Cardiovascular responsiveness to sympathoexcitatory stress in subjects with and without mild hypertension

Link to publication record in USC Research Bank:
http://research.usc.edu.au/vital/access/manager/Repository/usc:12808

Document Version:
Author accepted manuscript (postprint)

Citation for published version:

Copyright Statement:
Copyright © 2015 Wiley-Blackwell Publishing Ltd. This is the accepted version of the following article: Carthy, E. R., White, L., Russell, F. D., Holmes, M., Leicht, A. S., Brooks, P. R., Hitchen-Holmes, D. and Askew, C. D. (2015), Cardiovascular responsiveness to sympathoexcitatory stress in subjects with and without mild hypertension. Clinical Physiology and Functional Imaging, 35: 150–158. doi: 10.1111/cpf.12143, which has been published in final form at http://dx.doi.org/10.1111/cpf.12143

General Rights:
Copyright for the publications made accessible via the USC Research Bank is retained by the author(s) and / or the copyright owners and it is a condition of accessing these publications that users recognize and abide by the legal requirements associated with these rights.

Take down policy
The University of the Sunshine Coast has made every reasonable effort to ensure that USC Research Bank content complies with copyright legislation. If you believe that the public display of this file breaches copyright please contact research-repository@usc.edu.au providing details, and we will remove the work immediately and investigate your claim.
Full title: Cardiovascular responsiveness to sympathoexcitatory stress in subjects with and without mild hypertension

Elliott R. Carthy 1, Leigh White 1, Fraser D. Russell 1, Mark Holmes 1, Anthony S. Leicht 2, Peter R. Brooks 1, Deborah Hitchen-Holmes 1, Christopher D. Askew 1.

1. School of Health and Sport Sciences, Inflammation and Healing Research Cluster, University of the Sunshine Coast, QLD, Australia.

2. Institute of Sport and Exercise Science, James Cook University, QLD, Australia.

Short title: Cardiovascular responsiveness in mild hypertension

Address for correspondence:

Associate Professor Christopher D. Askew
School of Health and Sport Sciences
University of the Sunshine Coast
Maroochydore DC, Queensland 4558, Australia.

Email: caskew@usc.edu.au
Tel: +61 7 5456 5961
Abstract

Purpose: This study compared blood pressure, heart rate variability (HRV) and forearm blood flow, at rest and in response to sympathoexcitatory stressors between normotensive and mildly hypertensive participants.

Methods: Participants aged 30-79 years with normal blood pressure (n=49) or mild hypertension (n=17), with no history of taking anti-hypertensive medication, were recruited. Participants completed a cold pressor test (CPT) followed by an ischaemic handgrip test (IHGT). Blood pressure, HRV, forearm blood flow and vascular resistance were measured at rest and in response to each test.

Results: The CPT and IHGT evoked greater increases in mean arterial blood pressure in hypertensive participants (CPT: 10±2 mmHg, IHGT: 9±1 mmHg) compared with normotensive participants (CPT: 5±1 mmHg, IHGT: 3±1 mmHg; p<0.05). Resting high frequency power, which is a parameter of HRV associated with parasympathetic cardiac modulation, was lower in hypertensive participants (hypertensive: 31.73±4.07 nu; normotensive: 42.08±2.22 nu; p=0.026) and was negatively correlated with systolic blood pressure (r=-0.272, p=0.03) and mean arterial pressure across all participants (r=-0.258, p<0.05). There were no differences in HRV or forearm blood flow responses to the CPT or IHGT between groups.

Conclusion: This study demonstrated that sympathoexcitatory stress evoked by the CPT and IHGT induces an augmented blood pressure response in individuals with mild hypertension, which supports the notion that autonomic dysfunction is likely to contribute to the pathogenesis of hypertension. It remains to be determined whether the hypertensive response is mediated through alterations in cardiac activity, peripheral vascular resistance, or both.
Keywords: cardiac autonomic modulation, vascular resistance, blood pressure, cold pressor test, ischaemic handgrip test.
Introduction

Hypertension is a leading risk factor for cardiovascular disease, is associated with significant morbidity and mortality, and contributes substantially to the health care costs of most nations (Mancia et al. 2013). A better understanding of factors that contribute to the development and progression of hypertension would aid in the development of new detection, prevention and management strategies.

Autonomic nervous system (ANS) activity modulates transient changes in cardiovascular function, and autonomic dysfunction has been implicated in the pathogenesis of essential hypertension (Julius 1991). Increased sympathetic activity, characterized by elevated circulating catecholamines and muscle sympathetic nerve activity (MSNA) (Schlaich et al. 2004), combined with parasympathetic inhibition (Julius et al. 1971), may contribute to increased cardiac activity and/or peripheral vascular resistance. Although vascular resistance is usually normal under resting conditions in patients with borderline to mild hypertension, autonomic disturbances can contribute to the early development of hypertension through an elevated cardiac activity (Amerena & Julius 1995).

Heart rate variability (HRV) describes the cyclic variations in beat-to-beat intervals on an electrocardiogram, and provides a non-invasive assessment of autonomic modulation of cardiac activity. Excessive sympathetic tone associated with cardiac pathologies is characterized by lower HRV and an increased risk for lethal tachyarrhythmias (Kleiger et al. 1987). Resting HRV is lower in individuals with severe hypertension compared to patients with mild hypertension and healthy control participants (Mussalo et al. 2001). There is, however, some inconsistency in the
reported effects of mild hypertension on HRV, with some investigations showing significant reductions in all indices of resting HRV in this group compared with normotensive controls (Acampa et al. 2009).

Sympathetic dominance normally occurs during times of physical and mental stress, and in individuals with mild hypertension it is possible that disturbances in autonomic nervous system activity, and subsequent alterations in HRV and cardiovascular dynamics, may manifest themselves more clearly during periods of stress than under basal conditions. Little is known about the response of individuals with mild hypertension to periods of stress, and this is important to understand as an augmented sympathetic response may be a prognostic marker for stress-induced cardiovascular events (e.g. during physical exertion) (Stratton et al. 1983). The cold pressor test (CPT) and the ischaemic handgrip test (IHGT) are commonly used methods that evoke a sympathoexcitatory stress response (Mourot et al. 2009; Stewart et al. 2007). Augmented blood pressure in response to the CPT is predictive of the development of future hypertension (Kasagi et al. 1995). The increase in systolic arterial blood pressure during the IHGT is ~2-fold higher in people with moderate essential hypertension (61±21 mmHg) compared with those with normal blood pressure (28±4 mmHg) (Hamada et al. 1987). It is not known whether similar responses are observed in individuals with mild hypertension, or whether changes in blood pressure are accompanied by alterations in peripheral vascular resistance or HRV. The aim of this study was to compare the cardiovascular responsiveness to sympathoexcitatory stress induced by the CPT and IHGT between normotensive and mild-hypertensive participants.
Methods

Participants

Participants aged between 30 and 79 years were recruited, with eligibility conditional on having either mild hypertension (135/85 to 145/95 mmHg) or normal blood pressure (<135/85 mmHg), and not currently taking blood pressure lowering medications. Individuals with a history of other cardiovascular disease or major illnesses were not considered for participation. The Human Research Ethics Committee at the University of the Sunshine Coast approved all protocols (ECOO297 A/08/167), and informed written consent was obtained from each participant prior to entry into the study.

Study and experimental overview

Participants were categorised as either hypertensive or normotensive based on home-based blood pressure measurements recorded daily over a 3 week period using an automated sphygmomanometer (Medel Check, Torille, Italy). Participants collected four repeat measurements of blood pressure, each preceded by a 2 min rest period. Blood pressure was stable for measurements 2-4, and these were used to determine mean daily systolic and diastolic blood pressures. This rigorous home based measurement protocol eliminates white coat phenomena, and generally results in lower blood pressure measures than those obtained during patient consultation with medical practitioners (Mancia et al. 2011). A hypertensive cut-off of 135/85 mmHg was used, consistent with that recommended for home-based measurements (Parati et al. 2008). Following the 3-week lead-in period, and after being familiarised with all test procedures, participants attended the laboratory to perform the CPT and IHGT,
where blood pressure, HRV and limb blood flow responses were recorded. Participants were asked to avoid physical activity on the day of testing.

Testing was conducted with the participant lying supine with the right arm abducted to approximately 90° and the left arm placed comfortably by their side. An automated sphygmomanometer was used for brachial blood pressure measurements in the left arm (Pressure Perfect, Suntech Medical Instruments, Raleigh NC, USA). Blood flow was measured in the right forearm using strain gauge plethysmography; the arm was elevated slightly above the level of the heart to facilitate venous return, and a mercury-in-silastic strain gauge (Hokanson, Bellevue, WA, USA) was placed around the forearm at the point of widest girth. A rapid-inflation blood pressure cuff was placed around the right upper arm for venous occlusion and a small cuff was placed around the right wrist to exclude hand circulation from the measurements. Chest ECG electrodes were placed in a lead-II configuration. All signals were collected and stored at a sampling rate of 1 kHz using a PowerLab data acquisition system (16/30) and LabChart Pro 5.5.6 software (ADInstruments, Bella Vista, NSW). Participants initially rested for 5 min, followed by a further 10-min rest period during which ECG data were collected for resting HRV analysis. Forearm reactive hyperaemia was then assessed as the peak blood flow response immediately after a 5-min period of arm cuff-occlusion. Participants then completed the CPT, followed by the IHGT, each of which was preceded by further 7-min rest periods. Testing was conducted in a quiet room at a stable temperature (22°C), and participants were given standardized instructions to maintain a comfortable and regular breathing rate, to avoid the valsalva maneuver, and to avoid speaking or moving unnecessarily during the testing procedures.
Cold pressor test

The left forearm was immersed up to 5 cm below the crease of the elbow for 90 s in a standardised ice-water mix (≈4°C). ECG data were collected throughout the test, enabling HRV to be assessed throughout the period of immersion and during the 90 s following test cessation. Blood flow at the right forearm, and blood pressure at the left arm, were measured 3 min prior to arm immersion and immediately after removal of the arm from the water.

Ischaemic handgrip test

An isometric handgrip dynamometer (ADInstruments, Bella Vista, NSW) was calibrated prior to each test. Maximum voluntary contraction (MVC) force of the left hand was initially established over five repeated maximal contractions, each of which were 2 s in duration and separated by 60 s of rest. During the IHGT, participants were required to perform a sustained isometric contraction at 30% MVC force for 5 min or for as long as possible, whichever came first. Duration of the IHGT was not different between the groups (hypertensive: 4.71±0.65; normotensive: 4.57±0.50 min; p=0.36). HRV was assessed before, during and after the IHGT. Blood pressure and forearm blood flow were measured 3 min before and immediately following the IHGT.

Heart rate variability

Continuous ECG data were collected at rest and throughout the CPT and IHGT. R-wave position was determined using an event detection threshold of 0.1-0.2 mV below the average R wave peak. The data were inspected for ectopic beats, respiratory disturbances or noise artifact. Data exclusions were performed manually on a test-by-
test basis to ensure the inclusion of normal sinus rhythm data only (>99% of recording).

Frequency-domain and time-domain parameters of HRV were used to assess resting HRV. Resting HRV was measured over a 10 min period following the initial 5 min period of supine rest. The frequency-domain parameters were calculated using power spectral analysis (1024 point Fast Fourier Transformation (FFT) with a Welch window, 50% overlap) using LabChart Pro 5.5.6 software (ADInstruments, Bella Vista, NSW). The HRV parameters included total power (TP; 0.04-0.4Hz; total energy in the HRV spectrum), as well as high frequency power (HF; 0.15-0.4Hz; indicator of parasympathetic modulation) and low frequency power (LF; 0.04-0.15Hz; indicator of parasympathetic and sympathetic modulations including baroreflex function). Very low frequency power (VLF; risk factor for mortality (Hadase et al. 2004); 0-0.04Hz) was also measured, as was the LF/HF ratio (representing sympathovagal interactions on heart rate) (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996). Frequency-domain parameters were assessed using both absolute units (ms²) and normalised units (nu; absolute power of the components/(TP – VLF)x 100). The time-domain parameters included the standard deviation of all normal-to-normal RR intervals (SDNN; measure of overall HRV), and those representative of cardiac parasympathetic modulation, including standard deviation of the difference between adjacent NN intervals (SD\(\Delta NN\)), root mean square of successive differences in NN intervals (rMSSD), and the percentage of intervals >50 ms different from the preceding interval (pNN50) (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996).
Non-linear analysis techniques were used to assess the short-term cardiac autonomic responses to the CPT and IHGT, as these methods are more appropriate for the analysis of shorter, non-stationary data than conventional frequency-domain and time-domain analysis procedures (Tulppo et al. 2001). Recordings of RR intervals (90 s) immediately before and following both the CPT and IHGT were exported and later analysed for HRV using customised software (Kubios HRV v2.0, Biosignal Analysis and Medical Imaging Group, Department of Physics, University of Kuopio, Kuopio, Finland). The outcome measures included the Poincaré plot parameters of SD1 (reflective of cardiac parasympathetic activity), SD2 (reflective of long-term overall heart rate variability) and the SD1/SD2 ratio; sample entropy (SampEn; representative of the system’s overall complexity or randomness); and the fractal scaling component α-1 (reflective of overall HRV). The α-1 variable was determined using detrended fluctuation analysis and calculated as the slope of the relationship between the logarithmic based integrated and detrended fluctuation and window size (4-16 beats) (Tarvainen & Niskanen 2008). Sample entropy was preferentially examined over approximate entropy as it has been found to be independent of the ECG recording length and exhibits less bias (Lake et al. 2002; Richman & Moorman 2000). SampEn was determined using the input variables; m of 2 (the length of compared runs at each time point of the time series), and r of 20% (the tolerance for judging similarity of runs) (Tarvainen & Niskanen 2008).

**Forearm blood flow and vascular resistance**

Prior to each test the strain gauge was calibrated so that an increase of 1 mV was equal to a 1% increase in arm volume (i.e. 1 mL.100mL⁻¹). Each measurement of blood flow required a brief period of venous occlusion, achieved by inflating the right upper
arm cuff to 60 mmHg for 10 s. Blood flow was calculated as the relative change in forearm volume over the change in time (mL.100mL⁻¹.min⁻¹), and was assessed over two cardiac cycles that were free from movement or cuff artifact (Vaile et al. 2011). During each assessment the wrist cuff was inflated to 200 mmHg to prevent venous blood flow from the hand entering the forearm and thereby artificially elevating the measured blood flow (Lenders et al. 1991). For each forearm blood flow measure the corresponding forearm vascular resistance was calculated by dividing the mean arterial pressure by the blood flow (mmHg.mL⁻¹.100mL⁻¹.min⁻¹).

Data analysis

In response to the CPT and IHGT, the net change for each variable was calculated as the difference between values immediately before and after each test. Univariate one-way analysis of variance (ANOVA) was used to assess main effects and interactions between groups (hypertensive vs. normotensive) for each of the key baseline, net and post-test variables. Pearson’s Correlation Coefficient was used to explore the relationships between variables. Statistical analyses were performed using SPSS (IBM Statistics 19, SPSS Inc., USA), with significance for all comparisons set at p<0.05. Data are expressed as mean ± SEM except where indicated.

Results

Participants

A total of 66 participants (17 hypertensive and 49 normotensive) were recruited. Mass, height, body mass index (BMI) and all blood pressure parameters were significantly higher for hypertensive compared with normotensive participants (Table 1).
Blood pressure

The net blood pressure responses to the CPT and IHGT are shown in Figure 1. The increase in blood pressure evoked by the CPT was significantly greater in hypertensive than normotensive participants for both systolic blood pressure (SBP: \( p=0.011 \)) and mean arterial pressure (MAP: \( p=0.026 \)). A non-significant trend for an increased diastolic blood pressure (DBP) response to the CPT was also observed (see Figure 1A). During the CPT there was no significant difference in net heart rate (hypertensive: \(-2.33\pm1.15\); normotensive: \(-2.29\pm0.56\) bpm) or net pulse pressure (hypertensive: \(12.01\pm4.32\); normotensive: \(6.29\pm1.98\) mmHg) between groups.

Figure 1B shows that the IHGT evoked a significantly greater increase in DBP (\( p=0.001 \)) and MAP (\( p<0.001 \)) in hypertensive compared with normotensive participants. The net rise in SBP also tended to be greater in hypertensive than normotensive participants, although this was not significant (Figure 1B). Heart rate (hypertensive: \(-2.33\pm1.15\); normotensive: \(-0.99\pm0.72\) bpm) and pulse pressure (hypertensive: \(7.05\pm2.64\); normotensive: \(8.83\pm1.46 \) mmHg) not differ between groups during the IHGT.

Heart rate variability

Table 2 shows the resting frequency-domain and time-domain measures of HRV for hypertensive and normotensive participants. Resting HF power tended to be greater for normotensive compared with hypertensive participants, with this difference significant when expressed in normalised units (\( p=0.026 \)). Also, the LF/HF ratio was greater in hypertensive participants (\( p=0.008 \)). There were no significant differences between hypertensive and normotensive participants at rest for the time-domain HRV parameters. Changes in the short-term non-linear HRV parameters in response to the
CPT and IHGT were not different between hypertensive and normotensive participants (Table 3).

**Forearm blood flow and vascular resistance**

Forearm reactive hyperaemia did not differ significantly between the hypertensive (25.27±1.85 mL.100mL⁻¹.min⁻¹) and normotensive groups (21.84±1.02 mL.100mL⁻¹.min⁻¹). There was no difference in resting forearm blood flow (hypertensive: 3.38±0.26; normotensive: 3.33±0.28 mL.100mL⁻¹.min⁻¹) or vascular resistance (hypertensive: 34.09±2.40, normotensive: 34.94±3.16 mmHg.mL⁻¹.100mL⁻¹.min⁻¹) between the groups. Furthermore, there was no significant difference between groups in the net blood flow response to either the CPT (hypertensive: -0.19±0.27; normotensive: -0.21±0.21 mL.100mL⁻¹.min⁻¹) or IHGT (hypertensive: 0.63±0.33; normotensive: -0.01±0.34 mL.100mL⁻¹.min⁻¹), and similarly no difference in the net vascular resistance response to the CPT (hypertensive: -5.07±6.69; normotensive: 3.01±6.08 mmHg.mL⁻¹.100mL⁻¹.min⁻¹) or IHGT (hypertensive: -0.29±2.44; normotensive: 1.12±2.78 mmHg.mL⁻¹.100mL⁻¹.min⁻¹).

**Relationships with blood pressure**

As there were significant differences in blood pressure at rest and in response to the stress tests between the two groups, the relationships between blood pressure and other variables were investigated. The SBP and MAP responses to the IHGT were positively correlated with resting SBP (r=0.32 – 0.34, p<0.05) and resting MAP (r=0.27 – 0.28, p<0.05) across all participants. There was a weak negative correlation between resting HF power (nu) and both resting SBP (r=-0.272, p<0.05) and MAP (r=-0.258, p<0.05). The change in SD1 during the IHGT was negatively associated with both
baseline DBP (r=-0.62, p<0.01) and MAP (r=-0.66, P<0.01) in the hypertensive group. In this same group, the SD1 response to the CPT was positively correlated with baseline pulse pressure (r=0.63, p<0.01). SBP, DBP and MAP were all positively correlated with BMI across all participants (r=0.51, p<0.001), and these relationships were maintained when only the hypertensive group was analysed (r=0.41 - 0.47, p<0.05). Age of the hypertensive participants correlated positively with pulse pressure (r=0.69, p=<0.01) and negatively with DBP (r=-0.50, p<0.05). The net increase in pulse pressure during the IHGT also correlated positively with age (r= 0.67, p<0.01) in this group.

Discussion

Consistent with dysfunction of the ANS, we hypothesised that in response to sympathoexcitatory stress tests, participants with mild hypertension would display an augmented blood pressure response compared with normotensive individuals, and that this would be associated with changes in HRV that are indicative of a shift towards sympathetic modulation of heart rate and cardiac activity. Few studies have investigated the cardiovascular responses to the CPT and IHGT in patients with hypertension, and most have studied patients with well-established moderate-severe hypertension. We specifically recruited individuals with mild hypertension who had never taken blood pressure lowering medication, and we ensured that our baseline values accounted for daily variance and were free of a ‘white-coat’ effect by using a rigorous three-week home monitoring period. Compared with normotensive participants, the mean arterial blood pressure response to both the CPT and IHGT was significantly augmented in the mildly hypertensive group. This blood pressure response to the IHGT is consistent with the elevated systolic pressure response in a group of mild-moderate hypertensive subjects (Hamada et al. 1987), and confirms the
observation by others where there tended to be a greater rise in blood pressure in young male subjects with borderline hypertension, where blood pressure is occasionally within the hypertensive range, than in normotensive controls (Seals et al. 1985). In older adults (mean age: 70 y), there was no difference in the net pressure response during the IHGT between participants with established severe hypertension, severe isolated systolic hypertension, or normotension, although the peak systolic pressure attained was significantly higher in the hypertensive groups (Delaney et al. 2010). We observed a significant correlation where pulse pressure increased during the IHGT to a greater extent in older participants in the hypertensive group. This suggests that the exaggerated pressor response in hypertensive subjects might be augmented with increased age, although this remains to be clarified. In response to the CPT there was no difference in the blood pressure response between individuals with low, normal and high-normal blood pressure (Flaa et al. 2006). However, the cold pressor response was exaggerated in those with borderline hypertension (Lafleche et al. 1998), and our findings demonstrate that this is also true for individuals with mild hypertension.

Hypertensive participants demonstrated alterations in HRV that are indicative of an alteration in sympathovagal modulation of resting heart rate. Normalised HF power was significantly reduced in the hypertensive participants, and was negatively correlated with mean arterial pressure across all participants. This reflects a reduction in the parasympathetic influence on cardiac activity that becomes more pronounced with increases in resting blood pressure. Hypertensive participants also demonstrated a significantly higher LF/HF ratio, which reflects a greater sympathetic influence (Task Force of the European Society of Cardiology and the North American Society of Pacing
and Electrophysiology 1996). This supports the findings of Lucini et al., who found that with increases in resting blood pressure from normal to severe hypertension, there was a gradual shift in the HRV spectrum towards the LF range (Lucini et al. 2002). This is consistent with the notion that autonomic dysfunction mediates its effect in early stage hypertension through an alteration in cardiac activity (Amerena & Julius 1995).

We hypothesised that this autonomic dysfunction would also lead to an exaggerated HRV response during the sympathoexcitatory stress tests (CPT and IHGT) in participants with hypertension. In contrast, we found that there was no change in the indices of HRV in response to these stressors, and there was no difference in the response between hypertensive and normotensive participants. As we did not monitor respiratory rate, we cannot exclude the possibility that changes in respiratory activity during these stressors may have influenced HRV and masked any differences in the stress response between groups. We used nonlinear analyses to assess the short term HRV response as previously recommended (Tulppo et al. 2001). These nonlinear parameters are more reliable and better suited for test-retest comparisons than traditional HRV indices (Maestri et al. 2007). Use of the nonlinear methods requires large sample sizes to demonstrate differences or changes (Maestri et al. 2007). As the hypertensive group was relatively small (n=17), we also explored the data using frequency and time-domain analyses (data not shown) with similar findings to that of the non-linear analyses. To our knowledge, this was the first study to compare these short-term HRV responses in patients with hypertension. In healthy participants, the CPT was shown to increase MSNA, which was accompanied by reduced HF spectral power, and an increase in the LF/HF ratio and α-1 (Tulppo et al. 2005). Similarly, Mourot et al. showed that the increase in α-1 only occurred in healthy participants.
where there was a sustained rise in heart rate in response to the CPT, whereas $\alpha$-1 decreased in those participants where there was an initial rise, followed by a fall in HR during the test (Mourot et al. 2009). The authors suggested that this reflects a baroreflex co-activation of vagal outflow in response to the sustained increase in blood pressure (Mourot et al. 2009), which, if present, might be augmented in hypertensive participants.

To understand why there was no difference in the HRV stress responses between groups in the present study it would be necessary to directly assess sympathetic activity. In response to the CPT, Davis et al. found that circulating norepinephrine levels increased to a greater extent in participants with well-established hypertension, whereas there was no difference in the response of those with pre-hypertension (MAP: 95mmHg), compared with normotensive controls (Davis et al. 2012). This raises the possibility that any alterations in sympathetic tone and resulting cardiovascular function in mild hypertensives are small, and therefore difficult to detect. Hamada et al. reported a significantly greater systolic pressure response to the IHGT in hypertensive compared with normotensive individuals, with no significant difference in the plasma catecholamine response. However, there was a tendency for the norepinephrine response to be greater in the hypertensive group, suggesting that the pressor response may be mediated by greater sympathetic drive and/or increased sensitivity to norepinephrine (Hamada et al. 1987). Circulating catecholamines are influenced by rates of release and reuptake, and therefore MSNA is arguably a more robust and relevant marker of sympathetic activity. It was recently shown that MSNA burst frequency increased to a greater extent during a handgrip test in hypertensive compared with normotensive participants; however the MSNA response to the CPT
was not different between the groups (Delaney et al. 2010). Resting MSNA has been shown to be elevated with increasing BMI and body fat (Scherrer et al. 1994). In the present study, BMI was greater in the hypertensive group and may have contributed to the altered resting HRV, although any body composition effect might have also been expected to influence the HRV response to the stress tests. Examining the HRV response in conjunction with MSNA in future studies would provide a greater insight into the contribution of ANS mediated cardiac contributions to the pressor response seen in mildly hypertensive participants.

Forearm blood flow was measured in the present study as a marker of peripheral vasoconstrictor activity. Due to the differences in blood pressure between groups, both at rest and in response to the stress tests, vascular resistance was calculated to more accurately reflect vascular tone. There were no differences in resting forearm blood flow or vascular resistance between the normotensive and hypertensive groups, which is consistent with the notion that early stage hypertension is usually associated with elevated cardiac activity rather than vascular resistance (Amerena & Julius 1995). MSNA was shown to be increased in young hypertensive subjects compared with age-matched normotensives, and this corresponded with higher calf vascular resistance in the hypertensive group (Floras & Hara 1993). The average blood pressure of the hypertensive group in that study (151/95 mmHg) was higher than in the present study (141/89 mmHg), which may explain the difference in vascular resistance between the studies. Nonetheless, the relationship between MSNA and peripheral vascular resistance has also been demonstrated in normotensive individuals (Dinenno et al. 1999), and we are not aware of any studies that have directly assessed this relationship in individuals with mild hypertension.
There was no significant change in forearm blood flow or vascular resistance in response to the IHGT or CPT in both the hypertensive and normotensive groups. In one of only a few studies to examine limb blood flow responsiveness in hypertensives, it was previously shown that forearm vascular resistance increased in response to the CPT, and that this response was blunted in older (>55 y) patients (Sowers & Mohanty 1989). In another study of young patients (~25 y) who intermittently had blood pressure readings above 150/90 mmHg, both MAP and forearm vascular resistance increased in response to the CPT (Mark & Kerber 1982). However, this same response was observed in the normotensive control group, which raises questions about the interaction between age and hypertension severity on the vascular responses to such tests.

Our findings suggest that the augmented blood pressure response to the stress tests in the mild hypertensive group was not the result of an increased vascular tone. This is further supported by the lack of difference in reactive hyperaemia, which suggests that the forearm vasodilatory capacity of the groups was also similar. This is in contrast, however, to evidence that flow mediated dilatation is impaired in hypertensive subjects (Panza et al. 1990), even in those with high-normal blood pressure (Plavnik et al. 2007).

Conclusions
Alterations in resting HRV and an augmentation of blood pressure reactivity to sympatoexcitatory stress tests (cold pressor and ischaemic handgrip) in the present study provides support for the presence of autonomic dysfunction in patients with
mild hypertension. However, this was not reflected in the responses of HRV and forearm vascular resistance to the stressors, which were not different between hypertensive and normotensive groups. Alterations in the reactivity of these measures may become more pronounced in moderate to severe hypertension, and further studies are required to confirm these findings and elucidate whether the augmented blood pressure reactivity is mediated through alterations in cardiac activity, peripheral vascular resistance, or both.

**Acknowledgements**

This project was funded through a University of the Sunshine Coast research development grant. The authors have no conflicts of interest.
References


Mancia G., Bombelli M., Seravalle G. & Grassi G. Diagnosis and management of patients with white-coat and masked hypertension. *Nat Rev Cardiol*, (2011); **8**: 686-693.


Table 1: Participant characteristics

<table>
<thead>
<tr>
<th></th>
<th>Normotensive (N=49)</th>
<th>Hypertensive (N=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>50 ± 10</td>
<td>52 ± 11</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>16/33</td>
<td>13/4*</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>75.7 ± 14.5</td>
<td>95.1 ± 20.3*</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.69 ± 8.72</td>
<td>1.76 ± 10.68*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.4 ± 0.6</td>
<td>30.5 ± 1.2*</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>69 ± 9</td>
<td>66 ± 3</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>117 ± 10</td>
<td>141 ± 8*</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>73 ± 7</td>
<td>89 ± 9*</td>
</tr>
<tr>
<td>Pulse pressure (mmHg)</td>
<td>43 ± 1</td>
<td>53 ± 2*</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>87 ± 8</td>
<td>106 ± 8*</td>
</tr>
</tbody>
</table>

*Indicates a significant difference between hypertensive and normotensive groups (p<0.05). Data presented as mean ± SD.
Table 2: Resting heart rate variability

<table>
<thead>
<tr>
<th>HRV parameter</th>
<th>Normotensive</th>
<th>Hypertensive</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP (ms²)</td>
<td>2138±306</td>
<td>2245±541</td>
<td>0.863</td>
</tr>
<tr>
<td>HF (ms²)</td>
<td>490±96</td>
<td>278±65</td>
<td>0.224</td>
</tr>
<tr>
<td>HF (nu)</td>
<td>42.08±2.22</td>
<td>31.73±4.07*</td>
<td>0.026</td>
</tr>
<tr>
<td>LF (ms²)</td>
<td>584±104</td>
<td>713±311</td>
<td>0.611</td>
</tr>
<tr>
<td>LF (nu)</td>
<td>53.87±2.17</td>
<td>62.59±4.54</td>
<td>0.063</td>
</tr>
<tr>
<td>VLF (ms²)</td>
<td>1024±139</td>
<td>1216±306</td>
<td>0.525</td>
</tr>
<tr>
<td>LF/HF</td>
<td>1.64±0.17</td>
<td>3.54±1.11*</td>
<td>0.008</td>
</tr>
<tr>
<td>SDNN (ms)</td>
<td>43.9±3.0</td>
<td>43.0±5.0</td>
<td>0.877</td>
</tr>
<tr>
<td>SDΔNN (ms)</td>
<td>29.4±2.8</td>
<td>25.9±3.6</td>
<td>0.504</td>
</tr>
<tr>
<td>rMSSD (ms)</td>
<td>29.4±2.8</td>
<td>25.8±3.6</td>
<td>0.503</td>
</tr>
<tr>
<td>pNN50 (%)</td>
<td>10.0±2.23</td>
<td>12.5±4.52</td>
<td>0.587</td>
</tr>
</tbody>
</table>

Heart rate variability (HRV) frequency-domain parameters included total power of heart rate variability (TP), high frequency power (HF) in absolute (ms²) and normalised units (nu), low frequency power (LF), very low frequency power (VLF) and the low frequency power/high frequency power ratio (LF/HF). Time-domain parameters included standard deviation of all normal-to-normal RR (NN) intervals (SDNN), standard deviation of the differences between adjacent NN intervals (SDΔNN), root mean square of successive differences in NN intervals (rMSSD), and the percentage of intervals >50 ms different from the preceding interval (pNN50). *Indicates a significant difference between hypertensive and normotensive groups (p<0.05). Data are presented as mean ± SEM.
Table 3: Heart rate variability response to the cold pressor and ischaemic handgrip tests

<table>
<thead>
<tr>
<th>HRV parameter</th>
<th>Pre-test</th>
<th>Post-test</th>
<th>Net change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normotensive</td>
<td>Hypertensive</td>
<td>Normotensive</td>
</tr>
<tr>
<td><strong>Cold pressor test (CPT)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD1 (ms)</td>
<td>26.5±2.54</td>
<td>25.0±3.23</td>
<td>28.5±3.10</td>
</tr>
<tr>
<td>SD2 (ms)</td>
<td>75.0±4.24</td>
<td>74.3±13.1</td>
<td>79.5±5.57</td>
</tr>
<tr>
<td>SD1/SD2</td>
<td>0.35±0.02</td>
<td>0.38±0.05</td>
<td>0.35±0.02</td>
</tr>
<tr>
<td>SampEn</td>
<td>1.42±0.05</td>
<td>1.41±0.10</td>
<td>1.41±0.05</td>
</tr>
<tr>
<td>α-1</td>
<td>1.24±0.04</td>
<td>1.19±0.08</td>
<td>1.20±0.04</td>
</tr>
<tr>
<td><strong>Ischaemic handgrip test (IHGT)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD1 (ms)</td>
<td>25.7±2.82</td>
<td>20.8±2.47</td>
<td>30.3±3.30</td>
</tr>
<tr>
<td>SD2 (ms)</td>
<td>74.4±5.28</td>
<td>61.3±6.27</td>
<td>91.8±6.20</td>
</tr>
<tr>
<td>SD1/SD2</td>
<td>0.35±0.02</td>
<td>0.36±0.05</td>
<td>0.31±0.02</td>
</tr>
<tr>
<td>SampEn</td>
<td>1.46±0.06</td>
<td>1.36±0.10</td>
<td>1.27±0.06</td>
</tr>
<tr>
<td>α-1</td>
<td>1.23±0.04</td>
<td>1.17±0.06</td>
<td>1.21±0.03</td>
</tr>
</tbody>
</table>

Short-term heart rate variability (HRV) was assessed using nonlinear analysis procedures and the net HRV response for each test was calculated as the difference between the pre- and post-test values. The HRV parameters included the Poincaré plot parameters SD1 (cardiac parasympathetic activity), SD2 (long-term overall HRV) and the SD1/SD2 ratio; the fractal dimensional component α-1 (overall HRV); and sample entropy (SampEn; representative of the system’s overall complexity and hence HRV). Differences between groups and changes in response to each test were not significant. Data are presented as mean ± SEM.
**Figure caption**

**Figure 1:** The net change in systolic (SBP), diastolic (DBP) and mean arterial pressure (MAP) in response to the cold pressor test (A), and the ischaemic handgrip test (B). The net response was calculated as the difference in blood pressure from before to after each assessment. *Indicates a significant difference between hypertensive and normotensive participants (p<0.05). Data are presented as mean ± SEM.