International Journal of Psychophysiology 93 (2014) 340–348

Contents lists available at ScienceDirect

International Journal of Psychophysiology

journal homepage: www.elsevier.com/locate/ijpsycho

Vaccine-induced inflammation attenuates the vascular responses to mental stress

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ARTICLE INFO

Article history:
Received 31 January 2014
Received in revised form 27 June 2014
Accepted 28 June 2014
Available online 3 July 2014

Keywords:
Inflammation
Interleukin-6
Tumor necrosis factor-alpha
Vascular blood flow
Mental stress

ABSTRACT

Inflammation is associated with poorer vascular function, with evidence to suggest that inflammation can also impair the vascular responses to mental stress. This study examined the effects of vaccine-induced inflammation on vascular responses to mental stress in healthy participants. Eighteen male participants completed two stress sessions: an inflammation condition having received a typhoid vaccination and a control (non-inflamed) condition. Tumor necrosis factor-alpha and interleukin-6 (p's < .001) increased following vaccination, confirming modest increases in inflammation. Mental stress increased blood flow, blood pressure, heart rate, and cardiac output in both conditions (all p's < .001), but the blood flow response to stress was attenuated having received the vaccination compared to the control condition (p's < .05). These results further implicate the interaction between inflammation and the vasculature as a mechanism through which stress may trigger myocardial infarction.

1. Introduction

There is converging evidence that acute mental stress can precipitate myocardial infarction (MI). For example, survivors of MI have identified emotional stress as a trigger for their MI (Strike and Steptoe, 2005) and epidemiological studies have reported elevated MI incidence following stressful events, such as earthquakes, onset of wars, and even following international soccer matches (for review see Strike and Steptoe (2005)). Even though the underlying mechanisms are not yet fully understood, studies suggest that the effects of inflammation on the vasculature may play a role in stress-induced MI (Paine et al., 2012). Serological markers of inflammation and vasoconstriction were elevated in patients admitted for MI during the Football World Cup, compared to those admitted during a control period (Wilbert-Lampen et al., 2010). Similarly, laboratory studies have revealed that cardiac patients exhibiting mental stress-induced ischaemia (a laboratory proxy for MI) have higher basal inflammatory levels (Shah et al., 2006), and poorer vascular responses to mental stress (Burg et al., 2009; Goldberg et al., 1996; Jain et al., 1998).

Associations between inflammation and endothelial function have been studied in vivo. Endothelial function is assessed by measuring the vasodilatory response to a standardised stimulus (Sandoo et al., 2010), such as increases in blood flow. A reduced ability to vasodilate to such stimuli, i.e., endothelial dysfunction (Sandoo et al., 2010), is an indicator of atherosclerosis (Lerman and Zeiher, 2005), which is evident in patients with increased levels of inflammation (e.g., coronary artery disease (Fichtlscherer et al., 2004) and rheumatoid arthritis (Vaudo et al., 2004)). Transient endothelial dysfunction can also manifest in apparently healthy populations where inflammation has been induced by direct infusion of inflammatory cytokines (Bhagat and Vallance, 1997) or vaccination (Clapp et al., 2004; Hingorani et al., 2000; Kharbanda, 2002). Interestingly, in people without proven endothelial dysfunction, mental stress reliably causes vasodilation, indexed by increased blood flow (Joyner and Casey, 2009). However, stress-induced vasodilation is attenuated in those at risk for CVD (Hamer et al., 2007) and in those with heart failure (Middlekauff et al., 1997; Santos et al., 2005). Thus, it is important to examine the role of inflammation on stress-induced vasodilation, given that inflammation is evident in those with endothelial dysfunction and that endothelial dysfunction can lead to poorer vascular responses to stress (Sherwood et al., 1999).

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http://dx.doi.org/10.1016/j.ijpsycho.2014.06.016
0167-8760/

To our knowledge only two studies have examined the influence of inflammation on vascular responses to mental stress. Rheumatoid arthritis patients with high-grade systemic inflammation displayed increased vascular resistance in response to mental stress, which was not evident in patients with low-grade inflammation (Veldhuijzen van Zanten et al., 2008). A second study, involving a healthy population, observed that eccentric exercise-induced increases in IL-6 resulted in reduced calf, but not forearm, blood flow during mental stress (Paine et al., 2013a), further suggesting a possible role for inflammation in attenuating the vascular responses to mental stress.

However, one criticism of utilising an eccentric exercise protocol is that it typically yields increases in IL-6, but not other inflammatory markers (Febbraio and Pedersen, 2002; Steensberg et al., 2001, 2002). Other experimental paradigms are available, such as typhoid vaccination, which can induce elevations in other pro-inflammatory markers (e.g., TNF-α (Paine et al., 2013b)), which have been implicated in the development of atherosclerosis and subsequent cardiovascular disease (Anker and von Haehling, 2004; Blake and Ridker, 2002). Critically, the typhoid vaccination does not induce changes in mood, physical symptoms or sickness behaviour (Brydon et al., 2009; Paine et al., 2013b). However, this finding is not universal, with others demonstrating a relationship between mood and vaccination (Harrison et al., 2009a,b), as well as others demonstrating changes in mood as a result of vaccination administration (Strike et al., 2004; Wright et al., 2005). Therefore, the use of the typhoid vaccination allows us to use a vaccination paradigm whereby relatively substantial increases in inflammation are observed without altering these other psychological factors, allowing us to examine only the physiological alteration of blood flow in response to stress.

Therefore, the aim of the current study was to examine whether vaccination-induced inflammation, induced by the administration of the *Salmonella typhi* (typhoid) vaccination, influences the vascular responses to mental stress in healthy participants. It was hypothesised that inflammation would attenuate the vasodilatory response to mental stress.

2. Methods

2.1. Participants and study design

A total of 23 male university students were recruited, and randomised into one of two intervention groups: vaccination and saline. All participants completed two stress reactivity sessions scheduled at least 7 days apart — one in a 'control' condition, the other in an 'intervention' condition (vaccination or saline). In the intervention condition, participants completed a stress reactivity session having either received a typhoid vaccination or a saline injection. These were administered 6 h prior to the start of the stress reactivity session (Fig. 1). Eighteen male university students (mean age ± SD = 19.5 ± 0.9 years, mean body mass index (BMI) ± SD = 24.6 ± 2.8 kg/m^2) received the vaccination intervention (referred to as the vaccination group). The remaining five male students (mean age ± SD = 19.6 ± 1.34 years, mean BMI ± SD = 25.3 ± 4.6 kg/m^2) comprised a placebo (saline) group. A between-subject design rather than a counterbalanced within-subject design was chosen due to concerns regarding the length of time needed between receiving a vaccination and returning to a 'normalised' physiological state post-vaccination. For example, given that the peak antibody response to vaccination typically takes up to 28 days to occur and remain elevated for up to 20 weeks (Edwards et al., 2008; Rastogi et al., 1995), and that mental stress can also increase the antibody response to vaccination (Edwards et al., 2006, 2008), retesting participants within these time frames could lead to potential confounding. For this reason a between-subject design was administered.

None of the participants were suffering from an acute illness or infection, reported a history of inflammatory, cardiovascular or auto-immune disorders, had taken any medication in the last 4 weeks, or had received a typhoid vaccination in the last 12 months. Participants reported to the laboratory having refrained from vigorous exercise for at least 24 h, from alcohol for at least 12 h and food or caffeine in the 2 h prior to testing. The study was approved by the local research ethics committee and all participants gave written informed consent.

2.2. Procedures

2.2.1. Stress reactivity session

All testing was performed in a temperature controlled laboratory (18 °C). The stressor took place in the afternoon between 2:00 and 5:00 pm; the timing of the stressor was the same time for each participant for their two visits to the laboratory. Participants reported to the laboratory having refrained from vigorous exercise for at least 24 h, from alcohol for 12 h and food or caffeine in 2 h prior to testing. Upon arrival at the laboratory, the participant's height and weight were recorded, and they were instrumented for the cardiovascular assessment. The participant then assumed a supine position on a bed, where he remained throughout the session. An 18 gauge cannula (Insyte, Becton Dickinson) was inserted into an antecubital vein of each participant's dominant arm. After instrumentation, they rested for 20 min (baseline) and watched a nature documentary (*Life*; BBC). During minutes 13, 15, 17 and 19 blood flow was measured. Continuous recordings of impedance cardiography and blood pressure were taken, and minutes 13, 15, 17 and 19 were assessed. A resting blood sample was taken at the end of the baseline period. After practising the mental stress task, participants completed two 8 minute blocks of the mental stress task, with 1 minute rest in between. During minutes 1, 3, 5 and 7 of each block,

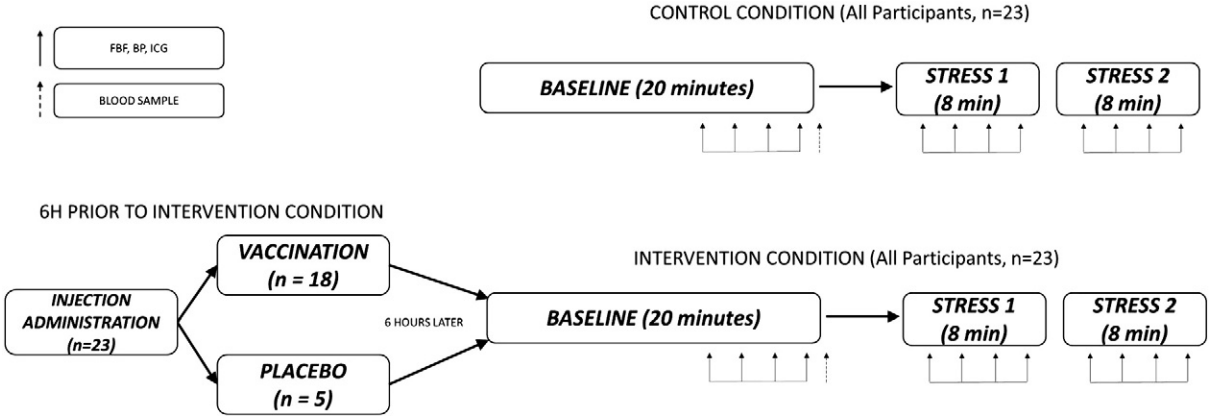

Fig. 1. Flow chart of the study protocol.

blood flow was measured, and impedance cardiography and blood pressure were analysed for those minutes. The procedures conducted during the stress reactivity session were identical in both conditions (e.g., control and intervention), and both groups (vaccination and placebo).

2.2.2. Vaccination/saline administration

Participants received either a 0.5 ml *S. typhi* capsular polysaccharide vaccine (0.025 mg in 0.5 ml, Typhim Vi, Sanofi Pasteur, UK) or a 0.5 ml saline injection via intra-muscular injection into the deltoid muscle of the non-dominant arm, by a registered nurse. All participants, who were blinded to the intervention group allocation, remained in the laboratory for 20 min post-injection for observation and then returned to the laboratory 6 h later to complete the stress session. At the start of this session, they rated the extent of any potential physical symptoms related to receiving the earlier vaccination or saline injection using a 6-point Likert scale (0 = not at all, 5 = severe), as described previously (Paine et al., 2013b). General reactions such as joint pain and pain at the site of the injection were also assessed using a 10-point scale (1 = very mild, 10 = very severe). The injection was administered 6 h before the start of the stress task to coincide with the IL-6 response to the vaccine (Padfield et al., 2010; Paine et al., 2013b).

2.2.3. Mental stress task

The paced auditory serial addition task (PASAT) was used as the mental stress task. The PASAT has been widely demonstrated to repeatedly produce a stressful stimulus, even when the task is repeated several times on separate days (Veldhuijzen van Zanten et al., 2005; Willemsen et al., 1998). During the PASAT, participants were presented with a series of single digit numbers, and were required to add each new number to the number presented previously (Gronwall, 1977; Ring et al., 2002). The task lasted a total of 16 min, split into two eight minute tasks, separated by one minute rest, which aimed to maintain task engagement and interest throughout the task. The numbers were delivered in four 2-minute blocks, with the numbers respectively presented every 3.2 s, 2.8 s, 2.4 s and 2.0 s for the first task, and every 2.4 s, 2.0 s, 1.6 s and 1.2 s for the second task, which resulted in a progressive increase in task difficulty. The experimenter, who sat 1 m adjacent to the participants, checked their responses against the correct answers. To enhance the stressfulness of the performance several features were added that had shown to enhance stress responses in previous studies (Veldhuijzen van Zanten et al., 2004). Participants heard a loud aversive noise once in each block of 10 numbers, with the noise presented after the participants' first incorrect response, or if they had not made an error, the noise was delivered at the end of the 10 number block. Participants were filmed with a video camera and were asked to look at their faces displayed on a television screen whilst performing the task, which they were told was to be analysed. If participants looked away from the screen, they heard the aversive noise and were reminded to continue to watch the screen. Each participant was told that a £10 gift voucher would be awarded for the highest score recorded on the task. A leader board with the highest five scores achieved by all the participants was displayed, so that each participant could compare themselves to the other participants' scores. An additional £10 voucher was awarded to the participant who recorded the greatest improvement in score between the two sessions. However, the latter of these rewards was only revealed before the start of the test in the intervention condition, to eliminate the risk of a poor performance in the control condition. These elements of social evaluation, competition, punishment and reward have been shown to enhance the provocativeness of the task (Veldhuijzen van Zanten et al., 2004). At the end of the stress task, participants rated the task in terms of perceived performance, arousal, stressfulness and engagement using a 7-point Likert scale (0 = not at all and 6 = extremely).

2.3. Physiological measurements

2.3.1. Cardiovascular measures

Beat-to-beat arterial blood pressure was recorded continuously during both baseline and stress tasks using a Finometer (Finapres Medical Systems; Amsterdam, The Netherlands). From this output, continuous data were recorded (via a Power1401 connected to a computer programmed in Spike2 version 6). Mean systolic (SBP) and diastolic (DBP) blood pressure were derived from the blood pressure waveform and used to calculate mean arterial pressure (MAP) during the periods of assessment. Indices of cardio-dynamic activity were recorded continuously by impedance cardiography using the Vrije Universiteit Ambulatory Monitoring System (VU-AMS, Amsterdam, The Netherlands) (Willemsen et al., 1996), in line with published guidelines (Sherwood et al., 1990). Ten second ensemble averages were calculated and used to determine heart rate (HR, bpm), pre-ejection period (PEP; ms), root mean of successive squared differences as a measure of heart rate variability (r-MSSD, ms) and cardiac output (as a product of stroke volume and HR) (CO; l/min).

2.3.2. Blood flow

Venous occlusion plethysmography, using a mercury-in-silastic-strain gauge, was utilised to measure forearm blood flow (FBF), with a full description found elsewhere (Paine et al., 2013a). One strain gauge (connected to a plethysmograph (EC6, Hokanson)) was fitted around the widest part of the non-dominant forearm, producing a calibrated output voltage proportional to limb circumference. Congestion cuffs were placed around the brachial region of the upper arm (SC10, Hokanson) and the wrist (TMC7, Hokanson). The brachial cuff was inflated for 5 s to above venous pressure (40 mm Hg), using a rapid cuff inflator (E20, Hokanson) attached to an automated air source (AG101, Hokanson). After 15 s, the brachial cuff was inflated again. This was repeated three times per minute, to give three measurements of blood flow which were averaged to give a mean estimate of blood flow per minute. Throughout the minute of assessment, the wrist cuff was manually inflated by a Sphygmomanometer (S300, Hokanson) to supra-systolic blood pressure (>200 mm Hg). Calibration and blood flow analysis was undertaken offline using Spike2 (CED), as has been described elsewhere (Paine et al., 2013a).

2.3.3. Blood sampling

Blood samples were taken at the end of the baseline period and were collected into three 6 ml and one 2 ml vacutainers containing potassium ethylene diaminetetraacetic acid (K3EDTA) (Becton-Dickinson, UK). The 6 ml samples were stored on ice until centrifugation (1500 *g* for 10 min at 4 °C) and plasma was stored at −80 °C for later assessment of IL-6 and TNF-α. The 2 ml samples were placed on a roller until analysis for full blood cell count (Coulter Analyser, Beckman Coulter, Inc.).

2.3.4. Assays

Plasma IL-6 and TNF-α were measured in duplicate using high-sensitivity ELISA (Quantikine HS Human IL-6 ELISA and Quantikine HS Human TNF-α ELISA, both R&D Systems, UK) in accordance with the manufacturer's instructions. The reported limit of detection of the assays was 0.039 and 0.106 pg/ml respectively, with recorded intra-assay and inter-assay variations both <10% for IL-6 and TNF-α.

2.4. Data analysis

For all cardiovascular variables, the four measurements taken during baseline, and the four measurements taken during each 8 minute block of the stress task were averaged to establish a Baseline, Stress 1 (1st 8 min of stress) and Stress 2 (2nd 8 min of stress) value, respectively. For the participants receiving the vaccination intervention, analyses of variance (ANOVAs) were conducted to examine differences between baseline values in the control and intervention conditions. To assess the

Table 1
Mean (SD) inflammatory markers for the vaccination group only (N = 18) during the control condition and the intervention condition.

Variable	Control condition	Intervention condition	
IL-6 (pg/ml)	0.90 (1.03)	2.97 (1.58)	$F(1,15) = 16.23$, $p = .001$, $\eta^2 = .52$
TNF-α (pg/ml)	4.93 (4.12)	16.94 (8.11)	$F(1,15) = 27.90$, $p < .001$, $\eta^2 = .65$
Granulocytes (10^9/l)	3.92 (1.16)	6.89 (1.23)	$F(1,17) = 106.53$, $p < .001$, $\eta^2 = .86$

effects of inflammation on the responses to mental stress, two Condition (Control, Intervention) by three Time (Baseline, Stress 1, Stress 2) repeated measures ANOVAs were conducted on all physiological measurements for the vaccination and placebo groups separately. Post-hoc analyses were undertaken through Newman–Keuls post-hoc comparisons. Where appropriate Greenhouse–Geisser correction was applied (Vasey and Thayer, 1987) and for all ANOVAs, eta squared (η^2) was used as a measure of effect size. Reactivity scores for all cardiovascular and vascular measures were calculated as the difference between the average stress value and the baseline value. Subsequently, Pearson correlations were conducted to examine the associations between resting inflammatory markers (IL-6, TNF-α, WBC) and vascular reactivity. These analyses were conducted for the control and inflammation conditions separately. Occasional missing data are reflected in the reported degrees of freedom.

3. Results

3.1. Manipulation checks

3.1.1. Inflammatory response to vaccination (S. typhi polysaccharide vaccine)

Table 1 displays the measures of inflammation at baseline in the intervention and control conditions in those who received the vaccination. A series of 2 Condition (Control, Intervention) ANOVAs confirmed that the vaccination induced an inflammatory response in those who received the vaccination intervention: TNF-α, IL-6, and granulocytes were all elevated during the vaccination intervention condition compared to the control condition (Table 1). No increases in IL-6, TNF-α or granulocytes would be expected in response to saline administration, given data from our laboratory which has demonstrated no significant increases in IL-6 after saline administration, which was in contrast to the increases in these inflammatory markers following *S. typhi* vaccination (Paine et al., 2013b). Finally, in comparison to those who received placebo (saline) injection, participants subjected to the vaccination intervention reported greater muscle ache (vaccination: 2.22 ± 1.35, placebo: 0.2 ± 0.48; $F(1,21) = 13.04$, $p = .002$, $\eta^2 = .38$), and localised pain at the site of the injection (vaccination: 3.17 ± 1.92, placebo: 0.2 ± 0.48; $F(1,21) = 13.18$, $p = .002$, $\eta^2 = .39$) at the start of the stress testing session, 6 h following the vaccination.

3.2. Task impact ratings

Table 2 presents the task self-report ratings for the control and intervention conditions for both the placebo and vaccination groups. There were no condition differences in perceived levels of stress, arousal, engagement or performance of the task, for either intervention group.

3.3. Physiological responses to mental stress in the vaccination group

3.3.1. Cardiovascular responses

Fig. 2 depicts the HR, CO, PEP, r-MSSD, SBP and DBP at rest and in response to mental stress in the control and intervention conditions. Two Condition (Control, Intervention) by three Time (Baseline, Stress 1, Stress 2) repeated measures ANOVA yielded main effects for time for HR ($F(2,13) = 28.21$, $p < .001$, $\eta^2 = .81$), CO ($F(2,13) = 24.50$, $p = .001$, $\eta^2 = .79$), PEP ($F(2,13) = 4.85$, $p = .006$, $\eta^2 = .43$), r-MSSD ($F(2,13) = 8.76$, $p = .012$, $\eta^2 = .57$), SBP ($F(2,13) = 22.22$, $p < .001$, $\eta^2 = .77$) and DBP ($F(2,13) = 15.02$, $p < .001$, $\eta^2 = .70$). Newman–Keuls post-hoc analyses revealed that HR, CO, SBP, and DBP increased in response to stress, whereas PEP and r-MSSD decreased in response to stress. No Condition effects or Condition by Time interaction effects were seen for CO, HR, PEP, r-MSSD, SBP and DBP (all *p*'s > .05).

3.3.2. Vascular responses

The forearm blood flow responses to mental stress are illustrated in Fig. 3. A 2 Condition by 3 Time (Baseline, Stress 1, Stress 2) ANOVA revealed a significant Condition by Time interaction effect ($F(2,16) = 3.43$, $p = .036$, $\eta^2 = .30$), whereby the stress-induced vasodilation was attenuated in the intervention condition, when inflammatory levels were elevated due to vaccination.

3.4. Physiological responses to mental stress in the placebo group

The HR, CO, PEP, r-MSSD, SBP and DBP responses to stress are depicted in Fig. 4. A series of 2 Condition (Control, Intervention) by 3 Time (Baseline, Stress 1, Stress 2) repeated measures ANOVAs yielded time effects for HR ($F(2,2) = 45.12$, $p < .001$, $\eta^2 = .94$). Time effects were also evident for FBF (Fig. 3) in response to stress ($F(2,3) = 12.54$, $p = .011$, $\eta^2 = .76$). No other Condition, Time or Condition by Time interaction effects were observed (*p*'s > .05), indicating that stress responses were similar during repeated stress testing.

Table 2
Mean (SD) task ratings of the mental stress task for the vaccination group (top; N = 18) and the placebo group (bottom; N = 5).

	Control condition	Intervention condition	
Vaccination group			
Perceived stress	4.56 (0.71)	4.44 (0.71)	$F(1,17) = .49$, $p = .50$, $\eta^2 = .03$
Perceived arousal	3.22 (1.35)	3.39 (1.50)	$F(1,17) = 1.00$, $p = .33$, $\eta^2 = .06$
Perceived performance	2.28 (0.96)	2.44 (1.04)	$F(1,17) = .55$, $p = .55$, $\eta^2 = .02$
Perceived engagement	3.78 (1.35)	3.67 (1.28)	$F(1,17) = .21$, $p = .65$, $\eta^2 = .01$
Placebo group			
Perceived stress	4.80 (0.84)	4.60 (0.55)	$F(1,4) = .29$, $p = .62$, $\eta^2 = .07$
Perceived arousal	2.60 (0.55)	2.60 (0.89)	$F(1,4) = 1.00$, $p = 1.00$, $\eta^2 = .00$
Perceived performance	2.40 (1.14)	3.00 (1.00)	$F(1,4) = 2.25$, $p = .21$, $\eta^2 = .36$
Perceived engagement	3.60 (1.14)	3.80 (1.64)	$F(1,4) = .17$, $p = .70$, $\eta^2 = .04$

Note: Ratings ranged from 0 (not at all) to 6 (extremely).

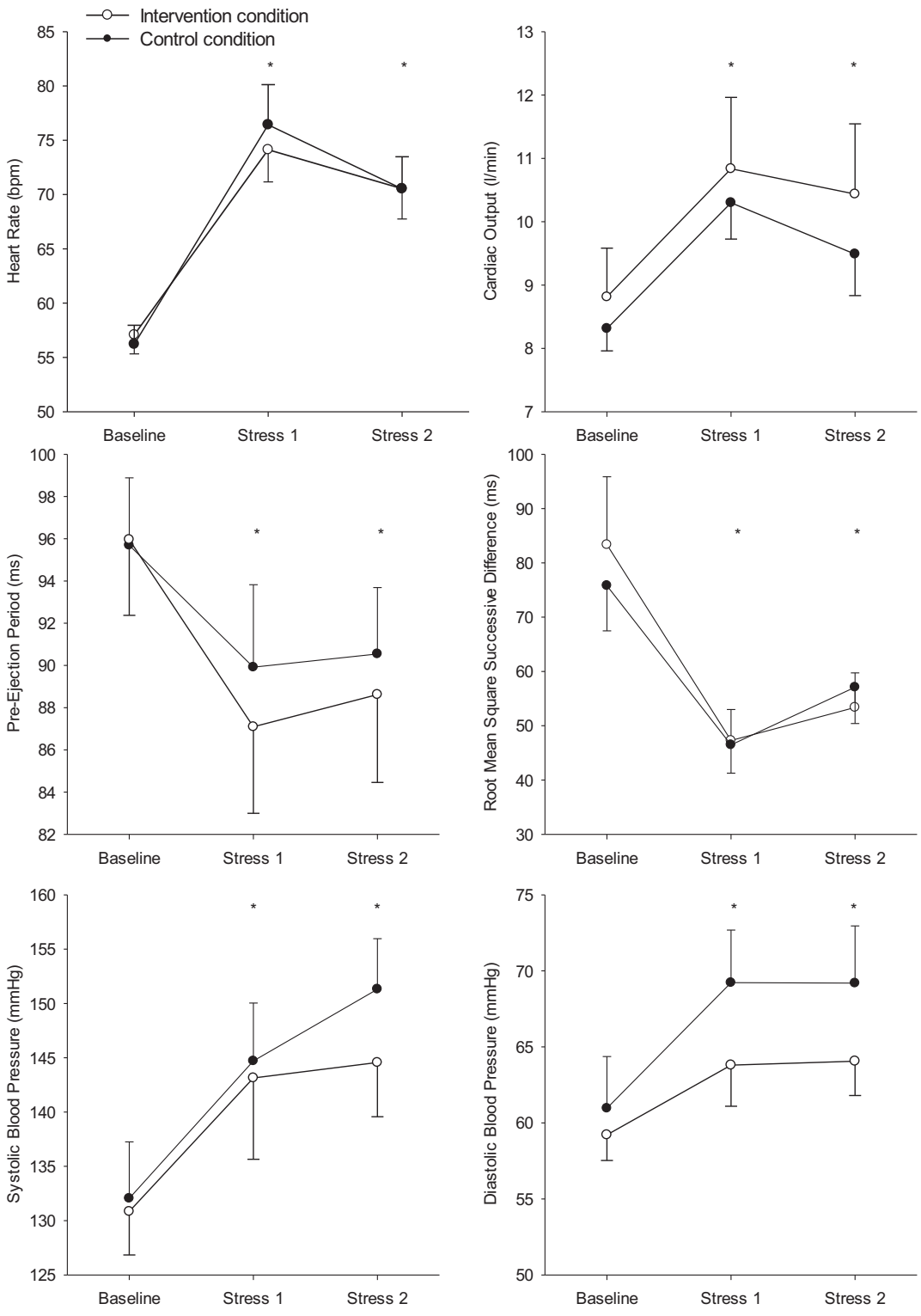

Fig. 2. Mean ± SE responses to mental stress for HR, CO, PEP, r-MSSD, SBP and DBP in the vaccination group (N = 18). Note: Time effects (for both conditions) are denoted with * indicating significantly different from baseline ($p < .05$).

4. Discussion

The aim of this study was to examine the impact of vaccine-induced inflammation on the vascular responses to mental stress. The *S. typhi* vaccination induced a systemic inflammatory response, evidenced by increased TNF-α, IL-6 and granulocytes. Elevated inflammation attenuated the vasodilatory response to mental stress. These findings are in line with previous findings where inflammation, induced through eccentric exercise, also induced an attenuation in the vascular response to mental stress (Paine et al., 2013a).

The vaccination model was chosen here to determine the influence of inflammation on the vasculature in a group without any manifest pathologies, therefore assessing the unique impact of inflammation. Even though the increases in IL-6 were modest, the levels are comparable to

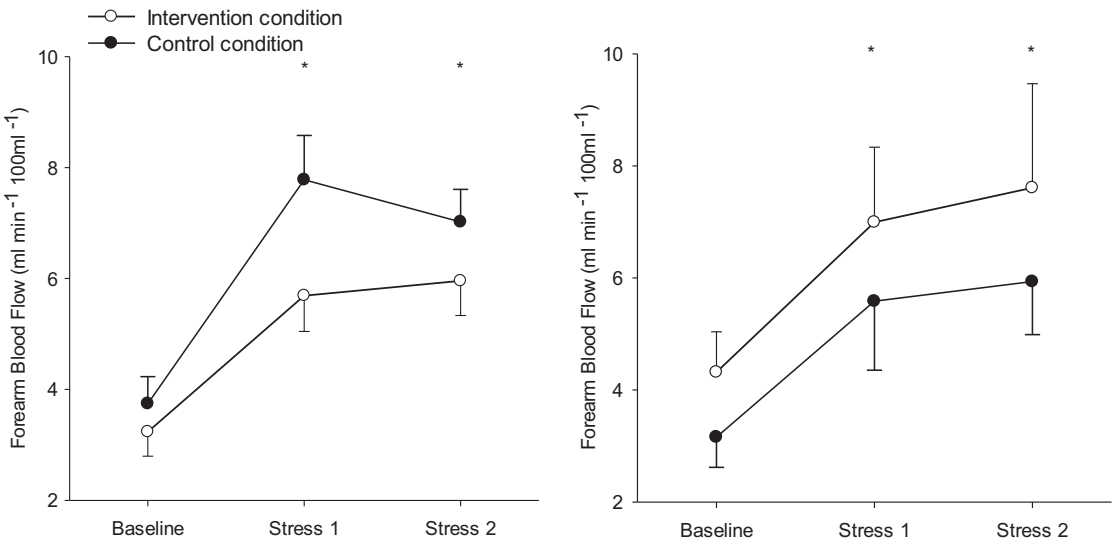

Fig. 3. Mean ± SE responses to mental stress for FBF in the vaccination group (left; N = 18) and the saline group (right; N = 5). Note: Time effects (for both conditions) are denoted with * indicating significantly different from baseline ($p < .05$).

those reported in patients with CAD (Kop et al., 2008) and in men who had suffered an MI (Ridker et al., 2000). In addition, the levels were similar to the inflammation induced through acute eccentric exercise task utilised in the only other study that has explored the effects of inflammation on the vascular responses to mental stress in healthy participants (Paine et al., 2013a). However, this particular inflammatory paradigm also yields increases in other pro-inflammatory markers (Paine et al., 2013b). Therefore, the levels of inflammation induced by the vaccination model used in this study are reflective of the inflammatory levels of individuals at risk for MI.

Stress-induced vasodilation has previously been shown to be dependent on nitric oxide (NO) bioavailability (Dietz et al., 1994; Joyner and Casey, 2009). NO has been implicated as a potential mechanism through which inflammation could cause endothelial dysfunction (Clapp et al., 2004; Goodwin et al., 2007; Hingorani et al., 2000; Hung et al., 2010; Kharbanda, 2002). Using the same vaccination protocol as the current study, Clapp et al. (2004) found that inflammation induced basal endothelial dysfunction via reduced NO bioavailability (Clapp et al., 2004). Inflammation can influence NO bioavailability through decreased eNOS activation (Goodwin et al., 2007), eNOS uncoupling (Rabelink and van Zonneveld, 2006), or increases in oxidative stress (Gao et al., 2007). Given these previous findings, it can be speculated that the attenuated stress-induced vasodilation in the current study during inflammation is due to reduced NO bioavailability. However, as direct measurement of NO in blood is difficult and often unreliable (Wadley et al., 2013), the reasons for a possible reduction in NO production must remain speculative.

The reduced stress-induced vasodilation observed in the inflamed state may have occurred via several mechanisms. Given the lack of changes in sympathetic activation (assessed by a reduction in PEP) or parasympathetic withdrawal (assessed by reduced r-MSSD) between sessions or conditions, it is unlikely the attenuated vasodilation in the current study can be attributed to the influence of inflammation on sympathetic activation, which has been considered as a mechanism for stress-induced vasodilation (Lindqvist et al., 1997).

In line with previous work, there were no correlations between inflammation and cardiovascular parameters (Paine et al., 2013a). Therefore, this suggests that increased TNF-α or IL-6 are not directly or uniquely responsible for the attenuation in vascular responses to stress, but might be indicative of an inflamed state. The only other study that used a vaccination to assess the effects of inflammation on responses to mental stress, reported correlations between IL-6 responses to a vaccine and blood pressure responses to a speech task (Brydon et al., 2009). However, comparison with this study is not possible, given the differences in time between vaccine administration and the stress task between the two studies. Whereas the current study conducted the stress task at peak IL-6 levels (Paine et al., 2013b), Brydon et al. (2009) completed the stress task at a time when IL-6 was still increasing. It is however, worth noting, that even at 2 h post-vaccination, inflammation did not influence the HR and BP responses to mental stress (Brydon et al., 2009), which is in line with the current study.

The finding that inflammation did not influence resting cardiovascular function seems to contradict previous findings (Brydon et al., 2009; Harrison et al., 2009a,b, 2013), which is most likely due to the timing of the assessments relative to the vaccine as described above. Changes in blood pressure have been reported 3–4 h after administering the vaccine, whereas, in line with the findings in the current study, no changes in blood pressure were found 6–8 h after the vaccine (Hingorani et al., 2000). However, it is plausible that assessment of blood pressure at earlier time points (such as 3–4 h post-vaccination) may have yielded similar effects of inflammation on blood pressure.

One limitation of this study was that it did not utilise a counterbalanced design. As the control and intervention conditions were not counterbalanced, it is possible that the attenuated responses to mental stress could be attributable to task habituation. Importantly, as it has been shown that with this particular stress task the largest cardiovascular and vascular responses to stress are observed during the first few minutes (Paine et al., 2013c), a reduction in blood flow during this period cannot be attributed to the stress task being at a lower intensity at the beginning of the task. This is important as attenuated vascular responses were evident during the first part of the stress task, which is during the same time frame as the appearance of mental stress-induced ischaemia has also been observed during the first minutes of a stress task (Blumenthal et al., 1995). The effect of inflammation was specific to the vasculature, as no effects of inflammation were evident for any cardiac measure. Importantly, this finding was apparent during the previous study which utilised an exercise protocol to induce increases in inflammation; despite the fact that the previous study adopted a counterbalanced design and the current one did not (Paine et al., 2013a).

Previous studies have found no attenuation with this stress task when or completed several times either within or between sessions (Veldhuijzen van Zanten et al., 2005; Willemsen et al., 1998). Critically, stress-induced vasodilation is not susceptible to task habituation and

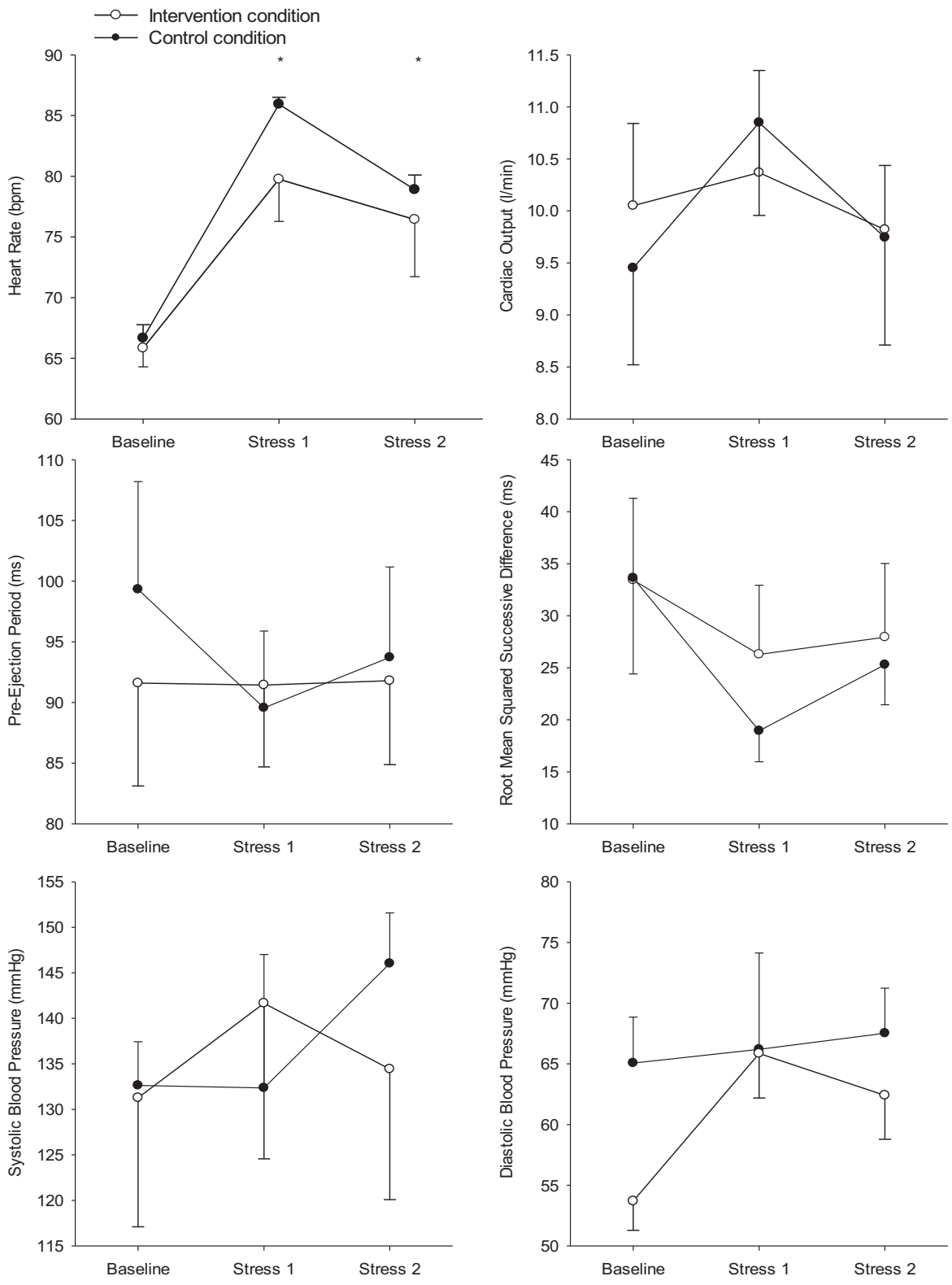

Fig. 4. Mean ± SE responses to mental stress for HR, CO, PEP, r-MSSD, SBP and DBP in the saline group (N = 5). Note: Time effects (for both conditions) are denoted with * indicating significantly different from baseline ($p < .05$).

that vasodilatory responses to repeated stress are robust, with significant test–retest correlations observed for both FBF and HR reactivity (Hamer et al., 2006). Here, there was no attenuating effect of inflammation on any of the other physiological responses, such as heart rate or blood pressure, or psychological responses; a finding that has been previously demonstrated (Paine et al., 2013a). Only the vascular responses to mental stress were affected during inflammation, and this high level of physiological selectivity is not consistent with mere habituation. It is noteworthy that for the placebo group who received the saline intervention there were no significant differences in the responses of any of the physiological parameters assessed. Therefore, the vascular attenuation observed in the inflammation intervention group is unlikely to

be attributed to task habituation. Despite being outside of the scope of the current study, future work may also wish to examine the inflammatory response to stress, and examine how the inflammatory response to stress is linked to the vascular responses to stress.

In sum, we observed an attenuation of the vascular responses to mental stress during a state of elevated inflammation which was achieved by a vaccination. This builds on previous evidence of attenuated vascular responses to stress through an acute, transient increase in inflammation in an otherwise healthy population. This interaction between inflammation and the vascular responses to mental stress might be one mechanism through which mental stress could trigger a myocardial infarction. Future work is needed that focuses on the associations between different inflammatory markers, and how reductions in inflammatory levels may reduce the potential risks associated with the vascular responses to mental stress.

Acknowledgements

The authors would like to acknowledge the valuable contributions of Oliver Dixon, Jessica Russell, Harpavan Shergill, Lucie Vickers and Alex Wadley in recruiting and testing participants, and Dr David McIntyre for technical assistance.

References

Anker, S.D., von Haehling, S., 2004. Inflammatory mediators in chronic heart failure: an overview. Heart 90, 464–470.
Bhagat, K., Vallance, P., 1997. Inflammatory cytokines impair endothelium-dependent dilatation in human veins in vivo. Circulation 96, 3042–3047.
Blake, G.J., Ridker, P.M., 2002. Inflammatory bio-markers and cardiovascular risk prediction. J. Intern. Med. 252, 283–294.
Blumenthal, J.A., Jiang, W., Waugh, R.A., Frid, D.J., Morris, J.J., Coleman, R.E., Hanson, M., Babyak, M., Thyrum, E.T., Krantz, D.S., O'Connor, C., 1995. Mental stress-induced ischemia in the laboratory and ambulatory ischemia during daily life. Circulation 92, 2102–2108.
Brydon, L., Walker, C., Wawrzyniak, A., Whitehead, D., Okamura, H., Yajima, J., Tsuda, A., Steptoe, A., 2009. Synergistic effects of psychological and immune stressors on inflammatory cytokine and sickness responses in humans. Brain Behav. Immun. 23, 217–224.
Burg, M.M., Graeber, B., Vashist, A., Collins, D., Earley, C., Liu, J., Lampert, R., Soufer, R., 2009. Noninvasive detection of risk for emotion-provoked myocardial ischemia. Psychosom. Med. 71, 14–20.
Clapp, B.R., Hingorani, A.D., Kharbanda, R.K., Mohamed-Ali, V., Stephens, J.W., Vallance, P., MacAllister, R.J., 2004. Inflammation-induced endothelial dysfunction involves reduced nitric oxide bioavailability and increased oxidant stress. Cardiovasc. Res. 64, 172–178.
Dietz, N.M., Rivera, J.M., Eggener, S.E., Fix, R.T., Warner, D.O., Joyner, M.J., 1994. Nitric oxide contributes to the rise in forearm blood flow during mental stress in humans. J. Physiol. 480, 361–368.
Edwards, K.M., Burns, V.E., Reynolds, T., Carroll, D., Drayson, M., Ring, C., 2006. Acute stress exposure prior to influenza vaccination enhances antibody response in women. Brain Behav. Immun. 20, 159–168.
Edwards, K.M., Burns, V.E., Adkins, A.E., Carroll, D., Drayson, M., Ring, C., 2008. Meningococcal A vaccination response is enhanced by acute stress in men. Psychosom. Med. 70, 147–151.
Febbraio, M.A., Pedersen, B.K., 2002. Muscle-derived interleukin-6: mechanisms for activation and possible biological roles. FASEB J. 16, 1335–1347.
Fichtlscherer, S., Breuer, S., Zeiher, A.M., 2004. Prognostic value of systemic endothelial dysfunction in patients with acute coronary syndromes. Circulation 110, 1926–1932.
Gao, X., Belmadani, S., Picchi, A., Xu, X., Potter, B.J., Tewari-Singh, N., Capobianco, S., Chilian, W.M., Zhang, C., 2007. Tumor necrosis factor-α induces endothelial dysfunction in Lepr^{db} mice. Circulation 115, 245–254.
Goldberg, A.D.M., Becker, L.C.M., Bonsall, R.P., Cohen, J.D.M., Ketterer, M.W.P., Kaufman, P. G.P., Krantz, D.S.P., Light, K.C.P., McMahon, R.P.P., Noreuil, T.M., Pepine, C.J.M., Raczynski, J.P., Stone, P.H.M., Strother, D.R., Taylor, H.M., Sheps, D.S.M., 1996. Ischemic, hemodynamic, and neurohormonal responses to mental and exercise stress: experience from the Psychophysiological Investigations of Myocardial Ischemia Study (PIMI). Circulation 94, 2402–2409.
Goodwin, B.L., Pendleton, L.C., Levy, M.M., Solomonson, L.P., Eichler, D.C., 2007. Tumor necrosis factor-α reduces argininosuccinate synthase expression and nitric oxide production in aortic endothelial cells. Am. J. Physiol. Heart Circ. Physiol. 293, H1115–H1121.
Gronwall, D.M., 1977. Paced auditory serial-addition task: a measure of recovery from concussion. Percept. Mot. Skills 44, 367–373.
Hamer, M., Boutcher, Y.N., Park, Y., Boutcher, S.H., 2006. Reproducibility of skeletal muscle vasodilatation responses to Stroop mental challenge over repeated sessions. Biol. Psychol. 73, 186–189.
Hamer, M., Boutcher, Y.N., Boutcher, S.H., 2007. Fatness is related to blunted vascular stress responsivity, independent of cardiorespiratory fitness in normal and overweight men. Int. J. Psychophysiol. 63, 251–257.
Harrison, N.A., Brydon, L., Walker, C., Gray, M.A., Steptoe, A., Dolan, R.J., Critchley, H.D., 2009a. Neural origins of human sickness in interoceptive responses to inflammation. Biol. Psychiatry 66, 415–422.
Harrison, N.A., Brydon, L., Walker, C., Gray, M.A., Steptoe, A., Critchley, H.D., 2009b. Inflammation causes mood changes through alterations in subgenual cingulate activity and mesolimbic connectivity. Biol. Psychiatry 66, 407–414.
Harrison, N.A., Cooper, E., Voon, V., Miles, K., Critchley, H.D., 2013. Central autonomic network mediates cardiovascular responses to acute inflammation: relevance to increased cardiovascular risk in depression? Brain Behav. Immun. 31, 189–196.
Hingorani, A.D., Cross, J., Kharbanda, R.K., Mullen, M.J., Bhagat, K., Taylor, M., Donald, A.E., Palacios, M., Griffin, G.E., Deanfield, J.E., MacAllister, R.J., Vallance, P., 2000. Acute systemic inflammation impairs endothelium-dependent dilatation in humans. Circulation 102, 994–999.
Hung, M.J., Cherng, W.J., Hung, M.Y., Wu, H.T., Pang, J.H., 2010. Interleukin-6 inhibits endothelial nitric oxide synthase activation and increases endothelial nitric oxide synthase binding to stabilized caveolin-1 in human vascular endothelial cells. J. Hypertens. 28, 940–951.
Jain, D., Shaker, S.M., Burg, M., Wackers, F.J., Soufer, R., Zaret, B.L., 1998. Effects of mental stress on left ventricular and peripheral vascular performance in patients with coronary artery disease. J. Am. Coll. Cardiol. 31, 1314–1322.
Joyner, M.J., Casey, D.P., 2009. The catecholamines strike back: what no does not do. Circ. J. 73, 1783–1792.
Kharbanda, R.K., 2002. Prevention of inflammation-induced endothelial dysfunction: a novel vasculo-protective action of aspirin. Circulation 105, 2600–2604.
Kop, W.J., Weissman, N.J., Zhu, J., Bonsall, R.W., Doyle, M., Stretch, M.R., Glaes, S.B., Krantz, D.S., Gottdiener, J.S., Tracy, R.P., 2008. Effects of acute mental stress and exercise on inflammatory markers in patients with coronary artery disease and healthy controls. Am. J. Cardiol. 101, 767–773.
Lerman, A., Zeiher, A.M., 2005. Endothelial function: cardiac events. Circulation 111, 363–368.
Lindqvist, M., Melcher, A., Hjemdahl, P., 1997. Attenuation of forearm vasodilator responses to mental stress by regional beta-blockade, but not by atropine. Acta Physiol. Scand. 161, 135–140.
Middlekauff, H.R., Nguyen, A.H., Negrao, C.E., Nitzsche, E.U., Hoh, C.K., Natterson, B.A., Hamilton, M.A., Fonarow, G.C., Hage, A., Moriguchi, J.D., 1997. Impact of acute mental stress on sympathetic nerve activity and regional blood flow in advanced heart failure: implications for 'triggering' adverse cardiac events. Circulation 96, 1835–1842.
Padfield, G.J., Tura, O., Haeck, M.L.A., Short, A., Freyer, E., Barclay, G.R., Newby, D.E., Mills, N.L., 2010. Circulating endothelial progenitor cells are not affected by acute systemic inflammation. Am. J. Physiol. Heart Circ. Physiol. 298, H2054–H2061.
Paine, N.J., Bosch, J.A., Veldhuijzen van Zanten, J.J.C.S., 2012. Inflammation and vascular responses to acute mental stress: implications for the triggering of myocardial infarction. Curr. Pharm. Des. 18, 1494–1501.
Paine, N.J., Ring, C., Aldred, S., Bosch, J.A., Wadley, A.J., Veldhuijzen van Zanten, J.J.C.S., 2013a. Eccentric-exercise induced inflammation attenuates the vascular responses to mental stress. Brain Behav. Immun. 30, 133–142.
Paine, N.J., Ring, C., Bosch, J.A., Drayson, M.T., Veldhuijzen van Zanten, J.J.C.S., 2013b. The time course of the inflammatory response to the *Salmonella typhi* vaccination. Brain Behav. Immun. 30, 73–79.
Paine, N.J., Ring, C., Bosch, J.A., McIntyre, D., Veldhuijzen van Zanten, J.J.C.S., 2013c. The effect of acute mental stress on limb vasodilation is unrelated to total peripheral resistance. Psychophysiology 50, 680–690.
Rabelink, T.J., van Zonneveld, A.J., 2006. Coupling eNOS uncoupling to the innate immune response. Arterioscler. Thromb. Vasc. Biol. 26, 2585–2587.
Rastogi, S., Gross, P.A., Bonelli, J., Dran, S., Levandowski, R.A., Russo, C., Weksler, M.E., Kaye, D., Levison, M., Abrutyn, E., 1995. Time to peak serum antibody response to influenza vaccine. Clin. Diagn. Lab. Immunol. 2, 120–121.
Ridker, P.M., Rifai, N., Stampfer, M.J., Hennekens, C.H., 2000. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. Circulation 101, 1767–1772.
Ring, C., Drayson, M., Walkey, D.G., Dale, S., Carroll, D., 2002. Secretory immunoglobulin A reactions to prolonged mental arithmetic stress: inter-session and intra-session reliability. Biol. Psychol. 59, 1–13.
Sandoo, A., Veldhuijzen van Zanten, J.J.C.S., Metsios, G.S., Carroll, D., Kitas, G.D., 2010. The endothelium and its role in regulating vascular tone. Open Cardiovasc. Med. J. 4, 302–312.
Santos, A.C., Alves, M.J.N.N., Rondon, M.U.P.B., Barretto, A.C.P., Middlekauff, H.R., Negrao, C. E., 2005. Sympathetic activation restrains endothelium-mediated muscle vasodilatation in heart failure patients. Am. J. Physiol. Heart Circ. Physiol. 289, H593–H599.
Shah, R., Burg, M.M., Vashist, A., Collins, D., Liu, J., Jadbabaie, F., Graeber, B., Earley, C., Lampert, R., Soufer, R., 2006. C-reactive protein and vulnerability to mental stress-induced myocardial ischemia. Mol. Med. 12, 269–274.
Sherwood, A., Allen, M.T., Fahrenberg, J., Kelsey, R.M., Lovallo, W.R., van Doornen, L.J., 1990. Methodological guidelines for impedance cardiography. Psychophysiology 27, 1–23.
Sherwood, A., Johnson, K., Blumenthal, J.A., Hinderliter, A.L., 1999. Endothelial function and hemodynamic responses during mental stress. Psychosom. Med. 61, 365–370.
Steensberg, A., Febbraio, M.A., Osada, T., Schjerling, P., van Hall, G., Saltin, B., Pedersen, B.K., 2001. Interleukin-6 production in contracting human skeletal muscle is influenced by pre-exercise muscle glycogen content. J. Physiol. 537, 633–639.

Steensberg, A., Keller, C., Starkie, R.L., Osada, T., Febbraio, M.A., Pedersen, B.K., 2002. IL-6 and TNF-alpha expression in, and release from, contracting human skeletal muscle. Am. J. Physiol. Endocrinol. Metab. 283, E1272–E1278.

Strike, P.C., Steptoe, A., 2005. Behavioral and emotional triggers of acute coronary syndromes: a systematic review and critique. Psychosom. Med. 67, 179–186.

Strike, P.C., Wardle, J., Steptoe, A., 2004. Mild acute inflammatory stimulation induces transient negative mood. J. Psychosom. Res. 57, 189–194.

Vasey, M.W., Thayer, J.F., 1987. The Continuing Problem of False Positives in Repeated Measures ANOVA in Psychophysiology: A Multivariate Solution. Psychophysiology 24, 479–486.

Vaudo, G., Marchesi, S., Gerli, R., Allegrucci, R., Giordano, A., Siepi, D., Pirro, M., Shoenfeld, Y., Schillaci, G., Mannarino, E., 2004. Endothelial dysfunction in young patients with rheumatoid arthritis and low disease activity. Ann. Rheum. Dis. 63, 31–35.

Veldhuijzen van Zanten, J.J.C.S., Ring, C., Burns, V.E., Edwards, K.M., Drayson, M., Carroll, D., 2004. Mental stress-induced hemoconcentration: sex differences and mechanisms. Psychophysiology 41, 541–551.

Veldhuijzen van Zanten, J.J.C.S., Thrall, G., Wasche, D., Carroll, D., Ring, C., 2005. The influence of hydration status on stress-induced hemoconcentration. Psychophysiology 42, 98–107.

Veldhuijzen van Zanten, J.J.C.S., Kitas, G.D., Carroll, D., Ring, C., 2008. Increase in systemic vascular resistance during acute mental stress in patients with rheumatoid arthritis with high-grade systemic inflammation. Biol. Psychol. 77, 106–110.

Wadley, A.J., Veldhuijzen van Zanten, J.J.C.S., Aldred, S., 2013. The interactions of oxidative stress and inflammation with vascular dysfunction in ageing: the vascular health triad. Age (Dordr.) 35, 705–718.

Wilbert-Lampen, U., Nickel, T., Leistner, D., Güthlin, D., Matis, T., Völker, C., Sper, S., Küchenhoff, H., Kääb, S., Steinbeck, G., 2010. Modified serum profiles of inflammatory and vasoconstrictive factors in patients with emotional stress-induced acute coronary syndrome during World Cup Soccer 2006. JACC 55, 637–642.

Willemsen, G., De Geus, E.J.C., Klaver, C.H.A.M., van Doornen, L.J., Carroll, D., 1996. Ambulatory monitoring of the impedance cardiogram. Psychophysiology 33, 184–193.

Willemsen, G., Ring, C., Carroll, D., Evans, P., Clow, A., Hucklebridge, F., 1998. Secretory immunoglobulin A and cardiovascular reactions to mental arithmetic and cold pressor. Psychophysiology 35, 252–259.

Wright, C.E., Strike, P.C., Brydon, L., Steptoe, A., 2005. Acute inflammation and negative mood: mediation by cytokine activation. Brain Behav. Immun. 19, 345–350.