Validation of pulse rate variability as a surrogate for heart rate variability in chronically instrumented rabbits

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Pellegrino PR, Schiller AM, Zucker IH. Validation of pulse rate variability as a surrogate for heart rate variability in chronically instrumented rabbits. Am J Physiol Heart Circ Physiol 307: H97–H109, 2014. First published May 2, 2014; doi:10.1152/ajpheart.00898.2013.—Heart rate variability (HRV) is a function of cardiac autonomic tone that is widely used in both clinical and animal studies. In preclinical studies, HRV measures are frequently derived using the arterial pulse waveform from an implanted pressure telemetry device, termed pulse rate variability (PRV), instead of the electrocardiogram signal in accordance with clinical guidelines. The acceptability of PRV as a surrogate for HRV in instrumented animals is unknown. Using rabbits implanted with intracardiac leads and chronically implanted pressure transducers, we investigated the correlation and agreement of time-domain, frequency-domain, and nonlinear indexes of HRV and PRV at baseline. We also investigated the effects of ventricular pacing and autonomic blockade on both measures. At baseline, HRV and PRV time- and frequency-domain parameters showed robust correlations and moderate to high agreement, whereas nonlinear parameters showed slightly weaker correlations and varied agreement. Ventricular pacing almost completely eliminated HRV, and spectral analysis of the PRV signal revealed a HRV-independent rhythm. After cardiac autonomic blockade with atropine or metoprolol, the changes in time- and non-normalized frequency-domain measures of PRV continued to show strong correlations and moderate to high agreement with corresponding changes in HRV measures. Blockade-induced changes in nonlinear PRV indexes correlated poorly with HRV changes and showed weak agreement. These results suggest that time- and frequency-domain measures of PRV are acceptable surrogates for HRV even in the context of changing cardiac autonomic tone, but caution should be used when nonlinear measures are a primary end point or when HRV is very low as HRV-independent rhythms may predominate.

heart rate variability; pulse rate variability; pulse interval; sympathovagal balance

HEART RATE (HR) variability (HRV) is a function of cardiac autonomic balance that is predictive of mortality in conditions ranging from chronic heart failure (20) to traumatic injury (8). While clinical standards dictate that HRV must be computed from an ECG signal (28a), dozens of human studies have investigated the acceptability of pulse wave recording, either by photoplethysmography (e.g., finger pulse oximetry) or blood pressure monitoring (i.e., Finapres) as a surrogate for HRV in various physiological and disease states (27). The differences between HRV indexes obtained by these two methods have led some authors to conclude that pulse wave monitoring does not provide an acceptable signal for HRV analysis (7).

Because of its prognostic value and widespread use in clinical literature, measures of HRV have become prevalent in animal studies, which lack unified guidelines like those established for humans (28a). Frequently, so-called HRV analysis in animals is performed on data derived not from an ECG signal but from the arterial pulse waveform obtained by a radiotelemetry implanted into a main artery (3, 4, 19). This is a much different pulse wave monitoring technique than has been investigated in clinical studies, and it is thus important to understand how well measures of pulse rate variability (PRV) obtained via chronically implanted radiotelemetry agree with measures of HRV obtained via ECG. The acceptability of PRV as a surrogate for HRV has never been systematically investigated in animals.

Measures of HRV are stratified into three categories: time-domain, frequency-domain, and nonlinear statistics. The SD of normal-to-normal intervals (SDNN) is the most widely used time-domain measure and has powerful predictive value in many diseases (18, 20). In the frequency domain, the low-frequency (LF) band comprises both sympathetic and parasympathetic oscillations, whereas the high-frequency (HF) band is specifically modulated by parasympathetic outflow. The LF-to-HF ratio (LF/HF), while somewhat controversial (12), remains widely used as an index of cardiac sympathovagal balance (28a). Nonlinear measures encompass a heterogeneous group of statistics, several of which quantify the entropy or complexity of the signal. Multiscale sample entropy is one such statistic that has been used to distinguish patients with cardiovascular pathologies from healthy subjects (10, 29, 30) and is predictive of mortality in trauma and heart failure patients (17, 21, 22, 26).

In the present study, we investigated the use of PRV as a surrogate for HRV using rabbits chronically instrumented with both intracardiac leads and arterial pressure (AP) radiotelemeters. We report the correlation and agreement of time-domain, frequency-domain, and nonlinear measures of HRV and PRV in the resting state. We also report the effect on PRV when HRV is driven to zero by ventricular pacing. Finally, since many studies use HRV measures as an index of cardiac autonomic status, it was important to examine the autonomic underpinnings of these two signals. By performing cardiac autonomic blockade experiments with atropine and metoprolol, we examined the effects of cardiac autonomic blockade on PRV and HRV.

MATERIALS AND METHODS

Animals

Experiments were carried out on male New Zealand White rabbits ranging in weight from 3.3 to 4.2 kg (Charles River Laboratories, Wilmington, MA). All experiments were reviewed and approved by the Institutional Animal Care and Use Committee of University of Nebraska Medical Center.
Surgical Instrumentation

All rabbits were instrumented with an implantable radiotelemeter and epicardial pacing leads as previously described (13). In brief, the tip of the telemetry catheter (model PA-C40, Data Sciences, New Brighton, MN) was advanced into the abdominal aorta via the right femoral artery and secured, allowing chronic recording of pulsatile AP and providing the signal for PRV analysis. As part of the same surgery, a left thoracotomy was performed, a platinum wire pacing electrode was placed on the left ventricle of the heart, and a ground wire was secured to the left atrial appendage. Wires were tunneled beneath the skin and exited in the midscapular region, providing the ECG signal for HRV analysis. The chest was evacuated, and the thoracotomy was closed in layers. Rabbits were allowed to recover for at least 2 wk before any experiments were conducted. During this time, animals were acclimatized to the procedure room.

Baseline Recordings

To investigate the correlation and agreement of HRV and PRV measures in chronically instrumented animals, baseline measurements were conducted on eight rabbits. Rabbits rested in a Plexiglas box in the dimly lit procedure room for at least 20 min. Pulsatile AP, calibrated by a Dataquest A.R.T. analog system (Data Sciences), and ECG were simultaneously digitized at a sampling frequency of 1 kHz via a 16-channel PowerLab system (AD Instruments, Colorado Springs, CO). Each rabbit was recorded on 4 separate days; however, due to an issue with the pressure telemeter in one animal, only 30 total recordings were analyzed. Five-minute, artifact-free sections of the recording were used for HRV and PRV analysis.

Ventricular Pacing

Ventricular pacing experiments were conducted on eight rabbits to examine PRV when HRV was driven to zero. For these experiments, the left chest, right chest, and right haunch of each rabbit were shaved, and ECG electrodes (TenderTrace Pediatric ECG electrodes, New Dimensions in Medicine, Dayton, OH) were applied to the shaved areas with conductive gel. This signal was differentially amplified to yield the skin ECG signal, and both skin ECG signal and pulsatile AP were recorded for at least 15 min. After this baseline recording, a

![Graphs and diagrams showing HRV and PRV measurements and analysis.](http://ajpheart.physiology.org/)
pulse generator was connected to the rabbit’s pacing leads, and HR was increased to 250 beats/min, a rate higher than the maximum baseline HR, and held at this rate for 10–15 min. Five-minute, artifact-free sections before and during pacing were used for analysis.

**Cardiac Autonomic Blockade**

Cardiovagal blockade with atropine, cardiac sympathetic blockade with metoprolol, and dual blockade with both atropine and metoprolol were performed to investigate the autonomic substrates of HRV and PRV. After local anesthesia with lidocaine, an intravenous catheter was inserted into a marginal ear vein to allow the intravenous administration of cardiac autonomic blockers. Each rabbit was allowed to rest for 10 min after the placement of the intravenous catheter, at which point a 1-ml bolus of saline was given and recording of pulsatile AP and ECG was initiated. Fifteen minutes later, a bolus of either atropine methyl bromide (0.2 mg/kg, Sigma-Aldrich), metoprolol bitartrate (1 mg/kg, Sigma-Aldrich), or both was given. We have previously used these doses to block cardiac parasympathetic and sympathetic outflow in normal and heart failure rabbits (16, 28). Five-minute, artifact-free sections before and at least 3 min after the bolus were used for HRV and PRV analysis, and changes in time, frequency, and nonlinear HRV and PRV statistics were analyzed for correlation and agreement. The fold change was used for LF/HF and the detrended fluctuation analysis scaling exponents.

**Interval Detection and Time-Domain Indexes**

Both R wave and pulse wave detection was performed using the HRV module of LabChart 7 software (AD Instruments). R wave detection was performed by finding the maximum of the ECG signal (Fig. 1A). The first time derivative of the AP signal (dAP/dt) was calculated using a three-point window and 45-Hz low-pass filtered for pulse wave detection (Fig. 1C). Because of the large impact of pulse wave detection on resultant PRV measures, three different fiducial points were screened for their time-domain, frequency-domain, and nonlinear fidelity to the R-R interval signal: 1) the relative maximum of the dAP/dt signal (maximum dAP/dt); 2) the zero of the dAP/dt signal before the maximum, corresponding to the diastolic minimum; and 3) the zero of the dAP/dt signal after the maximum, corresponding the systolic maximum. Per the LabChart HRV module, the timing of the maxima of the dAP/dt and R wave signals was computed via three-point quadratic interpolation and the timings of the zero crossings were linearly interpolated, both with 1-μs precision. The result-

![Graph](https://example.com/graph.png)
ing intervals were manually screened for artifacts and exported for subsequent time-domain analysis in Kubios HRV (Biosignal Analysis and Medical Imaging Group, University of Eastern Finland, Kuopio, Finland).

**Frequency-Domain Indexes**

Frequency-domain analysis was carried out in Kubios HRV. Briefly, the tachogram was linearly interpolated at 8 Hz, 50% overlapped 1,024-point windows were transformed into the frequency domain, and the resulting power spectra were averaged per Welch’s periodogram method. Based on previously published studies in rabbits, power from 0–0.0625 Hz was considered very LF (VLF), 0.0625–0.1875 Hz was considered LF, and 0.1875–2 Hz was considered HF (19, 23, 28).

**Nonlinear Indexes**

Multiscale entropy analysis used code publically available as part of PhysioNet (9, 15) with $m = 2$, $r = 0.15$, to compute all odd scale factors from 1 to 39. While many different parameters have been derived from multiscale entropy analysis (10, 17), only two were used in this study. To assess the fidelity of the tachograms of different pulse detection methods to nonlinear aspects of the R-R tachogram, the entropy difference (HRV – PRV) for each scale factor was computed for each baseline recording. For the remainder of the study, we used the sum of the entropy over all the computed scales to represent multiscale entropy because of its prognostic value in large patient studies (21, 22, 26). All other nonlinear indexes were computed in Kubios HRV, including Shannon, approximate, and sample entropy. Detrended fluctuation analysis was used to calculate short-term (4–16 samples) and long-term (16–64 samples) scaling exponents ($\alpha_1$ and $\alpha_2$, respectively).

**Statistical Analysis**

All group data are expressed as means ± SE. All $r^2$ values denote the square of Pearson’s correlation coefficient. As some correlations appeared to be driven by outliers, all points with a Cook’s distance of $>4/n$ were removed to give a corrected $r^2 (F^2)$ where applicable. Since Pearson’s correlation coefficient quantifies linear correlation, not agreement, we used Bland-Altman plots to show accuracy and precision. To allow easier comparison between indexes, all Bland-Altman plots are displayed with the percent difference on the y-axis, and we adopted a uniform terminology. A mean percent difference of $<5\%$, from $5\%$ to $15\%$, and $>15\%$ was considered as high, moderate, and poor accuracy, respectively. Precision, defined as 1.96 SDs, of $<10\%$, from $10\%$ to $20\%$, and $>20\%$ was considered as high, moderate, and poor, respectively. The percent difference in parameter $X$ was defined as follows:

$$\frac{X_{\text{HRV}} - X_{\text{PRV}}}{\frac{1}{2}(X_{\text{HRV}} + X_{\text{PRV}})} \times 100\%$$

Bias consistency was defined as the proportion of recordings that were biased in the same direction (i.e., $X_{\text{PRV}} < X_{\text{HRV}}$ or $X_{\text{PRV}} > X_{\text{HRV}}$) as the mean. The bias consistency was then tested against a uniform distribution using the Pearson $\chi^2$-test with $P < 0.05$ considered significant. This statistical significance formed the basis for any parameters denoted as consistently biased (e.g., consistently overestimated or consistently underestimated).

Statistical differences between pulse detection methods and pre- and postintervention were tested by repeated-measures ANOVA with the pulse detection method and, where relevant, the frequency or scale factor as within-recording factors. Post hoc tests were performed using the Holm-Sidak correction for multiple comparisons. Statistical differences between pre- and postintervention (i.e., autonomic blockade or pacing) were tested by paired $t$-tests or, where appropriate, repeated-measures ANOVA.

**Other Analysis**

The normalized cross-correlation, magnitude-squared coherence, and transfer function gain between HRV and PRV tachograms were compared using cross-correlation, cross-coherence, and cross-spectrum. A cross-spectrum of 0.95 or greater, as shown in a Bland-Altman plot, defined the mean bias to be consistent between the variables.

Table 1. Time-domain, frequency-domain, and nonlinear indexes of HRV and PRV for 30 baseline recordings

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HRV</th>
<th>PRV</th>
<th>Bland-Altman Interval, %</th>
<th>Bias Consistency</th>
<th>$r^2$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time domain</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total power, ms²</td>
<td>847 ± 165</td>
<td>883 ± 177</td>
<td>−3.32 ± 6.51</td>
<td>27/30‡</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td>VLF power, ms²</td>
<td>299 ± 55.7</td>
<td>302 ± 57</td>
<td>−0.48 ± 1.53</td>
<td>16/30</td>
<td>0.999</td>
<td>1.00</td>
</tr>
<tr>
<td>LF power, ms²</td>
<td>102 ± 18</td>
<td>105 ± 18</td>
<td>−3.06 ± 2.16</td>
<td>28/30‡</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td>Normalized units</td>
<td>25.8 ± 2.5</td>
<td>25.2 ± 2.4</td>
<td>−2.33 ± 9.99</td>
<td>21/30*</td>
<td>0.989</td>
<td>0.994</td>
</tr>
<tr>
<td>HF power, ms²</td>
<td>444 ± 103</td>
<td>473 ± 112</td>
<td>−6.5 ± 15.2</td>
<td>26/30‡</td>
<td>0.998</td>
<td>0.997</td>
</tr>
<tr>
<td>Normalized units</td>
<td>73.6 ± 2.5</td>
<td>74.3 ± 2.4</td>
<td>−1.07 ± 4.98</td>
<td>21/30*</td>
<td>0.988</td>
<td>0.994</td>
</tr>
<tr>
<td>LF/HF</td>
<td>0.413 ± 0.062</td>
<td>0.396 ± 0.059</td>
<td>3.39 ± 14.33</td>
<td>21/30*</td>
<td>0.986</td>
<td>0.996</td>
</tr>
<tr>
<td><strong>Frequency domain</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>MSE₂/1-39</td>
<td>29.9 ± 0.7</td>
<td>30.0 ± 0.7</td>
<td>−0.59 ± 5.86</td>
<td>16/30</td>
<td>0.947</td>
<td>0.970</td>
</tr>
<tr>
<td>Shannon entropy</td>
<td>3.81 ± 0.09</td>
<td>3.79 ± 0.09</td>
<td>0.59 ± 3.38</td>
<td>20/30</td>
<td>0.989</td>
<td>0.987</td>
</tr>
<tr>
<td>Approximate entropy</td>
<td>1.24 ± 0.04</td>
<td>1.26 ± 0.03</td>
<td>−1.83 ± 7.44</td>
<td>19/30</td>
<td>0.953</td>
<td>0.969</td>
</tr>
<tr>
<td>Sample entropy</td>
<td>1.26 ± 0.06</td>
<td>1.28 ± 0.06</td>
<td>−2.3 ± 12.2</td>
<td>19/30</td>
<td>0.949</td>
<td>0.964</td>
</tr>
<tr>
<td>$\alpha_1$</td>
<td>0.949 ± 0.032</td>
<td>0.914 ± 0.030</td>
<td>3.7 ± 22.7</td>
<td>21/30*</td>
<td>0.654</td>
<td>0.636</td>
</tr>
<tr>
<td>$\alpha_2$</td>
<td>0.948 ± 0.034</td>
<td>0.935 ± 0.033</td>
<td>1.36 ± 6.97</td>
<td>24/30†</td>
<td>0.971</td>
<td>0.986</td>
</tr>
</tbody>
</table>

Values are means ± SE. Bland-Altman intervals (mean bias ± 95% limits of agreement), bias consistency, and pairwise coefficient of determination ($r^2$) between heart rate (HR) variability (HRV) and pulse rate variability (PRV) values are shown. Where applicable, $r^2$ values after the removal of points with a Cook’s distance of $>0.133 (r^2)$ are shown. SDNN, SD normal to normal-intervals; RMSSD, root mean squared SD; VLF, very low frequency; LF, low frequency; HF, high frequency; MSE₂/1-39, sum of the entropy over odd scales from 1 to 39; $\alpha_1$, short-term scaling exponent of the detrended fluctuation analysis; $\alpha_2$, long-term scaling exponent of the detrended fluctuation analysis. *$P < 0.05$, †$P < 0.01$, and ‡$P < 0.001$ by Pearson’s $\chi^2$-test.

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calculated using MATLAB (The Mathworks, Natick, MA). The residual PRV power spectral density for each animal was calculated by subtracting the postpace HRV power spectrum from the postpace PRV power spectrum. The percentage of PRV power that was independent of HRV was defined as follows:

\[
1 - \frac{G(f) \times \text{HRV}(f)}{\text{PRV}(f)}
\]

where \(G(f)\) is the transfer function gain and \(\text{HRV}(f)\) and \(\text{PRV}(f)\) are the HRV and PRV autospectra. For all MATLAB frequency-domain calculations, tachograms were linearly interpolated at 8 Hz, and the corresponding spectra were computed by Welch’s periodogram method using 256-point Hamming-windowed segments with 50% overlap.

RESULTS

Pulse Interval Detection Method

Three different fiducial points derived from the dAP/dt signal were screened using time-domain, frequency-domain, and nonlinear measures to identify the best pulse interval detection method. In the time domain (Fig. 1D), the normalized cross-correlation was very close to 1 for all methods, indicating that the pulse interval tachograms are largely a time-lagged function of the R-R tachogram, with no statistically significant effect of fiducial point on cross-correlation. In the frequency domain (Fig. 1E), magnitude-squared coherence with the R-R spectra showed a similar trend for all methods, starting near 1 and falling as frequency increased. More important, the coherence was significantly higher for maximum dAP/dt than for the other two fiducial points. In the nonlinear domain (Fig. 1F), the pulse detection method did not significantly affect the difference in entropy from that of the R-R tachogram over multiple scale factors. Thus, because of its superiority in the frequency domain, the maximum of the dAP/dt signal was used to calculate PRV for the remainder of the study.

Baseline Time-Domain Indexes

As shown in Fig. 2A, tachograms obtained from ECG and arterial pulse signals are visually very similar. Figure 2B shows the very high correlation between PRV and HRV measures of SDNN. The Bland-Altman plot (Fig. 2C) reveals that PRV
consistently overestimated SDNN but that PRV SDNN was still highly accurate and precise. In the baseline condition, all time-domain indexes of PRV showed a strong correlation with HRV measures as well as high precision and accuracy (Table 1).

**Baseline Frequency-Domain Indexes**

Figure 3A shows the visual similarity between HRV and PRV power spectra for one recording. As would be expected from the coherence plots (Fig. 1E), spectra were very similar at lower frequencies and became more disparate at higher frequencies. As shown in Fig. 3B, PRV LF/HF strongly correlated with the HRV measure. The Bland-Altman plot (Fig. 3C) shows that PRV consistently underestimated LF/HF but still showed high accuracy and precision. The underestimation of LF/HF results from the fact that, while PRV consistently exaggerates the power in all frequency measures except the VLF range, this tendency is greatest for HF power. All other spectral measures of PRV showed high accuracy and precision in resting rabbits except HF power, which was only moderately accurate and precise (Table 1).

**Baseline Nonlinear Indexes**

Figure 4A shows the visual difference between HRV and PRV sample entropy at different scale factors for a single recording. These differences are not apparent in the group data (Fig. 4B). Although the correlation between HRV and PRV multiscale sample entropy was strong (Fig. 4C), it was clearly not as robust as the correlations for time- and frequency-domain indexes. Again, in contrast to time- and frequency-domain measures, PRV multiscale entropy was not consistently biased (Fig. 4D and Table 1). The Bland-Altman plot also showed high accuracy and precision for multiscale entropy (Fig. 4D). While most of the PRV nonlinear indexes showed a strong correlation with corresponding HRV indexes, these relationships tended to be notably weaker than those observed for time- and frequency-domain measures (Table 1). \( \alpha_1 \) showed a particularly poor correlation. In the baseline condition, all nonlinear measures had high accuracy, and all but \( \alpha_1 \) and sample entropy also had high precision.

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**Fig. 4.** Nonlinear statistics at baseline. A: PRV and HRV sample entropy at multiple scale factors for a single recording. B: PRV and HRV sample entropy at multiple scale factors for all recordings displayed as means ± SE \((n = 30)\). C: scatterplot of PRV multiscale entropy over all scales versus HRV multiscale entropy with a line of best fit (solid gray line) and line of equality (dotted black line). D: Bland-Altman plot showing the percent difference in multiscale entropy (HRV - PRV) versus the pairwise mean multiscale entropy with the mean bias (solid line) and upper and lower 95% limits of agreement (dashed lines).
**Ventricular Pacing**

Because PRV consistently overestimated both time- and frequency-domain measures of HRV at baseline, we investigated if PRV was simply amplifying HRV or if other, HRV-independent rhythms accounted for this increased variability. Figure 5A shows that transfer function gain was >1 from 0 to 0.375 Hz, indicating that PRV does indeed amplify HRV, albeit modestly, over the frequency range containing the vast majority of the HRV power. Interestingly, however, the ratio of PRV power to HRV power demonstrated that this amplification does not completely explain the tendency of PRV to overestimate HRV, and the percentage of PRV power that is independent of HRV increased with frequency until stabilizing at ~1.25 Hz.

Ventricular pacing experiments were conducted to assess the HRV-independent rhythms of PRV by driving HRV to zero. As shown in Fig. 5B, ventricular pacing almost completely eliminated HRV (postpace SDNN: 0.32 ± 0.04 ms) and significantly attenuated PRV. Still, PRV remained non-negligible, indicating a minimum PRV SDNN of 1.47 ± 0.13 ms. Figure 5C shows the magnitude-squared coherence for prepace and paced conditions. As expected, the coherence equaled 1 at 0 Hz, indicating that the mean rate was the same for PRV and HRV. With pacing, the coherence sharply declined until 0.25 Hz, beyond which point the coherence remained very low, indicating the absence of a relationship between paced PRV and HRV for these frequencies. Subtraction of the paced HR spectrogram from the paced pulse rate spectrogram yielded the residual PRV spectrum (Fig. 5D), which showed a peak at 1.25 Hz. This HRV-independent rhythm could explain some of the tendency of PRV to specifically overestimate the HR component of the HRV spectrum. It could also explain the trend of magnitude-squared coherence (Figs. 1E and 5C) to fall as frequency increased, and this HRV-independent variability constituted a greater proportion of PRV power.

**Cardiac Autonomic Blockade**

*Time domain.* Since many investigators use HRV as an index of cardiac autonomic balance, it is important that changes in cardiac autonomic tone are faithfully represented by PRV. Figure 6, A–C, shows the strong correlations between changes in PRV SDNN and HRV SDNN after cardiovagal blockade with atropine, cardiac sympathetic blockade with metoprolol, and combined blockade respectively. Bland-Altman plots showed a high accuracy of the change in PRV SDNN after autonomic blockade (Fig. 6, D–F). The precision was also high for atropine- and dual blockade-effected changes.

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**Fig. 5.** Transfer function analysis and ventricular pacing experiments. A: transfer function gain $|G(f)|$, the PRV-to-HRV ratio $\frac{\text{PRV}(f)}{\text{HRV}(f)}$, and HRV-independent power as a function of frequency at baseline ($n = 30$). B: effect of pacing on HRV and PRV SDNN ($n = 8$). C: effect of ventricular pacing on the coherence between HRV and PRV as a function of frequency ($n = 8$). D: residual PRV power spectrum for paced rabbits ($n = 8$).
Agreement and correlation of the changes in indexes of HRV and PRV after autonomic blockade are shown as Bland-Altman intervals (mean bias ± 95% limits of agreement) and $r^2$ values. Where applicable, $r^2$ values are also shown.
in SDNN but only moderate for metoprolol, mainly due to one outlier. Also of note, PRV consistently ($P < 0.01$) overestimated the atropine-mediated change in SDNN. Changes in HR showed high accuracy and precision, whereas root mean square of successive differences (RMSSD) showed high accuracy and moderate precision for each autonomic blockade intervention, and both showed strong correlations (Table 2). Overall, PRV accurately reflects changes in HRV time-domain measures that result from acute changes in cardiac autonomic tone.

Frequency domain. Atropine administration nearly eliminated HF power, and, as shown in Fig. 7A, the change in HRV and PRV HF power after atropine was very strongly correlated, but the slope of this relationship was greater than that of the line of equality. The Bland-Altman plot (Fig. 7D) shows that the change in PRV HF power was a moderately accurate and precise surrogate for the change in HRV HF power after atropine. More specifically, PRV consistently ($P < 0.05$) exaggerated the change in HF power, likely due to the increased PRV HF power at baseline. Similarly, the correlation between atropine-mediated changes in the absolute power of frequency-domain measures of HRV and PRV was very robust, and these measures showed high accuracy and precision. However, PRV HF power remained higher than HRV HF power (Table 3), likely due to the presence of intrinsic PRV, as revealed by our ventricular pacing experiment. While the magnitude of this intrinsic PRV was small, it constituted a large proportion of the greatly attenuated total power and exerted negative effects on normalized powers and LF/HF. As a result, the correlations and agreements of the change in normalized powers and LF/HF fold changes were very poor (Table 2).

Metoprolol administration did not have a robust, consistent effect on LF power in all animals; however, PRV reflected the changes in HRV LF power after metoprolol for each rabbit in a highly accurate and moderately precise manner (Fig. 7E). Here, the correlation between PRV and HRV spectral changes was strong for both normalized and absolute measures (Fig. 7B and Table 2). In addition, changes in other HRV and PRV spectral measures after metoprolol showed moderate to high accuracy and precision (Table 2).

Combined autonomic blockade attenuated total power, and the decrease in PRV total power correlated very well with the change in HRV power (Fig. 7C). The Bland-Altman plot showed the change in PRV power to be a highly accurate, highly precise estimate of the change in HRV power (Fig. 7D). It is also clear that PRV consistently ($P < 0.05$) exaggerated the decrease in power, likely due to the elevated PRV total power at baseline. Changes in other HRV and PRV frequency-domain statistics demonstrated robust correlations and high accuracy and precision with the notable exception of normalized measures and LF/HF. These measures showed very poor accuracy and precision.
as with atropine, this is likely due to the exaggerated influence of intrinsic PRV when HRV is dramatically decreased by autonomic blockade.

**Nonlinear measures.** Autonomic blockade-mediated changes in the nonlinear measure multiscale entropy differed considerably between PRV and HRV. As shown in Fig. 8, A–C, correlations between the change in PRV multiscale entropy and the change in HRV multiscale entropy were very modest for all three of the autonomic blockade conditions. Moreover, the slopes of the best-fit lines were visibly much less than those of the lines of equality. Bland-Altman plots showed very poor accuracy and best-fit lines were visibly much less than those of the lines of equality. Autonomic blockade-mediated changes in other measures of entropy, which were plagued by similarly disappointing performance was observed with nonlinear measures with high accuracy and moderate to high precision after single blockade. Correlations for all nonlinear measures varied widely between conditions but were clearly much lower than the time- and non-normalized frequency-domain measures (Table 2).

**DISCUSSION**

In general, we found that invasively acquired PRV overestimates corresponding HRV parameters in both the time and frequency domains in resting rabbits. Moreover, while PRV exaggerates HRV power in all frequencies, this effect is most pronounced in the HF range, and interrelated measures like normalized LF and LF/HF are accordingly decreased when assessed by PRV. While we have shown by transfer function analysis that some of this overestimation is due to amplification of HRV spectral components, we have also shown that HRV-independent oscillations are important as well. This additional variability must come from variations in either prejection time (i.e., the time after ventricular depolarization but before opening of the arterial valve) or pulse transit time (i.e., the time required for the arterial pulse to propagate from the aortic valve to the telemetry catheter). Preejection time is largely a function of contractility, preload, and afterload, whereas pulse transit time is affected by pulse pressure and arterial elasticity (1). The cyclic effects of respiration on venous return, preload, stroke volume, and thus pulse pressure are believed to account for much of the variability in prejection time and pulse transit time. This is supported by studies that observed peaks in the spectrum of the difference between pulse intervals and R-R intervals in the respiratory frequency range (7, 14) as well as the aforementioned tendency for PRV to overestimate HRV specifically in the HF range. Although respiration was not measured in this study, a spontaneous respiratory frequency of 1.25 Hz is reasonable for resting rabbits and is the most likely explanation for the residual PRV peak observed during ventricular pacing.

These findings agree with studies that used noninvasive methods to calculate time and frequency measures of PRV in resting human subjects (27). Compared with previous studies

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**Table 3. A selection of time-domain, frequency-domain, and nonlinear indexes of HRV and PRV before and after cardiac autonomic blockade with atropine, metoprolol, or both blockers**

<table>
<thead>
<tr>
<th></th>
<th>HRV Before Atropine</th>
<th>PRV Before Atropine</th>
<th>HRV After Atropine</th>
<th>PRV After Atropine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time Domain</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>178 ± 6</td>
<td>178 ± 6</td>
<td>233 ± 8†</td>
<td>233 ± 8‡</td>
</tr>
<tr>
<td>SDNN, ms</td>
<td>26.4 ± 2.4</td>
<td>27.2 ± 2.8</td>
<td>4.27 ± 0.70‡</td>
<td>4.48 ± 0.62‡</td>
</tr>
<tr>
<td><strong>Frequency domain</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total power, ms²</td>
<td>767 ± 155</td>
<td>820 ± 188</td>
<td>13.3 ± 3.3†</td>
<td>14.3 ± 3.3†</td>
</tr>
<tr>
<td>LF power, ms²</td>
<td>113 ± 20</td>
<td>118 ± 21</td>
<td>2.3 ± 1.0†</td>
<td>2.5 ± 1.1†</td>
</tr>
<tr>
<td>HF power, ms²</td>
<td>396 ± 121</td>
<td>444 ± 154</td>
<td>1.04 ± 0.30*</td>
<td>1.80 ± 0.31*</td>
</tr>
<tr>
<td><strong>Nonlinear</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSE</td>
<td>36.4 ± 3.1</td>
<td>36.7 ± 4.1</td>
<td>28.4 ± 5.3</td>
<td>24.1 ± 2.7</td>
</tr>
</tbody>
</table>

**Time Domain**

<table>
<thead>
<tr>
<th></th>
<th>HRV Before Metoprolol</th>
<th>PRV Before Metoprolol</th>
<th>HRV After Metoprolol</th>
<th>PRV After Metoprolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>191 ± 6</td>
<td>191 ± 6</td>
<td>186 ± 4</td>
<td>186 ± 4</td>
</tr>
<tr>
<td>SDNN, ms</td>
<td>20.8 ± 3.0</td>
<td>21.0 ± 3.0</td>
<td>16.4 ± 2.6*</td>
<td>16.6 ± 2.6*</td>
</tr>
<tr>
<td><strong>Frequency domain</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total power, ms²</td>
<td>477 ± 168</td>
<td>491 ± 175</td>
<td>272 ± 83</td>
<td>283 ± 89</td>
</tr>
<tr>
<td>LF power, ms²</td>
<td>50.1 ± 11.7</td>
<td>51.4 ± 12.1</td>
<td>56.5 ± 21.0</td>
<td>57.7 ± 21.6</td>
</tr>
<tr>
<td>HF power, ms²</td>
<td>252 ± 121</td>
<td>264 ± 128</td>
<td>121 ± 40</td>
<td>129 ± 44</td>
</tr>
<tr>
<td><strong>Nonlinear</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>MSE</td>
<td>39.1 ± 2.6</td>
<td>39.3 ± 2.8</td>
<td>45.6 ± 5.7</td>
<td>44.5 ± 4.66</td>
</tr>
</tbody>
</table>

**Time Domain**

<table>
<thead>
<tr>
<th></th>
<th>HRV Before Double Blockade</th>
<th>PRV Before Double Blockade</th>
<th>HRV After Double Blockade</th>
<th>PRV After Double Blockade</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>176 ± 5</td>
<td>177 ± 5</td>
<td>234 ± 7†</td>
<td>234 ± 7‡</td>
</tr>
<tr>
<td>SDNN, ms</td>
<td>28.0 ± 2.6</td>
<td>28.4 ± 2.7</td>
<td>1.51 ± 0.12†</td>
<td>1.82 ± 0.15†</td>
</tr>
<tr>
<td><strong>Frequency domain</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total power, ms²</td>
<td>994 ± 279</td>
<td>1,031 ± 295</td>
<td>1.45 ± 0.14*</td>
<td>2.25 ± 0.26*</td>
</tr>
<tr>
<td>LF power, ms²</td>
<td>116 ± 29</td>
<td>121 ± 31</td>
<td>0.133 ± 0.027†</td>
<td>0.149 ± 0.028†</td>
</tr>
<tr>
<td>HF power, ms²</td>
<td>603 ± 215</td>
<td>634 ± 227</td>
<td>0.445 ± 0.098*</td>
<td>1.22 ± 0.230*</td>
</tr>
<tr>
<td><strong>Nonlinear</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSE</td>
<td>39.0 ± 3.9</td>
<td>43.2 ± 4.5</td>
<td>34.3 ± 4.9</td>
<td>28.1 ± 3.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05, †P < 0.01, and ‡P < 0.001 vs. before blockade.
The effect of pulse detection on the correlation and agreement of noninvasive PRV and HRV measures in humans has been previously investigated by Posada-Quintero et al. (24). These authors found that a tangent method based on the timing of maximum $\frac{d\Delta P}{dt}$ was superior to both diastolic minimum and maximum $\frac{d\Delta P}{dt}$. Our results similarly show the frequency-domain superiority of maximum $\frac{d\Delta P}{dt}$ to the diastolic minimum and systolic maximum as a fiducial point, although we used more accessible and computationally simpler techniques than these authors.

While, at baseline, nonlinear PRV measures showed high accuracy but mixed precision in approximating corresponding HRV measures, none showed the highly robust correlations ($r^2 > 0.99$) like those observed for time- and non-normalized frequency-domain measures. Our attempts to augment the linearity of the relationship by plotting the log of PRV parameters versus the log of HRV parameters did not substantially improve these correlations (data not shown). Moreover, changes in most nonlinear measures of PRV after autonomic blockade were shown to be a very poor surrogate for changes in HRV nonlinear measures, suffering from poor accuracy, precision, and weak correlations. We thus do not recommend the use of PRV as a surrogate for HRV if nonlinear measures are a primary end point, especially in studies that manipulate autonomic tone.

One unexpected finding in our study was the lack of a strong change in LF power after $\beta$-blockade, which differs from a previous study in rabbits (19). While this dose of metoprolol results in profound bradycardia in rabbits with chronic heart
failure (16), it did not significantly decrease HR ($P = 0.180$) in this group of animals. Others have also reported the absence of a frequency-domain change after β-blockade in normal animals (4). We believe the lack of clear bradycardia and the spectral effect after metoprolol reflects low sympathetic tone in these healthy, resting rabbits.

It is important to point out that the criteria for high, moderate, and low accuracy and precision were fixed before analysis in an arbitrary manner. How much two methods can differ without becoming problematic depends on the intended application and is not a question that statistical methods can answer (6). Thus, it is important that investigators wishing to use PRV as a surrogate for HRV in chronically instrumented animals look critically at the data presented here and decide for themselves whether or not the demonstrated agreements and correlations are sufficient for their purposes.

For investigators interested in using invasively acquired PRV measures as an index of cardiac autonomic balance, these data also pose an important question of the necessity for agreement versus correlation. Clearly, changes in time-domain and absolute spectral power measures of PRV explain autonomic blockade-mediated changes in corresponding HRV measures in a very robust manner (i.e., coefficient of determination $> 0.99$). For studies in which the principal purpose of PRV analysis is the assessment of cardiac autonomic state, these robust linear relationships may be sufficient to justify the use of PRV. We advise special attention for using PRV in conditions in which HRV is very low, as residual PRV may constitute an unacceptably high proportion of total PRV, and in conditions or maneuvers that modify vagal tone, as PRV appears to amplify these changes.

Finally, we would like to point out some of the limitations of our study. Using pharmacological autonomic blockade is a convenient, acute method to manipulate cardiac sympathovagal balance, but these findings do not address the ability of PRV to reflect chronic changes in HRV. Similarly, it is difficult to generalize these findings in healthy animals to disease models, including those with autonomic dysfunction and cardiorespiratory disorders. Moreover, given the species-specific nature of HRV rhythms, it is unclear how the observed trends translate to other animal models like mice, rats, and dogs.

In summary, invasively acquired PRV is generally an accurate and precise surrogate for time, frequency, and some nonlinear measures of HRV at baseline. The tendency for PRV to slightly overestimate HRV parameters in the time and frequency domains can be explained both by amplification of HRV oscillations as well as its composition by HRV-independent rhythms. Furthermore, while robust correlations and moderate to high agreement exist between the changes in time- and absolute frequency-domain PRV and HRV parameters after autonomic blockade, most nonlinear parameters show very poor agreement after the administration of autonomic blockers. Thus, invasively acquired PRV is likely an acceptable surrogate for HRV except when nonlinear measures are a primary end point or when HRV is greatly reduced such that HRV-independence oscillations predominate. These findings are relevant to investigators who wish to use PRV to compute HRV measures as well as to the interpretation of countless studies that have already been published based on the assumption that PRV is an acceptable surrogate for HRV.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: P.R.P. performed experiments; P.R.P. analyzed data; P.R.P. and A.M.S. interpreted results of experiments; P.R.P. prepared figures; P.R.P. drafted manuscript; P.R.P., A.M.S., and I.H.Z. approved final version of manuscript; A.M.S. and I.H.Z. edited and revised manuscript; I.H.Z. conception and design of research.

REFERENCES


