Brain, Behavior, and Immunity 30 (2013) 133–142

Contents lists available at SciVerse ScienceDirect

Brain, Behavior, and Immunity

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

Eccentric-exercise induced inflammation attenuates the vascular responses to mental stress

Nicola J. Paine [a,*], Christopher Ring [a], Sarah Aldred [a], Jos A. Bosch [a,b,c], Alex J. Wadley [a], Jet J.C.S. Veldhuijzen van Zanten [a]

[a] School of Sport and Exercise Sciences, College of Life and Environmental Sciences, University of Birmingham, Birmingham B15 2TT, United Kingdom
[b] Department of Clinical Psychology, University of Amsterdam, Amsterdam, The Netherlands
[c] Mannheim Institute of Public Health, Social and Preventive Medicine (MIPH), Mannheim Medical Faculty, University of Heidelberg, Germany

ARTICLE INFO

Article history:
Received 26 October 2012
Received in revised form 15 January 2013
Accepted 23 January 2013
Available online 30 January 2013

Keywords:
Inflammation
Eccentric exercise
Vascular responses
Mental stress
Stress-induced vasodilation

ABSTRACT

Mental stress has been identified as a trigger of myocardial infarction (MI), with inflammation and vascular responses to mental stress independently implicated as contributing factors. This study examined whether inflammation moderates the vascular responses to mental stress. Eighteen healthy male participants completed a stress task under two counter balanced conditions. In the exercise condition, a morning bout of eccentric exercise (12 × 5 repetitions of unilateral eccentric knee extension at 120% intensity of concentric one repetition maximum) was used to increase levels of inflammatory-responsive cytokines during an afternoon stress session scheduled 6 h later. In the control condition, participants sat and relaxed for 45 min, 6 h prior to the afternoon stress session. Forearm blood flow, calf blood flow (measured in the leg which completed the exercise task), blood pressure, heart rate and cardiac output were assessed at rest and in response to mental stress. As expected, interleukin-6 was higher ($p = .02$) 6 h post exercise, i.e., at the start of the stress session, as compared to the no-exercise control condition. Mental stress increased forearm blood flow, calf blood flow, blood pressure, heart rate, and cardiac output in both conditions (p's < .001). Stress-induced calf blood flow was attenuated in the exercise condition compared to the control condition ($p < .05$) which was not the case for forearm blood flow. This study found that the inflammatory response to eccentric exercise attenuated the vascular responses to mental stress locally at the site of eccentric exercise-induced inflammation. The observed impairment in vascular responses to stress associated with increased levels of inflammation suggests a mechanism through which inflammation might increase the risk for MI.

© 2012 Elsevier Inc. All rights reserved.

1. Introduction

Acute mental stress has been identified as a possible of trigger myocardial infarction (MI). For example, survivors of MI have mentioned episodes of emotional stress as a trigger for their MI (Strike and Steptoe, 2005). Likewise, earthquakes (Leor et al., 1996a,b; Suzuki et al., 1995), war (Bergovec et al., 1992; Meisel et al., 1991), and even key football matches (e.g., Carroll et al., 2002; Wilbert-Lampen et al., 2010) have been associated with an increased incidence in MI. To explore the mechanisms through which stress might lead to MI, laboratory studies have assessed mental stress-induced ischaemia, which is predictive of future cardiac events and mortality (Babyak et al., 2010; Krantz et al., 1999). Mental stress-induced ischaemia is associated with higher resting levels of inflammation in cardiac patients (Shah et al., 2006). Also, mental stress-induced ischaemia has been associated with impaired vascular responses to stress, characterised by either increases in vascular resistance (Goldberg et al., 1996; Jain et al., 1998) or decreased peripheral dilation (Burg et al., 2009). Interestingly, serological markers of inflammation and vasoconstriction were increased in patients admitted to hospital for MI during the 2006 Football World Cup compared to those admitted for MI during a non-stressful period (Wilbert-Lampen et al., 2010). Therefore, both inflammation and vascular responses to mental stress appear to contribute to mental stress-induced MI (Paine et al., 2012c).

One mechanism that has received attention as a possible mechanism in stress-induced MI is inflammation. Both acute (Meier et al., 1998) and chronic (Levy et al., 2008; Ridker et al., 2000) levels of inflammation are associated with increased risk for MI. Both also play a critical role in development and progression of cardiovascular disease (CVD) (Hansson, 2005; Libby, 2006). The impact of inflammation on resting vascular function has been examined extensively; studies have revealed inverse associations between vascular function and inflammation in clinical populations (Fichtlscherer et al., 2004; Vaudo et al., 2004). However, the strength of the

* Corresponding author. Tel.: +44 121 414 4115; fax: +44 121 414 4121.
E-mail address: N.J.Paine@bham.ac.uk (N.J. Paine).

0889-1591/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbi.2013.01.082

vascular-inflammation relationship weakens when classic risk factors are controlled for (Cleland et al., 2000; Vita et al., 2004), and thus the sole influence of inflammation may be masked. Therefore, methods that experimentally induce inflammation in a healthy population have been used to directly examine the effects of inflammation on vascular function without the presence of potentially confounding factors. Studies using this approach have demonstrated that inflammation in healthy participants causes transient endothelial dysfunction at rest (Bhagat and Vallance, 1997; Clapp et al., 2004; Hingorani et al., 2000; Kharbanda et al., 2002).

In healthy participants, mental stress causes vasodilation (Blair et al., 1959; Dietz et al., 1994; Joyner and Halliwill, 2000), which is reduced in populations at risk for cardiovascular disease (Hamer et al., 2007) and patients with heart failure (Middlekauff et al., 1997; Santos et al., 2005). Despite different mechanisms being proposed as the putative cause of stress-induced vasodilation, the most consistent evidence implicates nitric oxide (NO) (Cardillo et al., 1997; Dietz et al., 1994; Joyner and Dietz, 2003; Joyner and Casey, 2009; Sarabi and Lind, 2001). In general, these studies reveal that pharmacologically blocking NO production results in an attenuated dilatory response to mental stress (Cardillo et al., 1997; Dietz et al., 1994; Joyner and Dietz, 2003; Joyner and Casey, 2009; Sarabi and Lind, 2001).

The role of NO in stress-induced vasodilation is interesting, given the reciprocal relationship between NO and inflammation at rest (De Martin et al., 2000; Hung et al., 2010; Verma et al., 2002). Thus, given the common link between NO for maintaining vascular function and stress-induced vasodilation, it has been hypothesised that inflammation may also attenuate the vasodilatory response to mental stress (Paine et al., 2012c), particularly given that the impairment in resting endothelial function as a result of increased inflammation has been attributed to a reduction in NO (Clapp et al., 2004). Very few studies have examined the influence of inflammation on stress-induced vasodilation, despite evidence for an association between basal inflammation and mental stress-induced ischaemia (Shah et al., 2006). In one of these studies, rheumatoid arthritis patients with high-grade systemic inflammation were found to display an increase in vascular resistance in response to mental stress, which was not evident in patients with low-grade inflammation (Veldhuijzen van Zanten et al., 2008). Interestingly, poorer vascular responses to mental stress have also been found in a healthy population when inflammation was induced using a typhoid vaccination (Paine et al., 2012a).

A potent, but less utilised, method to induce inflammation is eccentric exercise. Eccentric exercise involves increasing the tension in a muscle as it elongates (e.g., gradually lowering a weight against gravity) (Edwards et al., 2007; Proske and Morgan, 2001). Eccentric exercise initiates a localised inflammatory response through the ensuing myofibril damage in the muscle (Nosaka et al., 2002; Proske and Morgan, 2001). The extent of the inflammatory response has typically been characterised by the increase in interleukin-6 (IL-6) (Croisier et al., 1999; Depner et al., 2008; Jackman et al., 2010; Miles et al., 2008; Steensberg et al., 2002, 2000; Willoughby et al., 2003). IL-6 is an inflammatory-responsive cytokine that is involved in the acute-phase response that occurs as a result of injury (Willoughby et al., 2003). The peak IL-6 response is most commonly reported to be at 6 h post eccentric exercise (MacIntyre et al., 2001; Paulsen et al., 2005; Philippou et al., 2009; Willoughby et al., 2003). The magnitude of the response is dependent on exercise duration, exercise intensity, and size of the muscle used, such that extensive high intensity exercise using a large muscle mass should result in the most substantial increases in IL-6 (Febbraio and Pedersen, 2002). The exact sources from which IL-6 is released are likely to involve both resident leukocytes as well as the exercising muscle (Ostrowski et al., 1998; Willoughby et al., 2003).

The aim of the current study was to investigate the effect of inflammation induced by a bout of acute eccentric exercise on the vascular responses to mental stress in a healthy population. To ensure a robust response, exercise was performed in the quadriceps muscle of the non-dominant leg. A previous study has examined the impact of eccentric exercise on resting vascular function, revealing increases in resting arterial stiffness 48 h after performing eccentric exercise of the elbow flexors (Barnes et al., 2010). However, to our knowledge no study has investigated the effect of eccentric exercise on stress-induced vascular responses, which are of particular interest given their putative role in the triggering of MI. Therefore, it is hypothesised that eccentric exercise-induced inflammation will attenuate the vascular responses to mental stress compared to a no-exercise control condition.

2. Methods

2.1. Participants

Eighteen male participants were recruited from the University of Birmingham (mean age ± SE = 20.4 ± 1.2 years, mean body mass index (BMI) ± SD = 23.7 ± 0.6 kg/m^2). None were suffering from an acute illness or infection, reported a history of inflammatory, cardiovascular or auto-immune disorders, or had taken any medication in the last 4 weeks. None undertook any regular resistance exercise as greater amounts of muscle damage and inflammation have been demonstrated in those who are unaccustomed to eccentric exercise (Sorichter et al., 2006). 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. Eccentric exercise task

The eccentric exercise task was undertaken using a Cybex leg extension machine (Cybex International Medway, MA). The protocol was adapted from that used in a previous study (Jackman et al., 2010). First, measurements of limb circumference of both legs and assessments of muscle soreness were taken. After familiarisation with the equipment, each participant's one repetition maximum (1RM) of their non-dominant leg was determined. Each participant concentrically lifted the weight from a position of knee flexion to knee extension, holding it outstretched for 2 s, before lowering it. Weight was added until the participant could no longer lift the weight. Each 1RM was determined within five attempts to minimise fatigue. Once this was determined, the eccentric exercise task was explained before participants completed one set at a reduced weight (50% 1RM) to ensure understanding of the action. Participants were seated with their non-dominant leg positioned at 90 degrees to their torso. Two experimenters lifted the weight to its starting position (with the exercising leg extended to approximately 15 degrees of flexion), and the participant lowered the weight until their knee was flexed at approximately 110 degrees. Participants lowered the weight slowly over a 4 s period, resisting the weight at all times. If this was not achieved, the weight was replaced at the starting position and the action repeated. Participants completed 12 sets of five eccentric repetitions at 120% of their concentric 1RM. Five seconds rest separated each repetition, with a 1 min rest at the end of each set. At the end of the task, the 'Borg scale' (1970) was used to assess perception of physical exertion during the exercise task, using a 14 point scale, with anchors of 6 ('no exertion at all') and 20 ('maximal exertion').

2.3. Rest task

Participants rested quietly for 25 min; they completed a questionnaire pack (data not reported) at the start.

2.4. Mental stress task

A version of the paced auditory serial addition test (PASAT) was used as the mental stress task. The PASAT has been widely used to elicit a stress response, 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, delivered using an audio player, 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 (referred to as Stress 1 and Stress 2, respectively), separated by one minute rest. The numbers were delivered in four 2-min blocks, with the numbers presented every 3.2, 2.8, 2.4 and 2.0 s for the first task, and every 2.4, 2.0, 1.6 and 1.2 s for the second task. This resulted in a progressive increase in task difficulty. The experimenter, who sat 1 m adjacent to the participant, checked their responses against the correct answers. To enhance the stressfulness of the task several features were added that have been shown to enhance stress responses in previous studies (Veldhuijzen van Zanten et al., 2004). Participants heard a loud aversive noise blast 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 block of 10 numbers. Participants were filmed with a video camera and were asked to look at their faces displayed on a television screen while 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. They were 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, to compare participant scores. They were also told that another £10 voucher would be given to the participant recorded the greatest improvement in score between the two sessions. However, the latter of these rewards was only announced before the start of the task on the second day of testing, to eliminate the risk of a poor performance on the first day of testing. 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 were asked to rate the task in terms of perceived difficulty, arousal, stressfulness, engagement, and perceived performance using a 7-point Likert scale, with anchors of 1 ('not at all') and 7 ('extremely').

2.5. Procedure

Participants completed two conditions scheduled seven days apart (Fig. 1), with the order of the conditions counter balanced across participants. The control condition consisted of a 45 min rest session in the morning, whereas the exercise condition consisted of an eccentric exercise task in the morning. Six hours after the morning session, the participant completed the stress session. The time between the morning sessions and the stress reactivity sessions was chosen in order to start the afternoon session close to the peak inflammatory response to eccentric exercise (MacIntyre et al., 2001; Paulsen et al., 2005; Philippou et al., 2009; Willoughby et al., 2003). All procedures were performed in a temperature controlled (18 °C) laboratory. The start times for both sessions was the same for each participant, with morning session start times between 8:00 am and 10:00 am, and afternoon stress sessions starting between 2:00 pm and 4:00 pm.

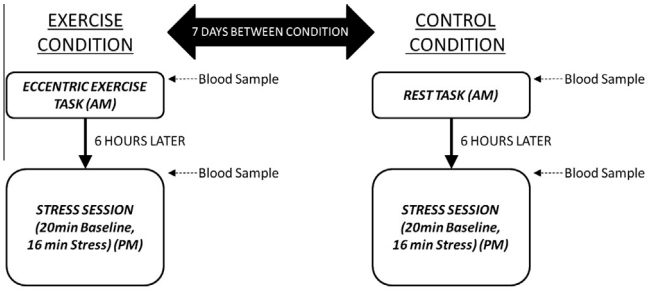

Fig. 1. The study protocol.

2.6. Morning session

Upon arrival in the laboratory, a blood sample was taken using a 21 gauge butterfly needle (Becton Dickinson, UK). Measures of limb circumference and muscle soreness were obtained and participants completed the eccentric exercise task or the rest task. After completion of these tasks, repeat measures of limb circumference and muscle soreness were taken. Both sessions lasted approximately 45 min.

2.7. Stress session

Upon arrival at the laboratory height and weight were measured, and the participant was instrumented with the cardiovascular equipment. The participant was then placed in a supine position on a bed, where they remained throughout the session. Endothelial function (a 12 min assessment) was assessed through flow mediated dilation (data not reported). An 18 gauge cannula (Insyte, Becton Dickinson) was inserted into an antecubital vein of the dominant arm of each participant. After the cardiovascular equipment was attached, the participant rested for 20 min (baseline rest period) while watching a nature documentary (Life; BBC). Blood flow was measured during minutes 13, 15, 17 and 19. A resting blood sample was taken at the end of the baseline rest period. After practice of the mental stress task, participants completed two 8 min blocks of the mental stress task, with 1 min rest in between blocks. During minutes 1, 3, 5 and 7 of each block, blood flow was recorded. ICG, ECG and blood pressure were recorded throughout the session, however, offline analyses were conducted on only the minutes during which blood flow was measured.

2.8. 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 was recorded via a Power1401 (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 analysed offline. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were calculated from each blood pressure waveform recorded during the minute of assessment, and then averaged for the periods of assessment. SBP and DBP were 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 et al., 1995; Willemsen et al., 1996).

This system used six Ag/AgCl spot electrodes (Invisatrace, ConMed Corporation) to record electrocardiography (ECG) and impedance cardiography (ICG), in line with published guidelines (Sherwood et al., 1990). Ten second ensemble averages were scored and used to determine Heart Rate (HR, bpm) and Cardiac Output (as a product of stroke volume and HR) (CO; l/min), Pre-Ejection Period (PEP; ms) a measure of sympathetic activation, and the root mean square of successive differences (rMSSD; ms) a measure of parasympathetic activation based on heart rate variability (HRV).

2.9. Blood flow

Venous occlusion plethysmography, using a mercury-in-silastic strain gauge, was used to measure forearm blood flow (FBF) and calf blood flow (CBF). A strain gauge was fitted around the widest part of the non-dominant forearm and calf. One congestion cuff was placed around brachial region of the upper arm and thigh (SC12, Hokanson) and another at the wrist and ankle (TMC7, Hokanson). The strain gauges were 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 (CED) connected to a computer programmed in Spike2 version 6 (CED). The brachial and femoral 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 allowed arterial inflow, while preventing venous return. After 15 s, the brachial and femoral cuffs were inflated again. This was repeated three times per minute. Throughout the minute of assessment, the wrist and ankle cuff was manually inflated by Sphygmomanometer (S300, Hokanson) to supra-systolic blood pressure (>200 mmHg), to prevent any blood flow into the hand and foot. Calibration and blood flow analysis was undertaken offline using Spike2 (CED). 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 first two pulses following cuff inflation. The slope was assessed using a least squares fit to the data to minimise the effects of outlying data points. Three measurements of blood flow (slopes in response to cuff inflation) occurred per minute, with these averaged to yield mean blood flow per minute.

2.10. Limb circumference and muscle soreness

Limb circumference measurements were taken from the non-dominant leg, with the participant asked to flex their quadriceps, to mark the widest part of the quadriceps. The participant then relaxed their leg in order to measure limb circumference. On a scale 1 (*pain-free*) to 100 (*worst pain imaginable*) (Melzack, 1987) participants rated the extent of their leg pain when resting (referred to as resting leg pain) and when asked to mimic the exercise actions (referred to as movement leg pain.

2.11. Blood sampling and analysis

Blood samples were taken at the start of each morning session using a 21 gauge butterfly needle (Becton Dickinson, UK) inserted to the antecubital vein of the non-dominant arm. During the stress sessions a blood sample was taken using an 18 gauge cannula (Insyte, Becton Dickinson) inserted into an antecubital vein of the dominant arm. Blood was collected into two 6 ml and one 2 ml vacutainers containing potassium ethylene diaminetetraacetic acid (K3EDTA) (Becton–Dickinson, UK). Both 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. Plasma IL-6 was measured in duplicate using high-sensitivity ELISA (Quantikine HS Human IL-6 ELISA, R&D Systems, UK) in accordance with the manufacturer's instructions. The reported sensitivity of the assays was 0.039 pg/ml, with recorded intra-assay and inter-assay variations both <10%.

2.12. Data reduction and statistical analysis

For all cardiovascular variables, each of the four measurements taken during baseline, Stress 1, and Stress 2 were averaged to establish a single Baseline, Stress 1, and Stress 2 value, respectively. Due to the skewed distribution of the IL-6 data, IL-6 levels were logarithmically transformed to the base 10 and subsequent analyses were conducted on the $\log_{10}$ values. Analysis of Variance (ANOVAs) was conducted on all baseline values in the control and exercise sessions. Two Condition (Control, Exercise) by three Time (Baseline, Stress 1, Stress 2) repeated measures ANOVAs were conducted on cardiovascular and vascular measurements, with Greenhouse-Geisser correction (Vasey and Thayer, 1987). For all ANOVAs, eta squared (η^2) was used as a measure of effect size, and, where appropriate, Newman–Keuls post hoc comparisons are reported. Occasional missing data are reflected in the reported degrees of freedom.

Reactivity (i.e., change) scores for all cardiovascular and vascular measures were calculated as the difference between the average stress value (calculated as the average between Stress 1 and Stress 2) and the baseline value. Subsequently, Pearson correlations were conducted to examine the associations between the resting inflammatory marker (IL-6) and vascular as well as cardiovascular reactivity. These analyses were conducted for the control and exercise condition separately.

3. Results

3.1. Responses to the rest and eccentric exercise task

Table 1 displays the self-reported pain ratings and changes in limb circumference, pre- and post-task for both the rest and exercise conditions, with a series of two time (pre-task, post-task) by two condition (rest, exercise) ANOVAs conducted. In the exercise condition, increases in limb circumference from pre- to post-task as a result of exercise were demonstrated ($F(1,17) = 22.86$, $p < 0.001$, $\eta^2 = .57$). Also higher self-reported pain in the exercising leg at rest ($F(1,17) = 20.38$, $p < 0.001$, $\eta^2 = .55$) and when mimicking the exercise action ($F(1,17) = 17.33$, $p = .001$, $\eta^2 = .51$) were reported in comparison to the non-exercising leg. The mean average self perceived exertion score for the exercise task was 15.9 ± 2.5, with the mean weight used for the task 72.1 ± 14.7 kg. As expected, no changes in limb circumference or pain were observed in the rest condition (p's > .05).

Analysis of the changes in IL-6 as a result of the exercise and rest tasks were conducted through a two condition (control, exercise) by two time (pre-task, baseline) repeated measures ANOVA, and is illustrated in Fig. 2.This analysis revealed a condition by time interaction effect ($F(1,15) = 7.94$, $p = .013$, $\eta^2 = .35$), with further interrogation of this effect revealing that IL-6 was higher in the exercise condition ($F(1,14) = 8.53$, $p = .011$, $\eta^2 = .38$) at the baseline period in the stress reactivity session. No increases in IL-6 were observed in the control (rest) condition. No difference in IL-6 were observed in the blood samples obtained before the start of the exercise and rest tasks (p's > .05).

3.2. Self-reported task ratings

No differences in the performance score, perceived performance, perceived levels of stress, arousal, and engagement were observed between conditions (p's > .05).

Table 1
Self reported pain responses and limb circumference changes to the task for both the exercise and rest condition.

		Pre-task	Immediately post-task	
EXERCISE TASK				
Movement pain	Non-exercising leg	2.44 (3.42)	3.72 (6.98)	$F(1,17) = 1.02$, $p > .05$, $\eta^2 = .06$
	Exercising leg	2.83 (3.26)	22.17 (21.30)	$F(1,17) = 17.33$, $p = .001$, $\eta^2 = .51$
Resting pain	Non-exercising leg	1.83 (2.57)	2.44 (5.10)	$F(1,17) = .72$, $p > .05$, $\eta^2 = .04$
	Exercising leg	1.83 (2.55)	15.11 (13.92)	$F(1,17) = 20.38$, $p < 0.001$, $\eta^2 = .55$
Limb circumference (cm)		50.27 (4.37)	51.16 (4.20)	$F(1,17) = 22.86$, $p < 0.001$, $\eta^2 = .57$
REST TASK				
Movement pain	Non-exercising leg	3.44 (4.34)	3.50 (4.18)	$F(1,17) = .07$, $p > .05$, $\eta^2 = .00$
	Exercising leg	3.11 (3.58)	3.22 (3.69)	$F(1,17) = .24$, $p > .05$, $\eta^2 = .01$
Resting pain	Non-exercising leg	2.33 (5.31)	2.61 (5.37)	$F(1,17) = 2.03$, $p > .05$, $\eta^2 = .11$
	Exercising leg	2.28 (4.96)	2.44 (5.04)	$F(1,17) = .81$, $p > .05$, $\eta^2 = .05$
Limb circumference (cm)		49.62 (4.89)	49.65 (4.80)	$F(1,17) = .46$, $p > .05$, $\eta^2 = .03$

Note: Values reported as mean ± SD. Movement pain refers to mimicking the exercise action; resting pain refers to the leg when resting in a stationary position with the knee flexed at 90 degree. Both pain scales rated using a scale of 0–100; 0 = no pain, 100 = worst pain imaginable.

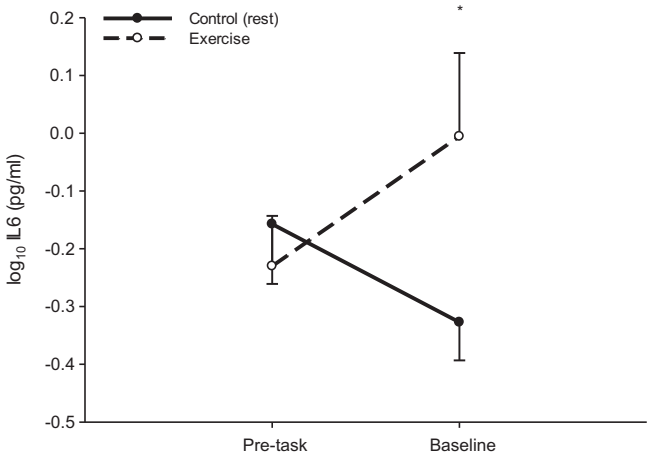

Fig. 2. The IL-6 responses to the rest and exercise tasks * indicates differences between condition ($p < .02$).

3.3. Physiological responses to mental stress

3.3.1. Cardiovascular responses to mental stress

Fig. 3 depicts the HR, CO, PEP, rMSSD, SBP and DBP responses to mental stress in the control and exercise conditions. Separate 2 Condition (Control, Exercise) by 3 Time (Baseline, Stress 1, Stress 2) repeated measures ANOVAs yielded main effects for time for HR ($F(2,16) = 31.47$, $p < .001$, $\eta^2 = .74$), CO ($F(2,14) = 8.87$, $p = .022$, $\eta^2 = .26$), PEP ($F(2,9) = 11.46$, $p = .002$, $\eta^2 = .52$), rMSSD ($F(2,14) = 16.15$, $p < .001$, $\eta^2 = .63$), SBP ($F(2,16) = 26.47$, $p < .001$, $\eta^2 = .61$) and DBP ($F(2,16) = 23.06$, $p < .001$, $\eta^2 = .54$). Newman–Keuls post hoc analyses revealed that HR, CO, SBP, and DBP increased, whereas PEP and rMSSD decreased in response to stress. No condition effects or condition by time interaction effects were seen for CO, HR, PEP, rMSSD, SBP and DBP (all p's > .05).

3.3.2. Vascular responses to mental stress

Forearm and calf blood flow responses to mental stress are illustrated in Fig. 4. The 2 Condition × 3 Time ANOVAs revealed significant main effects for time for forearm ($F(2,16) = 21.40$, $p < .001$, $\eta^2 = .66$) and calf ($F(2,15) = 8.08$, $p = .004$, $\eta^2 = .31$)) blood flow. No condition effects were evident for FBF or CBF, but a condition by time interaction effect was evident for CBF ($F(2,15) = 6.83$, $p = .002$, $\eta^2 = .32$). Newman–Keuls post hoc analysis of this interaction effect for CBF, revealed a greater stress-induced increase in CBF in the control condition compared to the exercise condition. No interaction effect was observed for FBF ($p > .05$).

3.4. Associations between inflammation and physiological responses to mental stress

Pearson correlation analyses were undertaken to examine the relationships between the marker of inflammation and the physiological responses to mental stress in both the control and exercise conditions. These analyses revealed no associations between IL-6 and any of the vascular assessments.

4. Discussion

The aim of this study was to determine whether exercise-induced inflammation moderated the vascular responses to mental stress, and thereby evaluate the possibility that inflammation might be a contributory factor for the triggering of myocardial infarction by stress. Our eccentric exercise task successfully induced an acute systemic inflammatory response: the eccentric exercise task caused an increase in IL-6 level reaching 2.5 pg/ml on average, which is similar to the levels of inflammation induced by a vaccination in healthy participants (Paine et al., 2012a). Importantly, these levels of IL-6 are also similar to those found in patients with coronary artery disease (Kop et al., 2008) and myocardial infarction (Ridker et al., 2000). Likewise, limb circumference and self-reported pain increased immediately post exercise, providing corroborative evidence for an inflammatory state. Importantly, prior eccentric exercise attenuated the vasodilatory response to mental stress in the calf (i.e. the limb where the exercise had been undertaken). However, exercise did not influence either blood flow responses in the forearm or cardiovascular reactions to stress. Taken together, our findings reveal that the effects of exercise-induced inflammation were confined to the site of muscle damage and were not widespread. Thus, we show here that eccentric exercise is capable of producing local rather than systemic inflammatory effects.

The effect of eccentric exercise-induced inflammation was specific to the measure of vascular function, as no changes in any of the cardiovascular measures (HR, SBP, DBP or CO) were observed. This provides further evidence that inflammation may not alter these variables at rest or in response to stress, as has been observed in other studies (Paine et al., 2012a). As the current study design was counter balanced and as no differences in the perception of the stress task were observed, the lack of any cardiovascular changes cannot be attributed to habituation to the task. Indeed, habituation is not a characteristic of this stress task (Paine et al., 2012a; Veldhuijzen van Zanten et al., 2009, 2005; Willemsen et al., 1998).

The attenuated calf blood flow responses to mental stress in the exercise-based exercise condition may be attributable to several

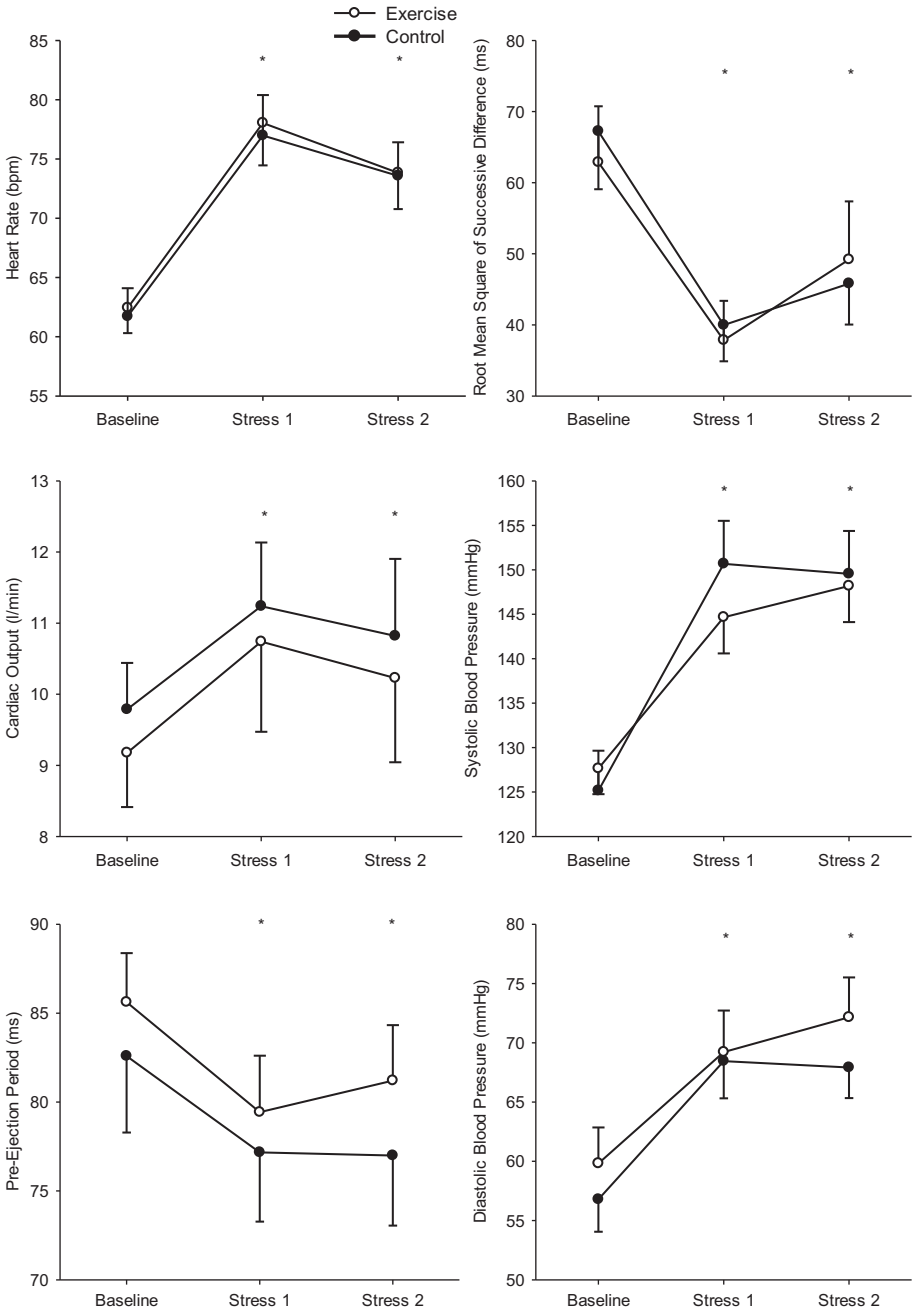

Fig. 3. Mean ± SE responses to mental stress for HR, CO, PEP, HRV, SBP and DBP. * represents significantly different from baseline ($p < .05$).

mechanisms. For example, sympathetic activation has been demonstrated to be involved in the vasodilatory response to mental stress (Lindqvist et al., 1997, 1999). However, no changes in cardiac sympathetic activation (reflected by a reduction in the cardiac preelection period) or parasympathetic withdrawal (reflected by our reduced measure of heart rate variability) were evident between the exercise and control conditions, and therefore it is unlikely that the attenuated dilation demonstrated here could be attributed to changes in sympathetic activation or parasympathetic withdrawal. In addition, the stress-induced changes in cardiac sympathetic and parasympathetic activity in both the control and exercise condition were not associated with changes in stress-induced blood flow. However, local sympathetic activation was not measured in this study, and as such the role of local sympathetic activation in stress-induced vasodilation at the site of the vasculature remains to be determined.

Another putative mechanism concerns NO. Its availability is the most consistently cited mechanism for stress-induced vasodilation (Joyner and Dietz, 2003; Joyner and Casey, 2009), with previous research establishing that blocking NO production results in an attenuated stress-induced vasodilation (Cardillo et al., 1997; Dietz et al., 1994; Joyner and Dietz, 2003; Joyner and Casey, 2009; Sarabi and Lind, 2001). Increases in blood flow in response to mental stress occur through up-regulation of eNOS in the vascular endothelium (due to increases in shear stress), which consequently increase NO availability (Nishida et al., 1992). Moreover, inflammation has been shown to reduce endothelial function at rest via reduced NO availability (Clapp et al., 2004). Unfortunately, to test the effect of inflammation on NO, direct measurements of NO would be needed, which are notoriously difficult and unreliable (Wadley et al., 2012) and therefore the direct influence of inflammation on NO *in vivo* must remain speculative. Indeed, it is cur-

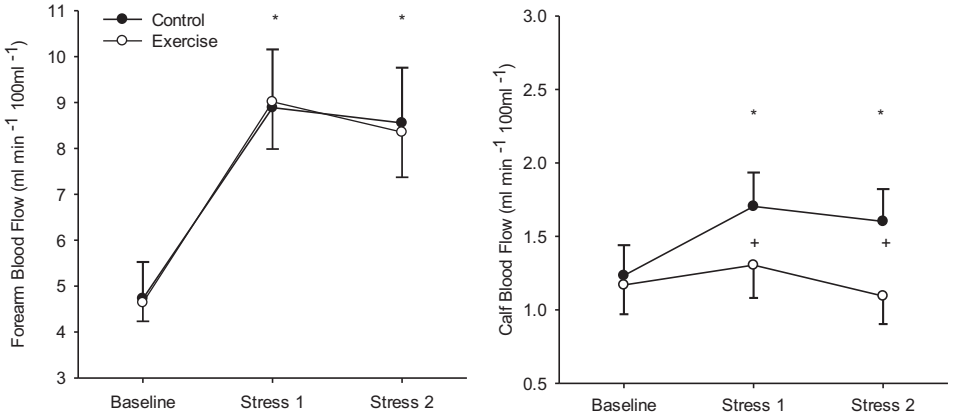

Fig. 4. Mean ± SE responses to mental stress for FBF and CBF. * indicates different from baseline ($p < .05$); +represents significantly different between conditions ($p < .05$).

rently not known if inflammation affects NO uptake or usability. NO levels may be lowered through enhanced NO removal through increases in oxidative stress (Gao et al., 2007), reductions in eNOS activation (Goodwin et al., 2007), or eNOS uncoupling (Rabelink and van Zonneveld, 2006). Elevations in oxidative stress, as a consequence of systemic inflammation (Forstermann, 2008), would lead to increased synthesis of reactive oxidative species (ROS) in the vascular wall and reduced NO (Forstermann, 2008). Eccentric exercise has previously resulted in increases in markers of oxidative stress such as free radicals, glutathione, lipids and protein oxidation (Nikolaidis et al., 2008; Theodorou et al., 2011), as well as increases in intra-muscular TNF-α (Peake et al., 2005b), which contributes to increases in oxidative stress (Zhang et al., 2009). Increases in superoxide production and reactive oxidative species (ROS) can lead to reduction in BH_4 (Cosentino and Luscher, 1999; Sindler et al., 2009; Vásquez-Vivar et al., 1998), which enhances eNOS uncoupling and reduces NO production. To our knowledge, little is known about the effects of acute eccentric exercise with BH_4. However, increases in vaccine-induced increases in inflammation can reduce BH_4 availability, and result in endothelial dysfunction (Antoniades et al., 2011). Therefore, a reduction in BH_4 availability is important, given the roles of BH_4 in maintaining endothelial function and protecting the vasculature from inflammation induced endothelial dysfunction (Antoniades et al., 2011). BH_4 is critical to NO production through other pathways such as eNOS activation, where L-arginine is converted to produced NO (van Zonneveld et al., 2010; Zhang, 2008), can only occur in the presence of BH_4 (Delp et al., 2008; Sindler et al., 2009). Therefore, a reduction in L-arginine would result in decreases in eNOS activation (Darley-Usmar et al., 1995; Förstermann and Münzel, 2006; Morris, 2000). Increases in inflammatory markers *in vitro* have been demonstrated to reduce L-arginine in aortic endothelial cells, and thus may be a mechanism for reduced NO (Goodwin et al., 2007; Zhang et al., 2009). However, despite increases in inflammation reducing NO levels through these outline pathways, the methodological issues with measuring NO in blood (Wadley et al., 2012) mean that these must remain speculative, and require further investigation.

In contrast to other protocols that have been used to determine the effects of inflammation on vascular responses to stress (Paine et al., 2012a), the current eccentric exercise protocol attenuated vascular response in the calf but not in the arm. The discrepant findings could be due to the different methods used to induce inflammation; vaccination versus eccentric exercise. The inflammatory response to vaccination occurs in response to the bacterial infection which is injected into the muscle, with the innate immune system instigating the response (Medzhitov, 2008). In contrast, eccentric exercise results in a more substantial localised inflammatory response (Bruunsgaard et al., 1997; Willoughby et al., 2003), than the response that occurs to vaccination (Medzhitov, 2008), and induces a localised inflammatory response as a result of myofibril damage and disruption of sarcomere excitation-coupling (Nosaka et al., 2002; Proske and Morgan, 2001). Increases in leukocytes in response to leg eccentric exercise have been demonstrated as soon as one hour posts exercise (Paulsen et al., 2005). As more is known about the inflammatory response to infection than to tissue injury (Medzhitov, 2008), the exact mechanisms through which muscle damage can induce an inflammatory response currently remain speculative.

The current study demonstrated an increase in the markers that indicate a localised inflammatory response (i.e., limb circumference and pain). Thus, the differences in the inflammatory responses to vaccination and exercise may be the reasons for the attenuation in blood flow, which was only evident at the site proximal to the site of the inflammatory stimulus (i.e., the leg muscles). However, it should be noted that IL-6 increased, indicating that a systemic response to eccentric exercise was also observed in addition to these local indicators of inflammation. This finding is in line with previous research (Edwards et al., 2007, 2010; Hirose et al., 2004). Thus, even though it is acknowledged that the inflammatory response to eccentric exercise is induced locally, an increase in inflammation is also detected systemically. This is in line with the work conducted in dental surgery, where an invasive local procedure results in a systemic increase in inflammation (D'Aiuto et al., 2005; Graziani et al., 2010; Tonetti et al., 2007). Interestingly, the incidence of acute cardiac events is increased following a dental procedure (Minassian et al., 2010), which is most likely due to the increase in inflammation.

To the best of our knowledge, only one study has examined the effects of acute eccentric exercise on the vasculature. This study revealed that both arm and leg eccentric exercise induced significant increases in pulse wave velocity, measured between the carotid and femoral artery, indicative of increases in arterial stiffness (Barnes et al., 2010). However, these increases were only relatively small (3–5% changes), which suggests that greater increases might have been detected if the vascular assessment was undertaken at a site more proximal to the source of inflammation.

The differences in the calf and forearm blood flow responses to stress observed in the current study may not be as a result of the inflammation and other changes evoked by eccentric exercise. Indeed, as other studies have also noted differences in the FBF and CBF responses to stress (Carter et al., 2005; Paine et al., 2012a). Such limb-related differences in blood flow have been hypothesised to be due to differences between vascular beds, with further

evidence of differences in the responses to stress of different organs (Hayashi et al., 2006). Likewise, limb vascular responses have been found to be unrelated to systemic vascular responses to stress (Paine et al., 2012b), and thus the observed differences among measures collected from the different areas of the vasculature may be an alternative reason for the differences in calf and forearm blood flow observed in this study.

No associations were observed between IL-6 and the vascular responses to stress. IL-6 was chosen as a marker of inflammation due its role in altering NO availability *in vitro* (Hung et al., 2010), as well as its prominent appearance after eccentric exercise (Croisier et al., 1999; Depner et al., 2008; Jackman et al., 2010; Miles et al., 2008; Steensberg et al., 2002, 2000; Willoughby et al., 2003). Given that the peak IL-6 response is most commonly reported to be at 6 h post eccentric exercise (MacIntyre et al., 2001; Paulsen et al., 2005; Philippou et al., 2009; Willoughby et al., 2003), associations between IL-6 and vascular measures taken in this study were examined when IL-6 levels were maximal. Associations between inflammatory markers and SBP have been demonstrated in a middle aged population in response to aerobic exercise (Hamer and Steptoe, 2012), however, the lack of associations between IL-6 and vascular responses to mental stress have been reported in another study (Paine et al., 2012a). Therefore, IL-6 increases may be representative of an inflamed state rather than being a direct cause for the attenuated stress-induced vascular responses.

Another reason for the lack of association between inflammation and the blood flow responses to stress may be down to the choice of inflammatory marker. Indeed, it is important to note that only IL-6 was assessed as an indication of increased inflammation and this could be considered a potential limitation. IL-6 was chosen for two reasons. First, the appearance of IL-6 in response to exercise should indicate the presence of other unmeasured pro-inflammatory cytokines, such as TNF-α and IL-1, since this key inflammatory-responsive cytokine is an important mediator of the acute inflammatory phase response (Moldoveanu et al., 2001). IL-6 also induces anti-inflammatory effects, such as increasing the production of C-reactive protein (CRP) (Ekstrom et al., 2008; Möller and Villiger, 2006), and thus is a suitable marker to indicate increases in inflammation. Second, IL-6 has been consistently shown to be present in the blood following eccentric exercise. The evidence for systemic increases in other inflammatory markers is more equivocal in contrast to their increases when measured intra-muscularly (e.g., TNF-α (Peake et al., 2005a; Ross et al., 2010; Steensberg et al., 2002)). Moreover, evidence shows that these other cytokines are frequently not elevated 6 h post exercise (e.g., CRP (Paulsen et al., 2005; Barnes et al., 2010)). Finally, a recent review suggested that concluded that increases in IL-6 in response to exercise may also be as a result of skeletal muscle contractions (Pedersen and Febbraio, 2012), in addition to the inflammatory response.

Despite this mixed evidence, a fuller investigation into the effects of other inflammatory cytokines on vascular responses to stress seems warranted. For example, previous observational studies have seen associations between inflammation (as indexed by CRP) and vascular function at rest (e.g., (Cleland et al., 2000; Verma et al., 2002, 2004). Moreover, the greatest increases in total vascular resistance in response to stress were demonstrated in patients with the greatest levels of CRP (Veldhuijzen van Zanten et al., 2008), while basal CRP levels have been linked to the presence of mental stress-induced ischaemia (Shah et al., 2006). However, elevations in CRP have only been noted at least 24 h post eccentric exercise (Barnes et al., 2010; Paulsen et al., 2005), and, therefore, we expected that CRP would not have been elevated 6 h post exercise, which was the time point at which the stress task was conducted. Indeed, IL-6 increases occur more quickly than CRP and can stimulate CRP production (Zakynthinos and Pappa, 2009). Whilst CRP is an acute phase protein, it has also been used in studies as a marker to indicate chronic levels of inflammation, and may therefore be indicative of an inflamed state (Libby, 2006). However, recently published large-scale studies have questioned the role of CRP as a predictor of cardiovascular disease (Emerging Risk Factors Collaboration et al., 2010; Heart Disease Genetics Collaboration CRPC, 2011), and thus the utility of CRP as a predictor of the responses to stress has still to be determined.

In conclusion, eccentric exercise-induced inflammation induced an attenuation of the vasodilatory response to mental stress. This key finding is similar to that found in a previous study that used vaccination to cause inflammation (Paine et al., 2012a). Here, the effect of prior exercise on the vasculature was only seen in the limb that had performed the exercise, indicating a localised effect of inflammation. However, even though inflammation was induced locally, an increase in systemic inflammation was detected. This is in line with evidence in dental studies, where a dental procedure locally induced inflammation that caused systemic inflammation, which is associated with an increased risk for acute cardiac events (D'Aiuto et al., 2005; Graziani et al., 2010; Minassian et al., 2010; Tonetti et al., 2007). The findings of the current study indicate that inflammation plays a role in influencing the vascular responses to stress. Given that elevated inflammation is a risk factor for myocardial infarction, the responses of the vasculature to mental stress observed during elevated levels of inflammation may represent a pathway through which mental stress could trigger myocardial infarction. Future studies assessing the effect of experimentally induced inflammation on stress-induced myocardial ischaemia are necessary to confirm this. In addition, future research should continue to explore the mechanisms through which elevations in inflammation may attenuate the vascular responses to stress. Studies designed to examine the effects of reducing inflammation on vascular responses to stress are now required particularly given evidence that the effects of acute inflammation on resting vascular function are reversible (Bhagat and Vallance, 1997).

Acknowledgments

The authors would like to acknowledge the invaluable help of Matthew Fergusson, Sarah Laight and Luke Orchard with recruitment and data acquisition.

References

Antoniades, C., Cunnington, C., Antonopoulos, A., Neville, M., Margaritis, M., Demosthenous, M., Bendall, J., Hale, A., Cerrato, R., Tousoulis, D., Bakogiannis, C., Marinou, K., Toutouza, M., Vlachopoulos, C., Leeson, P., Stefanadis, C., Karpe, F., Channon, K.M., 2011. Induction of vascular GTP-Cyclohydrolase I and endogenous tetrahydrobiopterin synthesis protect against inflammation-induced endothelial dysfunction in human atherosclerosis/clinical perspective. Circulation 124, 1860–1870.

Babyak, M.A., Blumenthal, J.A., Hinderliter, A., Hoffman, B., Waugh, R.A., Coleman, R.E., Sherwood, A., 2010. Prognosis after change in left ventricular ejection fraction during mental stress testing in patients with stable coronary artery disease. Am. J. Cardiol. 105, 25–28.

Barnes, J.N., Trombold, J.R., Dhindsa, M., Lin, H.f., Tanaka, H., 2010. Arterial stiffening following eccentric exercise-induced muscle damage. J. Appl. Physiol. 109, 1102–1108.

Bergovec, M., Mihatov, S., Prpic, H., Rogan, S., Batarelo, V., Sjerobabski, V., 1992. Acute myocardial infarction among civilians in Zagreb city area. Lancet 339, 303.

Bhagat, K., Vallance, P., 1997. Inflammatory cytokines impair endothelium-dependent dilatation in human veins *in vivo*. Circulation 96, 3042–3047.

Blair, D.A., Glover, W.E., Greenfield, A.D.M., Roddie, I.C., 1959. Excitation of cholinergic vasodilator nerves to human skeletal muscles during emotional stress. J. Physiol. 148, 633–647.

Bruunsgaard, H., Galbo, H., Halkjaer-Kristensen, J., Johansen, T.L., MacLean, D.A., Pedersen, B.K., 1997. Exercise-induced increase in serum interleukin-6 in humans is related to muscle damage. J. Physiol. 499, 833–841.

Burg, M.M.P., Graeber, B.M., Vashist, A.M., Collins, D.S., Earley, C.M., Liu, J.B., Lampert, R.M., Soufer, R.M., 2009. Noninvasive detection of risk for emotion provoked myocardial ischaemia. [article]. Psychosom. Med. 71, 14–20.
Cardillo, C., Kilcoyne, C.M., Quyyumi, A.A., Cannon, R.O., Panza, J.A., 1997. Role of nitric oxide in the vasodilator response to mental stress in normal subjects. Am. J. Cardiol. 80, 1070–1074.
Carroll, D., Ebrahim, S., Tilling, K., Macleod, J., Smith, G.D., 2002. Admissions for myocardial infarction and World Cup football: database survey. BMJ 325, 1439–1442.
Carter, J.R., Kupiers, N.T., Ray, C.A., 2005. Neurovascular responses to mental stress. J. Physiol. 564, 321–327.
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.
Cleland, S.J., Sattar, N., Petrie, J.R., Forouhi, N.G., Elliott, H.L., Connell, J.M., 2000. Endothelial dysfunction as a possible link between C-reactive protein levels and cardiovascular disease. Clin. Sci. 98, 531–535.
Cosentino, F., Luscher, T.F.M., 1999. Tetrahydrobiopterin and endothelial nitric oxide synthase activity. Cardiovasc. Res. 43, 274–278.
Croisier, J.L., Camus, G., Venneman, I., Deby-Dupont, G., Juchmès-Ferir, A., Lamy, M., Crielaard, J.M., Deby, C., Duchateau, J., 1999. Effects of training on exercise-induced muscle damage and interleukin 6 production. Muscle Nerve 22, 208–212.
D'Aiuto, F., Parkar, M., Tonetti, M.S., 2005. Periodontal therapy: a novel acute inflammatory model. Inflamm. Res. 54, 412–414.
Darley-Usmar, V., Wiseman, H., Halliwell, B., 1995. Nitric oxide and oxygen radicals: a question of balance. FEBS Lett. 369, 131–135.
de Geus, E.J.C., Willemsen, G.H.M., Klaver, C.H.A.M., van Doornen, L.J.P., 1995. Ambulatory measurement of respiratory sinus arrhythmia and respiration rate. Biol. Psychol. 41, 205–227.
De Martin, R., Hoeth, M., Hofer-Warbinek, R., 2000. The transcription of NF-kappa B and the regulation of vascular cell function. Arterioscler. Thromb. Vasc. Biol. 20, E83–E88.
Delp, M.D., Behnke, B.J., Spier, S.A., Wu, G., Muller-Delp, J.M., 2008. Ageing diminishes endothelium-dependent vasodilatation and tetrahydrobiopterin content in rat skeletal muscle arterioles. J. Physiol. 586, 1161–1168.
Depner, C.M., Gordon, L.K., Kidd, J.R., Miles, M.P.F., 2008. IL-6 bio-availability varies with basal crp concentration and is unaffected by eccentric exercise. Med. Sci. Sports Exerc. 40, S433.
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., Allen, L.M., McPhee, J.S., Bosch, J.A., Carroll, D., Drayson, M., Ring, C., 2007. Eccentric exercise as an adjuvant to influenza vaccination in humans. Brain Behav. Immun. 21, 209–217.
Edwards, K.M., Campbell, J.P., Ring, C., Drayson, M.T., Bosch, J.A., Downes, C., Long, J.E., Lumb, J.A., Merry, A., Paine, N.J., Burns, V.E., 2010. Exercise intensity does not influence the efficacy of eccentric exercise as a behavioural adjuvant to vaccination. Brain Behav. Immun. 24, 623–630.
Ekstrom, M., Eriksson, P., Tornvall, P., 2008. Vaccination, a human model of inflammation, activates systemic inflammation but does not trigger proinflammatory gene expression in adipose tissue. J. Intern. Med. 264, 613–617.
Emerging Risk Factors Collaboration, Kaptoge, S., Di Angelantonio, E., Lowe, G., Pepys, M.B., Thompson, S.G., Collins, R., Danesh, J., 2010. C-reactive protein concentration and risk of coronary heart disease stroke and mortality: an individual participant meta-analysis. Lancet 375, 132–140.
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., Schächinger, V., Dimmeler, S., Zeiher, A.M., 2004. C-reactive protein levels determine systemic nitric oxide bioavailability in patients with coronary artery disease. Eur. Heart J. 25, 1412–1418.
Forstermann, U., 2008. Oxidative stress in vascular disease: causes, defense mechanisms and potential therapies. Nat. Clin. Pract. Cardiovasc. Med. 5, 338–349.
Förstermann, U., Münzel, T., 2006. Endothelial nitric oxide synthase in vascular disease. Circulation 113, 1708–1714.
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 ischaemia study (PIMI). [article]. 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.
Graziani, F., Cei, S., Tonetti, M., Paolantonio, M., Serio, R., Sammartino, G., Gabriele, M., D'Aiuto, F., 2010. Systemic inflammation following non-surgical and surgical periodontal therapy. J. Clin. Periodontol. 37, 848–854.
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., 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.
Hamer, M., Steptoe, A., 2012. Vascular inflammation and blood pressure response to acute exercise. Eur. J. Appl. Physiol. 112, 2375–2379.
Hansson, G.K., 2005. Inflammation, atherosclerosis, and coronary artery disease. N. Engl. J. Med. 352, 1685–1695.
Hayashi, N., Someya, N., Endo, M.Y., Miura, A., Fukuba, Y., 2006. Vasoconstriction and blood flow responses in visceral artieries to mental task in humans. Exp. Physiol. 91, 215–220.
Heart Disease Genetics Collaboration CRPC, 2011. Association between C reactive protein and coronary heart disease: mendelian randomisation analysis based on individual participant data. BMJ 342, d548.
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.
Hirose, L., Nosaka, K., Newton, M., Laveder, A., Kano, M., Peake, J., Suzuki, K., 2004. Changes in inflammatory mediators following eccentric exercise of the elbow flexors. Exerc. Immunol. Rev. 10, 75–90.
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.
Jackman, S.R., Witard, O.C., Jeukendrup, A.E., Tipton, K.D., 2010. Branched-chain amino acid ingestion can ameliorate soreness from eccentric exercise. Med. Sci. Sports Exerc. 42, 962–970.
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., Dietz, N.M., 2003. Sympathetic vasodilation in human muscle. Acta Physiol. Scand. 177, 329–336.
Joyner, M.J., Halliwill, J.R., 2000. Sympathetic vasodilatation in human limbs. J. Physiol. 526 (Pt 3), 471–480.
Joyner, M.J., Casey, D.P., 2009. The catecholamines strike back: what NO does not do. Circ. J. 73, 1783–1792.
Kharbanda, R.K., Walton, B., Allen, M., Klein, N., Hingorani, A.D., MacAllister, R.J., Vallance, P., 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.
Krantz, D.S., Santiago, H.T., Kop, W.J., Bairey Merz, C.N., Rozanski, A., Gottdiener, J.S., 1999. Prognostic value of mental stress testing in coronary artery disease. Am. J. Cardiol. 84, 1292–1297.
Leor, J., Kloner, R.A., The, I., 1996a. The Northridge earthquake as a trigger for acute myocardial infarction. Am. J. Cardiol. 77, 1230–1232.
Leor, J., Poole, K., Kloner, R.A., 1996b. Sudden cardiac death triggered by an earthquake. N. Engl. J. Med. 334, 413–419.
Levy, L., Fautrel, B., Barnetche, T., Schaeverbeke, T., 2008. Incidence and risk of fatal myocardial infarction and stroke events in rheumatoid arthritis patients. A systematic review of the literature. Clin. Exp. Rheumatol. 26, 673–679.
Libby, P., 2006. Inflammation and cardiovascular disease mechanisms. Am. J. Clin. Nutr. 83, 456S–460S.
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.
Lindqvist, M., Melcher, A., Hjemdahl, P., 1999. Flow dependence of forearm noradrenaline overflow, as assessed during mental stress and sodium nitroprusside infusion. [article]. J. Hypertens. 17, 91–97.
MacIntyre, D.L., Sorichter, S., Mair, J., Berg, A., McKenzie, D.C., 2001. Markers of inflammation and myofibrillar proteins following eccentric exercise in humans. Eur. J. Appl. Physiol. 84, 180–186.
Medzhitov, R., 2008. Origin and physiological roles of inflammation. Nature 454, 428–435.
Meier, C.R., Jick, S.S., Derby, L.E., Vasilakis, C., Jick, H., 1998. Acute respiratory-tract infections and risk of first-time acute myocardial infarction. Lancet 351, 1467–1471.
Meisel, S.R., Dayan, K.I., Pauzner, H., Chetboun, I., Arbel, Y., David, D., Kutz, I., 1991. Effect of Iraqi missile war on incidence of acute myocardial infarction and sudden death in Israeli civilians. Lancet 338, 660–661.
Melzack, R., 1987. The short-form McGill pain questionnaire. Pain 30, 191–197.
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.
Miles, M.P., Andring, J.M., Pearson, S.D., Gordon, L.K., Kasper, C., Depner, C.M., Kidd, J.R., 2008. Diurnal variation, response to eccentric exercise, and association of inflammatory mediators with muscle damage variables. J. Appl. Physiol. 104, 451–458.
Minassian, C., D'Aiuto, F., Hingorani, A.D., Smeeth, L., 2010. Invasive dental treatment and risk for vascular events-a self-controlled case series. Ann. Intern. Med. 153, 499–506.

Moldoveanu, A.I., Shephard, R.J., Shek, P.N., 2001. The cytokine response to physical activity and training. Sports Med. 31, 115–144.
Möller, B., Villiger, P., 2006. Inhibition of IL-1, IL-6, and TNF-α in immune-mediated inflammatory diseases. Springer Semin. Immunopathol. 27, 391–408.
Morris, S., 2000. Regulation of arginine availability and its impact on NO synthesis. In: Ignarro, L.J. (Ed.). San Diego, pp. 187–197.
Nikolaidis, M.G., Jamurtas, A.Z., Paschalis, V., Fatouros, I.G., Koutedakis, Y., Kouretas, D., 2008. The effect of muscle-damaging exercise on blood and skeletal muscle oxidative stress: magnitude and time-course considerations. Sports Med. 38, 579–606.
Nishida, K., Harrison, D.G., Navas, J.P., 1992. Molecular cloning and characterization of constitutive bovine aortic endothelial cell nitric oxide synthase. J. Clin. Inest. 90, 2092–2096.
Nosaka, K., Newton, M., Sacco, P., 2002. Muscle damage and soreness after endurance exercise of the elbow flexors. [article]. Med. Sci. Sports Exerc. 34, 920–927.
Ostrowski, K., Rohde, T., Zacho, M., Asp, S., Pedersen, B.K., 1998. Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. J. Physiol. 508, 949–953.
Paine, N.J., Bosch, J.A., Aldred, S., Ring, C., Drayson, M., Veldhuijzen van Zanten, J.J.C.S., 2012a. The effect of inflammation on vascular responses to mental stress. Psychosom. Med. 74 (3), A-67 (Ref Type: Abstract).
Paine, N.J., Bosch, J.A., McIntyre, D., Ring, C., Veldhuijzen van Zanten, J.J.C.S., 2012b. Effects of mental stress on peripheral vascular resistance is unrelated to systemic vascular resistance. Psychosom. Med. 74 (3), A-57 (Ref Type: Abstract).
Paine, N.J., Bosch, J.A., Veldhuijzen van Zanten, J.J.C.S., 2012c. Inflammation and vascular responses to acute mental stress: implications for the triggering of myocardial infarction. Curr. Pharm. Des. 18, 1494–1501.
Paulsen, G., Benestad, H.B., Strom-Guidersen, I., Morkrid, L., Lappegard, K.T., Raastad, T., 2005. Delayed leukocytosis and cytokine response to high-force eccentric exercise. [article]. Med. Sci. Sports Exerc. 37, 1877–1883.
Peake, J., Nosaka, K., Suzuki, K., 2005a. Characterization of inflammatory responses to eccentric exercise in humans. Exerc. Immunol. Rev. 11, 64–85.
Peake, J.M., Suzuki, K., Wilson, G., Hordern, M., Nosaka, K., MacKinnon, L., Coombes, J.S., 2005b. Exercise-induced muscle damage, plasma cytokines, and markers of neutrophil activation. [article]. Med. Sci. Sports Exerc. 37, 737–745.
Pedersen, B.K., Febbraio, M.A., 2012. Muscles, exercise and obesity: skeletal muscle as a secretory organ. Nat. Rev. Endocrinol. 8, 457–465.
Philippou, A., Bogdanis, G., Maridaki, M., Halapas, A., Sourla, A., Koutsilieris, M., 2009. Systemic cytokine response following exercise-induced muscle damage in humans. Clin. Chem. Lab. Med. 47, 777–782.
Proske, U., Morgan, D.L., 2001. Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. J. Physiol. 537, 333–345.
Rabelink, T.J., van Zonneveld, A.J., 2006. Coupling eNOS uncoupling to the innate immune response. Arterioscler. Thromb. Vasc. Biol. 26, 2585–2587.
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.
Ross, M.L., Halson, S.L., Suzuki, K., Garnham, A., Hawley, J.A., Cameron-Smith, D., Peake, J.M., 2010. Cytokine responses to carbohydrate ingestion during recovery from exercise-induced muscle injury. J. Interferon Cytokine Res. 30, 329–337.
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.
Sarabi, M., Lind, L., 2001. Mental stress opposes endothelium-dependent vasodilation in young healthy individuals. Vasc. Med. 6, 3–7.
Shah, R., Burg, M., Vashist, A.M., Collins, D.S., Liu, J.B., Jadbabaie, F., Graeber, B.M., Earley, C.M., Lampert, R.M., Soufer, R., 2006. C-reactive protein and vulnerability to mental-stress-induced myocardial ischaemia. 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.
Sindler, A.L., Delp, M.D., Reyes, R., Wu, G., Muller-Delp, J.M., 2009. Effects of ageing and exercise training on eNOS uncoupling in skeletal muscle resistance arterioles. J. Physiol. 587, 3885–3897.
Sorichter, S., Martin, M., Julius, P., Schwirtz, A., Huonker, M., Luttmann, W., Walterspacher, S., Berg, A., 2006. Effects of unaccustomed and accustomed exercise on the immune response in runners. [article]. Med. Sci. Sports Exerc. 38, 1739–1745.
Steensberg, A., Keller, C., Starkie, R.L., Osada, T., Febbraio, M.A., Pedersen, B.K., 2002. IL-6 and TNF-α expression in, and release from, contracting human skeletal muscle. Am. J. Physiol. Endocrinol. Metab. 283, E1272–E1278.
Steensberg, A., van Hall, G., Osada, T., Sacchetti, M., Saltin, B., Pedersen, B.K., 2000. Production of interleukin-6 in contracting human skeletal muscles can account for the exercise-induced increase in plasma interleukin-6. J. Physiol. 529, 237–242.
Strike, P.C., Steptoe, A., 2005. Behavioral and emotional triggers of acute coronary syndromes: a systematic review and critique. Psychosom. Med. 67, 179–186.
Suzuki, S., Sakamoto, S., Miki, T., Matsuo, T., 1995. Hanshin-Awaji earthquake and acute myocardial infarction. Lancet 345, 981.
Theodorou, A.A., Nikolaidis, M.G., Paschalis, V., Koutsias, S., Panayiotou, G., Fatouros, I.G., Koutedakis, Y., Jamurtas, A.Z., 2011. No effect of antioxidant supplementation on muscle performance and blood redow status adaptations to eccentric training. Am. J. Clin. Nutr. 93, 1373–1383.
Tonetti, M.S., D'Aiuto, F., Nibali, L., Donald, A., Storry, C., Parkar, M., Suvan, J., Hingorani, A.D., Vallance, P., Deanfield, J., 2007. Treatment of periodontitis and endothelial function. N. Engl. J. Med. 356, 911–920.
van Zonneveld, A.J., de Boer, H.C., van der Veer, E.P., Rabelink, T.J., 2010. Inflammation, vascular injury and repair in rheumatoid arthritis. Ann. Rheum. Dis. 69, i57–i60.
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.
Vásquez-Vivar, J., Kalyanaraman, B., Martásek, P., Hogg, N., Masters, B.S.S., Karoui, H., Tordo, P., Pritchard, K.A., 1998. Superoxide generation by endothelial nitric oxide synthase: the influence of cofactors. Proc. Nat. Acad. Sci. 95, 9220–9225.
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., Carroll, D., Ring, C., 2009. Mental stress-induced haemoconcentration in women: effects of menstrual cycle phase. Br. J. Health Psychol. 14, 805–816.
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.
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.
Verma, S., Wang, C.H., Li, S.H., Dumont, A.S., Fedak, P.W.M., Badiwala, M.V., Dhillon, B., Weisel, R.D., Li, R.K., Mickle, D.A.G., Stewart, D.J., 2002. A self-fulfilling prophecy: C-reactive protein attenuates nitric oxide production and inhibits angiogenesis. Circulation 106, 913–919.
Verma, S., Wang, C.H., Lonn, E., Charbonneau, F., Buithieu, J., Title, L.M., Fung, M., Edworthy, S., Robertson, A.C., Anderson, T.J., 2004. Cross-sectional evaluation of brachial artery flow-mediated vasodilation and C-reactive protein in healthy individuals. Eur. Heart J. 25, 1754–1760.
Vita, J.A., Keaney, J.F., Larson, M.G., Keyes, M.J., Massaro, J.M., Lipinska, I., Lehman, B.T., Fan, S., Osypiuk, E., Wilson, P.W.F., Vasan, R.S., Mitchell, G.F., Benjamin, E.J., 2004. Brachial artery vasodilator function and systemic inflammation in the framingham offspring study. Circulation 110, 3604–3609.
Wadley, A., Veldhuijzen van Zanten, J., Aldred, S., 2012. The interactions of oxidative stress and inflammation with vascular dysfunction in ageing the vascular health triad. Age (Epub ahead of print).
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. J. Am. Coll. Cardiol. 55, 637–642.
Willemsen, G., Ring, C., Carroll, D., Evans, P., Clow, A., Hucklebridge, F., 1998. Secretory immunoglogulin A and cardiovascular reactions to mental arithmatic and cold pressor. Psychophysiology 35, 252–259.
Willemsen, G.H.M., De Geus, E.J.C., Klaver, C.H.A.M., Van Doornen, L.J.P., Carroll, D., 1996. Ambulatory monitoring of the impedance cardiogram. Psychophysiology 33, 184–193.
Willoughby, D.S., McFarlin, B., Bois, C., 2003. Interleukin-6 expression after repeated bouts of eccentric exercise. Int. J. Sports Med. 24, 15–21.
Zakynthinos, E., Pappa, N., 2009. Inflammatory biomarkers in coronary artery disease. J. Cardiol. 53, 317–333.
Zhang, C., 2008. The role of inflammatory cytokines in endothelial dysfunction. Basic Res. Cardiol. 103, 398–406.
Zhang, H., Park, Y., Wu, J., Chen, X.P., Lee, S., Yang, J., Dellsperger, K.C., Zhang, C., 2009. Role of TNF-α in vascular dysfunction. Clin. Sci. 116, 219–230.