Individual differences in the heart rate response to activation of the muscle metaboreflex in humans

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Watanabe K, Ichinose M, Fujii N, Matsumoto M, Nishiyasu T. Individual differences in the heart rate response to activation of the muscle metaboreflex in humans. Am J Physiol Heart Circ Physiol 299: H1708–H1714, 2010. First published September 17, 2010; doi:10.1152/ajpheart.00255.2010.—We tested the hypotheses that the heart rate (HR) response to muscle metaboreflex activation induced by postexercise muscle ischemia (PEMI) varies considerably among subjects and that individual differences in the HR response are associated with differences in cardiac autonomic tone and/or arterial baroreflex function during PEMI. Fifty-one healthy subjects (36 men and 15 women) performed a 1-min isometric handgrip exercise at 50% maximal voluntary contraction, which was followed by a 3.5-min period of imposed PEMI. We estimated cardiac autonomic tone using spectral analysis of beat-to-beat variation in the R-R interval (RRI). In addition, the sensitivity of the arterial baroreflex control of HR (BRS) was evaluated using transfer function analysis of systolic arterial pressure (SAP) and RRI. Although the mean RRI during the PEMI and subsequent recovery period did not differ from the resting value, the variance among the individual differences in RRI between the rest and PEMI periods was significantly greater than between the rest and recovery periods. The changes in RRI elicited by PEMI correlated significantly with changes in the spectral power of the RRI variability in the high-frequency range and the BRS. By contrast, no significant correlation was observed between changes in RRI and changes in mean arterial pressure or the power of the RRI variability in the low-frequency range. This suggests that, in humans, the HR response to PEMI-induced activation of muscle metaboreflex varies considerably from individual to individual and that these differences reflect changes in cardiac parasympathetic tone and spontaneous BRS during PEMI.

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ISOMETRIC HANDGRIP EXERCISE causes an increase in arterial blood pressure and heart rate (HR) (24, 26). Moreover, arresting the forearm blood circulation just before cessation of the exercise causes the blood pressure to remain above resting levels (2, 27, 28, 41). This suggests that the accumulation of metabolites within the muscles during the exercise stimulates chemosensitive afferents (primarily group IV, but also group III fibers) and reflexly raises arterial blood pressure (5, 21, 25). This reflex is commonly called the muscle metaboreflex, and it is thought to play an important role in cardiovascular regulation during exercise.

In animal experiments, drug-induced stimulation of the metaboreceptors in the muscles has positive chronotropic and inotropic effects on the heart (26). In addition, during submaximal dynamic exercise in both humans and dogs, activation of the muscle metaboreflex by reducing the blood flow to active muscles causes substantial increases in HR and cardiac output (CO), which accounts for most, if not all, of the rise in arterial pressure (4, 9, 22, 23, 40, 46, 50). On the other hand, when the reflex is activated as a result of postexercise muscle ischemia (PEMI), HR remains at the resting level, thereby limiting any rise in CO (4, 6, 7, 27). On the basis of those findings, several investigators have concluded that the muscle metaboreflex elicited during PEMI plays little, if any, role in the control of HR (39, 49).

Subsequently, however, it was found that both cardiac sympathetic and parasympathetic tone simultaneously increase during PEMI in both dogs and humans (11, 13, 27, 32). In addition, studies have shown that the muscle metaboreflex exerts its effects via changes in sympathetic nerve activity, with little direct influence on parasympathetic tone (32), and that the arterial baroreflex attenuates the pressor responses evoked by the muscle metaboreflex (22, 23, 45, 47). This suggests that the increase in cardiac parasympathetic tone during PEMI might reflect, in part, buffering by the arterial baroreflex of the increase in blood pressure induced by the muscle metaboreflex, as well as a sudden loss of central command (19, 27, 32). In addition, transfer function analysis showed that, in humans, PEMI-induced activation of the muscle metaboreflex increases the sensitivity of the spontaneous baroreflex control of HR (BRS) (13), which would enhance arterial baroreflex-mediated buffering of muscle metaboreflex. These findings suggest that maintenance of the resting HR during PEMI could be a result of an interaction between the effects of the sympathoexcitation via the muscle metaboreflex and parasympathetic activation induced by the arterial baroreflex and/or the other mechanisms, such as the removal of central command (19, 27, 32, 45).

Although we previously observed that the mean HR during PEMI did not differ from the resting HR in a group of subjects, we noted that in several subjects HR did increase or decrease from the resting levels during PEMI, suggesting that there are considerable individual differences in the HR response to PEMI (13–17, 27–29). To our knowledge, however, these individual differences have never been systematically investigated. Furthermore, the regulatory mechanism that determines the HR response to PEMI remains unknown. The aim of the present study was to test our hypothesis that the HR response to PEMI varies considerably from individual to individual and that the individual differences in the HR response are associated with cardiac autonomic tone and/or the function of the arterial baroreflex in the control of HR during PEMI. To test those ideas, we used spectral analysis of beat-to-beat changes in the R-R interval (RRI) to estimate cardiac autonomic tone. In addition, we used transfer function analysis of spontaneous
variations in systolic arterial pressure (SAP) and RRI to evaluate the dynamic properties of the cardiac component of the arterial baroreflex.

METHODS

Subjects. We studied 51 healthy volunteers (36 men and 15 women) with a mean age of 21.6 ± 0.2 yr, body weight of 62.0 ± 1.1 kg, and height of 168.8 ± 1.2 cm (means ± SE). None of the subjects was taking any medication, and none smoked. The study was carried out in accordance with the Declaration of Helsinki and was approved by the Human Subjects Committee of the University of Tsukuba. All subjects provided informed written consent.

Procedures. Before the actual experimental day, we familiarized each subject with the procedures during an orientation session in which they experienced static handgrip and PEMI. The subjects were asked to abstain from alcohol intake for at least 24 h before the experiment, from caffeine intake on the experimental day, and from everything except water during the 2 h before the experiment. On the experimental day, subjects entered the test room, which was maintained at 25°C, and assumed a supine position. They then performed a maximum voluntary contraction (MVC) using a handgrip dynamometer held in the right hand, which enabled us to determine the maximum MVC. Thereafter, a rapidly inflatable cuff for arterial occlusion was placed on the right upper arm (for the production of PEMI), and a respiratory mask was fitted. The subjects then had a rest period of at least 15 min before data collection began.

The subjects were instructed to maintain a constant respiration rate of 15 cycles/min and a constant tidal volume of 0.4–0.7 liters throughout the experiment. We previously established that this tidal volume did not cause dyspnea at a respiratory frequency of 15 cycles/min in any subject. Auditory signals and an oscilloscope display of the respiratory volume were supplied to assist the subject with this. Control data were acquired for 5 min before the handgrip exercise was started. The subjects then performed a 60-s isometric handgrip exercise at 50% MVC with visual feedback showing the achieved force on an oscilloscope display. Five seconds before cessation of the static handgrip, the occlusion cuff was inflated to the maximum voluntary contraction (MVC) using a handgrip dynamometer held in the right hand, which enabled us to determine the 50% MVC. Thereafter, a rapidly inflatable cuff for arterial occlusion was placed on the right upper arm (for the production of PEMI), and a respiratory mask was fitted. The subjects then had a rest period of at least 15 min before data collection began.

Measurements. HR and RRI were monitored via a three-lead ECG. Beat-to-beat changes in arterial blood pressure were assessed using finger photoplethysmography (Finometer; Finapres Medical Systems). The monitoring cuff was placed around the middle finger with the forearm and hand supported so that the cuff was aligned at the level of the heart. The subjects wore a mask connected to a respiratory flow meter (RF-H; Minato Medical Science) for the measurement of respiratory flow. The analog signals representing the ECG, blood pressure waveform, and respiratory flow were digitized at a sampling frequency of 100 Hz and stored in a personal computer (ThinkPad T30; IBM). The beat-to-beat HR, RRI, SAP, diastolic arterial blood pressure (DAP), and mean arterial blood pressure (MAP) were calculated using a data analysis program for measuring waveforms (LabVIEW6; National Instruments).

Data analysis. From the 5-min control (resting) recordings, we analyzed 3-min-long steady-state records as the control data. From the 3.5-min recordings made during the PEMI periods, we analyzed the final 3 min of the recordings as the PEMI data. Subjects with frequent ectopic beats (>1% of all beats; n = 5), periods in which the measured variables were obviously unstable (a linearly varying tendency or marked sudden changes; n = 2), or excessive respiratory sinus arrhythmia (RRI fluctuations of >400 ms with each respiratory cycle; n = 4) were eliminated from all analyses. The standard deviation of the RRI (SDRRI), which is a well-known marker of cardiac parasympathetic tone, was separately calculated for the control and PEMI periods. The beat-to-beat data for the RRI and SAP during the control and PEMI periods were interpolated and resampled. This process provided 512 points of equidistant time interval data. The data were then divided into five equal overlapping segments of 256 data points, and for each segment the linear trend was removed and a Hanning window was applied. Fast-Fourier transforms were implemented in each segment and then averaged to calculate the autospectra. The spectral resolution for these estimates was ~0.0011 Hz. The low-frequency (LF; ~0.03–0.15 Hz) and high-frequency (HF; ~0.15–0.35 Hz) powers of all of the variables were calculated from the integration of the autospectra. The HF power of the RRI variability reflects the level of cardiac parasympathetic tone, whereas the LF power is influenced by both cardiac sympathetic and parasympathetic nerve activities (1, 3, 12, 35, 48). We then employed transfer function analysis to evaluate the relationship between the SAP and RRI. The transfer function [H(f)] between the two signals was calculated as

$$H(f) = \frac{S_{xy}(f)}{S_{xx}(f)}$$

where $S_{xx}(f)$ is the autospectrum of SAP variability and $S_{xy}(f)$ is the cross-spectrum between SAP and RRI. The transfer function magnitude (gain), $H(f)$, and phase spectrum $\Phi(f)$ were obtained from the real $[H_r(f)]$ and imaginary $[H_i(f)]$ parts of the complex function as

$$H(f) = [H_r(f)^2 + H_i(f)^2]^{1/2}$$

$$\Phi(f) = \tan^{-1}[H_i(f)/H_r(f)]$$

and the squared coherence function $[\text{Coh}(f)]$ was estimated as

$$\text{Coh}(f) = \frac{|S_{xy}(f)|^2}{[S_{xx}(f)S_{yy}(f)]}$$

where $S_{yy}(f)$ is the autospectrum of changes in RRI. The squared coherence function reflects the fraction of the output power that can be linearly related to the input power at each frequency. Similar to a correlation coefficient, the squared coherence function varies from 0 to 1 and reflects the validity of the transfer function estimates.

The LF transfer function gain, phase, and coherence were estimated as mean values in the frequency range of ~0.05–0.15 Hz. For phase value interpretation, a negative phase suggests that changes in the input variable preceded changes in the output response, whereas a positive phase suggests the reverse. Previous studies have shown that the transfer function gain for the SAP-RRI relationship in the LF range reflects the BRS in the control of HR (34, 38).

Statistical analysis. Data are means ± SE. For mean values of SAP, DAP, MAP, RRI, and HR, as well as for derived variables from the time- and frequency-domain analyses, comparisons were made between the control and PEMI periods using paired t-tests. A one-way repeated-measures ANOVA with Tukey’s post hoc test was used to compare the hemodynamic responses among periods. Paired F-tests were used to assess the differences in the variance of individual changes in RRI (as a magnitude of the individual differences) from the control to the PEMI period and from the control to the recovery period. The relationships between selected physiological variables were evaluated using Pearson’s product-moment correlation analysis. Values of P < 0.05 were considered significant.

RESULTS

Baseline data. Table 1 shows the mean values for SAP, DAP, MAP, RRI, and HR during the control and PEMI periods. The changes in mean RRI and MAP during the control, exercise, PEMI, and recovery periods are shown in Fig. 1. Significant tachycardia and pressor responses occurred with the exercise. The RRI, and thus the HR, returned to control levels during PEMI, whereas MAP remained higher than control. In addition, neither RRI nor MAP differed from control during the recovery period.

Variance of RRI responses. Figure 2 shows the change in RRI from the control to the PEMI period for each subject.
Although there was no change in the mean RRI, RRI values varied considerably among individual subjects. In 20 subjects the RRI was higher than control during PEMI, whereas it was lower than control in the other 20 subjects. Figure 3 shows the standard deviations of the differences in RRI between the control and PEMI periods and between the control and recovery periods. The mean RRI during both the PEMI and recovery periods did not differ from control, but the standard deviation of the differences in RRI between the control and PEMI periods was significantly larger than that between the control and recovery periods. This suggests that the magnitudes of the individual changes in RRI elicited by PEMI were greater than those elicited by recovery. We performed the same analysis using HR instead of RRI and observed qualitatively similar results.

**Time- and frequency-domain analyses.** Table 2 shows the mean values for SD_{R,R} and the autospectral power for RRI and SAP variability, as well as the transfer function gain, phase, and coherence for the SAP-RRI relationship. During PEMI, the SD_{R,R}, LF and HF RRI power spectra, and transfer function gain (i.e., BRS) were significantly higher than control, whereas the HF SAP power was lower than control. The phase was negative and did not significantly differ between the two conditions. The coherence was above 0.5 during both the control and PEMI periods and did not differ among conditions.

**Relationships among RRI responses, pressor responses, cardiac autonomic tone, and arterial baroreflex functions.** Figure 4 shows the correlations for all subjects between PEMI-induced changes in RRI and changes in MAP, SD_{R,R}, LF and HF RRI power (indexes of cardiac autonomic tone), and BRS. The changes in RRI correlated positively with changes in SD_{R,R}, HF power of the RRI variability, and BRS. On the other hand, there were no significant correlations between changes in the RRI and changes in MAP and the LF RRI power. As expected, the changes in the two indexes of cardiac parasympathetic tone (i.e., SD_{R,R} and HF RRI power) were highly correlated with each other ($r = 0.862; P < 0.001$). Figure 5 shows the correlations between PEMI-induced changes in BRS and changes in SD_{R,R} and the LF and HF RRI power. The changes in the BRS correlated positively with changes in SD_{R,R} and the LF and HF RRI power.

**DISCUSSION**

To our knowledge, this is the first study focusing on individual differences in the HR response to PEMI-induced muscle metaboreflex activation. The major finding of this study is that, in humans, the HR response to PEMI varies considerably from individual to individual. Moreover, PEMI-induced changes in the cardiac period correlate positively with changes in cardiac...
parasympathetic tone and BRS. Together, these findings suggest that changes in cardiac parasympathetic tone and BRS during PEMI are associated with the large individual differences in the HR response to PEMI.

The type of exercise employed in this study (1-min isometric handgrip at 50% MVC) reportedly reduces intracellular pH from 7.1 to 6.5 in the exercising muscles (29), which is a sufficiently large reduction to stimulate chemosensitive afferent nerves (primarily group IV, but also group III fibers) through cellular efflux of hydrogen ions and/or production of metabolites associated with acidosis, thereby increasing sympathetic nerve activity and arterial blood pressure via the muscle metaboreflex (5, 21, 25). Furthermore, with the use of an occlusion cuff, the metabolites produced during the exercise were sequestered in the forearm, where they would be expected to activate the muscle metaboreflex throughout the PEMI in the absence of potential influences from either central command or the muscle mechanoreflex. Although MAP was higher during PEMI than during the control period, which was expected and is in agreement with previous studies (4, 6, 7, 13–17, 19, 27–29, 41, 49), PEMI-induced activation of the muscle metaboreflex had little effect on HR. These responses could be interpreted as indicating this reflex has no control over HR; however, earlier studies in humans and dogs provide evidence of unique changes in cardiac autonomic tone during PEMI that reflect simultaneous increases in both cardiac sympathetic and parasympathetic tone (11, 13, 27, 32). Consistent with these reports, we found that the LF and HF power of the RRI variability and the SDR-R were all increased during PEMI. This suggests that the effect of muscle metaboreflex activation

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<th>Control</th>
<th>PEMI</th>
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<tr>
<td>SDR-R, ms</td>
<td>61 ± 3.6</td>
<td>73 ± 4.6*</td>
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<tr>
<td><strong>Autospectral data</strong></td>
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<tr>
<td>RRI power, ms²</td>
<td></td>
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<tr>
<td>LF</td>
<td>681 ± 73</td>
<td>1,016 ± 169*</td>
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<tr>
<td>HF</td>
<td>2,507 ± 361</td>
<td>3,158 ± 447*</td>
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<tr>
<td>SAP power, mmHg²</td>
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<tr>
<td>LF</td>
<td>10.2 ± 1.3</td>
<td>8.5 ± 1.0</td>
</tr>
<tr>
<td>HF</td>
<td>3.6 ± 0.4</td>
<td>2.5 ± 0.3*</td>
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<tr>
<td><strong>Cross-spectral data</strong></td>
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<tr>
<td>LF gain, ms/mmHg</td>
<td>10.5 ± 0.9</td>
<td>12.5 ± 1.1*</td>
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<tr>
<td>LF phase, degrees</td>
<td>−44.7 ± 4.1</td>
<td>−36.8 ± 4.8</td>
</tr>
<tr>
<td>LF coherence</td>
<td>0.55 ± 0.02</td>
<td>0.56 ± 0.02</td>
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Values are means ± SE; n = 40 subjects. SDR-R, standard deviation of the R-R intervals; LF, low-frequency range; HF, high-frequency range. *P < 0.05 vs. control.

![Graphs](http://ajpheart.physiology.org/)
on cardiac sympathetic outflow can be masked by enhanced cardiac parasympathetic tone during PEMI, resulting in a HR that is unchanged from the resting levels. A recent study by Fisher et al. (11) reported that PEMI-induced robust activation of the muscle metaboreflex elicits sympathetically mediated tachycardia in humans. However, they also showed that in the case of cardiac parasympathetic blockade, there was a greater elevation in HR during moderate muscle metaboreflex activation (the pressor response was similar in magnitude to the response observed in the present study) than under normal conditions. These data support a role for cardiac parasympathetic activation in obscuring the enhancement of cardiac sympathetic activity elicited by moderate PEMI-induced activation of the muscle metaboreflex.

We observed large individual differences in the HR response to PEMI. Although the mean RRI values during the control, PEMI, and recovery periods were similar, the variance of the individual changes in RRI between the control and PEMI periods were significantly larger than between the control and the recovery periods (Fig. 3). This suggests that the lack of change from control in the average HR during PEMI reflects offsetting increases and decreases in HR elicited by the PEMI. Although the precise mechanism(s) underlying the large individual differences in the HR response to PEMI remains uncertain, our results provide important insight into the causes of the individual differences in the HR response. We found significant positive correlations between PEMI-induced changes in RRI and the changes in two indexes of cardiac parasympathetic tone (i.e., SD_{RRI} and HF power of the RRI variability). This suggests that individuals with greater enhancement of cardiac parasympathetic tone during PEMI had a greater bradycardic response to PEMI, and vice versa. By contrast, the changes in RRI did not correlate with the changes in the LF power of the RRI variability, which is thought to be influenced by both cardiac sympathetic and parasympathetic nerve activities. Consequently, the individual differences in the HR response to PEMI-induced activation of the muscle metaboreflex could be related primarily to changes in cardiac parasympathetic tone.

It has been demonstrated that the arterial baroreflex buffers muscle metaboreflex-induced pressor responses by inhibiting sympathetic nerve activity (22, 23, 45, 47). For example, activation of the muscle metaboreflex causes substantially greater increases in peripheral vasoconstriction and arterial blood pressure in barodenervated dogs than intact dogs (22, 23). In addition, Nishiyasu et al. (27) suggested that an increase in cardiac parasympathetic tone during PEMI might be part of a counterresponse by the arterial baroreflex to the elevation in arterial blood pressure induced by the muscle metaboreflex. Ichinose et al. (13) recently reported that BRS determined by transfer function analysis is increased during PEMI in humans. In the present study, we confirmed that finding and further showed that PEMI-induced changes in RRI correlate positively with changes in BRS. Our present results also indicate that a greater bradycardic response to PEMI is likely to occur in individuals who show greater increases in BRS during PEMI, and vice versa. Thus the change in arterial baroreflex-mediated control of the cardiac period during PEMI, rather than the level of arterial baroreflex loading, per se (i.e., PEMI-induced elevation of MAP, which can also be considered an output response of the muscle metaboreflex), would also contribute to the large individual variation in the HR response to PEMI-induced muscle metaboreflex activation. Our results support the concept that the control of HR during PEMI is a net result of the interaction between the muscle metaboreflex and the arterial baroreflex (19, 27, 32, 45). Bearing in mind that it has been shown that afferent input from arterial baroreceptors modifies sympathoexcitation evoked by activation of skeletal muscle afferents in the central nervous system (36), it seems likely that interaction of the muscle metaboreflex and arterial baroreflex occurs centrally.

Our findings do not enable us to provide a definitive explanation of the mechanism(s) responsible for the increase in BRS during PEMI. In contrast to our findings, recent studies have shown that imposed activation of the muscle metaboreflex during dynamic exercise attenuates BRS in dogs (18, 20, 42, 43). These contrasting observations raise the possibility that the influence of muscle metaboreflex activation on arterial baroreflex function differs depending on whether the muscle metaboreflex is engaged during the postexercise period (i.e., PEMI) or during dynamic exercise (although we should not ignore the possibility of a species difference). According to Ogoh et al. (30), BRS declines as workload increases during dynamic exercise, and reductions in BRS are associated with vagal withdrawal. Consistent with those findings, we observed...
individual differences in HR response to metaboreflex

We employed transfer function analysis to evaluate the dynamic properties of the cardiac component of the arterial baroreflex in a closed-loop relationship (based on spontaneous fluctuations in SAP and RRI). Because this analysis provides only an estimate of the spontaneous BRS around the operating point of the full arterial baroreflex stimulus-response curve (10, 37), it is possible that the increase in BRS seen during PEMI in this study was due to a shift in the operating point to a higher responsiveness portion of the stimulus-response relationship. In addition, transfer function estimates are limited by a fundamental assumption of linearity between changes in two variables and are reliable only if the squared coherence values are near or above 0.5 (31, 44). In the present study, the coherence for the SAP-RRI relationship in the LF range was sufficiently high to confirm the validity of using this technique to assess the gain and phase for that relationship.

We performed correlation analyses to evaluate relationships among cardiovascular responses, cardiac autonomic tone, and arterial baroreflex function in the control of the cardiac period. This method provides insight into the linear interrelationship between two variables; it does not evaluate causality, however. Consequently, any interpretation of relationships must be made with caution. Moreover, some correlations were not very close. For example, the correlation coefficient between PEMI-induced changes in RRI and the changes in BRS was -0.5. If the BRS response does in fact cause changes in RRI, the contribution ratio (coefficient of determination; $r^2$) was only ~24%. Our results highlight the complexity of integrative cardiovascular regulation and indicate that individual differences in cardiac responses depend on several factors, including cardiac autonomic tone and BRS.

An important feature of the present study was the relatively large sample size. However, considering that only healthy and relatively young subjects were recruited in this study, it is uncertain whether our findings can be translated to other populations, and further studies are needed to address this issue.

In conclusion, our findings demonstrate that, in humans, the HR response to PEMI-induced activation of the muscle metaboreflex varies considerably from individual to individual. They also suggest that changes in cardiac parasympathetic tone and spontaneous BRS during PEMI are associated with the large individual differences in the HR response to PEMI.

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Disclosures

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

references


