Manuscript ijerph-1702329 **"**Chronotropic response and heart rate variability before and after a 160m walking test in young, middle-aged, frail, and non-frail older adults**"**

Response to Reviewer 3

Please find the attachment

This paper presents the chronotropic response (Heart rate and Heart rate variability) to a walking test.
The author used a clinical frailty scale to distinguish between frail and non-frail older adults. They transformed the time domain HRV signals into the frequency domain and used some well-known features. This study is interesting however, there is some significant observations that author must address the followings:

Comment: Author must need to provide a valid clinical aspect of using 160 meter walking test.

Response: Thank you for your comments, which helped us to improve our manuscript.

We added the information in lines 146-149.

Comment: Is it clinically valid to measure frailty for older adults?

Response: There are different strategies to quantify frailty. Three strategies standout: i) clinical scales or indexes that integrate signs and symptoms about physical, psychological, emotional, social functionality and the clinical history of the patient, ii) deviation of genetic, cellular or biochemical biomarkers from their reference values and iii) dynamical markers of resilience such as the recovery time after major health events such as falls or stroke (Varadhan et al., 2009). Nowadays, the most widely used are the first ones. As the first 'formal' description of frailty was a physical phenotype which was quantified by 5 clinical aspects of the older adults (slow performance, weight loss, exhaustion, physical activity and strength) (Fried et al., 2001). Clinical scales have the advantages of being non-invasive, low cost and practical to be implemented in a clinical consultation. It is worth mentioned that there are several clinical scales and indexes that has been proposed. The scales used in this manuscript (FRAIL scale and clinical frailty scale) are widely used and are validated (Lee et al., 2005; López et al., 2012; Rockwood et al., 2005).

Comment: This study has used only 80 Mexican participants. Only 80 participants were insufficient to justify the research and use of machine learning in this small sample size. The author must need to be used more samples or provide some valid reasons.

Response: We agree with the impact of our small sample on the statistical power of the study, as is mentioned in lines 548-549.

Thank for your comment. Machine learning application to heart rate variability analysis has been gaining interest as way to improve classification and prediction in diverse circumstances and

pathologies (Ishaque et al., 2021) including frailty (Eskandari-Nojehdehi et al 2022; Ruberto et al., 2022). Although we measure HRV indices as potential "parameters" or "features" that may be useful to classify frail and non-frail patients, our approach in this first exploratory study was to apply traditional statistical tests to identify HRV indices that had different median values when comparing frail and non-frail patients.

Then we explored the potential value for classification of frail and non-frail by calculating sensitivity and specificity using the basic ROC curve analysis. We selected a candidate for cut-off value" by the simplest method: calculating the minimum orthogonal distance between the optimum point of the ROC curve (0,1) and each point within the ROC curve. This simple approach was enough to show the potential value of HRV analysis during a 160m walking test to identify frail patients based on a physiological measurement (the cardiac response to the test). These results, based on a small sample of participants are enough to warrant further studies in larger populations where machine learning methods would improve the classification of frail and non-frail patients.

This is now mentioned in the revised lines (529-541).

Comment: ECG recording and bit detection algorithm must be elaborated so that readers can understand the algorithm.

Response: The ECG signal was recorded with the Wearable Physiological Monitoring device Bioharness 3.0, which has been used in other scientific papers [Sbrollini et al., 2019] and has been validated [Nazari et al., 2018]. All HRV calculations were performed with a reliable and validated software specialized in HRV analysis [Tarvainen, et al., 2002; Niskanen et al., 2004; Tarvainen, et al., 2014; Lipponen Tarvainen, 2019, https://www.kubios.com/downloads/Kubios_HRV_Users_Guide.pdf].Kubios software uses an automatic algorithm to detect beats, which is based on Pan-Tompkins algorithm [Pan & Tompkins 1985]. Firs, the algorithm filters the ECG signal. Then squares it to increase the amplitude of the R waves and then applies a moving average filtering. After that, a decision rules phase begins with an amplitude threshold and comparing the adjacent R-waves with the expected values. This phase is adaptative when a R-wave is detected.

This is now mentioned in lines (162-174).

Comment: Similarly, HRV analysis must provide detail mathematical notations.

Response: The manuscript is citing the references where the HRV methods are described thoroughly: all time domain and frequency domain HRV methods are described in the guidelines for HRV analysis (TaskForce) and the nonlinear methods were described in the original papers cited in this work (Marple 1987; Tulppo et al. 1996; Richman & Moorman 2000; Lake et al., 2002; Peng et al., 1995). Considering that the mathematical notation and detailed definition of the methods is previously described, as well as the scope of this journal, we consider more adequate to avoid detailed mathematical notations.

Comment: There is no system architecture or study flow diagram. Adding these diagrams would be very beneficial to understanding the paper.

Response: We added a study flow diagram in Figure 1. Line 121

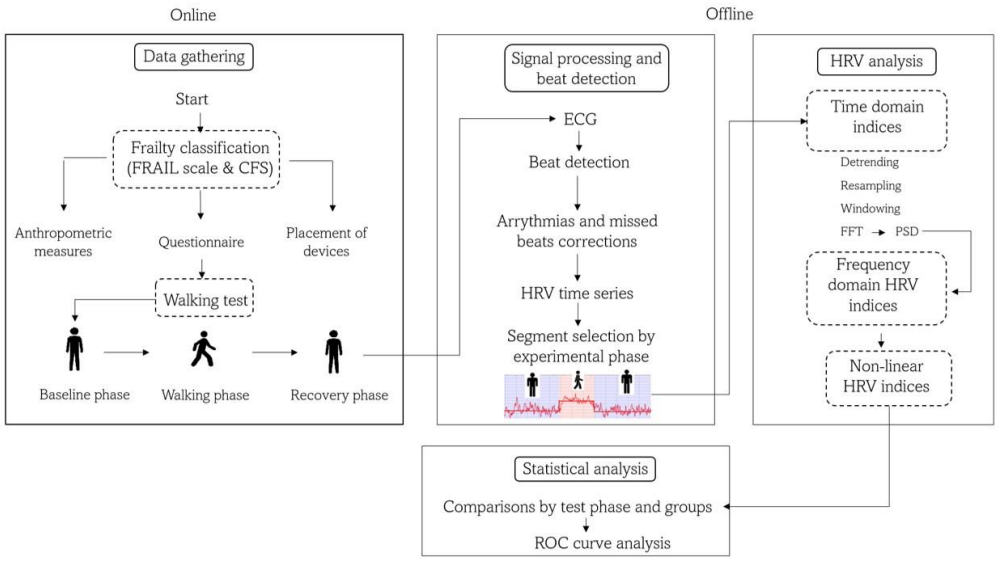


Figure 1. Flow diagram of the study protocol. FRAIL = fatigue, resistance, ambulation, illness and low performance scale; CFS = clinical frailty scale; HRV = heart rate variability; FFT = Fast Fourier Transform; PSD = power spectrum density; ROC = receiver operator characteristic.

Comment: Calculating an AUC value with a 95% competence interval on 80 samples may mislead the results, . Therefore author should provide a learning curve for this.

Response: The sample of patients allowed us to explore the value of the HRV indices to discriminate frail and non-frail older adults. However, the ROC curve estimation and the cut-off values we identified cannot be generalized to all frail and non-frail patients since a much larger sample is required. We applied the orthogonal distance method, which is simple but useful to avoid qualitative criteria in the selection of each cut-off value. Our exploratory results warrant future studies with considerably larger populations where the optimum cut-off values are defined by more robust methods, including those based on machine learning algorithms. This is now mentioned in the study limitations (lines 529-541).

Comment: There are several typos and grammatical mistakes, ing a 160-walking test. It must be 160m walking test etc.

Response: Thank you for your comment. We have carefully checked for typos and mistakes.

References:

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