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Impact of high-intensity interval exercise and moderate-intensity continuous exercise on the cardiac troponin T level at the early stage of training

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TITLE:

Impact of High-Intensity Interval Exercise and Moderate-Intensity Continuous Exercise on the Cardiac Troponin T Level at an Early Stage of Training

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KEYWORDS:

High-Intensity Interval Exercise, Sprint Interval Exercise, Repeated Sprint Exercise, Moderate-Intensity Continuous Exercise, Cardiac Troponin T, Cardiac Biomarker

SUMMARY:

Here, we present protocols of high-intensity interval and moderate-intensity continuous exercise to observe the response of circulating cardiac troponin T (cTnT) concentration to acute exercise over 10 days. The information may assist with clinical interpretations of post-exercise cTnT elevation and guide the prescription of exercise.

ABSTRACT:

An elevation in cardiac troponin T (cTnT), as a highly specific biomarker of cardiomyocyte damage, after moderate-intensity continuous exercise (MCE) has been described. The exercise-induced cTnT response distorts the diagnostic role of the cTnT assay. Although high-intensity interval exercise (HIE) is growing in popularity and concerns remain about its safety, available data related to cTnT release after HIE is limited, which hampers the use of HIE as a health intervention. Here, we present three representative HIE protocols [traditional HIE (repeated 4 min cycling at 90% $\dot{V}O_{2max}$ interspersed with 3 min rest, 200 kJ/session); sprint interval exercise (SIE, repeated 1 min cycling at 120% $\dot{V}O_{2max}$ interspersed with 1.5 min rest, 200 kJ/session); and repeated sprint exercise (RSE, 40 x 6 s all-out sprints interspersed with 9 s rest)] and one

representative MCE protocol (continuous cycling exercise at an intensity of 60% $\dot{V}O_{2max}$, 200 kJ/session). Forty-seven sedentary, overweight young women were randomly assigned to one of four groups (HIE, SIE, RSE, and MCE). Six bouts of respective exercise were performed by every single group, with each being 48 h apart. Meanwhile, for four groups, the duration of the entire testing period was identical, being 10 days. Before and after the first and final exercise bouts, an assessment was conducted of cTnT. The current study provides a frame of reference giving a clear picture of how a specific exercise session affects the circulating cTnT concentration at the early stage of training. The information may assist with clinical interpretations of post-exercise cTnT elevation and guide the prescription of exercise, especially for HIE.

INTRODUCTION:

The benefits of regular exercise on the heart are well-documented[1]. However, the risk of cardiac events, such as acute myocardial infarction (AMI), transiently increases during an intense exercise[2,3]. Individuals with low levels of regular physical activity show higher risk towards AMI[2,3]. Cardiac troponin T (cTnT) is the biochemical gold standard in the diagnosis of AMI[4]. However, there is a burgeoning evidence that the cTnT is elevated after continuous prolonged exercise, which undoubtedly distorts the diagnostic role of the cTnT assay[5].

The repetitive bouts of relatively intense exercise interspersed with short breaks are a typical element of high-intensity interval exercise (HIE), which is growing in popularity in various fields such as cardiac rehabilitation, health and fitness[6,7]. The widespread interest in HIE is due in part to the ability of HIE training to elicit beneficial physiological adaptations similar or superior to the traditional moderate-intensity continuous exercise (MCE) training, despite a reduced total exercise volume and time commitment[6]. However, concerns related to the safety of HIE have been expressed due to the high cardiac demand[8]. To date, available data related to cTnT release upon HIE is limited. Moreover, no previous integrated study has investigated the effect of various modalities of HIE and traditional MCE on the appearance of cTnT with exercise. Thus, it is unclear whether, with equalization of total mechanical work between HIE and MCE, different exercise formats will lead to the distinction in cTnT concentrations and what the range of the elevated cTnT values is. Given that exercise conducted at higher intensities might lead to a higher risk of cardiac events[2,3], it is pertinent to develop a representative HIE and MCE proposals with the known range of cTnT responses. The evaluation of exercise-associated cTnT elevation could potentially be helpful in clinical decision-making and assist clinical physiologists in developing more effective and safe exercise prescriptions.

Consequently, we outline protocols of the three representative types of HIE and one representative type of MCE to gather physiological data while observing cTnT responses. Considering that the risk of acute cardiac events is higher in people who do not engage in regular exercise[2,3] and the overall release of cTnT induced by exercise reduces with regular training[9], this study recruited sedentary, overweight females who completed a 10-day training program. This provided the prospect to work in the early stage of training and target an under-researched group.

PROTOCOL:

The protocol (No. 31771319) was approved by the Hebei Normal University Review Board and conformed to the Declaration of Helsinki. All participants provided written informed consent before participating in the testing described.

1. Participant screening and preparation for the experiment

1.1. For recruitment, ensure that the participants satisfy the following inclusion criteria: aged between 18 and 25, a minimum body mass index (BMI) of 23 kg/m, which is the overweight cut-off for Asian adults[10], consistent body weight (± 2 kg) for the most recent three months, no exercise training or regular physical activities, no record of hormonal, orthopaedic or cardiovascular diseases, diabetes, hyperlipidaemia, hypertension or polycystic ovary syndrome, as well as, no current use of prescribed medications (including contraceptive pills) and no history of smoking.

1.2. Randomly assign 47 eligible participants to one of the following four groups: traditional HIE (n = 12), sprint interval exercise (SIE, n = 11), repeated sprint exercise (RSE, n = 12) or MCE (n = 12) group.

1.3. Adjust and record the appropriate seat height on the cycling ergometers so that the participant pedals with a slight knee bend (~ 10°) at full downstroke of the pedal.

1.4. Instruct the participants to perform two initial exercise sessions (as described in step 3) to familiarize them with the respective type of cycling exercise (HIE, SIE, RSE or MCE).

2. Experimental procedures

2.1. First, instruct each participant to perform a continuous incremental test on a stress testing cycle ergometer to assess maximal oxygen uptake ($\dot{V}O_{2max}$).

2.1.1. Warm up for 5 min at 25 W. Then start the test by performing continuous 2 min stages (20 W increment per stage) starting at 50 W with a pedal frequency of 60 rpm until volitional exhaustion.

2.1.2. Use a breath-by-breath metabolic analyzer to measure the oxygen consumption during the exercise.

2.1.3. Calculate the $\dot{V}O_{2max}$ based on the highest 30 s average value. Then, calculate a power output that elicits 60%, 90% and 120% $\dot{V}O_{2max}$ in the MCE, HIE and SIE groups, respectively, using the equation of linear regression through plotting the steady state $\dot{V}O2$ versus power output[11].

2.2. At least 5 days after pre-intervention assessments, instruct the HIE, SIE, RSE and MCE groups to commence their respective training.

NOTE: Start all exercise tests at the same time of the day (e.g., 11:00 A.M.). Meanwhile, ensure that the tests are conducted in a laboratory with temperature and humidity-controlled settings (20 °C and 50% relative humidity). Ask all participants to stick to both their daily activities and eating habits throughout the experiment.

2.2.1. Instruct the participant to refrain from any strenuous exercise for 48 h, after a routine warm-up, instruct the HIE, SIE, RSE and MCE groups to engage in their respective exercise session on a cycle ergometer as arranged. Perform the exercise protocol as detailed in step 3.

2.2.2. Perform 6 exercise sessions carried out over a time span of 10 days for all four groups. Select the 1st (1ST) and 6th (6TH) exercise sessions to observe the cTnT response to the acute exercise (**Figure 1**).

[Place **Figure 1** here]

2.4. Record a continuous electrocardiogram (ECG) during exercise via a portable electrocardiograph (ECG) monitor.

2.5. Draw venous blood samples before and immediately after exercise, as well as 3 h and 4 h after the selected exercise session to assess the serum cTnT. With the subjects in a seated position, draw 5 mL of venous blood from the antecubital vein for each sample.

NOTE: Post-exercise blood samples timings conformed to prior work conducted in the laboratory, which demonstrated that blood cTnT concentrations reached their peak 3 or 4 h following acute exercise in a laboratory-based study[12].

2.6. For the separation of the serum, allow the blood to clot at room temperature. Centrifuge the blood samples at 3,500 *x g* for 20 min.

2.7. Aspirate the serum and store at -80 °C for the subsequent analysis of cTnT.

2.8. Use an analyzer to perform the quantitative measurement of the cTnT with a high sensitivity immunoassay based on electrochemiluminescence technology. Take 1 mL of the serum and put it into a special test tube for measuring cTnT. Then insert the tube in the analyzer and press the start button.

NOTE: The human cTnT protein itself consists of 288 amino acids. Two monoclonal antibodies are used for the assay which are specifically directed against human cardiac troponin T. The antibodies recognize the central part of cardiac troponin T protein[4], specifically targeting two epitopes at amino acid positions 125-131 and 136-147.

3. Exercise protocols

3.1. In each exercise session, instruct the 4 groups to follow the steps below.

3.1.1. Complete an identical 10 min warm-up at 50-60% of HRmax (percentage of individual maximal heart rate during exercise session) and 5 min cool down at 20 W.

3.1.2. Following the warm-up. Have a 2 min recovery period, where participants remain seated but stationary on the cycle ergometer.

3.1.3. Direct the participants to accelerate as soon as possible at the beginning of each exercise bout to reach the intended intensity. During this time a researcher counts down, "5-4-3-2-1-Go!". At the command of "Go!", drop the weight and activate the computerized system.

NOTE: For the MCE, HIE and SIE, accelerate to the planned power (see step 2.1.3), i.e. 60%, 90% and 120% $\dot{V}O_{2max}$, respectively. For the RSE, accelerate to “all-out” exercise (see step 3.4). The weights are in the cycle ergometer, and the ergometer is linked to a PC computer with specific software.

3.1.4. Instruct the participants to remain seated while cycling and secure their feet to the pedals using toe clips, and verbally encourage the participants to give a maximal effort to exercise at the desired intensity throughout each session.

3.2. HIE protocol: Repeat 4 min bouts of exercise on a stress testing cycle ergometer at an intensity of 90% $\dot{V}O_{2max}$, followed by a 3 min passive recovery (complete rest) until the targeted 200 kJ of work is achieved.

3.3. SIE protocol: Repeat 1 min bouts of exercise on a stress testing cycle ergometer at an intensity of 120% of $\dot{V}O_{2max}$, followed by a 1.5 min passive recovery until the targeted 200 kJ of work is achieved.

3.4. RSE protocol: Repeat 6 s “all-out” sprints interspersed with 9 s passive recovery periods on a Wingate testing cycle ergometer, with a resistance of 1.0 kg until the targeted 40 repetitions are achieved, and the total mechanical work was recorded.

3.5. MCE protocol: Perform continuous cycling exercise, until a target of 200 kJ of work is achieved, at an intensity of 60% $\dot{V}O_{2max}$.

4. Statistical analyses

4.1. Perform data analysis using a statistical software package. Evaluate the normality of the data using the Kolmogorov–Smirnov test[12]. Use $P < 0.05$ for assessing statistical significance.

4.2. Compare the differences in HR_{mean} (mean heart rate during exercise session) and $\%HR_{max}$ across the four groups (HIE, SIE, RSE and MCE) and the two observed exercise sessions (1ST and

6TH) using two-way ANOVA, with repeated measures. Use the Newman–Keuls post-hoc test for cases in which the main effect is significant.

4.3. Compare cTnT across the time points (pre-exercise and peak post-exercise) and two observed exercise sessions (1ST and 6TH) using the non-parametric Wilcoxon signed ranks test because of the skewed distribution of the cTnT data. Further, the Kruskal-Wallis test was used to assess the statistical significance of differences in cTnT levels across the four groups (HIE, SIE, RSE and MCE), and the Mann-Whitney U test was used for pairwise comparisons where appropriate.

REPRESENTATIVE RESULTS:

All participants (n = 47) completed the study, and no adverse cardiac events (e.g., chest pain and sign of myocardial ischemia on ECG) were found during testing in the four groups. As expected, the acute exercise heart rate (HR) data, including HR_{mean} and $\%HR_{max}$, at the 1ST assessment is similar (all $P > 0.05$) to those in the 6TH assessment in all four groups. Further, the HR data in the RSE and MCE groups is the highest and lowest among the four groups, respectively, but is similar between the HIE and SIE groups (**Table 1**).

[Place **Table 1** here]

Figure 2 reveals the acute exercise cTnT data on all four groups across the 10-day period, which are shown in the form of individual data points for pre-exercise (Pre-exe) and peak post-exercise (Post-exe) values. The cTnT concentration is discovered to be on the rise following acute exercise ($P < 0.05$) at the 1ST and 6TH assessments in all four groups. Moreover, no differences in cTnT concentration are found among the groups except for the smaller response after RSE at the 1ST. Moreover, the cTnT concentration at the 6TH assessment before MCE is higher than that at the 1ST assessment before MCE and at the 6TH assessment before RSE (both $P < 0.05$).

[Place **Figure 2** here]

FIGURE AND TABLE LEGENDS:

Figure 1: Schematic diagram of study procedures. HIE, high-intensity interval exercise; SIE, sprint interval exercise; RSE, repeated sprint exercise; MCE, moderate-intensity continuous exercise

Figure 2: Pre-exercise (Pre-exe) and peak post-exercise (Post-exe) cardiac troponin T concentrations (cTnT, ng/L). HIE, high-intensity interval exercise; SIE, sprint interval exercise; RSE, repeated sprint exercise; MCE, moderate-intensity continuous exercise; 1ST, the 1st exercise session; 6TH, the 6th exercise session. The scale is log plotted because of the data spread, and individual data points are presented by circles with values for the same participant connected by lines for each condition. The horizontal dotted line is the upper reference limit and the double-arrow line is the median of the cTnT values at pre-exercise (Pre-exe) or Post-exercise (Post-exe). ○, single subject; ^{n}●, n subjects. * Significantly different from the corresponding Pre-exe value, $P < 0.05$; † Significantly different from the corresponding RSE value, $P < 0.05$; ‡ Significantly

different from the corresponding value of 1ST, $P < 0.05$. This figure has been modified from Nie et al.[13] and Zhang et al.[14].

Table 1. Acute exercise data. Data are presented as the mean ± SD. HIE, high-intensity interval exercise; SIE, sprint interval exercise; RSE, repeated sprint exercise; MCE, moderate-intensity continuous exercise; 1ST, the 1st exercise session; 6TH, the 6th exercise session; $Power_{exe}$, power output during exercise; $Time_{exe}$, total exercise duration; $Work_{exe}$, work output during exercise; HR_{mean}, mean heart rate during exercise session; $\%HR_{max}$, percentage of individual maximal heart rate during exercise session.*Significantly different from the corresponding value of 1ST, $P < 0.05$; †Significantly different from the corresponding value of HIE, SIE, and RSE, $P < 0.05$; ‡Significantly different from the corresponding value of HIE and SIE, $P < 0.05$; This table has been modified from Nie et al.[13] and Zhang et al.[14].

DISCUSSION:

The repetitive short to long bouts of rather high-intensity exercise interspersed with recovery periods are involved in HIE, which is subdivided into traditional HIE ("near maximal" efforts) and SIE ("supramaximal" efforts), using a common classification scheme[6]. In addition, RSE is a particularly intense form of SIE, where the activity is "all-out" but only lasts for 3 to 7 s[6]. To the best of our knowledge, this is the first integrated study to outline protocols of three representative types of HIE and one representative type of MCE to gather physiological data while observing cTnT responses. The current protocols are noteworthy especially when considering the study design, where the specific observation window (i.e., the early stage of exercise training) was selected. To this end, in order to derive a clean training background and avoid the effects exerted by the prior training experience, the previously sedentary subjects were selected. Also, the post-exercise cTnT level at the 1ST assessment was like that in the 6TH assessment in all four groups (**Figure 2**). The current findings reflect an overview of exercise-induced cTnT in the previously sedentary subjects who have just initiated an exercise training regime. As our recent study[15] demonstrated, these subjects had improved cardiorespiratory fitness and same absolute intensity during exercise abolished exercise-induced elevation in cTnT. Moreover, this experiment also seems to support that the participants had relatively stable cardiorespiratory fitness during the 10-day period due to the lack of a significant difference observed in acute exercise HR data (see **Table 1**).

Theoretically, interval exercise is infinitely variable when the intensity and duration of work and relief intervals are manipulated, but here we selected three distinct, representative protocols based on the usual classification scheme[6]. As shown by our current data, despite the varying exercise intensities employed, HIE, SIE and MCE elicited similar cTnT elevations under the circumstance where identical total mechanical work was completed during the 1st cycling trials. The rising level of cTnT in RSE was found to be less than that in HIE or SIE, which was likely attributed to the much lower total mechanical work of RSE (RSE vs. HIE or SIE: ~50 vs. 200 kJ). However, the mechanical work might not be the only determinant, as the acute exercise in four groups induced a similar cTnT elevation during the 6th cycling trials, despite the completion of lower mechanical work in RSE. Therefore, additional studies are still warranted to clarify the role

of total work accomplished in post-exercise cTnT elevation.

In the present study, following exercise, almost all participants showed an increase in cTnT and the absence of symptoms or signs of myocardial ischemia based on an ECG, suggesting that exercise-induced cTnT elevation is largely obligatory, and thus, likely physiological in nature. The current study provides a frame of reference giving a clear picture of how a specific exercise session affects the circulating cTnT concentration at the early stage of training. This holds great clinical importance, considering some post-exercise cTnT data (9%) are above the population upper reference limit of 14 ng/L in the current study, and concerns related to the safety of high-intensity exercise, especially in less-trained exercisers[8]. Specifically, on one hand, clinicians should be aware that elevated cTnT after low-volume, high-intensity exercise is common, and the frame of cTnT release aids clinicians faced with the challenge of interpreting these data clinically in the post-exercise setting. On the other hand, the current data provides templates of different exercise protocols and a potential way to predict the cTnT response when considering initiating exercise regimes. The information may have practical implications for exercise prescriptions in sedentary populations, especially for HIE.

Here, we have included a young population, a limitation of this study is that we did not assess the cTnT levels in the elderly population. A higher risk of cardiac events typically occurs in the elderly population with cardiovascular risks and/or diseases[16]. An increased risk of cardiac events typically occurs among the elderly with cardiovascular risks and/or diseases[16]. At present, it remains unclear whether cTnT has similar responses to acute exercise in groups with cardiovascular disease or risk, which makes it worthy of further research using the exercise protocols developed in the present study. Accordingly, it is of significance to be aware that HIE has been made prevalent in recent years among the patients with cardiovascular diseases. However, the safety of the acute response to a single session of high-intensity exercise for these cohorts remains concerning[16].

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DISCLOSURES:

The authors declare that they have no competing financial interests.

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Figure1

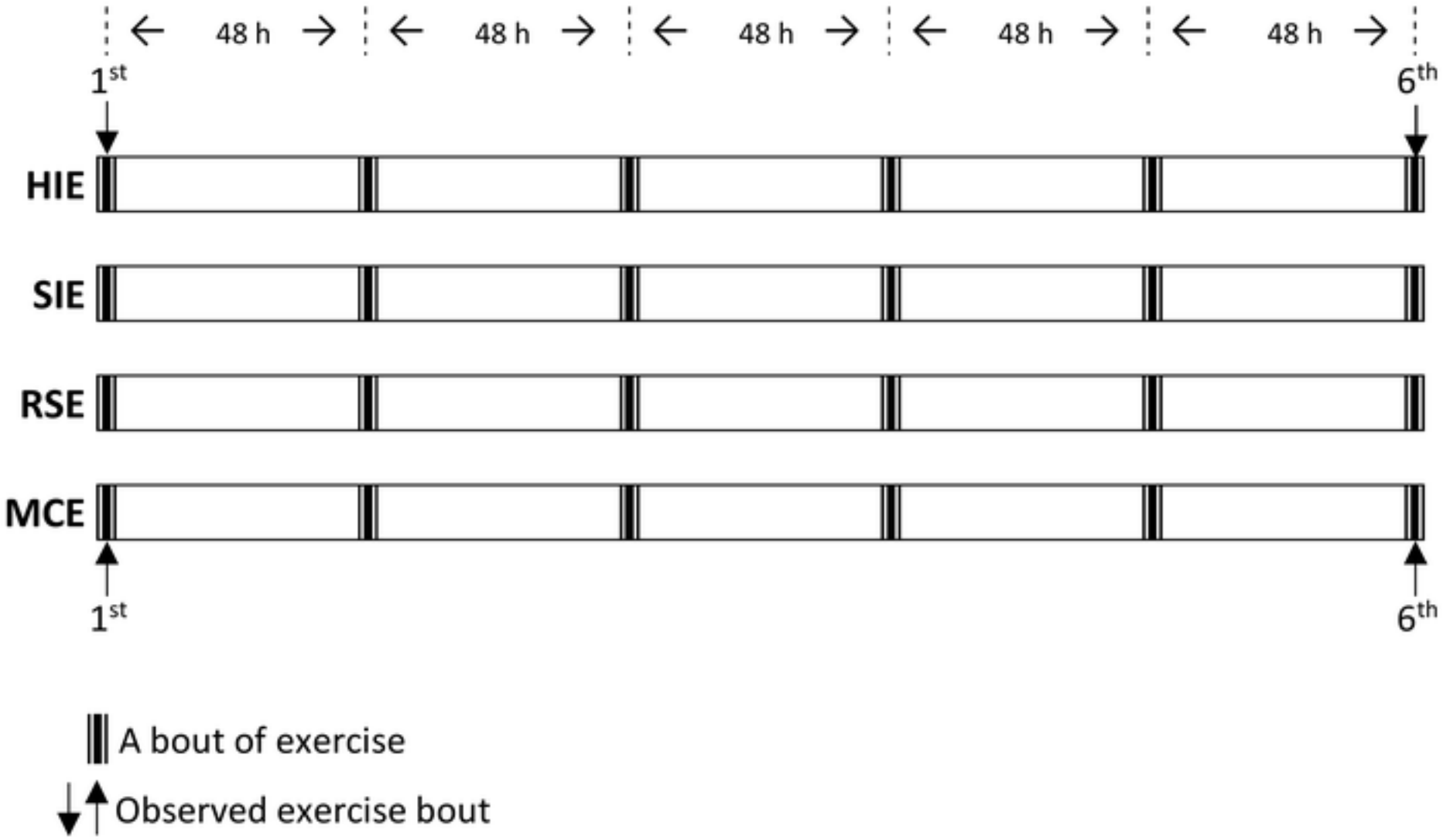

Figure2

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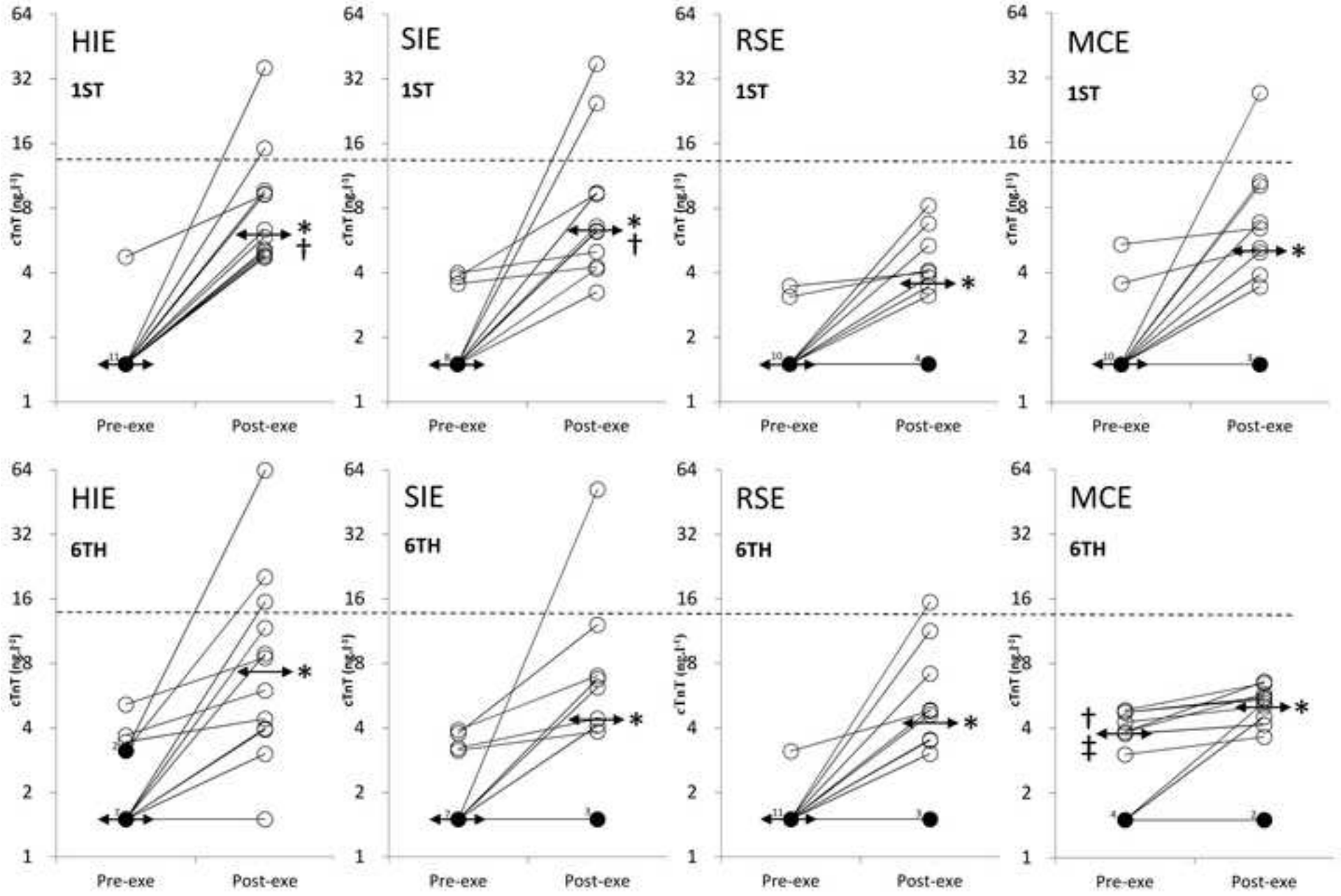

Table1 Click here to access/download;Table;Table1.xlsx

	$Power_{exe}$ (W)	$Time_{exe}$ (min)	$Work_{exe}$ (KJ)	HR_{mean} ($beat.min^{-1}$)	$\%HR_{max}$
HIE (n=12)					
1ST	119 ± 12	28 ± 3	200 ± 0	157 ± 9	85 ± 4
6TH	119 ± 12	28 ± 3	200 ± 0	155 ± 6	84 ± 4
SIE (n=11)					
1ST	160 ± 18	21 ± 2	200 ± 0	148 ± 11	85 ± 4
6TH	160 ± 18	21 ± 2	200 ± 0	147 ± 7	85 ± 5
RSE (n=12)					
1ST	193 ± 17‡	4 ± 0‡	46 ± 4‡	169 ± 5‡	94 ± 7‡
6TH	204 ± 15*‡	4 ± 0‡	49 ± 4*‡	171 ± 8‡	95 ± 6‡
MCE (n=12)					
1ST	54 ± 10†	63 ± 12†	200 ± 0	140 ± 12†	76 ± 6†
6TH	54 ± 10†	63 ± 12†	200 ± 0	137 ± 11†	74 ± 6†

Name of Material/ Equipment	Company	Catalog Number
Cobas E 601 analyser	Roche Diagnostics, Penzberg, Germany	
Monark 839E Stress Testing Cycle Ergometer	Monark Exercise AB, Vansbro, Sweden	
Monark 894E Wingate Testing Cycle Ergometer	Monark Exercise AB, Vansbro, Sweden	
Quark-PFT-ergo Metabolic Analyser	Cosmed, Rome, Italy	C09072-02-99
SPSS Statistics 20.0 software package	IBM Corp., Armonk, USA	
Zephyr BioHarness 3.0	Zephyr Technology, Auckland, New Zealand	9800.0189/9600.019 0

	Comments/Description
Used for measuring the circulating cardiac troponin T concentration	
Used for all exercise protocols except repeated sprint exercise	
Only used for repeated sprint exercise protocol	
Electrocardiograph Monitor	

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Dear Editor,

We thank you for the detailed and insightful comments regarding our manuscript. We have updated the paper considerably and provided a point-by-point rebuttal below. We now feel the paper is stronger and more focused. We look forward to your comments.

Regards,

Jinlei Nie

(on behalf of all authors)

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