#### **Original Article**

# One-Minute Deep Breathing Assessment and its Relationship to 24-h Heart Rate Variability Measurements

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## **Abstract**

**Background:** Heart rate variability (HRV), the change in the time intervals between successive pairs of heartbeats, is influenced by interdependent regulatory systems operating over different time scales to adapt to psychological challenges and environmental demands. Low ageadjusted HRV is predictive of upcoming health challenges in healthy people as well as a wide range of diseases in patients and correlates with allcause mortality. 24h HRV recordings are considered the "gold standard" and have greater predictive power on health risk than shortterm recordings. However, it is not typically costeffective or practical to acquire 24h HRV recordings. This has led to the growing use of shortterm recordings in research and clinical assessments. **Objective:** The first study examined the correlations between a 10min restingstate period, a 1min paced deep breathing protocol, response to handgrip, and 24h HRV measures in 28 healthy individuals. Based on the results of the initial study, the primary study examined the correlations between the 1min paced deep breathing assessment and 24h measures in a general population of 805 individuals. **Results:** The highest correlations for the HRV variables were with the vagally mediated sources of HRV. The 1min paced deep breathing was positively correlated with 24h highfrequency power (r = 0.60, P < 0.01), root mean square of successive difference (r = 0.62, P < 0.01), lowfrequency (LF) power (r = 0.64, P < 0.01), veryLF power (r = 0.57, P < 0.01) total power (r = 0.42, P < 0.01), standard deviation of normaltonormal interval (SDNN) index (r = 0.59, P < 0.01), and SDNN (r = 0.41, P < 0.01). **Conclusions:** The findings from this study suggest that the 1min paced deep breathing protocol is an ideal shortterm assessment that can be used in a health risk screening context. When low values are observed, it is recommended that a 24h assessment be conducted.

Keywords: Deep breathing, heart rate variability, risk assessment

# INTRODUCTION

The investigation of heart rate variability (HRV)<sup>[1]</sup> has rapidly expanded in more recent years. HRV is thus considered a measure of neurocardiac function that reflects heart—brain interactions and autonomic nervous system dynamics.<sup>[2,3]</sup> An appropriate overall amount of HRV for one's age reflects an intrinsic capacity for self-regulatory capacity, adaptability and resilience, and healthy function.<sup>[3-9]</sup> Whereas too little variation indicates pathology, chronic stress, and dysregulation of self-regulatory control systems.<sup>[1,10]</sup> HRV declines with age, and age-adjusted values should be used in the assessment of health risk.<sup>[11]</sup>

Reduced HRV was shown to be a greater risk predictor of postmyocardial infarction death than other known risk factors, [12] and is associated with diabetic autonomic neuropathy before the onset of symptoms. [13-15] Low age-adjusted HRV is an independent predictor of upcoming health challenges in

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healthy people as well as a wide range of diseases in patients and correlates with all-cause mortality. [16,17] Numerous studies have found that lowered HRV is associated with high levels of inflammation in patients without heart disease. [18] Low levels of HRV are also found in cases of autonomic dysfunction, depression, anxiety, asthma, and sudden infant death syndrome. [19-22]

Normal HRV levels indicate behavioral flexibility and psychological resiliency and capacity to effectively self-regulate and adapt to the changing environmental and social demands. [23,24] Vagally mediated HRV has been linked with self-regulatory capacity, [8,9,25] emotional regulation, [26,27]

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social interactions, <sup>[6,28]</sup> one's sense of coherence <sup>[29]</sup> the character traits of self-directedness, <sup>[30]</sup> and coping styles. <sup>[31]</sup>

# HEART RATE VARIABILITY ANALYSIS

The most commonly used analytical approaches to assessing HRV are frequency-domain (power spectral density) and time-domain analysis. The main advantage of spectral analysis is that it provides both amplitude and frequency information on the various rhythms that occur within the HRV waveform, allowing these oscillations to be quantified over any time. The international Task Force standardized the HRV oscillations into the following four primary frequency bands: high frequency (HF), low frequency (LF), very LF (VLF), and ultra LF (ULF). [32] The values are expressed as the power spectral density. [33]

The HF range reflects parasympathetic or vagal activity and is frequently called the respiratory band because it corresponds to the variations related to respiratory sinus arrhythmia.

The LF range primarily reflects baroreceptor activity while at rest. [34] In 24-h ambulatory recordings, it has been observed that this band reflects various degrees of sympathetic activity. [35] The LF/HF ratio has been proposed to reflect the balance between sympathetic and parasympathetic activities. [36-38] However, this perspective has challenged by a number of researchers who have argued persuasively that in resting states, the LF band reflects only baroreflex activity, not sympathetic activity. [33,39-42] In long-term ambulatory recordings, the LF band fairly approximates sympathetic activity when increased sympathetic activity occurs.

The VLF is the power in the range between 0.0033 and 0.04 Hz, and 24-h measures reflecting low VLF power are linked with an increased risk of adverse outcomes. The VLF band has a stronger association with all-cause mortality than the LF and HF bands. [17,43-45] Experimental evidence suggests that the source of VLF rhythm is generated intrinsically by the heart's intrinsic cardiac nervous system and that the amplitude of these oscillations is modulated by sympathetic efferent activity. [33]

The ULF range is activity below 0.0033 Hz. The circadian rhythm in heart rate (HR) is the primary source of the ULF rhythm, although other slower regulatory processes can affect this band.

#### TIME-DOMAIN MEASUREMENTS

Time-domain measure quantifies the variance in the inter-beat-intervals (IBIs) using statistical measures. The most commonly reported time-domain measures are the standard deviation of normal-to-normal interval (SDNN), root mean square of successive differences (RMSSD), and the SDNN index. The SDNN is the standard deviation of the normal-to-normal (NN) sinus-initiated IBIs measured in milliseconds. This measure reflects the contribution of all the sources that add to HRV. In short-term resting-state recordings, the source of the variations is primary vagally mediated. The SDNN index (mean of the standard deviations of all the NN

intervals for each 5-min segment) estimates variability due to factors affecting HRV within a 5-min period.

The RMSSD is the root mean square of successive differences between normal heartbeats. The RMSSD is the primary time-domain measure used to estimate the vagally mediated HRV and is correlated with HF power.<sup>[2]</sup>

The mean HR range (MHRR) is calculated by averaging the differences between the maximum HR during inspiration and the minimum HR during expiration for each breathing cycle over the 1-min test duration, typically 5–6 breaths. The mean IBI range (MIBIR) is calculated the same as MHHR only using IBIs in milliseconds. This avoids the potential influence of the rate transformation to beats-per-minute used in the calculation of MHHR.

The expiratory-to-inspiratory ratio (E:I ratio) is the ratio of the longest R-R interval during expiration to the shortest R-R interval during inspiration. The mean of the ratios for each breathing cycle over the 1-min test duration was used in this study.

# RECORDING LENGTHS

HRV recording lengths can be obtained over periods that range from 1-min to weeks, although the most common short-term recording length is 5-min, while the most common long-term period is 24-h. The length of the recording period significantly affects HRV values, [46] and it is inappropriate to compare any HRV metrics when they are obtained from different recording lengths. [47] In addition, the context in which the recording is made also significantly affects the values, such as resting state or ambulatory, seated or supine. 24-h HRV recordings provide the best assessment of the VLF and ULF rhythms. [48]

Obviously, longer recording periods provide more information regarding autonomic function, health status, stress reactions, and environmental influences than is possible in short-term recordings. For example, 24-h HR, responses to stressors, workloads, different aspects of circadian rhythms, differences in day–night HR, sleep–wake cycles, and dream activity can only be observed in 24-h recordings. Thus, 24-h HRV recordings are considered the "gold standard" for clinical HRV assessment<sup>[47]</sup> and have greater predictive power or health risk than short-term recordings,<sup>[48,49]</sup> which typically do not correlate well with 24-h recordings.<sup>[50]</sup>

Of course, it is not typically cost-effective or practical to obtain 24-h recordings in research, clinical, mental health, or large-scale health risk assessment contexts. Therefore, short-term recordings are currently being used in research and clinical contexts and more recently in consumer applications.

Another approach to short-term HRV assessments evolved from protocols developed for autonomic function assessments in diabetic patients called HR response to deep breathing. [51,52] For this assessment, the patient sits quietly and breathes deeply and evenly at a rate of six breaths per minute for three

successive breathing cycles. The maximum and minimum HRs during each breathing cycle are measured and expressed as the maximum and minimum differences in HR. This assessment was found to have a better diagnostic utility than the Valsalva maneuver in diabetic patients, lying-to-standing HR response, postural blood pressure change, and sustained handgrip test.<sup>[51]</sup> It was found that using a 1-min paced deep breathing at six breaths per minute protocol as a prognostic index after myocardial infarction as an assessment of HRV was a good predictor for all-cause mortality and sudden death in this population.<sup>[53]</sup> Therefore, it is considered one of the most reliable tests of cardiovagal function.<sup>[54]</sup>

The two most widely used metrics for deep breathing assessment are the MHRR and the E:I ratio. The MHRR is assessed from a series of deep breaths, at a rate of six breaths per minute. The difference between the minimum and maximum HR during each cycle is expressed as the mean of the HR differences in beats per minute.<sup>[55]</sup> The E:I ratio assesses the ratio of the longest R-R interval during expiration to the shortest R-R interval during inspiration.<sup>[56]</sup> In essence, the 1-min deep breathing assessment is a type of "challenge test" that assesses the maximum amount of parasympathetic-mediated HRV the autonomic system is capable of producing at the time of the measurement. In a study with 293 participants ranging in age from 10 to 82 years, the 5-min resting HRV and 1-min paced deep breathing assessment were compared on both time- and frequency-domain measures and the MHHR and E:I ratios. It was found that the maximum variation of HR measures in the 1-min deep breathing test had the highest negative correlations with age as compared to all the HRV parameters in the 5-min resting assessment.[57] In a pilot study, the MHRR of 486 healthy workers was measured with the 1-min paced breathing protocol and compared with the outcomes of a worker's health assessment. In this cross-sectional study, the MHRR statistically significantly correlated (P < 0.001) with multiple health risks and had interesting diagnostic value for screening of metabolic syndrome and reported general health risks.<sup>[58]</sup>

We are only aware of one study that has examined the correlations between short-term and 24-h measures of HRV, which was conducted in a population of patients with confirmed myocardial infarction. The correlation between a 5-min resting state recording and 24-h measures was relatively poor (r=0.51), although it was significant. At 1-year follow-up, both the short- and long-term measures were significantly lower in patients who died than in survivors. However, the long-term assessment was clearly superior to the resting-state, short-term assessment in predicting risk. The authors suggested that short-term recordings should be used for all patients and a 24-h assessment should be conducted in people with depressed short-term HRV values. [50]

# METHODS AND PROCEDURES

In the studies reported here, we examined the correlations between short-term resting-state HRV measures, the 1-min paced

deep breathing assessment, and 24-h measures. Two studies were conducted. The first was a smaller laboratory-based pilot study (n = 28) with healthy individuals that compared a 10-min resting-state period, 1-min resting state (1-min average of the 10-min recording), HRV response to handgrip exercise, the 1-min paced deep breathing assessment, and 24-h measures. The second primary study examined the correlations between the 1-min paced deep breathing assessment and 24-h measures in a general population (n = 805) of individuals, independent of health status.

#### **PARTICIPANTS**

The participants in the pilot study (n = 28) were healthy volunteers who were employees of one of the two HeartMath organizations located in Boulder Creek, CA, USA. Nearly 70% were female (17 females and 11 males). The group as a whole had a mean age of 55 years (range, 25–64 years). Those with a known health disorder, or who took medications known to affect autonomic function, were excluded from the study.

For the primary study, the participants (n = 805) were recruited from a general population of individuals, independent of health status, who were attending one of a series of self-development conferences in various cities such as Cabo, Bon, and Tacoma. Nearly 73% were female (596 females and 213 males). The mean age was 50.1 years (range, 19–89 years). There were no exclusion criteria, other than agreeing to sign the informed consent form. The research met all applicable standards for the ethics of experimentation in accordance with the Declaration of Helsinki. All participants signed informed consent and were free to withdraw from the study at any time.

# HEART RATE VARIABILITY DATA COLLECTION

All participants in both studies underwent 24-h ambulatory HRV recordings (Bodyguard2, Firstbeat Technologies Ltd., Jyväskylä, Finland). The participants were instructed how to stop the recorder at the end of the 24-h recording period. Ambu Blue Sensor L microporous breathable disposable electrodes (Columbia, MD, USA) were used for all the recordings. The electrodes were placed in a modified V5 position. The HRV recorder calculates the IBI from the electrocardiogram sampled at 1000 Hz. The RR interval data were stored locally in the device memory and downloaded to a computer workstation at the completion of the recordings.

All the HRV recordings were analyzed using DADiSP 6.7 (Newton, MA, USA). IBIs greater or less than 30% of the mean of the previous four intervals were considered artifacts and were removed from the analysis record. Following an automated editing procedure, all the recordings were manually reviewed by an experienced technician and, if needed, corrected. Daily recordings were processed in consecutive 5-min segments in accordance with the standards established by the HRV Task Force.<sup>[59]</sup> Any 5-min segment with >10% of the IBIs either missing or removed in editing was excluded from the analysis.

# PILOT STUDY

The participants completed a 10-min resting-state recording while seated upright in a comfortable chair, after which they did the 1-min HRV deep breathing assessment, followed by a 2-min handgrip exercise shortly after the 24-h HRV recorder was connected. The ECG was recorded (Biopac MP 30, Goleta, CA, USA) at a sample rate of 250 Hz during each segment of the protocol. For the resting-state recording, the participants were instructed to sit quietly for 10-min without talking, chewing gum, reading, etc., trying to remain as still as possible without sacrificing comfort. They were instructed not to meditate or use other similar practices and not to engage in intense mental or emotional activity and to keep their eyes open to help avoid falling asleep. For the 1-min paced deep breathing, the participants were instructed to breathe as deeply as they comfortably can at the rhythm shown on a breath-pacing screen (BreathPacer, Larva Labs Ltd., New York, USA) on an i-Pad screen, which was a 10-s rhythm (5 s on the inbreath and 5 s on the outbreath). The pacing period lasted for 1-min (six respiration cycles). Some people needed a practice session before successfully completing the deep breathing aspect of the protocol. For the handgrip segment of the protocol, each participant's maximal grip strength was first determined (Biopac MP3X dynamometer) from two brief contractions with their nondominant hand. Subsequently, the participants performed a sustained handgrip for 2-min at 35% of their maximal grip strength. This was typically an exhaustive exercise.

## PRIMARY STUDY

For the primary study, all participants were fitted with and wore an ambulatory HRV recorder for 24 h. At the start of the recording period, the participants were instructed the 1-min paced deep-breathing protocol as described above. The only difference was that the participants were not given a practice session.

#### **Statistics**

Correlation coefficients and *P* values were calculated for all 24-h and short-term HRV measures (IBM SPSS version 22, Armonk, New York, USA). Correlations for the pilot study are presented in Table 1, and correlations for the primary study are presented in Table 2.

# RESULTS Pilot study

As shown in Table 1, all the HRV assessments tested had significant, negative correlations with age. The highest

	1	2	3	4	5	6	7	8
Age, y	1	-0.08	-0.48**	-0.65**	-0.59**	-0.62**	-0.48*	-0.56**
24 h								
Mean IBI, ms	-0.08	1	0.34	0.41*	0.39*	0.44*	0.62**	0.56**
SDNN, ms	-0.48**	0.34	1	0.68**	0.68**	0.71**	0.82**	0.81**
RMSSD, ms	-0.65**	0.41*	0.68**	1	0.92**	0.76**	0.71**	0.79**
Ln 5-min High frequency, ms <sup>2</sup>	-0.59**	0.39*	0.68**	0.92**	1	0.86**	0.78**	0.86**
Ln 5-min Low frequency, ms <sup>2</sup>	-0.62**	0.44*	0.71**	0.76**	0.86**	1	0.88**	0.95**
Ln 5-min Very low frequency, ms <sup>2</sup>	-0.48*	0.62**	0.82**	0.71**	0.78**	0.88**	1	0.98**
Ln 5-min Total power, ms <sup>2</sup>	-0.56**	0.56**	0.81**	0.79**	0.86**	0.95**	0.98**	1
1-min Deep Breathing								
Mean IBI, ms	0.10	0.52**	0.40*	0.13	0.11	0.06	0.45*	0.32
SDNN, ms	-0.57**	0.19	0.66**	0.71**	0.74**	0.72**	0.64**	0.70**
RMSSD, ms	-0.56**	0.22	0.69**	0.71**	0.72**	0.74**	0.67**	0.72**
MHRR, bpm	-0.49**	0.26	0.58**	0.73**	0.77**	0.75**	0.66**	0.72**
10-min Resting								
Mean IBI, ms	-0.07	0.56**	0.37	0.14	0.13	0.23	0.52**	0.42*
SDNN, ms	-0.51**	0.17	0.55**	0.46*	0.52**	0.61**	0.60**	0.61**
RMSSD, ms	-0.68**	0.19	0.63**	0.70**	0.74**	0.78**	0.65**	0.73**
Ln High frequency, ms <sup>2</sup>	-0.53**	-0.05	0.48**	0.60**	0.71**	0.70**	0.51**	0.60**
Ln Low frequency, ms <sup>2</sup>	-0.41*	0.08	0.31	0.30	0.38*	0.50**	0.39*	0.44*
Ln Very low frequency, ms <sup>2</sup>	-0.07	0.25	0.36	0.18	0.20	0.20	0.46*	0.36
Ln Total power, ms <sup>2</sup>	-0.39*	0.12	0.42*	0.38*	0.49**	0.50**	0.52**	0.53**
Handgrip Test (1-min)								
Mean Grip Strength, Lbs.	-0.28	0.03	0.20	0.12	0.21	0.26	0.36	0.33
Mean IBI, ms	0.08	0.44*	0.24	0.10	0.05	0.06	0.31	0.23
Ln High frequency, ms <sup>2</sup>	-0.53**	0.00	0.46*	0.55**	0.58**	0.58**	0.46*	0.53**
Ln Low frequency, ms <sup>2</sup>	-0.46*	0.28	0.37	0.56**	0.51**	0.58**	0.54**	0.58**
Ln Total power, ms <sup>2</sup>	-0.50**	0.31	0.40*	0.63**	0.58**	0.63**	0.57**	0.61**

n=28, \*P<0.05, \*\*P<0.01

correlations were with the 24-h measures of LF and HF power (r = -0.62 and -0.59, respectively, P < 0.01) followed by total power (TP) (r = -0.56, P < 0.01), and VLF power (r = -0.48, P < 0.05). The 1-min deep breathing assessment had the next highest negative correlations: SDNN (r = -0.57, P < 0.01), RMSSD (r = -0.56, P < 0.01), and MHHR (r = -0.49, P < 0.01). The correlations in the 10-min resting state and handgrip assessment with age had similar results for HF power (r = -0.53, P < 0.01). The LF power was r = -0.41, P < 0.01, for the 10-min resting state, and r = -0.46, P < 0.01, for the handgrip assessment. The VLF power for the resting state did not significantly correlate with age.

#### One-minute paced deep breathing

Overall, the 1-min paced deep breathing had the highest correlations with the 24-h measures. The SDNN correlated with 24-h HF power (r=0.74, P<0.01), LF power (r=0.72, P<0.01), VLF power (r=0.64, P<0.01), TP (r=0.70, P<0.01), RMSSD (r=0.71, P<0.01), and SDNN (r=0.66, P<0.01). Similarly, the RMSSD correlated with HF power (r=0.72, P<0.01), LF power (r=0.74, P<0.01), VLF power (r=0.67, P<0.01), TP (r=0.72, P<0.01), and SDNN (r=0.69, P<0.01). The MHHR also highly correlated with HF power (r=0.77, P<0.01), LF power (r=0.75, P<0.01), VLF power (r=0.73, P<0.01), LF power (r=0.72, P<0.01), RMSSD (r=0.73, P<0.01), and SDNN (r=0.58, P<0.01).

#### **Ten-minute resting state**

In the 10-min resting-state assessment, the HF power correlated with 24-h HF ( $r=0.71,\ P<0.01$ ), LF ( $r=0.70,\ P<0.01$ ), VLF ( $r=0.51,\ P<0.01$ ), TP ( $r=0.60,\ P<0.01$ ), RMSSD ( $r=0.60,\ P<0.01$ ), and SDNN ( $r=0.48,\ P<0.01$ ). LF power correlated with 24-h LF ( $r=0.50,\ P<0.01$ ), VLF power ( $r=0.39,\ P<0.05$ ), and TP ( $r=0.44,\ P<0.05$ ) but did not correlate with the 24-h RMSSD or SDNN. The only correlation of the VLF power in the resting-state recording was

with 24-h VLF (r = 0.46, P < 0.05). The TP correlated with 24-h HF (r = 0.49, P < 0.01), LF (r = 0.50, P < 0.01), VLF (r = 0.52, P < 0.01), TP (r = 0.53, P < 0.01), RMSSD (r = 0.38, P < 0.05), and SDNN (r = 0.42, P < 0.05).

#### Handgrip

During the handgrip assessment, the HF power correlated with 24-h HF (r=0.58, P<0.01), LF (r=0.58, P<0.01), VLF (r=0.46, P<0.01), TP (r=0.53, P<0.01), RMSSD (r=0.55, P<0.01), and SDNN (r=0.46, P<0.05). LF power correlated with 24-h HF (r=0.51, P<0.01), LF (r=0.58, P<0.01), VLF power (r=0.54, P<0.05), TP (r=0.58, P<0.05), and RMSSD (r=0.56, P<0.01). The TP correlated with 24-h HF (r=0.58, P<0.01), LF (r=0.63, P<0.01), VLF (r=0.72, P<0.01), TP (r=0.61, P<0.01), RMSSD (r=0.63, P<0.01), and SDNN (r=0.40, P<0.05).

Based on the results of the pilot study, we chose to use the 1-min paced deep breathing protocol in the primary study.

#### **Primary study**

All the HRV assessments with the exception of IBIs in the 24-h assessments had significant, negative correlations with age [Table 2]. The highest correlations were with the LF (r = -0.521, P < 0.01) and HF power (r = -0.506, P < 0.01) followed by TP (r = -0.455, P < 0.01), SDNN index (r = -0.436, P < 0.01), RMSSD (r = -0.427, P < 0.01), and VLF power (r = -0.377, P < 0.05).

For the correlations between the 1-min paced deep breathing assessment and 24-h measures, the highest correlations were with mean IBIs (r = 0.761, P < 0.01) and its related measure HR (r = 0.756, P < 0.01).

The highest correlations for the HRV variables were with the vagally mediated sources of HRV. The 1-min paced deep breathing RMSSD positively correlated with 24-h HF power (r = 0.60, P < 0.01), RMSSD (r = 0.62, P < 0.01), and LF power (r = 0.64, P < 0.01). It also correlated with

Table 2: HRV	Correlations	for P	rimary	Study
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		1-Minute HRV Deep Breathing							
	Age	HR	IBI	SDNN	LnRMSSD	MIBIR, ms	MHRR, bpm	Elratio	
Age	1	-0.21**	0.18**	-0.41**	-0.40**	-0.51**	-0.50**	-0.42**	
24-h HRV									
Heart Rate, BPM	-0.14**	0.76**	-0.76**	-0.23**	-0.25**	-0.19**	0.39**	0.13**	
Inter-Beat Interval, ms	0.09**	-0.73**	0.76**	0.27**	0.29**	0.22**	-0.35**	-0.10**	
SDNN, ms	-0.27**	-0.27**	0.29**	0.40**	0.41**	0.36**	0.11**	0.24**	
SDNN index	-0.44**	-0.32**	0.35**	0.56**	0.59**	0.51**	0.21**	0.38**	
5-min Ln RMSSD	-0.43**	-0.27**	0.30**	0.55**	0.62**	0.52**	0.27**	0.42**	
Ln Total Power	-0.28**	-0.24**	0.27**	0.41**	0.42**	0.37**	0.15**	0.27**	
Ln Ultralow frequency, ms2Hz	-0.23**	-0.21**	0.23**	0.34**	0.35**	0.31**	0.11**	0.22**	
Ln 5-min Total Power, ms <sup>2</sup> Hz	-0.46**	-0.33**	0.36**	0.59**	0.62**	0.54**	0.25**	0.41**	
Ln 5-min Very low frequency, ms <sup>2</sup> Hz	-0.38**	-0.41**	0.43**	0.53**	0.57**	0.49**	0.13**	0.32**	
Ln 5-min Low frequency, ms <sup>2</sup> Hz	-0.52**	-0.18**	0.20**	0.61**	0.64**	0.58**	0.40**	0.52**	
Ln 5-min High frequency, ms2Hz	-0.51**	-0.11**	0.13**	0.55**	0.60**	0.52**	0.40**	0.49**	
Ln Low frequency/High frequency ratio	0.15**	-0.01	0.00	-0.18**	-0.24**	-0.18**	-0.18**	-0.19**	

n=805, \*P<0.05, \*\*P<0.01

VLF power (r = 0.57, P < 0.01), TP (r = 0.42, P < 0.01), SDNN index (r = 0.59, P < 0.01), and SDNN (r = 0.41, P < 0.01).

The MIBIR, expressed in ms, also highly correlated with the 24-h vagally mediated variables such as HF power (r = 0.52, P < 0.01), RMSSD (r = 0.52, P < 0.01), and LF power (r = 0.58, P < 0.01). It also correlated with VLF power (r = 0.49, P < 0.01), 5-min TP (r = 0.54, P < 0.01), TP (r = 0.37, P < 0.01), SDNN index (r = 0.51, P < 0.01), and SDNN (r = 0.36, P < 0.01).

The 1-min paced deep breathing SDNN correlated with 24-h HF power (r = 0.55, P < 0.01), LF power (r = 0.61, P < 0.01), VLF power (r = 0.53, P < 0.01), TP (r = 0.59, P < 0.01), RMSSD (r = 0.55, P < 0.01), SDNN index (r = 0.56, P < 0.01), and SDNN (r = 0.40, P < 0.01).

# DISCUSSION AND CONCLUSIONS

We examined the correlations between HRV measures during a short-term resting state, 1-min paced deep breathing, handgrip, and 24-h measures. In the pilot study, which was conducted at our laboratory with known healthy individuals, we were able to ensure that all the protocols were carefully followed. This was especially important for the 1-min paced deep breathing assessment, as it is important that the participants breathe as deeply as they comfortably can during the assessment. We found that many of the participants required a practice session before being able to get familiar with breathing as deeply as they comfortable could at the six breaths per minute rhythm.

In essence, the 1-min paced deep breathing assessment determines the practical maximum HRV the cardiorespiratory system is capable of producing at the time of the assessment. This requires the participant to breathe at the resonant frequency of the cardiorespiratory system and to breathe as deeply as they comfortably can to maximize respiratory drive. [60] Resonance occurs in an oscillatory system when there is a large sudden increase in amplitude at a specific frequency. Most mathematical models show that the resonance frequency of the human cardiovascular system is determined by the feedback loops between the heart and brain<sup>[61-63]</sup> and is approximately 0.1 Hz. Resonance is an aspect of the HRV coherence state, which is associated with a shift in autonomic balance toward increased parasympathetic activity, increased heart-brain synchronization, increased vascular resonance, and entrainment between diverse physiological oscillatory systems.[3,4,39]

Overall, the findings from the controlled pilot study suggest that the 1-min paced deep breathing assessment not only had the highest correlations with the 24-h measures of vagally mediated HRV, but also had slightly better correlations with VLF power than the 10-min resting HRV.

The primary study was undertaken to increase generalizability of the pilot study's findings with respect to the 1-min paced deep breathing assessment. The SDNN, RMSSD, and MIBIR

had stronger correlations with the 24-h measures than the more widely used MHHR and E:I ratios. As the SDNN and RMSSD are commonly used and easily calculated, they should be considered as important variables when using the 1-min deep breathing assessment in risk assessment contexts. The RMSSD had a 0.60 correlation with 24-h HF power, a 0.64 correlation with LF power, and a 0.57 correlation with VLF power. This is an important factor as the low power in the VLF rhythm has stronger associations with all-cause mortality than the LF and HF bands, [17,45] and is associated with arrhythmic death, [64] posttraumatic stress disorder, [65] and high inflammation. [66,67]

In conclusion, the findings from this study suggest that the 1-min paced deep breathing protocol is a useful and a potentially important test that can be used in health risk assessment context for screening patients. When low values are found, it is recommended that a 24-h assessment be conducted.

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#### **Conflicts of interest**

There are no conflicts of interest.

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#### McCraty, et al.: 1-Min, deep breathing HRV assessment

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