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Acute Cardiac Biomarker Responses to High-Intensity Interval and Continuous Running Under Matched Physiological Load in Middle-Aged Recreational Men

ABSTRACT

Objective: High-intensity interval exercise (HIIE) is gaining popularity due to its efficiency and cardiovascular benefits. However, it remains unclear whether intermittent supramaximal exercise bouts induce greater acute cardiac biomarker responses than continuous exercise (CE) when overall physiological workload is matched. This study aimed to compare the acute cardiac biomarker responses to HIIE and CE in recreational runners under equivalent exercise conditions.

Methods: Fifteen male recreational runners (41.8 ± 8.3 years; maximal oxygen uptake [VO_2max] 46.8 ± 4.1 mL·kg^{-1}·min^{-1}) completed 2 1-hour outdoor running sessions in a randomized crossover design with 1-2-week washout. Protocols were CE (~72% of velocity at VO_2max [vVO_2max]) and HIIE (12 × 1-minute intervals at ~107% vVO_2max, 4-minutes recovery jogs). Blood samples were collected before (PRE), immediately after (POST), and 3 hours post-exercise (POST+3) to assess serum high-sensitivity cardiac troponin I (hs-cTnI), N-terminal pro-B-type natriuretic peptide (NT-proBNP), creatine kinase-MB, and myoglobin. Heart rate, speed, and percentage vVO_2max were comparable between protocols.

Results: High-sensitivity cardiac troponin I increased significantly at POST+3 versus PRE in both protocols ($P < 0.001$), with no between-protocol differences. Creatine kinase-MB and myoglobin rose significantly at POST and POST+3 in both conditions ($P < .05$). N-terminal pro-B-type natriuretic peptide showed no significant changes between protocols. No biomarker differed between HIIE and CE at any interval, and hs-cTnI and NT-proBNP remained within clinical reference ranges throughout.

Conclusion: When duration and workload are matched, HIIE does not elicit greater acute cardiac biomarker responses than CE, suggesting intermittent supramaximal exercise does not impose greater acute myocardial stress than CE in recreationally active middle-aged men.

Keywords: Cardiac biomarkers, CK-MB, continuous exercise, high-intensity interval exercise, hs-cTnI, myoglobin, NT-proBNP, running

INTRODUCTION

Regular aerobic exercise is a well-established strategy for promoting cardiovascular health and preventing chronic diseases.[1] Accordingly, the World Health Organization recommends regular engagement in moderate to vigorous intensity aerobic activity to enhance cardiorespiratory fitness and reduce cardiometabolic risk.[2] Among aerobic activities, running remains one of the most practiced forms, with participation increasing substantially in recent years.[3] Most training volume in running is typically performed at lower intensities as continuous running, while a smaller proportion consists of high-intensity interval exercise (HIIE), which has been increasingly incorporated into runners' training programmes. High-intensity interval exercise is characterized by repeated bouts of vigorous to supramaximal effort interspersed with recovery periods of lower intensity.[4] Current evidence indicates that HIIE can induce improvements in cardiorespiratory fitness, vascular function, and metabolic health that are comparable or superior to those achieved

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ORIGINAL INVESTIGATION

Yiğitcan Menderes[1]
Şenay Akın[2]
Yeşim Somay Selbes[3]
Carl J. Lavie[4]
Yunus Emre Ekinci[5]
Ömer Faruk İlicepınar[6]
Yiğitcan Karanfiil[6]
Haydar A. Demirel[7]

[1]*Unit of Sports Medicine, Muğla Training and Research Hospital, Muğla, Türkiye*
[2]*Department of Exercise and Sport Sciences, Division of Exercise and Sport Physiology, Hacettepe University, Faculty of Sport Sciences, Ankara, Türkiye*
[3]*Hacettepe University, Turkish Doping Control Center, Ankara, Türkiye*
[4]*John Ochsner Heart and Vascular Institute, Department of Cardiovascular Diseases, Ochsner Clinical School - University of Queensland School of Medicine, New Orleans, LA, USA*
[5]*Department of Exercise and Sport Sciences, Hacettepe University, Faculty of Sport Sciences, Training and Movement Sciences, Ankara, Türkiye*
[6]*Division of Sports Medicine, Hacettepe University, Faculty of Medicine, Ankara, Türkiye*
[7]*Near East University, Center for Sport Sciences, Nicosia, Cyprus*

Corresponding author:
Haydar A. Demirel
✉ haydar.demirel@neu.edu.tr

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with continuous exercise (CE), despite requiring lower overall exercise volumes.[5] These advantages have contributed to the growing interest in interval-based exercise strategies in both performance and health contexts.[6]

Notwithstanding these demonstrated benefits, the cardiovascular safety of HIIE remains an area of ongoing investigation. The rapid and repeated elevations in heart rate (HR) and cardiac output inherent to HIIE transiently increase myocardial workload, raising concerns regarding acute cardiac stress and potential adverse cardiovascular responses, including ventricular remodeling and arrhythmogenesis.[7,8] These observations align with evidence supporting a J shaped dose–response relationship, where excessive or prolonged endurance exercise may increase the risk of arrhythmias and other cardiac events.[9] Acute elevations in circulating cardiac biomarkers such as cardiac troponins and natriuretic peptides are frequently observed following strenuous exercise, raising important questions about whether these responses represent physiological adaptations to exercise or early indicators of myocardial injury. Circulating cardiac biomarkers therefore provide indirect insight into transient myocardial stress and are widely used to evaluate acute cardiac responses to exercise. While elevations in cardiac biomarkers have been well-documented following prolonged endurance events, comparatively fewer studies have examined such responses following HIIE.[10] Moreover, prior comparative work examining CE and HIIE has primarily focused on cardiac troponin T (cTnT), whereas cardiac troponin I (cTnI), another clinically relevant marker of myocardial injury, has received less attention.[11]

However, several important gaps remain in the current literature. Most existing evidence has been derived from young or highly trained individuals.[12] Middle-aged recreational runners, who may present a less favorable cardiovascular risk profile than younger athletes, have received considerably less attention in studies examining exercise-induced cardiac biomarker responses. Furthermore, several previous investigations did not include blood sampling during the time window in which circulating troponin concentrations typically peak, approximately 3-4 hours after exercise.[13] Consequently, the temporal profile of cTnI responses to HIIE, particularly in middle-aged recreational runners, remains insufficiently characterized.

To address these gaps, the present study compared the acute effects of 1 hour of CE and HIIE on circulating levels of high-sensitivity cTnI (hs-cTnI), N-terminal pro-B-type natriuretic peptide (NT-proBNP), creatine kinase-MB (CK-MB), and myoglobin in middle-aged recreational runners. By evaluating both immediate and delayed biomarker responses and matching key physiological parameters, including average HR, running speed, and percentage of velocity at VO_2max (vVO_2max), the present design enabled a controlled comparison of interval and CE under comparable overall physiological workload.

HIGHLIGHTS

- Physiologically matched interval and continuous running elicited similar cardiac biomarker responses.
- High-intensity interval exercise did not increase high-sensitivity cardiac troponin I (cTnI) more than continuous exercise.
- Both cTnI and N-terminal pro-B-type natriuretic peptide concentrations remained below clinically relevant thresholds.

METHODS

Study Design

This study was conducted in middle-aged recreational men. The protocol was approved by the Hacettepe University Ethics Committee (approval number GO 23/521), and all procedures were conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants. The study consisted of 3 laboratory and field visits conducted over a period of 3-4 weeks using a randomized crossover design. During the first visit, participants completed anthropometric assessments and performed a maximal incremental treadmill test to determine VO_2max. They also completed a standardized questionnaire detailing their training history and weekly running routines. The exercise trials were carried out during the second and third visits. Each participant performed 2 running sessions on a 400-m outdoor track in a randomized crossover design: a 1-hour continuous running session and a HIIE session.

Participants

Fifteen male recreational runners (mean age: 41.8 ± 8.3 years) voluntarily participated in this study. All participants had a history of recreational endurance running (7.87 ± 6.28 years) and regularly participated in long-distance events such as marathons, ultramarathons, or trail races. Preexercise questionnaires confirmed that all participants had prior experience with HIIE as part of their usual training. Exclusion criteria included a history of cardiovascular disease, cardiac symptoms, hypertension, chronic illnesses, or the use of medications known to affect cardiovascular function (e.g., β-blockers, angiotensin-converting enzyme inhibitors, or other antihypertensives). Prior to inclusion, preparticipation screening was based on medical history and physical examination; resting 12-lead electrocardiography and echocardiography were not performed. Participants also completed a standardized questionnaire regarding their training history and weekly training routines.

Baseline Assessment

One week before the exercise trials, participants underwent baseline assessments including anthropometric measurements and maximal oxygen uptake (VO_2max) testing. Participants were instructed to refrain from strenuous physical activity, alcohol, and caffeinated beverages for at least 24 hours before testing and to maintain their usual diet. Body weight and body composition were assessed using a bioelectrical impedance analyzer (Tanita TBF-401A, USA), and height was measured with a wall-mounted stadiometer (Holtain Ltd., UK). VO_2max testing was conducted between

10:00 AM and 12:00 PM. Heart rate was recorded at 1-second intervals using a HR monitor (Polar V800, Polar Electro, Kempele, Finland). After a 5-minute warm-up at 6 $km \cdot h^{-1}$ on a motorized treadmill (Ergo ELG2, Woodway, Germany), the incremental running test began at 7 $km \cdot h^{-1}$, with running speed increased by 1 $km \cdot h^{-1}$ each minute until volitional exhaustion. The treadmill was set at a 1% incline to simulate outdoor running conditions.[14] Oxygen uptake was measured using a breath-by-breath gas analysis system (Quark CPET, Cosmed, Italy). VO_2max was defined as the highest VO_2 value averaged over the final 30 seconds of the last completed stage. VO_2max was considered achieved when at least 2 of the following criteria were met: (i) a plateau in VO_2, defined as an increase of <150 mL min^{-1} between the final 2 stages; (ii) a respiratory exchange ratio > 1.10; (iii) attainment of ≥90% of age predicted maximum HR (220 − age); and (iv) a rating of perceived exertion (RPE) ≥ 17. All participants satisfied at least 2 of these criteria.

Exercise Trials

The exercise trials were conducted on a 400-m outdoor track between 10:00 AM and 12:00 PM, with ambient temperatures ranging from 15 to 20°C. Participants were instructed to abstain from strenuous physical activity and alcohol for 48 hours before each trial and to avoid caffeine for 24 hours prior to testing, while water intake was allowed ad libitum. Before each exercise trial, participants performed a 10-minute warm-up consisting of light jogging and dynamic stretching.

Exercise intensity was quantified as a percentage of the vVO_2max based on individual VO_2max results. Running speed and HR were continuously monitored using a HR monitor (Polar V800, Polar Electro, Kempele, Finland) to verify pacing consistency and support post-session intensity analysis. The CE session consisted of 1 hour of uninterrupted running performed at a self-selected pace representative of the participants' habitual training intensity. Post-exercise analysis of running speeds indicated that this pace corresponded to approximately 71.7% of vVO_2max and 103 ± 8% of the velocity at the ventilatory threshold. The HIIE protocol included 12 repetitions of 1-minute fast running followed by 4-minute self-paced jogging. The fast intervals corresponded on average to 107.2% of vVO_2max, indicating supramaximal effort. The order of the exercise protocols was randomized, with a washout period of at least 1 week (maximum 2 weeks) between sessions.

Blood Sampling and Biomarker Analysis

For both exercise trials, venous blood samples were collected from the antecubital vein of the non-dominant arm at 3 time points: before exercise (PRE), within 10 minutes after exercise (POST), and 3 hours after exercise (POST+3). Samples were allowed to clot at room temperature for 30 minutes and were then centrifuged at 1500 rpm for 15 minutes using a refrigerated centrifuge (Universal Centrifuge Z 323 K, Hermle Labortechnik, Germany) to separate serum. Aliquots of 200 µL were transferred into microcentrifuge tubes and stored at -80°C (Forma 8800 Series, Thermo Scientific, USA) until biomarker analysis. Serum concentrations of hs-cTnI, NT-proBNP, CK-MB, and myoglobin were measured using a fully automated chemiluminescent immunoassay system (ADVIA Centaur CP, Siemens Healthcare Diagnostics, Germany) with manufacturer-provided reagents and calibrators. The detection ranges for each biomarker were as follows: hs-cTnI (2.5-25 000 pg/mL), NT-proBNP (35-35 000 pg/mL), CK-MB (0.18-300 ng/mL), and myoglobin (3-1000 ng/mL). According to the manufacturer, the respective upper reference limits were 125 pg/mL for NT-proBNP, 47 pg/mL for hs-cTnI, 5 ng/mL for CK-MB, and 110 ng/mL for myoglobin.[15,16] All samples were analyzed in duplicate, and the mean value was used for statistical analysis. Assay calibration and internal quality control procedures were performed according to the manufacturer's instructions.

Statistical Methods

A priori power analysis was performed using G*Power (version 3.1.9.7). Effect size estimates were derived from previous studies examining exercise-induced cardiac biomarker responses.[13,17] Assuming a conservative effect size ($f = 0.25$), $\alpha = 0.05$, and power = 0.80, the analysis indicated that a minimum of 14 participants were required. Data are presented as median (interquartile range). Normality was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk tests. Since cardiac biomarker concentrations were not normally distributed, non-parametric tests were applied. Within-protocol changes across PRE, POST, and POST+3 were evaluated using the Friedman test with Wilcoxon signed-rank post-hoc comparisons. To account for inter-individual and inter-occasion variability inherent to the crossover design and to isolate the effect attributable to each exercise protocol, between-protocol comparisons were performed on delta (Δ) change scores (Δ POST−PRE, Δ POST+3−PRE, and Δ POST+3−POST) rather than on raw biomarker concentrations. Between-protocol differences in Δ values were assessed using the Wilcoxon signed-rank test, appropriate for the paired, within-subject structure of the crossover design. For all analyses other than the Bonferroni-corrected comparisons, a $P < .05$ was considered statistically significant. Correlations were assessed using Pearson or Spearman tests as appropriate. To quantify the magnitude of biomarker responses across time points, effect sizes (r) were calculated using the method described by Cohen.[18] Analyses were performed using SPSS (Statistical Package for the Social Sciences) (version 23, IBM Corporation, Armonk, NY, USA).

RESULTS

Participant characteristics and training history are presented in Table 1.

Session Parameters

Heart rate and running speed data recorded during the CE and HIIE sessions are presented in Table 2. Average HR, running speed, and percentage vVO_2max were comparable between the 2 protocols. The fast intervals in the HIIE protocol were performed above vVO_2max, representing brief supramaximal bouts within an otherwise matched exercise load.

Table 1. Participant Characteristics

Variables	Mean ± SD
Age (years)	41.8 ± 8.3
Body weight (kg)	75.9 ± 9.1
Height (cm)	175.1 ± 4.3
Body fat percentage	12.1 ± 3.4
VO_2max (mL/kg/min)	46.8 ± 4.1
Years of running	7.87 ± 6.28
Weekly running sessions (hours)	3.27 ± 1.44
Duration per session (minutes)	65.33 ± 12.31
Distance per session (km)	11.13 ± 2.26
Weekly running distance (km)	36.93 ± 17.11

Biomarker Responses

The effects of CE and HIIE sessions on cardiac biomarkers are summarized in Figure 1, with detailed statistical outcomes presented in Table 3.

High-sensitivity cardiac troponin I concentrations increased over time in both exercise protocols ($P < .001$), with a significant immediate rise observed after CE ($P < .01$) and a delayed elevation at POST+3 following HIIE. At POST+3, hs-cTnI levels were comparable between protocols, with large effect sizes ($r > 0.85$).

N-terminal pro-B-type natriuretic peptide increased significantly immediately after HIIE ($r = 0.66$, $P = .011$), while the increase after CE ($r = 0.61$, $P = .018$) did not reach the corrected significance threshold ($P < .017$).

Creatine kinase-MB and myoglobin increased significantly at both post-exercise time points in both exercise conditions, with moderate to large effect sizes ($r = 0.66$-0.88).

Between-Protocol Comparisons

Between-protocol differences in biomarker change scores (Δ POST−PRE, Δ POST+3−PRE, Δ POST+3−POST) are summarized in Table 4. No statistically significant differences were found for any biomarker at any interval.

Clinical Threshold Exceedances

High-sensitivity cardiac troponin I and NT-proBNP concentrations remained within clinical reference limits in both exercise protocols, except for 1 elevated baseline NT-proBNP value. Creatine kinase-MB and myoglobin exceeded their clinical thresholds in several participants, particularly at POST and POST+3, in both CE and HIIE sessions.

Correlation Analyses

Correlation analyses between participant characteristics, exercise parameters, and biomarker responses are summarized in Table 5, with corresponding scatter plots provided in the Supplementary Material.

DISCUSSION

The present study evaluated acute cardiac biomarker responses to 1-hour CE and HIIE in middle-aged recreational runners. The principal finding was that intermittent supramaximal bouts did not induce greater cardiac biomarker responses than CE when exercise duration and overall physiological intensity were matched. Importantly, hs-cTnI and NT-proBNP did not exceed established clinical reference limits, suggesting that the observed responses likely represent transient physiological responses to exercise rather than clinically relevant myocardial injury. Collectively, these findings suggest that, when overall physiological workload is matched, HIIE does not appear to impose greater acute myocardial stress than CE in recreationally active middle-aged men.

High-Sensitivity Cardiac Troponin I

In the present study, hs-cTnI exhibited a delayed temporal response, characterized by modest immediate changes and more pronounced elevations at 3 hours post-exercise. This temporal pattern is consistent with previous reports showing that exercise-induced cardiac troponin elevations typically peak several hours after exercise rather than immediately upon cessation,[19-21] highlighting the importance of post-exercise sampling timing when interpreting acute hs-cTnI responses. The inclusion of a 3-hour post-exercise sampling

Table 2. Heart Rate and Running Speed Parameters During HIIE and CE Sessions

Parameters	CE (Mean ± SD)	HIIE (Mean ± SD)	*P*
HRmax during VO_2max test (bpm)	178.7 ± 8.2		
Average HR (bpm)	141.4 ± 17.1	147.5 ± 11.1	.254
HR during fast running phase (bpm)	-	158.2 ± 10.9	
HR during slow running phase (bpm)	-	144.9 ± 11.5	
Average speed (km/h)	10.5 ± 1.1	10.4 ± 0.9	.649
Speed during fast running phase (km/h)	-	16.1 ± 1.5	
Speed during slow running phase (km/h)	-	8.95 ± 1.05	
Percentage of vVO_2max average	71.7 ± 1.4	73 ± 4	.249
Percentage of vVO_2max during fast running phase	-	107.2 ± 4.9	
Percentage of vVO_2max during slow running phase	-	64.4 ± 5.0	

Fast running phase: high-intensity intervals of the HIIE session; slow running phase: recovery intervals of the HIIE session.
No significant differences were observed between CE and HIIE in terms of average heart rate ($P = .254$), average running speed ($P = .649$), or average %vVO_2 max ($P = .249$).
bpm, beats per minute; CE, continuous exercise; HIIE, high-intensity interval exercise; HR, heart rate; HRmax, maximum heart rate.

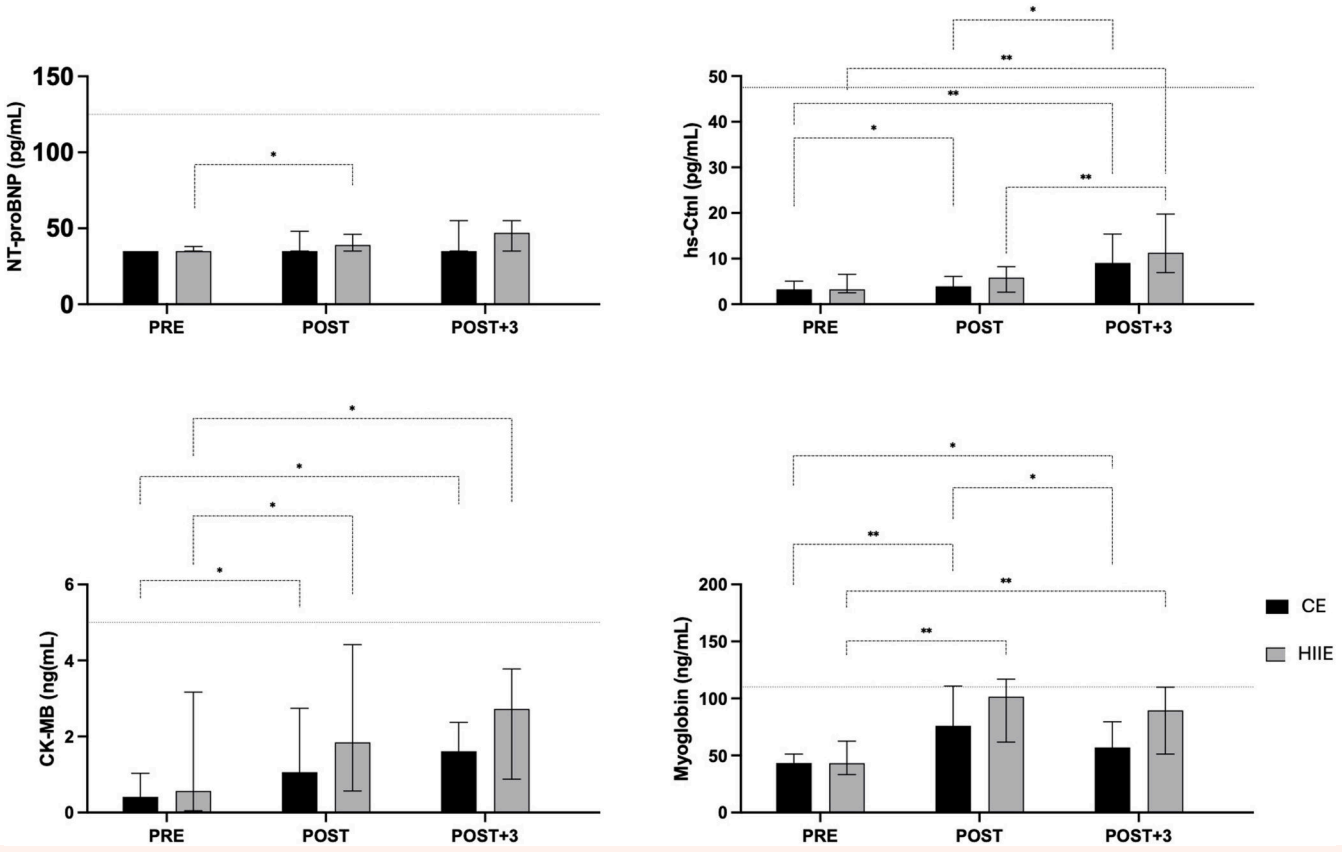

Figure 1. Biomarker concentrations at baseline (PRE), 1 hour (POST), and 3 hours (POST+3) following CE and HIIE protocols are presented as median and interquartile range. Dotted line indicates the clinical reference threshold. ****P* < .05, *P* < .001. **CE, continuous exercise; CK-MB, creatine kinase myocardial band; cTnI, cardiac troponin I; HIIE, high-intensity interval exercise; NT-proBNP, N-terminal pro-brain natriuretic peptide; POST, immediately after exercise; POST+3, 3 hours after exercise; PRE, before exercise.

point may have allowed capture of peak circulating troponin concentrations, which may not be detected when relying solely on immediate post-exercise measurements.

Previous studies comparing interval and CE have predominantly focused on cTnT responses,[11] while evidence specifically addressing cTnI remains limited. To date, only 2 studies have examined cardiac biomarker responses to HIIE and CE using broadly comparable designs.[12,13] In line with these findings, these studies reported no differences between the 2 exercise conditions; however, their generalizability to middle-aged individuals engaged in endurance exercise remains limited, particularly given that cardiovascular events during exercise are more frequently reported in this population.[22] This limitation is further influenced by methodological factors, including reliance on immediate post-exercise sampling,[13] which may fail to capture peak troponin responses occurring approximately 3-4 hours post-exercise,[23] as well as differences in participant characteristics, such as younger age and higher aerobic fitness (VO_2max ~64.3 mL·kg^{-1}·min^{-1}).[12]

In the present study, no differences in hs-cTnI responses were observed between HIIE and CE when overall physiological load was matched. This suggests that, under matched conditions, intermittent supramaximal efforts do not elicit greater troponin release than CE.

However, this does not preclude a role for exercise intensity at the individual level. Earlier studies have shown that exercise-induced cTnI release correlates positively with HR during exercise.[24] Consistent with this, the results showed a positive association between mean HR and the percentage increase in hs-cTnI concentrations.

Building on this, Bjørkavoll-Bergseth et al[25] suggested that cTnI elevations may also be influenced by exceeding critical HR thresholds (approximately 140-150 bpm). In line with this, the association observed between HR during high-intensity intervals and changes in hs-cTnI response suggests that transient exposure to higher cardiac strain may influence the magnitude of the troponin response, even when overall physiological load is comparable. From a mechanistic perspective, these findings are consistent with reversible cardiomyocyte stress and increased membrane permeability rather than irreversible myocardial injury. Additionally, exploratory analyses revealed no significant associations between age and hs-cTnI levels or their changes in the present study, suggesting that exercise-induced troponin release may be more strongly driven by acute physiological strain rather than age-related cardiac characteristics.[26]

N-Terminal Pro-B-type Natriuretic Peptide

N-terminal pro-B-type natriuretic peptide is released from cardiomyocytes in response to myocardial wall stress

Table 3. Changes in Plasma Cardiac Biomarker Levels in Response to CE and HIIE

Biomarkers	Exercise Type	Median (IQR)			*P* (Effect Size)		
		PRE	POST	POST+3	PRE-POST	PRE-POST+3	POST-POST+3
hs-cTnI (pg/mL)	HIIE	3.29 (2.5-6.55)	5.86 (2.66-8.21)	11.33 (6.4-19.76)	.039 (.53)	**<.001 (.88)**	**<.001 (.82)**
	CE	3.27 (2.5-5.05)	3.95 (2.5-6.09)	9.06 (4.02-15.38)	**.006 (.71)**	**<.001 (.85)**	**.002 (.80)**
NT-proBNP (pg/mL)	HIIE	35 (35-38)	39 (35-46)	47 (35-55)	**.011 (.66)**	.021 (.60)	.262 (.29)
	CE	35 (35-35)	35 (35-48)	35 (35-55)	.018 (.61)	.018 (.61)	.362 (.24)
CK-MB (ng/mL)	HIIE	0.56 (0.04-3.16)	1.84 (0.56-4.41)	2.72 (0.87-3.77)	**.008 (.69)**	**.011 (.66)**	.346 (.24)
	CE	0.41 (0.14-1.03)	1.06 (0.12-2.74)	1.61 (0.63-2.37)	**.007 (.70)**	**.004 (.75)**	.861 (.05)
Myoglobin (ng/mL)	HIIE	43.2 (33.26-62.66)	101.53 (61.81-116,99)	89.6 (51.22-109.96)	**<.001 (.88)**	**<.001 (.88)**	.027 (.57)
	CE	43.42 (34.93-51.26)	76.1 (58.06-110.85)	57.11 (44.18-79.55)	**<.001 (.88)**	**.009 (.68)**	**.012 (.65)**

IQR represents the range between the 25th and 75th percentiles.
Post hoc comparisons within groups were made using the Wilcoxon signed rank test with Bonferroni correction ($P < .017$).
Significant *P*-values are shown in bold.
r denotes the effect size calculated from the Wilcoxon signed-rank test and interpreted as small (0.1), medium (0.3), and large (0.5) according to Cohen's criteria.
CE, continuous exercise; CK-MB, creatine kinase-MB; HIIE, high-intensity interval exercise; hs-cTnI, high-sensitivity cardiac troponin I; IQR, interquartile range; NT-proBNP, N-terminal pro-B-type natriuretic peptide; POST, immediately after exercise; POST+3, 3 hours after exercise; PRE, before exercise.

associated with increased cardiac filling pressures and afterload.[27] In the present study, NT-proBNP concentrations increased immediately following both exercise protocols, but no differences were observed between HIIE and CE at any interval. These findings suggest that myocardial wall stress during exercise was comparable between the 2 protocols.

Previous studies have suggested that exercise duration and overall cardiovascular load may be stronger determinants of natriuretic peptide release than the distribution of exercise intensity.[28] The comparable NT-proBNP responses observed in the present study are consistent with this interpretation.

In addition, age was associated with NT-proBNP responses in the present study. N-terminal pro-B-type natriuretic

Table 4. Between-Protocol Comparison of Biomarker Change Scores (Δ) Following CE and HIIE Trials

		CE		HIIE				
		Median	IQR	Median	IQR	*Z*	*P*	Effect size (*r*)
Hs-cTnI	Δ POST-PRE	0.28	0.00-1.76	0.16	0.00-1.22	−.534	.594	0.14
	Δ POST+3-POST	3.13	0.39-6.56	6.65	1.88-9.49	−1.704	.088	0.44
	Δ POST+3-PRE	4.77	0.57-7.79	5.53	2.69-9.52	−1,817	.069	0.47
Nt-pro-Bnp	Δ POST-PRE	0	0-11	4	0-7	−.051	.959	0.01
	Δ POST+3-POST	0	0-9	0	0-14	−.237	.813	0.06
	Δ POST+3-PRE	0	0-16	0	0-20	−.211	.833	0.05
CK-MB	Δ POST-PRE	0.67	0.00-0.99	0.67	0.33-1.70	−1.538	.124	0.40
	Δ POST+3-POST	0,00	−0,13-0.15	0.16	−0,32 -0,56	−1.601	.109	0.41
	Δ POST+3-PRE	0.59	0.00-1.02	0.92	0.61-1.98	−2.040	.041	0.53
Myoglobin	Δ POST-PRE	31.17	11.26-61.81	39.45	29.26-68.58	−1.079	.281	0.28
	Δ POST+3-POST	−8.01	−17.29-−2.81	−7.94	−17.86- 1.56	−.568	.570	0.15
	Δ POST+3-PRE	18.99	−0.35-50.20	23.50	12.77-62.92	−1.931	.053	0.50

IQR represents the range between the 25th and 75th percentiles. Between-protocol comparisons of Δ change scores were performed using the Wilcoxon signed-rank test.
r denotes the effect size calculated from the Wilcoxon signed-rank test and interpreted as small (0.1), medium (0.3), and large (0.5) according to Cohen's criteria.
CE, continuous exercise; CK-MB, creatine kinase-MB; HIIE, high-intensity interval exercise; hs-cTnI, high-sensitivity cardiac troponin I; IQR, interquartile range; NT-proBNP, N-terminal pro-B-type natriuretic peptide; POST, immediately after exercise; POST+3, 3 hours after exercise; PRE, before exercise; Δ, change score.

Table 5. Associations Between Exercise Volume, Participant Characteristics, and Cardiac Biomarker Responses

Variable	Variable	*r*	*P*
Age	NT-proBNP concentrations at POST+3 in CE	0.523	.045
Age	NT-proBNP concentrations at POST+3 in HIIE	0.674	.006
Age	% Change in NT-proBNP from PRE to POST in the HIIE session	0.610	.016
Average heart rate	% Change in hs-cTnI from POST to POST+3 in the CE session	0.636	.011
Average heart rate	% Change in hs-cTnI from POST to POST+3 in the HIIE session	0.543	.037
Average heart rate during the high-intensity intervals of the HIIE	% Change in hs-cTnI POST to POST+3	0.531	.042
Average heart rate during the high-intensity intervals of the HIIE session	hs-cTnI levels measured at POST+3	0.696	.004
% Change in CK-MB from PRE to POST in CE session	% Change in myoglobin from PRE to POST in CE session	0.596	.024

CE, continuous exercise; CK-MB, creatine kinase-MB; HIIE, high-intensity interval exercise; hs-cTnI, high-sensitivity cardiac troponin I; NT-proBNP, N-terminal pro-B-type natriuretic peptide; POST, immediately after exercise; POST+3, 3 hours after exercise; PRE, before exercise.

peptide concentrations at 3 hours post-exercise were positively correlated with age in both exercise conditions, and the percentage increase from PRE to POST was significantly associated with age during HIIE. These findings are consistent with previous reports indicating greater natriuretic peptide responses to exercise in older individuals, potentially reflecting age-related changes in myocardial compliance or neurohormonal regulation.[13,29]

Creatine kinase-MB and Myoglobin

Creatine kinase-MB and myoglobin concentrations increased following exercise and remained elevated during early recovery in both exercise conditions. These biomarkers are present in both cardiac and skeletal muscle, which limits their specificity for myocardial injury.[30] The increases observed in the present study therefore likely reflect skeletal muscle stress associated with prolonged running rather than cardiac injury. No statistically significant between-protocol differences were observed for CK-MB or myoglobin, suggesting broadly comparable muscular stress between the 2 exercise modalities.

Absence of Clinically Relevant Myocardial Injury

Elevations in cardiac biomarkers exceeding clinical reference limits are frequently observed following prolonged endurance events such as marathons and ultramarathons, with some individuals exceeding clinical thresholds for troponin and NT-proBNP.[29,31] In contrast, none of the participants in the present study exceeded the upper reference limits for hs-cTnI or NT-proBNP following either exercise protocol. N-terminal pro-B-type natriuretic peptide concentrations were below the clinical threshold in all participants, except for 1 individual who presented with an elevated baseline value prior to the HIIE session. These findings are consistent with previous evidence suggesting that exercise-induced troponin elevations may be influenced by exercise intensity, with clinically significant elevations more likely to occur under higher competitive demands.[32] Additionally, hs-cTnI has been suggested to be more sensitive than hs-cTnT in detecting subtle myocardial responses;[33,34] however, despite this increased sensitivity, no clinically relevant elevations were observed in the present study.

The precise mechanism underlying exercise-induced cardiac troponin release remains unclear, although alterations in myocardial cell membrane permeability are widely considered the primary pathway.[11] Cardiac biomarker release following exercise has been reported to be modified by several factors, including exercise intensity, duration, age, and sex, with exercise intensity consistently identified as the primary determinant.[26,32] In the present study, HIIE and CE were matched on average intensity indices, including HR and %vVO_2max; this relative similarity in average intensity between protocols, despite their different distribution of exercise intensity, may reflect a similar cumulative myocardial wall stress between conditions and could explain why both hs-cTnI and NT-proBNP responses were comparable following the 2 exercise modalities.

Strengths and Limitations

A key strength of the present study is the inclusion of middle-aged recreational runners, an age group in which cardiovascular events during endurance exercise are more frequently reported.[22] Additionally, matching the exercise protocols for average HR, running speed, and percentage of vVO_2max enabled a controlled comparison of interval and CE under comparable overall physiological load. Furthermore, the 3-time-point biomarker sampling design provided a more comprehensive characterization of the temporal profile of exercise-induced cardiac biomarker responses than studies relying solely on immediate post-exercise measurements.

Several limitations should also be acknowledged. Cardiac evaluation was restricted to circulating biomarkers, without additional cardiac assessments such as electrocardiography (ECG), echocardiography, or cardiac magnetic resonance imaging, limiting the comprehensiveness of the cardiovascular assessment. Exercise intensity was not individually prescribed relative to ventilatory or lactate thresholds, and cumulative internal training load [e.g., TRIMP (Training Impulse) or session-RPE] was not quantified. Average HR alone may not completely reflect total physiological stress during interval exercise, and these factors together may have contributed to inter-individual variability in physiological strain. Lastly, the study included only recreationally

active men without known cardiovascular disease, limiting the generalizability of the findings to women, less active populations, and individuals with structural heart disease or coronary artery disease. Future research should extend these findings by investigating biomarker responses in more diverse populations and by examining long-term adaptations to different exercise training modalities.

CONCLUSION

When exercise duration and average physiological intensity were matched, HIIE elicited cardiac biomarker responses comparable to those observed following continuous running in middle-aged recreational runners. Importantly, neither exercise modality resulted in hs-cTnI or NT-proBNP concentrations exceeding established clinical reference limits. These findings indicate that, under the experimental conditions of the present study, well-structured HIIE did not elicit a greater acute cardiac biomarker response than workload-matched CE in regularly active recreational runners. However, given the absence of ECG-based cardiac assessment and echocardiographic evaluation, these findings should not be interpreted as evidence of clinical cardiovascular safety or extrapolated to individuals with known or suspected cardiovascular disease.

Ethics Committee Approval: This study was approved by the Ethics Committee of Hacettepe University (Approval No.: GO 23/521; Date: 20.06.2023).

Informed Consent: Verbal and written informed consent was obtained from the patients who agreed to take part in the study.

Peer-review: Externally peer-reviewed.

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Author Contributions: Concept – Y.M., S.A., H.A.D.; Design – Y.M., S.A., H.A.D.; Supervision – H.A.D., C.J.L.; Resources – Y.M., Y.E.E.; Materials – Y.M., H.A.D., Y.S.S.; Data Collection and/or Processing – Y.M., Y.S.S., Y.E.E., Y.K., O.F.I.; Analysis and/or Interpretation – Y.M., S.A., Y.S.S..; Literature Search – Y.M., H.A.D.; Writing – Y.M., H.A.D., Y.S.S., C.J.L.; Critical Review – Y.M., H.A.D., S.A., C.J.L.

Declaration of Interests: The authors have no conflicts of interest to declare.

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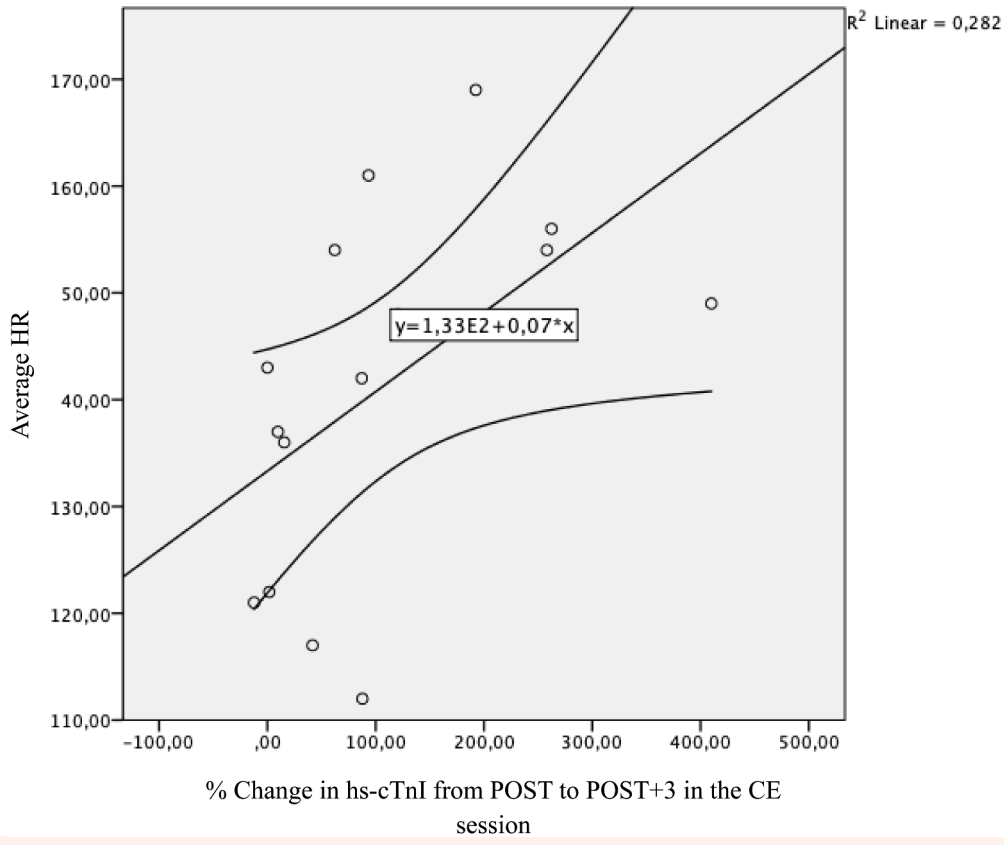
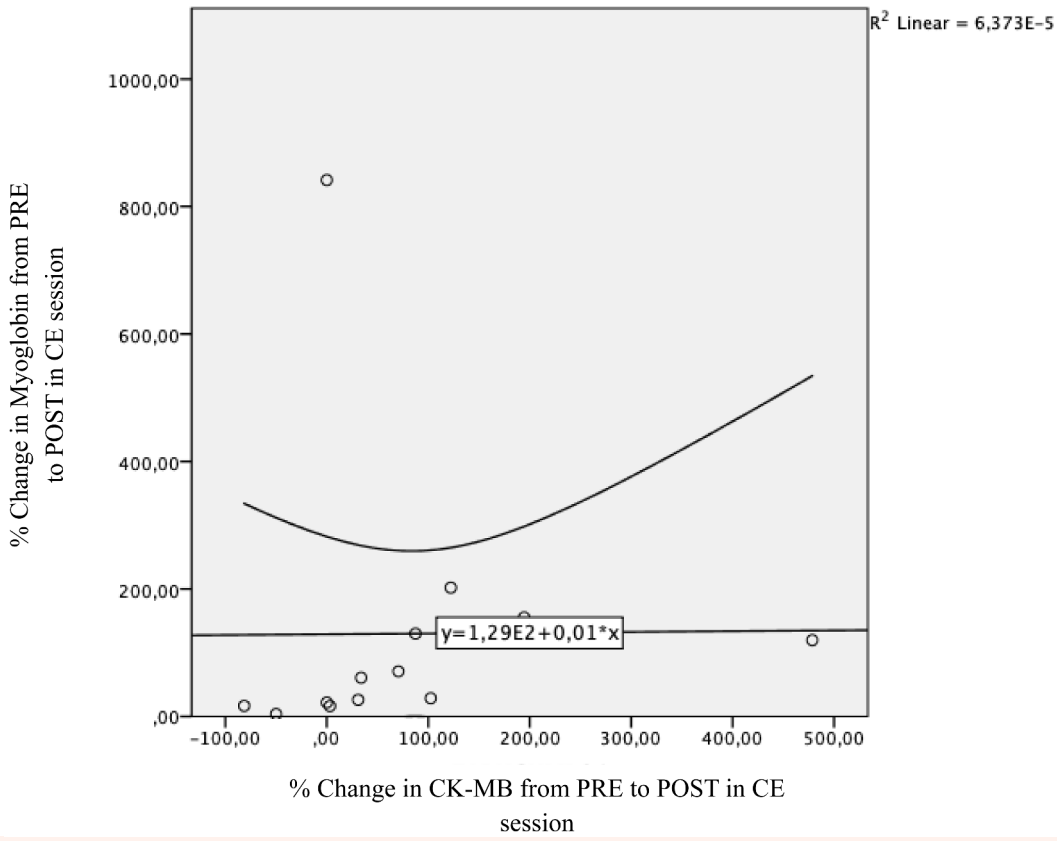
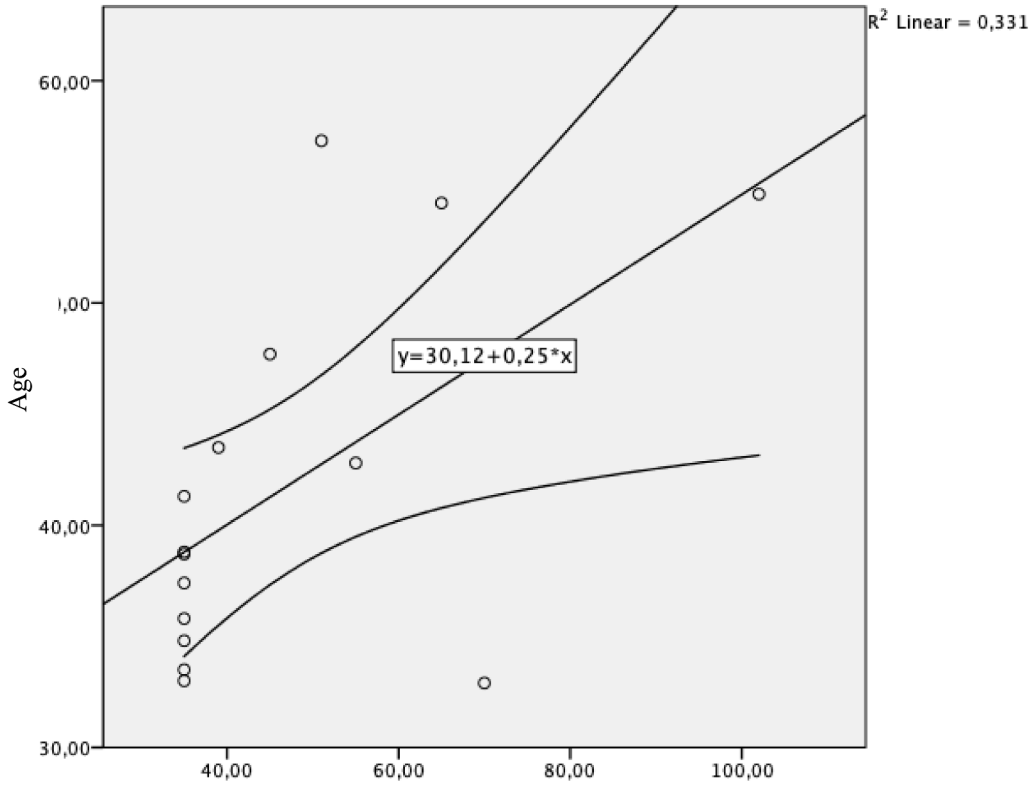


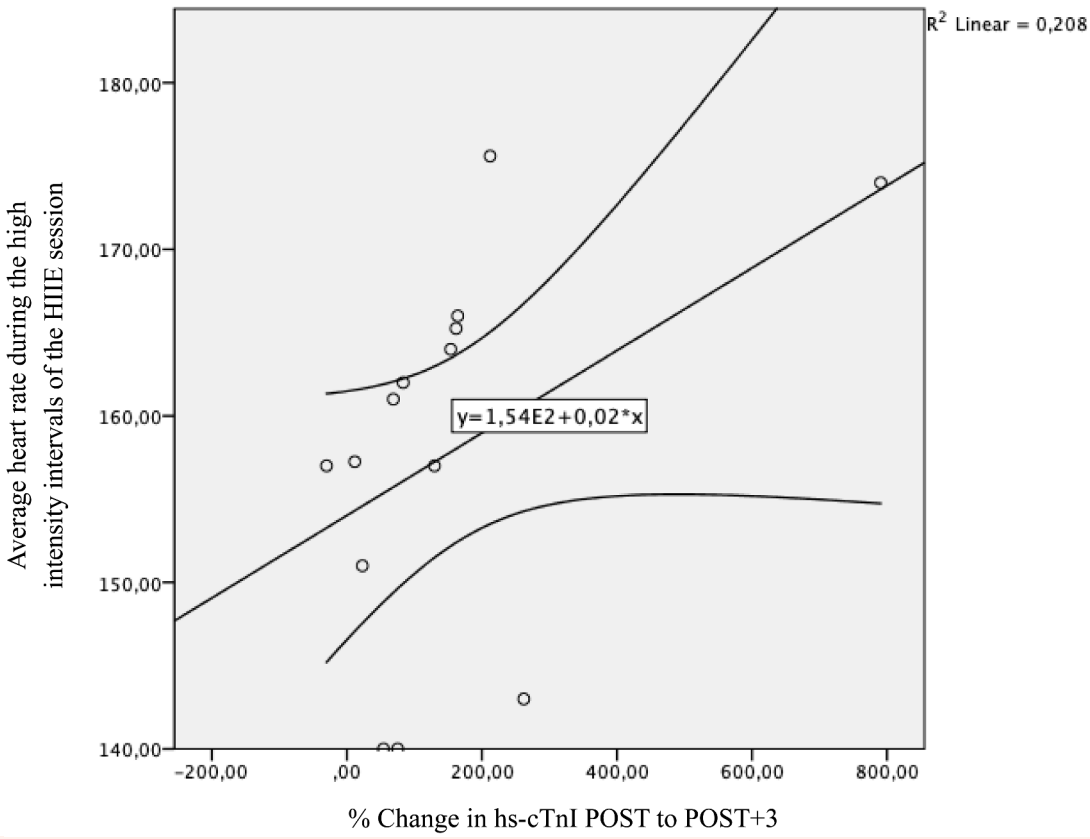
Supplementary Figure 1. Scatter plot showing the correlation between the percentage change in hs-cTnI from POST to POST+3 and average heart rate during the continuous exercise (CE) session. The solid line represents the linear regression fit and the curved lines indicate the 95% confidence interval. hs-cTnI, high-sensitivity cardiac troponin I; POST, immediately post-exercise; POST+3, 3 hours post-exercise.



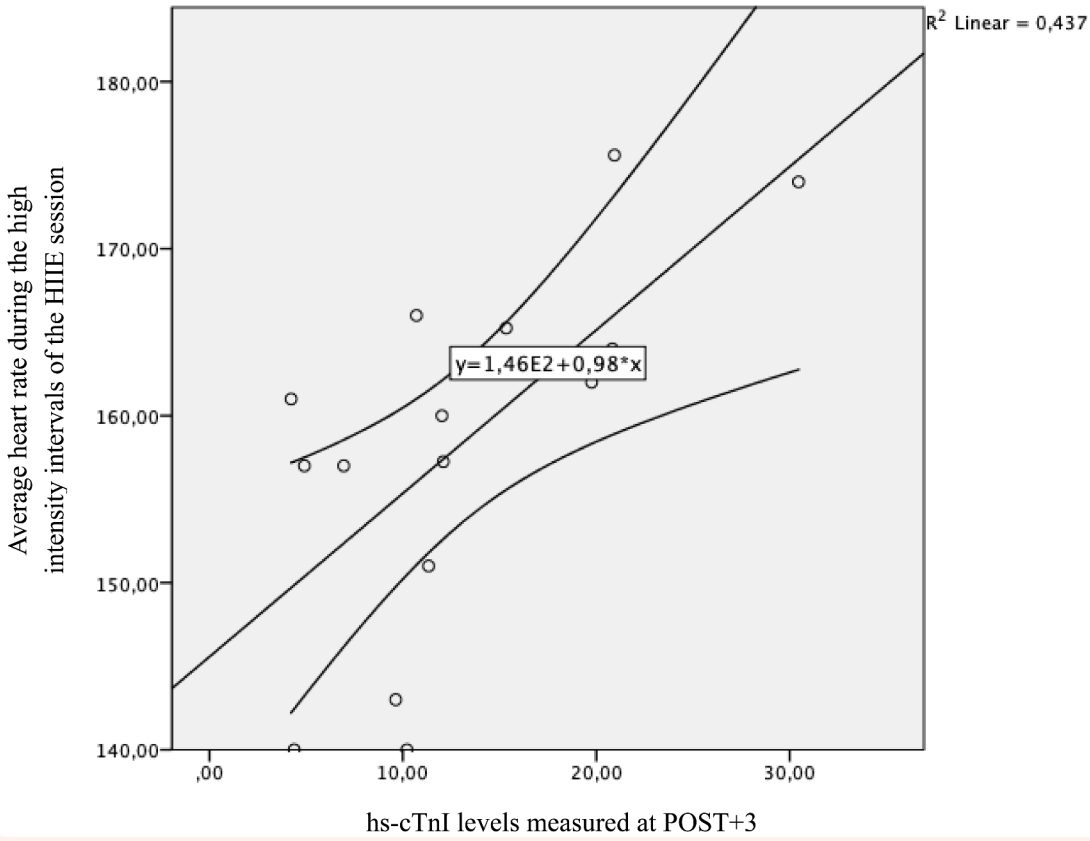
Supplementary Figure 2. Scatter plot showing the correlation between the percentage changes in CK-MB and myoglobin from PRE to POST in the continuous exercise (CE) session. The solid line represents the linear regression fit and the curved lines indicate the 95% confidence interval. CK-MB, creatine kinase-MB; PRE, pre-exercise; POST, immediately post-exercise.



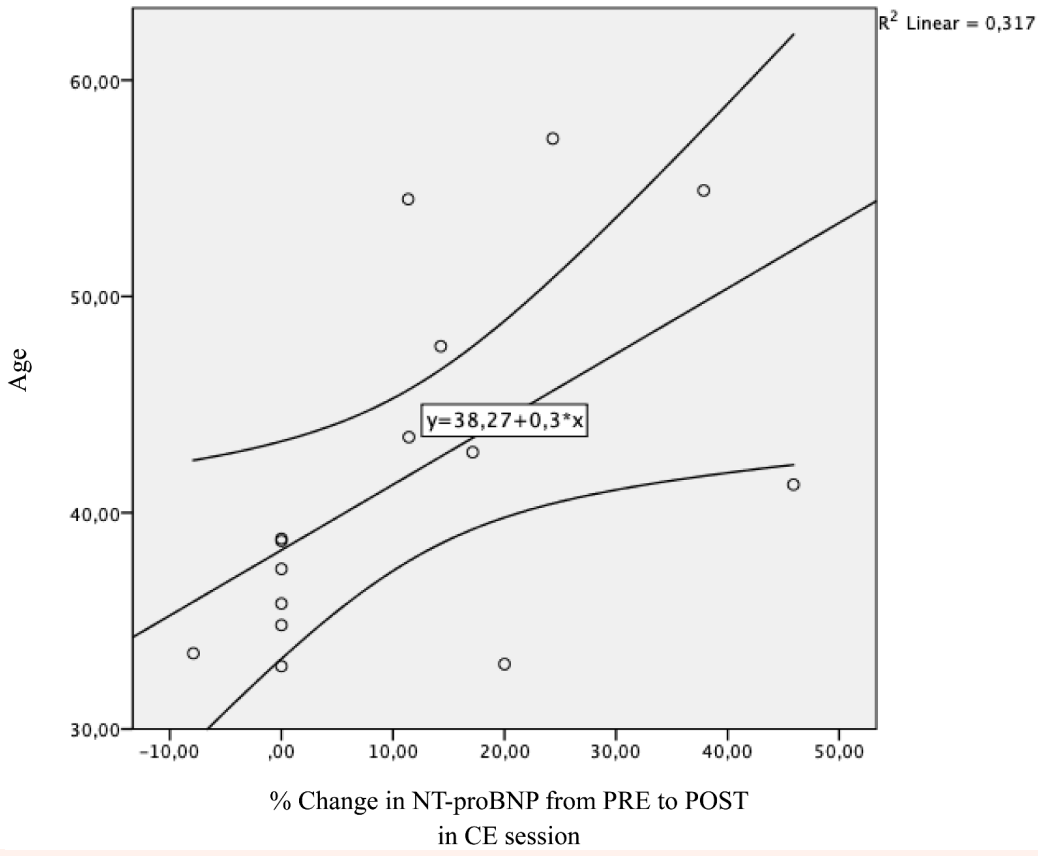
Supplementary Figure 3. Scatter plot showing the correlation between NT-proBNP concentrations at POST+3 in the continuous exercise (CE) session and age. The solid line represents the linear regression fit and the curved lines indicate the 95% confidence interval. NT-proBNP, N-terminal pro-B-type natriuretic peptide; POST+3, 3 hours post-exercise.



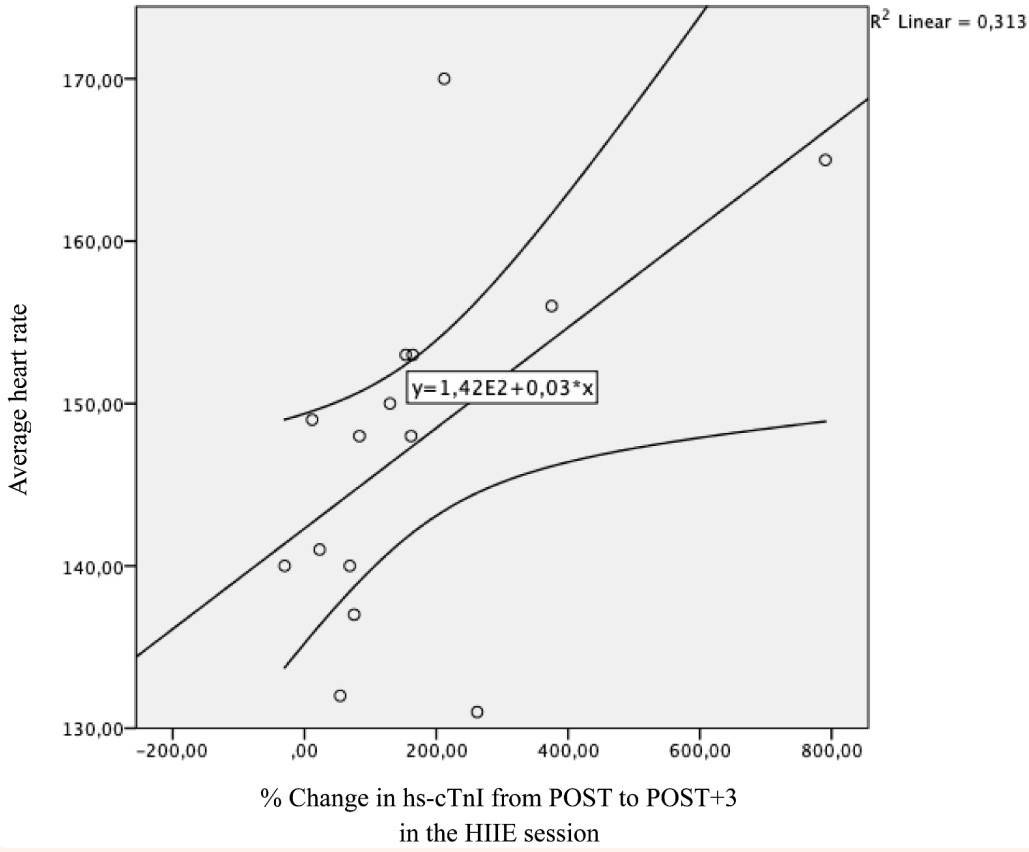
Supplementary Figure 4. Scatter plot showing the correlation between the percentage change in hs-cTnI from POST to POST+3 and average heart rate during the high-intensity intervals of the high-intensity interval exercise (HIIE) session. The solid line represents the linear regression fit and the curved lines indicate the 95% confidence interval. hs-cTnI, high-sensitivity cardiac troponin I; POST, immediately post-exercise; POST+3, 3 hours post-exercise.



Supplementary Figure 5. Scatter plot showing the correlation between hs-cTnI levels at POST+3 and average heart rate during the high-intensity intervals of the high-intensity interval exercise (HIIE) session. The solid line represents the linear regression fit and the curved lines indicate the 95% confidence interval. hs-cTnI, high-sensitivity cardiac troponin I; POST+3, 3 hours post-exercise.



Supplementary Figure 6. Scatter plot showing the correlation between the percentage change in NT-proBNP from PRE to POST in the continuous exercise (CE) session and age. The solid line represents the linear regression fit and the curved lines indicate the 95% confidence interval. NT-proBNP, N-terminal pro-B-type natriuretic peptide; PRE, pre-exercise; POST, immediately post-exercise.



Supplementary Figure 7. Scatter plot showing the correlation between the percentage change in hs-cTnI from POST to POST+3 and average heart rate during the high-intensity interval exercise (HIIE) session. The solid line represents the linear regression fit and the curved lines indicate the 95% confidence interval. hs-cTnI, high-sensitivity cardiac troponin I; POST, immediately post-exercise; POST+3, 3 hours post-exercise.