STUDY PROTOCOL

Effect of Transcranial Direct Current Stimulation associated with aerobic exercise on the autonomic modulation of hemiparetic individuals due to stroke: a study protocol for a double-blind randomized controlled trial [version 1; peer review: 1 approved with reservations, 1 not approved]

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Abstract

Background: Individuals after a stroke have an imbalance in the autonomic nervous system, which increases the risk of death or recurrent episodes of stroke. Transcranial Direct Current Stimulation (tDCS) combined with aerobic exercise has shown an effect on the modulation of this system.

Objective: The Heart Rate Variability (HRV) and the distance traveled on the exercise bike will be assessed to verify the additional impact of tDCS combined with aerobic exercise on individuals with chronic stroke sequelae.

Methods: The 34 adult individuals with diagnoses of chronic stroke will be randomized into two aerobic exercise intervention groups: G1 (with active tDCS) and G2 (with sham tDCS), three times a week, for 12 weeks.

Procedures: tDCS will be implemented during the aerobic exercise with the anodal electrode positioned over the lateral dorsal prefrontal left cortex, and the cathodal electrode over the contralateral supraorbital region, with 2mA, for 20 minutes. Assessments will be
carried out pre, immediately after the intervention, and on the 12th, 24th, 36th interventions, and 30 days later. The HRV data that are pulse interval (PI), square root of the mean of the squares of the differences between adjacent normal RR intervals (rMSSD), absolute high frequency (HF), absolute low frequency (LF), high and low frequency ratio (LF / HF) will be collected using a cardio frequency meter. The analysis of the distance traveled on the exercise bike before and after interventions will be analyzed in meters.

**Discussion:** The autonomic control via brain networks after a stroke can be altered and can promote an increase in sympathetic tone, and a higher risk of sudden death or relapse of stroke. It is crucial to demonstrate the effectiveness of available treatments to improve the autonomic function.

**Trial registration:** The study is registered as a [BRAZILIAN CLINICAL TEST RECORD (ReBEC): U1111-1222-4588](https://www.aneacs.org.br/Platano/impacto-treinamento/) on the 2018/10/16

**Keywords**
Stroke, Autonomic Modulation, Transcranial Direct Current Stimulation, Physical Exercise, Hemiplegia

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**Competing interests:** No competing interests were disclosed.

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Abbreviations

tDCS: transcranial direct current stimulation
F3: Left dorsolateral prefrontal cortex
HRV: heart rate variability
DLPFC: dorsolateral prefrontal cortex
DT: linear time-domain
FD: frequency-domain
IP: Pulse interval
rMSSD: square root of the mean of the squares of the differences between adjacent normal RR intervals
HF: Absolute high frequency
LF: Absolute low frequency
LF / HF: low frequency and high-frequency ratio
ACE: Addenbrooke Cognitive Exam questionnaire
SSQOL: stroke specific quality of life questionnaire
BDI: beck depression inventory
MMSE: mini mental state examination quiz

Introduction

The contribution of complications to the mortality of patients with stroke is variable between 12.5% to 22.7%.

Individuals with stroke have depressed parasympathetic activity mainly in the acute phase, exacerbated cardiovascular responses with increased sympathetic activity, unbalanced heart rate and blood pressure, decreased Heart Rate Variability (HRV), arrhythmias, and a higher risk of sudden death.

Dorrance & Fink (2015) observed that post-stroke Autonomic Nervous System (ANS) dysfunction increases the circulation of catecholamine levels in the heart, and enhances post-stroke patients’ morbidity and mortality. However, it is not yet clear whether this dysfunction is an effect of increased sympathetic nervous system (SNS) activity, reduced parasympathetic nervous system (PNS) activity, or a change in the balance of the two.

Thus, a great concern about the impact of physical fitness on stroke survivors to prevent cardiovascular risks has been observed in recent times, which shows the importance of physical exercise. It is notorious that physical exercise can prevent and mitigate heart problems. However, it requires prudence, since physical activity itself can increase cardiac output and increase blood pressure in these patients, even with the use of medications.

Therefore, supportive therapies that can improve the rehabilitation process are relevant, such as Transcranial Direct Current Stimulation (tDCS). Transcranial direct current stimulation associated with aerobic exercise has shown significant effects on autonomic modulation in athletes and healthy individuals, as noted by some researchers. Heinz et al. (2019) observed that the application of tDCS in individuals with stroke sequelae tends to modulate parasympathetic action. However, the effectiveness of tDCS was not noticed by Nguyen et al. (2015) in this same population.

Rossi et al. (2016) showed in a systematic review that tDCS performance is a therapeutic option in autonomic modulation, allowing instantaneous (‘online’) and lasting (‘offline’) modulation in cortical excitability. However, the tDCS protocol to obtain the better performance of the autonomic balance of individuals affected by stroke remains unclear.

The proposed study

- To investigate the effect of adding tDCS to aerobic training in the autonomic modulation of chronic stroke survivors immediately after the first therapy, after the 12th, 24th, and 36th interventions, and 30 days after the end of interventions.
- To evaluate the additional effect of tDCS on aerobic training in the distance covered (meters), quality of life, and cognition after the 12th, 24th, and 36th interventions and 30 days after the end of the interventions.

Hypotheses

This study assumes that the application of tDCS over the left dorsal prefrontal cortex (DPFC) will stimulate this area and, therefore, increase the effects of aerobic training on the autonomic modulation of individuals with stroke sequelae. This effect may occur because the left DPFC, when activated, has the function of inhibiting the sympathetic excitatory circuit of the amygdala, helping with autonomic regulation.

The hypothesis is that after a single stimulation session, it will be possible to verify some effect on the ANS, as observed by Heinz et al. (2019), Okano et al. (2015), Petrocchi et al. (2017). However, these may be even more significant after a 3-month aerobic training, a time described in the literature to obtain effects on cardiovascular conditioning. Another hypothesis is that these results will remain one month after the end of the training.

It is possible to observe effects on cognition since exercise can improve it and because the area that will be stimulated with tDCS is also responsible for behavior modulation, planning, temporal sequencing, language, and memory.

Through the possible beneficial effects of exercise for the body, the improvement in the quality of life can be a consequence.

Methods

Study design

This is a protocol for a double-blind study (evaluator and participants), controlled by sham and randomized that will follow the recommendations of the Consolidated Standards of Reporting Trials (CONSORT) (Figure 1) and the recommendations of the standard protocol items for clinical trials (SPIRIT).
(Reporting guidelines). The study was approved by the ethics committee of Universidade Nove de Julho, São Paulo, Brazil (CAAE: 97475718.5.0000.5511) - and registered in the Brazilian Registry of Clinical Trials (ReBEC) (U1111 -1222-4588). Participants will be informed about the research, procedures, risks, and benefits. If they agree, they will sign an informed consent form (Extended data: Appendix 1).

**Sample recruitment and selection**

There will be enrolled 34 participants of both sexes at the physiotherapy clinics at Nove de Julho University, in São Paulo.

**Inclusion and exclusion criteria**

The inclusion criteria are as follows: individuals of both sexes, aged between 21 and 74 years old, minimum of six months of stroke injury, medical authorization to participate in the study, with the functional capacity of lower limbs that allow them to pedal the exercise bike, even if with the help of the therapist. The participants who usually ingest beta-blockers will not be excluded, but after the end of the research, an analysis will be carried out to compare the HRV of the individuals who use the medication with those who do not use them. Exclusion criteria include individuals with cognitive...
impairment (≤17) assessed by the mini-mental status exam (MMSE), severe heart problems, use of a pacemaker, and/or contraindications to the use of tDCS.

Withdrawal and discontinuation
Participants can freely withdraw from assessment and therapy at any time. Criteria for the termination in the study include participants who were absent more than a week away from the start date; who became ill or acquire any injury making it impossible to perform physical activity.

Sample size
The sample size was calculated utilizing a pilot study with 8 individuals (4 for the active tDCS group and 4 for the tDCS sham group) and, with the sample power considering the rMSSD outcome variable by linear time-domain (DT) methods, assuming α of 0.05 and β of 0.80. Using the sample calculation tool on the website: calculoamostral.bauru.usp.br, the difference between two means with independent groups (t-test) was calculated. A total N of 15 individuals, considering possible losses, 10% was added totaling N of 17 individuals for each group and, therefore, 34 individuals were recruited, with an effect size of d = 0.000077 by Cohen (Figure 2).

Randomization
The allocation of individuals to Group 1 (active tDCS combined with aerobic exercise on the stationary bike) and Group 2 (tDCS sham combined with aerobic exercise on the stationary bike) was calculated utilizing a pilot study with 8 individuals (4 for the active tDCS group and 4 for the tDCS sham group) and, with the sample power considering the rMSSD outcome variable by linear time-domain (DT) methods, assuming α of 0.05 and β of 0.80. Using the sample calculation tool on the website: calculoamostral.bauru.usp.br, the difference between two means with independent groups (t-test) was calculated. A total N of 15 individuals, considering possible losses, 10% was added totaling N of 17 individuals for each group and, therefore, 34 individuals were recruited, with an effect size of d = 0.000077 by Cohen (Figure 2).

**Figure 2.** Representation of the sample calculation by the site: calculoamostral.bauru.usp.br from Test t: by the difference between the two averages with independent groups, with α of 0.05 and a β of 0.80 between 2 groups in 4 periods and an effect size by Cohen’s d.
bike) will take place using the website www.randomized.com by a researcher not involved in the evaluation and intervention.

**Study assessments schedule**
A study evaluation schedule with standard protocol items is provided in Table 1.

**Intervention**
The assessments and interventions will be carried out in the morning, always at the same time, to minimize the effects of the circadian cycle. The recommendations will be to continue to use the medications in their regular schedule, to have a light diet on the test days, to abstain from caffeine or alcoholic beverages, and smoking, and to avoid moderate or excessive efforts on the day before the test day.

**Transcranial direct current stimulation.** The therapy of tDCS DC-Stimulator Plus (NeuroConn) (active or sham) will be combined with aerobic exercise on the stationary bike. The anode electrode will be placed over the left dorsolateral prefrontal cortex (F3), and the return electrode (cathode) will be placed over the contralateral supra-orbital region, defined by the 10/20 electroencephalogram system. The intensity of current will be 2mA, applied for 20 minutes, 10-second linear ramp up / down.

The electrodes used will be of conductive rubber, anode 5x5 cm, and cathode 5x7 cm, wrapped in cellulose sponge moistened in 0.9% saline solution.

For sham stimulation, all electrode placement procedures will be performed equally with the active tDCS. Still, the stimulator will only be on for 30 seconds, considered a valid method for a control in tDCS studies.

**Blinding**
The NeuroConn DC-STIMULATOR PLUS device has settings that allow the selection of the active or sham stimulation mode by inserting codes. A researcher not involved in the procedures will allocate the participant. The external functioning

| Table 1. Standard protocol items: recommendations for interventional trials of this study. |
|----------------------------------|-------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **TIME POINT**                   | **Enrollment (T1)** | **Allocation (T0)** | **Pre-intervention (T1)** | **Intervention** | **Post-intervention** |
|                                  |                   |                  |                 |                 |                 |                 |                 |                 |
|                                  |                   |                  |                 | T2              | T3              | T4              | T5              | T6              | T7              |
| ENROLLMENT:                      |                   |                  |                 |                 |                 |                 |                 |                 |
| Eligibility screen              | X                 |                  |                 |                 |                 |                 |                 |                 |
| Informed consent                | X                 |                  |                 |                 |                 |                 |                 |                 |
| Demographic information         | X                 |                  |                 |                 |                 |                 |                 |                 |
| Allocation                       | X                 |                  |                 |                 |                 |                 |                 |                 |
| INTERVENTION:                   |                   |                  |                 |                 |                 |                 |                 |                 |
| active (anodal) tDCS associate aerobic training | | | | | | | | |
| Sham tDCS associate aerobic training | | | | | | | | |
| ASSESSMENTS:                    |                   |                  |                 |                 |                 |                 |                 |                 |
| Neurological and cardiovascular assessments | | | | | | | | |
| Primary outcome: HRV            | X                 |                  |                 |                 |                 |                 |                 |                 |
| SECONDARY OUTCOMES:             |                   |                  |                 |                 |                 |                 |                 |                 |
| Cognition assessments           | X                 |                  |                 |                 |                 |                 |                 |                 |
| Depression assessments          | X                 |                  |                 |                 |                 |                 |                 |                 |
| Quality of life assessments     | X                 |                  |                 |                 |                 |                 |                 |                 |
| Type of stimulation, real or sham tDCS and adverse effects | | | | | | | | |

Note: Heart Rate Variability (HRV); T1: one week before intervention; T0: one day before intervention; T1: 20 minutes before intervention; T2: 1st-day session; T3: 12th-day session; T4: 24th-day session; T5: 36th-day session; T6: immediately after the session; T7: 30th-day follow-up after the last training session.
of the device will not perceive the stimulus mode. Therefore, neither the researcher who will apply the intervention nor the individual will know what treatment will be used (double-blind).

After using the tDCS is carried out, participants and researchers will be asked to complete questionnaires about blinding (Extended data: Appendix 2⁴⁷), adverse effects (Extended data: Appendix 3⁴⁸), and satisfaction of therapy (Extended data: Appendix 4⁴⁹).

**Aerobic activity**
The participant will perform the aerobic activity on a Reebok® RT 445 model N° RBEX49021 exercise bike, 30 minutes a day, with the initial 5 minutes of warm-up, 20 minutes of aerobic activity associated with active tDCS or sham, and the final 5 minutes of cooling.

The treatment will be carried out three times a week, for 12 weeks, totaling 36 sessions⁵¹. The initial intensity will be 50% of the reserve heart rate, as tolerated, and will be rising until 5% of the reserve heart rate each week⁵². The target aerobic intensity will be 50% to 70% of the reserve heart rate.

The following formula [% reserve HR = [(maximum HR - resting HR) x%] + resting HR] will be used to obtain the reserve heart rate (HR). If one of the individuals uses β-blockers, the maximum corrected HR should be calculated using the following formula [(the dosage taken with the drug + 95.58) / 9.74 =% that should be removed from the maximum HR]. The maximum heart rate (HRmax) will be estimated using the Karvonen formula⁵³.

The HR and oxygen saturation (SpO₂) will be monitored as a protective measure by a portable pulse oximeter UT-100 Polar V800 frequency meter every two minutes of exercise; as well as blood pressure (BP) and the perception of dyspnea and fatigue of the lower limbs by the modified Borg questionnaire⁵⁴.

**Assessments**
The evaluations will be carried out before, after the 12th, 24th, and 36th interventions and 30 days after the end of the interventions. Personal data about the individual and the disease will be collected (Extended data: Appendix 5⁵⁵). The other data will be:

**Evaluation of heart rate variability (HRV).** The CardioSeries software (http://www.danielpenteado.com/cardioseries) will be used to identify correct premature ectopic beats, and undesirable transients will be removed using linear interpolation that alters the signal stationarity. The variances of the pulse interval (IP) will be evaluated in the domain of time and frequency by the linear method.

HRV will be measured using the Polar® V800 heart rate monitor device. The evaluation of cardiac modulation will be performed by recording the RR interval, processed using the Flow software (https://flow.polar.com), calculating the transducer indices of cardiac cycle fluctuation, high-frequency waves (0.15 and 0.4 Hz), low frequency (0.04 to 0.15 Hz), and the interrelation between low frequency and high frequency (0.15 and 0.4 Hz). The raw, unfiltered data will be exported, converted, and stored in an Excel file, used later for the domain of time and frequency.

The indexes obtained by analyzing the RR intervals in the time domain will be the average of the RR pulse interval utilizing absolute variance and the square root of the squared mean of the difference between the normal adjacent RR intervals (rMSSD), expressed in ms². As for the frequency domain, the data will be analyzed through the analysis of absolute high frequency (AF), low absolute frequency (BF), and the vagal sympathetic balance between low frequency and high frequency (BF / AF).

**Traveled distance.** The distance covered will be measured at the end of the 30 minutes of aerobic exercise performed by the participant on the exercise bike.

The results of the distance covered will be compared intragroup (active and sham tDCS), for each moment pre, post 12th, 24th, 36th interventions, and 30 days after the end of the interventions to verify the evolution in both.

**Cognitive Performance.** The cognitive performance of chronic stroke patients will be assessed using the Addenbrooke Cognitive Exam (ACE)⁵⁵ questionnaire. The evaluator will apply the questionnaire in three moments:

The first moment will be before randomization for the intervention groups, the second will be after the 36th intervention, and the third moment will be 30 days after the end of the training.

**Quality of life assessment.** Quality of life will be measured by the Stroke Specific Quality of Life questionnaire (SSQOL)⁵⁶.

**Determination of potential confounding factors**

**Depressive symptoms.** The Beck Depression Inventory (BDI)⁵⁷ will be used to assess the depressive symptoms. The results will be correlated with the performance of the physical activity⁵⁸.

**Statistical analysis.** The program SPSS Statistic version 17.0 will be used for statistical analysis.

For measures of central tendency and dispersion will be used descriptive statistical analysis. To measure the parametric variables will be used the mean and standard deviation, to measure the non-parametric variables will be used the median and the interquartile range, and to measure the categorical variables will be used the frequency and percentage.

HRV data (linear methods) will be analyzed in the time domain with the variable rMSSD and in the frequency domain with absolute and normalized high frequency. The data will be submitted to the Shapiro-Wilk normality test, using the unpaired t-test for parametric data and Mann-Whitney for non-parametric data, considering the significance level p≤0.05 for all conditions.
Discussion
This article provides a detailed description of a prospective, randomized, controlled, double-blind clinical trial designed to demonstrate the effects of combining tDCS and aerobic training with an exercise bike on the autonomic modulation of individuals with hemiparesis due to chronic stroke.

We will publish the results, and the evidence found can contribute to the cardiovascular rehabilitation process of this population. In this sense, if positive, it allows a better prognosis in the cardiovascular rehabilitation of these individuals and reduces the likelihood of a stroke with more severe recurrence.

Evaluation status
So far, participants have been enrolled, and the allocation is being made from the perspective of completion of collections in June 2021.

Dissemination of results
The results will be communicated to the public through publication as a data set and original research in the relevant scientific journals.

Study limitations
We consider some topics as possible limitations of our study:
- The possible difficulty in recruiting patients due to their limited mobility;
- A possible analysis difficulty due to a decrease in vagal withdrawal and use of B-blockers;
- Absence of control over modifiable risk factors.
- Difficulty getting complementary examinations for an accurate diagnosis regarding the location and extent of the lesion.

Data availability

Underlying data
No underlying data are associated with this article

Extended data
Harvard Dataverse: Effect of Transcranial Direct Current Stimulation associated with aerobic exercise on the autonomic modulation of hemiparetic individuals due to stroke: a study protocol for a double-blind randomized controlled trial, https://doi.org/10.7910/DVN/MUNWDB

This project contains the following extended data:
- Appendix 1: Clarified Free Consent Term
- Appendix 2: Blinding Questionnaire tDCS Researcher
- Appendix 3: Adverse effect tDCS
- Appendix 4: Evaluation of treatment satisfaction
- Appendix 5: Personal data about the individual
- Register approved by the ethics committee
- Registered in the Brazilian Registry of Clinical Trial (ReBEC)

Reporting guidelines

Data are available under the terms of the Creative Commons Zero “No rights reserved” data waiver (CC0 1.0 Public domain dedication).

Acknowledgments
The authors thank the Brazilian fostering agency Coordination for the Improvement of Higher Education Personnel (CAPES) for granting a scholarship to the first author and the University Nove de Julho. Funding provided by CAPES.

References
Brenton Hordacre
Innovation, IMPLementation And Clinical Translation in Health (IIMPACT in Health), Allied Health and Human Performance Academic Unit, University of South Australia, Adelaide, Australia

Thank you for the opportunity to review this study. It is an interesting concept and I have raised a few points the authors should consider below.

1. In general, this manuscript will need language editing. There are sections which do not make sense and paragraphs that are not well formulated. For example, the first two paragraphs of the introduction are 1 sentence long. These could be combined to a single paragraph. Equally, it is highly unusual to see dot points within an academic manuscript. I'd suggest they are removed from the introduction and discussion.

2. The introduction lacks the relevant background information. As a reader, I am unaware why tDCS or aerobic exercise as therapies delivered over several sessions can modulate the autonomic nervous system. I suggest the authors expand the introduction, possibly utilising references 13-15, rather than just saying the evidence has been noted by some researchers.

3. I am unclear what the primary and secondary aims of the study are. Is the first dot point the primary aim?

4. The hypotheses do not align with the aims. Why is there a hypothesis that you are stimulating the left DLPFC? Similarly, why is there a hypothesis that it is possible to observe effects on cognition – the hypothesis should be directional. Eg. we hypothesise that anodal TDCS to the left DLPFC combined with aerobic exercise will increase cognitive function compared to sham tDCS and aerobic exercise.

5. I am unclear why the consort diagram is provided if no data is collected. Interestingly, it appears 34 participants have already been randomised.

6. Why is there an upper age limit of 74 years for inclusion criteria?

7. The cohen's D value for the power calculation is extremely low – raising concerns. Please
have a biostatistician review. In fact, I cannot replicate the power calculation. I have had a biostatistician perform the calculation and they determined 56 participants are required based on the data provided.

8. Who is performing the outcome measures?

9. What is the primary outcome measure(s) and why. What is the reliability(validity and sensitivity of these measures?

10. The statistical analysis section is inadequate. If there are multiple outcome measurement times and two groups, a repeated measures anova or linear mixed model is appropriate. T-tests are going to increase your error.

11. The discussion is inadequate. What gap in the literate is this study likely to address and what is the clinical value of running this study. What potential implications could it have for the field. How do you see this study informing clinical practise of scientific knowledge?

Is the rationale for, and objectives of, the study clearly described?
No

Is the study design appropriate for the research question?
Partly

Are sufficient details of the methods provided to allow replication by others?
Partly

Are the datasets clearly presented in a useable and accessible format?
Not applicable

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Stroke recovery, neuroplasticity, brain stim

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 07 April 2021

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The present study has aimed to investigate the effect of transcranial direct current (tDCS) stimulation associated with aerobic exercise on the autonomic modulation of hemiparetic individuals due to stroke. It is a double-blind randomized controlled trial. The control group will receive sham tDCS. Both groups of patients will do aerobic exercise on an exercise bike for 30 min a day, 3 days a week for 12 weeks. Autonomic modulation will be assessed using heart rate variability (HRV).

I find the study protocol interesting and noble. It has both research and clinical significance. The title of the study protocol is well written, clearly summarizing the main objective of the study. Similarly, the rationale and some portions of the methods of the study are well written/explained. However, I have some comments that need to be discussed for the further improvement of the study.

Comments

**Related to the Objectives of the study:**

It would be better to **modify the objectives of the study** (page 3) for better clarity. It can be modified as:

1. To investigate the effect of transcranial direct current (tDCS) stimulation associated with aerobic exercise on the autonomic modulation of chronic stroke survivors immediately after the 1st, 12th, 24th, and 36th interventions, and 30 days after the end of the intervention.

2. To evaluate the effect of tDCS on the distance covered during aerobic training, quality of life, and cognition after the 12th, 24th, and 36th interventions and 30 days after the end of the intervention.

**Related to the methods of the study:**

1. In the inclusion and exclusion criteria of the study, nothing is mentioned about patients with chronic stroke along with other complications like diabetes mellitus, chronic kidney disease, Parkinsonism, and some other complications (page 4, last para & page 5, first para).

2. If patients with chronic stroke along with other complications (like diabetes mellitus, chronic kidney disease, Parkinsonism, and some other complications) are enrolled in the study, it needs to be noted down because these complications decrease HRV. If possible patients with these complications can be excluded from the study. Otherwise, a similar number of such patients enrolled in both the groups may nullify the effects.

3. For the intra-group comparisons of related data, it would be better to use repeated measure ANOVA for the parametric data and Friedman test for the non-parametric data (under statistical analysis page 7).

4. It is mentioned that the results of the depressive symptoms will be correlated with the performance of the physical activity (page 7). However, no statistical tool has been discussed under the statistical analysis. Please mention it.
5. Whether participants will perform aerobic exercise 3 times/days a week successively or on an alternate day (page 7, para 4). It would be better to mention it.

6. Please mention how you will measure distance traveled (page 7).

7. When will the quality of life and depressive symptoms be assessed? Not mentioned clearly in the protocol. It would be better to mention the time point for the assessment. Please check for the assessment of the other parameters also and improve them.

8. Evaluation of heart rate variability is written well. However, it needs some minor modifications. It is written that “rMSSD, expressed in ms\(^2\)” (page 7). It needs to be corrected as “rMSSD is expressed in ms.” It would be better to use the standard abbreviations for the absolute high-frequency and absolute low-frequency powers and their ratios (page 7). Absolute powers are expressed in ms\(^2\). It can be mentioned.

9. It would be better to add two more time-domain parameters of HRV; SDNN (standard deviation of R-R intervals) and pNN50 (percentage of the number of pairs of adjacent R-R intervals differing by more than 50 ms). Adding these parameters may strengthen the results.

**Is the rationale for, and objectives of, the study clearly described?**
Yes

**Is the study design appropriate for the research question?**
Yes

**Are sufficient details of the methods provided to allow replication by others?**
Yes

**Are the datasets clearly presented in a useable and accessible format?**
Not applicable

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Assessment of cardiovascular autonomic function, heart rate variability, Blood pressure variability, baroreflex sensitivity, exercise physiology, yoga, electroencephalography

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.
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