Orthostatic Hypercoagulability
A Novel Physiological Mechanism to Activate the Coagulation System

Muhammad Masoud, Galit Sarig, Benjamin Brenner, Giris Jacob

Abstract—Orthostatic stress causes significant plasma shift and raises transmural pressure in lower extremities, resulting in an increase in endothelial activation and plasma proteins concentrations, possibly including coagulation factors. This may lead to activation of the coagulation system during standing. To test this hypothesis, we recruited 18 healthy volunteers (9 females and 9 males; mean age: 25±1.2 years; body mass index: 21.7±0.5 kg/m²). Hemodynamics, plasma shift (extrapolated from sequential hematocrit concentration), plasma proteins, and coagulation tests, including procoagulants; fibrinogen, factor V, and factor VIII activity; prothrombin fragments 1 and 2; and endothelial activation–related factors (tissue factor and von Willebrand factor), as well as protein C global pathway, were determined at rest supine and at 15 minutes, 30 minutes, and 60 minutes of still standing. Thirty minutes of standing caused a decrease in plasma volume by 12.0±0.5% and an increase in plasma protein by 13.0±0.7%. Fibrinogen, factor V, and factor VIII activity rose by 12.0±1.2%, 13.0±1.0%, and 40.0±6.0% (P<0.002 for all), respectively. Prothrombin fragments 1 and 2 were elevated by 150.0±30.0%. Tissue factor and von Willebrand factor increased by 30.0±9.0% and 17.4±51.0% (P<0.02 for both), respectively. However, protein C assay results decreased from 0.95±0.20 to 0.83±0.16 (P<0.001). We hereby introduce a novel physiological mechanism, “orthostatic procoagulation,” that should be considered during coagulation tests. Furthermore, it could be extrapolated to the pathophysiology of stasis and venous thromboembolism. (Hypertension. 2008;51:1545-1551.)

Key Words: prolonged standing ■ coagulation ■ protein C global ■ endothelial activity

Hypercoagulable states predispose to the development of venous thromboembolism (VTE). This could result from either pathological or physiological situations, eg, cancer, major surgery, pregnancy, and prolonged stasis. Pathophysiologic mechanisms underlying these predisposing conditions are summarized in the triad of Virchow: alteration in coagulation factors, vascular endothelial injury, and blood stasis.

Standing is known to cause pooling of a large quantity of blood with a subsequent increase of orthostatic pressure in the lower part of the body. As a result, ≥12% of intravascular fluid transfers from the intravascular space to surrounding tissues. This plasma shift can be estimated indirectly by measuring sequential changes in hematocrit (Hct) or total protein (TP) concentration. Although much has been reported about the homoconcentration effect on total plasma protein level, no data are available regarding this effect on a variety of specific circulating proteins, including coagulation factors.

During prolonged standing, orthostatic pressure is increased in both venous and arterial vasculature of the lower limbs. This equally involves pulsatile pressure and shear stress, acting on the vessel wall, including the endothelial monolayer. Consequently, several endothelial homeostatic mechanisms are physiologically activated, which, along with vasodilatory function, play a pivotal role in the hemostatic processes. Under physiological conditions, endothelial cells have balanced anticoagulant and procoagulant properties. Once exposed to injuries, such as, high blood pressure or increased shear stress, their procoagulant activity prevails.

Although intensive exercise was shown to be associated with increased coagulability through a mechanism involving endothelial activation, virtually no data are available regarding the effect of still standing on the coagulation cascade.

Taking into account all of the aforementioned observations, we hypothesized that prolonged standing might activate the coagulation system via endothelial induction mainly because of increasing the orthostatic shear stress on the endothelial monolayer, especially in the lower extremities. To prove this hypothesis, we studied the effect of prolonged still standing on concentrations and activities of procoagulant proteins, an anticoagulant system, coagulation indices, and endothelial-dependent coagulation factors in healthy subjects.

Methods

Subjects
Subjects were enrolled in the study if they met the following eligibility criteria: age between 21 and 45 years, body mass index of...
Experimental Design
All of the investigational procedures were performed after overnight fasting in a human physiological laboratory under controlled environment conditions in a quiet and partially darkened room with an ambient temperature of 24°C, at the Recanati Autonomic Dysfunction Center.

During the study, each subject was asked to assume a rest supine posture, after which a large antecubital intravenous heparin-free lock (18 gauge) was inserted. Heart rate and blood pressure (BP) were monitored continuously using 3-lead ECG and the oscillometric cuff applied on the contralateral arm (DATEX). Thereafter, a 30-minute rest supine was allowed. During supine rest and at 15, 30, and 60 minutes of still standing, hemodynamics (heart rate and BP) were measured, 2 to 3 mL of waste blood were sampled, and then 12 mL were drawn for the assessment of Hct, TP, and coagulation studies. While standing, the subjects were limited to move within a 1-ft² area on a towel placed on the floor and were not allowed to stretch their lower extremities.

Plasma Shift
Acute plasma volume (PV) shift during quiet standing was estimated using quadruplicate microcapillary venous Hct measurements (Micropipettable device and Micro MB centrifuge, IEC), corrected for trapped plasma (0.96) and whole-body Hct (0.91), (0.96 × 0.91 = 0.87). Hct was measured after centrifugation for 10 minutes at 11,500 rpm and read on a microcapillary tube reader. Acute dynamic percent changes (Δ) in PV were calculated from Hct, where Hct denoted the baseline value, and Hct, indicated the tested value. Dynamic ΔPV (%)=100×(Hct−Hct)/(Hct×(1−Hct)). Percentage of change in the total plasma protein (triplicates measured with Refractometer, Leica) was calculated to further confirm the PV% changes in Hct.

Coagulation Studies
Extensive procoagulant and anticoagulant profiles were assessed in addition to the endothelial activation-related factors. Procoagulant pathway included prothrombin time (PT), activated partial PT time (aPTT), fibrinogen levels, and factor V and factor VIII activities. The anticoagulant pathway, ie, ProC Global assay, protein C activity, and free protein S antigen, was also assessed. More specific markers for the coagulation activation, fragments F1 and 2 and D-Dimer as a marker for the fibrin formation and fibrinolysis, were taken. Plasma levels of endothelial activation-related factors, including tissue factor (TF) and von Willebrand factor (vWF) antigens, were determined.

Coagulation Assays
Blood samples were collected into 3.2% sodium citrate tubes and centrifuged at 2000g for 15 minutes. PT, aPTT, fibrinogen, D-dimer, and ProC Global assays were performed on fresh plasma samples, whereas all of the other coagulation assays were done using thawed frozen plasma samples. Plasma samples were frozen after a second centrifugation at 2000g for 15 minutes in aliquots at −70°C. Before testing, plasma aliquots were thawed in the 37°C water bath for 15 minutes. PT, aPTT, fibrinogen, D-dimer, and ProC Global assays were performed on the STA-R evolution analyzer (Diagnostica Stago) using recombinant human thromboplastin Dade Innovin (Dade Behring Marburg GmbH) for PT assay and STA-PTT, STA-FIBRINOGEN, and STA-LIATEST D-DI kits (Diagnostica Stago) for aPTT, fibrinogen, and D-dimer assays, respectively.

The ProC Global assay (Dade Behring) was performed as described previously. Endogenous activated protein C activity was estimated by an aPTT base-clotting assay. The clotting time was measured with and without protein C activation, and the ratio between these values was calculated and expressed as the protein C activation time-normalized ratio (PCAT-NR).

Levels of protein C activity, free protein S antigen, and vWF antigen were determined on the STA-R analyzer using STACHEROM, STA-Liaiset Free Protein S, and STA-LIAATEST D-DI kits (Diagnostica Stago), respectively (Diagnostica Stago). Levels of coagulation factors V and VIII activity were determined by a 1-stage assay using factor V and VIII, respectively, deficient plasma (Diagnostica Stago).

Prothrombin fragment F1 + 2 concentration was measured by an enzyme immunoassay (ELISA) using Enzygnost® F1 + 2 (monoclonal; Dade Behring). TF level was determined by IMUBIND Tissue Factor ELISA kit (American Diagnostica Inc).

Statistical Analysis
Results were expressed as means ± SEMs. We used 1-way ANOVA for repeated measurements to test the effect of standing time on the evaluated parameters. Tukey’s multiple comparison test was applied for posthoc analysis. Paired 2-tailed r test was used to assess interindividuals for continuous data. One-phase exponential decay or association curve was used to illustrate eventual increments or decrements over time of the various parameters. Linear correlation was applied for testing the eventual association between 2 parameters. Data were analyzed with Excel software (version 2002, Microsoft) and GraphPad Prism (GraphPad Software Inc, version 4.03, 2005). A P value of <0.05 was considered to be statistically significant.

Results
General characteristics of participants are presented in Table 1. Unintentionally, equal numbers of males and females were enrolled in the study. During standing, systolic BP remained unchanged, whereas a significant increase was observed in diastolic BP (by 6±2 mm Hg; P<0.005) and heart rate (by 13±2 bpm; P<0.005). Vital signs (BP and heart rate) were stable at different time points during standing. All of the subjects completed the study protocol except for 1, who developed dizziness at 45 minutes of standing.
Rest supine-corrected Hct and plasma TP were 39.4±0.7% and 7.6±0.1 g/dL, respectively. After 30 minutes of still standing, a steady state was reached; the Hct increased to 42.1±0.8% and plasma TP to 8.6±0.1 g/dL. Plasma shifts, calculated from changes in corrected Hct and plasma TP, are presented in Figure 1 (top). A powerful linear correlation was found between the changes in Hct and plasma TP at various sampling points \((r=0.96; P<0.001)\). Plasma fibrinogen concentration (large macromolecular weight protein) increased similar to plasma TP and Hct changes; it increased from 260±10 mg/dL to 291±11 mg/dL at 30 minutes of standing \((=13\%)\). A linear correlation was observed between the percentage changes in fibrinogen and the corresponding changes in plasma \((r=0.59; P<0.001; \text{Figure 1, bottom}).\)

Evidence for thrombin formation was provided by a significant increase in prothrombin activation fragment F1+2. After 60 minutes of standing, it rose steeply from 150±20 pmol/L to 375±60 pmol/L \((P=0.002; \text{Figure 2). D-}\) dimer reached significant increase at only 30 minutes \((P=0.02; \text{Figure 2, bottom}).\) Moreover, aPTT and PT reduced significantly as shown in Figure 2 (second row), with the aPTT decrease of \(\approx 2.25±0.38 \text{ seconds} \,(P<0.001)\). For coagulant factors V and VIII, activity increased by 13% and 40%, respectively, after 60 minutes of still standing \((\text{Figure 2, third row}).\)

Analysis of the protein C anticoagulant pathway revealed that PCAT-NR levels significantly decreased after 15 minutes of standing \((0.95±0.2 \text{ to } 0.83±0.16; P<0.001)\) and reached a nadir within 30 minutes \((0.75±0.17; P<0.001; \text{Figure 3, top}).\) However, free protein S antigen and protein C activity levels significantly increased with standing and reached a plateau after 15 and 60 minutes, respectively \((\text{Figure 3, middle and bottom}).\) Of note, only 3 of all of the participants were found to have a PCAT-NR <0.8 (cutoff normal values) in the rest supine, and 2 were heterozygote for factor V Leiden mutation.

Simultaneous to the decrease in PCAT-NR levels, a significant increase in the endothelial activation–related factors, ie, TF and vWF antigen, was demonstrated \((\text{Figure 2, top})).\) TF antigen levels significantly rose after 15 minutes of standing \((59±41 \text{ to } 68±36 \text{ pg/mL}; P=0.007)\) and decreased gradually to 64±50 pg/mL after 60 minutes. However, the vWF antigen level increased significantly during the first 30 minutes of standing \((92±32 \to 110±10 \text{ mg/dL}; P<0.001)\) and remained at a level of 113 mg/dL after standing for another 30 minutes. Percentage changes in both factors are demonstrated in Table 2 and Figure 2 (top). It is noteworthy that maximal changes in the levels of vWF and TF antigens amounted to 21.6±7.0% and 44.0±13.0%, respectively.

Moreover, during 60 minutes of still standing, a significant correlation was demonstrated between the levels of vWF antigen and factor VIII activity \((r=0.75; P<0.002)\). Although factor VIII activity increased by 51.0±8.0%, that of vWF antigen rose by 21.6±7.0% only. Furthermore, throughout still standing, an inverse correlation was found between PCAT-NR and F1+2 \((r=−0.52; P=0.03)\) and D-dimer levels \((r=−0.51; P=0.03)\).

**Discussion**

Although much is known about the effect of orthostatic stress on hemodynamics and plasma shift,10,11 little, if any, information is available about the coagulation system in this setting. To the best of our knowledge, the present study has shown for the first time that prolonged still standing significantly activates the coagulation cascade.

Orthostatic stress causes an increase in transcapillary orthostatic pressure. As a result, \(\approx 12\%\) of plasma (water and micromolecules) crosses into the extravascular compartment. This hemoconcentration phenomenon leads to an increase in blood viscosity and stands behind the increment in Hct and TP.8,10,21 According to the data obtained in the current study, it also increases the concentration of fibrinogen \((=13\%)\) and protein S \((=10\%)\) and contributes, at least partially, to the rise in other proteins in the coagulation system \((\text{see Table 2}).\) Interestingly, vWF antigen, factor VIII activity, and TF increase by 22%, 51%, and 45%, respectively, which is far beyond the expected hemoconcentration effect. This suggests that other mechanisms are also involved in the latter phenomenon.

Persistent increase in fibrinogen, factor VIII activity, and most likely other procoagulant factors, is associated with high incidence of thrombotic and cardiovascular events.22,23 However, no data are available regarding the effect of hyperacute physiological increment on these components. Although hemoconcentration affects blood rheology by increasing viscosity24 and coagulant proteins concentrations, it is not the sole determinant in the present remarkable coagulant stimulation. It is noteworthy that the viscosity level measured in lower limbs while standing or sitting is higher compared with that seen in the upper limbs.25 The increase in viscosity could contribute to activation of the coagulation system through
various mechanisms, including augmentation of shear stress.\textsuperscript{26}

Undoubtedly, orthostatic pressure and shear stress are increased in the lower extremities during standing.\textsuperscript{10} Previous studies have shown that orthostatic stress enhances endothelial activation. Moreover, shear stress promotes activation of the coagulation cascade and fibrinolytic system. It stimulates endothelial cells, enhances the expression of the TF,\textsuperscript{27} modulates the release of endothelial vWF from Weibel Palade bodies,\textsuperscript{28} and also increases the levels of tissue plasminogen activator and plasminogen activator inhibitor-1. In the current study, on 15 minutes of still standing, the TF antigen first significantly increased and subsequently decreased (see Figure 2). TF is believed to be shed into the circulation from activated cells, including platelets and endothelium,\textsuperscript{29} or from the injured surrounding tissue, consequently creating hypercoagulable blood.\textsuperscript{30} The orthostatic stress-induced increase in TF in our healthy subjects is associated with a remarkable rise in vWF. This simultaneous elevation in both biomarkers and tissue-type plasminogen activator (unpublished data) is in further support of the suggestion of orthostatic endothelial activation.

The early increase in TF on 15 minutes of still standing, according to the current study, leads to coagulation activation (extrinsic pathway) and to the conversion of prothrombin into active thrombin, as expressed by the remarkable increment in the prothrombin fragment $F1+2$. Thrombin promotes further procoagulant effects by activating coagulation factors V and VIII,\textsuperscript{31,32} as demonstrated by the present study. Such increment in both factors is inversely correlated with the orthostatic progressive shortening of aPTT and PT. The shortening in aPTTT has been suggested to be a predictor of VTE.\textsuperscript{33}
together, these findings, along with the increase in D-dimer, confirm that prolonged standing (at least after 30 minutes) activates the coagulation cascade and causes a hypercoagulable state.

Interestingly, although the main anticoagulation system, protein C pathway, is activated, its function decreases continuously with prolonged standing. In fact, despite the increment in the levels of free protein S antigen and protein C activity, the global activity of protein C pathway (as measured by PCAT-NR) decreases remarkably (Figure 3). This reduction might be because of the ongoing increase in factor VIII activity. The increment in factor VIII activity is almost 4-fold compared with the increase in protein C and S levels. This may overwhelm the ability of the protein C pathway to inactivate factor VIIIa, as demonstrated by the continuous decrease in PCAT-NR during standing.34 Factor V activity, which is enhanced during standing, could also contribute to the decrease in PCAT-NR, because factor Va, similar to factor VIIIa, is inactivated by the protein C pathway.35

ProC Global assay has emerged as an important test in the evaluation of the risk of VTE,19,36 recurrent VTE, and decrease in PCAT-NR during standing.34 Factor V activity, the global activity of protein C pathway (as measured in the levels of free protein S antigen and protein C activity), is inactivated by the protein C pathway.35

ProC Global assay serves a detailed investigation in the future.

Limitations
Our hypothesis was based on previous studies that reasonably stated that orthostatic pressure is definitely increased in lower extremities while standing. Therefore, we did not measure intravascular pressure during standing. Also, we did not perform simultaneous measurements of vWF and TF, the

In summary, both endothelial-dependent procoagulant and anticoagulant systems are activated during prolonged still standing. Apparently, although significantly activated, the protein C system fails to counteract completely the ongoing thrombin generation, thereby leading to a hypercoagulable state.

Moreover, other mechanisms could be involved in this orthostatic hypercoagulability. Orthostatic stress causes activation of the sympathetic nervous system, renin-angiotensin system, and increases plasma levels of vasopressin.21 Vasopressin releases vWF via V2 endothelial receptor activation,38 and catecholamines promote the procoagulant state possibly through a β2-adrenergic mechanism,39 yielding a net procoagulant effect. However, the renin-angiotensin system activates both coagulation and fibrinolytic pathways by increasing TF expression and plasma plasminogen activator inhibitor -1 levels.16 These interesting interrelationships deserve a detailed investigation in the future.

Table 2. Maximal Percentage Changes in Plasma Shift, TPs, and Coagulation Macromolecule Concentrations or Activities While Standing

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Molecular Weight</th>
<th>Supine Values</th>
<th>Δ% Maximal</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma shift</td>
<td>NA</td>
<td>NA</td>
<td>−12.3±0.6</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>TP, g/dL</td>
<td>NA</td>
<td>7.6±0.1</td>
<td>14±0.8</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>Factor V activity, U/mL</td>
<td>300 000</td>
<td>81±3</td>
<td>15.5±1.3</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>Factor VIII activity, U/mL</td>
<td>300 000</td>
<td>110.5±7.2</td>
<td>51±8</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>vWF, U/mL</td>
<td>255 000 million</td>
<td>92±6.6</td>
<td>21.6±7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Fibrinogen, mg/dL</td>
<td>330 000</td>
<td>260±10</td>
<td>14.9±1</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>TF, pg/L</td>
<td>40 000</td>
<td>57±8.6</td>
<td>44.6±13.4</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Protein S, U/mL</td>
<td>69 000</td>
<td>94±2.8</td>
<td>13.6±1.2</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>Protein C activity, U/mL</td>
<td>62 000</td>
<td>98±3.4</td>
<td>10.7±0.6</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>Protein C global assay</td>
<td>NA</td>
<td>0.93±0.03</td>
<td>−19.3±3.8</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>D-dimer, mg/L</td>
<td>~100 000</td>
<td>0.17±0.02</td>
<td>77±39</td>
<td>0.02</td>
</tr>
<tr>
<td>Prothrombin F1 + 2, pmol/L</td>
<td>45 000</td>
<td>149.4±22</td>
<td>155±41</td>
<td>&lt;0.000</td>
</tr>
</tbody>
</table>

Δ% maximal changes is unrelated to the time. NA indicates not applicable.

*PCAT-NR.
markers of endothelial activation, in both the forearm and leg. These measurements, in addition to the evaluation of other specific markers of endothelium, such as P-selectin, adhesion molecules (vascular cell adhesion molecule and intercellular adhesion molecule), tissue-type plasminogen activator, and plasminogen activator inhibitor-1, could strengthen our findings. In addition, detailed exploration of the fibrinolytic system and the cellular component of the coagulation are warranted in this regard.

Blood-borne TF is believed to be shed into circulation from activated cells, including monocytes, platelets, and endothelium, or from the injured surrounding tissue, consequently creating a hypercoagulable state. The present results demonstrate that orthostatic stress induces an increment in the TF antigen level but do not promote identification of the type or source of the TF antigen. This question might be elucidated in future studies.

Perspectives and Clinical Implications

Prolonged orthostatic stress, ie, still standing, significantly activates the coagulation cascade through endothelial activation, possibly because of an increased shear stress in the lower extremities. In parallel, the main counterregulatory system, protein C pathway, fails to neutralize this ongoing orthostatic thrombogenesis.

The present study suggests that posture should be considered a significant factor affecting coagulation studies, including such tests as PT and aPTT. A simple in vivo method (prolonged still standing) to activate the procoagulant and anticoagulant pathways is proposed. Possible pathophysiological projections of the obtained findings could be further considered. Prolonged recumbency and sitting were shown to activate the coagulation cascade significantly.

According to the present findings, prolonged standing, but of much shorter duration, produces a similar effect. Therefore, occupations requiring prolonged standing, such as cashiers, military services, and guards, could predispose to increased hypercoagulability. The economy-class syndrome (trans-Atlantic flights), activates the coagulation cascade through endothelial activation or injury may contribute to this setting.

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Disclosures

None.

References


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