

# Relationship between the proficiency level and anxiety-reducing effect in a one-time heart rate variability biofeedback

## A randomized controlled trial

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### Abstract

**Introduction:** Previous studies have reported that the proficiency level of heart rate variability biofeedback (HRVBF) contributes significantly to the anxiety-reducing effects in continuous HRVBF interventions. Meanwhile, anxiety-reducing effects have been confirmed in one-time HRVBF interventions as well as continuous HRVBF; however, no study has analyzed the relationship between the proficiency level of a one-time HRVBF and its anxiety-reducing effects. To pursue the effectiveness of a one-time HRVBF intervention, it is necessary to clarify whether the proficiency level is an important predictor of anxiety-reducing effects from a dose-response relationship between these 2 variables. The purpose of this study was to examine the dose-response relationship between the proficiency level and anxiety-reducing effects of a one-time HRVBF.

**Methods:** This study was a single-blinded, randomized, controlled trial with stratification based on trait anxiety of the State-Trait Anxiety Inventory-JYZ. In total, 45 healthy young males aged 20 to 30 years were allocated to the HRVBF or control group with simple breathing at rest. The intervention was performed for 15 minute in each group. The state anxiety score of the State-Trait Anxiety Inventory-JYZ was measured to evaluate the anxiety-reducing effect before and after training.

**Results:** The results showed no significant linear relationship between the proficiency level and anxiety-reducing effect, and variations in the proficiency level were observed post-intervention in the HRVBF group. A significant anxiety-reducing effect was only observed in the HRVBF group ( $P = .001$ , effect size  $r = 0.62$ ).

**Conclusions:** These results suggest that there is no close relationship between the proficiency level and anxiety-reducing effect in one-time HRVBF and that HRVBF is effective in reducing anxiety regardless of individual differences in the proficiency level. Therefore, a one-time HRVBF may be a useful breathing technique for reducing state anxiety without specific education and breathing techniques.

**Trial registration:** University Hospital Medical Information Network Clinical Trial Registry (UMIN000041760).

**Abbreviations:** HF = high frequency, HRV = heart rate variability, HRVBF = heart rate variability biofeedback, LF = low frequency, LF/HF = ratio of low frequency to high frequency, STAI-JYZ = State-Trait Anxiety Inventory-JYZ, TP = total power.

**Keywords:** anxiety, biofeedback, heart rate variability, mental health

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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## 1. Introduction

Heart rate variability (HRV) is the changing cardiac beat-to-beat interval and reflects the autonomic nervous system.<sup>[1]</sup> HRV is associated with various physical and psychiatric symptoms, and higher HRV is associated with good health conditions.<sup>[2,3]</sup> One of the most representative methods for increasing HRV is heart rate variability biofeedback (HRVBF). HRVBF is a breathing technique that requires paced breathing at approximately 6 breaths per minute, thereby achieving cardiac coherence.<sup>[4]</sup> A recent meta-analysis has reported its effectiveness in various physical and mental conditions.<sup>[5]</sup> The 3 crucial factors, namely restoring autonomic homeostasis, central effects by the vagal afferent nerve, and the cholinergic anti-inflammatory system, have been postulated as its underlying mechanisms.<sup>[6]</sup>

Another recent meta-analysis has shown the anxiety-reducing effects of not only continuous HRVBF, but also one-time HRVBF.<sup>[7]</sup> However, the meta-analysis indicated variable effect sizes. Previous studies have suggested that the proficiency level of HRVBF may contribute to anxiety-reducing effects.<sup>[8,9]</sup> The proficiency level of HRVBF is based on physiological coherence

in the intervention. Physiological coherence is used to describe the degree of order, harmony, and stability in the various rhythmic activities within living systems over any given time period.<sup>[10]</sup> The goal of HRVBF is to achieve higher physiological coherence, and a higher proficiency level means higher physiological coherence. A state of higher physiological coherence activates vagal afferent pathways, which are known to affect brain regions involved in emotional control (the locus coeruleus, orbitofrontal cortex, insula, hippocampus, and amygdala).<sup>[11,12]</sup> This corresponds to the central effects of the vagal afferent nerve in the HRVBF mechanism described above, and it is thought to contribute toward the control of emotions, such as anxiety.<sup>[6,11]</sup> Zauszniewski et al.<sup>[18]</sup> showed that the proficiency level accomplished in the final session is negatively correlated with the psychometric variables at 2 and 8 weeks of follow-up, after a 4-week intervention of HRVBF. This suggests that there is a dose-response relationship between the anxiety-reducing effect and the proficiency level in continuous HRVBF. In regard to one-time HRVBF, only Sherlin et al.<sup>[9]</sup> demonstrated that the higher proficiency level group achieved lower state anxiety than the lower proficiency level group. However, there have been no studies that examined the dose-response relationship between the proficiency level and anxiety-reducing effects in a one-time HRVBF intervention. To pursue the effectiveness of a one-time HRVBF intervention, it is necessary to clarify whether the proficiency level is an important predictor of anxiety-reducing effects from a dose-response relationship between these 2 variables.

The purpose of this study was to examine the dose-response relationship between the proficiency level and anxiety-reducing effects of a one-time HRVBF intervention, focusing on the effect of HRVBF on anxiety induced by an anticipatory anxiety event. We hypothesized that a significant linear relationship between the proficiency level and anxiety-reducing effect would exist in a one-time HRVBF intervention, as the prerequisite of the fact that one-time HRVBF would have sufficient anxiety-reducing effects<sup>[9,13,14]</sup> and physiological effects on vagally mediated HRV.<sup>[15]</sup>

## 2. Methods

### 2.1. Participants

Forty-five healthy young males (mean age:  $22.07 \pm 1.92$ ; age range: 20-28) were recruited in the present study (See “2.2. Sample size estimation” for sample size). The inclusion criteria were as follows: age range of 20 to 30 years and male sex, considering the influence of age differences on HRV (the decline in HRV with age),<sup>[2]</sup> sex differences on HRV (higher vagal HRV in females than in males),<sup>[16]</sup> and the stress response-related neuroendocrinological system (male-female differences in the activity of the hypothalamic-pituitary-adrenal (HPA) axis).<sup>[17]</sup> The exclusion criteria were as follows: a medical history of brain disease, head injury, psychiatric disorder, heart disease, or respiratory disease; a history of dizziness and consciousness disorder due to slow breathing; and consuming drugs that affect the autonomic nervous system. No participants were excluded, and all 45 healthy participants were included in the study. Participants were recruited in Hokkaido University from October 2020 to March 2021. The study protocol was approved by the Ethics Committee of the Faculty of Health Sciences, Hokkaido University (Approval number: 20-43-1), and all

experiments were conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

### 2.2. Sample size estimation

The required sample size for this study was calculated by using a priori power analysis using G\*power 3.1.<sup>[18]</sup> Regarding input parameters, the effect size (Cohen's  $d=0.89$ ) calculated from a one-time HRVBF study<sup>[9]</sup> and correlation coefficient referenced from a continuous HRVBF study<sup>[8]</sup> ( $r=0.50$ ) were adopted. The sample size estimate for 2-group comparison based on significance probability,  $\alpha=0.05$ ; statistical power,  $1-\beta=0.80$ ; and effect size,  $d=0.89$ ; resulted in  $n=17$  per group. The sample size estimate for correlation analysis was based on significance probability,  $\alpha=0.05$ ; statistical power,  $1-\beta=0.80$ ; correlation coefficient,  $r=0.50$ ;  $n=26$ . Based on these estimation results, the sample size was planned as the HRVBF group ( $n=27$ ) and control group ( $n=18$ ), and the allocation ratio of the HRVBF group to the control group was set to 3:2. This allocation ratio is preferred for unbalanced designs.<sup>[19]</sup>

### 2.3. Study design

The present study was based on the prerequisite that a one-time HRVBF intervention would have anxiety-reducing effects that would help examine the dose-response relationship between the proficiency level and anxiety-reducing effects. For this reason, it was necessary to clarify whether the anxiety-reducing effects of a one-time HRVBF intervention could be observed. The control group was set to obtain more robust results which prove that the within-subject factor “time” in the control group has no significant differences.

The present study was conducted as a single-blinded randomized controlled trial with stratification based on the Trait Anxiety Scale of State-Trait Anxiety Inventory-JYZ (STAI-JYZ), which is a self-assessment scale to measure participants' trait anxiety.<sup>[20]</sup> The participants were blinded to the interventions. The stratified randomization was conducted according to whether trait anxiety scores were above or below the standard mean, stratifying into 2 categories with the allocation ratio of the HRVBF group to the control group of 3:2. The random allocation sequence was created by the computer and the allocation was conducted by sequentially numbered containers. This study followed the Consolidated Standards of Reporting Trials (CONSORT) guidelines, and the CONSORT flow diagram can be seen in Figure 1. This study was registered at the University Hospital Medical Information Network Clinical Trial Registry (UMIN-CTR) (Registered number: UMIN000041760).

### 2.4. Intervention

A HRVBF device, emWave Pro (HeartMath LLC, Boulder Creek, CA), was used to measure the pulse wave. The pulse wave data were displayed as the HRV waveform. Paced breathing was performed using a breath pacer, which showed the timing of inhalation and exhalation. In the HRVBF group, the resonance frequency, which is the breathing pace to maximize HRV, was determined based on the HRVBF training protocol<sup>[4]</sup> before the intervention. The optimal resonance frequency was set in the breath pacer. Participants were instructed to perform paced breathing using the breath pacer and to check the smoothness of

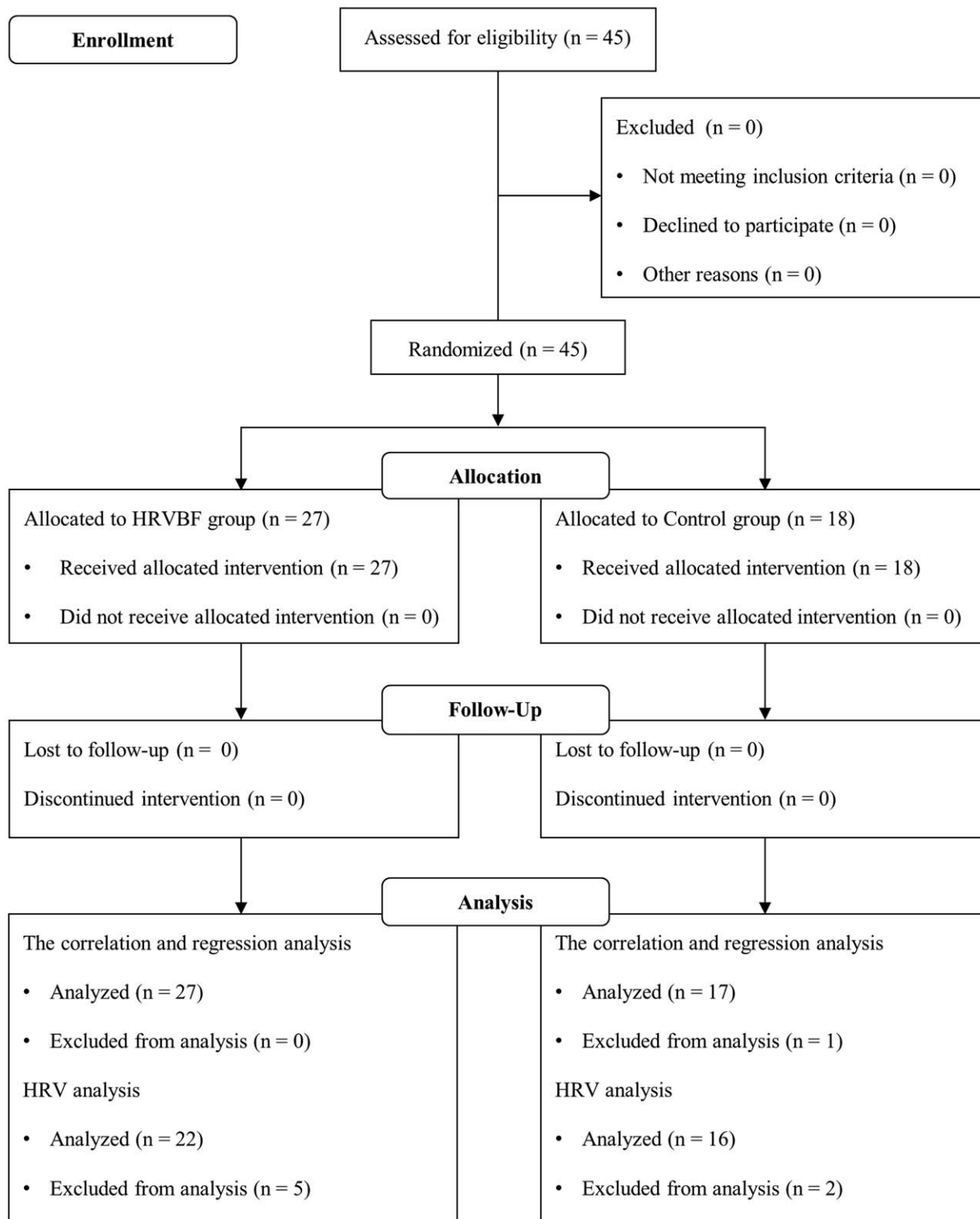


Figure 1. CONSORT flow diagram. HRV=heart rate variability, HRVBF=heart rate variability biofeedback.

the HRV waveform. The intervention time was set to 15 minute based on a previous study.<sup>[9]</sup> In the control group, the participants were instructed to breathe at rest for 15 minute while only checking the HRV waveform on the screen.

**2.5. Measurement**

**2.5.1. Psychological measurement.** The STAI-JYZ,<sup>[20]</sup> a modified Japanese version of the STAI Form Y,<sup>[21]</sup> was used to measure anxiety. The STAI-JYZ measures state and trait anxiety.

**2.5.2. Physiological measurement.** During the training, the coherence score was calculated every 5 seconds. The coherence score evaluates physiological coherence from patterns of the HRV waveform during the training. After completing the training, the average coherence score was calculated. The average coherence score was calculated through the whole training period and was used as the parameter of the proficiency level of the HRVBF.

The portable electrocardiograph (ECG), Check My Heart (Daily Care Biomedical Inc., Taiwan), was used to record ECG data. ECG data were recorded for 5 minute in the resting position with both forearms in supination on the table, and the RR interval data were extracted at a sampling frequency of 250 Hz. The RR interval data were analyzed using an HRV analysis software (Kubios HRV Premium version 3.4.2; University of Eastern Finland, Kuopio, Finland). All preprocessing was conducted in line with the default preprocessing pipeline in Kubios HRV Premium. After artifact correction and resampling were performed during preprocessing, the data were transformed into HRV frequency-domain parameters<sup>[11]</sup>: low frequency (LF; sympathetic and parasympathetic activity), high frequency (HF; parasympathetic activity), total power (TP; overall autonomic activity), and the ratio of low to high frequency (LF/HF; sympathetic activity) using fast Fourier transform (FFT). TP, LF, and HF ( $\text{ms}^2/\text{Hz}$ ) were natural log-transformed to adjust for unequal variances.

## 2.6. Procedure

Experimentation was conducted between 13:00 and 17:00, considering the influence of the time of day on autonomic activity.<sup>[22]</sup> To minimize differences, the participants were asked to get enough sleep the day before the experiment, not to consume alcohol or caffeine on the day of the experiment, and not to engage in intense exercise.

The experimental procedure is illustrated in Figure 2. A questionnaire about basic information and lifestyle was used to confirm the age, body mass index (BMI), smoking status, exercise habits, and sleeping time because these items are known to affect HRV.<sup>[2]</sup> HRV data collection and the State Anxiety Inventory assessment were performed 3 times: at baseline, pre-intervention, and post-intervention. The anticipatory anxiety event that was used to increase an individual's state anxiety was the instruction of a speech task.<sup>[23]</sup> The participants were instructed to speak on the topic to be announced later. In this study, the speech task was not actually conducted because the purpose of this event was only to evoke state anxiety. Thus, the participants were told that the speech task would not be administered at the end of the post-intervention measurement.

## 2.7. Statistical analysis

Basic characteristics, including demographic data, trait anxiety scores, and average coherence scores were compared between the 2 groups using an independent sample *t* test or Mann–Whitney *U* test according to the normality of the data distribution checked by the Shapiro–Wilk test. Correlation and regression analyses were performed to examine the dose-response relationship between the average coherence score and the change in the state anxiety score (post- minus pre-intervention score) using Pearson correlation and a simple linear regression with the average coherence score as the independent variable and the change in the

state anxiety score as the dependent variable. The normality of the residuals was examined by the quantile-quantile plots (QQ-plots). In regression models, it is known that checking the normality of the residuals is an appropriate prerequisite and the QQ-plots are a much better way to check the normality.<sup>[24]</sup> Furthermore, to confirm the influence of trait anxiety, a subgroup correlation analysis was also performed based on trait anxiety scores above or below the mean in this study.

Repeatedly measured state anxiety scores and all HRV parameters were checked for the normality of the data distribution using the Shapiro–Wilk test. To properly examine the effect of a one-time HRVBF in this study design, it was necessary to examine the interaction by analysis of variance (ANOVA). In addition, ANOVA has been shown to be robust to violations of normality.<sup>[25]</sup> Thus, this study conducted ANOVA regardless of the assumption of the normality. A  $2 \times 3$  mixed-design ANOVA with group (HRVBF or control) and time (baseline, pre or post) as the between- and within-subject factors, respectively, were separately performed for the state anxiety score and all HRV parameters. The Bonferroni method was used for multiple comparisons. Furthermore, planned comparisons were performed to compare intragroup and intergroup differences post-intervention. The intragroup differences were compared using a paired sample *t* test or Wilcoxon signed-rank test, and the intergroup differences were compared using an independent sample *t* test or Mann–Whitney *U* test, according to the normality of the data distribution. The effect size (Cohen's *d* or effect size *r*) was also calculated according to the normality of the data distribution. All statistical analyses were performed using SPSS (version 26.0; IBM Corp., Armonk, NY). The statistical significance level was set at 0.05.

## 3. Results

### 3.1. Group comparisons of basic characteristics

Group comparisons of the smoking status of participants were not performed because only the HRVBF group comprised smokers ( $n=5$ ). The Shapiro–Wilk test showed that the assumption of normality was met only for trait anxiety scores ( $P=.162$ ) among the basic characteristics. An independent sample *t* test revealed no significant differences between the HRVBF and control groups in trait anxiety scores ( $P=.719$ ). For other variables, the Mann–Whitney *U* test revealed no significant differences between the HRVBF and control group, in regard to factors including age ( $P=.485$ ), BMI ( $P=.487$ ), exercise habits ( $P=.427$ ), and sleeping time ( $P=.235$ ); however, a significant difference in the average coherence score was observed (HRVBF group: median, 4.2, interquartile range (IQR), 3.6–4.9; control group: median, 1.2, IQR, 1.0–1.5;  $P=.000$ ) (Table 1).

### 3.2. Regression between the proficiency level and effect on anxiety

Prior to the correlation and regression analysis, 1 control participant was excluded as an outlier. The normality of the residuals was graphically verified by the QQ plots. The correlation analysis revealed no significant correlation between the average coherence score and the change in the state anxiety score in either the HRVBF ( $r=-0.18$ ,  $P=.365$ ) or control groups ( $r=0.27$ ,  $P=.295$ ). Regression analysis revealed no significant

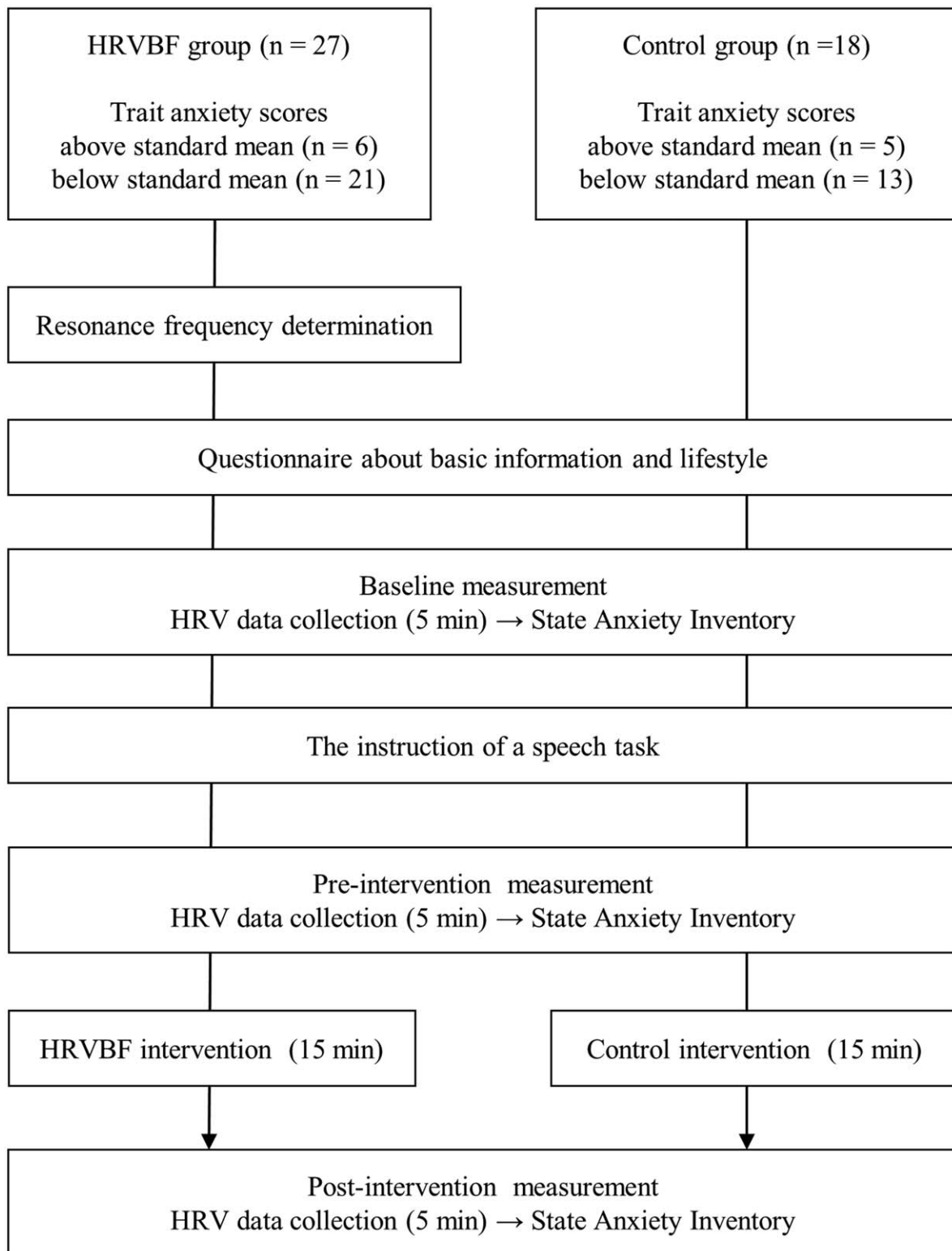


Figure 2. The experimental procedure. HRV=heart rate variability, HRVBF=heart rate variability biofeedback.

linear relationship between them in either the HRVBF ( $R^2=0.03$ ,  $P=.365$ ) or control group ( $R^2=0.07$ ,  $P=.295$ ) (Fig. 3). Subgroup correlation analysis revealed no significant correlations between these 2 variables.

**3.3. State anxiety**

The Shapiro–Wilk test showed that the assumption of normality was met only for state anxiety at baseline ( $P=.094$ ). A  $2 \times 3$  mixed-design ANOVA for anxiety scores was performed

**Table 1**  
Group comparisons of basic characteristics.

| Variables                            | Groups                   |                              |                      |                        | P value           |
|--------------------------------------|--------------------------|------------------------------|----------------------|------------------------|-------------------|
|                                      | HRVBF group (n=27)       |                              | Control group (n=18) |                        |                   |
|                                      | Mean ± SD                | Median (IQR)                 | Mean ± SD            | Median (IQR)           |                   |
| Age (yrs)                            | 22.26 ± 2.07             | 22.00 (21.00–23.00)          | 21.78 ± 1.70         | 21.50 (20.00–23.00)    | .485*             |
| BMI (kg/m <sup>2</sup> )             | 21.82 ± 2.41             | 21.89 (20.08–22.98)          | 22.41 ± 3.13         | 22.63 (19.63–24.20)    | .487*             |
| Smoking status                       |                          |                              |                      |                        |                   |
| Smoking history (mo)                 | 22.6 ± 24.5 <sup>‡</sup> | 14.0 (9.5–40.0) <sup>‡</sup> | NA                   | NA                     | NA                |
| Number of cigarettes smoked per day  | 5.4 ± 3.1 <sup>‡</sup>   | 4.0 (3.0–8.5) <sup>‡</sup>   | NA                   | NA                     | NA                |
| Average exercise time per week (min) | 176.11 ± 197.93          | 120.00 (0.00–240.00)         | 237.78 ± 296.82      | 120.00 (60.00–330.00)  | .427*             |
| Average sleeping time (min)          | 411.11 ± 53.16           | 420.00 (360.00–450.00)       | 431.67 ± 61.00       | 435.00 (382.50–480.00) | .235*             |
| Trait anxiety score                  | 42.52 ± 8.40             | 43.00 (34.00–47.00)          | 43.50 ± 9.67         | 43.50 (35.75–50.00)    | .719 <sup>†</sup> |
| Average coherence score              | 4.2 ± 1.1                | 4.2 (3.6–4.9)                | 1.4 ± 0.6            | 1.2 (1.0–1.5)          | .000*             |

BMI = body mass index, HRVBF = heart rate variability biofeedback, IQR = interquartile range, SD = standard deviation.

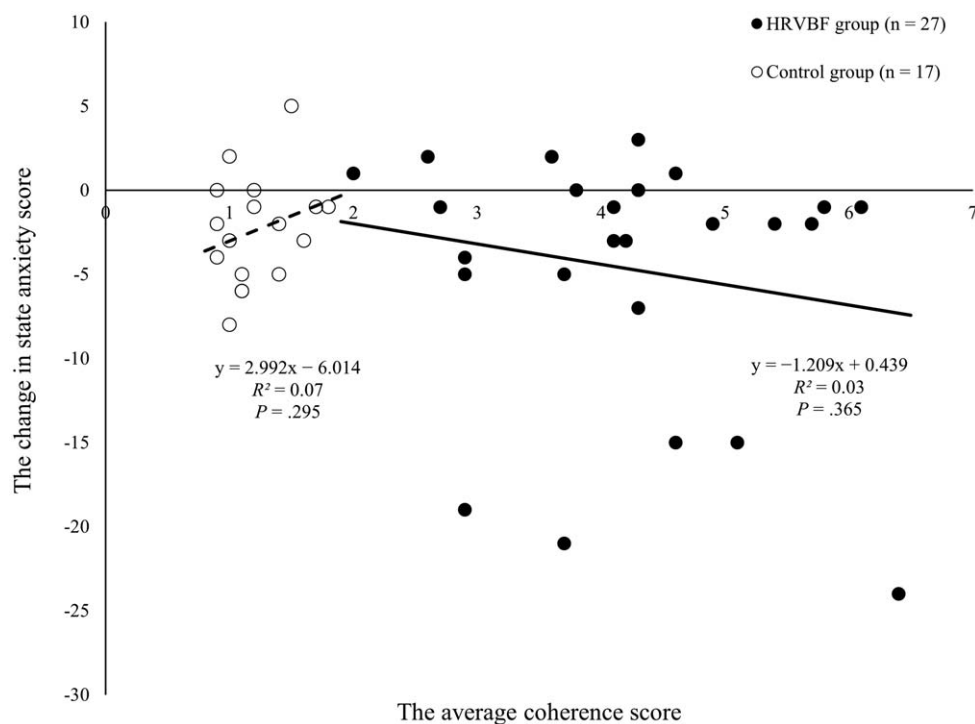
\* Mann–Whitney *U* test.

<sup>†</sup> Independent sample *t* test.

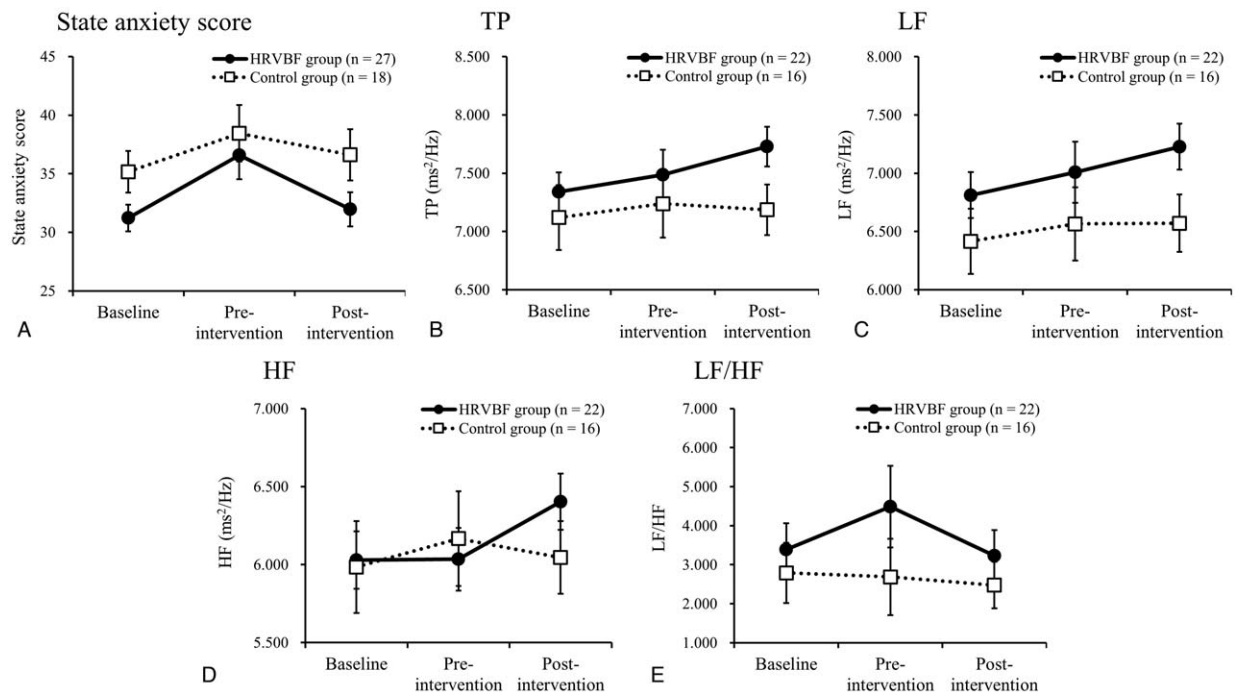
<sup>‡</sup> Smokers were in only HRVBF group (n=5).

with Greenhouse–Geisser correction because Mauchly sphericity test showed that the assumption of homoscedasticity was not met ( $P=.002$ ). ANOVA revealed no interaction ( $F(1.58, 68.10)=1.00, P=.357$ ) and no main effect of the group ( $F(1, 43)=2.20, P=.145$ ); however, a significant main effect of time ( $F(1.58, 68.10)=9.57, P=.001$ ) was observed (Fig. 4A). Multiple comparisons of the main effect of time revealed significantly higher pre-intervention scores than baseline ( $P=.004$ ) and significantly lower scores post-intervention than pre-intervention ( $P=.004$ ). In the planned comparisons, the intra-

group comparisons using the Wilcoxon signed-rank test revealed a significantly lower score at post-intervention, as compared with that at the pre-intervention in the HRVBF group (pre-intervention: median, 37.00, IQR, 27.00–43.00; post-intervention: median, 31.00, IQR, 24.00–39.00;  $P=.001$ , effect size  $r=0.62$ ), and a significantly lower tendency at post-intervention compared with pre-intervention in the control group (pre-intervention: median, 35.00, IQR, 30.00–47.25; post-intervention: median, 37.00, IQR, 28.50–42.75;  $P=.055$ , effect size  $r=0.45$ ).



**Figure 3.** The relationship between the proficiency level and the anxiety-reducing effect in the intervention. The average coherence score was a parameter of the proficiency level in the intervention. The change in the state anxiety score was calculated by subtracting the pre-intervention scores from the post-intervention scores. The correlation and regression analyses revealed no significant correlation and no significant linear relationship between the proficiency level and anxiety-reducing effect in either the HRVBF or control groups. HRVBF = heart rate variability biofeedback.



**Figure 4.** Temporal changes in state anxiety score and HRV parameters. (A) State anxiety score. A  $2 \times 3$  mixed-design ANOVA revealed significant main effect of time (baseline, pre-intervention, post-intervention) ( $P = .001$ ). Multiple comparisons revealed significantly higher score of pre-intervention than baseline ( $P = .004$ ) and significantly lower score in post-intervention than pre-intervention ( $P = .004$ ). (B) TP, (C) LF, (D) HF, and (E) LF/HF. A  $2 \times 3$  mixed-design ANOVA revealed no interaction and no main effect in all HRV parameters.  $P < .01$ . Error bars indicate standard errors. HF=high frequency, HRV=heart rate variability, HRVBF=heart rate variability biofeedback, LF/HF=ratio of low frequency to high frequency, LF=low frequency, TP=total power.

### 3.4. HRV parameters

Five patients in the HRVBF group and 2 in the control group were excluded from the analysis based on the criteria of Kubios HRV Premium. The Shapiro–Wilk test showed that the assumption of normality was met for TP ( $P = .506-.999$ ), LF ( $P = .478-.860$ ), and HF ( $P = .053-.331$ ) at baseline, pre- and post-intervention. Mauchly sphericity test showed that the assumption of homoscedasticity was met ( $P = .063-.698$ ) regardless of the HRV parameters. A  $2 \times 3$  mixed-design ANOVA revealed no interaction ( $F(2, 35) = 0.46-1.41$ ,  $P = .257-.637$ ) and no main effect of group ( $F(1, 36) = 0.10-2.55$ ,  $P = .119-.751$ ) or time ( $F(2, 35) = 1.68-2.20$ ,  $P = .126-.202$ ) on any of the HRV parameters (Fig. 4B, C, D, E). The intragroup comparisons in the HRVBF group revealed a significant increase in HF using a paired sample  $t$  test ( $t(21) = -2.91$ ,  $P = .008$ , 95% CI:  $-0.63, -0.11$ , Cohen's  $d = 0.41$ ), and a significantly decreased tendency in LF/HF by the Wilcoxon signed-rank test (pre-intervention: median, 3.072, IQR, 1.259-6.240; post-intervention: median, 2.168, IQR, 1.346-3.942;  $P = .077$ , effect size  $r = 0.38$ ). The intergroup comparisons revealed a significantly higher LF and a significantly higher tendency of TP in the HRVBF group than in the control group (LF:  $t(36) = 2.11$ ,  $P = .042$ , 95% CI: 0.02, 1.29, Cohen's  $d = 0.69$ ; TP:  $t(36) = 1.99$ ,  $P = .054$ , 95% CI:  $-0.01, 1.10$ , Cohen's  $d = 0.65$ ).

## 4. Discussion

The correlation and regression analyses revealed no significant linear relationship between the proficiency level and anxiety-reducing effect of a one-time HRVBF (Fig. 3). However, the

HRVBF group only showed a significant decrease in state anxiety scores between pre- and post-intervention time points (Fig. 4A). This suggests that the anxiety-reducing effect is independent of the proficiency level in a one-time HRVBF. However, this study's results differ from those of previous studies showing proficiency level effects<sup>[8,9]</sup> and are contrary to our hypothesis. Two reasons could be considered for such results. First, population characteristics can affect the relationship between proficiency level and effect. Previous studies have targeted individuals with high levels of anxiety, while this study targeted healthy individuals.<sup>[8,9]</sup> A recent systematic review suggested that HRVBF is more beneficial for patient populations or individuals with particular profiles (e.g., individuals exposed to stressful environments) than the healthy population.<sup>[26]</sup> This suggests that the effect of HRVBF on the healthy population may be different from that on other populations. The second reason pertained to individual differences in the variability of the average coherence scores and susceptibility to state anxiety. Variations in the average coherence scores were observed in the HRVBF group (Fig. 3). Usually, a continuous HRVBF training protocol requires learning breathing techniques such as pursed lip breathing and abdominal breathing<sup>[4]</sup>; however, considering the difficulty in learning these techniques in 1 HRVBF session, they were not taught. Therefore, the proficiency level might have been inconsistent because of differences in breathing methods among the participants. It is possible that the correlation between proficiency level and anxiety-reducing effects was not detected owing to the large variety of proficiency levels. Further studies are needed to investigate the influence of population characteristics on the dose-response relationship, considering standardized breathing techniques.

Regarding the state anxiety score (Fig. 4A), a significant difference between baseline and pre-intervention scores indicates that an anticipatory anxiety event worked effectively. A significant difference between pre- and post-intervention scores showed that both groups decreased their state anxiety after the intervention. However, the differences in the effect size in planned comparisons suggest that the HRVBF more effectively reduced anxiety compared to controls. This result supports the effectiveness of the anxiety-reducing effect of a one-time HRVBF.<sup>[9,13,14]</sup> Additionally, this is the first study to reveal the effectiveness of the HRVBF in anticipatory anxiety. Previous studies have not focused on anxiety-reducing effects in situations exposed to anticipatory anxiety.<sup>[9,13,14]</sup> This may suggest that a one-time HRVBF may be useful as a coping strategy for anxiety during everyday life where anticipatory anxiety persists.

Regarding HRV parameters (Fig. 4B, C, D, E), no statistical significance was observed in the omnibus ANOVA. However, planned comparisons revealed a significant increase in HF and a decreased tendency in LF/HF in the HRVBF group at post-intervention compared to pre-intervention, and a significantly higher LF and a tendency of higher TP at post-intervention in the HRVBF group, compared to the control group. These results support previous findings showing a one-time HRVBF effect of an increase in overall autonomic activity,<sup>[13]</sup> an increase in parasympathetic activity,<sup>[14,15]</sup> and a decrease in sympathetic activity.<sup>[14]</sup> Anxiety is generally associated with increased sympathetic activity and decreased parasympathetic activity.<sup>[27]</sup> Thus, the anxiety-reducing effect of HRVBF is likely to be caused by inducing parasympathetic dominance over sympathetic activity.

This study has a few limitations. The first pertains to not measuring the sleep hours on the day before the experiment. Since sleep deprivation is associated with increased levels of state anxiety,<sup>[28]</sup> it is possible that sleep hours on the previous day affected the evocation of state anxiety and the change in state anxiety before and after the intervention. However, this bias was thought to be minimized because participants were asked to get enough sleep the day before the experiment, and a randomization method was recruited in this study. The second pertains to the population. This study limited age and gender to account for psychophysiological influences. It should be noted that the results were based on healthy young men alone, which limits the generalizability of the results. The third pertains to the level of state anxiety in both groups. The level of trait anxiety in both groups was controlled by using a stratified randomization method. Trait anxiety indicates the tendency of an individual to become anxious, and it is known that trait anxiety strongly correlates with state anxiety.<sup>[20,21]</sup> For this reason, it was thought that the stratified randomization by trait anxiety could control the baseline level of state anxiety. However, the level of state anxiety in both groups was not sufficiently controlled (Fig. 4A). The difference in the level of state anxiety in both groups may have affected the change in state anxiety due to the interventions. Further studies are needed to investigate the influence of the population, such as females or elderly, on the relationship between the proficiency level and anxiety-reducing effect after controlling for influencing factors such as sleep hours on the previous day and the level of state anxiety at baseline.

In conclusion, the findings of this study demonstrated that although one-time HRVBF has an anxiety-reducing effect, the effect has no dose-response relationship with the level of proficiency level. The overall interpretation of these findings

suggests that one-time HRVBF has an anxiety-reducing effect even without a consistent proficiency level, and it can be a useful intervention to reduce state anxiety even in novice users without specific education on breathing techniques. This study might indicate adaptability beyond the existing protocol, although more detailed studies are required to investigate the adaptability of the HRVBF.

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## References

- [1] Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology. Heart rate variability—standards of measurement, physiological interpretation, and clinical use. *Eur Heart J* 1996;17:354–81.
- [2] Acharya UR, Joseph KP, Kannathal N, Lim CM, Suri JS. Heart rate variability: a review. *Med Biol Eng Comput* 2006;44:1031–51.
- [3] Bassett D. A literature review of heart rate variability in depressive and bipolar disorders. *Aust N Z J Psychiatry* 2016;50:511–9.
- [4] Lehrer P, Vaschillo B, Zucker T, et al. Protocol for heart rate variability biofeedback training. *Biofeedback* 2013;41:98–109.
- [5] Lehrer P, Kaur K, Sharma A, et al. Heart rate variability biofeedback improves emotional and physical health and performance: a systematic review and meta analysis. *Appl Psychophysiol Biofeedback* 2020;45: 109–29.
- [6] Gevirtz R. The promise of heart rate variability biofeedback: evidence-based applications. *Biofeedback* 2013;41:110–20.
- [7] Goessl VC, Curtiss JE, Hofmann SG. The effect of heart rate variability biofeedback training on stress and anxiety: a meta-analysis. *Psychol Med* 2017;47:2578–86.
- [8] Zauszniewski JA, Musil CM, Variath M. Biofeedback in grandmothers raising grandchildren: correlations between subjective and objective measures. *Biofeedback* 2015;43:193–9.
- [9] Sherlin L, Gevirtz R, Wyckoff S, Muench F. Effects of respiratory sinus arrhythmia biofeedback versus passive biofeedback control. *Int J Stress Manag* 2009;16:233–48.
- [10] McCraty R, Shaffer F. Heart rate variability: new perspectives on physiological mechanisms, assessment of self-regulatory capacity, and health risk. *Glob Adv Health Med* 2015;4:46–61.
- [11] Lehrer PM, Gevirtz R. Heart rate variability biofeedback: how and why does it work? *Front Psychol* 2014;5:756.
- [12] Grundy D. Neuroanatomy of visceral nociception: vagal and splanchnic afferent. *Gut* 2002;51(Suppl 1):i2–5.
- [13] Prinsloo GE, Rauch HGL, Lambert MI, Muench F, Noakes TD, Derman WE. The effect of short duration heart rate variability (HRV)



- biofeedback on cognitive performance during laboratory induced cognitive stress. *Appl Cogn Psychol* 2011;25:792–801.
- [14] Wells R, Outhred T, Heathers JAJ, Quintana DS, Kemp AH. Matter over mind: a randomised-controlled trial of single-session biofeedback training on performance anxiety and heart rate variability in musicians. *PLoS One* 2012;7:e46597.
- [15] Plans D, Morelli D, Sütterlin S, Ollis L, Derbyshire G, Cropley M. Use of a biofeedback breathing app to augment poststress physiological recovery: randomized pilot study. *JMIR Form Res* 2019;3:e12227.
- [16] Koenig J, Thayer JF. Sex differences in healthy human heart rate variability: a meta-analysis. *Neurosci Biobehav Rev* 2016;64:288–310.
- [17] Goel N, Workman JL, Lee TT, Innala L, Viau V. Sex differences in the HPA axis. *Compr Physiol* 2014;4:1121–55.
- [18] Faul F, Erdfelder E, Lang AG, Buchner A. G\*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods* 2007;39:175–91.
- [19] Pocock SJ. *Clinical Trials: A Practical Approach*. New York: John Wiley & Sons; 1983.
- [20] Hidano T, Fukuhara M, Iwawaki S, Soga S, Spielberger CD. *Manual for the State-Trait Anxiety Inventory – Form JYZ*. 2002; Jitsumu Kyoiku Shuppan, Tokyo, Japan. [in Japanese].
- [21] Spielberger CD, Gorsuch RL, Lushene RE, Vagg PR, Jacobs GA. *Manual for the State-Trait Anxiety Inventory: STAI (Form Y)*. Palo Alto, CA: Consulting Psychologist Press; 1983.
- [22] Vandewalle G, Middleton B, Rajaratnam SMW, et al. Robust circadian rhythm in heart rate and its variability: influence of exogenous melatonin and photoperiod. *J Sleep Res* 2007;16:148–55.
- [23] Masamoto K, Ichikawa Y, Hidaka I, Yoda A. Responses during speech: physiological reactivity and subjective tension, and relationship between these responses. *Health Behav Sci* 2003;2:27–34.[in Japanese].
- [24] Ernst AF, Albers CJ. Regression assumptions in clinical psychology research practice—a systematic review of common misconceptions. *PeerJ* 2017;5:e3323.
- [25] Blanca MJ, Alarcón R, Arnau J, Bono R, Bendayan R. Non-normal data: is ANOVA still a valid option? *Psicothema* 2017;29:552–7.
- [26] Tinello D, Kliegel M, Zuber S. Does heart rate variability biofeedback enhance executive functions across the lifespan? A systematic review. *J Cogn Enhanc* 2021;30:1–17.
- [27] Friedman BH. An autonomic flexibility-neurovisceral integration model of anxiety and cardiac vagal tone. *Biol Psychol* 2007;74:185–99.
- [28] Pires GN, Bezerra AG, Tufik S, Andersen ML. Effects of acute sleep deprivation on state anxiety levels: a systematic review and meta-analysis. *Sleep Med* 2016;24:109–18.