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Research Article

Comparison of Breathing Exercises and Auricular Vagus Nerve Stimulation Effects on Autonomic Nervous System Activity and Respiratory Functions in Healthy Adults: An Active Comparative Controlled Study

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Introduction: Vagal modulation is achieved directly by transcutaneous auricular vagus nerve stimulation, whereas breathing exercises stimulate arterial baroreceptors. In this study, we aimed to compare these two methods, which have similar effects.
Methods: 88 healthy participants aged 18–35 were randomly divided into breathing exercises (Group BE) and vagus stimulation (Group VNS). Thoracic expansion exercise was performed in the BE group. In the VNS group, biphasic electrical stimulation was applied to both ears with a pulse width of 300 ms, a frequency of 10 Hz, and 20 min. Pulmonary function tests were measured on the first and last days. Heart rate, systolic and diastolic blood pressure, RMSSD, PNN50, LF/HF, LF Power, and HF Power values were measured before and after each of the 10 sessions for both groups.
Results: Heart rate decreased significantly in both groups, with significant superiority in the BE group compared to that in the VNS group. In both groups, blood pressure values decreased significantly. RMSSD, PNN50, and HF values increased significantly in the VNS group, while LF and LF/HF values decreased significantly in the BE group. In pulmonary function test results, the FEV1 value increased significantly in both groups. A significant increase in the FVC value was observed in both groups, but the BE group was superior. The two groups had no significant superiority in the FEV1/FVC value.
Conclusion: As a result, auricular vagus stimulation seems superior to breathing exercises in increasing the parasympathetic system activity, reducing sympathetic activity, and partially increasing respiratory functions.

Clinical Trial Registration: NCT06531954

Keywords: auricular vagus nerve stimulation; autonomic nervous system; breathing exercises; pulse rate variability; respiratory functions

1. Introduction

The autonomic nervous system (ANS), composed of the sympathetic and parasympathetic branches, unconsciously controls physiological processes such as heart rate, blood pressure, respiration, digestion, and sexual arousal. The sympathetic part is related to the fight-or-flight response and stimulates the body in response to stress and new demands. The parasympathetic nervous system mainly supports the "rest and digest" process and lowers the heart rate and blood pressure. The most essential function of the ANS is to maintain homeostasis. Due to the control of physiological processes, ANS dysfunction is involved in many systemic disease pathogenesis [1].

The most critical component of the parasympathetic nervous system is the vagus nerve, the 10th and longest of the cranial nerves, which is also involved in the connection between the brain and the gut. In the 1880s, vagus nerve stimulation (VNS) began to be seen with manual massage applied to the neck; afterward, electrical stimulation was discovered in the 1930s [2]. Nowadays, VNS includes both invasive (surgically implanted) and noninvasive (transcutaneous) techniques (tVNS). Noninvasive stimulation can be performed through the ear or neck [3]. In our study, bilateral transcutaneous auricular VNS (taVNS) was preferred to stimulate the vagus fibers in the outer ear.

Breathing exercises significantly improve respiratory function, exercise capacity, and quality of life [4]. Thoracic expansion exercises, which utilize proprioceptive stimulation of the chest wall and apply pressure to the appropriate areas, expand the lungs better and increase oxygenation [5]. Slow breathing significantly modulates autonomic motor activity. It increases heart rate variability (HRV) and baroreflex sensitivity, indicating elevated parasympathetic activity, and decreases blood pressure and muscle sympathetic nerve activity. The positive effects of regular slow breathing on the baroreflex are explained by causing neuroplastic changes in the baroreflex [6].

Breathing exercises provide vagal modulation by stimulation of arterial baroreceptors, while auricular vagus stimulation electrically affects the vagus nerve. These two methods affecting the parasympathetic system are considered promising additional therapeutic approaches for anxiety, depressive disorders, chronic pain, cardiovascular diseases, and insomnia [7, 8]. In the literature, very few studies compare breathing exercises and VNS despite their similar effects on ANS. For this purpose, in our study, the effects of breathing exercises, including slow and deep breathing and vagus stimulation, on the ANS activity and pulmonary function were examined, and the short-term effects of these two methods were compared.

2. Methods

The study was planned as a randomized controlled design; however, since both groups received active interventions, it functionally corresponds to an active comparative controlled trial.

2.1. Participants. Healthy individuals between the ages of 18 and 35, without orthopedic disability, without any chronic disease and regular medication use, who had not smoked for the last year, and who had no problems in reading, writing, or comprehension were included in our study. The study did not include individuals with active or chronic respiratory diseases and communication problems because they may adversely affect pulmonary function testing (PFT).

In our study, we calculated that 88 cases would be needed based on the LF/HF parameter of HRV with the G∗Power version 3.0.10. Program to obtain 80% power with an effect size of 0.40 at a statistical significance level of p: 0.05 [9]. The 88 volunteer cases were divided into two groups by randomization. By randomization, according to the order of participation in the study, odd-numbered people (such as first, third, fifth) were included in the breathing exercise group (Group BE); even-numbered people (such as second, fourth, sixth) were included in the VNS group (Group VNS). It was conducted by the physiotherapist responsible for implementing the randomization procedures. After randomization, 44 cases in Group BE and 44 cases in Group VNS were evaluated. All participants completed the study. The flow diagram of the study is shown in Figure 1. The study was terminated once a sufficient number of participants was reached.

All protocols and methods were approved by the Istanbul Yeniyüzyıl University Clinical Research Ethics Committee with decision number 30.092022/5, and written informed consent was obtained from all participants.

2.2. Study Plan. Our study was conducted as a randomized prospective study. However, since both groups received active interventions, the design corresponds to an active comparative controlled trial. In both groups, the interventions were performed for 10 sessions for 2 weeks, 5 days a week. In both groups, HRV, heart rate, and blood tension were measured before and after each session, and the PFT was evaluated the day before and the day after the 10 sessions of applications. Since the study was conducted in healthy participants, PFTs were examined before and after aerobic training to see possible changes. The training on the treadmill consisted of 5-min warm-ups, 20 min of loading, and five 5-min cool-down periods. Participants performed warm-up and cool-down periods at a treadmill speed of 4 km/h. After determining the maximum heart rate according to the 220-age formula, the loading intensity was performed at 60% of this value [4]. Intergroup comparisons were made as a result of the evaluations in both groups. The data were collected at Bursa ROMATEM Hospital by the same physiotherapist.

The flow diagram of the study was as follows:

Day 1: Demographic data were collected. PFT was measured before and after the exercise.

Two weeks (10 sessions): Group BE performed breathing exercises; Group VNS received auricular VNS. Pulse rate variability (PRV), pulse, and blood pressure values were measured before and after each application.

The day after 10 sessions: PFT was measured before and after exercise again.

Breathing exercise group: The content of breathing exercises was planned as thoracic expansion exercises. These exercises were applied to the lower, middle, and upper lobes in three sets of 3×10 (90 times breathing in one area) and lasted for an average of 20 min. These exercises were first taught by the physiotherapist and performed under her supervision. Participants were asked to inhale as deeply as possible into the relevant area, hold their breath for 3 s, and then slowly exhale the entire breath.

VNS group: Vagus stimulation was applied bilaterally through the ear. taVNS was performed with the Vagustim device. The stimulation pulse width was 300 ms, the

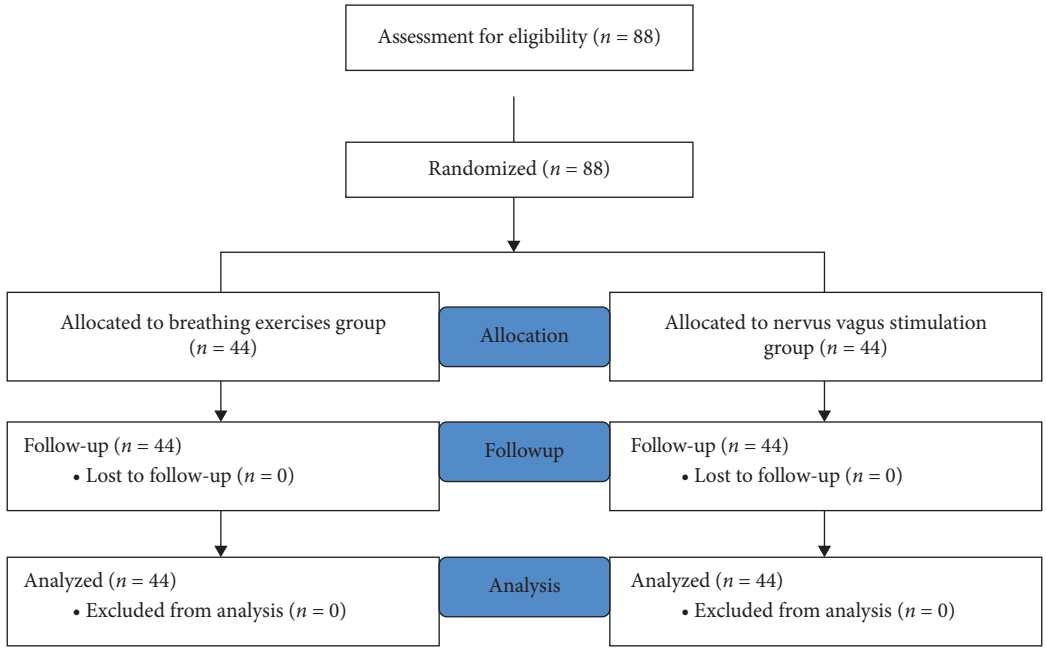

Figure 1: Consort flow diagram.

frequency was 10 Hz, and it was biphasic with a 20-min duration. Electrodes were placed on the concha and tragus parts of the ear, and the current was increased through these electrodes until the participant felt pain. When the participant felt pain, the intensity was reduced to a comfortable sensation. The VNS device and the application position are shown in Figure 1.

No blinding procedures were implemented in this study; the researcher who performed the interventions also conducted the outcome assessments.

2.3. Outcome Measures. In our study, 88 volunteer cases were randomly divided into two groups. Each volunteer filled out a demographic information form *ω*, and their age, gender, height, and body weight were recorded.

2.3.1. ANS Activity Measurement. Autonomic measurements of each individual participating in the study before and after the intervention were performed with HRV parameters. For this purpose, the Elite HRV device was used (Figure 2). The device has a finger sensor and an application compatible with the phone. Elite HRV application is a software that can synchronize with a personal monitor by collecting peak-to-peak intervals for instant analysis of PRV [10]. Studies show that Elite HRV, a smartphone application, is a valid application platform for examining RMSSD [11]. In addition to all these assessments, blood pressure measurements of the participants were also evaluated using an Omron M2 electronic sphygmomanometer on the right arm.

Values measured by Elite HRV other than pulse are the following:

- The root of the mean square of successive differences (RMSSD) is used to snapshot the parasympathetic branch of the ANS and reflect the parasympathetic activity.
- PNN50 expresses NN50 divided by the total number of NN (R-R) intervals and reflects parasympathetic activity.
- Low-frequency (LF) power is the frequency activity in the range of 0.04–0.15 Hz. It represents sympathetic activity [12].
- High-frequency (HF) power is the activity of the frequency between 0.15 and 0.40 Hz. It reflects parasympathetic activity [13].
- The LF/HF ratio measures sympathovagal balance [13].

2.3.2. Pulmonary Function Measurement. Participants in both groups underwent PFT evaluation on the first and last days. Since the study was conducted in healthy individuals, it was deemed appropriate to look at the PFT before and after exertion to see possible changes. A Medwelt SP10 spirometer was used for PFTs in our study. Each individual was measured in a sitting position using a separate mouthpiece. Three measurements were made, and the highest value was recorded [14]. The measured forced expiratory volume in the first second (FEV1), forced vital capacity (FVC), and FEV1/FVC values were recorded on the participant follow-up form.

All procedures including randomization, delivery of the interventions, and data collection were carried out by the same physiotherapist.

2.4. Statistical Analysis. Descriptive statistics were used to define continuous variables when analyzing the data processes in our study. Mean + standard deviation values were given for parameters suitable for normal distribution, and

Figure 2: ANS activity measurement with the Elite HRV device and taVNS application with Vagustim device.

median (minimum–maximum) values were given for parameters not ideal for normal distribution. The suitability of continuous variables to normal distribution was examined by the Wilks test. The difference between two independent groups of continuous variables that did not conform to normal distribution was analyzed by the Whitney *U* test. The difference between two independent groups of continuous variables that conform to normal distribution was analyzed by Student's *t*-test. The relationship between categorical variables was analyzed using the chi-squared test (or continuity correction where appropriate). The difference between two groups of continuous variables that do not conform to normal distribution was analyzed by the Wilcoxon signed rank test. The difference between two groups of continuous variables that conform to normal distribution was analyzed by paired samples *t* test. The statistical significance level was set as 0.05. Analyses were performed using MedCalc Statistical Software version 12.7.7 (MedCalc Software, Ostend, Belgium; http://www.medcalc.org; 2013).

3. Results

The study was terminated once a sufficient number of participants was reached. All participants completed the study. In our study, no side effects were observed in the participants during the applications and exertion.

Basic characteristics of participants are shown in Table 1. Descriptive statistics regarding the demographic information of the study participants were made with continuity correction, Mann–Whitney *U* test, and Fisher's exact test. When the demographic data of the individuals participating in the study were analyzed, no statistically significant difference was observed between the groups regarding gender, height, and weight. The mean age of the participants was 24.4 ± 4.7 years in the VNS group, which was statistically significantly higher than 21.8 ± 4.9 years in the BE group.

The participants' PRV, pulse, and blood pressure measurements were made before and after the applications; pretest and posttest evaluations were analyzed with the Wilcoxon signed ranks test and paired samples *t*-test. Statistical significance between the groups was analyzed with the Mann–Whitney *U* test and independent samples test. The results of the analysis are shown in Tables 2 and 3.

When the pulse rate values of the individuals participating in the study were analyzed, a statistically significant decrease was observed in both BE and VNS groups after the application. When the change between the groups is analyzed, the BE group decreases the heart rate value statistically significantly compared to the VNS group.

Systolic blood pressure value changes were similar to heart rate changes; a statistically significant decrease was observed in BE and VNS groups before and after the application; however, there was no statistical difference between the groups in terms of change.

Diastolic blood pressure values decreased significantly in both BE and VNS groups after the application. When the change between the groups was analyzed, the VNS group was statistically significant in decreasing the DBP value compared to the BE group.

RMSSD, PNN50, and HF values were lower in the BE group after the applications, while a statistically significant increase was observed in the VNS group.

LF values statistically significantly decreased in the BE group, while there was no statistically significant change in the V group. When the change between the groups is analyzed, the BE group is statistically significant in reducing the LF value compared to the V group.

Table 1: Demographic variables of participants.

	Group BE ($n = 44$)	Group VNS ($n = 44$)	Total	p
Gender				0.660[1]
Male	18 (%40.9)	15 (%34.1)	33 (%37.5)	
Female	26 (%59.1)	29 (%65.9)	55 (%62.5)	
Age (years)				< 0.001[2]
Mean ± SD	21.8 ± 4.9	24.4 ± 4.7	23.1 ± 5	
Med (min-max)	19.5 (18–35)	23.5 (19–34)	20 (18–35)	
Height (m)				0.550[2]
Mean ± SD	1.69 ± 0.1	1.68 ± 0.1	1.68 ± 0.1	
Med (min-max)	1.68 (1.55–1.90)	1.65 (1.55–1.90)	1.65 (1.55–1.90)	
Weight (kg)				0.515[2]
Mean ± SD	65.9 ± 14.1	63.8 ± 12.6	64.9 ± 13.3	
Med (min-max)	68 (44–96)	62.5 (45–100)	64 (44–100)	

Note: Bold values indicate statistical significance.
Abbreviations: BE, breathing exercise; SD, standard deviation; VNS, vagus nerve stimulation.
[1]Continuity correction.
[2]Mann–Whitney *U* test.

Table 2: Intragroup and intergroup analyses of pulse and blood pressure.

	Before ($n = 88$)	After ($n = 88$)	Difference	p
Pulse				**< 0.001**[4]
Group BE				
Mean ± SD	73.6 ± 3.7	69.2 ± 3.7	−4.4 ± 2.1	
Med (min-max)	74 (65–82)	70 (59–76)	−4 (−12–(−2))	
Pulse				**< 0.001**[4]
Group VNS				
Mean ± SD	74.5 ± 4.2	71.1 ± 4.5	−3.4 ± 1.5	
Med (min-max)	74.5 (64–82)	71 (60–79)	−3 (−8–(−1))	
p	0.310[1]	**0.035**[1]	**0.008**[1]	
SBP				**< 0.001**[3]
Group BE				
Mean ± SD	125.3 ± 9.6	120.9 ± 9.9	−4.4 ± 4	
Med (min-max)	122.5 (105–147)	118.5 (105–145)	−4.5 (−17–7)	
SBP				**< 0.001**[3]
Group VNS				
Mean ± SD	119.8 ± 8.6	114.6 ± 8.3	−5.3 ± 2.8	
Med (min-max)	120 (106–134)	114 (99–130)	−5 (−16–0)	
p	**0.024**[2]	**0.016**[2]	0.236[1]	
DBP group BE				**0.001**[3]
Mean ± SD	77.7 ± 7.9	75.5 ± 7.3	−2.2 ± 4.4	
Med (min-max)	76 (64–94)	74 (65–92)	−2 (−11–9)	
DBP				**< 0.001**[3]
Group VNS				
Mean ± SD	81.2 ± 5.7	75.9 ± 6.1	−5.3 ± 2.4	
Med (min-max)	82.5 (72–92)	76 (66–89)	−5.5 (−13–2)	
p	**0.016**[2]	0.441[2]	**< 0.001**[1]	

Note: Bold values indicate statistical significance.
Abbreviations: BE, breathing exercise; DBP, diastolic blood pressure; SBP, systolic blood pressure; SD, standard deviation; VNS, vagus nerve stimulation.
[1]Independent samples test.
[2]Mann–Whitney *U* test.
[3]Wilcoxon signed ranks test.
[4]Paired samples T-test.

LF/HF values were also decreased in the BE group, while there was no statistically significant change in the VNS group. However, the change between the groups was not statistically significant.

FEV1, FVC, and FEV1/FVC ratios were evaluated, and the pretest and post-test evaluations were analyzed by Wilcoxon signed ranks test, while the statistical significance between the groups was analyzed by the Mann–Whitney *U* test and independent samples test. The results of the analysis are shown in Tables 4 and 5.

FEV1 values of the individuals significantly increased in the BE and VNS groups between the first and last post-exertion; however, there was no statistically significant difference between the groups regarding change.

Table 3: Intragroup and intergroup analyses of PRV.

	Before ($n = 88$)	After ($n = 88$)	Difference	p
RMSSD group BE				**< 0.001**[3]
Mean ± SD	88.6 ± 38	73 ± 32.7	−15.6 ± 26.8	
Med (min-max)	82.8 (28.5–172.3)	68.1 (15.6–198.2)	−9.5 (−80–25.9)	
RMSSD group VNS				**< 0.001**[3]
Mean ± SD	59.4 ± 24.8	73.5 ± 16	14.1 ± 22.6	
Med (min-max)	46.6 (35.1–167.3)	67.1 (47–100.7)	14.6 (−73–56.8)	
p	**< 0.001**[2]	0.374[2]	**< 0.001**[1]	
PNN50 group BE				**< 0.001**[3]
Mean ± SD	43.5 ± 22.3	35.3 ± 16.1	−8.1 ± 13.9	
Med (min-max)	38.5 (9–112)	32.5 (8–97)	−7 (−49–18)	
PNN50 group VNS				**< 0.001**[3]
Mean ± SD	27.8 ± 13	36.2 ± 10	8.3 ± 11.8	
Med (min-max)	23 (13–78)	33 (24–57)	8 (−24–38)	
p	**0.001**[2]	0.542[2]	**< 0.001**[1]	
LF group BE				**< 0.001**[3]
Mean ± SD	7082.3 ± 4452.9	4656.1 ± 3353.7	−2426.3 ± 3118.1	
Med (min-max)	6365 (564–18070)	4282 (231–15098)	−1364 (−10899–1866)	
LF group VNS				0.080[3]
Mean ± SD	4526.4 ± 3757.3	5123.5 ± 3844.5	597 ± 3087.4	
Med (min-max)	2959 (1048–13006)	3849 (953–14567)	495 (−8384–9049)	
p	**0.002**[2]	0.686[2]	**< 0.001**[1]	
HF group BE				**0.008**[3]
Mean ± SD	2560.9 ± 1553	2016.7 ± 1213.5	−544.2 ± 1166.6	
Med (min-max)	2277 (424–6545)	1736.5 (186–5345)	−334.5 (−3465–1578)	
HF group VNS				**0.001**[3]
Mean ± SD	1655.8 ± 1030.1	2285.2 ± 1162.9	629.4 ± 1309.7	
Med (min-max)	1534 (672–5672)	1988 (910–6577)	469 (−1206–5897)	
p	**0.005**[2]	0.145[2]	**< 0.001**[1]	
LF/HF group BE				**0.003**[3]
Mean ± SD	3.1 ± 1.6	2.7 ± 2.4	−0.4 ± 2.2	
Med (min-max)	3 (0.5–7.2)	2 (0.2–14.9)	−0.4 (−3.2–11.5)	
LF/HF group VNS				0.484[3]
Mean ± SD	2.9 ± 2.3	2.5 ± 2.2	−0.4 ± 1.9	
Med (min-max)	1.9 (1–10.4)	1.8 (0.5–11.9)	−0.1 (−8.7–2.5)	
p	0.066[2]	0.381[2]	0.163[1]	

Note: Bold values indicate statistical significance.

Abbreviations: BE, breathing exercise; PRV, pulse rate variability; SD, standard deviation; VNS, vagus nerve stimulation.

[1]Independent samples test.

[2]Mann–Whitney *U* test.

[3]Wilcoxon signed ranks test.

[4]Paired samples *t* test.

When the FVC values were analyzed, the BE and VNS groups observed a statistically significant increase between the first postexertion and the last postexertion. When the change between the groups is analyzed, the BE group increases the FVC value statistically significantly compared to the VNS group.

When FEV1/FVC values were analyzed, while there was no statistically significant change in the BE group, a statistically significant increase was observed in the VNS group, and this increase was statistically significant between the groups.

4. Discussion

Our study aimed to compare the efficacy of auricular VNS (VNS group) and breathing exercises (BE group) on pulmonary function and ANS activity. Since both groups received active treatments, the study design corresponds to an active comparative controlled trial, which should be taken into account when interpreting the findings. The autonomic activity was measured before and after each application (10 sessions in total), while PFT was measured before and after exertion on the first and last days. Pulse decreased significantly in both groups, but more in the breathing exercise group than in the vagus stimulation group. There was a significant decrease in blood pressure values in both groups. It can be said that auricular vagus stimulation is superior to breathing exercises in increasing parasympathetic system activity, and breathing exercises are superior to vagus stimulation in decreasing sympathetic activity and partially increasing respiratory functions.

Transcutaneous VNS can be administered through the external ear (ear branch of the vagus nerve) or the neck (cervical branch of the vagus nerve) and may provide an alternative to invasive cervical VNS. Functional imaging

Table 4: Intragroup and intergroup analyses of PFT before aerobic training.

	Before first aerobic training ($n = 88$)	Before last aerobic training ($n = 88$)	Difference p^3	
FEV1 group BE			< 0.001	
Mean ± SD	2.7 ± 0.6	2.8 ± 0.5	0.2 ± 0.2	
Med (min-max)	2.6 (1.2–4.5)	2.8 (1.6–4.5)	0.1 (−0.3–0.9)	
FEV1 group VNS				0.047
Mean ± SD	2.7 ± 0.5	2.8 ± 0.5	0.1 ± 0.3	
Med (min-max)	2.6 (2–4.6)	2.8 (2–4.3)	0.1 (−0.5–0.7)	
p	0.438[1]	0.643[1]	0.109[2]	
FVC group BE				< 0.001
Mean ± SD	3 ± 0.7	3.2 ± 0.7	0.2 ± 0.2	
Med (min-max)	2.9 (1.6–5.4)	3 (2.1–5.5)	0.2 (−0.4–0.9)	
FVC group VNS				< 0.001
Mean ± SD	3.1 ± 0.6	3.2 ± 0.6	0.1 ± 0	
Med (min-max)	2.9 (2.3–4.8)	3.1 (2.3–4.8)	0.1 (−0.1–0.7)	
p	0.263[1]	0.805[1]	< 0.001[2]	
FEV1/FVC Group BE				0.078
Mean ± SD	88.8 ± 9.7	88 ± 8.1	−0.8 ± 8.1	
Med (min-max)	91.7 (57.4–98.7)	90.7 (65.6–98.7)	−1.1 (−14.5–29.4)	
FEV1/FVC Group VNS				0.718
Mean ± SD	88.3 ± 5.8	87.7 ± 8.5	−0.6 ± 5.9	
Med (min-max)	89.4 (72.6–95.3)	89.7 (62.1–96.2)	−0.3 (−15.8–13.3)	
p	0.094[1]	0.562[1]	0.267[2]	

Note: Bold values indicate statistical significance.
Abbreviations: BE, breathing exercise; PFT, pulmonary function test; SD, standard deviation; VNS, vagus nerve stimulation.
[1]Independent samples test.
[2]Mann–Whitney *U* test.
[3]Wilcoxon signed ranks test; *p:* intragroup analyses.

Table 5: Intragroup and intergroup analyses of PFT after aerobic training.

	Before first aerobic training ($n = 88$)	Before last aerobic training ($n = 88$)	Difference p^3	
FEV1 group BE			< 0.001	
Mean ± SD	2.8 ± 0.6	3 ± 0.6	0.2 ± 0.2	
Med (min-max)	2.6 (1.2–4.5)	2.9 (1.9–4.8)	0.2 (0–0.7)	
FEV1 group VNS				< 0.001
Mean ± SD	2.7 ± 0.6	2.9 ± 0.6	0.2 ± 0.3	
Med (min-max)	2.6 (1.9–4.6)	2.9 (2–4.5)	0.1 (−0.1–1.2)	
p	0.780[1]	0.854[1]	0.223[2]	
FVC group BE				< 0001
Mean ± SD	3.2 ± 0.7	3.5 ± 0.7	0.3 ± 0.2	
Med (min-max)	3 (1.9–5.5)	3.2 (2.6–5.7)	0.2 (0.01–0.9)	
FVC group VNS				< 0001
Mean ± SD	3.2 ± 0.6	3.3 ± 0.6	0.2 ± 0.2	
Med (min-max)	3 (2.3–4.9)	3.1 (2.3–5)	0.1 (−0.01–1.1)	
p	0.877[1]	0.331[1]	< 0.001[2]	
FEV1/FVC roup BE				0.053
Mean ± SD	87.5 ± 8	86.6 ± 5.3	−0.9 ± 5.8	
Med (min-max)	90.2 (63.5–97.8)	88.5 (74.2–93.5)	−1.4 (−10.0–20.8)	
FEV1/FVC Group VNS				0.008
Mean ± SD	85.9 ± 7.8	88.7 ± 6.3	2.7 ± 6.4	
Med (min-max)	88.4 (67.3–95.5)	90.4 (68.1–95.7)	2.7 (−9.1–20.6)	
p	0.083[1]	0008[1]	< 0001[2]	

Note: Bold values indicate statistical significance.
Abbreviations: BE, breathing exercise; PFT, pulmonary function test; SD: standard deviation; VNS, vagus nerve stimulation.
[1]Independent samples test.
[2]Mann–Whitney *U* test.
[3]Wilcoxon signed ranks test; *p:* intragroup analyses.

studies have shown that taVNS activates structures, including the nucleus solitarius and nucleus coeruleus in healthy adults and has similar effects to other methods of VNS. Moreover, this method can be a simple and inexpensive alternative to invasive vagus stimulation with a few side effects [15]. For these reasons, we preferred transcutaneous auricular stimulation of the vagus nerve in our study.

Slow breathing significantly modulates autonomic motor activity. It increases HRV and baroreflex sensitivity, decreases sympathetic nerve activity, and lowers blood pressure and muscle tone [16]. Although the effects of breathing exercises and taVNS on the ANS are similar and known, comparative studies are scarce. In addition, there are differences in the application protocols of breathing exercises and taVNS. taVNS parameters (frequency, pulse width, stimulation time, etc.) can vary widely in the literature. Regarding the stimulation frequency, some studies say that the range of 20–30 Hz has never been confirmed in terms of therapeutic effects [17]. Following studies showing that 50 Hz and above stimulation frequencies can cause massive and irreversible damage to the vagus nerve during invasive VNS, stimulation frequencies between 20 and 30 Hz were arbitrarily chosen to limit stimulation-related adverse events and were subsequently approved by the Food and Drug Administration [18]. Subsequently, stimulation frequencies lower than 20 Hz were also investigated. It was found that 10 Hz taVNS three times a day for 20 min for 6 months reduced the number of seizures, while an 8 Hz stimulation caused activation in frontal and limbic brain areas as measured by fMRI [19]. In a study conducted by Badran et al. in 2018, taVNS was performed on 15 healthy individuals at frequencies of 1, 10, and 25 Hz at each visit, and the effectiveness of different application parameters was compared. As a result of the study, it was found that applications performed at frequencies of 10 and 25 Hz significantly reduced heart rate. When these two parameters were compared with each other, it was reported that the most effective result was obtained at a frequency of 10 Hz [20]. As a result of all these findings, we applied a 10 Hz frequency in our study and found that this frequency caused a significant decrease in heart rate.

In a study conducted in 2022 with healthy young volunteers, 24 participants received vagus stimulation through the ear, and the interaction of the VNS with the circadian rhythm was examined. Stimulation was administered in the morning and the evening 2 hours before sleep. Although there was no difference between morning and evening VNS, an increase in RMSSD, PNN50, and HF values and a decrease in LF/HF values were found in both applications [21]. In our study conducted in healthy individuals, positive results were obtained toward increasing the parasympathetic system with taVNS, which is parallel with the study of Geng et al.

In 2019, Borges et al. conducted a study on 61 healthy volunteers and compared the effects of auricular VNS on cardiac vagal activity with sham application. As a result of the study, they stated that the application increased vagal activity, but there was no significant difference between the sham group [22]. In 2022, Tarasenko et al. investigated the potential effects of taVNS on stress processing. In the findings obtained, only the LF/HF ratio showed a statistically significant change in response to stimulation. Although the LF/HF ratio was expected to increase during stress exposure, taVNS during stress exposure prevented this increase and even decreased the LF/HF ratio [23]. In 2015, Buchholz et al. conducted a study on rabbits in which it was mentioned that VNS applications may cause a parasympathetic response while sympathetic coactivation mechanisms may be activated [24]. Our study found differences in the LF/HF ratio change of both methods affecting the autonomic system. While the LF/HF ratio decreased in both groups, this change was significant only in the respiratory exercise group. Despite the instantaneous increase in parasympathetic indicators in the vagus stimulation group, an increase in the LF value representing sympathetic activity was also observed. This suggests that the two treatments in our study may have similar and different effects on ANS.

A systematic review conducted in 2021 to investigate the clinical effects of auricular vagus stimulation included 38 double-blind, randomized controlled trials with a high level of clinical evidence. There were conflicting results between studies when looking at changes in heart rate and HRV, but it was suggested that taVNS may affect both. In the studies that reported changes in heart rate, a modest mean decrease of two to three beats per minute was measured in the active group. However, almost half of the 11 studies reporting heart rate effects reported no significant difference in impact between control and active stimulation. Similarly, conflicting results were found on HRV. Various stimulation methods, durations, and frequencies have been suggested as one of the reasons for the difference in results [25]. In our study, electrodes were placed on the concha and tragus parts of the ear; the stimulation pulse width was 300 microseconds, the frequency was 10 Hz, and biphasic vagus stimulation was performed bilaterally for 20 min. Significant results were found in heart rate and HRV. Comparison of studies is difficult when different methods, treatment durations, and measurement and application methods are used and may be different.

Since slow breathing modulates the activity of the vagus nerve and is used in behavioral medicine to reduce psychophysiological arousal, a study in 2022 reviewed studies investigating the effects of tVNS and slow breathing separately. As a result, both tVNS and slow breathing were recognized as promising additional therapeutic approaches for anxiety, depressive disorders, chronic pain, cardiovascular diseases, and insomnia. Therefore, it has been concluded that tVNS can produce additive or synergistic beneficial effects when combined with slow breathing [26]. Similarly, in our study, both applications positively affected the parasympathetic system. In 2017, a review aimed to provide a comprehensive overview of the physiological effects of normal breathing and slow breathing techniques in healthy humans and the impact of breathing on HRV and sympathovagal balance was examined. It was found that the slow breathing technique, which refers to taking six breaths per minute, decreases the LF value, causes a decrease in the

LF/HF ratio, increases vagal activity, provides a transition to parasympathetic dominance, optimizes sympathovagal balance, decreases heart rate and may decrease blood pressure, and also increases tidal volume [27]. In our study, a significant decrease was observed in LF and LF/HF values in the respiratory group, indicating a transition to parasympathetic activity. However, a decrease was found in values indicating parasympathetic activity, such as RMSSD, PNN50, and HF values. This may have been because PRV was measured instead of HRV, and PRV measurement was limited to 1 min. Deep and slow breathing is likely more effective on HRV than PRV due to intrathoracic pressure changes.

In a 2017 meta-analysis study investigating the heart rate and blood pressure effects of breathing exercises in people with cardiovascular disease, six studies with 269 participants were analyzed. In studies with trial durations ranging from 2 weeks to 6 months, the implementation of breathing exercises resulted in a statistically significant heart rate reduction. Compared to controls, reductions were seen in SBP and DBP [28]. In our study, similar to this study, performing breathing exercises resulted in a statistically significant decrease in heart rate. In addition, the reduction in SBP and DBP was also statistically significant.

In a study of asthmatic patients, 25 were evaluated in a prospective, nonrandomized study of patients treated in the emergency department for moderate to severe acute asthma, with FEV1 values between 25% and 70% of normal. During an acute asthma attack, cervical invasive vagal nerve stimulation administered at 25 Hz for 60 min improved FEV1 values by up to 80% at 15, 30, and 60 min, with moderate heart and respiratory rate reductions [29]. In 2013, another study by Steyn et al. examined the effects of noninvasive cervical vagal stimulation in treating acute asthma. In asthmatics treated in the emergency room setting, 21 patients with FEV1 values below 60% of the average value were included in the study. Subjects received two tVNS treatments, 30 min apart, lasting 60 s. During this time, subjects continued to receive standard pharmacologic treatment. The treatment was successful in three out of four patients based on a 73% increase in FEV1 from baseline, improvement in dyspnea scoring, and no device-related adverse events [30]. In our study, vagus stimulation was administered in 10 sessions of 20 min, once a day, in healthy subjects. Although there are differences between the studies, our research parallels these studies, with a significant increase in FEV1 and FVC values.

Although there are separate studies in the literature examining the effects of breathing exercises and VNS on HRV and respiratory functions, only some studies compare the two methods. In 2022, diaphragmatic breathing and auricular VNS were compared in treating patients with fibromyalgia. Sham groups and active application groups were formed, and the participants at home performed applications for 14 days for 15 min in/the morning and 15 min in/the evening. HRV data were measured with a finger photoplethysmography device at rest for 1 min before and after the interventions. At the end of the study, RMSSD, HF, and PNN50 values were analyzed; however, no significant HRV change was found between the groups [31]. In our study, a significant decrease was observed in these three values as a result of respiratory exercises, while a significant increase was observed after vagus stimulation. In our study, taking the values before and after each application and performing the applications under the supervision of a physiotherapist in terms of correct application may be essential and may be effective in different results.

Regarding the weaknesses of our study, we need long-term follow-up data to determine the durability of the observed effects; therefore, evaluating these outcomes in the medium and long terms would provide more comprehensive insight. Additionally, the use of PRV instead of HRV and the reliance on 1-min recordings constitute methodological limitations. However, a strength of the study is that PRV, pulse, and blood pressure values were measured before and after each session, which likely enhanced measurement accuracy. Furthermore, although the study was randomized, both groups received active interventions; thus, the design should be considered an active comparative controlled study rather than a classical randomized controlled trial. Another limitation is the absence of blinding, as the same physiotherapist performed the randomization, administered the interventions, and conducted all outcome assessments, introducing the possibility of observer bias.

It is interesting to observe significant changes in respiratory function parameters in healthy subjects in the VNS and BE groups. More detailed research on the effect of taVNS on respiration is needed.

5. Conclusion

In our study, comparing taVNS and breathing exercises in healthy subjects, we found that taVNS was superior to breathing exercise in increasing parasympathetic activity, while breathing exercise was superior to taVNS in decreasing sympathetic activity and partially increasing respiratory function. In conclusion, both interventions have similar but divergent effects on ANS activity and pulmonary function.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Statement

The study was approved by the Istanbul Yeniyuzyil University Clinical Research Ethics Committee on September 30, 2022, with decision number 5 and file number 30.092022/5. All the participants gave informed consent, and procedures were followed according to the Declaration of Helsinki.

Conflicts of Interest

Ali Veysel Ozden is one of the cofounders of the Vagustim Company, which produces VNS devices.

Author Contributions

Gulay Yalcin: writing–original draft, methodology, investigation, data curation, formal analysis, and conceptualization.

Ali Veysel Ozden: writing–review and editing, supervision, formal analysis, and conceptualization.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section
Supporting Information. The supporting information files include the CONSORT checklist. . *(Supporting Information)*

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