The effect of natural habituation on coagulation responses to acute mental stress and recovery in men

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Summary

Blood coagulation activation might be one mechanism linking acute mental stress with coronary events. We investigated the natural habituation of coagulation responses and recovery to short-term mental stress. Three times with one-week intervals, 24 men (mean age 47 ± 7 years) underwent the same 13-min stressor (preparation, job interview, mental arithmetic). During each visit venous blood was obtained four times (baseline, immediately post-stress, 45 min of recovery, 105 min of recovery). Eight blood coagulation parameters were measured at weeks one and three. Acute stress provoked increases in von Willebrand factor antigen, fibrinogen, clotting factor FVII activity (FVII:C), FVIII:C, FXII:C (p's ≤0.019), and D-dimer (N.S.). All coagulation parameters experienced full recovery except FVIII:C (p = 0.022). Stress did not significantly affect activated partial thromboplastin time and prothrombin time. At all time points FVIII:C and FXII:C levels were significantly higher at week one compared to week three (p's ≤0.041). Before catheter insertion, systolic blood pressure (p = 0.001) and heart rate (p = 0.026) were relatively higher at week one. Unlike the magnitude of systolic blood pressure response to stress (p = 0.007) and of cortisol recovery from stress (p = 0.002), the magnitude of all coagulation responses to stress and the recovery from stress were similar in week one and week three. Sympathetic activation with anticipatory stress best explained increased baseline activity in FVIII and FXII at week one. An incapacity of the coagulation system to adapt to stress repeats is perhaps a consequence of evolution, but might also contribute to increased coronary risk in some individuals, particularly in those with cardiovascular diseases.

Keywords

Blood coagulation, cardiovascular disease, psychological stress, recovery, habituation

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Introduction

Shear stress increases when blood pressure (BP) peaks during mental arousal (1) and intense emotions (2), thus enhancing the odds of rupture of atherosclerotic plaques (3). Upon exposure of prothrombotic plaque material to the blood stream, a procoagulant milieu elicited by sympathetic nervous system (SNS) activation (4) may accelerate and sustain coronary thrombus growth (5, 6). For instance, subjects reporting an intense outburst of anger had a more than double risk of developing an acute myocardial infarction in the two hours following anger onset (2). Similarly, numerous studies have shown that, subsequent to catastrophic stressors such as an earthquake (7) or a missile attack (8), the risk of acute myocardial infarction significantly increases. It is suspected that among other mechanisms, changes in hemostatic factors explain a substantial part of this risk (9).

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Aside from increases in fibrinogen (10-12), and clotting activities of factor VII (FVII:C) (10), factor VIII (FVIII:C) (10, 13), and the von Willebrand factor antigen (VWF:Ag) (10, 13, 14), acute mental stress also elicits an increase in tissue-type plasminogen activator activity (10, 15, 16). The more sensitive markers of coagulation activation, thrombin / antithrombin III complexes and fibrin D-dimer, were also elevated in plasma after acute mental stress (17, 18). Increase in D-dimer suggests that, despite the concomitant activation of coagulation and fibrinolysis with acute mental stress, procoagulant mechanisms outweigh anticoagulant forces even in healthy subjects (19).

Ineffective adaptation of physiological systems to environmental stress may contribute to cardiovascular disease (20). Hence, the inability of procoagulant responses to adapt or habituate to repeated stressors of the same type, or to sufficiently shut off or recover after stress termination, might possibly enhance coronary risk. As of yet, the question as to whether coagulation responses habituate to repeated stress has not been investigated. In addition, the few studies investigating recovery of coagulation changes from stress, namely in VWF:Ag, fibrinogen, FVIII:C, and D-dimer, are inconsistent (12, 13, 21).

Therefore, the first and primary aim of this study was to investigate habituation of stress responses in plasma VWF:Ag, fibrinogen, FVII:C, FVIII:C, FXII:C, D-dimer, the activated partial thromboplastin time (APTT), and the prothrombin time (PT) by subjecting male volunteers to the same stressor three times with one-week intervals. The second aim was to further elucidate recovery from stress-induced coagulation changes by extending previous recovery measures beyond 45 min. The third aim was to test for the stress responsiveness of FXII:C, APTT, and PT, all of which have not been investigated in previous work on the effects of a single acute stress period on blood coagulation.

Materials and methods

Study design and participants

Study participants were recruited by regular mail from a population of 1,802 permanently employed staff members of the Federal Institute of Technology, Zurich, Switzerland, over 35 years of age, i.e. when cardiovascular risk begins to increase (22). The Institute's ethics committee approved the study protocol. Incentive offered was a written report in terms of the individual cardiovascular risk factor profile based on a medical (health questionnaire, screening blood pressure, anthropomorphic data) and laboratory (serum lipids, glycosylated hemoglobin A1c, high-sensitive C-reactive protein) work-up by standard procedures (Synlab, Augsburg, Germany).

One hundred and thirty-two men and women expressed their written consent to volunteer in a study on "The Relationship Between Acute Stress and Blood Coagulation". The purpose of the present study was to include a consecutive sample of 27 middle-aged men in reasonably good health. Women were excluded because the female cycle may influence both basal hemostatic activity (23) and hemostatic response to acute stress (15). Exclusion criteria for men were verified based on personal history and a physical exam by two trained medical students (L.Z., K.M.), and by close review of the laboratory charts by a board-certified internist (R.v.K.). They were the following: any haematological, pulmonary, liver, renal, gastrointestinal, heart, cerebrovascular, or psychiatric disease; any history of a thromboembolic event, any current major or minor infection, any trauma or surgery within the preceding six months, body mass index ≥ 29 kg/m^2, high-sensitive C-reactive protein ≥ 1 mg/dl. All subjects were unmedicated and were required to not take any non-steroidal anti-inflammatory drug or aspirin for at least ten days before testing and throughout the habituation protocol.

Stress protocol

The 27 male participants were scheduled three times with an approximate one-week interval, and were all subjected to the same stress protocol. However, due to time restrictions, three subjects dropped out from the protocol after the first testing session, resulting in a total of 24 subjects who completed all three visits. After the first testing session (week one), subjects were informed that the two subsequent testing sessions (week two and week three) would be identical. Two or three subjects were tested each morning with an interval of 30 to 45 min between subjects. The first subject arrived at the laboratory at 7:30 am. After a rest period of 10 min, an intensive care unit physician (J.F.) or nurse inserted a 20-gauge indwelling venous forearm catheter. Thereafter, subjects received a light, standardized, non-caffeinated breakfast and remained seated for another 30 min until further instruction on the nature of the stress protocol.

We used the Trier Social Stress Test (TSST) as introduced by Kirschbaum et al. (24). In brief, the TSST combines a 3-min preparation phase followed by 5-min free speech (job interview) and 5-min mental arithmetic before an audience and elicits robust physiological stress responses (25). To avoid effects of learning and repetition, the theme of the free speech and the initial number for the serial subtraction tasks were minimally modified across the three stress sessions (26). After completion of the task, subjects remained seated for another 105 min in a quiet room. Four blood samples were obtained: i) immediately before the preparation phase ("baseline"), ii) immediately after stress ("post-stress"), iii) 45 min after stress ("recovery 1"), and iv) 105 min after stress ("recovery 2").

Hemodynamic and cortisol measures

Heart rate (HR), BP, and salivary cortisol were assessed: i) before inserting the venous forearm catheter to obtain a proxy index of anticipatory stress/arousal (27), ii) at baseline, iii)

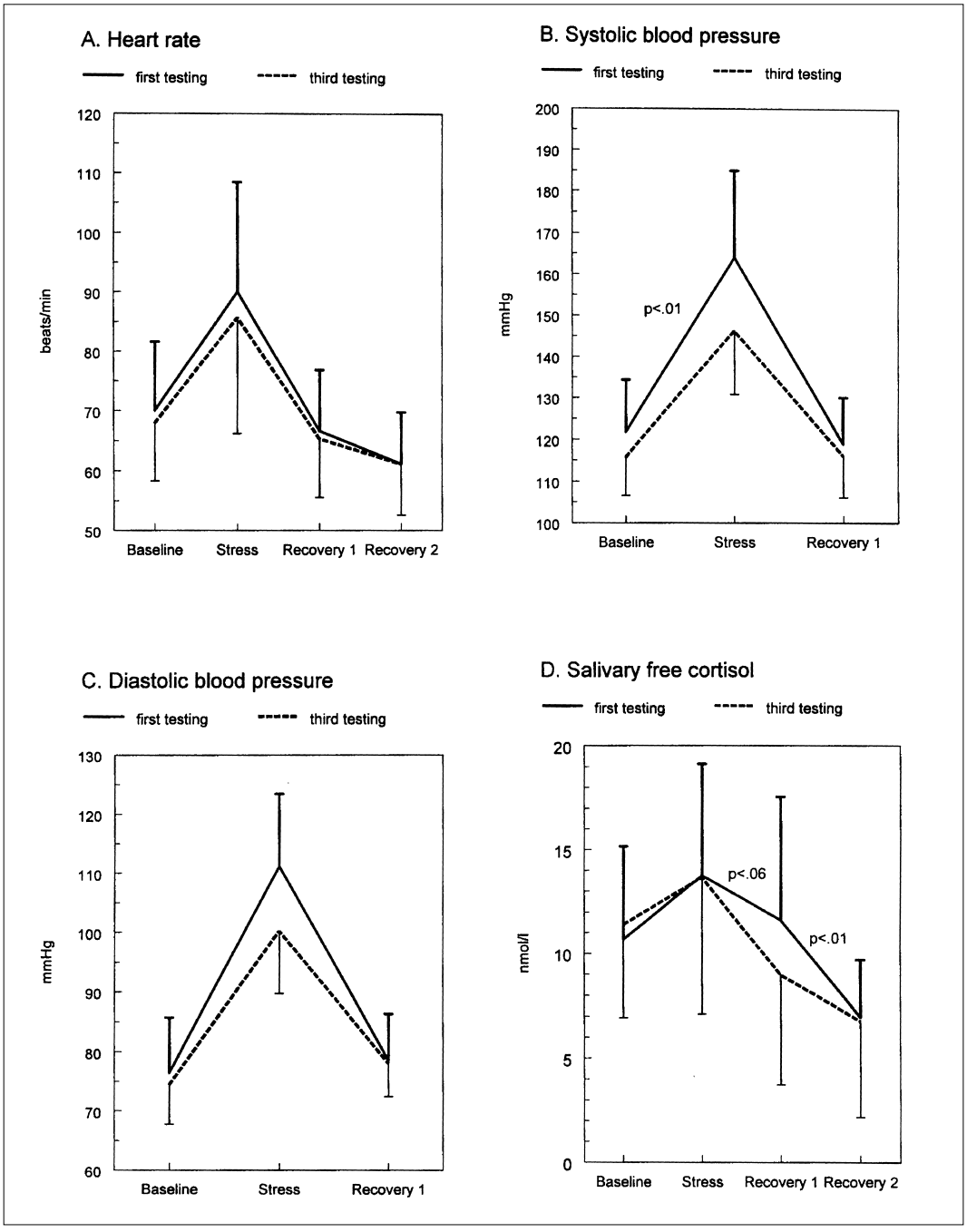

Figure 1: Hemodynamic and cortisol responses to stress. There was a significant main effect for "stress" with regard to heart rate (panel A) and diastolic blood pressure (BP) (panel C) and a significant "stress-by-habituation interaction" with regard to systolic BP (panel B) and salivary free cortisol (panel D) between baseline, immediately post-stress (stress), recovery 1 (45 min after stress), and recovery 2 (105 min after stress). Systolic BP increase from baseline to stress was attenuated (panel B) and recovery of cortisol after stress occurred more rapidly (panel D) at week three compared to week one, suggesting habituation of these responses. Values given are mean ± SD. Cf. text for further details.

immediately post-stress, and iv) at recovery 1. HR and cortisol were additionally measured at v) recovery 2. For logistic reasons, BP could not be measured at recovery 2.

Average HR was computed from a digitally recorded electrocardiogram lead (400 Hz). From a stable period of 30 sec not showing any artefacts or extra-systoles, ten consecutive R-R-intervals were selected and HR was computed as 10*60,000/t, where t is the time in ms of the ten R-R-intervals. During stress, systolic and diastolic BP readings were obtained from continuous measures at the left radial pulse using the Vasotrac model APM205A (Medwave Inc., St. Paul, MN, USA). The peak BP during stress was calculated as the highest mean value determined over two minutes from at least five valid readings from the Vasotrac device and adjusted to readings obtained by sphygmomanometry at the other three time points. Saliva samples were obtained in polypropylene Eppendorf tubes and kept at room temperature throughout one test session. They were then stored at -20° C. All samples were centrifuged at 3000 rpm for 5 min to provide clear supernatant fractions. Free cortisol was by Luminescence Immuno Assay (LIA) kit supplied by IBL (Hamburg, Germany) and expressed as nM.

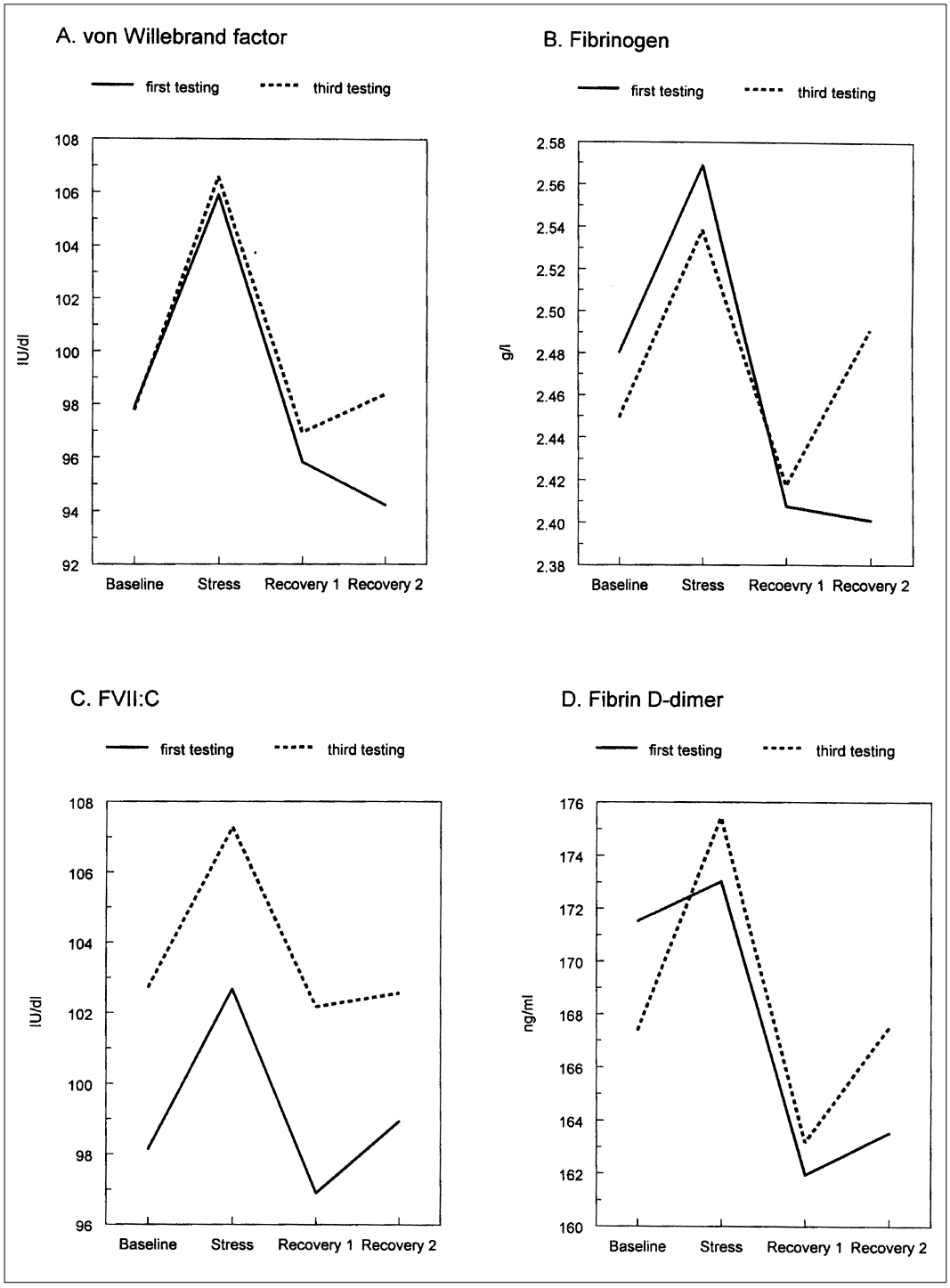

Figure 2: Coagulation measures showing a main effect for stress. There was a significant main effect for "stress" in terms of von Willebrand factor antigen (VWF:Ag) (panel A), fibrinogen (panel B), clotting factor VII activity (FVII:C) (panel C), and D-dimer (panel D) across the four time points assessed (baseline, immediately post-stress – stress, 45 min after stress – recovery 1, 105 min after stress – recovery 2). The geometric mean is given. Cf. text for further details.

Coagulation assays

After discarding the first 2 cc's, whole blood was drawn into 5 cc polypropylene tubes containing 3.8% of sodium citrate (Vacutainer Becton Dickinson Biosciences, Allschwil, Switzerland). Immediately following the draw, the blood was spun for 10 min at 3,000 X g. Platelet-poor plasma was aliquoted into polypropylene Eppendorf tubes and stored in a freezer at -80°C until analysed.

Due to restricted funding, coagulation measures were assessed in plasma obtained at week one and week three, but not at week two. All coagulation assays were performed in the Thrombosis Research Laboratory of the University Hospital Berne, Switzerland using the BCS® Coagulation Analyser from Dade Behring, Liederbach, Germany. Determination of FVII:C, FVIIIC, FXII:C, APTT, and PT was by standard coagulometric methods using factor-deficient standard human plasma and

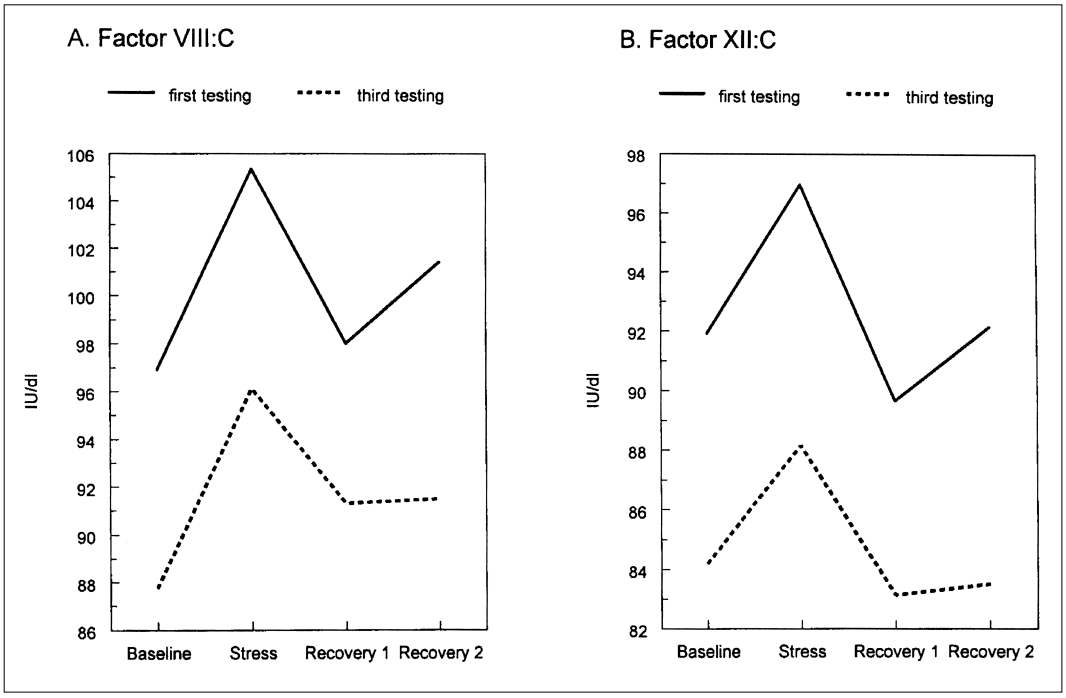

Figure 3: Coagulation measures showing main effects for stress and habituation. There were significant main effects for both "stress" and "habituation" for clotting factor VIII activity (FVIII:C) (panel A) and FXII:C (panel B) across the four time points assessed (baseline, immediately post-stress – stress, 45 min after stress – recovery 1, 105 min after stress – recovery 2). The geometric mean is given. Cf. text for further details.

reagents (Dade Behring). Quantitative determination of plasma fibrinogen levels followed a modified Clauss method (Multifibren U, Dade Behring) (28). Plasma D-dimer (29) and VWF:Ag (30) were measured by a turbidimetric method (Dade Behring). In brief, small polystyrene particles to which specific antibodies against D-dimer or vWF:Ag have been attached by covalent bonding are agglutinated when mixing plasma samples containing the respective coagulation molecules. Agglutination is detected turbidimetrically via an increase in turbidity, which is proportional to the concentration of D-dimer or VWF:Ag in the test sample.

For the eight time points assessed, data were complete for all 24 subjects in terms of the APTT, FVII:C, FVIII:C, and FXII:C. Data for the PT, VWF:Ag, and fibrinogen was complete in 23 subjects, and data for D-dimer was complete in 22 subjects. Inter-assay coefficients of variation were <10% for all coagulation parameters.

Statistical analyses

Data was analysed using the SPSS (Version 9.0) statistical software package (Chicago, Illinois, USA). Results were considered statistically significant at the $p \leq 0.05$ level, and all testing was two-tailed. All coagulation measures and C-reactive protein were logarithmically transformed before analyses. Data are given as mean ± SD except in figures 2 and 3, where the geometric mean of coagulation measures is presented to allow for comparison with previous study findings (13). Pearson's correlation analyses, Student's *t*-test, and univariate analysis of variance (ANOVA) were used to test for associations between health characteristics and coagulation measures at baseline. Student's *t*-test was also applied to test for a difference in HR, BP, and cortisol before catheter insertion between visits.

We applied repeated measures ANOVA to test for a significant within-subject change in coagulation measures (4 time points), HR (4 time points), BP (3 time points for SBP and DBP each), and salivary cortisol (4 time points) across baseline, stress, and recovery (termed "stress" effect) and between week one and week three (termed "habituation" effect). Note that a significant "stress-by-time-interaction" would mean that the magnitude of the response in a particular variable from baseline to post-stress and/or its recovery from stress would differ in the two testing sessions. Multivariate testing for "stress", "habituation", and "stress-by-habituation interaction" was by Roy's Largest Root. To account for violations of the sphericity assumption, we applied the Greenhouse-Geiser correction of degrees of freedom. Post hoc analyses were by Fisher's Least Significant Difference.

Results

Sample characteristics

The health characteristics of the 24 male participants who completed the protocol suggest that our sample was in reasonably good health and of middle to high socioeconomic status (Table 1). The majority of subjects showed favourable health behaviour, and, despite the fact that our subjects were middle-aged (range 38 to 59 years), the cardiovascular risk factor panel assessed suggested relatively low cardiovascular risk. Indeed, the Wilson-Framingham algorithm (31) predicted an average

Table 1: Health characteristics of 24 healthy men studied (mean ± SD).

Age (yrs)	47.1 ± 7.4
Level of education[1]	0, 2, 6, 16
Marital status[2]	5, 16, 3, 0
Smoking status[3]	3, 2, 4, 15
Alcohol consumption (days/week)	2.38 ± 1.58
Physical exercise[4]	10, 4, 5, 5
Body mass index (kg/m^2)	25.1 ± 2.4
Waist-to-hip ratio	0.88 ± 0.07
Total serum cholesterol (mg/dl)	201.1 ± 34.0
Low-density lipoprotein cholesterol (mg/dl)	126.5 ± 29.2
High-density lipoprotein cholesterol (mg/dl)	50.5 ± 10.5
Serum triglycerides (mg/dl)	120.3 ± 46.8
Screening systolic blood pressure (mmHg)	125.5 ± 13.9
Screening diastolic blood pressure (mmHg)	82.4 ± 7.9
Glycosylated hemoglobin A1c (%)	4.93 ± 0.51
High-sensitive C-reactive protein (mg/dl)	0.11 ± 0.15

[1] Did not finish primary school, graduated from primary school, graduated from secondary school, graduated from high school; [2] unmarried, married, divorced, widowed; [3] current smoker, quit smoking >5 yrs ago, quit smoking >10 yrs ago, life-long non-smoker; [4] exercises regularly >2 hrs/wk, exercises regularly, 1-2 hrs/wk, exercise regularly <1 hr/wk, no exercise.

risk of 6.4 ± 4.0% to develop coronary artery disease over a period of 10 years (not shown in detail).

Health characteristics and baseline coagulation measures

The number of days per week subjects consumed alcoholic beverages was inversely associated with FXII:C (r = -.41, p = 0.045) and with fibrinogen (r = -.52, p = 0.010). Age was positively associated with FVII:C (r = .43, p = 0.034). The PT was significantly shorter in subjects who exercised more than 2 hours per week, as opposed to individuals exercising less than 2 hours per week (p = .018). All other relationships between the various coagulation measures and health characteristics listed in table 1 were not statistically significant.

Measures of anticipatory arousal

Before catheter insertion SBP (129.1 ± 16.4 vs. 119.5 ± 13.5 mmHg, p = .001) and HR (73.6 ± 13.1 vs. 67.6 ± 11.5 beats/min, p = 0.026) were both significantly higher at week one than at week three. In addition, ANOVA showed that SBP significantly decreased thereafter till baseline of the stress test at week one, but not at week three (p <.001), with a similar trend towards significance seen for HR (p <0.09). DBP and salivary free cortisol levels before catheter insertion were not significantly different between week one and week three.

Hemodynamic and cortisol responses to stress

Figure 1 summarizes the stress responses of hemodynamic and cortisol measures from baseline to immediately post-stress and recovery for the two visits. There were main effects for "stress" in terms of HR ($F_{40.1}$ = 6.10, p <.001; panel A) and DBP ($F_{150.6}$ = 18.8, p <.001; panel C) suggesting that the magnitude of the significant increase in DBP (p <.001) and HR (p <.001) from baseline to post-stress did not differ between weeks one and three. A "stress-by-habituation interaction" emerged for SBP ($F_{7.69}$ = .96, p = 0.005; panel B) and salivary free cortisol ($F_{5.37}$ = 0.77, p = 0.007; panel D). Post-hoc analyses revealed that SBP increase from baseline to post-stress was greater at week one than at week three (p = 0.002). Recovery of cortisol levels between post-stress and 45 min (p = .058) and between 45 min and 105 min (p = .004) was more readily achieved at week three.

Effect of stress on coagulation responses and recovery

With the exception of APTT and PT (data not shown), repeated measures ANOVA showed a significant main effect for "stress" between baseline, immediately post-stress, and the two recovery periods in terms of VWF:Ag ($F_{6.53}$ = .98, p = .003), fibrinogen ($F_{6.72}$ = 1.01, p = .003), FVII:C ($F_{9.88}$ = 1.41, p <0.001), FVIII:C ($F_{7.11}$ = 1.02, p = .002), FXII:C ($F_{8.25}$ = 1.18, p = .001), and D-dimer ($F_{4.38}$ = .69, p = .017).

Post hoc analyses revealed a significant increase in VWF:Ag (Fig.1, panel A) from baseline to stress (p = 0.004), and significant decreases from stress to recovery 1 (p <0.001) and 2 (p = 0.001). A similar reaction pattern was observed for fibrinogen, FVII:C, and FXII:C. Fibrinogen (Fig. 1, panel B) increased with stress (p = 0.019), and decreased from stress to recovery 1 (p <0.001) and 2 (p = 0.015). FVII:C (Fig. 1, panel C) increased with stress (p = 0.004), and had recovered at 45 min (p <0.001) and at 105 min (p = 0.012). FXII:C (Fig. 2, panel B) significantly increased from baseline to stress (p = 0.018) with a subsequent decrease from stress to recovery 1 (p <.001) and 2 (p = 0.025). Plasma levels of VWF:Ag, fibrinogen, FVII:C, and FXII:C were not significantly different between baseline and either of the two recovery times. Also, these measures showed no significant difference between the two recovery times.

Changes in D-dimer and FVIII:C over time were somewhat different from those observed for VWF:Ag, fibrinogen, FVII:C, and FXII:C. Although the absolute value of D-dimer (Fig. 1, panel D) increased from baseline to stress, this finding did not reach statistical significance (p = 0.221). While there was an almost flat response curve of D-dimer between baseline and stress with the first testing session (p = 0.814), D-dimer showed a trend towards significant increase between baseline and stress with the third testing session (p = 0.087). In contrast, D-dimer significantly decreased between stress and recovery 1 (p = 0.001), and showed a similar trend to decrease from stress to recovery 2 (p = 0.062). There were no significant differences between baseline and recovery D-dimer values or between the two recovery values of D-dimer.

FVIII:C (Fig. 2, panel A) significantly increased from baseline to stress ($p < .001$) with a subsequent decrease from stress to recovery 1 ($p = 0.001$) and 2 ($p = 0.055$). Interestingly, FVIII:C levels at recovery 1 ($p = 0.095$) and at recovery 2 ($p = 0.022$) were higher than at baseline suggesting sustained elevation of FVIII:C up to 105 min after the end of the stressor. The two recovery values of FVIII:C did not differ significantly.

Effect of habituation on coagulation responses and recovery

No significant "stress-by-habituation-interaction" emerged for any coagulation measure. In other words, the magnitude of the acute stress response from baseline to stress, as well as the time required for coagulation activation to recover from stress did not differ significantly between the two testing sessions.

However, FVIII:C ($F_{17,9} = 0.78$, $p < 0.001$) and FXII:C ($F_{12,7} = 0.55$, $p = 0.002$), but not the other measures, showed a significant main effect for "habituation". Post hoc analyses revealed that for both factors values at baseline (FVIII:C, $p < .001$; FXII:C, $p = .041$), at stress (FVIII:C, $p < 0.001$; FXII:C, $p = 0.001$), at recovery 1 (FVIII:C, $p = 0.014$; FXII:C, $p = 0.011$), and at recovery 2 (FVIII:C, $p = 0.026$; FXII:C, $p = 0.026$) were all higher at week one than at week three (Fig. 2, panel A and B).

Discussion

We corroborated the responsiveness of VWF:Ag, fibrinogen, FVII:C, and FVIII:C to an acute standardized mental stressor (10-16). In extension to previous studies we measured the acute stress responsiveness of APTT, PT, and FXII:C. Hence, a novel finding was that acute stress elicited an increase in FXII:C, suggesting that stress may affect processes at the very beginning of both the extrinsic and intrinsic pathways of blood coagulation. APTT and PT were unchanged after stress, perhaps because they are measures too crude to mirror changes in the other, more sensitive components of the coagulation cascade. Contrary to our previous studies (17, 18), the increase in D-dimer from baseline to stress did not reach statistical significance. Possible explanations could be poor sensitivity (32) and agreement (33) of the D-dimer assay applied compared to that used previously (17, 18). Another explanation may relate to acceleration of clotting because of SNS activation with anticipatory arousal (34, 35). Indeed, the SNS activity markers SBP and HR were higher before catheter insertion at week one compared to week three. The flat response curve of D-dimer at week one may thus reflect that most of D-dimer was generated before baseline blood collection, whereas the trend towards a significant D-dimer response to stress at week three may reflect relatively less anticipatory anxiety because subjects felt more familiar with the stress protocol.

Given the consistency of the abovementioned hemostatic findings with the literature, we are confident that the stress protocol was a feasible test for our main study aim, i.e. whether coagulation responses to stress and recovery from stress adapt to three stress repeats one week apart. Notably, the issue whether coagulation responses would adapt to repeated stress has not yet been investigated and, therefore, the present study greatly expands previous research investigating effects of single stress episodes on hemostasis (19). We found that the magnitude of stress-induced changes and subsequent recovery showed no habituation in any coagulation variable. Since all three visits spanned a period of just two weeks, we feel that a seasonal effect is unlikely to account for higher FVIII:C and FXII:C at visit one. Besides a sample size issue not allowing us to detect a significant habituation effect, changes in coagulation to stress could be more robust in experiencing habituation than those in SBP (i.e., the SNS) and cortisol (i.e., the hypothalamus-pituitary-adrenal axis). Consistent with previous studies (26, 36), SBP response was attenuated and cortisol recovery was brisker at week three than at week one. Studies extending the number of visits to more than three are necessary to test whether the coagulation system would eventually adapt. However, an evolution paradigm posits that a hypercoagulable state during and after stress protected our ancestors from exaggerated bleeding when hurt in "fight-or-flight" (4, 19). Accordingly, habituation of coagulation activation to very similar "fight" or "flight" stress would confer a disadvantage rather than a benefit in terms of survival of the human species.

Whereas there was no response and recovery habituation of the various coagulation changes, FVIII:C and FXII:C at week three were lower for all four time points than at week one. Aforementioned anticipatory stress might underlie elevated basal activity of FVIII:C and FXII:C at week one. Although we did not measure catecholamines, arousal accompanying discharge of epinephrine mainly from the adrenal medulla robustly and dose-dependently increases FVIII:C in human plasma (37). However, whether catecholamine spillover provokes FXII:C increase is unknown. Notably, salivary free cortisol concentration before catheter insertion did not differ between weeks one and three. Differences in basal activities of FVIII and FXII between visits were thus not likely driven by the hypothalamus-pituitary-adrenal axis.

With the exception of FVIII:C, all coagulation measures had fully recovered 45 min after termination of stress. While recovery of FVII:C and FXII:C have not been investigated so far, our findings in terms of vWF antigen and FVIII:C recovery 45 min after stress confirm a recent study by Steptoe et al. (13). We additionally found that FVIII:C remained higher even after 105 min of recovery compared to baseline, suggesting that the duration of stress-induced FVIII:C increase is perhaps of clinical importance. On the other hand, we could not confirm another study by Steptoe et al. (12) who found fibrinogen remained significantly increased 45 min after stress compared to baseline. While that study showed greater fibrinogen

responses in women than in men, we only studied men. D-dimer had significantly recovered between post-stress and 45 min of recovery in the present study. In contrast, studying elderly subjects, D-dimer had further increased between post-stress and 14 min after stress (21). However, many of those subjects had atherosclerosis, which is regularly accompanied by impaired endothelial anticoagulant function (38). Subjects with atherosclerosis, as compared to those without, show an exaggerated procoagulant stress response (17, 39-42).

Apart from the small sample size, three further limitations of our study need to be addressed. First, we investigated men in reasonably good health, and, therefore, our results may not generalize cardiovascular disease populations and women. Second, factor assays for week one were performed in an earlier batch than factor assays for week three. To further analyse whether this affected the study results, eleven paired specimens from weeks one and three were thawed and re-run in the same batch for FVIII and FXII. Results for FXII:C remained the same as the initial analysis, confirming the findings for FXII:C. The repeat results for FVIII:C confirmed the findings for response to stress, but the habituation results could not be interpreted due to FVIII degradation. Third, changes in coagulation measures were modest, and the bulk showed full recovery 45 min after stress. Whether, and under which conditions the extent and duration of the observed coagulation activation are of clinical significance is unclear. However, in the case of at-risk individuals, particularly those with cardiovascular diseases and the elderly, an incapacity of the coagulation system to adapt to repeated stress exposure might result in exaggerated and prolonged procoagulant responses to daily stressors. This could provide one explanation for the epidemiological finding of an increased risk of coronary events following anger and other intense emotions.

To summarize, we confirmed the acute responsiveness of VWF:Ag, fibrinogen, FVII:C, and FVIII:C to a single mental stressor, and showed for the first time, that FXII:C (but not APTT and PT) is also responsive to acute mental stress. We further confirmed that recovery of elevated VWF:Ag following stress requires 45 min while FVIII:C recovery is not achieved at 45 min. We additionally showed that 45 min sufficed for FVII:C, FXII:C, and D-dimer to recover. In contrast to one previous study, fibrinogen had also recovered 45 min after stress in our subjects. A novelty of our study was the extension of the recovery time beyond 45 min. We found that FVIII:C remained significantly higher 105 min after stress compared to baseline. The present study was the first to investigate whether coagulation responses adapt to repeated stress suggesting that anticipatory stress may affect FVIII and FXII, but that the magnitude of the stress response in coagulation measures does not habituate.

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