Increased fibrinogen responses to psychophysiological stress predict future endothelial dysfunction implications for cardiovascular disease?

Elizabeth A Ellins[a,b], D Aled Rees[a], John E Deanfield[c], Andrew Steptoe[d], Julian P Halcox[b]

[a]Neurosciences and Mental Health Research Institute, School of Medicine, Cardiff University, Cardiff, CF24 4HQ, United Kingdom

[b]Institute of Life Sciences, Swansea University Medical School, Swansea University, Singleton Park, Swansea, SA2 8PP, United Kingdom

[c]UCL Institute of Cardiovascular Science, UCL

[d]Institute of Epidemiology and Health Care, UCL

Corresponding author: Julian Halcox Institute of Life Sciences 2, Swansea University Medical School, Swansea University, Singleton Park, Swansea. SA2 8PP.

Tel: +44 (0)1792 602938 Email: J.P.J.Halcox@swansea.ac.uk

Abstract:

Stress influences the risk of cardiovascular disease. Acute mental stress can induce both low-grade inflammation and endothelial dysfunction. The relationship between inflammatory responses to stress and future endothelial function is unexplored. We investigated the relationship between inflammatory responses to an acute mental stress challenge and future endothelial function. Interleukin-6 (IL-6), tumor necrosis factor α (TNFα) and fibrinogen were assessed at baseline, immediately following standardized behavioural tasks and 45 minutes post task in 158 men and women aged 52±3 years. Blood pressure and heart rate responses were also measured. Endothelial function was assessed by flow-mediated dilatation was measured 3 years later. Fibrinogen and IL-6 increased post stress ($p=<0.001$ &0.003) but TNF α was unchanged ($p=0.09$). FMD was lower in participants with the greatest change in fibrinogen assessed at 45 minutes post task ($p=0.006$). An independent negative association between FMD and change in fibrinogen at 45 minutes ($\beta=-0.047$ $p=0.016$) remained after multiple adjustment (baseline fibrinogen, baseline diameter, reactive hyperaemia, age, gender and other cardiovascular risk factors). There was no association between FMD and change in IL-6 or TNFα. Participants were divided into four groups by adverse and normal lipid profile and above and below median fibrinogen response. FMD differed between the four groups ($F=3.71$ $p=0.013$) after adjustment for baseline fibrinogen, baseline diameter and reactive hyperaemia. Post-hoc testing showed that those with an above median fibrinogen response had lower FMD than those with a below median fibrinogen response regardless of lipid status. We conclude that elevated fibrinogen responses to stress are associated with future endothelial dysfunction which may reflect increased cardiovascular risk.

Keywords: Fibrinogen; flow-mediated dilatation; psychophysiological stress; lipids

Highlights:

- Participants with elevated recovery fibrinogen in response to a stress challenge had poorer endothelial function 3 years later
- The relationship remained after adjustment for cardiovascular risk factors
- The presence of mild dyslipidaemia did not influence the association between fibrinogen response and endothelial function

1. Introduction:

There is substantial evidence for an association between psychosocial stress and the development of cardiovascular disease, which has led to it being considered as an important cardiovascular risk factor (Everson-Rose and Lewis, 2005; Steptoe and Kivimaki, 2013; Yusuf et al., 2004). However, despite recent advances, the pathophysiological pathways connecting the two are not yet fully understood.

Inflammation plays a key role in the initiation, development and destabilisation of atherosclerotic plaques (Hansson et al., 2015). Low grade systemic inflammation is associated with adverse cardiovascular risk in those with and without cardiovascular disease (Liuzzo et al., 1994; Ridker et al., 1997). Acute stressors trigger inflammatory responses which may play a role in the pathogenesis of cardiovascular disease (Steptoe and Brydon, 2009; Steptoe et al., 2007).

Endothelial function is a well-established measure of vascular health. Flow-mediated dilatation (FMD), a non-invasive measure of endothelial function, is diminished in the presence of traditional cardiovascular risk factors and also in the setting of inflammatory conditions (Celermajer et al., 1994; Celermajer et al., 1992; Di Minno et al., 2015; Woo et al., 2004). Furthermore, both acute inflammation, for example post vaccination, and acute mental stress have been shown to cause transient endothelial dysfunction in otherwise healthy people (Ghiadoni et al., 2000; Hingorani et al., 2000; Spieker et al., 2002). We have also previously shown that those with the most pronounced inflammatory responses (interleukin-6 [IL-6], tumor necrosis factor α [TNF α] and fibrinogen) to acute mental stress had greater arterial stiffness and increases in ambulatory systolic blood pressure when assessed three years later (Brydon and Steptoe, 2005; Ellins et al., 2008). Therefore the inflammatory response to acute

mental stress characterised in this way could serve as an individual biomarker of risk for the development of endothelial dysfunction and subsequent cardiovascular events.

Dyslipidaemia is associated with increased cardiovascular risk and endothelial dysfunction as well as a raised inflammatory profile (Andersson et al., 2014; Celermajer et al., 1992; Ueland et al., 2006). The relationships between lipids and stress responses are not yet fully understood. In particular, it is not yet clear how the stress response might contribute to dyslipidaemia or conversely if an unfavourable lipid profile enhances the inflammatory and haemodynamic responses to stress. There are very few studies which have looked at the immediate response to an acute mental stress challenge on endothelial function in those with pre-existing cardiovascular risk factors and the results have been conflicting. Ghiadoni et al found no change in FMD following a stress task in patients with type 2 diabetes, whilst Cardillo et al also saw no change in NO-dependent vasodilatation as assessed by forearm plethysmography in hypercholesterolaemic subjects, but did see a reduction in vasodilatation in hypertensive patients during mental stress (Cardillo et al., 1998; Ghiadoni et al., 2000) . However, the relationships between inflammatory responses to acute mental stress and future endothelial function, and the potential influence of dyslipidaemia have not been investigated. The aim of this study was (a) to investigate associations between inflammatory responses to acute mental stress and endothelial function assessed at three years, and (b) to evaluate the potential influence of an adverse lipid profile on this association.

2. Materials and Methods:

2.1. Participants

The participants were from the Whitehall II epidemiological cohort who took part in the psychobiology sub-study and underwent psychophysiological testing in 1999-2000 (Marmot et al., 1991; Steptoe et al., 2002). Participants (123 men, 105 women) within the sub-study

were of white European origin, aged 45-59 years, lived in London, and were in full time work, with no history or indicators for coronary heart disease or hypertension. Selection had been stratified by employment grade to ensure a wide range of socio-economic status. 158 participants (52±3 years) underwent endothelial function assessment during Phase 7 of the cohort study, 3 years after psychophysiological stress testing.

2.2 Psychophysiological stress testing

Studies took place in the morning or afternoon in a temperature-controlled laboratory. Participants were asked to refrain from drinking alcohol or exercising on the evening before or the day of testing, and to not drink caffeine or smoke for 2 hours prior to the study. Blood pressure and heart rate were continuously monitored during the study using a Partapress-2 (Finapress Medical Systems, Amsterdam, NL). Participants rested for 30 minutes following the insertion of a cannula for blood sample collection. During the last 5 minutes of the rest period, baseline blood pressure and heart rate were recorded and a baseline blood sample was drawn. Following this, two moderately stressful tasks were administered in a random order with a 5 minute inter-task interval. These tasks (computerized colour-word interference task and mirror tracing) have previously been used in cardiovascular stress research (Jennings et al., 2004). The rationale for using these tasks is explained elsewhere (Steptoe and Marmot, 2002).

The two tasks each lasted 5 minutes. A second blood sample was taken immediately post the second task and participants were left to rest quietly, reading or watching wildlife videos. Two 5 minute post-stress blood pressure and heart rate recordings were made at 15-20 minutes and 40-45 minutes. A final blood sample was taken after 45 minutes. The study was approved by the UCL/UCLH Committee on the Ethics of Human Research.

2.3 *Blood assays*

Blood samples were collected in EDTA tubes and serum gel tubes, and centrifuged immediately at 2500 rpm for 10 min at room temperature. The plasma was removed and stored at -80 ^{o}C until analysis. We have shown in previous studies that fibrinogen responds immediately to psychological stress, while increases in IL-6 and TNFα emerge after 30–45 min (Steptoe et al., 2007; Steptoe et al., 2003). Fibrinogen was therefore assayed from all three samples, whilst IL-6 and TNFα were only assessed from the baseline and 45 min post-stress samples. C-reactive protein (CRP) was measured from baseline samples only. Clottable fibrinogen was measured by an automated Clauss assay in a MDA-180 coagulometer (Oragon Teknika, Cambridge, UK). The coefficient of variation (CV) was <8%. IL-6 and TNFα were measured using high sensitivity two-site ELISAs from R&D Systems (Oxford, UK). The limit of detection of the human TNFα assay was 0.10 pg/ml and intra- and inter-assay CVs were 6.9% and 8.4%. For IL-6, the limit of detection was 0.09 pg/ml, and intra- and inter-assay CVs were 5.3% and 9.2%. CRP was detected using a sensitive, two-site ELISA with antibodies from Dako diagnostics (Ely, Cambs, UK). The inter- and intra-assay CVs were <10%.

The serum was snap frozen at -70^{o}C until analysis. Samples were taken at baseline only. Total cholesterol and triglycerides were measured in a centrifugal analyser by enzymatic colorimetric methods and HDL cholesterol was determined after dextran sulphate-magnesium chloride precipitation of non-HDL cholesterol. LDL was computed using the Friedewald equation.

2.4 Other measures

Height, weight, waist and hip circumference were assessed and used to calculate body mass index (BMI) and waist/hip ratio. Socio-economic status was determined by questionnaire as was smoking status.

2.5 Endothelial function assessment

Flow-mediated dilatation was assessed in the right brachial artery using high resolution ultrasound (Prosound 5500 ALOKA) as previously described (Donald et al., 2006). Analysis of changes in brachial artery diameter was done using automated edge detection software (Brachial Tools, Iowa City, Iowa). FMD was expressed as absolute change in diameter from baseline. Reactive hyperaemia was calculated from the baseline and maximal velocity time integral in the first 15 seconds following cuff release and expressed as a percentage.

2.6 Statistical analysis

SPSS version 20 was used for the analyses. Data were checked for normality and those non-normally distributed variables were transformed using ln transformation. Five participants were excluded from the analysis as they had starting taking lipid or blood pressure medication since undergoing stress testing. Relationships between FMD and conventional risk factors were assessed using partial correlations adjusted for age, gender, baseline diameter and reactive hyperaemia. Haemodynamic and inflammatory responses to psychological stress testing were assessed using repeated measures analysis of variance with trial as the within-subject factor. Post-hoc comparisons were made using Tukey's least significant differences (LSD) test.

For comparison of FMD in those with a high haemodynamic or inflammatory response versus none or low response to mental stress testing, the participants were divided into two

groups by being in the top and bottom 40% of responders and non-responders. Comparisons were made using one-way analysis of variance (ANOVA) and analysis of co-variance (ANCOVA) with adjustments for baseline haemodynamic or inflammatory variable, baseline brachial artery diameter and reactive hyperaemia. Presence of associations between endothelial function and inflammatory responses were also tested using multiple linear regression. Adjustments were made for baseline inflammatory variable, baseline brachial artery diameter, reactive hyperaemia, age, gender, BMI, waist/hip ratio, systolic and diastolic blood pressure, HDL and LDL cholesterol, glucose, CRP, socioeconomic status and smoking.

2.6.1 Dyslipidaemia

Participants were classified as having dyslipidaemia if they met one of the following criteria. Total cholesterol ≥ 6 triglycerides ≥ 1.7 HDL <1 LDL≥ 4 (mmol/L). Comparisons were made between those with and without dyslipidaemia using independent t-tests. To look at the additional influence of the inflammatory responses to the mental stress testing, the population was divided into four groups. Initially they were divided into above and below median inflammatory response and then by their lipid status. The four groups were: dyslipidaemia and high inflammatory response, dyslipidaemia and low inflammatory response, normal lipids and high inflammatory response, normal lipids and low inflammatory response. ANOVA was used for between group comparisons, further analyses were made using ANCOVA with adjustments for baseline inflammatory variable, baseline brachial artery diameter, and reactive hyperaemia.

3. Results:

3.1 Participant characteristics

Table 1 shows the characteristics for those participants included in the study. Males were older with a greater waist/hip ratio, blood pressure, triglycerides, LDL, Total/HDL ratio and brachial artery diameter, whilst HDL was significantly lower ($p<0.03$).

Characteristic	All (n=153)	Male (n=84)	Female (n=69)	p
Age (yrs)	52 ± 3	53 ± 3	52 ± 3	***0.021***
Smoker (Y)	9 (5.9%)	6 (7.1%)	3 (4.2%)	*0.47*
SES				
Higher	60 (39.2%)	34 (40.5%)	26 (37.7%)	*0.84*
Intermediate	50 (32.7%)	28 (33.3%)	22 (31.9%)	
Lower	43 (28.1%)	22 (26.2%)	21 (30.4%)	
BMI (kg/m2)	25.23 ± 3.52	25.36 ± 3.25	25.08 ± 3.85	*0.63*
Waist/hip ratio	0.84 ± 0.09	0.90 ± 0.07	0.78 ±0.06	***<0.001***
SBP(mmHg)	114 ± 12	118 ± 11	109 ± 12	***<0.001***
DBP(mmHg)	69 ± 9	71 ± 9	67 ± 9	***0.005***
HR(bpm)	64 ± 8	64 ± 9	66 ± 8	*0.14*
TC(mmol/L)	5.38 ± 0.87	5.42 ± 0.84	5.32 ± 0.91	*0.47*
Trigs (mmol/L)	1.34 ± 0.71	1.45 ± 0.65	1.21 ± 0.76	***0.003***
HDL (mmol/L)	1.57 ± 0.39	1.43 ± 0.30	1.74 ± 0.41	***<0.001***
LDL (mmol/L)	3.20 ± 0.81	3.34 ± 0.79	3.02 ± 0.82	***0.018***
Total/HDL	3.65 ± 1.13	3.96 ± 1.01	3.24 ± 1.13	***<0.001***
Glucose (mmol/L)	5.30 ± 0.79	5.34 ± 0.72	5.25 ± 0.88	*0.54*
CRP (mg/L)	1.02 ± 1.29	0.92 ± 0.97	1.13 ± 1.59	*0.8*
BL dia (mm)	3.60 ± 0.71	4.02 ± 0.61	3.08 ± 0.41	***<0.001***
FMD (mm)	0.19 ± 0.10	0.19 ± 0.10	0.20 ± 0.10	*0.81*
RH% (%)	658 ± 260	648 ± 249	665 ± 270	*0.61*

Table 1: Participant characteristics for all and by gender

3.2 Associations with conventional risk factors

FMD was correlated with diastolic blood pressure (DBP) and heart rate (HR) following adjustment for age, gender, baseline diameter and reactive hyperaemia (DBP $r= 0.18$ $p=0.037$ & HR $r=0.23$ $p =0.007$). No other conventional risk factors, including systolic blood

pressure, BMI, waist/hip ratio, total cholesterol, LDL, HDL, triglycerides, glucose and CRP, were associated with FMD.

3.3 Responses to acute mental stress

Systolic- and diastolic-BP and HR all increased in response to the mental stress challenge (all p<0.001). Both fibrinogen and IL-6 increased post-stress (p <0.001 & 0.003 respectively). The increase in TNFα levels post stress did not reach significance (p= 0.09) (Table 2).

	Baseline	Stress tasks	Recovery 1	Recovery 2	F	p
SBP (mmHg)	114	138*	119$^{*\neq}$	119$^{*\neq}$	250.3	***<0.001***
DBP (mmHg)	69	83*	73$^{*\neq}$	74$^{*\neq}$	268.3	***<0.001***
HR (bpm)	65	72*	62$^{*\neq}$	63$^{*\neq\circ}$	234.7	***<0.001***
Fbg (g/L)	2.81	2.87*		2.85*	13.4	***<0.001***
IL-6 (pg/ml)	1.12			1.20*	9.2	***0.003***
TNFα (pg/ml)	2.12			2.18	2.9	*0.09*

Table 2: Haemodynamic and inflammatory responses to a psychophysiological challenge. Means ± standard deviation. Values that have different superscripts are significantly different *= from baseline, $^{\neq}$=from task, ^{O}=from recovery1 (p<0.05).

3.4 Change in inflammatory markers and association with future endothelial function

To investigate the influence of the haemodynamic and inflammatory responses to an acute mental stress challenge on future endothelial function, the responses were divided into the highest and lowest 40% of responders for each variable. Changes in heart rate and blood pressure post-stress were not associated with endothelial function (data not shown). However, those participants with the greatest change in fibrinogen at 45 minutes had lower FMD than those with the lowest fibrinogen at 45 minutes post-stress, after adjustment for baseline fibrinogen, baseline arterial diameter and reactive hyperaemia (F (1,113) 7.85 p= 0.006) (Figure 1). There were no associations between change in fibrinogen immediately post task or changes in IL-6 and TNFα with FMD.

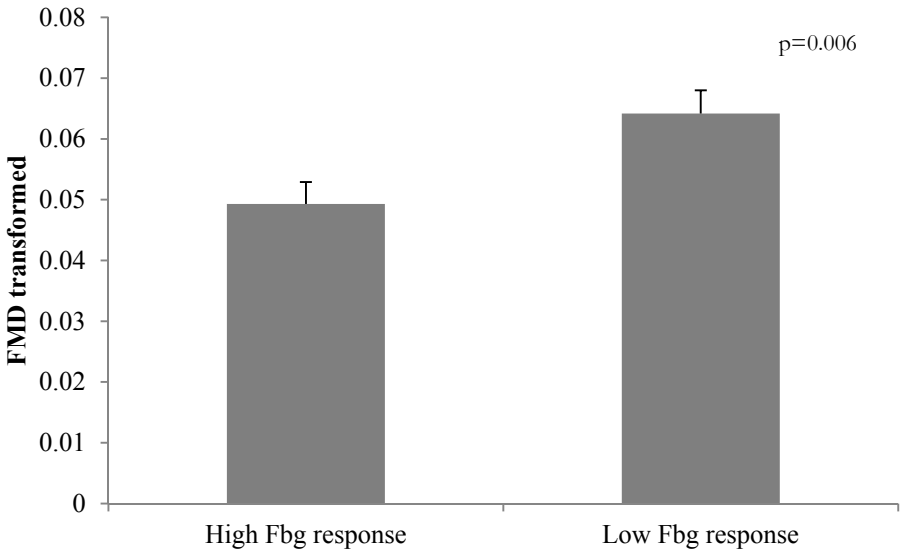

Figure 1: FMD by high and low fibrinogen response 45 minutes after an acute mental stress challenge, adjusted for baseline arterial diameter, baseline fibrinogen and reactive hyperaemia. Data are mean ± standard error.

Multiple linear regression modelling between FMD and inflammatory stress response variables (as continuous variables), demonstrated a significant negative association between FMD and elevated fibrinogen at 45 minutes after adjustment for baseline fibrinogen, baseline arterial diameter, reactive hyperaemia, age, gender, waist/hip ratio, BMI , SBP, DBP, glucose, HDL and LDL (β= -0.046 [0.019] p= 0.017). This association was still present after the addition of socio-economic status and smoking status to the model (β= -0.047 [0.019] p= 0.016), indicating that those participants with a greater change in fibrinogen at 45 minutes had lower FMD 3 years later.

3.5 Dyslipidaemia, inflammatory responses to acute mental stress and endothelial function

62 (21 females) participants met the criteria for dyslipidaemia, with the majority of participants only having one abnormal lipid variable (see supplemental table 1). Those with dyslipidaemia were more likely to be male (p=0.025) and to have greater BMI, waist hip ratio

and CRP levels (p<0.001). There was no difference in FMD between those with and without dyslipidaemia (p=0.75).

There were no differences in haemodynamic and inflammatory responses to the psychophysiological stress challenge between the two groups (p>0.05).

Inflammatory variable used for grouping with presence or absence of dyslipidaemia	Model	FMD	
		F	p
Fbg change	Unadjusted	0.99	*0.4*
	BL Fbg	0.97	*0.41*
	BL dia & RH%	0.73	*0.54*
	BL dia, RH% & BL Fbg	0.77	*0.51*
Fbg 45 change	Unadjusted	1.1	*0.35*
	BL Fbg	1.09	*0.35*
	BL dia & RH%	3.66	***0.014***
	BL dia, RH% & BL Fbg	3.71	***0.013***
IL-6 change	Unadjusted	0.25	*0.86*
	BL IL-6	0.37	*0.78*
	BL dia & RH%	0.65	*0.58*
	BL dia, RH% & BL IL-6	0.76	*0.52*
TNFα change	Unadjusted	0.26	*0.86*
	BL TNFα	0.21	*0.21*
	BL dia & RH%	1.2	*0.31*
	BL dia, RH% & BL TNFα	1.09	*0.36*

Table 3: The effect of dyslipidaemia and inflammatory responses to psychophysiological challenge

To investigate whether the presence of dyslipidaemia might influence the relationship between inflammation and FMD the group were divided into four categories by above and below median inflammatory response and by presence and absence of dyslipidaemia. As shown in table 3 there were no initial differences in FMD for any of the inflammatory variables. Following adjustment for baseline diameter and reactive hyperaemia there was a significant association in FMD and fibrinogen responses at 45 minutes which was maintained following additional adjustment for baseline fibrinogen level, (F (3,139) 3.71 p=0.013). Post

hoc analysis using Bonferroni found that those with a high fibrinogen response without dyslipidaemia had a lower FMD than those with a low fibrinogen response with dyslipidaemia (p=0.022) (figure 2). There was also a borderline significant difference between the two groups without dyslipidaemia with those having a greater fibrinogen response having a lower FMD than those with a low fibrinogen response (p=0.056). No other relationships between inflammatory markers and endothelial function in the presence or absence of dyslipidaemia were seen.

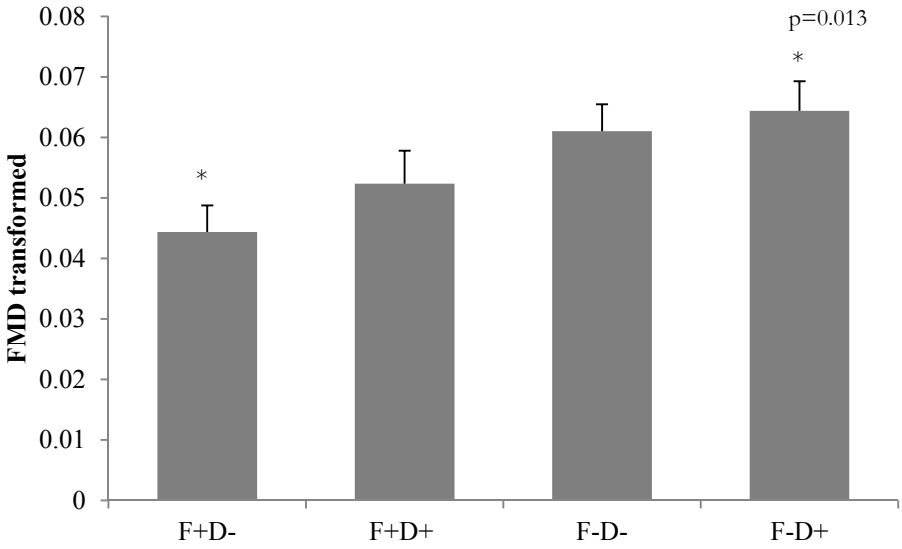

Figure 2: FMD by fibrinogen response at 45 minutes and the presence or absence of dyslipidaemia with adjustment for baseline arterial diameter, baseline fibrinogen and reactive hyperaemia. F+ high (above median) fibrinogen response; F- low fibrinogen (below median) response; D+ dyslipidaemia, D- normal lipids. Data are mean ± standard error

4. Discussion:

The main finding of this study is that those participants who had a higher fibrinogen response to a psychophysiological stress challenge had less well preserved endothelial function when assessed 3 years later following adjustment for key relevant cardiovascular risk factors. This complements our previous analyses in this cohort which found increased arterial stiffness and

ambulatory blood pressure in those participants who had increased inflammatory responses to a stress challenge (Brydon and Steptoe, 2005; Ellins et al., 2008).

Endothelial dysfunction is an early indicator of atherogenesis which can be assessed by FMD. A poor FMD has been associated with numerous cardiovascular risk factors and an increased risk of cardiovascular events (Celermajer et al., 1994; Celermajer et al., 1992; Clarkson et al., 1996; Green et al., 2011; Woo et al., 2004). Acute mental stress causes endothelial dysfunction both during a stress challenge and immediately afterwards but the mechanisms for this are not yet fully understood (Eriksson et al., 2007; Ghiadoni et al., 2000). Inhibition of cortisol and the endothelin-A receptor have both prevented the impairment of FMD induced by mental stress suggesting potential roles for both of these factors (Broadley et al., 2005; Spieker et al., 2002).

Inflammation plays a major role in the initiation, development and progression of atherosclerosis and is associated with endothelial dysfunction (Hansson et al., 2015). Although acute inflammation and acute mental stress have been shown to cause acute endothelial dysfunction there is limited work investigating whether and to what extent the acute inflammatory response to mental stress is implicated in the associated endothelial dysfunction (Clapp et al., 2004; Hingorani et al., 2000). One study by Ghiadoni et al measured the inflammatory markers IL-6, IL-1 & TNFα at baseline and 60 mins post stress challenge but did not see a significant change in these markers or any relationship between cytokine levels and FMD or change in FMD (Ghiadoni et al., 2000). Therefore our study linking fibrinogen at 45 minutes to FMD after three years is the first study to show an association between an inflammatory response to acute mental stress and future endothelial function.

Fibrinogen, an acute phase protein induced by IL-6 in the inflammatory pathway and a major component of the coagulation cascade, is associated with increased risk of coronary heart disease and stroke (Danesh et al., 2005). It has been implicated in the development of vascular dysfunction and atherosclerosis through its effects on plaque composition, blood viscosity, endothelial and smooth muscle cell activation, platelet aggregation and activation, and immune cell recruitment (Lominadze, 2010; Tousoulis et al., 2011).

Increased fibrinogen may affect endothelial function both by mechanical and biochemical processes. Elevated fibrinogen increases blood viscosity which raises shear stress and activates endothelial cells (Davies et al., 2003; Lominadze, 2010; Lowe et al., 1997). This stimulates expression and activation of adhesion molecules and integrins, resulting in the attraction and adhesion of monocytes to endothelial cells and the greater production of vasoconstricting agents which may further affect endothelial function and vascular tone (Languino et al., 1993; Lominadze et al., 2005; Suehiro et al., 1997).

Vascular injury triggers the coagulation cascade which results in fibrinogen being converted to fibrin which in turn forms a thin monolayer covering the damaged area. This layer attracts platelets which are also activated by fibrinogen causing further platelet aggregation, inflammatory responses and endothelial dysfunction. As the injury heals the platelets can also become part of the developing lesion/plaque (Badimon, 2014). Therefore elevated levels of fibrinogen through reactions to acute stressors could further exacerbate this process. The lack of association between IL-6 and future endothelial function may suggest that it is these haemostatic/prothrombotic properties of fibrinogen that could be more important in this setting than its inflammatory properties.

The presence of dyslipidaemia did not appear to have any influence on the haemodynamic or inflammatory responses to an acute mental stress challenge. Although a little surprising, the

severity of dyslipidaemia was only mild. However, when the cohort was divided into presence or absence of dyslipidaemia and high or low inflammatory response to stress, a difference in FMD was noted between the groups categorised according to dyslipidaemia and change in fibrinogen at 45 minutes. However, the differences between the groups seemed to be driven more by the size of the fibrinogen response rather than the presence of dyslipidaemia. Somewhat surprisingly, those with dyslipidaemia had a tendency to better FMD after three years than those without. This finding together with that of Steptoe and Brydon, that a greater increase in total cholesterol in response to stress is predictive of having higher total cholesterol 3 years later, could be suggestive that the stress response may influence future incidence of dyslipidaemia rather than current dyslipidaemia affecting the stress response (Steptoe and Brydon, 2005). It is worth noting that our study participants were generally very healthy and those with dyslipidaemia typically only had mild lipid abnormalities, which may account for why differences were not found between groups. However, another study found that males with LDL cholesterol> 4.1 mmol/l and triglycerides >2.8 mmol/l had similar haemodynamic or lipid responses to stress to those with normal lipid levels, further indicating that dyslipidaemia may well not influence these stress responses (McCann et al., 1995).

There are a number of limitations to this study. Endothelial function was not assessed at the time of stress testing so it is unknown whether those participants with poorer FMD 3 years later already had endothelial dysfunction at the point of stress testing. The population were originally selected for being free of cardiovascular disease so the dyslipidaemia group mainly consists of those with mild lipid abnormalities. More adverse lipid profiles such as in familial hypercholesterolaemia may well give different findings. Additionally, blood samples for the mental stress study were not taken in a fasted state. Therefore, some participants may have

been classified as dyslipidaemic due to elevated triglycerides which may have been within the normal range if the participant had fasted.

In conclusion participants with an elevated fibrinogen response at 45 minutes post-stress had impaired endothelial function 3 years later; this could be a pathway through which stress contributes to the development of cardiovascular disease.

Acknowledgments

This research was supported by the British Heart Foundation.

References

Andersson, C., Lyass, A., Vasan, R.S., Massaro, J.M., D'Agostino, R.B., Robins, S.J., 2014. Long-term risk of cardiovascular events across a spectrum of adverse major plasma lipid combinations in the Framingham Heart Study. The American Heart Journal 168, 878-883.e871.
Badimon, L.a.V.G., 2014. Thrombosis formation on atherosclerotic lesions and plaque rupture. Journal of Internal Medicine 276, 618--632.
Broadley, A.J.M., Korszun, A., Abdelaal, E., Moskvina, V., Jones, C.J.H., Nash, G.B., Ray, C., Deanfield, J., Frenneaux, M.P., 2005. Inhibition of Cortisol Production With Metyrapone Prevents Mental Stress-Induced Endothelial Dysfunction and Baroreflex Impairment. Journal of the American College of Cardiology 46, 344-350.
Brydon, L., Steptoe, A., 2005. Stress-induced increases in interleukin-6 and fibrinogen predict ambulatory blood pressure at 3-year follow-up. J Hypertens 23, 1001-1007.
Cardillo, C., Kilcoyne, C.M., Cannon Iii, R.O., Panza, J.A., 1998. Impairment of the nitric oxide-mediated vasodilator response to mental stress in hypertensive but not in hypercholesterolemic patients. Journal of the American College of Cardiology 32, 1207-1213.
Celermajer, D.S., Sorensen, K.E., Bull, C., Robinson, J., Deanfield, J.E., 1994. Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction. J Am Coll Cardiol 24, 1468-1474.
Celermajer, D.S., Sorensen, K.E., Gooch, V.M., Spiegelhalter, D.J., Miller, O.I., Sullivan, I.D., Lloyd, J.K., Deanfield, J.E., 1992. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherocslerosis. Lancet 340, 1111-1115.
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.
Clarkson, P., Celermajer, D.S., Donald, A.E., Sampson, M., Sorensen, K.E., Adams, M., Yue, D.K., John Betteridge, D., Deanfield, J.E., 1996. Impaired vascular reactivity in insulin-dependent diabetes mellitus is related to disease duration and low density lipoprotein cholesterol levels. Journal of the American College of Cardiology 28, 573-579.
Danesh, J., Lewington, S., Thompson, S.G., Lowe, G.D., Collins, R., Kostis, J.B., Wilson, A.C., Folsom, A.R., Wu, K., Benderly, M., Goldbourt, U., Willeit, J., Kiechl, S., Yarnell, J.W., Sweetnam, P.M., Elwood, P.C., Cushman, M., Psaty, B.M., Tracy, R.P., Tybjaerg-Hansen, A., Haverkate, F., de Maat, M.P., Fowkes, F.G., Lee, A.J., Smith, F.B., Salomaa, V., Harald, K., Rasi, R., Vahtera, E., Jousilahti, P.,

Pekkanen, J., D'Agostino, R., Kannel, W.B., Wilson, P.W., Tofler, G., rocha-Pinango, C.L., Rodriguez-Larralde, A., Nagy, E., Mijares, M., Espinosa, R., Rodriquez-Roa, E., Ryder, E., ez-Ewald, M.P., Campos, G., Fernandez, V., Torres, E., Marchioli, R., Valagussa, F., Rosengren, A., Wilhelmsen, L., Lappas, G., Eriksson, H., Cremer, P., Nagel, D., Curb, J.D., Rodriguez, B., Yano, K., Salonen, J.T., Nyyssonen, K., Tuomainen, T.P., Hedblad, B., Lind, P., Loewel, H., Koenig, W., Meade, T.W., Cooper, J.A., De, S.B., Knottenbelt, C., Miller, G.J., Cooper, J.A., Bauer, K.A., Rosenberg, R.D., Sato, S., Kitamura, A., Naito, Y., Palosuo, T., Ducimetiere, P., Amouyel, P., Arveiler, D., Evans, A.E., Ferrieres, J., Juhan-Vague, I., Bingham, A., Schulte, H., Assmann, G., Cantin, B., Lamarche, B., Despres, J.P., Dagenais, G.R., Tunstall-Pedoe, H., Woodward, M., Ben-Shlomo, Y., Davey, S.G., Palmieri, V., Yeh, J.L., Rudnicka, A., Ridker, P., Rodeghiero, F., Tosetto, A., Shepherd, J., Ford, I., Robertson, M., Brunner, E., Shipley, M., Feskens, E.J., Kromhout, D., Dickinson, A., Ireland, B., Juzwishin, K., Kaptoge, S., Lewington, S., Memon, A., Sarwar, N., Walker, M., Wheeler, J., White, I., Wood, A., 2005. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. JAMA: The Journal of the American Medical Association 294, 1799-1809.

Davies, P.F., Zilberberg, J., Helmke, B.P., 2003. Spatial Microstimuli in Endothelial Mechanosignaling. Circulation Research 92, 359-370.

Di Minno, M.N.D., Ambrosino, P., Lupoli, R., Di Minno, A., Tasso, M., Peluso, R., Tremoli, E., 2015. Clinical assessment of endothelial function in patients with rheumatoid arthritis: A meta-analysis of literature studies. European Journal of Internal Medicine 26, 835-842.

Donald, A.E., Charakida, M., Cole, T.J., Friberg, P., Chowienczyk, P.J., Millasseau, S.C., Deanfield, J.E., Halcox, J.P., 2006. Non-Invasive Assessment of Endothelial Function: Which Technique? Journal of the American College of Cardiology 48, 1846-1850.

Ellins, E., Halcox, J., Donald, A., Field, B., Brydon, L., Deanfield, J., Steptoe, A., 2008. Arterial stiffness and inflammatory response to psychophysiological stress. Brain, Behavior, and Immunity 22, 941-948.

Eriksson, M., Johansson, K., Sarabi, M., Lind, L., 2007. Mental Stress Impairs Endothelial Vasodilatory Function by a Beta-Adrenergic Mechanism. Endothelium: Journal of Endothelial Cell Research 14, 151 - 156.

Everson-Rose, S.A., Lewis, T.T., 2005. Psychosocial factors and cardiovascular diseases. Annu.Rev.Public Health 26, 469-500.

Ghiadoni, L., Donald, A.E., Cropley, M., Mullen, M.J., Oakley, G., Taylor, M., O'Connor, G., Betteridge, J., Klein, N., Steptoe, A., Deanfield, J.E., 2000. Mental Stress Induces Transient Endothelial Dysfunction in Humans. Circulation 102, 2473-2478.

Green, D.J., Jones, H., Thijssen, D., Cable, N.T., Atkinson, G., 2011. Flow-Mediated Dilation and Cardiovascular Event Prediction: Does Nitric Oxide Matter? Hypertension 57, 363-369.

Hansson, G.K., Libby, P., Tabas, I., 2015. Inflammation and plaque vulnerability. Journal of Internal Medicine 278, 483-493.

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.

Jennings, J.R., Kamarck, T.W., Everson-Rose, S.A., Kaplan, G.A., Manuck, S.B., Salonen, J.T., 2004. Exaggerated blood pressure responses during mental stress are prospectively related to enhanced carotid atherosclerosis in middle-aged Finnish men. Circulation 110, 2198-2203.

Languino, L.R., Plescia, J., Duperray, A., Brian, A.A., Plow, E.F., Geltosky, J.E., Altieri, D.C., 1993. Fibrinogen mediates leukocyte adhesion to vascular endothelium through an ICAM-1-dependent pathway. Cell 73, 1423-1434.

Liuzzo, G., Biasucci, L.M., Gallimore, J.R., Grillo, R.L., Rebuzzi, A.G., Pepys, M.B., Maseri, A., 1994. The prognostic value of C-reactive protein and serum amyloid a protein in severe unstable angina. N.Engl.J Med 331, 417-424.

Lominadze, D., Tsakadze, N., Sen, U., Falcone, J.C., Souza, S.E., 2005. Fibrinogen and fragment D-induced vascular constriction. American Journal of Physiology - Heart and Circulatory Physiology 288, H1257-H1264.
Lominadze, D.a.D.W.L.a.T.S.C.a.R.A.M., 2010. Mechanisms of fibrinogen-induced microvascular dysfunction during cardiovascular disease. Acta Physiologica 198, 1--13.
Lowe, G.D.O., Lee, A.J., Rumley, A., Price, J.F., Fowkes, F.G.R., 1997. Blood viscosity and risk of cardiovascular events: the Edinburgh Artery Study. British Journal of Haematology 96, 168-173.
Marmot, M.G., Smith, G.D., Stansfeld, S., Patel, C., North, F., Head, J., White, I., Brunner, E., Feeney, A., 1991. Health inequalities among British civil servants: the Whitehall II study. Lancet 337, 1387-1393.
McCann, B.S., Magee, M.S., Broyles, F.C., Vaughan, M., Albers, J.J., Knopp, R.H., 1995. Acute psychological stress and epinephrine infusion in normolipidemic and hyperlipidemic men: effects on plasma lipid and apoprotein concentrations. Psychosom Med 57, 165-176.
Ridker, P.M., Cushman, M., Stampfer, M.J., Tracy, R.P., Hennekens, C.H., 1997. Inflammation, Aspirin, and the Risk of Cardiovascular Disease in Apparently Healthy Men. N Engl J Med 336, 973-979.
Spieker, L.E., Hurlimann, D., Ruschitzka, F., Corti, R., Enseleit, F., Shaw, S., Hayoz, D., Deanfield, J.E., Luscher, T.F., Noll, G., 2002. Mental Stress Induces Prolonged Endothelial Dysfunction via Endothelin-A Receptors. Circulation 105, 2817-2820.
Steptoe, A., Brydon, L., 2005. Associations between acute lipid stress responses and fasting lipid levels 3 years later. Health Psychology 24, 601-607.
Steptoe, A., Brydon, L., 2009. Emotional triggering of cardiac events. Neuroscience & Biobehavioral Reviews 33, 63-70.
Steptoe, A., Feldman, P.J., Kunz, S., Owen, N., Willemsen, G., Marmot, M., 2002. Stress responsivity and socioeconomic status. A mechanism for increased cardiovascular disease risk? European Heart Journal 23, 1757-1763.
Steptoe, A., Hamer, M., Chida, Y., 2007. The effects of acute psychological stress on circulating inflammatory factors in humans: A review and meta-analysis. Brain, Behavior, and Immunity 21, 901-912.
Steptoe, A., Kivimaki, M., 2013. Stress and cardiovascular disease: an update on current knowledge. Annu Rev Public Health 34, 337-354.
Steptoe, A., Kunz-Ebrecht, S., Owen, N., Feldman, P.J., Rumley, A., Lowe, G.D.O., Marmot, M., 2003. Influence of Socioeconomic Status and Job Control on Plasma Fibrinogen Responses to Acute Mental Stress. Psychosomatic Medicine 65, 137-144.
Steptoe, A., Marmot, M., 2002. The role of psychobiological pathways in socio-economic inequalities in cardiovascular disease risk. Eur Heart J 23, 13-25.
Suehiro, K., Gailit, J., Plow, E.F., 1997. Fibrinogen Is a Ligand for Integrin α5β1 on Endothelial Cells. Journal of Biological Chemistry 272, 5360-5366.
Tousoulis, D., Papageorgiou, N., Androulakis, E., Briasoulis, A., Antoniades, C., Stefanadis, C., 2011. Fibrinogen and cardiovascular disease: Genetics and biomarkers. Blood Reviews 25, 239-245.
Ueland, T., Vissers, M.N., Wiegman, A., Rodenburg, J., Hutten, B., Gullestad, L., Ose, L., Rifai, N., Ridker, P.M., Kastelein, J.J., Aukrust, P., Semb, A.G., 2006. Increased inflammatory markers in children with familial hypercholesterolaemia. Eur J Clin Invest 36, 147-152.
Woo, K.S., Chook, P., Yu, C.W., Sung, R.Y.T., Qiao, M., Leung, S.S.F., Lam, C.W.K., Metreweli, C., Celermajer, D.S., 2004. Overweight in children is associated with arterial endothelial dysfunction and intima-media thickening. Int J Obes Relat Metab Disord 28, 852-857.
Yusuf, S., Hawken, S., Ôunpuu, S., Dans, T., Avezum, A., Lanas, F., McQueen, M., Budaj, A., Pais, P., Varigos, J., Lisheng, L., 2004. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. The Lancet 364, 937-952.

Supplement

	Dyslipidaemia categories				Nº of categories met		
	TC≥6	**Trigs ≥1.7**	**HDL <1**	**LDL ≥4**	**1**	**2**	**3**
Yes	40	36	2	33	27	21	14
No	112	116	150	119			

Supplement table 1: Number of participants who met each category for dyslipidaemia and the number of participants who met one or more of the categories