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Original Article

Sex differences in heart rate recovery and cardiac vagal reactivation following high-intensity functional training

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Abstract

Background: Heart rate recovery is key indicator of cardiovascular health, influenced by vagal reactivation and sympathetic withdrawal. However, research on sex differences in cardiac vagal reactivation following high-intensity functional training (HIFT) is limited. Aim: This study aimed to test the hypothesis that women exhibit slower heart rate recovery compared to men after HIFT workout, attributed to reduced cardiac vagal reactivation. Material and Methods: Young recreational HIFT practitioners, all apparently healthy individuals, voluntarily participated in this study. All subjects performed a HIFT session, the "workout of the day" (WOD), based on the structure of the training session: "as many repetitions as possible" (AMRAP) "Cindy", which consisted of as many rounds possible of five pull-ups, 10 push-ups, and 15 air squats in a 20-minute period. Anthropometric measurements, body composition, hemodynamic parameters, and heart rate variability (HRV) were analyzed at rest and during HIFT. Following this, cardiac vagal reactivation was indexed by the fall in heart rate and the HRV metric over the 5-minute period in 30-second intervals after the end of the HIFT workout. Results: The men showed lower values of the resting heart rate compared to women ($p = 0.023$). Nonlinear time series analysis of baseline HRV evidenced that men had higher resting short-term variability ($p < 0.001$, □2p: 0.771) and 2V ($p = 0.013$, □2p: 0.105) indices in comparison to women, as well as lower 0V variation indices ($p = 0.048$, □2p: 0.250). In addition, the male group showed lower vagal withdrawal (short-term variability [$p = 0.012$, □2p: 0.375] and 2V [$p < 0.001$, □2p: 0.885] indices), sympathetic outflow (0V variation [$p < 0.001$, □2p: 0.773]) and sympathovagal balance (short- and long-term variability ratio [$p < 0.001$, □2p: 0.748]) to the heart than women during HIFT performance. Women presented a slower recovery in heart rate than men regardless of the time point ($p < 0.001$, □2p: 0.724). Post-HIFT vagal tone metrics (pNN50 and RMSSD) were faster and increased by a greater magnitude in men compared to women ($p = 0.004$, □2p: 0.460 and $p = 0.005$, □2p: 0.437; respectively). Conclusion: Our findings suggest that women had a slower heart rate recovery compared to men after recreational HIFT training session, suggesting a sex-related difference in cardiac vagal reactivation.

Keywords: Heart rate variability, Cardiovascular system, Parasympathetic modulation, Sex differences, Exercise.

Introduction

The human heart is involved in the response to the energy demand of the body and its regulation is mainly driven by the balance between the sympathetic and parasympathetic branches of the autonomic nervous system (ANS). The activity and relative level of these two neural networks cause cardiac dynamic changes in response to external stimuli (Weissman & Mendes, 2021). Over the previous few decades, the acute and chronic effects of physical exercise on the cardiovascular physiology of the human body have been investigated through studies and are identified as responses to exercise that include influences on heart rate acceleration in transient exercise and adaptations to physical training, such as salutary effects on ANS function and consequently

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bradycardia at rest and lower heart rate for the same intensity of physical exertion (Matei et al., 2022). In addition to the assessment of physiological variables during exercise, post-exercise measurement is equally important. One way to assess cardiovascular responses after physical exercise is through the analysis of heart rate recovery (HRR) and vagal reactivation (Morshedi-Meibodi et al., 2002).

The HRR immediately after physical exercise may be an important mechanism in preventing cardiac stress and is a function of reactivation of the parasympathetic system and decrease in the sympathetic activity. However, an impaired HRR after physical exertion is indicative of attenuated cardiac vagal reactivation and was considered as predictor of mortality (Cole et al., 1999; Elshazly et al., 2018).

The decrease in heart rate after exercise does not replace other forms of measuring cardiac autonomic activity but works as a complement when carrying out the clinical and/or physical assessment of an individual. At the cessation of exercise, attention should be paid to the heart rate behavior, since its smaller reduction in the first minute of recovery after exercise test represents an unfavorable prognosis in terms of relative risk of cardiovascular mortality (Shetler et al., 2001). Thus, the smaller the variation of heart rate, the greater this relative risk.

Post-exercise HRR is an easily measured parameter that is believed to be mediated by intrinsic, neural, and humoral factors. The HRR in the first 30 s after exercise (fast phase) reflects the balance of reactivating vagal and withdrawal of sympathetic outflow. The last phase of HRR (slow phase) has been attributed to parasympathetic reactivation (Michael et al., 2017). As a result of threshold values of HRR are commonly espoused for differentiating a normal from an abnormal response (Cole et al., 1999), so it is relevant to question whether HRR is dependent on sex and exercise modality.

Researchers have largely ignored the potential impact of demographic variables, such as sex, could have on the association between heart rate variability (HRV) and HRR. In this regard, Koenig & Thayer (2016) showed women to have greater vagally-mediated HRV and higher heart rate compared to men. These findings represent paradoxical cardiovascular activity, as higher HRV is typically associated with lower heart rate. Therefore, it is plausible that the association between HRV and HRR may differ significantly between men and women. Recent studies have found sex differences in metabolic adaptation among endurance athletes, with women and men reducing body fat, increasing oxygen uptake, and increasing left ventricular mass after different lengths of training and to different extents (Regitz-Zagrosek et al., 2017). However, the literature to date is unclear on whether the HRR and cardiac vagal reactivation in women is purportedly higher, similar or lower than that in men. Conversely, the magnitude and time-course cardiac autonomic recovery depends on the preceding exercise intensity, as well as the sport modality (Pierpont et al., 2000). To study HRR most researchers have investigated cardiovascular dynamics following moderate or maximal aerobic activities (Goulopoulou et al., 2009). Restoration of HRV seems to be slower and less complete after acute bouts of high intensity compared with long-lasting low intensity exercise (Javorka et al., 2002) and minimal investigations addressed resistance training. In brief, it is unclear to what extent the exercise modality may influence the acute responses of cardiovascular autonomic control, as reflected by HRR and HRV markers. Thus, the extent to which post-exercise parasympathetic reactivation depends on the intensity and exercise modality, remains unclear and warrants further investigation. Furthermore, there are fewer data on cardiovascular recovery following high-intensity exercise, like Hight-Intensity Functional Training (HIFT).

HIFT is a training modality that emphasizes multi-joint functional movements that can be modified at any fitness level and helps to mobilize more muscles than traditional exercises. Due to its popularity, studies have aimed to investigate the physiological responses to HIFT in the respiratory and muscular systems, and mainly on cardiovascular behavior (Posnakidis et al., 2022). In this sense, it becomes necessary to investigate whether sex-specific differences collectively result in a different cardiac performance for women and men after HIFT. Therefore, whether these previous findings about sex differences are already manifested immediately upon the cessation of the exercise, which is known to be primarily mediated by cardiac vagal reactivation, remains to be determined. Thus, the purpose of the present study was to test the hypothesis that women present a slower HRR than men at the cessation of HIFT workout due to an attenuated cardiac vagal reactivation.

Material & methods

Sample

This study consisted of eighteen apparently healthy subjects (nine women) recreational HIFT practitioners with 27.4 ± 4.6 years old. The sample size used is validated by a previous study, which reported that nine is the minimum recommended number of subjects to draw statistically significant conclusions from a biomedical study (Ristić-Djurović et al., 2018). Participants were recruited through on social media and local HIFT gyms. To be included, subjects had to be training on 3-5 days per week for at least one year of HIFT participation and not be using any controlled medication or ergogenic aids. The exclusion criteria precluded individuals with sinus arrhythmia, those who smoked and/or drank alcohol and those with history or symptoms of cardiopulmonary, metabolic, or neurological diseases. All women in this study were nonusers of any contraceptive for at least six consecutive months and were studied during the early follicular phase, defined as counted as the first day of 6th the menstrual cycle until the day, as informed by the participants.

All the experimental procedure was explained to the participants, both verbally and in writing, and all subjects gave their full consent to take part in the study. This study was approved by the Institutional Ethics and Human Research Committee of the University of Uberaba (nº. 4370713/2020) and conducted according to the principles established in the Declaration of Helsinki.

Study protocol

This was a cross-sectional study design that was carried out in two phases (Figure 1). The first phase consisted of a visit by the participants to our laboratory to characterize the sample obtaining data on anthropometry (height, body mass and body mass index [BMI]), body composition (body fat and lean body mass), hemodynamic parameters (heart rate, blood pressure, double-product and breathing rate) and cardiac autonomic modulation at rest. For this purpose, all volunteers were previously instructed to abstain from stimulant drugs, caffeine, tobacco, alcohol, high-fat food, and physical activity for at least 24 hours before. The experimental protocols were carried out under baseline conditions (always at approximately the same time of day [between 8:00 a.m. and 11:00 a.m.], to negate any effects of circadian variation) in a noiseless room with average daytime temperature of around 21ºC, two hours after their usual breakfast. For the autonomic tests, an electrocardiogram signal was recorded for five minutes with participants in supine position (subjects were not allowed to sleep, move, or talk) by an electrocardiographic system (Matlab 6.1.1.450 Release 12.1.2001, ADInstruments, Australia). After two days, a standardized acute workout that consists of sequence of a 5-10-15 ascending repetition scheme was performed (second phase). Thereafter, post-exercise heart rate and blood pressure analysis were performed. All tests during the second visit were performed on a single day in the morning at the HIFT training center.

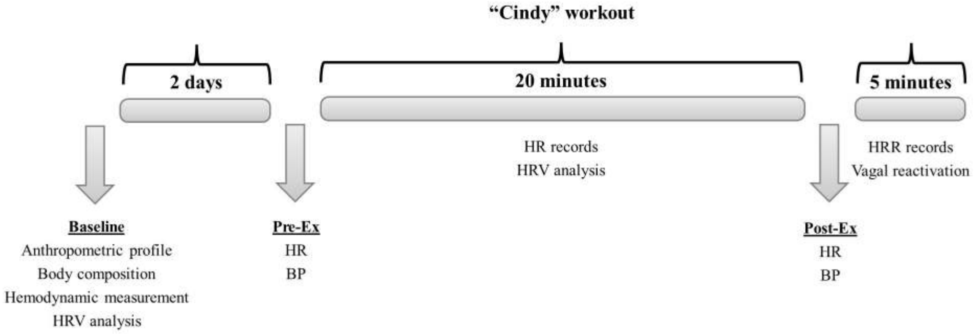

Figure 1. Time points of data collection. Pre-Ex: before exercise; Post-Ex: immediately after exercise; HR: heart rate; BP: blood pressure; HRV: heart rate variability; HRR: heart rate recovery.

Anthropometric profile and body composition

The total body mass, height and BMI were measured using a body weight scale (Tanita HD-350®) and a portable stadiometer (Prime Med®; 0.1 cm accuracy), mounted on a wall. The volunteers were barefoot and wearing light clothes. BMI was calculated as a ratio between weight and square height (kg/m^2). Waist circumference was measured using an anthropometric tape measure (Sanny, São Paulo, Brazil) at the midpoint between the anterior superior iliac crest and the last rib, with an accuracy of 0.01 m.

The Jackson-Pollock equations using three-site skinfold (chest, abdomen, and thigh on men and triceps, suprailiac, and thigh on women) were used to determine body density, which was used to determine overall body fat % values. These analyses were taken for every participant by the same trained researcher to ensure proper interrater reliability. The measurements were all taken on the right side of the body. A reading was taken a total of three times at each site prior to moving on to the next anatomical location.

Hemodynamic parameters measurements

The blood pressure was noninvasively measured on the left arm after five minutes of rest in the supine position using an automatic oscillometric cuff (M3 Intellisense HEM-7051-E; Omron Healthcare, Kyoto, Japan) with digital display. Using the systolic and diastolic blood pressure data, the mean arterial blood pressure was calculated. The heart rate was monitored by lead II of an electrocardiogram (Labchart Pro version 7.3.4, Brazil) on a beat-by-beat basis in the CM5 position and analyzed by the Matlab software (version 6.1.1.450, release 12.1.2001). The breathing rate was estimated using a metronome guide. All subjects were instructed to slow down their breathing following a metronome at 10 breaths per minute to slow down their respiratory rate.

Linear and nonlinear analyses of HRV

For the overall variability analysis of RR intervals (iRR), we considered the last 300 beats from the RR time series within the last five minutes in the resting condition. HRV was assessed in the time domain by means of the time-series variance. Subsequently, frequency domain analysis of HRV was performed with an autoregressive algorithm, order determined by Akaike's criterion in a range from 5 to 14, with a Hanning interval (50% overlap) on the same sequences used for symbolic dynamics of HRV.

The time domain analysis provided information on ultra-short variability of the signal as root mean square of successive iRR differences (RMSSD) and percentage of successive iRR that differ by more than 50 ms (pNN50). All these measurements of ultra-short-term variation estimate high-frequency variations in heart rate that is primarily mediated by vagus nerve activity (Task Force, 1996).

In the analysis of the frequency domain, the included components of the overall spectrum were the normalized units (nu) of LF and HF bands. The sympatho-vagal balance was calculated as the normalized units LF/HF ratio from the RR series (Ferreira et al., 2022).

The Poincaré Plot and symbolic dynamics were used as HRV nonlinear analysis. For the quantitative analysis of Poincaré were calculated the short-term variability (SD_1), long-term variability (SD_2). It has been shown that SD_1 is correlated with short-term HRV and is mainly influenced by parasympathetic modulation, while SD_2 is a measure of long-term variability and reflects sympathetic activation. The SD_2/SD_1 ratio indicates higher sympathetic outflow (Rahman et al., 2018).

In symbolic dynamics, a nonlinear index, the RR time series was converted into a sequence of symbols that were divided into 3-beat patterns. Patterns were classified into three families referred to as: 0V, patterns without variation, i.e., all 3 symbols were equal (e.g., 2-2-2) and 2V, patterns with 2 unlike variations, all symbols are different from the previous one but not in a consequent order (e.g., 1-5-2). The percentage of the patterns 0V is a marker of cardiac sympathetic outflow and 2V is a marker of cardiac vagal activity (Porta et al., 2007). The Shannon entropy of the distribution of the patterns was calculated to provide a quantification of the complexity of the pattern distribution. All analyses were performed using a custom software (HeartScope II AMPS, ITA).

High-intensity functional training

Each training session lasted approximately 40 minutes including a warm-up period, the acute phase of the HIFT workout, and a cool-down. Prior to HIFT workout, all participants completed a 10-minute active warm-up period led by an instructor and which included workout-specific exercises. Although intensity was not measured during the warm-up, participants were encouraged to maintain a light to moderate intensity and focus on working through the full range of motion rather than at high intensity. The HIFT training protocol used in this study was the "as many repetitions as possible" (AMRAP) method, which consists of a type of workout known as the "workout of the day" (WOD) that combines HIFT exercises for a predetermined time, where participants complete the maximum number of repetitions or rounds within the set period. The WOD model used was the "Cindy" training. This "Cindy" WOD consisted of as many rounds as possible of five pull-ups, 10 push-ups and 15 air squats in 20 minutes. Each round had to be properly executed according to preestablished minimum standards in order to continue onto the next round. One of the team's researchers was responsible for counting rounds using a hand-held counter for further comparison.

Autonomic analysis during HIFT performance

Interbeat intervals were recorded on a beat-to-beat basis using a cardiac monitor throughout the acute HIFT session (RS800cx™; Polar Electro Oy, Kempele, Finland), which uses a sampling frequency of 1000 Hz. The entire series was transferred to a microcomputer for offline data processing and analysis of iRR variability using the Kubios HRV analyzer™ version 2.0 (Kuopio, Finland). Before processing the HRV data, all iRR series were visually verified on a beat-to-beat basis to identify ectopic beats and artifacts. When present, spurious beats were deleted from the series without adding new intervals, which accounted for less than 2% of the data in every individual. After removing the artifacts, the signals were processed by the autoregressive model using the Welch method and a Hanning window with 50% overlap, through a custom algorithm (SinusCor, version 1.0.1) in Matlab (Matlab 6.0, Mathworks Inc., USA). The iRR series were then converted to equally spaced time series with 200 ms intervals using cubic spline interpolation (Task Force, 1996).

HRR and cardiac vagal reactivation after HIFT workout

The cardiac vagal reactivation after HIFT exercise was indirectly evaluated by the HRR (Peçanha et al., 2017). The oscillations between consecutive iRR were recorded through a cardiac monitor (RS800cx™; Polar Electro Oy, Kempele, Finland) during the five minutes of post-HIFT workout recovery. To assess the vagal reactivation, the RMSSD and pNN50 indices were analyzed in 30-second intervals.

Statistical analysis

The data are expressed as means ± standard deviation, 95% confidence interval, coefficient of variation and standard error of the mean. After testing the normality of the data distribution using the Shapiro-Wilk test, the SDNN index did not show a normal distribution, therefore, was logarithmically transformed (Ln). Participants' characteristics were compared by independent sample t-test. Two-way analysis of variance (ANOVA) for repeated measures followed Bonferroni post-hoc test was used to investigate significant interactions. Partial eta squared (η^2_p) was adopted as effect size analysis. η^2_p was classified as "small" ($0.001 \leq \eta^2_p < 0.06$), "medium" ($0.06 \leq \eta^2_p < 0.14$), and "large" ($\eta^2_p \geq 0.14$). Statistical significance was set at $p < 0.05$. All statistical analyses were conducted using *jamovi* software (version 0.9.2.9) and figures were plotted using Prism GraphPad Software (version 8.0, Inc., San Diego, United States).

Results

Both groups were matched for HIFT experience (46.7 ± 8.4 months for women vs. 46.3 ± 8.9 months for men; p = 0.278) and training scope per week (5.6 ± 0.7 hours for women vs. 5.4 ± 1.3 for men; p = 0.438). The mean in total number of complete rounds of 'CINDY' exercise did not differ between groups (women: 15.9 ± 0.8 and men: 16.2 ± 0.7; p = 0.344). All participants' baseline characteristics are presented in Table 1. Men and women had age and similar BMI, albeit women had shorter height, body mass and lean body mass and higher body fat ($p < 0.05$; respectively).

Table 1. Descriptive characteristics of recreational HIFT practitioners at baseline.

Variables	Men (n = 9)			Women (n = 9)		
	Mean ± SD	CV (SEM)	95%CI	Mean ± SD	CV (SEM)	95%CI
Age (years)	28.4 ± 5.0	16.2% (1.9)	23.8-33.0	26.7 ± 4.4	15.7% (1.5)	23.3-30.1
Body mass (kg)	80.1 ± 11.0	12.7% (4.2)	69.9-90.3	64.1 ± 6.4*	9.5% (2.1)	59.1-69.0
Height (m)	1.75 ± 0.07	3.7% (0.03)	1.7-1.8	1.64 ± 0.06*	3.7% (0.02)	1.6-1.7
BMI (kg/m^2)	26.2 ± 2.3	8.2% (0.9)	24.1-28.3	23.9 ± 2.5	9.7% (0.8)	22.1-25.8
Body fat (%)	8.5 ± 2.3	26.6% (0.8)	6.4-10.6	29.2 ± 6.9*	23.7% (2.3)	23.9-34.6
Body fat (kg)	6.9 ± 2.5	37.0% (1.0)	4.5-9.2	18.9 ± 5.4*	28.5% (1.8)	14.8-23.1
Lean body mass (kg)	73.2 ± 9.3	12.7% (3.5)	64.7-81.8	45.1 ± 4.2*	9.4% (1.4)	41.9-48.4

SD, standard deviation; CV, coefficient of variation; SEM, standard error of the mean; HIFT, high-intensity functional training; CI, confidence interval; BMI, body mass index. *$p < 0.05$ vs. men group.

Physiological responses of hemodynamic parameters at rest for individuals of both sexes are illustrated in Figure 2. Significant sex differences were not observed in in systolic, diastolic and mean blood pressure, double-product and breathing rate. Nonetheless, men showed lower values of the heart rate compared to women ($p = 0.023$).

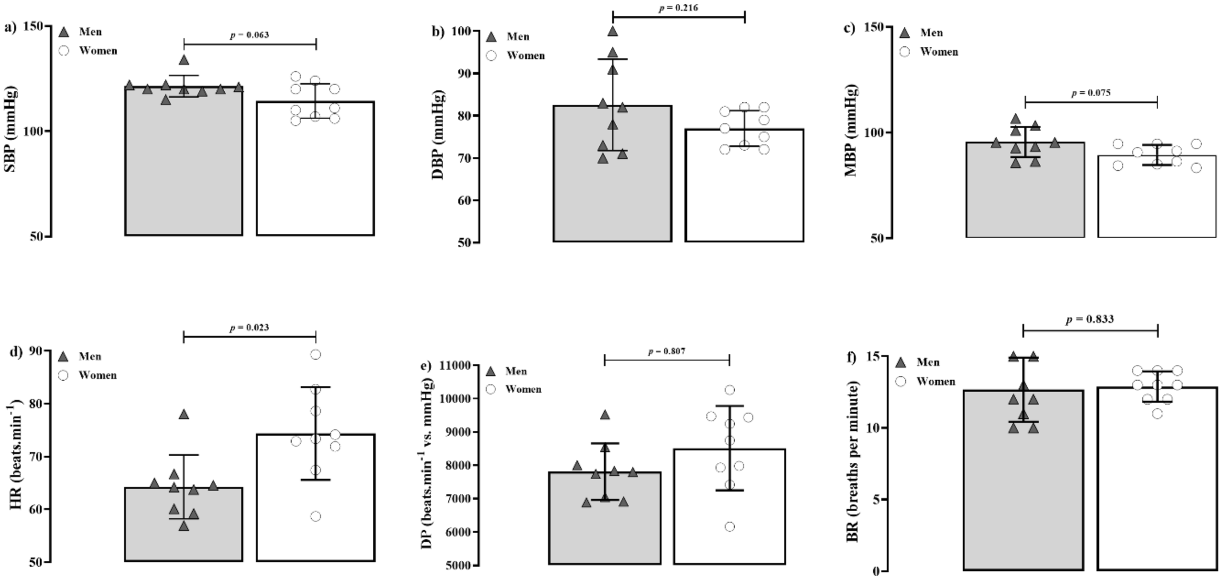

Figure 2. Differences between sexes in hemodynamic parameters of the (A) SBP, (B) DBP, (C) MBP, (D) HR, (E) DP and (F) BR at rest. Values are displayed as mean and standard deviation. SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; DP, double product; BR, breathing rate.

The cardiac autonomic variables at rest and during HIFT performance are presented in Table 2. Resting iRR values and during HIFT session were lower in women compared to men and both groups showed a similar decline in the acute phase of exercise. Men and women had similar resting HRV indices in time and frequency domain. However, in frequency domain of HRV, the analysis using the delta of variation of the HIFT exercise relative to the rest condition showed significant interaction (sex*time) for the normalized LF band (sympathetic outflow) and positive effects for temporal analysis. However, post-hoc analyses revealed no significant differences in comparisons between sex. Conversely, in the non-linear analyzes of HRV, it was found that men had higher resting SD_1 and 2V indices in comparison to women, as well as lower 0V variation indices. In addition, the men's group showed lower vagal withdrawal (SD_1 and 2V indices), sympathetic outflow (0V variation) and simpatho-vagal balance (SD_2/SD_1 relation) to the heart than women during HIFT performance.

Table 2. Heart rate variability metrics by linear and non-linear methods at rest and during session of HIFT in men and women groups.

	Men (n = 9)	Women (n = 9)	ANOVA	F	η^2_p	*p* value
RRi (ms)						
Rest	942.3 ± 87.2	817.3 ± 100.0†	sex	8.91	0.389	0.010
Exercise	327.6 ± 9.7‡	304.4 ± 6.2†‡	time	596.2	0.977	<0.001
Δ %	-64.9 ± 3.1	-62.3 ± 4.3	interaction	4.86	0.258	0.045
LnSDNN (ms)						
Rest	1.8 ± 0.3	1.6 ± 0.2	sex	3.21	0.186	0.095
Exercise	0.9 ± 0.2‡	0.8 ± 0.2‡	time	252.2	0.896	<0.001
Δ %	-1.7 ± 0.0	-1.6 ± 0.3	interaction	2.01	0.179	0.125
RMSSD (ms)						
Rest	48.4 ± 19.9	47.1 ± 17.5	sex	0.229	0.016	0.640
Exercise	8.5 ± 5.1‡	5.4 ± 2.2‡	time	66.42	0.826	<0.001
Δ %	-76.6 ± 22.8	-79.7 ± 16.6	interaction	6.02	0.002	0.864
pNN50 (%)						
Rest	28.3 ± 7.2	24.6 ± 8.0	sex	3.56	0.203	0.080
Exercise	6.7 ± 2.3‡	3.1 ± 1.0‡	time	110.0	0.887	<0.001
Δ %	-79.7 ± 7.9	-78.2 ± 9.6	interaction	0.004	0.000	0.982
LFnu (%)						
Rest	50.9 ± 5.1	53.6 ± 8.2	sex	0.120	0.009	0.734
Exercise	69.0 ± 10.9‡	63.6 ± 8.4‡	time	83.72	0.857	<0.001
Δ %	35.7 ± 15.8	21.0 ± 8.0†	interaction	7.08	0.336	0.019
HFnu (%)						
Rest	47.9 ± 12.8	46.1 ± 13.9	sex	2.57	0.155	0.131
Exercise	40.4 ± 3.9	28.5 ± 6.2†‡	time	19.29	0.508	<0.001
Δ %	-12.8 ± 9.6	-33.2 ± 11.1†	interaction	3.14	0.183	0.098
LFnu/HFnu						
Rest	1.1 ± 0.2	1.2 ± 0.2	sex	3.79	0.213	0.072
Exercise	1.7 ± 0.3	2.3 ± 0.4	time	1.64	0.105	0.221
Δ %	59.1 ± 32.8	93.6 ± 46.2†	interaction	2.62	0.158	0.128
SD$_1$ (ms)						
Rest	45.4 ± 7.5	36.3 ± 12.9†	sex	47.2	0.771	<0.001
Exercise	45.9 ± 12.8	13.1 ± 2.2†‡	time	8.39	0.375	0.012
Δ %	-8.5 ± 57.3	-60.1 ± 17.4†	interaction	9.37	0.401	0.008
SD$_2$ (ms)						
Rest	56.1 ± 15.2	57.1 ± 9.5	sex	0.016	0.001	0.901
Exercise	66.5 ± 16.7‡	67.1 ± 7.8†	time	115.18	0.892	<0.001
Δ %	18.9 ± 5.7	19.1 ± 14.5	interaction	0.503	0.004	0.821
SD$_2$/SD$_1$						
Rest	1.3 ± 0.4	1.8 ± 0.7†	sex	37.5	0.728	<0.001
Exercise	1.5 ± 0.5	5.3 ± 1.4†‡	time	41.5	0.748	<0.001
Δ %	25.4 ± 7.5	194.4 ± 73.3†	interaction	31.0	0.689	<0.001
0V						
Rest	32.2 ± 10.7	35.7 ± 5.7†	sex	4.67	0.250	0.048
Exercise	41.5 ± 12.8‡	42.3 ± 6.9‡	time	47.70	0.773	<0.001
Δ %	29.5 ± 6.4	19.5 ± 15.5†	interaction	7.08	0.336	0.019
2V						
Rest	36.9 ± 4.5	26.4 ± 5.7†	sex	1.64	0.105	0.013
Exercise	23.6 ± 3.5‡	13.6 ± 4.3‡	time	107.9	0.885	<0.001
Δ %	-36.3 ± 9.3	-48.5 ± 10.1†	interaction	22.3	0.614	<0.001
Shannon entropy						
Rest	3.4 ± 0.3	3.4 ± 0.4	sex	0.059	0.004	0.811
Exercise	1.4 ± 0.2‡	1.4 ± 0.2‡	time	355	0.962	<0.001
Δ %	-58.6 ± 9.9	-59.5 ± 7.1	interaction	0.004	0.001	0.962

Values are displayed as mean and standard deviation. HIFT, high-intensity functional training; ANOVA, analysis of variance; RRi, electrocardiogram RR intervals; LnSDNN, natural logarithmic transformation of the standard deviation of the normal RR intervals; RMSSD, root mean square of successive RRi differences; pNN50, percentage of successive RRi that differ by more than 50 ms; LFnu, normalized units of the low-frequency spectral component; HFnu, normalized units of the high-frequency spectral component of HRV; SD$_1$, short-term variability; SD$_2$, long-term variability; 0V, percentage of patterns without variation; 2V, percentage of patterns with two variation; Δ %, relative change. †p < 0.05 vs. men and ‡p < 0.05 vs. rest within group.

Figure 3 displays the recovery of heart rate, cardiac vagal reactivation represented by pNN50 and RMSSD indices and mean blood pressure at the cessation of HIFT exercise. HRR was slower in women compared to men regardless of the time point ($p < 0.001$, η^2_p: 0.724; Figure 2A). In Figure 2B, regarding hemodynamic variables, no significant effects in mean blood pressure were observed between the groups immediately after and within the five-minutes recovery period post-exercise. The increase in pNN50 (Figure 2C) and RMSSD (Figure 2D) indices was lower in women than in men after HIFT performance ($p = 0.004$, η^2_p: 0.460 and $p = 0.005$, η^2_p: 0.437; respectively).

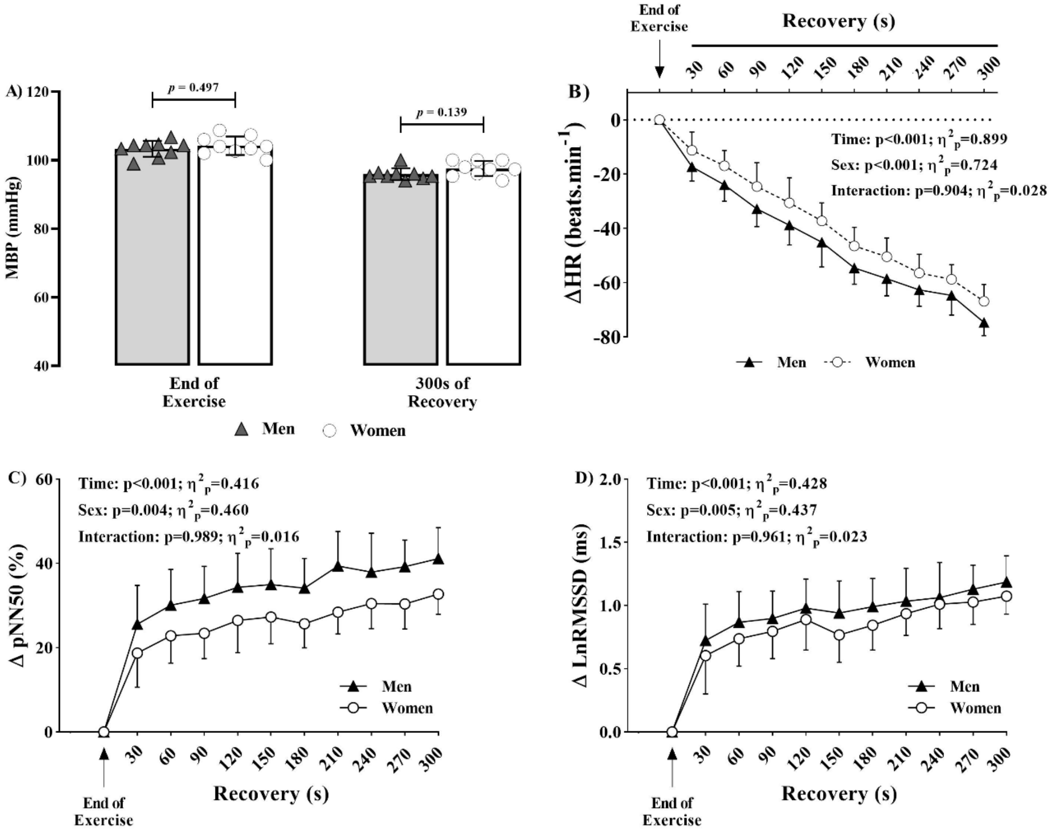

Figure 3. MBP (A) measured immediately and after five minutes of high-intensity functional training session. Changes in HR (B) and vagal tone indices (C and D) assessed at the end of the high-intensity functional training session and during a 5-minute recovery period. Values are displayed as mean and standard deviation. MBP, mean blood pressure; ΔHR, change in heart rate; ΔpNN50, change in the percentage of successive RRi that differ by more than 50 ms; ΔLnSDNN, change in the natural logarithmic transformation of the standard deviation of the normal RR intervals.

In the resting condition (Figure 4A), for both male and female individuals, the RMSSD index was not related to heart rate ($r = -0.443$; $p = 0.086$). By contrast, during the time course of recovery following the HIFT session (Figure 4B), the RMSSD index correlated significantly with heart rate ($r = -0.505$; $p < 0.001$).

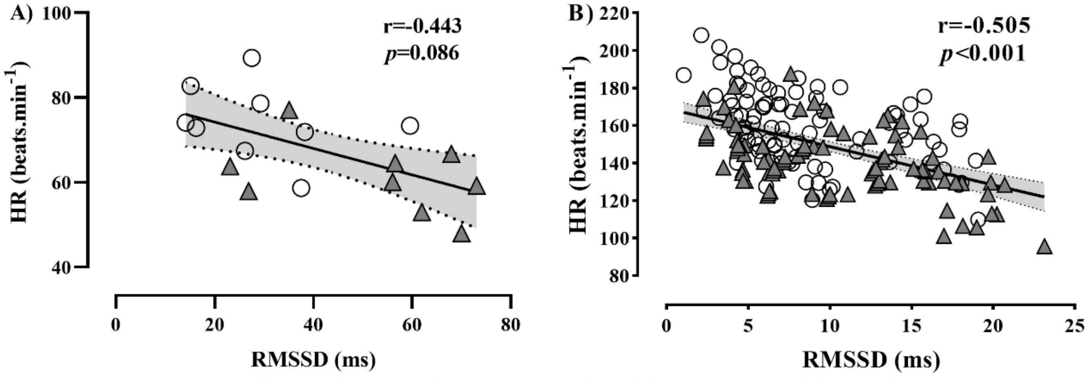

Figure 4. Correlation coefficient between the RMSSD index of heart rate variability and HR at rest (A) and HR on recovery moment after the high-intensity functional training session. RMSSD, root mean square of successive RRi differences.

Discussion

This study initially proposed testing the influence of single HIFT session on HRR and post-exercise cardiac vagal activity response among male and female recreational practitioners in this modality. We hypothesized that HRR and cardiac vagal reactivation following HIFT session would depend on the individual's sex, as previous studies have indicated sex differences in cardiac autonomic modulation (Samora et al., 2020). Based on our findings, the main evidence of the study is as follows: (i) women presented a slower HRR than men in the subacute phase of the HIFT session; (ii) increases in both pNN50 and RMSSD indices (representative of vagal tone) after HIFT practice were lower in women compared to men; (iii) men showed lower heart rate and vagal withdrawal compared to women during HIFT session; and (iv) cardiac vagal modulation (i.e., RMSSD) correlated significantly with heart rate during the recovery phase following the HIFT session. Overall, these findings suggest a sex-related difference in cardiac vagal reactivation after an acute HIFT session.

There are indications that men and women have different responses to cardiovascular control mechanisms (Ives et al., 2013; Xiong et al., 2015). Our findings align with the literature, which indicates sex-related differences in HRV. Previous studies suggest that attenuated cardiac vagal reactivation in women is confirmed by lower HRV indices compared to men at the cessation of isometric handgrip exercise (Samora et al., 2020). Furthermore, it was previously demonstrated that cardiac baroreflex sensitivity increases in men but not in women (Samora et al., 2019). Other researchers have also shown that males have higher iRR and HF-HRV indices, while females have higher heart rate and %LF-HRV. These findings suggest that sex differences should be considered when conducting baseline physiological assessments (Shafiq et al., 2023).

Considering HRR, measurements obtained after physical effort, a condition used in clinical practice to identify the reduction in HRR (Cole et al., 1999; Peçanha et al., 2014), revealed a slower HRR (30s, 60s and 300s) in women compared to men. These results demonstrate reduced HRR in women, in both fast and slow phases, indicating slower cardiac vagal reactivation and sympathetic withdrawal after exercise in women. Previous studies have also verified a lower reduction in HRR in women (Nunes et al., 2014), which, like baseline autonomic dysfunction, is associated with worse prognosis and serves as an independent predictor of mortality (Cole et al., 1999; Morshedi-Meibodi et al., 2002). These results, therefore, corroborate previous findings, demonstrating reduced HRR in the studied sample.

The blood pressure measurement methodology used in the present study (digital sphygmomanometer) does not allow us to assess the behavior of baroreflex sensitivity. However, no sex differences in blood pressure response were observed immediately or during the 5-minute recovery period following the HIFT session (Figure 2B). Could this result be attributed to attenuated cardiac vagal reactivation rather than arterial baroreflex mediation of heart rate responses? This slower cardiac vagal reactivation in women was also confirmed by attenuated pNN50 (Figure 2C) and RMSSD (Figure 2D) indices compared to men after HIFT session. Samora et al. (2019) demonstrated an increase in cardiac baroreflex sensitivity in men but not in women after isometric exercise. Therefore, women exhibit slower cardiac vagal reactivation, which is observed after the cessation of HIFT session.

As previously mentioned, delayed HRR is an independent predictor of mortality. However, the literature presents certain inconsistencies regarding the impact of sex on HRR following physical exercise. A study by Mendonca et al. (2017) reported no sex difference in heart rate at the first minute of recovery after aerobic exercise. Despite these discrepancies, a sex-depended effect on HRR has been proposed (Nunes et al., 2014). Physical exercise is a type of stress that shifts the sympatho-vagal balance toward sympathetic predominance and vagal withdrawal.

Previous studies enrolled individuals that had never engaged in competitions (Tibana et al., 2016) and individuals with intermediate level of physical fitness (Maté-Muñoz et al., 2017; Maté-Muñoz et al., 2018), as observed by the number of rounds. However, these studies included only men and younger adults. In assessing the average heart rate achieved during the 'Cindy' workout, we found mean values of 175.4 ± 10.6 bpm for women and 164.2 ± 5.1 bpm for men (88.9 ± 2.3 % and 90.2 ± 4.6 % of HRmax; respectively). According to the American College of Sports Medicine guidelines, these percentages of HRmax suggest physical exercise at near maximum-intensity (Garber et al., 2011). Studies using the 'Cindy' workout in interventions also found high cardiovascular demand (Kliszczewicz et al., 2014). Therefore, we can consider that our intervention through a HIFT session was effective.

At exercise start of physical exercise, the heart rate kinetics characteristics under parasympathetic blockade suggest that the sudden withdrawal of vagal tone may occur concomitantly with an increase in cardiac output (Lador et al., 2006). Recently, vagal withdrawal has also been suggested to explain early changes in baroreflex sensitivity at exercise onset. In the acute phase of physical exercise, heart rate increases according to the intensity of physical effort and is partially mediated by vagal withdrawal, mainly due to central command activation (Taboni et al., 2022). In fact, heart rate increases with parasympathetic withdrawal and sympathetic activation, driven by proprioceptors, baroreceptor reflex, and chemoreceptors (e.g., low pO_2, high pCO_2 and H^+ in bloodstream) (O'Leary, 1996). Thus, heart rate increase depends on movement time, muscle mass involved, and exercise intensity. In the present study, men exhibited a smaller cardiac response to stress (i.e., heart rate) during HIFT compared to women. This result may be explained by lower cardiac vagal withdrawal in the male group during exercise, represented by non-linear HRV indices of HRV (SD_1 and 2V variation) (Table 2). Ferreira et al. (2022) also found that thermal stress induces greater cardiac vagal withdrawal in women than in men. This response is likely because women's hearts rely more on vagal autonomic control, while men's hearts depend more on sympathetic control. Physiologically, sex hormones are involved in regulation of calcium homeostasis, contributing to sex differences in the cardiac excitation-contraction coupling pathway (Parks & Howlett, 2013). Regarding cardiac autonomic function, women exhibit greater vagal control over sympathetic responsiveness than men (Dart et al., 2002).

In summary, our findings demonstrated sex differences in cardiac vagal reactivation after a HIFT session. These results suggest that the autonomic control of circulation during and after physical exercise is affected by sex (Samora et al., 2020). However, further research is warranted to explore sex differences in HRR and cardiac autonomic control after different types and protocols of physical exercise.

Study limitations

Although the present study contributes to understanding sex differences in cardiac autonomic responses after HIFT workout, this study faces several limitations, with the primary constraint being the sample size. Larger studies are necessary to reach more robust conclusion regarding the autonomic profile of male and female recreational HIFT practitioners. It is worth noting that since our participants scaled each workout to their ability, the exact intensity and complexity of exercise may have differed among individuals. Unfortunately, we could not provide an exact workout “dose” for each participant, but we believe that these individuals, having extensive experience with HIFT, required fewer changes in exercise intensity, thus remaining consistent with the workout’s specific goals. Future studies should account for this limitation and document each participant’s workout to provide a more accurate representation of the actual training “dose”.

Additionally, daily protein and caloric intake are known to influence changes in body composition and lean mass (Jäger et al., 2017). Although these variables were not monitored or controlled in the present investigation, significant dietary changes were not expected, as participants had been consistently training for over a year. Nevertheless, future investigations should include assessments of nutritional and caloric intake. Furthermore, the evaluation of cardiovascular control mechanisms in HRR was performed using non-invasive maneuvers. Different results might be obtained with supraphysiological stimulation or pharmacological blockade. However, we choose these methods to assess these mechanisms’ performance in a physiological situation, so our results represent their function in real-world physical exercise conditions.

We studied only young, healthy, and physically active participants, which does not allow extrapolation of our results to other populations and/or diseased individuals. Brachial arterial blood pressure was measured using an automated digital sphygmomanometer, not a beat-to-beat continuous method by photoplethysmography. Therefore, we cannot comment on baroreflex sensitivity behavior. Unfortunately, we were unable to verify this mechanism’s action in post-exercise responses with the tools available. Lastly, breathing rate was standardized using a guide-metronome, although using a respiratory pneumatic belt would have been ideal. Respiratory normalization, however, is unnecessary when the goal is to evaluate HRV variation (Bloomfield et al., 2001).

Conclusion

Within the experimental condition employed, our results suggest that women have a slower cardiac vagal reactivation compared to men after recreational HIFT session. These findings have direct implications for the interpretation of the sex-related difference in vagal reactivation in healthy young adults. Moreover, post-exercise parasympathetic reactivation seems to be influenced by resting vagal control, whereby subjects characterized by higher vagal modulation at rest tend to exhibit better parasympathetic reactivation and faster HRR. From a practical perspective, resting vagal modulation appears to play a key role in post-exercise parasympathetic activation. These findings have direct implications for understanding the influence of intensity, exercise modality, and resting vagal modulation on HRR. Finaly, it is imperative that researchers consider demographic factors (especially sex) in HRV studies. Therefore, we must continue to be careful and mindful in our HRV measurements, practices, and analyses.

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Conflict of interest

The authors have no conflicts of interest to declare.

References:

Bloomfield, D. M., Magnano, A., Bigger, J. T. Jr., Rivadeneira, H., Parides, M., & Steinman, R. C. (2001). Comparison of spontaneous vs. metronome-guided breathing on assessment of vagal modulation using RR variability. *American journal of physiology. Heart and circulatory physiology*. 280(3), H1145-H1150. https://doi.org/10.1152/ajpheart.2001.280.3.H1145.

Cole, C. R., Blackstone, E. H., Pashkow, F. J., Snader, C. E., & Lauer, M. S. (1999). Heart-rate recovery immediately after exercise as a predictor of mortality. *The New England journal of medicine*, 341(18), 1351-1357. https://doi.org/10.1056/NEJM199910283411804.

Dart, A. M., Du, X. J., & Kingwell, B. A. (2002). Gender, sex hormones and autonomic nervous control of the cardiovascular system. *Cardiovascular research*. 53(3), 678-687. https://doi.org/10.1016/s0008-6363(01)00508-9.

Elshazly, A., Khorshid, H., Hanna, H., & Ali, A. (2018). Effect of exercise training on heart rate recovery in patients post anterior myocardial infarction. *The Egyptian heart journal: (EHJ): official bulletin of the Egyptian Society of Cardiology*. 70(4), 283-285. https://doi.org/10.1016/j.ehj.2018.04.007.

Ferreira, F. C., Padilha, M. C. S. V., Tobadini, E., Bellocchi, C., Carandina, A., Montano, N., Soares, P. P. S., & Rodrigues, G. D. (2022). Women have a greater cardiac vagal withdrawal to heat stress compared to men. *Temperature*. 10(4), 444-453. https://doi.org/10.1080/23328940.2022.2135354.

Garber, C. E., Blissmer, B., Deschenes, M. R., Franklin, B. A., Lamonte, M. J., Lee, I. M., Nieman, D. C., Swain, D. P., & American College of Sports Medicine (2011). American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. *Medicine and science in sports and exercise*. 43(7), 1334–1359. https://doi.org/10.1249/MSS.0b013e318213fefb.

Goulopoulou, S., Fernhall, B., & Kanaley, J. A. (2009). Hemodynamic responses and linear and non-linear dynamics of cardiovascular autonomic regulation following supramaximal exercise. *European journal of applied physiology*. 105(4), 525-531. https://doi.org/10.1007/s00421-008-0930-4.

Jäger, R., Kerksick, C. M., Campbell, B. I., Cribb, P. J., Wells, S. D., Skwiat, T. M., Purpura, M., Ziegenfuss, T. N., Ferrando, A. A., Arent, S. M., Smith-Ryan, A. E., Stout, J. R., Arciero, P. J., Ormsbee, M. J., Taylor, L. W., Wilborn, C. D., Kalman, D. S., Kreider, R. B., Willoughby, D. S., Hoffman, J. R., Krzykowski, J. L., Antonio, J. (2017). International Society of Sports Nutrition Position Stand: protein and exercise. *Journal of the International Society of Sports Nutrition*. 14, 20. https://doi.org/10.1186/s12970-017-0177-

Javorka, M., Zila, I., Balhárek, T., & Javorka, K. (2002). Heart rate recovery after exercise: relations to heart rate variability and complexity. *Brazilian journal of medical and biological research*. 35(8), 991-1000. https://doi.org/10.1590/s0100-879x2002000800018.

Kliszczewicz, B., Snarr, R. L., & Esco, M. (2014). Metabolic and cardiovascular response to the crossfit workout 'Cindy': a pilot study. *Journal of Sports and Human Performance*. 2(2):1-9. DOI: 10.12922/jshp.0038.2014.

Koenig, J., & Thayer, J. F. (2016). Sex differences in healthy human heart rate variability: A meta-analysis. *Neuroscience & Biobehavioral Reviews*. 64, 288-310. https://doi.org/ 10. 1016/j.neubiorev.2016.03.007.

Lador, F., Azabji Kenfack, M., Moia, C., Cautero, M., Morel, D. R., Capelli, C., & Ferretti, G. (2006). Simultaneous determination of the kinetics of cardiac output, systemic O_2 delivery, and lung O_2 uptake at exercise onset in men. *American journal of physiology. Regulatory, integrative and comparative physiology*. 290(4), R1071-R1079. https://doi.org/10.1152/ajpregu.00366.2005.

Maté-Muñoz, J. L., Lougedo, J. H., Barba, M., García-Fernández, P., Garnacho-Castaño, M. V., & Domínguez, R. (2017). Muscular fatigue in response to different modalities of CrossFit sessions. *PloS one*. 12(7), e0181855. https://doi.org/10.1371/journal.pone.0181855.

Maté-Muñoz, J. L., Lougedo, J. H., Barba, M., Cañuelo-Márquez, A. M., Guodemar-Pérez, J., García-Fernández, P., Lozano-Estevan, M. D. C., Alonso-Melero, R., Sánchez-Calabuig, M. A., Ruíz-López, M., de Jesús, F., & Garnacho-Castaño, M. V. (2018). Cardiometabolic and Muscular Fatigue Responses to Different CrossFit® Workouts. *Journal of sports science & medicine*. 17(4), 668–679.

Matei, D., Catalina, L., Ilie, O., Paula, M., Daniel-Andrei, I., & Ioana, B. (2022). Effects of exercise training on the autonomic nervous system with a focus on anti-inflammatory and antioxidants effects. *Antioxidants*. 11(2), 350. https://doi.org/10.3390/antiox11020350.

Mendonca, G. V., Teodósio, C., & Bruno, P. M. (2017). Sexual dimorphism in heart rate recovery from peak exercise. *European journal of applied physiology*. 117(7), 1373-1381. https://doi.org/10.1007/s00421-017-3627-8.

Michael, S., Graham, K. S., & Davis, G. M., Oam (2017). Cardiac autonomic responses during exercise and post-exercise recovery using heart rate variability and systolic time intervals-a review. *Frontiers in physiology*. 8, 301. https://doi.org/10.3389/fphys.2017.00301.

Morshedi-Meibodi, A., Larson, M. G., Levy, D., O'Donnell, C. J., & Vasan, R. S. (2002). Heart rate recovery after treadmill exercise testing and risk of cardiovascular disease events (The Framingham Heart Study). *The American journal of cardiology*. 90(8), 848-852. https://doi.org/10.1016/s0002-9149(02)02706-6.

Nunes, R. A., Barroso, L. P., Pereira, A. C., Krieger, J. E., & Mansur, A. J. (2014). Gender-related associations of genetic polymorphisms of α-adrenergic receptors, endothelial nitric oxide synthase and bradykinin B_2 receptor with treadmill exercise test responses. *Open heart*. 1(1), e000132. https://doi.org/10.1136/openhrt-2014-000132.

O'Leary D. S. (1996). Heart rate control during exercise by baroreceptors and skeletal muscle afferents. *Medicine and science in sports and exercise*. 28(2), 210–217. https://doi.org/10.1097/00005768-199602000-00009.

Parks, R. J., & Howlett, S. E. (2013). Sex differences in mechanisms of cardiac excitation-contraction coupling. *Pflugers Archiv: European journal of physiology*. 465(5), 747-763. https://doi.org/10.1007/s00424-013-1

Peçanha, T., Bartels, R., Brito, L. C., Paula-Ribeiro, M., Oliveira, R. S., & Goldberger, J. J. (2017). Methods of assessment of the post-exercise cardiac autonomic recovery: A methodological review. *International journal of cardiology*. 227, 795-802. https://doi.org/10.1016/j.ijcard.2016.10.057.

Peçanha, T., Silva-Júnior, N. D., & Forjaz, C. L. (2014). Heart rate recovery: autonomic determinants, methods of assessment and association with mortality and cardiovascular diseases. *Clinical physiology and functional imaging*. 34(5), 327–-339. https://doi.org/10.1111/cpf.12102.

Pierpont, G. L., Stolpman, D. R., & Gornick, C. C. (2000). Heart rate recovery post-exercise as an index of parasympathetic activity. *Journal of the autonomic nervous system*. 80(3), 169-174. https://doi.org/10.1016/s0165-1838(00)00090-4.

Porta, A., Tobaldini, E., Guzzetti, S., Furlan, R., Montano, N., & Gnecchi-Ruscone, T. (2007). Assessment of cardiac autonomic modulation during graded head-up tilt by symbolic analysis of heart rate variability. *American journal of physiology. Heart and circulatory physiology*. 293(1), H702-H708. https://doi.org/10.1152/ajpheart.00006.2007.

Posnakidis, G., Aphamis, G., Giannaki, C. D., Mougios, V., Aristotelous, P., Samoutis, G., & Bogdanis, G. C. (2022). High-intensity functional training improves cardiorespiratory fitness and neuromuscular performance without inflammation or muscle damage. *Journal of strength and conditioning research*. 36(3), 615-623. https://doi.org/10.1519/JSC.0000000000003516.

Rahman, S., Habel, M., & Contrada, R. J. (2018). Poincaré plot indices as measures of sympathetic cardiac regulation: Responses to psychological stress and associations with pre-ejection period. *International journal of psychophysiology*. 133, 79-90. https://doi.org/10.1016/j.ijpsycho.2018.08.005.

Regitz-Zagrosek, V., & Kararigas, G. (2017). Mechanistic pathways of sex differences in cardiovascular disease. *Physiological reviews*. 97(1), 1-37. https://doi.org/10.1152/physrev.00021.2015.

Ristić-Djurović, J. L., Ćirković, S., Mladenović, P., Romčević, N., & Trbovich, A. M. (2018). Analysis of methods commonly used in biomedicine for treatment versus control comparison of very small samples. *Computer methods and programs in biomedicine*. 157, 153-162. https://doi.org/10.1016/j.cmpb.2018.01.026.

Samora, M., Teixeira, A. L., Sabino-Carvalho, J. L., & Vianna, L. C. (2020). Sex differences in cardiac vagal reactivation from the end of isometric handgrip exercise and at the onset of muscle metaboreflex isolation. *Autonomic neuroscience: basic & clinical*. 228, 102714. https://doi.org/10.1016/j.autneu.2020.102714.

Samora, M., Teixeira, A. L., Sabino-Carvalho, J. L., & Vianna, L. C. (2019). Spontaneous cardiac baroreflex sensitivity is enhanced during post-exercise ischemia in men but not in women. *European journal of applied physiology*. 119(1), 103-111. https://doi.org/10.1007/s00421-018-4004-y.

Shafiq, M. A., Ellingson, C. A., Krätzig, G. P., Dorsch, K. D., Neary, J. P., & Singh, J. (2023). Differences in heart rate variability and baroreflex sensitivity between male and female athletes. *Journal of clinical medicine*. 12(12), 3916. https://doi.org/10.3390/jcm12123916.

Shetler, K., Marcus, R., Froelicher, V. F., Vora, S., Kalisetti, D., Prakash, M., Do, D., & Myers, J. (2001). Heart rate recovery: validation and methodologic issues. *Journal of the American College of Cardiology*. 38(7), 1980-1987. https://doi.org/10.1016/s0735-1097(01)01652-7.

Taboni, A., Fagoni, N., Fontolliet, T., Vinetti, G., & Ferretti, G. (2022). Dynamics of cardiovascular and baroreflex readjustments during a light-to-moderate exercise transient in humans. *European journal of applied physiology*. 122(11), 2343-2354. https://doi.org/10.1007/s00421-022-05011-4.

Task Force heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. (1996). *Circulation*, 93(5), 1043-1065. https://doi.org/10.1161/01.CIR.93.5.1043.

Tibana, R. A., de Almeida, L. M., Frade de Sousa, N. M., Nascimento, D.daC., Neto, I. V., de Almeida, J. A., de Souza, V. C., Lopes, M.deF., Nobrega, O.deT., Vieira, D. C., Navalta, J. W., & Prestes, J. (2016). Two Consecutive Days of Crossfit Training Affects Pro and Anti-inflammatory Cytokines and Osteoprotegerin without Impairments in Muscle Power. *Frontiers in physiology*. 28(7), 260. https://doi.org/10.3389/fphys.2016.00260.

Weissman, D. G., & Mendes, W. B. (2021). Correlation of sympathetic and parasympathetic nervous system activity during rest and acute stress tasks. *International journal of psychophysiology*. 162, 60-68. https://doi.org/10.1016/j.ijpsycho.2021.01.015.

Xiong, J., Lian, Z., Zhou, X., You, J., & Lin, Y. (2015). Investigation of gender difference in human response to temperature step changes. *Physiology & behavior*. 151, 426-440. https://doi.org/10.1016/j.physbeh.2015.07.037.