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Influence of respiration frequency on heart rate variability parameters: A randomized cross-sectional study

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Abstract.
BACKGROUND: Many patients visiting physiotherapists for musculoskeletal disorders face psychosocial challenges which may form a large barrier to recover. There are only a limited number of evidence based psychosocial therapies, but they are mainly based on breathing exercises.
OBJECTIVE: to study which respiration frequency would lead to the highest relaxation, reflected in vagal tone derived from the heart rate variability (HRV) in healthy subjects.
METHODS: A randomized controlled cross sectional study was performed. Respiration cycles of four, five, six, seven and eight breaths per minute (BPM) were delivered in randomized order for two minutes each. HRV metrics were measured during the sessions with electrocardiogram (ECG). Repeated Measures ANOVA's were performed to analyze differences between breathing frequencies.
RESULTS: 100 healthy volunteers were included (40 male). Standard Deviation of inter beat intervals (SDNN) values were significantly highest at 5 BPM, whereas the Root Mean Square of Successive Differences (RMSSD) values appeared highest at 7 breaths per minute ($p < 0.01$). High Frequency (HF) power was lowest at 4 BPM, whereas Low Frequency (LF) power was not significantly influenced by respiration frequency.
CONCLUSIONS: Breathing at a frequency of 5 to 7 breaths per minute leads to highest HRV values, but there is no single respiration ratio that maximizes all metrics. Physiotherapists may use five to seven BPM as guidance to determine ideal breathing frequencies.

Keywords: Heart rate variability, breathing frequencies, relaxation, stress, physiotherapy

1. Introduction

Physiotherapists increasingly treat patients who suffer from psychosocial problems besides physical problems, because of transition from secondary to primary care and because of the aging population, leading to more complex pathology. These psychosocial problems cause increased arousal and hamper quick recovery. It is known that many patients with chronic (pain) conditions, including patients with fibromyalgia, whiplash associated disorders or low back pain, suffer from psychosocial (stress) problems which is amongst other factors, reflected in a decreased autonomic nervous sys-

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tem function, leading to decreased heart rate variability (HRV) [1]. Decreased HRV can be regarded as a physiological indicator of lower self-regulatory capacity [2]. A decreased HRV correlates with less adaptive abilities and a higher HRV is a sign of good health and vitality [3]. Additionally, patients with autonomic dysfunctions and complaints, such as anxiety [4] or depression [5,6], show a decreased HRV compared to healthy populations, however, a recent systematic review concluded that HRV biofeedback may reverse these HRV values and has good effects on treating anxiety, depression, anger and athletic performance [7].

It is widely accepted that respiration mediates the HRV via baroreflex directly [8,9]. During inspiration the heart rate increases, whereas during exhalation the heart rate decreases, called respiratory sinus arrhythmia [10,11]. Several studies show that a respiration frequency of 6 BPM demonstrate a positive influence on the HRV [12–14] and deep and slow breathing may have positive effects on pain perception and autonomic activity [15]. The influence of inspiration to expiration ratio (i/e ratio) has previously been studied and from a theoretical perspective, lower inspiration compared to expiration time would produce higher parasympathetic activity and would increase HRV. According to Hejjel and Wang et al., however, the i/e ratio has no significant effect on HRV [14,16]. There is uncertainty about unobserved frequencies that might also influence the HRV parameters positively and within the slow breathing region (four to eight BPM), it is unclear which breathing frequency produces the highest HRV values.

As many physiotherapists already use breathing exercises and direct biofeedback instruments are frequently unavailable, it is important to investigate the influence of different respiration frequencies on the HRV to advise them in their choice of treatment. Increasingly, physiotherapists use breathing apps to help patients pace their breathing rhythm, however, the used breathing pace is often based on experience of the therapist. The objective of this study is to study in a high quality design, which respiration frequency would lead to the highest relaxation, reflected in vagal tone derived from the heart rate variability (HRV) in untrained healthy subjects.

2. Methods

2.1. Participants

Participants in this study were students and employees of Saxion University of Applied Sciences in the Netherlands. Included participants were above 18 years old and in good physical wellbeing. Participants not doing regular breathing training, including yoga or mindfulness training, were included. The exclusion criteria were heart- and lung diseases, including hypertension, upper respiratory tract infection and the use of antiarrhythmic or anticoagulant treatment. Prior to the study, participants were informed about the aim and content of the study and gave their written consent for anonymous reporting of their data. Because this study did not serve a medical purpose and was conducted with non-invasive freely available products, a medical ethical approval was not required.

2.2. Design and procedures

The study is designed as a randomized cross-sectional study. The session started with the participant drawing a lot with the randomized set of paced-breathing periods to control for any order effects (total of 120 possible orders to rule out order effects). Next, the participant was asked to sit down in a comfortable chair in upright position and be as relaxed as possible for the two minutes baseline measurement of individual, non-paced breathing to be carried out. Subjects were instructed not to use their telephone, fall asleep or meditate. Hereafter, the subject was connected to the HRV measurement followed by the measurement of the randomized set of 4, 5, 6, 7 and 8 BPM for two minutes each. Between each session, subjects had a one minute rest period. Subjects were instructed to inhale through their nose and exhale through their mouth. The respiration frequencies were guided by a visual stimulus of a Paced Breathing App, set at an i/e ratio of 1:2. The Paced Breathing App is freely available and developed for android gadgets. The paced breathing periods were separated by a one minute break of recovery and wash-out of the last session.

2.3. Measures

The measurements were administered by two physiotherapy students (4th grade) of Saxion University of Applied Science in the context of a research assignment. Prior to the data collection, the students were extensively trained by the first author, who is a physiotherapist, human movement scientist (PhD) and licensed Heartmath coach, in using the protocol for data collection and handling of HRV recordings.

Physiological recordings

1. Per set, two minute paced breathing recordings were obtained. These two minute time frames were chosen, firstly, because it has been studied to produce reliable results on the time [17,18] and frequency domains of interest [19]. Secondly, in a pilot conducted in advance of this study, we experienced that prolonged slow breathing of 4 BPM was difficult to maintain in untrained subjects. The HRV was measured with Nexus 10 MKII (Mindmedia, Herten, The Netherlands). The HRV was analyzed in frequency and time domains. Regarding the frequency domain, the Low Frequency power (LF) and the High Frequency power (HF) were calculated. The LF power (spectrum range 0.04–0.15 Hz) reflects sympathetic, parasympathetic and baroreceptor activity [20]. In long-term measurements, the sympathetic activity was reflected dominantly compared to short-term measurements, in which predominantly vagal activity is measured. The HF power (spectrum range 0.15–0.4 Hz) reflects the vagal or parasympathetic activity and corresponds to the respiratory sinus arrhythmia [20]. Higher HF power would lead to higher parasympathetic regulation and hence for more relaxation. Regarding time domain measures, the SDNN was calculated as a Standard Deviation of the RR tops expressed in milliseconds (ms). It reflects the intrinsic ability of the heart to respond to hormonal influences [20]. Higher SDNN is correlated to higher health status. The RMSSD was calculated as the Root Mean Square of Successive Differences between the normal heart beats, expressed in milliseconds (ms). It reflects the short-term variations in the heart frequency and estimates predominantly activity of vagally mediated changes reflected in HRV [20], therefore, higher RMSSD would reflect in higher relaxation. Additionally, respiration rate was recorded to control that subjects breathed as intended by the protocol. When deviation was larger than 0.25 breaths per minute, the measurement was considered invalid and the test was repeated.

2.4. Statistics

First, all HRV data were checked on artifacts, which were removed manually. Descriptive statistics were compiled and analyzed. All HRV values were calculated with Nexus software BioTrace+ (Mindmedia, Herten, The Netherlands). Data were checked on normality and Repeated Measures ANOVA's were conducted to investigate differences in HRV while breathing in different breathing cycles. Gender was inserted as a between subject factor [15]. Age was not inserted as covariate because of limited variation between subjects. As effect size, the partial eta^2 was calculated. An alpha < 0.05 was considered statistically significant. Least Significant Difference (LSD) corrections were applied for all post-hoc analyses. All statistics were calculated with IMB SPSS version 23.

Table 1
Participant characteristics

Subjects	Outcome ($N = 100$)
Gender: N (%;female)	100 (60%)
Age (years): mean (sd)	22 (2.9)
Weight (kg): mean (sd)	71.1 (11.0)
Length (cm): mean (sd)	175.7 (8.9)

3. Results

A total of 100 participants, with a mean age of 22.1 years $\pm$ 2.9 and a range between 18 and 30 years were included in the study. Participant characteristics are presented in Table 1.

In Fig. 1 differences of HRV parameters between respiration cycles are presented. The SDNN shows a reversed U shape form, with a maximum at 5 BPM, that is significantly larger than all other conditions ($p < 0.01$) with an explained variance of 24% (see Table 1). For RMSSD, a similar inversed U shape can be detected. The largest value was at 7 BPM ($p < 0.01$), with all other conditions having significantly lower values. In frequency domain measures, LF did not significantly differ between breathing conditions. The HF values increase significantly between 4 and 5 BPM and between 6 and 8 BPM. The results of the repeated measures ANOVAs are presented in Table 2.

4. Discussion

The objective of this study was to assess effects of different breathing frequencies on HRV metrics. The results show that there is no single respiration frequency that maximizes all values, however we found positive effects of low respiration frequencies (5–7 BPM) on time domain values RMSSD and SDNN and HF. The results showed significant differences in breathing frequencies in the RMSSD, however in much smaller mag-

Table 2
Influence of different breathing cycles on HRV parameters

Variables	4 BPM (mean/sd)	5 BPM (mean/sd)	6 BPM (mean/sd)	7 BPM (mean/sd)	8 BPM (mean/sd)	F (p)	Post hoc LSD	Partial eta^2
SDNN (ms)	113.5 (39.1)	118.9 (38.4)	113.4 (37.8)	108.9 (39.8)	95.1 (37.2)	30.2 (< 0.01)	4<5,7,8; 5>6,7,8, 6>7,8; 7>8	0.24
RMSSD (ms)	76.9 (37.0)	81.7 (37.4)	82.4 (35.4)	88.2 (42.7)	79.5 (40.0)	4.1 (< 0.01)	4<5,7; 6<7;8<7	0.04
LF	5507 (1412)	5616 (1378)	5683 (1498)	5619 (1511)	5691 (1556)	1.07 (0.37)	n.s.	0.01
HF	36.9 (28.3)	45.6 (29.6)	40.3 (22.7)	51.3 (40.1)	62.6 (38.0)	18.1 (< 0.01)	4<5,7,8; 4,6<5>8; 6<7,8; 7<8.	0.16

BPM: Breaths per minute; SDNN: standard deviation of N-N intervals; RMSSD: root mean square of successive difference; LF: low frequency power; HF: high frequency power; Post hoc LSD; Least Significant Difference: significant differences are presented; n.s. no significant post hoc tests.

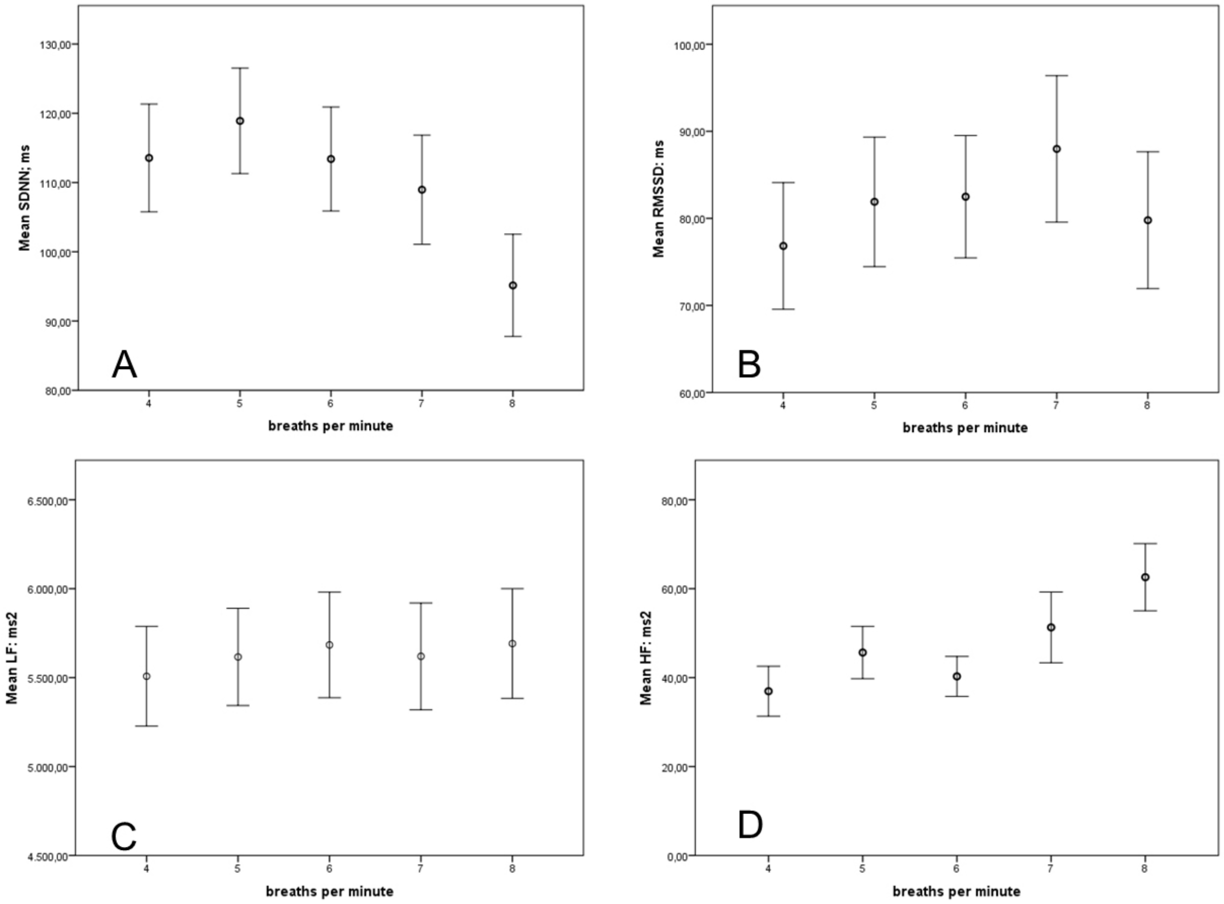

Fig. 1. Graphical presentation of mean HRV parameters in different respiration cycles. (A); SDNN values. (B); RMSSD values. (C); Low Frequency power values. (D); High Frequency power values. Error bars indicate 95% confidence intervals.

nitude compared to SDNN, which contradicts earlier studies [21,22]. The SDNN is significantly higher with lower breathing frequencies than with higher breathing frequencies. In general, the activity of the sympathicus is associated with lower frequencies in long term recordings and activity, but in short term recordings, during deep and slow breathing under a breathing frequency of 8.5 breathing cycles per minute, the vagal nerve causes oscillations in the heart rhythm which are reflected as increased LF values [3]. In a previous study, LF, HF and LF/HF ratio increased with low breathing frequency of 6 breathing frequencies, however, in another study, LF/HF ratio did not change between breathing frequencies (0.10–0.20 Hz.), which was in line with the results of our study [23]. The reason for not finding significant differences in LF power may be because time of measurement was too short, and low breathing frequency may have led to changes in the very low frequency domain. It is known from literature that in an ultrashort 1 minute HRV protocol, SDNN and RMSSD correlate moderately with HRV metrics [24]. Furthermore, in this study, significant increases in HF power were observed with higher breathing frequencies which was as expected. In evaluating training success, increased LF and decreased HF are to be expected.

In the study by Lehrer et al., best results in HRV parameters were reached at 6 BPM [25]. A reason for this could be the application of a fixed order of breathing frequencies, starting with 4.5 and 5 BPM. Participants could experience difficulties when changing from normal breathing to a very slow breathing frequency. In this present study the order of respiration frequency was randomized which allows a better evaluation of the effects of different breathing frequencies on HRV parameters. Furthermore, in the study of Lehrer and colleagues, participants practices the breathing exercise with different breathing frequencies for a period of one week, twice a day [25]. This may have led to differences in results. Next, we found higher RMSSD values on average in the present study compared to earlier [15]. This might have to do with the procedure as well as the healthy cohort that was used, consisting of students and employees at a health science department.

4.1. Limitations

In this study, the primary aim was to identify within subject changes of HRV to differing respiration frequencies. In order to warrant sufficient concentration of the subjects performing five conditions, two minute measurements were chosen. There is still a discussion about whether HRV values, especially power frequencies can be measured validly during a two minute measurement. Especially, the LF seems to be more valid at a measurement of minimal five minutes [26]. Another limitation of this study was possible omitted variable bias. We might have missed important covarying factors on HRV, including physical activity, body mass index, stress [27,28], and menstruation cycle, however, because this was a cross sectional study, we do not think these factors influenced the within subject variance. Besides respiration, those factors may effect HRV. The results of this study may be used for further study, in particular for studying long term effects of breathing exercise on stress and HRV parameters.

5. Conclusion

In summary, the study's output shows a positive effect of low respiration frequencies on the HRV values, especially on the SDNN. There is no single respiration frequency that leads to a maximum in all HRV metrics, however, 5 to 7 breath cycles per minute present the best results on HRV. This outcome could be recommended in physiotherapeutic practices for stress reduction purposes. Future research should focus on the correlation between subjective stress, HRV and other physiological parameters including skin temperature, skin conductance as additional proxies for stress.

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Conflict of interest

None to report.

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