International Journal of
Disabilities Sports and Health Sciences
e-ISSN: 2645-9094

RESEARCH ARTICLE

Effects of Auricular Vagus Nerve Stimulation on Cardio-Respiratory Functions After Aerobic Exercise

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Abstract
The aim of our study is to find out whether the return rate of parasympathetic activity (PA) can be accelerated with auricular transcutaneous vagus nerve stimulation (TVNS). Pulmonary function test, ANS activity, pulse and blood pressure measurements were made. Individuals ran for 15 minutes. Those in the TVNS group received 5 minutes of auricular VNS. In the control group, headphones were attached but no current was given. After the end of their procedures, they were compared with pre-exercise. In the TVNS group, the pulse parameter was higher than in the initial measurement ($p=0.001$). There was a significant ($p=0.007$) rise in the FEV1 value in the VNS group. The sympathetic nervous system (SNS) index ($p<0.05$) increased in both groups when compared to the initial measurement, although there was no significant difference in the index values between the groups. The TVNS group showed an increase from the first measurement ($p<0.05$), but there was no significant difference in the parasympathetic nervous system (PNS) Index between the groups ($p>0.05$). The groups' values for the first and second measures were equal ($p>0.05$), and the root mean square of the successive differences (RMSSD) values did not reveal a significant difference. TVNS can improve respiratory parameters in healthy individuals in the acute period after exercise, and it seems that it can also increase activation in both the sympathetic and parasympathetic system. It can be stated that TVNS may lead to variable results in different circumstances in sports

Keywords
Vagus Nerve, Stimulation, Respiratory Function, Exercise Recovery

INTRODUCTION

The autonomic nervous system is comprised of the parasympathetic and sympathetic nervous systems (ANS). The parasympathetic and sympathetic nerve systems in the body normally play opposing roles, with the activity of one system increasing and the other decreasing as needed. After engaging in physical activity, such as exercise, the body's sympathetic nervous system rises and eventually achieves a maximal activity plateau. After physical exertion, parasympathetic activity which was inhibited throughout the activity—begins to rise, and eventually the brain returns to resting state (Coote, 2010). According to Chen et al. , the primary factor influencing reorganization and recovery (restoration) following exercise is parasympathetic capability (Chen et al. 2011). It is well established that endurance training improves parasympathetic regulation both before and after running (Boullosa et al., 2009). When post-exercise alterations return to baseline levels, recovery from exercise has taken place.

The vagus nerve is widely distributed throughout the body, making cervical invasive vagus nerve stimulation (VNS), an approved technique for treating depression and epilepsy

Received: 22 December 2023 ; **Revised** ; 04 March 2024 ; **Accepted:** 02 May 2024; **Published:** 25 May 2024

How to cite this article: Güçlüer, A.O., Özden, A.V., Ural, H.İ., Alptekin, H.K., and Hatik, S.H. (2024). Effects of Auricular Vagus Nerve Stimulation on Cardio-Respiratory Functions After Aerobic Exercise. *Int J Disabil Sports Health Sci*;7(3):494-501. https://doi.org/10.33438/ijdshs.1406505

since 2005, nonetheless interesting for a variety of physiological and pathological disorders. According to Mullers et al., VNS can influence cardiovascular parameters during activity as well as at rest (Mulders et al., 2015). Auricular TVNS has the ability to lower sympathetic activity, according to Clancy et al. (Clancy et al., 2014). Furthermore, as reported by Antonino et al., transcutaneous VNS enhances autonomic regulation and heart baroreflex sensitivity (Antonino et al., 2017).

Due to their negative effects on exercise performance and increased risk of injury, post-exercise weariness and delayed recovery are important. With appropriate exercise routines, one can eventually raise parasympathetic activity following exercise (Gifford et al., 2018). When assessed abruptly, parasympathetic reactivation and the decline in sympathetic activity following exercise slow down with increasing exercise intensity (Michael et al., 2017; Michael et al., 2018; Ebersole et al., 2020). Recovery from exercise can be accelerated and improved by ANS modulation. The auricular vagus nerve can be electrically stimulated to boost parasympathetic activity following exercise, which could aid in recuperation. Our study aimed to evaluate the recovery status through pulse, blood pressure, respiratory functions and ANS activity after exercise and whether this recovery can be achieved faster with auricular TVNS.

MATERIALS AND METHODS

Study Design and Participants

Forty healthy male individuals between the ages of 18-40 were included in the study. Those who had any disease (Those with cardiovascular diseases, autoimmune diseases, musculoskeletal disorders, psychiatric and neurological disorders, and those taking medication for any reason) and pain, who were on medication, who had tachycardia (>90/min) and hypertension (diastolic>90 or systolic>140 mm Hg) were excluded from the study. This study was carried out in the Physiotherapy and Rehabilitation Unit of Istanbul Fatih Medicalpark Hospital. Age, weight and height of the individuals included in the study were evaluated. First, the measurements were made within the scope of the study. Pulmonary function test was performed with a spirometer (spirolab-III). An Omron M2 basic electronic device was used to measure the subject's heart rate and blood pressure on the left arm. To gauge ANS activity, the individuals' heart rate variability (HRV) was monitored for five minutes. The investigations were conducted using the eMotionFaros 180 equipment with Kubios standard software.

After these procedures, the participants were made to run for 15 minutes (at a speed of 70% of the maximum heart rate) on the treadmill (Figure-1). Maximum heart rate was determined to be 220-age. The participants rested for 5 minutes at the end of the run. After the rest, TVNS was applied from both ears for 5 minutes to those in the experimental group, while those in the control group wore earbuds for 5 minutes, but no stimulation was applied. HRV measurement was performed during VNS, the other measurements were repeated after VNS.

Spirometry is a simple test used to measure static lung volumes at rest - slow vital capacity (sVC), forced vital capacity (FVC) - and dynamic volumes - forced expiratory volume in 1 second (FEV1), flow-volume loops. Spirometry provides clinically relevant parameters of the patient's functional status. The highly reproducible acquisition of these lung function parameters, together with the caveat mentioned above, are advantages of spirometry and are particularly valuable in serial measurements (Behr et al., 2008).

Pulmonary function testing is used for lung function measurements. In the study, a spirolab-III spirometer was used for pulmonary function testing. A separate mouthpiece was used for each individual and three measurements were made and the highest value was recorded on the participant follow-up form.

Heart rate variability (HRV) analysis is often used in cardiovascular research and to evaluate the functioning of the autonomic nervous system (ANS). HRV is a result of the influence of the sinoatrial (SA) node on the ANS. Sympathetic activity tends to increase HR and decrease HRV, while parasympathetic activity tends to decrease HR and increase HRV. Kubios HRV is another software program used for heart rate variability (HRV) analysis. The software supports electrocardiogram (ECG) and beat-to-beat RR interval data. It calculates HRV parameters and various nonlinear parameters in the entire time domain and frequency domain. The ECG-derived

respiratory frequency is also calculated; This makes the analysis results reliable **(**Tarvainen et al., 2014).

In the study, E-motion Faros 180 was used for autonomic measurement. Kubios software was used to analyze the obtained SNS index, PNS index, RMSSD and stress index data.

Intervention

VNS: Stimulation was performed for 5 minutes using the Vagustim device (Figure 2), from the tragus and concha sites of both ears. Current characteristics were set biphasically with a pulse width of 100 is and a frequency of 10 Hz (Hatık et al., 2023).

Evaluation Criteria

The ANS was evaluated using the values of the SNS index for sympathetic activity and the RMSSD (Root mean square of the successive differences) and PNS index for parasympathetic activity.

The pulmonary function test uses two measures: forced expiratory volume in one second (FEV1) and forced vital capacity (FVC). The maximum value of the three measurements was recorded using a mouthpiece specific to each individual in the spirometer. Blood pressure and heart rate: measured in millimeter-hours (mmHg) for both the diastolic and systolic pressures.

Randomization

The study was randomized by having the participants draw numbers from a sealed envelope containing even and odd numbers. A total of 40 individuals were included in the study, 20 in the control group in which ear electrodes were placed but no current was applied, and 20 in the TVNS group.

Statistical Analysis

After being recorded using the measurements, the data was loaded into a computer and examined using SPSS 20.0 (SPSS Inc., Chicago, IL). The two groups' differences were evaluated using the Independent Samples T-test and Mann-Whitney U Test; the dependent groups' differences were evaluated using the Dependent Sample T-test and Wilcoxon Rank Test. A normality test and frequency analysis were used to determine the demographic traits. A significance level of $p<0.05$ was established for the study.

Permission

The study was planned in compliance with the Declaration of Helsinki's principles. The study was approved by the Istanbul Yeni Yuzyil University Clinical Research Ethics Committee on November 14, 2019, with decision number 14.11.2019/27.

RESULTS

The study included forty healthy participants: twenty in the TVNS group and twenty in the control group. The control group's mean age was 27.40, while the TVNS group's was 23.45. The control group's mean age was considerably greater ($p=0.024$). Height, weight, and body mass index did not significantly differ between the groups ($p>0.05$). (Table 1).

When the first and second heart rate measurements of the participants were compared, the TVNS group showed a significant rise ($p=0.001$), whereas the control group did not ($p=0.059$). TVNS group showed a statistically significant increase ($p=0.007$) in the mean scores of the first and second FEV1 measures when compared to the control group, which showed no significant difference ($p=0.102$). The TVNS group showed a substantial rise ($p=0.001$) in the mean scores of the first and second FVC measures, while the control group showed no significant difference ($p=0.366$). Both groups' first and second systole measures showed a substantial decline ($p=0.001$ and $p=0.001$). There was no discernible difference between the groups when the diastole values from the first and second measurements were compared (TVNS group $p=0.194$, control group $p=0.108$). A substantial rise was discovered when the first and second SNS index measurements' mean scores in the two groups were compared (TVNS group $p=0.002$, control group $p=0.006$). The first and second measurement values of the PNS index were found to be identical in the control group ($p=0.083$), although the PNS index value was found to be greater in the TVNS group in the second measurement ($p=0.010$). In both groups, there was no discernible difference in the mean score from the first and second RMSSD measurements (TVNS group $p=0.100$, control group $p=0.467$). (Table 2).

In the first and second measurement, it was noted that the pulse, FEV1, FVC, systole, diastole, SNS index, PNS index, and RMSSD values were comparable between the TVNS and control groups. ($p>0.05$) (Table 3).

Table 1. Intra-group comparison

	TVNS group (n=20)				Control group (n=20)			
	First Measurement Mean±SD / Median	Second Measurement Mean±SD / Median (Min-Max)	U/Z/T TEST	P	First Measurement Mean±SD / Median (Min-Max)	Second Measurement Mean±SD / Median (Min-Max)	U/Z/T TEST	P
Pulse	76,95±13,39	85,55±11,794	-6,212	0,000[a]	82,65±14,321	86,30±10,598	-2,012	0,059
FEV1	3,13±0,768	3,40±0,746	-3,029	0,007[a]	3,12±0,905	3,32±0,794	-1,717	0,102
FVC	3,31± 0,786	3,70±0,787	-3,965	0,001[a]	3,51±0,840	3,63±0,800	-,926	0,366
Systole	125,00±10,6	114,50±10,26	5,532	0,000[a]	120,35±14,88	109,05±10,01	4,746	0,000
Diastole	70,85±8,658	68,05 ±8,035	1,347	0,194[a]	72,10±8,705	69,90±8,789	1,688	0,108
SNS index	1,07±1,684	2,15±1,479	-3,483	0,002[a]	1.496/0.805 (-1,18/5,4)	1.267/1,705 (-1,14/4,24)	-2,722	0,006[d]
PNS index	2.016/-0,855 (-2,28/6,49)	0.914/-1.77 (-2.97/-0.03)	-2,576	0,010[d]	1.617/-0,425 (-2,75/3,76)	1.267/1.705 (-1.14/4,24)	-1,736	0,083[d]
RMSSD	47.915/36.8 (11,6/231,4)	18.626/26.9 (10.5/75,4)	-1,643	0,100[d]	39.924/46,7 (8,3/165,8)	43.778/34,8 (13,9/187,1)	-0,728	0,467[d]

[a]Paired Samples T-test / [b]Independent Samples T-test / [c]Mann Whitney U / [d]Wilcoxon Rank

Table 2. Comparison between groups

	First Measurement				Second Measurement			
Parameters	**TVNS Group (n=20) Mean±SD / Median (Min/Max)**	**Control Group (n=20) Mean±SD / Median (Min/Max)**	**U/Z/T TEST**	**P**	**TVNS Group (n=20) Mean±SD / Median (Min-Max)**	**Control Group (n=20) Mean±SD / Median (Min/Max)**	**U/Z/T TEST**	**p**
Pulse	13.39/71 (55/112)	14.32/80.50 (61/113)	151,50	0,189[C]	85,55±11,794	86,30±10,598	-0,212	0,834[b]
FEV1	3,130±0,768	3,127±0,905	0,013	0,990[b]	3,405±0,746	3,327±0,794	0,322	0,749[b]
FVC	3,318±0,786	3,519±0,840	-0,781	0,440[b]	3,708 ±0,787	3,636±0,800	0,285	0,777[b]
Systole	125±10,603	120,35±14,88	1,138	0,262[b]	114,50±10,26	109,05±10,0	1,699	0,097[b]
Diastole	70,85±8,658	72,10±8,705	-0,455	0,651[b]	68,05±8,035	69,90±8,789	-0,695	0,491[b]
SNS	1.684/0.77 (-2,29/4,48)	1.496/0.805 (-1,18/5,4)	192,00	0,829[c]	2,15±1,479	1,83±1,267	0,473	0,315[b]
PNS	2.017/-0.855 (-2,82/6,49)	1.6171/-0.425 (-2.75/3,76)	168,00	0,387[c]	0.914/1,77 (-2,97/-0,03)	1.641/-1.295 (-2,97/4,16)	166,00	0,358[c]
RMSSD	47.915/36,8 (11,6/231,4)	39.924/46,7 (8,3/165,8)	176,50	0,529[c]	18.6270/26,9 (10,5/75,4)	43.779/34,8 (13,9/187,1)	165,00	0,344[c]

[a]Paired Samples T-test / bIndependent Samples T-test / cMann Whitney U / dWilcoxon Rank

Table 3. Demographic characteristics of the participants

	Mean±SD		T TEST	df	P
	TVNS Group	Control Group			
Age (Years)	23.45±5.094	27.40±5.529	-2.350	38	0.024[b]
Height (m)	1.791± 0.056	1.772±0.059	1.036	38	0.307[b]
Body weight (kg)	73.65± 9.885	77.05±11.124	-1.022	38	0.313[b]
Body mass index (kg/m^2)	22.945±2.926	24.518±3.221	-1.616	38	0.114[b]

[b]Independent Samples T-test

DISCUSSION

Our study found that there may be acute changes in physiological parameters such as pulse, respiratory capacity and HRV with auricular VNS after exercise. ANS enables these vital functions to be regulated with the activities of sympathetic and parasympathetic sub¬branches that complement and balance each other. The balance between the sympathetic system, which is active during stress, and the parasympathetic system, which becomes evident at rest, leads to homeostasis (Ozden, 2023). Being the most important part of PNS, the vagus nerve can be stimulated noninvasively from the auricular branch, or invasively or noninvasively from the cervical branch. VNS is a new method that can be used in the treatment of disorders associated with ANS dysfunction by leading to functional changes in the brain and ANS (Yuan et al., 2016; Busch et al., 2013). There are studies showing that VNS improves functional status after trauma or injury. In one study, it was observed that median and ulnar nerve transection and repair resulted in significant and long-term recovery of somatosensory function (Darrow et al., 2021). We preferred auricular TVNS in the study because it is noninvasive and easy to apply, and we aimed to examine the physiological effects of this technique, which has attracted more attention in recent years, in healthy male individuals aged 18-40 years after exercise. We did not include healthy female individuals in our study, considering that menstruation may affect ANS.

It is known that aerobic exercises are effective on cardiovascular, metabolic, psychological and respiratory adaptations. ANS function decreases in parallel with diseases, aging and decrease in exercise capacity (Machhada et al., 2017). Dominance of sympathetic activity increases with aging and accordingly, the risk of developing heart diseases increases. This demonstrates that cardiovascular parameters change with aging. PS increases and SA decreases with VNS, resulting in positive effects on cardiovascular parameters (Deuchars et al., 2018). Buchheit et al. (2007) revealed that sympathetic activity increases during aerobic exercise and the activation of the parasympathetic system continues for 24 hours or more after exercise, depending on exercise intensity (Buchheit at al., 2007). In another study, Stanley et al. (2013) stated that parasympathetic restoration is achieved in the first 1 hour after physical activity, and that performance is low in individuals with high sympathetic activity. In our study, we made the participants do aerobic exercise by running and increased sympathetic activity as the body's physiological response (Stanley et al., 2013). In a study on the effects of a single session of vagus nerve stimulation on sports performance in elite athletes, including 60 people in the control group, it was concluded that although there was no statistically significant improvement in athletic performance, it showed a moderate benefit (Çalı et al., 2023). One of the parameters used to evaluate cardiovascular functions is pulse. Metabolism and cardiac output increase depending on the increase in oxygen used in skeletal muscle with aerobic exercise. The increase in this output during exercise increases pulse (Wang et al., 2018). Our participants were standardized by being made to run until reaching seventy percent of their maximum heart rate (220-years) and then rested for 5 minutes. Although there was no difference between the groups in the measurements in the study, the heart rate values increased more significantly in the TVNS group compared to the control group. This suggests that auricular TVNS may increase sympathetic activity after exercise.

Respiration is closely related to ANS activity. With deep and slow breathing, sympathetic activity decreases while parasympathetic activity increases (Joseph et al., 2005). An increase in respiratory rate and a decrease in tidal volume may occur with sympathetic activity (Breskovic et al., 2010). Vagal fibers innervate bronchial muscles and glands, causing bronchoconstriction and secretion, while sympathetic fibers exert a bronchodilator effect. In their study on the effect of VNS applied to acute asthma patients, Feng et al. (2012) revealed that it caused a positive increase in FEV1 value (Feng et al., 2012). In our study, a significant difference was observed in the TVNS group, but no significant difference was observed in the control group. An increase was observed in the FEV1 and FVC values in the TVNS group. While PNS causes bronchoconstriction, the increase in FEV1 and FVC values due to TVNS may be related to the reactive increase in sympathetic system activity or the selective effects of stimulation.

Essential hypertension exhibits the consequences of sympathetic nervous system activation (Mancia et al., 2014). Cardiac vagal nerve activity actually increases and is crucial for maintaining cardiac function during exercise. It modulates coronary artery blood flow during exercise (Shanks et al., 2023). Research indicates that elevated sympathetic nervous system activity is mostly linked to elevated systolic blood pressure (Pal et al., 2013). In a pilot study examining the acute effect of vagus nerve stimulation on the bicycle ergometer test and physiological parameters in healthy young individuals, it was observed that although it did not affect the bicycle ergometer test, it could affect parameters such as respiratory rate and blood pressure (Hatık et al., 2022). During aerobic exercise, systolic blood pressure increases while diastolic blood pressure decreases. When we analyzed the effect of our study on blood pressure statistically, we found that systolic blood pressures were lower in the second measurement than in the first measurement. No significant difference was found in the TVNS and control group in terms of diastolic blood pressure, and the first and second measurements were equivalent. The fact that this decrease in systolic pressure was also present in the control group indicates that it may be related to the physiological recovery of the body after exercise.

The most commonly used method to evaluate ANS functions is HRV. The devices measuring HRV are ECG-based devices that allow us to distinguish between time and frequency-dependent measurement methods and parasympathetic and sympathetic effects. While RMSSD and PNS index, which are the parameters used in the study, give the activation of the parasympathetic nerve, SNS Index is the data showing the activation of the sympathetic nerve numerically (Shaffer et al., 2017). PNS and SNS indices were determined based on the results of the study Nunan et al. (2010) conducted with 21 thousand individuals (Nunan et al., 2010). PNS and SNS indices available in Kubios software were established based on their study. We used e¬motionFaros 180 to obtain HRV parameters and Kubios software to obtain the other data. In our study, the SNS Index value increased in the TVNS and control group compared to the first measurement. This result can be attributed to the increased activation of the sympathetic nervous system during aerobic exercise. However, the participants in the TVNS group also showed an increase compared to the first measurement value of PNS Index. This indicates that auricular TVNS can increase parasympathetic activity. However, there was no significant difference between the TVNS and control groups in terms of RMSSD values, and the first and second measurement values were found to be equivalent to each other. This may be due to Kubios software or because the increase in the parasympathetic system is not obvious.

In their study with rat subjects, Brighina et al. stated that low-frequency VNS affects the central nervous system and efferent fibers. In their study conducted with healthy individuals, Clancy et al. (2014) used auricular VNS and discovered that the activation of the parasympathetic system increased in autonomic functions (Clancy et al., 2014). We also used low-frequency (10 Hz) bilateral auricular TVNS in our study. When compared to the control group, auricular TVNS may increase both sympathetic and parasympathetic activity after exercise, and this effect may be selective. To our knowledge, our study is the first to evaluate the effect of auricular.

VNS in the early post-exercise period. Although parasympathetic activity is the main factor in recovery after exercise, high sympathetic activity seems to be important in this period, too. According to Buchholz et al. (2015), in which they mentioned sympathetic coactivation, that VNS can increase parasympathetic activity may also increase sympathetic activity reactively (Buchholz et al., 2015). Something similar may have occurred in our study.

Concluıon

Parasympathetic system activity is of significance in recovery after exercise. Auricular TVNS can accelerate sports recovery by increasing PA after exercise. The short duration of the study, that TVNS was performed for 5 minutes, the measurement of ANS activity together with TVNS, the small number of participants, and the low number of measurement repetitions can be considered among the limitations of the study. We are of the opinion that further randomized controlled studies with a larger sample are needed to examine the effect of TVNS on sports performance and recovery. Our study found that auricular TVNS can acutely affect parameters such as pulse, respiratory function, and ANS activity after exercise. The effects of TVNS may vary

according to the physiological needs of the body at the time. TVNS performed in the acute period after exercise may cause an increase in sympathetic activity together with parasympathetic activity.

Conflict of Interest

During the development and publication of this work, the authors did not reveal any conflicts of interest.

Funding Information

The authors declared that throughout the course of conducting and writing this study, they did not receive any grants.

Acknowledgement

We would like to express our gratitude to every volunteer who participated in the research and Prof. Dr. Mehmet ÜNAL.

Author Contibution

Study Design, E.O.G., A.V.O., H.I.U., H.K.A.; Data Collection, E.O.G, A.V.O, H.I.U.; Statistical Analysis, E.O.G., A.V.O., H.I.U., H.K.A., S.H.H.; Data Interpretation, E.O.G., A.V.O., H.I.U., H.K.A., S.H.H.; Manuscript Preparation, E.O.G., A.V.O., H.I.U., H.K.A., S.H.H.; Literature Search, E.O.G., A.V.O., H.I.U., H.K.A., S.H.H. All authors have read and agreed to the published version of the manuscript.

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