The cTnT response to acute exercise at the onset of an endurance training program: Evidence of exercise preconditioning?

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Running Head: exercise preconditioning and cardiac troponin

Abstract

Purpose Exercise induces a cardioprotective effect referred to as "preconditioning". Whether the preconditioning impacts upon the cardiac troponin T (cTnT) response to subsequent exercise bouts is unclear. This study investigated the effects of an initial exercise bout, a second exercise bout 48h later, as well as subsequent exercise every 48 h for 4 days or a single identical exercise bout after 8 days of inactivity gap on cTnT response to acute exercise.

Methods Twenty-eight sedentary overweight young women were randomly assigned to either six bouts of exercise each separated by 48 h or three bouts of exercise with 48 h between the first two bouts and 8 days between the second and third bouts. All exercise bouts were identical (60% $\dot{V}O_{2max}$, 200 kJ) and the total testing period (10 days) was the same for both groups. cTnT was assessed before and after the 1st, 2nd, and final exercise bouts.

Results cTnT increased (129%, P<0.05) after the first bout of exercise in both groups (peak post-exercise cTnT, median [range], ng.l^{-1}: 3.43[<3.00−27.26]) with no between-group differences in the response. The second exercise bout had no significant (P>0.05) effect on post-exercise cTnT (<3.00[<3.00−21.96]). The final exercise bout resulted in an increase (190%, P<0.05) in cTnT (4.35[<3.00−13.05]) in both groups.

Conclusions A single bout exercise resulted in a temporary blunting of cTnT response to acute exercise 48h later. The effect of exercise preconditioning was not preserved, regardless of whether followed by repeated exercise every 48 h or a cessation of exercise for 8 days.

Key Words: cardiac troponin T; cardiac biomarker; endurance exercise; exercise preconditioning

Abbreviations

3BOUTS	Three repeated bouts of exercise
6BOUTS	Six repeated bouts of exercise
ANOVA	Analysis of variance
cTn	Cardiac troponin
cTnI	Cardiac troponin I
cTnT	Cardiac troponin T
CVD	Cardiovascular disease
HR	Heart rate
RPE	Rating of perceived exertion
$\dot{V}O_{2max}$	Maximal oxygen uptake

Introduction

A growing body of evidence suggests that continuous prolonged exercise may result in the elevation of cardiac troponins (cTn, including cTnT and cTnI) (Gresslien and Agewall 2016), which are highly specific biomarkers of a cardiomyocyte insult (Wu et al. 1999). Conversely, a marked cardioprotective effect (e.g. smaller infarct size) may be evoked by a single or small number of exercise bouts and has been termed "exercise preconditioning" (Thijssen et al. 2018). Whether the cardioprotective effect of exercise preconditioning includes changes in the cTn response to subsequent acute exercise has not been systematically investigated. This information may be important to help elucidate the mechanisms involved in the exercise-induced cTn response, and to inform clinical decision-making regarding the evaluation of exercise-associated cTn elevation, considering the influence of exercise upon cTn may hamper or distort the diagnostic and prognostic role of the cTn assay (Gresslien and Agewall 2016).

A typical exercise preconditioning effect has a characteristic biphasic cardioprotective response, i.e. "early protection", which occurs immediately after one bout of exercise and lasts for ~4 h, and "late protection", which reappears 36 h after one bout of exercise and can last ~60 h (Thijssen et al. 2018). Furthermore, when exploring the sustainability of the effect, several bouts of exercise in animals preserved cardioprotection up to 7 to 9 days (Thijssen et al. 2018). Previous work from our laboratory demonstrated that when subjects did two identical 45 min runs at ventilatory threshold separated by 4 h of recovery, significant elevations in cTnT after the first run were markedly attenuated after the second run (Nie et al. 2011b). This finding supports the notion that exercise preconditioning at an "early protection" stage may include a blunting or abolition of the cTnT response to acute exercise. Although emerging evidence, mainly from animals, suggests that *late protection* appears 36−60 h after the first exercise and subsequent episodes of exercise provides continuous protection against myocardial injury (Thijssen et al. 2018), there is no current data describing whether the exercise preconditioning at stage of late and sustained cardioprotection are also apparent to the cTn response to acute exercise. Taken together, an integrated study in humans in which the effects of *late protection* (36−60 h) and further sustained protection (7−9 days) on cTn appearance with exercise is analyzed is warranted.

Obesity represents an important and common cardiovascular risk factor (Lavie et al. 2014). With the prevalence of obesity still rising, exercise training is recommended as a component of

weight management (Lavie et al. 2014). It has been suggested that obesity is associated with higher resting cTn concentrations (Daniels 2013), but it is not known what cTn responses would be apparent in overweight individuals in the early stages of an endurance exercise training program.

Consequently, we designed a randomized controlled study in young, overweight, untrained females. Both groups completed two initial exercise bouts separated by 48 h. Both exercise bouts involved identical work and serial blood samplings were performed to measure cTnT pre- and post-exercise. After this one group received, (1) four identical exercise bouts all 48 h apart with serial cTnT measured in the final bout, or (2) one more identical exercise exposure 8 days (192 h) later, outside of the late protection window, where cTnT data were assessed serially as before. By designing the study in this way, we could assess, (a) whether an initial exercise bout induced a blunting of cTnT response to the second bout of exercise undertaken 48 h later, and (b) whether this effect was maintained with the next series of regular, repeated exercise exposures compared to a period of inactivity.

Materials and Methods

Participants

One hundred and twenty-seven volunteers were recruited publicly through local advertisements to participate in the study. In total, 46 females were eligible according to the following inclusion criteria: 1) age range of 18–25 years, 2) body mass index (BMI) $\geqslant$23 kg.m^{-2}, which is the overweight cut-off for Asian adults (WHO 2000), 3) body weight remained constant (± 2 kg) during the past three months, 4) no regular physical activities or exercise training, 5) no history of smoking, and 6) no history of hormonal, orthopaedic, or cardiovascular diseases, diabetes, hyperlipidaemia, hypertension, or polycystic ovary syndrome, and no current use of prescribed medications (including contraceptive pills). Eighteen eligible participants declined to enter the study for personal reasons, the remaining 28 participants (Table 1) were randomly assigned to one of two groups performing either 6 (6BOUTS, n=14) or 3 (3BOUTS, n=14) bouts of exercise. After receiving a thorough briefing, the participants gave their written informed consent to participate. The experiment was approved by the local ethics committee of the Hebei Normal University.

Insert Table 1 here

Experimental design and procedures

The experimental design is illustrated in Fig. 1. Briefly, two initial familiarization sessions were held in the laboratory to acclimate the participants to cycling exercise. At least 3 days later, anthropometric measurements analysis was completed and maximal oxygen uptake ($\dot{V}O_{2max}$) was determined. One week after pre-intervention assessments both 3BOUTS and 6BOUTS groups commenced their repeated exercise bouts.

In the 6BOUTS group six bouts of exercise were all separated by 48 h. For the 3BOUTS group the first 2 bouts of exercise were separated by 48 h and the second and final bout were separated by 8 days (192 h) of inactivity. In both groups the 1st, 2nd, and final bouts included serial blood samples for the determination of cTnT.

For each observation, after having refrained from strenuous exercise for 48 h and subsequent to a general warm-up, 6BOUTS and 3BOUTS groups performed identical exercise bouts on a cycle ergometer (Monark, 839E, Sweden). Heart rate (HR) was recorded continuously via a portable HR monitor (Zephyr BioHarness 3.0, Zephyr Technology, Auckland, New Zealand). Immediately afterward, the participants rated the test for perceived exertion (RPE, Borg scale 6–20). Venous blood samples were drawn before exercise (Pre-exe), immediately after (0HR) as well as 3 h (3HR) and 4 h (4HR) after the exercise to assess serum cTnT. The timing for the post-exercise blood samples were in accordance with our previous work that demonstrated that blood cTnT concentrations peaked 3 or 4 h after exercise in a laboratory-based study (Tian et al. 2012). All exercise tests started at 11:00 a.m. and were performed in an air-conditioned laboratory (20 °C and 50% relative humidity). All participants were asked to maintain their daily activity and avoid altering their eating habits during the experimental period.

Insert Fig. 1 here

Protocol and measurements

Graded exercise test. $\dot{V}O_{2max}$ was determined using a graded cycling exercise protocol as described previously (Zhang et al. 2017). The participants began at 50 W with a pedal frequency of 60 rpm on a cycle ergometer (Monark, 839E, Sweden); power output was increased by 20 W

every 2 min until volitional exhaustion. Oxygen consumption during the exercise test was measured using a Cosmed breath-by-breath metabolic analyser (Quark-PFT-ergo, Cosmed, Rome, Italy). $\dot{V}O_{2max}$ was calculated as the highest 30-s average value. Following the graded exercise test, a power output that elicited approximately 60% $\dot{V}O_{2max}$ in the 6BOUTS and 3BOUTS groups was selected from the linear relationship of steady-state $\dot{V}O_2$ versus power output.

Acute exercise and blood sampling procedures. In each exercise bout all participants performed identical continuous cycling exercise at an intensity of 60% $\dot{V}O_{2max}$ until a target of 200 kJ of work was achieved. The pedal frequency was maintained at 60 rpm during cycling. An identical 10 min warm-up and 5 min cool down at 50–60% of HR_{max} were completed with each exercise bout. All exercise sessions were supervised who provided verbal encouragement during the exercise bouts and ensured that the participants exercised at the intended intensity. For each blood sample, 5 mL of venous blood was drawn from the antecubital vein by venipuncture with the subjects in a seated position. To separate serum, the blood was allowed to clot at room temperature and then centrifuged at 3500 g for 20 min. The serum was drawn off and stored at -80 °C for later analyses of cTnT. cTnT was measured quantitatively with a new high-sensitivity immunoassay based on electrochemiluminescence technology using a Cobas E 601 analyser (Roche Diagnostics, Penzberg, Germany). This assay has a range from 3 to 10,000 $ng.l^{-1}$ with a lower limit of detection of 3 $ng.l^{-1}$. Serum cTnT concentrations that were below the limit of detection were reported as 1.5 $ng.l^{-1}$ (Kong et al. 2017; Tian et al. 2012). The coefficient of variation of the mean cTnT concentration of 13.5 $ng.l^{-1}$ was 5.2%. The upper reference limit for cTnT, defined as the 99th percentile of healthy participants, was 14 $ng.l^{-1}$ (Giannitsis et al. 2010).

Statistical analysis

A 2 × 3 two-way ANOVA with repeated measures was used to examine the differences in HR_{mean}, $\%HR_{max}$, and RPE across the two groups (6BOUTS and 3BOUTS) and three exercise sessions observed (1st, 2nd, and final). *Post-hoc* analyses using Newman–Keuls were performed for cases in which the main effect was significant.

The Kolmogorov–Smirnov test was used to evaluate the normality of the data. Non-parametric Friedman's test was used to compare cTnT across the time points (Pre-exe, 0HR, 3HR, and 4HR) and three assessment points (1st, 2nd, and final) because of the skewed distribution of the cTnT data. Wilcoxon signed ranks tests were completed for pairwise comparisons where

appropriate. Moreover, cTnT in the 6BOUTS and 3BOUTS groups were compared using the Mann-Whitney U test. The percentages of subjects with cTnT exceeding the limit of detection of 3 $ng.l^{-1}$ (cTnT positive rate) at each assessment point were compared using Fisher's exact test. Statistical significance was assumed at a level of $P < 0.05$. Data analysis was performed using the statistical software package SPSS 20.0 (IBM Corp., Armonk, NY, USA).

Results

All participants (n = 28) completed the study, and no adverse events were reported during testing or exercise in either group. As expected, the acute exercise data, including HR_{mean}, $\%HR_{max}$, and RPE, at the 1st assessment were similar (all $P > 0.05$) to those in the 2nd and final assessments in both groups.

Insert Table 2 here

The acute exercise cTnT data for the two groups are presented as cohort data in Table 3 and as individual data points for pre-exercise (Pre-exe) and peak post-exercise (Post-exe) values in Fig. 2. cTnT values increased ($P < 0.05$) after the first exercise bout in both 6BOUTS and 3BOUTS groups with no between-group differences. Accordingly, there was an increase ($P < 0.05$) in cTnT positive rates in both groups compared to the prior assessment bout. After the second bout, 48 h after the initial exercise exposure in both groups, there was no significant elevation in cTnT post-exercise cTnT (both groups $P > 0.05$) with a reduced frequency of individuals positive for cTnT. In the final exercise bout there was an increase ($P < 0.05$) in cTnT post-exercise in both the 3BOUTS and 6BOUTS groups.

Insert Table 3 and Fig. 2 here

Discussion

Overview of findings

To the best of our knowledge, this is the first study to characterise the cTnT responses to acute exercise across the early stages of a health-related endurance training programme in previously inactive participants. The main findings of this study are that in previously sedentary young overweight females 1) the initial bout of acute endurance exercise in the training programme induced a blunting of cTnT response to the second bout of acute endurance exercise undertaken 48 h later, and 2) the subsequent adoption of either repeated and identical endurance exercise bouts every 48 h for eight days, or no activity, before a final endurance exercise bout did not result in a continuation or augmentation of this initial blunting.

When the two groups were combined, we observed that most of our participants (61%, 17 of 28) demonstrated an increase in cTnT after the 1st exercise bout of the endurance training programme, but only 14% (4 of 28) of them exceeded the upper reference limit (14 ng.l^{-1}). The prevalence (14%) was lower than that (83%) from a previous meta-analysis (Sedaghat-Hamedani et al. 2015) that used the same high-sensitivity assays. Given that a higher cardiac load is likely to result in a greater cTnT elevation (Fu et al. 2009), the findings are not surprising, as the total work performed in the present study was very low (200 kJ, 60% $\dot{V}O_{2max}$, ~1 h) when compared with previous studies that used tasks over many hours, days, and even weeks (Gresslien and Agewall 2016; Sedaghat-Hamedani et al. 2015).

"Late protection"

This is the first study to demonstrate that when previously sedentary participants perform two identical endurance exercise bouts, separated by 48 h, the significant elevation in cTnT after the first bout was markedly attenuated after the second bout. A similar phenomenon was reported in our earlier investigation, but in which the two exercise bouts were separated by 4 h (Nie et al. 2011b). Our prior finding suggests exercise preconditioning at "early protection" stage may include a blunting of the cTnT response to acute exercise, and the current data extend this to "late protection" stage. Of note, whilst all participants (100%, 12 of 12) lacked a cTnT response to the second run in this prior study (Nie et al. 2011b), the current study observed 32% (9 of 28) of our participants did experience some increase in cTnT following the second bout of exercise. This

suggests that early protection may be a more robust phenomenon than late protection in this scenario. Indeed, it remains unclear whether the different non-response rate (100% vs. 68%, Fisher's test, $P = 0.026$) in the two studies reflects characteristic features of the cTnT response in stages of early and late protection due to exercise preconditioning, as other factors apart from the time between the exercise bouts, such as age of participants and exercise mode, may potentially contribute to the individual differences. Further studies should continue to compare exercise-induced cTn appearance during the early and late protection time windows in different groups and with different exercise stimuli.

"Sustainability of cardioprotection"

Contrary to expectations, this study did not find a significant attenuation of post-exercise cTnT response at the final endurance exercise bout in either the 6BOUTS or 3BOUTS groups. These findings suggest that blunting post-exercise cTnT response during late protection cannot be preserved over longer time periods regardless of whether followed by repeated exercise training or not. That is to say, continuous cardiac preconditioning effects evoked by repeated exercise do not include a blunting of the cTnT response to acute exercise, and the reason(s) remains unclear. The standardised use of lab-based settings in the current study largely excludes the possibility that the effects of exercise intensity, duration, total mechanical work, and environment influenced exercise preconditioning. In addition, altered cardiorespiratory fitness is an unlikely explanation of our current findings, as our recent study (Nie et al. 2018) demonstrated, with improved cardiorespiratory fitness, exercise-induced elevation in cTnT will be largely abolished when exercise is performed at the same absolute intensity. Besides, the lack of a significant difference in acute exercise HR data also suggested that five (6BOUTS) or two (3BOUTS) repeated exercise bouts did not lead to rapidly improved cardiorespiratory fitness in the present study.

Considering the efficacy of classic preconditioning is attenuated in the presence of risk factors for cardiovascular disease (CVD), such as obesity (Thijssen et al. 2018), we may speculate that the fact that the female participants were overweight may have impaired or shortened the period of late cardioprotection. This speculation is supported by a field-based study by Eijsvogels et al. showing in subjects with CVD risk factors that moderate-intensity long-distance exercise-induced increases in cTnI levels were comparable across four consecutive exercise days (Eijsvogels et al. 2010). Nevertheless, to further support such an assertion, future studies in

laboratory-based settings, which take risk factors of CVD into account, will need to be undertaken. In addition, it is worth noting that both the current study and that of Eijsvogels et al (2010) employed only continuous endurance exercise. Given emerging evidence suggesting that interval-type exercise may yield larger cardioprotection due to exercise preconditioning than endurance exercise (Thijssen et al. 2018), further research is required to determine whether interval exercise can strengthen and/or lengthen the attenuation of post-exercise cTnT response.

Baseline values for cTnT

We observed higher baseline values for cTnT after five repeated exercise bouts in the 6BOUTS group, which also differentiated this group from the 3BOUTS cohort. This supports data from a previous study, which demonstrated that after 14-week endurance training, baseline cTnT was significantly higher compared with pretraining and the control group (Legaz-Arrese et al. 2015b). The authors speculated that this was as a consequence of physiological hypertrophy of the heart (Legaz-Arrese et al. 2015b) although no data was presented to support this. The current data shorten the training duration to 10 days and suggests that higher baseline values for cTnT may appear at the early stage of training before heart physiological hypertrophy occurs. The reason for this is not clear, but the consistent timing of blood sampling in the present study excludes the possibility of influence of diurnal rhythm as a potential confounder (Klinkenberg et al. 2014).

Individual variability in cTnT response

This study confirmed the findings of previous studies that considerable inter-individual variation exists in the cTnT response to acute exercise (Legaz-Arrese et al. 2015a; Legaz-Arrese et al. 2015c; Nie et al. 2011a; Nie et al. 2011b; Nie et al. 2011c). Moreover, our results also suggest a degree of intra-individual variability in the response of cTnT with exercise across three bouts in two groups (see Figure 2), which supports previous data from male triathletes (Legaz-Arrese et al., 2015). Some studies suggest that exercise-induced cTn elevation is likely dependent on various, potentially interacting factors and as such is extremely difficult to predict (Eijsvogels et al. 2015). Our current results support this notion, as the marked inter- and intra-subject variability in the exercise-associated cTnT response could not be explained in our data by power output and/or HR during exercise, time of day, environment, or subject characteristics including age, body mass, and $\dot{V}O_{2max}$. Nevertheless, almost all of the previous studies simply employed a design with a single bout of exercise, and the subjects had training experience (Gresslien and Agewall 2016). Thus, the

current study adds new information by detailing the inter- and intra-subject variability apparent across the early stage of exercise training.

Implications

The mechanism(s) responsible for exercise-induced cTn elevation remains poorly understood. Nevertheless, it has been proposed that due to the almost obligatory nature of this phenomenon that there is little or no immediate clinical relevance to the health of the participant (Gresslien and Agewall 2016). Most cTnT data post-exercise are below the population upper reference limit and together with the absence of symptoms or signs of myocardial ischemia during exercise in the current experiment supports the likelihood that this is a benign physiological process.

A blunting of the cTnT response to acute exercise has now been reported in time periods typical of early and late cardioprotection, which seems to suggest that the attenuation of cTnT elevation may share a common mechanism(s) with exercise preconditioning, although we cannot provide direct evidence of shared mechanistic processes. The "classic" mediator of exercise preconditioning is believed to be elevated myocardial antioxidant capacity (Hamilton et al. 2003). Interestingly, a previous animal experiment in our lab suggested that exercise-induced increases in myocardial free radicals may be related to cTnT release from cardiomyocytes (Nie et al. 2010). Our current results add another dimension to support this notion and also promotes the idea that it may be insightful to manipulate myocardial antioxidant capacity, via gene transfer or use of exogenous agents, and simultaneously determine exercise-induced cTn appearance. Moreover, recent evidence suggests that the exercise preconditioning may also be mediated by opioid receptor activation and upregulation of mitochondrial/sarcolemmal K_{ATP} channels (Miller et al. 2015), which also provide novel clues to gain a better understanding of the mechanism(s) of exercise-induced cTn elevation in future research.

This study also adds to our understanding of the cTnT response to acute exercise by selecting a particular observation window, i.e. the early stage of exercise training. Specifically, a temporary blunting of the cTnT response can be expected, albeit heterogeneously. Such information will assist clinicians if faced with interpreting exercise-associated cTnT in patients who have just initiated an exercise training regime.

Limitations

There are a small number of limitations that should be considered. The sample size was relatively small and pertained only to young, overweight female participants. As such the generalisability of the data is confined to this group and the specific exercise task. The recruitment of young females was predicated on the relative lack of use of female participants in prior research and the availability of data for analyses of cTnT from a broader study on cardiovascular health in this sample. Further, although we attempted to control for menstrual cycle health (no oral contraceptive users and no one with menstrual dysfunction) in the female participants, we could not constrain testing to specific phases of the menstrual cycle on each exercise session observed. This could have some influence upon post-exercise cTnT concentrations and a specific menstrual cycle phase study would be useful. In addition, we did not measure the plasma volume. Such measurements could have broadened our understanding of the cTnT elevation due to acute exercise and should be undertaken in future studies. Finally, we selected previously sedentary subjects in order to obtain a "clean" training background and preclude the effects of prior training experience. For this reason, our work was limited to assessing the cTnT response following an exercise of relatively low load that would be achievable in these participants.

Conclusion

In conclusion, in previously sedentary, young overweight females a single bout of exercise induced a blunting of the cTnT response to an identical acute endurance exercise that occurred 48 h after the first bout. This is within the time window of late cardioprotection. This effect was not preserved in another bout of identical endurance exercise 8 days later, regardless of whether followed by repetition or cessation of exercise. Clinicians should be aware that a temporary attenuation of post-exercise cTnT response may appear, albeit heterogeneously, at the early stage of exercise training.

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Author contributions

HZ, JN, ZK and KG conceived and designed research. HZ, JN, ZK, WC, XZ and ZZ conducted experiments. HZ, JN, ZK and KG analyzed data. HZ, JN, ZK and KG wrote the manuscript. All authors read and approved the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Table 1 Participant characteristics (mean ±SD)

	All Participants (n=28)	6BOUTS (n=14)	3BOUTS (n=14)
Age (yr)	20.9 ±2.1	20.7 ±2.2	21.1 ±2.0
Height (cm)	159.6 ±6.3	159.4 ±6.2	159.8 ±6.6
Weight (kg)	67.3 ±8.6	66.0 ±8.6	68.7 ±8.8
Body mass index ($kg.m^{-2}$)	26.4 ±2.6	25.9 ±2.7	26.8 ±2.5
Body fat (%)	32.0 ±3.1	31.6 ±3.1	32.4 ±3.1
Fat mass (Kg)	21.7 ±4.7	21.0 ±4.7	22.4 ±4.7
$\dot{V}O_{2max}$ ($ml.kg^{-1}.min^{-1}$)	29.2 ±2.9	29.0 ±2.9	29.5 ±2.9

6BOUTS, six repeated bouts of exercise; 3BOUTS, three repeated bouts of exercise

Table 2 Acute exercise data (mean ±SD) from a 200-kJ cycling in six repeated bouts of exercise (6BOUTS) and three repeated bouts of exercise (3BOUTS) groups

	$Power_{exe}$ (W)	**$Time_{exe}$** (min)	**HR_{mean}** ($beat.min^{-1}$)	**$\%HR_{max}$**	**RPE**
6BOUTS (n=14)					
1^{st}	55 ±9	62 ±11	140 ±11	76 ±6	15 ±2
2^{nd}	55 ±9	62 ±11	137 ±11	74 ±6	15 ±2
6^{th}	55 ±9	62 ±11	135 ±8	73 ±4	14 ±2
3BOUTS (n=14)					
1^{st}	57 ±15	63 ±19	146 ±12	80 ±7	15 ±3
2^{nd}	57 ±15	63 ±19	143 ±12	79 ±7	14 ±3
3^{rd}	57 ±15	63 ±19	143 ±13	79 ±6	14 ±2

1^{st}, 2^{nd} and 6^{th} in 6BOUTS indicate the 1^{st}, 2^{nd} and 6^{th} exercise bout in 6BOUTS, respectively; 1^{st}, 2^{nd}, and 3^{rd} in 3BOUTS indicate the 1^{st}, 2^{nd} and 3^{rd} exercise bout in 3BOUTS, respectively. $Power_{exe}$, power output during exercise bout; $Time_{exe}$, exercise duration; HR_{mean}, mean heart rate during exercise bout; $\%HR_{max}$, percentage of individual maximal heart rate during exercise bout; RPE, rating of perceived exertion at end of exercise

Table 3 Serum cardiac troponin T [ng.l^{-1}, median (range)] before (Pre-exe) and immediately (0HR), 3h (3HR) and 4 h (4HR) after a 200-kJ cycling in six repeated bouts of exercise (6BOUTS) and three repeated bouts of exercise (3BOUTS) groups

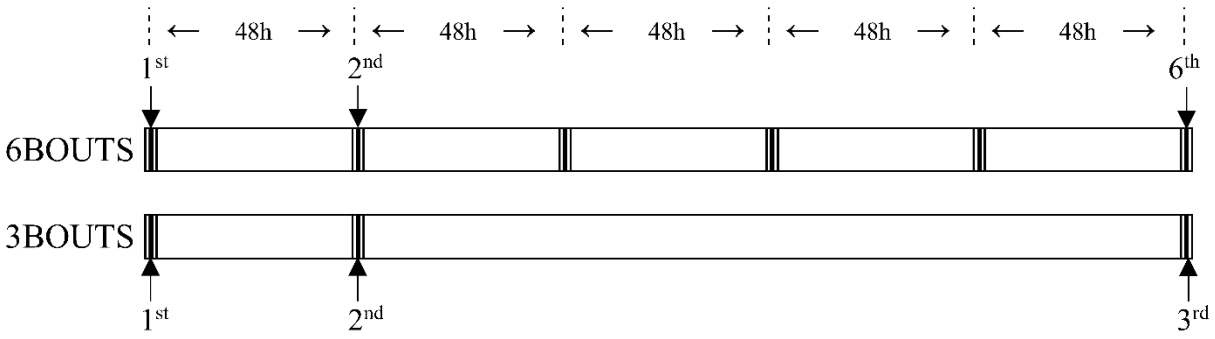
	Pre-exe	0HR	3HR	4HR
6BOUTS (n=14)				
1st	1.50 (1.50-5.40)	1.50 (1.50-5.93)	3.10 (1.50-10.31)	3.55 (1.50-27.26)*
2nd	1.50 (1.50-3.05)	1.50 (1.50-1.50)	1.50 (1.50-13.65)	1.50 (1.50-11.52)
6th	3.81 (1.50-5.62)†	4.10 (1.50-5.46)	4.27 (1.50-6.13)*	4.49 (1.50-6.60)
3BOUTS (n=14)				
1st	1.50 (1.50-1.50)	1.50 (1.50-1.50)	1.50 (1.50-20.38)	2.27 (1.50-21.92)*
2nd	1.50 (1.50-4.04)	1.50 (1.50-1.50)	1.50 (1.50-21.96)	1.50 (1.50-16.54)
3rd	1.50 (1.50-1.50)	1.50 (1.50-1.50)	1.50 (1.50-13.05)	2.35 (1.50-10.09)*

1st, 2nd and 6th in 6BOUTS indicate the 1st, 2nd and 6th exercise bout in 6BOUTS, respectively; 1st, 2nd, and 3rd in 3BOUTS indicate the 1st, 2nd and 3rd exercise bout in 3BOUTS, respectively.

* Significantly different from corresponding Pre-exe value, $P < 0.05$

† Significantly different from corresponding 1st value, $P < 0.05$

Fig. 1 Schematic diagram of study protocol



6BOUTS, six repeated bouts of exercise; 3BOUTS, three repeated bouts of exercise

A bout of continuous cycling exercise (60% $\dot{V}O_{2max}$, 200 kJ)

Observed exercise bout

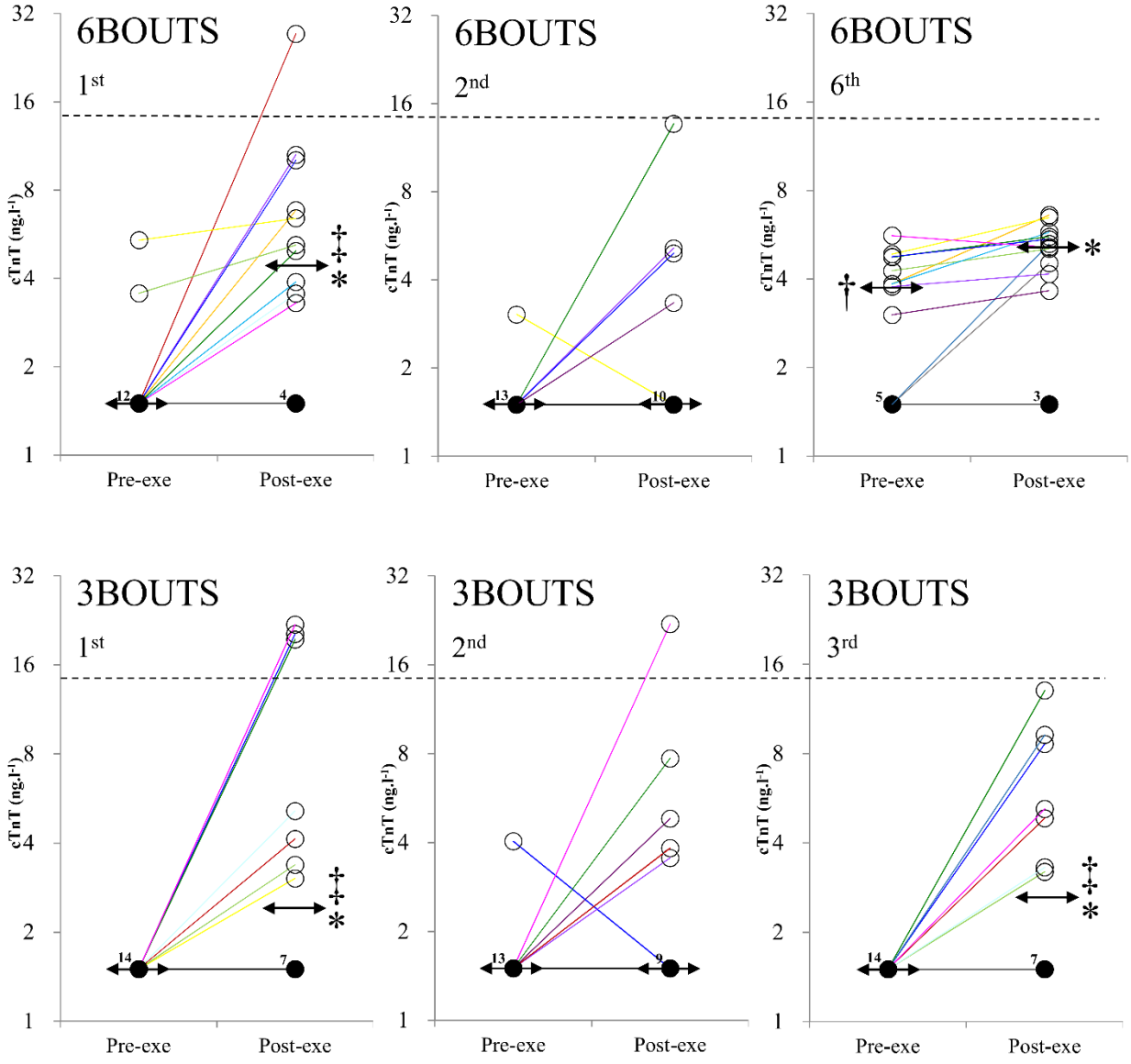
Fig. 2 Pre-exercise (Pre-exe) and peak post-exercise (Post-exe) cardiac troponin T (cTnT, $ng.l^{-1}$) after a 200-kJ cycling bout in six repeated bouts of exercise (6BOUTS) and three repeated bouts of exercise (3BOUTS) groups (scale is log plotted because of the data spread). Individual data points are presented by circles with values for the same participant connected by lines for each condition. Within each group, each subject has same color individual line across three exercise bouts.

Note: 1^{st}, 2^{nd} and 6^{th} in 6BOUTS indicate the 1^{st}, 2^{nd} and 6^{th} exercise bout in 6BOUTS, respectively; 1^{st}, 2^{nd}, and 3^{rd} in 3BOUTS indicate the 1^{st}, 2^{nd} and 3^{rd} exercise bout in 3BOUTS, respectively; The horizontal dotted line is the upper reference limit (14 $ng.l^{-1}$); The double-arrow line is the median of cTnT values at pre-exercise (Pre-exe) or Post-exercise (Post-exe); ○, single subject; ^{n}●, n subjects

* Significantly different from corresponding median of Pre-exe, $P < 0.05$

‡ Significantly different from corresponding positive rate (the percentages of subjects with cTnT exceeding the limit of detection of 3 $ng.l^{-1}$) of Pre-exe, $P < 0.05$

† Significantly different from Pre-exe value of 6BOUTS at other two assessments, and 3BOUTS at all three assessments, $P < 0.05$


6BOUTS
1st
6BOUTS
2nd
6BOUTS
6th
3BOUTS
1st
3BOUTS
2nd
3BOUTS
3rd
cTnT (ng.l-1)
32
16
8
4
2
1
Pre-exe
Post-exe
12
4
13
10
5
3
14
7
13
9
14
7