Published in final edited form as:

Clin Physiol Funct Imaging. 2009 November ; 29(6): 427–430. doi:10.1111/j.1475-097X.2009.00890.x.

Early activation of the coagulation system during lower body negative pressure

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Summary

We considered that a moderate reduction of the central blood volume (CBV) may activate the coagulation system. Lower body negative pressure (LBNP) is a non-invasive means of reducing CBV and, thereby, simulates haemorrhage. We tested the hypothesis that coagulation markers would increase following moderate hypovolemia by exposing 10 healthy male volunteers to 10 min of 30 mmHg LBNP. Thoracic electrical impedance increased during LBNP (by $2.6 \pm 0.7 \Omega$, mean $\pm$ SD; $P < 0.001$), signifying a reduced CBV. Heart rate was unchanged during LBNP, while mean arterial pressure decreased (84 ± 5 to 80 ± 6 mmHg; $P < 0.001$) along with stroke volume (114 ± 22 to 96 ± 19 ml min⁻¹; $P < 0.001$) and cardiac output (6.4 ± 2.0 to 5.5 ± 1.7 l min⁻¹; $P < 0.01$). Plasma thrombin–antithrombin III complexes increased (TAT, 5 ± 6 to 19 ± 20 µg l⁻¹; $P < 0.05$), indicating that LBNP activated the thrombin generating part of the coagulation system, while plasma D-dimer was unchanged, signifying that the increased thrombin generation did not cause further intravascular clot formation. The plasma pancreatic polypeptide level decreased (13 ± 11 to 6 ± 8 pmol l⁻¹; $P < 0.05$), reflecting reduced vagal activity. In conclusion, thrombin generation was activated by a modest decrease in CBV by LBNP in healthy humans independent of the vagal activity.

Keywords

haemorrhage; hypovolemia; pancreatic polypeptide; thrombin generation; vagal activity

Introduction

We considered that the coagulation system is activated by a reduction of the central blood volume (CBV). Both patients with a ruptured abdominal aortic aneurysm (Skagius *et al.*, 2008) and trauma demonstrate a procoagulant state that could render the patients better protected against continued haemorrhage (Innes & Sevitt, 1964; Kaufmann *et al.*, 1997). It is, however, unknown whether even moderate hypovolemia affects the coagulation system by an increase in either parasympathetic or sympathetic activity (Kuznik & Mishchenko, 1975a; von Känel & Dimsdale, 2000).

Lower body negative pressure (LBNP) pools blood in the abdomen and lower extremities thereby reducing the CBV (Murray *et al.*, 1968) as indicated by an increase in thoracic electrical impedance and reduced leg impedance (Cai *et al.*, 2000; Kitano *et al.*, 2005). Hence, LBNP is a non-invasive human model for central hypovolemia (Cooke *et al.*, 2004) and provides a means to assess the effect of a reduced CBV on coagulation markers. We tested the hypothesis that a moderate decrease of CBV during LBNP activates the coagulation system in healthy humans.

Methods

The study was conducted in accordance with the Helsinki declaration and approved by the Ethical Comity of Copenhagen and Frederiksberg (KF 11 312841) and 10 healthy male volunteers (26 ± 3 years, 80 ± 11 kg and 182 ± 6 cm) provided informed consent prior to commencement of study related activities. The subjects reported to the laboratory at 9 AM after an overnight fast. A cannula (1.1 mm ID, 20 gauge) was placed in the brachial artery of the non-dominant arm for blood sampling and measurement of mean arterial pressure (MAP) with a Bentley transducer (Uden, The Netherlands) positioned at the level of the midaxillary line and connected to a Dialogue 2000 monitor (IBC-Danica, Copenhagen, Denmark). A three-lead electrocardiogram recorded heart rate (HR), while stroke volume (SV) and cardiac output (CO) were estimated with a FinometerTM (Finapres Medical System BV, Amsterdam, The Netherlands). Such assessment of CO has been successfully validated both in healthy subjects and in patients during surgery and sepsis (Jellema *et al.*, 1999; Bogert & Van Lieshout, 2005; Nissen *et al.*, 2009). Cardiovascular variables were obtained as averaged data from 10–15 min before LBNP, at 5 and 10 min of LBNP, and ~5 min thereafter. Thoracic electrical impedance indicates changes in CBV; i.e. when the blood volume decreases, electrical impedance increases (Cai *et al.*, 2000). Paired electrodes were placed on the right sternocleidomastoid muscle and on the upper left rib at the mid-axillary line and evaluation was based upon a 200 μ A current at 1.5 and 100 kHz (C-Guard, Danmeter, Denmark).

After instrumentation, the subject was placed supine in the LBNP chamber sealed at the level of the iliac crest. To reduce the risk of lower-back pain and stiffness and to reduce body movements, a wedge shaped pillow was placed under the knees. Following 30 min of supine rest, the subject was exposed to 10 min of 30 mmHg LBNP. Arterial blood (~3 ml) was obtained 10–15 min before LBNP and at 5 and 10 min of LBNP, and sampled into citrate tubes.

Blood samples were centrifuged at 1000 *g* for 10 min at 20°C within 30 min of sampling and plasma was stored at –80°C for less than 3 months. Plasma was analysed for markers of hemostatic procoagulant activity: Thrombin–antithrombin III complexes (TAT; Enzygnost[®]; Dade Behring, Marburg, Germany) as a measure of thrombin generation; D-dimer (Tina-quant, cobas[®]; Roche Diagnostics GmbH, Mannheim, Germany) that is a fibrinolytic product; and for prothrombin time (PT; Nycotest[®]; PT, Axis-Shield, Oslo, Norway) evaluating the tissue factor dependent pathway of the coagulation system, and by activated partial thromboplastin time (aPTT; Platelin[®]; LS, bioMérieux, Inc., NC, USA) to evaluate the platelet dependent pathway (Futura, Instrumentation Laboratory, Milan, Italy). Plasma pancreatic polypeptide was determined by radioimmunoassay with a polyclonal rabbit-antibody to indicate vagal activity (Schwartz *et al.*, 1978; Damholt *et al.*, 1997).

Data analysis

Cardiovascular variables were obtained with a Biopac acquisition system (MP100 Acknowledge, Biopac Systems Inc., CA, USA). Data were evaluated by one-way repeated measures ANOVA followed by a Student–Newman–Keuls *post hoc* test using SigmaStat (version 3.0). If the normal distribution test failed, a Friedman repeated measures ANOVA on

Ranks was applied. A P -value <0.05 was considered to represent a statistical significant difference and data are presented as mean $\pm$ SD.

Results

Thoracic electrical impedance increased during LBNP (by $2.6 \pm 0.7 \Omega$; $P < 0.001$). While HR was unaffected at 55 ± 7 bpm, there was a decrease in MAP (84 ± 5 to 80 ± 6 mmHg; $P < 0.001$) along with SV (114 ± 22 to 92 ± 21 ml min⁻¹; $P < 0.001$) and therefore CO (6.4 ± 2.0 to 5.5 ± 1.7 l min⁻¹; $P < 0.01$). Thoracic electrical impedance was still elevated 5 min after termination of LBNP ($0.5 \pm 0.3 \Omega$; $P < 0.01$), but the cardiovascular variables had returned to baseline levels (84 ± 5 mmHg, 115 ± 25 ml min⁻¹; and 6.6 ± 2.3 l min⁻¹). While plasma pancreatic polypeptide decreased after 10 min of LBNP (13 ± 11 to 6 ± 8 pmol l⁻¹; $P < 0.05$), plasma TAT complexes increased ~5-fold (5 ± 6 to 27 ± 36 µg l⁻¹; $P < 0.05$) after 5 min of LBNP but decreased slightly hereafter (to 19 ± 20 µg l⁻¹; $P < 0.05$) (Fig. 1). Plasma aPTT decreased marginally after 10 min of LBNP (31.3 ± 3.2 to 30.7 ± 3.1 s; $P < 0.05$), while plasma PT and D-dimer were unchanged (0.80 ± 0.10 U ml⁻¹ and 0.10 ± 0.08 µg ml⁻¹ respectively).

Discussion

Haemorrhage following injury is a major cause of death and establishing hemostasis is an important survival strategy. The coagulation system is activated following injury (Meissner *et al.*, 2003) and the vasovagal syncope may be taken to represent a last line of defence against bleeding (Diehl, 2005), because vagal activity provokes activation of the coagulation system (Kuznik & Mishchenko, 1975a,b). Indeed, some trauma patients in hemorrhagic shock demonstrate parasympathetic activation as indicated by bradycardia and increased plasma pancreatic polypeptide (Sander-Jensen *et al.*, 1986b). We evaluated the displacement of blood to the lower extremities by thoracic electrical impedance and estimated that 0.3–1.0 l of fluid was displaced from the chest to the splanchnic region and the legs (Murray *et al.*, 1968; Cooke *et al.*, 2004).

LBNP reduced SV and CO by 20% and 15% respectively, and also MAP, but HR was unaffected. Plasma pancreatic polypeptide decreased indicating that vagal activity was reduced in accordance with the initial response to head-up tilt (Shi *et al.*, 2000). Nevertheless, upon exposure to LBNP, plasma TAT complexes increased to a level similar to that reported in patients with deep vein thrombosis or suspected disseminated intravascular coagulation (Hoek *et al.*, 1988), indicating elevated thrombin generation. Thrombin is a component in clot formation and, therefore, indicates the activity of the coagulation system (Roberts *et al.*, 2006). Notably, the plasma D-dimer concentration did not change indicating that the elevated thrombin generation did not provoke stable clot formation. D-dimer is a degradation product of crosslinked fibrin, i.e. a stable clot and is applied for the diagnosis of deep-vein thrombosis and venous thromboembolism (Bounameaux *et al.*, 1994). Also, PT was unaffected and aPTT decreased slightly suggesting that increased thrombin formation did not result in a significant consumption of coagulation factors.

Plasma pancreatic polypeptide indicated that vagal activity decreased during LBNP, thus parasympathetic activity cannot explain the increase in plasma TAT complexes. The sympathetic nervous system may also enhance coagulation, e.g. adrenaline enhances hemostatic activity (von Känel & Dimsdale, 2000), and the plasma adrenaline level may increase 3-fold during LBNP and head-up tilt (Sander-Jensen *et al.*, 1986a, 1988). While it is unlikely that adrenaline was increased to such a level in our test subjects, the applied level of LBNP is sufficient to increase muscle sympathetic nerve activity (Rea & Wallin, 1989).

The blood pooling in the legs during LBNP also resembles the pooling that happens during prolonged upright immobility, e.g. prolonged sitting during air and bus travel, where 0.2–0.5 l of blood may pool in the lower extremities (Mittermayr *et al.*, 2003; Schobersberger *et al.*, 2004). During such long-distance travel, the coagulation system is activated and there is an increase in venous thromboembolism (Homans, 1954; Schobersberger *et al.*, 2004). While the activation of the coagulation system is shown after hours of travel, only 15 min of still standing activates the coagulation (Masoud *et al.*, 2008), which is in accordance with the activation of the coagulation after 5–10 min of LBNP in the present study. During pooling of the blood in the lower extremities, the coagulation may be activated because of increased orthostatic stress on the endothelial monolayer in the lower extremities that stimulates the expression of tissue factor, release of von Willebrand factor, and increases the levels of tissue plasminogen activator and plasminogen activator inhibitor-1 (Masoud *et al.*, 2008).

In summary, LBNP-induced reduction in CBV, similar to mild to moderate haemorrhage, activates the coagulation system as indicated by increased plasma TAT complex formation unrelated to parasympathetic activity. Thus, like the circulatory system, the coagulation system responds early to a reduced CBV.

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Figure 1.

Thrombin–antithrombin III (TAT) complexes, D-dimer, activated partial thromboplastin time (aPTT), and prothrombin time (PT) for 10 healthy male volunteers before and following 5 and 10 min of 30 mmHg lower body negative pressure (LBNP). Values are mean $\pm$ SEM. *Different from baseline ($P < 0.05$).