



Hemoconcentration during a prolonged stress task: Associations with hemodynamic reactivity and microvascular permeability

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ABSTRACT

This study explored the association between stress-induced hemoconcentration and plasma colloid osmotic pressure, hemodynamic reactivity, and microvascular permeability during a protracted stress task in 26 healthy, young participants. Microvascular permeability was measured during rest using venous congestion plethysmography in a subsample of 13 participants. The task increased hematocrit, colloid osmotic pressure, blood pressure, and heart rate and decreased R-wave to pulse interval. Resting microvascular permeability was not correlated with hemoconcentration. Colloid osmotic pressure and diastolic blood pressure were associated with stress-induced hemoconcentration throughout the task. The association with systolic blood pressure as well as heart rate, however, was more evident during the initial 8 min of the task than throughout the total task duration. These findings suggest that factors associated with hemoconcentration vary with task duration.

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Coronary heart disease remains the major cause of death in the United States and the United Kingdom, and mortality is most frequently the result of acute myocardial infarction (Allender et al., 2006; American Heart Association, 2007). Psychologically stressful events have been implicated as precursors, with almost half of surviving patients attributing their myocardial infarction to an environmental or behavioral trigger, such as emotional distress (Tofler et al., 1990). Further, an increase in the incidence of and mortality from myocardial infarction has been associated with exposure to traumatic events, such as air raids (Bergovec et al., 1992), missile attacks (Meisel et al., 1991), earthquakes (Kloner et al., 1997) and even key international football matches (Carroll et al., 2002).

The issue arises as to how psychologically stressful events might precipitate myocardial infarction. Laboratory studies have established, in healthy participants, that brief mental stress tasks change blood rheology, as indexed by increased hematocrit and decreased plasma volume (e.g., Patterson et al., 1995b; Ring et al., 2008; Ross et al., 2001; Veldhuijzen van Zanten et al., 2005). Such hemoconcentration is likely to be particularly important at sites in the vessel where there is a vulnerable atherosclerotic plaque. The stress-induced hemoconcentration causes an increase in shear stress, which can lead to plaque disruption and clot formation (Falk et al., 1995; Worthley et al., 2001). Interestingly, an association

between hemoconcentration and mental stress-induced myocardial ischemia has been reported in patients with coronary artery disease; those who displayed mental stress-induced ischemia showed greater hemoconcentration compared to those with no mental stress-induced hemoconcentration (Bacon et al., 2006).

Even though stress-induced hemoconcentration is well established, limited attention has been paid to the underlying mechanisms. Factors influencing changes in fluid filtration, thus affecting hematocrit, are described by the Starling equation for fluid movement in exchange vessels:

$$J_v = K_f[(P_c - P_i) - \sigma(\pi_p - \pi_i)]$$

where J_v = filtration rate, K_f = vascular permeability, P_c = capillary hydrostatic pressure, P_i = interstitial hydrostatic pressure, σ = osmotic reflection coefficient for plasma proteins, π_p = plasma colloid osmotic pressure, and π_i = interstitial colloid osmotic pressure (Landis, 1927; Starling, 1896).

Of the Starling factors, the main focus so far has been on the relation between stress-induced hemoconcentration and blood pressure, as a possible index of down-stream changes in hydrostatic pressure at the microvascular interface (Berne and Levy, 1993). Several studies have reported an association between stress-induced changes in blood pressure and hemoconcentration: the higher the increase in blood pressure, the greater the increase in hematocrit (De Boer et al., 2006, 2007a,b; Patterson et al., 1995a,b,c, 1998; Veldhuijzen van Zanten et al., 2002). However, this association has not been found in all studies (Jern et al., 1989; Ross et al., 2001; Zraggen et al., 2005).

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Another Starling force that has received recent attention is plasma colloid osmotic pressure, which is the suction force that is applied by the particles that are too large to pass through the semipermeable blood vessel wall (e.g., proteins). An increase in plasma colloid osmotic pressure reflects an increase in concentration of particles in the blood relative to the interstitium. The impact of the difference between plasma and interstitial osmotic pressure on the filtration rate is multiplied by the osmotic reflection coefficient. This coefficient indicates the ratio of the proteins to pore size of the vasculature, and ranges from 0 (freely permeable to proteins) to 1 (not permeable to proteins) (Levick, 1991). A brief mental stress has been shown to induce increases in plasma colloid osmotic pressure (De Boer et al., 2007a,b). In addition, plasma colloid osmotic pressure was associated with the amount of hemoconcentration during brief stress tasks (De Boer et al., 2007a,b). Interestingly, the stress-induced changes in plasma colloid osmotic pressure were found to be a stronger predictor of hemoconcentration than changes in mean arterial pressure (De Boer et al., 2007b).

As yet, the vascular permeability factor in the Starling equation has largely been neglected in this context. To our knowledge only one group has investigated this factor during mental stress, using mast cell degranulation as an indirect measure. Mast cell degranulation has been shown to influence vascular permeability (Theoharides et al., 1998) and in one of the two studies mast cell degranulation was associated with hemoconcentration during mental stress (Veldhuijzen van Zanten et al., 2004). More directly, microvascular permeability can be assessed by a venous congestion plethysmography protocol developed by Gamble et al. (1993). Measurement of limb circumference in response to small incremental cuff pressures enables the quantification of permeability (Gamble et al., 1993). Although this measure has been used to assess resting permeability in both healthy and patient populations (Anim-Nyame et al., 2003; Bethell et al., 2001; Christ et al., 1998; Gamble et al., 1997), resting permeability has never been examined in relation to stress-induced hemoconcentration.

Most studies that have investigated factors associated with stress-induced hemoconcentration have conducted between-subject correlational analyses. Typically, in these studies only one blood sample was taken at the end of the stress task. Some studies, however, examined the mechanisms that could underlie hemoconcentration over time by using a within-subject design to brief mental stress tasks (De Boer et al., 2006, 2007a,b; Patterson et al., 1995b). This research revealed a reliable association between the increase in blood pressure and hemoconcentration over time. However, the total task duration of these time course studies was relatively brief, ranging from 4 to 10 min. Only two studies have explored factors associated with hemoconcentration with longer stress tasks (Muldoon et al., 1995; Veldhuijzen van Zanten et al., 2004). In one, Muldoon et al. (1995) employed a 20 min stress task and found an association between hemoconcentration and a combined heart rate and blood pressure index. In the second study, Veldhuijzen van Zanten et al. (2004) reported no association between blood pressure and hemoconcentration during 28 min of stress. However, in both studies only one blood sample was taken at the end of the stress task, thus only between-subject correlational analyses were conducted. As such, it remains to be determined whether the mechanisms underlying hemoconcentration vary over the course of a prolonged stress task.

Previous research has not been limited to the examination of Starling factors; cardiac measures have also been examined in relation to hemoconcentration. The results of the studies considering heart rate are equivocal, with some (De Boer et al., 2006; Jern et al., 1989; Patterson et al., 1995d, 1998; Ring et al., 2008; Veldhuijzen van Zanten et al., 2002) but not all (Patterson et al., 1995a,b; Ross et al., 2001; Zraggen et al., 2005) studies

finding an association. Cardiac contractility, either measured directly by pre-ejection period or by R-wave to pulse interval, as a proxy for pre-ejection period, has been shown to be associated with hemoconcentration in several studies (De Boer et al., 2006, 2007a,b; Veldhuijzen van Zanten et al., 2002). However, as before, these studies only employed stress tasks of short duration, lasting up to 12 min. Interestingly, hemodynamic activity underlying stress-induced increases in blood pressure has been shown to change during the course of a long stress task. At the start of the task, it was driven by cardiac factors, whereas towards the second part of the task, blood pressure increase was supported by vascular mechanisms (Ring et al., 2002a). The associations between this shifting hemodynamic activity and hemoconcentration during a prolonged stress task have yet to be examined.

Thus, the current study was undertaken to explore plasma colloid osmotic pressure and hemodynamic reactions as well as resting microvascular permeability as factors associated with stress-induced hemoconcentration. Several blood samples were taken during a prolonged 32 min stress task permitting for examination of the time course of stress-induced hemoconcentration. This design also permitted the investigation of the mechanisms underlying hemoconcentration during a prolonged stress task.

1. Method

1.1. Participants

Twenty-six participants (17 men, 9 women, mean age = 22.8, $SD = 3.8$ years; mean body mass index = 23.7, $SD = 3.0$, kg/m^2) from the University of Birmingham participated. None smoked, had a history of cardiovascular disease, or were currently taking medication. All female participants reported having regular menstrual periods, were not taking oral contraceptives, and were tested in the follicular phase of the menstrual cycle (between days 4 and 9 of the cycle). All participants were asked to abstain from vigorous exercise and alcohol for 12 h, and from food and caffeine for 2 h, prior to testing. All participants gave informed consent and the study was approved by the local ethics committee.

1.2. Mental arithmetic stress

Participants were presented with a series of single digit numbers and were required to add each number to the number presented previously (Gronwall, 1977; Ring et al., 2002b). Numbers were delivered using an audio tape player and participants called out their answers. Participants completed four 8-min tasks, with a 1-min break between tasks. The first task consisted of four consecutive 2-min periods of 33, 38, 43, and 50 digits, respectively, at inter-stimulus intervals of 3.6, 3.2, 2.8 and 2.4 s. The inter-stimulus intervals during the initial 2-min period of the second, third, and fourth tasks were 3.2, 2.8 and 2.4 s, respectively, and, as in the first task, the inter-stimulus intervals decreased by 0.4 s every 2 min. The experimenter, who sat beside participants, checked their responses against the correct answers. Participants were punished by a loud, aversive noise once during every 10 additions. In each block of 10 digits, the noise was presented after the participants' first incorrect response (either a wrong answer or no answer given); however, if they had not made an error, the noise was delivered at the end of the block. Participants were filmed with a video camera and were asked to look at themselves displayed on a television screen while performing the task. If participants looked away from the screen, they were reminded to continue to watch the screen. They were informed that the recording would be analyzed by two senior academics for body and facial composure during the task, although, in reality, no such analysis was undertaken. They were truthfully told that a £20 gift voucher would be awarded for the best performance on the task. These demands (increasing time pressure, social evaluation, punishment, and reward) have been found to increase the provocativeness of the task (Veldhuijzen van Zanten et al., 2004).

1.3. Rheological measures

A catheter (18 gauge, Insyte, Becton Dickinson) was introduced into an antecubital arm vein and connected to a stopcock (Posiflow, Becton Dickinson). On each draw, the first 3 ml of blood was collected in a syringe and discarded. Then, 2 ml was collected into a tube containing potassium ethylene diaminetetraacetic acid (EDTA K3, Becton Dickinson), which was analyzed for hematocrit and hemoglobin using a Coulter Analyzer (Beckman Coulter, Inc.). A further 2 ml was collected into a tube with lithium heparine (Becton Dickinson). This tube was centrifuged at room temperature for 5 min at $1500 \times g$. The plasma was stored at $-70^\circ C$. These samples were later analyzed for plasma colloid osmotic pressure (mmHg) using an osmometer (Osmomat 050, Gonotec) with a membrane

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