Temporal evaluation of the effects of controlled ventilation maneuver and transcutaneous auricular vagal stimulation on cardiac autonomic control in healthy subjects

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1 INTRODUCTION

The autonomic nervous system (ANS) is a division of the central nervous system (CNS) and controls most of the visceral functions of the human body. Among the main functions of this system are the control of blood pressure, heart rate, gastrointestinal motility, and body temperature (Prakash et al., 2021).

The ANS is divided into two branches: sympathetic and parasympathetic (or vagal). The sympathetic system controls the "fight or flight" responses, preparing the body for stressful activities, for example. In these situations, there is an increase in the production of adrenaline and noradrenaline, causing an increase in heart rate, inhibition of gastrointestinal activity, among other effects. The parasympathetic system, in turn, regulates the body functions related to rest and digestion. Parasympathetic stimulation causes a slight decrease in blood pressure and heart rate (McCorry, 2007).

The control of the cardiovascular system is performed by the ANS through afferent and efferent nerves of the heart (Shen et al., 2014), and the adjustment of cardiovascular function to control blood pressure and heart rate can be performed by reflex responses from mechanical and chemical mechanisms, receptors, among others. Among the reflex responses, arterial baroreceptors are nerve

endings with an important role in regulating heart rate in the face of blood pressure variations (Fisher et al., 2015).

The vagus nerve, the tenth pair of cranial nerves, is the main component of the ANS and is of great importance in the transmission of sensory information from the body to the brain, as well as being the protagonist of the ANS in performing functions such as controlling heartbeat, blood pressure, and regulating the function of glands and other involuntary muscles. The vagus nerve is the largest of the cranial nerves, distributing from the neck to the abdomen (Clancy et al., 2013; Howland, 2014). Its activity can be assessed through heart rate variability (HRV), which is a measure regarding the regulation of heart rate by the CNS, allowing visualization of the dynamic variation of HR under the control of the nervous system (Wang et al., 2014).

The use of vagus nerve electrical stimulation (VNS) was approved by the US *Food and Drug Administration (*FDA) in 1997 for patients with refractory epilepsy and in 2005 for patients with antidepressant-resistant chronic depression (Cai et al., 2014). Recent studies also explore the therapy's potential as a treatment for heart failure (Asad et al., 2019), Alzheimer's disease (Vargas-Caballero et al., 2022), obesity (Dai et al., 2020), and chronic pain (Moisset et al., 2020). VNS therapy is implemented by implanting an electrostimulator in the chest region, connected by a set of electrodes to the cervical branch of the vagus nerve in the neck (LivaNova, 2021). In the last decade, more than 60,000 patients have been treated with VNS therapy (De Couck et al., 2017). Despite the clinical potential of vagus nerve stimulation, the need for surgical intervention hinders the dissemination of the therapy, as the risks of post-surgical complications (Yakunina et al., 2017) and side effects such as hoarseness, dysphagia, coughing, and pain are taken into consideration (Elliot et al., 2011).

Given the benefits of VNS therapy and the risks associated with the surgical procedure, transcutaneous vagus nerve stimulation (t-VNS) has emerged as a non-invasive alternative and potential replacement for vagus nerve stimulation. Transcutaneous stimulation therapy is performed with surface electrodes, which apply electrical pulses to the auricular branch of the vagus nerve in the outer ear (Stefan et al., 2012). The treatment has similar efficacy to invasive treatment (Murray et al., 2016) and has been explored in patients with coronary artery disease (Zamotrinsky et al., 1997), epilepsy (He W et al., 2013), chronic pain (Napadow et al., 2012), and depression (Fang et al., 2016).

In healthy individuals, studies show that t-VNS is able to activate the region of the CNS responsible for autonomic control, in addition to increasing mechanical pain threshold and reducing pain level (Frangos, 2015; Busch, 2013). Recent studies present data that also point to a reduction in blood pressure (Tobaldini et al., 2019), HRV (Clancy et al., 2014), and salivary cortisol (Agostini, 2021) for these individuals.

The activity of the vagus nerve can also be modulated by behavioral habits, such as decreased respiratory rate, called slow breathing or controlled ventilation. Due to the coupling between the

respiratory and cardiovascular systems, controlled ventilation increases the activation of arterial baroreceptors during expiration (Russo et al., 2017; Szulczewski, 2022). Reduced respiratory rate is related to reduced BP and directly affects HRV (Bernardi et al., 2001; Jennings et al., 1996). Studies also show that slow breathing techniques are effective in treating cardiovascular disease and reducing HR (Krasnikov et al., 2013).

2 OBJECTIVE

This study aims to evaluate the effects of vagus nerve stimulation with the commercial electronic device VITOS and the controlled ventilation assisted via application. The analysis will be performed after interventions with the electronic device and with slow breathing techniques in healthy individuals, performed in a controlled environment and following the proposed protocol. The comparison between the responses obtained will be performed by means of vital parameters and autonomic control.

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3 METHODOLOGY

This study was carried out at Instituto de Ciência e Tecnologia da Universidade Federal de São Paulo (ICT - UNIFESP), located in São José dos Campos. Undergraduate students of the ICT UNIFESP, who self-declared that they were healthy, participated in the research. The invitation was made by public call, through posters scattered around campus, social media posts, and WhatsApp groups. Those interested were invited to attend a meeting held at UNIFESP, in which the objectives, dynamics and criteria for carrying out the work were explained.

The following inclusion criteria were used: to be a student regularly enrolled in a course at the ICT - UNIFESP to be healthy and be between 18 and 40 years old. And those who had been taking medications that interfered with autonomic control, anxiolytics, and antidepressants in the last six months were excluded from the study.

The study was approved by the Research Ethics Committee of UNIFESP (6299622.3.0000.5505). The selected volunteers received an informative material containing the explanation of the procedures used, the recommendations for the days of signal collection, and the Informed Consent Form. In this term, the objectives of the study, the risks and benefits to which the students would be submitted were exposed. After reading the document, the volunteers were able to clarify any doubts and, after their acceptance, two copies of the term were signed: one copy given to the volunteer and the other kept with the researchers. The volunteers had the right to refuse to participate in the study at any time, without any prejudice to them.

The volunteers were instructed about abstinence from alcohol and caffeine at least 12 hours before the protocol. The protocol consisted of two sessions: Conventional Transcutaneous Stimulation

(EVT) and Controlled Ventilation Maneuver (CVM). Cardiac data were collected using the POLAR V800 Monitor, which consists of a chest strap that collects pulse interval data through a sensor. These data are sent via telemetry to a watch, allowing the visualization of heart rate values in real time and storage for the evaluation of autonomic control.

The experimental sequence followed was:

Day 1

Accommodate and position the volunteer properly, instructing him/her to remain at rest for 05 minutes, instructing him/her to remain seated and avoid talking or making large movements. Adjust the POLAR monitor to the volunteer's thorax and verify signal capture.

- Collect saliva for cortisol measurement and initial vital data.
- Start protocol by performing controlled ventilation maneuvers for 20 minutes.
- At the end of the 20 minutes, the volunteer was instructed to remain at rest for another 05 minutes to record the post-maneuver effect and to collect saliva and vital data again.
- Remain with the POLAR monitor for 4 hours after the intervention.

Day 2

- On the second day of testing with the same volunteer, the steps from Day 1 were repeated, this time performing transcutaneous auricular stimulation.

Ventilation Maneuver Intervention Controlled by Mobile Application

For the controlled breathing intervention, the volunteer was instructed to use a cell phone with the Keep application, provided by the researcher, programmed to perform 10 ventilatory incursions per minute (10 bpm), for 20 minutes.

Transcutaneous Vagus Nerve Stimulation Intervention

For the vagus nerve transcutaneous auricular electrostimulation we used the commercial VITOS device from Cerbotec. The device has a programmer and a wire with an electrode, which was positioned in the auditory canal of the volunteer's external ear. The electrostimulation was performed for 20 minutes, at a frequency of 25 Hz. The current was adjusted from 0.3 to 1.2 mA according to the sensitivity and well-being of each subject, ensuring a painless intervention.

Autonomic Control Assessment

The time series data extracted from the POLAR system were evaluated for stationarity by separating the different recording phases:

A - Baseline Registration

B - During the intervention (Inter)

C - 1 Hour after intervention

D - 2 Hours after intervention

E - 3 Hours after the intervention.

Spectral analysis of the heart rate time series (tachogram) was performed to assess variability and characterization into specific frequency components. Rhythmic heart rate oscillations have three distinct ranges: the High Frequency (HF) range, similar to normal respiratory activity; o Low Frequency (LF) and o Very Low Frequency (VLF). These oscillations can vary in amplitude and frequency according to behavior, physiological and pathological conditions (Montano, Ruscone et al., 1994; Montano, Porta et al., 2009).

Heart rate variation measurements were evaluated by means of indices obtained by spectral decomposition of the tachogram, using an autoregressive model. The analysis was performed in stationary series of 200 beats, evaluated in the HF (0.15-0.04 Hz), LF (0.04-0.15 Hz) and VLF (0-0.04 Hz) bands.

Statistical Analysis

The data were evaluated by parametric methods, compared by 2-way ANOVA test with repeated measures, considering the intervention session and the protocol step as factors. Multiple comparisons were performed using Tukey's test. P values lower than 0.05 were indicative of statistical significance.

4 DEVELOPMENT

The results presented refer to the analysis of 9 participants: 7 female and 2 male. According to the selection criteria, all participants recruited were undergraduate students at UNIFESP. Each volunteer participated in two intervention sessions: controlled ventilation maneuver (CVM) and use of transcutaneous vagal auricular stimulator (TVE).

The intervention sessions took place between September and November 2022, in the Physiology Laboratory of the Institute of Science and Technology of the Federal University of São Paulo (ICT - UNIFESP), located in São José dos Campos. All sessions were held between 7 AM and 9 AM, with removal of the polar device 4 hours after the end of the interventions.

Autonomic Control Assessment

During the two sessions, the participants had their cardiac parameters monitored through the POLAR system. The tachograms extracted from the system were evaluated for stationarity, separating the different phases of recording, as shown below:

A - Baseline Registration

B - During the intervention (Inter)

C - 1 Hour after intervention

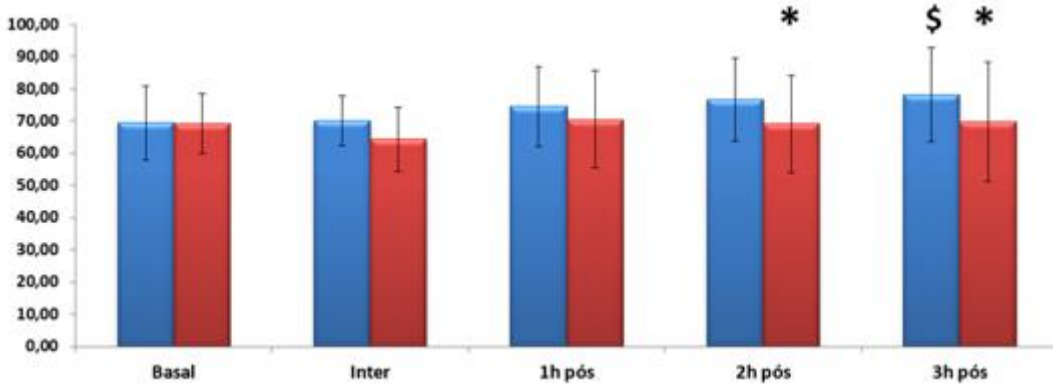
D - 2 Hours after intervention

E - 3 Hours after intervention

The data collected was stratified to perform the analyses and comparisons between the sessions, controlled ventilation maneuver and the session in which the participant used the transcutaneous vagal auricular stimulator.

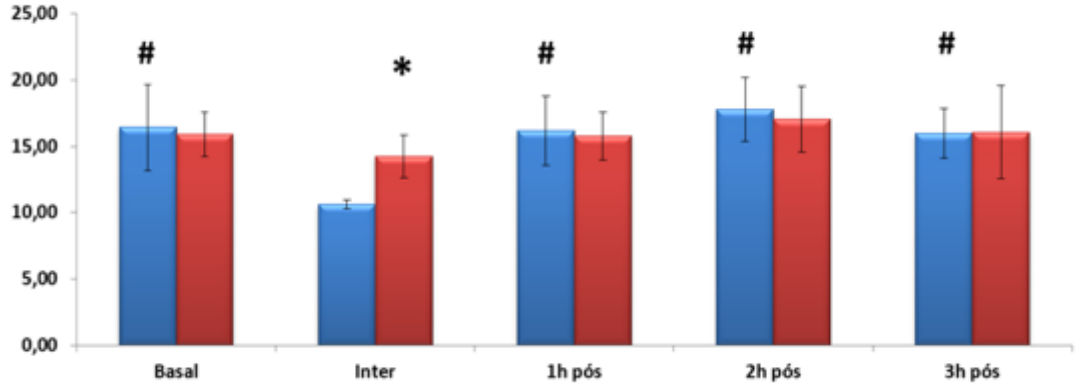
In Figure 1, the average heart rate values at each stage are shown. The heart rate values were not changed during the interventions. However, 3 hours after the protocol, heart rate in the MVC group was higher than its baseline situation (p=0.036), differing even from the EVT group (p=0.026). There was also a statistical difference between the MVC and EVT groups 2h after the intervention (p=0.041).

Figure 1: Mean values of heart rate, in beats per minute, at different protocol steps, MVC group (blue) vs EVT group (red)
* different from MVC compared to same step; $ different from baseline step in same protocol.



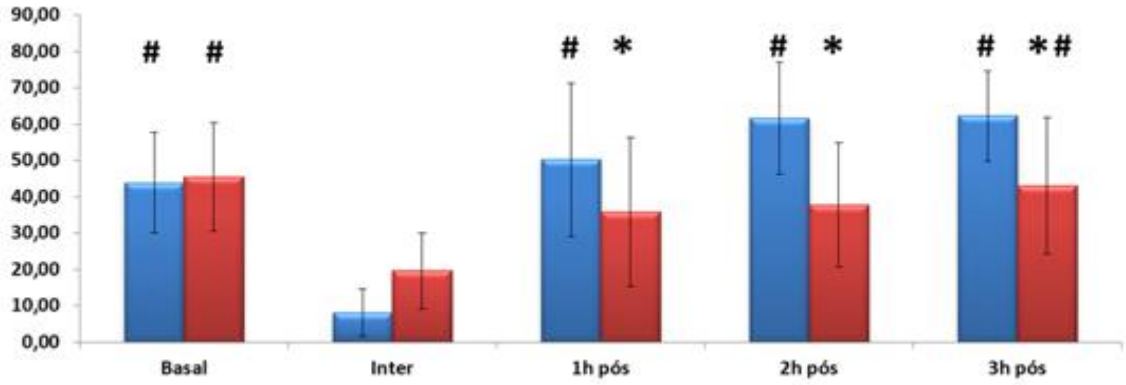
In Figure 2 it is possible to visualize the measured values of the mean respiratory rate, given in ventilatory inspirations per minute (bpm) in each step. There was a statistical difference between the MVC intervention step and all steps of the MVC protocol (p<0.001), as well as in relation to the EVT group in the intervention step (p=0.002). It is possible to verify that the controlled breathing maneuver was correctly performed, since the MVC intervention step has a mean close to 10 bpm, as adjusted in the protocol. The respiratory rate showed no significant change in the intervention with EVT in any of the steps.

Figure 2: Mean values of respiratory rate, in breaths per minute (bpm), in the different stages of the protocol, MVC group (blue) vs EVT group (red). * different from MVC compared to the same stage; # different from the intervention stage in the same protocol.



In Figure 3, the results of the quantification of power in the LF (Low Frequency) band, relative to sympathetic modulation, are presented in normalized units. There was significant difference regarding the protocol with MVC or EVT and the steps of each protocol in relation to the intervention ($p<0.001$ for MVC and=0.009 for EVT), with interaction between factors ($p=0.003$).

Figure 3: Normalized power values in the LF (Low frequency) range of the heart rate variability spectrum, extracted by spectral analysis of the tachogram, relative to cardiac sympathetic modulation at the different protocol stages, MVC group (blue) vs EVT group (red). * different from MVC compared to the same stage; # different from the intervention stage in the same protocol.



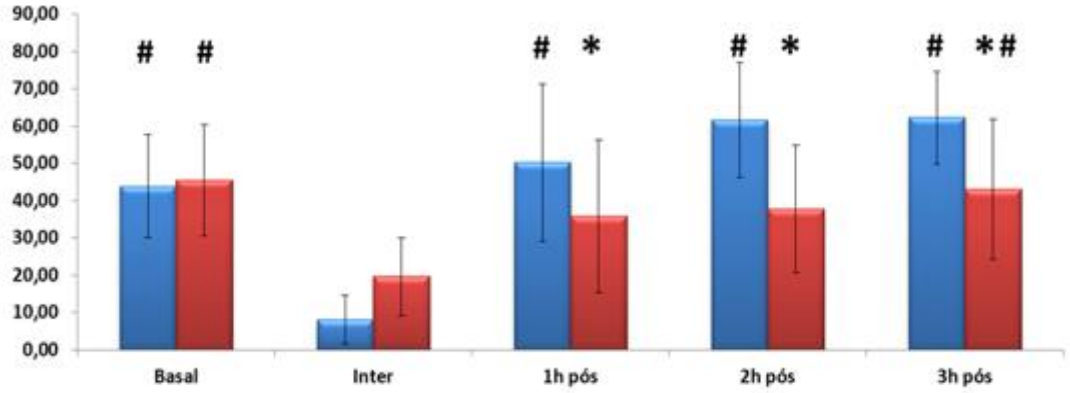
As expected, sympathetic modulation decreases significantly in the intervention phase in both protocols. It is noted, however, that sympathetic modulation rises again in the following steps only in the MVC protocol, which now presents a higher sympathetic modulation than the same step in the EVT protocol. In the steps of the EVT protocol after the intervention, a moderate increase in sympathetic activity is observed, only 3 hours after the intervention.

It is possible to observe that there was a significant difference in the stages related to 1 hour, 2 hours and 3 hours after the intervention, with $p=0.033$, $p=0.001$ and $p=0.006$, respectively. Taking into account that after the intervention sessions the participants continued with their routines, with classes and activities, an increase in sympathetic modulation would indeed be expected, due to the normal

circadian alteration. However, EVT was able to maintain, in the following steps, the sympathetic modulation below the level it was at at CVM. Thus, it is notable that the protocols showed different parameter results at the times after the intervention, even though they both decreased sympathetic modulation at the time of the stimulus.

Figure 4 shows the powers in the HF range (high frequency) that concerns cardiac vagal modulation, the parasympathetic. In this parameter, there was a significant difference in relation to the protocol with MVC or EVT (p=0.038) and the steps of each protocol in relation to the intervention, with interaction between the factors (p=0.002).

Figure 4: Normalized power values in the LF (Low frequency) range of the heart rate variability spectrum extracted by spectral analysis of the tachogram, relative to cardiac vagal modulation, at different times of the protocol, MVC group (blue) vs EVT group (red). * different from MVC compared to the same stage; # different from the intervention stage in the same protocol.



From these results, it was found that vagal modulation increases significantly during both interventions, being significantly higher in MVC than in EVT. Furthermore, it is observed that vagal modulation decreases 1 hour after MVC, but only 3 hours after the intervention. In the later stages, vagal activity is considerably higher in response to the protocol with transcutaneous vagal stimulation. It is observed, therefore, that the effect that the controlled ventilation intervention had on vagal modulation was more applied at the time of intervention, decreasing considerably in the following stages. This behavior is not observed in response to the vagal stimulation intervention, which despite producing a smaller effect at the time of intervention, maintained the levels in the following steps above the MVC protocol.

According to the results presented for heart rate variability, it is possible to state that the interventions of controlled ventilation and transcutaneous vagal stimulation were able to modulate autonomic control in healthy UNIFESP students, inducing an increase in vagal modulation and reducing sympathetic modulation at the time of stimulus. These results agree with the work of Clancy et al. (2014) and Oneda et al. (2010), who also observed, respectively, that transcutaneous vagal stimulation and controlled ventilation reduce sympathetic activity.

The intervention with controlled ventilation showed superiority in vagal activation at the moment of stimulus, being a potential technique for autonomic control in a short period of time. Transcutaneous vagal stimulation, however, had an advantage in the hours following the intervention, maintaining vagal activity higher than baseline.

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5 CONCLUDING REMARKS

According to the data presented, it is possible to conclude that the interventions of controlled ventilation assisted by a mobile application and transcutaneous vagal stimulation through a commercial device were efficient in modulating autonomic control in young college students. Controlled ventilation proved to be a potential solution for instantaneous vagal modulation, whereas transcutaneous vagal stimulation showed superiority in maintaining modulation for a long period of time.

The MVC intervention showed greater vagal activation during the maneuver, being a potential technique for vagal activation acutely. EVT, however, showed advantageous beneficial effects during the post-intervention period, maintaining vagal modulation higher than baseline for hours after the session.

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