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Sampling frequency of the RR interval time series for spectral analysis of heart rate variability

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Spectral analysis of heart rate variability (HRV) is an accepted method for assessment of cardiac autonomic function and its relationship to numerous disorders and diseases. Various non-parametric methods for HRV estimation have been developed and extensive literature on their respective properties is available. The RR interval time series can be seen as a series of non-uniformly spaced samples. To analyse the power spectra of this series using the discrete Fourier transform (DFT), we need to interpolate the series for obtaining uniformly spaced intervals. The selection of sampling period plays a critical role in obtaining the power spectra in terms of computational efficiency and accuracy. In this paper, we shall analyse the RR interval time series from selected subjects for different sampling frequencies to compare the error introduced in selected frequency-domain measures of HRV at a constant frequency resolution for a specific duration of electrocardiogram (ECG) data. It should be pointed out that, although many other error causes are possible in the frequency-domain measures, our attention will be confined only to the performance comparison due to the different sampling frequencies. While the choice of RR interval sampling frequency (f_s) is arbitrary, the sampling rate of RR interval series must be selected with due consideration to mean and minimum RR interval; $f_s = 4$ Hz was proposed for a majority of cases. This is an appropriate sampling rate for the study of autonomic regulation, since it enables us to compute reliable spectral estimates between dc and 1 Hz, which represents the frequency band within which the autonomic nervous system has significant response. Furthermore, resampled RR intervals are evenly spaced in time and are synchronized with the samples of the other physiologic signals, enabling cross-spectral estimates with these signals.

Introduction

Heart rate variability (HRV) analysis is an extensively used tool in contemporary biomedical and psychophysiological research to assess cardiac autonomic control [1–3]. Low values in a variety of HRV measures have been linked with manifold pathophysiological and psychopathological conditions such as cardiovascular (CV) disease, diabetes, anxiety disorders, smoking, obesity, lack of physical exercise and attentional deficits [1, 2, 4]. Similarly, reductions in HRV have been found in depression, generalized anxiety disorder and post-traumatic stress disorder [5]. The role of spectral analysis in the evaluation of HRV in Parkinson's disease has also been established [6].

Power spectral density (PSD) estimate of HRV is commonly used as a noninvasive test of the neural control of the cardiovascular system, since it is related to the sympathetic and parasympathetic regulation of the sino-atrial (SA) node. In the last two decades, frequency domain analysis has contributed to improve the understanding of HRV [7–10]. PSD methods attempt to infer the spectrum of neural control signals from the beat occurrence times, usually from the heart rate (HR) or the RR intervals, also known as heart period signals. The power spectrum of RR interval fluctuations, obtained by standard spectral analysis such as DFT, presents two principal components: a low frequency (LF) component around 0.1 Hz (in a range between 0.04 and 0.15 Hz) and whose changes in power have been related to the sympathetic activity on the basis of pharmacological and clinical experiments [2, 11], and a high frequency (HF) component, in synchrony with respiration rate (in the range between 0.15 and 0.4 Hz), which is considered to be an expression of the respiration disturbances mediated by the vagal activity [12]. A portion of the spectral power is also concentrated in a very low frequency (VLF) band (from 0.001 to about 0.04 Hz), which is probably due to slow mechanisms of regulation such as humoral and thermoregulatory factors [2].

The spectrum of HRV signals is generally calculated either from the RR interval tachogram, by interpolating the RR interval series, or by calculating the spectrum of counts [13, 14]. The amount of energy present in the VLF range is dependent on the processing of the RR interval series required by the algorithm used to perform spectral analysis, i.e. the resampling of the time series is associated with a significant reduction in VLF power [15]. In order to standardize the methods, the use of regularly sampled interpolation of RR interval series with non-parametric methods has been suggested [2]. Previously, time series have been resampled using wide range of sampling frequencies, i.e., 1, 1.2, 2, 2.17, 2.4, 4, 5.12, 7, 8 Hz [13, 16–26]. Despite the large number of studies using resampled RR interval series for spectral estimation, there is no consistent approach for uniformly accepted sampling frequency of RR interval series. Thus, to analyse the frequency domain measures of HRV using non-parametric techniques, there was a need to suggest an

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optimal sampling frequency of RR interval series. There is often a tendency to reduce the sampling frequency in order to increase the speed of analysis and to limit data storage. On the other hand, a low sampling frequency results in loss of information. In this paper, we present a comparison of frequency domain measures in HRV studies obtained with resampling of non-equispaced RR interval series obtained from ECG recorded in premier Autonomic Function Laboratory at All India Institute of Medical Sciences (AIIMS), New Delhi. To our knowledge such a quantitative evaluation of sampling frequency of RR intervals for spectral analysis in the application of beat-to-beat variability of heart period has not been previously published. Vinod Kumar and others [27–30] have discussed the importance of ECG data reduction/compression for efficient handling of a huge quantity of data for storage, transmission and analysis, which is available in the form of RR interval time series in HRV studies.

Defining the problem

The classical time domain heart period signal samples, i.e. RR intervals, are defined as $I_n = t_n - t_{n-1,}$ where t_n is the time instant at which the nth QRS peak occurs. For reasons to be discussed later these values will be referred to as samples. From figure 1, it becomes immediately clear that the RR intervals are non-equispaced in time.

The PSD of RR interval series can be estimated with the analytical expressions derived in [31], but even though this estimate is superior to others it is impractical to realize [32]. Estimation of the PSD of RR interval series by classical methods cannot be done directly from the time series signal. Instead, it requires resampling to achieve uniform time intervals [32, 33]. However, this resampling introduces low-pass filtering and possible artifacts in the estimated spectrum. DFT analysis requires the RR intervals to be equispaced in time. We may approximate the original series to be equispaced by mean RR interval [14], i.e. sampling is a function of beat number. The approximation is fairly good for small deviations from the mean RR interval. However, if fluctuations in RR intervals becomes larger, i.e. the coefficient of variance of RR intervals is greater than 0.1, harmonic distortion will contaminate the spectrum [34]. However, in practice, it is not practical for the coefficient of variance of RR intervals to be less than 0.1, particularly in cardiac patients.

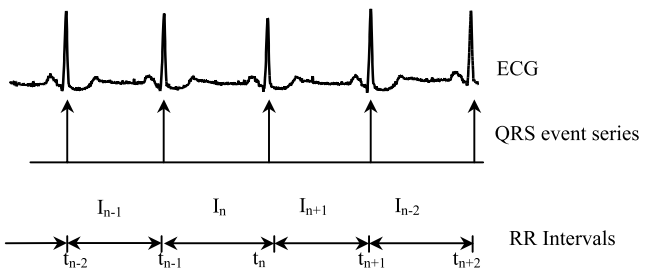

Figure 1. ECG and cardiac event series.

Also, when sampling is a function of beats, rather than seconds, the sampling rate will lack inter-individual and inter-task consistency due to differences in RR intervals. Interpolating and resampling the data using equal intervals prior to signal processing has been recommended to avoid this possible bias [35]. The short-term variability, i.e. 5-minute RR interval data sections, were interpolated with the linear interpolation method and resampled at 2, 4, 6, 8 and 10 Hz, respectively, to create an equispaced time series for an ECG recording of 256 seconds. The number of samples was chosen such that the frequency resolution after DFT analysis remains the same for a fixed data length in time to compare the results for the various sampling frequencies of RR interval series.

In discrete spectral analysis, there are three basic problems: (a) scaling and possible aliasing due to the sampling of the continuous signal, (b) scaling and possible spectral leakage due to the truncation of the sampled function within a limited window, and (c) possible erroneous interpretation due to the fact that the spectrum is only computed for discrete frequencies [36].

Windowing

To obtain the power spectrum of RR intervals, DFT is applied on N points of data. Leakage may appear in the spectrum if the signal entering the rectangular window is not periodic, or at least if amplitudes of the end points are not equal. In order to remove such leakage, the data is usually convolved with some kind of smoothing window, such as Hamming, Hann, or Blackman windows [37]. Their role is to taper the windowed data in order to make the two amplitudes of end points smoothly equal. They are defined according to the following relations:

$$\text{Hamming:} \quad W(x) = 0.54 - 0.46 \cos \frac{2\pi x}{N-1} \tag{1}$$

$$\text{Hann:} \quad W(x) = 0.50 - 0.50 \cos \frac{2\pi x}{N-1} \tag{2}$$

$$\text{Blackman:} \quad W(x) = 0.42 - 0.50 \cos \frac{2\pi x}{N-1} + 0.08 \cos \frac{4\pi x}{N-1} \tag{3}$$

Besides the leakage removal, these tapering windows also improve the time resolution of the time-dependent spectral analysis. When using such tapering windows, the influence of the edges of the analysed portion of the signal is reduced while the influence of central points is increased [19].

Data analysis

ECG signals were recorded from patients of the All India Institute of Medical Sciences, New Delhi (India). The signals were A/D converted at 500 Hz sampling

frequency, 12-bit resolution, and then stored and processed on a PIII-processor based machine. The recognition of the QRS complexes in the ECG and the detection of the R-wave were performed by means of new wavelets and an artificial neural network [38–40]. No interpolation of the original signal was applied and short-term recordings free of ectopy, missing data and noise were used. The RR interval time series was obtained as the time difference between consecutive R waves. The series were evaluated for quality assessment through visual inspection and editing. Figures 2(a), 3(a), 4(a) and 5(a) show variability signals derived from the original ECG; the interval tachogram is the series of the RR intervals expressed in milliseconds as a function of the beat number. The selection of subjects for this analysis was made keeping in view the different RR_{mean} and RR_{min} intervals, variances and shape of PSD plots. The statistical parameters of RR interval series of the subjects studied are given in the form of summary in table 1. Spectral analysis of the RR interval time series of all subjects was performed within the 5-minute rest period under lying conditions, using DFT.

The RR intervals of the 5-minute rest period were converted to a smoothed interval series at different sampling frequencies. Samples falling between QRS complexes were computed by linear interpolation. The intervals I_k were first normalized as $\acute{I}_k = (I_k - I_{mean})/I_{mean}$ and a Hann window applied in the time domain. A discrete Fourier transform [41] was performed and the squared magnitude computed. Each frequency component was multiplied by 2.66 to correct for Hann window, yielding the interval power spectral density in the units of $s^2\,Hz^{-1}$ [14], which was applied to calculate the power spectral distribution with the DFT. The power in three frequency bands was determined by integrating the power spectrum. The frequency resolution of this program was 0.004 Hz. The power spectrum was expressed as the squared modulation index $\times\ 10^6$ Hz; the power spectra of RR intervals was normalized so

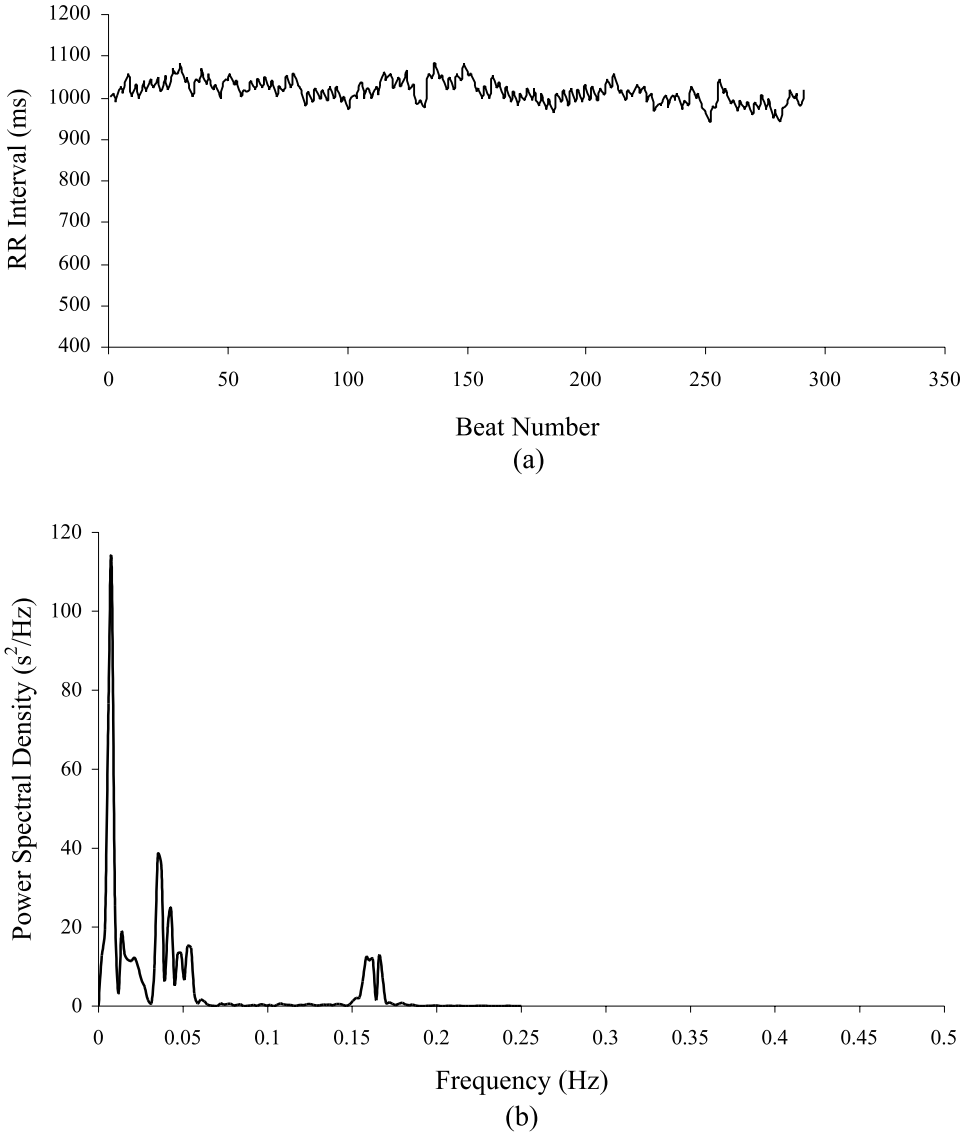

Figure 2. (a) Interval tachogram of 291 consecutive RR values of subject 1. (b) The plot shows the power spectral density of RR interval series of subject 1 at sampling frequency, $f_s = 2$ *Hz.*

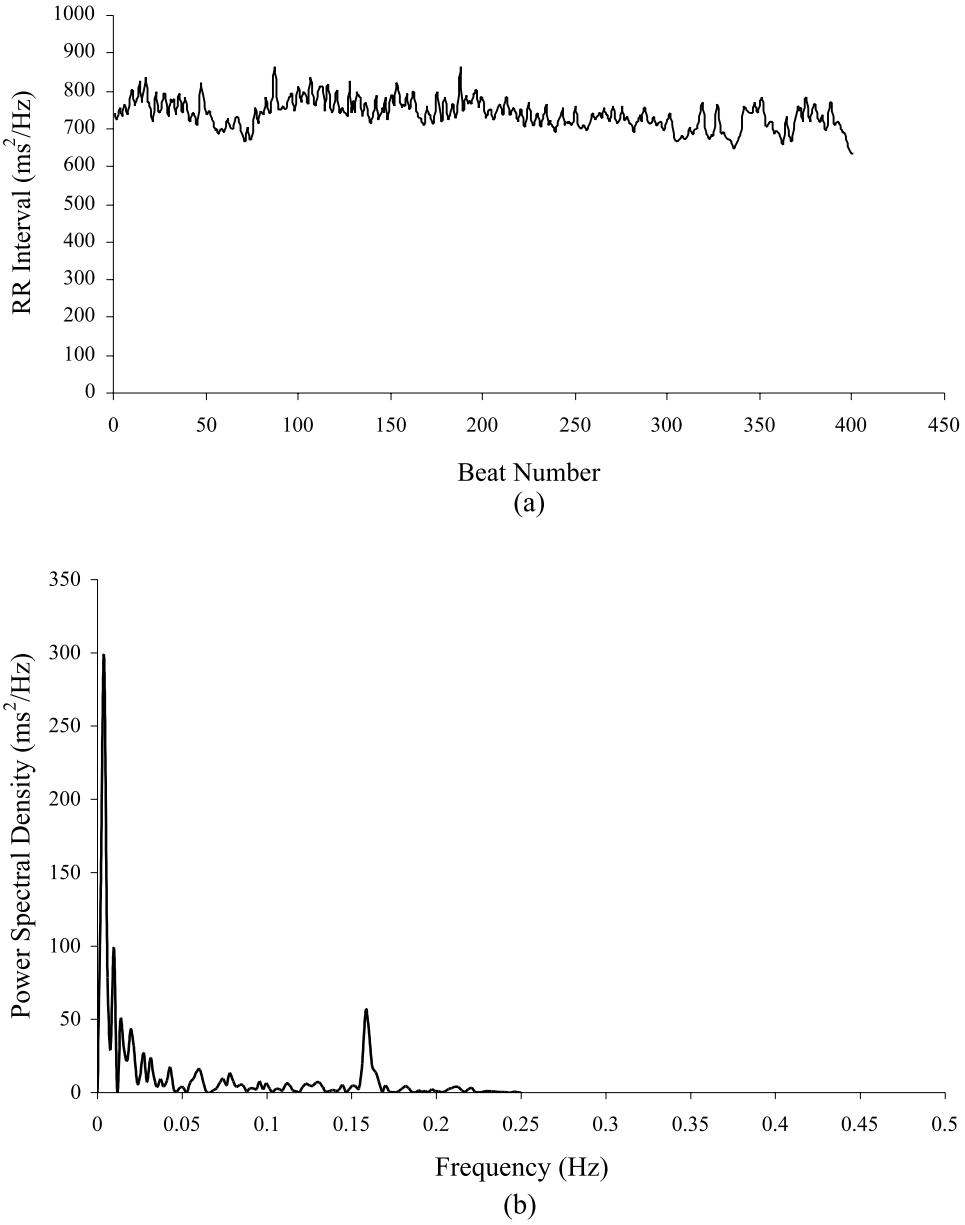

Figure 3. (a) Interval tachogram of 401 consecutive RR values of subject 2. (b) The plot shows the power spectral density of RR interval series of subject 2 at sampling frequency, $f_s = 2$ *Hz.*

that the total area under the spectral curve equalled the variance of original RR intervals for the period of 256 seconds, i.e. the time for which the spectrum is estimated. Thus, the DFT decomposes the variance of the input data into the variance attributable to each specific frequency. Table 2 shows the values of the HRV power spectral density in absolute units as well as in normalized units, i.e. as a percentage of total power minus the VLF component.

Test results and discussion

Table 2 shows the various frequency domain measures of HRV for the subjects selected, keeping in view a large range of the various statistical parameters, in order to generalize the observations for a broad category of patients. As discussed earlier, an important consideration in generating a uniformly sampled RR interval time series is the selection of an optimal sampling interval. By definition, an RR interval represents a single sample per beat and has a maximum frequency content of half the sampling frequency, or 0.5 cycles/beat. For an average heart rate of 60 bpm (beats/minute), i.e. 1 Hz, the maximum frequency content is 0.5 Hz. To avoid aliasing, the required sampling rate must be at least twice the lowest resolvable frequency (i.e. 1 Hz), and a sampling rate of four times the target frequency is more appropriate [3]. Therefore, a sampling frequency of at least 2 Hz i.e. 500 ms should be used for the spectra of interest, i.e. information at 0.5 Hz. This has been substantiated by our work as shown in tables 1 and 2. In all subjects studied, the peaks at the very low, low and high frequency regions of the PSD spectrum are the

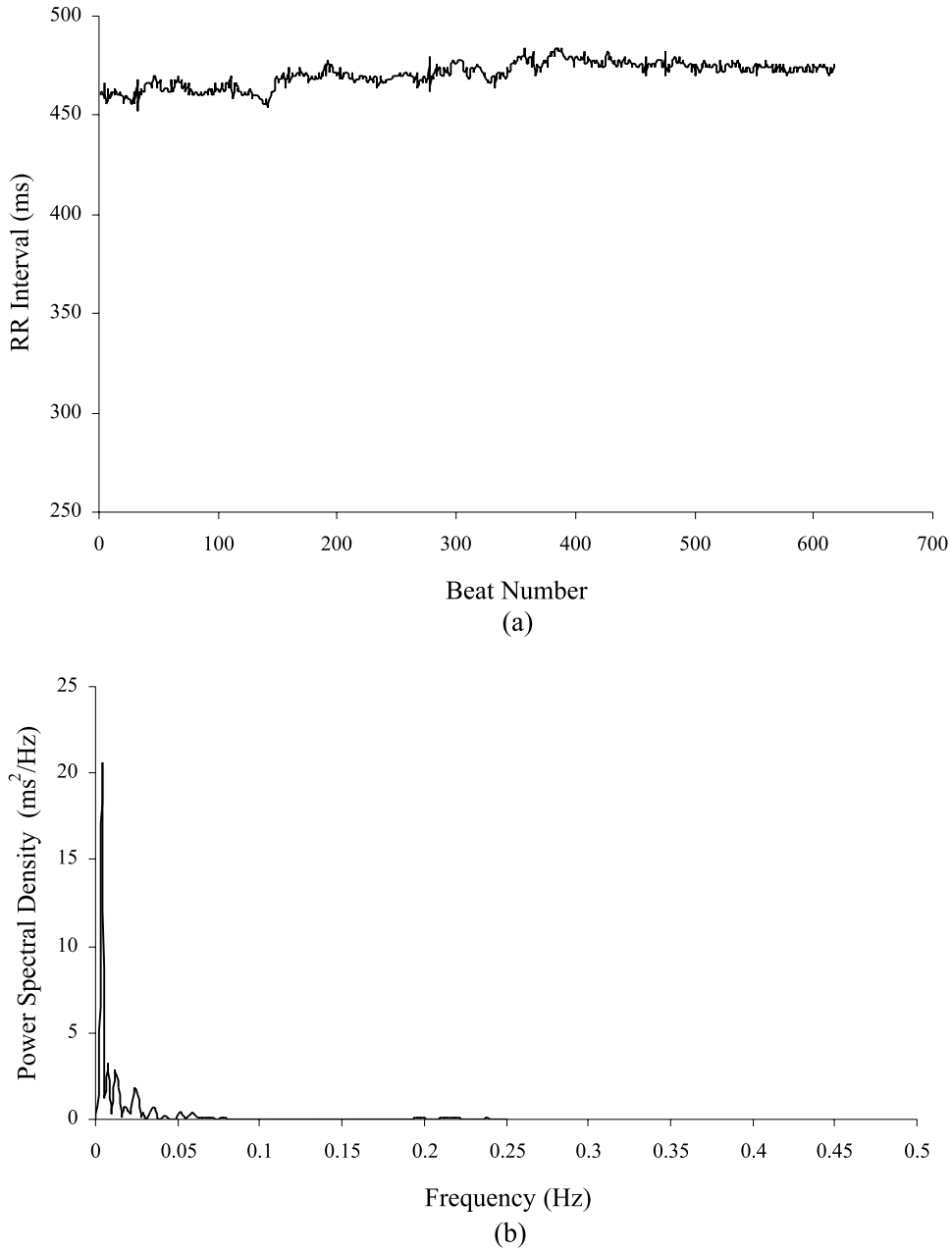

Figure 4. (a) Interval tachogram of 618 consecutive RR values of subject 3. (b) The plot shows the power spectral density of RR interval series of subject 3 at sampling frequency, $f_s = 2$ *Hz.*

same frequencies as the sampling frequencies of 2, 4, 6, 8 and 10 Hz as computed by our program using DFT under the conditions mentioned earlier in this paper. However, for subject 1, having $RR_{mean} = 1015.26$ ms and $RR_{min} = 942$ ms, the VLF, LF and HF regions power as well as the LF/HF ratio are in close agreement for various sampling frequencies, i.e. sampling intervals of 500 ms, 250 ms and 125 ms. In case of subject 2 having $RR_{mean} = 737.95$ ms and $RR_{min} = 634$ ms, frequency domain parameters are in better agreement for the sampling frequencies of 4, 6, 8 and 10 Hz than 2 Hz, which is further substantiated by subject 3 having $RR_{mean} = 470.76$ ms and $RR_{min} = 452$ ms. It is clear that as RR_{min} decreases, as in subjects 1, 2 and 3, the difference in various frequency domain measures increases, while in subject 4, having $RR_{mean} = 886.74$ ms and $RR_{min} = 704$ ms, this difference again diminishes with increased mean and minimum RR interval.

We have analysed the choice of RR interval sampling frequency on a large number of subjects, but here the results are presented for four subjects only. Another prominent observation is about the LF/HF ratio, which clearly conveys that it is in close agreement for various sampling frequencies, except at 2 Hz for subjects 1 and 4, and the differences becomes prominent in subject 3 with the lowest RR_{mean} and RR_{min}, where the LF/HF ratio for sampling frequencies of 6, 8 and 10 Hz is in better agreement as compared to sampling frequencies of 2 and 4 Hz. This observation has been further

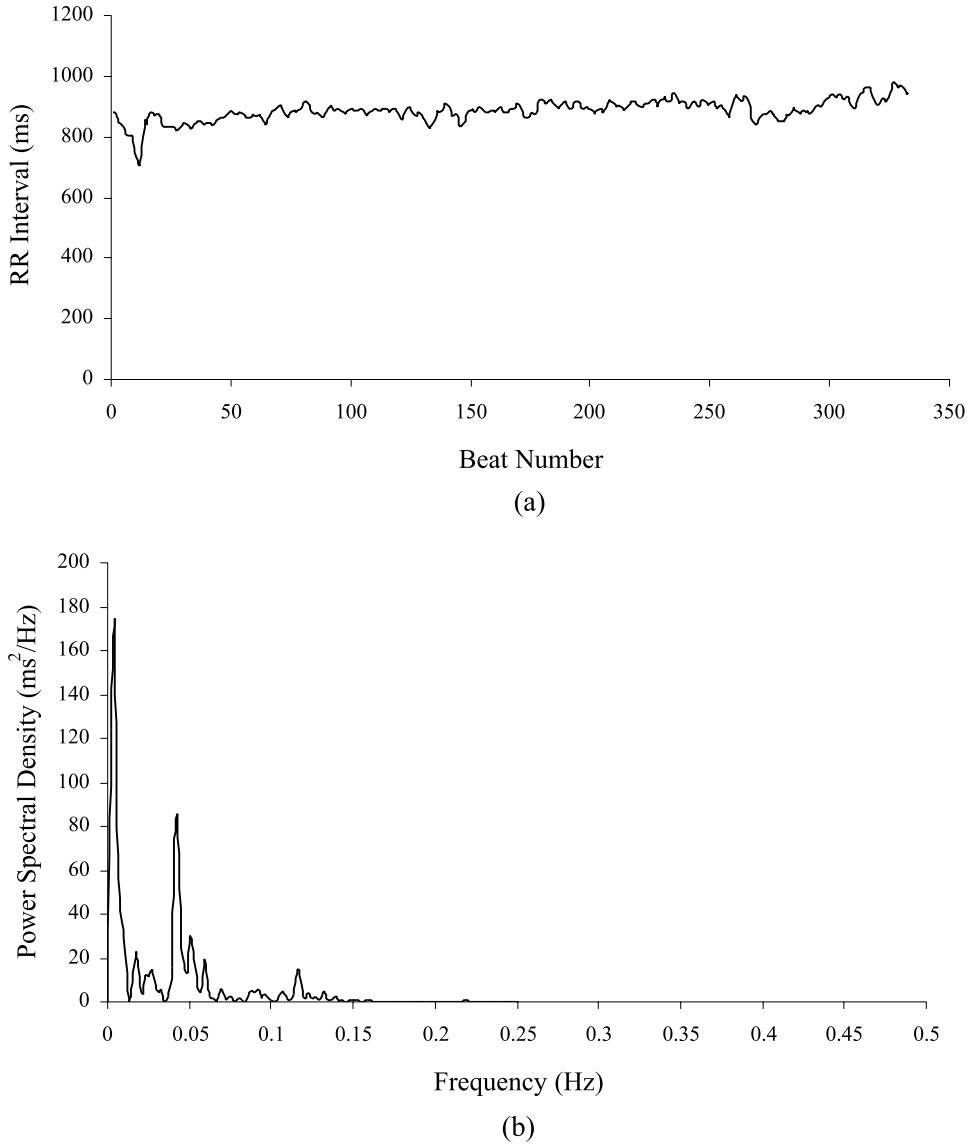

Figure 5. (a) Interval tachogram of 333 consecutive RR values of subject 4. (b) The plot shows the power spectral density of RR interval series of subject 4 at sampling frequency, $f_s = 2$ *Hz.*

Table 1. Summary of selected time domain measures of HRV for the selected subjects.

Subject number	1	2	3	4
Duration (s)	295.44	295.92	290.93	295.29
No. RR intervals	291	401	618	333
RR_{max} (ms)	1084	862	484	982
RR_{min} (ms)	942	634	452	704
RR_{max}/RR_{min}	1.15	1.36	1.07	1.39
RR_{mean} (ms)	1015.26	737.95	470.76	886.74
RR_{median} (ms)	1016	740	472	888
95% confidence interval	3.08	3.75	0.49	3.78
Coefficient of variance (%)	2.64	5.19	1.33	3.97
Variance $(ms)^2$	719.23	1465.09	39.04	1236.82
SDNN (ms)	26.82	38.28	6.25	35.17
Standard error (ms)	1.57	1.91	0.25	1.93
SDSD (ms)	21.73	28.26	3.15	14.27
RMSSD (ms)	21.69	28.22	3.14	14.25

RR_{max} = maximum value of RR interval.
RR_{min} = minimum value of RR interval.
SDNN = standard deviation of all RR intervals.
SDSD = standard deviation of differences between adjacent RR intervals.
RMSSD = the square root of the mean of the sum of squares of differences between adjacent RR intervals.

Table 2. Summary of various frequency domain measures of HRV for the considered subjects.

	f_s(Hz)	VLF_{max} (Hz)	LF_{max} (Hz)	HF_{max} (Hz)	VLF $(ms)^2$	LF $(ms)^2$	HF $(ms)^2$	Total $(ms)^2$	VLF (n.u.)	LF (n.u.)	HF (n.u.)	Total (n.u.)	LF/HF
Subject 1	2	0.012	0.066	0.328	317.30	235.67	85.67	638.64	98.74	73.34	26.66	198.74	2.751
	4	0.012	0.066	0.328	316.86	234.45	87.32	638.62	98.48	72.86	27.14	198.48	2.685
	6	0.012	0.066	0.328	317.26	234.71	86.69	638.65	98.72	73.03	26.97	198.72	2.707
	8	0.012	0.066	0.328	317.16	234.62	86.87	638.65	98.65	72.98	27.02	198.65	2.701
	10	0.012	0.066	0.328	317.21	234.67	86.78	638.66	98.68	73.00	26.99	198.68	2.704
Subject 2	2	0.004	0.051	0.312	818.77	216.99	335.28	1371.03	148.26	39.29	60.71	248.26	0.647
	4	0.004	0.051	0.312	816.33	215.67	337.89	1369.89	147.47	38.96	61.04	247.47	0.638
	6	0.004	0.051	0.312	817.55	215.30	337.47	1370.32	147.90	38.95	61.05	247.90	0.638
	8	0.004	0.051	0.312	817.13	215.69	337.40	1370.22	147.73	38.99	61.00	247.73	0.639
	10	0.004	0.051	0.312	816.27	215.16	338.42	1369.85	147.45	38.87	61.33	247.45	0.636
Subject 3	2	0.004	0.043	0.152	33.96	7.56	1.19	42.71	388.17	86.45	13.55	488.17	6.381
	4	0.004	0.043	0.152	34.04	7.60	1.15	42.78	389.39	86.91	13.09	489.39	6.638
	6	0.004	0.043	0.152	34.05	7.60	1.13	42.76	389.77	87.03	12.97	489.77	6.709
	8	0.004	0.043	0.152	34.06	7.61	1.13	42.79	389.80	87.05	12.96	489.80	6.719
	10	0.004	0.043	0.152	34.05	7.61	1.13	42.79	389.66	87.08	12.92	489.66	6.742
Subject 4	2	0.004	0.082	0.230	523.68	398.41	101.52	1023.60	104.75	79.69	20.31	204.75	3.925
	4	0.004	0.082	0.230	523.31	399.08	101.07	1023.47	104.63	79.79	20.21	204.63	3.948
	6	0.004	0.082	0.230	523.11	399.11	101.28	1023.49	104.54	79.76	20.24	204.54	3.941
	8	0.004	0.082	0.230	522.94	399.22	101.31	1023.47	104.48	79.76	20.24	204.47	3.941
	10	0.004	0.082	0.230	522.85	399.27	101.37	1023.47	104.44	79.75	20.25	204.44	3.939

VLF_{max} = frequency corresponding to power spectral density peak in VLF region; VLF = VLF power; n.u. = normalized units.

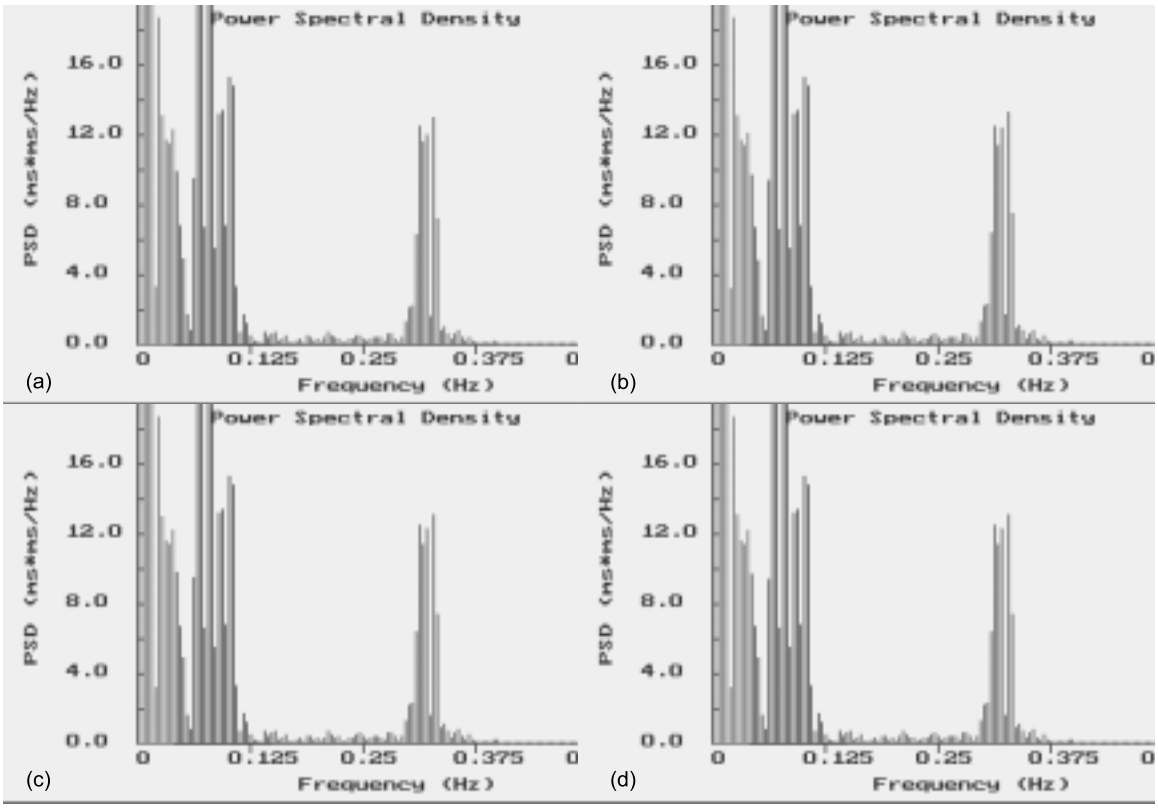

Figure 6. Screen dumps of power spectral density plots for subject 1. (a) $f_s = 2$ *Hz, (b)* $f_s = 4$ *Hz, (c)* $f_s = 6$ *Hz, (d)* $f_s = 8$ *Hz.*

substantiated by subject 2. Figure 6 shows the PSD plots for subject 1, indicating the relative change in spectra for a frequency resolution of 0.003906 Hz/line at different sampling frequencies of 2, 4, 6 and 8 Hz.

Conclusions

While the choice of RR interval sampling frequency is arbitrary, we generally choose $f_s = 4$ Hz. This is an appropriate sampling rate for the study of autonomic regulation, since it enables us to compute reliable spectral estimates between DC and 1 Hz, which represents the frequency band within which the autonomic nervous system has a significant response. Furthermore, resampled RR intervals are evenly spaced in time and are synchronized with the samples of the other physiologic signals, enabling cross-spectral estimates with these signals. However, as noted in the previous section, the sampling rate of RR interval series must be selected with consideration to mean and minimum RR intervals. For newborns [42] and other individuals with high heart rate i.e. HR $>$ 117 bpm, the f_s should be 8 Hz and above; however, for subjects with $90 \leqslant \text{HR} \leqslant 117$ bpm, $f_s = 6$ Hz and for HR < 90 bpm $f_s = 4$ Hz would suffice. Oversampling may create the illusion of high temporal resolution; it does not enhance the basic resolution afforded by the timing of actual RR intervals and the duration of recording, and is simply inefficient. Thus RR interval time series should neither be undersampled nor oversampled and, in general, a sample interval equal to one-fourth of RR_{min} is appropriate.

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