Does Paced Breathing Improve the Reproducibility of Heart Rate Variability Measurements?

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Abstract The effects of paced breathing (PB) on the reproducibility of heart rate variability (HRV) measurements were examined in 55 male subjects (age range: 20–54 years). Spectral components of HRV were measured under a combination of two respiratory conditions (spontaneous and 4s PB) and two postures (standing and supine). The procedures were repeated 3 weeks after the first measurement. Log-transformed low-frequency (lnLF) and high-frequency (lnHF) components of HRV were calculated from a 205s electrocardiography (ECG) recording. The coefficients of interindividual variations of lnHF and lnLF (ca. 13–16%) and the intraindividual variations of the frequency components (5–6%) were not significantly affected by PB. The coefficients of intraindividual variation of heart rate, lnHF and lnLF did not correlate with age in either posture. Effect sizes of PB on the intraindividual variation ranged from 0.04 to 0.13. Although intraclass correlation coefficients (ICCs) were slightly improved by PB in some cases, the differences were negligible. The above results suggest that PB provides a limited improvement in the reproducibility of HRV measurements, and metronome-guided respiration is not necessarily required for HRV measurement if subjects are reminded to avoid irregular respiration. J Physiol Anthropol 28(5): 225–230, 2009 http://www.jstage.jst.go.jp/browse/jpa2 [DOI: 10.2114/jpa2.28.225]

Keywords: paced breathing, controlled respiration, heart rate variability, reproducibility, reliability

Introduction

The relationship between respiration and heart rate (HR) has extensively been studied for years. The phenomenon of HR fluctuations with respiration, which has been documented since the mid 19th century (Ludwig, 1847), is known as respiratory sinus arrhythmia (RSA) or as the high-frequency (HF) component of heart rate variability (HRV). RSA or the HF component of HRV is also considered an index for monitoring parasympathetic activity, because atropine (an acetylcholine receptor blocker) decreases the component to near zero.

Both the depth and frequency of respiration affect RSA. The relationship between the tidal volume and the amplitude of RSA assumes a linear tendency with deeper breathing producing higher RSA (Kobayashi, 1998). However, the relationship between respiratory frequency and the amplitude of RSA is markedly nonlinear, where slower breathing produces higher amplitude for RSA. However, the maximal amplitude approximates 0.1 Hz (6 breaths/min) even if the tidal volume remains constant (Hirsch and Bishop, 1981; Kobayashi, 1997).

In general the respiratory rate in humans is approximately 0.2–0.4 Hz at rest; however, the respiratory rate often decreases to the low-frequency (LF) band (0.04–0.15 Hz). Bernardi et al. (2000) have reported that the respiratory rate decreases during conversation or mental arithmetic loading, resulting in LF increases accompanied by HF decreases. Because low-frequency (ca. 0.1–Hz) respiration produces maximal RSA (see above), the respiratory interference may induce unfavorable repercussions in HRV derivation.

To avoid respiratory interference on HRV components, the respiratory rhythm (and tidal volume, whenever and wherever applicable) of subjects should be kept constant during HRV measurement. Therefore, subjects were reminded to synchronize their breathing rhythm with an auditory signal. This procedure has been called paced breathing (PB), which is also known as metronome-guided breathing or controlled respiration.

It is unclear who first applied PB to HRV measurements. Studies advocating PB as a conditional prerequisite in HRV monitoring have been published since the 1990s (e.g., Fallen et al., 1988; Brown et al., 1992; Kobayashi et al., 1999; Camman and Michel, 2002; Tripath, 2004). On reviewing the number of HRV studies incorporating PB, Brown et al. (1992) found that 24 of 64 relevant studies (37%) had actually adopted respiratory rate control. This finding suggests that attention among scholars was already focused on the significance of PB on HRV in the early 1990s.
Many previous investigations of the effects of PB on HRV components have demonstrated PB to diminish the LF component. In other words, respiratory disturbances affect not only the HF-band but also the LF-band of the HRV spectrum. However, the effects of PB on the HF component were found to be inconsistent among the studies. The discrepancies might be attributable to a variation in the target-frequency of PB.

An article written by Pagani et al. (1986) is one of the most influential studies in the field of HRV; they suspected PB would have an effect on HRV because PB also produces vagal activation. However, studies (Hayano et al., 1994; Bloomfield et al., 2001; Pinna et al., 2006) demonstrated that a change of the HRV components induced by PB was not accompanied with a change in the parasympathetic tone.

In evaluating the benefits of PB, it is therefore more important to establish improvements in the reproducibility of HRV measurements rather than the effects on HRV indexes per se. As such, we quantitatively investigated the effects of PB on HRV measurements, especially the effects on inter- and intra-individual variations in the present study.

Methods

The heartbeat intervals in 55 healthy Japanese male subjects (age range: 20–54 years) were monitored (see age distribution of subjects in Fig. 1). The height and weight of the subjects (mean±S.D.) were 173.1±5.8 cm and 68.6±10.0 kg, respectively.

The heartbeat intervals were measured in two postures (standing and supine) under two respiratory conditions: spontaneous breathing (SB) and paced breathing (PB). Under the PB condition, subjects synchronized their breathing rhythm with 4s auditory signals, and they were instructed to avoid irregular breathing under both SB and PB conditions. The order of the experimental conditions between the experimental periods was adjusted to minimize the effect of circadian variation. Measurements were conducted after lunch (11:00 hr) between 13:00 and 16:00 hr.

A wristwatch-type HR monitor (Polar S801i; Finland) recorded heart beat intervals for 4 min at 1 ms resolutions. The heart beat data (including beat-detection errors) were either corrected or excluded from analysis. The sequences of heart beat intervals were interpolated as 5 Hz equidistant data. The HR was calculated from 1,024 interpolated data points (204.8s).

The power spectra of HRV based on the 1,024 interpolated interval sequences (204.8s) were calculated using Fast Fourier transformation (FFT) processing. HF and LF components were obtained by integrating the power spectra over their respective ranges of 0.15–0.35 and 0.04–0.15 Hz. The natural logarithms of the HRV indexes (lnHF and lnLF) were then calculated.

We performed similar measurements at two time-points a day, and averaged the two readings for analysis. The same procedures were repeated 3 weeks after the first measurement in order to examine the reproducibility of the same measurements on two different days.

We calculated the coefficients of interindividual variation (interCV) and intrainsividual variation (intraCV) to examine the extent of individual variations. Coefficients of interCV and intraCV were derived from the following formulae:

\[
\text{interCV} = 100 \frac{\sigma_b}{\bar{x}}
\]

\[
\text{intraCV} = \frac{1}{n} \sum_i 100 \frac{\sigma_{ei}}{\bar{x}_i}
\]

or

\[
\bar{x}_i = \frac{1}{r} \sum_{j} x_{ij}
\]

\[
\bar{x} = \frac{1}{n} \sum_i \bar{x}_i
\]

\[
\sigma_b = \left[ \frac{1}{n-1} \sum_i (\bar{x}_i - \bar{x})^2 \right]^{1/2}
\]

\[
\sigma_{ei} = \left[ \frac{1}{r-1} \sum_{j} (x_{ij} - \bar{x}_i)^2 \right]^{1/2}
\]

where \(n\), \(r\) and \(x_{ij}\) are the number of subjects, number of repeats and, and data (HR, lnHF or lnLF) of subject \(i\) on day \(j\), respectively.

The effects of PB were statistically verified by the paired \(t\)-test for mean and intraCVs. The effects of PB on interCVs were statistically examined by an approximate method described by Miller (1991) and Zar (1999). Furthermore, we
calculated the effect size (ES; Cohen’s *d*) to examine the effects of PB on intraCVs. The intraindividual reproducibility was also evaluated by an intraclass correlation coefficient (ICC), where higher ICC represents better reproducibility.

**Results**

According to the means (eq. 4), interCVs (eq. 1), and intraCVs (eq. 2) of HR, lnHF, and lnLF during SB and PB (Table 1), HR was significantly (*p*<0.01) increased by PB in the standing posture but not the supine posture. Frequency components of HRV (lnHF and lnLF) were significantly (*p*<0.01) reduced by PB in both postures. The interCVs of HR (16.14–18.39%) were not significantly affected by PB in both postures. Similarly, the interCVs of the frequency components of HRV (lnHF and lnLF) registered 11.63–17.42% and were not influenced by PB. The intraCVs of HR and frequency components of HRV registered 4.83–6.36% and were not significantly altered by PB in either posture.

In addition, we analyzed the ES of PB on intraCVs (Table 2) where the positive and negative values of ES respectively represent decreases and increases of intraCV induced by PB. The ES values of PB on intraCVs of the HR components ranged from −0.04 to 0.13. According to the interpretation by Cohen (1988), an ES may be categorized as either small (*d*=0.2), medium (*d*=0.5), or large (*d*=0.8). In other words, the effect of PB was small if it was statistically significant.

Based on the effects of PB on the ICCs of the HR and HRV components (Table 3), the ICCs of the HR and HRV components were 0.83–0.87 and 0.71–0.88 respectively. The HRV components showed slightly higher ICCs during PB than those during SB in most cases, although the improvements under PB were small (0.01–0.06).

The correlations of intraCVs of HR, lnHF, and lnLF with age in the supine position (Fig. 2) showed that the coefficients of determination (*r*²) were small (*r*²<0.03) in both breathing (SB and PB) conditions. Similar results were obtained in the standing position (*r*²<0.06).

**Discussion**

**Efficiency of paced breathing (PB): improving the reproducibility of HRV measurements**

In some studies conducted on the effect of PB on the reproducibility of HRV measurements, Sinnreich et al. (1998) have reported that intraCVs of lnLF and lnHF are slightly

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**Table 1** Effect of paced breathing (PB) on the means (eq. 4), and the coefficients of interindividual variations (interCVs, eq. 1) and intraindividual variations (intraCVs, eq. 2) of the heart rate (HR) and natural logarithms of the low-frequency (lnLF) and high-frequency (lnHF) components of heart rate variability (HRV). Differences in mean and intraCVs of the indexes were statistically verified by paired t-test. Differences between the two interCVs were statistically examined by an approximate method described by Miller (1991) and Zar (1999). Where *p*<0.05 (*), *p*<0.01 (**), or ns (not significant).

<table>
<thead>
<tr>
<th>Indexes</th>
<th>Standing</th>
<th></th>
<th></th>
<th>Supine</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>SB</td>
<td>PB</td>
<td>SB</td>
<td>PB</td>
<td></td>
</tr>
<tr>
<td>HR mean (bpm)</td>
<td>87.11</td>
<td>90.33**</td>
<td>69.41</td>
<td>69.67 ns</td>
<td></td>
</tr>
<tr>
<td>interCV (%)</td>
<td>16.14</td>
<td>16.33 ns</td>
<td>17.96</td>
<td>18.39 ns</td>
<td></td>
</tr>
<tr>
<td>intraCV (%)</td>
<td>5.35</td>
<td>4.84 ns</td>
<td>4.94</td>
<td>5.11 ns</td>
<td></td>
</tr>
<tr>
<td>lnHF mean (ln-ms²)</td>
<td>3.37</td>
<td>3.13**</td>
<td>3.89</td>
<td>3.77**</td>
<td></td>
</tr>
<tr>
<td>interCV (%)</td>
<td>15.85</td>
<td>17.42 ns</td>
<td>15.11</td>
<td>14.44 ns</td>
<td></td>
</tr>
<tr>
<td>intraCV (%)</td>
<td>6.36</td>
<td>5.91 ns</td>
<td>5.65</td>
<td>5.04 ns</td>
<td></td>
</tr>
<tr>
<td>lnLF mean (ln-ms²)</td>
<td>3.83</td>
<td>3.62**</td>
<td>4.00</td>
<td>3.82**</td>
<td></td>
</tr>
<tr>
<td>interCV (%)</td>
<td>14.88</td>
<td>15.11 ns</td>
<td>13.17</td>
<td>11.63 ns</td>
<td></td>
</tr>
<tr>
<td>intraCV (%)</td>
<td>4.83</td>
<td>4.64 ns</td>
<td>4.88</td>
<td>5.07 ns</td>
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**Table 2** Effect sizes (Cohen’s *d*) of paced breathing (PB) on the coefficient of intraindividual difference (intraCV) in natural logarithms of the high-frequency (lnHF) and low-frequency (lnLF) components of heart rate variability (HRV) in standing and supine positions. Positive and negative values respectively represent decreases and increases of intraCV by PB.

<table>
<thead>
<tr>
<th></th>
<th>Standing</th>
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</thead>
<tbody>
<tr>
<td>HR</td>
<td>0.12</td>
<td>−0.04</td>
</tr>
<tr>
<td>lnHF</td>
<td>0.09</td>
<td>0.13</td>
</tr>
<tr>
<td>lnLF</td>
<td>0.05</td>
<td>−0.04</td>
</tr>
</tbody>
</table>

**Table 3** Effects of paced breathing (PB) on intraclass correlation coefficients (ICCs) of heart rate (HR) and natural logarithms of the low-frequency (lnLF) and high-frequency (lnHF) components of heart rate variability (HRV). Higher ICC values represents better reproducibility.

<table>
<thead>
<tr>
<th></th>
<th>Standing</th>
<th>Supine</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR</td>
<td>0.83</td>
<td>0.86</td>
</tr>
<tr>
<td>lnHF</td>
<td>0.79</td>
<td>0.85</td>
</tr>
<tr>
<td>lnLF</td>
<td>0.84</td>
<td>0.88</td>
</tr>
</tbody>
</table>

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decreased; viz., from 11.5 to 10.7% and from 12.1 to 10.5% by 4s PB. Furthermore, the ICCs of lnLF and lnHF improved from 0.68 to 0.75 and from 0.76 to 0.82, respectively. In addition, according to Carasco et al. (2003), 5s PB improved the ICCs of the LF and HF components from 0.86 to 0.92 and from 0.86 to 0.93, respectively. Furthermore, 4s PB moderately improves the ICCs of lnLF from 0.79 to 0.86 (Pinna et al., 2007) to eventually yield marked improvements in nuLF (LF/total power) and LF/HF. Although PB (16 breaths/min) improves the ICCs of the lnHF component from 0.48 to 0.65, the ICCs of the lnLF component are reduced to 0.60 from 0.77 (Pitzalis et al., 1996). In a subsequent study using 3s PB, the

Fig. 2 Correlations of intraindividual variations (intraCVs) of heart rate (HR), natural logarithms of the low-frequency (lnLF) and high-frequency (lnHF) components of heart rate variability (HRV) with age in the supine position. Left and right columns represent spontaneous breathing (SB) and paced breathing (PB). Coefficients of determination ($r^2$) were small (up to 0.03) under both breathing conditions. Results in the standing posture resembled those obtained in the supine position ($r^2<0.06$).
ICC of LF decreases from 0.88 to 0.79 with a slight improvement of lnHF (Reland et al., 2005). In a study by Turkiainan et al. (2005), poor stability of HRV components (lnHF, lnLF) is observed during 5s PB compared with during SB.

From the above findings, although some researchers have been advocating the necessity of incorporating PB in HRV measurements, cumulative evidence for the improvement of reliability by PB remains incomplete.

Studies on the effects of PB on the interindividually variation of HRV are limited. In the present study of the measurement of HRV components, PB did not consistently diminish the interindividual variations. Kollai and Mizsei (1990) assumed that variations in the respiratory cycle and depth among the subjects might be factors contributing to interindividual variation of RSA amplitude. As such, a decrease in interindividual variation of the HF component by PB was expected, although the variation was not significantly suppressed by PB in this study. However, since the statistical test used to examine differences in interCV was an approximate analysis, statistical verification might be insufficient.

**Effect of age on intraindividual variation of HRV indexes**

It is well known that the HRV indexes are affected by age, viz., the LF and HF components of HRV show a decreasing tendency with age (Kuo et al., 1999; Agelink et al., 2001). However, little is known about the effect of age on the intraindividual variation of HRV. In the present study, the effects of age on the intrainCVs of HR, lnHF, and lnLF were negligible in either posture. This suggests that the relative intraindividual variations of the HRV indexes are independent of age. In short, the intraindividual standard deviation (S.D.) varies by a constant proportion to the individual mean. However, it remains possible that the correlation would appear in a sample-population with a broader age variation.

**What is the significance of PB in practice?**

In this study, the effects of PB on the reproducibility of HRV measurements were insignificant and inconsistent. A possible reason for this outcome might be the inability of subjects to completely control their respiration during the measurement. As the rhythm and depth of the breathing were not measured in this study, the accuracy of PB was unconfirmed. However, since the values of ICCs during PB in this study were comparable with previous results, this may indirectly suggest that the accuracy of PB was adequate.

When Driscoll et al. (2000) observed that 5s PB suppressed the intrainCVs of HF and LF components from 46.2 to 25.8% and from 47.6 to 23.8% respectively, they concluded that the incorporation of PB was effective in deriving HRV.

In this study, the intrainCVs of lnHF and lnLF components were approximately 5–6%. On comparing our results with the data of Driscoll et al. (2000) by incorporating calculated raw (non-logarithmic transformed) components of HRV (where intrainCVs of the raw components during SB and PB were 18–19% and 16–19% respectively), intrainCVs during PB were of almost the same values in both the studies, although marked differences occurred in the results during SB. The intrainCVs during SB obtained by Driscoll et al. (2000) were 46–48%, whereas we were able to observe 18–19% in this study.

In our present study, subjects were instructed to avoid irregular respiration during measurement under both PB and SB conditions. This may be a factor accounting for the discrepancy in results between this and other studies concluding that PB is essentially useful for HRV measurements. Therefore, a proper interpretation of the present results is a high stability without PB rather than a low stability during PB; i.e., PB is not necessarily needed in all cases.

Recently, HRV measurement has been used for various purposes; however, it is impractical to conduct PB in monitoring measurements of some cases. Thus, reliable measurements without PB can in fact expand the utility spectrum of HRV measurement.

Although we do not deny the fact that the reliability of HRV measurements strongly depends on respiration regularity, all subjects may not be able to practically control their respiration with perfect accuracy. Therefore, the results of this study demonstrated a practical limit of accuracy with PB.

Some researchers have tried to control the rhythm and depth of breathing simultaneously (e.g., Hirsch and Bishop, 1981; Kobayashi, 1996; Cooke et al., 1998). However, this method has not been popular, because of the need for complex equipment and utmost concentration on and by the subjects. In this study, only the rhythm and not the tidal volume of breathing was controlled during PB. It is possible that the full control of breathing is more efficient for improving the reproducibility of HRV measurements. Future studies are warranted to improve the extent of the reproducibility of HRV measurements by fully controlling the breathing.

**References**


spectra is largely ignored. J Appl Physiol 75: 2310–2317


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