

# The effects of a three-day cycling race on the autonomic nervous system (ANS) and heart rate variability in amateur cyclists

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## Research Article

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# Abstract

## Aims

Increased endurance exercise at high intensities may cause an acute reduction in cardiac function, causing a physiological cascade that releases cardiac biomarkers. Heart rate variability (HRV) is a physiological measurement used to evaluate the autonomic nervous system (ANS) state. This study set out to determine the changes in the ANS by participating in a three-day cycling event using HRV as an outcome measure.

## Methods

Sixteen healthy participants, participated in a three-day cycling event underwent five-minute resting electrocardiography (ECG) recordings in a supine position. In addition, heart rate variability measurements were recorded two days before the race (baseline testing), after each race day, and at 24 hours post-event (recovery).

## Results

Time-domain and frequency domain measures showed significant changes from baseline HRV parameters after each race day ( $p \leq 0.05$ ). These changes reflected an increase in sympathetic activity and parasympathetic withdrawal each day after the event. In addition, our data revealed that the mean heart rate (HR) and R-R variability variables did not return to baseline values after 24 hours of recovery, reflecting ANS dysfunction, and that changes persisted for at least 24 hours post-event.

## Conclusion

Our study demonstrated that competing in an cycling event led to diminished vagal activity and decreased HRV throughout the event, which persisted for at least 24 hours post-event. This physiological stress causes a disturbance in homeostasis and an increase in ANS dysfunction. This dysfunction has implications for further research, including dysrhythmia risk and monitoring of athletes in advising participation and returning to strenuous activity post-endurance events.

## Introduction

Every belief, substantiated by research, is that exercise is good for one. The evidence is that the impact of exercise leads to healthy physiological adaptations and that most endurance athletes benefit from these adaptations.<sup>1</sup> The healthy heart of an endurance athlete can respond effectively to acute exercise and can delay fatigue during prolonged exercise.<sup>1</sup> George et al.<sup>1</sup> studied the acute and chronic adaptation of endurance athletes' hearts and showed that increased endurance exercise might cause an acute reduction in cardiac function, causing a physiological cascade that leads to the release of cardiac biomarkers. This evidence indicates that some endurance athletes may develop a pathophysiological cascade. Athletes and clinicians should be mindful of this and react adequately to this pathophysiological phenomenon.<sup>1</sup> Considering the potential for exercise to cause significant cardiac conduction variations due to stimulation from the autonomic nervous system (ANS), this study set out to determine the effect of participation in a three-day endurance mountain bike cycling event on athletes' hearts using heart rate variability (HRV) as an outcome measure.

Allostasis and allostatic loads are physiological responses due to stress (positive or negative). Allostasis represents the adaptive process of maintaining homeostasis through complex physiological changes and therefore achieving a stable environment in the body.<sup>2,3</sup>

A deteriorated and cumulative allostatic process refers to an allostatic load that the physiological system cannot adapt to.<sup>2,4</sup> In addition, enduring challenges cause impairment of physiological regulating systems, which influences the sympathetic nervous system and the immune system.<sup>5</sup>

Therefore, exercise over time has implications, which can be either good stress (eustress) or bad stress (distress), but the individual's reaction to the stressor causes a physiological change.<sup>2</sup>

The ANS regulates the body's internal functions; the central nervous system (CNS) transmits impulses to peripheral organs, one of which is the cardiovascular system.<sup>6</sup> The ANS controls heart rate (HR), heart contraction force, vasoconstriction, vasodilatation of the blood vessels, and contracting and relaxation of smooth muscles in various organs.<sup>6</sup> The ANS is divided into two systems. The sympathetic and parasympathetic (vagal) systems transmit automatic signals to the organs. The sympathetic system increases metabolic function to cope with challenges outside the body, while the parasympathetic (vagal) system increases functions associated with growth and repair in the body.<sup>7-10</sup>

Heart rate variability is measured using the time between the R-R intervals of the QRS complex on an electrocardiogram (ECG) recording and determining the variability between the consecutive R waves.<sup>8,11,12</sup> In addition, heart rate variability is a physiological measurement used to evaluate the autonomic nervous system (ANS).<sup>8,11,12</sup> Heart rate variability monitoring is a valuable indicator in the diagnosis and prevention strategies of overreaching in athletes.<sup>13</sup> Meeusen et al.<sup>13</sup> defined overreaching as an 'accumulation of training and/or non-training stress resulting in a short-term decrement in performance capacity with or without related physical and psychological signs and symptoms of maladaptation in which restoration of performance capacity may take from several days to several weeks'.

The sympathetic and parasympathetic nervous system stimulates the heart variably and influences heart rate.<sup>8</sup> When the consistency of the R-R intervals has maintained an equilibrium, the sympathetic and parasympathetic nervous systems are in homeostasis.<sup>8</sup> A high HRV indicates an adaptation to exercise, which improves the function of the ANS. A lower HRV indicates a lack of adaptation or inadequate adaptation to exercise, which will impair the ANS.

Often, overreaching is related to several warning signs, one of which includes ANS dysfunction and imbalances.<sup>8</sup> In addition, due to increased exercise, the physiological system is compromised, either from the increased intensity or the duration of exertion.<sup>7</sup> Thus, this study aimed to determine the effect of participation in a three-day endurance mountain bike cycling event on the heart of athletes using the outcome measure of HRV as a marker of autonomic function. The hypothesis was that there would be significant variations in HRV during and after the three days of competing in an endurance mountain bike cycling event, and HRV would not fully recover to baseline values within 24 hours.

## Methods

### Study design and population

The study used a prospective quantitative research design, which involved collecting and analysing numerical data. A second party received no sources of funds, and the authors declare no competing interests.

All participants were amateur mountain bike cyclists with an experience of at least three years and a weekly training volume greater than eight hours per week at a moderate intensity greater than 6 METs. Amateur athletes are athletes who freely participate or do not participate in competitions either for hedonism or health reasons and do not receive material incentives for any representation.<sup>14</sup> Sixteen participants (male and female) took part in a three-day mountain bike cycling event through a sample of convenience. The Standard Bank Klein-Aus Vista mountain bike cycling challenge (Namibia) race organizers approved and provided written permission to conduct the study during their event.

Participants had the opportunity to volunteer to participate in the study after race organizers sent an invitation via email. The researcher then arranged a meeting and presented the details of the study to the participants. During the meeting, an informed consent form was available, and participants had the opportunity to read, question, understand, and sign the consent form before being enrolled in the study. As a result, the Biomedical Research Ethics Committee (BREC) and Research Management Committee (RMC), the Ministry of Health and Social Services in Namibia, and the Humans Research Ethics Committee (Medical) of the University of the Witwatersrand, South Africa, approved the study (clearance certificate M171037).

All participants were encouraged to avoid drinking alcoholic beverages and avoid smoking before and during the study. In addition, no participant reported taking any medication or sympathomimetic drugs that could have affected the cardiovascular system.

## **Characteristics of the three-day mountain bike cycling race**

The event consisted of a three-day mountain bike cycling event covering 12 km with an elevation of 398 m on day one, 65 km with an elevation of 1415 m on day two, and 65 km with an elevation of 1258 m on day three. This event is considered highly technical and only advised for highly experienced riders. The event took place during high temperatures of 37–40 degrees Celsius, and the average total finishing time for the three days was nine hours.

## **Heart rate variability variables**

The recording duration has influenced HRV parameters; the recommended duration for short-term recording is 5 min<sup>15</sup> to ensure comparison of results across studies and laboratories. Due to the physiological origin, the study used time-domain and frequency-domain measures for HRV.<sup>16</sup> Time-domain measures measure the R-R intervals between successive normal complexes, which are responsible for variability in the recording period.<sup>7,17,18</sup> The frequency domain consists of various spectral methods to calculate and estimate the R-R intervals in series, which calculates the absolute and relative power distribution into different frequency bands. These variables provide simple data on how power distributes as a function of frequency.<sup>7,17,18</sup> Table 1 presents a summary of time-domain and frequency-domain measures.

Table 1  
Heart rate variability parameters and their physiological origin.

Time domain measures		
Variable	Description	Physiological origin
HR	Mean Heart Rate	Autonomic modulation of the sinus node
RR-Variability	Mean RR-variability	Autonomic modulation of the sinus node
SDNN	Standard deviation of the sequence of NN intervals	Components responsible for heart rate variability
NN50	Number of pairs of successive RR interval which differ more than 50 ms	Vagal tone
RMSSD	Square root of the mean squared differences between successive RR intervals	Vagal tone
Frequency domain measures		
Variable	Description	Physiological origin
TP	Total power of overall RR-variability	Overall autonomic activity
HF	High-frequency power (0.15-0.40Hz)	Vagal tone
LF	Low-frequency power (0.04-0.15Hz)	Reflects baroreceptor activity, combination of both sympathetic and vagal activity
VLF	Very low-frequency power (< = 0.04Hz)	Inflammation, thermoregulation and hormonal mechanisms
LF/HF	The ratio between low-frequency power / High-frequency power	Balance between sympathetic and vagal activity

## HRV analysis

The Welch Allyn PC-based stress ECG system obtained resting 12-lead standard ECG tracing. In addition, the HRV module in Cardio Perfect (CP) software (version 1.6.6.1146) analyses short-term (five-minute recording) HRV in a series of heartbeat intervals up to a maximal resting ECG recording of five minutes. The CP system provides valid and reliable measures of HRV as established from previous validity and reliability studies.<sup>19-21</sup>

The technical requirements and recommendations for analysis include the need for a proper sampling rate.<sup>15</sup> The optimal range is between 250 and 500 Hz or higher.<sup>17</sup> The Welch Allyn PC-based stress ECG system and CP software have a sampling rate of 300, 600, and 1200 Hz,<sup>21</sup> and the sampling for the study was at 600 Hz.

The CP module automatically detected the QRS complexes and the R-R intervals. Prior to data processing, all ECG tracings were automatically corrected for artifact removal through CP software for ectopic and missed beats; therefore, the normal-to-normal interval (NN). The CP systems default setting for beat rejection is set for a difference of 10% between sequential beats. Therefore, the CP system replaced abnormal beats with linearly interpolated NN intervals based on preceding intervals.<sup>19</sup>

## Data collection

All recordings took place under controlled thermoneutral and quiet conditions. Before baseline testing measures, the researcher informed all participants about the testing procedures and what to expect during the testing and was requested to refrain from any physical activities two days before the baseline testing. Two days before the event, participants' body composition was measured using the International Society for the Advancement of Kinanthropometry (ISAK) standards.<sup>22</sup>

During early morning hours (i.e., from 7:00 am to 9:00 am), baseline testing and 24-hour post-event testing procedures were performed, whereas daily procedures were performed directly after each race, depending on the individual athlete's finishing time. Electrode placement followed the standard 12-lead ECG electrode placement method.<sup>23</sup> Before all ECG recordings, participants underwent a pre-reading stabilization rest period for five minutes to ensure stabilization of heart rate and blood pressure. In addition, the researcher instructed participants to breathe spontaneously during all recordings to avoid any influence on HRV.<sup>24–26</sup>

The resting ECG was recorded in a supine position for five minutes, providing baseline (pre-event) values. The athlete lay still during the five-minute recording to decrease interference and noise.

## Daily procedures

Participants presented to a designated area once crossing the finishing line after each stage for the post-event measurement procedures. They were escorted to the testing facility within two minutes after crossing the finishing line and underwent the pre-reading stabilization period for five minutes before recording HRV by performing a five-minute resting ECG recording in a supine position.

## 24-hour post-event testing procedures

The day after the participants completed the third and final stage of the event, the researchers recorded the 24-hour post-event measurement. Then, participants underwent the pre-reading stabilization period of five minutes of rest before recording HRV by performing a five-minute resting ECG recording in a supine position.

## Statistical analysis

The descriptive data are expressed and summarized as the means  $\pm$  SD and were analysed using the statistical package STATISTICA (version 13.2). The Shapiro-Wilk normality test confirmed non-normality for the normality of HRV distribution. In addition, the study used non-parametric tests, including Friedman's two-way analysis of variance and the Wilcoxon signed-rank test, with results expressed as median and interquartile ranges. Statistical significance was set at  $p \leq 0.05$ .

## Results

The baseline data of the 16 participants (male and female) included age ( $48.75 \pm 7.41$  years), weight ( $78.48 \pm 12.18$  kg), height ( $174.31 \pm 6.90$  cm), BMI ( $25.71 \pm 2.64$ ), body fat ( $12.94 \pm 3.28\%$ ), and waist-to-hip ratio ( $0.86 \pm 0.06$ ).

## Time domain measures

Compared with baseline testing, mean heart rate increased, and R-R variability decreased significantly (Table 2) and continued to increase and decrease, respectively, until after days two and three of the event but did not recover back to baseline measurements 24 hours post-event. At 24 hours post-event, the mean HR and R-R variability were significantly higher and lower, respectively, than the baseline measurements (Table 2). The box-and-whisker plot in Fig. 1 and Fig. 2 represents HR and R-R variability indicating minimum, quartile one, median, quartile three, reflecting significance from baseline through 24 hours post-event.

Table 2  
Changes in Time domain measures from baseline to 24-hour post-event (n = 16).

Time domain measures										
Measurement	HR (bpm)		RR-Variability (ms)		SDNN (ms)		NN50 (no.)		RMSSD (ms)	
	Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value
Baseline	59.50 ± 8.45	-	1031.81 ± 150.00	-	47.63 ± 21.57	-	47.25 ± 50.01	-	43.69 ± 30.16	-
Day 1	82.00 ± 8.66	0.00***	738.90 ± 71.83	0.00***	23.81 ± 10.18	0.00***	5.56 ± 13.79	0.01**	38.06 ± 103.32	0.84
Day 2	89.00 ± 9.00	0.00***	681.75 ± 69.04	0.00***	20.44 ± 11.12	0.00***	2.56 ± 3.58	0.00***	11.56 ± 5.53	0.00***
Day 3	88.06 ± 8.65	0.00***	688.88 ± 70.23	0.00***	22.06 ± 17.33	0.00***	7.69 ± 19.31	0.01*	16.50 ± 17.49	0.01**
24-Hour post- event	65.38 ± 8.40	0.03*	931.75 ± 125.36	0.03*	43.44 ± 21.69	0.57	27.60 ± 41.07	0.10	34.44 ± 25.18	0.23
Level of significant differences from *p ≤ 0.05, ** p ≤ 0.01, ***p ≤ 0.001. Abbreviations: HR = Heart rate; SDNN = Standard deviation of the sequence of normal-to-normal intervals; NN50 = Number of pairs of successive RR interval which differ more than 50 ms; RMSSD = Square root of the mean squared differences between successive RR intervals, ms = milliseconds, no.= number.										

The interval measures of both SDNN and NN50 decreased significantly from day one compared to baseline (Table 2) and continued to decrease until after day two. After day three, there was a slight increase compared to day two but significantly lower than baseline values (Table 2). The 24-hour recovery revealed no significant difference between baseline and 24-hour post-event.

The RMSSD decreased significantly (Table 2) on day two compared to baseline and decreased until after day three. After day three, there was a slight increase in the RMSSD compared to that on day two, but the RMSSD was significantly lower than the baseline values (Table 2). The 24-hour post-event RMSSD value revealed no significant difference between baseline and 24-hour post-event.

## Frequency domain measures

Changes in TP, HF, VLF, and LF/HF, indicative of cardiac conduction variations, were associated with a significant (Table 3) change in HRV from day one compared to baseline and continued to change significantly until after day three. There were no statistically significant differences between baseline and 24-hour post-event. The LF decreased significantly (Table 3) from baseline after day one, but no further changes were measured after days two and three or at 24-hour hours post-event.

Table 3  
Changes in Frequency domain measures from baseline to 24-hour post-event (n = 16).

Frequency domain measures										
Measurement	TP (ms)		HF (ms)		LF (ms)		VLF (ms)		LF/HF (%)	
	Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value	Mean ± SD	p-value
Baseline	2847.06 ± 3127.77	-	930.56 ± 1133.74	-	1012.44 ± 1271.96	-	904.13 ± 1230.69	-	1.43 ± 0.88	-
Day 1	512.56 ± 499.47	0.00***	60.25 ± 67.39	0.01**	215.69 ± 180.74	0.01**	236.56 ± 268.88	0.02*	6.23 ± 6.73	0.02*
Day 2	465.06 ± 624.08	0.01**	35.19 ± 38.23	0.01**	299.31 ± 534.66	0.07	130.69 ± 135.55	0.03*	8.24 ± 6.93	0.00***
Day 3	562.94 ± 945.72	0.01**	137.94 ± 347.65	0.02*	296.13 ± 522.87	0.07	129.00 ± 133.87	0.02*	5.14 ± 4.37	0.00***
24-Hour post-event	1807.38 ± 1770.36	0.23	523.88 ± 705.22	0.19	739.75 ± 1175.16	0.50	543.75 ± 445.89	0.30	2.09 ± 1.46	0.11

Level of significant differences from \*p ≤ 0.05, \*\* p ≤ 0.01, \*\*\*p ≤ 0.001. Abbreviations: TP = Total power of overall RR-variability; HF = High-frequency power; LF = Low-frequency power; VLF = Very low-frequency power; LF/HF = Ratio between low-frequency power and high-frequency power, ms = milliseconds.

## Discussion

Well-documented research exists in the field of HRV assessing the consequences of endurance exercise responses.<sup>7,8,10,27</sup> The consequences of endurance exercise responses have led to an increased interest in research. In recent studies by Kaikkonen et al.,<sup>27</sup> Martinmäki et al.,<sup>28</sup> and Perkins et al.,<sup>29</sup> the key factors determining post-exercise HRV appeared to be the exercise intensity and exercise duration.

## Effects on heart rate response after an endurance event

In our study, the HR at post-event measures on day one, day two, day three, and 24-hour post-event remained higher than that of the baseline values. This resultant sympathetic dominance influences the cardiac conduction variations in the heart through the sympathetic nerves. Our findings suggest that there is some form of continued sympathetic stimulation after an endurance event and that the sympathetic drive continues well into the recovery phase. This sympathetic dominance and parasympathetic withdrawal implies a cardiac physiological or a possibly pathophysiological cascade from day one up to at least 24 hours post-event.

## Effects on Time domain measures

Time-domain measures showed sympathetic dominance and parasympathetic withdrawal at rest after each race day, which significantly changed (decreased) up to the final day. In addition, two other studies reported a decrease in RR intervals, SDNN, NN50, and RMSSD, during endurance events, indicative of sympathetic dominance and parasympathetic withdrawal,<sup>30,31</sup> in line with the findings of our study.

## Effects on frequency domain measures

Our results further showed a significant decrease in TP over the event's three days. As previously noted, the resting HR post-event remained higher than the baseline values. The increased workload, physiological stress on the body, and sympathetic dominance lead to an increased resting heart rate. Thus, the resultant relative tachycardia reduced TP, leading to a significant decrease in HRV across the three days of the event.

Low VLF power not only predicts autonomic dysfunction but also indicates an increase in inflammation.<sup>32</sup> This increase in inflammation is often seen due to the sympathetic response and promotes the repair of exercise-induced myocardial and skeletal muscle damage.<sup>32</sup> Our study notices the change after day two, where there is a slight change from sympathetic dominance to parasympathetic dominance. The sympathetic system increases metabolic function to cope with exogenous challenges, while the parasympathetic (vagal) system will start to dominate to increase functions associated with growth and repair in the body.<sup>32</sup>

Therefore, although there was a decrease in power throughout the study period and the power started to plateau, one may link this to the increase in inflammation reflected in the VLF power. This inflammation may indicate inflammatory modulation, promoting healing and repairing exercise-induced myocardial and musculoskeletal damage from day two and continuing through day three.

The LF/HF ratios measuring greater than 2.0 express the sympathovagal balance and prevalence of sympathetic dominance.<sup>33</sup> Our study showed a disturbance and significant sympathetic dominance as reflected by the LF/HF ratio after days one, two, and three. This sympathetic dominance may result from decreased performance and increased inflammation, indicated by ANS dysfunction. Therefore, adequate recovery is necessary to promote repair and healing and maintain/improve performance. Our study showed that the most significant disturbance in LF/HF occurred after day two ( $8.24 \pm 6.93$ ). The ANS began to improve after day three ( $5.15 \pm 4.37$ ) but remained blunted, which indicates that although the participants were at rest, their ANS remained in a state of sympathetic dominance. The decrease in HF power found in our study indicates decreased vagal activity, reflecting an increase in an undesirable stimulus, possibly due to the extreme conditions and the duration and intensity of the endurance event.

## Recovery time and HRV

Both Hautala et al.<sup>34</sup> and Dong<sup>10</sup> have addressed the implication that may arise during persistent abnormal changes in autonomic regulation: that there is an increased risk of sudden cardiac death in athletes who present with diminished vagal activity, indicated by a decrease in HRV with the variability becoming constant.<sup>10</sup> Thus, recovery to baseline is prudently significant.

## Limitations

Our study presented the following limitations. First, there was a small number of participants. Second, assessment up to 24 hours post-event and not beyond, which in our study showed that the vagal activity remained blunted up to at least 24 hours after recovery. Bernardi et al.,<sup>35</sup> Hautala et al.,<sup>34</sup> and Gratze et al.<sup>36</sup> showed that cardiac parasympathetic activity returned to baseline levels after 24 hours of recovery and that complete cardiac autonomic recovery can take up to 48 hours of recovery. Finally, the study only used the time-domain and frequency-domain measures. Perhaps including Nonlinear HRV analysis would have added higher merit to the study outcome.

## Conclusion

Our study provides the following information regarding HRV and a three-day mountain bike cycling event:

- The data show that 24 hours is not an adequate period for the full recovery of the ANS after an endurance event. Therefore, when assessing recovery after an endurance event, one should be wise to assess recovery beyond 48 hours.

- HRV can be used to analyse the stress of the endurance event on the ANS, which in our study was significant after day one and continued through days two and three of the event.
- ANS dysfunction can correlate with sudden cardiac arrest, suggesting that healthcare and emergency personnel should be particularly aware of increased risk on day two of an endurance event.

Therefore, we recommend using HRV as a measuring tool to assess cardiac autonomic activity. As a result, sports physicians, athletes, and coaches can assess the stress of endurance events on the ANS and plan for correct recovery strategies after a single endurance event. Furthermore, we recommend that HRV should not only be used to assess recovery but should also be part of a screening tool where sports physicians can assess the state of the ANS before an endurance event. The reasoning is that if the ANS is in a state of dysfunction, susceptible amateur athletes might be at risk for a cardiovascular event. Further research is needed to show that cardiac events can be reduced during sporting events when assessing the state of the ANS through HRV measurements, especially when individuals present with an increased risk of cardiac disease.<sup>19</sup>

## Declarations

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### Conflict of interest

None declared.

### Authors' contributions

A. Swart participated in the design of the study, contributed to data collection, data analysis, and interpretation of results, and contributed to the writing of the manuscript; D. Constantinou participated in the writing of the manuscript and provided supportive feedback and supervision during the research study. All authors have read and approved the final version of the manuscript and agree with the order of presentation of the authors.

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## Figures

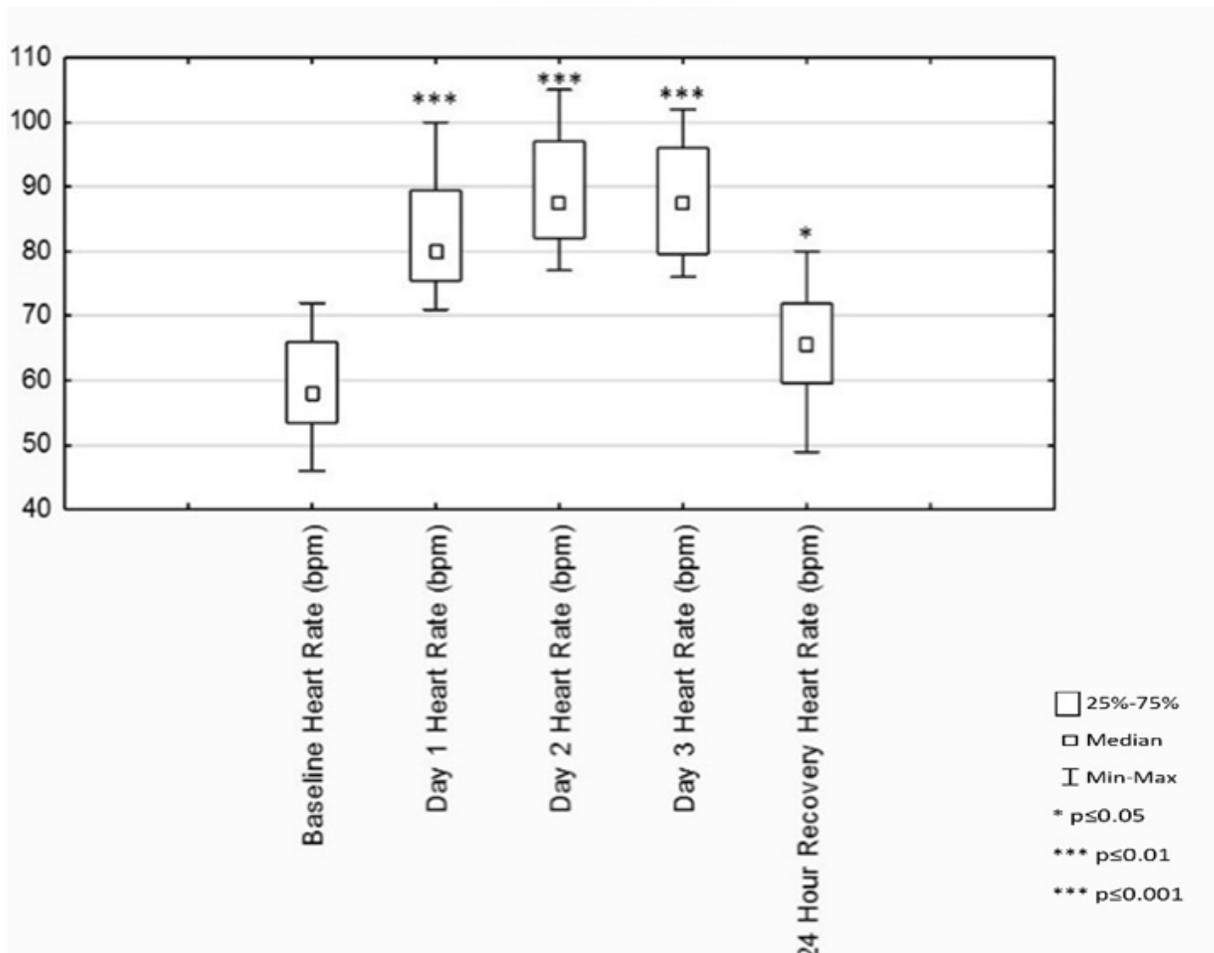


Figure 1

Box & Whisker Plot of Heart Rate (n=16)

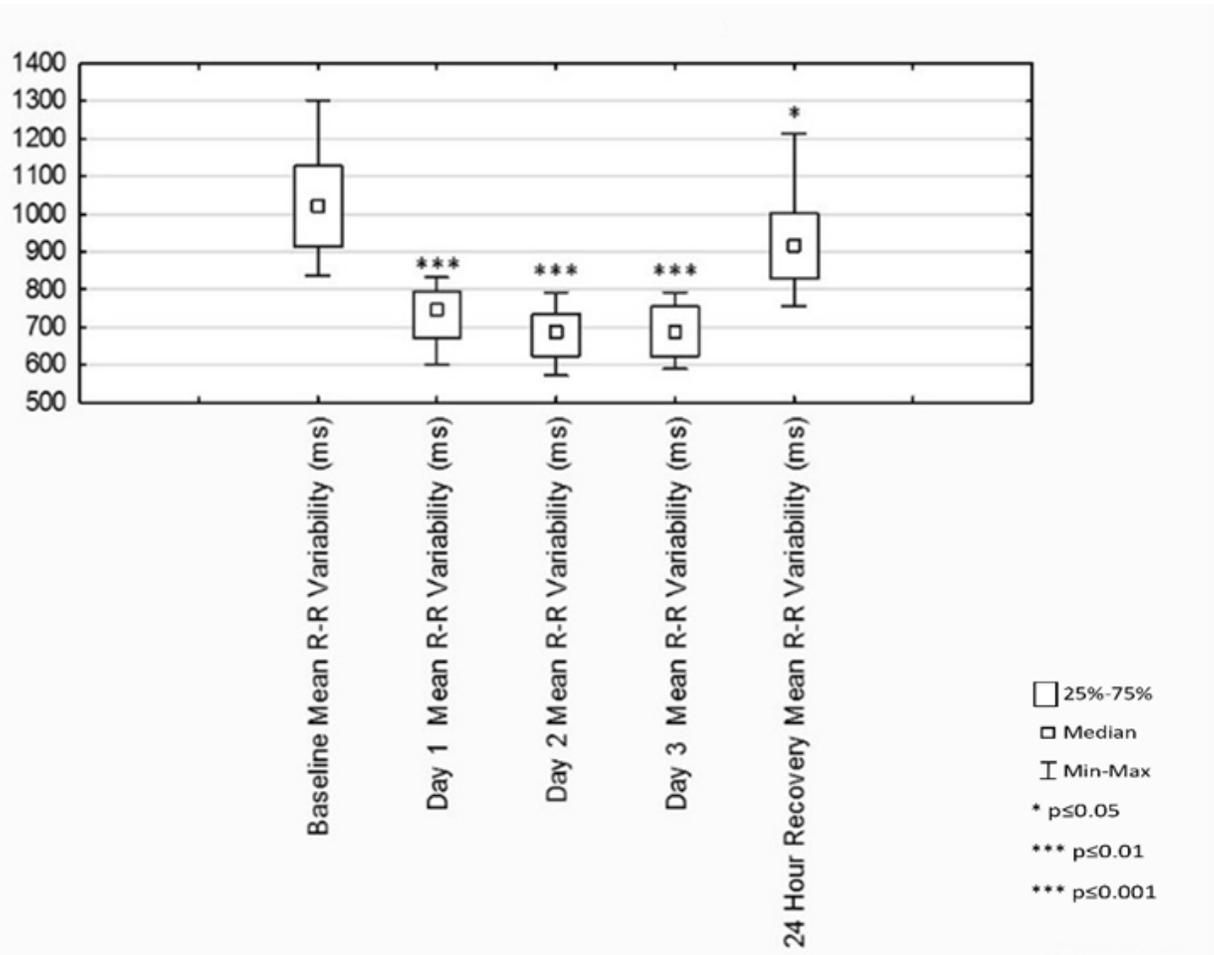
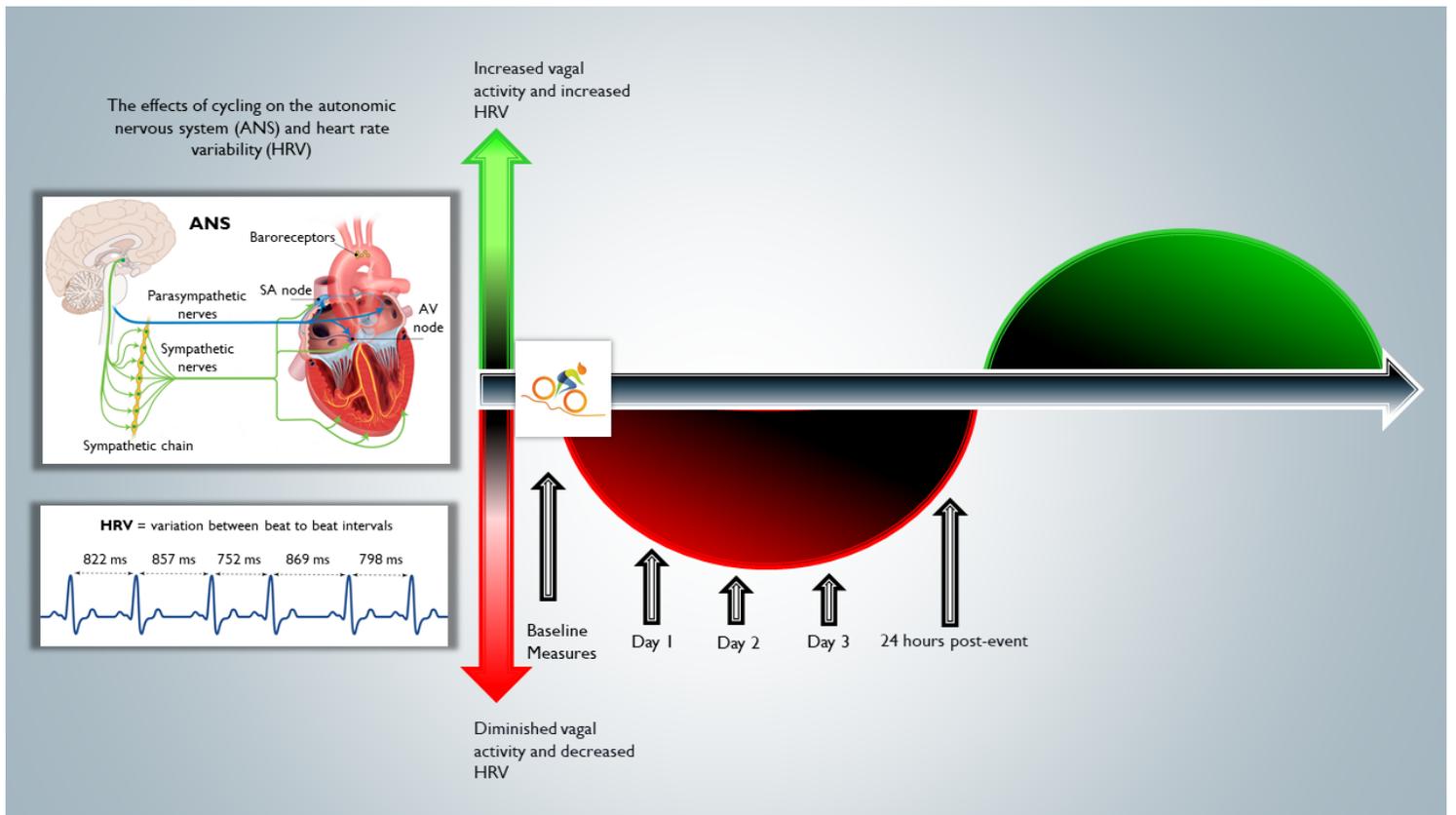


Figure 2

Box & Whisker Plot of the Mean R-R Variability of HRV (n=16)



**Figure 3**

Graphical abstract