Arterial-cardiac baroreflex function: insights from repeated squat-stand maneuvers

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Am J Physiol Regul Integr Comp Physiol 297: R116–R123, 2009. —To assess baroreflex function under closed-loop conditions, a new approach was used to generate large and physiological perturbations in arterial pressure. Blood pressure (BP) and R-R interval were recorded continuously in 20 healthy young (33 ± 8 yr) and eight elderly subjects (66 ± 6 yr). Repeated squat-stand maneuvers at the frequencies of 0.05 and 0.1 Hz were performed to produce periodic oscillations in BP to provoke the baroreflex. To assess the effects of the muscle reflex and/or central command on the baroreflex, passive squat-stand maneuvers were conducted using a pulley system to assist changes in body position. Transfer function between changes in BP and R-R interval was estimated to assess the arterial-cardiac baroreflex. Relative to resting conditions, large and coherent oscillations in BP and R-R interval were produced during both active and passive squat-stand maneuvers. However, changes in BP were smaller during passive than during active maneuvers. Changes in R-R interval were reduced commensurately. Therefore, transfer function gain did not change between the two maneuvers. Compared with the young, transfer function gain was reduced and the phase became more negative in the elderly, demonstrating the well-known effects of aging on reducing baroreflex sensitivity. Collectively, these findings suggest that the changes in R-R interval elicited by BP perturbations during squat-stand maneuvers are mediated primarily by a baroreflex mechanism. Furthermore, baroreflex function can be assessed using the transfer function method during large perturbations in arterial pressure.

Baroreflex function during repeated squat-stand maneuvers

Zhang R, Claassen JAR, Shibata S, Kilic S, Martin-Cook K, Diaz-Arrastia R, Levine BD. Arterial-cardiac baroreflex function: insights from repeated squat-stand maneuvers. Am J Physiol Regul Integr Comp Physiol 297: R116–R123, 2009. First published May 6, 2009; doi:10.1152/ajpregu.90977.2008.—To assess baroreflex function under closed-loop conditions, a new approach was used to generate large and physiological perturbations in arterial pressure. Blood pressure (BP) and R-R interval were recorded continuously in 20 healthy young (33 ± 8 yr) and eight elderly subjects (66 ± 6 yr). Repeated squat-stand maneuvers at the frequencies of 0.05 and 0.1 Hz were performed to produce periodic oscillations in BP to provoke the baroreflex. To assess the effects of the muscle reflex and/or central command on the baroreflex, passive squat-stand maneuvers were conducted using a pulley system to assist changes in body position. Transfer function between changes in BP and R-R interval was estimated to assess the arterial-cardiac baroreflex. Relative to resting conditions, large and coherent oscillations in BP and R-R interval were produced during both active and passive squat-stand maneuvers. However, changes in BP were smaller during passive than during active maneuvers. Changes in R-R interval were reduced commensurately. Therefore, transfer function gain did not change between the two maneuvers. Compared with the young, transfer function gain was reduced and the phase became more negative in the elderly, demonstrating the well-known effects of aging on reducing baroreflex sensitivity. Collectively, these findings suggest that the changes in R-R interval elicited by BP perturbations during squat-stand maneuvers are mediated primarily by a baroreflex mechanism. Furthermore, baroreflex function can be assessed using the transfer function method during large perturbations in arterial pressure.

Blood pressure; heart rate; spectral analysis

Baroreflex is essential for short-term arterial pressure stability (10). Impairment of baroreflex function may lead to orthostatic hypotension, resulting in brain hypoperfusion, falls, or syncope, a key problem in the elderly and patients with autonomic disease (9, 17, 39).

Assessment of baroreflex function is challenging for human studies. Baroreflex is a closed-loop feedback control system and is modulated by other reflex and nonreflex mechanisms (6). In this regard, a neck pressure chamber method has been developed to assess the carotid baroreflex function (5). Conversely, vasoactive drugs or performance of a Valsalva maneuver have been used to evaluate the integrated baroreflex function, which may involve multiple baroreceptors (25, 27). Recently, by taking the advantage of spontaneous oscillations in cardiovascular variables, baroreflex function has been assessed under resting conditions, using the sequence or transfer function methods (26, 29).

However, these methods, one way or another, have limitations. For example, the neck chamber method has been used extensively for research (6). However, the sophisticated techniques required for its use limit its value for daily practice in clinical medicine. Moreover, the linear regression used to link beat-to-beat changes in pulse interval (or heart rate) to changes in arterial pressure during vasoactive drug infusion or a Val-salva maneuver is static in nature (memory-less) and may not reveal dynamic properties of the baroreflex function. In addition, under closed-loop conditions, assessing baroreflex function from spontaneous changes in cardiovascular variables may be biased because of lack of sufficient external stimuli to provoke the baroreflex (16, 21). Finally, baroreflex function assessed under supine resting conditions may not be readily transferrable to the regulation of arterial pressure or heart rate during body postural change or other perturbations in daily life.

Squat followed by standing elicits transient changes in central blood volume and peripheral vascular resistance, leading to dramatic changes in arterial pressure and heart rate (23, 34, 39). Recently, it has been used as a model for studying the cardiovascular control during orthostatic stress (3, 39). In addition, heart rate response to changes in arterial pressure during a single squat-stand maneuver has been measured to assess the arterial-cardiac baroreflex function in patients with diabetes mellitus (22). However, assessment of baroreflex function during squat-stand maneuvers may involve not only arterial, but also cardiopulmonary, baroreflex due to changes in central blood volume/pressure (39).

In this study, we explored whether arterial-cardiac baroreflex function can be assessed during repeated squat-stand maneuvers at different frequencies. We hypothesized that coherent changes in heart rate (or R-R interval) would be produced in response to oscillations in arterial pressure, reflecting mainly a baroreflex mechanism. To reduce the potential effects of the muscle reflex and/or central command on the assessment of baroreflex function, passive squat-stand maneuvers were performed by using a pulley system to assist changes in body position. Finally, using this method, we demonstrated the well-known effects of aging on reducing baroreflex sensitivity in the elderly.
METHODS

Subjects. Ten healthy young subjects with a mean age of 30 yr (range 23–39 yr, 6 women) and eight elderly subjects with a mean age of 66 yr (range 58–78 yr, 5 women) participated in one study of performing only active squat-stand maneuvers. Another group of 10 healthy subjects with a mean age of 35 yr (range 23–50 yr, 6 women) participated in a second study of performing both active and passive squat-stand maneuvers to assess the influence of activation of the muscle reflex and/or central command on the baroreflex function. Elderly subjects with normal cognitive function were recruited from the University of Texas Southwestern Alzheimer’s Disease Center. No subject smoked or had known medical problems. Subjects were screened carefully with a medical history and a physical examination including 12-lead ECG. Two elderly subjects with leg or knee problems that prevented them from performing the squat-stand maneuvers were excluded. The protocol for this study was approved by and all subjects signed an informed consent form approved by the Institutional Review Boards of the University of Texas Southwestern Medical Center and the Presbyterian Hospital of Dallas.

Instrumentation and procedures. An electrocardiogram (ECG) was recorded using a three-lead system (Hewlett-Packard). Beat-to-beat hear rate was obtained from each R-R interval identified from the QRS complexes. Arterial pressure was measured noninvasively at the middle finger of the right hand using Finapres (Ohmeda). The finger pressure cuff was positioned carefully at heart level with the hand being held in the left midaxillary line. The hand and arm were supported securely and comfortably with a custom-made vest-sling system using Velcro to ensure the stability of the pressure recordings during squat-stand maneuvers. Intermittent arterial pressure was measured in the left arm by electrosphygmomanometry (Suntech) to corroborate the measurement of finger arterial pressure. Respiratory CO₂ was measured via a nasal cannula with capnography (Criticare Systems) to monitor the breathing frequency.

All experiments were performed in the morning in an environmentally controlled laboratory with an ambient temperature of 22°C. Subjects refrained from heavy exercise and caffeinated or alcoholic beverages at least 24 h before the tests. For active squat-stand maneuvers, first, a single squat-stand maneuver was performed. After 10 min rest in the sitting position, subjects stood up for 2 min for stabilization followed by a squat for 1 min and then stand for 2 min (Fig. 1). During squatting, subjects could take either a tiptoe or a feet-flat position, depending on their choice for a comfortable performance. This maneuver was repeated three times separated by two 3-min recovery intervals. The purpose of performing this single squat-stand maneuver was to confirm the temporal characteristics of changes in arterial pressure and heart rate as observed in previous studies (22, 30). This maneuver was performed only in eight young and five elderly subjects because changes in arterial pressure and heart rate were highly reproducible. After a 10-min recovery in the sitting position, spontaneous changes in arterial pressure and R-R interval were recorded for 5 min for spectral analysis of cardiovascular variability to compare with those during repeated squat-stand maneuvers.

Subjects were instructed to perform repeated squat-stand maneuvers at the frequency of 0.1 Hz (5-s squat followed by 5-s stand) for 5 min. After a 10-min recovery, the maneuver was repeated at 0.05 Hz (10-s squat followed by 10-s stand) for 5 min (Fig. 2). During these tests, subjects were instructed to breathe normally to avoid performing a Valsalva maneuver while standing up. The breathing pattern was monitored continuously with respiratory CO₂ (Fig. 2). When necessary, a stand was provided to the elderly to grab with the left hand to reduce the physical strain each time when they stood up from squatting (13). In addition, shorter durations of 3 min for 0.1 Hz and 4 min for 0.05 Hz were used to reduce their effort to perform these maneuvers.

For passive squat-stand maneuvers, a pulley system attached to a rock-climbing harness worn by the subjects was used to support the body weight to reduce the leg muscle contractions during changes in body position. During the experiment, subjects were lowered down during squatting and pulled up during standing and were instructed to make no subjective effort to minimize the effects of muscle contraction and/or central command on arterial pressure and heart rate. The frequencies used for these maneuvers were the same as for active maneuvers. The order of active and passive maneuvers was randomized for each subject. A time interval of at least 20 min between passive and active maneuvers was used for recovery.

There are several reasons for us to select the 0.1 and 0.05 Hz frequencies for these maneuvers. First, these frequencies were below...
Fig. 2. Changes in ABP, HR, and respiratory CO$_2$ in a young subject under resting conditions and during active squat-stand maneuvers at 0.1 and 0.05 Hz. Note the large and coherent oscillations in ABP and HR during these maneuvers relative to resting conditions.

the respiratory frequencies of the subjects (Tables 1–3). Therefore, respiration should have minimal effects on changes in arterial pressure and heart rate (or R-R interval) at these frequencies. Second, oscillations in arterial pressure are likely to be enhanced at these frequencies because they are close to the baroreflex-mediated resonance frequencies observed in humans (11, 38). Finally, transfer function gain estimated during the 0.1-Hz maneuvers could be compared directly with that under resting conditions at the frequencies around 0.1 Hz (14).

Data analysis. For the single squat-stand maneuver, increases in systolic blood pressure (SBP) and decreases in heart rate during squatting were obtained as the mean value during squat minus the baseline data (averaged 30-s data before squat). During standing, maximal reduction in SBP was obtained as the averaged 30-s data before standing minus the nadir of SBP after standing (Fig. 1). Maximal increases in heart rate were obtained similarly. The ratio of maximal changes in R-R interval to maximal changes in SBP was calculated to reflect baroreflex function. The use of R-R interval rather than heart rate to reflect baroreflex function would be consistent with most previous studies (14). In addition, animal studies indicate that changes in R-R interval rather than heart rate are related linearly to efferent vagal activity (15). It should be noted that assessment of baroreflex function using this index is explorative in this study. The purpose is to determine whether the effects of aging on the baroreflex function also can be revealed during a single squat-stand maneuver.

For repeated squat-stand maneuvers and those under resting conditions, the data segments of arterial pressure, heart rate, and respiratory frequency were averaged to assess the steady-state hemodynamics. Spectral power of SBP and R-R interval was estimated to quantify SBP and R-R variability in different frequency ranges. Transfer function between changes in SBP and R-R interval was estimated to quantify baroreflex function using the method described in our previous studies (14, 24). Briefly, under resting conditions, spectral power of SBP and R-R interval was estimated to quantify baroreflex function using the method described in our previous studies (14, 24). Briefly, under resting conditions, spectral power of SBP and R-R interval was estimated to quantify baroreflex function using the method described in our previous studies (14, 24). Briefly, under resting conditions, spectral power of SBP and R-R interval was estimated to quantify baroreflex function using the method described in our previous studies (14, 24). Briefly, under resting conditions, spectral power of SBP and R-R interval was estimated to quantify baroreflex function using the method described in our previous studies (14, 24). Briefly, under resting conditions, spectral power of SBP and R-R interval was estimated to quantify baroreflex function using the method described in our previous studies (14, 24). Briefly, under resting conditions, spectral power of SBP and R-R interval was estimated to quantify baroreflex function using the method described in our previous studies (14, 24).

Table 1. Spontaneous cardiovascular variability under resting conditions

<table>
<thead>
<tr>
<th>Variables</th>
<th>Young, $n = 10$</th>
<th>Elderly, $n = 8$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP, mmHg</td>
<td>120±10</td>
<td>143±14*</td>
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<tr>
<td>DBP, mmHg</td>
<td>68±10</td>
<td>70±6</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>71±16</td>
<td>71±14</td>
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<tr>
<td>RF, Hz</td>
<td>0.29±0.06</td>
<td>0.27±0.09</td>
</tr>
<tr>
<td>ETCO$_2$, mmHg</td>
<td>37±4</td>
<td>38±3</td>
</tr>
<tr>
<td>SBPVLF, mmHg$^2$</td>
<td>13±9</td>
<td>16±16</td>
</tr>
<tr>
<td>SBPLF, mmHg$^2$</td>
<td>8±5</td>
<td>10±13</td>
</tr>
<tr>
<td>SBPHF, mmHg$^2$</td>
<td>2±1</td>
<td>3±3</td>
</tr>
<tr>
<td>RRVLF, ms$^2$</td>
<td>908±812</td>
<td>133±62*</td>
</tr>
<tr>
<td>RRHF, ms$^2$</td>
<td>845±619</td>
<td>226±372*</td>
</tr>
<tr>
<td>Gain-VLF, ms/mmHg</td>
<td>5.7±4.4</td>
<td>2.6±1.7</td>
</tr>
<tr>
<td>Gain-LF, ms/mmHg</td>
<td>9.3±4.0</td>
<td>3.7±2.4*</td>
</tr>
<tr>
<td>Gain-HF, ms/mmHg</td>
<td>11.9±7.7</td>
<td>5.2±4.6*</td>
</tr>
<tr>
<td>Gain-SF, ms/mmHg</td>
<td>9.8±3.8</td>
<td>3.6±2.2*</td>
</tr>
<tr>
<td>Phase-VLF, rads</td>
<td>-0.9±0.6</td>
<td>-0.9±1.0</td>
</tr>
<tr>
<td>Phase-LF, rads</td>
<td>-0.9±0.4</td>
<td>-1.3±0.3</td>
</tr>
<tr>
<td>Phase-HF, rads</td>
<td>-0.1±0.4</td>
<td>-0.3±0.5</td>
</tr>
<tr>
<td>Phase-SF, rads</td>
<td>-0.9±0.3</td>
<td>-1.4±0.5*</td>
</tr>
<tr>
<td>Coherence-VLF</td>
<td>0.38±0.11</td>
<td>0.45±0.13</td>
</tr>
<tr>
<td>Coherence-LF</td>
<td>0.65±0.09</td>
<td>0.62±0.15</td>
</tr>
<tr>
<td>Coherence-HF</td>
<td>0.61±0.10</td>
<td>0.48±0.15</td>
</tr>
<tr>
<td>Coherence-SF</td>
<td>0.70±0.18</td>
<td>0.66±0.16</td>
</tr>
</tbody>
</table>

Values are means ± SD. SBP and DBP, systolic and diastolic blood pressure. HR, heart rate. RF, respiratory frequency. ETCO$_2$, end-tidal CO$_2$. SBPVLF, SBPLF, SBPHF, RRVLF, RRLF and RRHF, spectral power of SBP and R-R interval at the very low (VLF), low (LF), and high frequencies (HF). Transfer function gain, phase, and coherence were estimated in the VLF, LF, and HF ranges and in the specific frequency range from 0.078 to 0.125 Hz (SF). *P < 0.05, comparisons between the young and elderly.
Table 2. Cardiovascular variability during active squat-stand maneuvers in young and elderly subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>Young, n = 10</th>
<th>Elderly, n = 8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1 Hz</td>
<td>0.05 Hz</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>134±15</td>
<td>129±15</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>67±12</td>
<td>67±10</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>88±17</td>
<td>82±17</td>
</tr>
<tr>
<td>RF, Hz</td>
<td>0.37±0.05</td>
<td>0.37±0.06</td>
</tr>
<tr>
<td>ETCO2, mmHg</td>
<td>38±5</td>
<td>36±4</td>
</tr>
<tr>
<td>SBP-SP, mmHg²</td>
<td>253±119</td>
<td>374±191</td>
</tr>
<tr>
<td>RR-SP, ms²</td>
<td>8075±8930</td>
<td>24839±13829†</td>
</tr>
<tr>
<td>Gain, ms/mmHg</td>
<td>5.4±2.9</td>
<td>7.6±2.8†</td>
</tr>
<tr>
<td>Gain-P, ms/mmHg</td>
<td>5.3±2.9</td>
<td>8.6±3.4†</td>
</tr>
<tr>
<td>Phase, rads</td>
<td>−0.8±0.3</td>
<td>−0.5±0.2</td>
</tr>
<tr>
<td>Phase-P, rads</td>
<td>−0.8±0.3</td>
<td>−0.4±0.2†</td>
</tr>
<tr>
<td>Coherence</td>
<td>0.82±0.07</td>
<td>0.79±0.08</td>
</tr>
<tr>
<td>Coherence-P</td>
<td>0.97±0.03</td>
<td>0.98±0.01</td>
</tr>
</tbody>
</table>

Values are means ± SD. Transfer function gain, phase, and coherence were estimated in the frequency range from 0.078 to 0.125 Hz and from 0.031 to 0.078 Hz. Gain-P, Phase-P, and Coherence-P were estimated at the peak coherence. *P < 0.05, comparisons between young and elderly at the same frequency. †P < 0.05, comparisons between the frequencies for the same group.

Statistics. An unpaired t-test was performed between the young and elderly to determine the effects of aging on spontaneous cardiovascular variability under resting conditions. Two-way ANOVA was used to determine the effects of aging and the frequencies of squat-stand maneuvers on the measurements. Two-way ANOVA also was used to determine the effects of the active and passive maneuvers as well as the frequencies of the maneuvers on the assessment of baroreflex function. Mann-Whitney rank sum tests were performed if the variables were not normally distributed or had unequal variances. The relationship between the transfer function gain in the frequency range from 0.078 to 0.125 Hz under resting conditions and those during squat-stand maneuvers at 0.1 Hz was examined using Pearson’s linear correlation, and the group means were compared with paired t-tests. This correlation was not conducted at 0.05 Hz because most subjects had low coherence at the very low frequencies for spontaneous oscillations (Table 1). Pearson’s correlation also was used to examine the relationship between the estimates of transfer function gain between the active and passive squat-stand maneuvers. Statistical data analysis was performed using SigmaStat (version 3.1). Data are presented as means ± SD. The significance level was set to P < 0.05.

RESULTS

Representative changes in arterial pressure and heart rate during a single squat-stand maneuver are presented in Fig. 1. During squatting, arterial pressure increased acutely, then fell slightly, and stabilized at a level above baseline. Mean values of increases in SBP were 13 ± 5 mmHg for the young and 9 ± 16 mmHg for the elderly. In response to increases in arterial pressure, heart rate decreased by 8 ± 8 beats/min in the young and by 1 ± 4 beats/min in the elderly. During standing up from squat, SBP fell substantially by 42 ± 14 mmHg in the young and by 49 ± 10 mmHg in the elderly. In response to these large falls in pressure, heart rate increased by 37 ± 10 beats/min in the young but only by 8 ± 9 beats/min in the elderly. As expected, the ratio of maximal change in R-R interval divided by maximal change in SBP was reduced in the elderly (young, 7.8 ± 2.5 ms/mmHg; elderly, 1.3 ± 1.4 ms/mmHg, P = 0.0003), indicating reduced baroreflex sensitivity with aging.

Under resting conditions, relative to the young, R-R variability was reduced in the elderly, despite similar BP variability (Table 1). As expected, transfer function gain was reduced. During active squat-stand maneuvers, marked oscillations in arterial pressure and heart rate (or R-R interval) were produced at the frequencies of 0.05 and 0.1 Hz (Figs. 2 and 4, Tables 2 and 3). When compared with the young, induced R-R variability was reduced substantially in the elderly, consistent with reductions in transfer function gain (Table 2). In addition, the estimated phase was more negative in the elderly than in the young (Table 2). Finally, a weak but significant linear correlation was observed between the estimates of transfer function gain under resting conditions and those during squat-stand maneuvers in the frequency range from 0.078 to 0.125 Hz (r² = 0.48, P = 0.001). However, gain in this frequency range under resting conditions was significantly higher than that during squat-stand maneuvers in both young and elderly subjects (P < 0.05, Tables 1 and 2).

Passive squat-stand maneuvers at both 0.1 and 0.05 Hz also generated marked oscillations in arterial pressure and heart rate (or R-R interval) (Figs. 3 and 4). However, these oscillations were significantly smaller than those during active maneuvers (Fig. 4, Table 3). Notably, no difference in transfer function gain was observed between the two maneuvers (Table 3). Finally, a significant linear correlation of transfer function gain between the two maneuvers was observed (Fig. 5).

In contrast to the trend toward a reduction in gain for lower frequencies under resting conditions, transfer function gain during squat-stand maneuvers was higher at 0.05 Hz than 0.1 Hz associated with a reduction in phase (Tables 2 and 3). However, despite a higher gain (higher baroreflex sensitivity), spectral power of BP at 0.05 Hz was larger than at 0.1 Hz (Tables 2 and 3).

Peak value of coherence was between 0.89 to 0.99 during squat-stand maneuvers under all conditions (Tables 2 and 3). Gain and phase at the peak value of coherence were similar to the mean values averaged over each of the corresponding frequency ranges (Tables 2 and 3).

DISCUSSION

The main findings of this study are threefold. First, repeated squat-stand maneuvers at the frequencies of 0.1 and 0.05 Hz produced large and coherent oscillations in arterial pressure and R-R interval. Second, oscillations in arterial pressure and R-R interval were smaller during passive than during active maneuvers. However, transfer function gain did not change between the two maneuvers. Finally, when compared with the young, transfer function gain was reduced and the phase became more negative in the elderly, demonstrating the well-known effects of aging on reducing baroreflex sensitivity. Collectively, these findings suggest that the changes in R-R interval elicited by BP perturbations during squat-stand maneuvers are mediated primarily by a baroreflex mechanism and that baroreflex function can be assessed using the transfer function method during large perturbations in arterial pressure.
Arterial pressure and heart rate during squat-stand maneuvers. Squatting involves leg muscle contraction and compression of leg arteries and veins (23, 34). In addition, it brings the heart closer to the level of the feet, thus removing the force of gravity on the circulation below heart level, similar to the supine position (23). Thus, cardiac output increases due to increases in central blood volume and stroke volume (12, 19, 23). Interestingly, peripheral vascular resistance either was reduced slightly or did not change despite compression of the leg blood vessels (12, 19). Therefore, increases in arterial pressure and heart rate during squat-stand maneuvers are likely due to changes in cardiac output rather than changes in peripheral resistance.

Fig. 3. Changes in ABP, HR, and respiratory CO2 in a young subject under resting conditions and during passive squat-stand maneuvers at 0.1 and 0.05 Hz. Note the large and coherent oscillations in ABP and HR during these maneuvers relative to resting conditions.

Fig. 4. Group averaged autospectra of systolic blood pressure (SBP) and R-R interval variability obtained from young subjects under resting conditions and during squat-stand maneuvers at 0.1 and 0.05 Hz (n = 10). Solid lines are from active squat-stand maneuvers; dotted lines are passive squat-stand maneuvers. Note the scale differences in the y-axes under resting conditions and during squat-stand maneuvers. Large scales of y-axis are used to represent large oscillations in SBP and R-R during squat-stand maneuvers. The apparent coherent oscillations in SBP and R-R at 0.3 Hz under resting conditions suggest the synchronization of spontaneous changes in SBP and R-R with respiration.
Arterial pressure and R-R interval both were reduced when tractions or the involvement of central command during change central command on the baroreflex, passive squat-stand maneuver excluded (30, 39).

The increases in arterial pressure suggests a baroreflex mechanism. However, the pressure (20). The increase in heart rate in response to the fall output was increased, rather than decreased, during fall in cardiac output since cardiac myogenic responses to BP oscillations and/or the activities of muscle pump also may play a role (39). Conversely, the commensurate reduction in R-R oscillations most likely reflect a baroreflex mechanism in response to smaller changes in arterial pressure.

Assessment of baroreflex function using the transfer function method. Transfer function analysis of spontaneous changes in arterial pressure and R-R interval has been used for assessing baroreflex function (14, 29). However, the magnitude of spontaneous changes in BP and R-R interval may be small and variable among individual subjects and may not be mediated exclusively by the baroreflex (4, 6). In addition, changes in R-R interval may have feed-forward and/or feedback effects on arterial pressure, thus formulating a closed-loop relationship under resting conditions (36). Not surprisingly, using the open-loop transfer function method to assess baroreflex function was biased in animal studies (16). In this regard, both theoretical analysis and experimental data suggest that the accuracy of assessing baroreflex function could be improved by introducing external perturbations to arterial pressure (16, 21).

Based on this knowledge, repeated squat-stand maneuvers were employed to elicit large and clinically relevant perturbations in arterial pressure to provoke the baroreflex. As discussed above, changes in arterial pressure during squat-stand maneuvers are determined mainly by changes in central blood volume and peripheral vascular resistance (12, 39). Therefore, changes in heart rate (or R-R interval) should have minimal compared with active maneuvers. However, estimation of transfer function gain between these variables did not change. These findings suggest that activation of the muscle reflex and/or central command during squat-stand maneuvers do not alter arterial-cardiac baroreflex function.

The reduced pressure oscillations during the passive maneuvers could be attributed to a smaller change in cardiac output and/or peripheral vascular resistance or possibly less central command and less muscle reflex activity. In addition, vascular myogenic responses to BP oscillations and/or the activities of muscle pump also may play a role (39). Conversely, the commensurate reduction in R-R oscillations most likely reflect a baroreflex mechanism in response to smaller changes in arterial pressure.

Cardiovascular variability during active and passive squat-stand maneuvers in young subjects

<table>
<thead>
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<th>Variables</th>
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<tbody>
<tr>
<td>SBP, mmHg</td>
<td>133±13</td>
<td>128±11</td>
<td>138±17</td>
<td>128±11†</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>62±12</td>
<td>62±10</td>
<td>68±13</td>
<td>64±10</td>
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<td>HR, beats/min</td>
<td>86±9</td>
<td>79±9†</td>
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<td>RF, Hz</td>
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<td>0.32±0.06</td>
<td>0.40±0.09</td>
<td>0.37±0.09</td>
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<tr>
<td>ETCO2, mmHg</td>
<td>39±3</td>
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<td>39±3</td>
<td>39±3</td>
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<tr>
<td>SBP-SP, mmHg</td>
<td>223±90</td>
<td>343±248†</td>
<td>75±59*</td>
<td>113±74*</td>
</tr>
<tr>
<td>RR-SP, ms²</td>
<td>4,224±2,872</td>
<td>16,933±6,236†</td>
<td>1813±2130</td>
<td>6227±3608*†</td>
</tr>
</tbody>
</table>

Gain, ms/mmHg

- 4.3±1.5 to 6.8±2.4†
- 4.4±2.5 to 7.0±2.5†

Gain-P, ms/mmHg

- 4.1±1.8 to 7.7±2.6†
- 4.5±2.5 to 8.3±4.2†

Phase, radians

- 0.9±0.6 to -0.6±0.3
- -0.9±0.5 to -0.9±0.5

Phase-P, radians

- 0.1±0.6 to 0.5±0.42†
- -1.1±0.5 to -0.8±0.3*†

Coherence

- 0.70±0.06 to 0.77±0.08
- 0.73±0.17 to 0.69±0.09

Coherence-P

- 0.99±0.01 to 0.99±0.01
- 0.96±0.09 to 0.96±0.05

Values are means ± SD (n = 10). Transfer function gain, phase, and coherence were estimated in the frequency range from 0.078 to 0.125 Hz and from 0.031 to 0.078 Hz. Gain-P, Phase-P and Coherence-P, estimated at the peak coherence. *P < 0.05, comparisons between the frequencies under the same conditions.
effects on changes in arterial pressure, providing justifications to use the open-loop transfer function method to assess baroreflex function (16, 21). Peak value of coherence was ≈ 0.89 during squat-stand maneuvers at both the frequencies of 0.05 and 0.1 Hz and for both young and elderly subjects, further supporting the validity of using the transfer function method.

In line with this discussion, a weak but significant linear correlation was observed between the estimates of transfer function gain under resting conditions and during squat-stand maneuvers. However, gain under resting conditions was significantly higher than that during squat-stand maneuvers. It is possible that estimation of transfer function gain under resting conditions may be biased if changes in R-R interval had significant feed-forward and/or feedback effects on spontaneous changes in arterial pressure (16, 21, 36).

Furthermore, we found that transfer function gain during squat-stand maneuvers at 0.05 Hz was higher than that at 0.1 Hz, associated with a reduction in phase. These findings are consistent with the low pass filter properties of baroreflex function (1, 32). However, despite a higher gain (higher baroreflex sensitivity), oscillations in BP were larger at 0.05 Hz than at 0.1 Hz, reflecting possibly an enhanced myogenic vascular response and/or larger changes in cardiac output at the 0.05 Hz (39). These observations also are consistent with the argument that changes in heart rate (or R-R interval) mediated by the baroreflex are not likely to be essential for buffering changes in BP during squat-stand maneuvers.

Effects of aging on baroreflex function. In the elderly, not only was the magnitude of R-R interval response to changes in arterial pressure decreased, but also the estimate of phase between these variables became more negative relative to the young. The decreased R-R interval or heart rate response to changes in BP (reduction in transfer function gain) most likely reflect the well-known effects of aging on reducing baroreflex sensitivity (8). However, the underlying mechanisms for changes in phase are not clear.

The phase lag between changes in BP and R-R interval is likely to be determined not only by the baroreflex latency, but also the dynamic components (low pass filter) of the baroreflex function (7). Arterial-cardiac baroreflex latency is determined mainly by the vagal nerve activity (6). Moreover, heart rate response to sympathetic nerve stimulation is much slower than to vagal activity (1, 32). Aging is associated with a reduction in vagal activity and increases in sympathetic neural activity (33). Thus, the increased phase lag (more negative phase) in the elderly suggests that heart rate response to changes in BP was delayed, mediated by changes in autonomic neural activity (18, 33, 35).

However, despite a substantially decreased heart rate response, BP oscillations during squat-stand maneuvers were not enhanced significantly in the elderly (Table 2). Assuming similar changes in cardiac output during squat-stand maneuvers between the young and elderly subjects, these observations would suggest that a preserved arterial baroreflex and/or cardiopulmonary reflexes in control of the sympathetic neural activity, and hence vascular resistance, may play a more important role than the arterial-cardiac baroreflex for buffering changes in BP in the elderly (8). In addition, consistent with previous studies, these observations also suggest that changes in heart rate may not play an important role in orthostatic hypotension in the elderly (8, 13, 37).

Study limitations. First, we cannot exclude completely the possibility that muscle reflex and/or central command may influence heart rate during squat-stand maneuvers either directly or indirectly through interactions with the baroreflex (28, 31). Particularly, activation of muscle reflex and/or central command may cause an overall vagal withdrawal and increase in sympathetic neural activity, leading to increases in steady-state heart rate and arterial pressure. However, reducing the potential effects of these confounding factors with passive squat-stand maneuvers did not alter the dynamic relationship between beat-to-beat changes in arterial pressure and R-R interval. These results are consistent with previous studies showing that R-R interval response to sinusoidal changes in neck pressure remained unchanged during dynamic knee-extension exercise (40). Furthermore, leg pulling and compression in the supine position (squat-lying) or squat in water produced little or no effect on arterial pressure and heart rate (2, 23). Thus, we suspect that activation of muscle reflex and/or central command plays a minor, if any, role in the regulation of heart rate in response to changes in arterial pressure during squat-stand maneuvers.

Second, similar to the Valsalva maneuver or infusion of vasoactive drugs, assessment of baroreflex function during squat-stand maneuvers may involve multiple receptors, including both arterial and cardiopulmonary baroreceptors (6). Contributions from each of the individual baroreceptors to changes in heart rate cannot be determined in this study. Thus, assessment of baroreflex function during squat-stand maneuvers is likely to reflect an integrated baroreflex function associated with changes in body posture in the daily life. In addition, we do not know the specific mechanisms for autonomic control of arterial pressure and R-R interval. Further studies using autonomic blockade may provide insight on these issues (22).

Finally, the frail elderly, obese individuals, or people with severe orthostatic hypotension may not be able to perform squat-stand maneuvers. Practically, a less stressful sit-stand maneuver may be suitable for generating oscillations in arterial pressure to assess the baroreflex function (39).

Perspectives and Significance

Large and coherent oscillations in BP and R-R interval were produced during repeated squat-stand maneuvers at different frequencies. Similar transfer function gain between changes in BP and R-R interval was observed during active and passive maneuvers. These findings suggest that changes in heart rate are mediated primarily by a baroreflex mechanism in response to changes in BP. Thus, transfer function analysis can be used to assess baroreflex function during large perturbations in arterial pressure. Application of this method to the elderly confirmed the well-known effects of aging on reducing baroreflex sensitivity. Further work is warranted to demonstrate the reproducibility and to compare this method with other established methods for assessing the baroreflex function. The new method presented in this study potentially may improve the accuracy as well as the reliability for assessing baroreflex function in human studies. Furthermore, in-depth understanding the mechanisms for changes in arterial pressure and heart rate during squat-stand maneuvers may shed light on the clinical implications of these findings.
REFERENCES


