Heart rate variability in preterm neonates with and without abnormal cardiorespiratory events.

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Abstract

The heart rate variability (HRV) of preterm neonates undergoing a polysomnography is analyzed in relation to the occurrence of abnormal cardiorespiratory events. The goal of the study is to identify those neonates who experience abnormal cardiorespiratory events, based only on the heart rate recordings during periods without abnormal events. In addition to standardized HRV parameters, recently introduced methods such as detrended fluctuation analysis (DFA) and sample asymmetry analysis (SAA) are used to quantify different aspects of HRV. The methodology for calculating these HRV parameters is adapted to neonatal heart rate data. All HRV parameters are calculated for 30 preterm neonates, divided in three groups according to occurrence of abnormal events during the polysomnography and/or the eventual home monitoring. For a number of HRV parameters, statistical significant differences between the 3 groups are observed. Due to the large variability of all HRV parameters within each group, however, discrimination between the 3 groups is not possible. The values of the HRV parameters obtained in the present study are compared to other data reported in literature.

Keywords: heart rate variability, neonates, polysomnography, ALTE, spectral analysis, detrended fluctuation analysis, sample asymmetry analysis,

1 Introduction

Polysomnography is the continuous recording of physiological parameters such as heart rate (HR), respiration and peripheral oxygen saturation (SaO_2) during several hours. In preterm neonates and infants having experienced an apparent life threatening event (ALTE), subsequent life threatening events can be predicted with a single polysomnography [1]. Polysomnographies can thus be used to identify risk infants who need cardiorespiratory home monitoring (ECG, respiration and eventually SaO_2). In the University Hospitals Leuven (Belgium), the criteria for an abnormal polysomnography are central or obstructive apnea for more than 15 s combined with a bradycardia below 60 bpm and/or oxygen saturation below 80%, or a bradycardia below 50 bpm for at least 4 s. The home monitoring is also evaluated with bradycardia below 50 bpm for at least 4 s as criterium for abnormal follow-up [1]. According to the results of the polysomnographies (PS) and the follow-up (FU), the infants can thus be divided in 3 groups: NN (normal PS, normal FU), AN (abnormal PS, normal FU), AA (abnormal PS, abnormal FU). The goal of our study is to investigate if these possibly life-threatening cardiorespiratory events can be predicted from the heart rate during periods without abnormal bradycardia (below 50 bpm for at least 4 s). The underlying hypothesis is that the deficiencies in the autonomic nervous system that cause the abnormal cardiorespiratory events are reflected in the heart rate not only by abnormal bradycardia but also by more subtle changes. Specifically, it will be investigated if the aforementioned groups (NN, AN, AA) can be distinguished using measures of heart rate variability (HRV) calculated on segments without abnormal bradycardia. This would allow to improve the identification of risk infants, and could provide insight in the mechanisms causing abnormal cardiorespiratory events.

Heart rate variability (HRV) represents a non-invasive marker of autonomic activity. Numerous studies confirmed the potential of HRV with respect to several diseases and clinical

conditions, although the practical use of HRV is hitherto mainly oriented towards the assessment of diabetic neuropathy and the prognosis of risk after acute myocardial infarction in adults [2, 3, 4, 5]. Throughout the last decades, a multitude of methods have been developed to quantify HRV. In 1996, an international Task Force proposed standards for the measurement and calculation of a set of time- and frequency-domain HRV parameters, referred to as 'standard' HRV parameters in this paper [2]. In addition to these standard HRV parameters, two recently introduced methods, detrended fluctuation analysis (DFA) [6] and sample asymmetry analysis (SAA) [7] are used to quantify other aspects of HRV.

The main contribution of this paper is the comparison of HRV in preterm neonates with and without abnormal cardiorespiratory events. Methodological considerations to deal with the specific characteristics of neonatal heart rate data and the relatively low ECG sampling rate are pointed out. The results of recently introduced HRV parameters are related to physiological phenomena. The paper is organized as follows. In section 2.1, the subjects and measurements are described. The methodology used for the HRV analysis is outlined in section 2.2 and in Appendix A. Section 3 contains the results, which are interpreted and compared to other studies in section 4.

2 Materials and methods

2.1 Measurements

For the present study, polysomnographies of ALTE infants and preterm neonates recorded in the University Hospitals Leuven (Gasthuisberg) between 2000 and 2003 were considered. The subjects were divided in three groups (NN, AA, AN) according to evaluation of the polysomnography and the follow-up as described in the introduction. As there were only 9 ALTE infants in our data set, the present study is limited to the preterm infants. An abnormal polysomnog-

raphy was recorded in 39 preterm infants, 15 of whom experienced further abnormal events during the follow-up. As maturation is an important determinant of heart rate, age-matched AN and AA-groups were composed, consisting of 10 subjects each. Ten age-matched preterm infants were retained from the NN group. The mean post-conceptional age (PCA) at the time of the polysomnography was 36.4 weeks for the NN preterms, 36.3 weeks for the AN preterms and 36.4 weeks for the AA preterms. The mean gestational age (GA) was 32.3 weeks, 31.9 weeks and 32.3 weeks respectively for the NN, AN and AA preterms.

The physiological parameters measured during the polysomnographies included electrocardiogram (ECG), thoracic and abdominal respiratory movements, nasal flow (thermistor), SaO_2, chin electromyogram (EMG) and electro-oculogram (EOG). The RR intervals were derived from the ECG signal, which was usually sampled at 100 Hz as were all other signals. Ten ECG signals were sampled at 1000 Hz in order to study the effect of the ECG sampling rate on the HRV measures. The R peaks were detected by an automatic peak detection algorithm based on the second derivative of the digitized ECG signal. To avoid errors due to faulty peak detection, all RR interval series were inspected for false and/or missed peaks, and corrected if necessary (based on the ECG signal). The mean duration of the ECG recordings was 8 hours, yielding RR interval series containing on average 75000 RR intervals.

A typical neonatal RR interval series is shown in Fig. 1. A closer look at the data reveals several *rhythmical fluctuations*. Respiratory sinus arrhythmia (RSA) is a periodic fluctuation in the heart rate associated with respiration [8, 9]. Since the respiration rate in neonates, typically between 40 and 80 breaths/minute, is sometimes larger than half the heart rate, usually between 120 and 160 bpm, RSA can not always be observed in neonates (Nyquist theorem). Respiratory events such as sighs [10], respiratory pauses (apneas) [11] or periodic breathing also influence the heart rate. Furthermore, slower rhythmical fluctuations with a period around

30 heart beats (10 s) can be observed in most RR interval series. Although this '10s-rhythm' has been described in many other studies, its origin is not exactly known. It is believed to be related to the Mayer waves observed in blood pressure and is attributed to baroreceptor reflex [9]. Some other phenomena such as non-nutritive sucking cause similar rhythms in the RR interval series [12]. In addition to RSA and the 10s-rhythm, in some RR interval series even slower rhythmical fluctuations are observed, due to thermoregulation [13] or body movements.

The *behavioural state* has an important effect on heart rate. Heart rate is lower during quiet sleep than during active sleep or wakefulness [9, 14]. Long term HRV is reported to be higher during active sleep than during quiet sleep, which can partly be explained by body movements. Short term HRV, on the other hand, is suggested to be higher during quiet sleep than during active sleep. The decreased (long-term) HRV during quiet sleep (QS) periods can be observed for example in Fig. 1(a) between RR intervals 10500 and 12500. As such, QS periods can usually be identified in the RR interval series itself. Using other signals that were recorded during the polysomnography (EMG, EOG, respiration, EEG), it was verified that those segments correspond to QS. The duration of the QS periods depends on age. For our recordings, the duration of QS periods is between 10 and 30 minutes, which corresponds to approximately 2000 to 4000 RR intervals.

Another specific heart rate pattern, which is not typical for preterm neonates but is also found in full-term neonates and sometimes in adults, can be observed Fig. 1(c). Throughout this RR interval series, a number of *spikes* can be observed. Two kinds of spikes can occur in RR interval series: spikes due to cardiac arrhythmias such as premature beats which affect only one or two RR intervals, and short HR decelerations. Faulty peak detection (missed RR peak or false detection) causes similar spikes as certain cardiac arrhythmias. As all faulty peak detection were manually corrected (based on the ECG recording), however, such spikes

are not present in our RR interval series. The spikes shown in Fig. 1(c) are short heart rate decelerations.

2.2 Data Analysis

2.2.1 Standard HRV parameters

Four standard time-domain HRV parameters, SDNN, RMSSD, TI and SDANN, were calculated as recommended by the Task Force [2]:

- SDNN: standard deviation of normal-to-normal (NN) intervals;
- RMSSD: the root mean squared differences of successive NN intervals;
- TI: triangular index, defined as number of NN intervals divided by the maximum of the density distribution of all NN intervals;
- SDANN: standard deviation of the average NN intervals calculated over 5 minute periods.

For the frequency-domain analysis of HRV, the power of HRV in different frequency bands was calculated: the high-frequency component (HF: 0.15-1.5 Hz) reflecting the effect of the respiration on the heart rate (RSA), the low-frequency (LF: 0.04-0.15 Hz) component related to the Mayer waves, the very low frequency (VLF: 0.003-0.04 Hz) component presumably caused by thermoregulation [15] and the ultra low frequency component (ULF: $<$0.003 Hz). Note that the upper limit of the HF band is higher than in the guidelines of the Task Force to account for the higher breathing rate in neonates. The power spectra were estimated from an equidistant sampled (sampling rate: 8 Hz) interpolation of the HR sequences obtained as the reciprocal of the RR interval series [16]. A methodological complication in the calculation of HRV parameters stems from the relatively low sampling rate, typically 100 Hz, of ECGs recorded during polysomnographies. The low ECG sampling rate limits the accuracy of the RR intervals and consequently the accuracy of the HRV parameters, especially those quantifying short

term variability. Based on the statistical properties of the errors on the RR intervals, however, corrections can be calculated to compensate for the bias introduced by the low ECG sampling as described in Appendix A. It was shown that these corrections allow accurate estimation of all HRV parameters from RR interval series derived from ECGs sampled at 100 Hz. For each RR interval series, both short-term and long-term variability was assessed. For the short-term HRV, quiet sleep (QS) and active sleep (AS) periods were analyzed separately. Quiet sleep periods were identified as periods characterized with regular respiration, low EOG and low EMG amplitude. Following each QS period, periods of approximately the same duration, characterized by irregular breathing and limited EMG and EOG activity, were selected as AS periods. The short term HRV parameters were calculated on all QS and AS periods. For the long term HRV, the complete recordings, containing on average 75000 RR intervals, were analyzed. The HRV measures calculated on the complete RR interval series are referred to as "global" HRV measures. Abnormal bradycardias, according to the criteria discussed in section 1, were removed from the long-term RR time series by linear interpolation prior to the calculation of the HRV measures. The following parameters were calculated both globally and on the separate sleep states: SDNN, RMSSD, VLF, LF, HF, LFN (normalized), HFN (normalized) and (LF/HF). TI, SDANN and ULF were only calculated for the complete RR interval series because they can not be estimated accurately on short segments.

2.2.2 Detrended fluctuation analysis

Fractal analysis, which aims at quantifying the scaling behaviour of time series, has proven to be a useful tool in several HRV studies [6, 17, 18, 19]. Detrended fluctuation analysis (DFA) is a time-domain method that characterizes the power-law scaling relationship of the variance with the length of observation using one or more scaling exponents [6]. In order to calculate the scaling exponents, the time series (total length N) is first integrated and divided in segments of length n. Each segment is then detrended by subtracting the best linear fit. Finally, the

fluctuation function $F(n)$ is calculated as the root mean square of the detrended time series as a function of the segments size n. If the time series is self-similar, the fluctuation function $F(n)$ increases by a power-law: $F(n) \propto n^{\alpha}$. The scaling exponent α can be estimated by a linear fit on the log-log plot of $F(n)$ versus n. The scaling coefficient α is related to the spectral index β, defined as the slope of the power spectrum in logarithmic coordinates. For perfectly self-similar time-series such as fractional Brownian motion, $\alpha = (\beta+1)/2$. Also for arbitrary signals, it can be shown analytically and by numerical simulations that DFA and spectral analysis are closely related [20, 21]. So, despite often being presented as a 'nonlinear' measure, the DFA scaling exponent is actually a 'linear' statistic in the sense that it only uses information present in the power spectrum [22]. As real neonatal RR time series typically exhibit some deviations from perfect self-similarity, the effect of these deviations on DFA was investigated [23]. It was shown that the DFA scaling exponents are not constant in time, but mainly depend on behavioural state. Spikes (ectopic beats, HR decelerations) are shown to affect the scaling behaviour at small scales, and should therefore be removed prior to applying DFA if one is interested at quantifying the scaling behaviour at small scales. Periodic fluctuations are shown to introduce a crossover in the scaling behaviour at a scale corresponding to the average period of the fluctuations. Therefore, scaling exponents estimated as the linear slope on scaling ranges near the crossover scale do not reflect the properties of a possible underlying fractal component. In order to characterize the latter, it is more appropriate to use all available scales. In Appendix A, the effect of the ECG sampling rate on DFA is also investigated, and corrections are proposed based on the statistical properties of the errors on the RR intervals. As behavioural state has an important effect on the scaling properties of the RR interval series, DFA measures were calculated separately for quiet and active sleep segments. Two scaling exponents α_1 and α_2 were estimated as a linear fit over respectively short ($4 \leq n < 16$) and intermediate ($32 \leq n < 200$) scales. Similarly to the standard HRV measures, these scaling exponents were calculated on the first QS and the first AS segment. DFA was also applied to the complete RR interval series

to determine the scaling exponent α_3 at large scales ($500 \leq n < 4000$). The scaling exponents α_1 and α_2 at small and intermediate scales were also calculated on the complete RR interval series, as estimates of the average scaling behaviour at these scales over time. Additionally, one scaling exponent α_0 was calculated using the complete scaling range ($16 \leq n < 4000$).

2.2.3 Sample asymmetry analysis

As discussed in section 2.1, some neonatal RR interval series are characterized by a number of transient heart rate decelerations. These HR decelerations are usually relatively small and short: an average increase in RR interval between 0.1 s and 0.4 s during 5 to 20 heartbeats. Although these HR decelerations are not considered as abnormal bradycardias according to the criteria described in the introduction, they might be clinically relevant. Therefore, sample asymmetry analysis (SAA) [7] is used to quantify these HR decelerations. SAA was designed to quantify the contribution of accelerations and decelerations in heart rate recordings to detect early stages of neonatal sepsis and systemic inflammatory response syndrome [7]. The HRV parameter obtained with SAA is a ratio, SAA-R, that quantifies the asymmetry of the histogram of RR intervals caused by reduced accelerations and/or transient decelerations. SAA-R was calculated using the same methodology and parameters as in [7]. Note that for this parameter choice SAA-R is approximately equal to the ratio of the sum of squares of the RR intervals larger than the median to the sum of squares of the RR intervals smaller than the median. As SAA is specifically used in the present study to quantify short decelerations -not asymmetry due to slower variations- the RR interval series was detrended prior to constructing the histogram. To this end, the RR interval series were detrended using a high-pass filter with a cut-off frequency corresponding to 500 RR intervals. As a bin size of 10 ms was used, which is small enough to accurately describe the histogram, there were no difficulties with different ECG sampling rates (i.e. 1000 Hz or 100 Hz).

3 Results

For all HRV measures calculated on the complete RR interval series, the mean values ± standard deviations for each group of subjects is shown in Table 1.

The groups were compared pairwise for each HRV parameter individually. The Wilcoxon rank-sum test was used to assess the statistical significance of the differences between the groups. None of the global standard or DFA measures yielded significant differences between the three groups. SAA-R is larger for AA than for NN and AN, although only the difference with AN is significant ($p < 0.05$). The SAA-R values of each subject are shown in Fig. 2. Note that there is a large overlap between the three groups.

The HRV parameters were also calculated separately on QS and AS segments. The HRV parameters calculated on the first QS segment (QS1) were compared to those of the first AS segment (AS1), as shown in Table 2. Regarding the standard time-domain HRV parameters, no differences between groups are observed in QS1 or in AS1. No significant differences in the spectral parameters VLF, LF or HF can be observed, although LF tends to be smaller for AA than for AN and NN, especially in QS1. This is also reflected in the LFN, HFN and (LF/HF). During QS1, HFN is larger for AA than for NN ($p < 0.05$) and AN (n.s.). Regarding the scaling exponents, α_1 tends to be lower in AA than in NN and AN although the differences are not significant. During QS1, α_2 is significantly larger for AA than for both NN ($p < 0.05$) and AN ($p < 0.05$). The HFN and α_2 values of each subject during QS1 are shown in respectively Fig. 3(a) and Fig. 3(b). For both parameters, there is a large overlap between the different groups.

For each subject, the differences in HRV measures between behavioural states were evaluated

using all QS and AS segments of each subject. The Wilcoxon signed rank test for matched samples was used to assess the statistical significance. Heart rate (1/RR) and all absolute HRV measures (SDNN, RMSSD, VLF, LF and HF) are significantly lower in QS than in AS ($p < 0.001$). HFN is significantly larger in QS than in AS ($p < 0.001$), while (LF/HF) is smaller in QS than in AS. Both scaling exponents α_1 and α_2 are significantly smaller in QS than in AS ($p < 0.001$).

4 Discussion

The *mean heart rate* is not significantly different between NN-, AN- and AA-neonates. The heart rate during QS is significantly lower than during AS. The mean heart rate values in this study are comparable to the values reported in literature for healthy preterm infants with similar age (PCA, GA) [24, 25, 26, 27].

No significant differences in *standard time-domain HRV measures* (SDNN, TI, SDANN, RMSSD) were found between NN-, AN- and AA-neonates, neither globally neither in QS or AS separately. SDNN agrees with the values found in literature for preterm infants of comparable age [24]. Regarding the other standard time-domain HRV measures, to our knowledge, no comparable data is available in literature. Compared to normal full-term neonates with postnatal age less than 72 h, all standard time-domain parameters are smaller [28]. This finding agrees with results from studies on the maturation of the cardiac control in neonates [25, 24]. SDNN and RMSSD are significantly smaller in QS than in AS, a finding which was also observed in other studies [24, 27, 29, 30].

With respect to the global *spectral HRV measures*, no significant differences between NN-, AN- and AA-infants were found. During QS, VLF and LF tend to be smaller for AA infants than for NN or AN infants. No differences in HF were observed between groups, neither globally

nor in QS or AS. It should be noted that there is considerable variation in the absolute spectral HRV measures between subjects (in each group) as well as within subjects (different segments of the same behavioural state), a finding which was also reported in other studies [31, 26]. As in the few studies that reported absolute spectral HRV measures of comparable infants, different spectral estimation methods were used, no direct comparison with our results is possible. The *normalized spectral HRV measures* calculated on the complete RR interval series were not different between NN-, AN- and AA-infants. During QS, LF tends to be higher in NN- and AN- than in AA-infants. The tendency towards higher LF reflects the more pronounced Mayer waves in NN- and AN-infants. As a consequence, LFN and LF/HF tend to be smaller, and HFN larger in AA-infants. Only the difference in HFN^{QS1} between AA and NN is significant however. Due to the large overlap between all groups, shown in Fig. 3(a), however, the spectral HRV measures are not sufficient for discriminating NN-, AN- and AA-infants. Comparison of spectral HRV measures with the values obtained in other studies is hampered by differences in the used frequency bands. From the data in [26], it can be derived that if the same frequency bands as in the present study were used, the LF/HF values are comparable for neonates with similar age. In [32], comparable frequency bands were used for the spectral analysis for HRV of full-term neonates. The average values of LFN during QS (48 ± 17) is comparable to the value we obtained, but the LF/HF ratio is much larger (15.7 ± 15.4). Another study, which used the frequency bands recommended by the Task Force, reported a lower LF/HF ratio (2.7) in full-term infants [28]. As the HF band (0.15-0.4 Hz) was not adjusted to account for the high respiration rate in neonates, however, the difference in LF/HF with our results should not only be attributed to maturation. Several studies using 0.2 Hz as the border between LF and HF typically report higher LF/HF ratios, especially during AS [9, 33]. Studies in which the spectra were calculated from the RR interval series itself (instead of the heart rate), yield roughly comparable values for LFN and HFN, although comparison is complicated by the fact that the frequency bands are expressed in cycles per beat [25, 34]. Regarding the differences in

spectral measures between behavioural states, the absolute power in all three frequency bands is significantly larger during AS than during QS. Considering the normalized powers, both LFN and HFN are larger during QS than during AS, while the LF/HF ratio is significantly smaller during QS than during AS. These differences between AS and QS are in accordance with several other results in preterm as well as in full-term neonates [25, 34, 35].

Neither the global *DFA scaling exponents*, nor the DFA scaling exponents during AS were significantly different between NN-, AN- and AA-infants. During QS, α_1 tended to be lower and α_2 higher in AA-infants compared to NN- and AN-infants: α_2^{QS1} was significantly higher in AA-infants compared to both NN- and AN-infants. Due to the large overlap between the groups, however, discrimination of the different groups is not possible based on DFA scaling exponents. The differences in α_1 and α_2 can be related to differences in spectral HRV parameters in the corresponding frequency ranges. The LF range, where the normalized power is smaller in AA infants compared to NN- and AN-infants, corresponds approximately to the scaling range $16 < n < 60$ (assuming a mean heart rate of 150 *bpm*). This scaling range corresponds approximately to the crossover range between the ranges used to calculate α_1 and α_2. The lower LFN in AA-infants therefore results in a steeper slope in the fluctuation function (or in the spectrum) towards larger scales, i.e. larger α_2. Similarly, the lower LF/HF ratio in AA infants explains the smaller α_1. To our knowledge, scaling exponents of neonatal heart rate time series have so far only be reported in [36], where dispersional analysis was used to estimate the Hurst coefficient on segments of 256 RR intervals. Their results correspond to scaling exponents between 1 and 1.5 in the scaling range ($4 < n < 16$), which is in agreement with our results [36]. Both α_1 and α_2 were found to be significantly lower during QS than during AS. Similar observations have been reported regarding differences between REM and deep sleep in adults [37, 38].

With respect to the sample asymmetry analysis, SAA-R tends to be higher in AA-infants

compared to NN- and AN-infants, although only the difference between AA and AN is significant ($p < 0.05$). This suggests that AA infants have more and/or larger heart rate decelerations that are not considered as abnormal bradycardia according to the criteria presented in section 1. Due to the large overlap between SAA-R values of the different groups, as shown in Fig. 2, discrimination of the different groups is not possible using SAA-R only. The only other study that used SAA to analyze HRV values of preterm infants (GA: 34 w), reports SAA-R values from 3.3 ± 1.6 (5 days before sepsis) to 4.2 ± 2.3 (1 day before sepsis) [7].

5 Conclusions

In this study, the heart rate variability (HRV) of preterm infants during polysomnography was analyzed. In addition to standard time-and frequency domain HRV parameters, detrended fluctuation analysis (DFA) and sample asymmetry analysis (SAA) were used to quantify other aspects of the heart rate. The methodology for calculating these HRV parameters was adapted to deal with the specific characteristics of neonatal heart rate data and the relatively low ECG sampling rate (100 Hz). All HRV parameters were calculated on RR interval series derived from ECGs recorded during polysomnographies at the University Hospitals Gasthuisberg. According to the results of the polysomnography (PS) and the eventual home monitoring (follow-up - FU), the infants can be divided into three groups: NN (normal PS, normal FU), AN (abnormal PS, normal FU) and AA (abnormal PS, abnormal FU). The significant difference in SAA between AN and AA infants suggests that AA infants have more small HR decelerations. In addition, a tendency for lower LF power during QS was observed in AA- compared to NN and AN-infants, which was also reflected in the normalized spectral HRV parameters (lower LFN, higher HFN, lower LF/HF). This suggest that the Mayer waves are less pronounced in AA-infants. The observed differences in scaling exponents during QS in AA infants compared to NN and AN neonates (lower α_1, higher α_2) can also be attributed to the relatively smaller proportion of LF-power. Due to the large variability of all HRV parameters within each group, however,

none of these HRV parameters allows discrimination of the 3 groups. The values of the HRV parameters reported in the present study as well as the differences between behavioural states, are in agreement with the literature.

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Appendix A

The finite sampling frequency of the ECG introduces an error on the measured RR intervals, as illustrated in Figure 4. If the errors on the beat occurrence times are denoted $\Delta R(i-1)$ and $\Delta R(i)$, the error on the ith RR interval $\Delta RR(i)$ can be written as:

$$\Delta RR(i) \equiv RRk(i) - RR(i) = \Delta R(i) - \Delta R(i-1) \tag{1}$$

where $RR(i)$ represents the exact RR interval and $RRk(i)$ the RR interval determined from the digitized ECG. Assuming that $\Delta R(i)$ is a sequence of independent, identically distributed random variables uniformly distributed on the interval $-1/(2f_s) \leq \Delta R(i) \leq 1/(2f_s)$, where f_s represents the ECG sampling frequency, the sequence of errors $\Delta RR(i)$ can be considered as a coloured noise sequence (transfer function $H(z) = 1 - z$). As the statistics of the error sequence $\Delta RR(i)$ depend only on the ECG sampling frequency f_s, the resulting error on the HRV parameters introduced can be compensated for. The standard deviation of the exact RR interval series $R(i)$ can be estimated from the finite resolution RR interval series $RRk(i)$ as

follows:

$$\sigma_{RR} \approx \sqrt{\sigma^2_{RRk} - 1/(6f_s^2)} \overset{fs=100\ Hz}{=} \sqrt{\sigma^2_{RRk} - (1/6).10^{-4}} \tag{2}$$

This correction was used to calculate SDNN from 100 Hz RR interval series. Similarly, a correction for the RMSSD can be derived:

$$RMSSD_{RR} \approx \sqrt{RMSSD^2_{RRk} - 1/(2f_s^2)} \overset{fs=100\ Hz}{=} \sqrt{\sigma^2_{RRk} - 5.10^{-5}} \tag{3}$$

The accuracy of these corrections depend on the number of RR intervals used to calculate the HRV measures. The larger the number of RR intervals, the better the statistical properties of the sample distribution of the actual errors on the beat occurrence times $\Delta R(i)$ will match those of the assumed uniform distribution and the more accurate the corrections will be. In order to verify if the proposed model for the error $\Delta RR(i)$ and the accuracy of the corrections, 10 ECGs were recorded with a sampling rate of 1000 Hz, which introduces only a negligible error in the HRV parameters. The HRV parameters calculated on these 1000 Hz RR interval series were compared to those calculated on the 100 Hz downsampled RR interval series with the proposed corrections. It was observed, that even for the segments with the lowest variability (quiet sleep) and the shortest RR interval segments considered, the relative error was always smaller than $< 4\%$ [23].

Assuming that the sequences $RR(i)$ and $\Delta RR(i)$ are uncorrelated, the effect of the error sequence $\Delta RR(i)$ on the power spectrum can be described by the addition of coloured noise with a high-pass characteristic. If the uneven sampling of the RR interval series is disregarded, the power spectrum of the error sequence can be calculated analytically:

$$S_{\Delta RR}(f_{RR}) = 1/(6f_s^2)(1 - cos(2\pi f_{RR})) \tag{4}$$

where f_{RR} represents the frequency variable in "cycles per beat". As the power spectrum was estimated from the regularly sampled interpolation of the heart rate signal, however, the "correction" spectrum of Eq. (4) can not simply be subtracted from the spectrum estimated

from the 100 Hz RR interval series. Therefore, an artificial RR interval series was synthesized by adding a coloured noise sequence according to a given ECG sampling rate, simulating the error on the RR intervals, to a constant value equal to the mean of the RR interval series under analysis. This artificial RR interval series, which contains variations due to the finite sampling rate of the ECG, was interpolated in exactly the same way as the real RR interval series. The power spectrum of the resulting signal was used as a correction for the heart rate power spectra. The accuracy of the correction was evaluated using the 1000 Hz RR interval series. Although there are relatively large errors in the corrected spectrum for the individual frequency components, the corrections drastically improve the accuracy of the estimated HRV parameters, especially the HF power. The mean relative error of the HF power and LF/HF-ratio calculated from the 100 Hz RR interval series with correction, compared to the HF power respectively LF/HF ratio calculated from the 1000 Hz RR interval series is smaller than 3%; The errors on ULF, VLF and LF power are negligible.

As explained above, the errors in the RR intervals, and hence the DFA fluctuation function of $\Delta RR(i)$ is only dependent on the sampling frequency. Assuming that the sequences $RR(i)$ and $\Delta RR(i)$ are uncorrelated, the superposition principle can be used to correct the fluctuation function of the sampled RR interval series $RRk(i)$ [39]. The fluctuation function of true interval series can be approximated as:

$$F_{RR(i)}(n) \approx \sqrt{F_{RRk(i)}(n)^2 - F_{\Delta RR(i)}(n)^2} \tag{5}$$

where $F_{\Delta RR(i)}(n)$ represents the fluctuation function of the errors $\Delta RR(i)$, which can be calculated from the coloured noise sequence.

With respect to SAA, no corrections for the ECG sampling rate were necessary because a bin size of 10 ms was used, which is small enough to accurately describe the histogram.

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Table 1: *Summary of global HRV measures calculated on the complete RR interval series. Significant differences ($p < 0.05$) between groups are indicated between brackets.*

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Table 1: Summary of global HRV measures calculated on the complete RR interval series. Significant differences ($p < 0.05$) between groups are indicated between brackets.

	NN mean ± std	AN mean ± std	AA mean ± std
RR (ms)	405 ± 21	401 ± 22	396 ± 15
SDNN (ms)	41.4 ± 9.5	42.4 ± 9.9	43.6 ± 8.7
TI	9.1 ± 2.6	8.7 ± 2.3	9.7 ± 2.2
SDANN (ms)	27.0 ± 8.2	26.0 ± 4.8	29.5 ± 6.8
RMSSD (ms)	10.3 ± 2.4	10.1 ± 3.2	10.8±3.9
PWR ($10^6 bpm^2$)	2.8 ± 1.2	3.4 ± 0.8	3.1 ± 1.2
ULF ($10^6 bpm^2$)	1.17 ± 0.17	1.29 ± 0.68	1.27 ± 0.58
VLF ($10^6 bpm^2$)	1.11 ± 0.52	1.50 ± 1.00	1.24 ± 0.50
LF ($10^6 bpm^2$)	0.45 ± 0.17	0.49 ± 0.29	0.45 ± 0.14
HF ($10^6 bpm^2$)	0.12 ± 0.06	0.10 ± 0.06	0.11 ± 0.07
VLFN	39.8 ± 11.1	42.8 ± 8.0	40.4 ± 4.0
LFN	17.0 ± 7.5	14.9 ± 4.3	15.5 ± 4.6
HFN	4.6 ± 2.6	3.2 ± 1.1	3.7 ± 2.2
α_0	1.02 ± 0.07	1.03 ± 0.04	1.06 ± 0.03
α_1	1.51 ± 0.09	1.53 ± 0.06	1.50 ± 0.12
α_2	1.06 ± 0.11	1.10 ± 0.10	1.08 ± 0.10
α_3	0.99 ± 0.16	0.95 ± 0.11	1.01 ± 0.11
SAA-R	2.27 ± 1.48	$1.90 \pm 0.86_{(AA)}$	$2.85 \pm 1.50_{(AN)}$

Table 2: Summary of HRV measures calculated on the first quiet sleep segment and the first active sleep segment. Significant differences ($p < 0.05$) between groups are indicated between brackets.

	NN mean ± std	AN mean ± std	AA mean ± std
RR^{QS1} (ms)	432 ± 32	426 ± 44	431 ± 25
SDNN^{QS1} (ms)	12.2 ± 4.9	10.7 ± 5.4	9.9 ± 3.8
RMSSD^{QS1} (ms)	7.2 ± 2.6	5.9 ± 3.0	6.8 ± 3.9
VLF^{QS1} $(10^3 bpm^2)$	0.95 ± 0.50	0.97 ± 0.70	0.67 ± 0.41
LF^{QS1} $(10^3 bpm^2)$	3.5 ± 2.6	2.3 ± 1.4	1.5 ± 0.9
HF^{QS1} $(10^3 bpm^2)$	1.2 ± 0.8	0.9 ± 0.6	1.2 ± 1.2
LFN^{QS1}	57.7 ± 12.4	52.6 ± 11.9	46.4 ± 8.5
HFN^{QS1}	$22.5 \pm 7.3^{(AA)}$	23.7 ± 12.6	$30.3 \pm 14.3^{(NN)}$
$(\text{LF/HF})^{QS1}$	2.95 ± 1.45	3.15 ± 2.26	2.18 ± 1.80
α_1^{QS1}	1.33 ± 0.19	1.33 ± 0.21	1.23 ± 0.27
α_2^{QS1}	$0.61 \pm 0.15^{(AA)}$	$0.60 \pm 0.15_{(AA)}$	$0.75 \pm 0.15^{(NN)}_{(AN)}$
RR^{AS1} (ms)	408 ± 25	407 ± 33	412 ± 18
SDNN^{AS1} (ms)	26.7 ± 5.8	27.1 ± 11.3	25.6 ± 9.5
RMSSD^{AS1} (ms)	8.0 ± 1.6	7.7 ± 2.4	8.3 ± 3.5
VLF^{AS1} $(10^3 bpm^2)$	18.2 ± 12.0	16.0 ± 12.0	13.5 ± 7.7
LF^{AS1} $(10^3 bpm^2)$	9.4 ± 4.3	10.2 ± 4.9	8.5 ± 3.8
HF^{AS1} $(10^3 bpm^2)$	2.1 ± 1.1	2.1 ± 1.1	2.5 ± 2.0
LFN^{AS1}	33.2 ± 9.2	38.9 ± 10.7	37.1 ± 11.8
HFN^{AS1}	8.0 ± 3.9	8.2 ± 3.0	10.2 ± 4.5
$(\text{LF/HF})^{AS1}$	4.70 ± 1.77	5.05 ± 1.76	4.26 ± 1.97
α_1^{AS1}	1.56 ± 0.08	1.60 ± 0.09	1.49 ± 0.11
α_2^{AS1}	1.02 ± 0.17	1.00 ± 0.17	0.99 ± 0.19

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Figure 4: *Effect of the ECG sampling frequency on the accuracy of the RR intervals.*

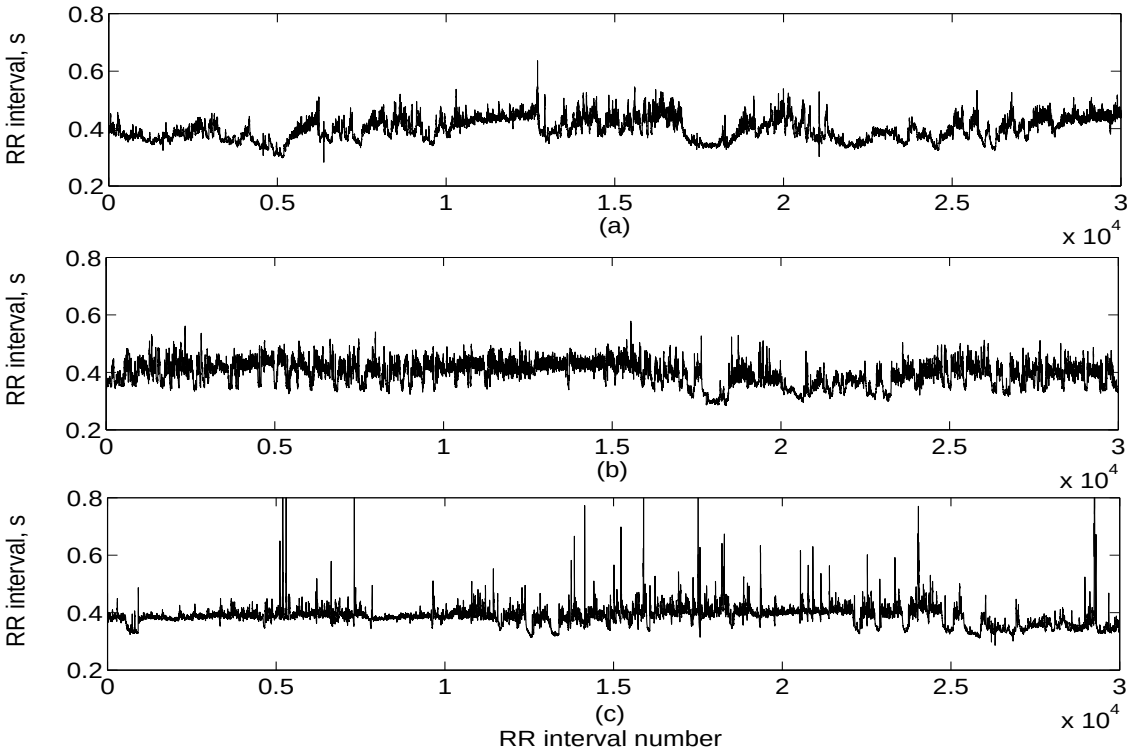


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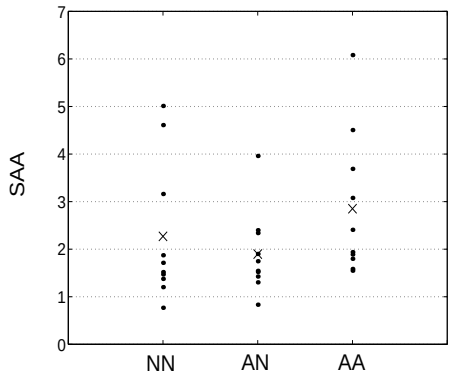


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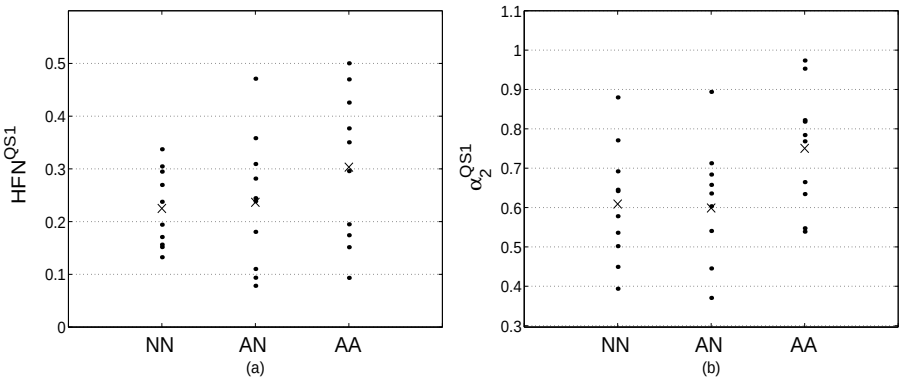


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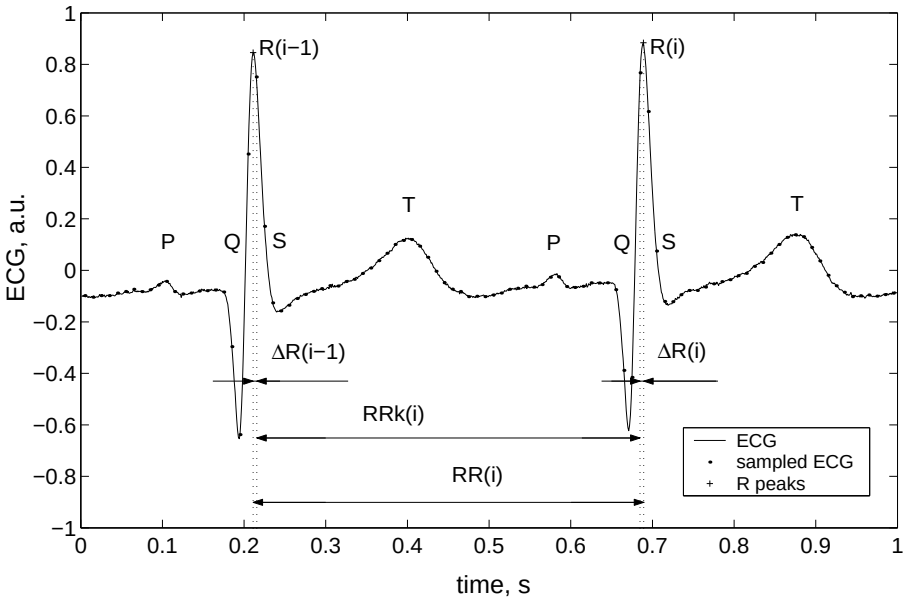


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