

Aus dem Max-Planck-Institut für Psychiatrie

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**Coagulation responses to acute and chronic stress – a comparison
between remitted depressed patients and healthy controls**

Dissertation

**zum Erwerb des Doktorgrades der Medizin
an der Medizinischen Fakultät der
Ludwig-Maximilians-Universität München**

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Tag der mündlichen Prüfung: 22.12.2016

für meine Familie

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Summary

An increased risk of cardiovascular morbidity and mortality has been demonstrated in studies on depressed individuals.

One of the mechanisms discussed to explain this relationship is an imbalance in the hemostatic system.

From an evolutionary perspective it appears plausible that stress-procoagulability is a beneficial adaptation to protect an organism from excessive bleeding in fight-flight situations. However, an imbalance of this system is harmful as a shift toward a hypercoagulable state contributes to atherosclerosis formation and acceleration of existing atherosclerotic plaques.

In depression, such hypercoagulable states have been frequently described – both at rest and in response to acute stress. Depression as a stress-related disease is often concurrent with chronic stress, as well as with hypothalamic-pituitary-adrenal (HPA)-axis alterations. A central question of this work is whether chronic stress and depression are independent factors influencing basal and stress-induced coagulation, or if they potentially have an interacting effect.

In the present study the procoagulant factors fibrinogen, D-Dimer, Von-Willebrand-Factor (VWF) and the antifibrinolytic factor plasminogen-activator-inhibitor (PAI-1) were measured in 63 subjects from an epidemiological sample, who were recruited for presence and absence of a history of major depression into a remitted major depression group (MD) and a control group. Further, the participants were divided into groups of high and low chronic stress (CS). All four coagulation factors were measured at baseline and all but PAI-1 were measured also following a psychosocial stress test. To evaluate chronic stress the Trier inventory of chronic stress (TICS) was employed while acute stress was elicited using a well-established psychosocial stress test, the Trier Social Stress Test (TSST). This 13-minute test combines preparation, a mock job interview and a mental arithmetic task in front of an evaluating audience.

The following results were observed: fibrinogen was higher in the remitted depression group at baseline and depression history had a significant effect also on stress-induced changes in fibrinogen. A large body of research has identified fibrinogen as a potential marker for an increased cardiovascular risk in depression, and even a marker for depression. The latter was supported by our findings. Furthermore, a trend toward higher D-Dimer levels at rest in chronically stressed subjects was observed. CS had an effect on stress-induced D-Dimer increases, which was independent from a history of major depression.

Unlike previous research this work does not observe differences in VWF and PAI-1 plasma concentrations between the MD and CS groups, neither at rest nor following the stress test. Novel in this study was that the recruited subjects were in remission from major depressive disorder (as diagnosed using DSM-IV) for at least 6 months. This work adds to the existing knowledge regarding fibrinogen as a potential marker for depression in that it identified fibrinogen as also being increased in cases of remitted depression.

Further research is needed to investigate the role of fibrinogen in remitted depression.

In particular, interventional studies examining the effect of known treatments of depression on fibrinogen levels are needed. It would be advisable that physicians treating depression are aware of their patients' cardiovascular risk factors and that they give depression, and also remitted depression, the same amount of attention as a traditional cardiovascular risk factor. Monitoring fibrinogen in depressed patients should be considered a real option in the future.

Abbreviations

ACTH	Adrenocorticotrophic hormone
BDI-II	Beck Depression Inventory, version II
BMI	Body Mass Index
BSKE	Multidimensional adjectives questionnaire regarding current state (BSKE: Befindlichkeitsskalierung nach Kategorie von Eigenschaftswörtern)
CRH	Corticotropin releasing hormone
CRP	C-reactive Protein
CS	Chronic stress
DNA	Desoxyribonucleic acid
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders (4 th edition)
EDTA	Ethylendiaminetetraacetat
ELISA	Enzyme linked immunosorbent assay
HDL	High-density lipoprotein
HPA	Hypothalamic-pituitary axis
IL	Interleukin
LDL	Low-density lipoprotein
M-CIDI	Munich- Composite International Diagnostic Interviews
MCH	Mean corpuscular hemoglobin. Hemoglobin amount per erythrocyte count
MCV	Mean corpuscular volume. A measure of the average volume of a red blood cell
MD	Remitted major depression
RNA	Ribonucleic acid
SAM	Sympathetic Adrenal Medulla
SCSS	Screening scale for the Trier inventory of chronic stress
SNS	Sympathetic Nervous System
SSRI	Selective serotonin reuptake inhibitor
t-PA	Tissue plasminogen activator
TICS	Trier inventory of chronic stress
TNF α	Tumor necrosis factor alpha
TSST	Trier Social Stress Test
VWF	Von-Willebrand-Factor

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1. Introduction

Depression is a debilitating disease with a high socioeconomic impact. Its lifetime prevalence ranges between 10 and 15% (Lépine & Briley, 2011) and the World Health Organization projects that by 2020, depression will be the second leading cause for disability (Murray & Lopez, 1996). An overall relative risk of 1.64 for the development of coronary heart disease in depressed subjects has been described (Rugulies, 2002) and the risk for cardiac mortality is increased in patients with major depressive disorder (Carney & Freedland, 2008; Cuijpers & Smit, 2002; Nemeroff & Goldschmidt-Clermont, 2012; Von Känel et al., 2013).

One factor that might account for the elevated risk for cardiac events in depression is an imbalance between coagulation and fibrinolysis (Bacon et al., 2006; Hemingway & Marmot, 1999; Surtees et al., 2008; Von Känel, Dimsdale, et al., 2004).

While coagulation is an interaction of various factors that causes „thickening of the blood“, expressed colloquially, fibrinolysis reverses this process by “dissolving coagulated blood“. Coagulation and fibrinolysis work together in order to ensure optimal functioning of blood flow and to repair vessel wall lesions. In many healthy subjects, coagulation, as well as fibrinolysis are activated simultaneously as a response to acute mental stress, with the balance shifted toward a procoagulant milieu (Hjemdahl & Von Känel, 2012). From an evolutionary standpoint a procoagulant response is biologically sensible in that it may protect the organism from possible blood loss during a fight-or-flight situation.

In patients with impaired endothelial function, i.e. arteriosclerosis, coagulation constantly outweighs fibrinolysis thereby resulting in a hypercoagulable state that increases the risk for cardiovascular events such as myocardial infarction or ischemic stroke. Several studies suggest that in depression, there also exists a shift towards a hypercoagulable state (Lahlou-Laforet et al., 2006; Tsai, Hong, Liou, Yu, & Chen, 2008; Von Känel, Bellingrath, & Kudielka, 2009a; Wium-Andersen, Orsted, & Nordestgaard, 2013) which could explain the increased cardiovascular mortality in these depressed patients (Tsai et al., 2008).

Despite the fact that well known risk factors such as hypertension, dyslipidemia, and diabetes history are not generally seen in depression, the cardiovascular risk is still increased. A common element seen in both classical risk factors and depression is an elevation in fibrinogen. The relationships between impaired hemostasis, stress and depression are not yet fully understood. This work attempts to make a contribution to understanding them better.

2. Theoretical background and current research

This chapter gives a summary of the theoretical background of the study. First, the major stress response systems, the sympathetic nervous system (SNS) and the hypothalamus-pituitary-adrenocortical (HPA) axis will be introduced, followed by a description of Virchow's triad: it serves as the foundation for understanding how changes in blood composition, alterations in blood flow, and vascular endothelial injury, can elevate the risk for thrombosis. Since a major aspect of this triad is hypercoagulability, the coagulation system will be described. Finally, the current state of research regarding the role of depression, acute and chronic stress on the coagulation system will be summarized.

2.1. The physiology of the stress reaction: the SNS and the HPA-axis

One of the pioneers in the area of stress research, Hans Selye described “stress as the failure to respond appropriately to emotional or physical threats to the organism, whether actual or imagined” (Selye, 1956 , as cited in Hamer & Malan, 2010). A breaking down of efficient adaptation mechanisms to chronic stress over long periods of time is harmful and can lead to disease. The two major stress response systems are the sympathetic adrenal medulla (SAM) axis as part of the autonomic nervous system, and the hypothalamic pituitary adrenal (HPA) axis (Houtveen, 2001). An understanding of these stress systems is relevant for this work because overactivities of both systems have been described in depression (Von Känel & Bacon, 2013) and because a dysregulation of the HPA-axis may also influence the vascular system negatively. In cases of chronic stress and depression, HPA-axis alterations may be responsible for disturbed hemostatic and endothelial function (Von Känel & Bacon, 2013), which may explain increased cardiovascular morbidity in these patient groups.

The Sympathetic-Adrenal-Medulla (SAM) axis

The hypothalamus as the subordinate structure of the autonomic nervous system regulates both the sympathetic and parasympathetic nervous systems. It initiates the release of norepinephrine from post-ganglionic sympathetic neurons and the release of adrenaline and norepinephrine from adrenal medulla (SAM).

The main function of this system is to maintain an equilibrium that ensures external demands on the organism can be met. This equilibrium, which Cannon termed ‘homeostasis’ (Cannon, 1915) is important, for example, when the body faces increased energy demands during exercise or in a ‘fight-flight’ situation. The responsiveness of the SAM system is immediate and

allows the organism to react without delay, for example by raising blood pressure and heart rate and initiating vasoconstriction.

The Hypothalamic-Pituitary-Adrenal (HPA) axis

In response to stress, corticotropin-releasing hormone (CRH) is released from neurosecretory nerve terminals in the hypothalamus, then transported to the anterior pituitary. Here CRH, together with vasopressin, acts to stimulate secretion of ACTH into the bloodstream. Once at the adrenals, ACTH initiates the synthesis of corticosteroids such as cortisol. Cortisol's functions include raising of blood sugar, modulation of the immune system and anti-inflammatory actions. Further, cortisol is a catabolic and lipogenic hormone.

The HPA-axis is regulated by a negative feedback mechanism by which cortisol receptors in the brain and at the pituitary inhibit further synthesis and release of both CRH and ACTH, respectively. The humoral HPA-axis response is markedly slower than the nervous SAM response. Furthermore, cortisol acts via translocation of the activated glucocorticoid receptor on the transcriptional or genomic level and therefore its effects are further delayed.

In depression, a desensitization of the glucocorticoid receptor for cortisol leads to an insufficient negative feedback and to increased CRH, cortisol and arginine-vasopressin levels (Binder, 2009). Depressed patients show impairments in HPA-axis regulation, as studies involving the combined Dexamethasone/CRH-Suppression-Test have shown: depressed individuals respond to a CRH injection under the suppressive effects of dexamethasone with exaggerated ACTH and cortisol levels, whereas the CRH effects under dexamethasone suppression are considerably blunted in healthy subjects (Ising et al., 2007).

Cortisol and the CRH can further influence synthesis and release of serotonin, norepinephrine and dopamine and modulate their receptors. Hypersecretion of these hormones can lead to the clinical manifestations of depression and anxiety, mainly through actions on limbic and neocortical structures (Holsboer & Ising, 2010). A dysregulation of the HPA-axis may also influence the vascular system: in chronic stress and depression, HPA-axis alterations may be responsible for disturbed hemostatic and endothelial function (Von Känel & Bacon, 2013). In a placebo-controlled trial, Brotman et al., (2006) showed that a 5-day treatment course of dexamethasone in healthy men increased circulating levels of fibrinogen, and the procoagulant factors VII, VIII and XI.

Although SAM and HPA systems seem to serve different purposes, aspects of interdependency of the two systems exist: cortisol as part of the HPA-system may be involved in modification of sensitivity of adreno-receptors (Houtveen, 2001; Kvetnansky et al., 1995), which belong to the SAM system, and further, CRH gene expression can be altered by catecholamines (Kaminski & Watts, 2013).

2.2. Virchow's triad and plaque formation

The psychophysiological mechanisms linking depression and stress to coronary artery disease, and ultimately cardiovascular events such as stroke or myocardial infarction, have not been fully elucidated (Von Känel, Bellingrath, et al., 2009a). Recent research suggests that a hypercoagulable state may contribute to the progression of atherosclerosis and atherosclerotic plaque rupturing, and ultimately leading to myocardial infarction (Von Känel & Bacon, 2013). Such a hypercoagulable state, i.e. increased clotting, decreased fibrinolytic activity and impairments of the endothelium, has also been described in depression and may be one important element to link depression with increased cardiovascular risk. Particularly autonomic dysfunction and alteration in the hypothalamic pituitary adrenal axis might mediate perturbed hemostatic and endothelial function under chronic stress and negative affect, e.g., depression or anxiety (Von Känel & Bacon, 2013).

Hypercoagulability, hemodynamic changes and endothelial dysfunction, also known as 'Virchow's triad' (Figure 1), describe properties of the vascular system that constitute risk factors for thrombosis and, ultimately, cardiovascular events such as myocardial infarction or stroke caused by thromboembolisms (Chung & Lip, 2003). It is important to note that Virchow's triad is complemented by inflammatory processes to ultimately increase the risk for cardiovascular events, as Figure 1 shows.

Hypercoagulability

Hypercoagulability describes a shift toward a 'thickening of the blood' through activation of platelets and the synthesis and activation of procoagulant factors. The procoagulant factors, thrombin and fibrin are formed in a complex cascade-like manner that involves multiple factors, as Figure 2 Shows. Triggers for hypercoagulability may be an activation of the sympathetic nervous system (Von Känel & Dimsdale, 2000)

Hemodynamic changes

Whenever blood flow is slowed down, the chance of turbulences and therefore the risk for thrombus formation is increased. Causes for slowing of blood flow can be shifts in the composition of blood, for example through exsiccosis, increases in platelet count (thrombocytosis), or obstruction of the vessel lumen through atherosclerotic plaques.

Endothelial damage

The healthy endothelium possesses anticoagulant, antiplatelet and fibrinolytic mechanisms and releases vasodilative nitric oxide to maintain vascular tone and structure and proper blood flow (Davignon & Ganz, 2004).

These mechanisms can be disturbed in a variety of ways: through inflammatory processes, increased permeability of the endothelium, a procoagulant milieu and diminished production of nitric oxide, which acts anti-atherogenic by inhibiting cellular adhesion, migration and proliferation (Ross, 1999). In major depression lower nitric oxide activity has been reported together with increased levels of reactive oxygen species (Chrapko et al., 2004); both of which point to a compromised endothelium (Von Känel & Bacon, 2013).

The acute phase fibrinogen which is studied in the present work has been identified as being involved in the pathogenesis of atherosclerosis in that it increases endothelial permeability, platelet reactivity, monocyte migration, LDL accumulation and formation of foam cells (Borissoff, Spronk, & Cate, 2011). The large population-based, prospective, observational CARDIA study (Green et al., 2010) revealed a significant association between fibrinogen levels and enhanced rate of coronary-artery calcification and carotid intima-media thickness over a 13-year study interval. Young subjects with elevated fibrinogen levels were more likely to exhibit these two precursory signs for atherosclerosis in middle age.

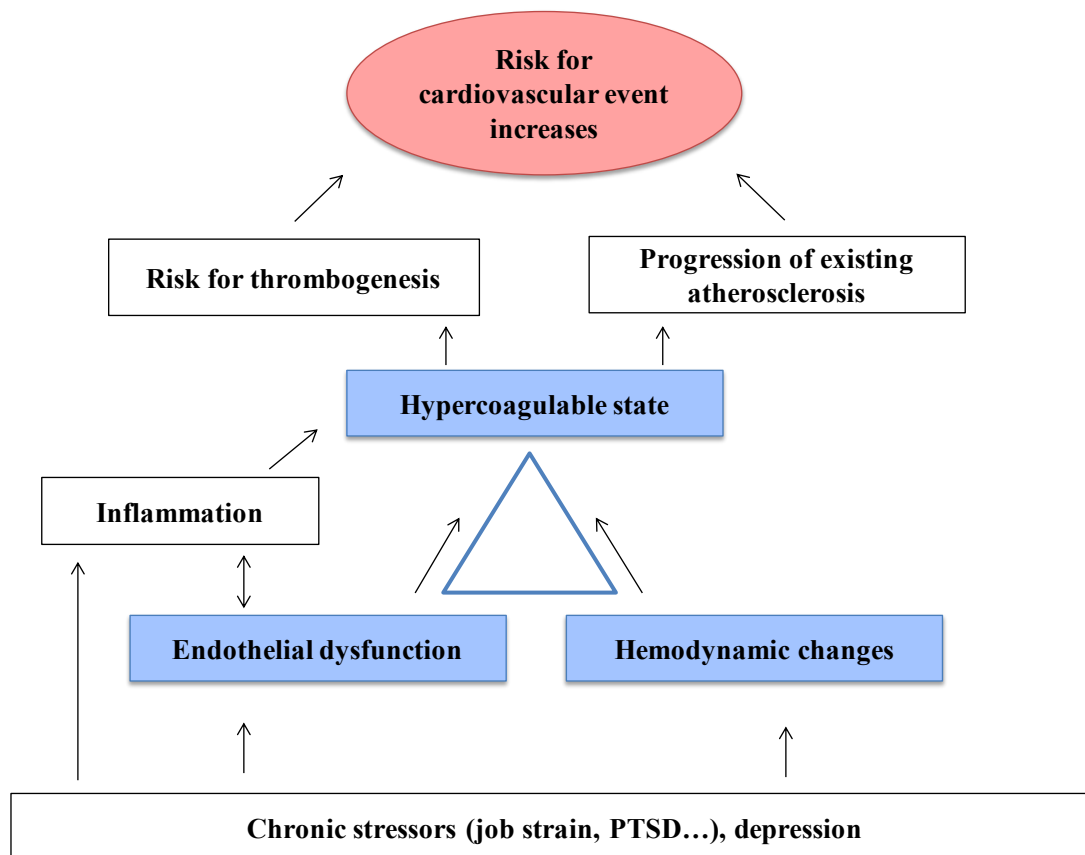


Figure 1. Virchow's triad and cardiovascular risk. The relationships between depression, stress and the hemostatic system. Blue boxes indicate that these elements belong to 'Virchow's triad'. Based on: Austin, Wissmann, & Von Känel (2013).

The hemostatic system and atherosclerotic plaque formation

When the endothelium lining the inner wall of a blood vessel is damaged, for example through small lesions caused by hypertension, the protein VWF becomes exposed. Platelets present in the blood that bypass this damaged site bind to VWF via their glycoprotein Ib-alpha receptors. Once adherent to the site of injury, platelets secrete mediators such as cytokines, chemokines, growth factors and the intrinsic components of the coagulation system. These interact with leukocytes and dendritic cells and commence the complicated process of atherosclerotic plaque formation. (Borissoff et al., 2011). It is believed that a procoagulant environment will gradually promote pre-existing atherosclerosis through deposits of fibrin in the vessel wall and through inflammatory processes (Borissoff et al., 2011; Falk & Fernández-Ortiz, 1995). Ruptured plaques are carried away with the bloodstream and may occlude coronary arteries and cerebral vessels, causing myocardial infarction and stroke, respectively.

2.3. Hemostasis, coagulation and fibrinolysis

Hemostasis describes the end result of the interaction of the platelet system with coagulant and anti-coagulant influences. It aims to support a dynamic equilibrium that allows proper blood flow and, ensures the vessel wall is sealed to prevent blood loss in case of injury (Furie & Furie, 2008). Since hemostasis is a very complex system, its components relevant to the present work shall be described here in a simplified manner. There are four steps involved in hemostasis: first, primary hemostasis as the initiation of the platelet plug, second, the coagulation cascade with its extrinsic and intrinsic paths; third, the cessation of further thrombus formation by antithrombotic mechanisms; and fourth, fibrinolysis, which removes the clot (Leung, 2014). The coagulation cascade, which consists of the intrinsic and extrinsic systems, leads to formation of fibrin polymers which consolidate the platelet plug that had been formed during primary hemostasis (Halperin & Reber, 2007).

The intrinsic pathway of coagulation

When blood comes into contact with damaged tissue, platelets become activated. An example where damaged tissue is exposed are minute lesions that happen many times daily even in healthy persons. These activated platelets expose negatively charged surfaces that activate the plasma protein Factor XII. Factor XII, like all components of the intrinsic system, is always present in the blood. As figure 2 depicts, activation of Factor XII sets into motion a cascade of activation of other plasma proteins that result in the formation of activated factor X, the end product of both the extrinsic and intrinsic systems.

The extrinsic pathway of coagulation

The extrinsic path is a fast-response system that is set into action once tissue factor (Factor VII) in the endothelial cells becomes exposed through injury. Activated Factor VII then also activates Factor X, at which point the two pathways merge.

Both pathways can also be activated without overt injury to the endothelium, which is particularly the case in stress-induced coagulation, as is examined in this work.

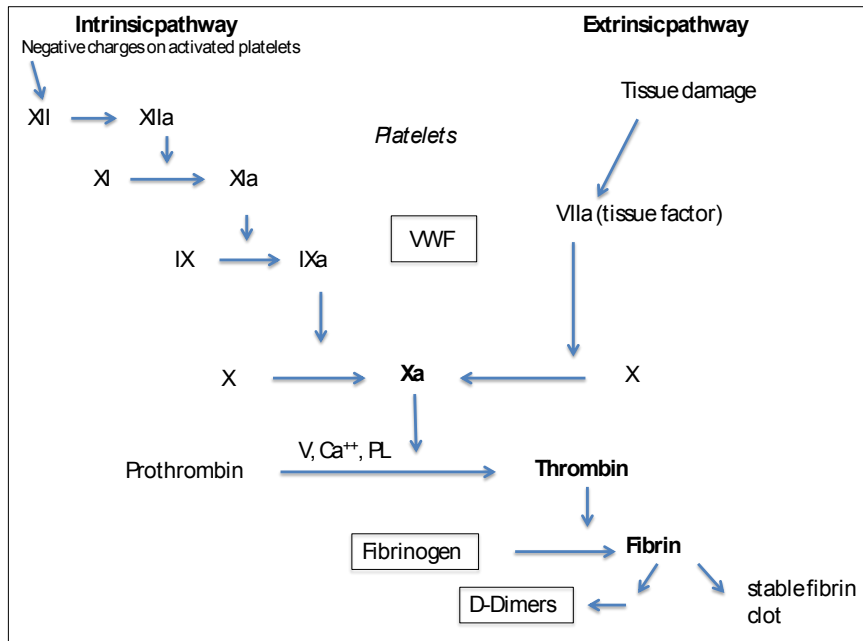


Figure 2. The hemostatic pathways. The fast-acting extrinsic and slower intrinsic coagulation pathways converge at Factor Xa, which ultimately converts pro-thrombin to thrombin. Thrombin, in turn converts fibrinogen to fibrin which binds platelets to one another to form a thrombus. Factor V, calcium ions (Ca⁺⁺ and phospholipids of platelet membrane (PL) act as co-activators in thrombin formation. The steps in which fibrin is broken down into fibrin degradation products, or D-Dimers is known as fibrinolysis. The factors examined in this work (VWF, Fibrinogen, D-Dimers) are marked by boxes. ‘a’ stands for the activated form of the coagulation factor.

Both systems are connected via the common Factor X, as shown in figure 2. Moreover, VWF facilitates the adherence of platelets to injury sites. VWF and platelets belong neither to the extrinsic nor the intrinsic pathway, they can be seen more as links between the two systems. From an evolutionary point of view, hemostasis serves as a beneficial adaptation mechanism, in that it protects the organism from bleeding in fight-flight situations. As a response to acute stress, epinephrine and norepinephrine as part of the SAM-axis stimulate the release of certain procoagulant factors (FVIII, VWF and tissue plasminogen activator) via action on beta-adrenergic sympathetic endothelial receptors. Further, epinephrine and norepinephrine activate platelets via alpha2-adrenergic receptors (Von Känel & Dimsdale, 2000).

Fibrinolysis

The opposing mechanism to coagulation is fibrinolysis. Its participating components are depicted in Figure 3. Without this plasma protein system, a thrombus would continue to get larger, as it further stimulates platelets and the coagulation cascade in a positive-feedback loop (Austin, Patterson, & Von Känel, 2011).

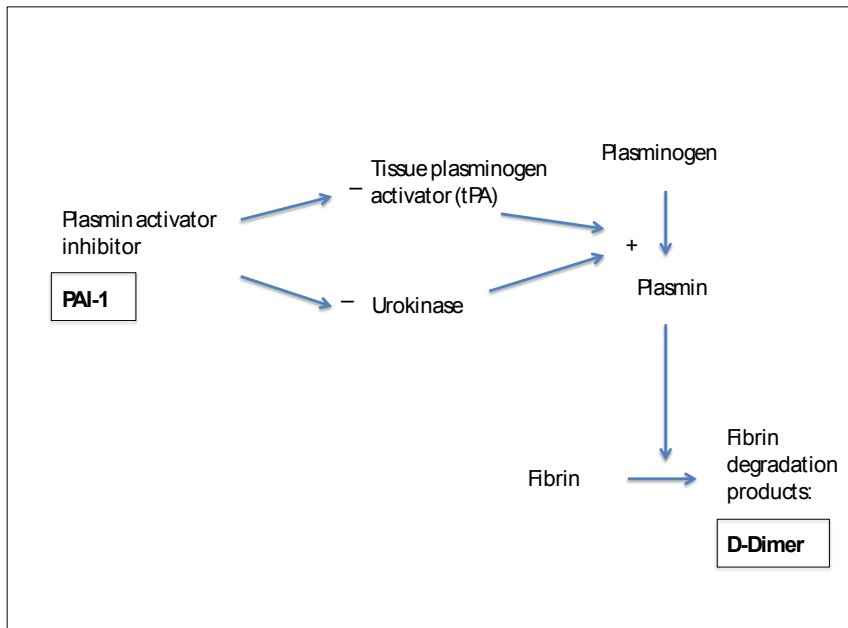


Figure 3. Fibrinolysis as the opposing mechanism to coagulation. As plasmin acts on fibrin, a thrombus is prevented from growing and is broken down. Plasminogen-activator inhibitor acts as a procoagulant factor via its inhibitory effects on t-PA and Urokinase.

Before describing some of the important research regarding the selected coagulation markers and their relations to depression and stress, an overview of the changes that previous research has described is presented (See Table 4).

Table 4. Overview of previous findings regarding the effects of depression, acute and chronic stress on hemostatic markers.(↑Increases with stressor, - no response); as in Mills & Von Känel (2010).

	Major Depression	Acute stress	Chronic stress
	HPA-axis dysregulation	Mainly SNS activation Psychosocial stress: also HPA-axis activation	Predominantly HPA-axis activation
Fibrinogen	Resting levels↑	↑	↑
D-Dimer	Attenuated response to acute stress and prolonged recovery ↑post stress	↑	↑
VWF	-	↑	↑/-
PAI-1	Resting levels ↑	-	↑

2.4. Depression and the coagulation system

Depression is associated with increased levels of fibrinogen (Martins-de-Souza et al., 2014; Wium-Andersen, Orsted, et al., 2013; Wium-Andersen, Ørsted, & Nordestgaard, 2013) and increases in depressive symptoms have been described to correlate with elevated fibrinogen levels in an examination of healthy school teachers over a 2-year period (Von Känel, Bellingrath, & Kudielka, 2009b). Further, studies on platelets and psychiatric disease revealed altered platelet functioning in depression. Increased aggregability to the stimulators thrombin and collagen (Lederbogen et al., 2001), as well as heightened reactivity to orthostatic stress (Musselman et al., 1996) were demonstrated in major depression patients compared to controls. Interestingly, platelets store large amounts of serotonin (Williams, 2012), and in depression, storage and metabolism of this neurotransmitter, as well as noradrenergic and serotonergic receptor density have been shown to be altered (Musselman et al., 1996). Research by von Känel et al. (2009) showed associations of severity of depression symptoms and vital exhaustion with attenuated D-Dimer responses to acute stress, but a delayed D-Dimer recovery (Von Känel et al. 2009a). A study on elderly persons found that depressive symptoms were associated with D-Dimer increases after exposure to an acute psychosocial stressor (Von Känel, Dimsdale, et al., 2004). Eskandari et al. (2005) conducted a study on premenopausal women with major depressive disorder (MDD) and report increased resting levels of the antifibrinolytic PAI-1.

2.5. Acute and chronic stress and the coagulation system

Acute and chronic stress do not affect the hemostatic system in the same way (Mills & Von Känel, 2010). These differences are explained in the following paragraphs.

Acute stress

Acute stress activates the sympathetic nervous system, which affects the coagulation system in the following way: catecholamines are able to activate vascular endothelial beta-2 receptors, resulting in increases of FVIII, VWF and t-PA. Catecholamines can further activate platelets (Halperin & Reber, 2007). Acute stress also causes hemoconcentration, a process in which increasing blood pressure causes efflux of plasma to the interstitial space. In hemoconcentration, larger non-diffusible molecules including coagulation markers remain inside the vascular system thereby increasing in concentration (Austin et al., 2011, 2013).

The HPA-axis is activated by acute stress that includes a psychosocial component, as seen in plasma ACTH and cortisol (Bellingrath & Kudielka, 2008; Dickerson & Kemeny, 2004; Höhne et al., 2014) However, chronic stress seems to elicit larger reactions in the HPA-axis. The research literature describes a variety of ways to elicit acute stress in study participants in a laboratory setting, such as mental arithmetic tasks, speech tasks, the mirror star tracing task, and the Stroop color-word conflict test (Austin et al., 2013).

Examinations of healthy subjects revealed increases of procoagulant markers in response to acute stress. Such markers were FVII, FVIII, FXII, fibrinogen, VWF antigen and platelets (Austin et al., 2011; Hjemdahl & Von Känel, 2012; Thrall, Lane, Carroll, & Lip, 2007; Von Känel et al., 2013; Von Känel, Mills, Fainman, & Dimsdale, 2001; Wirtz et al., 2008). However, the pro-fibrinolytic and anticoagulant factor tissue plasminogen activator (t-PA) was increased through acute stress as well in these studies, which indicates that both coagulation and fibrinolysis are activated at the same time (Austin et al., 2013; Hjemdahl & Von Känel, 2012). However, it is suggested that procoagulant mechanisms outweigh fibrinolytic mechanisms, which would result in net hypercoagulability. After exposure to acute stress, levels of coagulation factors returned to normal after 20-45 minutes, as two studies (Von Känel, Preckel, et al., 2004; Wirtz et al., 2008) have described.

Chronic stress

The literature describes different categories of chronic psychological stress, such as work stress, the stress of caregiving and post-traumatic stress disorder (PTSD), all of which have been associated with changes in coagulation (Austin et al., 2011).

Cross-sectional studies have revealed associations of capacity (Hansen, Larsen, Rugulies, Garde, & Knudsen, 2009; Von Känel et al., 2001) Persons caring for their spouse with Alzheimer's disease presented with higher resting D-Dimer levels than non-caregivers matched for age and sex (Von Känel, Dimsdale, et al., 2006). Research on PTSD patients showed an increased morbidity and a higher and more premature mortality due to cardiovascular disease and thromboembolism (Robicsek, Makhoul, Klein, Brenner, & Sarig, 2011). Higher levels of procoagulant hemostasis factors were described in PTSD patients compared to controls, for example VWF antigen and Factor VII (Robicsek et al., 2011). In addition, platelets from PTSD patients showed an increased reactivity to epinephrine/ADP stimulation (Vidović et al., 2011) suggesting increased stress sensitivity.

Furthermore, socioeconomic class has been found to also influence the hemostatic system: fibrinogen, FVII:C and VWF:AG were found to be increased in persons with low socioeconomic status, which was measured by constructs such as occupation and education (Von Känel et al., 2001). Low socioeconomic status has been treated as a form of chronic stress, however, one cannot make the claim that low social status always equates to chronic stress. Nonetheless, there exists some research that found connections between socioeconomic status and hemostatic changes. Von Känel et al., 2001, for example, summarize in their review on psychological stress and coagulation, that socioeconomic status is inversely related to plasma fibrinogen levels. A large public health survey by Berlin's Robert Koch Institute on German adults, found that a high stress burden was more prevalent in persons with low socioeconomic status (Hapke et al., 2013). In this work, chronic stress was assessed by a questionnaire that focuses on work and social stress primarily. Although associations between socioeconomic status and the hemostatic system exist, this work does not examine social status of the subjects.

Regarding changes in coagulation, chronic stress seems to differ from acute stress in that it is more involved in HPA-axis activation and in decreased parasympathetic activity (Hjemdahl & Von Känel, 2012; Von Känel & Bacon, 2013). How cortisol affects the coagulation system has not been studied as extensively as catecholamine effects. In women with coronary artery disease, morning serum cortisol was modestly associated with prothrombotic activity (fibrinogen and VWF) suggesting that by causing a hypercoagulable state, cortisol might be in part responsible for the formation atherosclerosis (Von Känel, Mausbach, Kudielka, & Orth-Gomér, 2008).

One coronary angiography study revealed that patients with hypercortisolism had excess coronary calcifications and atherosclerotic coronary plaques (Neary et al. 2013). Erem et al. (2009) found that in patients suffering from Cushing's syndrome increased platelet count, fibrinogen and PAI-1, and decreased tissue factor pathway inhibitor were measured, which, in summary, confirm a hypercoagulable and hypofibrinolytic state. This evidence strengthens the argument for an association between cortisol and augmented risk for atherosclerosis. Our study sample did not include any persons with clinical hypercortisolism. Nonetheless, the relationship between a disturbance in HPA-axis regulation, which is manifest in hypercortisolism, and atherosclerosis is worth mentioning here, because patients with depression also show alterations in HPA-axis regulation.

2.6. Coagulation markers for acute and chronic stress

In light of recent research in the area of acute stress and coagulation, the following parameters were selected for examination in the present work: fibrinogen, D-Dimer, VWF and PAI-1.

The subsequent section explains the functions of these parameters and summarizes their role in recent research.

Fibrinogen

Fibrinogen is a glycoprotein that is always present in plasma and plays a key role in thrombocyte aggregation and fibrin synthesis. Receptors on activated thrombocytes bind fibrinogen so that it can cross-link thrombocytes. The serine-protease thrombin cleaves fibrinogen, thus converting it to fibrin. Fibrin forms strands of insoluble protein that bind to thrombocytes and are cross-linked with the help of Factor XIII. Fibrin then contracts and hardens and together with aggregated thrombocytes forms a clot that can seal the site of injury.

A large-scale study of the general population by Wium-Andersen et al. (2013) found that elevated plasma fibrinogen levels were associated with depression and psychological distress after adjusting for the confounders age, gender, smoking, BMI, physical activity and chronic stress. Beside its role in blood clotting, fibrinogen is also an important acute-phase-protein, i.e. a plasma protein that rises in concentration in response to inflammation. One of the functions of an acute phase protein is to help the immune system fend off microbes. Although the mechanisms are not yet fully understood, fibrinogen is also related to increased cardiovascular risk: a meta-analysis showed that the hazard ratio per 1 g/L increase in normal fibrinogen levels was 1.8 for coronary heart disease and stroke (Danesh, Lewington, & Thompson, 2005). As previously mentioned, fibrinogen levels have been found to be increased in depression, and it is responsive to both acute and chronic stress. An increase in fibrinogen triggered by psychophysiological stress was shown to be associated with stiffness of the carotid arteries (Ellins et al., 2008), which further underlines the role of stress as a mediator for cardiovascular risk.

D-Dimer

D-Dimer, which is a product of fibrin-degradation in fibrinolysis, is an overall indicator of activation of coagulation and fibrinolysis or fibrin turnover (Von Känel & Bacon, 2013). During coagulation the enzyme thrombin connects soluble fibrin molecules to form a fibrin-

meshwork. This meshwork is further stabilized through Factor XIII, which creates links between fibrin fibrils at their D-fragment sites, creating a meshwork structure that serves as the foundation of a thrombus. During thrombus degradation, plasmin cleaves this mesh at various sites, resulting in polymers of different sizes. Sites in which two fibrin proteofils were linked (the D-fragment sites) remain intact and can be detected by an immunoabsorbant assay using monoclonal antibodies to a specific epitope on the D-Dimer fragment.

In clinical practice D-Dimer serves as a marker for ruling out the diagnoses pulmonary embolism and thromboembolism. Levels below 500ng/ml make it unlikely that a thrombus is in the process of degradation.

D-Dimer plays a pro-atherosclerotic role in macrophage function by enhancing the inflammatory response during foam cell formation (Zhou, Yang, Zhou, & Rui, 2007).

In a series of studies Von Känel et al. were able to show increases of D-Dimer in response to acute psychosocial stress (Von Känel, Preckel, et al., 2004; Von Känel, Bellingrath, et al., 2009a; Wirtz, Redwine, Ehlert, & Von Känel, 2009). It has been further shown that it is increased in inflammation (Borissoff et al., 2011; Zhou et al., 2007).

Von-Willebrand-Factor (VWF)

VWF is a very large protein released from stimulated or damaged endothelial cells and from thrombocytes. It triggers platelet adhesion to places of vascular injury in areas of high shear stress and stimulates the aggregation of platelets.

In acute-stress tasks, including the Trier Social Stress Test (TSST), an increase of VWF has been observed (Mills & Von Känel, 2010). Von Känel and Dimsdale (2000) report a dose-dependent stimulation of VWF antigen during a 15- to 40-min infusion of epinephrine, suggesting VWF production can be stimulated by sympathetic nervous system activation. Positive associations between severity of PTSD symptoms and VWF levels have been reported (Von Känel, Hepp, et al., 2006, 2008).

Plasminogen activator inhibitor (PAI1 or SERPINE1)

Plasminogen activator inhibitor (PAI-1) is a protein that acts on tissue plasminogen activator (t-PA) and Urokinase and prevents these enzymes from promoting plasmin production. Its action thereby prevents fibrinolysis and amplifies or prolongs coagulation. Figure 3 depicts the connection between PAI-1 and the coagulation cascade. Normally, the liver, adipocytes, smooth muscle cells and platelets synthesize PAI-1. In pathological conditions however, such as atherosclerosis, endothelial cells and other inflammatory-stimulated cells secrete increased

amounts of PAI-1 (Binder, 2002). Recent studies suggest a crucial role for PAI-1 in mediating stress-induced hypercoagulability and thrombosis (Jiang, Gingles, Olivier, Miles, & Parmer, 2011). A study by Eskandari et al. (2005) on premenopausal women with major depressive disorder showed increased resting levels of PAI-1. Further, an independent association between high PAI-1 and long term psychiatric symptoms in middle-aged men has been described by Huotari et al. (2010). Lahlou-Laforet et al. (2006) report an elevated PAI-1 activity in depressed patients after adjusting for the presence of chronic heart disease, smoking, triglyceride concentration, hypertension, and body mass index. Subjects with vital exhaustion showed increased baseline PAI-1 levels (Von Känel, Frey, & Fischer, 2004). Furthermore, two of six known polymorphisms for the PAI-1 gene have been found more frequently in patients with major depressive disorder (Tsai et al., 2008). Tsai and his team conclude that increased PAI-1 levels may be one factor in explaining the link between metabolic syndrome and depression. With respect to stress, PAI-1 activity increases have been observed in chronic stress, but it does not seem to be affected by acute stress (Mills & Von Känel, 2010).

3. Research questions and hypotheses

The focus of this work lies on the examination of the influence of a depression history and chronic stress on basal and stress-related coagulation.

Depression symptoms and chronic stressors, such as job strain, may influence vascular health by creating a low-grade hypercoagulable state (Austin et al., 2013). Net hypercoagulability within the vasculature may contribute to increasing the risk for thrombogenesis and elicit progression of atherosclerotic plaques. The result is an increased risk for cardiovascular events, such as stroke or myocardial infarction. The following two sets of research questions will be addressed to help to better understand these associations.

The first set of research questions deals with resting coagulation parameters, while the second set focuses on stress-induced changes of coagulation parameters. Within each set, the effects of depression history and of chronic stress burden are examined independently, as well as their interacting effect.

3.1. Hypotheses regarding basal coagulation

3.1.1. The effects of remitted depression on basal coagulation

Of the coagulation markers studied, fibrinogen stands out to have the strongest association with depression. Major depression has been associated with elevated resting levels of fibrinogen in a variety of studies (Martins-de-Souza et al., 2014; Wium-Andersen, Orsted, et al., 2013) and increases in depressive symptoms and in fibrinogen levels were significantly correlated in an examination of healthy school teachers over a 2-year period (Von Känel, Bellingrath, et al., 2009b).

Another marker that has been extensively studied in relation to depression is PAI-1. A study by Eskandari et al. (2005) on premenopausal women with major depressive disorder (MDD) showed increased resting levels of PAI-1. Further, an independent association between high PAI-1 and long-term psychiatric symptoms in middle-aged men has been described by Huotari et al. (2010). Lahlou-Laforet et al. (2006) report an elevated PAI-1 activity in depressed patients after adjusting for the presence of chronic heart disease, smoking, triglyceride concentration, hypertension and body mass index. In the current study we examine remitted depression in relation to coagulation markers, which remains relatively unexplored. Baune et al. (2012) report that elevated PAI-1 levels were associated with remitted depression in a sample of elderly persons. Kling et al. (2007) examined CRP and amyloid A, which, like fibrinogen

belong to the family of acute-phase-proteins. Both these markers were significantly elevated in a sample of remitted depressed women compared to BMI-matched healthy controls. One can deduce that a pro-inflammatory state that has also been described elsewhere persists even after symptomatic recovery from depression (Kling et al., 2007). This suggests that fibrinogen may also be elevated in remitted depression. Accordingly, the following hypothesis is deduced:

H1.1: Baseline fibrinogen and PAI-1 levels are higher in remitted depressed subjects compared to controls.

3.1.2. The effects of chronic stress on basal coagulation

There are different concepts of what constitutes chronic stress. The two frequently discussed models regarding the workplace are the effort/reward imbalance model (Siegrist, 1996) and the demand/control/support model (Karasek & Theorell, 1990), also termed ‘job strain’. In the former, high demands at work are combined with low security and few career opportunities, while in the latter, the affected person experiences high psychological demands but at the same time has a low decision latitude or job control (Kivimäki et al., 2002).

This combination of high demand and low control has been associated with increased cardiovascular mortality risk (Kivimäki et al., 2002) in a longitudinal study among employees who were free from cardiovascular disease at baseline. The cardiovascular mortality was 2.2-fold. Further, job strain predicted future BMI and cholesterol concentrations. In persons with high job strain, caregivers, or persons suffering from PTSD, increases in the following hemostasis factors have been described: fibrinogen, D-Dimer, FVII, FVIII and VWF. Lower socioeconomic status has been associated with higher resting plasma viscosity, VWF and Factor VIII (Steptoe, Kunz-Ebrecht, Rumley, & Lowe, 2003). A few studies have found that low socioeconomic status is associated with changes in fibrinogen, D-Dimer, FVII, FVIII and VWF. PAI-1 increases at rest have also been associated with chronic stress in a range of studies (Eskandari et al., 2005; Huotari et al., 2010; Lahlou-Laforet et al., 2006; Mausbach et al., 2007; Tsai, 2006). Furthermore, one study found that fibrinogen was elevated in unemployed persons (Kaptoge et al., 2007a). Based on these findings, the following hypothesis is derived:

H1.2.: Subjects under chronic stress have elevated baseline levels of the procoagulant factors fibrinogen, DD, VWF and PAI-1 compared to controls.

3.1.3. The effects of the interaction between remitted depression and chronic stress on basal coagulation

As seen in studies on Dexamethasone/CRH tests, major depression has been shown to have altered stress responsiveness, which is why it is often called a stress-related disease.

So far, interaction effects between remitted depression and chronic stress on resting coagulation levels have not been examined. The hypothesis is therefore a non-directional one.

H1.3.: Baseline procoagulant markers in remitted major depression are altered, depending on the presence of chronic stress.

The second part of this work looks at hemostatic responses to an acute stressor, the Trier Social Stress Test (TSST). Again, the effects of remitted depression and chronic stress, as well as their combined effect are analyzed.

3.2. Hypotheses regarding stress-induced coagulation

3.2.1. The effects of remitted depression on stress-induced coagulation

Regarding healthy subjects, previous research has described an increase in procoagulant factors with acute stress with the following markers showing significant increases: FVII, FVIII, FXII, fibrinogen, VWF antigen and platelets (Austin et al., 2011; Hjemdahl & Von Känel, 2012; Thrall et al., 2007; Von Känel et al., 2013, 2001; Wirtz et al., 2008). For stress coagulability in depressed individuals the literature is rather scarce. Neither of the studies examined includes major depression patients or remitted depressed patients (as diagnosed using the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders)). In a study similar to ours, which also used the TSST with healthy, middle-aged school teachers, increased levels of vital exhaustion and depression were associated with attenuated D-Dimer response to acute stress but with prolonged D-Dimer recovery (Von Känel, Bellingrath, et al., 2009a), indicating prolonged hypercoagulability after stress cessation.

Another study found that depressive symptoms were associated with exaggerated D-Dimer increases after the TSST in elderly subjects (Von Känel, Dimsdale, et al., 2004). Taken together, the literature suggests that D-Dimer changes over time are affected by depressive mood. The following hypothesis is derived:

H2.1.: Remitted major depression has an effect on acute psychosocial stress (TSST)-induced changes in D-Dimer levels.

3.2.2. The effects of chronic stress on stress-induced coagulation

Only few studies have examined how chronic stress influences reactions of the coagulation systems to an acute-stressor. Also, the way in which chronic stress was defined and assessed was not uniform. The results of a study on dementia caregivers showed that the number of negative life events experienced before a speech task was directly correlated with changes in a pro-coagulability score that included the thrombin/antithrombin III complex, D-Dimer, VWF, t-PA and PAI-1 (Von Känel, Dimsdale, Patterson, & Grant, 2003). Another study investigated social support with regards to stress-reactivity. Low levels of social support may also give rise to chronic stress. Wirtz et al. (2009), reported that high levels of social support, as assessed by a questionnaire, were associated with lower fibrinogen and D-Dimer before and after acute stress (TSST). Lower social support was associated with higher fibrinogen and D-Dimer levels, both at baseline and following the stressor. These results were independent of BMI, age, and mean arterial blood pressure (Wirtz et al., 2009). Based on this research, it is hypothesized that

H2.2.: High chronic stress modulates procoagulant responses to acute psychosocial stress.

3.2.3. The effects of the interaction between remitted depression and chronic stress on stress-induced coagulation

Whether chronic stress (CS) and remitted major depression (MD) have a combined effect on stress-induced coagulation has so far not been examined in the literature.

Since major depression is a stress-related disease, an interaction effect is likely. However, a non-directional hypothesis needs to be postulated:

H2.3.: Whether MD+ and MD- differ in their procoagulant responses to acute stress depends on the presence or absence of chronic stress.

4. Methods

The experiment conducted for this study consisted of a preliminary interview of each participant that lasted approximately two hours, and an experimental assessment that took place during an afternoon between 1pm and 5pm at the Max Planck Institute for Psychiatry, Munich. During the afternoon two psychosocial stress tests were carried out with each subject, of which only the first one is of relevance for this dissertation. Participants gave their written consent to take part in the study, which was approved by the local Ethics Committee of Munich's Ludwig Maximilians University. Participants were also informed that they had the right to withdraw their consent at any time. A compensation of EUR 175 was paid for participation.

4.1. Subject Sample

A total of 63 subjects between the ages of 31 and 42 years took part in the study. Mean age was 35.22 years overall; 36.08 (SD: 3.57) years in the control group and 34.7 (SD: 3.48) years in the remitted depression group. The remitted depression group included 40 subjects, the control group 24 subjects. Four subjects in the depression group were taking antidepressant medication. There were 26 males, and 37 females, 13 of which were taking hormonal contraception. Characteristics of the study sample are listed in Table 3 in the Results section.

All subjects were recruited from the Early Developmental Stages of Psychopathology (EDSP) study, a prospective-longitudinal investigation that commenced in 1994. It was designed to investigate prevalence, incidence, risk factors, course of disease and comorbidity of mental disorders and includes a random sample of the city of Munich's population register. In 1994, 3021 participants between the ages of 14 and 24 were interviewed using the Munich- Composite International Diagnostic Interviews (M-CIDI)(Wittchen & Pfister, 1997), which detects psychological disorders using DSM-IV. Between 1995 and 2005 participants were interviewed using the M-CIDI at four different time points. (2005: N = 2210, response rate 73%. 403 participants from the EDSP database were eligible for inclusion as potential participants. They were contacted and informed about the procedure. In case of approval to take part in the study, candidates underwent a screening interview via telephone to assess whether they were eligible for the study.

In recruitment of subjects for the study, the aim was not to have a representative cross-section of the study cohort (EDSP) but to recruit an equal amount of subjects with and without a de-

pression history. Subjects were therefore selected according to this information. During the experimental phase of the study, however, more subjects turned out to have suffered from depression, causing an imbalance between patient and control groups.

At the time of the stress test, the hormonal system, the coagulation system and the immune system were not to be modified by influences other than the experimental stressor. Therefore the following exclusion criteria were defined for all subjects:

- Hormonal disorders such as Morbus Cushing or Morbus Addison, untreated thyroid illnesses
- A history of cardiovascular events such as myocardial infarction or stroke within the past 6 months
- Organ transplant within the past 6 months
- Treatment with cancer chemotherapy within the past 12 months
- Chronic respiratory tract diseases
- Autoimmune diseases
- Diseases of the hematopoietic system or treatment with anticoagulant drugs
- Glucocorticoid treatment or treatment with other pharmaceuticals that influence HPA-axis functions
- Pregnancy or breast-feeding
- Professional athletes

Further, persons were excluded who were diagnosed, at present or in the past, with the following psychiatric disorders (DSM-IV):

- Schizophrenia and other psychotic disorders
- Social phobia
- Substance-abuse
- Needle-related anxiety
- Bipolar affective disorder
- Dysthymia

Subjects in the remitted depressed group had been diagnosed with *Major Depressive Disorder*, according to DSM-IV, but were in remission for a minimum of six months at the time of the experiment. In the control group, all affective disorders, including generalized anxiety disorder, whether at present or in the past, were further exclusion criteria.

In case of acute infections/inflammation or an overseas flight within the past two weeks, the appointment for the experiment was postponed or the situation re-evaluated. The same was true for persons under high physical strain or suffering from symptoms of depression. Shift workers' assessments were scheduled to take place on a day after a holiday or during a time of day shifts. Hormonal contraception and antidepressant medication were not among the exclusion criteria. 35% of participating women were taking hormonal contraception. For female subjects who were not taking hormonal contraception, the day of the examination was chosen to coincide with the luteal phase of the monthly cycle (days 14-28), because sex differences in HPA-axis reactivity are known to be least during this time (Kirschbaum et al., 1999). Four subjects included in the analyses were taking antidepressant medication. All four were taking SSRIs: citalopram, escitalopram and paroxetine. To control for a potential influence of the antidepressant medication, the presence of such medication was included as a covariate in all analyses. Initially, 66 subjects were recruited. Two subjects were excluded from analyses due to depressive symptoms within the past six months prior to the examination day. One subject was excluded due to missing data for chronic stress. Therefore N = 63. Three subjects presented outliers for some of the coagulation parameters that were above or below two standard deviations from the mean. The respective values, but not the three subjects, were excluded for analysis.

Groups

In order to test for effects of remitted depression and chronic stress on coagulation factors, subjects were assigned to a MD+/- and CS+/- group, which resulted in a 2x2 design:

MD+		MD-	
CS+	CS-	CS+	CS-

Remitted Major Depression (MD+) vs. healthy controls (MD-)

Subjects were assigned to two groups with respect to their disease history. Subjects without history of any psychiatric disorders were defined as healthy control group (MD-, N = 24), while subjects with a lifetime history of major depression were assigned to the depression group (MD+, N = 39).

Participants in the MD+ group had to have a cumulative lifetime diagnosis of major depression (according to DSM-IV criteria as determined using the M-CIDI) and had been in remission for a period of at least six months at the time of examination. Group membership was determined using longitudinal data gained from regular M-CIDI-interviews.

High chronic stress (CS+) vs. low chronic stress (CS -)

Chronic stress in study participants was evaluated with the Trier Chronic Stress Inventory, TICS, (Schulz & Schlotz, 1999). Based on the total score of this questionnaire, the sample was split into two groups. Using the median split a group with high chronic stress and a group with low or no chronic stress was formed (CS+, N= 34; CS-, N=29).

4.2. Experimental procedure

Diagnostic assessment

Participants of the EDSP study received a letter of invitation and were contacted by telephone to discuss their further involvement in the study.

After giving their consent to participate and if inclusion criteria were fulfilled (as checked through an additional phone call), the eligible subjects were invited for a diagnostic assessment. Here, the computerized Munich version of the Composite International Diagnostic Interview (M-CIDI) was used to assess symptoms and diagnoses of DSM-IV disorders and their onset, duration and severity (Wittchen & Pfister, 1997) . The same interview had been used at all assessments during the EDSP study. A variety of other questionnaires were completed at this assessment, some of which are described further on.

Trained interviewers conducted the interval version of the M-CIDI covering the time period between the previous assessment and the current evaluation.

Beck's Depression Inventory II (BDI-II) was used to exclude the presence of acute symptoms of depression within the last 14 days. Participants received an invitation for the experimental part of the study if their BDI-II score was at or below 14.

Setting

The experiments took place in the sleep laboratory at the Max Planck Institute, which proved to be a good choice since it shielded the participants from outside disturbances. A principal investigator and a lab technician were present. A detailed protocol regarding instructions for the study participants, a schedule for blood collections and questionnaires was established so

that the conditions were as similar as possible for each study subject. The study protocol is presented in Table 2.

The subjects were invited to arrive at the institute with enough time to minimize stress and get used to the new surroundings. There was also enough time for the stress caused by placing a venous catheter to subside before beginning the actual experiments.

Trier Social Stress Test (TSST)

As it was our priority to carry out the experiments in a most accurate and standardized manner, a detailed protocol was prepared as part of the study design so that conditions were the same for all participants (see Table 2). It listed in great detail the steps that the investigator and technical assistants were to go through during the afternoon. Instructions for the test subjects regarding the TSST were read out loud to minimize inconsistencies and ambiguities due to the investigator's interaction with the test subjects.

For the induction of acute stress we used the Trier Social Stress Test (TSST), a standardized experimental protocol developed by Kirschbaum et al. (1993). In the course of the test afternoon, the TSST was used twice, but for coagulation parameters only the first TSST was relevant. Several blood tests were performed for measurement of coagulation parameters.

In a first instruction 40 minutes prior to the TSST, subjects were informed that they were to take part in a psychosocial stress test that consists of two tasks to be performed in front of a jury. They were told that they first had to hold a speech that was to be videotaped and recorded. The material would then be used in an analysis of language and behaviour. The subjects were told to present themselves in the most convincing manner possible, so as to receive a good evaluation.

In a second instruction 25 minutes before the TSST, subjects were informed that their specific task during the stress test was to give a presentation for a job application or for a promotion. The subjects were able to choose the field this job was in, however, it was pointed out to them to choose a realistic job that could fit into their current lives. Within this 5-minute talk, the subjects would have to convince the audience of their suitability for this job/promotion. They would have to make sure to be within the video-camera's range. The jury could interrupt and ask further questions if needed. During this second instruction, the participants were informed that after their talk they would have to perform a second task, and that they would receive instructions then. Between the first and second instruction for the TSST, participants had to do a computerized neuropsychological test (Stroop color-word conflict test) measuring attention and concentration. Participants were informed that their results on this test would be taken

into consideration for a final evaluation. In this work, however, performance on the Stroop test was not analyzed and served as another stressful element.

After a 10-minute preparation time, subjects were led to a different room in which the jury was waiting for them. They were not allowed to take their written notes along.

The room was equipped with a video camera connected to a screen in which the subjects could see themselves. A microphone was handed to them at the beginning of their speech.

The jury consisted of two persons wearing laboratory coats. They were not to give any feedback or reassurance and had to keep neutral facial expressions.

Upon arrival of the participant, the video camera was switched on. After handing the microphone to the participant, the jury leader asked the participant to start the speech and started a stopwatch.

Five minutes into the speech the jury interrupted the participant and gave instructions on the second task, a mental arithmetic task. In steps of 13, the participant was asked to subtract from 1678 in their fastest and most accurate manner. Should participants make a mistake, they had to start over at 1678. After this second task the participants were led to the previous room where they were allowed to read a magazine or newspaper.

4.3. Assessment of the stress response

Heart rate as a response of the sympathetic nervous system or SAM-axis was measured using a pulse measurement belt (Polar© RS400) that participants placed around their chest. It transmitted heart rate to a pulse watch that was worn around the wrist.

To test for responses in the HPA-axis, cortisol levels were measured.

Blood for cortisol measures was collected at six times during the test afternoon: at baseline, directly before the TSST, directly after, and +15, +30 and +45 minutes after the TSST. 5ml EDTA aliquots were used, centrifuged at 4000x/min, their supernatant (approximately 1ml) separated and frozen at -20°C until further analyses were carried out using a radioimmunoassay kit (CT Cortisol RIA, DRG Diagnostics, Marburg, Germany) This kit has a detection limit at 1.7 ng/mL.

Multidimensional adjectives questionnaire (BSKE) regarding current state

At seven different time points subjects completed the short form of the multidimensional adjectives questionnaire (BSKE). This test examines subjects' current mental state using a likert-scale from 1 (= not at all) to 6 (= agree fully) and examines, among other categories, anxiety and inner restlessness (Janke, Debus, Erdmann, & Hüppe, 1995). The 12-item form

was used at several time points during the test afternoon: directly before the TSST instructions, directly before the TSST, after the TSST (retrospectively regarding the TSST) and three times during the recovery period after the TSST. The participants' subjective reactions to the stressor, i.e. anxiety and inner restlessness, were considered for statistical analyses.

4.4. Sampling procedure and laboratory analyses

At the beginning of the experiment, the principal investigator or medical student placed a venous catheter in the subjects' forearm. This catheter was connected to a flexible tube system, which connected through the wall to the lab technician's bench. After each collection, the tube was flushed with 10ml 0.9% sodium chloride to prevent clotting. Between blood collections, the subject received a 0.9%-sodium-chloride drip (50ml/h, Secura Infusomat, B-Braun, Melsungen) in order for the catheter to remain permeable. As participants were lead to the other room for the TSST, the tube was removed and a stylet was placed to clog the catheter. Routine blood samples were taken twice during the afternoon and included count of leukocytes, erythrocytes and thrombocytes, hematocrit and hemoglobin, MCV and MCH liver enzymes and creatinine. One 2.5ml EDTA tube and one 7.5ml serum tubes were drawn (Monovetten Sarstedt, Nürnberg). The EDTA tube was centrifuged at 4000x/min for 7 minutes and its supernatant separated and stored at +4°C until further analyses at the institute for clinical chemistry. A urine sample was taken to exclude drug use (benzodiazepines, opioids, cannabis).

Measurements of coagulation parameters took place at rest, 15 min post-stress and 45 min post-stress. PAI-1 was measured at rest only. For the final 22 subjects of the examination routine we decided to add another time point for blood collection for additional analyses: directly post-stress. Table 2 shows the exact time points in which blood samples for coagulation analyses were obtained.

2x 3ml venous blood was collected into 0.3ml citrate buffer tubes (Monovetten Sarstedt, Nuremberg). Aliquots were centrifuged at 4000x/min and the plasma containing the clotting factors was separated, then kept at +4°C temperature, and frozen at -20°C until they were shipped to a laboratory in Switzerland for further analysis. For the transport -78°C dry ice was used. Fibrinogen, vWF and PAI-1 levels were measured using a multiplexed particle-based flow cytometric cytokine assay (Vignali, 2000). Assay kits were used from Millipore (Zug, Switzerland) and R&D Systems (Oxon, UK). The analysis was conducted with a conventional

flow cytometer (Guava EasyCyte Plus, Millipore, Zug, Switzerland). For D-Dimer measures, an ELISA from Technoclone (Vienna, Austria) was used. Further 2x 7.5ml blood was drawn into DNA-aliquots (Monovette Sarstedt K3E) for other analyses. In addition, RNA aliquots for 2.5ml whole blood were drawn, pivoted and kept at room temperature until they were frozen at -20°C on the next day. RNA was analyzed for the study of polymorphisms in the FKBP5 gene and its association with major depression.

4.5. Questionnaires

Before the study day, either at home or the morning before, the subjects were asked to fill out the Munich version of the Composite International Diagnostic Interview questionnaire (M-CIDI), a questionnaire regarding symptoms, syndromes and diagnoses of DSM-IV disorders (Wittchen & Pfister, 1997). The same interview was used in the longitudinal EDSP study at all assessments. Trained interviewers conducted the interval version of the M-CIDI covering the time period between the previous assessment and the current evaluation.

Beck Depression Inventory (BDI-II)

During the diagnostic appointment, the BDI-II (Beck & Steer 1996; German version by Hautzinger et al. 2006) was used to assess the presence and severity of acute depression symptoms within the last 14 days. There are 21 items including questions on sadness, feelings of guilt, loss of energy and appetite that the participant answers on a scale from 1-4 points, which are added to yield the total score. 0-13 points are considered as minimal, 14-19 point as mild, 20-28 as moderate and 29-63 points as severe depression. Participants received an invitation for the experimental part of the study if their total score was below 14. The BDI-II questionnaire was also filled out a second time on the test day before the stress protocol.

Trier inventory for the assessment of chronic stress (TICS)

This 57-item questionnaire developed by Schulz and Schlotz (1999) evaluates the following dimensions of chronic stress: work overload, pressure to succeed, excessive demand at the workplace, work discontent, social tension, lack of social recognition, social stress. A 12-item screening scale (SCSS) serves as a comprehensive measure of chronic stress. Internal consistency (Cronbach's Alpha) of this scale has been reported at $\alpha = .87$, indicating good to very good reliability (Petrowski, Paul, Albani, & Brähler, 2012).

The frequency by which stress events were experienced or stressful feelings were perceived in these areas over the past three months, from 0 (never) to 4 (very often), permits the investiga-

tor to estimate the subjects' level of stress chronicity. Examples for questions found in the TICS questionnaire are listed in Table 1. In the present study the questionnaire was completed during the diagnostic interview.

Table 1. Examples of questions asked in the Trier inventory for the assessment of chronic stress for each category, as found in Petrowski, Paul, Albani, & Brähler (2012).

Category	Example question
Work Overload	"I have too many tasks to perform. "
Social Overload	"I must frequently care for the well-being of others. "
Pressure to Perform	"I have tasks to fulfill that pressure me to prove myself. "
Work Discontent	"Times when none of my tasks seem meaningful to me."
Excessive Demands at Work	"Although I try, I do not fulfill my duties as I should"
Lack of Social Recognition	"Although I do my best, my work is not appreciated"
Social Tensions	"I have unnecessary conflicts with others."
Social Isolation	"Times when I have too little contact with other people. "
Chronic Worrying	"Times when I worry a lot and cannot stop."

Table 2. The study protocol used for the examination. Overview of time points and tasks of investigator and technical assistant.

Time	Main investigator		Technical assistant	Participant (P)
	Questionnaires	Blood collection		
13:15			Urine sample Pulse-meter on	Welcome P at lobby
13:30	BDI-II	Routine blood work 1		- Instruction - Placement of venous catheter - P can read newspaper or book
13:55				Practice run Emotional Stroop
14:00	Baseline	1. BSKE	1. Blood collection RNA, Plasma, Cytokines, Coagulation parameters , Cortisol	Heart rate ____
14:05				- Instruction I for TSST - Emotional Stroop
14:15		2. BSKE		Heart rate ____

14:18					- Instruction II for TSST - 10 min preparation for TSST
14:30	0 min pre stress	3. BSKE	2. Blood collection RNA, Plasma, Cortisol	Heart rate ____ Time on pulse watch ____ (Pre-TSST)	
14:35				Disconnect participant from blood collection	TSST: take P to other room, TSST from 14.35 to 14.50
14:50	0 min post stress	4. BSKE- retrospectively	3. Blood collection Coagulation parameters (n=22) , Cortisol	Heart rate ____ Time on pulse watch ____ (Post-TSST)	
					Switch on movie
15:05	15 min post stress	5. BSKE	4. Blood collection Coagulation parameters , Cortisol	Heart rate ____	
15:20	30 min post stress	6. BSKE	5. Blood collection RNA, Plasma, Cortisol	Heart rate ____	
15:35 END of experiment	45 min post stress	7. BSKE	6. Blood collection Routine blood work 2 Coagulation parameters , Cortisol	Heart rate ____	Switch off movie

4.6. Statistical analyses

Data were analyzed using PASW Statistics for Windows (Version 18.0., SPSS Inc., released 2009, Chicago, IL, USA).

For statistical analyses of variables the level of significance was set at 5% (two-tailed). Group differences with a p-value of less than .10 are reported as suggestive trends.

Demographics

Differences between groups in quantitative and categorical demographic variables were evaluated with analyses of variance or Chi²-Tests, respectively. The presence of group interaction effects between the categorical variables was analyzed using a log-linear model.

Stress induction: effects of the TSST stress paradigm

To determine whether the stress task was effective, we compared the pre- and (maximum of the) post-stress measure of heart rate, cortisol and subjective arousal and anxiety (BSKE sub-scores ‘inner restlessness’, ‘anxiety’) in a repeated 3 way measures ANCOVA including two independent factors, MD and CS, and the respective baseline value as covariate. To localize the source of potential repeated measures effects, simple contrasts to the first assessment are

reported. To assess whether the TSST caused any overall effect on the three coagulation parameters, paired samples t-test were used for baseline and post-stress measures of all subjects.

Baseline effects on coagulation parameters

To examine the effect of depression and chronic stress on the levels of the coagulation factors PAI-1, Fibrinogen, DD and VWF at baseline, a two-factorial ANCOVA with the independent factors MD and CS was used. Age, gender, hormonal contraception, smoking status, antidepressant medication, hematocrit, hemoglobin, thrombocytes, leukocytes, CRP and smoking status were included as covariates. Smoking status was defined as consumption of ≥ 5 cigarettes per day.

Stress effects over different time points

To examine the effect of depression and chronic stress on the levels of the coagulation factors at the different time points (resting state, 15 minutes post-stress, 45 minutes post-stress) a repeated measures ANCOVA with two independent factors, MD and CS was used. Age, gender, hormonal contraception, antidepressant medication, smoking status, hematocrit, hemoglobin, thrombocytes, leukocytes and CRP were included as covariates. The Greenhouse-Geisser correction of degrees of freedom was applied to account for violations of the sphericity assumptions. For identifying the source of a potential repeated measures effect, simple contrasts to baseline were calculated.

Separate repeated measures ANCOVAs with the factor MD and CS, respectively, were used to examine separately the effects of depression and chronic stress on coagulation markers directly post-stress (N=22).

Due to the small sample size for the measurement of coagulation markers directly following the stressor, the 2x2 design would have created groups with too few members. Therefore MD and CS were evaluated separately and not their interaction in the N=22 subsample.

Associations of current depressive symptoms and chronic stress scores with coagulation factor levels

Partial correlation analyses were carried out to search for associations of the scores on the Beck depression inventory (BDI-II) and on the screening scale and subscales of the chronic stress questionnaire (TICS) with coagulation factor levels, correcting for the respective other group variable (CS for BDI-II associations, and MD for TICS associations).

Sensitivity Analysis

A sensitivity analysis was conducted using G*Power v. 3.1 (Faul et al., 2009) with alpha and beta errors set to .05 and .20, respectively. Given the sample size of $N=63$ and the 2×2 factorial design with repeated measures (resting state, 15 min post stress, 45 min post stress, assuming a moderate correlation of $r = .5$ between repeated measures), we calculated a detectable effect size of $f = .19$. Thus, the detectable effect size of our analysis ranges between the borders of a small ($f = .10$) and medium effect ($f = .25$) according to the effect size conventions reported by Cohen (1988).

5. Results

Source data for all Figures presented in this section can be found in Appendix I.

5.1. Demographics

The demographic characteristics of the study participants are listed in Table 3. Remitted depressed and control subjects did not differ with respect to age, gender, BMI, years of education (primary and secondary education), relationship status, antidepressant treatment, age at onset of first depression episode, hormonal contraception (% of females) and BMI score on the test day.

Subjects with high CS compared to low CS, as determined using the 12-item screening scale of the TICS questionnaire (Trier inventory of chronic stress) did not differ significantly with respect to age, gender, BMI and years of education. On average, subjects had between 1 and 38 points on the TICS questionnaire with a mean of 12 (± 8.6) points, with 48 being the maximum number of points.

Persons with MD and CS together did not differ from the control group with respect to the abovementioned characteristics.

Regarding relationship status, it is noteworthy that single persons were more often chronically stressed than married persons ($p=.006$).

On the BDI-II, subjects had between 0 and 14 points with an average of 2.0 (± 3.0) points. No difference was found between subjects in the depression group and controls.

However, chronically stressed subjects had significant higher BDI-II scores than controls, with mean scores at 3.2 (± 3.7) versus .6 ($\pm .8$); $p=.001$.

Chronically stressed individuals scored higher on the Beck Depression Inventory (BDI-II) on the test day ($p=.001$) than did individuals with low chronic stress. Subjects in the MD+ group had higher chronic stress scores than MD- subjects ($p=.007$).

Since smoking is known to increase the risk for deep vein thrombosis, it was taken as a covariate in all analyses. Smoking status was defined as ≥ 5 cigarettes per day. Out of the 63 subjects, 12 fell into this category, with a consumption of a minimum of 5 to a maximum of 20 cigarettes per day. In none of the analyses of covariance smoking status affected the results.

Table 3. Demographic characteristics of the study participants. CS+: chronic stress, MD+: major depression. CS-, MD- negative for chronic stress and major depression. ANOVA and Chi²-Tests were calculated for group comparison in quantitative and qualitative variables, respectively. SSRI-antidepressant treatment: selective-serotonin-reuptake-inhibitor, (N=4): escitalopram, citalopram, paroxetine.

Variable (n) %	MD+ N=39		MD- N=24		P values (ANOVA / Chi ²)		
	CS+ (20) 51.3	CS- (19) 48.7	CS+(14) 58.3	CS- (10) 41.7	MD	CS	MD x CS
Age in years (SD)	34.5 (3.5)	35.0 (3.5)	36.1 (4.0)	36.1 (3.1)	.159	.779	.802
Female (%)	14 (60.9)	9 (39.1)	9 (64.3)	5 (35.7)	.948	.120	.802
Years of Edu- cation (SD)	12.1 (1.9)	11.9 (1.5)	12.2 (1.8)	12.8 (1.5)	.272	.719	.408
BMI (SD)	24.5 (3.2)	23.5 (2.4)	24.0 (2.8)	24.7 (5.0)	.664	.876	.330
Relationship status n (%)							
Single	12 (60)	8 (40)	8 (72.7)	3 (27.3)	.511	.006	.415
Married	4 (26.7)	11 (73.3)	5 (41.7)	7 (58.3)			
Divorced	4 (100)	-	1 (100)	-			
Depression history							
Antidepressant treatment (SSRI) n (%)	1 (5.0)	1 (5.3)	2 (14.3)	0 (0)	.594	.383	.975
Age at onset, years (SD)	19.1 (4.1)	19.3 (4.9)					
Hormonal contraception n (% female)	3 (21.4)	4 (44.4)	4 (44.4)	2 (40.0)	.443	.443	.436
Amount Smokers (%)	3 (15)	4 (21)	4 (29)	1 (10)	.936	.736	.552
BDI-II score on test day (SD)	3.3 (5.5)	.7 (.8)	3.1 (4.1)	.5 (.7)	.794	.001	.974
TICS screen (SD)	20.2 (8.7)	6.9 (2.4)	14.5 (6.1)	3.9 (2.0)	.007	<.001	.394

5.2. Resting coagulation parameters

5.2.1. Fibrinogen

The remitted depression group and control group differed significantly in baseline levels of fibrinogen as shown in Figure 4 (left). Members of the remitted depression group had higher fibrinogen levels at baseline: 111.19 mg/dl in the control group versus 126.74 mg/dl, in the remitted depression group, which equals a difference of 12% ($F_{1,50}=7.738$, $p=.008$). There was no effect of CS ($F_{1,50}=.139$, $p=.711$) or of the interaction of CS and MD ($F_{1,50}=.184$, $p=.670$) on baseline levels of fibrinogen.

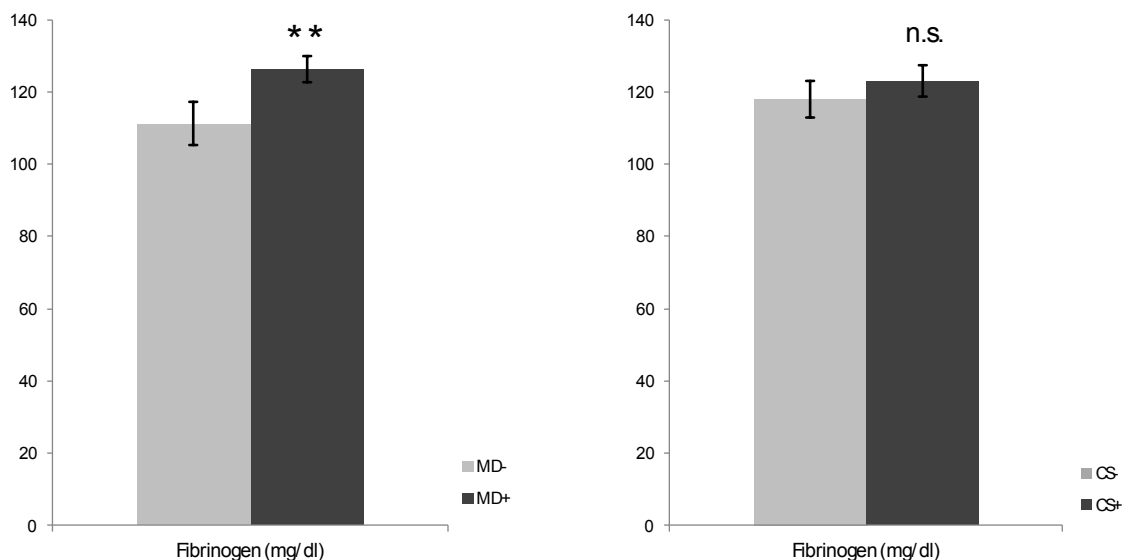


Figure 4. Mean baseline concentrations of fibrinogen. A) Comparison between subjects with remitted depression and without a history of depression. B) Comparison between subjects with and without chronic stress. **: $p<.01$, n.s.: non-significant.

5.2.2. D-Dimer

The remitted depression and control group showed no significant differences in baseline levels of D-Dimer ($F_{1,48}=.209$, $p=.649$). Subjects with chronic stress showed baseline D-Dimer concentrations that were on average 45% higher than in those without chronic stress.

Although this difference was not significant ($F_{1,48}=2.96$, $p=.092$), this can be seen as a suggestive trend toward higher resting D-Dimer levels in chronically stressed subjects (see Figure 5). No interaction effect of MD and CS was observed ($F_{1,48}=2.570$, $p=.115$).

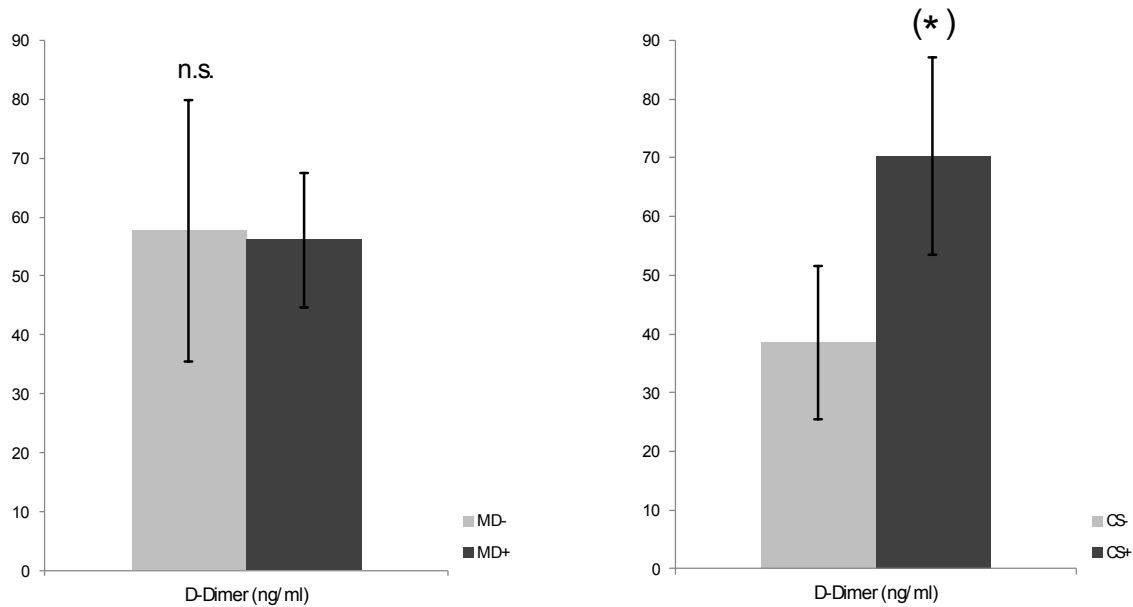


Figure 5. Mean baseline concentrations of D-Dimer. Left: comparison between subjects with remitted depression and without a history of depression. Right: comparison between subjects with and without chronic stress. Depicted are means and standard error bars. (*): $p < .10$, n.s.: non-significant.

5.2.3. VWF

No significant differences in resting levels of VWF were found between the MD and control group ($F_{1,49} = .203$, $p = .654$), as well as between subjects with chronic stress and without chronic stress ($F_{1,49} = .450$, $p = .505$). See Figure 6. An interaction effect of MD and CS cannot be reported ($F_{1,49} = .626$, $p = .432$).

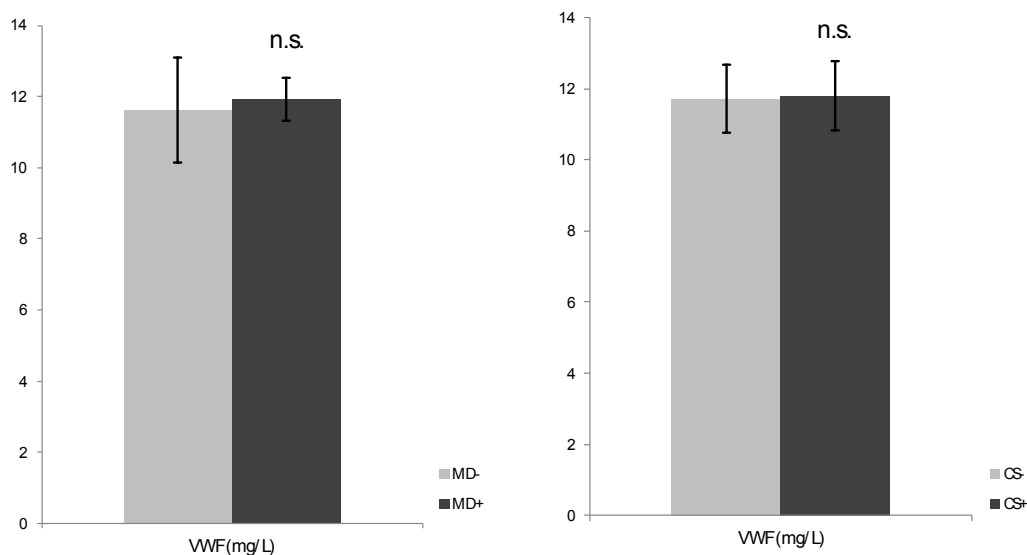


Figure 6. Mean baseline concentrations of VWF. Left: comparison between subjects with remitted depression and without a history of depression. Right: comparison between subjects with and without chronic stress. Depicted are means and standard error bars. n.s.: non-significant.

5.2.4. PAI-1

PAI-1 resting levels were neither significantly different between remitted depressed subjects and controls ($F_{1,50} = .246, p = .622$) nor between chronically stressed subjects and controls ($F_{1,50} = .837, p = .365$), as Figure 7 shows. There were no interaction effects of MD and CS in the abovementioned analyses of hemostasis factors ($F_{1,50} = .308, p = .581$).

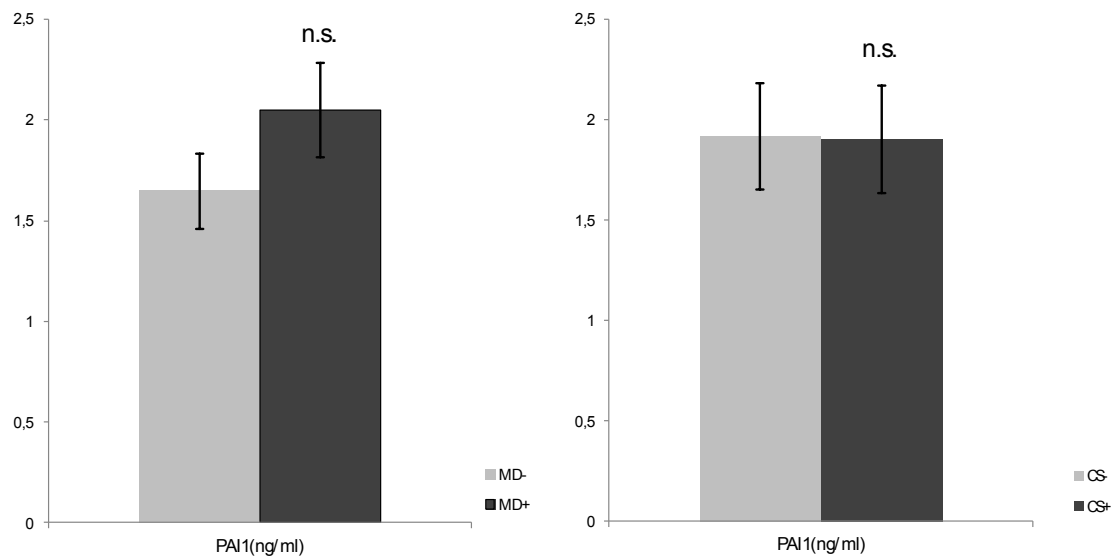


Figure 7. Mean baseline concentrations of PAI-1. A) Comparison between subjects with remitted depression and without a history of depression. B) Comparison between subjects with and without chronic stress. Depicted are means and standard errors. n.s.: non-significant.

5.3. Stress induction: effects of the TSST paradigm

The TSST psychosocial stress protocol elicited an effective stress reaction in all four study groups, as seen in heart rate, as a measure of SAM-axis activity, and in cortisol increases, as a sign of HPA-axis induction.

5.3.1. Heart rate response

Heart rate increased from an average at baseline of 80.26 beats per minute to an average of 98.13 beats per minute at the beginning of the TSST and 100.06/min at the end of the TSST. The general linear model revealed a non-significant change over all heart-rate measures with correction for baseline differences ($F_{4,200} = 1.679, p = .159$). However, a significant stress effect could be seen for the pre-to post-TSST heart rate ($F_{1,52} = 7.218, p = .01$) and for the heart rate pre-TSST to +30 min ($F_{1,52} = 4.123, p = .047$). No significant effect of depression history ($F_{1,52} = 3.179, p = .080$), chronic stress ($F_{1,52} = .581, p = .449$) or their interaction ($F < .001, p = .988$) was found. Heart rate curves for all four groups are depicted in Figure 8.

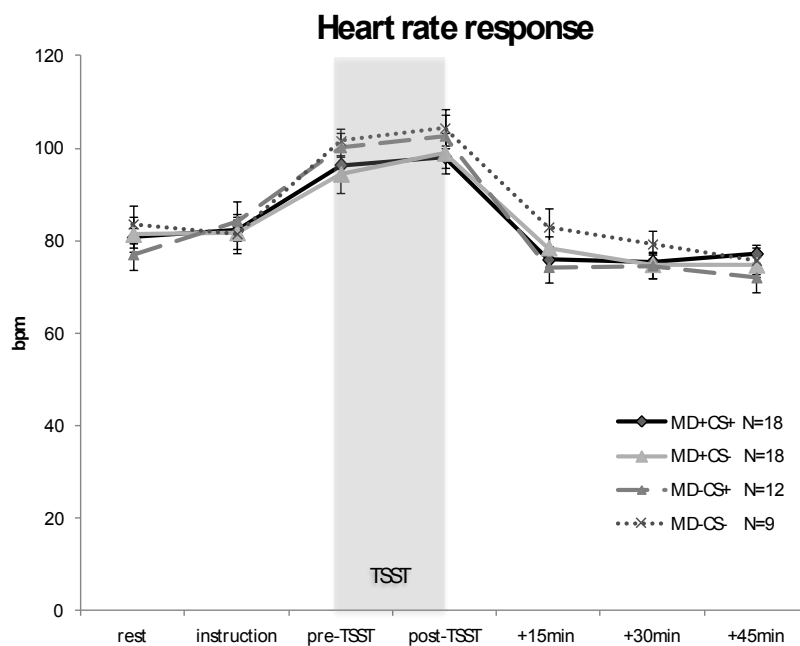


Figure 8. Mean heart rate measures of the four groups MD+CS, MD, CS and MD-CS- before and after the TSST. The figure shows means and standard errors corrected for baseline differences.

5.3.2. Cortisol response

On average, the TSST had a significant effect on cortisol levels ($F_{2,2, 123,1}=15.5, p<.001$), as shown in Figure 9. This effect was not due to group membership, that is, membership to one of the following: MD+CS+, MD+CS-, MD-CS+ and MD-CS-.

An increase of cortisol levels from 127.1 ng/ml at rest to 150.8 ng/ml right after the stress test was measured, equalling an increase of 15.7%.

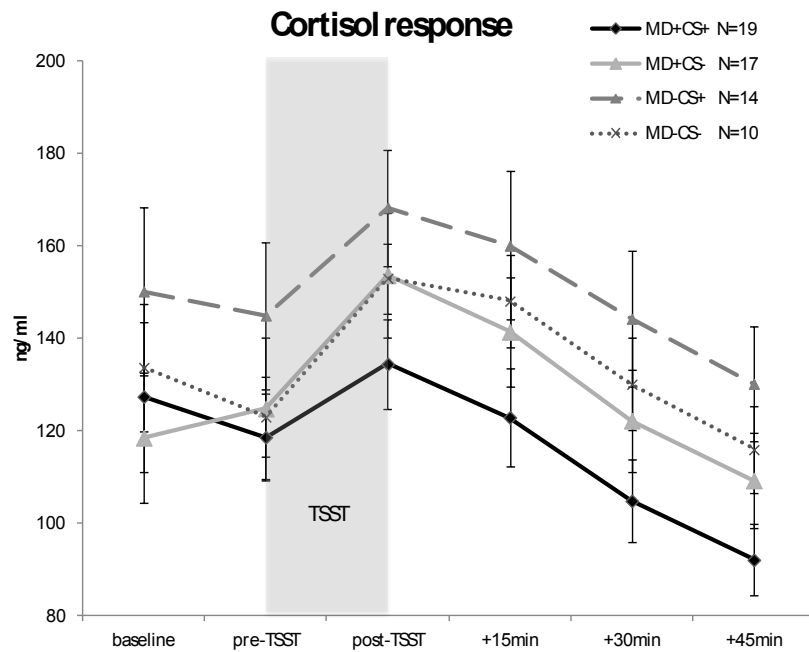


Figure 9. Cortisol response curves separate for each of the four groups. MD+: major depression. CS+: chronic stress. The figure shows means and standard errors corrected for baseline differences.

5.3.3. Perceived stress

The TSST evoked a noticeable change in the subjects' feelings of perceived stress. The subscore 'inner restlessness' as measured by the BSKE questionnaire increased significantly from baseline to the stressor ($F_{3,6, 198.8}=55.208, p<.001$); see Figure 10. Group effects (MD, CS, CS x MD) were not observed.

Subjects also perceived their anxiety levels, as measured using the BSKE 'anxiety' subscore, to rise with the stressor task ($F_{2,4, 133.6}=25.666, p<.001$) as shown in Figure 10. Again, group effects were not detected.

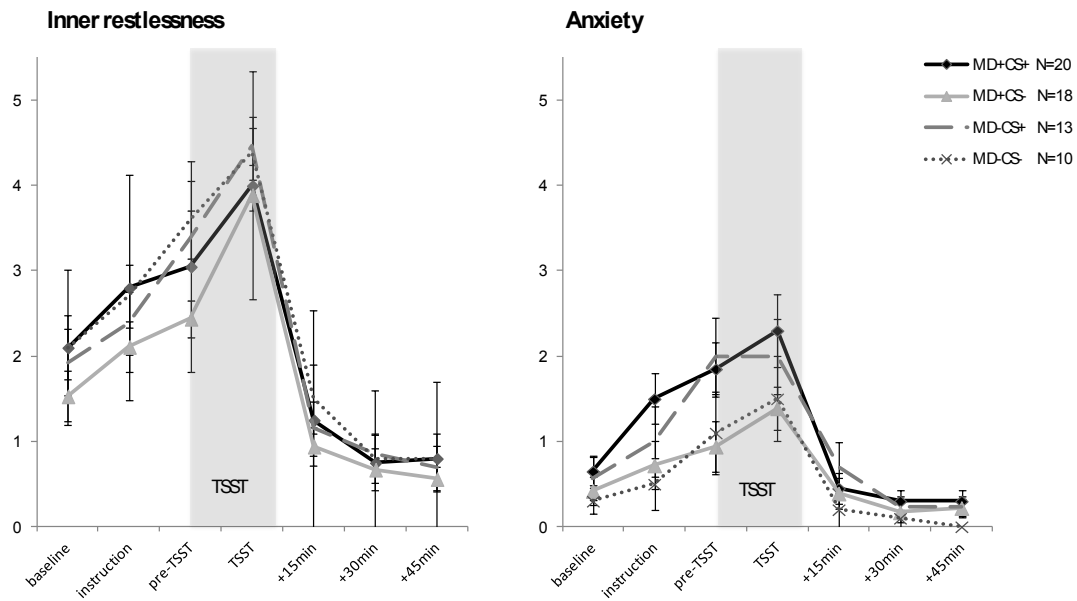


Figure 10. Changes in perceived anxiety during the stress protocol, as measured using the BSKE questionnaire. **A:** inner restlessness subscore. **B:** anxiety subscore. The figure depicts estimated means and standard errors corrected for baseline differences.

5.4. Stress-induced coagulation changes

Figure 11 gives an overview of the descriptive changes observed over the time points for the three markers (fibrinogen, D-Dimer and VWF), independent of group, with N=63. None of the changes between pre- and post-stress reached significance, however, for D-Dimer and VWF trends were observed ($p=.054$ and $p=.057$, respectively).

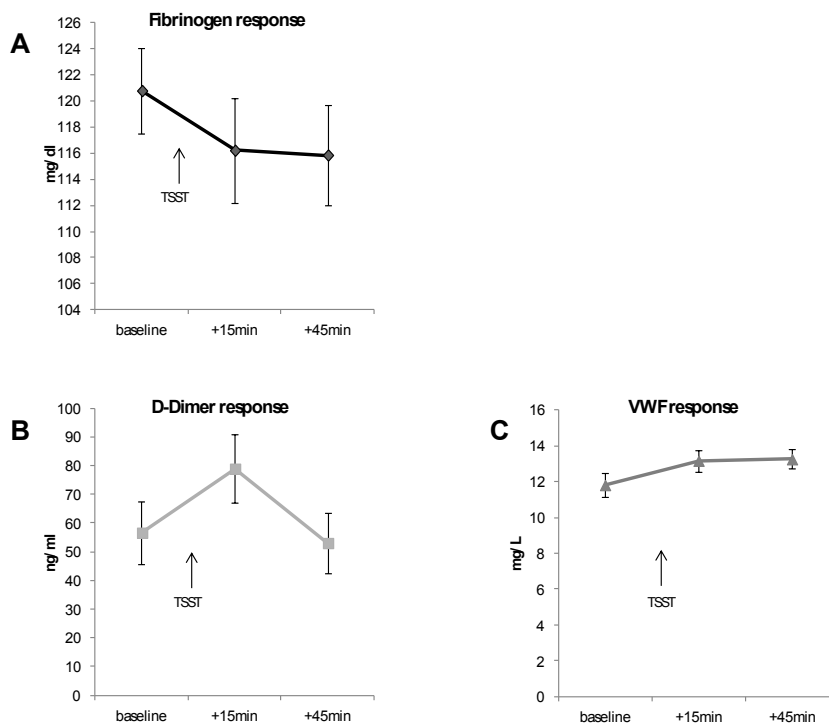


Figure 11. Changes in the three coagulation parameters in response to the TSST (N=63), independent of group. The figure depicts means and standard errors for all subjects.

A: Fibrinogen changes over time
B: D-Dimer changes over time
C: VWF changes over time

5.4.1. Fibrinogen

On a purely descriptive level, overall fibrinogen levels decreased from 120.8 mg/dl (± 26.2) at baseline to 116.2 mg/dl (± 31.8) post-stress, and at +45 minutes decreased to 115.8 mg/dl (± 30.1); see Fig. 11A.

Multivariate analyses revealed that the psychosocial stress induced by the TSST caused a significant change in fibrinogen levels ($F_{1,94.2}=4.076$ $p=.021$) over time, more precisely from baseline to 15 min post-TSST to 45 min post-TSST. Depression history accounts for this overall significant effect of TSST on fibrinogen levels ($F_{1,49}=7.850$, $p=.007$).

Chronic stress does not significantly account for the effect of TSST on fibrinogen levels ($F_{1,49}=.052$, $p=.820$). The interaction of chronic stress and depression did not have an effect on fibrinogen either ($F_{1,49}=.000$, $p=.993$).

When considering baseline fibrinogen levels and the fibrinogen levels measured immediately after the TSST, the effect of TSST on the fibrinogen reaction was non-significant ($F_{1,10}\leq .453$, $p\geq .516$) although the previously observed depression effect remains ($F_{1,10}=6.042$, $p=.034$); see Figure 13. The effect of chronic stress, however, was not significant ($F_{1,10}=.506$, $p=.493$). As mentioned earlier, interaction effects were not calculated due to small group sizes in the comparison of baseline to directly post-TSST.

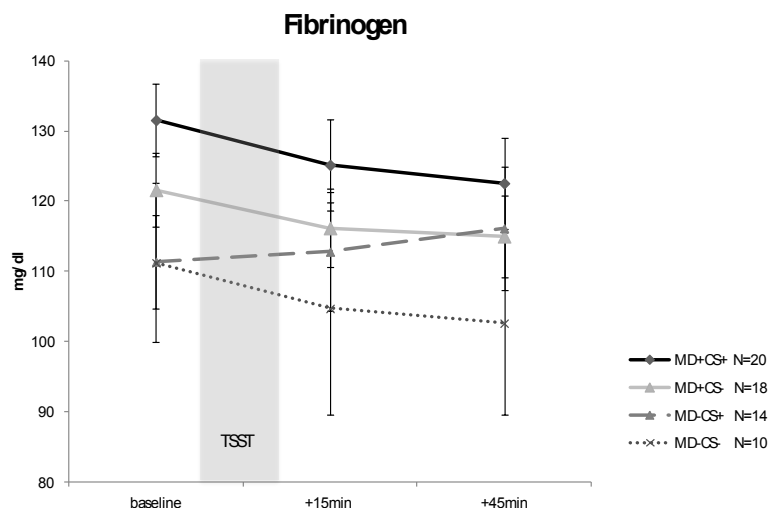


Figure 12. Baseline to post-stress measures of fibrinogen in the four groups. Presented are means with standard errors. +positive, -negative for MD: major depression, CS: chronic stress.

Fibrinogen

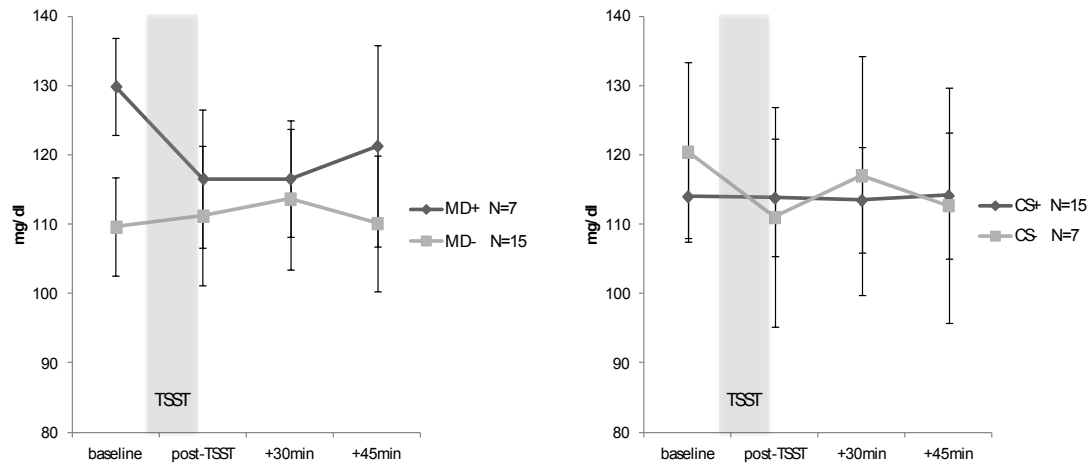


Figure 13. Baseline to post-stress measures of fibrinogen, including another post-stress time point (immediately post-TSST). Left: by MD: depression history. Right: by CS: chronic stress. Depicted are means and standard errors.

5.4.2. D-Dimer

A trend toward a change in D-Dimer levels was observed during the TSST. Average D-Dimer levels of all subjects increased from 56.6 ng/ml (± 85.6) at baseline to 79ng/ml (± 93.7) ng/ml at 15 min ($p=.054$). 45 minutes following the TSST they had decreased again to 53ng/ml (± 82.7). The difference between baseline and 45 minutes post-TSST was not significant ($p=.633$), see Fig. 11B.

In terms of repeated measures, the psychosocial stress test did not affect D-Dimer levels since the overall effect of the stressor was not significant ($F_{1,6,76.6}=.099$, $p=.868$). Depression did not have an effect on D-Dimer levels ($F_{1,47}=.008$ $p=.930$). Chronic stress however, had a significant effect ($F_{1,47}=4.107$ $p=.048$), and a trend can be observed regarding the interaction of chronic stress and a history of depression ($F_{1,47}=3.880$ $p=.055$) on D-Dimer levels. As Figure 14 shows, D-Dimer levels rose in all subjects after the TSST, but the increase was most pronounced when chronic stress was present, i.e. in the MD-CS+ group (increase by 38%) and the MD+CS+ group (increase by 25%). For repeated measures in the baseline to directly post-stress with the smaller sample of $N=22$, the TSST again did not seem to affect stress-coagulability ($F_{1,10} \leq 1.617$, $p \geq .232$). There was also no effect of depression history ($F_{1,10}=2.560$, $p=.141$) on D-Dimer levels. The CS effect seen for the larger sample could not be reproduced in the smaller sample over the two tested time points ($F_{1,10}=2.627$, $p=.136$).

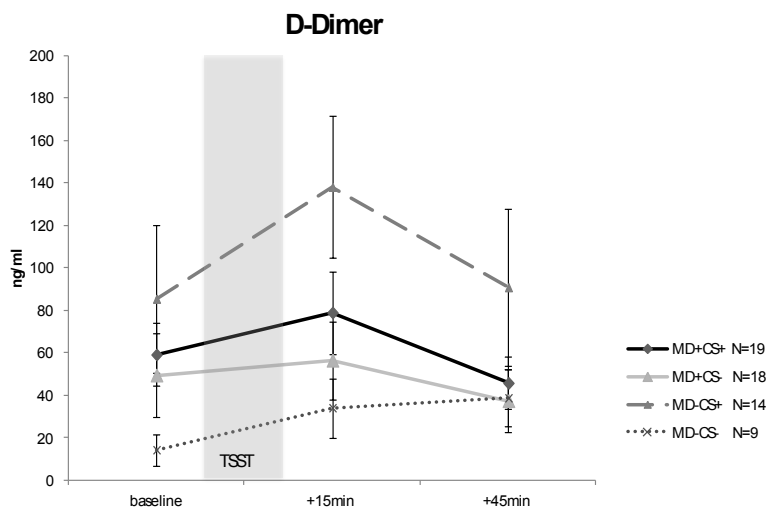


Figure 14. Baseline to post-stress measures of D-Dimer in the four groups. Depicted are means with standard errors. MD: major depression, CS: chronic stress.

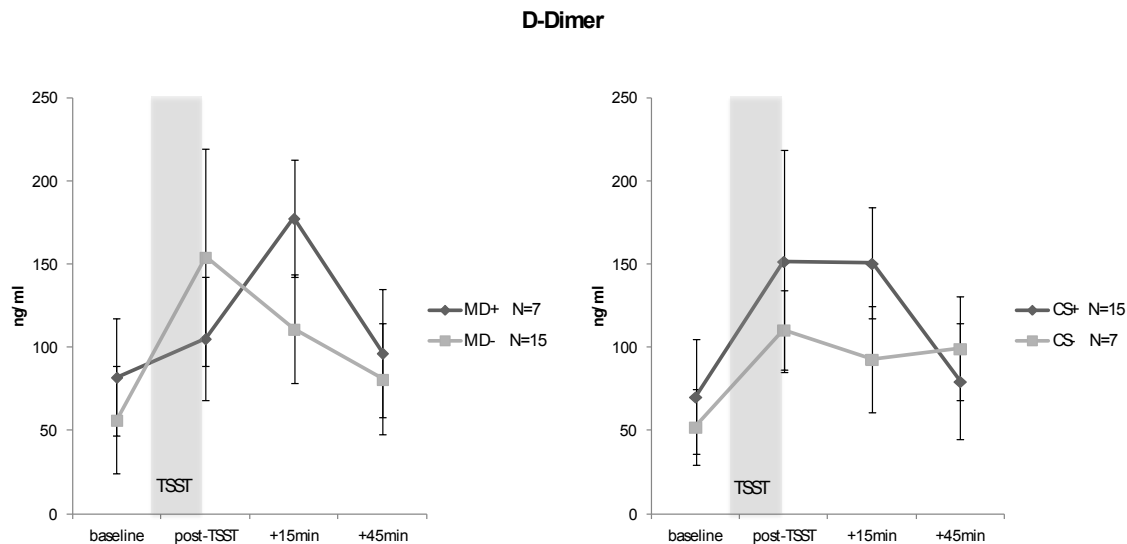


Figure 15. Baseline to post-stress measures of D-Dimer, including another post-stress time point (immediately post-TSST). Left: by MD: depression history. Right: by CS: chronic stress. Depicted are means and standard errors.

5.4.3. VWF

In all subjects, plasma levels of VWF increased on average from 11.80 mg/L (± 5.32) at rest to 13.16 mg/L (± 4.81) 15 minutes post-stress. While this difference did not reach significance ($p=.057$), it suggests a trend. After 45 minutes, average levels increased again to 13.25 mg/L (± 4.23). The difference between baseline and the recovery measurement 45 minutes after the stressor reached significance (11.80 mg/L to 13.25 mg/L $p=.026$); see Fig. 11C. Repeated measures revealed, however, that the psychosocial stressor did not affect VWF levels significantly ($F_{1.5,70.9}=1.643$, $p=.205$) and that neither the presence of depression history ($F_{1,48}=.724$, $p=.399$) nor chronic stress ($F_{1,48}=.302$, $p=.585$) were significant. Interaction effects were also not significant ($F_{1,48}=.048$, $p=.828$).

The same was observed for the baseline to immediate post-TSST measures ($N=22$). Neither the effect of TSST ($F_{1,9}\leq 1.722$, $p\geq .222$), nor the effect of depression ($F_{1,9}=.603$, $p=.457$) nor the effect of chronic stress ($F_{1,9}=.281$, $p=.609$) were significant.

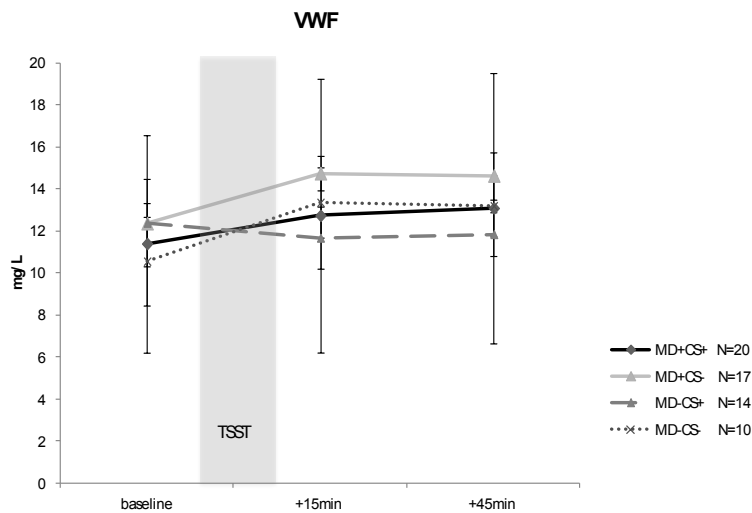


Figure 16. Baseline to post-stress measures of VWF. Presented are means plus standard errors, separate for the four groups. +positive, -negative for MD: major depression, CS: chronic stress.

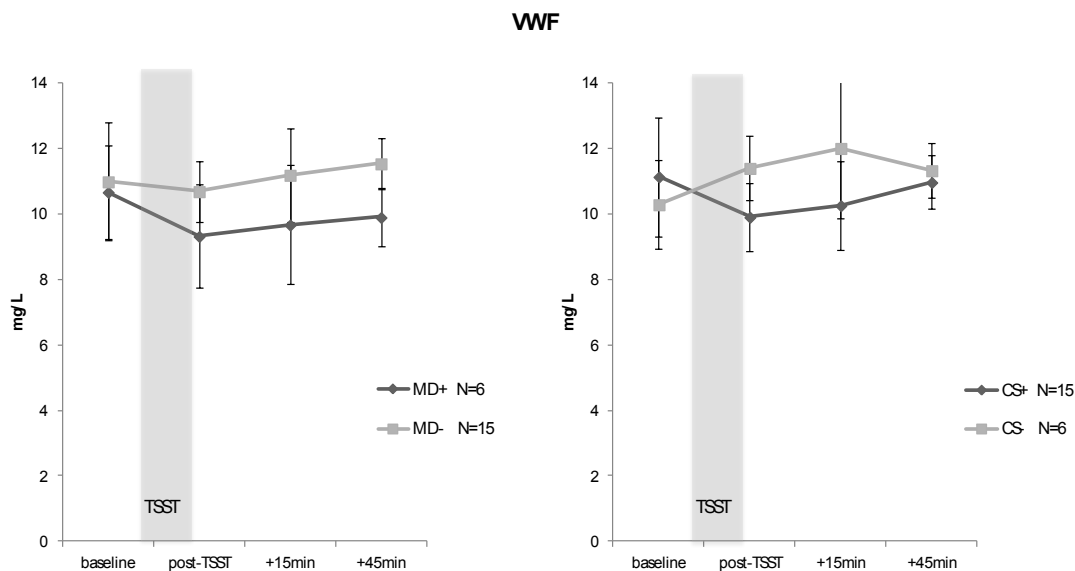


Figure 17. Baseline to post-stress measures of VWF, including another time point (immediately post-TSST). Left: by MD: depression history. Right: by CS: chronic stress. Depicted are means and standard errors.

5.5. Associations between depression symptoms and chronic stress load, and hemostatic markers

Partial correlation analyses were carried out to additionally examine the effects of depression symptoms and different aspects of chronic stress on hemostatic parameters. The same covariates as in the ANCOVA analyses were used plus CS or MD to further control for the respective other group effects (CS for depression symptoms, MD for chronic stress load).

Coagulation parameters and depression symptoms

Partial correlation analyses between fibrinogen, D-Dimer, VWF, PAI-1 levels and the scores on Beck's Depression Inventory II at the test day did not reveal any significant associations. The results of this correlation analysis are listed in the appendix.

Coagulation parameters and chronic stress

Associations between the TICS screening score and the nine subscores are listed in Table 5.

Fibrinogen levels directly post-stress correlated negatively with overall stress ($r=-.682$, $p=.030$) and positively with the TICS subscore chronic worrying ($r=.811$, $p=.004$).

Fibrinogen levels at 15 minutes post-stress showed a weak negative correlation with the subscore 'Excessive demand at the workplace' ($r=-.297$, $p=.038$) and 'Social tensions' ($r=-.279$, $p=.050$).

D-Dimers measured directly post-stress correlated positively with TICS' section 'pressure to succeed' ($r=.637$, $p=.047$).

As for PAI-1 levels and overall chronic stress, a negative association was discovered, albeit only a weak one ($r=-.246$) and as a trend, i.e. non-significant ($p=.082$). The subscore 'social tensions' showed a weak negative correlation with PAI-1 ($r=-.313$, $p=.025$).

Table 5. Pearson’s correlation coefficients for correlations between hemostatic measurements and TICS scores. The TICS consists of nine subscores and one screening score for overall measurement of chronic stress load. ‘post-stress’ stands for measurements that were taken immediately following the TSST and ‘+15 min’ for those taken 15 minutes after the TSST.

	TICS screening score	TICS Work overload	TICS social overload	TICS pressure to perform	TICS work discontent	TICS Excessive demand at workplace	TICS Lack of social recognition	TICS social tensions	TICS social isolation	TICS chronic worrying
Fibrinogen baseline	-.152 (p=.286)	-.048 (p=.739)	-.058 (p=.688)	-.117 (p=.418)	-.164 (p=.249)	-.136 (p=.249)	-.264 (p=.061)	-.168 (p=.239)	-.188 (p=.186)	-.167 (p=.241)
Fibrinogen (N=22) post-stress	-.682 (p= .030)	-.618 (p=.057)	.034 (p=.927)	.270 (p=.450)	-.601 (p=.066)	-.409 (p=.275)	-.256 (p=.475)	-.261 (p=.467)	-.278 (p=.436)	-.811 (p= .004)
Fibrinogen (N=63) +15 min	-.257 (p=.072)	-.121 (p=.399)	.033 (p=.821)	-.093 (p=.525)	-.270 (p=.058)	-.297 (p= .038)	-.268 (p=.060)	-.279 (p= .050)	-.149 (p=.301)	-.231 (p=.106)
D-Dimer baseline	.073 (p=.616)	.065 (p=.659)	.190 (p=.191)	.176 (p=.230)	-.017 (p=.906)	.059 (p=.693)	.204 (p=.159)	.175 (p=.229)	-.009 (p=.950)	.056 (p=.702)
D-Dimer (N=22) post-stress	.443 (p=.199)	.337 (p=.341)	.399 (p=.253)	.637 (p= .047)	.193 (p=.593)	.612 (p=.080)	.352 (p=.318)	.341 (p=.335)	.058 (p=.873)	.433 (p=.211)
D-Dimer (N=63) +15 min	.103 (p=.488)	.168 (p=.253)	-.004 (p=.980)	.192 (p=.195)	-.001 (p=.993)	.119 (p=.425)	.192 (p=.192)	.140 (p=.344)	.093 (p=.528)	.074 (p=.619)
VWF baseline	-.011 (p=.939)	.076 (p=.599)	.074 (p=.610)	.219 (p=.130)	-.118 (p=.416)	-.011 (p=.941)	.110 (p=.449)	-.038 (p=.795)	.104 (p=.470)	-.051 (p=.726)
VWF (N=22) post-stress	-.128 (p=.742)	-.276 (p=.472)	.097 (p=.804)	-.122 (p=.754)	-.101 (p=.796)	.322 (p=.437)	.224 (p=.563)	-.339 (p=.372)	-.063 (p=.872)	-.131 (p=.736)
VWF (N=63) +15 min	-.197 (p=.176)	r=-.269 (p=.061)	-.020 (p=.892)	.037 (p=.800)	-.041 (p=.777)	-.137 (p=.354)	.037 (p=.803)	-.120 (p=.413)	-.076 (p=.602)	-.178 (p=.220)
PAI-1 (N=63)	-.246 (p=.082)	-.214 (p=.132)	-.113 (p=.428)	-.087 (p=.550)	-.117 (p=.313)	-.214 (p=.135)	-.153 (p=.283)	-.157 (p=.270)	-.313 (p= .025)	-.254 (p=.072)

6. Discussion

This work's focus lies on whether or not a history of major depression or current chronic stress affect the hemostatic system at rest and after an acute stressor. Furthermore, the question whether depression history and chronic stress influence coagulation independent of each other or have interacting effects was examined.

In summary, the key findings were that subjects with a history of depression had higher resting fibrinogen levels and, additionally, that acute stress-induced changes in fibrinogen were affected by a history of depression. Furthermore, it was shown that chronic stress load significantly affected D-Dimer changes brought about by the TSST. The combination of CS with MD showed a trend toward significance in influencing stress-induced D-Dimer changes.

The following sections will elaborate on the hypotheses that are listed in part 3 of this work, together with the related findings, closing with a discussion of the limitations of this study, as well as an outlook to further required research.

6.1. Coagulation markers at rest

6.1.1. The effects of remitted depression on basal coagulation

The remitted depression group presented with higher baseline fibrinogen levels than the control group, which confirms our hypothesis H1.1. for fibrinogen. This hypothesis stated that baseline fibrinogen and PAI-1 levels are higher in persons with a history of depression, compared to controls. For PAI-1 levels, however, there were no differences between the groups.

The present data supports hypothesis H1.1. for fibrinogen, but not for PAI-1.

Previous research on fibrinogen also found a positive association between resting fibrinogen levels and depression (Hjemdahl & Von Känel, 2012; Wium-Andersen, Orsted et al., 2013) and interestingly, according to the results of the present study, this association seems to persist after remission of depression. The subjects in the remitted depression group experienced their last episode on average 10.5 years prior to this assessment, but still, fibrinogen remained elevated even when depressive symptoms were absent or at subclinical levels. These findings add to the hypothesis that fibrinogen might serve as a marker for depression, as other

researchers suggest (Martins-de-Souza et al., 2014; Okwuosa et al., 2013; Wium-Andersen, Orsted et al., 2013). Increased fibrinogen levels in remitted depression may confer an increased risk for cardiovascular disease, without the presence of the traditional cardiovascular risk factors. Notably, it has been reported that there exists a strong independent correlation between changes in traditional cardiovascular risk factors and changes in mean fibrinogen. (Okwuosa et al., 2013). It remains open whether these relationships hold true also for persons with depression or depression history.

Regarding PAI-1, there were no differences between remitted depressed subjects and controls. PAI-1 has a diurnal variation, with highest levels in the morning (Angleton, Chandler, & Schmer, 1989) and since the TSST was carried out in the afternoon, this might be one reason for our negative result. Another reason for our negative result may be that PAI-1 appears to be only associated with remitted depression in aged patients, as one recent study indicates. The subjects in that study were between the ages of 70 and 90 years (Baune et al., 2012). However, in our work, subjects were on average in their mid-thirties. The exact role of PAI-1 in remitted depression needs to be further investigated.

6.1.2. The effects of chronic stress on basal coagulation

At rest, persons with high chronic stress showed a trend toward higher D-Dimer plasma concentrations.

Hypothesis H1.2., which states that persons under high chronic stress show elevated coagulation parameters at rest, cannot be supported for any of the considered markers, namely fibrinogen, D-Dimer, VWF and PAI-1, albeit a trend is seen for D-Dimer.

Increased D-Dimer concentrations suggest that chronically stressed persons may have increased fibrin turnover. Elevated D-Dimer levels have been reported in a variety of diseases, such as venous thrombosis (Cushman et al., 2003), ischemic heart disease (Lowe et al., 2004) and even in cancer patients (Ay et al., 2012). Recently, a large study found that even in a healthy population free of cardiovascular risk factors, elevated D-Dimer levels were independently associated with increased mortality (multivariable hazard ratios ranged between 1.06 and 1.97). These findings linking increased D-Dimer plasma concentrations to increased mortality together with the results from our study suggest that further research on the connections between chronic stress and D-Dimer is needed.

Our results could not reproduce the findings regarding fibrinogen, the endothelial-dysfunction marker VWF and the anti-fibrinolytic marker PAI-1 that other studies have reported. This may have various reasons: the other studies did not assess chronic stress in the same way as was done in this study, i.e. using the TICS questionnaire. They studied the stress of caregiving for demented patients, job stress and traumatic life events, separately, and did not assess overall chronic stress, as the TICS does.

6.1.3. Interaction effects between remitted depression and chronic stress on basal coagulation

In hypothesis 1.3., it was stated that in previously depressed subjects, procoagulant parameters at baseline are altered, depending on the presence of chronic stress.

In this study no interaction effects between chronic stress and a history of major depression on baseline procoagulant markers could be found, and thus, hypothesis 1.3. cannot be supported.

It appears that in its effect on basal coagulation, a positive depression history is independent from chronic stress. Because depression is often accompanied and worsened by chronic stress, and in turn, chronic stressful life situations can increase the risk of developing depression, it was plausible to speculate that interacting effects of CS and MD existed. However, in remitted depression, as examined in this work, this interaction does not seem to exist regarding baseline levels.

6.2. Stress-induced changes

The stress test elicited a reliable stress reaction as indicated by rising cortisol levels and the subjects' feelings of perceived stress. On average, heart rate changed significantly from pre- to post-stress as an indicator of an activation of the SAM-system. When looking at the repeated measures analysis of covariance, heart rate changes over all time points were not significant. This result is not surprising, since TSST is known to more reliably trigger HPA-axis responses than responses of the sympathetic nervous system (Dickerson & Kemeny, 2004). Cortisol increases as part of a positive HPA-axis response were indeed significant. There are many elements in the TSST which seem uncontrollable to the subjects and cortisol is known to respond more with uncontrollable situations than with controllable ones (Hamer & Malan,

2010). Although not significant, the observation that the chronic stress group without a history of depression (MD-CS+) had the highest overall cortisol levels is noteworthy. Whereas chronic stress without a depression history shows a higher cortisol curve, the curve for the combination of chronic stress plus depression history runs lower. Beside its effects on autonomic functions, the TSST also influenced subjects' anxiety levels considerably. As expected, subjects reported that they felt more anxious during the stress test than before and that their anxiety decreased again during the recovery period.

The TSST also elicited changes in the hemostatic system: in all subjects unrelated to group, a trend toward an increase in D-Dimer levels was observed during the TSST, with average levels increasing by almost one third from pre- to post-TSST. A possible reason for why this increase was not significant may be that the post-stress measurement took place 15 minutes after, and not directly post-stress. It is possible that D-Dimer levels had already decreased again after this time.

6.2.1. The effects of remitted depression on stress-induced coagulation

To date, no other studies have examined remitted depression in relation to stress-induced changes of coagulation parameters. In the present study, fibrinogen levels decreased slightly in all but one of the four groups as a reaction to the TSST. In the MD-CS+ group, levels increased slightly. Depression history accounted for this change.

In a smaller sample of subjects for which the three parameters of interest were measured directly after the TSST (N=22), this depression effect remained. However, a stress-induced increase of fibrinogen, as reported by other studies, could not be seen here. Instead, a slight decrease was observed. A possibility for fibrinogen levels not increasing with the stressor could be an increased consumption of fibrinogen due to in-situ fibrin formation on the surface of blood vessels (Von Känel, Kudielka et al., 2009). However, despite the fact that fibrinogen did not increase, depression history had a significant effect on stress-induced fibrinogen levels over time: in subjects with a depression history, fibrinogen levels decreased after the stress test.

No effects of remitted depression on any of the other procoagulant markers could be observed.

Hence, hypothesis 2.1., which states that a history of depression has an effect on stress-induced D-Dimer changes, is not supported.

Previous findings focussing on D-Dimer stress-responsiveness and depression provided a mixed picture as both an attenuated response immediately post-stress (Von Känel, Bellingrath, et al., 2009b) and increases (Wirtz et al., 2008) have been reported. These studies included subjects with depression symptoms at the time of the experiment (TSST). We were not able to reproduce any of the previous results. In this work depression history did not have an effect on D-Dimer changes, possibly because this study examined persons without current depression symptoms. Nevertheless, the effect of depression history on fibrinogen levels was a significant one, as is described above.

6.2.2. The effects of chronic stress on stress-induced coagulation

Individuals with high chronic stress had significantly higher D-Dimer changes following the stress test than those without chronic stress.

Hypothesis H2.2., which states that high chronic stress modulates procoagulant responses to acute psychosocial stress, is therefore supported. D-Dimer was the only parameter that was affected by chronic stress, while fibrinogen and VWF were unaffected.

Notably, the effect of chronic stress on D-Dimer was only true for our larger sample for which three measurements were taken. In the smaller sub-sample, for which four measurements were taken, the effect of chronic stress on D-Dimer changes was no longer significant. This might be explained by the relatively small size of the sub-sample.

A D-Dimer increase indicates augmented production of thrombin and fibrin like during the formation of a thrombus. Also, as a thrombus is broken down, fibrin dissolves into D-Dimers and D-Dimer concentration increases. D-Dimer can be viewed as a marker for both fibrin formation and degradation. D-Dimer levels rose in all subjects after the TSST, independent of group, indicating that a hypercoagulable state had been reached briefly. The increase was most pronounced when chronic stress was present, i.e. in the MD-CS+ group and the MD+CS+ group.

Fibrin D-Dimer is considered an independent marker of the risk of cardiovascular disease and is also related to atherosclerosis severity (Zhou et al., 2007). In light of this known

relationship, the present results suggest that persons suffering from chronic stress may be more prone to develop atherosclerosis or that pre-existing atherosclerotic lesions may develop faster.

6.2.3. Interaction effects between remitted depression and chronic stress on stress-induced coagulation

With respect to our hypothesis regarding the interaction effects between remitted MD and CS on stress-induced coagulation, we found that there was a trend only for D-Dimer levels to be affected by the CS/MD interaction. None of the other markers that were examined were affected by the CS/MD interaction. Those subjects with both chronic stress and a history of depression tended to have greater D-Dimer changes over the different time points, but the interaction did not reach significance.

Hypothesis H2.3., which states that interacting effects of CS and MD history on stress-induced coagulation exist, is not supported for fibrinogen, D-Dimer and VWF.

Nonetheless, a trend for D-Dimer changes to be affected by a CS x MD interaction was found. This result suggests that chronic stress may be a mediator of the association between depression and D-Dimer change. In turn, depression history could be a mediator of the association between chronic stress and D-Dimer change following an acute stressor.

6.3. Associations between chronic stress and hemostasis

We carried out observational partial correlation analyses to examine if chronic stress load and current depressive symptoms were associated with any of the hemostatic factors at rest and post-stress. The score on the BDI-II, which was used to detect depression symptoms, was not associated with any of the coagulation parameters, possibly because all subjects were in remission and had sub-clinical scores. Some elements of the TICS questionnaire showed significant correlations with the measured coagulation factors.

Perhaps the most interesting association that was observed here is that fibrinogen and the screening score for chronic stress show an inverse relationship: the chronic stress screening score correlated negatively with fibrinogen measured immediately post-stress, as well as 15 minutes post-stress. A high chronic stress burden could be associated with lower fibrinogen levels. However, an interpretation of these results is difficult, because for the significant association the sample size was rather small (N=22) to draw conclusions, while for the

15 minutes post-stress measurement (N=63), the correlation was weak and did not reach significance. However, it is possible that a high chronic stress load somehow suppresses fibrinogen levels after an acute stressor, perhaps by increasing its turnover to fibrin.

Previous research on the anti-fibrinolytic marker PAI-1 has found it to be positively associated with chronic stress but we were not able to reproduce these results.

As mentioned earlier, we intended these correlation analyses to have explorative character.

Larger sample sizes would be required to reveal further effects.

6.4. Strengths and limitations

As already mentioned in the preceding chapters, interpretation of our findings must take into account certain choices made and limitations present. These are summarized in the following, addressing sample selection, study protocol, technical protocol execution and potential influences of stress hemoconcentration.

Having recruited subjects from the EDSP study, which includes a well-characterized epidemiological sample, one can assume an overall high quality of the subsample used in this study.

The influence of smoking and oral contraceptives were not defined as exclusion criteria since it would have created great difficulties in the recruitment of subjects. However, both have known influences on the hemostatic system. Fibrinogen and PAI-1 activity have been shown to be higher in previous and current smokers compared to persons who never smoked (Lahlou-Laforet et al., 2006) and women taking oral contraceptives are more prone to deep venous thrombosis. Contraception and smoking status were therefore used as covariates in the analyses. In the present sample there were no statistical differences in the number of smokers and women taking oral contraceptives between the groups. The inclusion of the four subjects who were taking antidepressant medication merits critical attention as well since an influence on the coagulation system is possible. We therefore accounted for its influence by using antidepressant medication as a covariate in all analyses.

All persons involved in the experimental part of the study adhered to a strict protocol that listed all steps. Instructions regarding the TSST were read out loud to minimize inconsistencies and ambiguities due to the investigator's interaction with the test subjects. This ensured

that the TSST was carried out in a highly standardized manner. Other strong points of this work were the location of the experiment in a soundproof room and a system that allowed for blood collection through the wall without disturbing the participant.

Regarding the time points for measurement of the four coagulation markers, one limitation was that in the initial study protocol, the first post-TSST blood sample would be taken 15 minutes after the TSST. Based on expert consultations during the study, we added another time point to the study protocol immediately post TSST. The amended study protocol with the additional measurement of coagulation factors was then implemented for the remaining 22 subjects of the sample (of a total of 63). For these 22 subjects, however, group distribution was uneven, with only 7 subjects in the remitted depression group. Statistical analyses were carried out for both, the complete and the N=22 sample, as described in chapter 4. For the N=22 sample, both the small sample size, as well as the uneven distribution among the MD/CS groups decreases the statistical power of the outcomes and any interpretations regarding these results should be viewed with a degree of caution. Still, the results from the complete and the N=22 samples are directionally equal for the following analyses: the depression effect on fibrinogen changes, the negative effect of TSST on D-Dimer levels, the negative effect of depression on D-Dimer levels, and all analyses regarding VWF. The two samples differed in the following results: the CS effect on D-Dimer and the TSST effect on fibrinogen were significant for the larger sample, but not for the smaller one.

Technical problems and delays could not be prevented in some cases. Baseline measures were taken after a 30-minute time period during which the subjects were able to acclimatize to the new setting. In some cases this time period may have been shortened, e.g. due to difficulties with placing the venous catheter or because subjects experienced circulatory problems. Thus, there were minor deviations in the subjects' starting conditions. Further, some of the post-stress blood collections may have been slightly delayed. Clogging of the catheter were likely reasons for this, and in some cases the placement of a new catheter became necessary.

Hemoconcentration is another possible process that may have an influence on stress-induced coagulation since it may influence the concentration of hemostatic components. As coagulation factors are relatively large molecules they cannot move passively through the vessel wall to the extravascular space (Austin et al., 2011; Hjendahl & Von Känel, 2012). As plasma shifts from the intra- to the extravascular space during acute stress, the concentration of

non-diffusible molecules rises (Allen & Patterson, 1995). This process is likely caused by an increase in blood pressure through SNS activation. It is important to think about this concept when studying coagulation changes since rising coagulation factor concentrations may be genuine effects of stress but they may also be due to plasma loss (Hjemdahl & Von Känel, 2012). A review of the relevant literature (Austin & Patterson, 2013) concludes that stress-induced changes in coagulation are due to both hemoconcentration and actual activation of coagulation and that the intrinsic pathway (as seen in FXIII) is likely genuinely activated by acute stress. It is not yet clear to what extent hemoconcentration needs to be accounted for and adjusted for arithmetically when studying stress-induced coagulation parameters. There are different methods for hemoconcentration-correction: an arithmetic correction, a baseline plasma reconstitution method and a reconstitution method using saline (Austin & Patterson, 2013). The most accurate method is currently being debated, and correction techniques have consequently not been included in this study.

6.5. Conclusion and outlook

Depression and chronic stress often form a vicious cycle, with chronic stress increasing depressive symptoms and depressive symptoms causing further stress. It is therefore relevant to determine whether chronic stress and depression are independent in their effect on the hemostatic system or whether they interact to create a hypercoagulable environment.

The present study thus intended to find out if patients who had suffered from major depression in the past but were in remission at the time of the experiment also showed alterations in hemostatic function, at rest and following acute stress. Furthermore, the influence of chronic stress and the combination of remitted depression and chronic stress was examined in the same way. First, the findings regarding fibrinogen are discussed, and then the findings regarding D-Dimer.

This work found that remitted depressed subjects in their mid-thirties had higher fibrinogen levels at rest than controls, with no change in D-Dimers. With regard to interaction effects between remitted depression and chronic stress on resting coagulation markers, our results are negative, implying that depression history and chronic stress are independent factors to influence the hemostatic system.

When remitted depressed participants were subjected to an acute psychosocial stressor, we found that their stress-responsiveness was different from healthy controls, i.e. that different effects on the concentration of coagulation factor fibrinogen and its development over time were observed. Our findings therefore support the association between major depression and elevations of fibrinogen levels – while other studies focused on acute depression, this study found this association to also hold true in remitted depression.

High blood pressure, dyslipidemia, smoking status and diabetes are known cardiovascular risk factors. Heightened fibrinogen has been identified as one of them and has been shown to also be elevated in depression. Notably, in depression, the risk for cardiovascular disease is increased, independent of the other cardiovascular risk factors. Fibrinogen therefore appears to be a common denominator in both classical risk factors and depression. The present results demonstrate that depression history is associated with altered hemostasis function when the two potentially confounding biological variables, BMI and smoking, both of which are related to metabolic syndrome, were taken into account. Positive correlations between fibrinogen levels and the known cardiovascular risk factors high BMI, high triglycerides and LDL cholesterol, low HDL, diabetes history and hypertension history have been reported (Kaptoge et al., 2007b). In a meta-analysis on healthy middle-aged adults, Danesh et al. (2001) also report associations between plasma fibrinogen levels and the risks for coronary heart disease and stroke. Again, fibrinogen generally increased with BMI, LDL-cholesterol, log triglycerides and diastolic blood pressure and fell with increasing physical activity and HDL-cholesterol (Danesh et al., 2001).

Since fibrinogen is a major acute-phase-protein, an increase could also indicate a state of inflammation. Whether increased resting fibrinogen levels reflect an increased state of inflammation or whether fibrinogen is elevated through pathophysiological processes caused by depression is unclear. Work on inflammatory markers revealed elevated levels of the cytokines TNF-alpha and IL-6 in depressed patients (Dowlati et al., 2010) and another study concluded that the inflammation marker C-reactive protein (CRP) and the cytokines IL-1 and IL-6 were associated with depression (Howren, Lamkin, & Suls, 2009). Furthermore, a strong positive correlation between CRP levels and fibrinogen has been described (Kaptoge et al., 2007b).

Our finding regarding increased baseline fibrinogen in remitted depression lends further support to fibrinogen as being a potential depression marker. A recent study by Martins-de-Souza et al. (2014) investigated whether fibrinogen may serve as a marker for antidepressant treatment response. Non-responders to antidepressant medication had higher fibrinogen levels compared to responders. Successful treatment of depression may therefore possibly lower the cardiovascular risk by lowering fibrinogen. Behavioral changes, such as smoking cessation and weight loss have been shown to also decrease fibrinogen levels in young, healthy adults (Okwuosa et al., 2013). As a consequence, it would be advisable that physicians treating depression have knowledge about their patients' cardiovascular risk factors and that they give depression, and also remitted depression, the same amount of attention as a traditional cardiovascular risk factor. One should also consider monitoring fibrinogen in depressed patients as a real option in the future.

Our second major finding was that a trend was seen for subjects under chronic stress to have increased D-Dimer levels at rest, however for fibrinogen there was no such elevation seen. Once all subjects underwent the acute stress test, their average D-Dimer concentrations increased significantly, with the factor chronic stress having a significant effect on these changes.

Previous research using the TICS questionnaire, which was also used in this study, reports that depressive symptoms, burnout syndrome and sleep disturbances are more common in people who have high levels of chronic stress than in those without high levels of stress. (Hapke et al., 2013). Whether scores on the TICS are also positively related to increased cardiovascular events remains open and would be worth examining. Adults experiencing social isolation and workplace stress situations that can be considered chronic stress factors, have been shown to have an increased risk for coronary heart disease (Steptoe & Kivimäki, 2012). These facts make the assumption plausible that high TICS scores are associated with increased cardiovascular disease. A possible reason for chronic stress being linked to coronary heart disease may be a hypercoagulable state, which is underlined by higher D-Dimer levels, as was found here. Prospective studies are needed to assess whether persons with high chronic stress, as measured by the TICS, have increased cardiovascular risk, particularly since they also showed alterations of D-Dimer levels.

The risk of cardiovascular disease and insults in chronically stressed individuals may be considerably reduced through interventional measures such as relaxation techniques and stress management, however, more studies in this area are needed. Social support seems to be another key factor to protect against chronic stress and its negative effects on health. Social support and chronic stress show an inverse relationship (Hapke et al., 2013), which needs to be considered as a key element in public health measures. The existing studies on pharmacological interventions to restore normal hemostatic function appear to be somewhat limited in scope, as a review by Austin et al. (2013) showed. More research on pharmacological, as well as behavioral measures would be useful to further assess whether these can influence stress-related hypercoagulability, and thus possibly sever the psychosomatic connections between stress and cardiovascular risk.

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Appendix I.

The following tables show the raw data presented in the Results section (Section 5).

Source data for Figures 4, 5, 6 and 7.

Mean baseline concentrations of fibrinogen, D-Dimer, VWF and PAI-1. Comparison between subjects with remitted depression and without a history of depression. Comparison between subjects with and without chronic stress and interaction effects.

Fibrinogen (mg/dl)	MD- (N=24)	MD+ (N=39)		CS- (N=29)	CS+ (N=34)		MD*CS (interaction)
	111.192 (±5.988)	126.742 (±3.567)	$F_{1,50}=7.738$ $p=.008$	118.069 (±5.076)	123.235 (±4.403)	$F_{1,50}=.139$ $p=.711$	$F_{1,48}=.184$ $p=.670$
D-Dimer (ng/ml)	MD- (N=23)	MD+ (N=38)		CS- (N=28)	CS+ (N=33)		
	57.609 (±22.223)	56.077 (±11.518)	$F_{1,48}=.209$ $p=.649$	38.464 (±13.060)	70.303 (±16.820)	$F_{1,48}=2.96$ $p=.092$	$F_{1,48}=2.570$ $p=.115$
VWF (mg/L)	MD- (N=24)	MD+ (N=38)		CS- (N=28)	CS+ (N=34)		
	11.628 (±1.475)	11.915 (±.610)	$F_{1,49}=.203$ $p=.654$	11.711 (±.954)	11.805 (±.971)	$F_{1,49}=.450$ $p=.505$	$F_{1,48}=.626$ $p=.432$
PAI1 (ng/ml)	MD- (N=24)	MD+ (N=39)		CS- (N=29)	CS+ (N=34)		
	1.648 (±.187)	2.072 (±.234)	$F_{1,50}=.246$ $p=.622$	1.919 (±.266)	1.903 (±.209)	$F_{1,50}=.837$ $p=.365$	$F_{1,48}=.308$ $p=.581$

Source data for Figure 8.

Mean heart rate measures (bpm) of the four groups before and after the TSST. The figure shows means and standard errors corrected for baseline differences.

	Rest	Instruction	pre-TSST	post-TSST	+15min	+30min	+45min
MD+CS+ (N=18)	80.680 (±2.222)	82.280 (±2.181)	96.390 (±2.180)	98.170 (±2.324)	75.890 (±1.473)	75.390 (±1.732)	77.170 (±2.071)
MD+CS- (N=18)	81.370 (±3.686)	81.720 (±3.457)	94.390 (±3.918)	98.940 (±4.290)	78.440 (±2.381)	74.830 (±2.914)	74.720 (±2.670)
MD-CS+ (N=12)	77.000 (±3.213)	84.250 (±4.143)	100.170 (±4.026)	102.670 (±4.760)	74.250 (±3.170)	74.580 (±2.684)	72.000 (±3.245)
MD-CS- (N=9)	83.560 (±4.167)	81.440 (±4.240)	101.560 (±1.923)	104.330 (±4.187)	82.890 (±4.053)	79.220 (±3.045)	75.670 (±2.872)

Source data for Figure 9.

Cortisol response (ng/ml) separate for each of the four groups. Means and standard errors corrected for baseline differences.

	Baseline	pre-TSST	post-TSST	+15min	+30min	+45min
MD+CS+ (N=19)	127.296 (±28.464)	118.510 (±9.323)	134.360 (±9.789)	122.790 (±10.480)	104.720 (±8.828)	91.940 (±7.706)
MD+CS- (N=17)	118.378 (±27.158)	124.710 (±15.231)	153.550 (±13.444)	141.310 (±11.809)	122.070 (±11.038)	109.140 (±10.182)
MD-CS+ (N=14)	150.069 (±18.119)	144.850 (±15.953)	168.250 (±12.588)	159.970 (±16.073)	144.150 (±14.739)	130.070 (±12.505)
MD-CS- (N=10)	133.608 (±13.734)	122.890 (±8.684)	152.910 (±7.577)	147.980 (±9.949)	129.970 (±10.028)	115.830 (±9.284)

Source data for Figure 10.

Changes in perceived anxiety during the stress protocol. Presented are estimated BSKE score means and standard errors corrected for baseline differences.

A: subscore ‘inner restlessness’, B: subscore ‘anxiety’.

A: (effect of TSST: $F_{3,6, 198.8}=55.208, p<.001$);

	Baseline	Instruction	pre-TSST	TSST	+15min	+30min	+45min
MD+CS+ (N=20)	2.100 (±.204)	2.800 (±.296)	3.050 (±.276)	4.000 (±.299)	1.250 (±.289)	.750 (±.190)	.800 (±.200)
MD+CS (N=18)	1.530 (±.298)	2.110 (±.290)	2.440 (±.217)	3.890 (±.179)	.940 (±.221)	.670 (±.243)	.560 (±.145)
MD-CS+ (N=13)	1.930 (±.384)	2.380 (±.367)	3.380 (±.331)	4.460 (±.215)	1.150 (±.317)	.850 (±.222)	.690 (±.263)
MD-CS- (N=10)	2.100 (±.379)	2.700 (±.367)	3.600 (±.452)	4.400 (±.400)	1.500 (±.401)	.800 (±.291)	.800 (±.291)

B: (effect of TSST: $F_{2,4, 133.6}=25.666, p<.001$)

	Baseline	Instruction	pre-TSST	TSST	+15min	+30min	+45min
MD+CS+ (N=20)	.650 (±.167)	1.500 (±.295)	1.850 (±.318)	2.300 (±.430)	.450 (±.185)	.300 (±.128)	.300 (±.128)
MD+CS- (N=18)	.420 (±.163)	.720 (±.278)	0.940 (±.297)	1.390 (±.257)	.390 (±.183)	.170 (±.121)	.220 (±.101)
MD-CS+ (N=13)	.570 (±.260)	1.000 (±.408)	2.000 (±.453)	2.000 (±.438)	.690 (±.308)	.230 (±.122)	.230 (±.122)
MD-CS- (N=10)	.300 (±.153)	.500 (±.307)	1.100 (±.482)	1.500 (±.500)	.200 (±.200)	.100 (±.100)	.000 (±.000)

Source data for Figure 11.

Changes in the three coagulation parameters in response to the TSST (N=63), independent of group. The table depicts means and standard errors with F and p-values (repeated measures ANCOVA with two independent factors, MD and CS).

	baseline	+15min	+45min	effect of TSST	effect of MD	effect of CS	Effect of MD*CS
Fibrinogen (mg/dl)	120.781 (±3.270)	116.206 (±4.011)	115.841 (±3.855)	$F_{1,9,94.2}=4.076$ p=.021	$F_{1,49}=7.850$ p=.007	$F_{1,49}=.052$ p=.820	$F_{1,49}=.000$ p=.993
D-Dimer (ng/ml)	56.645 (±10.866)	78.951 (±12.000)	53.049 (±10.582)	$F_{1,6,76.6}=.099$ p=.868	$F_{1,47}=.008$ p=.930	$F_{1,47}=4.107$ p=.048	$F_{1,47}=3.880$ p=.055
VWF (mg/L)	11.805 (±.670)	13.162 (±.611)	13.246 (±.538)	$F_{1,5,70.9}=1.643$ p=.205	$F_{1,48}=.724$ p=.399	$F_{1,48}=.302$ p=.585	$F_{1,48}=.048$ p=.828

Changes in the three coagulation parameters in response to the TSST (N=22), independent of group. Repeated measures ANCOVA with two independent factors, MD and CS.

	effect of TSST	effect of MD	effect of CS
Fibrinogen	$F_{1,10} \leq .453$ p>=.516	$F_{1,10}=6.042$ p=.034	$F_{1,10}=.506$ p=.493
D-Dimer	$F_{1,10} \leq 1.617$ p>=.232	$F_{1,10}=2.560$ p=.141	$F_{1,10}=2.627$ p=.136
VWF	$F_{1,9} \leq 1.722$ p>=.222	$F_{1,9}=.603$ p=.457	$F_{1,9}=.281$ p=.609

Source data for Figure 12.

Baseline to post-stress measures of fibrinogen (mg/dl) in the four groups. Presented are means with standard errors.

	baseline	+15min	+45min
MD+CS+ (N=20)	131.55 (±5.177)	125.15 (±6.515)	122.55 (±6.451)
MD+CS- (N=18)	121.61 (±5.304)	116.17 (±5.623)	115 (±5.813)
MD-CS+ (N=14)	111.36 (±6.715)	112.79 (±8.507)	116.14 (±8.769)
MD-CS- (N=10)	111.2 (±11.347)	104.7 (±15.107)	102.6 (±12.974)

Source data for Figure 13.

Baseline to post-stress measures of fibrinogen (mg/dl), including another post-stress time point (immediately post-TSST). Presented are means and standard errors.

	baseline	post-TSST	+30min	+45min
MD+ (N=7)	129.857 (±6.978)	116.571 (±9.954)	116.571 (±8.375)	121.286 (±14.484)
MD- (N=15)	109.600 (±7.105)	111.200 (±10.087)	113.667 (±10.145)	110.133 (±9.805)
CS+ (N=15)	114.000 (±5.928)	113.800 (±8.478)	113.467 (±7.600)	114.133 (±9.151)
CS- (N=7)	120.429 (±12.934)	111.000 (±15.837)	117.000 (±17.191)	112.714 (±16.903)

Source data for Figure 14.

Baseline to post-stress measures of D-Dimer (ng/ml) in the four groups. Presented are means and standard errors.

	baseline	+15min	+45min
MD+CS+ (N=19)	59.160 (±14.811)	78.950 (±19.471)	45.790 (±12.338)
MD+CS- (N=18)	49.500 (±19.566)	56.500 (±18.425)	37.440 (±14.746)
MD-CS+ (N=14)	85.430 (±34.624)	138.140 (±33.369)	90.860 (±37.102)
MD-CS- (N=9)	14.330 (±7.474)	34.110 (±14.045)	38.780 (±13.534)

Source data for Figure 15.

Baseline to post-stress measures of D-Dimer (ng/ml). including another post-stress time point (immediately post-TSST). Presented are means and standard errors.

	baseline	post-TSST	+15min	+45min
MD+ (N=7)	82.230 (±34.981)	105.430 (±36.840)	177.857 (±35.273)	96.714 (±38.757)
MD- (N=15)	56.400 (±32.184)	154.400 (±65.490)	111.133 (±32.540)	80.933 (±33.330)
CS+ (N=15)	70.400 (±34.446)	151.930 (±66.786)	150.733 (±33.563)	79.667 (±34.874)
CS- (N=7)	52.230 (±22.557)	110.710 (±24.057)	93.000 (±32.146)	99.429 (±30.977)

Source data for Figure 16.

Baseline to post-stress measures of VWF (mg/L). Presented are means and standard errors, separate for the four groups.

	baseline	+15min	+45min
MD+CS+ (N=20)	11.400 (±.843)	12.740 (±1.152)	13.090 (±.999)
MD+CS- (N=17)	12.370 (±.987)	14.750 (±.832)	14.640 (±1.123)
MD-CS+ (N=14)	12.390 (±2.070)	11.680 (±1.466)	11.850 (±1.042)
MD-CS- (N=10)	10.560 (±2.114)	13.360 (±1.672)	13.200 (±1.436)

Source data for Figure 17.

Baseline to post-stress measures of VWF (mg/L), including another time point (immediately post-TSST). Presented are means and standard errors.

	baseline	post-TSST	+15min	+45min
MD+ (N=6)	10.680 (±1.428)	9.330 (±1.564)	9.680 (±1.817)	9.910 (±.882)
MD- (N=15)	11.000 (±1.815)	10.700 (±.926)	11.200 (±1.425)	11.550 (±.788)
CS+ (N=15)	11.150 (±1.819)	9.917 (±1.032)	10.270 (±1.350)	10.980 (±.827)
CS- (N=6)	10.300 (±1.353)	11.410 (±.980)	12.020 (±2.154)	11.340 (±.838)

Appendix II.

The following table shows further results of the partial correlation analysis between the coagulation parameters and the BDI-II score that were not presented in the text.

FIB: fibrinogen

DD: D-Dimer

VWF: Von-Willebrand-Factor

PAI1: plasminogen activator inhibitor type 1

bdi_tsst: score on the BDI-II on the test day

time points were G1: baseline, G2: +15 min, G3: +45 min, G4: immediately post-TSST.

Correlations			bdi_tsst
Control Variables			
chron_stress & K_SEX & age & ADeinnahme & HORMVERH & BMI & CRP_1 & haematokrit_1 & haemoglob_1 & thrombo_1 & leuko_1	FIB_G1	Correlation	,039
		Significance (2-tailed)	,787
		df	48
FIB_G4	FIB_G4	Correlation	-,016
		Significance (2-tailed)	,962
		df	9
FIB_G2	FIB_G2	Correlation	-,027
		Significance (2-tailed)	,852
		df	48
FIB_G3	FIB_G3	Correlation	,150
		Significance (2-tailed)	,299
		df	48
DD_G1	DD_G1	Correlation	-,060
		Significance (2-tailed)	,681
		df	47
DD_G4	DD_G4	Correlation	-,247
		Significance (2-tailed)	,464
		df	9
DD_G2	DD_G2	Correlation	-,013
		Significance (2-tailed)	,931
		df	46
DD_G3	DD_G3	Correlation	-,211
		Significance (2-tailed)	,149
		df	46
VWF_G1	VWF_G1	Correlation	-,105
		Significance (2-tailed)	,468
		df	48
VWF_G4	VWF_G4	Correlation	-,322
		Significance (2-tailed)	,365
		df	8
VWF_G2	VWF_G2	Correlation	-,191
		Significance (2-tailed)	,189
		df	47
VWF_G3	VWF_G3	Correlation	-,237
		Significance (2-tailed)	,102
		df	47
PAI1_G1	PAI1_G1	Correlation	,044
		Significance (2-tailed)	,760
		df	48

The following tables shows results of the partial correlation analysis carried out between scores on the TICS questionnaire and the selected coagulation markers. Measurements at +45 minutes post-stress were not shown in Table 5 of the results section, but are presented here.

FIB: fibrinogen levels

D-Dimer levels

VWF: Von-Willebrand-Factor levels

PAI1: plasminogen activator inhibitor type 1

bdi_tsst: score on the BDI-II on the test day

time points were G1: baseline, G2: + 15 min, G3: +45 min, G4: immediately post-TSST.

Control Variables			Correlations		
			Soziale Spannung	Soziale Isolation	Chronische Besorgnis
group & K_SEX & age & ADEinnahme & HORMVERH & BMI & CRP_1 & haematokrit_1 & haemoglob_1 & thrombo_1 & leuko_1 & Raucher	FIB_G1	Correlation	-,168	-,188	-,167
		Significance (2-tailed)	,239	,186	,241
		df	49	49	49
	FIB_G4	Correlation	-,261	-,278	-,811
		Significance (2-tailed)	,467	,436	,004
		df	8	8	8
	FIB_G2	Correlation	-,279	-,149	-,231
		Significance (2-tailed)	,050	,301	,106
		df	48	48	48
	FIB_G3	Correlation	-,246	-,188	-,314
		Significance (2-tailed)	,085	,191	,026
		df	48	48	48
	DD_G1	Correlation	,175	-,009	,056
		Significance (2-tailed)	,229	,950	,702
		df	47	47	47
	DD_G4	Correlation	,341	,058	,433
		Significance (2-tailed)	,335	,873	,211
		df	8	8	8
	DD_G2	Correlation	,140	,093	,074
		Significance (2-tailed)	,344	,528	,619
df		46	46	46	
DD_G3	Correlation	,137	-,043	,063	
	Significance (2-tailed)	,353	,774	,671	
	df	46	46	46	
VWF_G1	Correlation	,038	,104	-,051	
	Significance (2-tailed)	,795	,470	,726	

Correlations

Control Variables			Screeningskala	Arbeitsüberlastung	Soziale Überlastung	Erfolgsdruck	Arbeitsunzufriedenheit	Überforderung bei der Arbeit	Mangel an sozialer Anerkennung
group & K_SEX & age & ADeinnahme & HORMVERH & BMI & CRP_1 & haematokrit_1 & haemoglob_1 & thrombo_1 & leuko_1 & Raucher	VWF_G1	df	48	48	48	47	48	47	48
	VWF_G4	Correlation	-,128	-,276	,097	-,122	-,101	,322	,224
		Significance (2-tailed)	,742	,472	,804	,754	,796	,437	,563
		df	7	7	7	7	7	6	7
	VWF_G2	Correlation	-,197	-,269	-,020	,037	-,041	-,137	,037
		Significance (2-tailed)	,176	,061	,892	,800	,777	,354	,803
		df	47	47	47	46	47	46	47
	VWF_G3	Correlation	-,081	-,171	-,076	,054	,008	-,039	,142
		Significance (2-tailed)	,578	,241	,604	,715	,959	,795	,332
		df	47	47	47	46	47	46	47
	PAI1_G1	Correlation	-,246	-,214	-,113	-,087	-,117	-,214	-,153
		Significance (2-tailed)	,082	,132	,428	,550	,413	,135	,283
	df	49	49	49	48	49	48	49	

Correlations

Control Variables			Soziale Spannung	Soziale Isolation	Chronische Besorgnis
group & K_SEX & age & ADeinnahme & HORMVERH & BMI & CRP_1 & haematokrit_1 & haemoglob_1 & thrombo_1 & leuko_1 & Raucher	VWF_G1	df	48	48	48
	VWF_G4	Correlation	,339	-,063	-,131
		Significance (2-tailed)	,372	,872	,736
		df	7	7	7
	VWF_G2	Correlation	-,120	-,076	-,178
		Significance (2-tailed)	,413	,602	,220
		df	47	47	47
	VWF_G3	Correlation	-,170	-,103	-,085
		Significance (2-tailed)	,243	,483	,561
		df	47	47	47
	PAI1_G1	Correlation	-,157	-,313	-,254
		Significance (2-tailed)	,270	,025	,072
	df	49	49	49	

Eidesstattliche Versicherung

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Ich erkläre hiermit an Eides statt, dass ich die vorliegende Dissertation mit dem Thema

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Danksagung

Bedanken möchte ich mich sehr herzlich für die sehr gute Betreuung am Max-Planck-Institut, und für die Möglichkeit, meine Dissertation an solch einem renommierten Haus absolvieren können.

Insbesondere gilt mein Dank Herrn Dr. Marcus Ising und Frau Dr. Nina Höhne, die mir stets sehr hilfsbereit und kompetent zur Seite standen.

Frau Melanie Huber danke ich sehr für ihre hervorragende und sehr gewissenhafte Arbeit beim praktischen Teil der Arbeit.