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Comparison of the Acute Effects of Auricular Vagus Nerve Stimulation and Deep Breathing Exercise on the Autonomic Nervous System Activity and Biomechanical Properties of the Muscle in Healthy People

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Abstract: Background/Objectives: We aimed to examine the acute effects of deep breathing exercise and transcutaenous auricular vagus nerve stimulation (taVNS) on autonomic nervous system activation and the characteristics of certain muscle groups and to compare these two methods. **Methods:** 60 healthy adults between the ages of 18-45 were randomly divided into two groups to receive a single session of taVNS and deep breathing exercises. Acute measurements of pulse, blood pressure, perceived stress scale, autonomic activity and muscle properties were performed before and after the application. **Results:** A significant decrease was detected in the findings regarding the perceived stress scale, pulse and blood pressure values as a result of a single session application in both groups ($p < 0.05$). In addition, it was determined that the findings regarding autonomic measurement values increased in favor of the parasympathetic nervous system in both groups ($p < 0.05$). In measurements of the structural properties of the muscle, the stiffness values of the muscles examined in both groups decreased ($p < 0.05$), while the findings regarding relaxation increased ($p < 0.05$). As a result of the comparative statistical evaluation between the groups, the increase in parasympathetic activity was found to be greater in the deep breathing group (DB) ($p < 0.05$). In the measurements made with the Myoton®PRO device, a significantly higher decrease in the stiffness value of the erector spinae was detected in the respiratory group ($p < 0.05$). and an increase in the relaxation values was detected in the erector spinae and gastrocnemius muscles ($p < 0.05$). **Conclusion:** It has been observed that both methods can increase parasympathetic activity and muscle relaxation in healthy people in a single session. However, DB seems slightly superior to taVNS in this respect.

Keywords: Auricular vagus nerve stimulation; deep breathing exercises; heart rate variability; muscle relaxation; muscle tone

1. Introduction

The autonomic nervous system (ANS) is responsible for the regulation and integration of internal organ functions through the mutual actions of its sympathetic and parasympathetic branches. Evaluation of ANS functions is important to get information about the status of the wellness or disease and the activity of ANS can be affected by several methods such as deep breathing, valsalva maneuver, handgrip, cold pressor test [1]. Activity dysfunctions in the ANS have been associated with various clinical disorders such as heart failure, inflammatory bowel disease and chronic pain syndromes, and it has been reported that this condition is mostly related to the lack of parasympathetic activity and relatively higher sympathetic activity [2]. Therefore, regulation of ANS activity may contribute to recovery. Breathing exercises seem to increase parasympathetic activity assessed

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by heart rate variability (HRV) indexes even in one session. Simultaneous anxiety and stress levels may accompany this decline [3]. Deep breathing in diabetic patients can also decrease glycosylated hemoglobin levels and hypertension due to parasympathetic activity enhancement as shown by HRV [4]. Another new method to modulate the ANS activity is the vagus nerve stimulation (VNS) which can be done also non-invasively. VNS is a medical treatment method that can be used in the treatment of diseases such as epilepsy, depression and migraine. VNS works by applying electrical impulses to the vagus nerve and can be applied to both ears [5]. Transcutaneous auricular vagus nerve stimulation (taVNS) provides an increase in parasympathetic activity and significantly reduces the incidence of firing of sympathetic fibers [6]. There are few studies on the effect of deep breathing and VNS on muscle tone, and the results are inconclusive. Brief breathing exercises do not substantially affect muscle tension under psychological stress [7]. However, deep breathing exercises can reduce muscle tone by increasing parasympathetic activity. Similarly, taVNS appears to both reduce muscle tone and alter autonomic activity [8]. In our study, we hypothesized that taVNS and deep breathing exercises may increase parasympathetic activity and reduce muscle tone in healthy participants. So, we aimed to compare the effects of both methods and see their superiority over each other.

2. Materials and Methods

2.1. Study Design

The study was conducted at the Physical Therapy Laboratory of the Faculty of Health Sciences, Istanbul Gelisim University between February and August 2022, in accordance with the rules of the Declaration of Helsinki. The participants were asked to sign the informed consent form before participating in the study. The clinical trial registration number was NCT06740032.

2.2. Participants

Volunteer individuals participating in our study were randomly divided into two groups to receive "vagus nerve stimulation" and "deep breathing exercises". Immediately before and after the single-session applications, measurements of ANS activity and muscle properties were recorded in both groups, and the obtained values were compared between groups. Publication approval was obtained from the individuals participating in the study. Our study is a randomized prospective experimental study without a control group. Individuals were divided into two groups according to their order of arrival. The researcher informed the individual whose group was randomly determined, which method would be applied.

2.3. Study Protocol

The study included 60 healthy participants, 30 in each group, aged between 18–45 years who could read and write Turkish. The inclusion criteria were as follows: having no known acute or chronic disease, and no previous treatment with taVNS. All participants included in the study were selected from those who were at an intermediate level according to the International Physical Activity Fitness Survey (IPAQ). In this study, IPAQ short form and one-on-one interview method were applied to determine the physical activity levels of people. The exclusion criteria were previous vagotomy, myocardial infarction or arrest, cardiac conduction disorders, intracranial hemorrhage, history of cancer, using any medication supplement, and pregnancy during the study period. After the evaluation of 60 participants, statistical analysis of the data was started. The study was started and completed as planned.

2.4. Assessment

2.4.1. Vagus Nerve Stimulation

Participants in the VNS group were performed with the Vagustim brand device. Bilateral taVNS was applied to individuals in the VNS group for 20 minutes with a frequency of 10 Hz and a pulse width of 300 µs. The earsets are placed in line with the concha and tragus. (Figure 1)

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Figure 1. VNS right earset placement.

2.4.2. Deep Breathing Exercise

The definition of slow, deep breathing is expressed in its broadest sense as slower than the typical adult rate of 12-15 breaths per minute. Various yogic techniques target a specific frequency of 6 breaths per minute (0.1 Hz). This frequency maximizes heart rate variability (HRV) [9]. Participants performed deep breathing exercises for 9 minutes in a semi-sitting position, with one hand on the diaphragm and the other on the thorax, in a position where they felt comfortable, followed by relaxation exercises for 2 minutes, and then breathing exercises again for 9 minutes. Slow deep breathing exercises were applied with an inspiration time of 3 seconds and an expiration time of 7 seconds, resulting in 6 breaths in 1 minute.

2.5. Outcome Measures

2.5.1. Primary Outcome Measure

In our study, Primary outcome measures were HRV results and muscle properties such as stiffness, and relaxation.

Analysis of the Autonomic Nervous System

Measurements were made using self-gel electrodes. One end of the e-motion faros device was connected to the electrode just below the participants' right clavicle, the other end was connected to the bottom of the left clavicle, and the third end was connected to the electrode just above the lowest left rib. Measurements were made in a semi-sitting position where the individual felt comfortable. Participants were told not to drink coffee, tea or cigarettes for at least 2 hours before the measurements, not to drink alcohol for at least 12 hours and not to exercise in a way that would increase their heart rate. Measurements were taken 2-4 hours after (neither hungry nor full) the last meal. Those who had insomnia the night before were measured on another day. After 30 minutes of rest, autonomic measurements were taken before and after taVNS and deep breathing exercise methods. Pulse and blood pressure measurements were made after HRV in order not to affect it. During the 5-minute HRV measurement, the participant was asked not to move or talk and to sit comfortably with eyes closed. After taVNS and breathing exercise application, the measurement made with the e-motion faros device was repeated in the same way and the pulse, blood pressure was measured again. The measurement records made with the e-motion faros device were later analyzed using kubios software and the following values were obtained: SNS index, PNS index, stress index, LF/HF ratio, PNN50, RMSSD.

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- High frequency (HF): Shows more parasympathetic activity.

-Low frequency (LF): It indicates more sympathetic activity.

-RMSSD: It is the root mean square of consecutive RR interval differences. It reflects beat-to-beat variance in HR, indicating parasympathetic activity.

-Stress Index: It indicates sympathetic activity.

-SNS Index: It indicates sympathetic activity.

-PNS Index: It indicates parasympathetic activity.

-PNN50: It refers to the division of NN50 by the total number of NN (R-R) intervals. It indicates parasympathetic activity [10].

Working Flow Chart

HRV> Blood Pressure+Pulse+Scale> Muscle	VNS or DB	HRV> Blood Pressure+Pulse+Scale> Muscle

Analysis of the Structural Features of the Muscles

The Myoton®PRO device is a reliable and portable digital palpation device that can provide a better understanding of the relationship of biomechanical properties such as tone, stiffness and flexibility to muscle health and physical condition. Application of Myoton®PRO: The masseter, trapezius (upper part), erector spinae, gastrocnemius, biceps brachii muscles of the individuals included in the study were measured bilaterally. The individuals were allowed to lie relaxed in the supine position for 10 minutes to allow their muscles to rest, and while in this position the trapezius (upper part) in the sitting position, erector spinae and gastrocnemius muscles in the supine position, biceps brachii and masseter muscles in the prone position were evaluated bilaterally. (Figure 2)

Figure 2. Application of Myoton®PRO to m. erector spinae.

The specific evaluation position and point of the muscle was made according to the criteria and positions recommended by the Myoton®PRO team (Myoton®PRO, https://www.myoton.com/applications/, Date of access:01.05.2022). The device was placed on each muscle surface in an upright position and the tip of the probe was placed on the motor point of the measured muscle. Dynamic Stiffness (S) and Relaxation (R) data were used for muscle characteristics. The average bilateral measurements of each muscle group were evaluated.

2.5.2. Secondary Outcome Measure

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Individuals' pulse and blood pressure values and perceived stress scale results before and after the application were recorded. Pulse and blood pressure were measured with the Visomat brand device while the individuals sat quietly and motionless in a comfortable position for 60 seconds.

2.6. Statistical Analysis

Sample size was calculated by using G-Power. When T test family and Mann-Whitney U Test: repeated measures, within–between interaction test, effect size: 0.80, beta level: 0.20, power (1-β): 0.80, number of groups: 2, number of measurements: 2, correction among repeated measures: 0.5 was selected, the total sample size was calculated as 60 participants (30 participants in each group). All the statistical analyses were performed using IBM SPSS 22.0 software. Descriptive statistics of the variables used are n and frequency in categorical data; In continuous data, mean, standard deviation and minimum and maximum values were used. First of all, it was examined whether continuous variables had normal distribution according to the Shapiro-Wilk test. Nonparametric tests were applied for variables that did not have a normal distribution ($p<0.05$). The Wilcoxon Sign Rank Test was used to measure the difference between the means of two dependent groups, and the Mann-Whitney U test was used to measure the difference between the means of two independent groups. In all analyses, the significance level was given as 0.05.

3. Results

The gender distribution of the participants in the VNS and DB groups participating in the study is shown in the table below. It was determined that individuals in the VNS group were predominantly female ($p<0,05$). (Table 1)

Table 1. Comparison of intergroup differences in gender descriptive statistics evaluation.

Gender	**VNS**		**DB**		**Total**		Pearson Chi-Square	p
	n	% İntragroup	n	% İntragroup	n	% İntergroup		
Male	5	16,67	15	50,00	20	33,33	7,500	**0,006**
Female	25	83,33	15	50,00	40	66,67		
Total	30	100	30	100	60	100		

Considering the demographic information of the individuals, there was no significant difference in the average height and weight between the two groups, while the average age in the VNS group was found to be higher ($p<0,05$). (Table 2)

Table 2. Comparison of intergroup differences in age, height, weight, blood pressure, pulse, measurement of autonomic and structural properties of muscle parameters.

	DB N=30			**VNS** N=30		
N=60	Mean±SD	Z/t	p	Mean±SD	Z/t	p

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Height (cm)	171,97±9,34	-0,074	0,941	171,70±8,17	-0,074	0,941
Weight (kg)	66,60±12,54	-0,429	0,668	68,40±13,22	-0,429	0,668
Age	24,70±4,50	-2,859	**0,004**	31,27±8,23	-2,859	**0,004**

It was determined that there were significant decreases in the perceived stress scale, pulse, systolic and diastolic blood pressure values in both the DB and VNS groups compared to the first measurement average (p<0,05). (Table 3)

Table 3. Comparison of intragroup differences in blood pressure, pulse, measurement of autonomic and structural properties of muscle parameters.

		DB			VNS		
		Mean±SD	Z/t	p	Mean±SD	Z/t	p
Perceived Stress Scale	1st measurement	27,20±7,29	3,892	**0,001****	26,10±8,31	2,443	**0,021****
	2st measurement	25,97±7,26			24,80±7,81		
Pulse	1st measurement	78,53±9,73	4,556	**0,000****	79,53±9,90	6,009	**0,000****
	2st measurement	74,00±9,74			73,50±8,11		
Systolic Pressure	1st measurement	118,23±11,31	5,245	**0,000****	119,73±13,74	6,394	**0,000****
	2st measurement	112,33±12,47			112,37±13,76		
Diastolic Pressure	1st measurement	73,40±10,03	3,267	**0,003****	76,00±12,03	4,906	**0,000****
	2st measurement	70,13±10,08			70,50±13,57		
SNS Index	1st measurement	0,24±1,01	6,041	**0,000****	0,74±0,95	7,75	**0,000****
	2st measurement	-0,48±1,16			0,02±0,79		
PNS Index	1st measurement	0,35±1,32	-4,33	**0,000***	-0,46±1,13	-4,76	**0,000***
	2st measurement	9,34±17,59			0,55±1,74		
RMSSD	1st measurement	53,94±30,71	-4,78	**0,000***	47,03±34,88	-4,73	**0,000***
	2st measurement	232,60±421,98			75,83±59,47		
Stress Index	1st measurement	8,25±3,07	8,519	**0,000****	9,39±3,65	-4,58	**0,000***
	2st measurement	4,79±2,22			6,61±2,65		
PNN50	1st measurement	21,70±13,31	-6,23	**0,000****	13,23±15,54	-3,96	**0,000***
	2st measurement	40,01±21,46			20,63±16,84		

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LF/HF	1st measurement	1,37±1,62	-1,29	0,199	2,56±2,44	-0,83	0,405
	2st measurement	1,14±0,62			2,60±2,39		
Stiffness-Trapezius muscle	1st measurement	267,18±33,88	5,784	**0,000****	259,43±44,90	-4,38	**0,000***
	2st measurement	249,70±31,19			244,45±43,59		
Stiffness - Erector spinae muscle	1st measurement	235,53±87,88	-4,54	**0,000***	192,15±46,10	-3,67	**0,000***
	2st measurement	235,53±87,88			185,92±43,91		
Stiffness - Gastrocnemius muscle	1st measurement	262,80±53,84	3,466	**0,000****	236,68±38,18	5,163	**0,000****
	2st measurement	249,10±50,39			227,13±39,87		
Stiffness - Biceps brachii muscle	1st measurement	243,65±36,29	-4,78	**0,000***	229,68±31,29	-4,63	**0,000***
	2st measurement	230,60±37,59			216,18±23,66		
Stiffness - Masseter muscle	1st measurement	328,25±88,46	3,444	**0,000****	362,68±61,49	3,533	**0,001****
	2st measurement	313,47±76,18			343,65±57,96		
Relaxation-Trapezius muscle	1st measurement	18,37±1,94	-3,97	**0,000***	18,66±1,99	-4,47	**0,000****
	2st measurement	19,09±1,98			19,69±2,02		
Relaxation - Erector spinae muscle	1st measurement	22,98±5,22	-4,57	**0,000***	26,53±3,98	-4,25	**0,000***
	2st measurement	24,23±4,42			27,05±3,89		
Relaxation - Gastrocnemius muscle	1st measurement	19,04±4,05	-2,24	**0,014***	22,46±3,56	-5,71	**0,000****
	2st measurement	19,57±3,93			23,63±3,59		
Relaxation - Biceps brachii muscle	1st measurement	19,60±1,75	-4,92	**0,000***	20,84±2,26	-4,1	**0,000***
	2st measurement	20,60±1,36			21,72±1,79		
Relaxation - Masseter muscle	1st measurement	15,84±2,90	-1,53	0,138	15,67±2,73	-4,98	**0,000****
	2st measurement	16,18±2,70			16,39±2,70		

Note: *Wilcoxan Signed Ranks Test **Paired Sample T-Test Abbreviations: DB, deep breathing; VNS, vagus nerve stimulation. *p<0.05.

Considering the HRV values, it was revealed that there was a statistically significant increase in PNS index, RMSSD, PNN50 values in the DB group, while there was a significant decrease in SNS index and stress index values (p<0,05). Although there was a decrease in the LF/HF ratio, no statistical significance was observed (p>0,05). In the VNS group, while there was a statistically significant increase in PNS index, RMSSD and PNN50 values, it was revealed that there was a significant decrease

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in perceived stress scale, SNS index and stress index values (p<0,05). Although there was a decrease in the LF/HF value, no statistical significance was observed (p>0,05). (Table 3)

In the DB group, in the measurements of the structural properties of the muscle, a decrease was observed in the measurement values of all muscles in the stiffness parameter (p<0.05), while a statistically significant increase was observed in the relaxation parameter in all muscles except the masseter muscle, while the increase in the masseter was not found to be statistically significant (p>0,05). In the VNS group, while a decrease was observed in the measurement values of all muscles in the stiffness parameter (p<0.05), a statistically significant increase was observed in the relaxation parameter in all muscles (p<0,05). (Table 3)

There was no significant difference between groups in terms of perceived stress scale, pulse and systolic/diastolic blood pressure and SNS index values (p>0,05). When the RMSSD, PNN50, PNS index results were compared, it was found that the DB group was statistically higher than the VNS group (p<0,05). (Table 4)

Table 4. Comparison of intergroup differences in blood pressure, pulse, measurement of autonomic and structural properties of muscle parameters.

	DB	VNS		
	Mean±SD	Mean±SD	Z/t	p
Perceived Stress Scale	-1,23±1,74	-1,30±2,91	-0,44	0,660*
Pulse	-4,53±5,45	-6,03±5,49	-1,771	0,077*
Diastolic Pressure	-3,27±5,48	-5,50±6,14	-1,305	0,143**
Systolic Pressure	-5,90±6,16	-7,37±6,31	-0,096	0,923*
RMSSD	178,67±413,87	28,79±36,28	-2,506	**0,012***
Stress Index	-3,45±2,22	-2,77±2,63	-1,427	0,153
PNN50	18,31±16,10	7,40±9,14	-2,816	**0,005***
LF/HF	-0,23±1,60	0,04±2,29	-0,651	0,515
SNS Index	-0,73±0,66	-0,71±0,50	-1,774	0,927
PNS Index	8,99±17,25	1,008±1,08	-1,368	**0,014***
Stiffness-Trapezius muscle	-17,48±16,55	-14,98±17,23	-0,584	0,559
Stiffness - Erector spinae muscle	-26,72±48,77	-6,23±9,05	-2,56	**0,010***
Stiffness - Gastrocnemius muscle	-13,70±21,65	-9,55±10,13	-0,88	0,379
Stiffness - Biceps brachii muscle	-13,05±9,12	-13,50±19,42	-1,435	0,151
Stiffness - Masseter muscle	-14,78±23,51	-19,03±29,51	-0,925	0,355
Relaxation- Trapezius muscle	0,73±1,00	1,03±1,26	-0,889	0,374
Relaxation - Erector spinae muscle	1,25±1,49	0,52±0,58	-2,62	0,009*
Relaxation - Gastrocnemius muscle	0,53±1,30	1,17±1,13	-2,346	0,019*

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Relaxation - Biceps brachii muscle	0,99±1,11	0,88±1,11	-1,287	0,198
Relaxation - Masseter muscle	0,34±1,23	0,72±0,79	-0,473	0,636

Note: *Mann-Whitney U Test **Independent Sample T-Test Abbreviations: DB, deep breathing; VNS, vagus nerve stimulation. *p<0.05.

In the intergroup comparison, a significantly greater decrease in stiffness values was observed in the erector spinae in the DB group. As for relaxation values, a statistically significant increase was detected in erector spinae and gastrocnemius values in the DB group (p<0,05). (Table 4).

4. Discussion

In our study, in which we compared the effects of taVNS and breathing exercises on healthy people, a significant decrease was detected in the findings regarding the perceived stress scale, pulse and blood pressure values as a result of a single session of application in both groups, in accordance with literature data [4,11]. In addition, the findings regarding HRV values in both groups developed in favor of the parasympathetic nervous system, again in line with the literature [3,6]. When the effects on the structural properties of various muscle groups were examined in our study, a decrease in the stiffness value of the muscles was observed with both applications, while an increase in the relaxation coefficient was observed. Clancy et al. (2014) stated that ~~their~~ single-session stimulation of the tragus part of the ear using 200 µs pulses at 30 Hz 10-50 mA provided an increase in parasympathetic activity and reduced the incidence of firing of sympathetic fibers [6]. In our study, 10 Hz, 300 microsecond pulse width stimulation was applied bilaterally to the tragus and concha and it was revealed that parasympathetic activity increased.

ANS modulation via taVNS is proposed as a noninvasive therapeutic strategy for the treatment of cardiovascular diseases [12]. Billman (2013) gave a different perspective to the idea that the LF/HF ratio measures "sympatho-vagal balance". He reported that LF does not only reflect SNS activity, and half of the variability in this frequency band originates from the PNS [13]. It is also stated by other researchers that there is uncertainty about the contributions of PNS and SNS to the LF/HF ratio [10]. In our study, systolic/diastolic blood pressure and pulse measurement values decreased significantly after a single session of taVNS. Moreover no significant change in the LF/HF ratio as a result of a single session of taVNS. While other HRV parameters changed in favor of parasympathetic activity, the lack of change in the LF/HF ratio may have been related to the measurement time (5 minutes of measurement), the number of participants (60 people) and their characteristics (healthy people). As a matter of fact, Soltani et al (2023), evaluated the effect of taVNS on the LF/HF ratio in 15 studies conducted with 380 healthy participants, and in 7 studies, it was reported that there was no significant difference in the LF/HF ratio between the VNS group and the control group. In the same study, it was stated that there was a high correlation between the level of autonomic dysfunction and the response to taVNS [14]. In our study conducted on healthy individuals, no significant change was observed in the LF/HF ratio, which is in line with the literature.

The study of Ferstl et al (2022), in which 90 minutes of taVNS was performed from one ear (right or left) by randomization in 82 healthy participants, reported that taVNS improved positive mental state but was only effective in the acute phase after stimulation [15]. Cook et al. (2021), examined virtual reality-based deep breathing exercises in head injury rehabilitation. 15 participants were given 5-minute-deep breathing exercises with virtual reality applications, and participants reported a decrease in stress, tension, fatigue and confusion following the deep breathing exercise [16]. In our study, we found that short-term deep breathing practices led to a decrease in perceived stress scales. Deep slow breathing increases vagus nerve activity, which is indexed by HRV [17]. Jensen et al. (2022), compared the effect of taVNS and DB on vagal tone in healthy participants and patients with rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE), and vagal tone was evaluated using HRV parameters. 30 minutes of DB and 30 minutes of taVNS were applied to 42 healthy participants and 52 patients on separate days. While DB was associated with the highest increase in HRV

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parameters in healthy participants, both applications caused an increase in HRV parameters in favor of parasympathetic activity in patients [18]. As in our study, DB exercises seem to be more effective than taVNS in increasing parasympathetic activity in healthy individuals. However, this situation seems to change in patients with autonomic dysfunction. Studies have shown that slow breathing can reduce the stress response by encouraging the body to relax [19]. Kampusch et al. in their study, applied taVNS to a patient with cervical dystonia who was resistant to treatment for 20 months. As a result of the treatment, it was determined that there was a decrease in muscle tone in the right and left trapezius muscles [8]. However, there are also studies showing that a single session of taVNS increases muscle activation in healthy people [20]. We think that measuring muscle activation during muscle contraction with electromyography in their study of Konakoglu et al. may cause such a difference to emerge. In our study, it was determined that as a result of a single session of taVNS, there was a statistically significant decrease in the stiffness values of all muscles measured with the Myoton®PRO device and a significant increase in the relaxation parameter. The results obtained are consistent with those of Kampusch et al. (2015), who stated that taVNS reduces muscle tone by increasing parasympathetic activity [8]. In their study using Myoton®PRO they stated that the sitting position with lumbar lordosis assist caused a decrease in upper trapezius muscle tone in participants with head anterior tilt [21]. In another similar study using Myoton®PRO, Kim (2021) determined that there was a correlation between low mixing ability index of the masseter muscle and high muscle tone and low flexibility [22]. Skeletal muscles normally receive no parasympathetic innervation, the autonomic effects appear to be exclusively sympathetic in origin, mediated either by the neurally released noradrenaline or indirectly through circulating adrenaline released in the blood by the adrenal medulla. Thus, sympathetic activity can increase muscle contractility and excitability [23]. So, it can be said that, elevated parasympathetic and decreased sympathetic activity can reduce tension in the muscles and lead to relaxation as seen in our study.

This study has some limitations. The most important limitation is that the applications to individuals in both groups were carried out in a single session. In addition, the average age and gender distribution of the participants in the VNS and DB groups are not homogeneous. (Table 1) Relevant measurements were performed immediately after the application in the VNS and DB groups. Randomized, prospective studies with longer duration, using additional measurement methods, and conducted in healthy individuals or different patient populations are needed to develop data regarding autonomic responses and how long the changing properties of the muscle last.

5. Conclusions

In conclusion, we found that a single-session application of taVNS and DB was effective on HRV and biomechanical properties of the muscle in healthy individuals. However, deep breathing exercises were found to be more effective in increasing parasympathetic activity and reducing musle tension in certain parameters than taVNS. As a result, DB seems slightly superior to taVNS in this respect.

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Data Availability Statement: Data are available on request from the authors.

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Conflicts of Interest: A.V.O. is one of the co-founders of the Vagustim Company (Istanbul, Turkey) which produced the taVNS device used in this study.

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