Quantifying the Relationship of Bilateral Blood Flow in Glabrous Skin at Rest and During Sympathetic Perturbations

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Abstract

Sympathetic nervous system regulation of blood flow within glabrous skin occurs through control of vasoconstrictor tone, with vasodilation being a passive process. As bursts of sympathetic vasoconstrictor activity occur simultaneously at separate sites of the body, blood flow patterns should also be closely matched due to the direct connection between sympathetic nerves and peripheral microvessels. With sympathetic activity difficult and invasive to measure directly, the possibility of using blood conductance as an indirect measure seems promising. We investigated the relationship of bilateral blood conductance recordings of both middle fingers in ten (7M, 3F) healthy participants, while at rest and in response to perturbations known to elicit sympathetic activity. Cutaneous vascular conductance was measured from both middle fingers via laser Doppler flowmetry, while at rest in a thermoneutral room for 20 minutes and in response to 4 randomized sympathetic perturbations (2 breath holds and 2 cold stimuli) while centrally vasodilated via heating of the back. Correlation coefficients while at thermoneutral rest were high (0.80 ± 0.22) demonstrating a strong temporal relationship for blood conductance in both fingers. During the sympathetic perturbations, blood conductance in both fingers were more related during (0.93 ± 0.11) and post (0.87 ± 0.11) administration of the sympathetic perturbation than prior (0.67 ± 0.25) to the administration ($p = 0.002$). Taken together, these findings indicate that blood conductance patterns at separate sites of the body are significantly more related during vasoconstrictor activity and that blood conductance may have potential as a non-invasive measure of sympathetic activity.

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ATP = Adenosine Triphosphate

AVA = Arteriovenous Anastomoses

BH = Breath Hold

BP = Blood Pressure

CIVD = Cold Induced Vasodilation

CS = Cold Stimulus

CVC = Cutaneous Vascular Conductance

ECG = Electrocardiogram

eNOS = Endothelial Nitric Oxide Synthase

F_{mean} = Average CVC value in 10 s prior to start of sympathetic perturbation

F_{min} = Minimum value CVC value reached during the sympathetic perturbation

HIVC = Heat Induced Vasoconstriction

IG = Inspiratory Gasp

LDF = Laser Doppler Flowmetry

MSNA = Muscle Sympathetic Nerve Activity

NE = Norepinephrine

NO = Nitric Oxide

NPY = Neuropeptide Y

PNS = Parasympathetic Nervous System

RBC = Red Blood Cell

SA/SNA = Sympathetic Activity/Sympathetic Nerve Activity

SD = Standard Deviation

SkBF = Skin Blood Flow

SNS = Sympathetic Nervous System

SP = Sympathetic Perturbation

SSNA = Skin Sympathetic Nerve Activity

T_{core} = Core Temperature

$T_{decrease}$ = Time for blood conductance to reach F_{min} following the administration of the sympathetic perturbation

T_{LH} = Left Hand Temperature

T_{re} = Rectal Temperature

t_{regen} = Time for blood conductance to return from F_{min} to F_{mean}

T_{RH} = Right Hand Temperature

T_{skin} = Skin Temperature

VC = Vasoconstriction

Chapter 1 - Introduction

One of the primary effector organs under the direct control of the sympathetic nervous system (SNS) is the peripheral microvasculature, where SNS activity causes either vasoconstriction or vasodilation. A sympathetic signal originates in the rostral ventrolateral medulla (RVLM) and terminates at nerves in the periphery which form direct connections with peripheral microvessels (Flavahan, 2014; Morrison, 2016; Johnson and Kellogg Jr, 2018; McAllen and McKinley, 2018). Several factors influence how the SNS regulates peripheral microvascular activity, including individual characteristics like sex or age, mental or emotional stress, and temperature (Mano, 1998). Temperature changes result in the activation of two distinct sympathetic pathways, where decreases in skin or core temperature result in peripheral vasoconstriction to conserve heat and increases in skin or core temperature result in peripheral vasodilation to dissipate excess heat (Charkoudian, 2003; Wilson and Wilson-Metzler, 2018). An understanding of sympathetic nerve activity is therefore important for understanding the end organ responses involved in human thermoregulation.

Skin sympathetic nerve activity (SSNA) displays two important characteristics, the first is that it is matched across the body, and the second is that bursts of activity display rhythmicity. Matching of SSNA was shown in the 1980s by direct measures of nerve activity that demonstrated a similarity for both timing and magnitude of individual bursts of SSNA across separate sites of the body (Bini et al., 1980; Wallin and Fagius, 1988), suggesting that sympathetic vasoconstrictor nerve signals are sent out in a matched way and therefore should cause a closely matched response in peripheral microvessels at separate sites of the body. SNA also displays two distinct levels of rhythmicity, a high frequency oscillatory component, as well as a low frequency component (Malpas, 1998). The high frequency component sets a tonic level of vasoconstrictor tone in the vessels themselves, while the low frequency component is responsible for oscillations in blood flow that occur approximately 1-3 times per minute (Malpas, 1998). The direct connection between sympathetic nerves and the peripheral microvasculature means that blood flow patterns should also be closely matched at separate sites.

While sympathetic activity is one of the primary regulators of peripheral blood flow patterns, it is not the only modulator of blood flow. Peripheral blood flow is influenced by heart rate, respiratory activity, myogenic activity, sympathetic activity, and endothelial activity (Hodges, Mallette, and Cheung, 2018), while also being separately influenced by central (e.g., sympathetic activity) versus local control (e.g., nitric oxide modulation) (Johnson and Kellogg Jr, 2010). The control of blood flow by sympathetic activity is itself difficult to understand as sympathetic activity results in both a vasoconstrictor and vasodilator response in non-glabrous (hairy) skin which makes up most of the body, as well as regulating sudomotor responses (Mano, 1998). The only exception to this is in glabrous (non-hairy) skin, where there is no sympathetically mediated vasodilation, only vasoconstriction.

Glabrous skin, which is the non-hairy skin found on the hands, feet, and face of humans, contains an increased density of arteriovenous anastomoses (AVAs), and does not contain an active vasodilatory system (Wallin and Fagius, 1988; Walløe, 2016). Arteriovenous anastomoses bypass capillary blood flow and are much larger in diameter compared to other microvessels of the periphery, allowing for large increases or decreases in peripheral blood flow which results in much higher fluctuating blood flow values to glabrous skin compared to non-glabrous skin. AVAs are innervated by the sympathetic nervous system and thus respond strongly to sympathetic activity (Walløe, 2016). Since there are no vasodilator nerve fibres in glabrous skin, vasoconstriction is the only sympathetically mediated process, with vasodilation occurring via the passive release of vasoconstriction or local vasodilatory activity (Walløe, 2016). As a result of the decreased complexity, glabrous skin presents as an ideal candidate to assess sympathetically mediated changes in blood flow.

Sympathetic activity, through its direct connection to the peripheral microvasculature regulates blood flow at rest, causing oscillations that occur approximately 1-3 times per minute (Thoresen and Walløe, 1980; Walløe, 2016). These oscillations have been demonstrated to occur at separate sites of the body, and when a sympathetic block is administered, these oscillations cease (Janbu, 1989). The connection between sympathetic activity and changes in blood flow patterns has also been investigated as a potential clinical tool via the assessment of the vasoconstrictor response

to known sympathetic perturbations (Khan et al., 1991; Valley et al., 1993; Schürmann, Gradl, and Fürst, 1996). In these studies, perturbations known to elicit sympathetic activity are used and the vasoconstrictor response is measured, typically in a healthy population and a population of patients with a neurogenic impairment. These studies have furthered the understanding of how SNA influences overall changes in blood flow.

Peripheral blood flow is directly regulated by sympathetic activity and thus it is possible that by assessing changes in peripheral blood flow, information of SNS activity may be observable. Studies have investigated separate aspects of blood flow patterns such as the temporal relationship (Khanokh et al., 2004; Grinevich et al., 2019; Ho, Toska, and Wesche, 2020) or the vasoconstrictor relationship at separate sites of the body (Khan et al., 1991; Valley et al., 1993; Schürmann, Gradl, and Fürst, 1996). However, rarely do these studies focus on all aspects of peripheral blood flow patterns, instead focusing on just the vasoconstrictor response to sympathetic perturbations or cardiac physiology (e.g., heart rate and blood pressure), with blood flow as a secondary measure. As a result, these studies often do not take measures of blood flow at rest and in response to sympathetic perturbations and often do not standardize blood flow measures with techniques such as cutaneous vascular conductance (CVC) or by reporting as a percent max. With measures of sympathetic activity being costly and invasive, the potential of a non-invasive tool to assess sympathetic nerve activity is of great importance. With vasoconstriction in glabrous skin being the result of increased SNA, it presents itself as a suitable candidate for investigation. The goal of this research is a preliminary look into the potential of using glabrous skin blood flow as a non-invasive measure of sympathetic activity by quantifying the relationship of blood flow at separate sites and the vasoconstrictor activity in response to stimuli known to evoke a strong sympathetic response. The prediction is that blood flow will be highly related at separate sites of the body due to SA activity being matched at separate sites, and that blood flow will be more related at separate sites during vasoconstrictor activity.

Chapter 2 - Literature Review

2.1 Thermoregulation Via the Sympathetic Nervous System

The sympathetic nervous system (SNS) is involved in the maintenance of homeostasis in the human body. It is one branch of the autonomic nervous system, the other being the parasympathetic nervous system (PNS), with the SNS most often active during "fight or flight" scenarios and the PNS dominating during "rest and digest". The SNS plays a large role in the process of thermoregulation, where it regulates heat transfer between the body and environment through regulation of peripheral microvessel tone, changes in blood flow, and sweating (Mano, 1998; Charkoudian, 2003).

The ability to detect and respond to thermal stimuli is of vital importance, as even small deviations in core temperature can have deleterious effects in the body (Morrison and Nakamura, 2011). Thermal stimuli are detected by increases or decreases in skin or core temperature which can occur in response to varied environmental temperatures, the inability of thermoregulatory mechanisms to match the magnitude of thermal stress, or increased metabolic heat production (Morrison and Nakamura, 2011; Morrison, 2016; Wilson and Wilson-Metzler, 2018). The amount of thermal stress exerted on the body can vary, with a "thermal steady state" occurring if the magnitude of the thermal stress does not exceed the body's thermoregulatory capabilities (Wilson and Wilson-Metzler, 2018).

One of the primary mechanisms underlying sympathetically mediated thermoregulation is the control of peripheral vasculature, governed by distinct hot and cold pathways (Charkoudian, 2003). In response to cold stress, vasoconstriction decreases peripheral blood flow, causing blood to pool in the core of the body thereby minimizing further heat loss (Charkoudian, 2003; Cheung, 2015; Wilson and Wilson-Metzler, 2018). Peripheral vasodilation occurs in response to heat stress, allowing for warm blood to move out to the periphery and promote heat dissipation (Charkoudian, 2003; Wilson and Wilson-Metzler, 2018). Heat stress also elicits a sympathetically mediated sweat response, whereby sweat glands expressing adrenergic and cholinergic receptors increase evaporative heat loss (Wilson and Wilson-Metzler, 2018). The ability to dissipate heat through sweating is one of the primary factors that determines whether a thermal stress is

considered compensable or not, as humans heavily rely on sweating with one litre of sweat able to evaporate an estimated 580 kcal (Wegner, 1972).

The sympathetic nervous system contains two branches, which control changes to skin blood flow, the noradrenergic vasoconstrictor system and the sympathetic active vasodilator system (Charkoudian, 2003). These branches innervate the microvasculature of the periphery targeting receptors expressed on the smooth muscle cells of blood vessels (Charkoudian, 2003; Morrison, 2016). Therefore, they operate in a conflicting manner, with one often dominating over the other based on the overall central signal. The thermoregulatory pathway begins with thermal afferents mediated by thermal receptors found in the skin (Morrison, 2016). Transient receptor potential (TRPs) channels are believed to be the family of cation channels that sense skin temperature changes (Morrison and Nakamura, 2011). Distinct populations of TRP channels exist, which are either warm sensitive (e.g., TRPV3 and TRPV4) or cold sensitive (e.g., TRPM8 and TRPA1), mediating thermal sensation across a broad range of temperatures (Bautista, et al., 2007; Caterina, 2007; Morrison and Nakamura, 2011). These thermal afferent signals reach the hypothalamic preoptic area and either inhibit or excite premotor neurons (Morrison, 2016; McAllen and McKinley, 2018). Warm skin or warm core leads to descending GABAergic signalling that indirectly inhibit sympathetic premotor neurons in the medullary raphe, while cold skin or cold core temperatures activate a direct preoptic to raphe excitatory pathway (McAllen and McKinley, 2018). These premotor neurons drive cutaneous vasoconstriction via excitatory glutamatergic and serotonergic connection to spinal preganglionic neurons, and thus warm skin or warm core inhibits vasoconstriction while cold skin or cold core promotes vasoconstriction (McAllen and McKinley, 2018). The net input to the medullary raphe determines the sympathetic outflow and therefore cutaneous blood flow (Morrison, 2016; McAllen and McKinley, 2018).

The influence of the sympathetic nervous system on the peripheral microvasculature is not simply a thermoregulatory response, but also impacts blood flow patterns under thermoneutrality (Krupatkin, 2006). As previously mentioned, cold skin or cold core promotes additional or further vasoconstriction through an excitatory connection (McAllen and McKinley, 2018), however while this excitation increases the

vasoconstrictor activity, it does not cause it. The SNS induces a tonic level of vasoconstrictor tone throughout the periphery due to an always present, high frequency level of SNS activity as well as vasoconstrictor events which manifest as changes in blood flow through the previously described low frequency oscillations in SNS activity (Krupatkin, 2006). This tonic level of vasoconstrictor tone is part of what influences the vasodilatory response to heat, as elevations in temperature result in the vasoconstrictor tone being diminished thereby augmenting the vasodilatory effect. In addition to the high frequency SNS activity, there is also a lower frequency component which induces rhythmic changes in microvessel diameter in the periphery while an individual is thermoneutral (Krupatkin, 2006).

Several factors are known to cause variation in sympathetic function, which can lead to altered thermoregulatory responses (Wilson and Wilson-Metzler, 2018). Factors such as age (Greaney, Alexander, and Kenney, 2015), female reproductive hormone levels/menopause (Charkoudian et al., 2017), and neuropathies (Khan et al., 1991; Schürmann, Gradl, and Fürst, 1996) have all been shown to influence human thermoregulatory capabilities due to altered sympathetic activity (SA) and end organ response. Due to the importance of the SNS in the regulation of the peripheral microvasculature, many studies have been done measuring nerve activity responses to various environmental stimuli.

2.2 Recordings of Sympathetic Activity

2.2.1 Features of Sympathetic Recordings

Direct recordings of human sympathetic nerve activity were first taken in 1960 by Hensel and Boman (1960). These recordings were from a single nerve fibre, and since then methods have allowed for recordings from multiple nerves *in situ* and research has looked at how sympathetic activity is controlled and how it is influenced by external factors. Since these recordings are direct measurements of sympathetic activity, they quantify in real time measures of sympathetic traffic (Mano, 1998). As such, an understanding of the signals of the sympathetic nervous system are of great importance, particularly the neurotransmitters and post-synaptic receptors that mediate this activity.

Sympathetic vasoconstrictor activity directed to the microvasculature of the periphery results in the release of norepinephrine (NE) and neuropeptide Y (NPY) from postganglionic nerve fibres, among other possible co-transmitters such as ATP and NO (Johnson and Kellogg Jr, 2018). While NE is the primary neurotransmitter responsible for peripheral vasoconstriction, studies have shown that when NE release is blocked, NPY augments the vasoconstrictor response (Johnson and Kellogg Jr, 2018). This illustrates the importance of co-transmission in the SNS, as well as the built-in redundancy underlying the mechanisms of sympathetic activity. When the sympathetic signal reaches the peripheral nerve, it results in the formation of an action potential, which travels down the axon and leads to the release of the neurotransmitters into the synaptic cleft (Malpas, 1998) where they will then bind to post synaptic receptors. The family of post-synaptic receptors responsible for binding NE are the α adrenoreceptors (Flavahan, 2014). The α adrenoreceptors when activated translocate to the smooth muscle of the microvasculature, and when NE binds, they begin the cascading signal which results in peripheral vasoconstriction of the microvascular smooth muscle (Flavahan, 2014). NE and α adrenoreceptors receptors are upregulated by sympathetic activity, such as a cold stimulus, and may inhibit the expression of endothelial nitric oxide synthase (eNOS) and the formation of nitric oxide (NO), which is a potent vasodilator (Sun, 2010). This shows the specificity and control of actions underlying sympathetic activity, with built-in redundancy and inhibitory mechanisms, sympathetic nervous systems activity is highly regulated.

Studies taking measurements of sympathetic activity in peripheral nerves have shown two distinct types of measurable sympathetic activity, muscle sympathetic nerve activity (MSNA) and skin sympathetic nerve activity (SSNA) (Mano, 1998) which can be measured separately. MSNA and SSNA discharge independently based on regional differentiation, as MSNA innervates the vasculature of peripheral muscles, while SSNA innervates peripheral cutaneous vasculature (Mano, 1998). MSNA has been shown to be related to the cardiac cycle (Hagbarth et al., 1972), increases linearly with core temperature (Mano, 1998), and is negatively related to blood pressure (Mano, 1998). MSNA contains only active vasoconstrictor nerves, which makes measurements of MSNA fairly simple and a favourite to study for researchers (Wallin and Fagius, 1988).

The focus of sympathetic activity discussed in this literature review will be on SSNA, unless otherwise specified.

2.2.2 SSNA

Skin sympathetic nerve activity is a measure of the sympathetic nerve traffic from fibres that innervate the peripheral cutaneous vasculature (Mano, 1998). SSNA measurements are more complex than MSNA as SSNA contains 4 distinct nerve fibre types: vasoconstrictor, vasodilator, sudomotor, and pilomotor (Wallin, 1981; Mano, 1998), and can innervate two distinct types of skin: glabrous and non-glabrous (Mano, 1998). Identification of SSNA is based on the following discharge characteristics: 1) spontaneous arrhythmic efferent burst discharges, 2) that SSNA activity is followed closely by peripheral vasoconstriction or perspiration, and 3) that it is elicited with almost constant latency by mental stress and sensory stimuli (Mano, 1998; Iwase et al., 2016). SSNA can be expressed as burst rate, but seems to be better characterized as total SSNA, due to the irregularity in frequency, amplitude, and duration of SSNA bursts (Mano, 1998). Burst rate is a measure often expressed as the number of bursts per minute or the number of bursts per hundred heart beats, while the measure of total SSNA is a total of the observed activity over a given time, typically done by taking the integral of that time (area under the curve) (Guild et al., 2009). Unlike MSNA, SSNA does not show rhythmicity with the cardiac cycle (Wallin and Fagius, 1988; Iwase et al., 2016), however, it does seem to be influenced by respiration (Hagbarth et al., 1972; Eriksen and Lossius, 1995). Overall, measures of SSNA are often much more complex than MSNA due to its irregularity and that recordings involve multiple nerve fibre types.

Sympathetic activity is the complex output of the central nervous system, which provides control over a variety of end organ functions (Malpas, 1998). Synchronized bursts of efferent sympathetic impulses appear either spontaneously (tonically) or may be triggered by various peripheral stimuli (evoked) (Hagbarth et al., 1972). Unlike motor nerves, sympathetic nerves are continuously active, meaning that the innervated blood vessels of the skin are constantly under some level of constriction as previously described (Malpas, 1998). The tonic level of sympathetic activity within subjects varies between individuals, however it appears to be relatively stable over time within subjects (Malpas,

1998), indicating that there is a reliable index of sympathetic tone, which may be comparable across different groups (Malpas, 1998).

Simultaneous recordings of SSNA from separate peripheral nerves reveal a striking similarity in terms of both timing and magnitude of burst amplitudes, particularly in those nerves innervating the hands and feet (Bini et al., 1980b; Wallin and Fagius, 1988). In a study looking at sympathetic activity across separate sites of the body, sympathetic recordings were taken from pairs of nerves to compare their firing patterns (Bini et al., 1980b). The sites recorded included left and right median nerve, median and peroneal, and the left and right peroneal among others. This study revealed a close, time matched release of sympathetic activity in nerves at separate sites of the body, as well as nerves innervating different skin types (Figure 2.1). This indicates that skin sympathetic nerve activity is sent throughout the body in a way that appears to be closely matched at bilateral sites of the body. Differences in conduction velocity may explain some of the differences observed between glabrous and non-glabrous skin, as sudomotor nerves have a faster conduction velocity than vasoconstrictor nerves and are more influential in non-glabrous skin (Malpas, 1998). Overall, the matching of sympathetic activity across the body has been demonstrated for both timing and magnitude of individual bursts and shows good reproducibility (Bini et al., 1980b). This similarity of sympathetic activity at separate sites should therefore result in a nearly identical level of sympathetic tone and blood flow oscillation patterns at separate sites of the body.

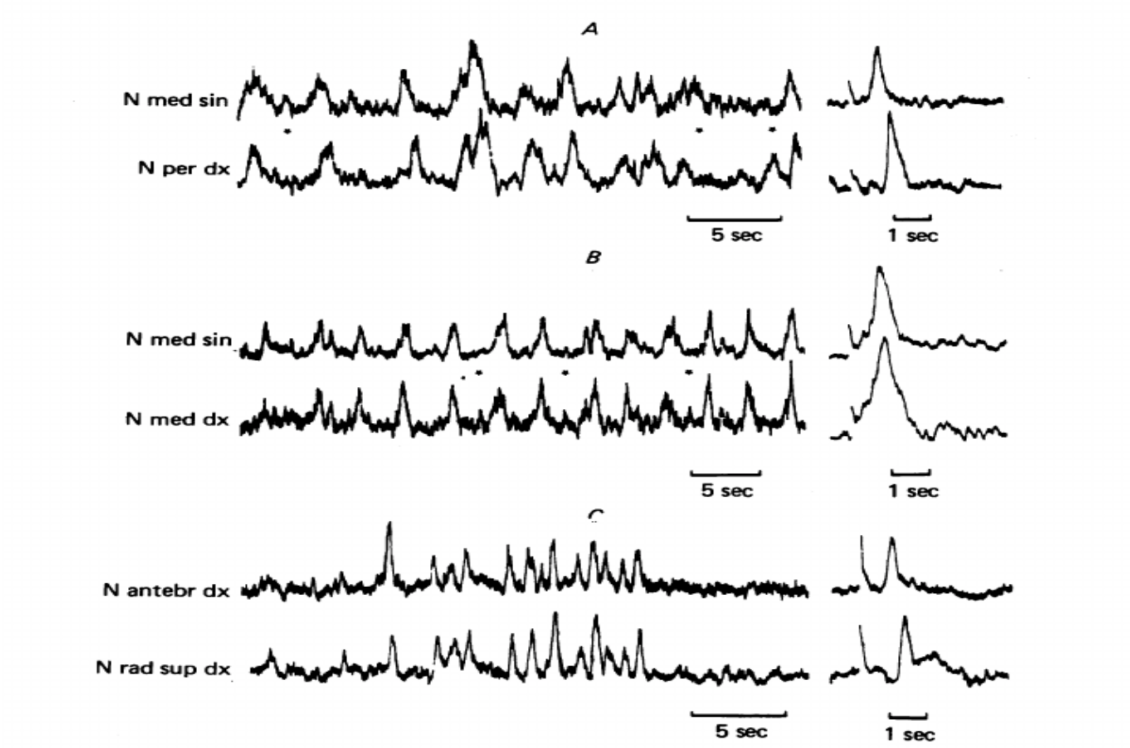

Figure 2.1 Recordings of sympathetic activity at separate sites displaying a strong relationship for both timing of individual bursts as well as for magnitude of individual bursts. From Bini et al., 1980b

A and B represent recordings of bursts of skin vasoconstrictor impulses from the left median (N med sin) and the right peroneal (N per dx) nerves (A) and from the two median nerves (B). C represents bursts of sudomotor impulses from the left antebrachial (N antebr dx) and the left superficial radial (N rad sup dx) nerves (C). Left side of image represents bursts of activity occurring spontaneously, while the right side represents reflex bursts elicited by electrical skin stimulus.

2.2.3 Influence of Environmental Stimuli

SSNA is particularly influenced by changes in ambient temperature (Mano, 1998). Separate components of SSNA (vasomotor and sudomotor) respond differently to changes in ambient temperature (Mano, 1998). When ambient temperature is raised outside of the thermoneutral zone (~34°C) both sudomotor and vasoconstrictor sympathetic outflow are elevated in the peroneal but were suppressed in the tibial nerve (Okamoto et al., 1994). The sudomotor and vasoconstrictor outflows were elevated in both nerves when temperatures were dropped to 18°C (Okamoto et al., 1994). Reflex thermoregulatory functions appear to be anatomically distinct, with SSNA to glabrous

skin being executed via vasoconstrictor fibres, while sudomotor fibres dominate in non-glabrous skin, indicating that SA is matched at separate sites, but may carry out unique end organ responses based on body region (Bini et al., 1980a). This sudomotor control to non-glabrous skin appears to also involve a vasodilatory response, as there appears to be a coupling of sudomotor and vasodilatory nerve activity in non-glabrous skin (Mano, 1998). This has not been shown to be the case in glabrous skin where there is only an active vasoconstrictor system, and thus the changes in blood flow are largely attributable to vasoconstriction and the release of that vasoconstriction (Mano, 1998). The total SSNA measured appears to be the lowest when an individual is in a thermoneutral state and increases with deviations in temperature in either direction (Figure 2.2) (Wilson and Wilson-Metzler, 2018).

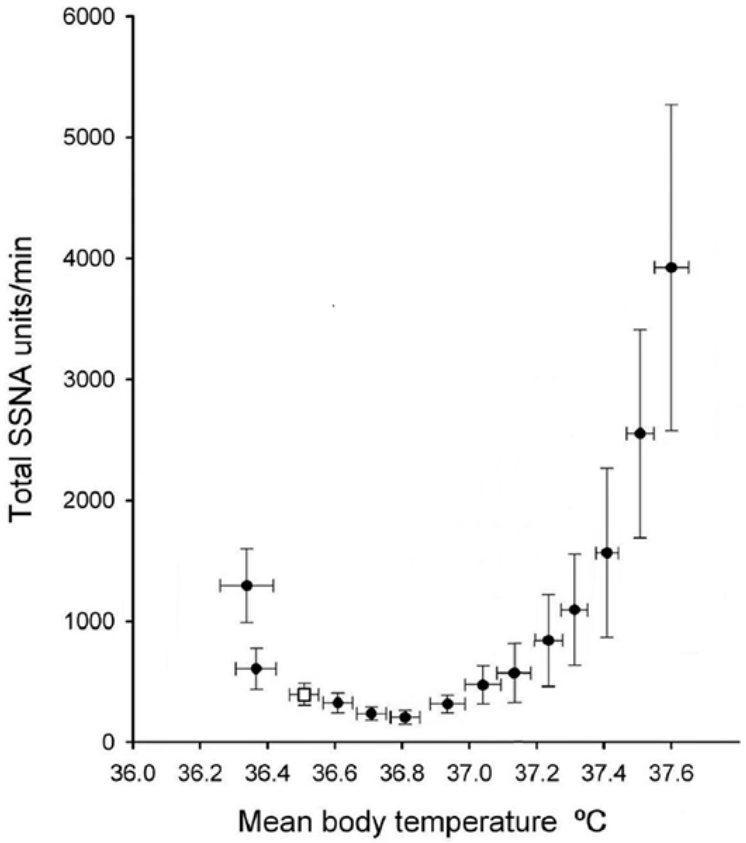

Figure 2.2 Total SSNA vs Mean Body Temperature. From Wilson and Wilson-Metzler, 2018

Mean body temperature (0.9 × internal temperature + 0.1 × mean skin temperature) manipulated by skin-surface cooling and whole body heating. Increases in SSNA to heat are due to sudomotor and related vasomotor components of skin SNA. Increases in SSNA to cooling are due to vasomotor and pilomotor components of skin SNA.

The effect of local manipulations in temperature also influences sympathetic activity however they are not as well understood. SSNA has been shown to increase to glabrous skin during local cold exposure, but not to non-glabrous skin (Mano, 1998) indicating a difference in the thermoregulatory functions of these distinct regions. A paradoxical mechanism known as cold induced vasodilation (CIVD), occurs in glabrous skin after a short period of time, whereby when exposed to local cooling, increases in finger blood flow and local tissue temperature are proposed to occur due to sympathetic withdrawal (Hodges, Mallette, and Cheung, 2018). A similarly paradoxical mechanism occurs in response to local heating where SSNA is shown to increase to glabrous skin in response to local elevations in temperature. The mechanism appears to be the exact opposite of CIVD and is referred to as heat induced vasoconstriction (HIVC) (Mano, 1998). When a subject was kept within their thermoneutral zone in a warm environment, local heating of the fingers resulted in increased sympathetic activity and a decrease in fingertip blood flow (Nagasaka et al., 1990). Overall, the effect of local manipulations in temperature appears easier to study for SSNA to glabrous skin because they contain only an active vasoconstrictor system and thus changes in activity and blood flow patterns are less complicated. The nerve fibre type differences between skin types are likely responsible for the differences observed in activity levels to local temperature manipulations.

Several other external stimuli are known to influence SSNA recordings. As previously mentioned, SSNA seems to be influenced by respiration (Hagbarth et al., 1972; Eriksen and Lossius, 1995). Various manipulations of breath hold perturbations have been used to elicit sympathetic activity in several studies (Schürmann, Gradl, and Fürst, 1996). The breath hold technique elicits a strong reflex burst of SSNA (Wallin and Fagius, 1998), which contains both vasoconstrictor and sudomotor impulses. Unexpected noise and vibrations lead to an increase in SSNA, in both glabrous and non-glabrous skin (Mano, 1998). This response of increased SSNA to unexpected noise may, however, habituate rather quickly (Mano, 1998). SSNA is sensitive to mental stress, such as arithmetic, and displays strong reflex bursts, in response (Wallin and Fagius, 1988). The idea of a “cold sweat” may in fact be elicited by SSNA in response to mental stress

(Wallin and Fagius, 1988). Measures of SSNA are complicated as they are sensitive to a variety of stimuli that, unless controlled for, can cause noise in the recordings.

2.2.4 Individual Variability

Several factors are known to influence an individual's level of sympathetic activity, or their response to sympathetic activity (Greaney, Alexander, and Kenney, 2015; Charkoudian et al., 2017). While research into sex-specific differences is drastically understudied, the studies that do exist show a distinct difference in the control of peripheral blood flow between males and females (Charkoudian et al., 2017). Females display distinct patterns of control across the menstrual cycle, as well as showing an increased reactivity of peripheral vasculature to strong sympathetic activity (Schürmann, Gradl, and Fürst, 1996; Charkoudian et al., 2017). Impairments to reflex vasoconstriction in response to cold exposure have been shown in aged skin (Greaney, Alexander, and Kenney, 2015). The impairment of this process has been demonstrated at several steps along the pathway, including decreased sympathetic outflow and altered end organ response (Greaney, Alexander, and Kenney, 2015). Females post-menopause display a drastically reduced sympathetic response compared to pre-menopause, often to values even lower than age-matched male counterparts (Charkoudian, 2017). This makes participant selection for studies incredibly important, as an understanding of the mechanisms underlying a response in different populations is of importance when characterizing results.

Those with neuropathies also display diminished sympathetic responses, whether these neuropathies be nerve lesions or impairments (Schürmann, Gradl, and Fürst, 1996). Some neuropathies result in altered sympathetic outflow, while others involve a diminished sympathetic response (Schürmann, Gradl, and Fürst, 1996). Measures of sympathetic activity have become increasingly important for diagnoses of these neuropathies, as the diminished sympathetic response is of great clinical importance and may occur prior to other complications resulting in earlier diagnoses and thus more positive outcomes (Schürmann, Gradl, and Fürst, 1996). Overall, those with an augmented or a diminished sympathetic response display variation in the reactivity of their microvasculature to variations in sympathetic activity.

2.3 Regulation of Skin Blood Flow

2.3.1 Sympathetic Control of Blood Flow

The peripheral vasculature is one of the primary effector organs of the sympathetic nervous system (Flavahan, 2014). The function of the peripheral vasculature includes delivery of dissolved substances, the removal of waste products, and is involved in thermoregulation. The regulation of blood flow through the peripheral vasculature is of great importance and is under the influence of both reflexive sympathetic control as well as local control. As previously mentioned, there are two branches of sympathetic control of skin blood flow, the noradrenergic vasoconstrictor system and the active vasodilator system (Charkoudian, 2003). These distinct branches rely on separate nerve fibre types to elicit their effects and as such the skin they innervate is of great importance to the signal which they carry. There are two distinct types on skin on the body as previously discussed, glabrous (non-hairy) and non-glabrous (hairy) skin. These two types of skin are anatomically and regionally distinct. Non-glabrous skin covers most of the body and has hair. It also contains four distinct types of nerve fibres those being: vasoconstrictor, vasodilator, sudomotor, and pilomotor. As previously mentioned there appears to be a coupling of sudomotor and vasodilator nerve activity in non-glabrous skin, and these are thought to be the primary effectors of thermoregulation in the non-glabrous regions of the body (Mano, 1998). The rest of this literature review will focus on blood flow patterns in glabrous skin unless otherwise specified.

2.3.2 Local Control of Blood Flow

While the primary focus of this literature review is on the sympathetic regulation of glabrous skin blood flow patterns, an understanding of the local control of blood flow is an important consideration as it may oppose or augment the effects of the sympathetic nervous system (Johnson and Kellogg Jr, 2010b). The primary substance that appears responsible for the control of local blood flow changes is nitric oxide (NO), both to local heating and cooling through stimulatory and inhibitory pathways respectively (Johnson and Kellogg Jr, 2010b). NO is a strong vasodilatory substance that has been studied largely for its ability to increase blood flow and has been indicated as a potential ergogenic aid (Johnson and Kellogg Jr, 2010b). With adrenergic activation there is an

observed downregulation of endothelial nitric oxide synthase (eNOS) and thus NO, and with eNOS activation and the formation of NO, there is an inhibition of adrenergic function (Johnson and Kellogg Jr, 2010b; Sun, 2010). This push-pull of activation and inactivation helps to demonstrate the interplay between the two mechanisms and why one cannot be considered without the other. The importance of local control of blood flow should not be underestimated, as local manipulations in temperature have been proven capable of eliciting near maximal vasoconstriction or vasodilation (Johnson and Kellogg Jr, 2010a). In fact, the application of local heating (42-44°C) is often used as a means of normalizing blood flow responses in studies as it elicits a near maximal vasodilatory response with good replicability (Johnson and Kellogg Jr, 2010a; Hodges et al., 2016). Local heating is often utilized by studies investigating microvascular reactivity as it has been shown to help standardize results, while not negatively influencing the vasoconstrictor response being investigated (Henricson et al., 2011). The impact of local control is made up of both adrenergic and endothelial (NO dependent or independent) interaction mechanisms that can work with or against reflexive control.

2.3.3 Glabrous Skin Blood Flow

Glabrous skin is anatomically unique from non-glabrous skin as it is hairless and makes up only a small percentage of the surface area of the body, being mainly found on the hands, feet, and face. It also contains two distinct nerve fibre types, vasoconstrictor and sudomotor, rather than the four found in non-glabrous skin (Wallin and Fagius, 1988). Thus, blood flow patterns in glabrous skin are less complex as they are brought about via sympathetically mediated vasoconstriction or a release of vasoconstriction, with no active neurogenic vasodilation occurring (Walløe, 2016). These vasoconstrictions therefore occur rather rapidly, while the passive dilation is a much slower process (Charkoudian, 2003; Walløe, 2016).

One of the most distinct anatomical differences between glabrous and non-glabrous skin is the presence of arteriovenous anastomoses (AVAs) in glabrous skin (Walløe, 2016). While there is some evidence indicating that AVAs are present in non-glabrous skin (Elstad et al., 2014), research is clear that they are found most abundantly in glabrous skin, particularly in the hands and feet of humans (Walløe, 2016). AVAs are

direct connections between small arteries and veins that bypass capillary flow and therefore are not a part of the nutritive blood flow supply to the periphery (Figure 2.3). Since AVAs are not a part of the nutritive flow they are not involved in the transportation of dissolved substances or the removal of waste products, meaning their only function appears to be thermoregulation (Walløe, 2016). During cold exposure, the primary mediator for the decrease in blood flow are the AVAs, allowing for a small amount of blood to still reach the capillaries, thus preserving the nutritive blood supply (Flavahan, 2014). Their influence in thermoregulation is clear from their anatomical make up as they are much larger in diameter than most vessels in the peripheral microvasculature allowing for a drastic rise in blood flow when they are open, and when they are closed, they decrease blood flow to practically zero (Walløe, 2016). They are densely innervated by adrenergic axons and thus respond strongly to sympathetic signals. AVAs are found to close in response to sensory signals occurring at other sites of the body, displaying the previously described matching of sympathetic activity across the body (Walløe, 2016). The constriction of AVAs occurs via sympathetic activity and the dilation, which occurs is the result of the passive release of the vasoconstriction (Walløe, 2016). A table presenting some of the most relevant similarities and differences between glabrous and non-glabrous skin is presented in Table 2.1.

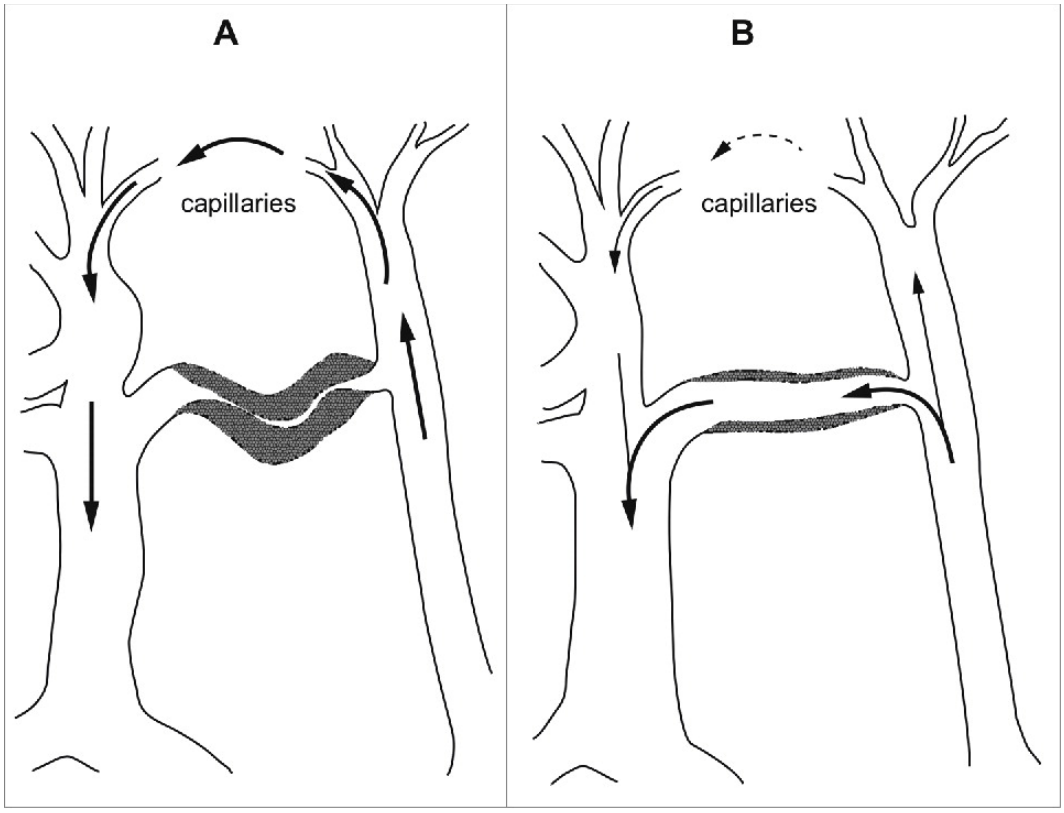

Figure 2.3 Representation of AVA structure and path of blood flow when AVAs are closed (A) vs open (B). From Walløe, 2016

AVAs are much larger in diameter than other peripheral microvasculature and as such when open, most blood flows through them causing a marked increase in flow through the area and less flow through the capillaries. When closed, blood flow to the area can decrease to near zero, and blood is forced to go only through the capillaries.

Table 2.1 Differences between glabrous and non-glabrous skin sympathetic nerve innervation and blood flow regulation

Skin Type	Nerve Types that Innervate	Control of Vasoconstriction	Control of Vasodilation	Factors which Influence Sympathetic Control
Glabrous	Vasoconstrictor Sudomotor	Sympathetic noradrenergic vasoconstrictor system (NE/NPY)	Inhibition/Release of vasoconstriction and local control (eNOS/NO)	Aging = (-) Cold = (+) VC Ex.Acute = (+) VC Ex.Training = (+) VD Heat = (+) VD Neuropathies = (-) Noise = (+) VC Sex = (+?) Stress = (+) VC
Non-Glabrous	Vasoconstrictor Vasodilator Sudomotor Pilomotor	Sympathetic noradrenergic vasoconstrictor system (NE/NPY)	Sympathetic cholinergic vasodilator system and local control (eNOS/NO/SubstanceP)	

NE = norepinephrine. NPY = neuropeptide Y. eNOS = endothelial nitric oxide synthase. NO = nitric oxide. VC = vasoconstriction. VD = vasodilation. Ex. = exercise. Glabrous skin also contains arteriovenous anastomoses (AVAs) compared to non-glabrous skin which does not. Glabrous skin does not contain an active vasodilator system and relies on local control and release of vasoconstriction to do so. In non-glabrous skin sudomotor and vasodilator activity are closely matched. Effects of sex related differences are less well known and females may display additional differences across phases of the menstrual cycle.

2.3.4 Blood Flow Patterns

Glabrous skin blood flow displays distinct oscillations brought about by a variety of physiological mechanisms. Much like SSNA these oscillations seem to display distinct frequencies that influence changes in blood flow. Some of the more relevant influential mechanisms on blood flow patterns include heart rate (Mano, 1998), respiration (Eriksen and Lossius, 1995), neurogenic/sympathetic (reflexive) (Söderström et al., 2003), and endothelial mediated changes (Hodges, Mallette, and Cheung, 2018). An example of a typical LDF tracing as well as the frequency bands is provided in Figure 2.4. The frequency range influenced by sympathetic activity was determined by measuring LDF signals simultaneously from surfaces of free microvascular flaps deprived of SSNA and adjacent intact skin (Söderström et al., 2003). This allowed for the determination of the frequency interval in which SSNA manifests by comparing the decreased frequencies present in the free microvascular flaps compared to the intact skin. This study also showed a decrease in power of even lower frequencies (Söderström et al., 2003), however

this frequency interval is known to correlate to endothelial control of blood flow (Hodges, Mallette, and Cheung, 2018) and thus the decrease observed is likely due to the interaction between sympathetic activity and local factors which influence endothelial control.

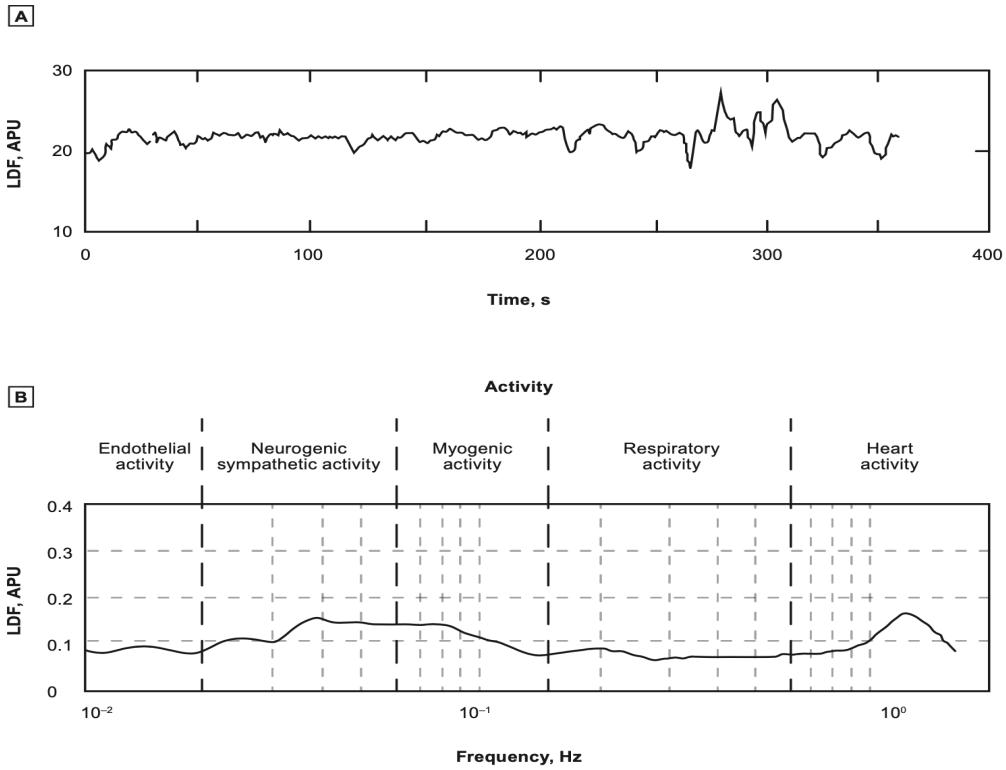

Figure 2.4 Representative signal of blood flow recordings via Laser Doppler Flowmetry (LDF; A) and example of transforming an LDF signal to a frequency domain (B). From Zegarra-Parodi et al., 2014
Typical ways to transform the LDF signal to a frequency domain include the Fourier transformation, the inverse Fourier transformation and the wavelet transform technique. APU = arbitrary perfusion units. B depicts the 5 distinct frequency bands known to influence peripheral blood flow patterns.

As early as 1939 the large oscillations in blood flow that occur approximately 1-3 times per minute were observed and attributed to thermoregulatory fluctuations arising from a tonic level of sympathetic activity (Burton, 1939). In these studies, utilizing finger plethysmography, values of blood flow seconds apart gave drastically different values and, over the course of several minutes, the patterns of blood flow showed characteristic rhythmic oscillations with a frequency of between approximately one and three

fluctuations per minute (Burton, 1939). These observed fluctuations were found to be closely related between the big toe and the finger of the same subject, and these fluctuations were not present in the fingers of a sympathectomized arm (Burton, 1939). This indicated that these fluctuations in blood flow patterns are mediated by the tonic level of sympathetic activity. These measures using plethysmography however, had some limitations, such as they could only be taken over relatively short periods of time, and the advent of more precise, time sensitive measures of blood flow allowed for further study.

In the 1970s laser Doppler flowmetry (LDF) and ultrasound Doppler allowed for the continuous measure of blood flow and lead to more sensitive research into the patterns of blood flow across the body (Walløe, 2016). In a study by Thoresen and Walløe using ultrasound Doppler measurements, they confirmed many of the same findings in the oscillatory patterns of blood flow as Burton had described but were able to detect that the fluctuations had a much greater amplitude than previously believed, due to their more precise measures (Thoresen and Walløe, 1980). They concluded that these large fluctuations could not be the result of vasomotion in the smaller vessels of the periphery and must be the result of opening and closing of the AVAs (Thoresen and Walløe, 1980).

Oscillations in blood flow patterns across the body appear to be closely matched (Figure 2.5), possibly due to the tonic level of sympathetic activity throughout the body. This has been found to be particularly true in glabrous skin blood flow oscillations, where the regulation of blood flow by sympathetic activity is less complex (Burton, 1939; Thoresen and Walløe, 1980). Glabrous skin blood flow patterns are less complicated as they only contain active vasoconstriction and their patterns in oscillation are simply due to sympathetic activity or the withdrawal of sympathetic activity (Mano, 1998). In one particularly fascinating study in 1989, blood flow patterns were taken from the radial and dorsalis pedis arteries of women during labor (Janbu, 1989). Prior to epidural block, the characteristic blood flow fluctuations were seen in both arteries (Figure 2.6), however for the group of women who received the epidural, blood flow values remained constantly high, while those who did not receive the epidural continued to display the oscillations in blood flow (Figure 2.6). This showed that the epidural block not only affected the pain fibres but also the sympathetic nerves resulting in a constantly high, non-fluctuating,

blood flow pattern to glabrous skin sites (Janbu, 1989). In another study looking at the fluctuations of blood flow at separate sites of the body at different temperatures, it was shown that the oscillatory patterns of blood flow align well across a range of temperatures (18-32°C), with the highest levels of coherence occurring within the thermoneutral zone (Elstad, Zilakos, and Bergersen, 2017). Blood flow fluctuations were shown to have positive correlation coefficients between 0.7 to 0.95 in subjects who are in the middle of their thermoneutral zone (Walløe, 2016). Due to the sympathetic control of glabrous skin blood flow, bursts of activity from adrenergic axons occurs simultaneously, and therefore blood flow fluctuations appear to be correlated at all glabrous skin sites (Walløe, 2016).

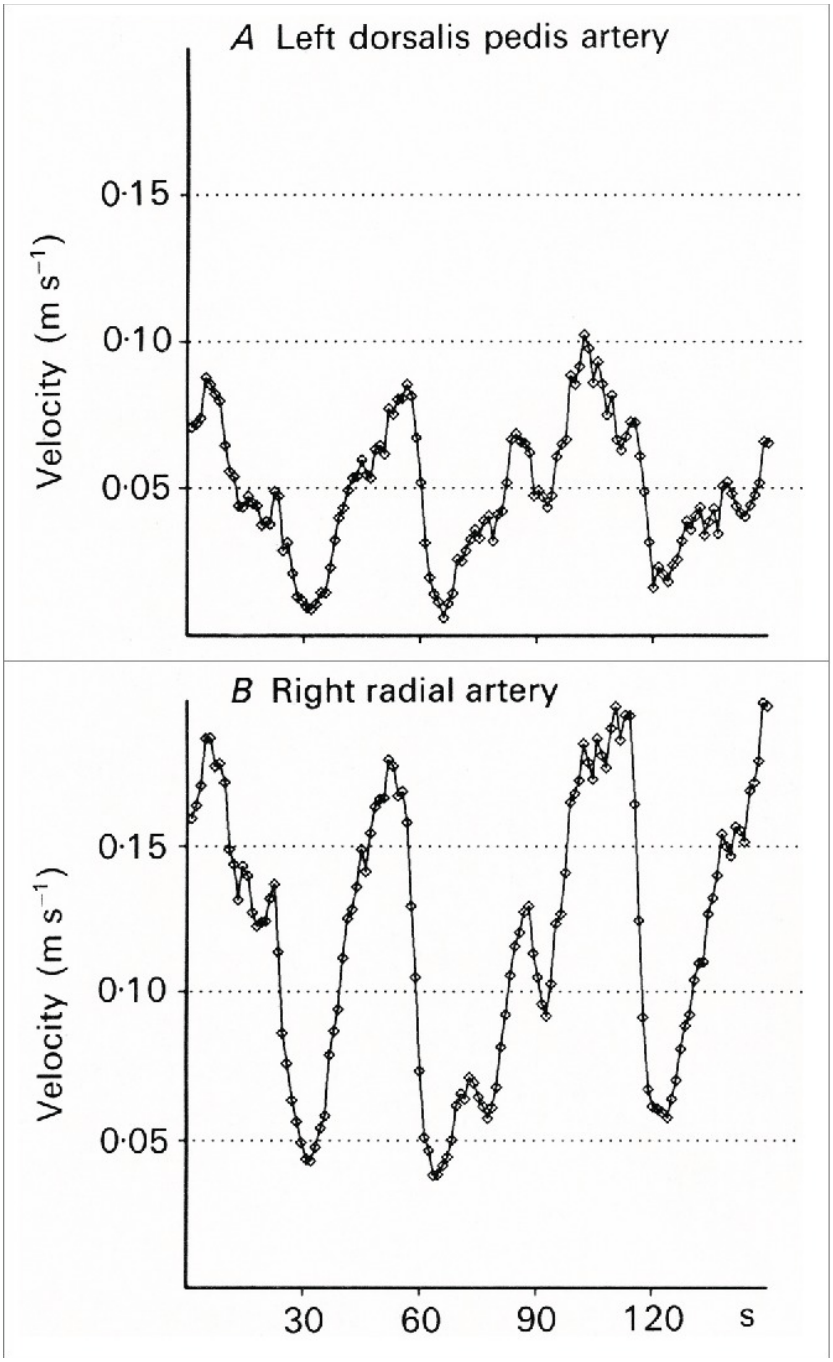

Figure 2.5 Simultaneous recordings of blood flow from the left dorsalis pedis artery (A) and the right radial artery (B). From Lossius, Eriksen, and Walløe, 1993
Measures taken at rest from separate sites of the body demonstrate the large fluctuations seen in blood flow and reflect the closely related nature of these fluctuations over time at separate sites of the body.

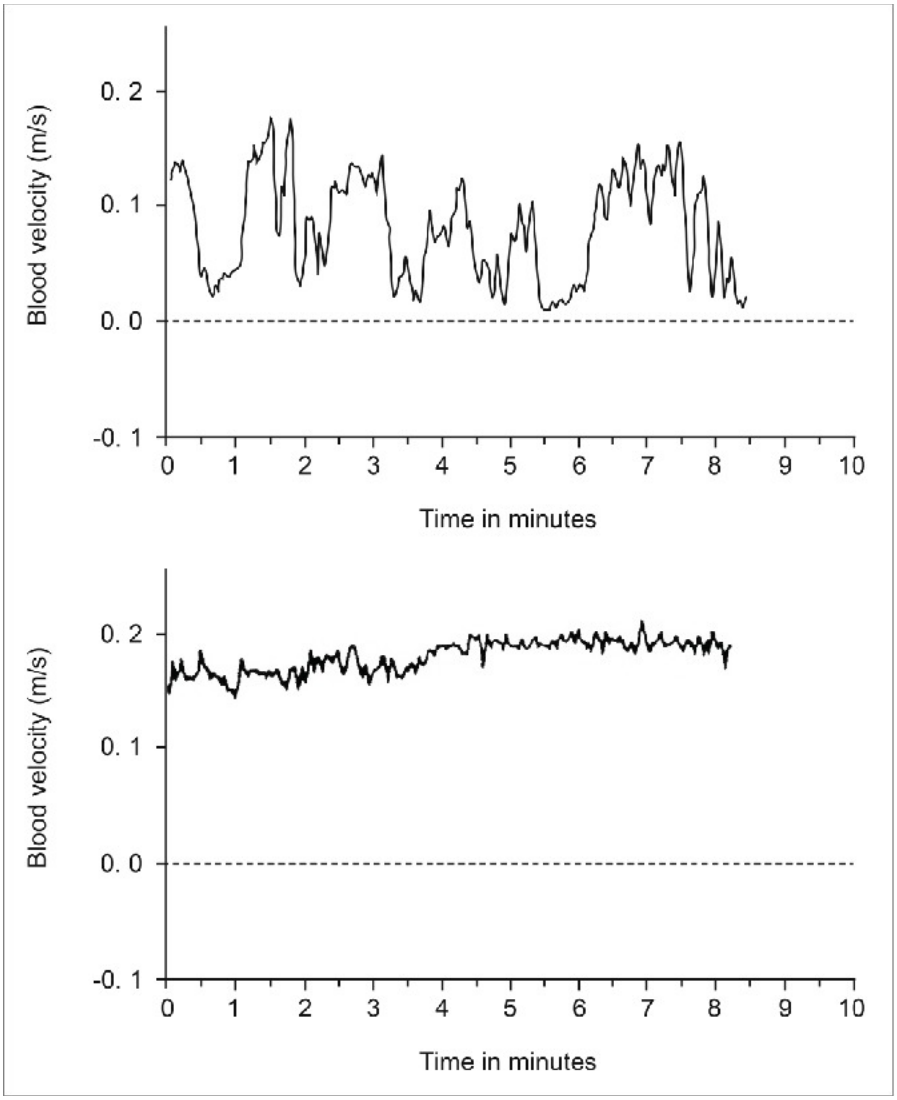

Figure 2.6 Recordings of blood flow from the right dorsalis pedis artery of women in labour, but between contractions. From Janbu, 1989
Fluctuations typical of temperature regulation in a warm delivery room (upper panel), same woman in the same environment after an epidural block no longer show the same fluctuations in blood flow (lower panel).

2.3.5 Influence of Environmental Stimuli

Many of the same environmental stimuli that influence sympathetic activity also influence blood flow, the most prominent of which is varied environmental temperatures. The peripheral vasculature is one of the most important thermoregulatory effector organs in the human body and as such it responds strongly and rapidly to changes in ambient temperature. AVAs seem to largely be involved in thermoregulatory activity within an individual's thermoneutral zone, being open at the upper ends and closed near the lower

ends (Walløe, 2016). The thermoneutral zone is considered the temperature range over which the basal metabolism is approximately constant (Figure 2.7) (Walløe, 2016). Factors such as subcutaneous adipose tissue and clothing can greatly alter the thermoneutral zone due to their insulative properties. Above the upper end of the thermoneutral zone, metabolic rate increases due to the production of sweat, while it increases below the lower end due to increased heat production from shivering and non-shivering thermogenesis (Walløe, 2016). The amplitude of fluctuations in blood flow patterns has been shown to be greater when an individual is within their thermoneutral zone (Thoresen and Walløe, 1980; Elstad et al., 2014). Outside of the thermoneutral zone AVAs appear to remain constantly open at the upper end and constantly closed at the lower end (Walløe, 2016).

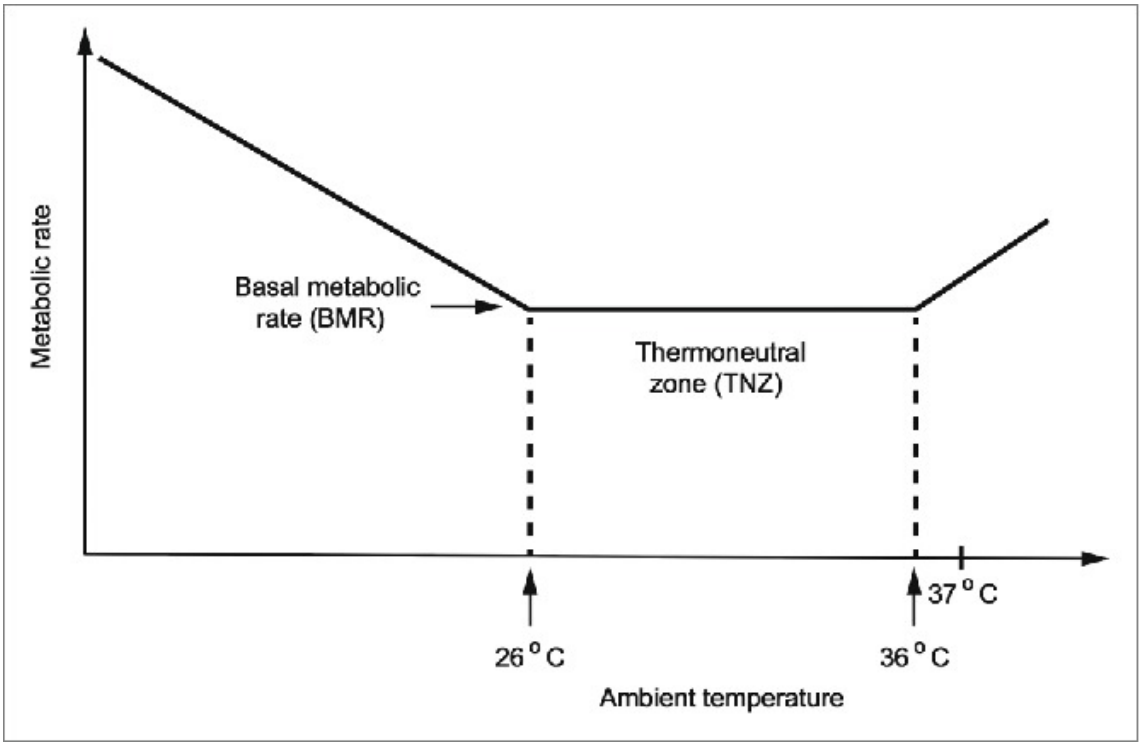

Figure 2.7 Representative graph of the thermoneutral zone in humans. The temperature (ambient) at which basal metabolic rate (BMR) is stable. From Walløe, 2016 Schematic is for the BMR of a resting naked human as a function of ambient temperature.

Sympathetic activity is altered by changes in ambient temperature, therefore the oscillations in blood flow patterns governed by sympathetic activity display altered

characteristic to these varied temperatures. The effects of cooling are known to elicit a strong sympathetic response and therefore decreases in peripheral blood flow (Cheung, 2015). Blood flow patterns to glabrous skin and non-glabrous skin were looked at during cooling from 32-17°C in a climatic chamber (Elstad et al., 2014). Fluctuation frequency, amplitude and synchronicity were found to be higher at 25°C compared to either 32°C or 17°C, corresponding to higher synchronicity when subjects were in their thermoneutral zone. The effect was a general fall in peripheral blood flow with decreasing temperature, with a dramatic drop in blood flow to glabrous skin observed at a median temperature of 24°C. The control of peripheral blood flow seems to be most impacted by core temperature. Several studies have demonstrated that when core temperature begins to decrease below baseline, peripheral blood flow remains continuously low (Daanen et al., 1997; Flouris et al., 2008; Vanggaard et al., 2012). Thus, while peripheral thermal sensation is important for governing blood flow patterns, the maintenance of core body temperature appears to override this at all costs, as seen by the diminished CIVD response to lower than normal body temperatures (Daanen et al., 1997; Flouris et al., 2008).

The impact of ambient temperatures may not only impact blood flow through sympathetic influence but may also involve endothelium-dependent vasodilation (EDV). While there is no reflexive control of vasodilation in glabrous skin, the endothelium does contain a vasodilatory mechanism, which is mediated through the activity of endothelial nitric oxide synthase (eNOS) and nitric oxide (NO) (Bergersen, Skytioti, and Elstad, 2017). In 2017, a study looked at how the SNS influences EDV by exposing subjects to thermoneutral (29°C) and cold (22°C) ambient temperatures on separate days and inducing an EDV response through the release of a suprasystolic pressure cuff (Bergersen, Skytioti, and Elstad, 2017). The results of this study showed that the relative EDV response was higher in the cold than in the thermoneutral condition in skin containing AVAs (fingertips), while in the dorsal wrist (absence of AVAs) absolute EDV response was smaller in the cold than in thermoneutral. This increase in EDV in fingertips is likely mediated by AVAs, which allow for a much larger quantity of blood flow, and the results of this study seem to indicate that the relaxation caused by EDV of the vascular smooth muscle is not affected by the increase in sympathetic activity brought

about by ambient cold temperatures (Bergersen, Skytioti, and Elstad, 2017). This increase in blood flow following occlusion by a cuff maintained at a suprasystolic pressure, termed post occlusive reactive hyperemia, was initially believed to be a measure of sympathetic activity (Schürmann, Gradl, and Fürst, 1996), however, recent studies have indicated that it is an important marker of cardiovascular reactivity and therefore cardiovascular health and is mediated by the activity of K_{IR} channels (Crecelius et al., 2013). The importance of these ion channels and others may also be involved in other events such as the myogenic control of blood flow, which is poorly understood but seems to involve an increase in intraluminal pressure which results in the activation of mechanosensitive ion channels in the smooth muscle membrane (Clifford, 2011). Importantly, this reactive hyperemia is typically studied using forearm blood flow, and therefore differences in patterns between the glabrous skin of the fingertips and non-glabrous skin of the forearms may play a role in a difference of control of this mechanism.

The effects of local temperature manipulations also influence blood flow patterns. In a study from 1995, subjects submerged both hands into water baths at a temperature of 35°C (Bergersen, Eriksen, and Walløe, 1995). From there, the experimental hand was then raised linearly to 43°C while the control hand was kept at 35°C. The blood velocity fluctuation in the control hand remained unaltered, while the mean velocity of the heated hand showed an increase with increasing water temperature (Bergersen, Eriksen, and Walløe, 1995). Despite this increase in velocity, the fluctuations remained closely correlated to those of the control hand, showing that local temperature manipulations do not appear to affect synchronicity (Bergersen, Eriksen, and Walløe, 1995). This seems to contrast with the observed increase in SSNA in response to local heating, however the study by Bergersen and colleagues took blood flow velocity recordings from the radial artery and indicated it as a proxy for flow through glabrous skin. In two separate studies from 1987 and 1990, it was indicated that HIVC may be mediated by AVAs brought about by this increase in SSNA activity, while the increase in blood flow observed by Bergersen and colleagues, may be a result of dilation of the capillaries and other microvasculature in the periphery (Nagasaka, Hirata, and Nunomura, 1987; Nagasaka et al., 1990). This conclusion was drawn based on the results of utilizing both LDF as well

as plethysmography and was based on the assumptions that LDF in the study by Bergersen, Eriksen, and Walløe did not measure blood flow deep enough to reach AVAs. In the study performed in 1990, they also used an anesthetic blockade of the median nerve which blocked the response of SSNA bursts as well as decreases in fingertip blood flow, indicating that the HIVC response is the result of increased SSNA to local heating (Nagasaka et al., 1990).

Local cooling also plays a significant role in the manipulations of blood flow fluctuations. There are several main effects of local cooling including the reduction in tonic NO formation due to the inhibition of eNOS, upregulation of α_2c adrenoreceptors to the smooth muscle cell membrane, increased NE release, and after a short period of time CIVD may occur in glabrous skin (Johnson and Kellogg Jr, 2018). The magnitude of the local cooling has been shown to be an important consideration for the amount of SNA elicited. In 2000, Sendowski and colleagues tested the effects of local cooling in 5°C water by immersing either the right index finger, right hand, or both left hand and right index finger (conditions 1, 2, and 3 respectively) (Sendowski et al., 2000). For both condition 2 and 3, an initial elevated heart rate was observed, as well as increased concentration of plasma NE (Sendowski et al., 2000). This elevated concentration of plasma NE indicates that the magnitude of the cold exposure elicits an increasingly greater sympathetic response based on the amount of skin exposed. In a study from 1997 subjects underwent local cooling from 35-19°C for their experimental hand while the control hand remained unchanged (Bergersen, Eriksen, and Walløe, 1997). The velocity fluctuations of the experimental hand remained nearly unchanged and related to those of the control hand during cooling to 21.5°C, below which the velocity fluctuations ceased abruptly. This indicates that there is a local thermal level below which there is an abrupt closure of all AVAs (Bergersen, Eriksen, and Walløe, 1997). This is in support of local cooling response to SSNA which displays an immediate increase in SSNA followed by a likely decrease mediating the response of CIVD. The study by Bergersen and colleagues did not allow adequate time for a CIVD response to occur, however in a follow up study they tested blood flow fluctuations during CIVD (Bergersen, Hisdal, and Walløe, 1999). They investigated blood flow oscillations during local cooling kept at a steady temperature of 3°C and found that velocity fluctuations in the finger artery during CIVD

were similar to those in the control hand just with smaller amplitude (Bergersen, Hisdal, and Walløe, 1999). Considering the findings in response to both local heating and local cooling of the periphery, blood flow fluctuations do show a strong correlation at separate sites of the body despite manipulations in local temperature.

Mental disturbances have also been shown to cause disruptions in blood flow patterns, as they result in the closure of AVAs and a decrease in blood flow to the periphery (Walløe, 2016). Respiratory perturbations (such as the breath hold), arousing or painful stimuli, and emotional stress have all been demonstrated to cause reflex changes in skin blood flow (Krogstad et al., 1995). The regulation of this reflexive control was investigated by placing LDF probes on both glabrous and non-glabrous skin and measuring the response to emotional stress and painful stimuli (Krogstad et al., 1995). In non-glabrous skin, no vasoconstrictions were observed in response to the reflexive stimuli indicating that AVAs are the major effectors of the vasoconstriction to reflexive stimuli (Krogstad et al., 1995).

2.3.6 Individual Variability

As seen with sympathetic activity, a variety of individual factors affect blood flow patterns, many of which are identical to the those that influence sympathetic activity. Factors such as biological sex, menstrual cycle status, and age have all been shown to influence blood flow patterns. During menopause, reproductive hormone levels change drastically and alter the thermoregulatory control of skin blood flow (Charkoudian, 2003). Estradiol and progesterone have both been shown to influence thermoregulation through changes in blood flow patterns, with estradiol promoting heat dissipation and progesterone tending to conserve higher body temperatures (Charkoudian et al., 2017). Estradiol is believed to be involved in the formation of NO, as despite limited research, a seemingly positive relationship between estradiol and NO levels has been observed in females (Charkoudian et al., 2017). The overall regulation of blood pressure in young women is typically better than in young men due to the reactivity of their blood vessels, with lower blood pressures and a smaller risk of hypertension observed (Charkoudian et al., 2017). The hot flashes that occur during menopause are believed to be mediated by hormonal influences on central neural control of skin blood flow and sweating

(Charkoudian et al., 2017). In general, the decrease in reproductive hormones post-menopause results in a decreased thermoregulatory response in aging women, however, this is not only the case in women, as age itself seems to influence blood flow patterns (Greaney, Alexander, and Kenney, 2015; Charkoudian et al., 2017). The decrease in reactivity of the vasculature with aging can be partially reversed through increased physical activity (Charkoudian et al., 2017), allowing for better thermoregulatory function with age, however the general change in blood flow patterns with age is a part of normal healthy aging and is not indicative of any pathology. The decreases in reactivity with aging can occur along various pathways including neural and local vascular responses, thus influencing blood flow patterns in a variety of ways (Greaney, Alexander, and Kenney, 2015; Charkoudian et al., 2017). Some recent work has argued that the decreased responsiveness of aged skin is due to the aging cutaneous arterioles themselves (Greaney, Alexander, and Kenney, 2015).

Patients with type 2 diabetes mellitus, Raynaud's phenomenon, and erythromelalgia, all display varied forms of altered blood flow patterns (Charkoudian, 2003). Whether these pathologies are the result of local or reflexive alterations to blood flow patterns can vary, however the most important consideration is the lack of responsivity of these vessels. It has been shown that these patients may not elicit the normal fluctuations in blood flow patterns observed inside or out of the thermoneutral zone, creating potential thermoregulatory complications (Schürmann, Gradl, and Fürst, 1996). The understanding of how these different pathologies influence blood flow patterns is of great concern to those in the medical field, and blood flow patterns and reactivity of the microvasculature has been a proposed mechanism of identifying some of these pathologies (Schürmann, Gradl, and Fürst, 1996).

2.4 Measurements of Sympathetic Activity

2.4.1 Original Measures/Microneurography

Methods to measure sympathetic activity have been around since the late 1800s, however, these original measures were relatively crude by today's standards. The conventional measures of sympathetic activity were indirect, often done by electrical stimulation of nerves, observing the response of the effector organs themselves, or by

measuring the changes in plasma norepinephrine (NE) levels (Mano, 1998). Some animal model work had been used as a proxy to understand the observed responses, however, the need for human measurements was clear (Hensel and Boman, 1960). One of the largest limitations of early sympathetic measurements was the lack of standardization, with procedures often not being able to be replicated from one study to the next (Vallbo, 2018).

Considering the limitations of recordings of sympathetic activity, the search for a novel measurement yielded success in the 1960s with the development of microneurography (Hensel and Boman, 1960; Hagbarth and Vallbo, 1968; Vallbo, 2018). Microneurography is a direct measure of sympathetic activity recorded from peripheral nerves *in situ*, and while it continues to be the gold standard for measurements of sympathetic activity it does come with several important limitations, with the most immediate and obvious limitation being the invasiveness of the measure among several other known limitations, including the difficulty of getting an adequate signal itself (Wallin and Fagius, 1988). The ability to accurately measure SNA is of vital importance to those in the medical field as increased SNA has been demonstrated in a host of cardiovascular diseases (Guild et al., 2009) and thus a search for other measures of SA, particularly for non-invasive, standardized methods is of growing interest as the understanding of the importance of sympathetic activity continues to grow.

2.4.2 The Search for Novel Measures of Sympathetic Activity

More recent studies have attempted to create novel measures of sympathetic activity based on the end organ response. Possible measures have included salivary α-amylase levels (Bosch et al., 2011), the skin wrinkling response following cold water exposure (Wilder-Smith, 2015), and monitoring of SSNA through recordings taken with an electrocardiogram (Medias, 2017). The rationale behind these methods in general is that an increased end organ response, is indicative of increased sympathetic activity and while these methods have shown some promise in their relation to overall SNA levels, the major limitation is that these methods often do not demonstrate a direct relationship in terms of the magnitude of sympathetic activity burst by burst, or temporal or regional

information. Therefore, research continues to look for standardized, non-invasive measures of sympathetic activity.

2.5 Measurements of Peripheral Skin Blood Flow

2.5.1 Other Measures of Blood Flow

Methods for measuring blood flow through the peripheral microvasculature present a series of methodological challenges in part due to the lack of spatial homogeny in vessel structure throughout the periphery (Allen and Howell, 2014). Another difficulty is the fact that perfusion through the microvasculature varies greatly, from near zero when vessels are closed, to up to 8 $L \bullet min^{-1}$ when maximally vasodilated (Charkoudian, 2003). As an understanding of the importance of the peripheral microvasculature continues to grow, an increased focus on reliable visualization tools of these vessels and measures of cutaneous blood flow is of growing interest to those in research and clinical fields. Measures of peripheral cutaneous blood flow include techniques which continue to be refined to this day such as plethysmography and newer more direct measures such as laser Doppler flowmetry (Allen and Howell, 2014). Different measures of peripheral blood flow have distinct advantages and disadvantages and the method chosen depends on the focus of the research being performed.

There are several unique methods of plethysmography which can be utilized to study peripheral blood flow patterns. Arterial occlusion plethysmography involves the application of a suprasystolic pressure to the digit being measured by inflating a pressure cuff which is connected to a plethysmograph (Mahbub and Harada, 2011). The cuff is then gradually released, ceasing the occlusion, and allowing for a resumption of blood flow which can then be measured via the plethysmograph (Mahbub and Harada, 2011). Another measure of plethysmography is the venous occlusion method where the cuff is inflated to a pressure above venous pressure but below arterial pressure (Mahbub and Harada, 2011). This allows blood flow into the finger but traps blood there as it cannot return through the venous system and thus overall blood volume increases which can then be measured as the amount of blood over time to give a value of blood flow (Mahbub and Harada, 2011). To this day novel measures of plethysmography continue to be developed such as the photoplethysmography method, which uses an LED to illuminate tissue and a

photodetector to measure the variations in light intensity associated with changes in perfusion (Jayanthy, Sujatha, and Reddy, 2011). Methods of plethysmography have several limitations including: the cost of the equipment, the complicated nature of this method, as well as that these measures often do not give a continuous measure of flow (Mahbub and Harada, 2011, Walløe, 2016).

2.5.2 Laser Doppler Flowmetry

Laser Doppler flowmetry (LDF) is a non-invasive, relatively inexpensive way of measuring peripheral skin blood flow (SkBF) (Schabauer and Rooke, 1994). LDF was developed after plethysmography was already an established technique, however LDF measurements addressed one of the largest limitations of plethysmography, which was that LDF measures could take continuous recordings of blood flow (Walløe, 2016). LDF recordings were first measured in 1972 when studying retinal perfusion in rabbits by Riva, Ross, and Benedek, which at the time was termed laser Doppler velocimetry (1971). The first use of LDF technology for assessments of skin blood flow in humans was performed by Stern in 1975, measuring blood flow in the fingertip with and without occlusion to demonstrate that the Doppler signal measured was the result of blood flow (Stern, 1975). Stern demonstrated that LDF measures are relatively easy to implement compared to other methods at the time, which has resulted in LDF being used in both research and clinical settings since its inception (Stern, 1975; Zegarra-Parodi et al., 2014).

LDF is a relatively easy to implement technique whereby when a probe is placed onto the skin, a low power laser shines infrared light carried by an optical fibre onto the tissue it is placed over (Vongsavan and Matthews, 1993; Zegarra-Parodi et al., 2014). An example figure of the LDF schematic is provided in Figure 2.8. The light undergoes a small shift in frequency due to the Doppler effect when it hits moving particles, such as red blood cells (RBCs) (Eriksson, Nilsson, and Sturesson, 2014), which is then scattered back and detected by a photoreceptor (Zegarra-Parodi et al., 2014). This Doppler shift only occurs when the light is reflected off a moving object, thus any reflected light from static tissue returns with the same frequency (Eriksson, Nilsson, and Sturesson, 2014). The average amount of change in frequency is proportional to the average speed and

concentration of the RBCs (Eriksson, Nilsson, and Sturesson, 2014). This infrared light penetrates to a depth of approximately 1.0-4.5 mm, measuring blood perfusion of the dermis. The depth the LDF measures is dependent on the wavelength of light emitted, and as such LDF can be used to assess blood flow to the nutritive capillaries (superficial) all the way down to the underlying arterioles and venules located deeper in the dermis (Allen and Howell, 2014). An example tracing of the LDF output is provided in Figure 2.4.

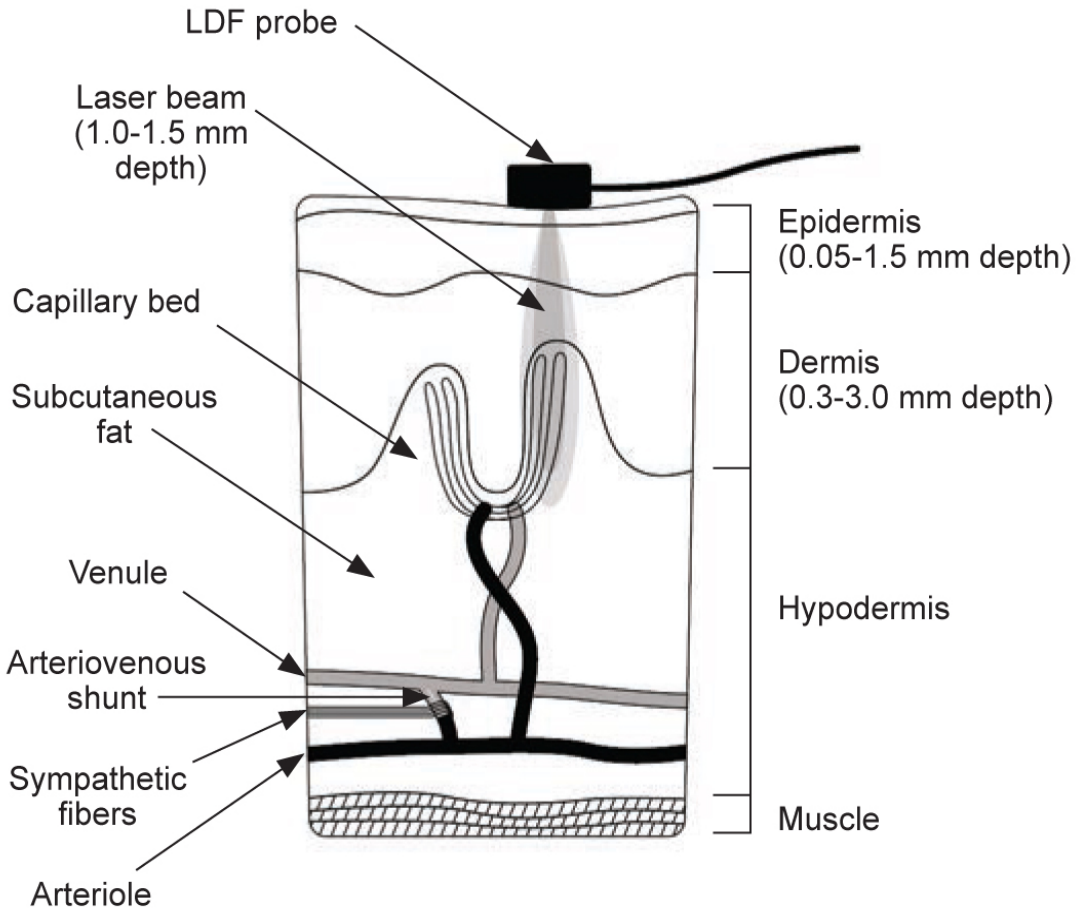

Figure 2.8 Representation of a typical LDF setup with the probe placed on the skin and the area being measured from being the cutaneous peripheral microvasculature. From Zegarra-Parodi et al., 2014
Laser beam penetrates the dermis to measure the number of moving red blood cells (RBCs) and their average velocity. The Doppler shift in the refracted light is used to measure blood flow by a proxy of red blood cell flux.

One important consideration of LDF is that it does not actually measure blood flow but rather monitors red blood cell velocity and concentration, thus providing an index of perfusion referred to as flux (Zegarra-Parodi et al., 2014). A limitation of this is that the flux signal increases linearly, but not proportionally, with flow (Walløe, 2016). In addition, due to the nature of the scattered light the recording will never be zero, even if there is no blood flow (Vongsavan and Matthews, 1992). Because of these two limitations, the value recorded is relative and not absolute (Jayanthy, Sujatha, and Reddy, 2011), however the use of LDF as a reliable measure has been demonstrated on several occasions (Dawson et al., 2015; Roeykens, Deschepper, and De Moor, 2016). The validity of LDF measures continues to be tested to this day and with novel standardization techniques and increasing knowledge on the factors which influence blood flow, the focus has become on presenting LDF measures in a standardized way to compensate for the highly variable values obtained from peripheral skin blood flow measures.

Two methods for processing of LDF recordings have been proposed: the time-domain analysis and the frequency-domain analysis (Zegarra-Parodi et al., 2014). When the time-domain analysis is used, LDF data are often presented as arbitrary perfusion units (APU) and values are averaged over a specific time and then expressed as a percent change from baseline values (Zegarra-Parodi et al., 2014). The LDF signal can also be filtered and transformed into a frequency domain using a power spectral analysis (Zegarra-Parodi et al., 2014). Spectral analysis is a tool by which, the relative power at distinct frequencies can be observed from an overall signal (Tankanag and Chemeris, 2008). Using a process of deconvolution of the time-based signal, the original signal can be summarised as an additive series of sine and cosine waves of varying frequency and amplitude within the signal which when observed overlaid on one another make up the entire recording (Tankanag and Chemeris, 2008). Within the time domain analysis, two methods of expressing and analyzing SkBF data exist: the maximal effect method and the integral measurement method (Zegarra-Parodi et al., 2014). The latter is the recommended method and is performed by averaging APU data over different lengths of time and the experimental and final rest period values are converted into percent change from baseline. The results of SkBF recordings can be reported as APU as previously

mentioned or as cutaneous vascular conductance (CVC), which is a more novel way of reporting blood flow as instead a measure of conductance. CVC is obtained by taking the measured APU value and dividing by mean arterial pressure (MAP) (Zegarra-Parodi et al., 2014). The utilization of CVC over APU is of growing importance in the field as it is a standardization technique which provides more reliable values for blood conductance, particularly over time (Dawson et al., 2015).

2.5.3 Application of Laser Doppler Flowmetry

LDF measures are often coupled with reactivity tests to elicit changes in blood flow which can then be used as tools for standardization or to understand the influence of these effects on blood flow patterns. The reactivity tests include mechanical, thermal, electrical, and pharmacological stressors, which elicit a physiological response that can then be measured. These reactivity tests include: the postocclusive reactive hyperemia test and the local thermal hyperemia test both of which have established validity in elevating blood flow above baseline to maximal or near maximal levels (Zegarra-Parodi et al., 2014). Evaluations of the association between SSNA and SkBF include the breath hold test, cold pressor test, and mental tests which are all recorded by LDF before, during and after the perturbations to determine how blood flow changes throughout the test (Zegarra-Parodi et al., 2014). LDF has also been used to investigate peripheral microvasculature disorders commonly seen in a variety of disease states, as there are often associated decrements to blood flow patterns (Allen and Howell, 2014; Zegarra-Parodi et al., 2014). The principle of these tests is a perturbation is applied which alters blood flow patterns in a known way and using LDF measures of peripheral blood flow are observed, looking for an abnormal response indicating the possibility of damaged microvasculature or a compromised sympathetic response. In fact, LDF has been a proposed clinical tool for quite some time now, with uses including the evaluation of burn depth and assessment of Raynaud's phenomena via the measurements of peripheral blood flow (Eun, 1995).

There are several individual variables which are known to influence measures of blood flow and must be considered using LDF to understand the values recorded. The placement of the LDF probe is an important consideration when measuring SkBF as there

are several factors known to influence recordings such as skin thickness which itself can be dependent on factors such as age and sex (Zegarra-Parodi et al., 2014). Areas with less adipose tissue or with more superficial, and therefore accessible microvessels, often display higher baseline values of blood flow, such as in the hands compared to the lower extremities (Bircher et al., 1994; Eun, 1995). The best data are often obtained when the LDF probe is placed over top of skin that has the highest density of AVAs superficial enough to be recorded from (Zegarra-Parodi et al., 2014). For example, when the probe is placed on the fingertip, the reproducibility is higher than if it is placed on the forearm, due to the higher concentration of AVAs in the fingertips compared to the forearms (Zegarra-Parodi et al., 2014). Factors such as age and race appear to not influence baseline values of LDF recordings, however there are mixed results on whether sex influences recordings of blood flow by LDF (Bircher et al., 1994). Whether this is due to more adipose tissue, thinner skin, or differences in blood flow patterns is unclear, however it is important to understand the differences between males and females in terms of regulating blood flow when interpreting blood flow measures (Bircher et al., 1994). LDF recordings are highly dependent on the temperature that the recordings are taken at, and some LDF devices have built in technology to adjust the local temperature at the probe allowing the researcher control of the temperature recorded at (Bircher et al., 1994). Recordings utilizing LDF can be highly variable even millimetres apart on the body due to the lack of spatial homogeny in the microvasculature of the area. Thus, it is often difficult to take recordings from the exact location on subsequent measures and as such it is difficult to standardize normal flow values even within subjects (Eun, 1995). Overall, the use of LDF as a tool to continuously monitor blood flow has shown good reproducibility and measures of blood flow display the previously described rhythmic oscillations that are observed when recorded via LDF which has led researchers to postulate about the possibility of LDF for other uses than just measuring blood flow.

2.6 Linking Sympathetic Activity and Measures of Blood Flow

2.6.1 LDF and Sympathetic Perturbations

With an understanding of how sympathetic activity influences peripheral skin blood flow and the advent of methods allowing for blood flow to be continuously

monitored, researchers have begun to look at the effect of sympathetic perturbations on blood flow patterns (Zegarra-Parodi et al., 2014). Several studies were done, beginning in the 1990s, which coupled measures of blood flow via LDF with some of the previously discussed perturbation tests known to elicit sympathetic activity to determine whether the changes in blood flow were indicative of altered sympathetic activity and therefore if this measure had future research or clinical implications. Some of the more commonly used perturbation stimuli include variations of the cold pressor test, breath hold, painful stimuli, and mental or emotional stressors which are known to increase sympathetic activity (Kistler, Mariauzouls, and von Berlepsch, 1998). Research began to assess the feasibility of this modality, as blood flow patterns are highly variable and influenced by a multitude of factors, including both vasoconstrictor and vasodilator nerves. To reduce these variables researchers often look at glabrous skin where the control of blood flow is less complex.

Due to the arrhythmic nature of fingertip blood flow one common methodology that has evolved to bring about some level of standardization is the use of body heating to induce central vasodilation, thereby obtaining relatively high, stable, and comparable fingertip blood flow values (Khan et al., 1991; Schürmann, Gradl, and Fürst, 1996). This method allows for the evaluation of decreases in blood flow brought about by sympathetically mediated vasoconstriction to be evaluated more easily as the large oscillations mediated by sympathetic activity are all but abolished with central vasodilation (Khan et al., 1991). Researchers have also investigated the potential of local heating, as studies have demonstrated that local heating increases blood flow to near maximal levels and all but abolishes the vasomotion brought about by neurogenic control (Kastrup, Bürlow, and Lassen, 1989). Due to the complex nature of local heating, such as rate of warming and the possibility of HIVC, central vasodilation is often the preferred method of standardization, however future research into the differences of central versus local heating are needed to better understand what mechanisms govern these differences. The reproducibility of perturbation tests such as inspiratory gasp and contralateral cooling when combined with central vasodilation has been tested and demonstrates good reproducibility over the course of several days, indicating that these are reliable tests to assess the autonomic nervous system through changes in blood flow patterns

(Schürmann, Gradl, and Fürst, 1996). Thus, while the patterns of peripheral blood flow are often difficult to interpret, the development of standardization techniques such as the induction of central vasodilation, which elevates peripheral blood flow to a relatively high, nonfluctuating signal, enables researchers to evaluate the response of peripheral blood flow patterns to sympathetic activity.

The evaluation of skin blood flow responses to different perturbations is often assessed in a healthy population and then compared to a population with a known sympathetic impairment to observe differences and determine the validity of the test (Khan et al., 1991; Schürmann, Gradl, and Fürst, 1996). The use of different populations allows for an understanding of how participants with varying levels of sympathetic activity respond to the perturbations, and thus how well the LDF captures these differences via an altered blood flow response. In a study from 1991, patients with autonomic neuropathy were used and compared to age-matched control subjects (Khan et al., 1991). Those with the autonomic neuropathy were shown to have a significantly lower vasoconstrictor reflex than the age matched controls in response to both contralateral hand cooling, as well as an inspiratory gasp (Khan et al., 1991). In a similar study done in 1996, patients with sympathetic reflex dystrophy (SRD) were compared to healthy age matched control subjects, and similar results were found (Schürmann, Gradl, and Fürst, 1996). Those with known autonomic impairments displayed a reduced vasoconstrictor response compared to healthy participants, despite having no differences in baseline blood flow values or elevated blood flow values to central vasodilation (Schürmann, Gradl, and Fürst, 1996). This showed that the difference lay specifically in the vasoconstrictor response and was characterized by the reduced microvascular reactivity. These studies also showed that this reduced sympathetic activity could be observed utilizing LDF through the lack of a response in blood flow patterns. Due to the difference between healthy subjects and those with autonomic disorders, LDF has been proposed as a non-invasive assessment of peripheral sympathetic activity with the ability to possibly diagnosis disorders in the early stages of the pathology (Schürmann, Gradl, and Fürst, 1996).

The purpose of these studies is often to determine the potential clinical applications of LDF technology, attempting to determine its potential of diagnosing

autonomic dysfunction in the periphery (Schürmann, Gradl, and Fürst, 1996; Bonelli and Költringer, 2000). The importance of these tools cannot be overlooked as fibre degeneration occurs in the smallest, most distal areas first and thus the ability to test the reactivity of the smallest fibres in the periphery could lead to earlier diagnoses with more positive outcomes (Bonelli and Költringer, 2000). One consideration of presenting these methods for clinical application is the need for standardization. The method must be reproducible and must be able to distinguish between a normal and an abnormal response. In the study from 1996, they developed two quotients that were meant to help standardize the measures and reduce interindividual variability by simply quantifying the magnitude of the response rather than the direct value (Schürmann, Gradl, and Fürst, 1996). The results of this study showed good reproducibility of the two quotients over time as well as high sensitivity and specificity indicating that they were reliable measures for distinguishing between those with and without autonomic dysfunction (Schürmann, Gradl, and Fürst, 1996). In a study done in 1993, researchers compared 3 separate perturbation tests, the inspiratory gasp, ice-water immersion, and pin prick, to determine their effect on the vasoconstrictive response (Valley et al., 1993). This study found that the inspiratory gasp (breath hold technique) and contralateral cooling (cooling stimulus) produced the largest and most reproducible vasoconstrictive responses and suggested these two as the principal tests going forward (Valley et al., 1993). Since then, many other studies have continued to use, and show the reliability of these two reactivity tests in eliciting robust sympathetic activity and strong vasoconstrictive responses, making them the most common perturbation tests due to their strong sympathetic response and relatively low level of discomfort caused to the participant (Zegarra-Parodi et al., 2014).

The tests assessing the ability of peripheral blood flow changes to approximate sympathetic activity have revealed distinct differences in separate populations. As previously discussed, those with autonomic disorders show little or no vasoconstrictive response to the perturbations (Khan et al., 1991; Schürmann, Gradl, and Fürst, 1996). Gender differences have also been observed, with young women displaying increased microvasculature reactivity compared to age matched male counterparts (Schürmann, Gradl, and Fürst, 1996). This appears to only be true for perturbations involving temperature however, as the increased reactivity was observed for contralateral cooling

but not for inspiratory gasp (Schürmann, Gradl, and Fürst, 1996). Whether this is due to an overall larger cold stimulus from the smaller area cooled or an actual difference in the response to the same cold stimulus is unclear, though women have been shown to display a larger cold response prior to menopause compared to men (Charkoudian et al., 2017). In the study by Schürmann, Gradl, and Fürst they showed a tendency for a decreased vasoconstrictor response with age; however, this finding did not reach statistical significance (Schürmann, Gradl, and Fürst, 1996). Despite this, recent research has clearly shown that the sympathetic response is diminished with age and thus it is an important consideration when performing studies such as these to consider the age and sex of the participant (Greaney, Alexander, and Kenney, 2015). One potentially overlooked variable is the influence of circadian rhythm on blood flow patterns (Smolander et al., 1993). Variations in internal temperature throughout the day result in differences in baseline blood flow values, and as such, studies should attempt to control for the time of day that participants are examined, while also measuring internal core temperature to better justify that these measures are truly taken within an individual's thermoneutral zone. Finally, the hand measured on does not appear to influence the overall response with no dominant hand effects being revealed in the study when they tested both the right and left hand of participants to multiple perturbation tests (Schürmann, Gradl, and Fürst, 1996).

In a unique study done in 1998, researchers attempted to use fingertip temperature rather than blood flow to determine if this was a possible measure for sympathetic activity, proposing that this method had even fewer limitations than blood flow measures (Kistler, Mariauzouls, and von Berlepsch, 1998). With the knowledge of the relationship between blood flow and skin temperature the study used infrared thermography to measure fingertip temperature and related the observed changes to a variety of stimuli known to elicit a sympathetic response, along with LDF to characterize the changes in skin blood flow (Kistler, Mariauzouls, and von Berlepsch, 1998). They showed that the stimuli produced an immediate decrease in blood flow brought about by peripheral vasoconstriction, and that with a lag phase of approximately fifteen seconds the reduced blood flow was reflected by a decrease in fingertip temperature (Kistler, Mariauzouls, and von Berlepsch, 1998). There were limitations to this method however, such as that

vasoconstriction of a short duration was not enough to cause a decrease in fingertip temperature as well as that the initial starting temperature of the fingertip needed to be greater than 32°C (Kistler, Mariauzouls, and von Berlepsch, 1998). Overall, the use of temperature rather than blood flow remains a possible tool for the non-invasive assessment of sympathetic activity, however further standardization as well as more simple ways to continuously monitor areas of interest with infrared thermography are needed.

2.6.2 Bilateral Blood Flow Studies

Peripheral blood flow patterns have been studied extensively over the past century, however with more recent advances in technology and standardization a great deal of research continues to be done to better understand this process. One of the more recent findings is that there are the distinct frequencies in blood flow oscillations, essentially boiling down to central and local control of peripheral blood flow patterns. At separate times and in separate scenarios one may be more influential than another, or these processes may work together to alter blood flow patterns. Central control of blood flow should indicate that peripheral blood flow patterns are closely matched at separate sites of the body and with these newer technologies, researchers have begun to investigate this possibility.

As early as the 1930s researchers realized that blood flow oscillations at separate sites of the body appeared to be similar, exhibiting large fluctuations that were demonstrated to be of sympathetic origin (Burton 1939; Thoresen and Walløe, 1980; Walløe, 2016). The vasoconstrictions that cause these oscillations in blood flow occur rhythmically, approximately 1-3 times per minute, and have a characteristic shape due to the rather rapid nature of the vasoconstriction and the subsequent gradual release of the vasoconstriction (Walløe, 2016). The fluctuations are most correlated when subjects are in the middle of their thermoneutral zone and have demonstrated positive correlation coefficients in the magnitude of 0.7-0.95 (Walløe, 2016).

Since the 1990s several studies have utilized photoplethysmography (PPG) to assess bilateral blood flow patterns and assess autonomic control (Bernardi et al., 1996). The PPG signal displays very low frequency fluctuations which have been shown to be

mediated by sympathetic activity (Khanokh et al., 2004). Researchers use aspects of these fluctuating signals, such as pulse amplitude and its baseline value, and compare these across separate sites of the body (Khanokh et al., 2004). In a study from 2004, bilateral measures utilizing PPG were done on the right and left index finger as well as the right and left big toe of participants (Khanokh et al., 2004). The baseline and amplitude fluctuations in the low frequency signal showed high (>0.89) correlation between left and right hand and feet (Khanokh et al., 2004). In a study done in the following year, researchers investigated whether this high correlation across the body remained for patients with diabetes (Buchs et al., 2005). In participants without diabetes the same high (>0.93) correlation at separate sites was observed, however for patients with diabetes the correlations were much lower (<0.78) (Buchs et al., 2005). Patients with diabetes exhibit decrements in the sympathetic activity over time and this study showed that patients who had been diabetic for a longer time had lower correlation values than those who had been recently diagnosed (Buchs et al., 2005). In another very similar study, the correlation across the body was looked at prior to and following thoracic sympathectomy, however this study looked at cardiac induced oscillations (Nitzan et al., 2001). The authors showed that prior to sympathectomy the correlation across the body was high (>0.90) and that following the procedure, values decreased significantly (<0.76) (Nitzan et al., 2001).

The limitations of the aforementioned studies are twofold; first they utilize PPG which is a less reliable measure of peripheral blood flow when compared to other measures such as LDF (Lindberg, Tamura, and Oberg, 1991), and the second is that they only assess the fluctuations known to be caused by sympathetic activity not blood flow oscillations as a whole. Some studies have utilized other technologies such as one done in 1996, where researchers investigated leg blood flow using nuclear magnetic resonance flowmetry with and without lower extremity arterial disease (LEAD) (Mayrovitz and Larsen, 1996). They found highly significant correlations between legs for pulsatile blood flow, particularly in healthy subjects compared to those with LEAD (Mayrovitz and Larsen, 1996). Finally, Tankanag and colleagues in 2017 looked at the influence of local temperature changes and the synchronization of blood flow oscillations (Tankanag et al., 2017). In this study, skin blood flow to the left and right forearms were observed at rest and when elevating the local temperature of the left forearm. At rest there was a high

correlation in oscillations between the right and left forearms, however, when the asymmetric local change in temperature was applied, there was a significant reduction in synchronization of the oscillations in skin blood flow indicating the importance of temperature on these oscillation patterns (Tankanag et al., 2017). Taken together these studies provide good evidence for the influence of sympathetic activity on the control of peripheral blood flow patterns, with future studies needed to observe overall blood flow dynamics to a range of scenarios.

Finally, the most recent bilateral blood flow studies utilize both the newest technologies, such as LDF, as well as complicated analytical tools to investigate the symmetry of blood flow at separate sites. In a study from 2019, spectral analyses were performed on blood flow recordings at separate sites of the body, and then averages of the peaks in each frequency were used to make histograms to create average power within specific frequency bands which can be compared at separate sites (Grinevich et al., 2019). By doing so they were able to show a strong linear correlation in the lower frequency components of blood flow oscillations (Grinevich et al., 2019). A separate study looked at the reproducibility of separate cardiac responses to various stimuli in healthy subjects (Ho, Toska, and Wesche, 2020). One of the variables measured was bilateral blood flow recorded with LDF. They showed a strong relationship between the two sites both while resting and in response to several minor stimuli (Ho, Toska, and Wesche, 2020). The limitations of the most recent blood flow synchronicity studies are their overall design. Studies either investigate only one aspect of bilateral blood flow relationships such as the spectral analysis, or they do not make the bilateral blood flow relationship the primary focus of the study (Ho, Toska, and Wesche, 2020). By doing so they do not employ the latest standardization techniques such as the utilization of CVC, they fail to control for variables that may influence the recordings, and most importantly they often do not quantify the relationship but rather simply state that both variables appear related. Therefore, despite the understanding of how peripheral blood flow patterns are influenced by the sympathetic nervous system, as well as knowledge that sympathetic activity is related at separate sites of the body, research has yet to directly quantify the patterns of bilateral blood flow, as characterization of just the sympathetic

response does not itself reveal whether measures of blood flow may one day be able to adequately be a surrogate for measures of sympathetic activity.

2.7 Future Directions

Despite the advent of LDF technology nearly half a century ago, along with an understanding of how peripheral blood flow patterns are governed by SSNA, very little research has investigated elucidating the influence of sympathetic control at bilateral sites of the body using LDF. As is evidenced by the multitude of studies investigating phenomena such as: the role of the sympathetic nervous system in regulating peripheral cutaneous blood flow, the closely matched sympathetic activity at separate sites of the body, and the linking of perturbations known to elicit SA with changes in blood flow, research in this field continues to an area of interest. Despite this, no studies have taken all the known literature on skin blood flow patterns, sympathetic perturbations, and directly measured bilateral blood flow correlation to determine the role of central control. One important consideration not addressed in many of these previous studies is that the sympathetic vasoconstrictor system is tonically active and therefore regulates skin blood flow while at rest. Therefore, it will be an important consideration to have measures of bilateral blood flow both at rest and in response to perturbations which will elicit increased sympathetic activity to gain a better understanding of the degree of correlation in blood flow at these separate sites. With an increasing knowledge of the importance of the peripheral microvasculature, the decades of research preceding this study, and the possibility of this research being the first step to determining whether LDF measures of blood flow can be used as an indirect measure of SSNA, the next step in the field is to determine the degree to which peripheral blood flow patterns are controlled by sympathetic activity.

Objectives, Research Questions and Hypotheses

The primary objective of this study is to determine the relationship of skin blood conductance at bilateral sites of the body, both at rest and during sympathetic perturbations, to better understand the role of the sympathetic nervous system in controlling peripheral blood conductance patterns. By measuring blood conductance at bilateral sites and determining the temporal relationship between the sites, the strength of that relationship could imply a strong relationship between peripheral blood conductance activity and sympathetic nerve activity, this could lead to future research investigating the possibility of utilizing peripheral blood conductance measures as non-invasive tools to assess sympathetic activity by combining measures of peripheral blood flow with measures of SSNA. This study will have two research questions:

1. The primary research question is “how well do bilateral blood conductance measures align temporally at rest and in response to sympathetic perturbations?”. The goal of this question is to determine how changes in blood conductance patterns are related in time at separate sites. The prediction is that there will be a positive correlation between bilateral blood conductance measures indicating that changes in these variables are closely matched in time, particularly for the vasoconstrictor activity.

2. The secondary research question is “how does the magnitude of vasoconstriction in response to sympathetic perturbations compare across bilateral sites over repeated tests?”. The goal of this question is to understand the magnitude and characteristics of sympathetic activity on the vasoconstrictor response in blood conductance patterns. The prediction is that the magnitude of vasoconstriction will be similar between sites to sympathetic perturbations with a tendency to increase with additional perturbations.

Chapter 3 - Methodology

3.1 Participants

The study was approved by Brock University's Bioscience Research Ethics Board (BREB # 20-049) and conformed to the standards set by the Declaration of Helsinki. All participants were screened prior to participation using a modified Physical Activity Readiness Questionnaire (PAR-Q) and were informed of the experimental protocol and associated risks. Consent, both verbal and written, was obtained from each participant.

Ten healthy males and females (7M, 3F) were recruited from the university and general public for this experiment. A power analysis done prior to recruitment gave a target of 16 participants, however due to this project occurring during the ongoing COVID-19 global pandemic, only 10 participants were able to come in during the data collection phase. Participants were free from cardiovascular and neurological disorders and were not currently taking any form of prescription medication, apart from an oral contraceptive form of birth control (n = 1 females).

3.2 Experimental Design

Participants underwent one virtual familiarization and one experimental session for a total of one at-home and one in-lab session. The familiarization session consisted of a one-on-one meeting between the participant and the experimenter going over the experimental timeline and familiarizing the participant with the sympathetic perturbations that would be administered during the trial. This familiarization session was done virtually as this project took place during the COVID-19 pandemic, and thus this decreased total time spent in close proximity to one another. The in-lab experimental session consisted of two distinct sections: 1) a resting- thermoneutral portion, and 2) a heated, sympathetic perturbation portion. The experimental session took place on one day and had a duration of approximately 2.5 hours.

3.3 Experimental Sessions

3.3.1 Pre-test Measures and Instrumentation

Figure 3.1 outlines the protocol for the experimental session. Participants were instructed to avoid strenuous exercise in the 24 hours prior, as well as alcohol and caffeine in the 12 hours prior to their experimental session. Upon arrival to the lab on the day of the experimental session, participants age (years), height (cm), and mass (kg) were measured. The mean (± SD) for age, height, and mass, of the participants was 24 ± 3 years, 174.6 ± 9.4 cm, 76.6 ± 14.9 kg respectively. Participants voided their bladder and urine specific gravity (USG) was measured with a refractometer (PAL-10S, Atago, Tokyo, Japan). Participants were considered euhydrated if USG was ≤ 1.020, or else participants were instructed to drink 600mL of water and the test was retaken in 30 minutes. If hydration was ≤ 1.020 the participant was considered euhydrated and the experimental session continued, if the value remained > 1.020 for a second time the experimental session was rescheduled.

Female participants were asked to come in during the early follicular phase of their menstrual cycle or the placebo pill phase of oral contraceptive use. This was self-confirmed by the participants and a pregnancy test was administered using a dipstick to confirm that female participants were non-pregnant.

Participants also had their baseline blood pressure measured twice prior to the start of the trial using an automated blood pressure cuff (BPM-100, BpTRU Medical Devices, Canada) placed on their upper left arm. Participants were dressed in a t-shirt, shorts and shoes that they brought in with them and were instrumented with a 3-lead electrocardiogram (ECG), a 4 -site skin thermistor setup (bicep, chest, thigh, and calf), rectal core probe, skin thermistors on the back (between the scapula), laser Doppler flowmetry (LDF) probes (finger pad of both second fingers), and a finger blood pressure cuff (right hand, third finger).

3.3.2 Resting, Thermoneutral Portion of Experiment

Participants then rested supine on a dental chair in a room with an average temperature of 21.6 ± 0.1°C and an average relative humidity of 34.5 ± 1.5%. Participants rested on the dental chair with both arms supported by tables placed beside them with a soft towel underneath their arms to keep both arms at approximately heart level. The

lights were turned off in the room, save for one warm overhead light, and all external noise was kept to a minimum to decrease ambient stimuli. 5-10 minutes of data were collected to allow time for participants to relax as well as to ensure data were stable and accurate. After ensuring that data were stable and accurate, the experimental session began with the resting, thermoneutral portion. Participants were instructed to remain relaxed for 20 minutes with no talking or moving.

3.3.3 Sympathetic Perturbation Portion of Experiment

At the end of the 20-minute thermoneutral portion, heating pads placed between the participant's back and the chair were turned on to elevate the local temperature of the participants back to 40-45°C to elicit central vasodilation and covered with a small, light blanket. During this time participants were able to move and readjust after the 20 minutes of silent, non-moving to ensure participant comfort. When local back temperatures reached 40-45°C participants were again instructed to remain calm, and with as little movement or talking as possible. Once LDF values had elevated and stabilized the sympathetic perturbation portion began. Participants received 4 total perturbations, consisting of 2 cold stimuli and 2 breath holds in a randomized order, via a random number generator. The cold stimulus was the application of ice packs directly to both shoulders for 10 seconds. The breath hold was a non-maximal breath hold on inspiration of approximately 15-30 seconds, depending on the participant's own level of comfort, with them having been told prior to exhale when they felt "the need to breathe". The time between perturbations was at least 2 minutes to ensure LDF data had returned to pre-perturbation levels and to confirm that values were again non-fluctuating.

3.3.4 Determination of Maximal CVC

Following the 4 randomized perturbations, the local temperature control in the LDF was set to 44°C to elicit maximal blood conductance in the fingers. Once blood flow values had elevated and plateaued, 10 minutes of data were collected to ensure the maximal value had been obtained. Finally, the experimental session ended, the participants were de-instrumented, and thanked for their participation.

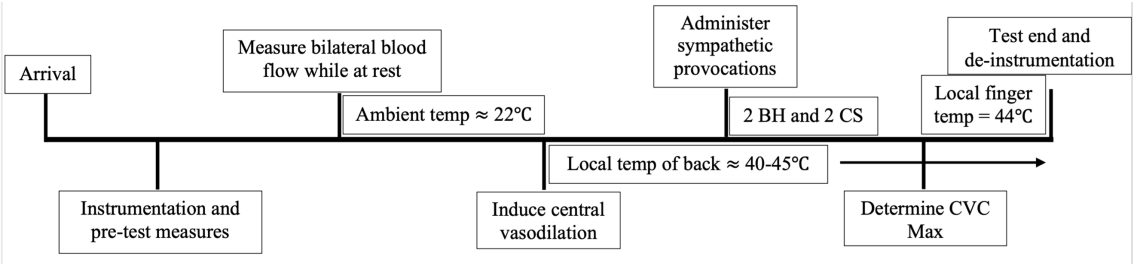

Figure 3.1 Experimental Protocol
BH = breath hold. CS = cooling stimulus. CVC = cutaneous vascular conductance. Measures of bilateral blood conductance while at rest for 20 minutes of data. Induction of central vasodilation via local heating of the back to elevate peripheral blood conductance. CVC max acquired approximately 10 minutes of data.

3.3.5 Blood Flow

Measures of blood flow were recorded via laser Doppler flowmetry (LDF) from the second finger on both the left and right hand (PeriFlux 5010 laser-Doppler perfusion monitor, Perimed, Järfälla, Sweden). An integrated laser-Doppler probe (Probe 413, Perimed) was used to measure blood flux (Arbitrary Perfusion Units; APU) and T_{LH} and T_{RH} (°C) (PeriFlux 5020 Temperature Unit, Perimed) on the distal phalanx (finger pad) of the second digit of both hands. LDF data were collected at 40 Hz (LabChart, ADInstruments, Colorado Springs, USA). Thermoneutral resting data is reported as 5 s averages every 5 s. Sympathetic perturbation data is reported as 1 s averages every 1 s.

3.3.6 Temperature

Throughout the study, core temperature (T_{core}) (°C) was monitored by using a thin, flexible, general-purpose thermistor which was self-inserted by the participant 15cm past the anal sphincter. Mean skin temperature ($\bar{T}_{sk}$) (°C) was recorded with thermocouples (VC-T-24-190, Omega Environmental Inc., CAN), which were affixed to the skin with tape (Transpore™, 3, St. Paul, USA) at 4 sites: the chest, bicep, thigh, and calf. $\bar{T}_{sk}$ was calculated using the weighted average of the four thermocouples (Ramanathan, 1964) determined using the equation:

$$\bar{T}_{sk} = 0.3T_{chest} + 0.3T_{thigh} + 0.2T_{arm} + 0.2T_{calf}$$

Finally, a thermocouple was also placed between the scapula on the back to record local back temperature (T_{back}) (°C). All temperature data were collected at 4Hz (LabChart, ADInstruments, Colorado Springs, USA).

3.3.7 Heart Rate

Heart rate measures were recorded via a 3-lead electrocardiogram (BioAmp, AD Instruments, USA) sampled at 1 kHz, with leads placed on the upper and lower left ribcage, as well as the upper right ribcage allowing for the measurement of R-R intervals, which were then used to determine heart rate (beats·min^{-1}).

3.3.8 Blood Pressure

Beat-by-beat blood pressure was measured continuously by photoplethysmography of the third digit on the right hand, resting on a table at heart level, by a finger cuff (Nexfin, BMEYE, Amsterdam, The Netherlands). Averaged mean arterial pressure (MAP) was calculated as 1/3 systolic pressure + 2/3 diastolic pressure. In addition, two baseline blood pressure measurements were collected by an automatic blood pressure cuff (BPM-100, BpTRU Medical Devices, Canada) to ensure a value of blood pressure was obtained in case of complications with the photoplethysmography signal throughout the experiment.

3.4 Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics software (Version 27; IBM Corp., USA). Statistical significance was set at a $p \leq 0.05$. Normality of data were assessed by checking the skewness, kurtosis, and by using the Shapiro-Wilk test. Outliers were checked for, using SPSS software, and if present, data was assessed with and without the outlier to determine if the outlier was affecting significance. No outliers were found to influence significance either way, and so any outliers were left in the final data. Blood flow standardization was performed by dividing the LDF data (APU) by the participant's MAP (mmHg) throughout to get cutaneous vascular conductance (CVC). Percent Max CVC was determined by then dividing their CVC value throughout by their determined CVC Max. Sympathetic perturbation characteristics (Table 3.1) were quantified using custom macros in LabChart, for example a search for F_{mean} was done by taking the 10 s average blood conductance prior to the administration of the sympathetic perturbation and F_{min} was determined by finding the minimum value blood conductance reached during the vasoconstrictor response (ADInstruments; Colorado Springs, USA). The equations for the quotients, SRF and QI, were performed as previously defined by

Schürmann, Gradl, and Fürst, and describe the magnitude of the vasoconstrictor response, with SRF representing the relative decrease of the curve following the perturbation and the QI being influenced by the maximum fall of the curve as well as the duration of the decrease (1996). All continuous data are expressed as mean ± SD.

3.4.1 Vasoconstrictor and Maximal Blood Flow Analysis

Paired samples t-tests were used to compare the maximal CVC in both right and left hands, the mean conductance for the 10s prior to- and the mean conductance of the 10 s during the sympathetic perturbation, and the duration of both the vasoconstrictor response and the return to pre-sympathetic perturbation values ($t_{decrease}$ vs t_{regen}). Comparison of the mean prior to and following the sympathetic perturbation was to ensure the SP had caused a significant vasoconstrictor response, comparison of maximal CVC was needed to ensure there was not a difference between sites measured, and comparison of $t_{decrease}$ vs t_{regen} was due to the known difference in sympathetic vasoconstriction versus the passive resumption of normal flow (Charkoudian, 2003).

3.4.2 Cardiovascular and Temperature Analysis

Differences in physiological variables (temperature measures, MAP, and HR) over the sympathetic perturbation (pre, during, and post) were assessed using a 1-way ANOVA. Comparisons were made using the 10 s average for each variable for each phase of the SP.

3.4.3 Analysis of Variables of Interest for the Sympathetic Perturbations

A 1×4 Repeated Measures ANOVA was performed to analyze the sympathetic perturbation variables to determine if there was an order effect, where the 4 conditions were the order the tests were administered. A 2×2 Repeated Measures ANOVA was performed to analyze the sympathetic perturbation variables to assess the conditions of hand (right vs left) and test (BH vs CS). An interaction effect was looked for first, but was not found for any of the variables of interest and so main effects were then looked at. Interactions deemed significant from the RM-ANOVA were analyzed using Bonferroni post hoc comparisons. Where data did not meet certain criteria (e.g., sphericity $p < 0.05$) appropriate corrections were made (e.g., Greenhouse-Geisser).

3.4.4 Analysis of Blood Conductance Correlation Over Time

To assess the relationship of blood conductance in both middle fingers over time, Pearson's Correlation Coefficients were used. This was done for both the relationship during the thermoneutral portion of data collection, as well as for the sympathetic perturbation portion. For the resting portion of the experiment, 5 s averages every 5 s of blood conductance were compared in both fingers. For the sympathetic perturbation portion of the experiment, 1 s averages every 1 s of blood conductance were compared in both fingers. A 1-way ANOVA was performed to check for statistical differences in the correlation coefficients over the phases of the sympathetic perturbation.

3.4.5 Assessment of Sympathetic Activity in Thermoneutral Blood Flow

An attempt at a preliminary look into possible sympathetically mediated vasoconstrictor activity was done by combining results from the SP portion of the experiment with thermoneutral blood flow. The average SRF for both hands was used and vasoconstrictor value that fell within 2SD of this value was looked for in thermoneutral blood flow. The rationale behind this decision was that the SRF value was not significantly different between hands and depends less on starting or ending values than other measures (e.g., F_{min} or magnitude) and that the 2SD should account for a large percentage of possible sympathetically mediated vasoconstrictor activity. From there thermoneutral blood flow was looked at for moments where vasoconstrictor events of this magnitude occurred simultaneously in both hands.

Table 3.1 Parameters of LDF Data During the Sympathetic Perturbations

Variable	Definition
Pre Phase	10 s prior to when the SP was administered
During Phase	10 s immediately after when the SP was administered
Post Phase	10s following the during phase
F_{mean}	Mean CVC value for 10s prior to sympathetic perturbation
F_{min}	Minimum CVC value reached during sympathetic perturbation
Magnitude	$F_{mean} - F_{min}$
SRF	$\frac{F_{mean} - F_{min}}{F_{mean}}$
QI	$\frac{\int pre\ phase}{\int during\ phase}$
$t_{decrease}$	Time to reach F_{min} from when sympathetic perturbation was administered
t_{regen}	Time to return to F_{mean} from F_{min}

SRF and QI are quotients used by Schürmann, Gradl, and Fürst (1996). SP = sympathetic perturbation. t = time.

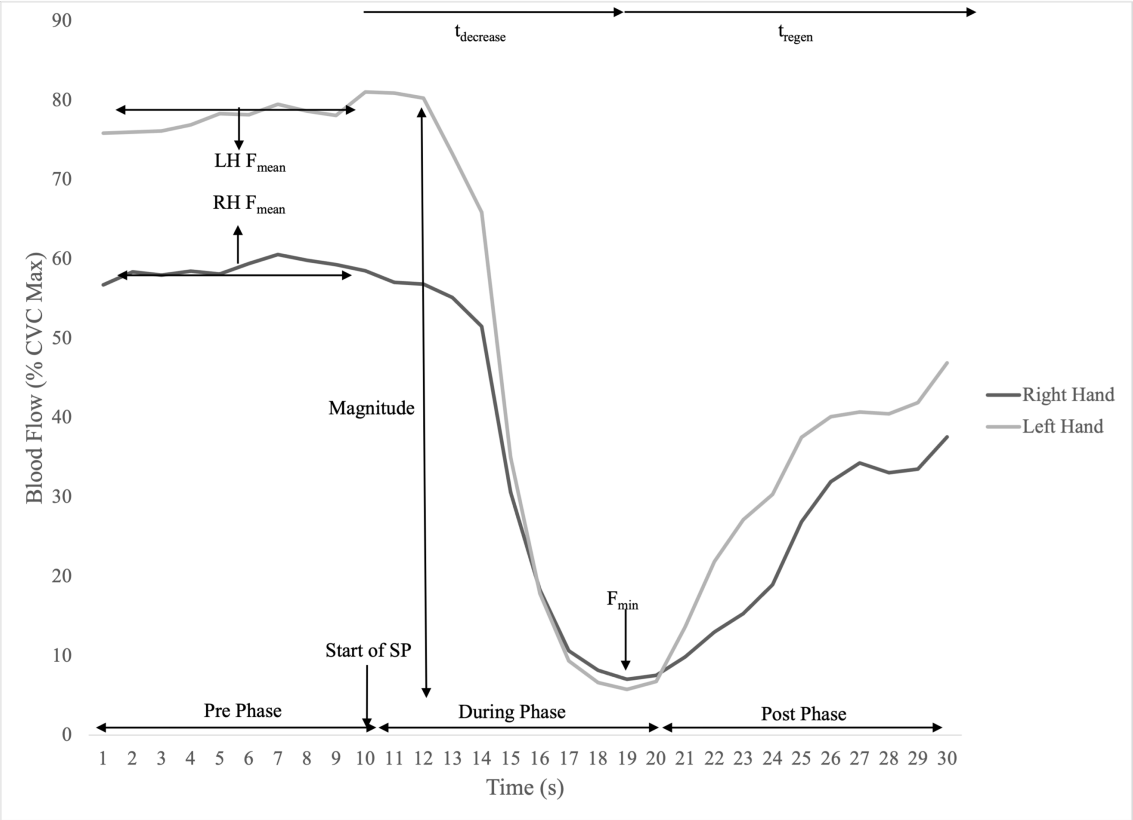

Figure 3.2 Example Tracing of LDF Response to Sympathetic Perturbation

Example tracing is actual data from participant in the study for a breath hold perturbation. SP = sympathetic perturbation. Data are 1 s averages over entire recording of the sympathetic perturbation converted to % CVC Max from APU.

Chapter 4 - Results

4.1 Skin Blood Conductance Responses

Maximal CVC, determined by local heating to 44°C was not different between the left (5.91 ± 1.61APU/MAP) and right hand (5.94 ± 2.1 APU/MAP) ($p = 0.921$). The average conductance in the 10 s period prior to the sympathetic perturbations (53.40 ± 8.95 %CVCMax) was significantly greater ($p < 0.001$) than the average blood conductance in the ten seconds following the sympathetic perturbation being administered (29.83 ± 5.06 %CVCMax), indicating that the sympathetic perturbations did cause a significant vasoconstrictor response. The time it took for blood conductance to reach its minimum (F_{min}) following the sympathetic perturbation ($t_{decrease}$; 8.57 ± 0.82 s) was significantly shorter ($p < 0.001$) than the time it took for the resumption of normal conductance (t_{regen}; 21.69 ± 3.52 s).

4.2 Cardiovascular and Temperature Responses

Cardiovascular and temperature responses were analyzed using a one-way ANOVA for time (pre, during, post phases) averaged over all 4 sympathetic perturbations. There was no effect for time ($p > 0.05$) on heart rate or any of the temperature responses (T_{core}, T_{skin}, T_{LH}, T_{RH}) during the sympathetic perturbations as presented in Table 4.1. As expected, MAP was significantly higher in the during phase of the sympathetic perturbation compared to the post phase (Table 4.1), likely due to the strong vasoconstrictor response that occurs in response to the perturbation (Table 4.1). The data were also checked with a 2×3 repeated measures ANOVA (2 = test, 3 = phase) to determine if the test administered significantly changed any of these results, however the same non-significant findings were observed for heart rate and temperature responses, and the same significant difference between during phase and post phase was observed for MAP regardless of test administered over each phase of the sympathetic perturbation.

Table 4.1 Cardiovascular and Temperature Variables Over the Phases of the Sympathetic Perturbations

Variable	***Pre Phase***	***During Phase***	***Post Phase***	***Result of RM-ANOVA***	***p Value***	***Power***
HR (bpm)	61.62 (7.63)	59.56 (5.45)	62.79 (7.65)	2.296	0.155	0.312
T_{core} (°C)	36.66 (0.31)	36.67 (0.30)	36.67 (0.30)	0.185	0.731	0.069
T_{skin} (°C)	31.52 (0.91)	31.52 (0.91)	31.46 (0.93)	0.847	0.382	0.131
T_{LH} (°C)	32.68 (0.48)	32.72 (0.47)	32.72 (0.46)	3.321	0.096	0.394
T_{RH} (°C)	32.66 (1.25)	32.72 (1.22)	32.71 (1.18)	4.309	0.065	0.47
MAP (mmHg)	84.86 (8.09)	**87.64 (9.52)**	**85.19 (10.50)**	3.85*	**0.047**	0.598

** Indicates a significant difference for main effect ($p < 0.05$). Bold is to draw attention to the significant difference between groups observed in post hoc tests ($p < 0.05$). HR = heart rate. T_{LH} = left hand temperature. T_{RH} = right hand temperature. MAP = mean arterial pressure. Result of RM-ANOVA is calculated F statistic. All variables have an n=10 except for MAP which was only recorded continuously on 8/10 participants. Data are presented as mean (±SD).*

4.3 Order Effect

To assess whether the order the tests were administered significantly affected the variables of interest, a repeated measures ANOVA was used. There was no significant effect of order in which the tests were administered across all the measured variables of interest ($p > 0.05$). This was found to be true both when variables were looked at across both hands and for when hands were averaged together, indicating that the order the tests were administered does not significantly influence the variables.

4.4 Magnitude Variables for Sympathetic Perturbations

4.4.1 Effect of Test Administered

When assessing the variables of interest for this study (Table 3.1), a significant difference was found between the breath hold and cooling stimulus perturbations for both F_{min} ($p = 0.021$) and for the SRF quotient ($p = 0.025$). As seen in Table 4.2, on average the absolute minimum value reached was lower during the breath hold perturbation (9.33 ± 6.50 %CVCMax) compared to the cooling stimulus (13.05 ± 6.55 %CVCMax), and the

calculated SRF was on average greater during the breath hold (0.80 ± 0.17 AU) when compared to the cooling stimulus (0.73 ± 0.17 AU). No significant difference was observed for any of the other variables of interest based on the test administered ($p > 0.05$).

Table 4.2 Effect of Test Administered Variables of Interest

Variable	***BH (%CVCMax)***	***CS (%CVCMax)***	***Main Effect Test***	***p Value***	***Power***
F_{mean}	53.30 (13.75)	53.61 (11.61)	0.02	0.89	0.052
F_{min}	**9.33 (6.50)**	**13.05 (6.55)**	7.852*	**0.021**	0.704
Magnitude	43.97 (16.96)	40.57 (16.36)	2.216	0.171	0.266
SRF	**0.80 (0.17)**	**0.73 (0.17)**	7.149*	**0.025**	0.664
QI	1.76 (0.43)	1.73 (0.68)	0.038	0.82	0.054
$t_{decrease}$	8.66 (1.16)	8.58 (0.94)	0.144	0.713	0.063
t_{regen}	22.85 (5.50)	21.46 (7.17)	0.47	0.51	0.094

** Indicates a significant difference for main effect ($p < 0.05$). Bold is to draw attention to the significant difference between groups observed in post hoc tests ($p < 0.05$). BH = breath hold. CS = cooling stimulus. LH = left hand. RH = right hand. Main effect is calculated F statistic (degrees of freedom = 1, 9).* Data are presented as mean *(±SD).*

4.4.2 Effect of Hand Measured

A significant difference was found for F_{mean} ($p = 0.012$) as well as in the magnitude of the vasoconstrictor response to the perturbation ($p = 0.028$) between the left and right hands. On average blood conductance prior to the perturbation was higher in the left (58.62 ± 12.35 %CVCMax) than the right hand (48.30 ± 8.75 %CVCMax), and the left hand also demonstrated larger magnitudes of vasoconstriction (47.86 ± 17.55 %CVCMax) when compared to the right hand (36.68 ± 11.57 %CVCMax) which can be seen in Table 4.3. No significant difference was observed in any of the other variables between hands ($p > 0.05$).

Table 4.3 Effect of Hand Measured for Variables of Interest

Variable	***LH (%CVCMax)***	***RH (%CVCMax)***	***Main Effect Hand***	***p Value***	***Power***
F_{mean}	**58.62 (12.35)**	**48.30 (8.75)**	9.982*	**0.012**	0.802
F_{min}	11.62 (6.72)	10.76 (6.94)	0.208	0.659	0.069
Magnitude	**47.86 (17.55)**	**36.68 (11.57)**	6.805*	**0.028**	0.643
SRF	0.74 (0.17)	0.79 (0.18)	1.383	0.27	0.184
QI	1.69 (0.70)	1.80 (0.60)	1.163	0.309	0.162
$t_{decrease}$	8.58 (2.78)	8.65 (1.02)	0.12	0.737	0.061
t_{regen}	22.17 (6.12)	22.14 (6.73)	0	0.985	0.05

** Indicates a significant difference for main effect ($p < 0.05$). Bold is to draw attention to the significant difference between groups observed in post hoc tests ($p < 0.05$). LH = left hand. RH = right hand. Main effect is calculated F statistic (degrees of freedom = 1, 9). Data are presented as mean (±SD).*

4.5 Bilateral Correlation

4.5.1 Thermoneutral

The oscillations in blood flow normally seen at rest were observable in participants in this study, as seen in Figure 4.1. Correlation coefficients investigating the relationship between right hand blood conductance-left hand blood conductance yielded expectedly high values of 0.80 ± 0.22, with most being greater than 0.9 (7 out of 10) and all Pearson's correlation coefficients were found to be significant ($p < 0.05$).

4.5.2 Sympathetic Perturbations

The relationship between left hand blood conductance and right hand blood conductance was investigated over the three phases of the sympathetic perturbation (pre, during, and post), as well as for the overall perturbation (Figure 3.2). Correlation coefficient values remained high for the right hand blood conductance-left hand blood conductance relationship much like at rest, with most values being above 0.65 (Table 4.4).

4.5.3 Strength of Correlation Between Bilateral Fingers Over Phases of the SP

No statistically significant difference was observed between the during, post, or overall phase of the sympathetic perturbation (Table 4.4). The strength of the relationship between the right hand and left hand blood conductance was significantly lower during

the pre-phase (0.67 ± 0.25) of the sympathetic perturbation compared to any of the other phases of the perturbation (during, post, or the overall perturbation) (Table 4.4).

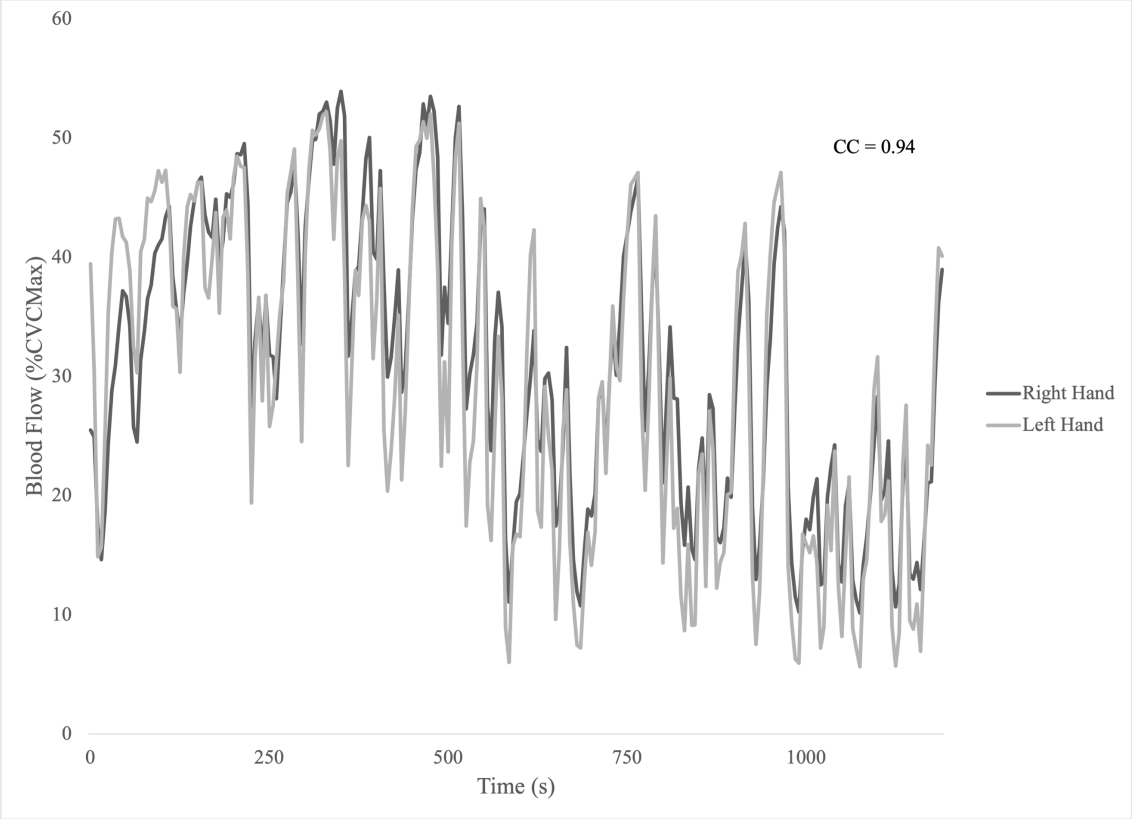

Figure 4.1 Example Tracing of LDF Response During Thermoneutral-Resting Portion Example tracing is actual data from one participant in the study. CC = correlation coefficient. Data are 5 s averages over the entire recording of LDF data converted to %CVC Max from APU.

Table 4.4 Comparison of Correlation Coefficients for Conductance Between Bilateral Fingers Throughout Phases of Sympathetic Perturbations

Phase of SP	***Correlation Coefficient***
Pre	**0.67 (0.25)***
During	0.93 (0.11)
Post	0.87 (0.11)
Overall	0.93 (0.10)

***** Indicates a significant difference ($p < 0.05$) from all other phases. SP = sympathetic perturbation. Data are presented as means *(±SD).*

4.6 Investigation of Possible Sympathetically Mediated Vasoconstrictor Events at Rest

Using the sympathetic perturbation portion of the experiment to define what possible sympathetically mediated vasoconstrictor events may look like, the thermoneutral portion of the data were investigated. Due to the highly variable nature of the thermoneutral, resting data, SRF was selected as the tool to investigate and vasoconstrictor events as it is less influenced by large variability (Schürmann, Gradl, and Fürst, 1996). Vasoconstrictor events that were within 2 standard deviations of the mean SRF (≥0.4) that occurred simultaneously in both hands were labelled as possible sympathetic vasoconstrictor events. When investigated, most participants, when following these defined criteria, exhibited the expected 1-3 sympathetically mediated vasoconstrictor events per minute (Figure 4.2).

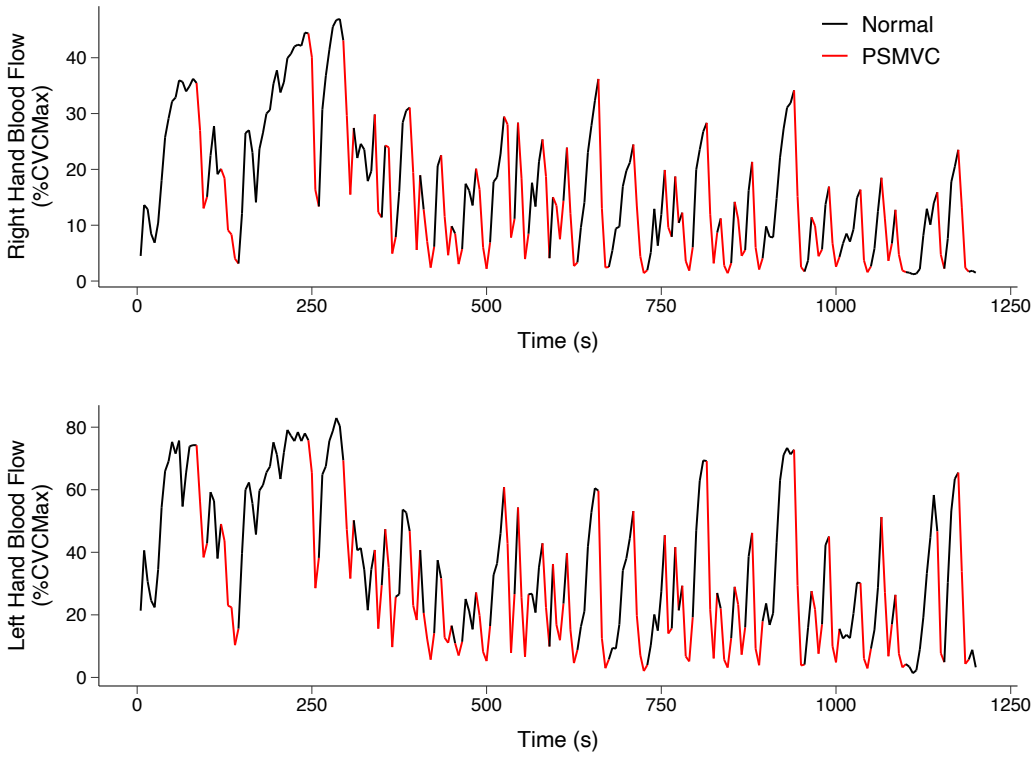

Figure 4.2 Preliminary Search for Possible Sympathetically Mediated Vasoconstrictor Activity in Resting Blood Conductance

PSMVC = possible sympathetically mediated vasoconstriction. Example tracing is actual data from one participant in the study. Data are 5 s averages over the entire recording of LDF data converted to %CVC Max from APU. PSMVC are vasoconstrictor events that occur simultaneously in both hands and are within 2 SD based on SRF data from sympathetic perturbation portion of the experiment.

Chapter 5 - Discussion

5.1 Temporal Relationship

Previous research has shown that sympathetic activity is closely matched at separate sites of the body, both in terms of the magnitude of the stimulus as well as for the timing of each burst (Bini et al., 1980b; Wallin and Fagius, 1988). With knowledge that sympathetic activity regulates the vasoconstrictor events in glabrous skin (Johnson and Kellogg Jr, 2010b), this led to the hypothesis that blood conductance patterns would also be significantly related across the body over time. This was assessed using correlation coefficients to measure the strength of the relationship of blood conductance at separate sites of the body which demonstrated strong correlation over time, both when at rest and throughout sympathetic perturbations. Previous studies investigating the relationship of blood flow at separate sites of the body have seen correlation coefficients above 0.7 (Walløe, 2016; Ho, Toska, and Wesche, 2020), which was similar to what was seen in this study, with an average of 0.80 (0.22), supporting the proposed hypothesis.

The function of the heating to cause central vasodilation during the sympathetic perturbation portion of the experiment was to abolish oscillations in blood flow caused by sympathetic activity thereby resulting in elevated, stable blood conductance patterns (Khan et al., 1991). As a result, the central influence of sympathetic activity on blood flow patterns should be diminished prior to the administration of the sympathetic perturbation and should increase during the vasoconstrictor activity. This was demonstrated by the significantly higher correlation of blood conductance at separate sites during the sympathetic perturbation compared to prior to it being administered (Table 4.4), a novel finding which has not been demonstrated in any previous study to my knowledge. Blood conductance in the fingertips became more correlated during a strong sympathetic burst compared to when the body is centrally vasodilated from a heating protocol. Thus, the increase in synchronicity from pre-phase to during-phase is likely due to the sympathetic activity which manifests at separate sites of the body in a closely related manner (Bini et al., 1980b). If true, it is the first example that blood conductance synchronicity is elevated during increased sympathetic activity and may provide further

credentials to the possibility of blood conductance as a non-invasive measure of sympathetic activity.

With the knowledge that during sympathetic bursts of activity, blood conductance appears to be more closely related at separate sites of the body, one unexpected finding of this study was that there was not a significant difference in the correlation values between when the vasoconstriction was occurring (during-phase) and the subsequent release of the vasoconstriction (post-phase). It is possible that the post phase was simply highly correlated due to it being only 10 s in duration, whereas the time it took to resume pre-sympathetic perturbation values was longer than the post-phase. However, due to the non-significant difference in the t_{regen} of both the left and right hand (Table 4.2), this seems unlikely. This finding is then, likely the result of one of two scenarios, the first being that the central signal causing peripheral vasoconstriction dissipates and the passive release of the vasoconstriction is a matched process across the body particularly with the central vasodilation, or the second being that following a sympathetic vasoconstrictor event, local control of blood conductance patterns helps to return blood conductance to normal values in a similar way at separate sites of the body. Either way, this appears to be a novel finding and will need to be further investigated to better understand the effects of passive vasodilation following a strong sympathetic stimulus.

5.2 Characteristics of Sympathetic Activity in Blood Conductance Recordings

Previous studies have demonstrated that not only are the bursts of sympathetic activity closely matched temporally at separate sites of the body, but also that they demonstrate closely matched magnitude of individual bursts (Bini et al., 1980b; Wallin and Fagius, 1988). With knowledge of how sympathetic bursts of activity result in the release of NE to the smooth muscle of peripheral microvasculature causing vasoconstriction (Johnson and Kellogg Jr, 2018), this related magnitude of sympathetic activity led to the prediction that the magnitude of vasoconstriction would also be related. The results of this study showed a significant difference for the magnitude of vasoconstriction at separate sites of the body contradicting this hypothesis. However, the left hand had a significantly higher F_{mean} compared to the right hand with no differences in F_{min} between hands (Table 4.2), meaning that the difference in magnitude was due to a

higher starting value in the left compared to the right hand. There was no difference in the value that they decreased to (F_{min}) during the vasoconstriction, and the calculated SRF value was also not found to not be significantly different between hands (Table 4.2). Thus, despite the difference in overall magnitude between hands, the vasoconstriction to a strong sympathetic stimulus, results in a similar absolute decrease in overall value between hands.

As for why the average conductance prior to the sympathetic perturbation was different between hands it is hard to say. One strong possibility, which is discussed in more detail in the limitations section is the lack of randomization for probe selection, as the LDF probe placed on the left or right hand was largely consistent throughout the study. Another possibility is the methodology from this study was a modified version of that done in a previous study (Schürmann, Gradl, and Fürst, 1996) with the only difference being that they not only heated the back to 40-45°C but also elevated the local temperature of the finger to 40°C. The reason that this local heating of the fingers was not done in this study is two-fold. The first being that in pilot studies done, the 40°C local heating when combined with heating the back diminished the vasoconstrictor response to the sympathetic perturbations leading to much more varied results and negatively impacting one of the primary variables of interest in this study. The second reason is the point of this was to cause central vasodilation diminishing the low frequency sympathetic activity causing blood flow oscillations, which was achieved with just local heating of the back. This is supported by animal model work showing that heating of the spinal cord elevates blood flow in areas with dense AVAs (Hales et al., 1978). Also, with additional knowledge on the control of local blood flow, particularly to local heating (Johnson and Kellogg Jr, 2010a; Hodges et al., 2016), this was considered the more appropriate way to best control for the variables this study was most interested in. Thus, while previous studies have employed the use of local heating as well, this is by no means a standardized or validated methodology and as such the difference in F_{mean} between hands could be due to differences in temperature, differences in AVA density in the area recorded from, handedness, which was recorded in this study with all participants being right hand dominant. As such, when a more consistent way to elevate blood flow to submaximal

levels is found, this study should be redone to assess for the F_{mean} and magnitude differences, since no difference was found by Schürmann, Gradl, and Fürst (1996).

Sympathetic activity is known to cause vasoconstriction in glabrous skin, however, there appears to be differences based on the type of stimulus. Two sympathetic perturbations were used in this study, a breath hold and a cooling stimulus. These two different perturbations resulted in different F_{min} values as well as different calculated SRF values. The breath holds on average resulted in decreases to a lower value (F_{min}) and with a larger ratio (SRF) as seen in Table 4.2. This could be due to a variety of reasons including that the cooling stimulus may have varied in strength between the participants due to differences in body anthropometrics. In fact, previous studies have shown differences between males and females for the cooling stimulus (Schürmann, Gradl, and Fürst, 1996), with females having a more significant vasoconstriction from the cooling stimulus. Due to the small sample size of women in this study, this could not be investigated, however, it appears that the way sympathetic activity manifests in terms of overall magnitude can vary both within individuals to different stimuli, and between individuals based on a variety of factors.

5.3 Search for Sympathetic Activity in Thermoneutral Blood Conductance Recordings

It is known that low frequency oscillations in sympathetic activity manifests in corresponding changes in blood flow at rest approximately 1-3 times per minute. Previous experiments have used perturbations known to elicit strong sympathetic bursts of activity, much like was done in this study, to track blood flow changes (Khan et al., 1991; Valley et al., 1993; Schürmann, Gradl, and Fürst, 1996. However, previous studies have not investigated the possibility of using these findings to assess thermoneutral, resting blood flow patterns for possible sympathetically mediated vasoconstrictor events. Therefore, to apply the findings of the sympathetic perturbation portion of this experiment to the thermoneutral portion, an analysis of the thermoneutral portion of the data was done by looking for vasoconstrictor events similar to those found to be elicited by sympathetic perturbations. This was done using the calculated SRF value, as it is least influenced by starting, or ending values of the vasoconstrictor event and looked through the thermoneutral data for moments where decreases were similar (within 2 standard

deviations) from the mean SRF (≥0.4). Only moments where these occurred simultaneously in both hands were accounted for since sympathetic activity should manifest in a synchronized manner. This resulted in a fairly robust finding where most participants had approximately 2 vasoconstrictions per minute greater than the defined value simultaneously in both hands (Figure 4.2). This is of course, still an early investigation and does not properly consider that sympathetic vasoconstriction may manifest differently at rest than in response to a strong stimulus. However, it continues to provide strong evidence that sympathetic activity may be directly observable in peripheral blood flow patterns, when an accurate and reliable methodology is found.

5.4 Limitations

This study had several limitations which will be outlined below. Measures were done to take these limitations into account and, where possible, steps were put in place to best alleviate the effect these limitations had on the study. The limitations were as follows; there was no direct measure of sympathetic activity, the study consists of a small sample size, probes were not randomized, and the lack of similar studies making direct comparisons difficult.

The first limitation is the lack of a direct measure of sympathetic activity. Sympathetic activity can be measured via microneurography and can measure burst by burst SSNA or MSNA. This study does not include a measure of sympathetic activity and as such the implied relationship between sympathetic activity and blood conductance cannot be directly quantified. This study used perturbations known to elicit strong sympathetic activity as has been done in previous studies (Khan et al., 1991; Valley et al., 1993; Schürmann, Gradl, and Fürst, 1996) as a way of generalizing the sympathetic response. This, however, does not give a temporal or magnitude measure to the sympathetic activity itself and as such, future research will need to combine measures of blood conductance with direct measures of sympathetic activity to build on the findings of this study.

The second limitation to this study is the relatively small, non-diverse, population of participants used in this study. Of note, this study took place in the later months of the year 2020, during the ongoing COVID-19 global pandemic and as such, the study ran

under more strict safety regulations. Due to this, despite a power analysis revealing that 16 participants would be needed only 10 were able to be taken through the entire study. Despite this, the power for calculated statistics was relatively high (Table 4.1, Table 4.2, and Table 4.3). As such, despite the small sample size the data are relatively robust and shows common trends throughout, and the researchers are thankful to all the participants and everyone else involved for their efforts during the unprecedented times. Males and females aged 18-29 were recruited however, only 3 females were able to complete the study due to the tight time constraint of when the study took place and the need for females to be in the early follicular phase of their menstrual cycle or the placebo pill phase of oral contraceptive use. Previous research has shown differences in the way females react to the cold stimulus perturbation (Schürmann, Gradl, and Fürst, 1996), however for this study with a limited number of females, sex related differences were not able to be investigated. Future research should continue to investigate any sex related differences in sympathetic response, as well as investigate over the course of the female menstrual cycle which has effects on circulating hormones known to influence temperature and blood flow regulation (Charkoudian et al., 2017). The selected age range for this study was due to the known diminished sympathetic activity that occurs with age (Greaney, Alexander, and Kenney, 2015). As such, with this study attempting to demonstrate a strong correlation in blood conductance, young healthy individuals were selected. Future research should investigate whether this relationship is diminished with factors such as age and sympathetic impairment.

Another limitation is the lack of randomization for which probe was placed on which hand. Largely throughout the study the same probe was placed on each participant's right or left hand, and as such, the difference in F_{mean} could be due to this lack of randomization for probe placement. This is a potential major limitation, as if there is a difference between the probes, it is possible that this is what caused the difference in F_{mean} and subsequently the magnitude of the vasoconstrictor response between hands.

A limitation of this study is it is a novel study with few (Khan et al., 1991; Valley et al., 1993; Schürmann, Gradl, and Fürst, 1996) other studies looking at the possibility of blood conductance as a measure of sympathetic activity. This is a limitation as there is little standardization in terms of methodology. For example, the central heating method

used was similar to that done by Schürmann, Gradl, and Fürst (1996) without the administration of local heating to the hand. Despite using a similar methodology, where they found no difference in F_{mean} between hands (Schürmann, Gradl, and Fürst, 1996), this study demonstrated a significant difference between hands. The reason local heating was not used is two-fold, the first being that local heating would later be used to elicit maximal blood conductance and as such 40°C local heating prior may affect heating later, and the second is that in pilot studies the local heating resulted in greater variability in the vasoconstrictor response (the primary focus of the study) to the sympathetic perturbation. This is just one example of the fact that there are many limitations that arise due to there being few other studies to compare methodology and results to. The hope is that future studies can continue to build off this and previous studies to bring standardization to this area of research.

5.5 Future Directions

Despite the limitations in this study, the results demonstrate a strong temporal relationship between blood conductance at separate sites of the body, both at rest and during sympathetic perturbations. This combined with previous research showing that sympathetic activity is closely related at separate sites of the body, particularly in glabrous skin, indicates that this area of research warrants further investigation.

Future studies should investigate the possibility of using blood conductance as a non-invasive measure of sympathetic activity by pairing measures of blood conductance with direct measures of sympathetic activity over a variety of scenarios. This should allow for an accurate and reliable way to observe how sympathetic activity manifests in recordings of blood conductance. From there researchers can continue to investigate this relationship and determine the ability and accuracy of blood conductance to detect changes in sympathetic activity over time, potentially opening the possibility of measures of blood conductance as a research and clinical tool.

One novel finding of this study was the close relationship of blood conductance at separate sites over time not only during the vasoconstriction caused by the sympathetic perturbation, but also during the release of that vasoconstriction and passive vasodilation during the return to F_{mean}. This warrants further investigation, as it leads to the idea that

either local factors take over after a sympathetically mediated vasoconstriction in a way that is similar across the body or that the tonic vasoconstriction that has been abolished by the central heating results in the passive release of vasoconstriction in a way that is matched across the body. No matter which is true, further investigation is required as the role of local versus central control of peripheral blood conductance is still a relatively poorly understood topic.

Chapter 6 - Conclusion

This study investigated blood conductance at separate sites of the body to determine the strength of the relationship in terms of both temporal correlation and overall magnitude. This study showed strong temporal correlation for blood conductance patterns measured in glabrous skin at rest in a thermoneutral environment. This was demonstrated by high (0.80 ± 0.22) correlation coefficient values that support previous research investigating the relationship of blood conductance at separate sites. A novel finding of this study was the increased strength of the relationship in blood conductance patterns at separate sites during the administration of a strong sympathetic perturbation, indicating that the relationship of blood conductance patterns at separate sites of the body is increased during burst of sympathetic activity. This finding makes sense as previous research has shown sympathetic bursts of activity manifest with in a closely related manner at bilateral sites of the body and therefore a centrally originating signal like sympathetic activity should manifest in increased correlation values when measured at separate peripheral sites. The overall magnitude of the vasoconstrictor response to known sympathetic perturbations was also found to be similar in this study, indicating that sympathetic activity may cause an observable, repeatable change in blood conductance that can be quantified and used to assess peripheral blood conductance patterns.

With an understanding of the role of sympathetic activity in governing overall blood flow patterns, particularly in glabrous skin and the knowledge that sympathetic bursts of activity are closely matched throughout the body, this close relationship of blood conductance at separate sites gives a good indication for the possibility of measures of blood conductance as a non-invasive tool to assess sympathetic activity. Measures of sympathetic activity are costly, invasive, and difficult to perform and assess. Measures of blood flow are comparably less expensive, non-invasive and are therefore an ideal candidate as an indirect measure of sympathetic activity. This research expanded on previous studies investigating this possibility, building on them by demonstrating the increased relationship of blood conductance during strong sympathetic activity and by beginning to assess the resting portion of blood conductance for ways to observe sympathetic activity.

Future research should continue to investigate this relationship by pairing measures of sympathetic activity (microneurography) with measures of blood conductance (LDF) to determine whether the manifestation of sympathetic activity in blood conductance recordings through measurable vasoconstrictions can be used as a non-invasive tool to assess sympathetic activity. Measures of sympathetic activity help to diagnosis neuropathies, aid in research of the autonomic nervous system and a non-invasive tool to do this would allow for this tool to be used in populations where invasive measures are unrealistic and research settings where previous tools to measure sympathetic activity are unavailable or unrealistic. This study demonstrates the strong possibility of blood conductance as an indirect tool to assess the autonomic nervous system in real time both at rest and to various stimuli, opening the door on many possible future research and clinical possibilities.

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