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EFFECTS OF CIRCADIAN RHYTHM AND DAILY PHYSICAL ACTIVITY ON SHORT-TERM HEART RATE VARIABILITY IN YOUNG HEALTHY ADULTS – A PILOT STUDY

UTICAJ CIRKADIJALNOG RITMA I DNEVNE FIZIČKE AKTIVNOSTI NA KRATKOTRAJNU VARIJABILNOST SRČANE FREKVENCIJE KOD MLADIH ZDRAVIH ODRASLIH OSOBA – PILOT STUDIJA

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Summary
Introduction. Heart rate variability is the leading non-invasive method used for assessing the activity of the autonomic nervous system. Investigation of the changes in the autonomic nervous system activity under the influence of circadian rhythm and daily physical activity can be beneficial to exercise at the best time of the day and at regular time intervals. Furthermore, it can be used to determine the optimal level of total daily physical activity. This study aimed to demonstrate the effects of circadian rhythm and daily physical activity on the autonomic nervous system at rest through short-term measurements of heart rate variability. **Material and Methods**. Fifteen young healthy adults participated in the study. Heart rate variability was measured on three separate occasions. During these visits, heart rate variability measurements were made in the morning, in the afternoon hours following a physically active day, and in the afternoon hours after a physically inactive day. **Results**. Our study showed no significant differences in the parameters of heart rate variability measured at different times of the day. A comparison of heart rate variability values after a physically inactive day and heart rate variability values after a physically active day did not show a significant difference in any of the heart rate variability parameters. **Conclusion**. Short-term measurements of heart rate variability showed no impact of circadian rhythm and daily physical activity on heart rate variability at rest.
Key words: Circadian Rhythm; Activities of Daily Living; Heart Rate; Young Adult; Autonomic Nervous System

Sažetak
Uvod. Varijabilnost srčane frekvencije je vodeći neinvazivni metod procene rada autonomnog nervnog sistema. Ispitivanje promena aktivnosti autonomnog nervnog sistema pod uticajem cirkadijalnog ritma i dnevne fizičke aktivnosti može biti od koristi u plasiranju vežbi u optimalno doba dana i u pravilnim vremenskim intervalima. Takođe se može koristiti za određivanje optimalnog nivoa ukupne dnevne fizičke aktivnosti. Ova studija je imala za cilj da pokaže uticaj cirkadijalnog ritma i dnevne fizičke aktivnosti na autonomni nervni sistem u mirovanju putem kratkotrajnih merenja varijabilnosti srčane frekvencije. **Materijal i metode.** U istraživanju je učestvovalo 15 mladih zdravih odraslih osoba. Varijabilnost srčane frekvencije je izmerena u tri odvojene posete. Tokom ovih poseta, merenja varijabilnosti srčane frekvencije vršena su u prepodnevnim satima, u popodnevnim satima nakon fizički aktivnog dana i u popodnevnim satima nakon fizički neaktivnog dana. **Rezultati.** Naša studija nije pokazala značajne razlike u parametrima varijabilnosti srčane frekvencije merenim u različito doba dana. Poređenje vrednosti varijabilnosti srčane frekvencije nakon fizički neaktivnog dana i vrednosti nakon fizički aktivnog dana nije pokazalo značajnu razliku ni u jednom od pokazitelja varijabilnosti srčane frekvencije. **Zaključak.** Kratkotrajna merenja varijabilnosti srčane frekvencije nisu pokazala uticaj cirkadijalnog ritma i dnevne fizičke aktivnosti na varijabilnost srčane frekvencije u mirovanju.
Ključne reči: cirkadijalni ritam; aktivnosti svakodnevnog života; srčana frekvencija; mlada osoba; autonomni nervni sistem

Introduction

Heart rate variability (HRV) is a physiological phenomenon of heart rate fluctuations over time that is evident in the oscillations in the length of R-R intervals [1]. These variations are a direct consequence of complex interactions between the parasympathetic and sympathetic branches of the autonomic nervous system (ANS) which makes measuring HRV one of the most reliable, simple, and non-invasive methods for measuring ANS activity intervals [1].

Increased HRV usually indicates a dominant parasympathetic activity, good health, and well-being, while reduced HRV is predictive of worse cardiovas-

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Abbreviations

HRV – heart rate variability
ANS – autonomic nervous system
CR – circadian rhythm
DPA – daily physical activity
SDNN – standard deviation of normal-to-normal RR intervals
RMSSD – root mean square of successive differences
HF – high-frequency power
LF – low-frequency power
SD1 – standard deviation of the projection of the Poincaré plot on the line normal to the line of identity
rANOVA – repeated analysis of variance

cular outcomes and greater risk of all-cause morbidity and mortality, as it is linked to predominant sympathetic activity intervals [1]. The HRV values are affected by factors other than ANS activity such as sex, age, circadian rhythm (CR), and daily physical activity (DPA) [2–6]. The CR causes daily fluctuations of HRV, where HRV is expected to be greater during sleep, remain at high values upon waking, and usually decreases during the day [7]. In addition, the circadian fluctuations of HRV are closely related to the DPA level, but this connection is still not fully explored [5]. Namely, DPA affects not only HRV but CR itself, which is explained by the "timer" or "zeitgeber" activity of DPA [8, 9]. Exploring the overall effect of CR and DPA on the human body and measuring it through HRV monitoring may help to determine the best time for physical activity. These findings can also be used to achieve maximum training effectiveness and the fastest post-workout recovery. Long-term HRV recordings have been shown to correctly predict unwanted outcomes such as myocardial infarction, cerebrovascular incidents, and all-cause mortality.

Despite the high prognostic value of long-term HRV measurements, it has not been broadly integrated into mainstream medical care or personal health monitoring, since it is time consuming and expensive [10, 11]. In contrast to long-term HRV analysis, short-term measurements (5 - 30 minutes) provide results almost immediately, making them suitable for patient monitoring and outpatient care [12]. Although short-term HRV has its advantages over long-term measurements, it is underutilized in medical care, because it is a much less-sensitive way for HRV monitoring [10]. While 24-hour HRV Holter measurements showed a diurnal variation of HRV [6, 8], no study has ever evaluated the possibility of detecting diurnal HRV variation using short-term measurements. Therefore, a question arose whether short-term HRV measurements can determine the best time to exercise and evaluate the impact of other stressors, such as DPA, on HRV. This pilot study aimed to demonstrate the effects of CR and DPA on HRV at rest by using short-term HRV measurements.

Material and Methods

The pilot study was conducted between October 2019 and February 2020, at the Functional Testing Laboratory, Department of Physiology, Faculty of Medicine Novi Sad, Republic of Serbia. Approval of the Institutional Ethics Committee was obtained for the pilot study (No. 01-39/139/1). The subjects provided informed written consent to participate in this pilot study.

Fifteen young (21 ± 1-year-old) healthy male adults were included in the study. All participants were non-athletes, that is, they did not compete in any sport that involved strength, speed, and/or endurance, and they practiced only recreational activities that involved light-to-medium physical activity three to five times a week. They were non-smokers and had no significant previous medical history (including family history). To exclude the risk of possible adverse outcomes, each participant underwent a general physical examination before being included in the study. Electrocardiography, resting heart rate, and blood pressure were examined.

Each participant came to the laboratory three times on three different days. On the first occasion, HRV was measured at 10.00 a.m. (morning HRV). On the day before, the subjects did not engage any kind of strenuous physical activity and did not consume alcohol. Also, the subjects had their usual breakfast and did not consume any caffeine on the day of the experiment. Polar Speed Sensor V800 Bluetooth Smart (Polar Electro Oy, Kempele, Finland; production date 2013) was used to obtain a five-minute recording of R-R intervals at rest. On the second visit, the afternoon HRV was measured at 6.00 p.m. after a physically active day (active afternoon HRV). The level of DPA was estimated by using the total amount of steps, measured via the "Pedometer" smart-phone application, the subjects had made prior to HRV measurement. Subjects were considered to have had a physically active day if they made between 7,000 and 10,000 steps. The third and final visit was intended to determine the afternoon HRV after a physically inactive day (inactive afternoon HRV). The measurement was done at 6.00 p.m., while the participants were required to meet a low level of DPA, less than 3,000 steps.

The HRV analysis was performed using the Kubios HRV 2.0 program (Kubios Oy, Kuopio, Finland; production date 2016). The time-domain and frequency-domain parameters, obtained by linear HRV analysis and Poincaré Plot for non-linear analysis, were used [13]. The time-domain parameters included: (1) standard deviation of normal-to-normal RR intervals (SDNN); (2) root mean square of successive differences (RMSSD); and (3) proportion of consecutive RR intervals that differ by more than 50 ms (pNN50). The frequency-domain parameters included: (1) high-frequency (HF); and (2) low-frequency (LF) spectra. The LF/HF ratio was also measured. Regarding parameters obtained by non-linear analysis, only the standard deviation of the projection of the Poincaré plot on the line normal to the line of identity (SD1) was used, since it describes the short-term variability [13].

Collected data were analyzed using the SPSS 28 software (IBM Corp., Armonk, NY, USA). The repeated analysis of variance (rANOVA) was used to test for differences between the relevant parameters. The frequency-domain parameters that were not normally distributed (LF and HF) were logarithmically transformed and then used for rANOVA. The level of significance was set at 5% ($p < 0.05$).

Table 1. Comparison (rANOVA) of the morning HRV, inactive afternoon HRV, and active afternoon HRV
Tabela 1. *Poređenje (rANOVA) jutarnje VSF, popodnevne VSF neaktivnog dana i popodnevne VSF aktivnog dana*

Parameter *Parametar*	Morning HRV *Jutarnja VSF*	Inactive afternoon HRV *Popodnevna VSF neaktivnog dana*	Active afternoon HRV *Popodnevna VSF aktivnog dana*	F	p-value *p-nivo*
SDNN (ms)	68.6 ± 31.3	67.9 ± 24.3	54.3 ± 14.6	2.77	0.080
RMSSD (ms)	43.3 ± 24.7	39.7 ± 19.9	33.3 ± 17.3	1.31	0.271
pNN50 (%)	18.2 ± 21.0	15.6 ± 15.9	14.5 ± 15.7	0.28	0.758
logHF [log (ms^2)]	6.3 ± 1.4	6.2 ± 0.9	5.9 ± 1.1	0.83	0.448
HF (n.u.)	24.1 ± 11.8	24.8 ± 11.0	23.6 ± 13.2	0.07	0.931
logLF [log (ms^2)]	7.5 ± 1.3	7.4 ± 0.5	7.1 ± 0.7	1.11	0.343
LF (n.u.)	75.9 ± 11.8	75.2 ± 11.0	76.4 ± 13.2	0.07	0.931
LF/HF	4.7 ± 2.0	4.1 ± 3.2	4.7 ± 3.2	1.03	0.369
SD1	33.7 ± 25.4	28.1 ± 13.6	23.2 ± 11.2	1.46	0.250

Legend: Morning HRV - morning heart rate variability; Inactive afternoon HRV - afternoon heart rate variability after a physically inactive day; Active afternoon HRV - afternoon HRV after a physically active day; F - ANOVA test; SDNN - standard deviation of normal-to-normal R-R intervals; RMSSD - root mean square of successive differences; pNN50 - percentage of consecutive R-R intervals that differ by more than 50 ms; HF - high-frequency power; LF - Low-frequency power; logHF - logarithmically transformed high-frequency power; logLF - logarithmically transformed low-frequency power; LF/HF: LF/HF ratio; SD1 - standard deviation of the projection of the Poincaré plot on the line normal to the line of identity; ms - milliseconds; n.u. - normalized units

Legenda: Jutarnja VSF – varijabilnost srčane frekvencije izmerena u jutarnjim časovima; Popodnevna VSF neaktivnog dana – varijabilnost srčane frekvencije izmerena u popodnevnim časovima nakon fizički neaktivnog dana; Popodnevna VSF aktivnog dana – varijabilnost srčane frekvencije izmerena u popodnevnim časovima nakon fizički aktivnog dana; F – ANOVA test; SDNN – standardna devijacija normalnih R-R intervala; RMSSD – srednji kvadrat uzastopnih razlika; pNN50 – Procenat konsekutivnih R-R intervala koji se razlikuju za više od 50 milisekundi; HF – visokofrekventna snaga; LF – Niskofrekventna snaga; logHF – Logaritamski transformisana visokofrekventna snaga; logLF – logaritamski transformisana niskofrekventna snaga; LF/HF – LF/HF odnos; SD1 – standardna devijacija projekcije Poinkareovog dijagrama na pravu koja je normalna na liniju identiteta; ms – milisekunde; n.u. – normalizovane jedinice

Results

Table 1 shows the mean values and standard deviations of the morning HRV, inactive afternoon HRV, and active afternoon HRV parameters. No significant difference was observed between any of HRV parameters.

Discussion

In a review article by Sammito et al. [14], the circadian patterns of all HRV parameters were observed. In most cases, values of time-domain HRV parameters were higher during the night and reached their peak value in the second half of sleep due to higher parasympathetic activity [14]. Upon waking, most parameters remained high and then began to decrease as a consequence of increased cortisol levels and higher sympathetic activity throughout the day [14]. Most studies have shown elevated SDNN values during the night, peak values in the early morning hours (between 5.00 a.m. and 7.00 a.m.), a decrease during the late morning hours, and the lowest values during the early afternoon [15–17]. The RMSSD and pNN50, another two indices of total HRV, fluctuated almost simultaneously. Their trend was similar to the SDNN [17]. According to the study of Jarczok et al. [7], the biggest difference in RMSSD values was between 11.00 a.m. and 10.00 p.m. In regard to frequency-domain HRV parameters, the data from other studies were as follows: HF, which reflects parasympathetic activity, reached the highest values during the night, then decreased during the morning hours with the lowest values in the afternoon, between 1.00 p.m. and 7.00 p.m. [18, 19]; LF and LF/HF values, which indicate sympathetic activity, showed an inverse correlation to HF values as their lowest values were during sleep, then rapidly increased on awakening, remained high during the day, and then started to decrease in the evening [18, 19]. Vandewalle et al. [20] and Yamasaki et al. [21] showed elevated LF and slightly elevated HF in the morning, compared to the early evening. The non-linear metric SD1 is identical to the RMSSD parameter [13]. Therefore, the discussion on the SD1 parameter is redundant. In our pilot study, HRV values measured by the short-time method did not show circadian rhythmicity. Two reasons may be responsible for this. Firstly, the sensitivity of short-term HRV measurements is lower than the sensitivity of long-term measurements [10]. Secondly, the time interval between the morning and afternoon HRV measurements was probably not long enough for the short-term measurement method to detect any changes. Other studies that have shown a difference in HRV parameters used longer time intervals between two measurements [14]. Thus, studies should consider measuring HRV values later in the day (after 10.00 p.m.) or earlier in the morning (before 10.00 a.m.) [7, 14]. The level of DPA did not significantly affect any of HRV parameters. Based on other studies, we expected a decrease in HRV as a consequence of the transient elevation of the sympathetic activity induced by exercise in non-athletes [4, 22, 23]. In our study, HRV did not change for two reasons. Firstly, the measuring may have been too short to detect any hormone-induced changes in HRV, so only acute changes in ANS activity could be detected. However, the acute fluctuations of ANS activity at rest were not significant, even after a long day of walking. Secondly, the intensity of DPA may have been too low to induce a sympathetic response. Therefore, the effect of higher levels of DPA (> 10,000 steps) should be explored. Also, more extended short-term HRV measurements (more than five minutes) could yield different results. Soares-Miranda et al. showed higher values of the mean 24-hour SDNN in people who spend more time walking long distances and doing recreational activities [22]. Accordingly, Zaffalon Júnior et al. showed lower values of RMSSD and increased values of LF/HF ratio in sedentary people [24]. These findings may be attributed to the chronic impact of high levels of physical activity which results in decreased sympathetic activity, an increased vagal tone, and subsequently increased HRV over time. This may be explained by cardiovascular adaptation to an exercise regimen via an increase in maximal cardiac output [25] and via an increase in cardiovascular health in general [26]. In our study, one day of physical activity was not long enough to induce such cardiovascular adaptive mechanisms, and consequently, HRV did not change. Therefore, studies should perform short-term HRV measurements after multiple days of increased DPA levels.

Study limitations

Firstly, this pilot study was limited by the number of participants. Therefore, other studies should include more subjects. Furthermore, a study with subjects of different age, gender, athletic performance, and chronotype should be considered. Finally, because the laboratory was not available at all times of the day, we were not able to measure HRV before 10.00 a.m. and after 6.00 p.m.

Conclusion

Our pilot study failed to detect any heart rate variability changes using short-term (five-minute) measurements. Further research should include different time points of measurements, before 10.00 a.m. and after 10.00 p.m. Also, short-term measurements longer than five minutes should be performed. Different levels of daily physical activity did not affect any heart rate variability parameters. To estimate the effects of daily physical activity on short-term heart rate variability, further research is necessary. Different levels of daily physical activity, longer duration of physical activity before heart rate variability measurements, as well as longer short-term heart rate variability measurements should be evaluated.

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