ASSOCIATION BETWEEN HOSPITAL ANXIETY DEPRESSION SCALE AND AUTONOMIC RECOVERY FOLLOWING EXERCISE

HRV & Hospital Anxiety Depression Scale

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ABSTRACT

The hospital anxiety depression scale (HADS) is a benchmark used to investigate possible and probable cases of psychosomatic illness. Its affiliation with autonomic recovery after exercise is unclear and, as a technique applied to evaluate cardiovascular risk. We assessed a possible link between HADS and autonomic recovery after exercise. We studied healthy subjects split into two groups: Low HADS (n=20) and High HADS (n=21). Subjects consented to moderate aerobic exercise on a treadmill at 60% to 65% of the maximum heart rate (HR) for 30 minutes. We studied HR variability (HRV) before and during 30 minutes after exercise. Subjects with higher HADS values presented delayed recovery of HR and RMSSD (root-mean square of differences between adjacent normal RR intervals) after submaximal exercise. RMSSD during recovery from exercise had a significant association with HADS. In summary, subjects with higher HADS presented slower vagal recovery following exercise.

Keywords: Anxiety; Autonomic Nervous System; Depression; Cardiovascular System; Exercise.

INTRODUCTION

The hospital anxiety and depression scale (HADS) is a benchmark applied to measure the symptoms of depression and anxiety. This technique is permitted to evaluate possible and probable cases of depression and anxiety (Zigmond and Snaith, 1983). It includes 14 questions, which effect in a concluding value between 1 and 21. Scores below 7 indicate improbable anxiety or depression, whilst scores between 8 and 11 represent possible anxiety or depression, then scores between 12 and 21 correspond to feasible levels of anxiety or depression (Zigmond and Snaith, 1983). The HADS is an important technique for evaluating individuals with psychosomatic illness, including coronary heart disorders and estimates of cardiovascular mortality (Doyle et al. 2010).

Hence, anxiety and depression can cause a significant impact on the cardiovascular system. Two prospective national studies reported the connection between anxiety and coronary heart disorders (Janszky et al. 2010; Nabi et al. 2010). Janszky *et al* (Janszky et al. 2010) evaluated 49321 young males between 18 and 20 years old. These authors demonstrated that although depression was unable to be considered a significant major risk factor in males for cardiovascular outcomes sooner or later, anxiety was strongly correlated with cardiovascular events in later life. Nabi and co-workers (Nabi et al. 2010) presented a prospective cohort study with a 7-year continuation that examined four different age groups: 20 to 24 years, 30 to 34 years, 40 to 44 years, and 50 to 54 years. It was recognized that anxiety in females was strongly related to increased risk of coronary heart disease whilst in males only somatic symptoms were related to a high risk of coronary heart disease.

Recently, Fangauf *et al.* (Fangauf et al. 2019) provided further evidence on how HADS has been linked to cardiovascular disease states. The authors assessed a HADS score and blood B-type natriuretic peptide levels in 570 subjects with coronary heart disease. As an important finding, the data demonstrated that higher baseline levels of blood B-type natriuretic peptide

were associated with lower levels of HADS-anxiety scores. Similarly, the C-terminal pro-arginine vasopressin was related to HADS in 1463 subjects with cardiovascular risk factors (Sadlonova et al. 2019). The study stated that the serum concentrations of C-terminal pro-arginine vasopressin was inversely linked with self-assessed anxiety in a cohort of subjects presenting with cardiovascular risk factors.

In this manner, cardiovascular risk can be assessed through heart rate (HR) variability (HRV) (Koopman et al. 2015; Kleiger et al. 1987), a simple, inexpensive and non-invasive technique enforced to evaluate HR autonomic regulation. HRV quantifies fluctuations between inter-beat intervals (IBI) and offers information concerning the autonomic nervous systems influence on the sinus node. Abnormal HRV patterns are related to the dysfunction of the parasympathetic and sympathetic nervous systems (Camm et al. 1996; Vanderlei et al. 2009).

HRV has been related to cardiovascular morbidity (Raimundo et al. 2013) and mortality (Chen et al. 2016). Cardiovascular and metabolic morbidity were associated with decreased HRV, as well as with metabolic syndrome (Stuckey et al. 2014) and stroke (Raimundo et al. 2013). Increased mortality was related to decreased HRV (Chen et al. 2016). Furthermore, HRV changes were similarly associated with anxiety levels (Goessl et al. 2017) and throughout stressful situations (Valenti et al. 2012). Increases in HRV were anticipated to improve self-reported stress and their anxiety levels (Goessl et al. 2017).

In a different way, assessment of cardiovascular performance can be achieved by exploring cardiac autonomic recovery following a single bout of exercise (Imai et al. 1994; Watanabe et al. 2001). The classical study of Imai and co-workers evaluated sedentary male subjects between 28 and 39 years old submitted to a pharmacological blockade during exercise tests. Amongst their key results, it was revealed that HR recovery within 30 seconds is mediated through parasympathetic reactivation following exercise and thus vagal recovery is quicker in athletes compared to patients with chronic heart failure. Similarly, Watanabe *et al* (Watanabe

et al. 2001) surveyed 5438 patients younger than 30 years old that completed a symptom-limited exercise testing according to the Bruce, modified Bruce, or Cornell protocols. The investigation anticipated that delayed HR recovery after exercise was an independent and potential predictor of mortality.

In the opening stage of the Bruce exercise test protocol, the subject walks at 1.7 miles per hour and a 10% grade; in the second stage the subject proceeds at 2.5 mph and 12% grade and in the third and final stage the subject moves at 3.4 mph and 14% grade. Yet, in the modified Bruce protocol, the subject originally moves at 1.7 mph and 0% grade, after that 1.7 mph and 5% grade and in the final stage the subject moves at 1.7 miles per hour and a 10% grade. Then, in the Cornell protocol, the initial stage is based on 1.7 mph and 0% grade, the second stage is based on 1.7 mph and 5% grade and then subsequently 1.7 mph and 10% grade (Gibbons et al. 1997).

According to the American College of Cardiology/American Heart Association Task Force (Gibbons et al. 1997), ST-segment depression after less than six minutes of test was associated with a risk of 3.6 in women and 6.7 in men, fewer than five minutes of exercise was connected with a risk of 14.7 in men and 5.6 in women of cardiac ischemia. The exercise test protocols have as an advantage, a steady and gradual elevation in work rate, providing improved estimations of functional capacity (Myers and Buchanan 1991). Recently, Qiu *et al.* (Qiu et al. 2017) demonstrated that slower HR recovery following exercise is associated with higher risk of cardiovascular complications and mortality, underpinning the recommendation of evaluating this variable for risk assessment. Once more, the scientific research literature upholds the connection between abnormal autonomic recovery and increased cardiovascular risk.

Thus, exercise has been proven to provide a beneficial effect in animals (Rossi et al. 2011) and humans (Martinez-Tellez et al. 2019). Exercise treatments for anxiety or depression

have been considered a helpful intervention (Wegner et al. 2014). As an alternative, we should be cautious with exercise intensity so as to circumvent cardiovascular problems after exercise in this population. This is reinforced by a previous study conducted by Laukkanen (Laukkanen et al. 2014), which stated that abnormal cardiovascular recovery following exercise is related to risk of sudden cardiac death. Bearing this in mind, we highlighted the theory that subjects with HADS>12 (viable anxiety or depression) would display delayed autonomic recovery following exercise. Consequently, we considered a possible association between HADS and autonomic recovery after exercise.

METHODS

Ethical approval and informed consent

All methods were approved by the Research Ethics Committee in Research from Faculdade de Juazeiro do Norte (Number 2.244.700). The experimental protocols were performed in accordance with the 466/2012 resolution of the National Health Council of 12/12/2012. Informed consent was obtained from all participants and they signed a confidential consent letter.

Study design

This is a prospective, observational and analytical study performed on 66 students (18 to 23 years old) with similar anthropometric profiles selected from the School of Juazeiro do Norte, Juazeiro do Norte, CE, Brazil. All subjects were physically active consistent with the International Physical Activity Questionnaire (IPAQ) (Pardini et al. 2001). The subjects were recruited through social network advertisements. We excluded 16 subjects because they presented excessive artifacts during the IBI recording (> 5%) and increased resting HR (>100 bpm). Artifacts were considered as an IBI not naturally present in the HR recording (Kaufmann et al. 2011). We omitted subjects with resting HR >100 bpm since this is related to

physiological instability (Al Bannay et al. 2013). We undertook a stratification with the purpose of attaining equal numbers in each group and, the initial analysis provided a total of 50 subjects. After excluding subjects with excessive artifacts and resting HR > 100bpm, the final sample was comprised of a total of 41 subjects. The high HADS score (HHDAS) group was founded on 21 subjects (15 women) without a clinical history of anxiety or depression, and scored > 12 according to the HADS (Hinz et al. 2014). The group with low HADS values (LHADS) was comprised of 20 subjects (15 women) who scored < 7 according to the HADS (Figure 1).

Non-inclusion criteria

We omitted subjects that offered cardiovascular, respiratory, neurological, previous history of anxiety or depression, other disorders that prevented the subjects undertaking experimental protocols and pharmacological treatments that influenced cardiac autonomic regulation. Females between the 10^{th} and 15^{th} days and within the 20^{th} and 25^{th} days of their menstrual cycle were excluded so as to remove potential influences of their luteal and follicular phase, respectively (Bai et al. 2009). We excluded sedentary and insufficiently active subjects according to IPAQ (Pardini et al. 2001).

HRV analysis

In order to measure IBI we applied the portable RS800CX HR monitor with a sampling rate of 1 kHz. The IBI data records were transferred to the Polar Precision Performance program (v.3.0, Polar Electro, Finland). This transmitter is required to detect all heartbeats in the left ventricular muscle and the data registered then transfers the signal to the computer through a wireless connection. The software permits the visualization and extraction of IBI files in the ".txt" format.

In 1996, the European Society of Cardiology and the North American Society of Pacing and Electrophysiology published directives to standardize specific methodological procedures to perform HRV analysis (Camm et al. 1996). Thus, HRV analysis was achieved according to

directions from the Task Force (Camm et al. 1996). Previous studies provided precise details of HRV analysis (Gonzaga et al. 2017; Carvalho et al. 2014).

To evaluate the parasympathetic regulation of HR we considered RMSSD (root-mean square of differences between adjacent normal IBI in a time interval) in the time domain. We enforced the Kubios® HRV v. 2.0 software to compute these metrics (Tarvainen et al. 2008).

The SDNN (standard deviation of all normal IBI) index represents sympathetic and parasympathetic components of HRV (Camm et al. 1996); consequently, this is not an adequate index to provide reliable physiological interpretation, since we cannot be confident about which autonomic component (sympathetic or parasympathetic) we are concerned with. Also, the sympathetic component of HRV is not isolated by time and frequency domain analysis. In this way, we were unable to apply SDNN as we intended to concentrate on the vagal HR control.

Experimental protocols

Before the initiation of the experimental events, subjects were recognized according to age (years), mass (kg), height (m), systolic (mmHg) and diastolic arterial pressure (mmHg) and body mass index (BMI).

The protocol was completed at identical times of the day (between 13:00 and 17:00) to standardize circadian impact on HRV in a room with humidity amid 40% and 60% and temperature between 21°C and 25°C. The subjects were told to avoid consumption of caffeine or ingestion of other autonomic stimulants for 24 hours prior to the data collection and to retain an empty bladder during the entire protocol.

Initially, the HR receiver (Polar RS800CX, Finland) was located on the subjects' chest to register HR beat-to-beat signals. Then, the subjects remained initially at rest and seated for 15 minutes. Next, they performed a brisk walk on a treadmill at 60% to 65% of the estimated maximum HR (220 – age (in years)) (Camarda et al. 2008). In the last part of the exercise

protocol, the subjects were once more located in the at rest, seated position and were monitored for over 30 minutes.

HR and HRV were studied during the following stages: M1: Rest before exercise; M2: 0-5 minutes after exercise; M3: 5-10 minutes after exercise; M4: 10-15 minutes after exercise; M5: 15-20 minutes after exercise; M6: 20-25 minutes after exercise and; M7: 25-30 minutes after exercise. Systolic (SAP) and diastolic arterial pressure (DAP) were logged before and for 30 minutes following exercise.

When the subjects were seated and not performing exercise, they were told to remain silent, awake whilst breathing normally.

Statistical Analysis

The sample size was reached by a calculation founded on a pilot test, wherein the online software provided by the website www.lee.dante.br was necessary considering the RMSSD index as a variable. The significant difference in magnitude assumed was 14.11 ms (milliseconds), with a standard deviation of 12.8 ms, per alpha risk of 5% and beta of 80%, with the sample size determined at a minimum of 13 individuals per group.

We computed the Shapiro-Wilk test to estimate the normality of distributions. For evaluation of the variables between HHADS and LHADS groups we computed an unpaired Student t-test for parametric distributions and Mann-Whitney statistical test for non-parametric distributions. We measured Cohen's *d* effect size for significant differences. Large effect size was accepted for Cohen's *d* greater than 0.8 and medium effect size for values between 0.8 and 0.5 (Fritz et al. 2012).

For examination of the moments (rest versus recovery times), the repeated measurements one-way analysis of variance (ANOVA1) technique followed by the Dunnett's test (parametric distribution) or the Friedman test followed by Dunn's test (non-parametric distribution) were necessary.

In an effort to assess the correlation between HAD score and HRV indices we computed the Pearson correlation coefficient for parametric distributions and the Spearman correlation coefficient for non-parametric distributions. Strong correlations were accepted for r greater than 0.75 and moderate correlations were considered for r between 0.5 and 0.75.

With the intention of investigating the effect of independent variables on dependent variables a simple linear regression model was finalized. The selection of the independent variables was achieved primarily by correlation analysis, considering only the variables with a significant correlation ($p<0.05$). A simple linear regression model was needed to model the HRV indices as outcome variables, predictors included the HAD score. The R^2 was estimated to verify the coefficient of determination of the percentage of variation explained by the model. The adjusted-R^2 was requisite to evaluate the stability of the model.

Statistical significance was considered at the level $p < 0.05$ (or <5%).

RESULTS

Table 1 illustrates descriptive statistics of age, height, mass, body mass index and HADS of the contributors. We achieved no significant changes between groups regarding age, height, mass and body mass index. As expected, the HADS was higher in the HHADS group.

SAP and DAP before and after exercise are stated in Figure 2. There was a significant decrease of SAP 30 minutes following exercise in the HHDAS (Cohen's *d*: 0.68, medium effect size) and LHDAS groups (Cohen's *d*: 0.41, small effect size).

In the LHADS set the RMSSD was significantly reduced ($p<0.0001$, $F=8.87$, $t=5.022$, Degrees of freedom: 139) 0 to 5 minutes after exercise (M2) compared to rest (Cohen's *d*: 0.79, medium effect size), while in the HHADS group the same index was significantly declined ($p<0.0001$; $F= 9.262$, $t=4.886$, Degrees of freedom: 146) 0 to 5 minutes (Cohen's *d:* 0.91, large effect size), 5 to 10 minutes (Cohen's *d*: 0.82, large effect size) and 10 to 15 minutes after

exercise (Cohen's *d*: 0.61, medium effect size). We found no significant differences regarding the other specific comparisons separately (Figure 3).

Recovery of HR was comparable between groups (LHADS: $p<0.0001$, $F= 31.376$, $t=10.797$, Degrees of freedom: 139; HHADS: $p<0.0001$; $F= 25.445$, $t=9.382$, Degrees of freedom: 146), as it was reduced 0 to 5 minutes (Cohen's *d*: 1.21, large effect size) and 5 to 10 minutes after exercise (Cohen's *d*: 0.53, medium effect size) compared to rest in the LHADS. HR was similarly diminished 0 to 5 minutes (Cohen's *d*: 1.19, large effect size) and 5 to 10 minutes after exercise (Cohen's *d*: 0.55, medium effect size) compared to rest in the HHADS. In relation to the other specific comparisons separately, we found no significant differences (Figure 3).

Considering the RMSSD as an independent variable, linear regression analysis specified that RMSSD had significant association with HADS at the same moments following exercise (Table 2).

DISCUSSION

Our study proposed to investigate the association between HADS and HR autonomic recovery after exercise. As the principal results, we established that 1) healthy subjects with higher HADS presented delayed HRV recovery following submaximal exercise; 2) anxiety or depression evaluated via HADS presented slight relationship with parasympathetic regulation of HR between 5 and 15 minutes after exercise. The relationship between RMSSD and HADS was evaluated through linear regression, which is a secondary support for delayed autonomic recovery.

Accordingly, HADS is an extensively applied psychometric approach to evaluate somatic disorders (Cosco *et al*. 2012). This technique has been applied to evaluate cardiovascular risk in patients with acute coronary syndrome (Doyle *et al.* 2006). A previous study included 25

suspected cases of acute myocardial infarction and applied the depression subscale of the HADS and the Beck Depression Inventory-Fast Screen Scale. Interestingly, the authors proposed that the HADS depression subscale offers more accurate information regarding acute coronary disease at elevated risk of one-year mortality compared to the Beck Depression Inventory-Fast Screen Scale.

Therefore, it has been recognized that depression may have a significant association with autonomic and cardiovascular functions. A prospective study defined a connection between irregular cardiac autonomic control and depression (Guinjoan *et al.* 2004). This mentioned study assessed mood, peripheral autonomic output through HRV and immune-inflammatory mediators (Tumor Necrosis Factor (TNF)-alpha, Interferon (IFN)-gamma, Interleukin (IL)-2, IL-4, IL-6 and IL-10) in older individuals presented with heart failure. These authors stated that autonomic dysfunction and mood were associated with higher levels of systemic inflammation, indicating that mood may affect the clinical outcome of heart failure.

Moreover, the association of anxiety on cardiovascular risk has been extensively reviewed by Tully *et al* (Tully et al. 2013). These authors emphasized the relationship between worry, coronary heart disease and the generalized anxiety disorder. More specifically, it was demonstrated that worry decreases HRV, general anxiety disorder increases blood pressure and anxiety was associated with recognized hypertension.

Equally, it was unclear thus far if cardiac autonomic function is related to HADS. So as to resolve this question, we evaluated HRV during recovery from aerobic exercise (Peçanha et al. 2014). This is a well-recognized technique that evaluates the possibility of developing cardiovascular disorders. Late return to basal levels was revealed to be linked with all-cause mortality (Kokkinos et al. 2012).

Considering that the research literature upholds abnormal autonomic responses in individuals with anxiety or depression (Hamilton and Alloy 2016; von Känel et al. 2009) and

that the HADS was revealed to provide relevant data about risk of mortality in patients with acute coronary syndrome (Doyle et al. 2006), we predicted the delayed HRV return to baseline levels after exercise in subjects with higher HADS. In agreement, we support this theory. According to our investigation, we observed delayed recovery of RMSSD during recovery from exercise in subjects with HADS > 12. In contrast, HR was significantly increased 0 to 10 minutes after exercise in both groups, indicating a similar recovery of HR. Our data indicates that RMSSD was more sensitive to detect HR autonomic changes.

Hence, an earlier study discovered that increased levels of anxiety were related to blunted HR recovery in patients with chronic heart failure. Unusually, the same study highlighted that depression was not significantly connected with autonomic recovery (von Känel et al. 2009). We draw attention to the situation that the subjects analyzed in our cohort did not exhibit cardiovascular, respiratory or metabolic diseases. Again, we hypothesize that the association between HADS and the autonomic nervous system is influenced by associated disorders, since we assessed healthy subjects.

Based on our results, systolic blood pressure was significantly decreased 30 minutes after exercise in subjects with low and high HADS values, supportive of the similarity between groups regarding cardiovascular recovery following exercise. Diminishing blood pressure between 5 to 7 mmHg after a single bout of aerobic exercise has been hitherto recognized (Augeri et al. 2009; Blanchard et al. 2006). This mechanism is termed post-exercise hypotension (Bruneau et al. 2016).

Slower autonomic return after aerobic submaximal exercise in subjects with higher HADS was supported by correlation tests and linear regression analysis. Statistical analysis identified a small association between HADS on HR autonomic regulation. We revealed a slight but significant association of HADS and RMSSD at 5-10 (10%) and 10-15 (12%) minutes after exercise. This endorses previous suggestions indicating a relationship between

depression, anxiety and cardiovascular risk. An elegant meta-analysis evaluated prospective cohort studies from 1980 to 2003 in an attempt to validate the impact of depression on mortality in patients with coronary heart disorder (Barth et al. 2004). The study emphasized that clinical depression and depressive symptoms present a negative influence on mortality in coronary heart disease patients.

Additionally, and recently, another review article revealed that anxiety-induced stress responses include vasoconstriction even in healthy arteries and at plaque formation sites, promoting cardiac arrhythmia and ischemia. Also, acute coronary events and acute myocardial infarction may be elicited by anxiety-induced stress because of increased levels of plasma catecholamine and platelet reactivity (Silverman et al. 2018).

Some points are worth highlighting in our study. We assessed a small sample, yet, statistical analysis provided significance. Nevertheless, we encourage further studies with larger samples. Autonomic function could be measured via electroneuromyography, but this is an invasive procedure and therefore more complicated. Blood samples would strengthen our data, then we could have access to a more detailed physiological responses, together with oxidative stress, heart and muscle injuries and inflammatory markers.

Our study affords important data for subjects with high levels of anxiety or depression evaluated through the HADS. Previously, Laukkanen *et al*. (Laukkanen et al. 2014) suggested that higher values of SAP during recovery from exercise is connected with increased risk of sudden cardiac death. The authors examined 2366 men between 42 and 61 years old. Based on our study, and considering that post-exercise cardiovascular autonomic recovery was delayed in subjects with high HADS values, our results highlight the requirement for a longitudinal study on exercise amongst subjects with high HADS scores. We similarly recommend that subjects with high HADS values submitted to an exercise program may improve autonomic recovery following exercise because of a beneficial effect of exercise training on the autonomic

nervous system (Pearson and Smart 2018). Until now, we identified significant association of HADS and autonomic HR recovery between 5 to 15 minutes after exercise, signifying that HADS scores have a minor but significant affiliation with HRV recovery.

CONCLUSION

Subjects with high HADS values presented delayed autonomic recovery following submaximal exercise compared to subjects with low HADS scores. We also stated that HADS has a significant impact on HR autonomic recovery after exercise. We support the importance of studies examining autonomic recovery during an exercise intervention for depressed individuals.

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COMPETING INTERESTS

The authors declare no competing financial and non-financial interests.

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Table 1. Descriptive statistics of age, height, mass, body mass index and HADS of the participants. Mean ± standard deviation (maximum-minimum).

Variables	HHADS	LHADS	p	Cohen's *d*
Age (years)	20.16 ± 1.51	20.68 ± 2.09	0.09	-
(min-max)	(18-23)	(18-25)		
Height (m)	1.63 ± 0.05	1.54 ± 0.08	0.45	-
(min-max)	(1.55-1.79)	(1.54-1.78)		
Mass (kg)	62.44 ± 9.30	59.68± 11.69	0.81	-
(min-max)	(45-80.3)	(42.7-93.4)		
BMI (kg/m^2)	23.25 ± 2.96	21.91 ± 4.38	0.62	-
(min-max)	(19.24-29.42)	(17.65-38.91)		
HAD score	16.9 ± 3.5	5.05 ± 1.67	<0.0001	4.32
	(12-26)	(1-7)		

m: meters; kg: kilograms; BMI: body mass index.

Table 2. Linear regression between HAD score and RMSSD at M3 and M4.

Models	β	95% C.I.	p	R^2-adjusted
RMSSD (M3)	-0.681	-1.335; -0.02535	0.04	0.1
RMSSD (M4)	-0.6922	-1.286; -0.09877	0.02	0.12

Legend: RMSSD: square root of the square mean of the differences between adjacent normal RR intervals; M3: 5-10 minutes after exercise; M4: 10-15 minutes after exercise.

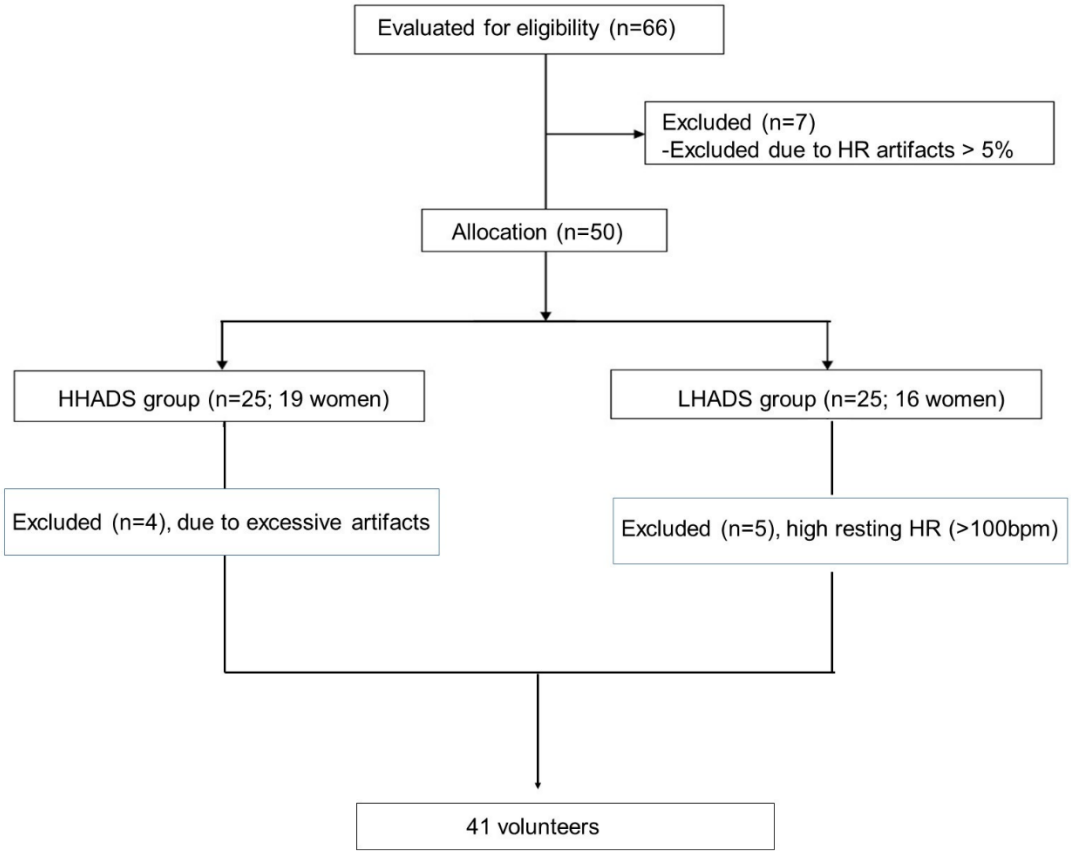

Figure 1: Flowchart.

Figure 2: Mean values and respective standard deviations of systolic (SAP) and diastolic arterial pressure (DAP) obtained at rest and during recovery. Pre: Rest before exercise; Post: immediately after exercise. *L: Values with significant differences ($p<0.05$) in relation to rest for the LHADS group; *H: Values with significant differences ($p<0.05$) in relation to rest for the HHADS group; mmHg (millimeters of mercury).

Figure 3: Mean values and respective standard deviations of RMSSD obtained at rest and during recovery. *H: Values with significant differences ($p<0.05$) in relation to rest for the HHADS group; *L: Values with significant differences ($p<0.05$) in relation to rest for the

LHDAS group; **H: Values with significant differences ($p<0.05$) in relation to M2 for the HHADS group; **L Values with significant differences ($p<0.05$) in relation to M2 for the LHADS group; RMSSD: square root of the square mean of the differences between adjacent normal RR intervals; ms: milliseconds; M1: Rest before exercise; M2; 0-5 minutes after exercise; M3: 5-10 minutes after exercise; M4: 10-15 minutes after exercise; M5: 15-20 minutes after exercise; M6: 20-25 minutes after exercise; M7: 25-30 minutes after exercise.