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Altered skin flowmotion in hypertensive humans

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Abstract

Essential hypertensive humans exhibit attenuated cutaneous nitric oxide (NO)-dependent vasodilation. Using spectral analysis (fast Fourier transformation) we aimed to characterize the skin flowmotion contained in the laser-Doppler flowmetry recordings during local heating-induced vasodilation before and after concurrent pharmacological inhibition of nitric oxide synthase (NOS) in hypertensive and age-matched normotensive men and women. We hypothesized that hypertensive subjects would have lower total power spectral densities (PSD), specifically in the frequency intervals associated with intrinsic endothelial and neurogenic control of the microvasculature. Furthermore, we hypothesized that NOS inhibition would attenuate the endothelial frequency interval. Laser-Doppler flowmetry recordings during local heating experiments from 18 hypertensive (MAP: 108 ± 2 mmHg) and 18 normotensive (MAP: 88 ± 2 mmHg) men and women were analyzed. Within site NO-dependent vasodilation was assessed by perfusion of a non-specific NOS inhibitor (N^G -nitro-L-arginine methyl ester; L-NAME) through intradermal microdialysis during the heating-induced plateau in skin blood flow. Local heating-induced vasodilation increased total PSD for all frequency intervals (all $p < 0.001$). Hypertensives had a lower total PSD ($p = 0.03$) and absolute neurogenic frequency intervals ($p < 0.01$) compared to the normotensives. When normalized as a percentage of total PSD, hypertensives had reduced neurogenic ($p < 0.001$) and augmented myogenic contributions ($p = 0.04$) to the total spectrum. NOS inhibition decreased total PSD ($p < 0.001$) for both groups, but hypertensives exhibited lower absolute endothelial ($p < 0.01$), neurogenic ($p < 0.05$), and total PSD ($p < 0.001$) frequency intervals compared to normotensives. These data suggest that essential hypertension results in altered neurogenic and NOS-dependent control of skin flowmotion and support the use of spectral analysis as a non-invasive technique to study vasoreactivity.

Keywords

skin blood flow; fast Fourier transformation; essential hypertension; endothelial signaling; neurogenic signaling; myogenic signaling

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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INTRODUCTION

The cutaneous circulation is an accessible vascular bed which allows for non-to minimally invasive studies of endothelial, neurovascular, and vascular smooth muscle vasoreactivity *in vivo* (Holowatz et al., 2008; Minson, 2010; Roustit and Cracowski, 2013). A number of skin vasoreactivity tests have been used to study microvascular function in populations that are at risk for or currently have cardiovascular disease (CVD). Local skin heating coupled with laser-Doppler flowmetry is a common test of microvascular function which elicits highly reproducible skin blood flow responses that have been mechanistically well characterized. Local skin heating causes a hyperemic response that is predominantly reliant on endothelium-dependent nitric oxide (NO)-mediated vasodilation (~70%)(Bruning et al., 2012; Kellogg et al., 2008). This response has been studied in a range of healthy (Bruning et al., 2012; Kellogg et al., 2008; Minson et al., 2001) and diseased populations (Holowatz and Kenney, 2011; Smith et al., 2011).

Our laboratory has examined cutaneous vasoreactivity in unmedicated stage 1 essential hypertensive men and women without other co-morbidities. This subject cohort exhibited endothelial dysfunction during local skin heating marked by a reduction in endothelial nitric oxide synthase (eNOS)-dependent vasodilation (Smith et al., 2011). There is growing evidence that spectral analysis of the low frequency periodic oscillations in blood flux measurements using laser-Doppler flowmetry can provide non-invasive mechanistic information on microvascular control mechanisms (Kastrup et al., 1989; Salerud et al., 1983; Stefanovska et al., 1999). These periodic oscillations, or skin flowmotion, represent the influence of heart beat (0.6–2.0Hz), respiration (0.15–0.6Hz), myogenic (~0.05–0.15Hz) (Stefanovska et al., 1999), neurogenic (~0.02–0.05Hz) (Kastrup et al., 1989; Soderstrom et al., 2003), and endothelial (~0.0095–0.02Hz) influences on vascular smooth muscle relaxation (Gustafsson et al., 1993; Kvandal et al., 2003; Kvernmo et al., 1999; Rossi et al., 2008a). Spectral analysis has been performed on populations with known microvascular dysfunction such as peripheral arterial obstructive disease (Rossi et al., 2005), chronic kidney disease (Rossi et al., 2008b), diabetes (Schmiedel et al., 2007), chronic smokers (Avery et al., 2009; Rossi et al., 2007), hypercholesterolemics (Rossi et al., 2009) and essential hypertensive men and women (Gryglewska et al., 2010a; Gryglewska et al., 2010b; Rossi et al., 2006). While these studies observed altered skin flowmotion responses, many of them utilized different skin vasoreactivity tests and subject populations in which the mechanisms mediating skin blood flow are not thoroughly understood. The one study that has examined cutaneous vasoreactivity during local skin heating in essential hypertensive men and women did not control local skin temperature throughout the entire protocol and used a different rate of skin heating (Gryglewska et al., 2010a) from what has been used in mechanistic studies examining NOS-dependent vasodilation in hypertensive humans (Smith et al., 2011). Further, no study has examined skin flowmotion in essential hypertensive men and women during local skin heating with concurrent pharmacological inhibition of NOS.

The aim of the present study was to utilize spectral analysis to evaluate skin vasoreactivity in unmedicated essential hypertensive and age-matched normotensive men and women before and after NOS inhibition. We hypothesized that essential hypertension would result in reduced total power spectral density (PSD) around the frequency intervals of interest during

local heating, due to reduced intrinsic endothelial and neurogenic signaling. Furthermore, we hypothesized that *within* site inhibition of NO production would result in reduced endothelial signaling in both essential hypertensive and normotensive subjects.

MATERIALS AND METHODS

Subjects

All experimental protocols were approved by the Institutional Review Board at The Pennsylvania State University and conformed to the guidelines set forth by the *Declaration of Helsinki*. Verbal and written consent were voluntarily obtained from all subjects prior to participation. We performed Fourier transform-based power spectral analysis on previously collected local skin heating studies in unmedicated essential hypertensive subjects from our laboratory (pilot data and (Smith et al., 2011)). Fast Fourier transformations (FFT) were applied to laser-Doppler recordings during local skin heating in 18 essential hypertensive and 18 age-matched normotensive men and women. Subject characteristics are presented in Table 1. Subject's blood pressures were classified in accordance with the guidelines set forth by the American Heart Association (Chobanian et al., 2003) during three separate visits and further explored using an ambulatory 24-hour blood pressure monitor. Subjects underwent a complete medical screening including a resting ECG, physical examination, lipid profile and blood chemistry (Quest Diagnostics, Pittsburgh, PA) and were otherwise healthy with the exclusion of stage 1 essential hypertension. All subjects were normally active, non-diabetic, non-smokers who were not taking any prescription medications with primary or secondary vascular effects, including antihypertensive pharmacotherapy, vitamins and supplements. Seventeen of the 18 essential hypertensive subjects were naïve to antihypertensive therapy, and 1 subject had stopped antihypertensive therapy > 1 year prior to participating in the protocol. All of the premenopausal women (n=4) were studied on days 2 to 7 of their menstrual cycle, and postmenopausal women (n=14) reported that it had been 1 year since the cessation of their last menses.

Local skin heating and assessment of skin blood flow

The local heating protocols were performed in a thermoneutral laboratory with the subject in a semi-supine position and the experimental arm at heart level. Intradermal microdialysis fibers (MD 2000, Bioanalytical Systems, West Lafayette, IN) were inserted into the ventral side of the forearm skin as previously described (Smith et al., 2011). Local heaters (SHO2, Moor Devon UK) were used to control skin temperature and mount the laser-Doppler flowmeter probes (MoorLAB, Moor Devon UK), which measured skin blood flux over the microdialysis sites. We performed Fourier transform-based power spectral analysis on control microdialysis sites from pilot data and a previously published study (Smith et al., 2011). The control site had lactated Ringer's solution perfused throughout insertion hyperemia (60–90 minutes), a baseline where local skin temperature was clamped at 33°C (20 min), during standardized local skin heating to 42°C (0.5°C every 5 s), and post local skin heating to record at least 30 minutes of a stable, heat-induced hyperemic skin blood flow response (Johnson and Kellogg, 2010; Kellogg et al., 1999; Minson et al., 2001; Smith et al., 2011). The latter skin blood flow plateau is predominantly mediated by NO from eNOS (Bruning et al., 2012; Johnson and Kellogg, 2010; Kellogg et al., 2008; Minson et al.,

2001). The NO-dependent vasodilation was assessed *within* each control site by perfusing 20mM of a non-specific NOS inhibitor (*N*^G-nitro-*L*-arginine methyl ester (L-NAME); Tocris) through the microdialysis fiber until the skin blood flow was reduced for a stable 20 minute period (L-NAME plateau). After the L-NAME plateau, local skin temperature was increased to 43°C and 28mM of sodium nitroprusside (SNP) was perfused to elicit maximal vasodilation for data normalization and to test endothelium-independent vasodilator responsiveness (Holowatz et al., 2005).

Power spectral density (PSD) analysis

The skin blood flow data was collected using a MoorLAB laser-Doppler flowmetry system. This system uses 785nm solid state laser diodes as the laser light source. In the MoorLAB device, the laser-Doppler signal was band-pass filtered in the range of 20Hz to 14.9kHz. The skin blood flow data from the local heating protocols were digitized and saved at a sampling frequency of 40Hz using WinDAQ software (Dataq Instruments, Akron OH). These files were later converted to Microsoft Excel files and each skin blood flow response was divided into four periods: baseline, local heating plateau, L-NAME plateau, and SNP plateau for further analysis. The regions of interest converted into Microsoft Excel files were determined by visually assessing 600 seconds (24001 data points) of stable, flat portions of the laser-Doppler recordings that were devoid of motion artifacts.

Estimating PSD—The frequencies of oscillations contained in the laser-Doppler recordings from the four periods were analyzed using Fourier transform-based power spectral analysis as described by Avery et al, 2009. All laser-Doppler recordings were measured in arbitrary flow units (AU) prior to analysis. Periodograms were derived from the FFT of the laser-Doppler recordings in MATLAB® (version 2013a) and the average of the periodograms were used to estimate the PSD (Avery et al., 2009). The power (in AU²) was calculated around the 0.01Hz (0.008–0.02Hz), 0.04Hz (0.02–0.05Hz) and 0.1Hz (0.05–0.15Hz) frequency intervals, considered to correspond to endothelial, neurogenic, and myogenic activity, respectively (Avery et al., 2009; Meyer et al., 2003; Rossi et al., 2006). During a given period (i.e. baseline, local heating plateau, etc.) the frequency intervals of interest were normalized relative to that period's total power to assess the contribution of each frequency during standardized physiological perturbations.

Statistical analysis

The Anderson-Darling test was used to assess normality of the data. All normally distributed data are presented as mean±SEM and non-normally distributed data are presented as median and interquartile ranges [IQR]. A one-way analysis of variance (ANOVA) was used to test the differences in mean PSD between baseline, local heating plateau, L-NAME plateau, and SNP plateau within a group (i.e. normotensive or hypertensive) when data were normally distributed, whereas the non-parametric Kruskal-Wallis test was used to assess differences in the median PSD when data were non-normal. The Wilcoxon rank sum test was used to determine differences between each group's frequency intervals where appropriate. The level of significance was set at $\alpha=0.05$, with post hoc Bonferroni corrections for the specific planned comparisons. Multivariate reverse regression analysis was used to detect the subject characteristics that are predictors of the laser-Doppler flux (AU) responses and the total

power, endothelial, neurogenic, and myogenic frequency intervals associated with skin flowmotion responses. The simplest significant model was selected. All statistical analyses were performed using Minitab statistical software (version 16.2.4, State College, PA).

RESULTS

Subject characteristics are presented in Table 1. Subject groups were well matched for age, body mass index, fasting glucose, hemoglobin A1c, triglycerides, total cholesterol, and high- and low-density lipoproteins. Hypertensive subjects had higher systolic, diastolic and mean arterial pressure (MAP) compared to the normotensive subjects ($p<0.001$) by design.

Table 2 presents the laser-Doppler flux responses during baseline, local skin heating, L-NAME plateau, and SNP-induced max. The hypertensive subjects had an attenuated laser-Doppler flux response during local skin heating versus normotensive subjects ($p=0.05$). There was a trend towards a lower baseline flux in the hypertensive men and women ($p=0.058$), but it did not reach significance. There were no differences in the L-NAME plateau or SNP-induced max between groups. In our previously published study, when skin blood flow is expressed as cutaneous vascular conductance and normalized to maximum ($\%CVC_{max}$), the essential hypertensive men and women had a significantly reduced local skin heating plateau compared to the normotensive men and women (Smith et al., 2011). Similar to the present analysis, we found that there was no difference in the baseline, L-NAME plateau, or SNP and heating-induced maximum vasodilation in our previously published study (Smith et al., 2011).

The mean PSD from FFT of the laser-Doppler recordings are presented in Figure 1 for a visual representation of how the mean PSD changes during a standardized local heating protocol in normotensive and essential hypertensive subjects. The mean PSD were lower across all frequency intervals during baseline versus the local heating plateau, L-NAME plateau, and SNP-induced max in the normotensive and essential hypertensive subjects. Mean PSD was also lower during L-NAME perfusion versus local skin heating and SNP perfusion for both groups ($p<0.001$). Visual inspection of the mean PSD plots illustrates that normotensive subjects had a higher PSD in the lower frequency intervals ($\sim 0.01\text{--}0.06\text{Hz}$) during the local heating plateau (figure 1b) and L-NAME plateaus (figure 1c) versus essential hypertensive subjects. However, after SNP+heating-induced maximum vasodilation (figure 1d), the PSD are nearly identical between normotensive and essential hypertensive subjects.

Figure 2 presents the absolute PSD calculated from the frequency intervals corresponding to endothelial, neurogenic, myogenic, and total power from the mean PSD plots (figure 1). Local heating, L-NAME perfusion (post heating), and SNP perfusion with heating increased PSD in all of the frequency intervals and total power versus baseline (figure 2a) in both groups ($p<0.001$). There were no differences in any of the frequency intervals of interest or total power between the normotensive and essential hypertensive groups during baseline ($p>0.05$). However, after locally heating the skin (figure 2b), the essential hypertensive subjects had a lower total power versus normotensive subjects ($p=0.03$). Specifically, the essential hypertensive subjects had lower absolute PSD in the neurogenic frequency intervals

($p < 0.01$) with a trend towards lower endothelial PSD ($p = 0.07$) compared to the normotensive group. During the L-NAME plateau PSD was lower across all frequency intervals and total power versus the heating plateau and SNP with heating-induced max ($p < 0.001$). After NOS-inhibition, the essential hypertensive subjects had a lower PSD in the endothelial ($p < 0.01$) and neurogenic frequency intervals ($p = 0.04$) as well as the in the calculated total power ($p < 0.001$) compared to the normotensive group. During vasodilation elicited from perfusion of SNP and heating, there were no differences in any of the frequencies of interest or total power in the normotensive or essential hypertensive groups ($p > 0.05$). However, the endothelial frequency interval was higher during the SNP+heating-induced max in the essential hypertensive group versus baseline, heating plateau, and L-NAME plateau ($p < 0.05$).

Figure 3 illustrates the endothelial, neurogenic, and myogenic PSD as a percentage of total power for each given period of the local heating response. The pie charts for the heating plateau, L-NAME plateau, and SNP+heating-induced max are drawn to scale according to their absolute total power. The baseline charts were increased in size for visual purposes. The normotensive group exhibited an elevated normalized neurogenic contribution to the total power during local skin heating versus any other phase of the local heating response ($p < 0.001$). However, there were no differences in the contribution of endothelial and myogenic frequency intervals to total power for any of the phases of local heating ($p > 0.05$). There were no differences in the endothelial, neurogenic, or myogenic contributions to the total power for any of the local heating phases in the hypertensive group ($p > 0.05$). However, the hypertensive subjects had a reduced neurogenic contribution to total power during the local heating response compared to the normotensive group ($p < 0.001$). The hypertensive group had a higher myogenic contribution to the total power during the local heating plateau versus the normotensive group ($p = 0.04$). After NOS inhibition, hypertensive individuals had less of an endothelial contribution to the total power versus the normotensive group ($p = 0.016$). There were no differences in vasomotor-control during baseline or SNP+heating-induced max between groups.

Multivariate regression analyses were performed to determine which subject characteristics were significant predictors of laser-Doppler flux, endothelial, neurogenic, myogenic and total power responses during the phases of the local skin heating protocol. There were no significant predictors for laser-Doppler flux and myogenic PSD responses. The significant predictors of the endothelial, neurogenic, and total power skin flowmotion responses are shown in Table 3. During the local skin heating plateau and L-NAME plateau, MAP was a predictor of the neurogenic (0.02–0.05Hz) skin flowmotion responses ($p = 0.008$). BMI was a predictor of the endothelial (0.008–0.02Hz, $p = 0.004$) and total power skin flowmotion responses ($p = 0.05$) during the SNP+heating-induced maximum. In addition, BMI was also a predictor of the total power responses after L-NAME perfusion ($p = 0.003$).

DISCUSSION

The principle finding from the present study is that hypertensive subjects exhibit attenuated skin flowmotion responses versus age-matched normotensive subjects (Smith et al., 2011). Specifically, hypertensive men and women had reduced total power and neurogenic

frequency intervals contributing to skin flowmotion during local skin heating. MAP was a predictor of neurogenic skin flowmotion responses during local skin heating, suggesting hypertension alters neural control of the cutaneous microvasculature. After NOS inhibition, hypertensive subjects exhibited lower total power, endothelial (0.0095–0.02Hz), and neurogenic (0.02–0.05Hz) frequency intervals versus normotensives, suggesting that hypertension results in altered neurogenic and NO-independent control of skin flowmotion. Similarly, MAP was a significant predictor of neural PSD responses during local skin heating with NOS inhibition, while BMI was a predictor of NOS inhibited and total power responses. These findings suggest that spectral analysis of laser-Doppler flux can detect altered cutaneous vascular function similar to those observed during pharmacological assessment of vasoreactivity.

Similar to our pharmacological analysis of skin blood flow in unmedicated essential hypertensive participants (Smith et al., 2011), we did not observe any difference in baseline vasoreactivity using spectral analysis. Other studies that have performed spectral analysis on laser-Doppler flux similarly found no difference in the PSD during baseline conditions in early stage (Gryglewska et al., 2010b; Rossi et al., 2006) or long standing essential hypertension (Rossi et al., 2006). In contrast, Gryglewska et al. 2010 found that individuals who have masked hypertension, or normal in-clinic blood pressure recordings but have consistently high ambulatory blood pressure readings, have altered basal blood flow suggesting they may have mechanistically-distinct vascular adaptations with their disease progression (Gryglewska et al., 2010b).

In the present study, essential hypertensive subjects had an attenuated total power with a reduction in the frequency interval associated with neurogenic signaling during local skin heating. This corroborates our previous finding analyzing skin blood flow normalized to absolute cutaneous vascular conductance (CVC; $CVC = \text{laser-Doppler flux}/\text{MAP}$) and when expressed as a percentage of CVC maximum (Smith et al., 2011). Gryglewska et al. did not observe any differences in PSD between normotensives and early stage essential hypertensives during local skin heating, but noticed a reduced total power and reductions in the frequency interval associated with myogenic activity in those with family history of hypertension (Gryglewska et al., 2010a). However, in the latter study only 8 minutes of laser-Doppler recordings were measured post-heating, which may not be enough time to reach full expression of local eNOS-mediated heating-induced vasodilation.

Our original hypothesis was that we would observe a significant reduction in PSD in the frequency interval associated with endothelial signaling in essential hypertensive men and women. However, we did not find a difference in the endothelial frequency interval even though essential hypertensives showed a trend toward lower endothelial signaling ($p=0.07$). We may not have observed a difference in the lowest frequency interval because the essential hypertensive participants were relatively healthy with the exception of their high blood pressure classification. In addition, this was a retrospective analysis of previously collected data so we were underpowered with only eighteen subjects based on our power calculations for detecting statistically significant differences in PSD for a given frequency interval. Our preliminary power analysis suggested that twenty seven subjects would be necessary to detect an effect size of 25% for a given frequency interval.

Interestingly, essential hypertensive subjects exhibited reductions in the frequency interval corresponding to neurogenic signaling when assessed as absolute and normalized to total power. Multivariate analysis revealed that MAP was a significant predictor of neurogenic PSD responses. Neurogenic regulation of skin blood flow involves activation of heat-sensitive afferent sensory nerves and/or sympathetic activity that is reliant on local skin heating rate (Hodges et al., 2009; Houghton et al., 2006). The standard rate of heating we used results in the well characterized biphasic response marked by an axon reflex-mediated peak followed by slower prolonged vasodilation that is primarily reliant on NO. The axon reflex is largely mediated by C-fiber afferent sensory nerves that are hypothesized to release substance P (Wong and Minson, 2011; Wong et al., 2005) and CGRP (Minson et al., 2001; Munce and Kenney, 2003; Stephens et al., 2001) which can then act on the endothelium and/or vascular smooth muscle to induce vasorelaxation. NO and endothelial derived hyperpolarization factors (EDHF) also appear to play a role in the axon reflex (Brunt and Minson, 2012). The secondary plateau of the skin blood flow response is largely dependent on eNOS production of NO for full expression (~60–70%) (Bruning et al., 2012; Kellogg et al., 2008; Minson et al., 2001) with the remainder coming from EDHF-dependent mechanisms (Brunt and Minson, 2012). There is growing evidence to suggest that NO-dependent vasodilation elicited during local heating interacts with sympathetic and/or sensory nerves for full expression, providing a putative mechanism for the observed lower neurogenic frequency interval in our hypertensive subjects (Carter and Hodges, 2011; Hodges and Sparks, 2013; Houghton et al., 2006; Tew et al., 2011). Additionally, essential hypertensive men and women had a larger myogenic contribution to vasomotor control, which may be indicative of less neurogenic-induced vascular smooth muscle relaxation or a compensatory means of regulating vasomotor tone. Further pharmacological studies are needed to elucidate if hypertension reduces sympathetic and/or sensory nerve-induced vasodilation because this could have clinical implications for the prevention of peripheral neuropathies in this population.

After NOS inhibition with L-NAME, there was a reduction in the total power and in all frequency intervals for both groups. When assessed relative to the total power, the local heating-induced elevation of the neurogenic frequency interval observed in the normotensive subjects was restored to baseline values after NOS-inhibition. This suggests there may be sympathetic and/or sensory nerve interaction with NO. Similar to local skin heating, MAP was a significant predictor of neurogenic PSD after NOS inhibition. We originally hypothesized that L-NAME would reduce PSD in the lowest frequency interval associated with endothelial signaling when expressed in absolute and relative terms. While we did observe a reduction in the endothelial frequency interval in essential hypertensives when expressed as absolute PSD, we did not observe this when normalized as a percentage of total power.

Other studies have used endothelium-dependent agonists to characterize the endothelial frequency interval in different populations. Iontophoretic application of Ach and SNP increases PSD in all of the frequency intervals governing vasomotor control, but when assessed relative to total power, Ach selectively increases the endothelial frequency interval (Kvandal et al., 2006; Kvernmo et al., 1999; Stefanovska et al., 1999). When NOS inhibition with N^G-monomethyl-L-arginine (L-NMMA) was intra-brachially infused after Ach and

SNP application, it abolished the difference between the Ach and SNP treatments in the endothelial frequency interval 0.0095–0.021 Hz (Kvandal et al., 2006). We may not have observed a selective reduction in the lowest frequency interval when normalized relative to total power after NOS inhibition for several reasons. One is that we used local heating instead of a pure endothelial-dependent agonist. We observed that the local heating response is highly neurogenic and likely causes vasodilation through a mechanistically different pathway than pure pharmacological stimulation of the endothelium with acetylcholine. Second, we also used a different method for delivering the NOS inhibitors than the aforementioned studies. In the present study, we used intradermal microdialysis to locally deliver the non-selective NOS inhibitor to the site where laser-Doppler flux was assessed, whereas in the latter studies brachial artery infusions of L-NMMA were used (Kvandal et al., 2006; Kvandal et al., 2003). Finally, the latter studies used Morlet wavelet analysis to assess skin flowmotion which uses an adjustable window length allowing for the frequency resolution to be varied across the frequency intervals of interest (Kvandal et al., 2006; Kvandal et al., 2003) instead of a fixed window length which is used during fast Fourier transformations.

Interestingly, NOS inhibition resulted in a lower contribution from the endothelial frequency interval to total power in the essential hypertensive group, but was not different than the other periods of the local heating response. We did not observe an increase in the neurogenic frequency interval during local skin heating. In our previous pharmacological assessment study (Smith et al., 2011), the impaired NO-dependent vasodilator system could be restored with inhibition of inducible NOS (iNOS) with *N*-(3-(Aminomethyl)benzyl)acetamidine (1400w) (Smith et al., 2011). Together these findings suggest that altered neurogenic PSD in essential hypertensive humans may be due to chronic inflammation which may 1) desensitize the sympathetic and/or sensory nerves to stimuli such as heat, 2) cause chronic neurogenic inflammation and thus reduce post synaptic control of neurogenic vascular signaling, 3) increase oxidative stress causing eNOS-uncoupling and reducing NO production, and/or 4) increased oxidative stress creating a favorable environment for the generation of pro-constrictor endothelium derived contracting factors.

Finally, when SNP was perfused in addition to local heating the skin to 43°C, the total power was restored in essential hypertensive subjects similar to the normotensive group. Further, when normalized as a percentage of total power, there was no difference between the groups. BMI was a significant predictor of both total power and endothelial PSD during SNP perfusion+heating, which is similar to findings by Gryglewska et al 2010 (Gryglewska et al., 2010a). In the present study our participants were within normal BMI ranges, suggesting that it is a strong predictor of skin flowmotion responses.

Limitations

Sex and ethnic differences exist in the pathophysiology of endothelial dysfunction associated with hypertension (Hart et al., 2012; Morris et al., 2013; Reckelhoff, 2001; Stein et al., 1997), and in the present study we included men and women as well as a mixture of ethnicities. In the present study, the groups were not evenly matched for sex and were predominantly Caucasian. We did not observe any sex differences, but we were

underpowered to make this comparison. Further, sex was not a significant predictor for laser-Doppler flux or any of the spectral analysis outcomes.

In conclusion, spectral analysis of the laser-Doppler recordings during local skin heating corroborates *in vivo* pharmacological assessment of the mechanisms mediating skin blood flow and may provide additional mechanistic information. Specifically, essential hypertension reduces the neurogenic and total power contributing to skin vasomotion during local skin heating and MAP was a significant predictor of neural PSD responses, suggesting hypertension attenuates neural control of vasomotion in the skin. Further, after NOS inhibition, essential hypertension reduced endothelial, neurogenic, and total power suggesting that they have reduced NO-independent redundant vasodilator mechanisms contributing to skin flowmotion during local thermal stress. Essential hypertension did not affect the skin vasomotor response to SNP, supporting that skin flowmotion can detect altered microvascular vasoreactivity before there is measurable vascular smooth muscle dysfunction.

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Highlights

- Hypertension reduces total skin vasomotion during local skin heating in humans.
- Hypertension reduces neurogenic spectral density during local skin heating.
- NOS inhibition lowers endothelial, neurogenic and total spectra in hypertensives.
- NO-independent skin vasomotion is preserved in newly hypertensive subjects.

Figure 1.

Absolute mean power spectral density plots for (A) baseline, (B) local skin heating, (C) non-specific nitric oxide synthase inhibition with L-NAME, and (D) SNP-induced maximum laser-Doppler recordings during a local skin heating protocol in normotensive (unfilled circles) and essential hypertensive (black circles) men and women. Spectra were estimated from the fast Fourier transformation of the laser-Doppler recordings. The frequency intervals 0.01Hz (0.008–0.02Hz), 0.04Hz (0.02–0.05Hz), and 0.1Hz (0.05–0.15Hz) are considered to correspond to endothelial, neurogenic, and myogenic activity, respectively.

Figure 2.

Power spectral density during (A) baseline, (B) local skin heating, (C) non-specific nitric oxide synthase inhibition with L-NAME, and (D) SNP-induced maximum laser-Doppler responses during local skin heating protocol in normotensive (white bars) and essential hypertensive (gray bars) men and women. The power was calculated from the fast Fourier transformation of the laser-Doppler signal around 0.01Hz (0.008–0.02Hz), 0.04Hz (0.02–0.05Hz), and 0.1Hz (0.05–0.15Hz) frequency intervals, which are considered to correspond to endothelial, neurogenic, and myogenic activity, respectively. The total power was calculated from the of the non-overlapping frequency intervals. Baseline spectral power in all frequency intervals were lower than all other periods of the local heating response in both groups ($p < 0.001$). L-NAME plateau spectral power was lower for all frequency intervals compared to the heating plateau and SNP-induced max ($p < 0.05$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ Significantly different than normotensive men and women. † $p < 0.05$ Significantly different than baseline, local skin heating, and reduction with L-NAME for the endothelial frequency interval in essential hypertensive subjects. Data are represented as box and whisker plots with the whiskers representing the 95th percentile. The solid lines within the box represent the median power spectral density and the dashed lines represent the average power spectral density.

Figure 3.

Pie chart representing the mean normalized PSD as a percent contribution of the calculated total power during (A) baseline, (B) local skin heating, (C) non-specific NOS inhibition with L-NAME, and (D) SNP-induced maximum laser-Doppler recordings. The relative energy contribution of each frequency interval was assessed around the 0.01Hz (0.008–0.02Hz), 0.04Hz (0.02–0.05Hz), and 0.1Hz (0.05–0.15Hz) frequency intervals, considered to correspond to endothelial, neurogenic, and myogenic activity, respectively. The total power was calculated from the of the non-overlapping frequency intervals. The size of the pie chart is proportional to the absolute total power with the exception of the baseline period which was increased in size so the data would be visible (proportional between the normotensive and essential hypertensive group). # $p < 0.05$ Significantly different than the neurogenic frequency interval in the baseline, L-NAME plateau, and SNP max periods. * $p < 0.05$ Significantly different than normotensives.

Table 1

Subject characteristics (mean±SEM).

	Normotensives	Essential Hypertensives
Subjects (male, female)	(5,13)	(13,5)
Age (years)	53±1	52±1
BMI	25.3±0.7	26.5±0.5
Systolic BP (mmHg)	113±3	139±2 ^{**}
Diastolic BP (mmHg)	76±2	92±2 [*]
MAP (mmHg)	88±2	108±2 ^{**}
Fasting Glucose (mg·dL ⁻¹)	91±2	93±2
HbA1c	5.5±0.1	5.5±0.1
HDL (mg·dL ⁻¹)	56±4	57±3
LDL (mg·dL ⁻¹)	107±5	110±5
Triglycerides (mg·dL ⁻¹)	94±12	91±16

BMI, body mass index; BP, blood pressure; MAP, mean arterial pressure; HDL, high-density lipoprotein, LDL, low-density lipoprotein.

^{*} P<0.01,

^{**} P<0.001 significantly different than normotensive men and women.

Table 2

Laser-Doppler flux responses during the phases of the local skin heating protocol.

	Normotensive laser-Doppler flux (AU)	Hypertensive laser-Doppler flux (AU)	<i>P-value</i>
Baseline	18±2	13±2	0.058
Heating plateau	174±18	139±13	0.050
L-NAME plateau	91±13	74±13	0.183
SNP Max	178±15	167±13	0.320

Table 3

Significant predictors of the endothelial, neurogenic, and total power skin flowmotion responses after multivariate reverse regression analysis.

Outcome	Phase of local heating protocol	Predictor	β coefficient	<i>P</i> -value
Endothelial Frequency Interval	SNP-induced max	BMI	−0.777	0.004
Neurogenic Frequency Interval	Local heating plateau	MAP	−0.421	0.008
Neurogenic Frequency Interval	L-NAME plateau	MAP	−0.125	0.007
Total Power	L-NAME plateau	BMI	−1.417	0.003
Total Power	SNP-induced max	BMI	−2.014	0.050

BMI, body mass index; MAP, mean arterial pressure.