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Respiratory sinus arrhythmia – testing the method of choice for evaluation of cardiovagal regulation

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ABSTRACT

Respiratory sinus arrhythmia (RSA) is an index of cardiovagal regulation, emotional and cognitive processing. RSA is quantified using heart rate variability (HRV) spectral analysis at respiratory-linked high-frequency band (HF-HRV) using Fast Fourier transformation (FFT) or autoregressive (AR) method, both requiring resampling of recordings - a potential source of error. We hypothesized that rarely used HRV time-frequency analysis with Lomb-Scargle periodogram (LSP) without resampling could be more sensitive to detect neurocardiac response to posture change than FFT and AR. Orthostasis (posture change from supine to standing) evoked significant decrease of HF-HRV well detectable by FFT, AR, and LSP. In contrast, during posture change from sitting to lying, significant increase of HF-HRV and peak HF was best detected using LSP. In regression analysis, the associations between RR-interval, HF-HRV, and peak HF were best detected when evaluated using LSP. Time-frequency HRV analysis with LSP could represent an important alternative to conventional FFT and AR methods for assessment of cardiovagal regulation indexed by RSA.

1. Introduction

Respiratory sinus arrhythmia (RSA) represents a physiological phenomenon of heart rate (HR) variations according to breathing – HR decreases during the expiratory phase and *vice versa,* HR increases during the inspiratory phase. From a physiological point of view, the RSA improves gas exchange associated with a rise in ventilation-perfusion ratio and a decrease in physiological dead space by maximal pulmonary blood flow in the inspiration (Hayano and Yasuma, 2003; Yasuma and Hayano, 2004). Regarding the RSA mechanisms, the first study of Anrep et al. (1936) described mechanical component due to changes in intrathoracic pressure, the effect of central respiratory neurons on cardiac outflow, and blood pressure impact on the HR mediated through reflex mechanisms (according to Larsen et al., 2010). Recently, both central and peripheral mechanisms have been intensively studied and discussed (e.g. Farmer et al., 2016; Larsen et al., 2010). Specifically, breathing influences the cardioinhibitory center represented by *nc. ambiguus* through many pathways including the interaction between respiratory and cardioinhibitory center, reflex responses to the intrathoracic and blood pressure changes or activation of the pulmonary stretch receptors with lung inflation (Mortola et al., 2016). This complex central-peripheral network modulates both preganglionic vagal and sympathetic motoneurons resulting in continuous respiratory-linked *„beat-to-beat“* HR oscillations – short-term heart rate variability (HRV), which is predominantly regulated by cardiac vagal outflow. Moreover, the RSA is considered as a non-invasive marker of cognitive and emotional processing influencing both respiratory and cardiovascular systems, what makes this mechanism a center of interest in psychophysiological research (Thayer and Lane, 2009, 2000). Notably, the RSA can be quantified by various methods such as HRV time-

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domain or frequency-domain analysis. The spectral (frequency)-domain HRV analysis at the respiratory-linked high-frequency band (HF-HRV) using Fast Fourier transformation (FFT) and autoregressive (AR) method represent the conventional methods for RSA magnitude assessment. Yet, both methods have specific limitations which could be potentially overcome using some of the distinct, more recent, algorithms. Therefore, this study aimed to compare the application of traditionally used FFT and AR spectral analyses with a generally available alternative method - the time-frequency analysis with the windowed Lomb-Scargle periodogram (LSP).

During recent years, several authors demonstrated that FFT overestimated the HF component compared to AR analysis (e.g. Pichon et al., 2006); thus, novel recommendations for HRV application in psychophysiological research prefer to use the AR method (Laborde et al., 2017). Both FFT and AR require the signal to be stationary and evenly sampled, therefore, resampling procedure is necessary before analysis. However, such pre-processing of the time series of RR-intervals is a potential source of error in the estimation of spectral components (Clifford and Tarassenko, 2005). This limitation of HRV spectral analysis can be overcome using methods which make no assumptions on the evenness of data sampling, such as rarely applied Lomb-Scargle periodogram (LSP). LSP is considered to be resistant to errors from ectopic beats removal and resampling, which is not required for this method, thus, it could, theoretically, offer a more exact power spectrum estimation compared to traditional spectral analysis using FFT and AR (Clifford and Tarassenko, 2005; Ramshur, 2010).

Today, emphasis is put on a detailed study of mutual interactions between the physiological mechanisms behind HRV. While the effects of resampling and other pre-processing procedures on the accuracy of spectral estimates have already been studied, the effects on the assessment of complex interactions between distinct aspects of HR regulation are yet unknown (Clifford and Tarassenko, 2005). Therefore, this study aimed to compare the applicability of the conventional FFT/AR analysis and a rarely applied LSP method in the assessment of the associations between the parameters reflecting physiological basis of HRV - respiratory rate, parameters of RSA, respiratory-linked peak frequency within HF-HRV, and mean duration of RR-interval.

We addressed the hypothesis that quantification of the RSA magnitude using time-frequency analysis with the windowed Lomb-Scargle periodogram (LSP) is a more sensitive method to detect neurocardiac reflex response to physiological stressor than traditionally used FFT and AR analyses. To the best of our knowledge, this is the first study to compare various HRV spectral analyses quantifying RSA magnitude in response to posture change in healthy subjects. Secondly, we addressed the hypothesis that LSP method could provide a more robust evaluation of the mutual relationships between intrinsically interrelated parameters of respiration, cardiac vagal control, and consequent effects on cardiac cycle represented by duration of RR-interval.

2. Methods

The study was approved by the Ethics Committee of Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava in accordance with the 1964 Helsinki declaration and its later amendments. All participants and their parents/legal representatives were carefully instructed about the study protocol and they gave informed written consent to participation in the study prior to the examination.

2.1. Subjects

The studied group consisted of 60 healthy adolescents (31 girls, age: 15.2 ± 2.2 years, body mass index: 21.4 ± 3.2 kg/m^2) recruited from local schools. All participants were normotensive non-smokers with normal weight, no history of recent acute illness or chronic cardiovascular, respiratory, endocrine, neurological, metabolic, or infectious diseases or mental disorders. The participants were instructed to avoid strenuous physical exercise and consumption of substances which could affect the cardiovascular or autonomic nervous system (e.g. medicaments, caffeine, and alcohol) 24 h prior to the examination.

2.2. Protocol

Continuous recordings of ECG signal were performed using Medical DiANS (Dimea, Czech Republic) with sampling frequency 1000 Hz. The applied system was developed and validated according to methodological recommendations of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (Salinger and Gwozdziewicz, 2008; Task Force, 1996). Respiratory rate was continuously recorded using FlexComp Infinity (Thought Technology Ltd., Canada).

The examinations were carried out under standard conditions: a quiet room with a standard temperature (23 °C) and humidity (45%–55%), minimization of stimuli, in the morning between 9.00 a.m. and 12.30 pm after normal breakfast 2 h prior to the examination. The participants were instructed to sit comfortably in a special armchair. After 15 min of rest required for autonomic nervous system stabilization and for the exclusion of a potential effect of stress, the participants remained in a sitting position. The protocol consisted of three periods: sitting position, supine position, and active orthostatic test (change of posture from supine to standing position). The time of each phase was 5 min recommended by Task Force standards (1996) for short-term HRV analysis.

2.3. HRV data analyses

The time series of RR-intervals were derived from the continuous ECG-recordings and carefully checked for the presence of artifacts. The artifact-free 5 min sequences of RR-intervals were analyzed by means of time- and frequency-domain methods using freely available open source HRV analysis software package HRVAS (Ramshur, 2010).

2.3.1. Time-domain analysis

In a time-domain analysis based on the calculation of beat-to-beat differences in the duration of RR-intervals, root mean square of successive differences (rMSSD, ms) and percentage of the number of successive heartbeats differing more than 50 ms (pNN50, %) were calculated. Parameters rMSSD and pNN50 are considered to represent the time-domain indices of RSA (Task Force, 1996).

2.3.2. Frequency-domain and time-frequency analysis

Prior to frequency-domain HRV analysis, the slow fluctuations were filtered using smoothness priors detrending with $\Lambda = 500$ (Tarvainen et al., 2002). Consequently, the spectral power in high-frequency band of HRV (HF-HRV, 0.15 - 0.40 Hz) was analyzed using three distinct methods: frequency-domain nonparametric analysis of Welch periodogram based on the Fast Fourier Transformation (FFT), frequency-domain parametric method of autoregressive (AR) spectral estimation using Burg periodogram, and time-frequency analysis with the windowed Lomb-Scargle periodogram (LSP). For the frequency-domain analyses using Welch and Burg periodograms, the time series of RR-intervals were resampled using cubic spline interpolation with the frequency 4 Hz to meet the assumption of a regularly time-sampled signal. Time-frequency method with LSP does not require an evenly sampled signal, therefore, time series of RR-intervals were analyzed without resampling (Laguna et al., 1998; Ramshur, 2010).

The Welch periodogram (FFT) with weighted window function was computed using window width 128 samples with 50% overlapping. Estimation of HF-HRV with Burg periodogram (AR) was performed using model order 16, which is recommended for the short series of RR-intervals with the sampling frequency 2–4 Hz (Boardman et al., 2002). For the time-frequency method with LSP, 30 s non-overlapping windows were used. LSP estimates the frequency spectrum using a

Table 1
Parameters of RSA and respiration during active change of posture.

Parameter	1 Sitting position	2 Supine position	3 Orthostatic test
RR-interval (ms)	781.7 (710.8 – 882.3)	878.9 (779.5 – 1011.0)	657.7 (592.1 – 730.5)
rMSSD (ms)	49.35 (29.55 – 64.70)	63.80 (44.40 – 78.10)	26.95 (19.25 – 35.45|
pNN50 (%)	26.60 (7.60 – 43.30)	42.60 (21.55 – 57.10)	5.50 (2.05 – 12.35)
lnHF-HRV FFT (ms^2)	6.98 (6.11 – 7.54)	7.22 (6.70 – 7.78)	5.94 (5.34 – 6.42)
lnHF-HRV AR (ms^2)	5.97 (5.12 – 6.62)	6.25 (5.73 – 6.84)	4.88 (4.19 – 5.40)
lnHF-HRV LSP (ms^2)	5.02 (4.18 – 5.56)	5.36 (4.89 – 5.90)	4.09 (3.52 – 4.53)
Peak HF FFT (Hz)	0.25 (0.22 – 0.30)	0.29 (0.23 – 0.33)	0.25 (0.20 – 0.31)
Peak HF AR (Hz)	0.27 (0.24 – 0.31)	0.30 (0.26 – 0.33)	0.23 (0.15 – 0.27)
Peak HF LSP (Hz)	0.26 (0.26 – 0.28)	0.28 (0.27 – 0.29)	0.24 (0.24 – 0.26|
Respiratory rate (/min)	15.5 (14.5 – 17.0)	16.8 (15.2 – 17.6)	14.8 (13.7 – 16.0)

rMSSD - root mean square of successive differences of RR-intervals, pNN50 - percentage of the number of successive RR-intervals differing more than 50 ms, lnHF-HRV – spectral power in high frequency band of heart rate variability evaluated using Fast Fourier transformation (FFT), autoregressive modelling (AR) or time-frequency method with Lomb-Scargle periodogram (LSP), peak HF - peak frequency within high-frequency band of heart rate variability. Values are expressed as median (interquartile range).

calculation of the least squares fit of sinusoids to the data, and in contrary to Welch periodogram, weighted window functions are not applied (Ramshur, 2010).

Reactivity (%) of the HF-HRV analysis methods to change of position from sitting to supine was calculated as $\frac{\text{(value during supine position)} - \text{(value during sitting position)}}{\text{(value during sitting position)}} \times 100$, and the reactivity to active orthostatic test as $\frac{\text{(value during orthostasis)} - \text{(value during supine position)}}{\text{(value during supine position)}} \times 100$.

Peak frequency within the high-frequency band (peak HF) was assessed for all the three methods of FFT, AR, and LSP HF-HRV analysis.

2.4. Statistical analysis

The data were analyzed using statistical software package SAS University Edition (SAS Institute Inc., Cary, NC, USA) and R (R Foundation for Statistical Computing; Vienna; Austria, 2017), version 3.4.1, using the libraries WRS2 (Mair et al., 2017) and robustbase (Maechler et al., 2017). The parameter HF-HRV evaluated using FFT, AR, and LSP method was logarithmically transformed prior to statistical analyses. Due to the non-sphericity of the data, the robust ANOVA (Mair et al., 2017) was used, which does not require the assumption of the homoscedasticity. Wherever the ANOVA hypothesis was rejected, it was followed by a robust post-hoc test, leading the 95% confidence interval (CI), adjusted to control the family-wise error (FWE) rate. A robust heteroscedastic analysis of the explanatory measure of effect size (ξ) was used for the main effects and post-hoc comparisons, where the values 0.10, 0.30, and 0.50 correspond to small, medium, and large effect sizes (Wilcox and Tian, 2011). Regression analysis was used to study the associations between variables. Due to the presence of a small number of influential observations, the robust regression, as implemented in (Maechler et al., 2017), was used. The associations between respiratory rate, peak HF evaluated using FFT, AR, and LSP method, and the parameters of RSA were analyzed using Spearman's rank-order correlation. Where applicable, the values of $p < 0.05$ were considered as significant.

3. Results

The parameters of RSA and respiration during sitting, supine, and standing position are presented in Table 1.

3.1. Effect of active change of posture on the parameters of RSA and respiration

By the robust ANOVA, the effect of active change of posture (sitting vs. supine vs. standing) was significant for lnHF-HRV evaluated using FFT ($F_{[2,68.80]} = 37.29, p < 0.001, \xi = 0.64$), AR ($F_{[2,68.64]} = 36.27, p < 0.001, \xi = 0.65$) and LSP method ($F_{[2,68.99]} = 33.16, p < 0.001, \xi = 0.62$), peak HF evaluated using FFT ($F_{[2,68.81]} = 4.50, p = 0.015, \xi = 0.28$), AR ($F_{[2,67.23]} = 16.29, p < 0.001, \xi = 0.62$) and LSP method ($F_{[2,69.95]} = 47.03, p < 0.001, \xi = 0.73$), RR-interval ($F_{[2,68.11]} = 45.48, p < 0.001, \xi = 0.76$), rMSSD ($F_{[2,62.19]} = 39.70,$

Table 2
Changes of the parameters of RSA and respiration during active change of posture.

Parameter	95% CI and effect size	Supine (2) vs. sitting (1) position	Standing (3) vs. supine (2) position
RR-interval (ms)	LL	31.57	−296.69
	UL	164.37	−169.50
	ξ	0.46	0.90
rMSSD (ms)	LL	2.92	−47.18
	UL	28.60	−25.10
	ξ	0.41	0.82
pNN50 (%)	LL	2.83	−43.17
	UL	26.71	−24.32
	ξ	0.39	0.84
lnHF-HRV FFT (ms^2)	LL	−0.042	−1.761
	UL	0.788	−0.983
	ξ	0.28	0.79
lnHF-HRV AR (ms^2)	LL	−0.087	−1.821
	UL	0.806	−1.006
	ξ	0.26	0.83
lnHF-HRV LSP (ms^2)	LL	0.018	−1.700
	UL	0.851	−0.917
	ξ	0.31	0.82
Peak HF FFT (Hz)	LL	0.000	−0.075
	UL	0.059	−0.004
	ξ	0.31	0.37
Peak HF AR (Hz)	LL	0.000	−0.116
	UL	0.051	−0.046
	ξ	0.30	0.61
Peak HF LSP (Hz)	LL	0.006	−0.044
	UL	0.024	−0.026
	ξ	0.56	0.98
Respiratory rate (/min)	LL	0.113	−2.552
	UL	1.811	−1.000
	ξ	0.36	0.64

The values represent the results of a robust post-hoc tests leading the 95% confidence interval, adjusted to control the family-wise error (FWE) rate. rMSSD - root mean square of successive differences of RR-intervals, pNN50 - percentage of the number of successive RR-intervals differing more than 50 ms, lnHF-HRV – spectral power in high frequency band of heart rate variability evaluated using Fast Fourier transformation (FFT), autoregressive modelling (AR) or time-frequency method with Lomb-Scargle periodogram (LSP), peak HF - peak frequency within high-frequency band of heart rate variability, CI - confidence interval, LL and UL - lower and upper limit, respectively, ξ - explanatory measure of effect size.

$p < 0.001$, $\xi = 0.66$), pNN50 ($F_{[2,55.07]} = 49.64$, $p < 0.001$, $\xi = 0.63$), and respiratory rate ($F_{[2,69.57]} = 15.43$, $p < 0.001$, $\xi = 0.45$).

Post hoc analysis showed that the change of posture from sitting to supine position resulted in significant increase of the parameter lnHF-HRV evaluated using LSP time-frequency method, significant increase of peak HF evaluated using FFT, AR, and LSP method, significant lengthening of the mean duration of RR-interval, and significant increase of the time domain parameters rMSSD and pNN50. Respiratory rate during supine position was significantly higher compared to sitting position. Changes of lnHF-HRV evaluated using FFT and AR methods did not reach statistical significance. Comparisons of the HRV parameters between the evaluated positions are presented with 95% CI and explanatory measure of effect size in Table 2.

The orthostatic test (change of posture from supine to standing position) resulted in significant decrease of the lnHF-HRV evaluated using FFT, AR, and LSP method, significant decrease of the peak HF evaluated using FFT, AR, and LSP method, significant shortening of the mean duration of RR-interval, and a significant decrease of rMSSD and pNN50 compared to supine position. Respiratory rate during active orthostasis was significantly lower compared to supine position. Comparisons of the HRV parameters between the evaluated positions are presented with 95% CI and explanatory measure of effect size in Table 2.

3.2. Regression analysis – prediction of mean RR-interval by HF-HRV and peak HF

The robust multivariate regression analysis showed that out of the three methods of HF-HRV analysis (FFT, AR, and LSP), mean duration of RR-interval was best predicted by lnHF-HRV evaluated using LSP method (regression coefficient 0.217 ms^{-2}, adjusted $R^2 = 0.560$, $p < 0.001$), compared to FFT and AR method ($p = 0.178$, $p = 0.478$, respectively; Table 3).

In univariate regression analyses, a significant association between mean duration of RR-interval and peak HF was detected using LSP (the slope 3.757 Hz^{-1}, 95% CI: 2.698–4.816, adjusted $R^2 = 0.241$ $p < 0.001$) and AR method (the slope 0.761 Hz^{-1}, 95% CI: 0.319–1.202, adjusted $R^2 = 0.053$, $p < 0.001$), but not using FFT algorithm (the slope 0.191 Hz^{-1}, 95% CI: -0.330 – 0.712, adjusted $R^2 = -0.003$, $p = 0.470$; Fig. 1).

3.3. Effect of HRV analysis method on HF-HRV reactivity and peak HF

By the robust ANOVA, no significant effect of the applied HRV analysis methods (FFT vs. AR vs. LSP) was found in the reactivity of lnHF-HRV during supine position ($F_{[2,68.75]} = 0.83$, $p = 0.439$, $\xi = 0.13$) and in response to orthostatic test ($F_{[2,68.81]} = 2.49$, $p = 0.090$, $\xi = 0.20$).

No significant differences between peak HF evaluated using FFT, AR, and LSP method were found during sitting position, supine position and orthostatic test ($F_{[2,54.44]} = 0.76$, $p = 0.473$, $\xi = 0.19$; $F_{[2,55.69]} = 2.13$, $p = 0.129$, $\xi = 0.18$; $F_{[2,50.44]} = 2.90$, $p = 0.065$, $\xi = 0.38$, respectively).

Table 3
Estimated effects of lnHF-HRV evaluated using FFT, AR, and LSP method on mean RR-interval (joint importance evaluated using multivariate robust regression analysis).

Parameter logRR-interval	Estimate	Std. Error	t - value	p - value
Intercept	6.155	0.129	47.552	< 0.001
lnHF-HRV FFT	−0.133	0.099	−1.351	0.178
lnHF-HRV AR	0.059	0.083	0.710	0.478
lnHF-HRV LSP	0.217	0.064	3.401	< 0.001

lnHF-HRV – spectral power in high frequency band of heart rate variability evaluated using Fast Fourier transformation (FFT), autoregressive modelling (AR) or time-frequency method with Lomb-Scargle periodogram (LSP).

3.4. Correlation analyses

LnHF-HRV evaluated using FFT, AR, and LSP method showed a significant positive correlation with peak HF evaluated using LSP method ($r_s = 0.390$, $p < 0.001$; $r_s = 0.381$, $p < 0.001$; $r_s = 0.396$, $p < 0.001$; respectively), but not with peak HF evaluated using FFT ($r_s = -0.097$, $p = 0.195$; $r_s = -0.094$, $p = 0.210$; $r_s = -0.047$, $p = 0.530$; respectively) and AR method ($r_s = 0.078$, $p = 0.300$; $r_s = 0.042$, $p = 0.580$; $r_s = 0.111$, $p = 0.138$; respectively).

Peak HF evaluated using LSP and AR method showed a significant positive correlation with rMSSD and pNN50 (LSP: $r_s = 0.517$, $p < 0.001$; $r_s = 0.508$, $p < 0.001$; respectively; AR: $r_s = 0.212$, $p = 0.004$; $r_s = 0.207$, $p = 0.005$, respectively). No significant correlation was found between peak HF evaluated using FFT method and the parameters of RSA.

Respiratory rate showed a significant positive correlation with peak HF evaluated using FFT, AR, and LSP method ($r_s = 0.532$, $p < 0.001$; $r_s = 0.684$, $p < 0.001$; $r_s = 0.649$, $p < 0.001$, respectively). No significant correlation was found between respiratory rate and the parameters RR-interval, rMSSD, pNN50, and lnHF-HRV.

4. Discussion

This study revealed that HRV time-frequency analysis with Lomb-Scargle periodogram was the most sensitive method to detect cardiovagal regulatory adjustment in response to posture changes (sitting, lying, and standing) compared to traditionally used HRV spectral methods (FFT, AR) in healthy subjects. Specifically, the increase in cardiovagal efferent outflow (parameters HF-HRV, peak HF) evoked by posture change from sitting to lying was best detected using HRV time-frequency analysis with LSP, providing thus potentially important alternative to conventionally used FFT and AR analyses in psychophysiological/psychiatric research. Moreover, the LSP algorithm best detected the associations between mean duration of RR-interval and cardiac vagal regulation indexed by HF-HRV and peak HF, as well as several other relationships between the distinct parameters of HRV.

Psychophysiological research focuses on cardiac vagal regulation indexed by RSA linked to self-regulation at the cognitive, emotional, social, and health levels (Laborde et al., 2017). The Neurovisceral Theory (Thayer and Lane, 2009, 2000) reported that both cognitive and emotional functions regulated by brain regions are also involved in the HRV regulation indexed by the respiratory-linked component of the HRV. Further, the Polyvagal theory (Porges, 2007, 1995) suggests that quantification of the RSA – as a direct measure of the vagal efferent outflow originating in the *nucleus ambiguus* that influences the nicotinic preganglionic receptors on the sinoatrial node - provides a unique non-invasive opportunity to assess the central regulation *via* peripheral measure of a neural circuit involved in the coordination of visceral state and the expression of emotion and social communication (Porges, 2007). The neurovisceral integration model is based on the assumption that prefrontal cortex plays an important inhibitory function on subcortical sympathoexcitatory centers through central autonomic network and *n. vagus* (Thayer et al., 2009). In other words, this theory assumes that the higher vagal modulation is associated with better executive cognitive performance, emotional and health regulation (Thayer et al., 2009). Furthermore, Porges' Polyvagal theory posits interaction between vagal tone and social functioning – if the vagal modulation is higher, the social functioning is better (Porges, 2007). The common basis of these theories is their focus on cardiac vagal modulation indexed by the respiratory-linked high-frequency component of the HRV (HF-HRV).

Body position significantly influences cardiac-linked autonomic regulation in humans. Specifically, the active orthostatic test, *i.e.*

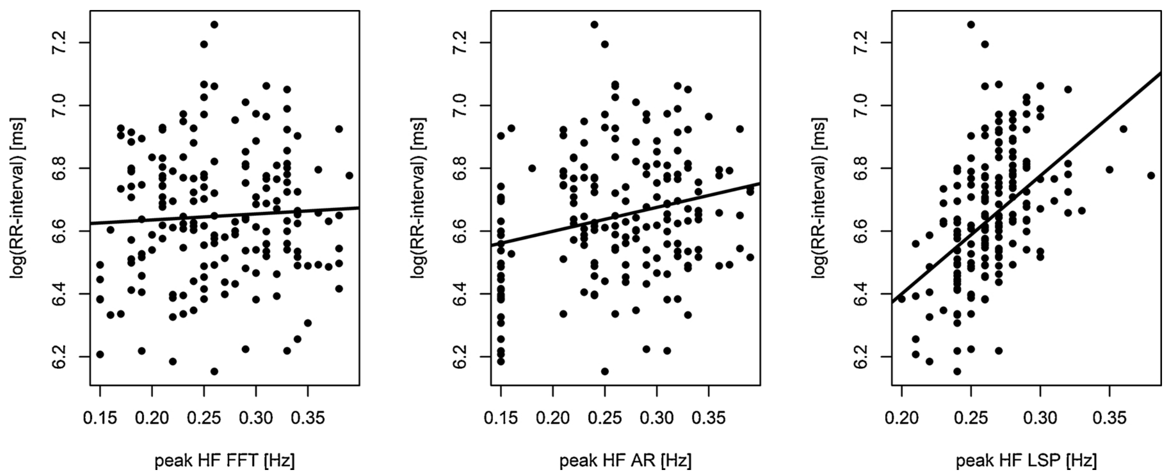

Fig. 1. Robust regression analysis of the associations between RR-interval and peak frequency within high-frequency band of heart rate variability (peak HF) evaluated using Fast Fourier transformation (FFT), autoregressive modeling (AR) or time-frequency method with Lomb-Scargle periodogram (LSP).

posture change from lying to standing, evokes a sequence of compensatory complex hemodynamic baroreflex-mediated responses leading to a vagal withdrawal associated with sympathetic activation (Freeman and Chapleau, 2013; Quintana et al., 2016; Tonhajzerova, 2016). HRV complex analysis was used to detect abnormal neurocardiac reflex control to orthostatic test in several diseases, such as major depression or ADHD (Tonhajzerova et al., 2016, 2012, 2010, 2009). Moreover, recent studies reported good reproducibility of HRV parameters during the orthostatic challenge (Schäfer et al., 2015), and the modified orthostatic load protocol (supine-standing-supine) improves the sensitivity of short-term HRV examination (Howorka et al., 2010).

Based on the recent recommendations for HRV assessment focusing on linear and nonlinear parameters (*e.g.* Quintana and Heathers, 2014), the autoregressive (AR) or Fast Fourier Transformation (FFT) methods have been recently recommended for psychophysiological research (Laborde et al., 2017). Previously, application of AR spectral analysis was limited due to greater computational demands compared to FFT. However, progress in hardware and software used for HRV analysis together with detailed knowledge about the effect of the selected AR model order allowed easy use of both methods with the preference of AR due to potentially greater accuracy of estimated parameters (Boardman et al., 2002; Pichon et al., 2006; Ramshur, 2010; Tarvainen et al., 2014). Despite the advances in pre-processing procedures and consequent HRV spectral analysis, the major problem of classical methods (FFT and AR) remains to be the low-pass effect of the resampling, which is necessary for obtaining evenly sampled signal (Laguna et al., 1998). This potential source of bias can be avoided using a less known method of spectral analysis - LSP, which may thus provide a more robust tool to assess the changes of HF-HRV (Laguna et al., 1998; Ramshur, 2010). In our study, significant changes of cardiac vagal regulation in response to orthostatic test were well detected with all the three evaluated methods of HF-HRV analysis. In contrast, discrete alterations in cardiovagal regulation after posture change from sitting to lying were best detected using the time-frequency method with LSP, supporting potential benefit of its application along with the conventional methods of FFT and AR under certain specific conditions. Moreover, regression analysis showed that mean duration of RR-interval was best predicted by HF-HRV and peak HF evaluated using LSP, compared to FFT and AR method. In addition, HF-HRV evaluated using FFT, AR, and LSP method correlated with the peak frequency within the high-frequency band (peak HF) only when assessed using LSP. The respiratory-linked indices HF-HRV and peak HF reflect the autonomic control of heart rate through vagal modulation of the activity of sinoatrial node. Thus, they are intrinsically interrelated and directly connected with the consequent mean RR-interval duration. Lack of these associations in the results of FFT and AR analyses in the present study underscores potential distortion of information from HRV analysis due to the application of resampling procedure in FFT and AR. Resampling itself adds erroneous data leading to significant errors in spectral estimates and under-estimation of HF components of HRV (Clifford and Tarassenko, 2005). The cubic spline interpolation method was found to behave erratically particularly in lower heart rates with lower density of data, and to produce more unstable oscillations with greater overall HRV (Clifford and Tarassenko, 2005). Specifically, it shifts the spectral peaks towards the low frequency band due to linear phase shifting (Saini et al., 2013). Moreover, the resampling affects the HRV metrics in means of resolution, spectral smoothness, and power distribution among the frequency bands (Saini et al., 2013). Although in general, both LSP and the spectral methods involving resampling (FFT, AR) provide similar results, detailed analysis of the associations between the distinct interrelated indices of cardiac vagal regulation points toward greater robustness of LSP method. This seems to be particularly relevant for the assessment of peak HF frequency.

Importantly, the breathing pattern characterized by respiratory rate and depth could affect the HF-HRV; this question is extensively discussed. With regard to respiratory depth, the RSA indexed by HF-HRV shows greater amplitude during higher tidal volume and lower respiratory rate (Hirsch and Bishop, 1981). However, the effect of tidal volume on HRV has been shown to account for less than 5% of the variance in the HRV measures (Lewis et al., 2012). Furthermore, the respiratory rate represents an important factor influencing the HF-HRV. In particular, HF-HRV corresponds to vagal modulation at the physiological range of respiratory rate for adults between 9 to 24 cycles per minute, *i.e.* 0.15 - 0.40 Hz (Laborde et al., 2017). In this aspect, novel recommendations regarding HRV and cardiac vagal tone in psychophysiological research prefer to use spontaneous compared to controlled breathing (Laborde et al., 2017; Larsen et al., 2010). As noted by Thayer et al. (2011), there is an evidence from genetics, neuroimaging, and psychophysiological studies suggesting that the removal of respiration-linked variation from HRV could remove variance associated with the common neural origin of cardiorespiratory coupling indexed by HRV. Nevertheless, respiration monitoring necessary for understanding of the neurobiological mechanisms and contextual factors responsible for the complex interaction between the respiratory and cardiovascular system is recommended (Laborde et al., 2017). In our study, the respiratory rate varied between 10.4 and 19.0 breaths per minute, thus fitting well in the HF-range of 0.15 - 0.40 Hz. Moreover, correlation analysis did not show significant associations between respiratory rate and HF-HRV, rMSSD, and pNN50, therefore, we do not assume significant effect on the evaluated parameters of RSA.

It is important to note that besides the Welch and Burg periodogram, which were applied in the present study, several different algorithms exist for FFT and AR methods of spectral analysis, e.g. Yule-Walker estimation for AR (Stavrinou et al., 2014). Similarly, there are other procedures than LSP for estimation of the HRV power spectrum directly without resampling, such as penalized sum-of-squares estimator, which could be superior to LSP due to diminished production of noise, or a robust period detection, which seems to be superior in analysis of ECG data with ectopic beats and artifacts (Krafty et al., 2014; Skotte and Kristiansen, 2014). In the present study, we aimed to compare the effects of the spectral analysis methods which are traditionally used in the widely available HRV software packages such as HRVAS, Kubios or SinusCor (Bartels et al., 2017; Ramshur, 2010; Tarvainen et al., 2014), and which can be applied by the researchers in the field of psychophysiology and HRV analysis, even without particular mathematical background. Future implementation of the recent modern methods for HRV spectral estimation could offer a more detailed information about bidirectional neuro-cardiac interactions.

5. Study limitations

In the present study, the studied population consisted of a homogeneous sample of healthy adolescents within a relatively narrow age range. Therefore, it may not be possible to extrapolate the findings to other populations and age ranges. Moreover, the reported differences between the three methods of HF-HRV spectral estimation are rather small, thus, a significance of our findings may be relevant only under specific conditions (e.g. low cardiovagal reactivity) or within a more detailed study of interactions between the distinct indices of cardiac vagal regulation. In addition, LSP method has also some limitations such as the production of noisy estimates. Application of the more recent methods capable of analysis of unevenly sampled ECG data without resampling could, theoretically, offer a better accuracy of the obtained power spectrum estimations.

6. Conclusion

The quantification of RSA magnitude using three distinct methods: frequency-domain nonparametric analysis of Welch periodogram based on the Fast Fourier Transformation (FFT), frequency-domain parametric method of autoregressive (AR) spectral estimation using Burg periodogram, and time-frequency analysis with the windowed Lomb-Scargle periodogram (LSP) revealed that neurocardiac reflex response to posture change (sitting to lying) was best detected by HRV time-frequency analysis with LSP. Moreover, LSP was the best method for evaluation of the associations between mean duration of RR-interval, HF-HRV, and peak HF. We suggest that HRV time-frequency analysis with the windowed Lomb-Scargle periodogram could represent an important alternative to conventionally used FFT and AR analyses for the assessment of cardiovagal regulation indexed by RSA.

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Declarations of interest

None.

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