Prolonged elevations in haemostatic and rheological responses following psychological stress in low socioeconomic status men and women

Andrew Steptoe¹, Sabine Kunz-Ebrecht¹, Ann Rumley², Gordon D. O. Lowe²

¹Department of Epidemiology and Public Health, University College London, England, UK
²Department of Medicine, University of Glasgow, Scotland, UK

Summary
Low socioeconomic status (SES) and psychological stress are associated with increased risk of coronary heart disease, and both may influence haemostatic responses. Von Willebrand factor (vWF), Factor VIII, plasma viscosity, haematocrit, blood viscosity, tissue plasminogen activator (t-PA) and fibrin D-dimer were measured at rest and following stressful tasks in 238 middle-aged British civil servants. SES was defined by grade of employment. Lower SES was associated with higher resting vWF, Factor VIII and plasma viscosity. Psychological stress stimulated increases in haemostatic and rheological factors. Initial stress responses did not vary with SES, but Factor VIII, plasma viscosity and blood viscosity remained more elevated 45 minutes post-stress in lower SES participants. High blood pressure stress reactivity was also associated with greater haemostatic responses. We conclude that lower SES is characterised by more prolonged elevations in procoagulant responses following psychological stress, and that these processes might contribute to increased cardiac risk.

Keywords
Socioeconomic status, haemostatic factors, psychological stress, behaviour

Introduction
There is a marked socioeconomic gradient in the incidence of coronary heart disease (CHD) and in subclinical atherosclerosis (1, 2). Haemostatic and rheological factors may contribute to this pattern. Socioeconomic gradients in fibrinogen have been described in the Whitehall II study (3) and in other cohorts (4, 5). Socioeconomic status (SES) is also inversely associated with the concentration of von Willebrand factor (vWF) antigen (4-7), and associations between low SES and raised levels of coagulation Factor VIII and plasma viscosity have been described (6, 8, 9). No association with SES has been observed for activated Factor VII or plasminogen activator inhibitor-1 (5), while evidence concerning relationships with blood viscosity, tissue plasminogen activator (t-PA) and fibrin D-dimer is very limited. Haemostatic factors are also sensitive to chronic and acute psychological stress (10). It has been postulated that one mechanism by which low SES status might increase cardiovascular disease risk is through activation of stress-related neuroendocrine and autonomic pathways (11). We therefore examined SES variations in haemostatic and rheological factors both at rest, and in response to psychological stress, in a sub-sample of the Whitehall II epidemiological cohort. We hypothesized that stress would induce a hypercoagulable state that was more pronounced in lower SES participants. We have recently observed that lower SES is associated with impaired blood pressure and heart rate recovery following stress (12), and postulated that a similar pattern might be present for haemostatic variables. There are marked variations in cardiovascular stress reactions between individuals (13), so the association between changes in haemostatic and rheological factors following psychological stress.
static and rheological measures and blood pressure reactivity was also assessed. Results relating to fibrinogen are described elsewhere (14), and the present analyses focus on vWF, Factor VIII, plasma viscosity, haematocrit, blood viscosity, t-PA and fibrin D-dimer. Recent meta-analyses of prospective studies have shown that these variables appear to be independent predictors of CHD events (15-17).

**Methods**

**Participants**
Participants in this study were 129 men and 109 women, drawn from the Whitehall II cohort. The Whitehall II cohort consists of 10,308 London-based civil servants, recruited in 1985-1988 to investigate demographic, psychosocial and biological risk factors for CHD (18). Volunteers for this substudy were recruited on the following criteria: white European origin, aged 45-58 years, based in the London area, not planning to retire for at least three years, no history of coronary heart disease, no previous diagnosis or treatment for hypertension, and willingness to take part in ambulatory blood pressure monitoring (not described here) as well as the laboratory session. SES was defined by grade of employment, since occupational status in the British civil service correlates highly with income and educational attainment. Participants were drawn from higher (administrative and professional, n = 90), intermediate (senior executive officers, n = 81), and lower (executive officers, clerical, office support, n = 67) official employment grades. Analyses of individual haemostatic factors were based on the following numbers: von Willebrand factor – 224, Factor VIII – 237, plasma viscosity – 235, haematocrit – 237, blood viscosity – 234, t-PA antigen – 222, D-dimer – 216.

**Measures**
Blood pressure and heart rate were monitored continuously from the finger using a Portapres-2, a portable version of the Finapres device (19). Cortisol was assessed from saliva samples collected using cotton dental rolls (Salivettes, Sarstedt, Leicester, UK), and analysed using a biotin-streptavidin immunoassay (20).

**Behavioural tasks**
Psychological stress was induced by two tasks, as detailed elsewhere (14). The first was a computerized colour-word interference task, involving the successive presentation of target colour words, printed in another colour. The second task was mirror tracing, involving the tracing of a star with a metal stylus which could only be seen in mirror image. Participants were asked to give accuracy priority over speed on both tasks.

**Procedure**
Participants were tested in the morning or afternoon in a light and temperature controlled laboratory. They were instructed not to have drunk tea, coffee, or caffeinated beverages, or to have smoked for at least two hours prior to the study, and not to have consumed alcohol or have exercised on the evening before or the day of testing. Participants who had taken over-the-counter anti-inflammatory or antihistamine medication over the previous two days were rescheduled. The study was approved by the UCL/UCLH Committee on the Ethics of Human Research.

The experimental session commenced with measurement of height, weight, waist and hip circumference using standard techniques. Following instrumentation and the insertion of a 21-gauge venous cannula for the periodic collection of blood samples, the participant rested for 30 minutes. A baseline blood sample was drawn, blood pressure and heart rate were recorded for a 5 min period, a baseline saliva sample was obtained, and participants rated how stressed they felt on a scale ranging from 1 (low) to 7 (high). The two tasks were then administered in random order. Each lasted for 5 min, during which blood pressure and heart rate were recorded continuously. Following each task, the participant rated stress, task difficulty and task involvement on 7-point scales ranging from 1 = low to 7 = high. A second blood sample (stress measure) was drawn immediately after the task period, together with saliva samples and mood and stress ratings. A further saliva sample was obtained 20 min following tasks. A third blood sample was drawn after 45 min quiet rest (recovery measure).

**Blood assays**
Venous blood samples were collected in citrated (0.109 mol/L; 9:1 v:v) and K$_2$-EDTA (1.5 mg/mL) tubes, centrifuged at room temperature, and the supernatant plasma was snap frozen at –70°C within one hour. Plasma viscosity was measured in the K$_2$-EDTA samples in a semi-automated capillary viscometer (Coulter) at 37°C. Factor VIII activity (one-stage assay), vWF antigen (ELISA, DAKO), t-PA antigen, and fibrin D-dimer (ELISAs, Biopool AB) were assayed in citrated plasma (9). Haematocrit was assessed immediately after each blood sample was drawn using a micro-haematocrit centrifuge and reader (Hawksley Gelman, Lancing, Sussex, UK). Blood viscosity was calculated from haematocrit and plasma viscosity as previously described (21).

**Statistical analysis**
Preliminary analysis indicated that haemostatic measures did not vary with the time of day of experimental sessions. Five measures (von Willebrand factor, factor VIII, plasma viscosity, t-PA and fibrin D-dimer) were logarithmically transformed prior to analysis, and geometric means are presented. SES differences were assessed by analysis of variance of baseline measures, with grade of employment (higher, intermediate, lower) and sex as between-subject factors. Age, body mass index, and smoking status were included as covariates. Linear trends across grades of employment, and contrasts between higher and lower SES groups were carried out. Responses to stress were assessed with
repeated measures analysis of variance. The relationship with cardiovascular stress reactivity involved comparing haemostatic responses in high and low systolic pressure reactors (defined by median split of systolic pressure stress responses). Associations between haemostatic responses, subjective stress and cortisol were analyzed using product-moment correlations. Analyses were conducted using SPSS v10.0.5.

Results

There were no SES differences in the proportion of men and women, body mass index, waist/hip ratio, use of hormone replacement therapy by women, or in resting blood pressure (Table 1). There were SES differences in age (P = .013) and sex (P = .017), since lower SES individuals tended to be slightly

| Table 1: Details of the three SES groups: means (s.e.m.) and percentages |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                 | Higher SES      | Intermediate SES | Lower SES       |
|                                 | Men N=49        | Women N=41       | Men N=44        | Women N=37      | Men N=36        | Women N=31      |
| Age (years)                     | 52.5 (0.38)     | 51.1 (0.43)      | 52.0 (0.40)     | 52.5 (0.44)     | 53.9 (0.44)     | 52.2 (0.48)     |
| Body mass index                 | 25.6 (0.54)     | 25.8 (0.59)      | 26.0 (0.57)     | 25.0 (0.62)     | 25.7 (0.63)     | 25.4 (0.68)     |
| Waist/hip ratio                 | .914 (0.01)     | .798 (0.01)      | .893 (0.01)     | .809 (0.01)     | .913 (0.01)     | .787 (0.02)     |
| Current smokers                 | 2.0%            | 7.3%             | 11.6%           | 8.1%            | 22.2%           | 6.7%            |
| Hormone replacement therapy     | 29.3%           | 29.7%            | 25.8%           |
| Resting systolic pressure (mmHg)| 119.8 (1.8)     | 111.9 (2.1)      | 118.2 (2.0)     | 108.9 (2.1)     | 119.4 (2.2)     | 113.6 (2.3)     |
| Resting diastolic pressure (mmHg)| 72.4 (1.4)     | 68.6 (1.6)       | 72.2 (1.5)      | 68.4 (1.6)      | 72.2 (1.7)      | 69.4 (1.8)      |

| Table 2: Haemostatic factors and socioeconomic status: means (s.e.m.) and geometric means |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                 | Higher          | Intermediate    | Lower           |
| Von Willebrand factor (log)     | 4.516 (.034)    | 4.518 (.036)    | 4.611 (.039)    | .065            | .034            |
| Geometric mean (IU/dl)          | 91.5            | 91.7            | 100.6           |
| Factor VIII (log)               | 4.805 (.022)    | 4.857 (.024)    | 4.895 (.026)    | .010            | .008            |
| Geometric mean (IU/dl)          | 122.1           | 128.6           | 133.6           |
| Plasma viscosity (log)          | 0.184 (.005)    | 0.181 (.005)    | 0.205 (.006)    | .008            | .006            |
| Geometric mean (mPa.s)          | 1.202           | 1.198           | 1.228           |
| Hematocrit (%)                  | 39.2 (.28)      | 39.0 (.29)      | 39.3 (.32)      | .76             | .81             |
| Blood viscosity (mPa.s)         | 2.84 (.023)     | 2.83 (.023)     | 2.90 (.026)     | .12             | .14             |
| t-PA (log)                      | 1.661 (.041)    | 1.745 (.043)    | 1.744 (.047)    | .19             | .15             |
| Geometric mean (ng/ml)          | 5.26            | 5.73            | 5.72            |
| Fibrin D-dimer (log)            | 3.818 (.064)    | 3.920 (.068)    | 3.752 (.073)    | .51             | .46             |
| geometric mean (ng/ml)          | 45.5            | 50.4            | 42.6            |

Values adjusted for age, sex, body mass index and smoking status
older on average, while women were slightly younger than men. The proportion of smokers was inversely associated with SES in men (P = .003), but not women. As expected, resting systolic and diastolic pressure and waist/hip ratio were higher in men than women (P < .01).

The associations between SES as indexed by grade of employment and haemostatic variables at rest are summarized in Table 2. The plasma concentration of vWF was greater in lower than higher status groups (P = .034) after adjustment for age, sex, body mass index and smoking. There were also significant SES effects for Factor VIII (P = .01) and plasma viscosity (P = .008), with higher levels in lower SES groups. Statistical adjustment for haematocrit and the use of hormone replacement therapy in women did not alter these results. By contrast, no SES differences were recorded in haematocrit, blood viscosity, t-PA antigen, or fibrin D-dimer.

**Acute psychological stress, SES, and haemostatic responses**

The behavioural tasks elicited mean increases of 22.6 mmHg systolic pressure (19.6% increase) and 13.6 mmHg diastolic pressure (19.2% increase). The increase in salivary cortisol was smaller, averaging 5.0%. Subjective stress ratings increased from an average 1.43 at baseline to 4.00 immediately after tasks, indicating that the tasks were perceived as moderately stressful. Stress ratings returned to baseline levels (mean 1.38) after 45 minutes, and subjective responses did not vary with SES.

The response to psychological stress was similar for vWF, Factor VIII, plasma viscosity, and blood viscosity (Fig. 1). For all four measures, the increases between baseline and stress (P < .001), and the decreases between stress and recovery (P < .01) were significant. The concentration of vWF in the recovery sample did not differ from baseline (P = .28). There were differences between baseline and recovery for Factor VIII (P = .52), plasma viscosity (P < .001), and blood viscosity (P < .001), indicating that post-stress recovery was incomplete by 45 minutes in these variables. There were also significant SES effects for vWF (P = .047), Factor VIII (P = .042), and plasma viscosity (P = .001), as the greater levels observed in the lower SES participants were maintained in stress and recovery trials. The SES groups did not differ in initial responses to stress, as indexed by increases from baseline to stress samples. However, stress
Stress, haemostasis, and socioeconomic status responses in Factor VIII, plasma viscosity and blood viscosity were more persistent in lower SES participants. Thus, the reduction between the stress and 45 min recovery sample was greater in higher and intermediate SES than lower SES groups for Factor VIII (P = .037), plasma viscosity (P = .018), and blood viscosity (P = .046). Men and women did not differ in these response patterns, and hormone replacement therapy did not affect stress responses in women. Inclusion of concurrent haematocrit as a covariate did not change the pattern of results, and SES differences also persisted when regular physical activity was taken into account (data not shown).

A stress response was also observed for haematocrit (P < .001), with values increasing from 39.1% (SD 3.1) at baseline to 39.8% (SD 3.1) during stress. The haematocrit in the recovery trial (39.6%, SD 3.1) was above baseline (P < .001), but did not differ from that recorded during stress (P = .069). Haematocrit responses did not vary with SES. Similar analyses were carried out for t-PA and D-dimer, but in neither case were there any systematic changes across trials. These two measures appear not to have been affected by acute psychological stress.

**Haemostatic stress responses and cardiovascular reactivity**

Associations between haemostatic stress responses and cardiovascular reactivity were evaluated by comparing high and low
systolic pressure reactors. The mean systolic pressure stress responses in high and low reactor groups averaged 33.5 and 11.7 mmHg respectively. After adjustment for age, body mass and smoking status, the increase between baseline and stress was greater in high than low systolic pressure reactors in vWF (P = .016), Factor VIII (P = .044), plasma viscosity (P = .021), haematocrit (P = .032) and blood viscosity (P = .004). The high systolic reactivity group showed greater haemostatic responses to stress (Figs. 2 and 3). This pattern did not differ by SES or sex. There were no effects of systolic pressure reactivity on t-PA or D-dimer.

The stress-induced increases in haemostatic measures were not associated with changes in saliva cortisol. Nor were haemostatic responses correlated with the subjective stress reports.

**Discussion**

The haemostatic and rheological variables analyzed in this study have all been associated with increased coronary heart disease risk (15-17). Von Willebrand factor is released from vascular endothelial cells and platelets, and promotes thrombus formation by stimulating platelet aggregation and adherence of platelets to damaged vessel walls. It binds to Factor VIII and protects it from proteolysis. Factor VIII serves as a co-factor for Factor IX in catalyzing the activation of Factor X, leading to the conversion of prothrombin to thrombin. Plasma viscosity is a major determinant of whole blood viscosity, and is itself affected by plasma fibrinogen and lipoprotein levels. These high molecular weight plasma proteins also increase red cell aggregation, which increases blood viscosity at low shear rates (22). Plasma and blood viscosity, haematocrit, and erythrocyte sedimentation rate (a measure of red cell aggregation) predict future CHD (15). High blood viscosity may increase CHD risk through effects on atherogenesis, thrombosis, or ischaemia. Tissue plasminogen activator is an activator of the fibrinolytic system that converts plasminogen into fibrin-cleaving plasmin, removing fibrin clots and thrombi by proteolytic degradation into soluble fragments such as D-dimer. Fibrin D-dimer is therefore a marker of fibrin turnover, and is elevated in conditions that are characterised by excessive activation of the coagulation system followed by fibrinolysis (16).

Many haemostatic factors are associated with gender, smoking, body mass index, age and (in women) hormonal status (9). Nevertheless, after taking these factors into account, we observed associations between lower SES and raised von Willebrand factor, Factor VIII and plasma viscosity (Table 2). SES differences in vWF have previously been described in the full Whitehall II cohort (7) and in other population studies (5, 6), while SES differences in Factor VIII and plasma viscosity have been inconsistent (8, 9). Our failure to identify any SES gradient in haematocrit, t-PA or D-dimer conforms with previous reports (8, 9).

Acute psychological stress stimulated increased vWF and Factor VIII, plasma viscosity, haematocrit, and blood viscosity (Fig. 1). The responses were small and short-lived, since values had begun to return to baseline levels by the 45 min post-stress sample. However, Factor VIII, plasma and blood viscosity remained above baseline even after 45 min. Previous studies of the impact of acute psychological stress on vWF, plasma and blood viscosity have produced variable results, with increases observed in some (23, 24) but not in all experiments (25). The present study is substantially larger than earlier investigations, and post-stress recovery effects of this duration have not previously been assessed. The increase in haemoconcentration in response to psychological stress is well established, and the raised concentration of plasma proteins might be due in part to reduced plasma volume (26). However, in this study the magnitude of haemostatic stress responses remained unchanged after statistical adjustment for changes in haematocrit.

Psychological stress did not affect t-PA antigen or fibrin D-dimer. Increased t-PA and D-dimer following stressors have been shown by some investigators (23, 25), although decreases in t-PA have also been observed (27). The responses we observed in the haemostatic measures are indicative of heightened coagulability (vWF, Factor VIII, fibrinogen) without a corresponding response in fibrinolytic pathways (t-PA, D-dimer), and suggest that acute stress induces a hypercoagulable state (10).

We hypothesised that lower SES would be associated with heightened stress responsivity. The results indicated that the initial stress response was similar across the social gradient, but that haemostatic and rheological activation was more prolonged in lower SES participants (Figure 1). Thus the reduction in Factor VIII concentration and in levels of plasma and blood viscosity between stress and 45-minute samples was smaller in the lower compared with intermediate and higher SES participants. This finding is potentially important, since prolongation of stress responses indicates that a mild hypercoagulable state was maintained for longer in the lower SES participants. The absence of differences in subjective stress ratings at 45 min suggests that this response pattern was not due to more persistent psychological distress or rumination in lower SES participants. The effect may therefore be caused by disturbances of autonomic and neuroendocrine regulatory processes due to chronic allostatic load (28). It is known that lower SES groups are also exposed to greater social stress in everyday life than more affluent people (18). Small differences in the duration of activation of procoagulant pathways may accumulate over years of differential stress exposure, leading to increased cardiovascular disease risk (11).

There are marked individual differences in cardiovascular stress reactivity, associated with factors such as genetic predispositions, cognitive appraisals, personality, physical fitness and background levels of life stress (13). Heightened cardiovascular
reactivity, either alone or in combination with chronic life stress, predicts future high blood pressure (29) and progression of carotid atherosclerosis (30). The stress-induced increases in vWF, Factor VIII, plasma viscosity, haematocrit and blood viscosity were all positively correlated with systolic pressure reactivity (Figs. 2 and 3), suggesting that sympathoadrenal activation underpinned the haemostatic responses (31).

This study was limited to participants of white Caucasian origin, so results may not generalize to other racial and ethnic groups. The changes in haemostatic and rheological measures were small, and probably not clinically significant in themselves. Nevertheless, if these results reflect the pattern prevailing in everyday life, then small but persistent differences in procoagulant pathways might exist across the social gradient, and be subject to more prolonged exacerbation by psychological stress in lower than higher SES groups. Such processes might contribute in the long term to higher risk for CHD in less privileged sectors of society.

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