The impact of exercise modality and menstrual cycle phase on circulating cardiac troponin T

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Abstract

Objectives: It is unclear whether exercise modality (moderate-intensity continuous [MCE]; high-intensity interval [HIE]) and menstrual cycle phase (follicular [FP]; luteal [LP]), individually or in combination, mediate the commonly observed exercise-induced elevation in cardiac troponin T (cTnT). This study examines cTnT responses to MCE and HIE during both the FP and LP.

Design: Randomised crossover study.

Methods: Seventeen healthy, eumenorrheic women completed four trials including MCE (60% VO_{2max} steady-state cycling until 300 kJ) and work-equivalent HIE (repeated 4-minute cycling at 90% VO_{2max} interspersed with 3-minute rest) during both the FP and LP. The FP and LP were verified based on ovarian hormones. Serum cTnT was assessed using a high-sensitivity assay before, immediately after, and 1 (1HR), 3 (3HR) and 4 (4HR) hours after exercise. cTnT values were corrected for plasma volume changes.

Results: cTnT was significantly elevated (p <0.05) post-exercise in both MCE (at 3HR and 4HR) and HIE (at 1HR, 3HR and 4HR). No statistically significant difference (p >0.05) in peak post-exercise cTnT, which mostly occurred at 3HR, was seen among the four trials (median [range], $ng.l^{-1}$: 5.2 [1.7-18.1] after MCE during FP; 4.8 [1.7-24.9] after MCE during LP, 8.2 [3.9-24.8] after HIE during FP and 6.9 [1.7-23.1] after HIE during LP).

Conclusions: A single 300 kJ bout of both MCE or HIE resulted in a significant post-exercise increase in cTnT, with no differences in peak cTnT response between menstrual cycle phases or between exercise modes, but the cTnT elevation occurs slightly earlier after HIE.

Key Words: Cardiac biomarker; Follicular phase; Luteal phase; Moderate-intensity continuous exercise; High-intensity interval exercise

Practical implications

- The exercise-induced cardiac troponin T elevation is largely obligatory, and we would hypothesise that this points to a physiological phenomenon.
- When applying the algorithm of acute coronary syndromes recommended by the European Society of Cardiology, clinicians need to be aware that prior exercise, both moderate-intensity continuous and high-intensity interval, might complicate the use of the 0h/3h algorithm. The 0h/1h algorithm might be affected by prior high-intensity interval exercise.
- Future studies assessing exercise-associated changes in cTnT in eumenorrheic, young women do not need to control from menstrual cycle phase in any trial design.

1. Introduction

There is a burgeoning evidence base to suggest that cardiac troponin (cTn; cTnT and/or cTnI), a biomarker pathognomic for cardiomyocyte damage[1], is elevated after exercise.[2, 3] To date, the mechanism(s) and exact determinant(s) that mediate post-exercise cTn elevation are poorly understood.[3, 4] This knowledge can contribute to any clinical decision-making associated with exercise-induced cTn changes such as a visit of a patient to a medical department following a bout of exercise in which extreme fatigue, shortness of breath and/or orthostatic intolerance have occurred.

Whether factors related to the exercise modality are associated with cTn response patterns is poorly understood.[3] A recent study from our group noted that two distinct kinds of high-intensity interval exercise (HIE), matched for total mechanical work, induced a similar elevation in cTnT after acute exercise.[5] It is unclear whether equalisation of total work between moderate-intensity continuous exercise (MCE) and HIE will result in similar cTnT perturbations.

Although little empirical data are available it has been widely speculated that exercise may cause reversible cardiomyocyte damage that temporarily increases the permeability of the cell membrane, leading to release of soluble cTn molecules from the cytosol.[2-4] Experimental studies have demonstrated that oestrogen can protect the heart from ischaemia-reperfusion injury such that cTnI is reduced.[6] It is thought that one of the ways in which oestrogen reduces myocardial damage and cTn release is a consequence of its antioxidant properties, which to help stabilise and protect the cell membrane.[6] We recently demonstrated that exercise-induced cTnT elevation was lower in

females compared to males,[7] which supports the hypothesis that the attenuated cTnT response may occur in the presence of high oestrogen. Interestingly, the exercise-induced release of creatine kinase from skeletal muscle cells is lower in the luteal phase (LP, high oestrogen) as compared to the follicular phase (FP, low oestrogen) of the menstrual cycle.[8] It is not known if the menstrual cycle is a determinant that could mediate any post-exercise cTn elevation. This information may be important to help elucidate the mechanisms involved in the exercise-induced cTn response.

Consequently, the aims of the present study were (1) to compare cTnT appearance following acute MCE and HIE, when matched for total work, and (2) to investigate the effects of menstrual cycle phase (LP and FP) on cTnT responses to acute exercise. We hypothesized that exercise modality (matched for work output) will not mediate exercise-induced cTnT. In line with the cardioprotective effect of oestrogen, we hypothesized that the cTnT response observed at post-exercise will be lower in LP than FP.

2. Methods

Sixty female volunteers were recruited through local advertisements to participate in the study. In total, 23 females were eligible according to the following inclusion criteria: 1) age range of 18–25 years; 2) eumenorrheic for the past six months; 3) no regular physical activities, based on International Physical Activity Questionnaire,[9] in the last 6 months; 3) no history of smoking; and 4) no history of hormonal, orthopaedic, or cardiovascular diseases, diabetes, hyperlipidaemia, hypertension or polycystic ovary syndrome; and no current use of prescribed medication (including contraceptive pills in the last 6 months), as assessed by a medical screen prior to the study. After receiving a thorough briefing, the participants gave their written informed consent to participate. The experiment was approved by the ethics committee of the Hebei Normal University and was conducted in accordance with the Helsinki declaration 1975 (revised 2013).

On the first and second visits separated by three days to the laboratory, MCE (70% of age-predicted HR_{max}/85min) and HIE (85% of age-predicted HR_{max}/45min) trials, which simulated the experimental trials, were performed to acclimatise the participants to corresponding cycling sessions, respectively. Three to five days later, the assessment of maximal oxygen uptake (VO_{2max}) was completed. The four experimental trials were completed in a fully randomised (by lottery) crossover design of exercise modality (MCE and HIE) and menstrual phase (follicular [FL] and luteal [LP]) and were completed within a 2-month window. The time range between the two

contiguous experimental trials was 7-15 days. During each exercise session, heart rate (HR) was recorded continuously via a portable HR monitor (Zephyr BioHarness 3.0, Zephyr Technology, Auckland, New Zealand). After a 5-min rest period in a seated position, venous blood samples were drawn before exercise (PRE), immediately after (0HR), as well as at 1 h (1HR), 3 h (3HR) and 4 h (4HR) after exercise to assess cTnT, haemoglobin (Hb) and haematocrit (Hct) as well as oestrogen and progesterone (only before exercise). Considering that the latest European Society of Cardiology (ESC) guidelines for the management of acute coronary syndromes recommends serial cTn testing and an algorithm of 0h/1h and 0h/3h rule in and rule out,[10] we chose the before, immediately after as well as 1 h, 3 h and 4 h after exercise to assess circulating cTnT. One day prior to each trial, participants were provided with the same meals at 20:00. The last meal before testing was a light snack (~400kcal) consumed at 08:00 and water was allowed *ad libitum*. All exercise tests started at 11:00 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 and sleeping habits during the experimental period.

VO_{2max} was determined using a graded cycling exercise protocol that has been described previously.[5, 11] 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 3 min until volitional exhaustion. Volitional exhaustion was defined as the point at which the subject could no longer maintain the required exercise intensity. Oxygen consumption during the exercise test was measured using a Cosmed breath-by-breath metabolic analyser (Quark-PFT-ergo, Cosmed, Rome, Italy). VO_{2max} was calculated as the highest 30-s average value. Following the graded exercise test, a power output that elicited approximately 60% and 90% VO_{2max} was used in the MCE and HIE, respectively.

The MCE protocol comprised continuous exercise on a cycle ergometer (Monark, 839E, Sweden) at an intensity of 60% VO_{2max} until a target of 300 kJ of work was achieved.[5] By contrast, the HIE protocol comprised of repeated 4-min exercise bouts on a cycle ergometer (Monark, 839E, Sweden) at an intensity of 90% VO_{2max}, followed by a 3-min passive recovery (seated) until the targeted 300 kJ of work was achieved.[5] In each exercise session, participants completed an identical 10-min warm-up and 5-min cool down at ~50% of HR_{max}.

The phases of the menstrual cycle were determined initially by measuring morning basal body temperature and urine luteinising hormone concentration using the home ovulation prediction kit (Runbio Biotech Co., Ltd., Shantou, China). Testing occurred 3–7 days after the onset of menses (i.e., early follicular phase, FP), and 4–10 days post a positive luteinising hormone reading (i.e., mid-luteal phase, LP). Additionally, blood samples taken before the commencement of exercise were analysed for oestrogen and progesterone to verify menstrual cycle phase. The verification of menstrual cycle phase is based on (gold standard),[12] (1) a minimum twofold increase in oestrogen from FP to LP, and (2) a progesterone level of > 9.5 nmol.l^{-1}. This provides good evidence that ovulation has occurred. Using these criteria, we excluded six participants from the final analysis, which left a total of 17 participants (mean±SD: aged 21.5±2.6 years, body mass 58.4±5.2 kg, height 158.8±5.5 cm, and VO_{2max} 33.8±3.6 ml.kg^{-1}.min^{-1}).

At each sample time point, 5 ml of venous blood was drawn from the antecubital vein by venepuncture with the participants in a seated position. An aliquot was obtained for the determination of whole blood Hb and Hct using a Sysmex XP-100 analyser (Sysmex Corporation, Kobe, Japan). The remaining 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 analysis of cTnT, as well as oestrogen and progesterone (only before exercise). cTnT, oestrogen (17β-oestradiol) and progesterone were measured based on electrochemiluminescence technology using a Cobas E 601 analyser (Roche Diagnostics, Penzberg, Germany). The cTnT assay is the 5th generation high-sensitivity immunoassay and has a lower detection limit of 3 ng.l^{-1} with an upper limit of 10,000 ng.l^{-1}. Serum cTnT concentrations that were below the limit of detection are reported as 1.5 ng.l^{-1}.[13] The coefficient of variation at a mean cTnT concentration of 13.5 ng.l^{-1} is 5.2%. The upper reference limit for cTnT, defined as the 99th percentile of healthy participants, was 14 ng.l^{-1}.[14] The intra-assay coefficients of variation for oestrogen and progesterone were 3.5 % and 2.1 %, whereas the limits of detection were 5 pg.l^{-1} and 0.095 nmol.l^{-1}, respectively. Results for cTnT and hormones were corrected for percent change in plasma volume, calculated from levels of Hb and Hct in four trials, as described elsewhere.[13, 15]

A 2 × 2 two-way ANOVA with repeated measures was used to examine the changes in oestrogen, progesterone, power during exercise, exercise duration, exercise mean HR and $\%HR_{max}$ across the two modalities (MCE and HIE) and two menstrual cycle phases (FP and LP). *Post-hoc*

analyses, using the Newman–Keuls test,[13] were performed for cases in which the main effect was significant. The Kolmogorov–Smirnov test was used to evaluate the normality of the data. The non-parametric Friedman's test was used to compare cTnT across the time points (PRE, 0HR, 1HR, 3HR and 4HR) and exercise sessions because of the skewed distribution of the cTnT data. Wilcoxon signed ranks tests were completed for pairwise comparisons where appropriate. The percentages of participants with a 50 % or greater increase in cTnT values from PRE and 0HR at subsequent assessment points were compared between four trials using Fisher's exact test. The criterium of a 50 % or greater change is based on a threshold that detects changes which are greater than the combined biological and analytical variation of cTnT according to ESC guidelines.[10] Statistical significance was assumed at a level of $p<0.05$. Values are reported as mean±SE unless otherwise indicated. Data analysis was performed using the statistical software package SPSS 20.0 (IBM Corp., Armonk, NY, USA).

3. Results

Seventeen participants completed the study (dropout: 6 of 23, 26%), and no adverse events were reported. Oestrogen and progesterone concentrations were significantly higher ($F_{(1,16)}$=94.52/98.98, both $p<0.001$, cohen's d=3.20/3.01) in the LP but not different ($F_{(1,16)}$=0.465/0.088, p=0.5051/0.771, cohen's d=0.02/0.08) for each exercise modality (**Fig. 1**). The acute exercise data, including power output during exercise (HIE, FP/LP: 110±4 W; MCE, FP/LP: 61±3 W), mean HR during exercise (HIE, FP/LP: 157±3/153±3 beat.min^{-1}; MCE, FP/LP: 139±4/134±4 beat.min^{-1}) and %HR_{max} during exercise (HIE, FP/LP: 89±2/86±1 %; MCE, FP/LP: 79±2/76±8 %), were similar ($p>0.05$) between menstrual phases but were higher ($p<0.001$) in HIE than in MCE. Accordingly, exercise duration was lower in HIE than in MCE (HIE, FP/LP: 46±2 min vs. MCE, FP/LP: 85±4 min, $p<0.001$).

Insert Fig.1 here

cTnT data for the four trials are presented as cohort data in **Table 1** and as individual data points in **Fig. 2**. cTnT values increased ($p<0.001$) after the exercise bout in all four trials with no between-trials differences (p=0.12) in peak post-exercise cTnT values, which mostly (78%) occurred at 3HR (**Fig. 2**). Accordingly, the percentages of participants with maximum cTnT elevations greater than combined biological and analytical variation were similar among the four trials (HIE, FP/LP: 82/82 % [14 of 17]; MCE, FP/LP: 65/59 % [11 of 17/10 of 17], p=0.186). A

small difference in cTnT kinetics was noted, with a significant elevation at 1HR after HIE compared to an initial significant elevation at 3HR in the MCE trials (**Table1**). Accordingly, the percentages of participants with cTnT elevations at 1HR from 0HR that were greater than the combined biological and analytical variation were significantly higher (p=0.0001) in HIE (FP/LP: 82/59 % [14 of 17/10 of 17]) than in MCE (FP/LP: 24/12 % [4 of 17/2 of 17]).

Insert Fig.2 and Table1 here

4. Discussion

The present study provides a direct comparison of the early kinetics of cTnT after energy-matched MCE and HIE during different phases of the menstrual cycle. Using serial sampling in a randomised crossover design, we demonstrated that acute MCE or HIE resulted in a significant post-exercise increase in cTnT with no between-modality differences in peak response. Interestingly the cTnT elevation tended to occur slightly earlier after HIE than MCE. Moreover, menstrual cycle phase did not significantly mediate the cTnT response to acute exercise, irrespective of exercise modality.

The fact that cTnT concentrations increase shortly after exercise in males is well known,[2, 3] and the current data add to this in almost the entire female cohort (16 of 17, 94%). Of further interest, most of the peak cTnT changes (49 of 68, 72%) were greater than the combined biological and analytical variation for cTnT.[10] This study confirmed the findings of previous studies that inter-individual variation exists in cTnT response to acute exercise,[3] but a large portion (>90%) of variations cannot be explained by current understanding of participant attributes.[16] The absolute cTnT changes in the current study are lower than those observed after a marathon or ultra-endurance race,[3] likely due to the lower cardiac load in the current study. In support of this interpretation, a recent investigation[17] showed that a single 40 min bout of both MCE or HIE resulted in a significant post-exercise increase in cTnT whose absolute change was similar to that observed in the present study. Uniquely, the current work is the first study to report that both MCE and HIE (matched for total work done) displayed similar peak post-exercise cTnT values. Moreover, the peak cTnT values were most commonly observed at 3 h post-exercise (**Fig.2**). The timing of peak data supports our previous lab-based work which demonstrated that, in male adults

and adolescents, cTnT concentrations peaked 3 or 4h after continuous endurance exercise.[13] Taken together, the two studies seem to suggest that the timing of peak post-exercise cTnT is similar across exercise modality, gender and age.

Whilst we recently showed that two distinct kinds of HIE, matched for total work, induced a similar elevation in cTnT after acute exercise,[5] the present study expands these findings to different exercise modalities (constant vs. variable intensity). Taken together, it would seem that total mechanical work may be an important determinant for any cTnT elevation. It should be noted that the current data is at odds with the study by Ranjbar et al., who showed that continuous exercise resulted in a higher cTnT elevation than intermittent exercise.[17] However, notably, this study employed HR during exercise to equalise the cardiac workload of the two exercise modalities. The comparison of results from this investigation above should be approached with some caution, considering that HR lag and inertia at exercise onset and cessation occurred during intermittent exercise.[18, 19]

While visual observation suggests consistency in the overall pattern of cTnT appearance during early recovery periods following MCE and HIE (**Fig.2**), statistical analysis implies the initiation of cTnT elevation occurred slightly earlier in HIE than in MCE. This finding supports the previous work in continuous exercise models showing that post-exercise cTnT elevation occurred sooner after more intense exercise, but with higher peak values.[20] We should note that, in the present study, *intermittent* higher intensity work did not elicit a higher peak post-exercise cTnT level than the work-equivalent continuous exercise. Thus, the present study expands on the understanding of the kinetics of exercise-induced cTnT rise by demonstrating a different timing of the initiation of cTnT elevation, but a similar timing and level of cTnT peak after HIE and MCE.

We determined that despite significantly higher oestrogen levels during the LP, the menstrual cycle phase had no independent or combined (with exercise modality) impact on exercise-induced cTnT. This runs contrary to our stated hypothesis that oestrogen may exert protective effects on the cardiomyocytes and thus induce a lower post-exercise cTnT concentration. Of note, in the current study, levels of progesterone are ~50 times greater during the LP than during the FP, whereas oestrogen is only ~4 times greater. The effects of progesterone on the cardiovascular system are controversial. The addition of progesterone to hormone treatment regimens has been reported to inhibit oestrogen's protective effects in some, but not all, models of

cardiovascular injury.[21, 22] It is unclear whether the similar post-exercise cTnT kinetics during LP and FP in the current data is related to the potential "antiestrogenic" effect of progesterone. Further work is required to determine whether the late FP, characterised by the pre-ovulatory surge in oestrogen and suppressed progesterone concentrations, could attenuate the elevation of post-exercise cTnT. It is, however, challenging to "capture" the short window with the highest oestrogen level, as the FP is more variable in length than the LP and, thus, it is difficult to predict the day of ovulation.[12, 23]

In the current study, almost all participants presented with an increase in cTnT following exercise, but most cTnT data are below the population upper reference limit of 14 $ng.l^{-1}$. These findings suggest that an exercise-induced cTnT elevation is likely to be obligatory and, thus physiological in nature. Considering the latest fourth universal definition of myocardial infarction recommends evaluation of cTn kinetics as a fundamental component of the clinical assessment of chest pain patients,[24] and the latest ESC guidelines present a 0h/1h and 0h/3h rule in and rule out algorithm,[10] the current pilot study provides some initial clinical insight and may be a base for future work. Here, we present characteristics of the kinetics of post-exercise cTnT within a 4 h recovery period across exercise modality and menstrual cycle phase. Such information provides clues to assist clinicians if faced with interpreting exercise-associated cTnT in women, e.g., both of MCE and HIE might complicate the use of the 0h/3h algorithm, and the 0h/1h algorithm might be affected by HIE, irrespective of menstrual cycle phase. Another practical implication of the current findings would be that in future studies related to exercise-associated change in cTnT, the inclusion of eumenorrheic women can be undertaken without a need to adjust trial design for menstrual phase.

An important limitation is that we selected sedentary, young, healthy subjects in a small sample size (n=17), which may limit the generalisability of the current findings to other groups, such as trained or middle-aged individuals. Nevertheless, this feature allows assessment of a homogeneous cohort, free of potential confounding issues associated with training status or health status.[25] In addition, a new stricter luteal phase verification limit of >16 $nmol \cdot l^{-1}$ for progesterone has just been proposed.[23] It is, therefore, recommended that future research follow this criterion to reduces the risk of inclusion of anovulatory or luteal phase deficient cycles in a large and heterogeneous cohort.

5. Conclusion

In conclusion, we reported that cTnT levels increased after a single 300 kJ bout of MCE or HIE, with no between-modality differences in the timing (at 3 h post-exercise) and magnitude of peak response, although the initial post-exercise cTnT rise began a little earlier after HIE. The menstrual cycle phase did not alter post-exercise cTnT kinetics, irrespective of exercise modality. The results remind clinicians when applying ESC guideline, prior exercise might complicate the use of 0h/1h and/or 0h/3h algorithms in young female patients, irrespective of menstrual cycle phase.

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Table 1. Serum cardiac troponin T [ng.l^{-1}, median (range) [95% confidence interval], n =17] before (PRE) and immediately (0HR), 1h (1HR), 3h (3HR) and 4 h (4HR) after a 300-kJ cycling in four bouts of exercise

	PRE	0HR	1HR	3HR	4HR
MCE					
FP	3.0 (1.5-8.0) [2.4-4.3]	3.1 (1.1-7.0) [2.2-4.1]	4.1 (1.2-12.2) [2.5-5.8]	4.3 (1.4-18.1)*† [3.6-8.8]	4.0 (1.5-15.3)* [3.1-6.9]
LP	2.7 (1.3-4.8) [1.9-3.1]	2.8 (1.2-7.5) [2.2-3.9]	3.7 (1.3-11.4) [2.4-5.3]	3.5 (1.4-24.9)*† [2.7-9.5]	3.8 (1.4-14.5)*† [2.8-6.0]
HIE					
FP	3.0 (1.1-6.3) [2.3-4.0]	3.5 (1.1-6.8) [2.4-4.1]	6.0 (2.9-16.4)*† [4.8-9.0]	8.2 (3.8-24.8)*† [7.3-14.0]	7.6 (1.5-20.9)*† [6.2-11.9]
LP	2.4 (1.1-5.0) [2.0-3.4]	2.9 (1.0-5.7) [2.1-3.6]	4.7 (1.1-19.4)*† [3.3-8.3]	6.5 (1.7-23.1)*† [5.2-11.8]	6.4 (1.1-19.9)*† [4.3-10.1]

MCE, moderate-intensity continuous exercise; HIE, high-intensity interval exercise; FP, follicular phase; LP, luteal phase

*Significantly different from corresponding PRE value, P <0.05

†Significantly different from corresponding 0HR value, P <0.05

Figure Legends

Fig. 1. Serum 17β-estradiol (**A**) and progesterone (**B**) levels in individuals (n =17) before the commencement of exercise.

Note: Individual data points are presented by symbols with values for the same participant connected by lines for each trial, and each participant has the same symbol and line across four trials. **FP**, follicular phase; **LP,** luteal phase; **MCE**, moderate-intensity continuous exercise; **HIE**, high-intensity interval exercise; The double-arrow line is the mean of hormone values; *Significantly different from the corresponding value of luteal phase, p <0.001

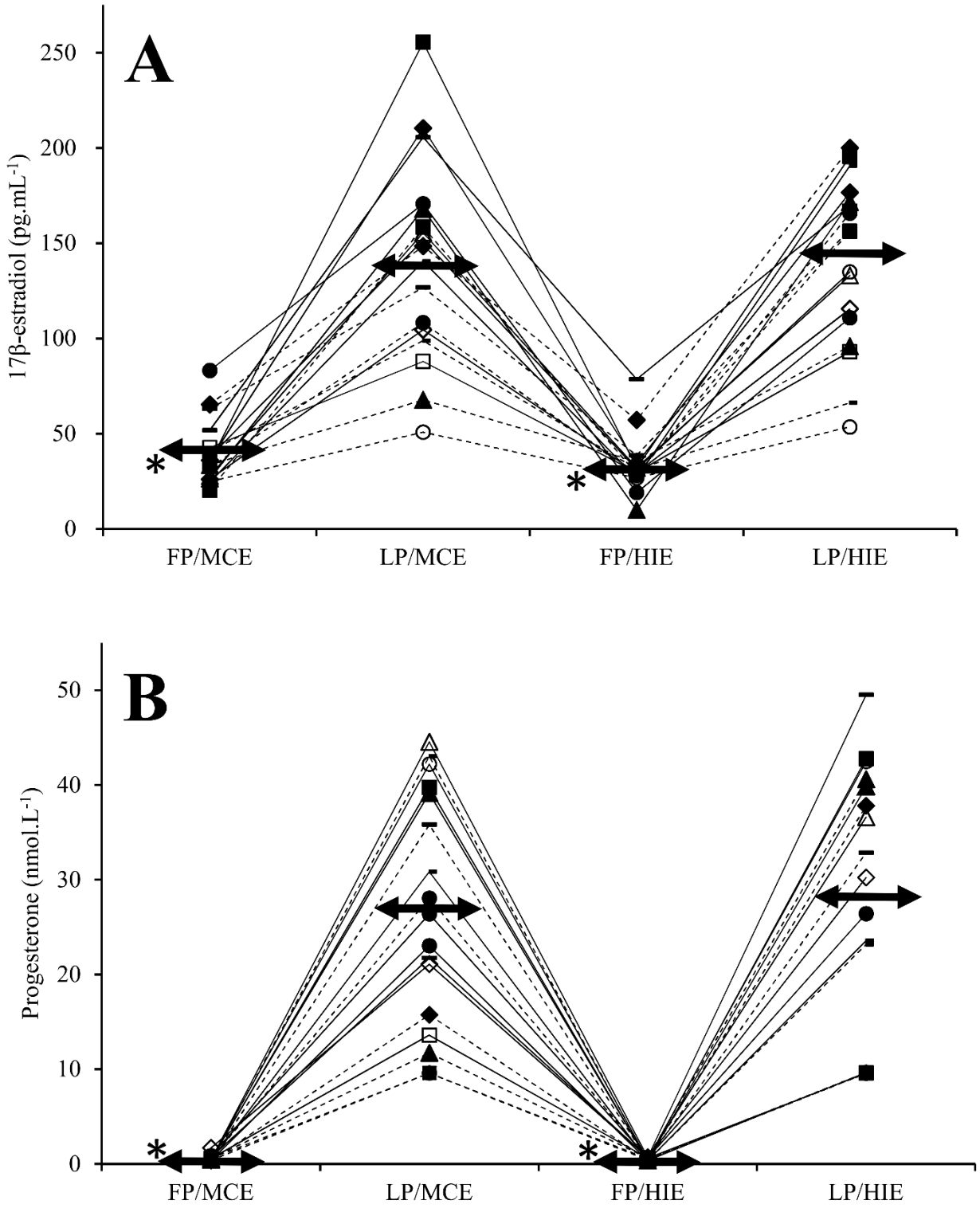
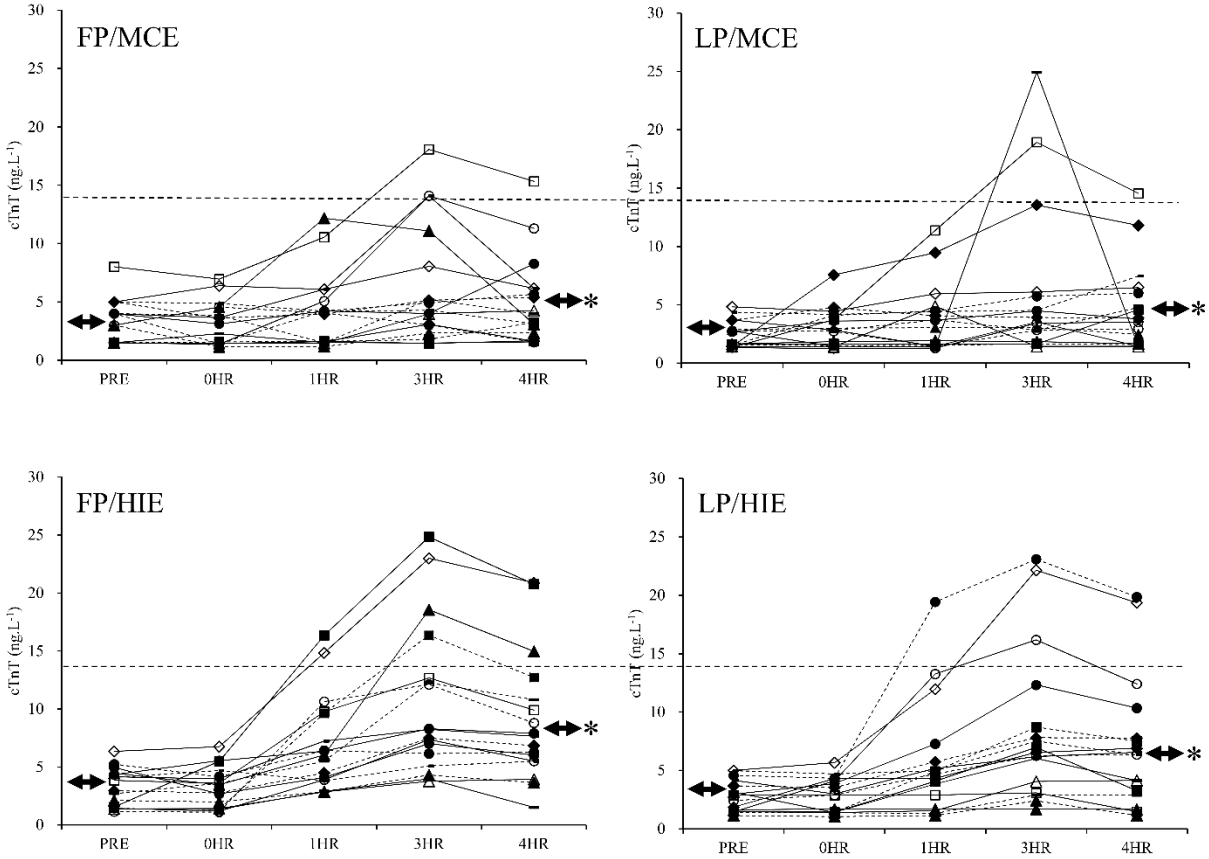

A
17β-estradiol (pg.mL-1)
250
200
150
100
50
0
*
*
FP/MCE
LP/MCE
FP/HIE
LP/HIE
B
Progesterone (nmol.L-1)
50
40
30
20
10
0
*
*
FP/MCE
LP/MCE
FP/HIE
LP/HIE

Fig. 2. The cardiac troponin T levels (cTnT, $ng.l^{-1}$) in individuals (n =17) before (PRE) and immediately (0HR), 1h (1HR), 3h (3HR) and 4 h (4HR) after a 300-kJ cycling in four trials.

Note: Individual data points are presented by symbols with values for the same participant connected by lines for each trial, and each participant has the same symbol and line across four trials. **FP**, follicular phase; **LP**, luteal phase; **MCE**, moderate-intensity continuous exercise; **HIE**, high-intensity interval exercise; 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) or peak post-exercise (mostly at 3HR); *Significantly different from corresponding PRE value, p <0.001


FP/MCE
LP/MCE
FP/HIE
LP/HIE
cTnT (ng.L⁻¹)
0
5
10
15
20
25
30
PRE
0HR
1HR
3HR
4HR
*