Influence of different breathing patterns on heart rate variability indices and reproducibility during experimental endotoxaemia in human subjects

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ABSTRACT

HRV (heart rate variability) analysis is a widely employed method to assess cardiac autonomic nervous system activity. Accurate HRV measurement is critical to its value as a diagnostic and prognostic tool. Different breathing patterns may affect HRV, but results obtained under static conditions are conflicting. HRV indices decrease considerably during systemic inflammation evoked by experimental endotoxaemia, enabling the determination of the effects of different breathing patterns on HRV in a dynamic setting. We investigated the impact of different breathing patterns on short-term HRV measurements during experimental endotoxaemia. Furthermore, we assessed whether paced breathing improved HRV reproducibility. Twelve healthy male volunteers received an intravenous bolus (2 ng/kg of body weight) of endotoxin [LPS (lipopolysaccharide), derived from Escherichia coli O:113] on two occasions with an interval of 2 weeks. Five-minute HRV recordings were performed just prior to LPS administration and hourly thereafter until 8 h post-LPS. Three breathing protocols were employed every hour: (i) spontaneous breathing, (ii) metronome-guided breathing at the subject’s normal respiratory rate (‘paced’) and (iii) metronome-guided breathing at 150% of the subject’s normal respiratory rate (‘mild hyperventilation’). LPS administration resulted in a sharp decrease in all of the HRV indices measured, which was similar during both LPS administrations. Neither paced breathing nor mild hyperventilation influenced HRV indices compared with spontaneous breathing. Paced breathing did not improve reproducibility as it did not exert a significant effect on intra-subject coefficients of variation and intra-class correlation coefficients (calculated between both visits). In conclusion, over a wide range of HRV magnitudes during experimental endotoxaemia, neither paced breathing nor mild hyperventilation affected HRV indices. Moreover, paced breathing did not result in a significant improvement in reproducibility. Therefore employing a paced breathing protocol is not required to obtain valid HRV data during endotoxaemia.

INTRODUCTION

HRV (heart rate variability) analysis is a widely employed method to assess cardiac autonomic nervous system activity [1]. Owing to its non-invasive nature, HRV, and especially short-term HRV recordings (5 min), are easily obtainable from patients or healthy volunteers. Changes in HRV, in particular a reduction in HRV,
are associated with increased mortality after myocardial infarction and heart failure [2–5]. In systemically inflamed critically ill patients, such as in sepsis, HRV is diminished and inversely correlated with disease severity [6–9]. Moreover, reduced HRV is a predictor of MODS (multi-organ dysfunction syndrome) and death in these patients [10,11]. Therefore HRV analysis could represent a valuable diagnostic and prognostic tool.

Accurate measurements of HRV are critical for its interpretation and clinical use. For example, it is well-documented that postural changes have a major impact on HRV [12–14]. Therefore all HRV measurements within a study should be performed in the same position and data cannot be compared with subjects measured in another position. Less attention has been paid to effects of different breathing patterns, although this is considered to have a significant impact on HRV. The parasympathetic component of HRV {respiratory sinus arrhythmia, reflected by HF (high-frequency) power and r-MSSD [root-mean-square differences of successive NN (normal-to-normal) intervals]} is thought to be predominantly mediated by respiration-induced blood pressure changes, which are sensed by carotid baroreceptors leading to (de)activation of cardiac vagal fibres [15,16]. An increased respiratory rate has been linked to a reduction in LF (low-frequency) and HF power [17]; however, increased HF and reduced LF power with increased breathing rate have also been reported [18]. Metronome-guided (paced) breathing appears to result in increased HF and reduced LF power compared with spontaneous breathing in some [19,20], but not all [21] studies. In addition, paced breathing may [19,22] or may not [23,24] increase HRV analysis reproducibility. These conflicting results indicate that the effects of different breathing patterns on HRV are not well established yet.

To date, all studies investigating effects of different breathing patterns on HRV have been performed under relatively static conditions, i.e. when HRV does not vary to a large extent. Experimental endotoxaemia [LPS (lipopolysaccharide) administration in healthy volunteers] is a well-characterized standardized model of systemic inflammation widely used to study the innate immune response in man [25]. HRV indices greatly fluctuate during experimental endotoxaemia [26–29], which makes it a very suitable model to investigate the effects of different breathing patterns on HRV in a dynamic setting. Therefore the aim of our present study was to investigate whether paced breathing or mild hyperventilation affect short-term HRV indices during experimental endotoxaemia. Furthermore, since subjects in our study were administered endotoxin twice with an interval of 2 weeks, we assessed whether paced breathing improved HRV measurement reproducibility during experimental endotoxaemia.

MATERIALS AND METHODS

Subjects

Twelve healthy young male non-smoking volunteers were enrolled in a crossover experimental endotoxaemia study (ClinicalTrials.gov identifier NCT00783068) in which they received LPS twice (LPS visits 1 and 2) with a mean interval of 14 days (range, 11–18 days). The study protocol was approved by the local ethics committee of the Radboud University Nijmegen Medical Centre and is in accordance with the Declaration of Helsinki (2000) of the World Medical Association. Written informed consent was obtained from all study participants. The findings of the physical examinations, electrocardiography and routine laboratory studies on all the volunteers before the start of the experiment showed normal results. Volunteers were not taking any prescription medications, and they were negative for hepatitis B surface antigen and HIV infection.

Experimental endotoxaemia model

Subjects refrained from food 12 h before the start of the experiment and from caffeine- or alcohol-containing substances 24 h before the start of the experiment. The experiments were performed at the research unit of the intensive care department, with subjects in supine position. After local anaesthesia (20 mg/ml lidocaine), the radial artery was cannulated using a 20-Gauge arterial catheter (Angiocath; Becton Dickinson) and connected to an arterial pressure monitoring set (Edwards Lifesciences LLC), connected to a Phillips IntelliVue MP70 monitor. The arterial line was used for continuous monitoring of blood pressure and blood sampling. A cannula was placed in the antecubital vein to permit infusion of 2.5 % glucose/0.45 % saline solution; subjects received a bolus of 1.5 litres during 1 h before LPS infusion (prehydration), followed by 150 ml/h until 6 h after LPS infusion and 75 ml/h until the end of the experiment. Heart rate was continuously monitored using a three-lead ECG. Body temperature was measured every 30 min using an infrared tympanic thermometer (FirstTemp Genius; Sherwood Medical). Leucocyte counts were determined using flow cytometry (Sysmex XE-2100; Goffin Meyvis). U.S. Reference Escherichia coli endotoxin [E. coli O:113, Clinical Center Reference Endotoxin, NIH (National Institutes of Health)] was used. Lot Ec-5 endotoxin, supplied as a lyophilized powder, was reconstituted in 5 ml of 0.9 % saline for injection and vortex-mixed for at least 10 min after reconstitution. The endotoxin solution was administered as an intravenous bolus injection at a dose of 2 ng/kg of body weight.

HRV measurements

HRV was measured hourly by 5-min recordings starting just before LPS administration (t = 0 or baseline) and
up to 8 h after LPS administration. HRV was measured in supine position and in a quiet environment. HRV measurements were performed at the same time of day during both visits ($t = 0$ at 11 am). Each subject’s normal spontaneous breathing rate was determined in rest before LPS administration. We employed three breathing protocols, repeated every hour: (i) 5 min of spontaneous breathing, (ii) 5 min of metronome-guided breathing at the subject’s spontaneous respiratory rate (paced) and (iii) 5 min of metronome-guided breathing at 150% of the subject’s spontaneous respiratory rate (mild hyperventilation). A three-lead ECG signal was obtained using a Medilog AR12 recorder (Huntleigh Healthcare). R-peak position was determined at a sample rate of 4096 Hz. HRV was analysed using dedicated software (Medilog Darwin HRV, Huntleigh Healthcare).

In each 5-min recording, QRS complexes were detected, and only NN beat intervals were tabulated, yielding an interval tachogram (from which the mean heart rate was calculated). Recordings with artefacts such as extrasystolic or supraventricular beats or other arrhythmias comprising more than 5% of the total epoch were discarded. After linear detrending, power spectral density was determined by fast Fourier transformation of interval tachograms using the Welch method and a FFT (fast Fourier transform) width of 1024. We chose to solely analyse ‘raw’ HRV indices, not calculated indices such as LF/HF, Lfnu (LF power in normalized units) and Hfnu (HF power in normalized units), since effects on calculated values are a direct consequence of their impact on raw values and therefore do not provide additional information. We analysed time domain indices SDNN (S.D. of NN intervals) and r-MSSD and frequency domain indices LF power, HF power and total power. VLF (very-LF) power (0.0033–0.04 Hz) was not analysed since this parameter cannot be reliably obtained from 5-min recordings [30]. The HRV indices were analysed; their definitions and their physiological correlates are listed (Table 1).

### Table 1 HRV indices definitions and physiological correlates

<table>
<thead>
<tr>
<th>Domain</th>
<th>HRV index (unit)</th>
<th>Definition</th>
<th>Physiological correlate</th>
</tr>
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<tbody>
<tr>
<td>Time domain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SDNN (ms)</td>
<td>S.D. of all NN intervals</td>
<td>Sympathetic and parasympathetic activity</td>
</tr>
<tr>
<td></td>
<td>r-MSSD (ms)</td>
<td>Root-mean-square differences of successive NN intervals</td>
<td>Parasympathetic activity</td>
</tr>
<tr>
<td>Frequency domain</td>
<td>LF (ms²)</td>
<td>LF power (0.04–0.15 Hz)</td>
<td>Sympathetic and parasympathetic activity</td>
</tr>
<tr>
<td></td>
<td>HF (ms²)</td>
<td>HF power (0.15–0.4 Hz)</td>
<td>Parasympathetic activity</td>
</tr>
<tr>
<td></td>
<td>Total (ms²)</td>
<td>Total spectral power (0–0.15 Hz)</td>
<td>Sympathetic and parasympathetic activity</td>
</tr>
</tbody>
</table>

Calculations and statistical analysis

None of the measured HRV indices was normally distributed (calculated using the Shapiro–Wilk test) and therefore they were log-transformed. Comparison of the HRV indices between between the two LPS visits were made by repeated measures two-way ANOVA. For Bland–Altman analysis, paced breathing was designated as the gold standard [31]. The Grubbs test (extreme studentized deviate method) was performed to test for significant outliers. Percentage intraCVs (intra-subject coefficients of variation) were calculated by the following formula: $100 \times \left( \frac{\text{S.D. (visit 1, visit 2)}}{\text{mean (visit1, visit 2)}} \right)$. We performed a power calculation to determine the power achieved in case of actual intraCV differences of 1 and 2%. Using a S.D. of 4% [32], a sample size of 108 observations (12 subjects measured at nine time points) and a two-tailed $\alpha$ of 0.05, we would achieve a 73.1% power to detect an intraCV difference of 1 and 99.9% to detect an intraCV difference of 2%. Percentage intraCVs between spontaneous and paced breathing were tested by paired, two-sided Student’s $t$ tests. A $P$ value < 0.05 was considered significant. Statistical analysis was performed using Graphpad Prism 5 and MedCalc 11.3.1.0.

## RESULTS

### Effects of endotoxin administration on haemodynamic, clinical, haematological and HRV parameters

LPS administration resulted in the expected changes in haemodynamic, clinical and haematological parameters (see Figure 1 for the data of visit 1), as well as increased plasma levels of pro- and anti-inflammatory cytokines (results not shown). Endotoxaemia also resulted in a typical sharp decrease in all of the HRV indices (see Figure 2 for the data from spontaneously breathing subjects during visit 1). There were no differences in the haemodynamic, clinical, haematological and HRV responses to LPS between both visits (results not shown).

### Influence of paced breathing on HRV during endotoxaemia

We constructed Bland–Altman plots of the difference between spontaneous breathing compared with metronome-guided paced breathing at a subject’s normal respiratory rate [mean rate, 11 (range 8–15) breaths/min] against their average values from 0 to 8 h post-LPS administration (Figure 3). All indices displayed a
symmetrical distribution around the zero line, indicating the absence of a systematic error. The bias was not dependent on HRV magnitude, which greatly decreased following LPS administration.

**Influence of mild hyperventilation on HRV during endotoxaemia**

As shown in Figure 4, there was no difference in HRV indices between paced breathing at a subject’s normal respiratory rate compared with paced breathing at 150% of this rate (mean rate 17 (range, 12–22) breaths/min) during experimental endotoxaemia. Again, the bias was not dependent on HRV magnitude. In the subject with the lowest normal respiratory rate (8 breaths/min), a large increase in HF power was observed during mild hyperventilation (12 breaths/min; bias: 20.6%, significant outlier compared with the 11 other subjects whose mean bias was −3.2% with a range of −11.6 to 3.6%).

**Effect of paced breathing on HRV measurement reproducibility**

IntraCVs (calculated between visits 1 and 2) did not differ between paced and spontaneous breathing (Table 2). Similarly, ICCs (intra-class correlation coefficients) of paced and spontaneous breathing were not significantly different.

**DISCUSSION**

In the present study, we investigated the effects of different breathing patterns on short-term HRV and its reproducibility in a dynamic setting during experimental endotoxaemia in humans. Compared with spontaneous breathing, neither paced breathing nor mild hyperventilation affected HRV indices significantly. Furthermore, paced breathing did not improve HRV reproducibility.

To the best of our knowledge, this is the first study assessing the effects of different breathing patterns on HRV during experimental endotoxaemia. The effects of endotoxin administration on HRV are well established. Similar to other studies [26–29], we observed a distinct decrease in all measured HRV indices. Consequently, the experimental endotoxaemia model uniquely allowed us to investigate the effects of different breathing patterns on HRV and its reproducibility in a wide range of HRV magnitudes, as opposed to previous studies that examined it during relatively static conditions [17–19,21–24,32,33].

Our first objective was to investigate the effects of different breathing patterns on HRV. We employed a paced breathing protocol in which subjects were...
Breathing patterns and heart rate variability

Figure 3  Bland–Altman plots of the percentage bias of HRV indices between spontaneous breathing and paced breathing against their average during experimental endotoxaemia

Percentage bias was calculated as $100 \times \frac{(\text{spontaneous} - \text{paced})}{\text{average}} \%$ on the y-axis. Dotted lines indicate upper and lower 95% limits of agreement. Data from 12 subjects who were administered LPS on two separate occasions (visits 1 and 2) with an interval of 2 weeks are depicted. HRV indices (SDNN and r-MSSD in ms; LF, HF and total power in ms$^2$) were measured during spontaneous and paced breathing every hour starting at $t = 0$ until $t = 8$, yielding a total of 216 data points. Mean $\pm$ S.D. bias is shown in each panel.

Paced at their normal respiratory rate, measured at rest before LPS administration. In all other investigations on the subject, a fixed respiratory rate of 12 or 15 breaths/min was employed [19,21,32–34]. Paced breathing has been shown to lead to an increased HF and a decreased LF power [19,32,33], whereas others found no differences [21,34]. The absence of a significant bias during spontaneous breathing in our present study could result from the fact that subjects breathed extremely regularly during the spontaneous breathing measurements, implying that breathing patterns were virtually identical during spontaneous and paced breathing measurements. However, we did not find any differences when the subjects were paced at 150% of their normal respiratory rate either. Interestingly, the only subject in whom a relatively large bias (increased...
HF power) was observed upon mild hyperventilation had a low spontaneous respiratory rate of 8 breaths/min. This observation can be explained by the fact that this subject’s respiratory rate corresponds to a frequency of 0.13 Hz, which is just outside of the HF power spectrum (0.15–0.4 Hz), whereas all other subjects’ spontaneous breathing frequencies were within the HF band. Mild hyperventilation moved this subject’s breathing frequency well within the HF band (0.2 Hz). These findings are corroborated by a recent investigation in which subjects, as part of a HRV biofeedback protocol, were paced at low respiratory frequencies outside the HF band (0.1 Hz) during endotoxaemia [35]. In these subjects, large differences in HRV parameters were found compared with subjects paced at 0.25 Hz. Effects of paced respiration (within the HF band) on HRV might also be observed in subjects breathing spontaneously at frequencies above 0.4 Hz, which has been reported during experimental endotoxaemia [26]. However, we can only speculate on this since we did not measure the spontaneous respiratory rate throughout the study protocol.

The second objective of the present study was to determine the effects of paced breathing on HRV measurement reproducibility during repeated experimental endotoxaemia. There is a large body of literature on the stability of HRV over time and the effect of paced breathing on it. Although the definition of a categorical rating of relative reproducibility based on ICC is still controversial, values found during spontaneous breathing in the present study can be considered moderate-to-good (0.69–0.83) [22,36,37]. These results are in line with those found by others, using intervals between HRV measurements of 1 day [22,23], 5 days [38], and 3 weeks [24]. Paced breathing did not result in significant reproducibility improvements. Our findings are in line with several other investigations, where either no differences or small non-significant ICC and intraCV improvements were found with measurement intervals of 1 day [22,23], 5 days [38], 3 weeks [24] and 2 months [33]. In one small study, significantly reduced intraCVs were found for LF and HF power for paced breathing (12 breaths/min) compared with spontaneous breathing [19]. However, in that study, indices were not log-transformed, which is surprising in light of the commonly described skewed distribution of HRV indices [22,33,38].

In the present study, we provide strong evidence for the lack of an effect of paced breathing or mild hyperventilation on HRV in healthy subjects exposed to experimental endotoxaemia. It is not clear, however, whether our findings can be extrapolated to different patient groups, since several conditions might alter the relationship between respiration and HRV. For instance, hypertension and diabetes are associated with a decreased baroreflex sensitivity, which may significantly affect the influence of respiration on the parasympathetic component of HRV [39,40].

In conclusion, over a wide range of HRV magnitudes during experimental endotoxaemia, paced breathing and mild hyperventilation did not affect HRV indices, provided that breathing frequencies lay within the HF band. Furthermore, paced breathing did not improve HRV measurement reproducibility. Therefore, employing a paced breathing protocol is not required to obtain valid HRV data during experimental endotoxaemia. In subjects whose respiratory frequency lies outside the HF band, paced

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**Table 2** Percentage intraCVs and ICCs of HRV indices between spontaneous and paced breathing during experimental endotoxaemia

Values from 12 subjects who were administered LPS on two separate occasions (visits 1 and 2) with an interval of 2 weeks are listed. HRV indices were measured during spontaneous and paced breathing every hour starting at $t=0$ until $t=8$, yielding a total of 108 paired observations from which ICCs and percentage intraCVs were calculated.

<table>
<thead>
<tr>
<th>HRV index</th>
<th>Reproducibility measure</th>
<th>Spontaneous breathing (95% CI)</th>
<th>Paced breathing (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>log SDNN</td>
<td>Percentage intraCV</td>
<td>5.78 (4.83–6.73)</td>
<td>6.69 (5.73–7.65)</td>
</tr>
<tr>
<td></td>
<td>ICC</td>
<td>0.78 (0.68–0.84)</td>
<td>0.78 (0.69–0.85)</td>
</tr>
<tr>
<td>log r-MSSD</td>
<td>Percentage intraCV</td>
<td>9.11 (7.53–10.70)</td>
<td>8.32 (6.90–9.74)</td>
</tr>
<tr>
<td></td>
<td>ICC</td>
<td>0.82 (0.74–0.87)</td>
<td>0.86 (0.79–0.91)</td>
</tr>
<tr>
<td>log LF</td>
<td>Percentage intraCV</td>
<td>9.45 (8.01–10.89)</td>
<td>8.45 (7.16–9.74)</td>
</tr>
<tr>
<td></td>
<td>ICC</td>
<td>0.69 (0.58–0.78)</td>
<td>0.79 (0.70–0.85)</td>
</tr>
<tr>
<td>log HF</td>
<td>Percentage intraCV</td>
<td>13.86 (11.08–16.65)</td>
<td>11.57 (9.12–14.01)</td>
</tr>
<tr>
<td></td>
<td>ICC</td>
<td>0.83 (0.76–0.88)</td>
<td>0.88 (0.83–0.92)</td>
</tr>
<tr>
<td>log total</td>
<td>Percentage intraCV</td>
<td>7.00 (5.85–8.15)</td>
<td>6.80 (5.78–7.81)</td>
</tr>
<tr>
<td></td>
<td>ICC</td>
<td>0.74 (0.63–0.81)</td>
<td>0.81 (0.72–0.87)</td>
</tr>
</tbody>
</table>
breathing at frequencies within the HF band might be considered.

**AUTHOR CONTRIBUTION**

Matthijs Kox, Jan Pompe, Cornelia Hoedemaekers and Peter Pickkers designed the study. Matthijs Kox and Jan Pompe performed the study. Matthijs Kox analysed the data and wrote the manuscript. Jan Pompe, Johannes van der Hoeven, Cornelia Hoedemaekers and Peter Pickkers corrected the paper. Johannes van der Hoeven, Cornelia Hoedemaekers and Peter Pickkers supervised the conduct of the study and writing of the paper.

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