Lower limb-localized vascular phenomena explain initial orthostatic hypotension upon standing from squat

Michael E. Tschakovsky, Kristine Matusiak, Catherine Vipond, and Lisa McVicar
School of Kinesiology and Health Studies, Queen’s University, Kingston, Ontario, Canada

Submitted 8 June 2011; accepted in final form 10 August 2011

Tschakovsky ME, Matusiak K, Vipond C, McVicar L. Lower limb-localized vascular phenomena explain initial orthostatic hypotension upon standing from squat. Am J Physiol Heart Circ Physiol 301: H2102–H2112, 2011. First published August 19, 2011; doi:10.1152/ajpheart.00571.2011.—The cause(s) of initial orthostatic hypotension (transient fall in blood pressure within 15 s upon active rising) have not been established. We tested the hypothesis that this hypotension is due to local vascular phenomena in contracting leg muscles from the brief effort of standing up. Seventeen young healthy subjects (2 males and 15 females, 22.5 ± 1.0 years) performed an active rise from resting squat after a 10-s squat, a 1-min squat, or a 5-min squat. Beat-by-beat arterial blood pressure, cardiac output, heart rate, and stroke volume (Finometer finger photoplethysmography) and right common femoral artery blood flow (Doppler and Echo ultrasound) were recorded. Data are means ± SE. Quiet standing before squat represented baseline. Peak increases in lower limb and total vascular conductance (ml·min⁻¹·mmHg⁻¹) upon standing were not different within squat conditions (10-s squat, 50.0 ± 12.4 vs. 44.3 ± 5.0; 1-min squat, 54.7 ± 9.2 vs. 50.5 ± 4.5; 5-min squat, 67.4 ± 13.7 vs. 58.8 ± 3.9; all P > 0.574). Mean arterial blood pressure (in mmHg) fell to a nadir well below standing baseline in all conditions despite increases in cardiac output. The hypotension predicted by the increase in leg vascular conductance accounted for this hypotension [observed vs. predicted (in mmHg): 10-s squat, −17.1 ± 2.1 vs. −18.3 ± 5.5; 1-min squat, −22.0 ± 3.8 vs. −25.3 ± 4.9; 5-min squat, −28.3 ± 4.0 vs. −29.2 ± 6.7]. We conclude that rapid contraction induced dilation in leg muscles with the effort of standing, along with a minor potential contribution of elevated lower limb arterio-venous pressure gradient, outstrips compensatory cardiac output responses and is the cause of initial orthostatic hypotension upon standing from squat.

Address for reprint requests and other correspondence: M. E. Tschakovsky, Human Vascular Control Laboratory, School of Kinesiology and Health Studies, Queen’s University, Kingston, Ontario, Canada (e-mail: mt29@queensu.ca).

INITIAL ORTHOSTATIC HYPOTENSION (IOH) is an exaggerated transient fall in blood pressure that occurs upon actively rising to a standing position. An active muscular effort to stand is required from lying down, sitting, or squatting to observe IOH. This differs from passive change to upright posture, where there is actually no initial reduction in blood pressure. IOH is a frequent cause of orthostatic complaints (14; 16, 34, 52, 56). Standing from a squat position as opposed to recumbent tends to evoke a greater hypotension (16, 35) and is a recognized trigger for a faint in daily life (16). Episodes can lead to injury, especially in older adults, and can have a negative impact on quality of life (49). Understanding the cause of IOH therefore represents an important step in development of prevention and countermeasures to limit the negative impact of IOH.

Previous attempts to identify the cause of IOH focused on beat-by-beat measurement of cardiac and total peripheral resistance responses and have established that cardiac output (CO) actually increases with the onset of standing, whereas total peripheral resistance falls markedly (3, 41, 42, 52, 53). More invasive measurements have identified immediate elevation in right atrial pressure (RAP) upon standing from supine (53), similar to what occurs at the onset of upright exercise. These observations support the hypothesis that a central shift in blood volume due to leg and abdominal vascular compression with the effort of standing evokes activation of cardiopulmonary baroreceptors and subsequent systemic sympathetic withdrawal (for review see Ref. 54). However, measurements of sympathetic nerve traffic upon standing remain impossible, and no regionally specific measurements of vascular resistance changes upon standing have been performed. As such, definitive support for this hypothesis remains elusive.

An alternative hypothesis is that the marked decrease in total peripheral vascular resistance is due to contributions from vascular phenomena localized to the active lower limbs. One such contribution could be rapid vasodilation in the contracting leg muscles with the effort of standing (54). In the past decade it has been firmly established both in human and animal model studies that skeletal muscle resistance vessels can dilate rapidly (within 1 s) in response to a single brief muscle contraction (9, 18, 29, 36, 48, 50). The magnitude of this dilation is proportional to contraction intensity (46).

Another potential contribution could be the immediate elevation in lower limb hydrostatic pressure on the arterial but not the venous side of the circulation, increasing the pressure gradient for lower limb perfusion (37). Because the effective downstream pressure cannot be determined across a vascular bed in vivo, vascular bed conductance is typically calculated from arterial pressure at the heart level and local blood flow. The term virtual vascular conductance (VVC) has been coined to acknowledge that without direct measurement of the effective local pressure gradient for flow, changes in calculated vascular conductance using heart level arterial blood pressure could reflect either changes in vasomotor tone, the local pressure gradient, or both (38, 47, 54). With this as a background, we tested the hypothesis that IOH upon standing from a squat is explained by vascular phenomena localized to the lower limbs.

MATERIALS AND METHODS

General Methods

Subjects. Seventeen young, healthy adults (2 males, 15 females, 22.5 ± 1.0 year), with no history of smoking, participated. The study was approved by the Health Sciences Human Research Ethics Board at Queen’s University according to the terms of the Declaration of Helsinki. Procedures followed were in accordance with institutional
guidelines. After receiving a complete verbal and written description of the experimental protocol and potential risks, each subject provided signed consent.

**Instrumentation.** Upon arrival at the laboratory, measurement of a subject’s right common femoral artery (CFA) diameter and right brachial artery (BA) diameter were performed with the subject supine. We used Echo ultrasound (see Data Acquisition and Analysis) to assess the diameter of the CFA during quiet standing, lying supine, and 10 s following return to upright in each of the three different squat duration conditions in four subjects before the start of the study to confirm that there were no detectable changes in the artery diameter and that these diameters were the same as in resting supine. The coefficient of variation within subjects across the five measurements ranged from 1.36% to 1.8%. Lack of change in a conduit vessel that is exposed to differences in hydrostatic pressure and in brief elevations in shear is a common finding in our laboratory and others (6, 51).

Subjects were then instrumented for measurement of heart rate (HR; ECG electrodes in standard CM5 placement), beat-by-beat arterial blood pressure (finger photoplethysmograph cuff on the middle finger of the left hand supported in a sling at heart level; Finomter MIDI; Finapres Medical Systems, Amsterdam, the Netherlands), and right CFA blood velocity (pulsed Doppler ultrasound, Model 500V TCD; Multigon Industries, Mt. Vernon, NY). Further details are in Data Acquisition and Analysis below. The Finomter MIDI provided non-invasive, beat-by-beat measures of finger arterial blood pressure, which are then transformed into brachial artery pressure using an antiresonance filter approach (1). This approach has been shown to result in excellent agreement between Finomter brachial artery pressure and directly measured (catheterized brachial artery) arterial pressure measurements during active and passive orthostatic stress maneuvers (1). The Modelflow method is then used to compute aortic flow [stroke volume (SV)] based on a three-element model of aortic input impedance. This approach also results in excellent agreement with thermodilution-derived measures of SV in response to varying changes in posture (19).

**Specific Experimental Protocol**

Subjects had a minimum of 4 h without food and abstained from alcohol, caffeine, or exercise for a minimum of 12 h before arriving at the laboratory for testing. The testing for each subject was completed during a single visit, but the visit time varied between subjects from morning through afternoon.

**Squat trials.** Figure 1 illustrates the sequence of squat to stand trials. It has been suggested that the act of squatting results in ischemia of the leg muscles, and therefore during the squat a buildup of vasodilatory metabolites could occur, contributing to leg vasodilation upon standing (54). To account for this contribution, we had subjects perform three different squat durations [10 s of squat (10-s SQ), 1 min of squat (1-min SQ), and 5 min of squat (5-min SQ)]. We reasoned that, if leg metabolite buildup-evoked vasodilation during squatting was a contributor to subsequent measures of leg vasodilation upon standing, the magnitude of this contribution should be increased with squat duration. Subjects began from a quiet standing baseline (SBL) with a 5-min lead in period. They then performed three squat and rise trials for each of the squat durations and were reminded for each to rise with equal effort in both legs and not to strain or hold their breath. CFA blood flow responses were obtained before squat and upon standing. Other hemodynamic variables were also measured throughout the squat trial. The order of squat duration trials was counterbalanced to prevent an order effect. Specifically, all trials of a given squat duration were performed in sequence, but the six potential orders of squat duration that can occur with three squat duration conditions were sequentially assigned to subjects on entry into the study. Four minutes of recovery during quiet standing occurred between each squat trial. Subjects maintained a relaxed, flat-footed posture in the squat. In a subset of eight subjects, the third trial for each of the squat durations was performed once the right arm BA above the antecubital fossa was instrumented for pulsed Doppler ultrasound measures of brachial artery blood flow. This served as a surrogate for detecting systemic changes in vascular tone, as is hypothesized to be responsible for IOH subsequent to cardiopulmonary baroreceptor-mediated sympathetic withdrawal (41, 53).

**Data Acquisition and Analysis**

Quantifying hemodynamic responses. SBL values for all variables were computed as an average of beat-by-beat values for 1 min preceding the onset of squat. The effect of squatting on hemodynamic variables was determined by quantifying the last 10 s of squat before standing for the 1- and 5-min squat tests (End SQ). For the 10-s squat condition, the last 5 s of squat before standing were averaged. The peak response magnitude for each hemodynamic variable was identified for each subject by finding the cardiac cycle that represented the nadir (lowest if direction of response was a decrease, highest if direction of response was an increase) and then averaged across the preceding to the following cardiac cycle (3 cardiac cycles).

As there was no difference in the time to peak response between VVC in the lower limbs (VVC_{lower limbs}) or total vascular conductance (TVC) or mean arterial pressure (MAP), we quantified the contribution of central and peripheral hemodynamic responses to the MAP nadir in each subject, by averaging the three cardiac cycles occurring at the subject-specific VVC_{lower limbs} and TVC nadirs. Because the peak CO often occurred after the MAP nadir, CO was obtained for the three cardiac cycles of the MAP nadir. For all of these, the response for a given subject was obtained by averaging the results of the multiple trials (3 or 2 or 1, depending on the variable and the subject, as described previously).

**CFA and brachial artery blood flow.** CFA blood flow was determined from baseline resting measurement of CFA diameter and mean blood velocity. In brief, the right CFA was imaged proximal to the identified bifurcation into the superficial and deep femoral arteries using a 10 MHz linear array ultrasound probe (GE Vingmed System Five; GE Medical) with the subject lying supine. One minute of
images was captured in DICOM format for automated offline analysis of diameter using automated edge detection software as described previously (32). The angle of the CFA relative to the skin surface was determined to allow correction for Doppler ultrasound measures of mean blood velocity (see explanation below). Upon standing, a flat 4 MHz pulsed Doppler ultrasound probe was affixed to the skin at the same ultrasound probe site and used for subsequent measures of CFA blood velocity during the experimental trials. This system does not have imaging capabilities but allows acquisition of arterial blood velocity immediately upon standing. Measurement of CFA blood velocity was obtained for 1 min of quiet standing (baseline) and again for 1 min following return to standing after squat. A quality signal could be re-established by the operator within 2 s of the subject rising from squat. For the BA, the procedure for measuring artery diameter and angle relative to the skin was the same and occurred proximal to the antecubital fossa. Subsequently, the flat 4 MHz pulsed Doppler ultrasound probe was affixed to the skin at this same location. The subject was reminded to let his or her arm hang by his or her side and remain completely relaxed during the squat trials.

As described previously our Doppler ultrasound systems have been calibrated by measurement of fluid with ultrasound reflecting particles flowing through tygon tubing of known internal diameter (32). This results in a linear relationship between ultrasound voltage output ($r^2 = 0.98$), which is linearly related to the Doppler frequency shift representing velocity and known mean flow velocity for the 4 MHz pulsed Doppler ultrasound probe angle of 57 degrees when it is parallel to the tube. By measuring the angle of the CFA relative to the skin surface, we quantified the actual insonation angle of the ultrasound probe and computed a correction factor, which accounted for differences between the actual insonation angle and the calibration insonation angle. For example, if the actual insonation angle of the pulsed Doppler probe on the CFA of a particular subject was steeper than the 57 degrees used for the calibration, the frequency shift for a given flow velocity would be less by a predictable amount based on the effect of the cosine of the insonation angle. This translated into a change in the slope of the velocity versus voltage calibration, which could then be applied to quantification of the actual CFA mean blood velocity for that subject. This ensured that we were obtaining accurate absolute measures of CFA blood flow across subjects. CFA blood flow (in ml/min) was then calculated as mean blood velocity (in cm/s) $\times$ 60 s/min $\times$ $\pi$ (diameter $^2$). The same approach was utilized for measures of brachial artery blood flow (in ml/min).

**MAP and estimation of SV.** As mentioned previously, a finger photoplethysmograph cuff placed on the middle finger of the left hand, with the hand maintained at heart level by supporting the arm in a sling, was used to obtain beat-by-beat MAP. SV estimates were obtained using the Modelflow method in which arterial pressure waveforms measured at the finger are used to compute an aortic flow waveform, which is then integrated to provide an estimate of left ventricular SV (2).

**Calculated hemodynamic variables.** All of the post-squat subscripts below indicate which hemodynamic variable nadir was used for the calculations. CO was calculated as SV $\times$ HR. TVC was calculated as MAP $\div$ CO. Because we measured CFA blood flow in one leg, we doubled this to represent lower limb vasodilation. VVC$_{lower}$ limits was therefore calculated as 2 $\times$ CFA blood flow $\div$ MAP. Forearm vascular conductance (FVC) was calculated as BA blood flow $\div$ MAP.$^\dagger$ We also wished to isolate the effect of a given hemodynamic variable as it would have occurred were it the only variable to change. The change in CO from SBL to its post-squat nadir upon standing that was due solely to increased SV was calculated as $HRS_{BL} \times$ SV$_{CO Post-Squat Nadir} - CO_{SBL}$. The change in CO from SBL to its post-squat nadir upon standing that was due solely to increased HR was calculated as $HR_{CO Post-Squat Nadir} \times$ SV$_{SBL} - CO_{SBL}$. The decrease in MAP from SBL upon standing from squat that would have been observed due to increased TVC if no CO increase had occurred (i.e., the independent effect of TVC) was calculated as $[CO_{SBL} + TVC_{Post-Squat Nadir} - MAP_{SBL}]$. The degree to which increases in CO blunted the hypotension that would have occurred due to the increased TVC upon standing from squat was calculated as $MAP_{CO Post-Squat Nadir} - MAP_{due to increased TVC}$.

To determine whether the observed peak $VVC_{lower}$ limits could account for the observed IOH we calculated the drop in MAP that would be due to $VVC_{lower}$ limits alone as follows: $CO_{MAP Post-Squat Nadir} \div (TVCS_{BL} + VVC_{VVClower limbs Post-Squat Nadir}) - MAP_{SBL}$.

By measuring lower limb blood flow, we were able to partition the TVC into $VVC_{lower}$ limits plus a lumped sum of all other vascular conductances. Differences between TVC and $VVC_{lower}$ limits would therefore reveal a net vasodilation or vasoconstriction, or no change, summed for all other vascular beds. It is important to recognize that observing no difference between TVC and $VVC_{lower}$ limits changes does not rule out the possibility of vasodilation in some other vascular beds, but such vasodilation would have to be exactly counterbalanced by vasoconstriction in additional vascular beds.

**Statistics.** Two-way repeated measures ANOVA was used to test for main effects of squat duration (10-s SQ, 1-min SQ, 5-min SQ) and time (SBL, End SQ, post-squat nadir, 1-min post-squat), as well as interaction effects, on the absolute values of all hemodynamic variables. To determine whether the peak TVC change in rising from a squat could be explained by changes in $VVC_{lower}$ limits and whether this was dependent on squat duration, a two-way repeated-measures ANOVA was used to test for a main effect of squat duration on the change in TVC and $VVC_{lower}$ limits from SBL to post-squat nadir, a main effect of conductance location (TVC vs. $VVC_{lower}$ limits), and significant interaction effects. To determine whether the magnitude of hypotension upon standing could be explained by the peak increase in $VVC_{lower}$ limits and whether this was dependent on squat duration, a two-way repeated-measures ANOVA was used to test for a main effect of squat duration, hypotension source (actual vs. $VVC_{lower}$ limits predicted), and significant interaction effects. One-way repeated-measures ANOVA was used to test the independent contributions of HR and SV to changes in CO and TVC and CO to changes in MAP as a function of squat duration, as well as the effect of squat duration on changes in CO and MAP upon standing. The level for significance was set at $P < 0.05$, and significant differences for ANOVA were further assessed using Student-Neuman-Keuls post hoc tests. All statistics were calculated using SigmaStat 3.1 (SPSS, Chicago, IL). All data are means $\pm$ SE.

**RESULTS.**

**Hemodynamic Changes in Response to Squat.** Table 1 summarizes the SBL and End SQ hemodynamic response. There were no differences in SBL between the squat conditions for any of the hemodynamic variables. By the end of the squat, SV, CO, and MAP had all increased significantly. In contrast, HR had decreased significantly. There was no significant change in TVC. The increase in MAP was affected by squat duration (5-min SQ $> 1$-min SQ $> 10$-s SQ; $P < 0.05$).

**Hemodynamic Changes After Rising From Squat.** Figure 2 presents the beat-by-beat arterial blood pressure and CFA blood flow velocity response in a single subject upon rising from 5-min SQ.

**Peak hemodynamic response.** Table 1 summarizes the hemodynamic response upon rising from squat. The peak re-
response for each variable is shown, as is the response after 1 min. Figure 3 presents the time course of hemodynamic variables upon rising from squat. Over the first 10 s upon standing, MAP pressure fell substantially in all three squat conditions. Squat duration had an impact on the magnitude of hypotension since MAP fell to a greater extent following the 5-min SQ versus both 1-min SQ and 10-s SQ (Fig. 4B). This effect was greatest in the 5-min SQ compared with both 1-min SQ and 10-s SQ (P < 0.05). The increase in CO blunted the impact of elevated TVC. The increase in CO from SBL to MAP nadir would have, on its own, elevated MAP by ~20 mmHg in all three squat conditions (Fig. 4B). The net result was therefore an actual reduction in MAP upon returning to SBL levels (i.e., removal of the squat effect), whereas the true hypotension is represented by reductions below SBL.

Table 2 summarizes the time it took to reach peak response following standing from squat for VVC_lower limbs, TVC, MAP, and CO. There was no difference in the time to peak change between VVC_lower limbs, TVC, and MAP within or between any of the squat durations. However, CO time to peak was significantly delayed versus VVC_lower limbs, TVC, and MAP in the 5-min SQ condition (P < 0.05).

The net result was therefore an actual reduction in MAP upon returning to SBL levels (i.e., removal of the squat effect), whereas the true hypotension is represented by reductions below SBL.

Table 1. Hemodynamic responses to squatting and returning to standing

<table>
<thead>
<tr>
<th>Squat Time</th>
<th>Standing Baseline</th>
<th>End Squat</th>
<th>Post-squat Nadir</th>
<th>1-min Post-squat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>84.0 ± 2.3</td>
<td>74.7 ± 2.3†‡</td>
<td>109.3 ± 3.0†‡</td>
<td>84.1 ± 3.0†‡</td>
</tr>
<tr>
<td>5 min</td>
<td>81.5 ± 2.1</td>
<td>63.7 ± 2.4†</td>
<td>99.9 ± 2.6†</td>
<td>78.4 ± 2.6</td>
</tr>
<tr>
<td>1 min</td>
<td>81.6 ± 2.1</td>
<td>71.4 2.7†</td>
<td>93.6 ± 2.5†</td>
<td>80.1 ± 1.4</td>
</tr>
<tr>
<td>Stroke volume, ml/bt</td>
<td>50.4 ± 3.3</td>
<td>76.0 ± 4.6*</td>
<td>80.1 ± 6.0*</td>
<td>63.6 ± 5.7*‡‡</td>
</tr>
<tr>
<td>5 min</td>
<td>53.4 ± 3.7</td>
<td>80.5 ± 5.1*</td>
<td>81.1 ± 6.6*</td>
<td>56.2 ± 4.6</td>
</tr>
<tr>
<td>1 min</td>
<td>52.2 ± 3.3</td>
<td>75.3 ± 6.0*</td>
<td>81.7 ± 7.4*</td>
<td>53.7 ± 3.3</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>4.1 ± 0.3</td>
<td>5.6 ± 0.4*</td>
<td>7.6 ± 0.5†‡</td>
<td>5.2 ± 0.3*‡</td>
</tr>
<tr>
<td>5 min</td>
<td>4.3 ± 0.3</td>
<td>5.0 ± 0.3*</td>
<td>7.0 ± 0.5*</td>
<td>4.3 ± 0.3*</td>
</tr>
<tr>
<td>1 min</td>
<td>4.2 ± 0.3</td>
<td>5.1 ± 0.3*</td>
<td>6.7 ± 0.5*</td>
<td>4.9 ± 0.7*</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>91.1 ± 3.1</td>
<td>110.1 ± 5.0†‡</td>
<td>62.8 ± 4.4†‡</td>
<td>91.9 ± 3.5</td>
</tr>
<tr>
<td>5 min</td>
<td>91.2 ± 3.6</td>
<td>102.2 ± 4.0†‡</td>
<td>69.2 ± 3.1*</td>
<td>91.7 ± 3.5</td>
</tr>
<tr>
<td>1 min</td>
<td>89.1 ± 3.8</td>
<td>97.0 ± 4.6*</td>
<td>72.0 ± 3.9*</td>
<td>91.0 ± 4.3</td>
</tr>
<tr>
<td>TVC, ml·min⁻¹·mmHg⁻¹</td>
<td>45.8 ± 3.2</td>
<td>51.7 ± 2.8</td>
<td>104.6 ± 6.2†‡</td>
<td>57.3 ± 4.1†‡</td>
</tr>
<tr>
<td>5 min</td>
<td>47.5 ± 3.1</td>
<td>49.5 ± 2.6</td>
<td>98.0 ± 6.1†‡</td>
<td>48.0 ± 3.8</td>
</tr>
<tr>
<td>1 min</td>
<td>48.1 ± 3.4</td>
<td>52.2 ± 3.1</td>
<td>92.4 ± 6.9†‡</td>
<td>47.3 ± 3.4</td>
</tr>
<tr>
<td>VVC_lower limbs, ml·min⁻¹·mmHg⁻¹</td>
<td>3.0 ± 0.7</td>
<td>—</td>
<td>70.4 ± 14.1†‡</td>
<td>9.9 ± 1.8†‡</td>
</tr>
<tr>
<td>5 min</td>
<td>3.3 ± 0.5</td>
<td>—</td>
<td>58.0 ± 9.6*</td>
<td>4.1 ± 1.0</td>
</tr>
<tr>
<td>1 min</td>
<td>3.0 ± 0.7</td>
<td>—</td>
<td>53.0 ± 12.9*</td>
<td>4.3 ± 0.9</td>
</tr>
<tr>
<td>2-LBF, ml/min</td>
<td>272 ± 50</td>
<td>—</td>
<td>3.914 ± 668*</td>
<td>1.041 ± 226‡</td>
</tr>
<tr>
<td>5 min</td>
<td>311 ± 50</td>
<td>—</td>
<td>3.996 ± 576*</td>
<td>374 ± 83</td>
</tr>
<tr>
<td>1 min</td>
<td>307 ± 67</td>
<td>—</td>
<td>3.661 ± 740*</td>
<td>325 ± 61</td>
</tr>
</tbody>
</table>

Values are means ± SE. CO, cardiac output; MAP, mean arterial pressure; TVC, total vascular conductance; VVC_lower limbs, virtual vascular conductance of the vascular beds supplied by the common femoral artery; 2-LBF, two-leg blood flow. *Significantly different from standing baseline within condition; †significantly different from 1-min squat within time; ‡significantly different from 10-s squat within time. All P < 0.05.
in the 1-min SQ, all \( P < 0.05 \), with no change in FVC in the 5-min SQ. The combination of changes in FVC and MAP resulted in significant reductions in FBF following 1-min SQ and 5-min SQ (\( P < 0.05 \)).

**DISCUSSION**

This study was the first to measure lower limb blood flow in combination with cardiac responses to distinguish between a cardiopulmonary baroreflex versus lower limb vascular phenomena as the cause of IOH during rising from squat. The key novel findings in this regard are as follows. First, SV was already maximally elevated during squat with no further increases upon standing. Second, the timing of peak changes in VVC\(_\text{lower limbs} \) and TVC upon standing from squat was not different from that for MAP. Third, peak (\( \sim 12\)-fold) increases in VVC\(_\text{lower limbs} \) explained all the increase in TVC upon standing from squat. Fourth, the hypotension post-squat that could be attributed to an independent effect of VVC\(_\text{lower limbs} \) explained all of the actual hypotension observed. Fifth, forearm measurements were consistent with a lack of systemic vasodilation as would be predicted based on a cardiopulmonary reflex-mediated withdrawal of sympathetic vasoconstrictor activity. These findings support lower limb-localized vascular phenomena, in particular rapid vasodilation as a result of the brief muscular effort to stand from a squat, as being responsible for initial orthostatic hypotension during this active posture change.

**Accounting for the Hemodynamic Effect of Squatting on IOH**

SBL arterial blood pressure represents the blood pressure regulation target upon returning to standing from squat. Therefore, evaluation of IOH magnitude must take into account a squat-specific influence on cardiovascular homeostasis. In this regard, our findings indicate that squatting results in an increase in MAP from SBL that is already manifest with brief (10 s) squatting and increases with squat duration (see Table 1 and Fig. 3). This hypertension appears to be due to an increase in CO during squat resulting from increased SV, despite a decreased HR. The SV increase was likely due to lower body compression-evoked translocation of venous volume to the central veins. The fact that this SV increase was already present in the 5- to 10-s period of the 10-s squat and equal to the other squat conditions supports this. The HR reduction would likely be a baroreflex-mediated compensation that blunts the hypertension with squatting.

**Mechanisms Localized to the Lower Limbs Explain IOH on Rising From Squat**

The observed rapid but transient fall in blood pressure with active standing indicates that blood flow out of the arterial space temporarily exceeded blood flow into the arterial space. The former is a function of resistance vessel caliber and the pressure gradient for peripheral blood flow, which are lumped together in the calculated VVC\(_\text{lower limbs} \). In this study, whereas the latter is a function of CO. By measuring the increase in CFA blood flow immediately upon standing, we were able to quantify how much of the increase in arterial outflow could be localized to the vascular beds supplied by the CFA (i.e., lower limb vascular beds; see Fig. 4). This allowed us to identify that all of the increase in TVC was accounted for by increases in VVC\(_\text{lower limbs} \). This also allowed us to identify that all of the observed hypotension could be explained by increases in VVC\(_\text{lower limbs} \) (Fig. 4).

Given that VVC\(_\text{lower limbs} \) can be pressure gradient and resistance vessel caliber dependent, the question arises as to which mechanism(s) contributed to IOH. Mechanisms that elevate the pressure gradient for blood flow through the CFA supplied vascular bed (37, 47) could include the reduction of venous pressure by the muscle pump (25) and the restoration of arterial hydrostatic pressure in the lower limbs upon standing (37). Mechanisms that increase resistance vessel caliber could include local vasodilator substances (8), mechanical vessel compression effects on vascular tone (9), and possibly resistance vessel mechanical distension via increased local hydrostatic pressure upon standing (51).

**Lower limb pressure gradient contributions to IOH.** As described in the introduction, limb vascular conductance calculated from measures of arterial pressure and limb blood flow is potentially virtual, meaning that an increase in blood flow observed at a given arterial pressure could reflect both vasodilation (increase in true vascular conductance) and changes in the effective pressure gradient for flow, which are hidden due to the inability to directly measure the relevant downstream pressure (17, 38, 47). In this regard, Sheriff et al. (37) observed a \( \sim 82\% \) increase in lower limb blood flow in response to passive 30° head up tilt (thought to be \( \sim 0.5 \) G) from supine, which had a similar time course to CFA blood flow in the present study. They attributed this hyperemia to an increase in arterial but not venous hydrostatic pressure upon tilt, due to venous valve prevention of immediate venous refilling.
In our study we observed a 12- to 14-fold increase in CFA blood flow upon rising from squat compared with SBL. How much of this might be explained by changes in the pressure gradient for lower limb blood flow? Before this question can be answered, two models of the microvasculature need to be considered. The conventional model, based on Poisseille’s Law, states that flow through a vascular bed is proportional to the pressure drop from arterial to venous vasculature, divided by the resistance of the vascular bed. An alternative model, often ignored despite being supported by a considerable body of evidence, is that the microvasculature acts like a Starling resistor (4, 22, 27, 31, 39, 40) where the resistance to flow requires arterial pressure to rise above a microvascular critical closing pressure (Pcrit) before flow can occur. Consistent with this are observations that flow through a vascular bed ceases when arterial pressure is still well above venous pressure (22, 27). The Pcrit is a characteristic of collapsible vessels such as blood vessels and can be easily understood by imagining a collapsible vessel traveling through a column of water, where the hydrostatic column pressure acts to collapse the vessel (i.e., the Pcrit). There will be no flow through this tube until the inflow pressure exceeds the Pcrit to force the vessel open. In this case, Pcrit represents the downstream pressure of the pressure gradient for flow through the vessel. Most impor-

Fig. 3. Time course of hemodynamic responses to squat and return to standing. Bln, standing baseline before squat. End SQ, last 10 s of squat (last 5 s for the 10-s squat condition). Gray vertical bar represents transition period from squat to standing. Data are presented in 2-s averaged intervals upon standing from squat, 10-s squat (black circles), 1-min squat (gray circles), and 5-min squat (white circles). A: MAP at heart level (top left). B: total vascular conductance (TVC; middle left). C: lower limb leg virtual vascular conductance for both legs (VVClower limbs, bottom left). D: cardiac output (CO; top right). E: stroke volume (SV; middle right). F: heart rate (HR; bottom right). bpm, Beats/min.
stantly, pressure on the outflow side has no impact on flow through the vessel until this downstream pressure exceeds \( P_{\text{crit}} \). In the microvasculature, vascular smooth muscle tone acts as the collapse pressure (39). Venous pressure would represent the outflow pressure, but not the downstream pressure of the pressure gradient for flow, until it exceeds the \( P_{\text{crit}} \).

Turning to the data, heart level arterial pressure in our study averaged \( 90 \) mmHg (Table 1). If we assume Poisseille’s Law as an accurate model of lower limb hemodynamics, even if venous pressure remained at \( 0 \) mmHg until the peak CFA blood flow \( 4 \) to \( 5 \) s after rising, then the addition of a hydrostatic column of \( 122 \) cm would be required simply to double the lower limb arterio-venous pressure gradient from \( 90 \) mmHg during quiet standing to immediate post-rise from squat. Most of the muscle mass engaged in rising from squat would be knee and hip extensors well above such a hydrostatic column relative to heart level. Therefore, an arterio-venous pressure gradient effect due to venous emptying in squat on its own could not have been responsible for more than a very small fraction of the \( 12 \)-fold increase in CFA blood flow and calculated VVC lower limbs upon rising from squat. If we assume a vascular waterfall model, then returning to an upright standing position from squat would merely restore the lower limb arterial pressure to the pre-squat baseline, thus restoring the arterial to \( P_{\text{crit}} \) pressure gradient so that we would not expect any increase in CFA blood flow and calculated VVC lower limbs.

To our knowledge, only one previous study has directly examined the effect of reducing and then restoring hydrostatic pressure in a limb vascular bed and isolating the contribution of the venous circulation to this effect. Tschakovsky and Hughson (45) examined the effect of elevating the forearm from below to above heart level (difference of \( 32 \) mmHg hydrostatic pressure column) and returning it to below heart level after 2 min. They did so under two conditions: one where forearm veins were allowed to empty with arm elevation and one where inflation of an upper arm cuff prevented forearm venous emptying with arm elevation. Arm elevation resulted in a

![Fig. 4](http://example.com/fig4.png)

**Fig. 4.** A: partitioning the change in CO from standing baseline to the CO nadir. The increase in CO above standing baseline that is due to SV is determined using the standing baseline HR and the SV at the CO nadir. The additional effect of HR is then calculated using the SV and HR at the CO nadir. B: the change in MAP due to the peak change in TVC if no change in CO occurred is shown on at left. The compensatory independent effect of CO on MAP, shown in the middle, is calculated using the CO at the MAP nadir and the peak TVC. The resultant MAP shown on at right is the difference between the. *Significantly different from 10-s squat; †significantly different from 1-min squat. All \( P < 0.05 \).

![Fig. 5](http://example.com/fig5.png)

**Fig. 5.** A: comparison of the peak change in TVC and VVC\textsubscript{lower limbs} upon standing. B: the actual change in mean arterial pressure (MAP) compared with the change in MAP that occurred due to VVC\textsubscript{lower limbs}. *Significantly different from 10-s squat; †Significantly different from 1-min squat. All \( P < 0.05 \).

### Table 2. Time to peak change from standing baseline

<table>
<thead>
<tr>
<th>Squat Time</th>
<th>VVC\textsubscript{lower limbs}</th>
<th>TVC</th>
<th>MAP</th>
<th>CO</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 min</td>
<td>4.9 ± 0.5</td>
<td>5.7 ± 0.4</td>
<td>6.2 ± 0.4</td>
<td>9.5 ± 1.2*</td>
</tr>
<tr>
<td>1 min</td>
<td>4.4 ± 0.5</td>
<td>5.8 ± 0.4</td>
<td>6.2 ± 0.5</td>
<td>6.6 ± 0.9</td>
</tr>
<tr>
<td>10 s</td>
<td>4.0 ± 0.3</td>
<td>4.9 ± 0.2</td>
<td>5.7 ± 0.4</td>
<td>5.7 ± 0.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. Time to peak is in seconds starting upon return to standing after squat. *Significantly different from other hemodynamic variables within squat time, \( P < 0.05 \).
Delayed minor vasodilation in both conditions. Poiseille’s model of the circulation would predict that upon arm lowering when venous emptying was allowed, forearm blood flow would increase compared with baseline in proportion to both the minor vasodilation and the temporarily increased local arterio-venous pressure gradient due to venous emptying, whereas when venous emptying was prevented, the increase in forearm blood flow would be smaller since there was no increased arterio-venous pressure gradient. The vascular waterfall model would predict that the increase in flow would be the same between conditions since both experienced the same minor vasodilation in the arm above heart position. Their observation of identical increases in flow upon arm lowering, regardless of whether veins had emptied, argues strongly against an effect of an arterio-Pcrit gradient will enhance the effect of a given increase in resistance vessel caliber. For example, an increase in true vascular conductance due to vasodilation of 10 ml·min⁻¹·mmHg⁻¹ at a local perfusion pressure of 100 mmHg will increase blood flow by 1,000 ml/min, whereas at a local perfusion pressure of 150 mmHg it will increase blood flow by 1,500 ml/min. This change is independent of the starting blood flow or vascular conductance.

In the present study, this effect would manifest as an increase in CFA blood flow for a given vasodilation that was greater at lower limb level than would be expected at heart level in proportion to the difference in heart versus lower limb arterial pressure (i.e., local hydrostatic pressure could increase blood flow by 1,500 ml/min. This change is independent of the starting blood flow or vascular conductance. The vascular waterfall model would predict that the increase in flow would be the same between conditions since both experienced the same minor vasodilation in the arm above heart position. Their observation of identical increases in flow upon arm lowering, regardless of whether veins had emptied, argues strongly against an effect of an arterio-venous pressure gradient per se on the local limb blood flow. Taken together, the present data combined with these previous findings would indicate very little, if any, contribution of a hydrostatic pressure gradient effect on VVC_lower limbs in the present study.

Lower limb contraction evoked rapid vasodilation contributions to IOH. It has been well established in a number of independent laboratories and a number of different experimental models, including in vivo human forearm contractions (5, 6, 23, 46, 48), in situ electrically stimulated animal muscle (30, 50), and in vitro isolated vessel preparations (9), that resistance vessels in skeletal muscle can dilate virtually immediately following a brief (as little as 0.3 s) muscle contraction. The time course of this dilation peaks approximately three to four cardiac cycles following contraction release (30, 48). The temporal profile of the increase in VVC_lower limbs in the present study is consistent with that of muscle contraction-induced rapid vasodilation. The magnitude of the response is contraction intensity dependent (46) and may be sensitive to aging (6) and to exercise training (28). The magnitude of this vasodilatory response has not been determined for lower limb muscle contractions, but can be up to a ~5-fold increase following 1-s forearm contractions performed at heart level in forearm trained athletes (28) or below heart level in untrained people (46).

Although the magnitude of hydrostatic pressure increase in the present study by itself could only explain a small portion, if any, of the observed increase in lower limb blood flow upon rising from squat, a greater local arterio-venous or arterio-Pcrit gradient will enhance the effect of a given increase in resistance vessel caliber. For example, an increase in true vascular conductance due to vasodilation of 10 ml·min⁻¹·mmHg⁻¹ at a local perfusion pressure of 100 mmHg will increase blood flow by 1,000 ml/min, whereas at a local perfusion pressure of 150 mmHg it will increase blood flow by 1,500 ml/min. This change is independent of the starting blood flow or vascular conductance.

In the present study, this effect would manifest as an increase in CFA blood flow for a given vasodilation that was greater at lower limb level than would be expected at heart level in proportion to the difference in heart versus lower limb arterial pressure (i.e., local hydrostatic pressure could modulate the effect of a given vasodilation on the increase in CFA outflow and therefore the calculated VVC_lower limbs).

Myogenic and sustained muscle contraction limb ischemia contributions to IOH. With squat, the possibility for lower limb vasodilation due to external vessel compression-evoked reductions in transmural pressure, a relative hypoperfusion due to sustained mechanical compression of resistance vessels, and/or minor muscular effort in maintaining squat also exists. We observed that the magnitude of IOH was not different when squat duration was 10 s or 1 min, but 5 min of squat resulted in a significantly greater IOH. Tschakovskiy and Hughson (45) and Rogers and Sheriff (33) provide evidence for myogenic relaxation with reduced transmural pressure in the resistance vasculature. It is possible that in the present study, there is a reduction in leg resistance vessel transmural pressure due to the compressive effect of squat. This would be expected to already be near maximal in the 10-s squat based on the findings of Rogers and Sheriff (33). Therefore, it is possible that such myogenic dilation also contributed to the CFA blood flow increase upon standing. Because the contraction intensity dependence of rapid vasodilatory mechanisms should be the same for all squat conditions as the effort of rising would be the same, these observations suggest that hypoperfusion mediated vasodilation (i.e., accumulation of metabolic vasodilators) contributed to the increase in VVC_lower limbs in the 5-min squat condition. The observation that there was not a difference in the peak VVC_lower limbs between the 10-s and 1-min squat.

**Fig. 6.** A: forearm vascular conductance (FVC) at standing baseline and at peak response upon standing from squat. B: Forearm blood flow (FBF) at standing baseline and at peak response upon standing from squat. *Significantly different from standing baseline within squat condition. All P < 0.05.
conditions upon standing further supports the existence of this particular hypoperfusion effect being limited to the 5-min squat condition.

Cardiopulmonary Baroreflex Does Not Explain IOH on Rising From Squat

The lack of increase in SV upon standing is consistent with previous findings (54). However, by tracking SV from presquat standing onward we have identified a critical temporal characteristic of the SV contribution to elevated CO that provides further insight into the potential role of a cardiopulmonary baroreflex contribution to IOH. Our data demonstrate that SV elevation occurs during the squat and is not associated with the act of standing up from squat. A number of investigators have previously proposed that IOH is due to cardiopulmonary baroreflex-mediated sympathetic withdrawal brought about by a sudden increase in RAP upon standing (3, 41, 42, 56). Some of these studies used a supine to standing transition (3, 21, 41, 56). Indeed, Wieling and colleagues (53) observed increases in RAP upon rising from supine without a change in SV. These investigators proposed that a lack of increase in SV reflected a balance between right atrial filling pressure and increased HR.

In contrast, we did not see an increase in SV in the first few seconds upon standing from squat when HR had not yet increased. If there had been an increase in RAP due to blood translocation from the periphery, an increase in SV should have been evident during those first few seconds. It was not. The large increase in SV during squat would suggest that central blood volume translocation was already manifest during squatting. Others have demonstrated that SV is actually also maximized in the supine position due to central blood volume translocation, as evidenced by virtually no increase in SV with the onset of supine exercise (15). Despite this evidence, we cannot conclusively exclude the possibility that there could be some elevation in SV when standing up from supine, which might make the central hemodynamic environment evoked by rising from supine slightly different than rising from squat. Regardless, it remains that all of the decrease in MAP upon standing from squat could be attributed to the increase in VVC\textsubscript{lower limbs}, and therefore any involvement of the cardiopulmonary reflex in IOH was so small as to be physiologically insignificant.

Finally, we also measured resting forearm blood flow and vascular conductance to assess the presence of systemic sympathetic withdrawal. We compared the FVC and FBF during SBL with the peak change in FVC and FBF immediately upon standing. During the squat there was an increase in SV and MAP. This might be expected to initiate baroreflex and cardiopulmonary reflex-mediated sympathetic withdrawal and therefore systemic vasodilation. However, if that were the case, then the comparison of SBL with return to standing FVC would indicate that, if anything, there was forearm vasoconstriction in going from squat to stand (Fig. 6). This is further evidence against a cardiopulmonary reflex mediated systemic sympathetic withdrawal explaining IOH in rising from squat and is consistent with our finding that all of the change in TVC was explained by changes in VVC\textsubscript{lower limbs}.

Arterial Baroreflex Regulation of MAP Upon Rising From Squat

The elevated CFA blood flow upon rising from squat represents a perturbation to arterial blood pressure homeostasis. Elevation in CO and vasoconstriction of other vascular beds are the two ways in which the arterial baroreflex response to the sensed hypotension could counteract this disturbance. Indeed, a further increase in CO output above levels during squat was evident upon rising from squat. However, unlike during squat, this increase in CO was due solely to an increase in HR, with no change in SV evident. Previous observations that parasympathetic blockade eliminates the rapid increase in HR upon standing would argue that it is due to immediate vagal withdrawal, and observations of peak HR by 3 s upon standing were also consistent with central command and peripheral muscle mecanoreflex activation being responsible (for review see Ref. 54). However, in our study HR increase did not peak until well after 3 s. Furthermore, it remained elevated for a prolonged period in the 5-min squat. We propose that the timing, direction, and duration of the HR change upon standing in this study represents a significant contribution of the arterial baroreflex to counteracting IOH.

Potential Limitations

There are a number of active posture changes that can occur during daily life. Rising from supine when we get out of bed and from sitting when we arise from a chair are common. However, so is rising from squat, which occurs whenever one is required to access something from, or close to, the floor (e.g., kitchen cupboards, doors), which would also occur on a daily basis for most people. We chose the squat to stand change because it evokes the greatest IOH and is actually used to assess active standing induced IOH (the Schellong test). Patients often complain of orthostatic dizziness and syncope on arising from the squatting position (W. Wieling, MD, personal communication). Although we cannot be certain that our findings with squat to stand posture change are completely representative of supine or seated to stand, rising from squat is a common posture change and therefore equally relevant to the issue of understanding causes of IOH. We do acknowledge that future directions would require confirmation of the mechanism underlying IOH in these other active posture changes.

We do not show hemodynamic measurements during the 2-s period of rising from squat, because of the high possibility of measurement error due to alterations in finger cuff position in the effort of maintaining balance while rising. It may be argued that changes in SV occurred during this brief period and that they are relevant for stimulation of the cardiopulmonary reflex. Again, immediately upon standing there was no difference in SV compared with squat. Given that changes in RAP would manifest in the left ventricle a few seconds afterward due to transit time through the lungs, we believe that the observations immediately upon standing are representative of what is happening upon the effort of standing.

Perspectives

Syncopal episodes have a negative impact on quality of life (26, 49). IOH is a frequent cause of orthostatic complaints (11–13, 20, 43, 44, 52, 55, 56). It is fairly common in young
adults (16) but does not appear to be worsened with age (55). Prevention or management of IOH is guided by understanding of the underlying physiological cause(s). The identification of lower limb vascular phenomena, primarily consisting of muscle contraction-evoked rapid vasodilation as the cause, has important implications for identification of at risk populations and for advising appropriate countermeasures. In this regard, it has been demonstrated that contraction-induced rapid vasodilation is actually blunted with age (6), but emerging evidence from our laboratory (28) indicates it may be enhanced with exercise training. Thus fitness may need to be considered in identifying at risk persons. Current suggested countermeasures include leg tensing upon standing (24) to attenuate IOH by mechanically restricting leg vasculature. Because muscular effort-evoked lower limb vasodilation is the cause of IOH, educating at risk populations to minimize muscle effort and to be aware that under conditions of strong effort that they need to bend over to lower their head for \(~10\) s upon standing (hydrostatic pressure elevation compensation for the period of systemic hypotension) may be advisable. Future work to identify factors that predispose to enhanced rapid vasodilation is therefore warranted.

Conclusions

In summary, evidence from this study supports arterial outflow through the CFA supplied vascular bed of the lower limbs as the cause of IOH. Rapid vasodilation is the primary determinant of the elevated CFA outflow, and its effects are enhanced by the greater hydrostatic pressure in the lower limbs relative to heart level. Additional vasodilation, likely in response to hypoperfusion relative to local metabolic demand, can contribute if squat duration is prolonged. Temporal characteristics of SV changes, as well as FVC in transition from standing to squat and back to standing, offer an additional argument against any contribution of the cardiopulmonary baroreflex to IOH in this type of active postural change.

ACKNOWLEDGMENTS

We thank the study participants for their time and dedication to the experiment.

GRANTS

M. E. Tschakovsky is supported by a grant from the Natural Sciences and Engineering Research Council of Canada (NSERC 250367-06) and Infrastructure Funding from the Canada Foundation for Innovation and the Ontario Innovation Trust. L. McVicar and K. Matusiak were supported by Undergraduate Student Research Awards from NSERC.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


