin. This has been demonstrated consistently in studies blocking vasoconstrictor responses in human skin with pre- or post-synaptic inhibition of NE or NPY (7). Further, intradermal perfusion of exogenous NE under baseline conditions elicits vasoconstriction (11), not vasodilation. However, the idea that the vasoconstrictor neurotransmitters are somehow involved in a vasodilator response in human skin is not new. In 1969, Mosley showed a marked reduction in the skin blood flow response to local skin warming following intra-arterial infusion of BT (21). Further, there have been several recently published
reports suggesting some interaction between vasoconstrictor nerve stimulation and vasodilation to local skin warming in humans (11, 30). Indeed, we have presented a hypothesis for this phenomenon, linking NE and NPY with NOS (7). Thus the observed effects of sympathetic nerve blockade on cutaneous vasodilation and the different regional responses are hard to mechanistically describe.

Limitations

The data from the present study cannot delineate among the neurotransmitters that could be involved in the sympathetic response to local warming.

CONCLUSION

The current study found that the basal sympathetic tone in the leg was higher than that of the arm confirming results found previously by our laboratory group (Study 1 and Study 2). However, when comparing arm skin blood flow to leg skin blood flow under conditions of sympathetic blockade the %max CVC was not different. We conclude that utilizing the microcirculation as an indicator of systemic vascular health should be done with caution.

DISCLOSURES

The authors have no disclosures or conflicts of interest.

ACKNOWLEDGMENTS

We thank the participants for their time and commitment to this study. As well we extend our greatest gratitude to Miss Ann B. Collins and Mr. Stephen J. Carter for their help with data collection and participant recruitment and screening.
REFERENCES


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FIGURE LEGENDS

Figure 1. Average baseline responses to local skin warming at arm and calf in %CVCmax from all 9 participants. Note the higher cutaneous vascular response at the leg sites compared to the arm skin sites. * indicates P < 0.05 compared to arm.

Figure 2. Average initial peak responses to local skin warming at arm and calf in %CVCmax from all 9 participants. Arm sites had higher CVC responses to the onset of heating. Note the higher response of the treated legs compared to that of the control legs. * indicates P < 0.05 compared to arm † indicates P < 0.05 compared to control.

Figure 3. Increase in %CVCmax in response to prolonged local skin warming at arm and leg sites in 9 participants. The leg skin sites had a higher CVC response to 42 °C than the arm skin sites. * indicates P < 0.05 compared to arm † indicates P < 0.05 compared to control.
Figure 1
Figure 2
Figure 3

![Graph showing cutaneous vascular conductance (% Max) for Arm and Leg, comparing Control and BT conditions.](image)
CHAPTER V

REFERENCES


APPENDIX

Office of Research
Institutional Review Board for the
Protection of Human Subjects

THE UNIVERSITY OF
ALABAMA
RESEARCH

December 6, 2011

Andrew Del Pozzi
Department of Kinesiology
College of Education
The University of Alabama

Re: IRB Protocol # 11-021-ME
"Skin Blood Flow in Humans with Spinal Cord Injury"

Mr. Del Pozzi:

The University of Alabama IRB has received the revisions requested by the
full board on 11/18/11. The board has reviewed the revisions and your
protocol is now approved for a one-year period. Please be advised that your
protocol will expire one year from the date of approval, 11/10/11.

If your research will continue beyond this date, complete the Renewal
Application Form. If you need to modify the study, please submit the
Modification of An Approved Protocol Form. Changes in this study cannot
be initiated without IRB approval, except when necessary to eliminate
apparent immediate hazards to participants. When the study closes, please
complete the Request for Study Closure Form.

Should you need to submit any further correspondence regarding this
proposal, please include the assigned IRB application number. Please use
reproductions of the IRB approved stamped consent/assent forms to obtain
consent from your participants.

Good luck with your research.

Sincerely,

John C. Higginbotham, Ph.D., MPH
Medical IRB Chair
The University of Alabama
UNIVERSITY OF ALABAMA
INSTITUTIONAL REVIEW BOARD FOR THE PROTECTION OF HUMAN SUBJECTS
REQUEST FOR APPROVAL OF RESEARCH INVOLVING HUMAN SUBJECTS

I. Identifying information

Principal Investigator: Andrew T. DeL Pozzi
Second Investigator: Gary J. Hodges
Third Investigator: None

Department: Kinesiology
College: Education
University: Alabama
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Telephone: 502-859-2050
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E-mail: ATDeL Pozzi@crimso.edu
ghodges1@bamaed.edu

Title of Research Project: Skin blood flow in humans with spinal cord injury

Date Submitted: 10/12/2011
Funding Source: None

Type of Proposal: New

☐ Revision
☐ Renewal
☐ Completed
☐ Exempt

Please attach a continuing review of studies form
Please attach any IRB # at the top of the page

UA faculty or staff member signature: ________________________________

II. NOTIFICATION OF IRB ACTION (to be completed by IRB):
Type of Review: ✓ Full board □ Expedited

IRB Action:
Rejected
Tabbed Pending Revisions
Date: _______________________
Approved Pending Revisions
Date: _______________________

This proposal complies with University and federal regulations for the protection of human
subjects.

Approval is effective until the following date: 11/10/12

Items approved: Research protocol (dated) _______________________
Informed consent (dated) _______________________
Recruitment materials (dated) _______________________
Other (dated) _______________________

Approval signature _______________________
Date 12/6/11
November 5, 2012

Andrew Del Pozzi
Department of Kinesiology
College of Education
The University of Alabama

Re: IRB Protocol # 11-021-ME-R1
“Skin Blood Flow in Humans with Spinal Cord Injury”

Mr. Del Pozzi:

The University of Alabama IRB has received the revisions requested by the full board on 10/22/12. The board has reviewed the revisions and your renewal application is now approved for a one-year period.

Your application will expire on October 11, 2013. You will receive a notice of the expiration date 90 days in advance. If your research will continue beyond this date, complete the renewal portions of the FORM: IRB Renewal Application. If you need to modify the study, please submit FORM: Modification of An Approved Protocol. Changes in this study cannot be initiated without IRB approval, except when necessary to eliminate apparent immediate hazards to participants. When the study closes, please complete the FORM: Request for Study Closure.

Please use reproductions of the IRB approved stamped consent form or information sheet to obtain consent from your participants.

Should you need to submit any further correspondence regarding this application, please include the above application number.

Good luck with your research.

Sincerely,

John C. Higginbotham, Ph.D., MPH
Medical IRB Chair
The University of Alabama
UNIVERSITY OF ALABAMA
INSTITUTIONAL REVIEW BOARD FOR THE PROTECTION OF HUMAN SUBJECTS
REQUEST FOR APPROVAL OF RESEARCH INVOLVING HUMAN SUBJECTS

I. Identifying information

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<tr>
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<td>205-348-2151</td>
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<tr>
<td>FAX</td>
<td><a href="mailto:ATDelPozzi@crimson.ua.edu">ATDelPozzi@crimson.ua.edu</a></td>
<td><a href="mailto:ghodges1@bamaed.ua.edu">ghodges1@bamaed.ua.edu</a></td>
</tr>
</tbody>
</table>

Title of Research Project: Skin blood flow in humans with spinal cord injury

Date Submitted: 10/12/2011
Funding Source: none

Type of Proposal: New

Please attach a renewal application
Please attach a continuing review of studies form
Please enter the original IRB # at the top of the page

UA faculty or staff member signature: ____________________________

II. NOTIFICATION OF IRB ACTION (to be completed by IRB):
Type of Review: Full board Expedited

IRB Action: Approved

Revised

Tabled Pending Revisions

Approved Pending Revisions

Approved-this proposal complies with University and federal regulations for the protection of human subjects.

Approval is effective until the following date: 10/14/13

Items approved:
- Research protocol (dated)
- Informed consent (dated)
- Recruitment materials (dated)
- Other (dated)

Approval signature: ____________________________ Date: 11/5/12