Brain, Behavior, and Immunity 60 (2017) 233–239

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

Full-length Article

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

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ARTICLE INFO

Article history:
Received 9 June 2016
Received in revised form 24 September 2016
Accepted 17 October 2016
Available online 18 October 2016

Keywords:
Fibrinogen
Flow-mediated dilatation
Psychophysiological stress
Lipids

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. Knowledge on the impact of other cardiovascular risk factors, such as dyslipidaemia, on such relationships is also limited We investigated the relationship between inflammatory responses to an acute mental stress challenge and endothelial function plus the influence of dyslipidaemia on the associations. Interleukin-6 (IL-6), tumor necrosis factor α (TNFα) and fibrinogen were assessed at baseline, immediately following standardized behavioural tasks and 45 min post-task in 158 participants. Blood pressure and heart rate responses were measured. Flow-mediated dilatation (FMD) was measured 3 years later. Fibrinogen and IL-6 increased post-stress ($p \leqslant 0.001$ & 0.003) but TNFα was unchanged ($p = 0.09$). An independent negative association between FMD and change in fibrinogen at 45 min ($\beta = -0.047$ $p = 0.016$) 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. There were no differences in the responses to stress between those with and without dyslipidaemia. However, there was an interaction between the presence of dyslipidaemia and immediate change in fibrinogen with stress which was associated with FMD. Those participants with dyslipidaemia who had a greater change in fibrinogen had lower FMD. We conclude that elevated fibrinogen responses to stress are associated with future endothelial dysfunction which may reflect increased cardiovascular risk.

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. Assessment of the dynamic cardiovascular and inflammatory responses to acute mental stress challenges provides the opportunity to understand more fully the potential mechanisms by which everyday psychological stresses influence the development of cardiovascular pathophysiology. In turn this may improve our understanding of how stress influences the presentation of clinical disease and may offer potential novel targets for treatment.

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 vasomotor function is a well-established measure of general 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, 1992; Di Minno et al., 2015; Woo et al., 2004). Furthermore, both

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http://dx.doi.org/10.1016/j.bbi.2016.10.017
0889-1591/

acute inflammation, for example post vaccination, and acute mental stress have been shown to cause transient impairment of endothelium dependent dilatation in otherwise healthy people (Ghiadoni et al., 2000; Hingorani et al., 2000; Spieker et al., 2002). This may be due to the effects of inflammatory cytokines and fibrinogen which activate the endothelium, reducing nitric oxide bioavailability (Hansson et al., 2015; Tousoulis et al., 2011). We have also previously shown that those with the most pronounced inflammatory responses (interleukin-6 [IL-6], tumor necrosis factor α [TNFα] 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). There is limited work looking at how responses to acute stress might be further influenced by the presence of cardiovascular risk factors such as dyslipidaemia. One small study in men with mixed dyslipidaemia saw no difference in the haemodynamic responses to acute stress challenges compared to controls with normal lipids (McCann et al., 1995). However, subjects with type II diabetes have been shown to have blunted blood pressure, heart rate and IL-6 responses to an acute mental stress (Steptoe et al., 2014). Studies looking at the effect of the presence of risk factors on the endothelial response to stress are also few in number and small in size, with mixed findings. They also do not look at how responses to stress might influence endothelial function over the longer term (Cardillo et al., 1998; Ghiadoni et al., 2000). We have previously shown that greater Fibrinogen and TNFα responses following a modest acute psychophysiological stress stimulus was associated with increased carotid arterial stiffness, a measure of structural arteriosclerotic/atherosclerotic changes, in middle aged civil servants. The relationships between inflammatory responses to acute mental stress and future endothelial function, a more dynamic measure of arterial (patho)physiology than arterial stiffness 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 Whitehall II epidemiological cohort consisted of 10,308 nonindustrial civil servants aged 35–55, who were recruited between 1985 and 1988 to investigate social and occupational influences on health and disease. Follow-up of these participants has occurred through clinic visits and self-administered questionnaires every 2–5 years. Between 1999 and 2000, 123 men and 105 women from the Whitehall II Study underwent psychophysiological testing as part of a psychobiology sub-study (Marmot et al., 1991; Steptoe et al., 2002a). Participants 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 h 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 min following the insertion of a cannula for blood sample collection. During the last 5 min 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 min 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 min. 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 min post-stress blood pressure and heart rate recordings were made at 15–20 min and 40–45 min. A final blood sample was taken after 45 min. 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 °C until analysis. We have shown in previous studies that fibrinogen responds immediately to psychological stress, whilst increases in IL-6 and TNFα emerge after 30–45 min (Steptoe et al., 2007, 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 2.5% and 4.1%.

The serum was snap frozen at −70 °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. Socioeconomic status (SES) based on current or last known grade of employment was determined by questionnaire as was smoking status.