Effects of lower-leg rhythmic cuff inflation on cardiovascular autonomic responses during quiet standing in healthy subjects

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2Graduate School of Bio-Applications and Systems Engineering, Tokyo University of Agriculture and Technology, Koganei, Tokyo; 3Department of Mathematical Information Science, Ashikawa Medical College, Ashikawa; and 4Department of Physical Therapy, Yamagata Prefectural University of Health Sciences, Yamagata, Japan

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Niizeki K, Tominaga T, Saitoh T, Nishidate I, Takahashi T, Uchida K. Effects of lower-leg rhythmic cuff inflation on cardiovascular autonomic responses during quiet standing in healthy subjects. Am J Physiol Heart Circ Physiol 300: H1923–H1929, 2011. First published March 4, 2011; doi:10.1152/ajpheart.01288.2010.—To determine the effects of muscle pump function on cardiac autonomic activity in response to quiet standing, we simulated the muscle pump effect by rhythmic lower-leg cuff inflation (RCI) with four cuff pressures of 0 (sham), 40, 80, and 120 mmHg at 5 cycles/min. The R-R interval (RRI) and beat-to-beat blood pressure (BP) were acquired in healthy subjects (6 males and 5 females, aged 21–24 yr). From the continuous BP measurement, stroke volume (SV) was calculated by a pulse-contour method. Using spectral and cross-spectral analysis, RRI and systolic BP variability as well as the gain of spontaneous cardiac baroreflex sensitivity (sBRS) were estimated for the low- and high-frequency (HF) bands. Compared with the sham condition, RCI with cuff pressures of 80 and 120 mmHg led to increases in the mean RRI (P < 0.01) and HF power of RRI fluctuation (P < 0.05 for 80 mmHg and P < 0.01 for 120 mmHg) during quiet standing. Reduction in SV during standing was suppressed, and the sBRS of the HF band for standing were increased by RCI for either cuff pressure (P < 0.05 for 80 mmHg and P < 0.01 for 120 mmHg). However, at 40 mmHg RCI, these remained unchanged. These results suggest that, during standing, RCI of the lower leg increases cardiac vagal outflow when the cuff pressure is raised enough to oppose the hydrostatic-induced venous pressure in the calf.

stroke volume; heart rate variability; baroreflex

DURING STANDING, BLOOD TRANSLOCATES from upper to lower body as a result of gravity, thereby reducing venous return (3, 24), and eventually provokes orthostatic intolerance when venous return is sufficiently impaired. Vasoconstriction of the arteriolar bed and activation of muscle pump are potential counterregulatory mechanisms against orthostatic intolerance that reduce venous pooling during standing (28). Vasoconstriction due to sympathetic outflow to the legs during postural stress may lead to a reduction in calf blood volume (27). Contraction of the lower extremity muscles squeezes venous blood from the leg upward, and contractions of the calf and thigh muscles are effective in reducing venous pooling (15). Physical countermaneuver of leg crossing with muscle tensing can prevent venous pooling in patients with orthostatic hypotension (14, 35, 39). Muscle pump function is impaired in postural tachycardia patients and is associated with low calf blood flow (30).

Lower-body positive pressure (LBPP) has been used to simulate the muscle pump. Fu et al. (9) investigated the effect of LBPP on muscle sympathetic nerve activity during 70° head-up tilt in healthy subjects and found that LBPP could reduce the baroreflex-mediated enhancement in sympathetic activity (9). The responses to rhythmical external pressure acting on the lower body could add to understand of muscle pump function. To accomplish this, noninvasive cardiovascular variables were recorded when muscle pump function was mimicked by rhythmic calf cuff inflation during quiet standing in healthy subjects.

METHODS

Subjects. Healthy subjects (6 males and 5 females) aged 21–24 yr (mean ± SD = 22 ± 0.9 yr) were recruited from the Yamagata University student body. No subject was taking any medications or had a history of cardiopulmonary diseases. The study was approved by the Human Subjects Review Committee of the Faculty of Engineering of Yamagata University, and each subject gave their written informed consent after a verbal explanation of the experimental procedures. The study was performed in accordance with the guidelines established by the Declaration of Helsinki. Subjects were requested to abstain from caffeinated beverages for 12 h and strenuous physical activity and alcohol for ≥24 h before the experiment.

Experimental procedures. Experiments were carried out in a quiet room with the temperature maintained at 22–24°C. Subjects were comfortably seated and wore a one-way breathing mask (model-7930; Hans-Rudolph, Kansas, MO) on which a hot-wire respiratory flowmeter (RF-2; Minato, Osaka, Japan) was mounted. Ag-AgCl electrodes were placed on the chest for obtaining bipolar electrocardiogram (ECG) leads, and a Finapres sensor (model-2300; Ohmeda, Englewood, CO) was placed around the index or middle finger of the subject’s left hand, which was supported at heart level. Subjects were instrumented with custom-designed BP cuffs (20 cm in bladder width) that were wrapped bilaterally around the middle portion of the calf muscle. The cuffs were secured with knee supporters so it would not slip off. After instrumentation, the subjects rested in the sitting position for 10 min. During this period, subjects became familiarized with the paced-breathing protocol at a frequency of 15 breaths/min (0.25 Hz) controlled across all testing sessions, because breathing frequency affects heart rate variability (HRV) (12). The subjects were instructed to follow a respiratory pacing stimulus displayed on the personal computer monitor to breathe at the given frequency with the inspiratory duty cycle set at 40%. The respiratory pacing stimulus was a “saw-tooth”-shaped line that was colored blue on each upward slope (expiration) and yellow on each downward slope (inspiration).

Rhythmic cuff inflation (RCI) was performed with an inflation/deflation cycle of 8 s/4 s (5 inflations/min) to mimic the mechanical...
muscle pump effect. The cuffs were inflated to either 0 (sham experiment), 40 (RCI40), 80 (RCI80), or 120 (RCI120) mmHg by a rapid cuff inflator (model-E20; Hokanson, Bellevue, WA). The administration of the calf cuff pressure was initiated at the onset of standing and continued throughout the test. The applied cuff pressure was recorded via a semiconductor transducer (P-2000; Copal, Tokyo, Japan) connected to the inside of the cuff, which was calibrated with a mercury manometer.

The order of RCI was randomized among the subjects, and each test was separated by a 15-min interval during which subjects removed their facemask and breathed at their own rate. During the standing period, subjects were instructed to remain as still as possible to reduce any influence of the muscle pump.

Signal acquisition. During the experiments, ECG by means of a wireless ECG (ZM-940P; Nihon Kohden, Tokyo, Japan) and respiratory flow were recorded. The ECG signal was amplified and differentiated to distinguish the R waves. The beat-to-beat blood pressure (BP) was obtained with the Finapres device (18). After achievement of a stable BP waveform, the servo-reset mechanism of the Finapres was turned off, allowing 8 min of uninterrupted data collection. All signals were digitized with a sampling frequency of 1 kHz with a PowerLab Data Acquisition System (model-8/SP; ADInstruments, NSW, Australia).

Data analyses. Beat-to-beat R-R intervals (RRIs) and systolic (SBP) and diastolic (DBP) BP values were derived, and pulse pressure (PP) was their difference. Beat-to-beat stroke volume (SV) was determined by the pulse-contour method (38). In this method, SV was defined by the integration of pressure wave over the ejection phase (pulsatile systolic area, PSA) divided by the effective characteristic impedance of the aorta (Zao). The PSA was obtained as the integral of the BP wave from the start of the upstroke to the incisura. Zao is decreased by aortic cross-sectional area and compliance, which depend on pressure and age, and increased by reflections from the pressure wave at the periphery related to heart rate (HR). We used the correction formula proposed by Wesseling et al. (37, 38) as given by

\[
SV = \frac{\text{PSA} \times (1.320 + \text{HR} \times 10 - \text{age})}{(0.28 \times \text{MAP} - 16})/2,000
\]

where the initial value for Zao (Zaoini) is (90 + age)/1,000, MAP is mean arterial pressure (in mmHg), and HR is computed as the inverse of the RRI (in beats/min) of the same beat. This methodology provides reproducible estimates of the change in SV during postural stress (11, 14, 31, 35). Cardiac output (CO) was SV times HR, and total peripheral resistance (TPR) was MAP divided by CO.

Time series of RRI and SBP were cubic-spline interpolated and then resampled at 10 Hz to create a uniform time series for spectral analysis. Before resampling, RRIs were inspected, and inadequate QRS detections were interactively corrected. To obtain a time series for the respiratory flow at corresponding sampling times, stored respiratory flow signals were also resampled at 10 Hz.

Frequency domain variability analysis. To obtain frequency-domain measures of HRV and SBP variability (SBPV), a power spectral analysis was performed based on Welch’s averaged periodogram technique. After trend elimination and Hanning windowing, power spectral density estimates were made from 512-point windows with 50% overlapping segments for each of the resting and standing data sets. The total spectral power was calculated for the low (LF: 0.04–0.15 Hz)- and high (HF: 0.15–0.4 Hz)-frequency bands by integration of the spectral components (32). For the analysis of the autonomic balance, the LF-to-HF ratio was also calculated.

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Fig. 1. Representative recordings from one subject under sham (left) and 120 mmHg rhythmic cuff inflation (RCI120, right) conditions 1 min before and during the first 3 min of standing. Broken line indicates the onset of standing. BP, blood pressure; RRI, R-R interval; SV, stroke volume; CO, cardiac output; TPR, total peripheral resistance.
Spontaneous baroreflex assessment. The spontaneous baroreflex sensitivity was estimated by the gain (α-index) between the HRV and SBPV calculated separately for the LF and HF bands using spectral and cross-spectral analyses (α_{LF} and α_{HF}, respectively). With this approach, we computed the coherence function, which describes the linear relationship between the SBPV and HRV at each frequency and has values between zero and one, and we also computed the phase shifts to estimate the delay between the two signals in the LF and HF bands. In line with previous studies (1, 19, 23), the α_{LF} and α_{HF} spontaneous cardiac baroreflex sensitivities (sBRS) were calculated

\[ \alpha_{LF} = \sqrt{\frac{P_{RRI(LF)}}{P_{SBP(LF)}}}, \quad \alpha_{HF} = \sqrt{\frac{P_{RRI(HF)}}{P_{SBP(HF)}}} \]

where P_{RRI(LF)} and P_{SBP(LF)} represent the spectral power density of the RRI and SBP for the LF region, respectively, and P_{RRI(HF)} and P_{SBP(HF)} represent those for the HF region, respectively. The α-index was accepted as valid only when the cross-spectral coherence exceeded 0.5 and the phase shift from SBP to RRI was positive.

Statistics. To quantify the variability of parameters, time domain measures of the mean and the SE over the 3-min resting period and the last 4 min of the quiet standing period were separately calculated for each subject and then averaged to obtain group mean values. The effect of RCI on each variable was assessed using a repeated-measures one-way ANOVA. Pairwise comparisons with Bonferroni corrections were used to examine significant interactions. \( P < 0.05 \) was regarded as statistically significant for all comparisons.

RESULTS

None of the subjects complained of any discomfort during the experiment, and all subjects succeeded in following the respiratory pacing signal. A representative trace from one subject during the sham and RCI_{120} protocols is shown in Fig. 1. To show the effect of RCI on cardiovascular variables, average RRI, SBP, PP, SV, CO, and TPR time series data for all subjects were compared between RCI_{120} and sham groups.
Cardiorespiratory variables and autonomic indexes during sitting and standing for sham and three different RCI conditions

<table>
<thead>
<tr>
<th>RCI Cuff Pressure, mmHg</th>
<th>0</th>
<th>40</th>
<th>80</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RRI, ms</strong></td>
<td>787 ± 30</td>
<td>735 ± 30</td>
<td>793 ± 35</td>
<td>746 ± 27</td>
</tr>
<tr>
<td><strong>SBP, mmHg</strong></td>
<td>120 ± 6.1</td>
<td>117 ± 5.7</td>
<td>124 ± 5.3</td>
<td>119 ± 6.0</td>
</tr>
<tr>
<td><strong>DBP, mmHg</strong></td>
<td>60.6 ± 4.0</td>
<td>63.0 ± 3.2</td>
<td>64.6 ± 4.9</td>
<td>65.0 ± 4.4</td>
</tr>
<tr>
<td><strong>PP, mmHg</strong></td>
<td>57.9 ± 2.1</td>
<td>51.6 ± 2.8</td>
<td>59.2 ± 1.3</td>
<td>53.7 ± 3.9</td>
</tr>
<tr>
<td><strong>SV, ml</strong></td>
<td>80.5 ± 4.3</td>
<td>68.0 ± 4.5</td>
<td>80.7 ± 4.5</td>
<td>71.7 ± 5.6</td>
</tr>
<tr>
<td><strong>CO, l/min</strong></td>
<td>6.18 ± 0.34</td>
<td>5.59 ± 0.36</td>
<td>6.14 ± 0.34</td>
<td>5.75 ± 0.45</td>
</tr>
<tr>
<td><strong>TPR, mmHg l−1 min−1</strong></td>
<td>13.73 ± 1.01</td>
<td>15.36 ± 1.01</td>
<td>14.35 ± 1.38</td>
<td>15.69 ± 1.66</td>
</tr>
<tr>
<td><strong>Vt, ml BTPS</strong></td>
<td>567 ± 37</td>
<td>559 ± 32</td>
<td>564 ± 37</td>
<td>565 ± 29</td>
</tr>
</tbody>
</table>

**HRV**

| **LF, ms²**              | 329 ± 50 | 335 ± 64 | 382 ± 115 | 402 ± 59 | 381 ± 73 | 403 ± 70 | 445 ± 93 | 600 ± 171 |
| **HF, ms²**              | 614 ± 183 | 296 ± 81 | 838 ± 271 | 402 ± 106 | 799 ± 213 | 458 ± 119* | 844 ± 247 | 526 ± 137** |
| **LF-to-HF ratio**       | 0.97 ± 0.29 | 2.78 ± 1.04 | 0.80 ± 0.18 | 2.47 ± 0.95 | 0.79 ± 0.18 | 1.57 ± 0.39 | 0.72 ± 0.13 | 1.68 ± 0.40 |

**SBPV**

| **LF, mmHg²**            | 16.6 ± 2.2 | 25.4 ± 4.5 | 16.3 ± 2.1 | 21.6 ± 4.5 | 14.3 ± 2.6 | 22.3 ± 3.9 | 14.6 ± 1.7 | 25.1 ± 3.0 |
| **HF, mmHg²**            | 11.7 ± 2.5 | 11.0 ± 2.2 | 12.5 ± 1.7 | 11.5 ± 2.4 | 11.8 ± 2.5 | 11.8 ± 2.4 | 10.3 ± 2.3 | 11.8 ± 2.4 |
| **LF-to-HF ratio**       | 2.32 ± 0.74 | 4.87 ± 1.87 | 1.65 ± 0.46 | 3.16 ± 1.01 | 1.86 ± 0.48 | 3.36 ± 1.01 | 2.35 ± 0.60 | 3.36 ± 1.01 |

Values are means ± SE. RCI, rhythmic lower-leg cuff inflation; RRI, R-R interval; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; SV, stroke volume; CO, cardiac output; TPR, total peripheral resistance; Vt, tidal volume; HRV, heart rate variability; SBPV, systolic blood pressure variability; LF, low frequency; HF, high frequency. RCI vs. sham: *P < 0.05 and **P < 0.01.
respiratory frequency tended to be augmented by RCI120 compared with those in the sham experiment. Moreover, the LF spectral power at the cuff inflation frequency was more prominent during RCI120. The mean values of the autonomic indexes of HRV and SBPV are summarized in Table 1. There was an effect of RCI on the HF component of the HRV [F(3) = 5.69, P = 0.0033], and differences were present for the HF component of HRV in RCI80 (P < 0.05) and RCI120 (P < 0.01) conditions compared with the sham condition.

In Fig. 4, the effect of RCI on the sBRS calculated separately for the LF and HF band using spectral and cross-spectral analysis is shown. RCI did not cause any differences in the LF band sBRS (αLF) during standing, whereas the HF band sBRS (αHF) was increased by the RCI80 (P < 0.05) and RCI120 (P < 0.01) protocols compared with that for the sham protocol. The αHF values were associated with the HF power of RRI fluctuation [αHF = 0.00679 × PRR(HF) + 3.48, r = 0.766, P < 0.0001].

**DISCUSSION**

The primary finding of the present study is that rhythmic lower-leg cuff inflation during quiet standing with cuff pressures of 80 and 120 mmHg results in significant changes in neural circulatory control. These changes are accompanied by prolonged RRI and increases in the HF component of HRV and spontaneous baroreflex sensitivity.

During the transition from sitting to standing, blood is translocated from upper to lower body as a result of the gravitational forces (3). Venous pooling occurs within the first 10 s due to filling of the veins caused by a rise in the hydrostatic pressure (8). The transfer is almost complete within 3–5 min of orthostatic stress (4, 15, 26), and we evaluated the time course of cardiovascular variables during the 5 min after the initiation of quiet standing.

As indicated by Table 1, the average differences in RRI between sitting and standing became smaller with increasing cuff pressure; these values were −52 (sham), −47 (RCI40), −30 (RCI80), and −18 (RCI120) ms. Compared with the sham experiment, the average RRI during standing was increased during the RCI80 and RCI120 interventions. Similarly, the HF component of HRV was increased during the RCI80 and RCI120 experimental conditions. These results led us to suggest that RCI enhanced cardiac vagal tone. Venous return to the heart is reduced during standing, which leads to a decrease in cardiac SV. In the present study, reduction in SV during standing was suppressed by RCI80 and RCI120. Therefore, we propose that inhibition of the decrease in RRI observed during standing with the application of RCI is due to the increase in venous return by the mechanical pumping effect of RCI. The application of LBPP to upright subjects causes an increase in SV (17). RCI might exert a similar influence on venous circulation and cause a translocation of blood from the lower to the central region. The greater venous return would increase afferent input from cardiopulmonary baroreceptors, which would inhibit efferent sympathetic nerve activity (24, 36). In fact, muscle sympathetic nerve activity is inversely related to CO and SV (6). Presumably, the inhibition of the reduction in RRI and the increase in the HF component of RRI variability elicited by applying RCI to upright subjects likely would be due to the increased baroreceptor activity, which would inhibit sympathetic nerve activity and, in turn, induce a shift of the sympathovagal balance toward a parasympathetic activation.

A significant effect of RCI on RRI and the HF component of HRV during standing was absent when the cuff pressure was 40 mmHg (RCI40). The intramuscular pressure in the lower limb increases up to 12–48 mmHg during quiet standing (15). This intramuscular pressure opposes the hydrostatic-induced venous pressure, lowering capillary and venous transmural pressure and thereby reducing venous pooling (5), and yet a pressure of 40 mmHg appears to be too low to oppose the hydrostatic pressure in the lower limb during standing.

In response to the postural change from sitting to standing, baroreflex sensitivity and vagally mediated cardiac variability become attenuated (29). The αHF was decreased from 8 during sitting to 5 ms/mmHg during standing in the sham protocol (Fig. 4). However, the αHF during standing in the RCI120 protocol (7 ms/mmHg) was comparable to that for sitting. A similar influence was observed in the RCI80 protocol. This suggests that the application of RCI reduced the inhibition of the HF component of baroreflex gain during standing. As indicated in Table 1, the HF component of RRI power during standing was increased in both the RCI80 and the RCI120 protocols, indicating an increased efferent cardiac parasympathetic activity. Therefore, the reduced inhibition of αHF observed during RCI is likely due to increased parasympathetic activity. A decrease in respiratory frequency and an increase in tidal volume would be responsible for the increase in αHF. There is evidence suggesting that slow breathing enhances sBRS both in healthy and chronic heart failure subjects (2). In
addition, tidal volume influences venous return by the alteration of intrathoracic pressure through the Frank-Starling reflex mechanism. However, we controlled breathing at 0.25 Hz, and we did not observe a significant difference in the tidal volume across the protocols (Table 1).

Active muscle contractions depend on central command, which increases sympathetic outflow (24). In our study, central command-mediated increase in sympathetic outflow is not to be expected, since experiments were carried out under the absence of voluntary calf contractions. However, the RCI activates mechanoreceptors by mechanically compressing the calf muscle (40). The afferent input from mechanoreceptors in the muscles could increase orthostatic enhancement of muscle sympathetic nerve activity (27) and alter the baroreflex response (16). However, there were no indications to suggest an increase in sympathetic nerve activity judging from the autonomic indexes tabulated in Table 1: significant differences in SBP, CO, and the LF-to-HF ratio of HRV were not observed between sham and RCI experiments during standing. If present at all, the muscle mechanoreflex-mediated sympathoexcitation might be overridden by increased baroreceptor activity.

There are several potential limitations of our study. First, the spectral and cross-spectral method for evaluating sBRS (ω-index) relies on the variations in RRI that are secondary to changes in SBP mediated by the baroreflex (21, 33, 34), and this causal association cannot be guaranteed in all situations because the underlying mechanisms for generating respiratory-induced variations in SBP and RRI are not clear. However, most of the approaches currently used for the analysis of sBRS are based on an open-loop description of the SBP-RRI relationship, but there are more comprehensive methods for assessing baroreflex function that provide a robust index of the cardiac-vagal reflex (19, 20, 22, 23, 25). Therefore, we used the spectral and cross-spectral method to explore changes in the cardiac-vagal reflex in response to the postural change from sitting to standing. Second, the effect of RCI on cardiovascular variables was examined only at the frequency of 5 cycles/min. The estimation of the baroreflex function as a transfer gain between RRI and SBP demonstrates frequency-dependent properties (7, 10). Because the magnitude of fluctuations of RRI and SBP is considered to depend on the rate of perturbation, studies altering RCI frequency may yield different response. Third, RCIs do not mimic the mechanical effect of muscle contractions perfectly. We used an identical duty cycle for the RCI in all experimental trials and found a different pressure effect on RRI, SV, and autonomic index. Our interpretations assume that the RCI produced similar muscle pumping in the calf and evoked an increase in venous return depending on the cuff pressure. Finally, age limitations for generalizing our findings may exist.

The present findings suggest the possibility that RCI may contribute to pathophysiological conditions in which sympathetic nerve activity is inappropriately elevated and/or upright systemic venous return is impaired. Hypovolemia is associated with a reduced baroreflex function and an augmented sympathetic nerve activity (13), and LBPP can reduce the symptoms of hypovolemia-related orthostatic dysfunction (9). Similar to the LBPP, the present study indicates that application of RCI with appropriate cuff pressure prevents excess venous pooling and reduces inhibition of cardiac vagal reflex that is often attenuated in patients with orthostatic dysfunction.

In summary, we examined the effects of rhythmic lower-leg cuff inflation and deflation, which mimics muscle pump function, in regulating cardiac autonomic activity during quiet standing. We demonstrated significant increases in RRI and SV as well as enhancements of the HF components of the HRV and sBRS by RCI with cuff pressures >40 mmHg. This suggests that RCI of the lower leg increases cardiac vagal outflow when the cuff pressure rises high enough to oppose the hydrostatic-induced venous pressure during standing. Our results contribute to understand the effectiveness of muscle pump function in cardiovascular control during orthostatic stress.

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**DISCLOSURES**

No conflicts of interest are declared by the authors.

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