Effects of Stress-Hemoconcentration on the Coagulation Cascade

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This dissertation titled
Effects of Stress-Hemoconcentration on the Coagulation Cascade

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ABSTRACT

ANTHONY W. AUSTIN, Ph.D., November 2011, Psychology

Effects of Stress-Hemoconcentration on the Coagulation Cascade

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Objective: When examining the effects of acute psychological stress on coagulation responses, researchers have typically used the Dill and Costill (1974) mathematical correction to adjust for stress-induced plasma volume shifts. While the correction is appropriate for adjusting concentrations of large blood constituents, it may be inappropriate for time dependent or functional coagulation assays. Two novel plasma reconstitution techniques for correcting hemoconcentration effects on stress-induced coagulation changes were compared to the Dill and Costill correction. Methods: Blood was collected from 40 men during the last minute of a 20-minute baseline period, a 6-minute mental arithmetic stressor and a 20-minute recovery period. For the reconstitution techniques, stressor plasma samples were reconstituted with either the person’s own plasma from baseline or physiological saline such that plasma volume at stress became equal to plasma volume at baseline. Results: Plasma volume increased significantly from baseline to stress, but returned to baseline levels at recovery. Uncorrected activated partial thromboplastin time (APTT) decreased, whereas Factor VII clotting activity (FVII:C), FVIII:C, prothrombin time (PT%), and fibrinogen concentration increased significantly from baseline to stress. Arithmetic correction produced a significantly greater decrease in APTT during stress compared to uncorrected APTT, whereas FVII:C, PT%, and fibrinogen concentration were not significantly different from baseline after
arithmetic correction. Arithmetic correction had no effect on FVIII:C. After saline reconstitution, APTT, PT%, FVII:C, and fibrinogen concentration were no longer significantly different from baseline, but FVIII:C remained elevated. After plasma reconstitution, fibrinogen was not significantly different from baseline, whereas FVII:C, FVIII:C, APTT and PT% were unchanged. Though D-dimer concentration increased in the expected direction from baseline to stress, this change was non-significant. D-dimer concentration was not significantly different from baseline after each plasma volume correction technique. With the exception FVIII:C, coagulation parameters returned to baseline levels at recovery. **Conclusions:** The baseline plasma reconstitution method does not seem to be a useful hemoconcentration correction technique, as the Dill and Costill formula adjusts equally well. The saline reconstitution method may be a more biologically pertinent correction technique when examining stress-hemoconcentration effects on clotting time and clotting activity, whereas the Dill and Costill formula does not seem appropriate. With the exception of FVIII:C, hemoconcentration appears to account for most of the stress-induced changes in coagulation parameters examined in this study. Given that stress-induced increases of FVIII:C survived all hemoconcentration correction techniques, the intrinsic pathway of the coagulation system appears to be actually activated during acute stress. Stress-induced changes in coagulation are a consequence of both hemoconcentration and actual activation of the coagulation system.

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INTRODUCTION

Cardiovascular disease (CVD) is the underlying cause of approximately 36% of all deaths in the United States, making it the nation’s number one killer (Kochanek, Xu, Murph, Minño, & Hsiang-Ching, 2009). Furthermore, CVD is the most prevalent disease in the United States; approximately 37% (79.4 million) of American adults have one or more types of CVD (Rosamund et al., 2007). Types of CVD include hypertension, heart failure, stroke, and coronary heart disease (CHD), and types of CHD include myocardial infarction and angina pectoris. Individually, CHD and stroke are the first and third leading causes of death; cancer is the second leading cause. Several risk factors have been identified for CHD, but five have been recognized as conferring the most risk: high blood pressure, elevated serum cholesterol, body mass index, diabetes and smoking (Rosamund et al., 2007). However, these traditional risk factors do not fully explain CHD risk (Greenland et al., 2003). Psychological stress has been purported to explain additional risk, but physiological mechanisms linking stress to CHD have not been delineated fully. One possible mechanism through which stress may be linked to CHD is via adverse effects on hemostatic processes such as platelet function (Markovitz & Matthews, 1991; Patterson & Krantz, 1995), hemorheology (Allen & Patterson, 1995; Patterson, Krantz, Gottdiener, Hecht, Vargot, & Goldstein, 1995) and coagulation (von Känel, Mills, Fainman, &Dimsdale, 2001; Thrall, Lane, Carroll, & Lip, 2007). In fact, alterations in various factors (e.g., fibrinogen and D-dimer) involved in hemostasis are also independent risk factors for CHD (Folsom, 2001).
The initiation of CHD, especially myocardial infarction, has been linked to acute psychosocial stress that can result from negative emotions, taxing social situations and traumatic events (Strike & Steptoe, 2005). Several pathophysiological mechanisms have been suggested, including hemoconcentration (i.e., when the ratio of blood cells to plasma increases) and activation of coagulation factors (Allen & Patterson, 1995; Rozanski, Blumenthal, & Kaplan, 1999; Rozanski, Blumenthal, Davidson, Saab, & Kubszansky, 2005; Bhattacharyya & Steptoe, 2007). Disruption of hemostatic balance (i.e., balance between clot formation and clot dissolution) is a critical factor in atherosclerotic development and CHD. After plaque rupture, a vessel occlusion is more likely if the ratio of prothrombosis activity (i.e., promoting clot formation) to fibrinolysis activity (i.e., promoting clot dissolution) is high. During psychological stress both prothrombosis and fibrinolysis activity increase, but prothrombosis activity usually increases to a greater extent than fibrinolysis activity. The resulting net hypercoaguability may put an individual at greater risk for plaque disruption, and thus contribute to CHD (von Känel, Mills, et al., 2001; Thrall et al., 2007). Other crucial factors suggested to accompany hypercoagulation are stress-induced hemoconcentration (von Känel, Kudiela, Haeberli, Stutz, Fischer, & Patterson, 2009) and increased blood viscosity (Patterson, Austin, Matthews, & Owens, in preparation), both of which exacerbate shear stresses imposed on atherosclerotic plaques and plaque ruptures (Allen & Patterson, 1995). Elucidation of how these mechanisms occur is central to understanding the relationship between stress and cardiovascular disease.
This study used an original and innovative method to examine the effects of hemoconcentration during acute psychological stress on various markers of coagulation (i.e., fibrinogen, D-dimer, factor VII, factor VIII, activated partial thromboplastin time, and prothrombin time). Specifically, a novel reconstitution method was used to determine whether the observed increases in clotting factors during acute psychological stress are due to actual activation of the clotting system or to hemoconcentration, or a combination of both.

This introduction has two main sections: Hemoconcentration and Hemostasis. The first section provides an overview of hemoconcentration with discussion of physiology, association with cardiovascular disease, and relationships between hemoconcentration and acute psychological stress. The second section is organized similarly, providing an overview of hemostasis with discussion of physiology, association with cardiovascular disease, and relationships between hemostasis and acute psychological stress, with particular emphasis on coagulation. Finally, the few studies examining the effects of stress-hemoconcentration on hemostasis are reviewed. The purpose of this overview is to provide an understanding of relationships between stress, hemostasis, hemoconcentration and cardiovascular disease.

**Hemoconcentration**

**Physiology of Hemoconcentration**

Hemoconcentration refers to conditions in which the ratio of cellular components of the blood (principally red blood cells) to the plasma volume increases (Allen & Patterson, 1995). As already stated, hemoconcentration is a potential mechanism linking
psychological stress to the initiation of CHD (Allen & Patterson, 1995; Rozanski et al., 1999, 2005; Bhattacharyya & Steptoe, 2007). Hemoconcentration mainly occurs in two ways. First, plasma volume can remain constant while the number of red blood cells increases. This form of hemoconcentration is seen in polycytemia vera, a rare tumorous condition in which red blood cells are produced to a greater extent than normal (Guyton & Hall, 1996). The second and more common form of hemoconcentration occurs when the red blood cell count remains constant while plasma volume decreases. This condition has been variously referred to as ‘stress’ polycytemia, relative polycytemia, pseudopolycytemia, spurious polycytemia and stress-hemoconcentration (Emery, Whitcomb, & Frohlich, 1974; Isbister, 1987; Allen & Patterson, 1995). Stress-hemoconcentration occurs when plasma shifts from the vascular compartment into interstitial spaces. Early studies of plasma volume shifts demonstrated this phenomenon with such factors as emotional stress (Lawrence & Berlin, 1952) and anxiety (Russell & Conley, 1964), and thus, the term ‘stress-hemoconcentration’ was coined (Allen & Patterson, 1995). The term stress-hemoconcentration is used from this point forward in this dissertation.

A fundamental measurement of the extent of hemoconcentration at any one time is hematocrit, or the percentage of whole blood that is made up of red blood cells (Allen & Patterson, 1995). In healthy adults during rest, hematocrit ranges from 40-55% in males and 36-48% in females (Brown, 1993). Hemoglobin, an oxygen-carrying molecule present in red blood cells, is closely related to hematocrit. Red cells are able to carry up to 34 g of hemoglobin per 100 ml of cells (Guyton & Hall, 1996). Consequently, in
normal healthy adults, the total hemoglobin concentration is determined by hematocrit and the quantity of hemoglobin in each cell, and ranges from 13-18 g/dl in males and 12-16 g/dl in females. In healthy individuals, the amount of hemoglobin carried by each red blood cell is near the maximum, so changes in hematocrit yield near parallel changes in hemoglobin concentration (Guyton & Hall, 1996).

Another indicator of hemoconcentration is the concentration of plasma proteins. Plasma passes easily into interstitial spaces through minute openings in the capillaries, but plasma proteins are large and pass through these pores with great difficulty, if at all. Notably, the presence of these large molecules creates a large colloidal osmotic pressure gradient. In other words, the plasma protein concentration is greater in the capillaries than in the interstitial fluid surrounding the capillaries, resulting in a constant pressure gradient for fluid moving into the capillaries. Even though fluid moves across capillary pores freely, a lower limit is imposed on how much fluid can leave the capillaries. Nevertheless, an acute increase in the plasma protein concentration can signify stress-hemoconcentration (Allen & Patterson, 1995).

A consequence of an increase in the plasma protein concentration is altered flow properties of the blood. Specifically, blood becomes thicker and more viscous. When plasma shifts into interstitial spaces, the resultant increase in total plasma protein concentration causes an increase in plasma viscosity. Whole blood viscosity, in turn, is determined mainly by plasma viscosity, the deformability of the red cells, the extent of cellular aggregation and the concentration of suspended particles (i.e., hemoconcentration). Increased blood viscosity ultimately contributes to blood pressure
by increasing total peripheral resistance (i.e., the resistance to blood flow in the arterial tree; Allen & Patterson, 1995).

Plasma viscosity and total plasma protein concentration are considered direct measures of hemoconcentration. Calculated plasma volume from hematocrit and hemoglobin, on the other hand, is an indirect measure of hemoconcentration. Calculated plasma volume is very closely related to plasma viscosity; therefore, correction factors based on calculated plasma volume are often used to assess hemoconcentration (Allen & Patterson, 1995; Patterson, Marsland, Manuck, Kameneva, & Muldoon, 1998). Early researchers assumed that under conditions of constant red cell volume a decrease in hematocrit corresponded to a proportionally equal increase in plasma volume (Bazett, Sunderman, Doupe, & Scott, 1940; Stein, Eliot, & Bader, 1948). Later information, however, suggested that hematocrit and plasma volume are never as proportional as was earlier predicted (van Beaumont, 1972), and therefore, hematocrit has not been shown to be directly related to plasma volume. Rather hematocrit is the ratio of red cell volume to blood volume. In other terms, hematocrit is the percentage of whole blood that is composed of red blood cells. For changes in hematocrit to accurately reflect changes in plasma volume, it is necessary for two assumptions to be satisfied (Greenleaf, Convertino, & Mangseth, 1979). First, the number of red blood cells must remain constant. In other words, red blood cells should not be added to or removed from the blood during the period under examination. Second, the volume of red blood cells must remain constant. However, as plasma volume decreases, the concentration of plasma proteins increases with a resultant increase in plasma osmolarity. As a result of an
increase in osmolarity, fluid may move out of the red blood cells, thus diminishing their size. In other words, events that change plasma osmolarity should also change red cell volume, which discredits the use of only hematocrit for estimating changes in plasma volume (Greenleaf, Convertino, et al., 1979). To more accurately estimate changes in plasma volume, Costill and colleagues (Costill, Branam, Eddy, & Fink, 1974; Costill & Fink, 1974; Dill & Costill, 1974) showed that such estimations should incorporate both hematocrit and hemoglobin. Dill and Costill (1974) developed an equation that has been widely used for estimating acute plasma volume shifts from changes in both hematocrit and hemoglobin values:

\[
\begin{align*}
BV_2 &= BV_1 \times \left( \frac{Hgb_1}{Hgb_2} \right) \\
CV_2 &= BV_2 \times \left( \frac{Hct_2}{100} \right) \\
PV_2 &= BV_2 - CV_2 \\
\Delta PV, \% &= 100 \times \left( \frac{PV_2 - PV_1}{PV_1} \right)
\end{align*}
\]

where \( BV = \) blood volume, \( CV = \) red cell volume, \( PV = \) plasma volume, \( Hgb = \) hemoglobin, \( Hct = \) hematocrit, subscript 1 refers to baseline sample, subscript 2 refers to stressor (mental or physical) sample, \( BV_1 \) is taken as 100, and \( PV_1 \) is 100 – \( Hct_1 \).

Given that the conditions employed by Costill and colleagues were rather severe (i.e., maximal exercise and severe dehydration), Greenleaf, Convertino, et al. (1979) examined whether the addition of hemoglobin was indeed necessary to estimate plasma volume shifts resulting from less extreme conditions. Under conditions causing modest
changes in plasma osmolarity (i.e., short-term heat exposure and orthostasis), hematocrit by itself estimated plasma volume changes as well as those made from the Dill and Costill (1974) equation that includes both hematocrit and hemoglobin. That is, the change in the size of red blood cells was not significant (Greenleaf, Convertino, et al., 1979). Nevertheless, researchers continue to use the Dill and Costill equation to ensure the most accurate approximations of plasma volume shifts.

_Hemoconcentration and Cardiovascular Disease_

Hematocrit is a basic measurement of the degree of hemoconcentration at any given time. It is valuable to examine factors that influence hemoconcentration given the close relationship between hemoconcentration and blood pressure and given that hematocrit seems to be an independent risk factor for all-cause mortality, CHD and stroke (Garcia-Palmeiri et al., 1978; Sorlie, Garcia-Palmeiri, Costas, & Havlik, 1981; Cirillo et al., 1992; Gagnon, Zhang, Brand, & Kannel, 1994). That is, hematocrit explains variance in all-cause mortality and the incidence of these diseases beyond that associated with traditional risk factors.

_Hemoconcentration, Coronary Heart Disease and Stroke_

Since the 1960s, several epidemiological studies have indicated that hematocrit is predictive of CHD. For example, the Puerto Rico Heart Health Program (PRHHP) was a prospective epidemiologic study of coronary heart disease over 8 years in more than 8700 middle-aged men (Garcia-Palmieri et al., 1978; Sorlie et al., 1981). In this study, hematocrit at baseline was related to all-cause mortality among all individuals. However, it was only related to incidence of myocardial infarction or death from CHD only among
urban dwellers, but not among rural inhabitants. Specifically, after adjustment for coexisting risk factors, an urban man with a hematocrit of 50% had a 50% higher chance of incident CHD during the 8-year follow-up than an urban man with a hematocrit of 40%. That is, the estimated relative odds for an absolute difference of 10% in hematocrit after controlling for risk factors was 1.5 among urban individuals. The authors speculated that such relationships were not observed in rural individuals because a smaller relative sample was used (2369 rural vs. 5720 urban residents; Sorlie et al., 1981). Nevertheless, the possibility that cultural variations accounted for the observed effects cannot be eliminated entirely by low statistical power.

More detailed analyses were conducted in over 5200 men and women in the Framingham Study (Gagnon et al., 1994) such that a quadratic effect was found for the effect of hematocrit on all-cause mortality over 34 years of follow-up in men and women. Specifically, participants were divided into quintiles based on their hematocrit levels. Those in the first quintile were compared to those in the third quintile; those in the third quintile were compared to those in the fifth quintile. Hematocrit was an independent risk factor for all-cause mortality among men (age 34-94) and older women (age 65-94) in both the lowest and highest quintiles, even after controlling for other risk factors (age, SBP, serum cholesterol, glucose intolerance, number of cigarettes smoked, and left ventricular hypertrophy). The middle quintile appeared to be related to the lowest mortality. Closer analyses indicated that mortality in the highest quintile was attributed mainly to CHD and stroke. However, among those in the lowest quintile, the cause of death was not usually associated with CHD, but was associated with stroke. That is, both
low and high hematocrit were independent risk factors for stroke, an effect seen especially in older women (Gagnon et al., 1994).

Similarly, Kiyohara and colleagues (1986) reported that low hematocrit was certainly, and high hematocrit was most likely, related to the incidence of stroke over a 16 year follow-up in Japanese women, but not in Japanese men. Kunnas and associates (2009) compared Finnish men with hematocrit greater than or equal to 50% to Finnish men with hematocrit less than 50%. Men with lower hematocrit had better survival rates than those with higher hematocrit. Likewise, in a Cox proportional hazards model, men with hematocrit greater than 50% had a 1.8-fold increased risk of death from CHD compared to those with hematocrit less than 50%. Interestingly, hematocrit was not related to survival from non-CHD diseases (Kunnas et al., 2009).

Taken together, the results of the above studies suggest that both elevated and depressed hematocrit relative to normal hematocrit probably puts an individual at greater risk for CHD and stroke. Elevated hematocrit may exert its effects through other risk factors or by increasing blood viscosity. The mechanisms by which low hematocrit exerts its effects are less clear; this effect was only seen in stroke (Kiyohara et al., 1996; Gagnon et al., 1994). One possibility for only seeing the effects in stroke may be that when hematocrit falls below a critical level, oxygen transport capacity is inadequate, increasing the risk for hypoxia. The brain is quite sensitive to hypoxia which may subsequently result in a stroke, especially in the elderly for whom the effect appears strongest (Gagnon et al., 1994).
When interpreting the findings from these epidemiological studies, one must bear in mind that hematocrit has a good deal of variation from day-to-day, month-to-month and season-to-season (Thirup, 2003). Specifically, two successive hematocrit values within individuals, measured at time intervals ranging from one day to six months, can vary as much as 6%. Given such variation and given that time of year of measurement (i.e., seasonal differences) was often not controlled in these studies, one must use prudence in interpreting these findings.

Hemoconcentration and Hypertension

Epidemiological and laboratory studies suggest that elevated hematocrit and contracted plasma volume are related to essential hypertension. For instance, among over 3700 Italian adults in the Gubbio Population Study (Cirillo, Laurenzi, Trevisan, & Stamler, 1992), resting hematocrit levels were significantly higher in untreated hypertensive individuals compared to normotensive individuals. Furthermore, after adjusting for age, body mass index, alcohol intake, cigarette smoking, heart rate, serum glucose, and serum cholesterol, hematocrit showed an independent association with systolic (SBP) and diastolic (DBP) blood pressure. However, participants were required to refrain from strenuous exercise for only 30 minutes before the measurement. Given that exercise causes acute plasma volume shifts (Ring et al., 2008), the results from this study must be interpreted cautiously, as it is not known whether participants exercised shortly before hematocrit measurement.

Several studies have also shown lower plasma volume to be related to essential hypertension (Tibblin, Bergentz, Bjure, & Wilhelmsen, 1966; Tarazi, Frohlich, & Dustan,
1968; Julius, Pascual, Reilly, & London, 1971), suggesting a common mechanism such as sympathetic activity. For example, the Men of 1913 Study (Tibblin et al., 1966), a population study of men born in 1913, found that when the men were 50 years old, those with DBP > 100 mmHg had significantly lower plasma volume at rest and during and after exercise than men with DBP < 100 mmHg. Such differences, however, are not due to loss of extracellular water. Hypertensive and normotensive individuals do not have different amounts of extracellular fluid, but rather have an altered distribution of the components of extracellular fluid. In other words, the ratio of intravascular fluid to interstitial fluid is lower in hypertensive than in normotensive individuals (Tarazi, Dustan, & Frohlich, 1969; Ibsen & Leth, 1973). Given that the total amount of extracellular fluid is not different between hypertensive and normotensive individuals, the reduction in this ratio cannot be expected to result from greater renal filtration and excretion of fluid. Instead, plasma is displaced from the intravascular to interstitial space (Ibsen & Leth, 1973). Furthermore, men with essential hypertension have a stable red cell mass, indicating that the observed increase of hematocrit among those with essential hypertension is due to a contracted plasma volume (Kobrin et al., 1984). Altogether, contracted plasma volume and elevated hematocrit suggest that essential hypertension may confer a chronic hemoconcentration state.

Hemoconcentration and Other Cardiovascular Risk Factors

Even though hematocrit and plasma volume are related to blood pressure, they also have much interdependence with other risk factors, especially those associated with sympathetic nervous system activity, or “sympathetic tone” (Smith, Julius, Jamerson,
Amerena, & Schork, 1994). Thus, decreases in plasma volume and elevations in hematocrit and blood pressure may result concurrently from other actions associated with greater activity of the sympathetic nervous system, such as increased heart rate and contractility of the heart and catecholamine secretion from the adrenal glands. Evidence for greater sympathetic activity among hypertensive individuals comes from studies that have examined the effects of catecholamine infusion on blood pressure, hematocrit and plasma volume. If hypertension is characterized by greater sympathetic nervous system activity, then infusion of catecholamines should mimic hypertension for a brief period. Indeed, infusion of catecholamines results in transient hemoconcentration by increasing blood pressure and hematocrit and decreasing plasma volume (Finnerty, Buchholz, & Guillaudeu, 1958; Kjeldsen et al., 1995). This effect probably occurs from increased capillary hydrostatic pressure due to increased venous tone and subsequent increased filtration of fluid out of the intravascular compartment into the interstitial space (Cohn, 1966; Allen & Patterson, 1995). Of note, catecholamine infusion studies examining changes in plasma volume provide a model for comparable changes that occur during acute psychological stress. Stress results in elevated sympathetic nervous system activity, with subsequent release of catecholamines from the adrenal glands. If catecholamines are responsible for many of the physiological changes caused by acute stress, then, in a sense, catecholamine infusion bypasses the need to activate the sympathetic nervous system in order to observe such changes. However, mental stress studies provide a more naturalistic method for observing stress-induced activation of the sympathetic nervous
system and subsequent physiological effects. The next section reviews studies that have examined the effects of acute psychological stress on hemoconcentration.

**Hemoconcentration and Psychological Stress**

Mental stress in the laboratory reliably increases various indices of hemoconcentration (i.e., hematocrit, hemoglobin, blood viscosity and total plasma protein concentration), apparently due to a reduction of plasma volume. These effects occur in various groups of people, including men and women, normotensive and hypertensive individuals, and individuals across different age groups. During mental stress, plasma moves from the intravascular space to the extravascular space, resulting in an increase in the concentration of blood components that cannot pass through capillary pores (for review, see Allen & Patterson, 1995). Stress-hemoconcentration is of particular concern for molecules greater than 69 kilodaltons (Bacon, Ring, Lip, & Carroll, 2004), because such molecules are too big to move with plasma through vascular pores to the extravascular space (Holliday, 1999). Thus, during acute mental stress, the concentration of these molecules necessitates adjustment for plasma volume shifts. Total plasma proteins, immune cells, lipids, platelets and many coagulation factors greatly exceed 69 kDa. It is clear that these non-diffusible blood constituents, perhaps with the exception of coagulation factors, become hemoconcentrated during acute mental stress. With regard to coagulation factors, it is not entirely clear whether acute mental stress causes greater concentration of the factors through hemoconcentration or through actual activation of the coagulation system. On the other hand, smaller substances, such as catecholamines (i.e., norepinephrine and epinephrine), are not affected by hemoconcentration, because
they can pass freely across capillary pores into the interstitial space (Patterson, Gottdiener, Hecht, Vargot, & Krantz, 1993). Following is a review of previous work examining plasma volume shifts during stress and the effects of such shifts on various large, non-diffusible blood components.

**Stress-Induced Changes in Indices of Hemoconcentration**

Physiologists in several early studies examining hemoconcentration effects subjected volunteers to various physical stressors such as heat exposure (Costill & Fink, 1974; van Beaumont, Young, & Greenleaf, 1974; van Beaumont, Underkofler, & van Beaumont, 1981), exercise (Greenleaf, Convertino et al., 1979; van Beaumont et al., 1981), altitude change (Jung, Dill, Horton, & Horvath, 1971; Greenleaf, Convertino, et al., 1979), prolonged bed rest (van Beaumont, Greenleaf, & Juhos, 1972; Greenleaf et al., 1977), water immersion (Greenleaf, Shvartz, Kravik, & Keil, 1980) and head tilt (Greenleaf, van Beaumont, Brock, Morse, & Mangseth, 1979). Plasma volume decreased during all of these stressors, ranging from an 8% change during heat exposure (van Beaumont et al., 1974) to an 18% change during exercise (Greenleaf, Convertino, et al., 1979). In addition to the physical aspect, it can be argued that many of these stressors have a psychological component. As such, these studies set the stage for the study of hemoconcentration effects resulting from psychological stressors as well as physiological challenges. However, psychological stress does not appear to induce as great of a change in plasma volume, with percent plasma volume change ranging from 1.3% (paced auditory serial addition test; Bacon et al., 2004) up to 12% (mental arithmetic with harassment; Patterson, Krantz & Jochum, 1995).
Changes in various indices of hemoconcentration have been examined after subjecting participants to acute psychological stress. For instance, Jern, Wadenvik, et al. (1989) investigated the hematological and cardiovascular effects of exposure to 10 minutes of mental arithmetic and subsequent recovery in healthy young males. Along with increases in heart rate, SBP and DBP, significant increases in hematocrit and hemoglobin relative to a pre-stress baseline period were observed during the mental arithmetic task. The mean increases in hematocrit and hemoglobin were 0.8% and 3 g/l, respectively. Moreover, both hematocrit and hemoglobin changes in response to mental arithmetic were significantly correlated with heart rate change. However, the correlations between DBP and SBP with hematocrit and hemoglobin were not significant. In an extension of this study, Jern, Jern and Wadenvik (1991) examined basal hematological levels in young Type A and Type B males and females and the effects of a 10-minute mental arithmetic task on hematological and cardiovascular measures. Type A individuals had significantly higher basal hematocrit (43.8 vs. 42.1%) and hemoglobin levels (150 vs. 147 g/L) than Type B individuals. Hematocrit and hemoglobin increased significantly during the stressor with magnitudes similar to that of Jern, Wadenvik, et al. (1989), but no Type A/B differences were observed in the magnitude of change. In both of these studies, hematocrit and hemoglobin returned to baseline levels after mental arithmetic during a 10 minute recovery period. This quick return to baseline levels implies that the hemoconcentration was due to reversible shifts in plasma volume.

Similar findings have been observed with other stressors and with additional measures of hemoconcentration. For example, compared to individuals resting quietly,
total plasma protein concentration increased significantly in individuals completing the Stroop Color-Word Interference Test and orthostatic challenge, but correcting for plasma volume changes rendered these effects non-significant (Muldoon et al., 1995). Furthermore, an index of cardiovascular “reactivity” (residualized heart rate and blood pressure change scores were standardized and summed) was positively correlated with stress-induced changes in total cholesterol level and hematocrit. In a similar study (Patterson et al., 1998), calculated plasma volume, plasma viscosity, total plasma proteins, blood pressure and heart rate were assessed in healthy, young men during a 30-minute rest period and a five minute speech stressor. Plasma volume decreased significantly during the stressor, which corresponded to an increase in plasma viscosity and total plasma protein concentration. In addition, change in plasma volume from baseline to stress had a significant, negative correlation with changes in heart rate and SBP. Similar relationships between hemoconcentration and hemodynamic measures were reported by Veldhuijzen van Zanten and associates (2002, 2004, 2005). Altogether, with the exception of the studies conducted by Jern, Wadenvik, et al. (1989) and Jern (1991), these lines of evidence suggest that increased hydrostatic pressure from sympathetic-induced cardiovascular responses to stress is responsible for movement of fluid into extravascular spaces and consequent concentration of non-diffusible blood components (Muldoon et al., 1995; Patterson et al., 1998). The reason why Jern and colleagues did not find blood pressure changes to be related to hematocrit changes is not entirely clear.

Although these studies strongly suggest that hemoconcentration occurs relatively quickly during acute stress, they do not address how long it takes for a
hemoconcentration state to return to normal (i.e., recovery). Patterson, Krantz, and Jochum (1995) reported hemoconcentration effects for total plasma proteins among males and females who completed a 10-minute stressful serial subtraction task with harassment compared to control individuals completing a benign reading task. Calculated plasma volume and mean arterial pressure (MAP) were measured every two minutes during the mental arithmetic task and every three minutes during a 30-minute recovery period. The percent change in plasma volume relative to baseline was negatively correlated with MAP across the mental arithmetic task and recovery period. Moreover, percent change in plasma volume remained depressed and MAP remained elevated during the first 9 minutes of the recovery period, but both returned to baseline levels after 12 minutes of recovery. It was concluded that stress-induced changes in plasma volume and blood pressure return to normal within 12 minutes and these changes coincide during the stress task and recovery period. Thus, further support was provided for the contention that blood pressure elevations are the driving force behind plasma volume losses during stress and subsequent hemoconcentration of non-diffusible blood components. However, as Patterson, Krantz, and Jochum (1995) acknowledge, increased renal function and diuresis following stress could account for plasma volume losses. Along the same lines, Muldoon and colleagues (1992) reported that plasma volume remained lower than baseline levels after a 30-minute recovery period following mental arithmetic and a Stroop task, so it was suggested that increased renal filtration could be responsible for prolonged plasma volume losses. However, blood pressure and plasma volume were only measured at the end of the recovery period, so one cannot rule out the possibility that they corresponded
inversely throughout the recovery period. It is also possible that plasma volume losses are driven by a combination of increased blood pressure and increased renal filtration.

de Boer and colleagues (de Boer, Ring, & Carroll, 2006; de Boer, Ring, Curlett, Ridley, & Carroll, 2007) further examined the time course and mechanisms of hemoconcentration in response to mental stress as well as relationships between hematological and cardiovascular variables. In the first study (de Boer et al., 2006), male participants completed a four minute paced auditory serial addition test (PASAT) and were led to believe their performance would be analyzed for body and facial composure during the task. Hematocrit increased gradually during the first 3 minutes of mental stress, plateauing in the fourth minute. Hematocrit was at its highest level in the first minute of recovery and steadily decreased back to baseline levels within 16 minutes of the recovery period, which is somewhat similar to 12 minutes following a 10 minute math stressor in the Patterson, Krantz, and Jochum (1995) study. Therefore, recovery time may be relatively independent of the length of the stress task, at least in the case of math stressors (i.e., serial subtraction and PASAT) lasting up to 10 minutes. It is possible, however, that recovery takes longer with stressors of greater duration or of different types. Such a possibility is supported by the findings of a study whereby hematocrit failed to return to baseline levels 30 minutes after a 20 minute combined Stroop-mental arithmetic task (Muldoon et al., 1992). Furthermore, within-subjects correlations between hemodynamic variables and hematocrit from baseline through recovery were significant (de Boer et al., 2006), which is consistent with between-subjects observations seen in previous studies (e.g., Patterson, Krantz, Gottdiener, et al., 1995; Patterson et al., 1998),
providing more support for the notion that blood pressure changes may underlie hemoconcentration during stress. Alternatively, recovery may be independent of blood pressure changes and may rely more on colloid osmotic pressure (de Boer, Ring, Curlett et al., 2007).

The aforementioned studies have all included normotensive individuals as participants, but a number of studies have assessed hemoconcentration and hemodynamic stress responses in hypertensive patients. For instance, Kitahara and colleagues (1988) studied men and women with mild hypertension between the ages of 38 and 72 who underwent a 10-minute period of speech and mental arithmetic followed by a 60-minute recovery. The mental stress period resulted in a significant increase of hematocrit (0.7%) and hemoglobin (2 g/l), which returned to baseline levels during the recovery period. Parallel increases in blood pressure and heart rate were observed during the stressor, with significant positive correlations between hematocrit change and both SBP change and heart rate change. Sakamoto, Imataka, Nishimura and Fujii (1992) conducted a very similar study utilizing the same stressor as well as isometric handgrip exercise and found comparable increases in hematocrit (0.5% for both tasks) and blood pressure.

The above studies reliably document hemoconcentration effects during a variety of mental stressors, whether it is observed as changes in hematocrit, hemoglobin, total plasma protein concentration or plasma volume. Stress-hemoconcentration is observed in men and women, normotensive and hypertensive individuals and individuals of different age groups. A large amount of evidence indicates that tasks producing sympathetic
nervous system activation result in large increases in hematocrit and hemoglobin and that such changes are likely produced by increases in blood pressure and heart rate.

The next section discusses changes in non-diffusible blood constituents during acute stress and the relationships between such changes and hemoconcentration. Particular attention is paid to those substances implicated in chronic disease—namely, lipids and immune cells.

*Stress-Induced Changes in Non-Diffusible Blood Components*

**Lipids.** Researchers in psychosomatic medicine have long been interested in whether a person’s emotional or motivational state affects lipid levels, and whether stress contributes to the development of atherosclerosis by increasing lipids. Thus, it is not surprising that lipids were among the first physiological parameters for which stress-hemoconcentration effects were corrected. Dimsdale and Herd (1982) reviewed studies looking at stress and lipids and concluded that free fatty acids and serum cholesterol generally increase during acute and chronic psychological stress. Conversely, Niaura and colleagues (1992) reviewed research from the next decade and reported that the majority of studies showed that free fatty acids as well as low-density lipoprotein-cholesterol (LDL-c) and high-density lipoprotein-cholesterol (HDL-c) increase during acute and episodic psychological stress, but not during chronic stress. Such differences between the two reviews may be due to differences in the nature of the types of chronic stress examined. Dimsdale and Herd reviewed studies that examined stressful life events, whereas Niaura and colleagues reviewed studies that examined occupational stress.
Both Dimsdale and Herd (1983) and Niaura et al. (1992) suggested that the observed increase in lipids with acute stress may be mediated by sympathadrenergic activity, but such a contention has not been clearly supported by lipid reactivity studies (e.g., Stoney, Matthews, McDonald, & Johnson, 1988; Patterson et al., 1993). Alternatively, the effects may be due to plasma losses during acute stress and subsequent hemoconcentration of lipids. Indeed, several studies have corroborated this assertion in healthy individuals (Muldoon et al., 1992, 1995; Patterson et al., 1993, 1995; McCann, Magee et al., 1995; Bachen, Muldoon, Matthews, & Manuck, 2002) and patients with suspected CHD (Bacon, Ring, Lip, & Carroll, 2004). These studies reported that, when mathematically correcting for plasma volume losses, the observed increases in total cholesterol, LDL-c, HDL-c and triglycerides during acute stress were no longer significant. However, one study reported that stress-hemoconcentration did not account for changes in triglycerides (Stoney, Niaura, & Bausserman, 1997). Triglycerides could be particularly resistant to hemoconcentration effects due to a transiently reduced ability of the body to clear triglycerides from the bloodstream during acute stress (Stoney et al., 2002). Alternatively, inter-laboratory differences in administering the stressors or differences in the methods of determining lipid levels could explain why Stoney et al. (1997) did not find stress-hemoconcentration effects for triglycerides.

Free fatty acids (FFA), on the other hand, consistently do not appear to be susceptible to hemoconcentration effects and remain statistically elevated during acute stress after correcting for plasma volume shifts. As opposed to other lipid sub-types, the observed increases in FFA during acute stress may indeed be mediated by
sympathadrenergic activity. Given that catecholamines have the ability to cleave FFA from triglycerides, it is possible that elevated catecholamines during acute stress may act on triglycerides to increase the release of FFA (Patterson et al., 1995).

Immune Cells. White blood cells, or leukocytes, are cells of the immune system that defend the body against infectious disease and foreign materials. Five types of leukocytes exist: neutrophils, eosinophils, basophils, monocytes and lymphocytes. Several studies have indicated that acute mental stress alters the concentration of various lymphocytes (e.g., T-suppressor/cytotoxic cells, T-helper cells, natural killer cells, and B-cells) in healthy individuals (Herbert et al., 1994; Bachen et al., 1995; Marsland, Manuck, Fazzari, Stewart, & Rabin, 1995; Marsland, Manuck, Wood, et al., 1995). For example, the concentration of T-suppressor/cytotoxic and natural killer cells generally increases within minutes after the initiation of conventional laboratory stressors (Herbert et al., 1994). On the other hand, T-helper and B-cells appear to be unchanged during acute stress. However, these early studies did not account for plasma volume shifts and subsequent stress-hemoconcentration. Marsland et al. (1997) reanalyzed these studies to determine the extent to which changes in the concentration of various lymphocyte subpopulations (T-suppressor/cytotoxic, natural killer, T-helper and B-cells) during acute mental stress were due to hemoconcentration. Increases in T-suppressor/cytotoxic and natural killer cells were indeed partly attributable to reduced plasma volume. Specifically, arithmetic correction for plasma volume changes reduced by 50% the magnitude of change in T-suppressor/cytotoxic cells in response to acute stress, whereas such correction decreased by 10% the magnitude of change in natural killer cells.
Furthermore, although the unadjusted concentrations of T-helper and B-cells were unchanged during stress, both actually decreased significantly during stress when correcting for plasma volume shifts. Thus, the reactions of certain immune cells may be controlled by opponent processes—one actively eliminating lymphocytes from the blood and one concealing such changes by concurrently reducing plasma volume (Marsland et al., 1997).

Whereas Marsland et al. (1997) examined individual lymphocytes, a more recent study (Mischler et al., 2005) examined the effects of hemoconcentration on total lymphocyte concentration in addition to total leukocyte concentration. Total leukocyte concentration was significantly greater immediately after stress than during baseline and remained significantly elevated when correcting for plasma volume shifts. Lymphocyte concentration was also significantly greater immediately after stress compared to baseline, but was no longer significantly elevated when adjusting for plasma volume shifts (Mischler et al., 2005). These results suggest that other leukocytes (e.g., granulocytes and monocytes) may be released into the bloodstream during acute stress but that lymphocytes may not be. Some support for this assertion comes from a study that did not report stress-hemoconcentration effects on lymphocytes, granulocytes and monocytes among patients with suspected CHD (Bacon et al., 2004). Therefore, the observed increases in granulocytes and monocytes among healthy individuals and patients with CHD and in lymphocytes among patients with CHD are likely the result of other processes, such as release from storage, migration, increased production or reduced
catabolism. It is possible that these processes are altered and that stress-hemoconcentration does not affect immune cells among those with CHD.

In sum, the concentrations of many leukocytes increase during acute mental stress in both healthy individuals and patients with CHD. However, increases in only the total lymphocyte concentration and the concentrations some subsets of lymphocytes (T-suppressor/cytotoxic and natural killer cells) appear to be attributable to hemoconcentration in healthy individuals. Other lymphocyte subsets (T-helper and B-cells) actually decrease during acute stress but this reduction is masked by hemoconcentration. Moreover, stress-induced increases in lymphocyte concentration among patients with CHD are not attributable to hemoconcentration. Finally, stress-induced increases in the concentration of other leukocytes, such as granulocytes and monocytes, survive correction for hemoconcentration. Together, these results suggest physiological processes other than hemoconcentration are responsible for the observed increases in leukocyte concentration among both healthy individuals and patients with CHD and in total lymphocyte concentration among patients with CHD.

**Summary**

Stress-hemoconcentration is a robust phenomenon that accounts for the observed changes in the concentrations of several large, non-diffusible blood components (e.g., total plasma proteins and various types of lipids and leukocytes) during acute psychological stressors. Notably, several substances involved in hemostasis, such as platelets and coagulation factors, are greater than 69 kDa and have been observed to increase in concentration during acute psychological stress. Alterations in hemostasis
factors provide a possible link between acute psychological stress and initiation of CHD. The next section provides an overview of hemostasis. First, normal hemostasis physiology is discussed. Second, evidence for the presence of hemostatic abnormalities in cardiovascular disease is presented. Finally, the effects of acute psychological stress and hemoconcentration on hemostasis factors are described with particular focus on coagulation.

Hemostasis

Hemostasis is a critical defense mechanism against bleeding whereby blood loss is prevented after a vessel is damaged (Guyton & Hall, 1996). Theorists have long associated psychological stress with accelerated hemostasis and thickening of the blood. Given that hemostasis factors are of primary importance to this dissertation, hemostasis is described in detail. Particular emphasis is placed on coagulation factors, leading to the central question addressed by this project—whether the apparent increase in coagulation activity during acute psychological stress is due to actual activation of the coagulation system or as a result of hemoconcentration, or a combination of both.

Physiology of Hemostasis

As mentioned, hemostasis is a complex process that stops bleeding, often changing blood from a fluid to solid state. The process of hemostasis is achieved by three fundamental processes—vascular spasm, platelet plugging and coagulation (Guyton & Hall, 1996; Riddel Jr., Aouizerat, Miaskowski & Lillycrap, 2007).
**Vascular Spasm**

First, a vascular spasm occurs to minimize the immediate loss of blood. The vessel walls contract immediately after a blood vessel suffers a trauma in order to minimize the amount of blood leaving the vessel. The vascular spasm can last for minutes to hours, during which time the other two mechanisms for halting blood loss occur (i.e., platelet plugging and blood coagulation).

**Platelets**

Platelets are minute round or oval anuclear cells that serve several hemostatic functions once activated. Activation occurs when platelets are stimulated by various agonists or when they contact a foreign surface or the subendothelial collagen of a damaged vessel wall. Once activated, they have granules that move to the platelet surface and release their contents into the plasma. Two types of granules are of primary interest: alpha granules and dense bodies. Alpha granules contain many important substances involved in thrombosis and atherosclerosis, including fibrinogen, platelet factor 4 (PF4), beta-thromboglobulin (BTG), Factor V, Factor VIII, von Willebrand factor, and platelet-derived growth factor (Bennett & Shattil, 1990; Holmsen, 1990a). Substances released from platelet granules, especially thromboxane B₂ (a metabolite of thromboxane A₂), PF4 and BTG, are often measured as markers of platelet activation (Owen & Kaplan, 1987; Markovitz & Matthews, 1991; Patterson & Krantz, 1993). Dense bodies hold adenosine diphosphate, calcium ions and thromboxane A₂, all of which activate other platelets.

Once activated, platelets undergo drastic changes to carry out the following functions: 1) formation of a platelet plug to occlude sites of vascular damage, 2) provide
a surface for the configuration and activity of coagulation proteins, 3) serve as the nucleus around which a fibrin clot forms, and 4) secrete various factors involved in vascular repair (Bennett & Shattil, 1990). Once activated, platelets swell, assume irregular forms, become sticky and activate other platelets. The stickiness of platelets causes them to adhere to each other. This process continues, attracting more and more platelets to the newly forming platelet plug. At first, the platelet plug is loose, but sufficiently blocks blood loss if damage to the vascular wall is minor. If a rupture is very small, it is often sealed only by a platelet plug. Minute ruptures in very small blood vessels occur hundreds of times daily. Thus, formation of the platelet plug is extremely important for closing these minute ruptures. If the rupture is severe, however, then platelets stimulate blood coagulation, the third step in hemostasis, through the release of activator substances (Guyton & Hall, 1996; Riddel Jr. et al., 2007). Another response of platelet activation is the expression of receptors for coagulation factors (Holmsen, 1990b). Coagulation factors, in turn, bind to and stimulate platelets in a positive feedback fashion to form the final hemostatic plug (Bennett & Shattil, 1990).

**Blood Coagulation**

Blood coagulation is the formation of a blood clot in a damaged vessel or through inappropriate activation such as during thrombus development. More than 50 substances affect coagulation. Whether or not blood will coagulate depends on the balance between anticoagulants and procoagulants. In normal states, anticoagulants predominate and clots do not develop (Dahlbäck, 2000). However, after a vessel ruptures, procoagulants become “activated” and override anticoagulants, resulting in the development of a blood
clot. Rapid clotting in such situations or during pain or psychological stress is adaptive, because it prevents fatal bleeding from occurring (Cannon & Mendenhall, 1914a).

Coagulation takes place in three essential steps: formation of prothrombin activator, conversion of prothrombin to thrombin, and the formation of a clot through the conversion of fibrinogen to fibrin (Guyton & Hall, 1996). These steps were described in the 1960s by two independent laboratories as a cascade (MacFarlane, 1964) and a waterfall (Davie & Ratnoff, 1964) in which activation of one clotting factor leads to activation of another, culminating in a burst of thrombin generation that catalyzes the conversion of fibrinogen to fibrin. Although these two groups labeled coagulation differently, they both described the same basic model. Eventually the waterfall label was abandoned but the cascade term has persisted. In this model, clotting factors are proenzymes (i.e., inactive precursors of enzymes) that are available to be converted to active enzymes. This model further suggests that clotting can be initiated by either the extrinsic or intrinsic pathway, each of which ultimately lead to a common pathway. The extrinsic and intrinsic pathways correspond to the first step of coagulation—the formation of prothrombin activator. At the end of this step, the two pathways merge into the common pathway. Once prothrombin activator is formed, it converts prothrombin to thrombin. Thrombin must then be present to convert fibrinogen to fibrin so that a clot can form (Guyton & Hall, 1996). The extrinsic pathway (Figure 1), intrinsic pathway (Figure 2) and common pathway (Figure 3) are described in detail.

Extrinsic Pathway. The extrinsic pathway is initiated by tissue damage, which triggers the release of a complex of factors (i.e., tissue factor) into the blood. Two
important parts of this complex are phospholipids from the cell membranes of the tissues and a lipoprotein complex that contains a critical enzyme. Once tissue factor is released, the lipoprotein complex acts enzymatically on Factor VII (FVII) resulting in activated Factor VII (FVIIa). FVIIa, in the presence of calcium, acts on Factor X (FX) to form FXa. FXa combines with the phospholipids of tissue factor and with FVa to form prothrombin activator (Dahlbäck, 2000; Guyton & Hall, 1996; Riddell Jr. et al., 2007). Clotting via the extrinsic pathway is fast, taking as little as 15 seconds (Guyton & Hall, 1996). Table 1 contains a list of clotting factors and their synonyms.

**Intrinsic Pathway.** The intrinsic pathway is so-named because all its components are present in the blood. The intrinsic pathway is most likely implicated in CHD, because CHD develops inside the vasculature. It is initiated by blood contact with a damaged interior vessel surface, which activates platelets. When platelets become activated, they release phospholipids and their membranes become negatively charged. Factor XII (FXII) is a plasma protein that becomes activated (FXIIa) when coming into contact with a negatively charged surface such as a glass test tube or the membrane of an activated platelet. Subsequently, FXIIa acts on FXI resulting in FXIa. FXIa acts enzymatically on FIX resulting in FIXa. FIXa acts in concert with FVIIIa to activate FX, resulting in FXa. FXa combines immediately with platelet phospholipids and FVa to form prothrombin activator (Dahlbäck, 2000; Guyton & Hall, 1996; Riddell Jr. et al., 2007). Clotting via the intrinsic pathway requires more steps and is much slower than via the extrinsic pathway and takes one to six minutes (Guyton & Hall, 1996).
**Table 1**

*Clotting Factors in Blood and Their Synonyms*

<table>
<thead>
<tr>
<th>Clotting Factor</th>
<th>Synonyms</th>
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<tbody>
<tr>
<td>Factor I</td>
<td>Fibrinogen</td>
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<tr>
<td>Factor II</td>
<td>Prothrombin</td>
</tr>
<tr>
<td>Factor III</td>
<td>Tissue factor; tissue thromboplastin</td>
</tr>
<tr>
<td>Factor IV</td>
<td>Calcium</td>
</tr>
<tr>
<td>Factor V</td>
<td>Proaccelerin; labile factor; Ac-globulin</td>
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<tr>
<td>Factor VII</td>
<td>Serum prothrombin conversion accelerator; proconvertin; stable factor</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>Antihemophilic factor; antihemophilic globulin; antihemophilic factor A</td>
</tr>
<tr>
<td>Factor IX</td>
<td>Plasma thromboplastin component; Christmas factor; antihemophilic factor B</td>
</tr>
<tr>
<td>Factor X</td>
<td>Stuart factor; Stuart-Prower factor</td>
</tr>
<tr>
<td>Factor XI</td>
<td>Plasma thromboplastin antecedent; antihemophilic factor C</td>
</tr>
<tr>
<td>Factor XII</td>
<td>Hageman factor</td>
</tr>
<tr>
<td>Factor XIII</td>
<td>Fibrin-stabilizing factor</td>
</tr>
<tr>
<td>High-molecular-weight kininogen</td>
<td>Fletcher factor</td>
</tr>
</tbody>
</table>

*Note.* Factors were numbered based on order of discovery. No Factor VI exists.
Figure 1. Extrinsic pathway of the coagulation cascade.
Figure 2. Intrinsic pathway of the coagulation cascade.
**Final Common Pathway.** After prothrombin activator is formed by the extrinsic or intrinsic pathway, it catalyzes the conversion of prothrombin to thrombin. In addition, platelets play an important role in the conversion of prothrombin to thrombin. Much prothrombin first binds to prothrombin receptors on platelets attached to damaged tissue. This binding, in turn, accelerates the formation of thrombin from prothrombin at the exact location where a clot is needed. Subsequently, thrombin has a number of important physiological functions. First, it activates platelets to help in the formation of a platelet plug. Second, it acts in a positive feedback fashion on the extrinsic and intrinsic pathways by increasing activation of FV and FVIII, respectively. Finally, it is essential for the conversion of fibrinogen to fibrin, the last step of the coagulation cascade (Guyton & Hall, 1996). Within 10-15 seconds of its formation, thrombin acts enzymatically to convert fibrinogen to fibrin monomers. Fibrin monomers have the automatic capability of joining together to form long fibrin fibers. These fibrin fibers then enmesh platelets, blood cells and plasma to form a clot. Additionally, fibrin fibers adhere to damaged surfaces of blood vessels. Thus, the blood clot adheres to any vascular opening, thereby preventing blood loss (Guyton & Hall, 1996).

**Fibrinolysis**

Once a clot has started to develop, it normally expands into the surrounding blood within minutes (Guyton & Hall, 1996). The clot initiates a vicious cycle (i.e., positive feedback) to promote more clotting by stimulating platelets as well as parts of the clotting cascade itself. The blood clot continues to grow until something stops its growth. Termination of clot growth is accomplished by the plasma protein system known as the
Figure 3. Final common pathway of the extrinsic pathway.

The main components of the fibrinolytic system are plasmin, plasminogen, and tissue plasminogen activator (t-PA). Briefly, when a clot is formed, a large amount of
plasminogen becomes trapped in the clot. Injured tissues and the vascular endothelium slowly release t-PA. Once the clot stops bleeding and after the vessel wall has had time to repair itself (approximately 24 hours), t-PA converts plasminogen to plasmin. Plasmin then acts as a proteolytic enzyme to digest several clotting factors, including fibrin fibers, fibrinogen, FV, FVIII, FXII and prothrombin (Guyton & Hall, 1996; Dobrovolsky & Titaeva, 2002). Fibrin disintegration is also regulated by a number of inhibitors of plasminogen activation, such as plasminogen activator inhibitor type-1, and by inhibitors of plasmin’s activity on clotting factors, such as α2-plasmin inhibitor. In the long run, the components of the fibrinolytic system interact with the coagulation system to keep the blood from becoming either too thick or too thin.

*Other Factors Involved in Hemostasis*

Numerous other physiological factors are involved in hemostasis. Of these, the most important and most studied with respect to acute mental stress are probably D-dimer and von Willebrand factor. Plasma D-dimer is a molecular marker of hemostatic activation (Fareed et al., 1999), whose level reflects fibrin turnover both from intravascular fibrin formation and from consequent fibrinolysis. As opposed to other hemostatic markers, D-dimer is more easily detected in plasma because of its comparatively longer half-life and because it is a marker of activation of both the coagulation and fibrinolysis systems (Lip & Lowe, 1995). Thus, it is likely a reliable indicator of *in vivo* activation of the hemostatic system (Boisclair, Ireland, & Lance, 1990).
von Willebrand Factor (vWF) is a very large molecule released from stimulated or damaged endothelial cells. Thus, the plasma level of vWF indicates endothelial cell dysfunction. Once released into the circulation, vWF plays an essential role in hemostasis by triggering platelet adhesion to places of vascular injury in areas of high shear stress (e.g., in arterioles and microcirculation) and by stimulating the aggregation of platelets to each other. Furthermore, vWF indirectly influences the coagulation cascade by acting as the carrier protein for FVIII in plasma (Bennett & Shattil, 1990; Reininger, 2008; Sugimoto & Miyata, 2002).

Hemostatic Abnormalities in Cardiovascular Disease

Platelets and Coronary Heart Disease

Platelets play an important role in the development of CHD. Their importance is emphasized by the effectiveness of aspirin, a platelet aggregation inhibitor, in reducing the incidence of myocardial infarction (Lewis et al., 1983; Steering Committee of the Physicians’ Health Study Research Group, 1989). Regarding pathophysiology, platelet-derived growth factor causes smooth muscle cells to grow and migrate into the intima of arteries. Smooth muscle is a primary component of mature atherosclerotic plaques (Ross, 1986). Furthermore, platelets themselves bind to foam cells that are exposed when the endothelium becomes damaged in the initial stages of plaque formation (Faggiotto, Ross, & Harker, 1984). Additionally, when platelets adhere to exposed endothelium at the site of a lesion, they increase the size of the lesion and capture leukocytes on the lesion surface (Massberg et al., 2002). These processes occur at the beginning of atherosclerotic
development, but morbidity and mortality occur in the later stages of CHD, in which platelets are also intimately involved.

At points of increased wall shear stress, plaques are more likely to fissure and/or rupture. It is at these sites to which platelets adhere, followed by subsequent platelet activation. Once activated, platelets release various substances involved in vessel occlusion. Platelet growth factors impair fibrinolysis by inducing the release of plasminogen activator inhibitor from endothelial cells (Fujii, Lucore, Hopkins, Billadello, & Sobel, 1989; Fujii & Sobel, 1990). Moreover, thromboxane A$_2$ is a potent vasoconstrictor and is likely to contribute to vascular occlusion when plaques are perturbed (Markovitz & Matthews, 1990).

Further showing the importance of platelets in CHD are epidemiologic studies showing increased levels of platelet markers (i.e., BTG and PF4) in patients with acute coronary syndromes, unstable angina and stable CHD (for reviews, see Markovitz & Matthews, 1990 and Brydon, Magid, & Steptoe, 2006). In the vast majority of studies, platelet markers have a positive relationship with cardiac disease. What is more, patients with stable coronary disease have elevated levels of circulating activated platelets and have platelets that are more reactive to platelet agonists compared to healthy controls (Furman et al., 1998).

**Blood Coagulation and Coronary Heart Disease**

Hypercoagulability spurs chronic development of CHD by providing an environment for gradual deposition of fibrin within atherosclerotic plaques (Davies, 1996). Despite the debate over whether hypercoagulability only indicates an underlying
atherosclerotic process or is also a cause of atherosclerosis (Lee, 1997; Koenig, 1998), many hemostatic factors have been associated with hypertension and CHD. Of these, it has become apparent that fibrinogen and D-dimer are the strongest risk markers (Folsom, 2001), although other hemostasis markers (e.g., FVII, FVIII and vWF) have been shown to have predictive value for CHD. Notwithstanding the long-term unfavorable effects on the vasculature eventually leading to atherosclerosis, a procoagulant environment also plays a central role in CHD by speeding thrombus enlargement after plaque disruption (Virmani, Kolodgie, Burke, Farb, & Schwarz, 2000). Thus, if periods of acute psychological stress stimulate hypercoagulability, then it is plausible that such stressful events could precipitate initiation of CHD (von Känel, Mills, et al., 2001). This section discusses the relationships between hemostatic factors and CHD.

**Fibrinogen.** Fibrinogen is associated with traditional cardiovascular risk factors. For instance, in the Prospective Epidemiological Study of Myocardial Infarction of men in their 50s (Scarabin et al., 1998), fibrinogen was positively related to age, smoking, waist/hip ratio and low-density lipoprotein cholesterol (LDL-c) level. Moreover, fibrinogen was negatively related to education level, physical activity, alcohol intake and high-density lipoprotein cholesterol (HDL-c) level. Similarly, in the Framingham study (Stec et al., 2000), fibrinogen was shown to be interrelated with several cardiovascular risk factors. Significant positive associations were found between fibrinogen and age, body mass index (BMI), smoking, diabetes mellitus, total cholesterol, LDL-c and triglycerides in men and women. Furthermore, a recent meta-analysis reported comparable findings across 31 studies and over 154,000 participants (Fibrinogen Studies
Collaboration, 2007). Fibrinogen levels in both sexes had continuous, positive and approximately linear associations with BMI, SBP, DBP, total cholesterol, and LDL-c and slightly curvilinear, positive relationships with triglycerides, albumin, amount of smoking and amount of alcohol intake. Additionally, fibrinogen levels had a negative, linear relationship with HDL-c. Across studies, it seems that fibrinogen has positive relationships with age, smoking, BMI and total and LDL-c and a negative relationship with HDL-c.

Despite interrelationships of fibrinogen with traditional cardiovascular risk factors, several prospective, epidemiological studies have demonstrated elevated fibrinogen levels to be an independent risk factor for various CHD outcomes (for reviews, see Ernst & Resch, 1993; Koenig, 2003). For instance, in the Framingham Study, each standard deviation increase (approximately 0.56 g/L) in fibrinogen was found to be associated with an increased risk of CHD of 20% in men and 30% in women (Kannel, 1997). Likewise, after adjusting for age, BMI, smoking, diabetes mellitus, total cholesterol and triglycerides, fibrinogen was reported to be higher in patients with CHD than in healthy controls (Stec et al., 2000). Moreover, a meta-analysis of 18 studies (Danesh, Collins, Appleby, & Peto, 1998) compared individuals in the top third of the fibrinogen distribution to those in the bottom third, showing that higher fibrinogen had a relative risk for any type of CHD of 1.8 (95% CI, 1.6-2.0). Most studies in this analysis made adjustments for smoking, blood lipid levels and other traditional CHD risk factors. In a more recent meta-analysis of 31 prospective studies and over 154,000 individuals (Fibrinogen Studies Collaboration, 2005), a one g/L increase in plasma fibrinogen level
was associated with a hazard ratio for any type of CHD of 2.42 (95% CI, 2.24-2.60), when controlling for age, sex and cohort. After additionally controlling for smoking status, total cholesterol, SBP and BMI, the hazard ratio was reduced to 1.93 (95% CI, 1.79-2.08). In 14 of the cohorts, data was available for HDL-c, LDL-c, triglycerides, alcohol consumption, and history of diabetes. When controlling for these additional risk factors, the hazard ratio was reduced to 1.82 (95% CI, 1.6-2.06). Nevertheless, the hazard ratio was still significant, indicating an independent relationship between increased fibrinogen and occurrence of CHD. Similar relationships were found for stroke, other vascular mortality (i.e., aortic aneurysm, heart failure, and acute pulmonary heart disease) and nonvascular mortality (i.e., cancer and other causes of death; Fibrinogen Studies Collaboration, 2005).

Given that fibrinogen is recognized as a risk factor for cardiovascular disease and as being associated with traditional cardiovascular risk factors, such risk factors may exert their pathophysiological effects through fibrinogen (Kannel, 2005). As one example, smoking increases risk of mortality from several types of cardiovascular disease (Ezzati, Henley, Thun, & Lopez, 2005). This increased risk may be partially mediated through fibrinogen, perhaps because fibrinogen levels increase with the number of cigarettes smoked per day, and fibrinogen levels decrease rapidly after smoking cessation (Fogari, Zoppi, Marasi, Vanasia, & Villa, 1994).

Greater levels of plasma fibrinogen likely increase cardiovascular risk through several physiological mechanisms. Fibrinogen is a main determinant of plasma and whole-blood viscosity. Greater viscosity itself is related to hypertension (Zannad &
Stoltz, 1992) and cardiac disease (Woodward, Rumley, Tunstall-Pedoe, & Lowe, 2003). Additionally, fibrinogen penetrates artery walls, activating and promoting platelet aggregation, and stimulates atherogenic cell proliferation, which leads to atherosclerotic plaque formation and, ultimately, blockage of arteries (Rauch et al., 2001).

**D-Dimer.** Compared with studies examining relationships between fibrinogen and CHD, fewer large-scale epidemiological studies have examined whether D-Dimer is related to CHD. Nevertheless, increased levels of D-dimer are associated with vascular disorders, such as stroke and ischaemic heart disease (Smith et al., 1997), as well as established cardiovascular risk factors, such as hypertension, overweight, and smoking (von Känel et al., 2001; Yarnell, Sweetnam, Rumley, & Lowe, 2000; for review, see Lip & Lowe, 1995; Lowe, 2005). In the Edinburgh Artery Study (Smith et al., 1997), elevated D-Dimer levels at baseline were associated with greater relative risk of myocardial infarction and stroke over a follow-up of 5 years. For myocardial infarction, D-Dimer levels at baseline were no longer associated with a greater relative risk when controlling for traditional risk factors (age, sex, SBP, LDL-c, cigarette smoking and baseline disease). However, D-Dimer levels at baseline were still associated with a greater relative risk of stroke over 5 years (RR=1.96; 95% CI, 1.12-3.41). In a meta-analysis of prospective studies that accounted for established CHD risk factors (Danesh et al., 2001), individuals in the highest D-dimer tertile had an odds ratio for a coronary event of 1.7 (95% CI, 1.3-2.2) over an average follow-up of 5 years compared to individuals in the lowest D-dimer tertile. Moreover, in the prospective Progetto Lombardo Atero-Trombosi study (Cortellaro et al., 1993), individuals who had an atherothrombotic event over one
year of follow-up had significantly higher levels of D-dimer at baseline compared to those who did not have an atherothrombotic event. Furthermore, it has been reported that D-dimer levels may predict coronary events in healthy individuals (Lowe et al., 1998).

Other hemostasis variables. Several other hemostatic and fibrinolytic variables have shown some prognostic value for coronary artery syndromes in healthy persons and in patients with CHD. Hemostatic factors with such predictive value include FVIIa (Meade, Ruddock, Stirling, Chakrabarti, & Miller, 1993), FVIIIa (Haines et al., 1983; Meade et al., 1994) and vWF antigen (Meade et al., 1994; Smith et al., 1997; Thögersen et al., 1998). In middle-aged, white men free of heart disease at baseline in the Northwick Park Heart study (Meade et al., 1993; Meade et al., 1994), both FVII and FVIII clotting activity significantly predicted fatal, but not non-fatal, coronary events over a 16-year follow-up period. In the Prospective Cardiovascular Münster study (Heinrich, Balleisen, Schulte, Assmann, & van de Loo, 1994), however, FVII levels were not related to coronary events (sudden cardiac deaths and fatal and nonfatal myocardial infarctions) in men over 6 years of follow-up, although a trend toward higher FVII levels was observed among those who suffered fatal coronary events. In the Edinburgh Artery Study (Smith et al., 1997), men and women who had higher FVII levels at baseline were more likely to experience angina over 5 years follow-up, but FVII levels were not related to the incidence of any vascular event. One possible explanation for inconsistent findings is the use of different assay techniques for determining FVII concentrations.

vWF antigen levels were related to the occurrence of myocardial infarction over nine years in a case-control study of Swedish adults, but only among those who had
already had an acute coronary event (Thögersen, et al., 1998). Similarly, those who suffered any vascular event (i.e., angina, myocardial infarction, and stroke) had higher vWF concentrations at the beginning of the Edinburgh Artery Study (Smith et al., 1997) compared to those who had no vascular event. However, when adjusting for other CHD risk factors (i.e., age, sex, SBP, LDL-c, cigarette smoking and baseline disease), the relative risk of vascular events was no longer greater among those with greater vWF levels at baseline.

**Blood Coagulation and Hypertension**

Essential hypertension is a major risk factor for the development of atherosclerosis and CHD (Greenland et al., 2003). Therefore, understanding the mechanisms underlying essential hypertension can provide insight into understanding the development of CHD. In essential hypertension, the endothelium of arterial walls is subjected to surges of blood under high pressure, but frequent complications of hypertension (such as atherosclerosis and CHD) are paradoxically prothrombotic (i.e., hypercoagulable) rather than hemorrhagic (Lip, 2003). A large number of studies have provided evidence suggesting that hypertension may confer a prothrombotic state (for review, see Lip & Beevers, 1994; Lee, 1997; Varughese & Lip, 2005). Indeed, hypertension satisfies the fundamental requirements of Virchow’s (1856) triad described over 150 years ago, in which the likelihood of thrombosis is enhanced when abnormalities in the vessel wall, blood components and blood flow are all present. Today, it is established that hypertension is related to endothelial damage or dysfunction, abnormalities in hemostatic and fibrinolytic factors, and abnormal blood viscosity,
respectively. Each of these irregularities maps onto the abnormalities originally described by Virchow. These effects are often seen in cardiovascular diseases that result from untreated hypertension, such as atrial fibrillation, heart failure and left ventricular hypertrophy (Varughese & Lip, 2005).

In hypertension, increased pressure forces impinge on arterial endothelium, causing endothelial injury and exposure of collagen to platelets. As described previously, platelets adhere to and aggregate on endothelial collagen, providing the platform and catalyst of a blood clot. Indeed, abnormalities of platelet activation and aggregation are associated with hypertensive status (e.g., Nyrop & Zweifler, 1988; Blann, Lip, Islim, & Beevers, 1997) and CHD (see discussion above). Additionally, studies have generally reported somewhat elevated fibrinogen, D-dimer and vWF in hypertensive individuals compared to normotensive individuals (for review, see Lee, 1997), though such differences are not always found (Trifiletti et al., 1995; Armas-Hernandez et al., 2007). Finally, blood viscosity is a main determinant of total peripheral resistance, which in turn is one of the two main determinants of blood pressure (the other is cardiac output). Elevated blood viscosity is associated with hypertension (Lee, 1997). While the main determinant of blood viscosity is hematocrit, fibrinogen also has a strong influence on blood viscosity, due to its effects on both plasma viscosity and red cell aggregation. Thus, elevated fibrinogen may be an underlying cause of hypertension by increasing blood viscosity and, in turn, total peripheral resistance. Notwithstanding the evidence just presented, it is not entirely clear whether endothelial dysfunction, hypercoagulability and abnormal rheology are causes or effects of essential hypertension (Lee, 1997).
When challenged by psychological stressors, individuals with essential hypertension may experience hyperreactivity across several physiological domains (Nyklicek, Bosch, & Amerongen, 2005). When faced with a variety of physical and mental stressors, hypertensive individuals show exaggerated cardiovascular (Fredrikson & Matthews, 1990; Manuck, Kasprowicz, & Muldoon, 1990), cortisol (Nyklicek et al., 2005; Wirtz, von Känel, et al., 2006), immune (Nyklicek et al., 2005) and lipid (Wirtz, Ehlert, Bärtschi, Redwine, & von Känel, 2009) responses compared to normotensive individuals. Likewise, hypertensive individuals appear to have hyperreactivity of platelets (Lande et al., 1988; Tomoda et al., 1999) and coagulation (Wirtz et al., 2007). Moreover, elevated coagulation activity is a possible mechanism linking acute psychological stress to the initiation of CHD (Allen & Patterson, 1995; Rozanski, 1999, 2005; Bhattacharyya & Steptoe, 2007). Therefore, the remainder of this review focuses on the effects of acute psychological stress on hemostasis activity.

Hemostasis and Acute Psychological Stress

Platelets and Stress

It has long been known that catecholamines (i.e., norepinephrine and epinephrine) are strong stimuli for platelet activation in vitro (Mills & Roberts, 1967; O’brien, 1963; Ponari et al., 1978) and that platelets contain alpha$_2$-adrenergic receptors (Alexander, Cooper, & Handin, 1978). Individuals with various psychological disorders, including general anxiety disorder, panic disorder, and posttraumatic stress disorder, have fewer platelet alpha$_2$-adrenergic receptors available for binding (i.e., catecholamines are already bound to a large number of receptors), which is consistent with the greater catecholamine
concentrations also seen in these conditions (for review, see Bremner, Krystal, Southwick, & Charney, 1996). Furthermore, epinephrine infusion in vivo may cause greater platelet activation and aggregation, and platelets from hypertensive men appear to be even more responsive to epinephrine compared to platelets from normotensive men (for review, see von Känel & Dimsdale, 2000). Emotional stress stimulates the sympathetic nervous system, which in turn causes the release of catecholamines (Bremner et al., 1996; Dimsdale & Moss, 1980) that have the potential to activate platelets.

A large body of research convincingly suggests that acute psychological stress is related to changes in platelet activity in individuals both with and without cardiovascular disease. The majority of studies examining the effects of acute psychological stress on platelet activation report increases in levels of circulating platelet factor 4 (PF4) and beta-thromboglobulin (BTG) among healthy individuals (Levine et al., 1985; Naesh, Haedersdal, Hindberg, & Trap-Jensen, 1993; Patterson & Krantz, 1993; Mundal & Rostrup, 1995; Patterson, Matthews, et al., 1995; Patterson, Zakowski, et al., 1995; Markovitz, Matthews, Kiss, & Smitherman, 1996) and among individuals with hypertension (Tomoda et al., 1990) and with angina pectoris (Wallén, Held, Rehnqvist, & Hjemdahl, 1997). However, Wallén and colleagues (1997) reported that acute stress did not elicit elevations in these platelet activation markers in elderly healthy volunteers. The authors speculated that the balance between platelet activation and inhibiting systems during tasks that elicit acute stress may be different in elderly compared to younger individuals.
Although platelet activation markers generally increase during acute psychological stress in both groups, hypertensive individuals have larger responses of these markers than normotensive individuals. For instance, platelet activation following epinephrine infusion is greater in hypertensive individuals than in normotensive individuals (Lande et al., 1988). Similarly, Tomoda and colleagues (1990) reported that the BTG response is dependent on hypertensive status. Normotensive individuals did not have BTG changes during a mental arithmetic stressor, whereas individuals with World Health Organization (1999) stage 1 (SBP 140-159 or DBP 90-99) and stage 2 (SBP 160-179 or DBP 100-109) hypertension had significant BTG increases. Individuals with stage 1 hypertension had greater increases during mental arithmetic than individuals with stage 2 hypertension, which is most likely due to platelet function already being enhanced in the resting period among those with stage 2. The temporary increase in platelet function during stress in the early phase of hypertension (i.e., stage 1) could bring about perpetual enhancement of platelet function in the more advanced phase of hypertension (i.e., stage 2; Tomoda et al., 1999).

A major limitation in the vast majority of platelet stress-reactivity studies is that adjustments for stress-induced plasma volume shifts have generally not been conducted. One exception (Patterson, Matthews, et al., 1995) suggests that the effects of acute stress on PF4 and BTG levels are independent of hemoconcentration. That is, elevations in PF4 and BTG survived correction for plasma volume shifts. Rather, changes in platelet activation are most likely related to increased catecholamine secretion (Patterson, Matthews, et al., 1995; Patterson, Zakowski, et al., 1995). Along these lines, the
adrenergic binding sites on platelets undergo changes and may become desensitized or have increased density following acute psychological stress (Freedman et al., 1990; Maes et al., 2002), suggesting catecholamine surge and subsequently greater platelet activation. Altogether, platelets seem to become more active during psychological stress, providing one possible mechanism through which stress may be linked to cardiovascular disease.

As previously discussed, activated platelets release activator substances that initiate coagulation activity, the third step of hemostasis. Once platelets become activated, they also express receptors for coagulation factors. Subsequently, coagulation factors bind to and further stimulate platelets. Thus, platelet activation initiates but also perpetuates clotting activity (Bennett & Shattil, 1990; Guyton & Hall, 1996; Holmsen, 1990b; Riddel Jr. et al., 2007). If psychological stress activates platelets, platelets may, in turn, stimulate blood coagulation. The following section reviews coagulation and its relationship to acute psychological stress.

**Blood Coagulation and Stress**

As early as the 1910s, Cannon and Mendenhall (1914b) showed that blood coagulation was accelerated in cats in “fight-flight” circumstances, such as pain and emotional excitement. Such conditions also increase adrenal secretion. In addition, Cannon and Gray (1914) showed that injecting adrenalin (i.e., epinephrine) shortened clotting time. Furthermore, Cannon and Mendenhall (1914a) reported that adrenalin secretion from the adrenal glands as occurs with splanchnic stimulation accelerated clotting. Taken together, these early findings indicate that sympathetic activity accelerates coagulation. Rapid clotting during pain or emotional excitement is adaptive,
defending the organism against fatal bleeding during injury (Cannon & Mendenhall, 1914a).

Cannon’s work set the stage for the next century of research that has examined the effects of emotion, stress, and anxiety on coagulation and fibrinolysis. Several naturalistic and laboratory studies, predominantly conducted from the 1940s to the 1970s, suggested that stressful and emotional conditions increase activity of both fibrinolysis and coagulation. MacFarlane and Biggs (1946) first reported that emotional distress (i.e., fear of a forthcoming operation) stimulated fibrinolytic activity. Other researchers also reported that emotional stressors accelerate fibrinolysis. Such stressors included an air raid warning (Latner, 1947), academic examinations (Truelove, 1951), induced fever by pyrogens, electroshock therapy in psychiatric patients (Sherry, Lindenmeyer, Fletcher, & Alkjaersig, 1959), announced blood tests and even hypnosis-induced agitation and anxiety (Ogston, McDonald, & Fullerton, 1962). Conversely, other investigations reported fibrinolysis to be unaffected during psychological stress, such as that resulting from academic examinations (Truelove, 1953) or from an unexpected tirade from a supervisor telling employees they were inept and inefficient (Sherry et al., 1959). Additionally, Sherry and colleagues (1959) suggested that increased fibrinolytic activity from induced fever and electroshock therapy was likely due to activation of plasminogen after clot formation occurred rather than to the release of preformed plasmin.

During this time, a number of authors also reported faster blood clotting during stressful situations and negative emotional states. Schneider and Zangari (1951) reported that the cold pressor shortened clotting time by 25% in participants classified as blood
pressure reactors (i.e., participants whose SBP increased by 20 mmHg or DBP increased by 15 mmHg during the cold pressor), whereas clotting time was unaffected in nonreactors. Other situations that have been reported to shorten clotting time include periods of heightened anxiety (Macht, 1952; Ogston et al., 1962; Aleksandrowicz, Dzikowski, & Schiffer, 1964), occupational stress in accountants during tax season (Friedman, Rosenman, & Carroll, 1958), academic stress in students during examinations (Dreyfuss, 1956), and actual and anticipated electroconvulsive therapy in psychiatric patients (Kast & Zweibel, 1954). On the other hand, some early studies reported that coagulation activity was unaffected by or attenuated during stressful situations. Ruxin, Bidder, and Agle (1972) reported that coagulation time was unchanged before anticipated electroconvulsive therapy. Both Ruxin et al. (1972) and Kast and Zweibel (1954) used different modifications of the Lee-White whole blood clotting time test, an often used clotting time test employed in coagulation studies of this time period. Thus, it is possible that the discrepancy observed between the study performed by Ruxin et al. and the study performed by Kast and Zweibel could be explained by slight methodological differences. Additionally, a 77-hour vigil, in which women were prevented sleep while required to “fire” an electronic rifle at small targets with amplified noise, resulted in decreases in FV, FVIII, FIX and fibrinogen concentrations (Palmblad et al., 1977). The decreases in these clotting factors could be a result of the considerably longer stressor used in this study relative to the short-term stimuli used in most other studies from this time period. However, such an explanation does not account for why clotting time was observed to
shorten during the occupational stress that accountants experience during tax season (Friedman et al., 1958).

It must be acknowledged that many instruments used to measure fibrinolysis and coagulation in early studies were rather imprecise, and many of these studies were conducted before the fibrinolysis and coagulation systems were fully described. Furthermore, several of these studies exercised questionable ethical practices. Thus, their results must be interpreted with caution. Nevertheless, the vast majority of these studies indicate that fibrinolysis and coagulation activity increase during psychological stress, which is in line with more recent findings using standardized laboratory stress protocols (e.g., Jern, Eriksson, et al., 1989; Jern, 1991). It eventually became known that, although both systems are concomitantly active during psychologically stressful situations, coagulation is stimulated to a greater extent than fibrinolysis, resulting in net hypercoagulability (von Känel, Mills, et al., 2001).

Investigations of the effects of acute psychological stress on coagulation have generated generally positive findings for markers of the intrinsic and extrinsic coagulation pathways as well as markers of the common pathway. Such findings are observed in healthy individuals and in patients with hypertension and CHD. FVII activity (FVII:C), FVIII:C, FXII:C (Jern et al., 1989; Steptoe, Kunz-Ebrecht, Rumley, et al., 2003; von Känel, Preckel, et al., 2004; Zgraggen et al., 2004; Steptoe & Marmot, 2006; Wirtz, Ehler, et al., 2006), fibrinogen concentration (Jern, Eriksson, et al., 1989; Davis, 1999; Wallén, Goodall, Li, & Hjemdahl, 1999; Steptoe, Kunz-Ebrecht, Owen, et al., 2003; von Känel, Dimsdale, et al., 2004; Steptoe & Marmot, 2006; Wirtz, Ehler, et al.,
2006) and D-dimer concentration (von Känel, Dimsdale, et al., 2001; 2002; von Känel, Dimsdale, et al., 2004; Wirtz, Ehlert, et al., 2006) increase during acute psychological stress. Elevations of FVIII:C and FXII:C levels suggest greater activity of the intrinsic pathway, whereas elevations of FVII:C levels suggest greater activity of the extrinsic pathway. Increased fibrinogen levels suggest activation of the common pathway of coagulation. Increases in D-dimer levels, a more sensitive marker of coagulation activation, suggest that procoagulant forces prevail over anticoagulant influences, even though coagulation and fibrinolysis are coexistent with acute mental stress (von Känel, Mills, et al., 2001).

In contrast, some studies have reported equivocal changes in clotting activity resulting from acute psychological stress. For example, vWF significantly increased during the Stroop color-word interference task and mental arithmetic in males (Jern, Eriksson, et al., 1989) but not in females (Jern et al., 1991). It is possible that this difference is due to hormonal differences in females during different phases of the menstrual cycle, indicating the need to control for phase of the menstrual cycle when examining coagulation responses to acute stress. Likewise, some studies have not found a statistically significant increase in D-dimer during acute mental stress in men (Steptoe, Kunz-Ebrecht, Rumley, et al., 2003; von Känel, Preckel, et al., 2004; von Känel, Kudielka, Hanebuth, Preckel, & Fischer, 2005; von Känel, Kudielka, et al., 2009) and women (Steptoe, Kunz-Ebrecht, Rumley, et al., 2003). Such discrepancies may be due to the use of different assays. The aforementioned studies that report significant increases in D-dimer (von Känel, Dimsdale, et al., 2001, 2002; von Känel, Dimsdale, et al., 2004;
Wirtz, Ehlert, et al., 2006) used enzyme-linked immunosorbent assays (ELISA), but other studies (von Känel, Preckel, et al., 2004; von Känel et al., 2005b; von Känel, Kudielka, et al., 2009) used a turbidimetric method, which may have poorer sensitivity (Freyburger et al., 1998) and agreement (Bozic, Blinc, & Stegnar, 2002) than ELISA. However, this does not explain why Steptoe, Kunz-Ebrecht, Rumley, et al. (2003) did not find a significant increase in D-dimer with acute stress using ELISA.

An alternative explanation for mixed findings is in the use of different stressors. Steptoe, Kunz-Ebrecht, Rumley, et al. (2003) used counterbalanced 5-minute Stroop and 5-minute mental arithmetic tasks, whereas von Känel, Preckel, et al. (2004), von Känel et al. (2005b), and von Känel, Kudielka, et al. (2009) used the Trier Social Stress Test (TSST; Kirshbaum, Pirke, & Hellhammer, 1993), an evaluative stressor in which participants give a speech and perform mental arithmetic in front of an audience. In a comprehensive meta-analysis, Dickerson & Kemeny (2004) concluded that psychosocial stress tasks that have unpredictable and social-evaluative threat components elicit the largest hormonal changes (namely, cortisol) and have the longest recovery times. The TSST is characterized by unpredictability and threat of social evaluation. However, there may not have been a social-evaluative threat element in the Stroop and mental arithmetic tasks in the study conducted by Steptoe, Kunz-Ebrecht, Rumley, et al. (2003). This argument is based on the presupposition that hormonal changes arising from social-evaluative threat are driving coagulation changes during acute stress. However, during a 148-minute stress protocol that included 30-minute baseline period, 13-minute TSST and 105-minute recovery period (von Känel et al., 2005b), D-dimer stress reactivity and
cortisol reactivity were unrelated. The lack of relationship between D-dimer reactivity and cortisol reactivity could be due to the approximate 30 minute lag in cortisol response to a stressor, whereas D-dimer tends to increase within minutes of the initiation of a stressor. Thus, cortisol and D-dimer have peaks that are completely out of phase with each other, and reactivity should not be expected to coincide. On the other hand, catecholamine reactivity might be related to D-dimer reactivity, especially if both have stress-responses that follow similar time courses. Altogether, it appears that D-dimer concentrations increase during acute stress when D-dimer is assessed via ELISA and the stressor is characterized by unpredictability and social-evaluative threat.

Equivocal findings have also been reported for measures of clotting time. Prothrombin time (PT) and activated partial thromboplastin time (APTT) are temporal measures of the extrinsic and intrinsic pathways of coagulation, respectively. Of the studies examining coagulation times, two studies have reported no change in APTT during acute psychological stress (de Boer, Ring, Wood et al., 2007; von Känel, Preckel, et al., 2004) while another reported a shortening of APTT (von Känel, Kudielka, et al., 2009). PT showed no change in one study (von Känel, Preckel, et al., 2004), a shortening trend in another (de Boer, Ring, Wood et al., 2007) and a significant shortening in yet another (von Känel, Kudielka, et al., 2009). One possible explanation for these incongruous findings is that the most recent study (von Känel, Kudielka, et al., 2009) aggregated prothrombotic changes across two stress sessions, while prior studies only examined responses at one stress session, and aggregation across multiple measurements
is more likely to yield more robust physiological effects than a single measurement (Kamarck, Debski, & Manuck, 2000).

How acute psychological stress elicits increased coagulation is not fully understood (von Känel, Mills, et al., 2001; von Känel, Kudielka, et al., 2009). One possible mechanism is stress-induced catecholamine spillover and altered adrenergic receptor functioning. Consistent with this explanation, infusion of epinephrine results in elevations of clotting activity of various coagulation markers. von Känel and Dimsdale (2000) reviewed such effects and found that blood clotting time (assessed by various means) shortened in 9 of 11 studies and elevations in vWF antigen were observed in 7 of 7 studies. Moreover, FVIII:C increased in all 15 studies reviewed and increased significantly in 14 of those studies. The mean peak response for FVIII:C was 206% and was seen on average after 19 minutes of infusion. However, in studies measuring recovery after termination of infusion, some noted that FVIII returned to baseline after 30 minutes (Libre, Cowan, Watkins, & Shulman, 1968) and 6 hours (Prentice, Forbes & Smith, 1972), whereas others showed incomplete recovery after 30 minutes (Gader, Clarkson & Cash, 1973) and 90 minutes (Mannucci, Aberg, Nilsson, & Robertson, 1975). Along the same lines, infusion of isoproterenol (a potent β-adrenergic stimulator) in hypertensive individuals leads to a dose-response increase in vWF (von Känel, Dimsdale, Adler, et al., 2003). The most likely explanation for these effects is that epinephrine binds to endothelial β2-adrenergic receptors, stimulating the release of FVIII and vWF (von Känel & Dimsdale, 2000).
von Känel and Dimsdale (2000) also reviewed the effects of epinephrine infusion on fibrinolysis. A consistent stimulation of fibrinolysis was observed in 31 of 32 studies with a dose-response relationship with the amount of epinephrine infused. A limitation of most studies they reviewed, however, is the lack of comparison between stimulation of coagulation and fibrinolysis. As mentioned, both coagulation and fibrinolysis become activated during acute psychological stress, but coagulation is heightened to a greater extent than fibrinolysis, resulting in net hypercoagulability (von Känel, Mills, et al., 2001). Furthermore, evidence exists for a catecholamine surge and a change in the hormonal environment following acute stress (Dimsdale & Moss, 1980; Bremner et al., 1996), which concurrently activates the coagulation and fibrinolytic systems through effects on endothelial β2- and platelet α2-adrenergic receptors. Thus, acute stress may lead to hypercoagulability through the release of catecholamines. Another limitation of the studies reviewed is that only the effects of epinephrine infusion on FVIII:C were examined. FVIII:C is a marker of the intrinsic pathway of blood coagulation, but the effects of epinephrine infusion on FVII:C (i.e., a marker of the extrinsic pathway) were not examined. Theoretically, only the intrinsic pathway should be activated by elevations of epinephrine, but the actual physical process of epinephrine infusion (i.e., catheter insertion) may activate the extrinsic pathway.

Notwithstanding sympathetic nervous system mechanisms, hemoconcentration may play a crucial role in the coagulation changes observed during acute psychological stress. Although a small number of studies have examined hemoconcentration effects in stress-coagulation studies, such effects have often been overlooked. This is the most
important limitation of the majority of studies examining the effects of psychological stress on coagulation. Of the few studies that have examined these effects, some have included hematocrit changes as a covariate when examining changes in the concentration of coagulation factors during acute psychological stress (Jern, Eriksson, et al., 1989; Steptoe, Kunz-Ebrecht, Owen, et al., 2003; Steptoe, Kunz-Ebrecht, Rumley, et al., 2003). As such, it was concluded in these studies that increases in coagulation factors during acute stress were independent of hemoconcentration. This approach, however, only controls for variation in changes in hematocrit, rather than mathematically correcting for plasma volume shifts. Another approach to examining stress-hemoconcentration effects on coagulation has been to calculate Spearman correlations between change scores of hemoconcentration (i.e., hematocrit, hemoglobin and plasma volume) and coagulation measures from baseline to immediately post-stress (Zgraggen et al., 2005). No significant relationships emerged with this method, and thus, the authors concluded that stress-hemoconcentration is an unlikely explanation for the coagulation response to stress. However, the relationships in this study pointed in the right direction for stress-hemoconcentration effects and the authors acknowledge that significant associations may have been detected with a larger sample. Furthermore, correlations are not nearly as powerful as arithmetic adjustment for plasma volume shifts (Muldoon et al., 1992).

Only one known coagulation study has arithmetically adjusted for stress-induced plasma volume shifts (von Känel, Kudielka, et al., 2009). In this study, it was reported that stress-hemoconcentration significantly altered plasma levels of prothrombotic measures in response to the TSST. Specifically, fibrinogen, FVII:C, FVIII:C, FXII:C and
vWF levels all became elevated immediately after the TSST relative to baseline when there was no adjustment for plasma volume shifts. However, when taking plasma volume shifts into account, none of these increases remained significant. In contrast, fibrinogen and FVII:C levels even decreased significantly when correcting for hemoconcentration, which is consistent with studies examining changes in these factors during exercise (van den Burg et al., 1995; El-Sayed, Jones, & Sale, 1999). Without correction for plasma volume shifts, activated partial thromboplastin time (APTT) showed a shortening trend in this study as well, which suggests faster initiation of the intrinsic pathway of blood coagulation during acute stress, but prothrombin time (PT) did not change, suggesting that acute stress had no effect on the clotting time of the extrinsic pathway. When adjusting for plasma volume shifts, however, a significant decrease was found for APTT, indicating even faster clotting via the intrinsic pathway. On the other hand, PT was longer when correcting for plasma volume shifts, suggesting slower clotting via the extrinsic pathway (von Känel, Kudielka, et al., 2009). The adjusted result for APTT is puzzling because one would expect a decrease in fibrinogen to be accompanied by a prolongation in APTT. That is, a reduction in a clotting factor should be associated with a slower clotting response. Moreover, the Dill and Costill formula is designed to mathematically adjust the concentration of a physiological parameter based on changes in plasma volume but may be of limited utility when correcting for plasma volume changes in time dependent functional assays such as clotting time tests.

When comparing baseline levels of coagulation measures to levels 45 minutes post-stress (i.e., recovery), similar observations were noted, with the notable exception of
FVIII, which was the only clotting factor with elevations during recovery that survived arithmetic correction (von Känel, Kudielka, et al., 2009). Moreover, FVIII:C remained higher when taking into account plasma volume changes during recovery. Together with the previous observation that FVIII:C remained elevated two hours post-stress compared to baseline levels (von Känel, Preckel, et al., 2004), these results suggest that FVIII is actually or “genuinely” activated during the time immediately after acute psychological stress. Correcting for plasma volume changes in APTT and PT from baseline to recovery resulted in changes opposite those seen between baseline and immediately post-stress. That is, APTT was prolonged and PT was shortened (von Känel, Kudielka, et al., 2009). Figure 4 illustrates the changes in APTT and PT that occurred in this study both with and without arithmetic correction for hemoconcentration. Again, given that APTT and PT are measures of time, these results may be misleading and must be interpreted with extreme caution. Again, Dill and Costill’s (1974) arithmetic correction for plasma volume shifts adjusts for the amount of plasma that leaves the vasculature during acute stress and should be used when examining changes in the concentration of a parameter. However, the arithmetic adjustment has no time component. Similarly, arithmetic correction may be inappropriate when examining the activity or activation of clotting factors. Changes in plasma volume may influence clotting time and clotting activity differently than the concentrations of clotting factors. Therefore, the use of such mathematical correction for these measures is questionable. The present study circumvents this limitation by employing a reconstitution technique that physically, rather than mathematically, corrects for plasma volume shifts.
Figure 4. Changes in activated partial thromboplastin time and prothrombin time immediately post-stress and after 45 minute recovery. Prothrombin time (PT) was measured as a percentage of normal. A decrease in the percent indicates an increase in the actual PT. From “Prothrombotic changes with acute psychological stress: combined effect of hemoconcentration and genuine coagulation activation” by R. von Känel, B.M. Kudielka, A. Haeberli, M. Stutz, J.E. Fischer and S.M. Patterson, 2009, Thrombosis Research, 123, p. 626. Copyright 2009 by Elsevier. Reprinted with permission.

Summary

Elucidation of mechanisms linking acute stress to CHD is a crucial issue in cardiovascular behavioral medicine. One promising link is through elevated coagulation activity. The ensuing hypercoagulable state after acute stress may be more likely to cause an atherothrombotic event, especially when atherosclerosis or endothelial dysfunction is
already present (von Känel & Dimsdale, 2000; von Känel, Mills, et al., 2001). Heightened sympathetic arousal could crucially shift hemostatic balance toward a hypercoagulable condition in patients with atherosclerosis due to prothrombotic and fibrinolytic irregularities (von Känel, Mills, et al., 2001). However, how heightened sympathetic arousal exerts its effects on hemostatic balance is unclear. Possibilities include activation of platelets and clotting factors by catecholamines and hemoconcentration of clotting factors resulting from increased blood pressure. Studies attempting to address this issue have shown that acute stress results in a hypercoagulable state and that such a prothrombotic state is probably exacerbated in hypertensive individuals. However, it is unclear whether the observed increases in coagulation activity during acute stress results from hemoconcentration or from actual activation of the coagulation system. Different studies employing differing methods for correcting for stress-hemoconcentration effects have reported equivocal findings. Therefore, this project aims to more fully explain whether stress-induced hypercoagulation is due to hemoconcentration, actual coagulation activation, or a combination of both.
OVERVIEW OF THIS PROJECT

Previous research has shown that acute psychological stress stimulates clotting activity in the blood. This effect is important because the initiation of CHD, especially myocardial infarction, has been linked to acute psychosocial stress (Strike & Steptoe, 2005). Two possible explanations for this stress-clotting effect are proposed. First, acute stress may cause actual activation of the clotting system. Alternatively, it may be that prothrombotic changes appear because plasma is pushed out of the vasculature during acute stress, resulting in stress-hemoconcentration of clotting factors.

Specific Aims

Specific Aim #1: To examine whether any observed increases in FVII:C, FVIII:C, fibrinogen and D-dimer in plasma during acute psychological stress could be reversed by using a person’s own plasma from baseline to reconstitute plasma obtained during stress or during recovery back to baseline plasma volume levels.

Hypothesis 1a: It was hypothesized that when reconstituted with the person’s own baseline plasma back to baseline plasma volume, plasma obtained during an acute math stressor or during recovery (i.e., 20 minutes post-stressor) would no longer have different FVII:C than plasma obtained during baseline.

Hypothesis 1b: In line with previous research (von Känel, Preckel, et al., 2004; von Känel, Kudielka, et al., 2009), it was hypothesized that when reconstituted with the person’s own baseline plasma back to baseline plasma volume, plasma obtained during an acute math stressor or during recovery would still have elevated FVIII:C than plasma obtained during baseline.
Hypothesis 1c: It was hypothesized that when reconstituted with the person’s own baseline plasma back to baseline plasma volume, plasma obtained during an acute math stressor or during recovery would no longer have a different concentration of fibrinogen than plasma obtained during baseline.

Hypothesis 1d: It was hypothesized that when reconstituted with the person’s own baseline plasma back to baseline plasma volume, plasma obtained during an acute math stressor or during recovery would no longer have a different concentration of D-dimer than plasma obtained during baseline.

Specific Aim #2: To examine whether any observed increases in FVII:C, FVIII:C, fibrinogen and D-dimer in plasma during acute psychological stress can be reversed by using physiological saline to reconstitute plasma obtained during stress or during recovery back to baseline plasma volume levels.

Hypothesis 2a: It was hypothesized that when reconstituted with physiological saline back to baseline plasma volume, plasma obtained during an acute math stressor or during recovery would no longer have different FVII:C than plasma obtained during baseline.

Hypothesis 2b: In line with previous research (von Känel, Preckel, et al., 2004; von Känel, Kudielka, et al., 2009), it was hypothesized that when reconstituted with physiological saline back to baseline plasma volume, plasma obtained during an acute math stressor or during recovery would have elevated FVIII:C than plasma obtained during baseline.
**Hypothesis 2c:** It was hypothesized that when reconstituted with physiological saline back to baseline plasma volume, plasma obtained during an acute math stressor or during recovery would no longer have a different concentration of fibrinogen than plasma obtained during baseline.

**Hypothesis 2d:** It was hypothesized that when reconstituted with physiological saline back to baseline plasma volume, plasma obtained during an acute math stressor or during recovery would no longer have a different concentration of D-dimer than plasma obtained during baseline.

**Specific Aim #3:** To examine whether stress-hemoconcentration influences clotting time of the intrinsic and extrinsic pathways.

**Hypothesis 3a:** It was hypothesized that APTT measured in plasma obtained during an acute math stressor or during recovery that is reconstituted with the person’s own baseline plasma back to baseline plasma volume would not be different than APTT in plasma obtained during baseline.

**Hypothesis 3b:** It was hypothesized that APTT measured in plasma obtained during an acute math stressor or during recovery that is reconstituted with physiological saline back to baseline plasma volume would not be different than APTT in plasma obtained during baseline.

**Hypothesis 3c:** It was hypothesized that PT measured in plasma obtained during an acute math stressor or during recovery that is reconstituted with the person’s own
baseline plasma back to baseline plasma volume would not be different than PT in
plasma obtained during baseline.

_Hypothesis 3d:_ It was hypothesized that PT measured in plasma obtained during
an acute math stressor or during recovery that is reconstituted with physiological saline
back to baseline plasma volume would not be different than PT in plasma obtained during
baseline.

_Specific Aim #4:_ To test a new reconstitution method for correcting for plasma volume
shifts against the usual arithmetic correction developed by Dill and Costill (1974).

_Hypothesis 4a:_ It was hypothesized that reconstituted plasma, whether with the
individual’s own baseline plasma or with physiological saline, would have concentrations
of fibrinogen and D-dimer similar to concentrations that are calculated by the Dill and
Costill method.

_Hypothesis 4b:_ It was hypothesized that reconstituted plasma, whether with the
individual’s own baseline plasma or with physiological saline, would have FVII:C and
FVIII:C similar to FVII:C and FVIII:C calculated by the Dill and Costill method.

_Hypothesis 4c:_ It was hypothesized that APTT and PT of reconstituted plasma,
whether with the individual’s own baseline plasma or with physiological saline, would be
similar to the APTT and PT produced by the Dill and Costill method.

A study using the following protocol was used to examine these aims. Blood
samples were collected at three time points: the last minute of a baseline period, the last
minute of a standardized mental arithmetic stressor, and the last minute of a recovery
period. The percentage of whole blood at baseline that is plasma was determined immediately after the blood sample was obtained. Then, plasma from blood samples obtained during the stressor task and recovery period were aliquoted into smaller portions and subjected to three conditions. First, given that the percentage of whole blood that is plasma is expected to decrease during psychological stress, plasma obtained during the math task was reconstituted with the participant’s actual plasma from baseline in vitro such that the percentage of blood that is plasma became equal to that of baseline. Second, plasma from the math task and recovery period was reconstituted with physiological saline in vitro such that the percentage of blood that is plasma became equal to that of baseline. Third, plasma from the math task and recovery period was not reconstituted.

If clotting activity, clotting time and concentrations of clotting factors are still different after reconstitution compared to levels prior to the stressor, then it can be considered that actual activation of the clotting system occurred independent of hemoconcentration. However, if levels are not different from baseline after reconstitution, then the effect may be explained by hemoconcentration. Previous researchers have corrected arithmetically for hemoconcentration effects, but the primary contribution of this study was the use of an original reconstitution method that physically adjusts for such effects. This study provides a novel approach to studying mechanisms underlying the relationship between stress and cardiovascular disease, which is a foremost issue in cardiovascular behavioral medicine research.
METHOD

This study was conducted as a 3 (Period: baseline, math task, recovery period) × 4 (Plasma manipulation: plasma reconstituted with baseline plasma, plasma reconstituted with physiological saline, plasma corrected arithmetically, plasma not corrected) within-subjects design. The dependent variables were FVII:C, FVIII:C, fibrinogen, D-dimer, APTT and prothrombin time percentage (PT%).

Power Analysis

One previous study (von Känel, Kudielka, et al., 2009) has corrected arithmetically for plasma volume shifts with the Dill and Costill (1974) equation. Therefore, estimates of effect size are taken from this study. Immediately post-stress relative to baseline, the effect sizes for of clotting measures corrected for hemoconcentration were as follows: FVII:C ($d = .31$); FVIII:C ($d = .09$); fibrinogen ($d = .33$); D-dimer ($d = .11$); APTT ($d = 0.91$); PT% ($d = 0.83$). Forty-five minutes post-stress relative to baseline, the effect sizes were as follows: FVII:C ($d = .18$); FVIII:C ($d = .35$); fibrinogen ($d = .11$); D-dimer ($d = .03$); APTT ($d = 0.30$); PT% ($d = 0.42$). Because there are no existing studies that have physically corrected for hemoconcentration effects, an effect size of $d = .331$ (the mean of these effect sizes) is hypothesized. G-Power Version 3.0.10 (Faul, Erdfelder, Lang, & Buchner, 2007) was used to perform a-priori sample size calculations. Using a one-way ANOVA with repeated measures to calculate power estimates, and based on an anticipated effect size of $d = .331$, alpha = .05 and the assumption that correlations among repeated measures are very high (i.e., at least .80), it was estimated that a total sample of 24 participants would be required to achieve a power
of 0.80. Moreover, according to Table 13.5 in Stevens (2002), based on an anticipated medium effect size, alpha = .05, correlations among repeated measures of 0.80, and three levels of the repeated measure (i.e., time), only 14 subjects are needed to achieve power of 0.80. However, given the possibility of missing data due to equipment or experimenter error, at least 40 participants were recruited.

Participants

Participants were 40 male university students over 18 years of age and enrolled in introductory psychology courses. Women were not examined in order to avoid difficulty in controlling for phase of the menstrual cycle. Exclusionary criteria were the following: (a) personal history of cardiovascular or other chronic disease, (b) any history of a thromboembolic event, (c) use of prescription anti-inflammatory or antidepressant medication, (d) use of prescription medication that would affect blood pressure (e.g., beta blockers, diuretics), (e) regular use of aspirin, (f) smokers, (g) obesity (body mass index [BMI] greater than 30 kg/m$^2$), (h) any current major or minor infection, (i) any trauma or surgery within the previous six months, (j) history of fainting during blood draws or blood donation, (k) history of fainting or passing out, (l) resting systolic blood pressure over 140 mmHg or under 85 mmHg, (m) diastolic blood pressure over 90 mmHg or under 55 mmHg.

Procedure

The study protocol was approved by the Biomedical Institutional Review Board at Ohio University (Protocol #09F017). Participants were asked to abstain from food and drink for four hours prior to their laboratory session but were allowed to drink water.
Participants were also asked to refrain from exercising and from drinking alcohol for 24 hours prior to their session. Upon arriving to the laboratory, participants provided informed consent and were informed of their right to discontinue participation at any time (Appendix A), and eligibility was verified using the Health Information Screening Questionnaire (Appendix B) and the Pre-Study Restrictions form (Appendix C). Next, to verify blood pressure, sitting, resting blood pressure was determined via the auscultatory method. To verify non-smoking status, participants followed instructions and blew into a calibrated piCO Smokerlyzer® (Bedfont Scientific Ltd, Kent, United Kingdom) and excluded if the carbon monoxide result was greater than 10 parts per million. To verify that participants had not consumed alcohol in the previous 24 hours, they followed instructions and blew into a calibrated AlcoHAWK® PT500 Breath Alcohol Tester (Q3 Innovations, Independence, IA) and excluded if the blood alcohol content was greater than 0.00%. To verify that BMI was less than 30, height and weight were measured with a calibrated stadiometer, and BMI was calculated (BMI = kg/m²).

Eligible participants sat in a comfortable chair in a quiet room. An indwelling venous 20-gauge catheter (Exel Safelet Cath, Exelint International Co., Los Angeles, CA) was inserted in a suitable vein in the antecubital fossa by the Health Psychology Research Nurse at Ohio University. A blood pressure cuff was placed on the opposite arm. Participants then rested quietly while listening to gentle music at low volume during a 20-minute baseline period. Following baseline, participants performed a standardized 6-minute mental arithmetic task with strong verbal encouragement from the experimenter. Each participant was instructed to subtract aloud by sevens from a pre-recorded 4-digit
number as quickly and as accurately as possible. A new 4-digit number was presented each minute. Throughout the task, the experimenter instructed the participant to be more accurate and to work more quickly. Such instructions were given regardless of the participant’s performance on the task. The math stressor was followed immediately by a 20-minute recovery period in which participants again rested quietly while listening to gentle music at low volume. Blood samples for hemoconcentration and hemostasis measures were collected during the last minute of the baseline period, the math task and the recovery period. As a manipulation check, heart rate and blood pressure were assessed at minutes 15, 17, and 19 of the baseline period, at minutes 0.5, 2.5, and 4.5 of the mental arithmetic task, and at minutes 1, 3, 5, 7, 9, 11, 13, 15, 17, and 19 during the recovery period. Finally, the catheter and blood pressure cuff were removed and participants were debriefed (Appendix D). Figure 5 summarizes the experimental protocol and all times of collection of blood samples.

Hemodynamic Measures

A Colin Press-Mate BP-8800 automated blood pressure monitor (Colin Medical Instruments) was used to measure heart rate, SBP and DBP at fixed intervals during baseline, the psychological stressor and recovery.

Blood Sampling

At baseline, blood was collected into one 4-ml EDTA vacutainer tube for hemoconcentration measures and three 4.5-ml 3.2% sodium citrate tubes for hemostasis measures. During the last minute of the math task and recovery period, blood was collected into one 4-ml EDTA vacutainer tube for hemoconcentration measures and four 3.2%
sodium citrate tubes for hemostasis measures. Tubes were completely filled. Immediately
after being obtained, the sodium citrate tubes for coagulation measures were centrifuged
for 10 minutes at 3000xg. Then, 1-ml portions of platelet-poor plasma were aliquoted
into Eppendorf tubes and put on ice until the plasma manipulation.

Hemoconcentration Measures

Hematocrit (Hct) and hemoglobin (Hgb) concentrations were determined in
triplicate from EDTA tubes with a Coulter Counter (AcT 10). Hct levels were calculated

Figure 5. Experimental protocol
from the red cell concentration, and Hgb concentrations were determined by the cyanmethemoglobin method (Streck Mini-Pack, Omaha, NE). Plasma volume changes were arithmetically calculated from baseline and task (i.e., math or recovery) Hct and Hgb values using the Dill and Costill method (1974).

Hemostasis Measures

Fibrinogen, FVII:C, FVIII:C, APTT and PT% were determined singularly with the Beckman Coulter ACL 1000 by standard coagulometric methods using factor-deficient standard human plasma and reagents. D-dimer was determined in duplicate using a microplate reader and an enzyme-linked immunosorbent assay (ZYMUTEST DDimer, HYPHEN BioMed, Neuville sur Oise, France). Standards and controls were assessed for each measure. Coefficients of variation for all hemostasis measures were less than 5%.

Plasma Manipulations

Plasma samples from the math task and recovery period were subjected to the following four conditions: 1) Plasma volume changes from baseline to math and recovery were corrected arithmetically by the Dill and Costill (1974) equation. 2) Plasma samples from the math task and recovery period were reconstituted back to baseline plasma volume with the participant’s actual plasma from baseline. 3) Plasma samples from the math task and recovery period were reconstituted back to baseline plasma volume with physiological saline. 4) Plasma samples from the math task and recovery period were not reconstituted or corrected arithmetically. After each plasma manipulation, plasma samples were frozen at -80°C until assayed.
Plasma Corrected by the Dill and Costill (1974) Equation

Plasma samples from the math task and recovery period were analyzed for coagulation and hemoconcentration measures after correcting arithmetically for plasma volume shifts. The calculation for estimating plasma volume changes incorporates both hematocrit (to determine the percentage of plasma volume) and hemoglobin (to control for hemoconcentration-induced changes in red cell volume) before and after each manipulation (Dill & Costill, 1974). The equation is as follows:

\[
BV_A = BV_B \times \left( \frac{Hgb_B}{Hgb_A} \right) \\
CV_A = BV_A \times \left( \frac{Hct_A}{100} \right) \\
PV_A = BV_A - CV_A \\
\%\Delta PV = 100 \times \left( \frac{PV_A - PV_B}{PV_B} \right)
\]

where \(BV = \) blood volume, \(CV = \) red cell volume, \(PV = \) plasma volume, \(Hgb = \) hemoglobin, \(Hct = \) hematocrit, subscript B refers to baseline sample, subscript A refers to the period (math or recovery) sample, \(BV_B = 100\), and \(PV_B = 100 - Hct_B\). Given that changes in the actual size of red blood cells can affect the packed cell volume of Hct, Hgb is used in the plasma volume equation to control for possible changes in mean corpuscular volume (van Beaumont, 1972).

The corrected period values for concentrations of coagulation measures were calculated from the measured levels during each period and the estimated percentage change in plasma volume. The equation is as follows:
\[ C_C = C_U [1 - (\% \Delta PV/100)], \]

where \( C_U \) = measured coagulation parameter during each period.

*Plasma Reconstituted with Baseline Plasma*

The 1-ml of plasma in each Eppendorf tube from the math task and recovery period was reconstituted with an amount of plasma from baseline such that plasma volume during the math task and recovery period became equal to plasma volume at baseline. This amount was determined from the following formula:

\[ \mu l \text{ per } 1 \text{ ml} = \left( \frac{PV_B}{PV_A} \right) \left( \frac{CV_A}{CV_B} \right) - 1 \right) / 1000, \]

where \( PV_B \) is plasma volume during baseline, \( PV_A \) is plasma volume during the math task or recovery period, \( CV_B \) is red cell volume during baseline and \( CV_A \) is red cell volume during the math task or recovery period. The mean reconstitution amount for the math task was 55.40 µl ± 36.24 and ranged from 6.76 µl to 150.27 µl. For example, if the reconstitution amount was 55 µl, then this amount of baseline plasma was added to 1 ml (1000 µl) of plasma from the math task, resulting in a final volume of 1055 µl.

It was expected that plasma volume would increase to above baseline levels during the recovery period in some individuals. If this occurred, plasma was not reconstituted. Though unexpected, plasma volume increased in eight participants during
the math task. The mean calculated reconstitution amount for these participants was -25.78 µl ± 20.33 and ranged from -61.63 µl to -2.07 µl.

**Plasma Reconstituted with Physiological Saline**

Plasma samples from math and recovery were reconstituted via the same method as in the plasma reconstitution manipulation. However, instead of plasma being reconstituted with the participant’s own plasma from baseline, it was reconstituted with physiological saline (0.9% NaCl; Hospira, Inc., Lake Forest, IL).

**Plasma Not Reconstituted or Corrected**

Plasma was not reconstituted or corrected arithmetically, However, it was still analyzed for all coagulation measures.

**Statistical Analyses**

Data were analyzed using SPSS (version 17.0) statistical software (Chicago, IL). All data are presented as mean ± SD. All tests are two-tailed with level of significance set at p < .05. The Kolmogorov-Smirnov was used to test for normality for hemoconcentration, hemostasis and hemodynamic measures. The Huynh-Feldt correction was applied to account for any violations of the sphericity assumption.

A series of repeated measures ANOVAs were conducted for heart rate, DBP, SBP, hematocrit, and plasma volume with time (baseline, post-stress and recovery) as the independent variable. Planned comparisons were used to compare post-stress and recovery to baseline, using the Bonferroni adjustment for multiple comparisons (α_{adj} = .05/2 = .025). Next, for coagulation variables, baseline variables with values equal to actual baseline values were created for each plasma manipulation condition. These
variables were needed in order to compare differences in plasma manipulations from
baseline to post-stress. Though it was unexpected that post-stress plasma volume would
be greater than baseline plasma volume, when this occurred values equal to uncorrected
post-stress values were included for each reconstitution condition at post-stress.
Moreover, based on previous findings (Patterson, Krantz, & Jochum, 1995), it was
expected that recovery plasma volume would not be significantly different from baseline
plasma volume. Therefore, when recovery plasma could not be reconstituted because
calculated plasma volume at recovery was greater than or equal to baseline plasma
volume, values equal to uncorrected recovery values were included for each
reconstitution condition at recovery. Then, 3 (Period: baseline, math task, recovery
period) x 4 (Plasma manipulation: plasma reconstituted with baseline plasma, plasma
reconstituted with physiological saline, plasma corrected arithmetically, plasma not
corrected) within-subjects ANOVAs were conducted to test for changes in each
coagulation measure. Twenty planned comparisons using paired-samples t-tests were
conducted to compare levels of hemostasis parameters after each plasma manipulation
during stress and recovery to levels at baseline and to each other, using the Bonferroni
adjustment for multiple comparisons ($\alpha_{adj} = .05/20 = .0025$). 95% confidence intervals of
the difference are reported. Marginal significance was set at $\alpha = .05$. 
RESULTS

Participant Characteristics

Sixty-four males were screened for testing. Twenty individuals were excluded for the following reasons: 4 for BMI greater than 30, 11 for eating within four hours prior to the study, one for exercising within 24 hours prior to the study, one for drinking alcohol within 24 hours prior to the study, one for history of fainting during blood draws/donation, one for high blood pressure, and one for having a sore throat. Four individuals were eligible but did not complete the study for the following reasons: syncope during baseline period, low blood pressure and lightheadedness during the baseline period, did not want a catheter in his arm, and research nurse was sick. Table 2 contains participant characteristics of the 40 participants included in the study.

Table 2.

*Participant Characteristics, N = 40*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>(Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>19.85 ± 2.56</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.02 ± 2.94</td>
</tr>
<tr>
<td>Screening SBP (mmHg)</td>
<td>114.38 ± 8.41</td>
</tr>
<tr>
<td>Screening DBP (mmHg)</td>
<td>74.11 ± 9.26</td>
</tr>
<tr>
<td>Carbon Monoxide (parts per million)</td>
<td>1.71 ± 1.60</td>
</tr>
<tr>
<td>Blood Alcohol Content (%)</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>
Hemodynamic Measures

All measures followed a normal distribution. All repeated measures analyses for hemodynamic and hemostasis parameters violated the sphericity assumption, whereas plasma volume and hematocrit did not. Thus, results for hemodynamic and hemostasis parameters are presented using the Huynh-Feldt correction, but results for plasma volume and hematocrit are presented with sphericity assumed.

Hemodynamic measurements were averaged at baseline, post-stress and recovery. For SBP, there was a significant main effect of time, $F(1.48, 57.59) = 174.22, p < .001$. Figure 6 shows that SBP increased significantly from baseline ($M = 122.19 \pm 8.85$) to post-stress ($M = 141.05 \pm 11.8$), $p < .001$, and remained elevated at recovery ($M = 124.63 \pm 9.06$), $p = .003$. For DBP, there was a significant main effect of time, $F(1.38, 53.63) = 138.11, p < .001$. DBP increased significantly from baseline ($M = 63.38 \pm 6.87$) to post-stress ($M = 76.43 \pm 8.04$), $p < .001$, and remained elevated at recovery ($M = 64.97 \pm 6.47$), $p = .021$. For HR, there was a significant main effect of time, $F(1.16,45.35) = 129.05, p < .001$. Figure 7 shows that HR increased significantly from baseline ($M = 63.6 \pm 10.31$) to post-stress ($M = 81.03 \pm 13.35$), $p < .001$, and remained marginally elevated at recovery ($M = 64.84 \pm 9.99$), $p = .029$.

Hematocrit and Plasma Volume

Repeated measures ANOVA indicated a significant main effect of time for hematocrit, $F(2, 78) = 21.04, p < .001$. Figure 8 shows that hematocrit increased significantly from baseline ($M = 42.26 \pm 2.21$) to post-stress ($M = 43.17 \pm 2.17$), $p <
Figure 6. Blood pressure change. *Greater than Baseline at $p < .001$. ^Greater than Baseline at $p < .025$.

Figure 7. Heart rate change. *Greater than Baseline at $p < .001$. ^Greater than Baseline at $p = .029$.  
.001, but was not significantly different from baseline at recovery ($M = 42.03 \pm 1.98), p = .477. Plasma volume had a main effect of time, $F(2, 78) = 28.22, p < .001. Figure 9 shows that plasma volume decreased significantly from baseline ($M = 57.74 \pm 2.21$) to post-stress ($M = 55.35 \pm 2.67), p < .001, but was not significantly different from baseline at recovery ($M = 58.17 \pm 2.69), p = .771. The calculated reconstitution value was negative for eight participants (mean reconstitution amount = -25.78 µl, range: -2.07 µl to -61.63 µl), despite a slight negative percent change in plasma volume from baseline to post-stress in two participants (-0.36% and -0.71%). Therefore, these eight participants were excluded from the reconstitution procedure.

Percent change in plasma volume from baseline to post-stress had a significant, negative correlation with SBP change, $r = -.392, p = .012, and with heart rate change, r =
Percent change in plasma volume from baseline to recovery had non-significant correlations with SBP change, $r = .065$, $p = .692$, DBP change, $r = -.019$, $p = .909$, and HR change, $r = -.065$, $p = .692$.

**Hemostasis Measures**

**Fibrinogen**

Repeated measures ANOVA indicated a significant time-by-plasma manipulation interaction, $F(3.34, 130.28) = 6.18$, $p < .001$. Figure 10 shows that fibrinogen marginally increased from baseline ($M = 285.4$ mg/dl ± 47.3) to post-stress when uncorrected ($M = 293.1$ mg/dl ± 44.1), $t(39) = -2.58$, $p = .015$, 95% CI [-13.70, -1.65], but was not significantly different from baseline when corrected with baseline plasma reconstitution ($M = 287.9$ mg/dl ± 43.7), $t(39) = -.51$, $p = .62$, 95% CI [-12.47, 7.47], when corrected.
Figure 10. Differences in fibrinogen change across conditions (mean ± SEM). *Different from Baseline at $p = .015$. ^Different from Uncorrected at $p < .001$.

with saline reconstitution ($M = 280.3$ mg/dl ± $43.8$), $t(39) = 1.18$, $p = .24$, 95% CI [-3.60, 13.75] or when corrected arithmetically ($M = 281.74$ mg/dl ± $42.9$), $t(39) = 1.01$, $p = .32$, 95% CI [-3.71, 11.08]. At post-stress, there was no significant difference between uncorrected fibrinogen and fibrinogen corrected with baseline plasma reconstitution, $t(39) = 1.55$, $p = .13$, 95% CI [-1.59, 11.94]. However, uncorrected fibrinogen was significantly greater than fibrinogen corrected arithmetically, $t(39) = 7.24$, $p < .001$, 95% CI [8.19, 14.53] and fibrinogen corrected with saline reconstitution, $t(39) = 4.13$, $p < .001$, 95% CI [6.51, 18.99]. Fibrinogen corrected with baseline plasma reconstitution was marginally greater than fibrinogen corrected with saline reconstitution, $t(39) = 2.15$, $p =$
.038, 95% CI [0.43, 14.71], but not significantly different from fibrinogen corrected arithmetically, \( t(39) = 1.80, p = .079, 95\% \text{ CI} [-0.76, 13.13] \). Fibrinogen corrected with saline reconstitution was not significantly different than fibrinogen corrected arithmetically, \( t(39) = -0.49, p = .63, 95\% \text{ CI} [-7.11, 4.33] \). Uncorrected fibrinogen at recovery (\( M = 283.5 \text{ mg/dl} \pm 45.8 \)) was not significantly different from fibrinogen at baseline, \( t(39) = 1.00, p = .33, 95\% \text{ CI} [-4.85, 14.25] \). At recovery, plasma manipulation did not have any effect on fibrinogen, \( p’s > .15 \).

A secondary repeated measures analysis was conducted that only included participants who had the expected decrease in calculated plasma volume from baseline to post-stress (\( N=32 \)). Results more or less mirrored the results from the primary analysis, with the only notable differences being that fibrinogen at baseline was marginally greater than fibrinogen corrected arithmetically at post-stress, \( t(31) = 2.06, p = .048, 95\% \text{ CI} [0.09, 16.64] \). Significance levels for all other comparisons were not notably different.

**D-Dimer**

Due to cost, plasma was analyzed for D-dimer only at baseline and post-stress in only 37 participants. Repeated measures ANOVA indicated a marginally significant time-by-plasma manipulation interaction, \( F(1.85,66.64) = 2.93, p = .064 \). Given that D-dimer was only analyzed at baseline and stress, only 10 pairwise comparisons were made, with an adjusted significance level of \( \alpha_{adj} = .005 \). Figure 11 shows that D-dimer non-significantly increased from baseline (\( M = 207.6 \text{ ng/ml} \pm 72.1 \)) to post-stress when
Figure 11. Differences in D-dimer change across conditions (mean ± SEM). *Different from Uncorrected and Plasma Reconstitution at $p < .05$. ^Different from Uncorrected at $p < .001$.

uncorrected ($M = 214.9$ ng/ml ± 73.1), $t(36) = -1.70, p = .097$, 95% CI [-15.87, 1.38] and when corrected with baseline plasma reconstitution ($M = 212.4$ ng/ml ± 79.5), $t(36) = -0.85, p = .40$, 95% CI [-16.11, 6.62], but non-significantly decreased from baseline when corrected arithmetically ($M = 206.9$ ng/ml ± 70.1), $t(36) = 0.17, p = .87$, 95% CI [-8.09, 9.59], and when corrected with saline reconstitution ($M = 201.6$ ng/ml ± 74.8), $t(36) = 0.82, p = .42$, 95% CI [-8.78, 20.75]. There was no significant difference between uncorrected D-dimer and D-dimer corrected with baseline plasma reconstitution, $t(36) = 0.48, p = .63$, 95% CI [-7.99, 13.00]. Likewise, there was no significant difference
between D-dimer corrected arithmetically and D-dimer corrected with saline reconstitution, \( t(36) = -0.92, p = .37, 95\% \text{ CI} [-16.81, 6.34] \). However, uncorrected D-dimer was marginally greater than D-dimer corrected with saline reconstitution, \( t(36) = 2.22, p = .03, 95\% \text{ CI} [1.15, 25.31] \), and significantly greater than D-dimer corrected arithmetically, \( t(36) = 6.30, p < .001, 95\% \text{ CI} [5.42, 10.57] \). D-dimer corrected with baseline plasma reconstitution was marginally greater than D-dimer corrected with saline reconstitution, \( t(36) = 2.33, p = .025, 95\% \text{ CI} [1.40, 20.06] \), but was not significantly different from D-dimer corrected arithmetically, \( t(36) = 1.09, p = .28, 95\% \text{ CI} [-4.70, 15.69] \).

A secondary repeated measures analysis was conducted that only included participants who had the expected decrease in calculated plasma volume from baseline to post-stress (N=29). Results mirrored those of the primary analysis, with significance levels for all comparisons not being notably different.

**Factor VIII Clotting Activity**

Repeated measures ANOVA indicated a non-significant time-by-plasma manipulation interaction, \( F(2.93, 114.2) = 2.13, p = .10 \), a non-significant main effect for plasma manipulation, \( F(1.9, 114.2) = 0.71, p = .49 \), and a significant main effect for time, \( F(1.6, 114.2) = 4.06, p = .03 \). Given that the interaction was non-significant, only uncorrected FVIII:C at stress and recovery were compared to baseline, with the adjusted significance level set to \( \alpha_{adj} = .025 \). Figure 12 shows that uncorrected FVIII:C significantly increased from baseline (\( M = 129.0\% \pm 51.9 \)) to post-stress (\( M = 140.0\% \pm \))
Figure 12. Differences in FVIII:C (mean ± SEM). *Different from Baseline at p = .005.

^ Different from Baseline at p = .02.

65.3), t(39) = -2.96, p = .005, 95% CI [-18.55, -3.50], and remained significantly elevated at recovery (M = 142.2% ± 68.5), t(39) = -2.43, p = .02, 95% CI [-24.30, -2.20].

A secondary repeated measures analysis was conducted that only included participants who had the expected decrease in calculated plasma volume from baseline to post-stress (N=32). Results mirrored those of the primary analysis, with significance levels for all comparisons not being notably different.
Repeated measures ANOVA indicated a significant time-by-plasma manipulation interaction, $F(3.38, 128.51) = 7.2, p < .001$. Figure 13 shows that FVII:C marginally increased from baseline ($M = 119.1\% \pm 45.2$) to post-stress when uncorrected ($M = 122.6\% \pm 49.8$), $t(39) = -2.73, p = .01$, 95% CI [-6.25, -0.93], and when corrected with baseline plasma reconstitution ($M = 123.1\% \pm 48.4$), $t(39) = -2.66, p = .011$, 95% CI [-7.00, -0.95], but was not significantly different from baseline when corrected arithmetically ($M = 117.8\% \pm 47.6$), $t(39) = .92, p = .37$, 95% CI [-1.45, 3.87], or when corrected with saline reconstitution ($M = 120.6\% \pm 48.2$), $t(39) = -0.92, p = .36$, 95% CI [-4.96, 1.86]. At post-stress, there was no significant difference between uncorrected FVII:C and FVII:C corrected with baseline plasma reconstitution, $t(39) = -0.58, p = .58$, 95% CI [-3.09, 1.76], or between uncorrected FVII:C and FVII:C corrected with saline reconstitution, $t(39) = 1.18, p = .24$, 95% CI [-1.15, 4.38]. However, uncorrected FVII:C was significantly greater than FVII:C corrected arithmetically, $t(39) = 6.34, p < .001$, 95% CI [3.26, 6.33]; FVII:C corrected with baseline plasma reconstitution was marginally greater than FVII:C corrected with saline reconstitution, $t(39) = 2.39, p = .022$, 95% CI [0.37, 4.48]; and FVII:C corrected with saline reconstitution was marginally greater than FVII:C corrected arithmetically, $t(39) = 2.35, p = .024$, 95% CI [0.44, 5.92]. Uncorrected FVII:C at recovery ($M = 116.7\% \pm 41.9$) was not significantly different than FVII:C at baseline, $t(39) = 0.99, p = .33$, 95% CI [-2.54, 7.39]. At recovery, FVII:C corrected with baseline plasma reconstitution ($M = 117.2\% \pm 6.7$) was marginally greater than FVII:C corrected with saline reconstitution ($M = 115.9\% \pm 6.6$) $t(39) = 2.04,$
Figure 13. Differences in FVII:C across conditions (mean ± SEM). *Different from Baseline at \( p < .02 \). \(^*\)Different from Uncorrected at \( p = .039 \), 95% CI [0.07, 2.38]. No other comparisons at recovery were significant, \( p 's > .13 \).

A secondary repeated measures analysis was conducted that only included participants who had the expected decrease in calculated plasma volume from baseline to post-stress (N=32). Results more or less mirrored the results from the primary analysis, with the only notable differences being that FVII:C corrected with plasma reconstitution at recovery was not significantly different from FVII:C corrected with saline.
reconstitution, $t(31) = 1.95, p = .06, 95\% \text{ CI } [-0.06, 2.77]$. Significance levels for all other comparisons were not notably different.

*Activated Partial Thromboplastin Time*

Repeated measures ANOVA indicated a significant time-by-plasma manipulation interaction, $F(3.82, 148.8) = 15.27, p < .001$. Figure 14 shows that APTT significantly decreased from baseline ($M = 31.26 \pm 4.7$) to post-stress when uncorrected ($M = 30.55s \pm 4.8$), $t(39) = 3.92, p < .001, 95\% \text{ CI } [0.35, 1.08]$, and when corrected arithmetically ($M = 29.4s \pm 5.1$), $t(39) = 7.28, p < .001, 95\% \text{ CI } [1.35, 2.38]$, and marginally decreased when corrected with baseline plasma reconstitution ($M = 30.62s \pm 4.8$), $t(39) = 2.56, p = .015, 95\% \text{ CI } [0.13, 1.15]$, whereas APTT was no longer significantly different from baseline when corrected with saline reconstitution ($M = 31.18s \pm 4.9$), $t(39) = 0.33, p = .74, 95\% \text{ CI } [-0.43, 0.60]$. At post-stress, there was no significant difference between uncorrected APTT and APTT corrected with baseline plasma reconstitution, $t(39) = -0.40, p = .69, 95\% \text{ CI } [-0.42, 0.28]$. However, uncorrected APTT was significantly greater than APTT corrected arithmetically, $t(39) = 6.98, p < .001, 95\% \text{ CI } [0.82, 1.49]$, and marginally less than APTT corrected with saline reconstitution, $t(39) = -2.88, p = .006, 95\% \text{ CI } [-1.07, -0.82]$. APTT corrected with baseline plasma reconstitution was significantly greater than APTT corrected arithmetically, $t(39) = 5.09, p < .001, 95\% \text{ CI } [0.74, 1.71]$, and marginally less than APTT corrected with saline reconstitution, $t(39) = -2.24, p = .031,$
Figure 14. Differences in APTT across conditions (mean ± SEM). *Different from Baseline and Arithmetic Correction at $p < .001$ and Saline Reconstitution at $p = .006$.

^Different from Baseline and Saline Reconstitution at $p < .04$. +Different from Baseline, Uncorrected, Plasma Reconstitution and Saline Reconstitution at $p < .001$.

95% CI [-1.06, -0.05]. APTT corrected with saline reconstitution was significantly greater than APTT corrected arithmetically, $t(39) = 5.86, p < .001, 95\% \text{ CI } [1.17, 2.39]$. Uncorrected APTT at recovery ($M = 30.67s \pm 4.7$) was not significantly different than APTT at baseline, $t(39) = 1.79, p = .082, 95\% \text{ CI } [-0.8, 1.27]$. At recovery, plasma manipulation did not have any effect on APTT, $p’s > .17$. 
A secondary repeated measures analysis was conducted that only included participants who had the expected decrease in calculated plasma volume from baseline to post-stress (N=32). Results more or less mirrored the results from the primary analysis, with the only notable differences being that APTT at baseline was marginally greater than uncorrected APTT at recovery, $t(31) = 2.55, p = .016, 95\% \text{ CI } [0.15, 1.32]$. Significance levels for all other comparisons were not notably different.

*Prothrombin Time*

Repeated measures ANOVA indicated a significant time-by-plasma manipulation interaction, $F(3.38, 131.99) = 7.51, p < .001$. Figure 15 shows that PT% significantly increased from baseline ($M = 93.4\% \pm 21.4$) to post-stress when uncorrected ($M = 96.7\% \pm 24.5$), $t(39) = -3.46, p = .001, 95\% \text{ CI } [-5.19, -1.36]$, and marginally increased when corrected with baseline plasma reconstitution ($M = 96.3\% \pm 23.3$), $t(39) = -2.62, p = .012, 95\% \text{ CI } [-5.14, -0.66]$, but was not significantly different from baseline when corrected with saline reconstitution ($M = 92.2\% \pm 21.7$), $t(39) = 0.99, p = .33, 95\% \text{ CI } [-1.32, 3.87]$, or when corrected arithmetically ($M = 93.0\% \pm 24.0$), $t(39) = 0.43, p = .67, 95\% \text{ CI } [-1.63, 2.50]$. At post-stress, there was no significant difference between uncorrected PT% and PT% corrected with baseline plasma reconstitution, $t(39) = 0.45, p = .65, 95\% \text{ CI } [-1.30, 2.05]$, or between PT% corrected with saline reconstitution and PT% corrected arithmetically, $t(39) = -0.92, p = .36, 95\% \text{ CI } [-2.68, 1.00]$. However, uncorrected PT% was significantly greater than PT% corrected arithmetically, $t(39) = 7.22, p < .001, 95\% \text{ CI } [2.67, 4.75]$, and PT% corrected with saline reconstitution, $t(39) = 4.05, p < .001, 95\% \text{ CI } [2.28, 6.82]$. PT% corrected with baseline plasma reconstitution was significantly
Figure 15. Differences in PT% across conditions (mean ± SEM). *Different from Baseline, Saline Reconstitution and Arithmetic Correction at \( p < .001 \). \(^\wedge\)Different from Baseline at \( p = .012 \) and different from Saline Reconstitution and Arithmetic Correction at \( p < .001 \).

greater than PT% corrected arithmetically, \( t(39) = 3.86, p < .001, 95\% \text{ CI} [1.59, 5.08] \), and PT% corrected with saline reconstitution, \( t(39) = 3.85, p < .001, 95\% \text{ CI} [1.98, 6.37] \).

Uncorrected PT% at recovery (\( M = 92.2\% ± 21.5 \)) was not significantly different than PT% at baseline, \( t(39) = 0.90, p = .37, 95\% \text{ CI} [-1.49, 3.89] \). At recovery, plasma manipulation did not have any effect on PT%, \( p \)'s > .15.

A secondary repeated measures analysis was conducted that only included participants who had the expected decrease in calculated plasma volume from baseline to
post-stress (N=32). Results mirrored those of the primary analysis, with significance levels for all comparisons not being notably different.

Table 3 shows effect sizes (Cohen’s $d$) for all conducted pairwise comparisons.
Table 3.

*Estimated Effect Sizes for Pairwise Comparisons*  

<table>
<thead>
<tr>
<th>Pairwise Comparison</th>
<th>Fibrinogen</th>
<th>D-Dimer</th>
<th>FVIII:C</th>
<th>FVII:C</th>
<th>APTT</th>
<th>PT%</th>
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<tbody>
<tr>
<td>Baseline - Stress(_U)</td>
<td>0.17</td>
<td>0.10</td>
<td>0.19</td>
<td>0.08</td>
<td>0.15</td>
<td>0.15</td>
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<tr>
<td>Baseline – Stress(_B)</td>
<td>0.05</td>
<td>0.06</td>
<td>0.19</td>
<td>0.09</td>
<td>0.13</td>
<td>0.13</td>
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<td>Baseline – Stress(_S)</td>
<td>0.11</td>
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<td>0.14</td>
<td>0.04</td>
<td>0.02</td>
<td>0.06</td>
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<tr>
<td>Baseline – Stress(_A)</td>
<td>0.08</td>
<td>0.01</td>
<td>0.10</td>
<td>0.03</td>
<td>0.38</td>
<td>0.02</td>
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<tr>
<td>Stress(_U) – Stress(_B)</td>
<td>0.12</td>
<td>0.03</td>
<td>0.02</td>
<td>0.01</td>
<td>0.02</td>
<td>0.02</td>
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<tr>
<td>Stress(_U) – Stress(_S)</td>
<td>0.29</td>
<td>0.18</td>
<td>0.05</td>
<td>0.03</td>
<td>0.13</td>
<td>0.20</td>
</tr>
<tr>
<td>Stress(_U) – Stress(_A)</td>
<td>0.26</td>
<td>0.11</td>
<td>0.08</td>
<td>0.10</td>
<td>0.23</td>
<td>0.15</td>
</tr>
<tr>
<td>Stress(_B) – Stress(_S)</td>
<td>0.18</td>
<td>0.14</td>
<td>0.05</td>
<td>0.05</td>
<td>0.11</td>
<td>0.19</td>
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<tr>
<td>Stress(_B) – Stress(_A)</td>
<td>0.14</td>
<td>0.08</td>
<td>0.08</td>
<td>0.11</td>
<td>0.25</td>
<td>0.14</td>
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<tr>
<td>Stress(_S) – Stress(_A)</td>
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<td>0.08</td>
<td>0.03</td>
<td>0.07</td>
<td>0.36</td>
<td>0.04</td>
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<tr>
<td>Baseline - Recovery(_U)</td>
<td>0.10</td>
<td>N/A</td>
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<td>0.05</td>
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<td>Baseline – Recovery(_B)</td>
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<td>0.21</td>
<td>0.07</td>
<td>0.11</td>
<td>0.08</td>
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<td>Baseline – Recovery(_A)</td>
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<td>N/A</td>
<td>0.24</td>
<td>0.04</td>
<td>0.05</td>
<td>0.02</td>
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</table>

*Note.* Subscript \(U\) refers to Uncorrected values. Subscript \(B\) refers to Baseline Plasma Reconstitution values. Subscript \(S\) refers to Saline Reconstitution values. Subscript \(A\) refers to Arithmetically Corrected values.
DISCUSSION

The purpose of this study was to examine a new method of studying the association between stress-hemoconcentration and stress-induced alterations in the coagulation system. Previous research in this area has examined such relationships using correlations (Zgraggen et al., 2005), analysis of covariance (Jern, Eriksson, et al., 1989; Steptoe, Kunz-Ebrecht, Owen, et al., 2003; Steptoe, Kunz-Ebrecht, Rumley, et al., 2003) or arithmetic correction (von Känel, Kudielka, et al., 2009). However, these methods have limitations upon which this study attempted to improve. For instance, correlations are not very powerful and analysis of covariance only controls for variation in changes in hematocrit and plasma volume. Moreover, arithmetic correction using the Dill and Costill (1974) formula is designed to correct for changes in concentrations of large, non-diffusible molecules that occur with plasma volume shifts, but may not correct appropriately for changes in clotting activity or clotting time. In this study, a novel, physical reconstitution procedure was tested against the Dill and Costill arithmetic correction for hemoconcentration effects. Specifically, plasma collected at the end of a mental arithmetic stressor and at the end of a 20-minute recovery period was reconstituted with the person’s own plasma collected at baseline and with physiological saline such that plasma volume was equivalent to plasma volume at baseline.

In general, the results of this study revealed that the mental arithmetic stressor evoked significant changes in hemodynamic, hemoconcentration and coagulation activity. Increases in blood pressure during acute stress force plasma into interstitial spaces. Therefore, the experimental manipulation produced the hemodynamic changes
necessary to produce the expected significant decrease in plasma volume. Specifically, hematocrit increased and plasma volume decreased during mental arithmetic, and both returned to baseline levels 20 minutes after the stressor. Without correction for stress-induced hemoconcentration, APTT decreased and FVIII:C increased from baseline to stress. Though non-significant, uncorrected APTT at recovery remained faster at recovery relative to baseline, whereas FVIII:C remained significantly elevated at recovery relative to baseline. Together, these results suggest heightened activity of the intrinsic pathway of the coagulation cascade during stress that remained elevated 20 minutes after termination of the stressor. Prothrombin time (%) and FVII:C increased from baseline to stress, and returned to baseline levels during recovery, suggesting a transient elevation of activity of the extrinsic pathway of the coagulation cascade during stress that quickly returned to baseline levels following the termination of the stressor. Fibrinogen concentration also increased significantly from baseline to stress and returned to baseline levels at recovery, while D-dimer concentration did not increase significantly from baseline to stress.

Altogether, the mental arithmetic stressor resulted in marked changes in coagulation. However, the specific aims of the current investigation were proposed to assess the efficacy of the reconstitution methods for correcting for plasma volume shifts and to test the reconstitution method against the usual arithmetic correction developed by Dill and Costill (1974).

The first specific aim of the study was to examine whether any observed increases in fibrinogen, D-dimer, FVII:C and FVIII:C during acute psychological stress could be reversed by using the person’s own plasma from baseline to reconstitute the
hemoconcentrated stress and recovery plasma back to baseline plasma volume levels. It was hypothesized that when reconstituted with the person’s own baseline plasma back to baseline plasma volume, plasma obtained during an acute math stressor or during recovery would no longer have different fibrinogen or D-dimer concentrations or different FVII:C than plasma obtained during baseline. Conversely, it was hypothesized that plasma obtained during the math stressor or recovery that was reconstituted with baseline plasma would still have elevated FVIII:C than plasma obtained during baseline.

As predicted, fibrinogen levels were no longer significantly different from baseline levels after baseline plasma reconstitution, though levels were still somewhat elevated compared to uncorrected levels, suggesting that reconstitution with the person’s own plasma from baseline incompletely reduced the fibrinogen concentration toward baseline levels. Though the changes in D-dimer from baseline to stress were non-significant for both uncorrected levels and for levels after baseline plasma reconstitution, they were in the expected direction and the pattern of results mirrored those of fibrinogen. However, these results are somewhat at odds with the only previous study examining these reconstitution methods, which reported that D-dimer concentration after the Trier Social Stress Test (TSST) remained greater than the baseline concentration after baseline plasma reconstitution (Austin, Wirtz, Patterson, Stutz, & von Känel, under review). Possible explanations for this discrepancy include the use of different study protocols. In the current study, participants were tested at various times throughout the day and had a 20 minute baseline period, whereas participants in Austin et al. (under review) were tested at the same time of day and had a 120 minute baseline period.
Therefore, the baseline in Austin et al. may have been a truer baseline than the baseline in the current investigation. These differences in the protocol may have produced the slight differences in the relationships observed. On the other hand, like the previous investigation (Austin et al.), uncorrected D-dimer concentration in the current study was not significantly different from D-dimer concentration after correction with baseline plasma reconstitution.

Contrary to fibrinogen and D-dimer, FVII:C and FVIII:C during stress did not change after baseline plasma reconstitution, suggesting that reconstitution with the person’s own plasma from baseline did not significantly alter the plasma’s environment. This result was predicted for FVIII:C, because previous research (von Känel, Preckel, et al., 2004; von Känel, Kudielka, et al., 2009; Austin et al., under review) suggests that the intrinsic pathway of the coagulation cascade (i.e., FVIII:C) is genuinely activated during acute stress. Conversely, these results did not support the prediction for FVII:C. More FVII is introduced to the plasma collected during stress, but the FVII that is introduced to the stressor plasma has less activity than the FVII (i.e., FVII:C) in the plasma collected during stress. However, this introduced FVII combined with the FVII in the stressor plasma does not appear to reduce the total activity of FVII. In other words, reconstitution with the person’s own plasma from baseline did not alter FVII:C during stress. No comparisons to previous research can be made, as Austin et al. (under review) did not examine FVII:C.

At recovery, fibrinogen and FVII:C returned to baseline levels when uncorrected, and baseline plasma reconstitution did not alter their levels. When uncorrected, FVIII:C
remained elevated at recovery, and, as hypothesized, baseline plasma reconstitution did not alter FVIII:C. Unfortunately, D-dimer was not assessed at recovery due to the cost-prohibitiveness of the ELISA. Altogether, after baseline plasma reconstitution, FVII:C remained elevated during stress and FVIII:C remained elevated during stress and during recovery, whereas fibrinogen and D-dimer were not significantly different from baseline. Thus, baseline plasma reconstitution removed hemoconcentration effects when examining concentrations but had no effect when examining clotting activity.

The second specific aim of this study was to examine whether any observed increases in fibrinogen, D-dimer, FVII:C and FVIII:C during acute psychological stress could be reversed by using physiological saline during stress or during recovery back to baseline plasma volume levels. Compared to the person’s own plasma from baseline, physiological saline is more likely to be representative of the filtrate that is lost through capillary pores during acute stress, as this filtrate is free of large, non-diffusible molecules. Similar to the hypotheses for baseline plasma reconstitution, it was hypothesized that when reconstituted with physiological saline, plasma obtained during an acute math stressor or during recovery would no longer have different fibrinogen or D-dimer concentrations or different FVII:C than plasma obtained during baseline, but would still have elevated FVIII:C than plasma obtained during baseline.

As predicted, fibrinogen levels were no longer significantly different from baseline levels after saline reconstitution. In fact, fibrinogen concentration after saline reconstitution was somewhat less than the baseline concentration, suggesting that the stress-induced increase in fibrinogen was completely wiped away and that the increase in
fibrinogen was due to hemoconcentration. Similar to the baseline plasma reconstitution, the changes in D-dimer from baseline to stress were non-significant for both uncorrected levels and for levels after saline reconstitution, but were in the expected direction and mirrored those of fibrinogen. These results are to be expected, given that this saline reconstitution technique dilutes the stressor plasma in such a way that the concentrations of fibrinogen and D-dimer should be equivalent to baseline levels. These results are in line with previous research (Austin et al., under review), which showed that saline reconstitution after the TSST resulted in D-dimer concentration no longer being significantly different from the baseline concentration.

Like fibrinogen and D-dimer, when reconstituted with saline reconstitution after stress, FVII:C was not significantly different than FVII:C at baseline. Though it is contrary to the result observed for baseline plasma reconstitution, this result was expected and suggests that saline reconstitution reduced the activity of FVII. When plasma becomes more diluted, FVII has less opportunity to interact with other factors in the coagulation system and with the endothelium, thereby reducing its activity. In effect, hemoconcentration seems to have been responsible for the increase in FVII:C. On the other hand, FVIII:C remained elevated at stress after saline reconstitution, suggesting actual activation of FVIII independent of hemoconcentration. This result was as predicted and is in line with previous research (Austin et al., under review; von Känel, Preckel, et al., 2004; von Känel, Kudielka, et al., 2009) which suggests that the intrinsic pathway of the coagulation system becomes genuinely activated during acute stress.
At recovery, fibrinogen and FVII:C returned to baseline levels when uncorrected, and saline reconstitution did not alter their levels. When uncorrected, FVIII:C remained elevated at recovery, and, as hypothesized, saline reconstitution did not alter FVIII:C. Altogether, after saline reconstitution, FVIII:C remained elevated during stress and during recovery, whereas fibrinogen, D-dimer and FVII:C were not significantly different from baseline. Thus, saline reconstitution removed hemoconcentration effects when examining concentrations and clotting activity of the extrinsic pathway but had no effect when examining clotting activity of the intrinsic pathway.

The third specific aim of this study was to examine whether stress-hemoconcentration influences clotting time of the intrinsic and extrinsic pathways of the clotting cascade. It was hypothesized that uncorrected APTT (i.e., clotting time of the intrinsic pathway) and PT% (i.e., clotting time of the extrinsic pathway) during stress or during recovery would not be different than APTT and PT% at baseline after reconstitution with either baseline plasma or physiological saline. Uncorrected, APTT decreased significantly from baseline to stress, indicating faster clotting of the intrinsic pathways. However, APTT corrected with baseline plasma reconstitution (30.62 seconds) was only marginally faster than APTT at baseline (31.26 seconds), despite being slightly slower than uncorrected APTT (30.55 seconds). This marginal finding is most likely due to the larger standard deviation observed in the paired t-test comparing APTT at baseline with APTT corrected with baseline plasma correction (SD = 1.58) than in the t-test comparing APTT at baseline with uncorrected APTT (SD = 1.15). However, uncorrected APTT and APTT corrected with baseline plasma reconstitution were not significantly
different from each other. Altogether, baseline plasma reconstitution appeared to have meager effects on APTT. On the other hand, saline reconstitution resulted in APTT at stress (31.18 seconds) being essentially the same as APTT at baseline (31.26 seconds), indicating that saline reconstitution removed the effects of stress on APTT. At recovery, APTT returned to baseline levels when uncorrected, and neither reconstitution procedure altered it.

Uncorrected, PT% increased significantly from baseline (93.4%) to stress (96.7%), indicating faster clotting of the extrinsic pathway, as PT% is inversely related to PT in seconds. However, PT% corrected with baseline plasma reconstitution (96.3%) was only marginally greater than PT% at baseline (93.7%), indicating that baseline plasma reconstitution caused a very small reduction in PT%. On the other hand, saline reconstitution resulted in APTT at stress (92.2%) not being significantly different from PT% at baseline (93.7%), indicating that saline reconstitution removed the effects of stress on PT%. At recovery, PT% returned to baseline levels when uncorrected, and neither reconstitution procedure nor arithmetic correction altered it.

Baseline plasma reconstitution likely did not influence APTT and PT% for many of the same reasons that it did not influence FVII:C and FVIII:C. That is, the plasma’s environment is slightly altered by introducing the person’s own plasma from baseline, but this alteration is not great enough to cause any significant change in clotting time. Conversely, saline reconstitution removed the stress effects on APTT and PT%, suggesting that faster clotting times during stress are a consequence of hemoconcentration. These results are directly in line with previous research that showed
that baseline plasma reconstitution had no effect on APTT and PT% at stress, whereas saline reconstitution resulted in APTT and PT% not being different from baseline (Austin et al., under review). However, this result is somewhat at odds with other research that suggests the intrinsic pathway is actually activated during acute stress (von Känel, Preckel, et al., 2004; von Känel, Kudielka, et al., 2009). If there was complete, actual activation of the intrinsic pathway, then APTT at stress should have survived correction with saline reconstitution. Nevertheless, one must bear in mind that these previous studies suggested that FVIII:C is genuinely activated, but not necessarily APTT.

The final specific aim of this study was to test the baseline plasma and saline reconstitution methods against the usual arithmetic correction developed by Dill and Costill (1974). The Dill and Costill formula appears to be appropriate when correcting for the effects of plasma volume shifts on concentrations of large, non-diffusible molecules, but seems to improperly adjust when examining clotting time and clotting activity, as was suggested by two previous studies (von Känel, Kudielka, et al., 2009; Austin et al., under review). When examining concentrations in the current investigation, arithmetic correction resulted in concentrations of fibrinogen and D-dimer during stress not being significantly different from baseline. During stress, fibrinogen corrected arithmetically was marginally less than fibrinogen corrected with baseline plasma reconstitution and not significantly different than fibrinogen corrected with saline reconstitution. However, both the uncorrected concentration and the concentration after correction with baseline plasma reconstitution were greater than baseline, whereas the arithmetically corrected concentration and the concentration after saline reconstitution were less than baseline. D-
dimer corrected arithmetically was not significantly different than D-dimer corrected with either reconstitution technique, though the pattern of results was similar to that of fibrinogen. Altogether, either arithmetic correction or correction with saline reconstitution appears to be appropriate techniques for correcting for the effects of plasma volume shifts (i.e., hemoconcentration) on the concentrations of fibrinogen and D-dimer. Conversely, correction with baseline plasma reconstitution appears to insufficiently correct for plasma volume shifts, because the composition of the baseline plasma is not sufficiently different enough from the composition of the plasma during stress. Furthermore, the amount of baseline plasma used to reconstitute one milliliter of plasma from stress was typically very small ($M = 55.4 \mu l$). Therefore, reconstitution with the person’s own plasma from baseline did not have substantial effects on the concentrations of fibrinogen or D-dimer. It is likely that arithmetic correction and correction with saline reconstitution are also appropriate and correction with baseline plasma reconstitution is inappropriate for other large, non-diffusible molecules, such as lipids, plasma proteins or other clotting factors.

Though there was no significant interaction between time and plasma manipulation, examination of the data suggests FVIII:C after arithmetic correction or after correction with saline reconstitution was less than uncorrected FVIII:C or FVIII:C corrected with baseline plasma reconstitution. However, both during stress and recovery, no correction technique completely removed the effects of stress-hemoconcentration on FVIII:C, suggesting that FVIII:C increases during stress independent of hemoconcentration. Thus, in line with previous studies (von Känel, Preckel, et al., 2004;
von Känel, Kudielka, et al., 2009; Austin et al., under review), the intrinsic pathway of the coagulation system appears to be truly activated during acute psychological stress.

The significant effects of stress on FVII:C were removed after arithmetic correction and after correction with saline reconstitution. In spite of this, closer examination of the data suggests that saline reconstitution only partially removed hemoconcentration effects, whereas only arithmetic correction completely eliminated hemoconcentration effects. Given the findings for fibrinogen, D-dimer and FVIII:C, however, one would expect saline reconstitution and arithmetic correction to exhibit equivalent correction effects, as saline reconstitution is basically the physical equivalent of arithmetic correction. Based on these findings, it is unclear which plasma volume correction technique is most appropriate for FVII:C.

When examining clotting time, both arithmetic correction and saline reconstitution removed the effects of stress on PT% in the current investigation. However, the arithmetically corrected finding is at odds with previous studies (von Känel, Kudielka, et al., 2009; Austin et al., under review), which showed that arithmetically adjusted PT% was less than PT% at baseline, suggesting that correction for hemoconcentration resulted in slower clotting time of the extrinsic pathway. Austin et al. suggested that the Dill and Costill (1974) formula over-corrected for changes in PT% associated with plasma volume shifts. It is not entirely clear, however, why the Dill and Costill formula did not over-correct in the current study.

Arithmetic correction appeared to improperly adjust for APTT in the current study. Similar to previous findings (von Känel, Kudielka, et al., 2009; Austin et al., under
review), instead of APTT being adjusted back toward its baseline level, it decreased, suggesting that clotting speed becomes faster when taking into account shifts in plasma volume. However, this finding is incommensurate with the finding that FVIII:C decreases slightly and fibrinogen is no longer different from baseline after arithmetic adjustment. One would expect a decrease in FVIII:C and fibrinogen to be accompanied by a prolongation in APTT. In other words, a reduction in a clotting factor should be associated with a slower clotting response. Additionally, the Dill and Costill (1974) formula is designed to mathematically adjust the concentration of a physiological parameter based on changes in plasma volume but appears to be of limited utility when correcting for plasma volume changes on time dependent functional assays such as clotting time tests. Baseline plasma reconstitution, on the other hand, did not have any effect on APTT, whereas saline reconstitution completely removed the effects of stress-hemoconcentration on APTT. Saline reconstitution appears to be the most biologically relevant method of correcting for the effects of hemoconcentration on APTT, as APTT reaches a level corresponding to FVIII:C and fibrinogen.

Summary of Results

In sum, the different hemoconcentration correction techniques yielded different results. Mathematical adjustment appears to be practical when examining the effects of plasma volume shifts on large molecular weight substances, such as D-dimer and fibrinogen, but produces results that are difficult to interpret when examining parameters measured via functional assays, such as clotting time and clotting activity. The baseline plasma reconstitution technique resulted in incomplete removal of the stress effects on
fibrinogen and D-dimer and had negligible effects on clotting time and clotting activity. Therefore, reconstituting a plasma sample with the person’s own plasma collected at baseline does not appear to be a meaningful hemoconcentration correction technique. The saline reconstitution technique appears to be the most biologically relevant correction method when examining stress-hemoconcentration effects on clotting time and clotting activity, but also emerged as an appropriate hemoconcentration correction technique for adjusting changes in concentrations of large, non-diffusible molecules (e.g., fibrinogen and D-dimer). FVIII:C was the only parameter under study whose stress-induced increase survived all three hemoconcentration correction techniques. Additionally, FVIII:C remained elevated 20 minutes after cessation of the stressor, whereas the other parameters had returned to baseline levels, indicating a prolonged intrinsic pathway response. Thus, as indexed by the findings for FVIII:C, strong support was found for genuine, actual activation of the intrinsic pathway during and in the time after an acute psychological stressor. On the whole, the results of the current investigation suggest that stress-induced changes in coagulation are a product of both hemoconcentration and actual stimulation of the coagulation system.

Atypical Responders

Plasma volume decreases in the majority of individuals who undergo an acute psychological stressor, most likely due to sympathetic-induced increases in hydrostatic pressure and subsequent movement of fluid into extravascular spaces and consequent concentration of non-diffusible blood components (Allen & Patterson, 1995; Muldoon et al., 1995; Patterson et al., 1998; Austin et al., in press). However, with the exception of
Austin et al. (under review), most previous studies (e.g., Muldoon et al., 1995; Patterson et al., 1995; Patterson et al., 1998; Veldhuijzen van Zanten et al., 2004) have not reported the number of participants who did not show the expected hemoconcentration differences. In the current investigation, eight of the forty participants showed an unexpected increase in calculated plasma volume, which is in contrast to only one out of thirty participants who showed an increase in plasma volume in Austin et al. (under review). Given that these eight participants showed the expected increases in blood pressure and heart rate, it is unclear why they did not have the expected changes in calculated plasma volume. Participants were requested to not consume any food in the four hours prior to their experimental session, but were allowed to consume water at any time leading up to their session. When dehydrated individuals consume a large amount of water, plasma volume tends to increase by approximately 1% within 30 minutes, which then lasts at least 70 minutes (Greenleaf, Jackson, Geelen, Keil, Hinghofer-Szalkay, & Whittam, 1998). Therefore, it is possible that these participants could have consumed a large amount of water immediately prior to their session, thereby temporarily increasing plasma volume which, in turn, could have outweighed plasma volume changes that would normally occur.

No reconstitution of these eight participants’ plasma could be performed, and the reconstituted values for coagulation measures were set to the uncorrected levels for the primary analyses. When omitting these participants, no outstanding changes in the results were observed. Nevertheless, future investigations are necessary to untangle why most, but not all, individuals are susceptible to hemoconcentration effects during stress.
Potential unmeasured behavioral, psychological or physiological variables may account for some of the variation in stress-induced changes in plasma volume and associated coagulation changes.

Potential Unmeasured Mediators and Moderators

Several unmeasured mediators and moderators could account for some proportion of the relationships observed in the current investigation. For instance, usual water consumption was not measured. If one does not typically drink the recommended daily amount of water, then one will likely have a reduced plasma volume initially and less plasma is likely to be lost during acute stress. Conversely, if one typically drinks large amounts of water, then one will likely have an expanded plasma volume initially and potentially more plasma will be lost during acute stress. Adjusting for hydration status via bioimpedance would help to control possible influences of one’s typical water consumption on plasma volume shifts.

Several physiological parameters could also play a role in the observed changes in plasma volume and coagulation observed in the present study. For example, as demonstrated by de Boer, Ring, Curlett, et al. (2007) and de Boer, Ring, Wood, et al. (2007), changes in colloid osmotic pressure parallel changes in hematocrit and arterial pressure and may act as an underlying mechanism of stress-hemoconcentration, especially during recovery. Whether colloid osmotic pressure accounts for any of the observed increases in coagulation during stress remains to be elucidated.

Nuclear factor kappa B (NF-κB), another factor that may play a mediating or moderating role, is a pro-inflammatory, pro-coagulant genetic transcription factor that is
activated by increased shear stress (Boon & Horrevoets, 2009) as well as by such cardiovascular risk factors as hypertension, obesity, diabetes mellitus, smoking and hyperlipidemia (Bierhaus, Humpert, & Nawroth, 2004). When an organism faces harmful situations, NF-κB directly and indirectly controls a wide variety of biological responses in cells. In chronic disease, pathophysiological factors like oxidative stress, cytokines and growth factors induce NF-κB activation and nuclear translocation in monocytes, macrophages, endothelial cells, and smooth muscle cells. After nuclear translocation, NF-κB binds to DNA sequences and stimulates gene expression for cytokines, adhesion molecules and coagulation factors, thereby promoting endothelial dysfunction (Collins & Cybulsky, 2001). Dysregulation of an autoregulatory mechanism to terminate NF-κB activation may further promote vascular dysfunction through continued through activity of cytokines, adhesion molecules and coagulation factors (Bierhaus et al., 2001). Acute psychological stress increases shear stress, thereby stimulating NF-κB activation. Therefore, NF-κB activation could be a key mediator between stress, elevated coagulation activity and atherogenesis (Bierhaus et al., 2003; 2004). Moreover, given that stress-hemoconcentration is associated with greater shear stress (Allen & Patterson, 1995), interrelationships between NF-κB, coagulation and hemoconcentration during stress should be examined in the future.

It should be acknowledged that many contemporary hematologists (e.g., Hoffman, 2003; Malý et al., 2007; Smith, 2009) have adopted a cell-based model of coagulation as opposed to the cascade model, because the cascade model has limitations as a model of hemostatic processes in vivo. In this model, procoagulant forces are restricted to sites of
vessel damage rather than extending through the vasculature. Such control of coagulation is accomplished by restricting procoagulant reactions to the site of injury on the surface of cells, chiefly platelets and tissue-factor bearing cells. These two types of cell interact to either inhibit or hasten clotting (Smith, 2009). However, existing assays used to assess tissue factor activity and clotting in vivo lack sensitivity and specificity (Kasthuri, Glover, Boles, & Mackman, 2010). Furthermore, an examination of acute clotting changes in vivo such as those examined in the current investigation is challenging and often unfeasible. Thus, stress research has been restricted to changes measured in vitro within the bounds of the cascade model (Austin et al., in press). However, as biomedical technology improves, cardiovascular behavioral medicine researchers should begin to examine clotting and hemoconcentration in vivo.

Clinical Implications

Acute stress has been suggested to initiate atherothrombosis and subsequent acute coronary syndromes (Strike & Steptoe, 2005; Thrall et al., 2007). Defining mechanisms of this link is a chief issue in cardiovascular behavioral medicine. One potential mechanism is through perturbed hemostasis activity. Disruption of hemostatic balance (i.e., balance between coagulation and fibrinolysis) is a crucial factor in atherosclerotic development and CHD. If a plaque ruptures, vessel occlusion is more likely if the ratio of prothrombosis activity to fibrinolysis activity is high. During psychological stress both prothrombosis and fibrinolysis activity increases, but prothrombosis activity typically increases more than does fibrinolysis activity. The resulting net hypercoagulability may put an individual at greater risk for thrombotic coronary occlusion after plaque
disruption, especially in individuals with established atherosclerosis or endothelial
dysfunction (von Känel, Dimsdale, et al, 2001; Thrall et al., 2007; Austin et al., in press).

The results of the present study suggest that hemoconcentration may contribute to
stress-induced changes in hemostasis activity. Contracted plasma volume may provide a
prothrombotic intravascular environment by temporarily allowing clotting factors to be
physically closer together and by exposing the endothelium to a greater amount of
prothrombotic molecules. By increasing the relative concentration, but not absolute
number of cells, the endothelium still “sees” more of that prothrombotic molecule.
Furthermore, when their concentrations increase, clotting factors have greater opportunity
to act on each other and on platelets, which in turn can accelerate clotting (e.g., APTT
and PT). Such an intravascular environment resulting from acute stress could promote
atherogenesis or trigger a coronary event, especially in patient populations, pointing to
the clinical significance of stress-hemoconcentration.

Limitations of the Present Study

Several limitations of the present study must be acknowledged. First, due to lack
of funding, D-dimer was not measured at recovery. However, given that Austin et al.
(under review) reported that D-dimer returned to baseline levels and that no plasma
volume correction had any effect on D-dimer at recovery and given that reconstitution
had no effect on any parameter at recovery in the current investigation, it is expected that
D-dimer would have returned to baseline levels and that reconstitution would not have
influenced its concentration. Second, power to detect effects for D-dimer could have been
especially low, as the effect size for uncorrected change in this ($d = .10$) and previous
studies (\(d = .06\); e.g., von Känel, Kudielka, et al., 2009) were quite low. More participants may have been necessary to detect D-dimer effects. Third, participants self-reported that they abstained from alcohol, exercise, food and drink. Though a breathalyzer was used to verify abstinence from consuming alcohol, no such mechanism was available to verify abstinence from exercise, food and drink. One participant’s plasma was visibly cloudy, suggesting recent ingestion of fatty food. However, this participant’s pattern of plasma volume and coagulation changes did not deviate from the overall pattern of changes, suggesting that, even if he had elevated blood lipids, it did not affect the hemoconcentration and coagulation mechanisms under study. Fourth, given the very small amounts of plasma and saline used in the reconstitution procedure, potential pipetting errors were possible, potentially skewing results. Fifth, race/ethnicity was not recorded. A few of the participants appeared to be of Asian descent and, during debriefing, reported that the stressor was especially difficult due to language barriers. Sixth, the results of the current study cannot be generalized to women, middle-aged and older men, or individuals with disease, as the study sample consisted solely of university students. This is especially important given that hypercoagulability may be particularly harmful in individuals with developed coronary heart disease. Seventh, as previously discussed, some individuals showed a “hemodilution” pattern of plasma volume shifts during stress. Finally, all values at baseline were the same across all four plasma manipulations, which could have possibly artificially created the relationships observed in this study. However, this is unlikely, because separate repeated measures analyses examining each correction technique individually with only time as the repeated measure
produced results commensurate with those observed in the 3(Time) X 4(Plasma Manipulation) ANOVA (analyses not shown).

Directions for Future Research

This study points to the need of a number of future investigations. Primarily, replication is necessary across different populations. As mentioned in the limitations section, similar research should be extended to women, older men, and heart disease populations. In addition, replication is needed across different stressors. The mental arithmetic task in the current study could be construed as an active coping task associated with a myocardial response, whereby blood pressure increases primarily due to an increase in cardiac output (Obrist, 1981). With the exception of a few early studies (Schneider & Zangari, 1951; Kast Zweibel, 1954; Bidder & Agle, 1972), most previous hemoconcentration and coagulation studies have also used active coping tasks (e.g., TSST, Stroop color-word interference test). It is not known if the hemoconcentration and coagulation responses observed in this study would be seen in a study employing an inhibitory-passive coping task, such as the cold pressor. Inhibitory-passive coping tasks are associated with a vascular response, whereby blood pressure increases primarily due to an increase in total peripheral resistance (Obrist, 1981). Thus, more research is needed to determine whether specific underlying aspects of blood pressure (i.e., cardiac output and total peripheral resistance) are the driving force behind plasma volume shifts during stress and subsequent hemoconcentration of coagulation molecules.

The cardiovascular reactivity hypothesis suggests that exaggerated cardiovascular responses to acute psychological stress may be a risk factor for cardiovascular disease
(Manuck, 1994). However, no known study has examined whether hemoconcentration or coagulation reactivity confers any risk for future development of cardiovascular disease. Acute stress could promote atherogenesis through contracted plasma volume and subsequent exposure of the endothelium to clotting activity. Future research should attempt to determine if such hemoconcentration and coagulation reactivity confers risk for development of atherothrombotic disease and for the occurrence of future coronary events. If stress-induced changes in hemoconcentration and coagulation do confer risk for future disease, then steps to reduce such reactions should be implemented in such individuals. For example, in those with strong hemoconcentration reactions, consumption of more water may be one simple behavioral change that could offset risk associated with stress-hemoconcentration, as consuming more water will increase plasma volume. Moreover, psychosocial factors, such as chronic stress (von Känel, Dimsdale, Patterson, & Grant, 2003; von Känel, Dimsdale, Adler, Patterson, Mills & Grant, 2005), overcommitment to work (von Känel, Bellingrath, & Kudielka, 2009), social support (Steptoe, Kunz-Ebrecht, Owen, et al., 2003, Wirtz, Redwine, Ehlert, & von Känel, 2009), job control (Steptoe, Kunz-Ebrecht, Owen, et al., 2003) and socioeconomic status (Steptoe, Kunz-Ebrecht, Rumley, et al., 2003), may moderate coagulation levels at rest and during stress, and future work should take into account such factors when examining whether hemoconcentration and coagulation responses to stress predict future health outcomes.

The protocols of acute stress studies using indwelling catheters typically involve blood being drawn either during the last minute or immediately after a stressor. However,
the extrinsic pathway clots blood in as little as 15 seconds (Guyton & Hall, 1996). Thus, it may be prudent to measure extrinsic pathway parameters (e.g., FVII:C and PT%) during the first minute of a stressor. Additionally, it is not known if hemoconcentration or coagulation responses acclimate, steadily increase or plateau during the course of a stressor. Moreover, stress responses of other coagulation parameters as well as fibrinolysis parameters should be examined in light of the current results. Finally, as detailed earlier, additional studies are needed to ascertain how to correct for such plasma volume increases, or “hemodilution” that may occur, as well as whether such correction is necessary.

Conclusions

Two novel reconstitution methods of correcting for hemoconcentration effects on stress-induced coagulation changes were examined in this study. Both the saline and baseline plasma reconstitution methods appeared to adequately adjust for plasma volume shifts when examining concentrations, but the baseline plasma reconstitution method had inconsequential effects on clotting time and clotting activity. Thus, the baseline plasma reconstitution method does not appear to be an informative hemoconcentration correction technique, because the Dill and Costill (1974) formula adjusts equally as well or better without having the extra step in the lab of reconstitution. The saline reconstitution method, on the other hand, may be the most biologically relevant correction technique when examining stress-hemoconcentration effects on clotting time and clotting activity, whereas the Dill and Costill formula does not seem appropriate. With the exception of FVIII:C, hemoconcentration appears to account for most of the stress-induced changes in
the coagulation parameters examined in this study. The intrinsic pathway of the coagulation system, however, is most likely genuinely activated during acute stress, as indicated by increases in FVIII:C surviving all hemoconcentration correction techniques. Altogether, stress-induced changes in coagulation are a consequence of both hemoconcentration and actual activation of the coagulation system.
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Title of Research: How does stress affect your body?

Researchers: Anthony W. Austin, M.A. & Stephen M. Patterson, Ph.D.

You are being asked to participate in research. For you to be able to decide whether you want to participate in this project, you should understand what the project is about, as well as the possible risks and benefits in order to make an informed decision. This process is known as informed consent. This form describes the purpose, procedures, possible benefits, and risks. It also explains how your personal information will be used and protected. Once you have read this form and your questions about the study are answered, you will be asked to sign it. This will allow your participation in this study. You should receive a copy of this document to take with you.

Exclusion/Eligibility:
The following are eligibility criteria for the study. By signing this document, you certify that:

   a) You are male.
   b) You are 18 years of age or older.
   c) You have no personal history of angina, heart disease, diabetes, or other chronic physical diseases.
   d) You have no history of a thromboembolic event (i.e., blood clot).
   e) You do not currently use prescription (e.g., Motrin) or non-prescription anti-inflammatory medications (e.g., Advil, Aleve, Tylenol), or anti-depressant medication (e.g., Zoloft).
   f) You do not currently use prescription or non-prescription medication or herbal remedies that would affect blood pressure (e.g., beta-blockers, diuretics, St. John’s Wort).
   g) You do not currently use anti-coagulant therapy drugs (e.g., Plavix, Coumadin).
   h) You do not regularly use aspirin.
   i) You do not currently have any major or minor infection.
   j) You have not had any severe trauma or surgery within the previous six months.
   k) You do not use nicotine.
   l) You do not have a history of fainting during blood draws/donation.
   m) You do not have a history of passing out or of fainting spells.
In line with the study prerequisites listed on the sign-up system, you also certify that:

a) You have refrained from eating or drinking for the past 4 hours.
b) You have refrained from exercise for the past 24 hours.
c) You have not consumed alcohol in the past 24 hours.
d) You have not consumed over-the-counter anti-inflammatory (e.g., Advil, Alleve, Tylenol) or blood pressure altering medication in the past 24 hours.

EXPLANATION OF STUDY

Previous research has shown that clotting of the blood increases during psychological stress. However, the exact mechanism for this effect is unknown. Therefore, the purpose of this study is to identify the mechanism by which psychological stress increases clotting of the blood. An initial screening procedure was conducted to identify people who are in good physical health. You have been asked to participate because you are in good health.

Qualifying individuals have been invited to participate in the present laboratory protocol that will last approximately 60-70 minutes and includes the following:

1) A 15-minute preparatory period
2) A 20-minute rest period while seated.
3) A 6-minute mental arithmetic task while seated.
4) A 20-minute recovery period while seated.

During the 15-minute preparatory period, you will breathe into a carbon monoxide detector and into an alcohol detector (i.e., breathalyzer) to verify that you do not use nicotine and that you have not consumed alcohol in the past 24 hours, respectively. Then, height and weight will be measured. Next, our research nurse, Lauren Mente, RN, BSN, will insert a catheter into your non-dominant arm for blood collection. This catheter will remain in your arm for the entire length of the experimental session, but blood will be collected only during the last minute of the rest period, the last minute of the mental arithmetic task and the last minute of the recovery period. The amount of blood drawn will be small (approximately 4.6 tablespoons) and provide minimal risk to you. In addition, a blood pressure cuff will be applied to your arm during the preparatory period. Blood pressure will be recorded three times during the last 5 minutes of the 20-minute rest period, 3 times during the 6-minute mental arithmetic task, and every two minutes during the 20-minute recovery period.

You may terminate participation at any time without penalty.

After you complete this study, your blood will be processed and frozen until a later date when it will be analyzed for various blood clotting parameters. The following clotting variables will be measured from your blood: fibrinogen, D-dimer, clotting factor VII, clotting factor VIII, activated partial thromboplastin time, prothrombin time, hematocrit and hemoglobin. These are molecules involved in blood clotting and measures
of the amount of time it takes blood to clot. It is unclear if stress-induced clotting is due
to actual activation of the clotting system or due to plasma losses. During acute stress,
the blood pressure increases and pushes plasma (the fluid portion of blood) out of the blood
vessels, which results in a greater concentration of cellular components such as clotting
molecules. Using an innovative research method, your blood will be processed to
determine if the various clotting measures examined in this study increase during acute
stress as a result of actual activation of the clotting system or due to changes in the
amount of blood plasma.

Risks and Discomforts

The risks posed by this study are no greater than those typically associated with
blood draws or blood donation. Most people feel fine during and after having blood
samples drawn or during blood donation. A few people may experience an upset
stomach, slight dizziness, or lightheadedness. In some cases, an individual may faint.
Our Research Nurse is trained to treat these difficulties should they occur. There may also
be possible discomfort, redness, swelling or bruising during the insertion and/or removal of
the catheter from the arm; however these effects are temporary and will subside in 2 or 3
hours. If any of these effects persist, you may come back to the laboratory and consult
with our research nurse, Lauren Mente, RN, BSN. Ms. Mente’s office hours are
Wednesday and Friday from 8:00 – 12:00 and Thursday from 8:00 – 4:00. During this
time she may be contacted at (740)597-1424. After hours, she may be contacted at
(740)594-7303.

Benefits

Your participation will help researchers better understand physiological responses
to psychological stress and how these responses may link stress with cardiovascular
disease. Specifically, your participation will benefit society by helping to determine the
mechanism(s) behind increased clotting activity during acute psychological stress. The
primary benefit to you is that you will gain knowledge about research and methodology
in the area of Psychophysiology.

Confidentiality and Records

All information obtained today will be kept strictly confidential, within limits
of the law. This information will be identified according to a code number known only to
those directly involved with this research project, and any personally identifiable
information will be kept in locked files in Stephen Patterson’s laboratory in Porter 116
accessible only to those persons directly involved in the study. You will be assigned a
unique 4-digit code. The code key and identifiable information will be kept in a locked
file cabinet accessible only to Anthony Austin and Stephen Patterson. The code key will
be de-identified following conclusion of the study. Additionally, after your blood samples
are analyzed, they will be disposed of properly in biohazard waste containers. By signing
this document, you consent to the use of your data for research and teaching purposes and understand that your identity will not be revealed in any description or publication that results from this research.

Additionally, while every effort will be made to keep your study-related information confidential, there may be circumstances where this information must be shared with:
* Federal agencies, for example the Office of Human Research Protections, whose responsibility is to protect human subjects in research;
* Representatives of Ohio University (OU), including the Institutional Review Board, a committee that oversees the research at OU;

Compensation
You will receive two experimental credits for your participation.

Contact Information
If you have any questions regarding this study, please contact Anthony Austin, M.A., (aa301306@ohio.edu; 740-593-0912) or Stephen Patterson, Ph.D., (patters@ohio.edu; 740-597-2717), if you have any questions either before or after participating in the study. If you have any questions regarding your rights as a research participant, please contact Jo Ellen Sherow, Director of Research Compliance, Ohio University, (740)593-0664.

By signing below, you are agreeing that:
• you have read this consent form (or it has been read to you) and have been given the opportunity to ask questions.
• known risks to you have been explained to your satisfaction.
• you understand Ohio University has no policy or plan to pay for any injuries you might receive as a result of participating in this research protocol.
• you are 18 years of age or older.
• your participation in this research is given voluntarily.
• you may change your mind and stop participation at any time without penalty or loss of any benefits to which you may otherwise be entitled.

Signature_________________________________________ Date_______

Printed Name________________________________________

Experimenter’s Signature____________________________

Experimenter’s Name______________________________
APPENDIX B: HEALTH INFORMATION SCREENING QUESTIONNAIRE

AGE: _______  Sex: _______

DO YOU HAVE ANY PERMANENT OR CHRONIC HEALTH PROBLEMS? (e.g., High Blood Pressure, Heart Condition, Arthritis, Diabetes, Cancer...): Y / N
   IF YES, SPECIFY: ____________________________________________

ARE YOU CURRENTLY TAKING ANY PRESCRIPTION MEDICATION?  Y / N
   IF YES, SPECIFY: ____________________________________________

DO YOU CURRENTLY HAVE ANY MAJOR OR MINOR INFECTION? (e.g., Common Cold, Sinus Infection, Influenza): Y / N
   IF YES, SPECIFY: ____________________________________________

HAVE YOU EVER HAD A THROMBOEMBOLIC EVENT? (e.g., a blood clot in the blood vessels, lungs, brain, gastrointestinal tract, kidneys, or leg): Y / N
   IF YES, SPECIFY: ____________________________________________

DO YOU TAKE ASPIRIN ON A REGULAR BASIS?  Y / N

HAVE YOU HAD ANY TRAUMA (e.g., bone fracture, severe wounding) OR SURGERY WITHIN THE PREVIOUS SIX MONTHS?  Y / N
   IF YES, SPECIFY: ____________________________________________

DO YOU USE NICOTINE?  Y / N

HAVE YOU EVER FAINTED DURING BLOOD DRAWS OR DONATION? Y/N

DO YOU HAVE A HISTORY OF PASSING OUT OR A HISTORY OF FAINTING SPELLS?  Y/N
APPENDIX C: PRE-STUDY RESTRICTIONS

1) What and when have you eaten in the last 12 hours?
________________________________________________________________________
________________________________________________________________________

2) Have you eaten within the last 4 hours?  Yes___  No___

3) Have you drunk any caffeinated beverages (i.e., coffee, tea, soda) or eaten anything containing caffeine (i.e., chocolate) in the past 4 hours?
   Yes___  No___

4) Have you drunk any alcohol in the past 24 hours?
   Yes___  No___  If yes, how much?  ________________

5) Have you taken any prescription or non-prescription drugs in the past 24 hours?
   Yes___  No___  If yes, what?  ________________

6) If you exercise regularly, when and for how long did you exercise last?
   _________________________________
Debriefing Statement

Thank you for participating in this research study. In this study, we were examining the effects of psychological stress on hematological functioning. We are attempting to discover if the apparent increases in clotting during acute stress are due to actual activation of the clotting system or due to a phenomenon known as hemoconcentration (i.e., the ratio of cellular components of the blood to plasma increases).

You completed a serial subtraction math task. This task is often used as a type of mental stressor in physiological reactivity studies. In no way was the math task designed to assess your math skills or ability.

The data that we collected from you today will be kept confidential and in a locked cabinet.

You may experience some redness, swelling or bruising on your arm because of the catheter, but this will subside.

Is there anything in this study that you suggest we change because we make you feel uneasy?

Do you have any questions at this time?

If at any time you have questions, please feel free to contact Anthony Austin at (740)593-0912, aa301306@ohio.edu or Stephen Patterson at (740) 597-2717, patters@ohio.edu or visit the Psychohematology/Psychophysiology Laboratory (Porter #116).

Thank you again for participating in this research project.