THE PENNSYLVANIA STATE UNIVERSITY
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DEPARTMENT OF KINESIOLOGY

Nitric Oxide Dependence Calculations: Determining Nitric Oxide Contributions for 39°C and 42°C Local Heating Protocols

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ABSTRACT

Endothelial microvascular function of skin blood vessels is predictive of the cardiovascular disease risk (Holowatz et al., 2008). This study aims to examine differences in nitric oxide- (NO) dependent vasodilation during 39°C and 42°C local heating protocols and determine if the method of calculating NO contribution influences findings. We hypothesized that NO would contribute more to vasodilation when the skin blood flow (SkBF) values are subtracted (traditional) rather than the contribution value calculated from the percent change (relative). In addition, we hypothesized that older subjects would have reduced NO-dependent vasodilation in both protocols and with both methods of analysis. We conducted a cross-sectional study of 31 young (12 male/19 female, 24 (4) years) and 18 older (5 male/13 female, 68 (7.517)) participants. NO-dependent vasodilation was determined by perfusing (intradermal microdialysis) 15 nM N-nitro-l-arginine methyl ester during the heating plateau of the 39°C and 42°C protocols. Red blood cell flux (laser-Doppler flowmetry) was used to calculate cutaneous vascular conductance (CVC; red blood cell flux/mean arterial pressure), and values were represented as a percentage of maximum (%CVCmax) (28 nM sodium nitroprusside + 43°C). We found that when NO contribution is analyzed with the traditional method, NO contribution was lower in the older group than the young group in the 39°C heating protocol ($p=0.030$) but not in the 42°C protocol ($p=0.262$). We also found that NO contribution was lower in the 39°C protocol in the older group ($p<0.001$), but there were no differences in protocols in the younger group ($p=0.967$). When NO contribution is analyzed with the relative method, there were no age-related differences for the 39°C heating protocol ($p=0.339$). We also found that the NO contribution was lower in the 39°C protocol compared to the 42°C for the older group ($p=0.039$).

In conclusion, age-related and protocol-related responses to local heating depend on how responses are analyzed.

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Chapter 1

Literature Review

Regulation of Skin Blood Flow: Neutral, Local, Vasoconstriction, Vasodilation

Thermoregulation is used to maintain normal body temperatures when the body encounters thermal stress (Romanovsky, 2014). When the skin experiences temperature changes, this information is relayed to the hypothalamus, eliciting the response of heat dissipation when the skin temperature increases, and the body responds with heat conservation when the skin temperature decreases. To regulate normal body temperatures, the body modifies the amount of heat released from the body and the amount of heat created. One important response to regulate body temperature changes is SkBF (Charkoudian, 2003).

The SkBF is controlled by neurons both centrally and locally. The non-glabrous skin is innervated by sympathetic vasodilator and sympathetic adrenergic vasoconstrictor nerves (Wong & Hollowed, 2017). Activation of sympathetic nerves can result in changes in either an increase or decrease in SkBF via vasodilation or vasoconstriction, respectively (Osilla et al., 2022). During thermoneutral states, the body maintains normal temperatures via the tonically active vasoconstrictor system. This system increases SkBF in response to heat exposure and decreases SkBF in response to cold exposure (Charkoudian, 2010).

This system's subtle changes allow the vasomotor tone to thermoregulate the skin and the body temperature. When there is an increase in body temperature, reflex vasodilation occurs. The initial reflex of vasodilation is considered "active" vasodilation. Active vasodilation is paired with a withdrawal of vasoconstrictor tone (Kamijo et al., 2005; D. L. Kellogg et al., 1998).

Acetylcholine and co-transmitters are also released from cholinergic sympatric nerve terminals (Dean L. Kellogg et al., 1995; Low et al., 2020).

The second phase of reflex vasodilation is largely mediated by NO (Coffman, 1989). The mechanism of active vasodilation requires responses from the endothelial and vascular smooth muscle cells, dependent on NO's bioavailability (Sandoo et al., 2010). NO is released from the endothelial cells via stimulation of the neurotransmitters, which initiates "active" vasodilation of the vascular smooth muscle (D. L. Kellogg et al., 1998).

Vasodilation increases SkBF in response to heat exposure or exercise. Metabolic heat production is caused by exercise. To decrease body core temperature, warmed blood from the core is transported to the body's skin at the periphery (Charkoudian, 2010). This blood flow facilitates dry heat loss from the skin to the environment (i.e., heat loss via conduction, convection, and radiation) (Périard et al., 2021). Minor adjustments to the body temperature are largely regulated by modulating SkBF; however, when the requirement for heat loss exceeds that which can be dissipated via dry heat loss alone. Sweating is initiated to facilitate evaporative heat loss. The sweating response is also an efferent response to increases in core temperature. Once sweating is initiated, the SkBF response primarily transports heat from the core to the skin, where it can be lost through the evaporation of sweat (Cramer et al., 2022). Sweating decreases the skin's temperature and cools the blood in the dilated vessels (Charkoudian, 2010). Increased SkBF and sweating will increase proportionally to the internal body temperature until the

internal body temperature returns towards resting values or stable core temperature (97-99°F) or heat balance is attained (Osilla et al., 2022).

Using the Skin as a Model of Circulation: Microvascular Function as a Predictor of Systemic Vascular Dysfunction

The human cutaneous circulation is a representative model vascular bed for assess of overall vascular health because this skin is accessible to multiple perturbations that can elicit vasodilation through specific pathways. Moreover, different minimally invasive approaches can be utilized to dissect specific signaling mechanisms pharmacologically. Local heating paired with intradermal microdialysis can be used to directly assess NO production, providing insight into microcirculatory function and dysfunction. Reductions in cutaneous response to local heating can indicate microvascular dysfunction, making it a predictor for some vascular diseases and an indicator of cardiovascular health (Low et al., 2020). Evaluation of SkBF response can also be used to follow the progression of a disease or the success of treatment for improving microvascular function (Holowatz et al., 2008). Microvascular dysfunction often precedes large vessel impairment, and altered skin circulation can determine the presence of cardiovascular disease. Endothelial and smooth muscle dysfunction can be measured in the cutaneous vascular beds, which rely on vasodilatory and vasoconstrictive function (Holowatz et al., 2008). (The relationship between endothelial cells and vascular smooth muscle is described in more detail below).

The skin has a high vasodilatory reserve, permitting substantial increases in vasodilation to occur when the skin is stimulated with thermal stress or pharmacological stimuli (Wong & Hollowed, 2016). To examine cutaneous circulation, minimally invasive techniques like laser-

Doppler flowmetry can provide insight into microvascular pathology and microcirculatory function. Vasodilation and vasoconstriction can be elicited in small areas of the skin using intradermal microdialysis to provoke hyperemia (Holowatz et al., 2008; Roustit & Cracowski, 2012). These techniques allow manipulation of the microvascular pathways involved in cutaneous microvascular dysfunction. Microdialysis involves continuously perfusing drugs into the intradermal layer of the skin (Figure 1), and the effects of these drugs on the microvascular vasodilatory response are measured via laser-Doppler flowmetry (Cracowski et al., 2006). The pairing of these two techniques provides insight into microvascular dysfunction in humans (Holowatz et al., 2008).

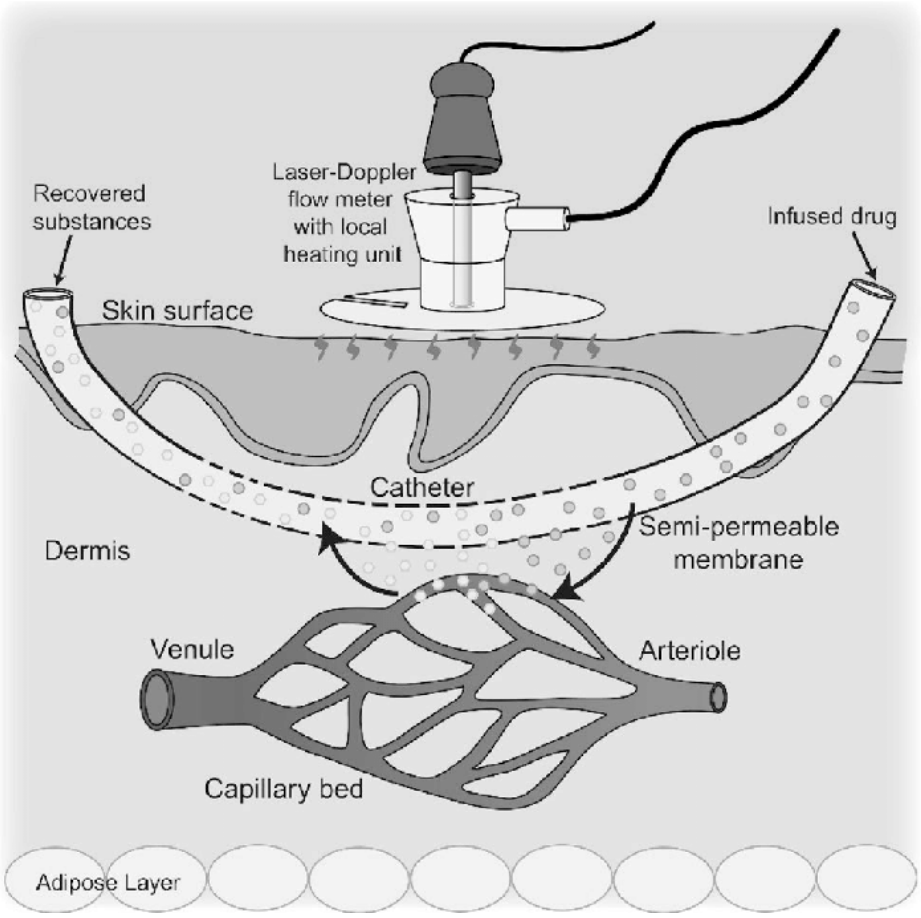

Figure 1 Intradermal Microdialysis Technique Paired with Laser-Doppler Flowmetry (Kenney, 2017).

Illustration of intradermal microdialysis technique. A dialysis membrane inserted into the skin acts as a capillary bed. Pharmacological agents are perfused into the skin with microdialysis. Skin blood flow can be measured when microdialysis is paired with laser-Doppler flowmetry.

Anatomy of the Skin: The Role of Endothelium in Heat Exchange

The skin contains a vascular network of vessels with terminal arterioles, papillary loops, and post-capillary venules. The papillary loops play a major role in determining heat exchange with the environment. These loops are located close to the dermal-epidermal junction, as seen in

Figure 2, with a high thermal gradient and blood flow. Innervated arterioles control blood flow in the papillary loops. These arterioles make up the lining of endothelial cells (Holowatz et al., 2008; Low et al., 2020).

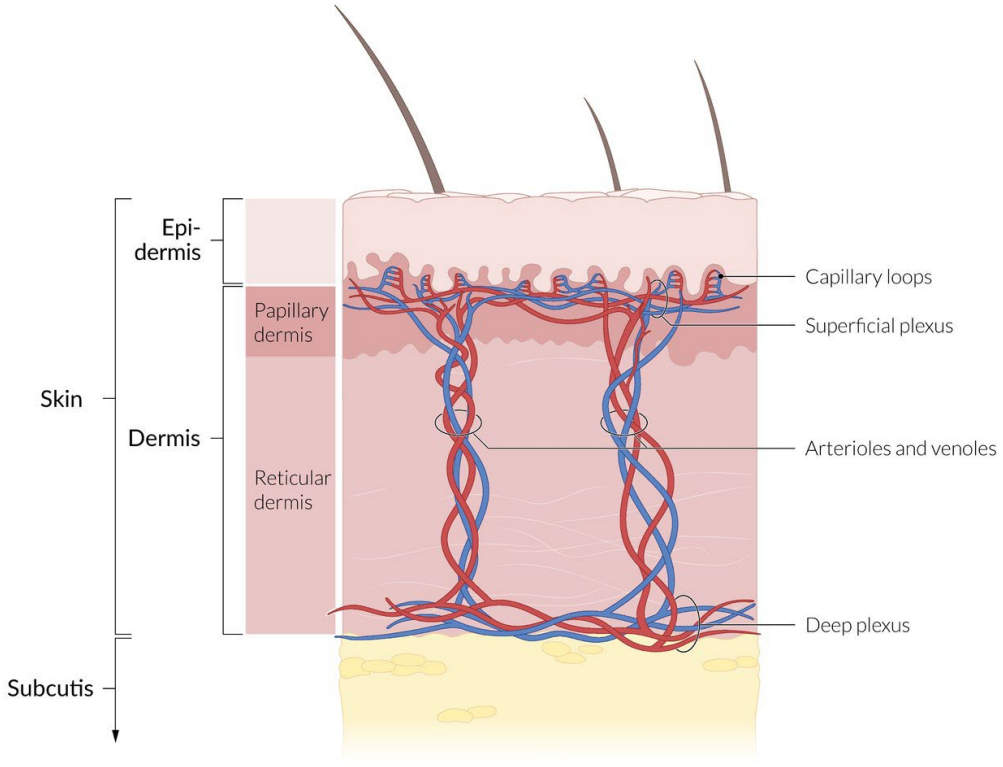

Figure 2 Layers of the Skin with Vasculature (Fuchs, 2007).

Schematic representation of the layers of the skin including capillary loops and the superficial plexus and arterioles of the dermis layer.

Laser-Doppler Flowmetry for Measurement of Cutaneous Blood Flow and Microvascular Function

Local heating, or cutaneous thermal hyperemia, produces vasodilation when the skin is heated to above resting temperatures. Laser-Doppler flowmetry measures SkBF as a response to vasodilation using pharmacological and nonpharmacological agents. As seen in Figure 3, laser-

Doppler flowmetry uses the frequency change in light reflected off moving objects like red blood cells (McGarr et al., 2023). The magnitude and frequency of the changes in light are related to the velocity and number of red blood cells, which are translated into electrical signals (Low et al., 2020).

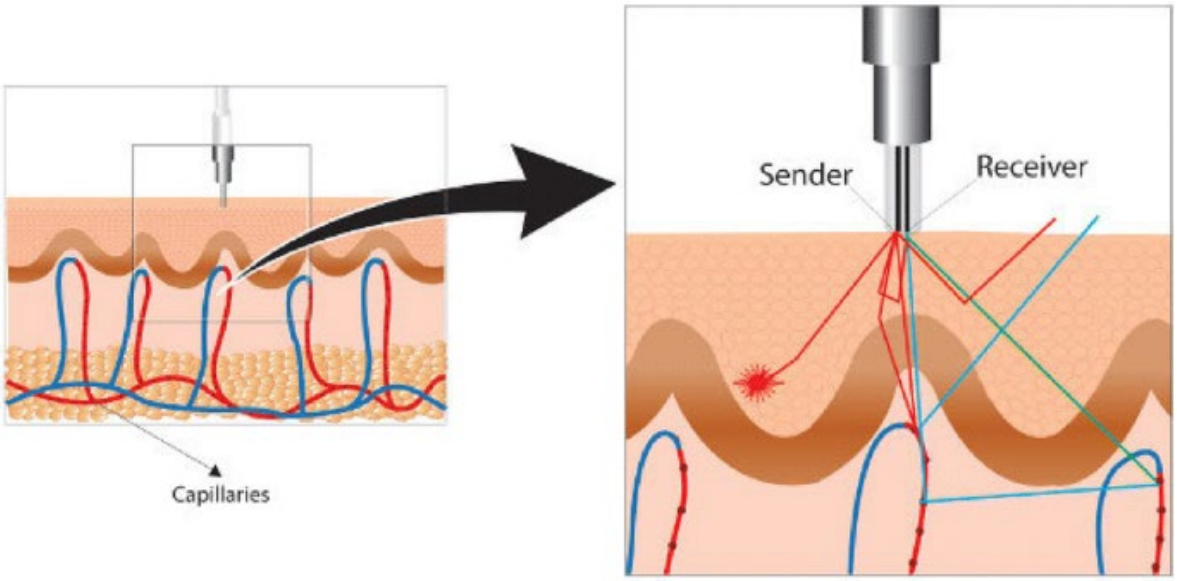

Figure 3 Laser-Doppler Flowmetry Laser Light Pathway (Low et al., 2020).

Laser-Doppler flowmetry determines skin blood flow as a fiber-optic probe emits light that enters the tissue (sender), and the blood cells that come into contact with the laser reflect it (receiver), known as a doppler shift.

Cutaneous vascular conductance (CVC) can be determined from the ratio between the laser-Doppler-flux red blood cells flux and mean arterial pressure (MAP) (Journeay et al., 2005). A %CVCmax was determined by determining CVC during maximal heating of 43°C and representing CVC values as a %CVCmax (Choi et al., 2014).

Nitric Oxide Contribution to the Local Heating Response: 39°C and 42°C Protocols

Two local heating temperatures have been previously used to assess the vasodilatory response, including heating the skin: 39°C and 42°C. The local heating response is mediated by two distinct mechanisms. The first is an initial axon reflex that is mediated by NO and endothelium-derived hyperpolarizing factors (Brunt & Minson, 2012; Minson et al., 2001). The secondary phase is largely dependent upon the generation of NO from the endothelial nitric oxide synthase (eNOS) (Mayer et al., 1989). This release of NO initiates "active" vasodilation of the vascular smooth muscle, as seen in Figure 4 (Dean L. Kellogg et al., 1995).

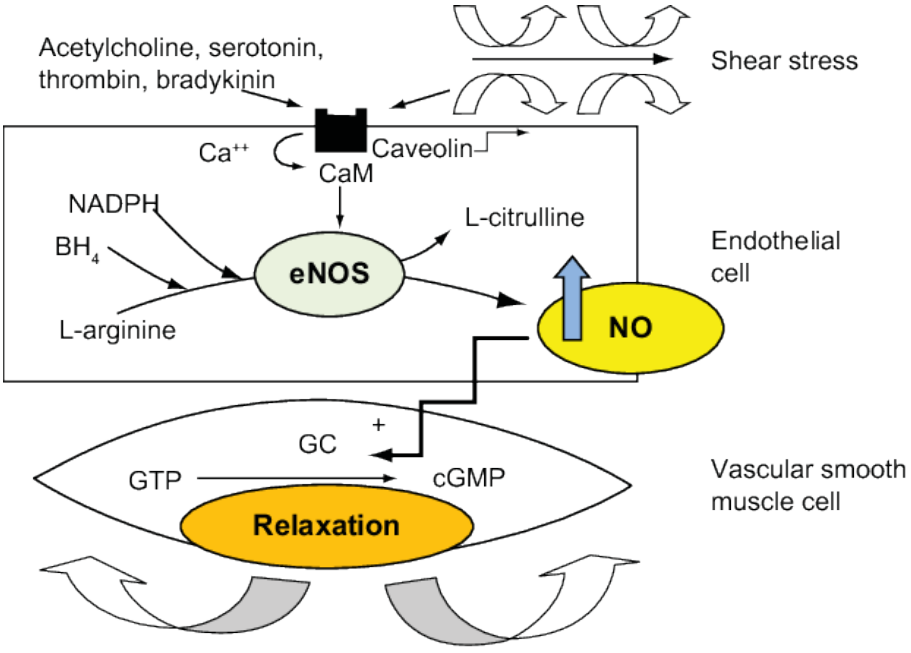

Figure 4 Nitric Oxide Synthesis in Endothelial Cells (Zhu et al., 2016).

Endothelial nitric oxide synthase's (eNOS) role in blood flow. eNOS synthesis is triggered by acetylcholine, sheer stress, etc. eNOS converts L-arginine into NO. In smooth muscle cells, eNOS reacts with guanylate cyclase (GC) and converts guanosine triphosphate (GTP) into cyclic guanosine monophosphate (cGMP), relaxing the smooth muscle.

The L-arginine methyl ester (L-NAME) is a pharmacological agent that acts as a competitive inhibitor for eNOS. Perfusing this agent into the skin via microdialysis once a plateau in SkBF is reached determines that the plateau is more reliant on NO at 39°C compared to 42°C. NO contribution was represented as a %CVCmax. Evaluating nitric oxide contributions in vasodilation once the plateau phase has been reached can determine NO-dependent vasodilation in humans and assess their cutaneous microvascular functions. NO contribution is important because it is a vasodilator and is vasoprotective. The availability of nitric oxide, therefore, contributes to microvascular function (Celermajer, 1997).

Examining Nitric Oxide Contribution Calculations as a Traditional or a Relative Value

In this study, NO contribution is determined by calculating the difference between the heating plateau and post-L-NAME perfusion values (traditional calculation). NO contribution was also examined as a percentage of absolute NO contribution (relative calculation). NO as a percentage is calculated by determining the percentage change in SkBF (recorded as %CVCmax) between the heating plateau and the post-L-NAME perfusion plateau, as seen in Figure 5.

$$Traditional\ NO\ Contribution = (Heating\ \%CVCmax) - (LNAME\ \%CVCmax)$$

$$Relative\ NO\ Contribution = \frac{(Heating\ \%CVCmax) - (LNAME\ \%CVCmax)}{Heating\ \%CVCmax} * 100$$

Figure 5 NO Contribution Analysis Methods: Traditional and Relative.

These equations describe the methods for determining nitric oxide (NO) contribution using traditional and relative analysis methods. These two equations use the heating and L-arginine methyl ester (L-NAME) SkBF values normalized to a percentage of maximum (%CVCmax).

Aims of the Thesis

Therefore, the primary aim of this thesis is to investigate whether the method of analysis, traditional versus relative NO-dependence calculations, modifies whether NO contribution differs depending on the heating protocol (i.e., 39°C and 42°C), NO contribution differs between young and older adults, and whether the interaction of these factors, protocol, and age, affects NO contribution in either of the analysis methods.

As a secondary aim, this thesis will explore contributing factors underlying any differences observed in the aims above. Specifically, this thesis will explore age and heating protocol-related differences between phases of the heating protocol to delineate the source of any differences related to the heating condition, age, and/or analysis method.

Chapter 2

Introduction

Vasodilation occurs when the smooth muscle walls of the blood vessels relax and increase in diameter. This increased diameter allows more blood to flow through the vessels (Ramanlal & Gupta, 2022). Endothelial cells make up the lining of blood vessel walls and play a central role in relaxation, as seen in Figure 6. During vasodilation, the mechanoreceptors of endothelial cells signal surrounding cells to relax smooth muscle and increase blood flow. The smooth muscle relaxation is partly due to the vasodilator NO. Endothelial cells produce NO by the amino acid L-arginine using the eNOS enzyme (Dinerman et al., 1993).

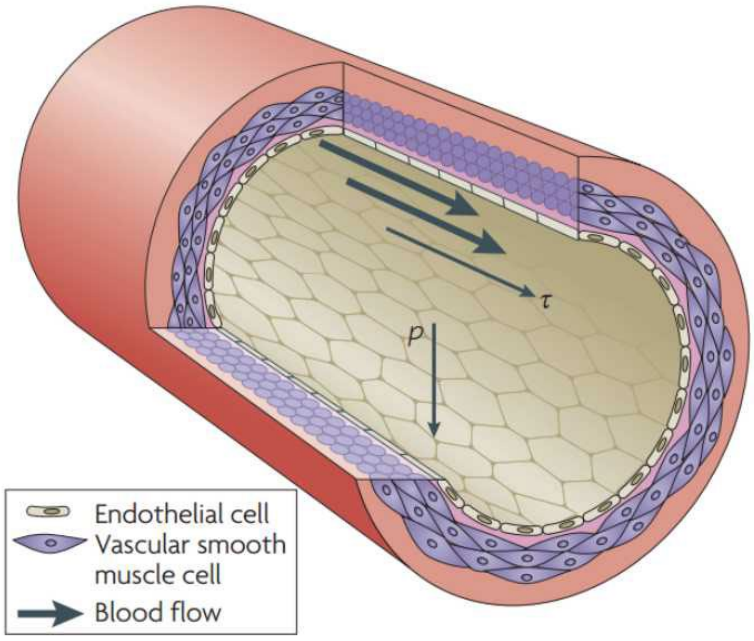

Figure 6 Blood Vessel Anatomy (Carciati, 2017).

In the depiction of the lining of a blood vessel, the inner layer is covered in endothelial cells adjacent to the middle layer of vascular smooth muscle cells. The contraction of vascular smooth muscle cells enables blood to flow through the vessels.

Thermoregulation causes cutaneous vasodilation when the body's core temperature increases. This vasodilation increases blood flow to transfer heat via convection and reduces body core temperature (Charkoudian, 2010; Wong & Hollowed, 2016). Similar to increased core temperature, local increases in skin temperature can cause local vasodilation at the area of skin being warmed. Blood vessels' ability to vasodilate in response to changes in skin and core temperature regulates the volume and rate of SkBF.

Common protocols to assess SkBF involve heating a local area of the skin of the forearm to 39°C and 42°C at a rate of 0.1°C per second (Choi et al., 2014). Pharmacological agents are perfused into the skin during local heating to determine NO contribution and maximal SkBF. This perfusion method is paired with laser-Doppler flowmetry to measure red blood cell flux and calculate an index of SkBF. This measurement technique is useful when studying the endothelial function of skin microvasculature. Ringer solution (isotonic control vehicle) is perfused through the microdialysis fiber during the initial gradual heating to 39°C or 42°C. When locally heating the skin, the SkBF response is characterized by an initial peak in SkBF due to a sensory nerve axon reflex (Minson et al., 2001). After a temporary decrease in blood flow, there is a second prolonged plateau of SkBF (i.e., often referred to as a heating plateau).

The secondary plateau when heating the skin to 39°C has been shown to be largely dependent on NO (Choi et al., 2014; Minson et al., 2001). This was observed by infusing L-NAME, an eNOS antagonist, into the skin using a microdialysis fiber. At 39°C, the blocking of eNOS via L-NAME decreased the heating plateau the greatest (82.8(4.2%)), meaning NO contributes most to increased SkBF or vasodilation at this secondary plateau. L-NAME had a greater effect on diminishing the heating plateau at 39°C compared to 42°C local heating (81.5(3.2%)) (Choi et al., 2014). The understanding that the increase in SkBF or vasodilation

from heating to 39°C is NO-dependent is important because NO availability underlies many vascular diseases and conditions of older individuals. Therefore, the measurement of NO-dependent microvascular function can be clinically valuable (Holowatz et al., 2008). In addition, it is common to use both 39°C and 42°C with local heating protocols.

This study also heated the skin to 43°C to provoke maximum SkBF. The skin is first perfused at this temperature with the Ringer solution through the microdialysis fiber, followed by sodium nitroprusside (SNP; a NO donor) to determine the maximum SkBF. The SkBF is recorded as %CVCmax. CVC is determined by dividing the SkBF or red blood cell flux measurements by the mean atrial pressure (MAP) (Choi et al., 2014).

The 39°C protocol was developed because the amount of vasodilation appears to be mostly reliant on the production of NO. Whereas in the 42°C protocol, other endothelium-derived vasodilators are involved. However, it is unknown if this was a factor of the method of calculation. To calculate NO contribution, one study took the SkBF value as a %CVCmax after L-NAME perfusion and subtracted it from the secondary plateau SkBF value as a %CVCmax (traditional method) (Minson et al., 2002). However, NO contribution can be calculated with a different method by finding the percent change in NO (relative method). This method of calculating percent change is determined by calculating the percent change between the secondary plateau SkBF value as a %CVCmax and the SkBF value as a %CVCmax after L-NAME perfusion as seen in Figure 5.

This thesis aims to determine if NO contribution differs depending on if the traditional or the relative analysis method is used for the 39°C and 42°C local heating protocols.

We hypothesized that NO would contribute more to vasodilation when the SkBF values are determined with the traditional analysis method, as shown in previous studies (Minson et al.,

2001, 2002; Roberts et al., 2017), rather than the contribution value calculated from the relative analysis method.

Chapter 3

Methods

Participants

Forty-nine individuals participated in this study (young: n=31, older: n=18). Subject characteristics are presented in Table 1. The characteristics of 6 participants were not obtained ([BMI]); n=[6], [Age]; n=[4]). Participants underwent medical screening and blood chemistry analysis (Quest Diagnostics, Pittsburgh, PA). Participants did not use tobacco and were not taking prescriptions with primary or secondary cardiovascular effects. All women were premenopausal. The menstrual phase was not controlled in women participants. Participants were told not to consume caffeine or alcohol nor participate in vigorous physical activity 12 hours before visiting the laboratory.

Table 1 Subject Characteristics. Data are Mean (SD)

	Young	Older	p-values
Age (years)	24 (3)	68 (8)	0.000
BMI (m^2*kg)	24 (3)	27 (4)	0.010
SBP (mmHg)	112 (12)	123 (18)	0.015
DBP (mmHg)	71 (6)	76 (9)	0.031

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Skin Blood Flow Procedures

Two microdialysis fibers (CMA Linear 31 probe, 55 kDa, Harvard Apparatus, Holliston, MA) were placed into the ventral aspects of the forearm for the delivery of pharmacological agents. The pharmacological agents were mixed before use and dissolved in Ringer solution (Acrodisc; Pall, Port Washington, NY). All solutions were perfused through the fiber at a rate of 2 μL/min (Bee Hive controller and Baby Bee micro-infusion pumps; Bioanalytical Systems, West Lafayette, IN). Fibers were placed under local heaters set to 39°C and the other to 42°C. After microdialysis fiber insertion, participants rested for 60-90 minutes to resolve hyperemia that results from needle insertion trauma. This increased blood flow can confound the determination of baseline SkBF (Holowatz et al., 2008). Brachial blood pressure was taken in the final minute of each stage of the protocol (baseline, heating plateau, L-NAME plateau, maximum) (Cardiocap; GE Healthcare, Milwaukee, WI; Connex Spot Monitor, Welch Allyn, Skaneateles Falls, NY).

Laser-Doppler flowmetry probes were placed over the heating sites to continually measure changes in SkBF (VP12 and VHP2; Moor Instruments, Wilmington, DE). Blood flow is measured as the flux in red blood cells through sub-capillary vascular beds and nutritional capillaries (Abularrage et al., 2005). The flux is determined by measuring a wavelength of light that meets a moving blood cell. The magnitude and frequency of changes in wavelength are related to the number and velocity of the blood cells (Cracowski et al., 2006).

Baseline SkBF values were taken as a 10-minute average before the local heating protocol of 39°C and 42°C. The initial increase in SkBF from localized heating is due to an axon reflex followed by a nadir and a blood flow plateau after 40 minutes. After the plateau had been established, 15 mM L-arginine methyl ester (L-NAME) was perfused into the skin to determine

NO-dependent vasodilation by inhibiting eNOS (Choi et al., 2014). After a plateau is reached with L-NAME perfusion, 28 mM of sodium nitroprusside (SNP) is perfused along with local heating to 43°C (Dillon et al., 2022). The maximum CVCmax is determined following the 39°C and 42°C local heating from these measurements of maximum skin vasodilation.

Data Analysis

Data were recorded at 40 hertz and analyzed in LabChart. Red cell flux (arbitrary perfusion units) was measured at each protocol phase. The baseline SkBF value was averaged between a 5 to 10-minute time period. The heating and L-NAME plateaus phases were averaged between a 2 to 5-minute time period. The maximal SkBF response was taken from a 30-second time period during the highest peak value during the protocol. Absolute CVC values (CVCraw) were calculated by dividing the MAP by the resulting perfusion unit values. Due to the heterogeneity of capillary density at each microdialysis site, CVC was normalized to %CVCmax. Percent of CVC maximum was calculated by normalizing measurements to the peak CVC value.

NO contribution was calculated using two methods. The traditional method determined NO contribution by subtracting the heating plateau SkBF from the L-NAME SkBF value and representing the value as a %CVCmax. The second method, or the relative calculation method, took the percent change between the heating plateau SkBF and the L-NAME plateau SkBF value and represented the value as a %CVCmax.

Statistical Analysis

To address our primary aim of investigating whether analysis methods modify heating protocol and/or age-related differences in NO-contribution, NO-contribution, derived via each analysis method, was analyzed using a linear mixed-effects model (Jamovi, Version 2.3.21.0) (Love et al., 2022). We used a multiplicative model, whereby fixed effects were inputted as a group (young, older), heating protocol (39°C, 42°C), and an interaction term.

To address our secondary aim of exploring the source of any potential differences in NO-contribution by investigating age and heating protocol-related differences in SkBF across phases of the protocol (baseline, heating, L-NAME, and, for non-normalized CVC values, maximum SkBF response), non-normalized CVCraw and %CVCmax values were also analyzed, adding protocol phase as a fixed effect. Given our aim to explore specific differences across protocol phases, a three-way interaction term was also added to the model, in lieu of any two-way interactions. Participant ID was added as a random effect (random intercept models) for all analyses. When a significant main or interaction effect was detected in our primary analysis, post-hoc pairwise contrasts were compared with Bonferroni corrections for multiple comparisons.

Due to the exploratory, hypothesis-building nature of the secondary analysis and the lack of statistical power for this analysis, these p-values were not corrected for multiple comparisons. Significance was set to $p<0.05$. Participant characteristics of age, BMI, systolic blood pressure, and diastolic blood pressure were analyzed using a two-tailed t-test (Microsoft Excel, Version 2301; Microsoft Corporation, 2023) ("Microsoft Excel," 2023). All results are displayed as mean (standard deviation).

Chapter 4

Results

Skin Blood Flow Responses

Figure 7 displays the NO contribution represented after the traditional and relative calculations for local heating for the 39°C and 42°C local heating protocols. Figure 8 displays the SkBF responses as CVCraw and %CVCmax at each phase for the 39°C and 42°C heating protocols.

Traditional Method Protocol and Age-Dependent NO Contribution Differences

It was determined if the analysis method modified whether NO contribution differed between protocols and age groups for the traditional analysis method. There was no overall effect of protocol ($p=0.750$) or group ($p=0.209$); however, there was a protocol*group interaction effect ($p=0.015$). The post-hoc contrasts revealed that NO contribution was 15.3 [3.0, 27.5] percent-points lower in the older group than the young group in the 39°C heating protocol ($p=0.030$) but not in the 42°C protocol ($p=0.262$). Further, NO contribution was 26.6 [13.2,40.0] percent-points lower in the 39°C protocol in the older group ($p<0.001$), but there was no difference between protocols in the young group ($p=0.967$).

Relative Method Protocol and Age-Dependent NO Contribution Differences

It was determined if the analysis method modified whether NO contribution differed between protocols and age groups for the relative analysis method. There was no overall effect of protocol (p=0.406) or group (p=0.973); however, there was a protocol*group interaction effect (p=0.029). The post-hoc contrasts revealed no difference in NO contribution between groups for either protocol (p>0.203). In addition, the older group's NO contribution was 11.1 [20.2, 1.92] percent-points lower in the 39°C compared to the 42°C protocol (p=0.039), but there was no difference between protocols for the young group (p=0.411) (Figure 7).

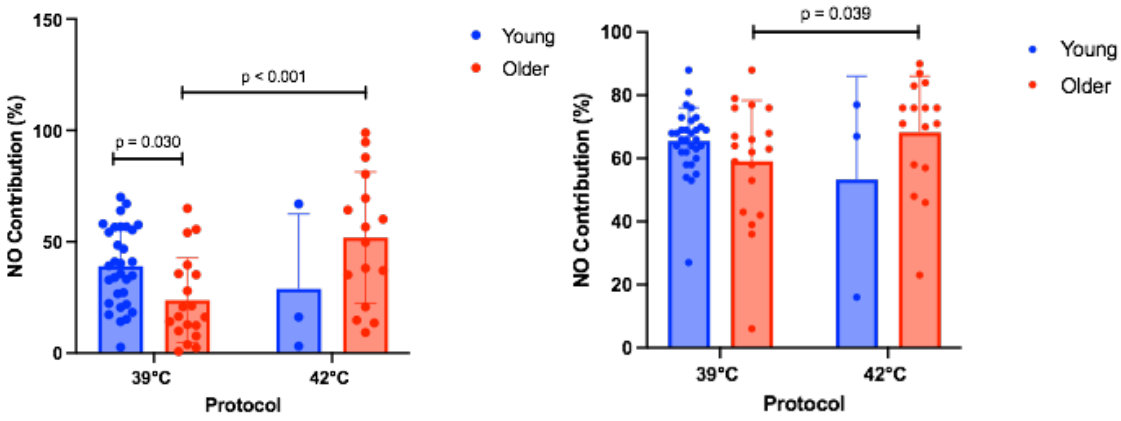

Figure 7 %CVCmax for 39°C and 42°C Local Heating Protocols: Traditional Calculations (Left) & Relative Calculations (Right).

Nitic oxide (NO) contribution during the 39°C and 42°C local heating protocol with the traditional and relative analysis method for the young and older groups.

CVCraw Phase Comparison Analysis Between Protocols and Ages

As expected, there was an overall effect of phase (p<0.001) across groups and protocols. There was also a main effect of protocol (p<0.001), but not group (p=0.894). However, there was

a three-way, group*phase*protocol interaction effect ($p<0.001$). Post hoc contrasts revealed that CVCraw values during the heating plateau were 0.32 [0.03, 0.62] PU/mmHg lower in the older group than the younger group in the 39°C protocol ($p=0.033$) (Figure 8) but were not different between groups during the heating plateau in the 42°C protocol ($p=0.405$), nor between groups during any other phase of either protocol ($p\geq0.117$). Further, CVCraw values during the heating plateau were 0.31 [0.01, 0.61] PU/mmHg lower in the 39°C than the 42°C protocol in the older group ($p=0.044$) but not in the young group ($p=0.344$) (Figure 8). CVCraw values were also 0.57 [0.27, 0.87] PU/mmHg higher during maximal SkBF (44°C+SNP) in the 39°C than the 42°C protocol in the older group ($p<0.001$), but not in the young group ($p=0.306$). There were no other between-protocol differences in either group at any other phase ($p\geq0.666$).

%CVCmax Phase Comparison Analysis Between Protocols and Ages

There was an overall effect of phase ($p<0.001$) across groups and protocols. There was no main effect of protocol ($p=0.149$) or group ($p=0.783$). There was a three-way, group*phase*protocol interaction effect ($p<0.001$). Post hoc contrasts revealed that the %CVCmax values during baseline were 0.114 [0.01, 0.21] PU/mmHg lower in the older group than the younger group in the 39°C protocol ($p=0.028$). The %CVCmax values during the heating plateau were 0.23 [0.13, 0.33] PU/mmHg lower in the older group than the younger group in the 39°C protocol ($p<0.001$). There was no difference between groups during the heating plateau ($p=0.062$) or the baseline ($p=0.898$) in the 42°C protocol, nor between groups during any other phase of either protocol ($p\geq0.115$). Further, %CVCmax values during the heating plateau were 0.33 [0.42, 0.24] PU/mmHg lower in the 39°C than the 42°C protocol in the

older group (p=0.044), but not in the young group (p=0.341) (Figure 8). There were no other between-protocol differences in either group at any other phase (p≥0.160).

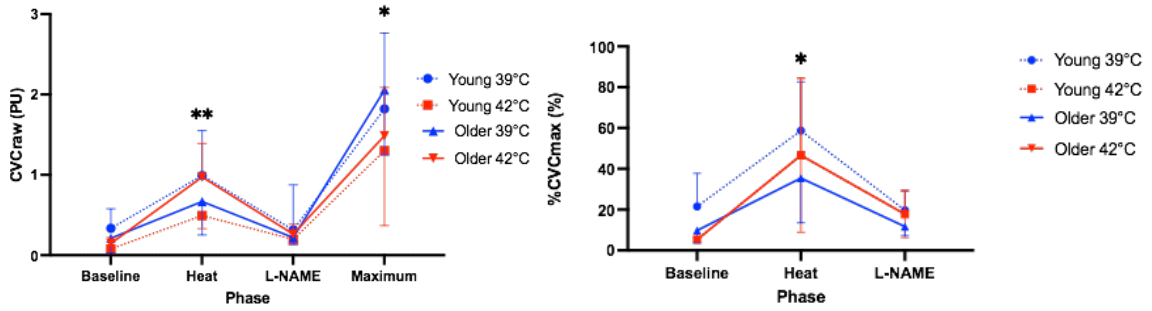

Figure 8 CVCraw and %CVCmax at each Phase of Local Heating Protocols.

The absolute cutaneous vascular conductance (CVCraw) and percentage of maximal cutaneous vascular conductance (%CVCmax) values during the phases of the 39°C and 42°C local heating protocols for the young and older groups.

Chapter 5
Discussion

This study investigated young and older participants' SkBF responses during two commonly used local heating protocols. The blood flow responses were measured during baseline, heating plateau, L-NAME plateau, and maximal SkBF. We then examined the difference in NO contribution values between the heating and plateau phases and represented those values in two forms: as a difference in values or as a percent change.

The main finding of this thesis is that age-related responses depend on how responses are analyzed. This means that depending on how NO is represented, with the traditional or relative method, affects the interpretation of whether young and older individuals exhibit differences in NO contribution between the protocols. We also found that protocol-related responses also depend on how responses are analyzed. This means that the way that NO contribution responses are presented also influences if NO contribution appears to be significantly different between protocols for the age groups, as age-related differences for protocols were only observed using the 39°C protocol.

Perspectives

Using a 39°C protocol and traditional analysis method reveals an age-dependent difference in NO contribution, whereby only the older group shows a lower NO contribution in the 39°C than the 42°C protocol, and the NO contribution was lower in the older group than the younger group in the 39°C condition only. In the young group, we observed no difference in NO contribution between the protocols. This is in contrast to Choi's study, which included young

adults only. Interestingly, we did see a protocol-dependent difference in the older group. However, this was also in contrast to Choi's finding, given that NO contribution was lower in the 39°C protocol.

The relative analysis method revealed an age-dependent difference in NO contribution, whereby only the older group showed a lower NO contribution in the 39°C protocol than in the 42°C protocol. This was also a finding from the traditional analysis method. An important difference between the relative and traditional analysis methods is that the relative analysis method revealed no difference in NO contribution between groups for either protocol, while the traditional method had an age-dependent difference in NO contribution within the 39°C protocol.

The CVCraw values during the heating plateau were lower in the older group than the younger group in the 39°C protocol. These differences were not present in the 42°C protocol heating plateau or any other phase of either protocol. This supports the finding that the age-related difference in NO contribution by traditional analysis methods is due to a reduced heating plateau, particularly in the 39°C condition. The CVCraw values during the heating plateau were lower in the 39°C than the 42°C protocol in the older group and not the younger group, which may mean that studies on younger populations may not have been affected by the difference in protocols and may only be visible in populations or interventions where SkBF is compromised. The CVCraw values were higher during maximal SkBF at 39°C than the 42°C in the older group but not in the younger group. The lower maximum value, therefore, affects the interpretation of %CVCmax data overall. An interpretation of this is that a lower maximal SkBF value can lead to values that appear as large percentages when normalized to %CVCmax.

When values were normalized to a %CVCmax, the heating plateau was lower in the 39°C protocol compared to the 42°C protocol in the older group, in addition to the age-related

difference in heating plateau during the 39°C protocol. This relates to higher CVCraw values during CVCmax in the 39°C protocol for the older group. Higher CVCraw values at maximum contribution to the SkBF values at different phases normalized to higher %CVCmax to be lesser values.

Limitations

Limitations of this study include the small number of participants. In addition, data taken for the same participants over multiple days will measure SkBF response for different vascular beds each day. Similarly, when a participant moves during the protocol, the laser-Doppler probe may measure the SkBF of various vascular beds instead of an individual vascular bed throughout the entire protocol.

These findings are important because they can give further insight into findings from previous literature, possibly due to how NO values were represented. Minson et al. found there to be significant NO contribution differences between older and younger subjects. This study presented NO contribution as the difference in CVC values in the heating and L-NAME perfusion plateau normalized to %CVCmax (Minson et al., 2002). Bruning et al. found there to be reduced NO-dependent vasodilation in middle-aged participants compared to younger participants when the NO-dependent vasodilation was calculated as the difference in plateau values (Bruning et al., 2012). We agree with the findings of these studies based on the results of this thesis showing that when NO contribution is determined with the traditional analysis method, significant differences in NO contribution are found between age groups for the 39°C protocol.

Conclusion

In summary, findings related to NO contribution during local skin heating depended on how this value is calculated. When the NO contribution was presented using the traditional analysis method, the NO contribution was lower in the older group compared to the younger group for the 39°C heating protocol; however, these age-related differences were not present with the relative analysis method. The results of this thesis may be useful when examining microvascular dysfunction in aging adults and those with risks of developing cardiovascular diseases.

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ACADEMIC VITA

Education

The Pennsylvania State University, University Park, PA Aug 2019 - May 2023

- Bachelor of Science in Kinesiology, Schreyer Honors College
- Dean's List 6 semesters

MGH Institute of Health Professions, Charlestown, MA Jun 2023 - Dec 2026

- Doctorate in Physical Therapy

Research Experience

Thesis Research, University Park, PA Oct 2022 – Present

- Analyzing skin blood flow during 39°C and 42°C local heating protocols
- Examining protocol and age-dependent differences in nitric oxide contribution for the local heating protocols

Research Assistant, Exercise Prescription Lab, University Park, PA Aug 2021 – Oct 2022

- Determined which types of resistance exercise results in the highest endocrine function in breast cancer survivors using mHealth and at-home saliva samples
- Administered resistance exercise protocols and aerobic exercise protocols to participants while tracking their heart rate
- Used the heart rate data to write an honors thesis relating aerobic heart rate adaptations to cannabis use disorder cravings

Physical Therapy Aide Experience

Professional Physical Therapy, South Yarmouth, MA May - Aug 2022

- Prepared patients for physical therapy and providing routine treatments, such as hot or cold packs
- Motivated, safeguarded, and assisted patients in practicing exercises and functional activities
- Collaborated with staff to maintain a clean work environment and progress toward treatment goals

Physical Therapy Observation Experience

South Shore Hospital, Weymouth, MA Jun - Jul 2022

- Shadowed for 16 hours in an inpatient rehabilitation setting; Observed post-operative patients with total hip replacements, total knee replacements, and spinal fusions

Boston Children's Hospital, Boston, MA May 2022

- Shadowed for 8 hours in an inpatient rehabilitation setting; Observed pediatric patients with diagnoses of cerebral palsy, spina bifida, muscular dystrophy, and developmental dysplasia

Cotting School, Lexington, MA Jul - Aug 2021

- Observed for 50 hours of physical therapists addressing students' motor skill performance, focusing on postural control, balance, muscle tone, muscle strength, and coordination of motion

Bay State Physical Therapy, Chelmsford, MA May - Jun 2021

- Observed 45 hours of tasks around the clinic, including hands-on soft-tissue work, stretching, and exercises for total joint replacements, rotator cuff tears, ankle fractures, and chronic back pain

Professional Physical Therapy, Tyngsborough, MA Sept - Dec 2020

- Shadowed physical therapists for 48 hours and learned about exercise prescriptions, surgical procedures, appointment frequency, billing, and documentation

Campus Involvement

- Pre-Physical Therapy Club: President, previous Alumni Relations Chair & Secretary
- Learning Assistant: Human Anatomy and Physiology
- Phi Epsilon Kappa: Vice President, previous Recruitment Chair
- Health Promotion and Wellness Department: Healthy Penn State Ambassador
- Global Brigades Benefitting THON: Outreach Chair

Skills/Certifications

- Adult & Pediatric CPR/AED/First Aid Certified: American Red Cross Jul 2022 - Jul 2024