

■ REVIEW

Short-term heart rate variability in resting conditions: methodological considerations

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A B S T R A C T

Low values on heart rate variability (HRV) derived parameters at resting have been used to predict cardiovascular diseases (CVD) and mortality. In this regard, short-term HRV recordings (usually from 5 to 15 minutes) are increasing their popularity because data acquisition can be performed under more controlled conditions than long-term recordings (e.g., 24 hours). However, different methodological aspects before, during, and after the HRV assessment could affect the quantification and the clinical interpretations of the HRV-derived parameters, as well as hamper comparisons across different studies. In the present study, we summarize these methodological aspects that should be considered in both the research and the clinical settings. These are: (1) the validity and reproducibility of the device used to assess the HRV; (2) the influence of the software used to perform the artefact correction; (3) previous conditions before the testing day; (4) establishing the proper conditions during the HRV assessment (e.g., controlled respiratory frequency); (5) after assessing the HRV, considering the "best" data selection and statistical analysis approach; and (6) the role of the heart rate on the associations between the different CVD risk factors outcomes (e.g., cardiorespiratory fitness) and the HRV-derived parameters.

Key words: electrophysiology, heart rate, parasympathetic, R-R interval, sympathetic

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INTRODUCTION

Heart rate variability (HRV) is a non-invasive indicator of cardiac autonomic modulation, which reflects the variation in the time intervals between consecutive normal-to-normal R-R intervals [1]. The HRV is commonly expressed using different parameters in time and frequency domains [1, 2]. In most cases, reduced HRV-derived parameters values, while the subject is in at resting state (e.g., lying on a bed), are associated with a higher risk of cardiovascular disease (CVD) and mortality [1, 3–5].

Different methodological aspects should be considered to properly derive the HRV parameters and to determine its clinical interpretations. An example could be the duration of the HRV assessment (e.g., 5 min period vs 24 h period of R-R signal recording), the method or the instrument used (e.g., heart rate monitor [HRM] vs electrocardiography [ECG]) for the assessment, and, in the final step, the procedure followed for the data selection and/or processing. In this regard, the duration of the HRV recordings could

directly influence the quantification of some of the HRV-derived parameters in the time domain such as the standard deviation of the normal R-R intervals (SDNN) [1]. This is mainly produced because the total variance of the HRV is directly related to the duration of the HRV recording [1]. Importantly, in the last years, short-term HRV recordings (commonly from 5 to 15 minutes periods) using HRM are increasing in popularity [2, 6, 7]. One of the reasons is that short-term HRV recordings allow to obtain meaningful and accurate data under more controlled conditions (e.g., reducing confounder factors, such as body positioning or respiratory rate, etc.), compared to the long-term or the 24 h period of R-R signal recordings [2, 6].

Another methodological example which has a direct impact on the quantification of the HRV-derived parameters is the researcher [6]. Prior to deriving the HRV parameters, the R-R signal requires the researcher to make certain "subjective decisions" that could impact the quantification of the HRV parameters [6]. However, this R-R

signal data processing is a mandatory step, as in most R-R signal recordings (independently of the instrument used for the recording, i.e., HRM or ECG) artifacts occur [8]. This procedure of removing (or interpolating) the R-R signal artefacts present in the signal is commonly known as the “artefact correction procedure” [7, 8]. If those artefacts are not removed (or interpolated), they could directly influence the HRV-derived parameters producing either under- or over-estimations up to 50% [9]. There are several ways to overcome these “artefacts-related” problems, e.g., in the Kubios software, i.e., one of the most commonly used the HRV analytical tools (HRV analysis, University of Eastern Finland), different levels of threshold-based artefact filters may be employed depending on the artefact amount (so-called “Kubios filters”) [7, 10]. However, it has been shown that the Kubios filters may influence the HRV-derived parameters from short-term recordings in both the time and frequency domains especially in young populations [7].

Lastly, an inverse and non-linear relationship between the HRV-derived parameters and the average heart rate (HR) exists [11]. Therefore, HR should be considered when the HRV analyses are performed [12]. Briefly, it has been shown that changes in HR might produce alterations in the HRV-derived parameters due to mathematical influences [11]. Moreover, it has been suggested that the inter-subjects differences in the HRV-derived parameters could be explained, to a greater or lesser extent, by their differences in HR [12, 13].

This narrative review aimed at summarizing different methodological considerations that may influence the quantification, and the clinical interpretation of the HRV-derived parameters assessed from resting short-term recordings.

HRV-DERIVED PARAMETERS IN TIME AND FREQUENCY DOMAINS

The parasympathetic and sympathetic nervous systems are considered the main determinants of the HRV-derived parameters in time and frequency domains [14]. However, human physiology is complex and HRV is considered a physiological variable that could reflect the complex interaction among several physiological systems such as endocrine, respiratory, and immunological, among other systems [15]. The most common HRV-derived parameters in the time domain (Table 1) are considered as indicators of vagal tone, and lower values at resting conditions are associated with a higher risk of CVD and mortality [3, 4, 15]. Importantly, although the HRV-derived parameters in the time domain reflect vagal tone, they cannot determine if reductions in the variations of the R-R intervals (or the HRV-derived parameters) are produced by an increase in the sympathetic tone or by a decrease in the vagal tone [15].

First, it should be noted that not all the parameters can be used “appropriately” when the data is derived from short-term recordings [1]. In Table 1 we introduced the most used HRV-derived parameters using data from short-

Table 1. Most used heart rate variability (HRV) derived parameters

HRV-derived parameter	Parameter description
Time domain	
RMSSD, ms	Square root of the mean of the sum of the squares of successive normal R-R interval differences
SDNN, ms	Standard deviation of all normal R-R intervals
pNN50, %	Percentage of pairs of adjacent normal R-R intervals differing by more than 50 ms in the entire recording
Frequency domain	
HF, absolute units; ms ²	The absolute power of the HF band (HF: 0.15–0.4 Hertz)
LF, absolute units; ms ²	The absolute power of the LF band (LF: 0.04–0.15 Hertz)
Ratio LF:HF	The ratio of LF to HF

All descriptions of HRV derived parameters are extracted from Plaza-Florida et al. [16] and/or Task Force Report [1].

Abbreviations: HF, high frequency; LF low frequency; R-R interval, the time between R peaks on electrocardiograms; ms, milliseconds

term recordings performed with the subject resting [1, 16]. The HRV-derived parameters in the frequency domain (Table 1) allow researchers to determine the cyclic fluctuations of the R-R intervals [2, 15]. For that purpose, the R-R signal is divided into different frequency bands. The high frequency (HF) band (0.15–0.40 Hz), is an indicator of vagal tone and is also called the “respiratory band” [2, 17]. Notably, the HRV-derived parameters that indicate vagal tone in the time domain — the squared root of the mean of the sum of the squares of successive normal R-R interval differences (RMSSD), and the percentage of pairs of adjacent normal R-R intervals differing by more than 50 milliseconds during the entire recording (pNN50) — are strongly and positively associated with the HF band [1, 18]. Then, we find the low frequency (LF) band (0.04–0.15 Hz), which is influenced by the parasympathetic and sympathetic control, making clinical and physiological interpretations difficult [1, 19, 20]. In the previous literature it has been suggested that the LF band could be used as an indicator of sympathetic tone [21]. However, the LF band has not been related to sympathetic nervous responses which are associated with different acute stimuli [21]. In this regard, exercise and myocardial ischemia reduced the LF band instead of increasing it, thus the LF band has been considered a “poor” indicator of sympathetic tone [20]. Similarly, the ratio of LF to HF (LF:HF) is commonly considered as an indicator of sympatho-vagal balance (e.g., sympathetic activation is accompanied by a reduction of vagal tone). Nevertheless, this “simple” interpretation does not consider the non-linear and complex relationship between the parasympathetic and the sympathetic nervous systems [19]. Therefore, considering these reasons, the ratio LF:HF should not be used as an indicator of sympatho-vagal balance [19].

Lastly, the non-linear analysis, and thus its derived parameters, could better describe the complex non-linear interactions between parasympathetic and sympathetic nervous system branches and the behavior of the variations among R-R intervals. However, understanding the

HRV-derived parameters in non-linear domains is more difficult (e.g., entropy and fractal parameters) than either time or frequency domains, and their physiological and clinical implications remain unclear [2, 22]. In this regard, more studies are needed to reveal the physiological and autonomic regulation mechanisms underlying the changes in different HRV-derived parameters in the non-linear domain under different conditions and scenarios (e.g., mental stress, diverse pathologies, physical exercise, etc.). In addition, non-linear parameters should be derived from long ECG traces, and indeed little is known about their clinical values from short-term recordings, so they are not discussed in detail in this article.

R-R SIGNAL ASSESSMENT: VALIDITY AND REPRODUCIBILITY OF HEART RATE MONITORS

The International Bureau of Weights and Measures defines *validation* as “verification (i.e., provision of objective evidence), where the specified requirements are adequate for an intended use” [23]. Therefore, the concept *validity* refers to how accurately a device, a method, or a technique “assess what is intended to be assessed”. On the other hand, for monitoring changes over time, the method used must be *reproducible* (a concept also found in literature as *reliable* or *reliability*) [24]. Although readers could find diverse definitions of *reproducibility*, for simplicity, we will herein be referring to it as “the degree of similarity between measurements performed under the same conditions at different times”. In other words, how repeated measures under the same testing conditions vary (or not) for the individuals.

The measurement of the R-R signal usually demands a high-quality ECG, which is considered the ‘gold standard’ (i.e., the reference) device [1] and an electrocardiogram (sampling rate ≥ 500 Hz), plus an algorithm that allows detecting the QRS complex accurately [1]. In recent years, and aiming at satisfying these necessities, diverse ambulatory ECG and Holter monitors have been developed and manufactured [25]. Unfortunately, the equipment’s relative difficulty and cost have, somehow, made the acquisition and processing of the R-R signal and the HRV parameters challenging outside the clinical and research settings [26]. To overcome these limitations, diverse user-friendly wireless HRMs equipped with an adjustable elastic electrode belt have been developed, allowing for both R-R signal detection and recording.

Most studies have reported a similar concordance between HRMs and ECGs. The most studied brand in the current literature is probably Polar® (Polar Electro OY, Kempele, Finland) and its different HRM models (e.g., Polar RS800CX™, S810™, etc.). In general terms, the aforementioned HRM models have shown an acceptable validity [27–33], or, in other words, a good level of agreement compared to the ‘gold standard’ method. Nevertheless, some other studies have observed a low validity [34] of the HRM. Importantly, it has been suggested that those results could be related to

the software used for deriving the HRV parameters instead of the HRM itself [35].

In summary, studies assessing both the validity and the concordance among different HRM models and brands are mandatory for a better understanding of the HRV-derived parameters (estimated from short-term recordings) and their clinical and practical implications. Importantly, the software used to process the R-R signal should be considered, as it may influence the conclusions [35]. It should be highlighted that other validation studies have also been performed in different conditions, for example, in non-healthy individuals (e.g., [36] — the authors observed that the R-R signal measured using HRM and a 12-lead electrocardiograph is comparable) or during exercise (e.g., [37, 38] — the authors observed that HRM is a valid device compared to a 12-lead electrocardiograph in endurance athletes while running [37], and that the Polar HRM measures are comparable to an electrocardiograph during different intensities exercises [38]). However, as we mentioned earlier, the present study is focused on methodological considerations for subjects at a resting state.

It should be noted that, in short-term HRV recordings (even employing ECG), the difficulty is that the same subject shows the exact same level of autonomic nervous system modulation at different times (e.g., day-to-day). However, achieving a high day-to-day reproducibility is fundamental for analyzing and detecting the magnitude of change in the HRV-derived parameters over a period of time (e.g., before vs after an intervention). Previous reviews have suggested that HRV-derived parameters are, to a greater or lesser extent, reproducible [39, 40], although this generalization might not be suitable for certain populations. For example, the HRV-derived parameters seem to be less reproducible in unhealthy compared to healthy populations [24]. Therefore, it is important to know the reproducibility of the HRV parameters, as this will allow to compare the results obtained at different time points (under the same conditions) with a certain degree of confidence.

A relatively recent study [28] tested the day-to-day reproducibility (each test was performed 2 weeks apart at the same time of day) of different HRV-derived parameters in time and frequency domains assessed using an HRM under diverse orthostatic conditions (sitting and standing). In brief, their results were similar to those reported by Sandercock et al. [24] in their systematic review. They also observed a moderate reproducibility of the HRV-derived parameters during the abovementioned conditions after reviewing the literature [24]. Comparing the conditions (i.e., sitting vs standing), Williams et al. [28] observed a better reproducibility for the LF band than the HF band (under free-breathing conditions), which agreed with the results showed by Pitzalis et al. [41]. Furthermore, Williams et al. [28] demonstrated that the reproducibility of frequency domain HRV-derived parameters may be influenced by the spectral analysis algorithm used (i.e., autoregressive [AR] vs fast Fourier Transform [FFT]). Therefore, caution

is needed when comparing reproducibility results across studies using different spectral analysis algorithms as the comparisons may be biased. These results were in agreement with those obtained by Pichon et al. [42] (healthy adults) and by Chemla et al. [43] (unhealthy populations). This reinforces the idea that although AR and FFT provide 'the same end-point' (i.e., analyze the R-R signal), they should not be considered as interchangeable options [28] due to the discrepancies showed in the aforementioned studies. However, other studies [44–46] have suggested that AR and FFT algorithms provided similar reproducibility results in adolescents and adults. Therefore, more studies are needed addressing this comparison of AR vs FFT algorithms across different cohorts to elucidate the source of these disagreements. On the other hand, previous studies [6, 44] have reported that short-term HRV-derived parameters in the time domain (e.g., RMSSD) have shown a higher reproducibility compared to the short-term HRV-derived parameters in the frequency domain (e.g., HF band) in young populations. Moreover, HRV-derived parameters repeatability is affected by changes in mean HR, and even a minimal change in mean HR can significantly change HRV-derived parameters, therefore this aspect should be taken into account when assessing R-R signal in the same patient [47, 48].

Finally, although some HRV-derived parameters seem more reproducible than others, studies analyzing this issue sometimes used different cohorts, devices for obtaining the R-R signal [24], and/or software for the data processing (i.e., selection and analysis) and artefact detection [6, 7, 49]. Therefore, this could indicate that such HRV differences (e.g., inter-days and/or intra-day differences) may be attributable, among other reasons, to the biological differences of subjects rather than to the HRV-derived parameter itself. In summary, and considering all together, there is a necessity to understand better whether some of the HRV-derived parameters are more reproducible than others. Thus, it is mandatory to identify the most reproducible HRV-derived parameters considering different issues that have a direct influence (the health status of participants, the device used to assess the R-R signal, among others) to establish them as 'good markers' independently of the period between assessments.

R-R SIGNAL ARTEFACT CORRECTION AND ITS INFLUENCE ON HRV-DERIVED PARAMETERS

Once the R-R signal has been measured, the data is transferred to a computer for further processing [8]. Importantly, both the sampling frequency and the accuracy of the algorithm that "searches" the R wave (i.e., R peak) are fundamental factors for obtaining an accurate R-R signal. Theoretically however, good algorithms are not so dependent on sampling frequency, and thus, a specific and high-quality HRV software is mandatory. This specific software allows the selection of the period (e.g., usually a 5 min period for short-term recordings) which will then be analyzed and

used to derive the HRV parameters [1]. However, as certain artefacts that could negatively influence the R-R signal (and thus the HRV-derived parameters) may appear, artefact correction procedure has to be performed [8].

In a perfect situation, the R-R signal would be measured including only normal-to-normal (N-N) intervals, or in other words, "pure" sinus beats. Unfortunately, the R-R signal obtained from either HRM or ECG presents, in most cases, a diverse number of artefacts that may have a "technical" or a "biological" source [8]. When using HRM, technical artefacts may be introduced by a poor placement of the HRM band, movements from the subject, or may even be caused by sweating during the assessment. However, these technical artefacts in short-term R-R signal recordings, under well controlled and stable conditions, may not be common. On the other hand, biological artefacts may be introduced by ectopic beats or "abnormal" heart rhythms, which may appear even in healthy individuals [7, 8]. ECG verification is necessary to determine the presence of sinus rhythm, the presence or absence of arrhythmia (the most difficult are "late" supraventricular premature beats), the presence or absence of artifacts, and the origin of the shortest and longest R-R intervals. Besides, HR turbulence (visible in R-R intervals), is an interesting indicator to identify patients with autonomic dysfunction or impaired baroreflex sensitivity. However, the current manuscript is not focused on heart rate turbulence. Regardless if artefacts are technical or biological, they represent an important problem as could influence either the HRV-derived parameters or their reproducibility [8]. In this scenario, short-term R-R recordings contains approximately 300 R-R intervals [8]. From these R-R intervals, and although a small amount of them ($\leq 5\%$) might be interpolated or deleted because of artefacts, they could affect the quantification of HRV-derived parameters in both time and frequency domain [8].

For the abovementioned reasons, an appropriate R-R signal artefact correction procedure is needed. Unfortunately, nowadays there is no agreement regarding these procedures and what is the best way to correct such artefacts [8]. To handle the R-R signal data processing, diverse software can be used (e.g., Kubios, gHRV, ARTiiFACT, KARDIA, etc.) [49]. The Kubios software [10], is probably one of the most-frequently used tool in both clinical and research settings [7]. The Kubios software may correct the artefacts using their "threshold-based artefact correction" (or Kubios filters) algorithms [10]. In brief, these "filter" algorithms compare each R-R interval value against a local average interval (obtained by median filtering the R-R interval time series) which is not influenced by outliers R-R intervals [7]. Therefore, if such an interval differs from the local average interval more than a pre-defined threshold value (depending on the "intensity" of threshold-based artefact correction algorithm used), the interval is considered as an artefact and is marked for correction by the software [10]. The influence of the different threshold-based artefact correction algorithms has been recently studied

[7] in three different cohorts [7]. In their study, Alcantara et al. [7] concluded that the application of the Kubios threshold-based artefact correction algorithms had a significant influence on the quantification of the HRV-derived parameters obtained from short-term recordings in both, time and frequency domains. Moreover, although the study design precluded the “definitive” recommendation of a threshold-based artefact correction algorithm [7], the study suggested to use the Very Low, Low or Medium threshold-based artefact correction algorithms in children and young adults. In contrast, any threshold-based artefact correction algorithm (with caution when using the Very Strong) may be employed for middle-aged adults.

Importantly, in most cases, each study uses a different software and artefact correction procedure, which could lead to problems in between studies comparisons [8]. These problems may be related to the greater or lesser amount of filtered/interpolated R-R intervals, which may vary between each software [49]. Furthermore, the subject's (e.g., age, sex, health status, etc.) [50, 51], the length of recordings (e.g., short-term recordings vs ultra-short or 24 h recordings) [1,2], and even a researcher involved in the data selection and analysis of R-R signals [6] may influence the the comparison between studies. Regarding the “researcher influence”, previous studies have shown that both, the intra- and inter-researcher differences (i.e., intra- and inter-researcher reproducibility) in the data selection and analysis (using short-term recordings) process are not large [6, 44]. However, the inter-researcher variability could induce significant differences, being them clinically relevant, in the time domain HRV-derived parameters [6, 44]. Therefore, the HRV signal data selection and processing should be carried out by the same researcher in order to obtain more reproducible HRV-derived parameters [6, 44].

Considering it all together, studies are needed to determine the best software and procedures to correct artefacts that may be present on R-R signals, and, consequently, in the HRV-derived parameters, to establish more standardized recommendations for both clinicians and researchers.

OTHER METHODOLOGICAL CONSIDERATIONS FOR SHORT-TERM HRV RECORDINGS AT RESTING

We have described some methodological aspects that should be considered when HRV is measured. However, other methodological issues deserved some attention.

Previous conditions. Commonly, the previous conditions before the R-R signal assessment [52] are the following: (1) avoiding food intake (i.e., fasting), and coffee, tea, or caffeinated drinks intake at least 2 hours before the assessment; (2) avoiding intense physical activity at least 2 hours before; (3) not drinking alcoholic beverages at least 24 hours before the assessment; (4) sleeping as normal as possible (i.e., sleep duration and sleep schedule). Importantly,

no control of these issues could bias the HRV-derived parameters [52].

Body positioning. Importantly, while resting, the body position (lying, sitting or standing) during the assessment could directly influence the quantification of the HRV-derived parameters [53, 54]. Specifically, the change of the position from lying supine or seated to standing rest decreased the HRV-derived parameters (mainly in frequency domain) and increased the HR in healthy young adults [53]. The reason for this is that in a vertical position, the sympathetic tone is higher than vagal tone. In contrast, in a lying supine position, the vagal tone predominates over the sympathetic tone [55].

Respiration. “Controlling” the subject's respiration (i.e., respiratory frequency; the number of breaths per minute) during the HRV assessment has been a methodological issue with certain disagreement among researchers. In fact, nowadays there is no consensus in the literature [52]. It should be noted that the HRV parameters quantification could be affected by the respiratory frequency and its depth (the amount of air taken into the lungs; deep vs shallow breathing) [1, 52]. For example, the HF or the “respiratory” band reflects vagal tone from 0.15 to 0.40 Hz, which corresponds to a respiratory frequency from 9 to 24 breaths per minute [1, 52]. Thus, a respiratory frequency below 9 or above 24 breaths per minute (0.15–0.40 Hz respectively) may impair the vagal tone quantification using the HF band [1, 52]. Considering this, the HRV-derived parameters that reflect vagal tone (while resting) in the frequency domain (e.g., HF band) are more influenced by the respiratory frequency compared to these derived from the time domain (e.g., RMSSD) [56]. However, “controlling” the respiratory frequency, or in other words “telling the subjects to breath at a specific frequency”, is controversial. In this regard, removing the inherent variance associated with the respiration pattern could “artificially” remove the variance associated with the common neural origin of the respiration and HRV [52, 57]. Otherwise, the impact of the respiration frequency on the quantification of the HRV-derived parameters that reflect vagal tone is minimal when the HRV assessment is performed at resting state [58, 59].

Room conditions (temperature, humidity, light, time of day). Prior to assessing the R-R signal we should consider the ambient conditions of the room where the assessment will be performed as they may affect it. For example, the HRV-derived parameters decrease in response to heat stress in healthy adults [60, 61]. Similarly, 2 hours of passive heat exposure produced a reduction of the HRV-derived parameters that reflect vagal tone (e.g., RMSSD and HF) in healthy children [62]. Besides, sweat-induced fluid loss may increase cardiovascular work [63] due to dehydration, while appropriate hydration (i.e., higher total body water) during resting is positively associated with vagal tone [64]. On the other hand, it is recommended to assess the R-R signal using dim lighting because bright lights may affect the autonomic nervous system activity during the resting

assessments [65]. Thus, modifications in light intensity and color may have an impact on the HRV parameters [66, 67]. Lastly, it is known that time of day (morning vs night) might influence the HRV-derived parameters because vagal tone is increased at nighttime compared to the rest of the day [68]. Therefore, in general terms, the HRV-derived parameters decrease by day and tend to increase at night [68]. Nevertheless, another study [54] suggests the contrary. In fact, Vila et al. [54] observed that the HRV parameters decreased from morning to night. Further, they advise to recording the R-R signal (early) in the morning as diverse factors that may affect the HRV could be avoided (e.g., food, coffee and/or alcohol ingestion, fatigue, etc.).

Time interval data selection procedure. Studies using short-term recordings usually record the R-R signal during a period of 10 to 15 minutes. Then, either a pre-fixed interval (e.g., from the 10th to the 15th min period) or the “best quality period” (e.g., the 5 minutes period or higher quality) is selected to derive the HRV parameters [6, 69]. Although the criteria to select the “best period” are somehow *subjective*, this data selection procedure may be interesting. In fact, using this approach, the researcher select (after a visual inspection of the R-R signal) the best period based on the following criteria [6]: (1) less amount of large R-R interval outliers included in the selected period (those included will be then corrected using a specific HRV software); (2) R-R intervals equidistance; and, (3) R-R intervals distribution graphs as similar as possible to Gaussian distribution. Interestingly, it has been shown that the duration of the signal (i.e., using different pre-fixed intervals [e.g., 2, 5, 10,

or 15 minutes]) used to derive the HRV parameters directly impacted their quantification (the shorter the interval, the higher the random measurement error) [70]. Lastly, and before performing any statistical analysis, we should consider that the HRV-derived parameters are not normally distributed [52]. Therefore, the HRV-derived parameters are commonly transformed using the natural logarithm [71], although other transformations or “normalization” procedures have been proposed (e.g., \log_{10} , normal scores) [16, 72].

These methodological aspects that have been described should be extensively studied to determine their “real” impact on various HRV-derived parameters and their validity and reproducibility, to further establish standardized recommendations among the scientific community and general HRV users.

THE ROLE OF HR ON HRV ANALYSIS

The HRV parameters derived from R-R intervals are negatively correlated with an average HR. However, this relationship is both physiologically and mathematically determined [11–13]. The physiological mechanisms are based on the autonomic nervous system activity, but the mathematical one is caused by the non-linear (inverse) relation between R-R intervals and HR — **Figure 1** [73]. For that reason, slow HR usually exhibits higher HRV (i.e., higher variability of R-R intervals) than fast HR, and hence, the HRV analysis may be mathematically biased [11]. Moreover, if HRV is so strongly associated with HR, some of its clinical and physiological meanings must originate from HR [73]. In this sense, a recent viewpoint [74] has been

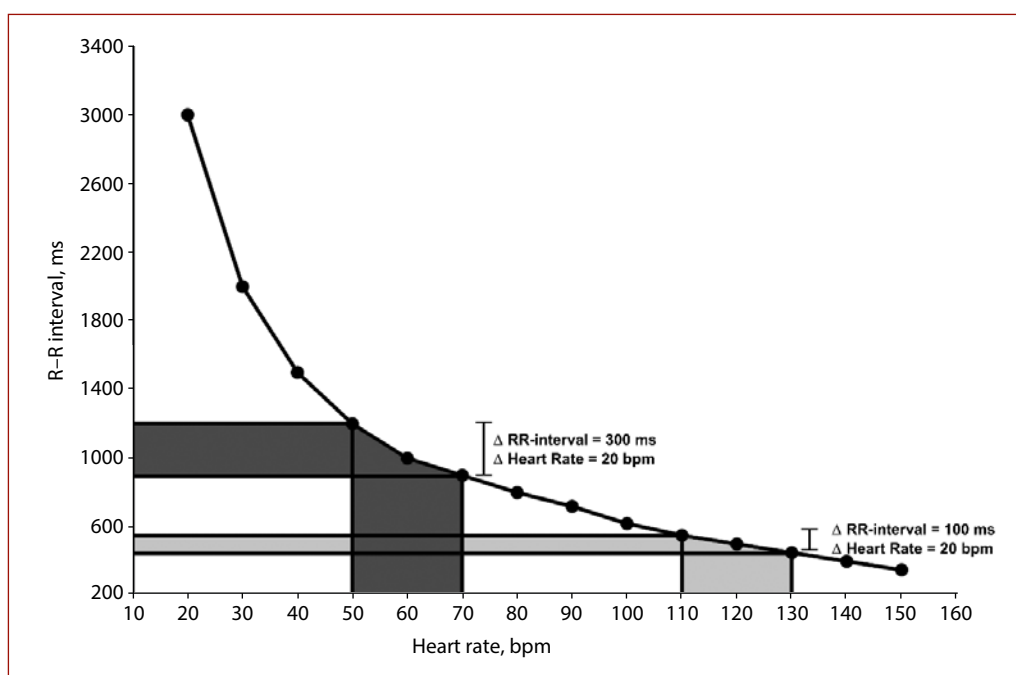


Figure 1. The non-linear (mathematical) relationship between R-R interval and heart rate. The oscillations of slow heart rate (x-axis, dark gray area; $\Delta 20$ bpm) result in greater oscillations of R-R intervals (y-axis, dark gray area; 300 ms) compared to the same heart rate oscillations of a fast heart rate (light gray area: x-axis $\Delta 20$ bpm and y-axis 100 ms) [13]. Modified from [12].

Abbreviations: ms, milliseconds; bpm, beats per minute

published highlighting this relationship between HRV and HR, where authors summarized that the HRV is essentially determined by HR and cannot be used “independently” of it to outline the cardiac autonomic tone [74]. Therefore, to explore the HR impact on HRV and check whether the differences in HRV between subjects presenting different HR are due to actual differences in variability or simply due to differences in HR, one should “correct” HRV for the prevailing HR (i.e., normalize the fluctuations with respect to the mean R-R interval) [73]. In fact, the mathematical bias may be removed by dividing the R-R interval signal by the average R-R interval, or by dividing the HRV parameters by appropriate powers of the average R-R interval (e.g., for spectral parameters the power should be 2) [11, 12]. However, in order to completely remove the HRV dependence on HR (i.e., even the physiological association), one should divide the HRV parameters by higher powers of the mean R-R interval [73]. On the other hand, one may also strengthen the HRV dependence on HR by multiplying R-R interval signals or HRV parameters by average R-R intervals [73]. Such approaches allow researchers to explore the HR contribution to the physiological and clinical significance of HRV [75, 76].

Several studies employed the abovementioned correction methods [73] to explore the interaction between HR

and its variability. In particular, the relationship between HRV and HR is crucial when studying cohorts with different HR, such as men and women. In fact, rapid HR has been shown to overshadow the prognostic value of HRV in women; however, it may be uncovered if the effect of HR is excluded [77]. In other words, the normalization procedure and the exclusion of the influence of HR can act as a “magnifying glass” for HRV in females with fast HR (Figure 2) and enables us to see more prognostic information. On the other hand, the removal of the HR impact on HRV may diminish or even eliminate the clinical value of HRV in conditions or populations where HR is a strong risk factor [73, 75, 77]. In such cases, the prognostic value of HRV can be improved by strengthening the relationship between HRV and HR, and it has indeed been shown that multiplying the HRV-derived parameters by the mean R-R interval increases the ability of HRV to predict mortality in men, where HR was a strong risk factor for cardiac death [77].

Some studies employed the correction method approach [78] to investigate the associations of HRV-derived parameters with CRF [16] and body composition measures [79] in overweight or obese children. Interestingly, after correcting the HRV-derived parameters by HR (i.e., HRV-derived parameters divided by average R-R interval) all the associations observed between HRV-derived parameters,

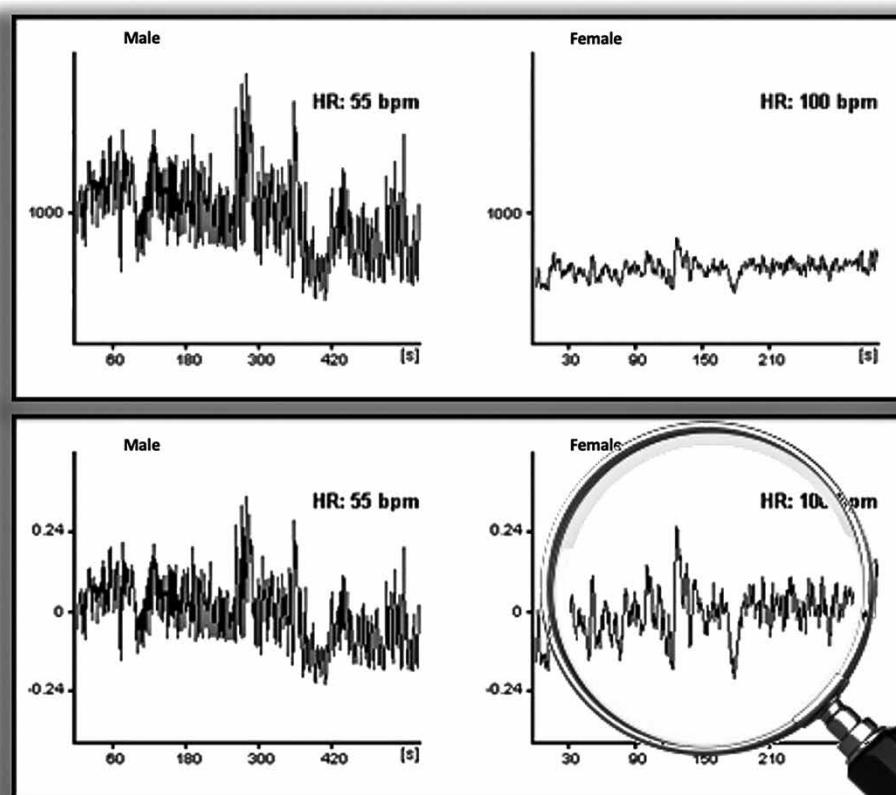


Figure 2. The R-R interval signals taken from a man and a woman. The upper panel shows the original signals where the male heart rhythm is slow (mean HR = 55 bpm), but the female one is fast (mean HR = 100 bpm), consequently, the R-R interval fluctuations have higher amplitudes (i.e., higher HRV) in the man than in the woman. However, after the normalization to the average R-R interval (lower panel), both rhythms present comparable amplitudes — the normalization procedure acts like a magnifying glass for the variability of fast HR

CRF, and body composition disappeared [16, 79]. Similarly, Grant et al. [80] concluded similar results addressing the associations between HRV-derived parameters corrected by HR (i.e., HRV-derived parameters divided by average R-R interval) and CRF in healthy young adults, meaning HR is more strongly associated with CRF compared to any HRV-derived parameter. The conclusion reported by Grant et al. [80] was recently confirmed by an experiment conducted in healthy young adults [81]. Likewise, another recent study showed how most of the associations between cardiometabolic syndrome markers (i.e., glucose, triglycerides, etc.) and HRV-derived parameters disappeared after including HR as a covariate in multiple regression models in three independent human cohorts (especially in children and young adults) [82]. In summary, the associations of HRV parameters with CRF, body composition and metabolic syndrome markers were partially explained by HR in children with weight disturbances and healthy young adults [16, 79–83].

CONCLUSION

In conclusion, researchers and clinicians should consider several methodological aspects to appropriately quantify and interpret the HRV-derived parameters at resting conditions using data from short-term recordings (Figure 3). Finally, and based on the possible influence that these methodological issues may have on the R-R signal deter-

mination and the HRV-derived parameters, we encourage researchers to describe the R-R signal acquisition and processing in detail, this would allow better comparisons across studies and robust conclusions when the short-term HRV-derived parameters are used.

Article information

Conflict of interest: None declared.

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Methodological considerations for short-term R-R interval signal recording using a heart rate monitor

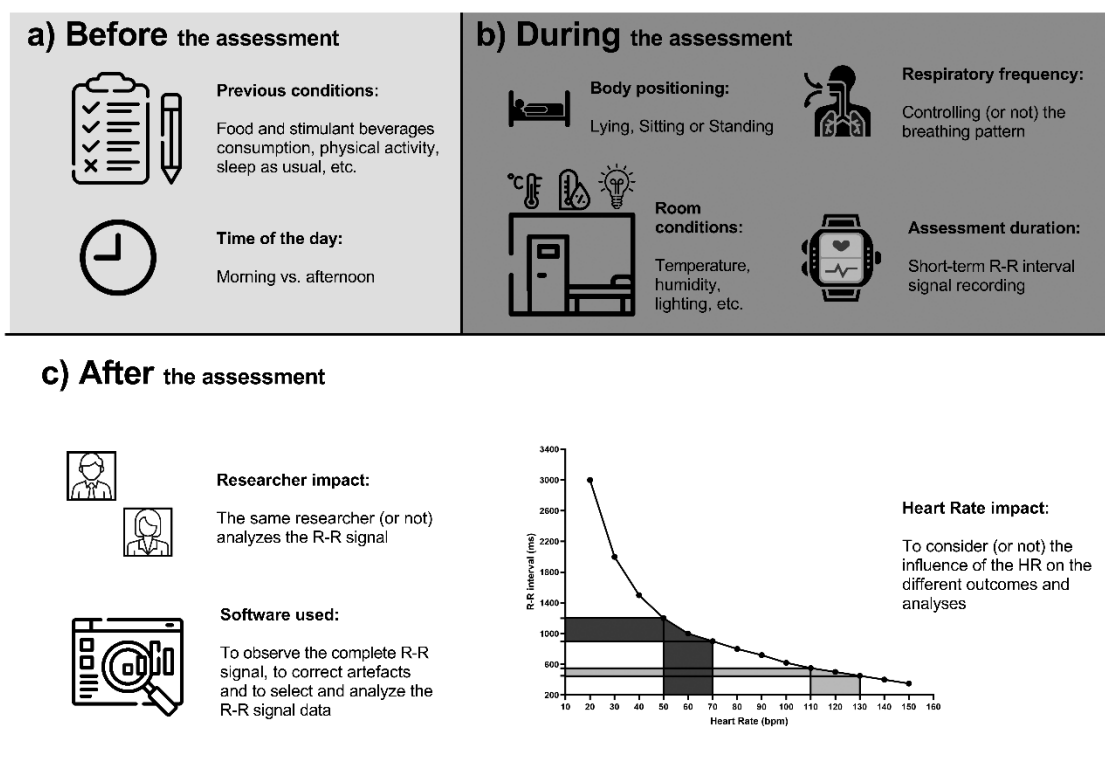


Figure 3. Methodological aspects that should be considered to appropriately assess the R-R signal; **A.** (light gray) before the assessment. **B.** (dark gray) during the assessment. **C.** (white) after the assessment

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