

The effect of acute mental stress on limb vasodilation is unrelated to total peripheral resistance

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Abstract

Mental stress can trigger myocardial infarction, with poor vascular responses to stress implicated as a pathway. Vascular stress reactivity can be assessed by different methods, such as total peripheral resistance (TPR) and forearm blood flow (FBF). Little is known about how these vascular assessments are linked. This was examined in two separate studies. Healthy men (Study 1: $N = 29$, Study 2: $N = 23$) completed rest and mental arithmetic (Study 1: 8 min, Study 2: 16 min). In both studies, heart rate, mean arterial pressure, and FBF increased in response to stress. In Study 1, no changes in TPR were seen, but Study 2 found stress-induced increases in TPR. FBF was not linked to TPR at any time (all $ps > .05$). It appears that limb vasculature and TPR responses to stress do not give the same information about impairments of the vasculature. These findings are relevant to the interpretation of prior research findings and the design of future studies on stress and vascular responses.

Descriptors: Mental stress, Forearm blood flow, Total peripheral resistance

Anecdotal and epidemiological studies have demonstrated that psychological stress may trigger a myocardial infarction (MI) (Bergovec et al., 1992; Carroll, Ebrahim, Tilling, Macleod, & Smith, 2002; Katsouyanni, Kogevas, & Trichopoulos, 1986; Meisel et al., 1991; Suzuki, Sakamoto, Miki, & Matsuo, 1995). Laboratory studies assessing mental stress-induced ischemia have implicated poor peripheral vascular responses to stress as a possible contributing mechanism (Burg et al., 2009; Goldberg et al., 1996; Jain et al., 1998). Studies that have examined endothelial function during mental stress have demonstrated improvement (Harris et al., 2000), no change (Dyson, Shoemaker, & Hughson, 2006), as well as impairment of endothelial function (Gottdiener et al., 2003; Jambrik, Sebastiani, Picano, Ghelarducci, & Santarcangelo, 2005; Sarabi & Lind, 2001) in relation to mental stress. Other studies have utilized arterial stiffness as a measure of vascular function (Ellins et al., 2008), with studies that have assessed the effects of mental stress on arterial stiffness yielding increases in arterial stiffness (Vlachopoulos et al., 2006). However, in contrast to resting vascular function, which is commonly assessed using flow-mediated dilation (FMD) or arterial stiffness, the more commonly used assessments to examine the peripheral vascular responses to stress are forearm blood flow (FBF) and total peripheral resistance (TPR). FBF and TPR allow for continuous assessment throughout the stress task, with assessments of TPR and reduced peripheral dilation have been associated with mental

stress-induced ischemia (Burg et al., 2009; Goldberg et al., 1996; Jain et al., 1998).

Blood Flow

First described in the 1950s (Whitney, 1953), FBF is typically assessed by venous occlusion plethysmography, which measures the total blood flow of a limb. By inflating a wrist cuff to supra-systolic pressure and inflating an upper arm cuff to below diastolic yet above venous pressure, arterial inflow still occurs while venous outflow is blocked. The subsequent changes in limb (arm/leg) circumference as detected by the strain gauge are indicative of blood flow (Joyner, Dietz, & Shepherd, 2001; Newton, Khan, & Belch, 2001). In this method, 70% of the measured flow is reflective of muscle blood flow and the remainder indicative of skin blood flow (Cooper, Edholm, & Mottram, 1955). Up to 10-fold increases in FBF in response to mental stress have been demonstrated in healthy populations (Blair, Glover, Greenfield, & Roddie, 1959; Dietz et al., 1994), with strong reproducibility (Hamer, Boutcher, Park, & Boutcher, 2006). Significantly, FBF during mental stress does not increase in heart failure patients, in contrast to healthy controls (Middlekauff et al., 1997; Santos et al., 2005) and is impaired in those at higher risk of MI (Hamer, Boutcher, & Boutcher, 2007), thus suggesting the role of the peripheral vasculature as a potential trigger for MI.

Several mechanisms have been postulated that might, either singularly or in combination, be responsible for increases in FBF in response to stress. Firstly, the vascular responses to stress were considered to be neurally driven (Blair et al., 1959); however,

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associations between muscle sympathetic nerve activity and blood flow responses to stress have not been consistently demonstrated (Carter, Cooke, & Ray, 2005; Carter, Durocher, & Kern, 2008; Carter, Kupiers, & Ray, 2005). Beta (β)-adrenergic mechanisms such as adrenaline have also been speculated to be the reason for stress-induced increases in blood flow (Kjeldsen, Petrin, Weder, & Julius, 1993; Lindqvist, Melcher, & Hjendahl, 1997). However, there appears to be more consistent support for the role of nitric oxide (NO) (Dietz et al., 1994; Halliwill et al., 1997; Joyner & Casey, 2009; Joyner & Dietz, 2003). Importantly, β -adrenergic driven vasodilation is attenuated when NO production is inhibited, suggesting NO plays an important role in β -adrenergic driven vasodilation, as without NO, reductions in dilation through an alternative mechanism are seen (Dawes, Chowienzyk, & Ritter, 1997). In addition to increases in forearm blood flow as a result of mental stress, other indicators of vasodilation such as decreases in forearm vascular resistance (FVR) may also occur (Pike, Elvebak, Jegede, Gleich, & Eisenach, 2009). FVR is calculated as mean arterial pressure/FBF (Pike et al., 2009). Therefore, these assessments allow a measure of resistance through the limb, in addition to blood flow.

Total Peripheral Resistance

TPR reflects whole-body vascular resistance, and can be assessed using impedance cardiography. Impedance cardiography is a technique that assesses the resistance of the thorax to an electrical current, which is in part determined by the amount of fluid in the aorta. Ejection of blood into the thoracic cavity during the systolic phase of contraction is associated with a reduction in impedance. Impedance cardiography identifies the start of the systole, the peak blood flow velocity, and, when blood stops flowing, allows us to calculate stroke volume (SV, ml) using the Kubicek formula (Kubicek et al., 1974). SV can then be used to derive cardiac output and subsequently TPR. TPR is measured in dynes-s/cm⁻⁵, and can be calculated as (mean arterial pressure/cardiac output) \times 80 (Sherwood et al., 1990). Cardiac output is calculated as heart rate \times stroke volume.

Stress-induced changes in TPR are important to measure from a clinical standpoint, because stress-induced myocardial ischemia is associated with stress-induced increases in TPR in those with coronary artery disease (Goldberg et al., 1996; Jain et al., 1998), but research of the effects of mental stress on TPR yield inconsistent results. Increases in TPR, as assessed by impedance cardiography, have been demonstrated in healthy populations in response to singular (Bacon, Keller, Lavoie, & Campbell, 2010) as well as successive (Gillin et al., 2008; Ottaviani, Shapiro, Goldstein, & Mills, 2007) acute stress tasks. Examination of both normotensive and mildly hypertensive individuals has demonstrated no changes in TPR in response to stress (Tsai, Yucha, Nichols, & Yarandi, 2003), whereas decreases in TPR have also been reported in response to both active and passive stress tasks (e.g., Sherwood, Johnson, Blumenthal, & Hinderliter, 1999; Sherwood et al., 2010). Increases in TPR have been speculated only to occur after a more prolonged stress task, as gradual increases in TPR have been observed during a 32-min stress task (Ring, Burns, & Carroll, 2002).

The FBF and TPR responses to mental stress have been studied extensively, but to our knowledge, no studies have simultaneously examined these two measures at rest and in response to mental stress. Cardiovascular measurements have been associated with measures of vascular function. Pike et al. (2009) showed a positive

correlation between heart rate and FBF, plus a negative association between heart rate and FVR. Even though FVR and FBF are known to be associated with heart rate (Pike et al., 2009), little is known about the relationship between TPR and heart rate responses to stress. Therefore, this study aims to examine the relationships and interactions between these measurements at rest and in response to mental stress in a healthy population. This association is examined in two separate studies with different task duration in order to explore the potential effects of task duration on the vascular responses to mental stress.

Method

Study 1

Participants. Twenty-nine white Caucasian male university students were recruited (mean age \pm SD = 19.2 \pm 0.7 years, mean body mass index (BMI) \pm SD = 22.2 \pm 2.1 kg/m²). None of the participants were suffering from an acute illness or infection, reported a history of inflammatory, cardiovascular, or autoimmune disorders, had taken any anti-inflammatory medication in the last 4 weeks or any regular prescribed medication. Participants reported to the laboratory after an overnight fast and having refrained from vigorous exercise and over-the-counter medication for at least 24 h and from alcohol for at least 12 h. The study was approved by the local research ethics committee. All participants gave written informed consent.

Procedure. The session started between 6.30–9.00 am and was performed in a temperature-controlled (18°C) environment. Upon arrival, the participant was familiarized with the procedures. Measurements of height and weight were taken, and the participant was instrumented for the impedance cardiography assessment. The participant was then placed into a supine position on a bed, where he remained throughout the session. After the blood pressure and blood flow equipment was attached, the participant rested for 20 min (baseline rest period) while watching a nature documentary (*Planet Earth*; BBC). During minutes 13, 15, 17, and 19, blood flow measurements were taken, and impedance cardiography and blood pressure were analyzed for these minutes. After explanation and practice, participants completed an 8-min mental stress task. During minutes 1, 3, 5, and 7, blood flow was recorded, and impedance cardiography and blood pressure were analyzed for these minutes. Hereafter, these minutes will be referred to as minutes of assessment for baseline and stress, accordingly.

Mental stress task. The mental stress task was the paced auditory serial addition task (PASAT). Participants were presented with a series of single digit numbers, which were delivered using a CD player. They were required to add each number they heard to the number presented previously, while retaining the last number to add it to the next number they heard (Gronwall, 1977; Ring, Drayson, Walkey, Dale, & Carroll, 2002). The numbers were delivered in four consecutive 2-min blocks, becoming progressively more challenging whereby the numbers were presented every 2.4 s, then 2.0 s, 1.6 s, and 1.2 s, respectively. The experimenter, who sat at a distance of 1 m adjacent to the participant, checked their responses against the correct answers. Participants were alerted by a loud, aversive noise after an incorrect response or once during every 10 additions in instances where no error was made. Further,

the participant was filmed and asked to view the live recording on a screen while performing the task. If the participant looked away, the aversive noise again sounded as a reminder. The participants were informed that the recording would be analyzed by two senior academics for body and facial composure (in actuality, no such analysis was undertaken). They were truthfully told that a £10 gift voucher would be awarded for the best performance on the task, and a leader board with the highest five scores achieved by the participants was displayed. The addition of elements of social evaluation, competition, punishment, and reward have been shown to enhance the provocativeness and physiological responsiveness to the task (Veldhuijzen van Zanten et al., 2004).

Physiological measurements

Cardiovascular measures. Beat-to-beat arterial blood pressure was recorded continuously during both the baseline and stress tasks using a Finometer (Finapres Medical Systems, Amsterdam, The Netherlands). A small cuff was placed around the middle finger of the dominant hand of each participant. From this output, continuous data were recorded via a Power1401 (Cambridge Electronic Design; CED) connected to a computer programmed in Spike2 version 6 (CED). Cardiovascular parameters were derived from the blood pressure waveform obtained from the recorded output, and this was then analyzed offline. Systolic and diastolic blood pressure were calculated from each blood pressure waveform recorded during the minute of assessment, and mean averaged for the periods of assessment. Systolic and diastolic blood pressure was then used to calculate mean arterial pressure (MAP). Indices of cardiodynamic activity were recorded continuously using the Vrije Universiteit Ambulatory Monitoring System (VU-AMS, Amsterdam, The Netherlands) (de Geus, Willemsen, Klaver, & van Doornen, 1995; Willemsen, De Geus, Klaver, Van Doornen, & Carroll, 1996). This system used six Ag/AgCl spot electrodes (Invisatrace, ConMed Corporation) to record electrocardiography and impedance cardiography, in line with published guidelines (Sherwood et al., 1990). It is important to note that the use of a spot electrode configuration is comparable to the more commonly used band electrode configuration (Boomsma, de Vries, & Orlebeke, 1989). Ten-second ensemble averages were calculated and used to determine the following measures: Heart rate (HR, bpm), pre-ejection period (ms), with root mean square of successive differences (r-MSSD, ms) calculated as a measure of heart rate variability (HRV) and cardiac output (as a product of stroke volume and HR) (CO; l/min).

Blood flow. Venous occlusion plethysmography, using a mercury-in-Silastic strain gauge, was utilized to measure FBF. A strain gauge was fitted around the widest part of the nondominant forearm. One congestion cuff was placed around the brachial region of the upper arm (SC12, Hokanson) and another at the wrist (TMC7, Hokanson). The strain gauge was connected to a plethysmograph (EC6, Hokanson), which produced a calibrated output voltage proportional to limb circumference with a frequency response of 0–25 Hz. The plethysmograph signal was digitized at 100 Hz with 16-bit resolution, via a Power1401 connected to a computer programmed in Spike2 version 6. The brachial cuffs were inflated for 5 s to above-venous pressure (40 mmHg), using a rapid cuff inflator (E20, Hokanson) attached to an automated air source (AG101, Hokanson). This procedure allows for arterial inflow, while preventing venous return. Following 15 s, the brachial cuff was inflated again. This was repeated three times per minute. Throughout the minute of assessment, the wrist cuff was manually

inflated by sphygmomanometer (S300, Hokanson) to suprasystolic blood pressure (> 200 mmHg), to prevent any blood flow into the hand. Calibration and blood flow analysis was undertaken offline using Spike2. Increases in limb circumferences associated with inflation of the cuffs were identified and, for each of these, the slope was measured between the upstroke of the first two pulses following cuff inflation. The slope was assessed using a least squares fit to the data to minimize the effects of outlying data points. Three measurements of blood flow (slopes in response to cuff inflation) occurred per minute, with these means averaged to give an estimate of blood flow per minute. FVR was calculated as MAP/BBF.

Data reduction and statistical analysis. For all cardiovascular and vascular variables, the average of the four measurements taken during rest was computed to obtain a baseline value. The 4 min of assessment during stress are referred to as Stress 1, Stress 2, Stress 3, and Stress 4. To examine the responses to stress, a series of five time (Baseline, Stress 1, Stress 2, Stress 3, Stress 4) repeated measures analyses of variance (ANOVA) were conducted on FBF, FVR, HR, TPR, and MAP. Greenhouse-Geisser correction was applied for all ANOVAs (Vasey & Thayer, 1987). Eta squared (η^2) was used as a measure of effect size. Where appropriate, Newman-Keuls post hoc comparisons are reported. Reactivity scores for all cardiovascular and vascular measures were calculated as the difference between the average stress value (calculated as the average of Stress 1, Stress 2, Stress 3, and Stress 4) minus the baseline value. Subsequently, Pearson correlations were computed to examine the associations between the responses at rest, during stress, and the stress-induced differences between the cardiovascular and vascular variables assessed. Occasional missing data are reflected in the reported degrees of freedom. Correction of TPR for body surface area was undertaken using Mosteller's formula (Mosteller, 1987).

Study 2

Participants. Twenty-three white Caucasian male university students were recruited (mean age \pm SD = 19.5 \pm 0.9 years, mean BMI \pm SD = 24.6 \pm 2.8 kg/m²). These participants were separate from those tested in Study 1. The participants met the same inclusion and adherence criteria as described in Study 1. The study was approved by the local research ethics committee, and all participants gave written informed consent.

Procedure. All sessions started between 2–6 pm and were performed in a temperature-controlled environment. The procedures were the same of those of Study 1 with the exception of a longer 16-min stress task, which was chosen because TPR changes have been reported in response to a longer stress task (Ring, Burns, & Carroll, 2002). Assessments were taken during minutes 1, 3, 5, and 7 of the first part of the task, and minutes 9, 11, 13, and 15 of the second part of the task, such that blood flow was recorded, and impedance cardiography and blood pressure were analyzed for these minutes.

Mental stress task. As with Study 1, the mental stress task was the PASAT. However, this version of the task lasted 16 min, and was divided in two 8-min tasks. For each 8-min block, the numbers were delivered in four consecutive 2-min blocks, the numbers presented every 3.2 s, then 2.8 s, 2.4 s, and then 2.0 s for the first

task, with numbers presented every 2.4 s, then 2.0 s, 1.6 s, and then finally 1.2 s for the latter of the two tasks. A 1-min rest period separated the two tasks.

Physiological measurements. Forearm blood flow, blood pressure, and cardiovascular measures were taken as described in Study 1.

Data reduction and statistical analysis. For all cardiovascular and vascular variables, the average of the four measurements taken during rest was derived to obtain a baseline value. Four measurements were taken during each part of the stress tasks, with the 4 min of assessment in the first stress task referred to as Stress 1–4 (as in Study 1). The second part of the stress task took an additional 4 min of assessment during minute 9 (Stress 5), minute 11 (Stress 6), minute 13 (Stress 7), and minute 15 (Stress 8). The same statistical analyses were conducted in this study, with the exception that the time factor of the analyses involved 9 levels instead of 5, to reflect the longer stress task. Correction of TPR for body surface area was undertaken using Mosteller's formula (Mosteller, 1987).

Results

Study 1

Overall responses to stress. Figure 1 illustrates the vascular and cardiovascular responses to the 8-min mental stress task. ANOVAs revealed significant time effects for FBF, $F(4,23) = 20.2$, $p < .001$, $\eta^2 = .44$; FVR, $F(4,23) = 7.03$, $p < .001$, $\eta^2 = .31$; HR, $F(4,23) = 26.31$, $p < .001$, $\eta^2 = .70$; and MAP, $F(4,25) = 27.6$, $p < .001$, $\eta^2 = .76$. No time effects were observed for TPR, $F(4,20) = .96$, $p > .05$, $\eta^2 = .02$.¹ As highlighted in Figure 1, post hoc analyses showed that FBF, HR, and MAP all increased in response to stress, whereas FVR decreased in response to mental stress. There was no change in TPR in response to mental stress.

Correlation analysis. Correlation analyses were undertaken to examine the extent to which the baseline, responses to stress, or stress-induced changes in vascular and cardiovascular responses to stress were associated. These correlations are presented in Table 1. Baseline FBF was negatively correlated with baseline FVR ($p < .001$). Baseline MAP was also negatively correlated with baseline FVR ($p < .05$). TPR was not correlated with either baseline FBF or FVR ($p > .05$), or baseline HR or MAP ($p > .05$) but was correlated with baseline CO ($p < .001$). As with the baseline values, FBF during stress was negatively correlated with stress FVR ($p < .001$). Stress MAP was also correlated with stress FVR ($p < .05$). Again, stress TPR was not correlated with either stress FBF or FVR ($p > .05$) nor with HR or MAP during stress ($p > .05$). Stress TPR was correlated with stress CO ($p < .001$). Stress-induced changes in FBF were associated with stress-induced changes in HR ($p < .05$) and MAP ($p < .05$). Again, stress-induced changes in TPR were not correlated with either stress-induced changes in FBF, FVR ($p > .05$), nor stress-induced changes in HR or MAP ($p > .05$). Stress-induced changes in TPR were correlated with stress-induced changes in CO ($p < .001$).

1. Correction for BMI and body surface area did not alter the time effects observed for TPR.

Study 2

Overall responses to stress. Figure 2 displays the vascular and cardiovascular responses to the 16-min mental stress task. ANOVAs revealed significant time effects for FBF, $F(8,12) = 12.42$, $p < .001$, $\eta^2 = .40$; FVR, $F(8,12) = 2.74$, $p < .001$, $\eta^2 = .29$; TPR, $F(8,10) = 2.78$, $p < .05$, $\eta^2 = .14$; HR, $F(8,12) = 41.54$, $p < .001$, $\eta^2 = .69$; and MA, $F(8,15) = 10.95$, $p < .05$, $\eta^2 = .14$. Post hoc analyses identified increases in FBF, HR, MAP, and a decrease in FVR in response to stress, which remained different to baseline throughout (see Figure 2). TPR decreased during the first 8 min of the stress task, before increasing during the second 8-min period (see Figure 2).

Correlation analysis. Correlation analyses were undertaken to examine whether the baseline, responses to stress, or stress-induced changes in vascular and cardiovascular responses to stress were associated. These correlations are in Table 2. Baseline FBF was negatively correlated with baseline FVR ($p < .001$). TPR was not correlated with either baseline FBF, FVR, HR ($p > .05$) but was associated with MAP ($p < .05$) and CO ($p < .001$). As with the baseline values, FBF was negatively correlated with stress FVR ($p < .001$). As with Study 1, stress TPR was not correlated with either stress FBF, FVR ($p > .05$), neither HR nor MAP during stress ($p > .05$). Stress TPR was correlated with stress CO ($p < .001$). Stress-induced changes in TPR were not correlated with stress-induced changes in FBF or FVR ($p > .05$). However, stress-induced increases in TPR were correlated with stress-induced changes in HR ($p < .05$), MAP ($p < .05$), and CO ($p < .001$).

Discussion

This study examined the associations among various assessments of cardiovascular responses to mental stress. During the 8-min task (Study 1), increases in HR, MAP, FBF, and decreases in FVR were seen in response to stress. During the longer 16-min task (Study 2), similar responses to mental stress were found, even though the responses tended to diminish as the task progressed. Gradual increases in TPR were seen only during the longer stress task. No associations were found between FBF and TPR regardless of task length at rest, during stress, or when assessed in terms of stress-induced changes. In sum, despite the different responses seen to the stress task, it appears that there are no links between the vascular responses when assessing whole body vascular responses (TPR) and the vascular responses of a limb (FBF).

This study demonstrated that the TPR responses at rest, during stress, or as a measure of stress-induced difference were not associated to the FBF or FVR responses at any of these time points. Indeed, these results were displayed regardless of task length, and may be due to differences in the method of assessment or the mechanisms that underlie these responses. TPR is a measure of constriction of the vasculature, which is derived from cardiac output and MAP and reflects the overall constriction in the body, whereas FBF is a direct measure of dilation in one particular limb. The TPR responses to stress have been reported to be more variable and dependent on other factors (e.g., time; Ring, Burns, & Carroll, 2002), and may even be due to other factors associated with calculation of this measurement, such as changes in cardiac output and

2. As with Study 1, correction for BMI and body surface area did not alter the time effects observed for TPR.

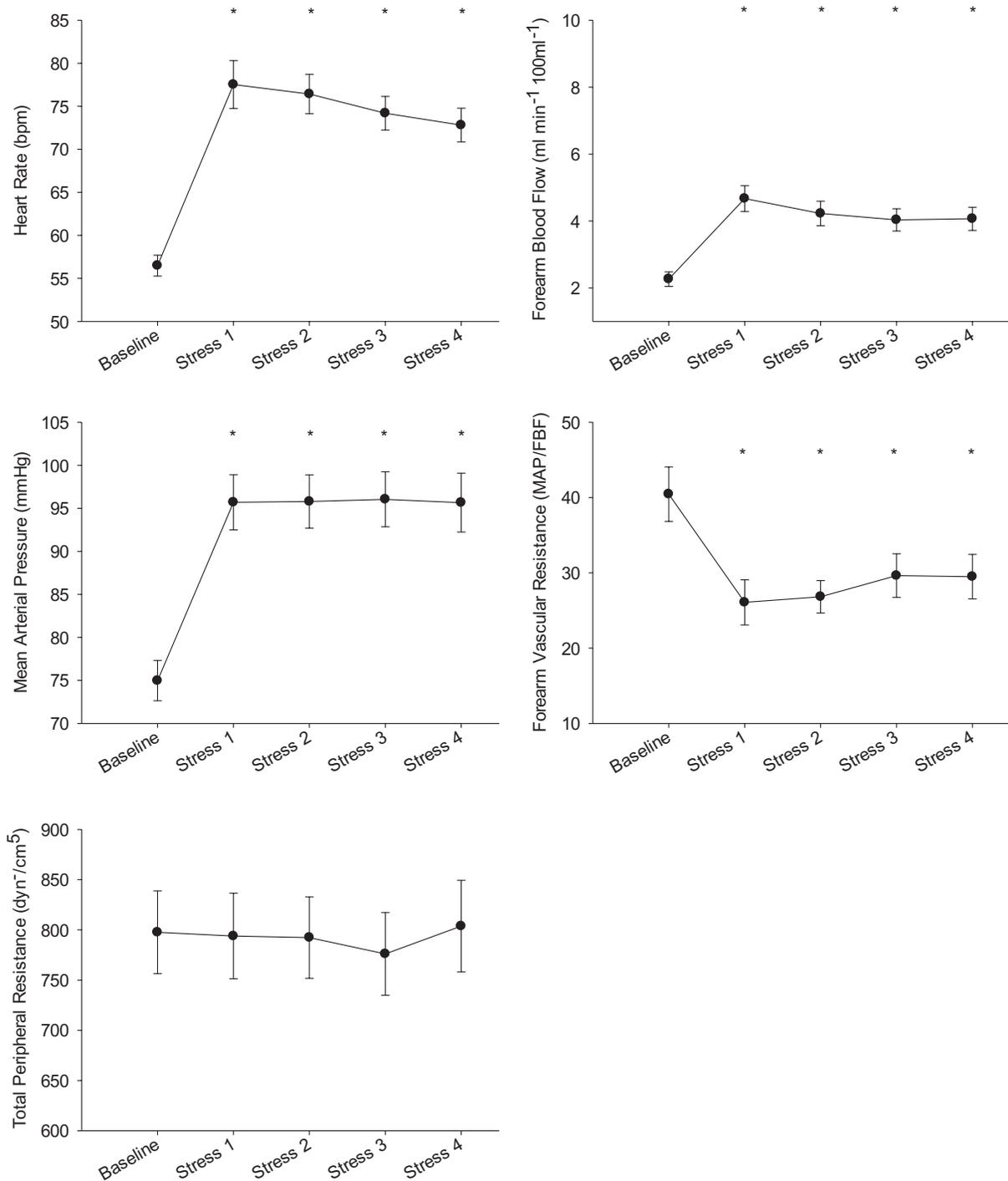


Figure 1. Mean \pm SE responses to stress for heart rate, mean arterial pressure, total peripheral resistance, forearm blood flow, and forearm vascular resistance. *significantly different to baseline ($p < .05$).

blood pressure in response to stress (Kelsey, Ornduff, & Alpert, 2007). We also found this to be the case in that the TPR response to stress was not as consistent as the other limb-related variables assessed.

However, it has been shown that differences in limb blood flow as a result of stress do not always reflect the whole body vasodilatory changes, or even responses between different anatomical locations. For example, in response to mental stress, increases in calf

and forearm blood flow are evident when measured by plethysmography (Carter, Kupiers, & Ray, 2005), but correlations between calf and forearm blood flow were not seen. Further correlations between forearm vascular resistance or conductance and the vascular resistance in calf are not demonstrated (Carter, Kupiers, & Ray, 2005). Differences in the forearm and calf responses to mental stress have been observed (Carter, Kupiers, & Ray, 2005; Hjerdahl et al., 1989; Rusch, Shepherd, Webb, & Vanhoutte, 1981), with

Table 1. Study 1 Pearson Correlation Coefficients Between Vascular Measures at Rest, During Stress, and as Stress-Induced Changes

	Baseline FBF	Baseline FVR	Baseline HR	Baseline MAP	Baseline CO
Baseline FBF	–	–.81**	.03	–.14	–.09
Baseline FVR	–	–	–.02	.42*	.20
Baseline TPR	.17	–.11	–.22	–.06	–.82**
	Stress FBF	Stress FVR	Stress HR	Stress MAP	Stress CO
Stress FBF	–	–.89**	.36	–.12	.09
Stress FVR	–	–	–.17	.40*	.05
Stress TPR	–.04	.10	–.10	–.07	–.79**
	Δ FBF	Δ FVR	Δ HR	Δ MAP	Δ CO
Δ FBF	–	–.37	.52**	.40*	.35
Δ FVR	–	–	–.19	–.05	–.17
Δ TPR	–.06	.05	.23	.19	–.63**

Note. Stress-induced changes calculated as Δ = baseline – stress.

* $p < .05$. ** $p < .001$.

stress-induced increases in forearm blood flow consistently seen whereas the results for calf blood flow are equivocal (Kuipers, Sauder, Carter, & Ray, 2008). Myogenic differences (i.e., differences in blood pressure due to posture) have been put forward as a potential reason for these stress-induced differences between limbs (Imadojemu et al., 2001); however, given the supine position observed in this study, this is unlikely to be a reason for the differences between FBF and TPR responses to stress. Importantly, assessment of FBF focuses on a small area of the vasculature, which is in contrast to TPR assessment, which gives an indication of vascular function over a larger area. Assessments of the relationship between the microvasculature and macrovasculature have shown inconsistent findings, with some showing associations between micro- and macrovascular function in rheumatoid arthritis patients (Foster, Carruthers, Lip, & Blann, 2010) and others showing no association (Arosio et al., 2007; Sandoo, Carroll, Metsios, Kitas, & Veldhuijzen van Zanten, 2011), highlighting differences in larger and smaller vessel function. Likewise, increases in renal vasoconstriction in response to mental stress have been demonstrated (Hayashi, Someya, Endo, Miura, & Fukuba, 2006), insinuating how different vascular responses to stress can occur depending on the vascular beds. Therefore, different organs and vascular beds might respond differently to stress.

It may be surprising that there are no associations between the responses of FBF and TPR at rest, to stress, and as a stress-induced change given the similarity between the mechanisms that are responsible for vasodilation. Vasodilation of the vasculature can occur through NO, where endothelial cells produce NO via endothelial nitric oxide synthase (eNOS), which is responsible for controlling vascular tone (Sandoo, Veldhuijzen van Zanten, Metsios, Carroll, & Kitas, 2010). Increased strain on the vessel wall as a result of mental stress causes an increase in shear stress, which in turn leads to increases in eNOS production (Nishida, Harrison, & Navas, 1992). However, due to the methodological difficulties in directly measuring nitric oxide in the blood and urine due to its highly transient nature *in vivo* (Beckman, Beckman, Chen, Marshall, & Freeman, 1990; Wadley, Veldhuijzen van Zanten, & Aldred, 2012), the effect of NO in stress-induced vasodilation has been examined by administering NO inhibitors. Inhibition of NO has resulted in elevations in resting vascular resistance (TPR) (Simonsen, Rasmussen, Johansen, Hoilund-Carlsen, & Bie, 2010), as well as attenuated FBF responses to mental stress (Cardillo, Kilcoyne, Quyyumi, Cannon, & Panza, 1997; Dietz et al., 1994). It has even been suggested that NO might be the limiting factor in the vasodilatory response to stress (Sarabi & Lind, 2001).

However, other mechanisms have been postulated to be responsible for the TPR and FBF responses to stress, such as β -adrenergic mechanisms impacting upon both TPR and FBF responses to stress. For example, in addition to the altering TPR responses via increases in adrenaline resulting in increases in HR (McEwen, 2007), and subsequently CO and TPR (Turner, 1994), increases in β -activation have been shown to cause increases in vasodilation and decreases in TPR (Joyner & Dietz, 2003). Interestingly, blockade of β -adrenergic receptors in the forearm decreases the blood flow responses to stress (Halliwill et al., 1997; Lindqvist et al., 1997), providing support for the suggestion that β -adrenergic vasodilation caused by increases in adrenaline might play an important role in vasodilation. Infusion of adrenaline (which acts on β -adrenergic receptors) increases resting blood flow, with a stepwise increase in adrenaline infusion resulting in similar increases in FBF as those found during mental stress (Kjeldsen et al., 1993). Further positive associations between adrenaline and forearm blood flow responses to mental stress have been demonstrated (Liu et al., 2006). However, the levels of adrenaline during mental stress are lower than the exogenous levels of adrenaline that were necessary to raise the same FBF response in the absence of stress, suggesting that the adrenaline produced during stress may not singularly be able to produce the vasodilatory response seen during mental stress (Lindqvist, Kahan, Melcher, Bie, & Hjemdahl, 1996). It is worth noting that a reduction in dilation through β -adrenergic mechanisms has been demonstrated when NO production was inhibited (Dawes et al., 1997), highlighting the evidence for NO playing a more prominent role in stress-induced vasodilation.

A final mechanism that may be responsible for the differences in FBF and TPR responses to stress is α -adrenergic activation. It has been demonstrated that blocking of α -adrenergic receptors does not alter the FBF response to mental stress (Barcroft, Brod, Hejl, Hirsjarvi, & Kitchin, 1960; Blair et al., 1959; Blair, Glover, Greenfield, & Roddie, 1960; Halliwill et al., 1997), whereas TPR increases with α -adrenoreceptor activation (Joyner & Dietz, 2003). Therefore, it may be possible that the differences in TPR and FBF seen in this study might be as a result of changes in α -adrenoreceptor activation. The initial decrease of TPR can occur as a result of either increased β -adrenoreceptor activation or decreases in α -adrenoreceptor activation (Joyner & Dietz, 2003) with increases in TPR seen towards the end of the task considered partly as a result of increases in α -adrenergic activation (Joyner & Dietz, 2003). The blood pressure responses to stress remain stable, initially as a result of increases in cardiac output, which is then

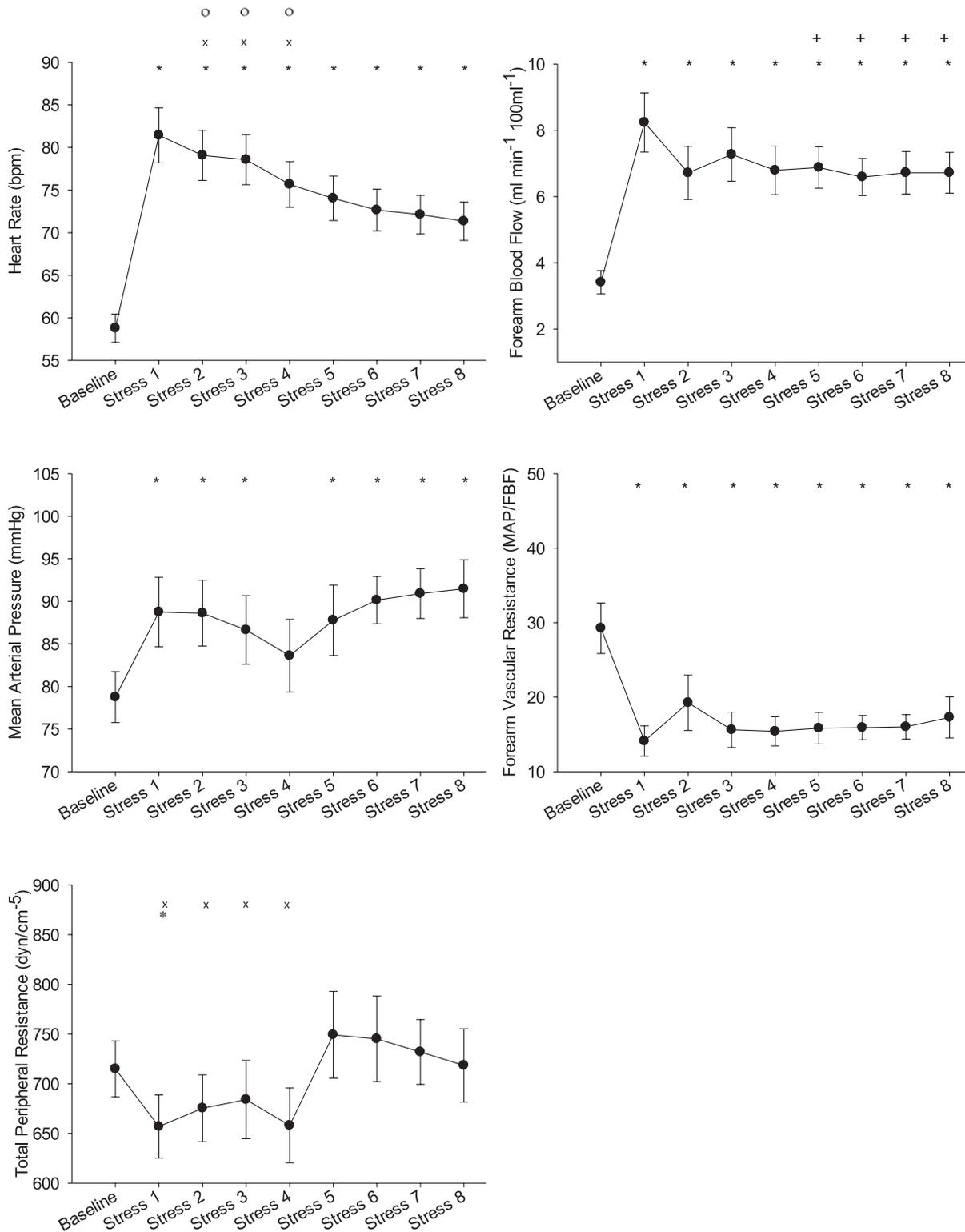


Figure 2. Mean \pm SE responses to stress for heart rate, mean arterial pressure, total peripheral resistance, forearm blood flow, and forearm vascular resistance. * indicates significantly different to baseline ($p < .05$); + indicates significantly different to stress 1; # indicates significantly different to stress 2; \times indicates significantly different to stresses 5–8; \circ indicates significantly different to stress 4 ($p < .05$).

Table 2. Study 2 Pearson Correlation Coefficients Between Vascular Measures at Rest, During Stress, and as Stress-Induced Changes

	Baseline FBF	Baseline FVR	Baseline HR	Baseline MAP	Baseline CO
Baseline FBF	–	–.83**	.06	.05	–.11
Baseline FVR	–.83**	–	–.00	.22	.05
Baseline TPR	.21	.02	–.20	.50*	–.68**
	Stress FBF	Stress FVR	Stress HR	Stress MAP	Stress CO
Stress FBF	–	–.83**	.13	.06	–.39
Stress FVR	–.83**	–	.14	.34	.21
Stress TPR	.20	.11	.24	.41	–.82**
	Δ FBF	Δ FVR	Δ HR	Δ MAP	Δ CO
Δ FBF	–	–.27	.24	.11	.27
Δ FVR	–.27	–	–.26	–.11	–.22
Δ TPR	.34	–.02	.34*	.44*	–.75**

Note. Stress-induced changes calculated as Δ = baseline – stress.

* $p < .05$. ** $p < .001$.

followed by increases in TPR as a result of α -adrenoreceptor activation (Ring, Burns, & Carroll, 2002). In line with this, studies of longer duration report increases in TPR seen towards the end of the task (Hamer & Steptoe, 2007; Ring, Burns, & Carroll, 2002), with constrictive influences on blood pressure and vascular resistance reported elsewhere with prolonged exposure to stress (Kelsey et al., 1999; Miller & Ditto, 1991) highlighting the role of task length in the TPR responses to mental stress.

One limitation of this study is that the studies were conducted at different times of the day and may be a reason for the differences in the TPR responses to stress. Few studies have examined circadian variation of TPR, finding lower TPR during night in comparison to daytime (Guo & Stein, 2003). However, given that blood pressure (which is used to calculate TPR) is higher in the morning (Guo & Stein, 2003), it could be assumed that there would be differences in TPR in the morning compared to the afternoon. Circadian variation has been demonstrated for FBF, with lower FBF demonstrated in the morning and attributed to lower α -adrenergic sympathetic activation (Panza, Epstein, & Quyyumi, 1991). However, the focus of the current study is not to examine and compare the circadian differences in the responses to stress, but rather to examine the relationships between FBF and TPR within each study. Therefore, as these factors ought to be equal and consistent, these differences in circadian variation should be irrelevant. However, the differences in the TPR responses to stress (e.g., reduced TPR in the first 8 min of the stress task in Study 2) might be due to differences in the delivery of the numbers in each task. The longer stress task begins with a slower number delivery than the shorter task and might be a reason for a reduced TPR response in the longer stress task (Study 2).

One criticism of using a longer mental stress task is that habituation to the task may occur. However, despite mild attenuation, HR and BP remained significantly elevated during the stress period above the baseline values reported. The continuous increase in task difficulty ensured that participants remained engaged and stressed throughout the task, which is in line with previous use of this task from our laboratory (Ring, Burns, & Carroll, 2002; Ring, Drayson et al., 2002; Veldhuijzen van Zanten, Carroll, & Ring, 2009; Veldhuijzen van Zanten, Thrall, Wasche, Carroll, & Ring, 2005). This version of the PASAT has been proven to consistently perturb cardiovascular activity throughout versions of the task of up to 32 min (Ring, Burns, & Carroll, 2002; Veldhuijzen van Zanten et al., 2005, 2009). Further, self-reported perceptions of the stress

task were not different despite the differences in task length, such that the task was perceived to be as difficult, stressful in nature, and engaging despite task length.

There are limitations and other considerations that should be acknowledged. Venous occlusion plethysmography is a technique that has been widely used and displays good reproducibility (Roberts, Tsao, & Breckenridge, 1986); however, this technique is very sensitive to movement artifacts. To counteract this, three assessments per minute of blood flow were taken, and movement artifacts deleted after careful inspection of the data, in order to calculate a more reliable average of blood flow for the minutes of assessment. Also, the nature of impedance cardiography involves an element of subjective scoring when determining the B point, dZ/dt_{max} , and X point of each impedance trace and, therefore, it is important that all the assessments and analysis are conducted by the same experimenter to minimize the amount of subjectivity involved.

Associations between peripheral and coronary endothelial function at rest have been demonstrated (Anderson et al., 1995; Takase et al., 2005, 1998) with the strongest association found when a similar stimulus was used (Takase et al., 2005). In addition, both exaggerated TPR responses to mental stress as well as impaired limb vasodilatory responses to mental stress have been associated with mental stress-induced ischemia in coronary artery disease (CAD) patients (Burg et al., 2009; Goldberg et al., 1996; Jain et al., 1998). However, in the current studies (Study 1 and 2), no associations between the local (FBF) and systemic (TPR) responses to mental stress were found, indicating that the vascular responses to mental stress may not be uniform across the whole of the vascular system. In view of this, questions remain about which peripheral assessment is the best predictor. Indeed, to our knowledge, no mental stress-induced ischemia studies have assessed both TPR and FBF; therefore, it is currently not known which measurement is the most appropriate peripheral predictor of myocardial responses to mental stress. In addition, to our knowledge, little is known about the associations between resting endothelial function (assessed with flow-mediated dilatation) and the vascular responses to mental stress. Poorer endothelial function at rest has been associated with a greater increase in TPR in healthy participants (Sherwood et al., 1999), and with mental stress-induced ischemia in CAD patients (Peix et al., 2006). Therefore, exploration of the associations between endothelial function at rest and the vascular responses to stress warrants further investigation.

In conclusion, this study examined the relationships between the cardiovascular responses to mental stress in a short (8-min) and longer (16-min) mental stress task. It appears that associations exist between some of the β -adrenergic driven responses to mental stress; however, these associations appear to be dependent on task length. The differences in the nature of the assessments may also

play a contributory role towards the lack of associations seen. As TPR was not associated with any of the limb vasodilatory responses to stress (e.g., FBF, FVR), it remains to be determined which peripheral assessment may be the best predictor of the myocardial responses to stress.

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