Title

Influence of maturational status in the exercise-induced release of cardiac troponin T in healthy young swimmers

Authors

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Abstract

Objective: To determine the influence of maturational status on the release of cardiac troponin T (cTnT) induced by a bout of 30 min, high-intensity, continuous exercise.

Design: Quasi-experimental, cross-sectional study.

Methods: Seventy male, young, well trained swimmers (age range 7–18 years, training experience 1–11 years) were classified by maturational stages: Tanner stage I (n = 14), II (n = 15), III (n = 15), IV (n = 13), and V (n = 13). Participants underwent a distance-trial of 30 min continuous swimming, and cTnT was measured before, immediately after and 3 h after exercise. Changes in cTnT over time were compared among groups, and associated with exercise load.

Results: Basal cTnT was higher in Tanner-V (3.8–8.1 ng/L) compared with I (*1.5–5.5 ng/L, p* < 0.001), II (*1.5–4.5 ng/L, p* < 0.001) and III (*1.5–6.8 ng/L, p* = 0.003), and in IV (1.5–6.3 ng/L) compared with II ($p = 0.036$). Maximal elevations of cTnT from baseline were notable ($p < 0.001$) and comparable among maturational stages ($p = 0.078$). The upper reference limit for myocardial injury was exceeded in 35.7% of the participants, without differences among groups ($p = 0.18$). Baseline cTnT correlated with participant characteristics, and maximal cTnT elevations from baseline with exercise internal load (%HRpeak, $r_s = 0.34$, $p = 0.003$; %HRmean, $r_s = 0.28$, $p = 0.02$).

Conclusions: Maturational status influences positively absolute pre- and post-exercise cTnT but not its elevation after a bout of 30 min, high-intensity, continuous exercise.

Keywords: Adolescent (MESH D000293), Biomarkers (MESH D015415), Child (MESH D002648), Exercise (MESH D015444), Puberty (MESH D011627); Troponin (MESH D014336)

Introduction

The exercise-induced release of cardiac troponin (cTn) is common in apparently healthy athletes of all ages,[1] despite the fact that, clinically, an elevation in cardiac troponin T (cTnT) or I (cTnI) has been associated to myocardial damage, and cTn is the preferred biomarker for the diagnosis of myocardial injury.[2] Although the physiological mechanisms underlying the exercise-induced release of cTn remain unclear, its kinetics has been thoroughly investigated, and related with individual and exercise characteristics.[1,3,4]

Since most of the studies were conducted in adults and research in younger populations is still scarce,[1] the association between exercise-induced cTn elevations and age is still under debate. In this regard, two recent meta-analyses found that age was positively associated with the release of cTn.[5,6] Both of these associations, however, were based on studies in adults. By contrast, some studies comparing adults with adolescents, found higher cTnT intra-group variability in the adolescent group.[7–9] Furthermore, one of these studies found higher values in the adolescents,[9] whereas the other two did not find differences when compared to adults.[7,8]

It has been hypothesized that the higher elevations of cTn in young athletes might be related to maturation, since the myocardium might be more vulnerable to injury in clinical situations during its development.[9,10] In this regard, Tanner stages are a commonly used criteria based on genitalia assessment that allows participant classification in five maturational categories (I to V).[11] To the best of our knowledge, only three studies compared cTnT in children and/or adolescents with

indicators of maturational status.[7,9,12] Further, when comparing baseline cTnT, none of these studies found significant group differences among maturational stages III-IV, II-IV and III-V. [7,9,12] In addition, all three coincided that exercise induces significant elevations of cTnT. However, whilst two of them did not report group differences among maturational stages in terms of peak post-exercise concentrations (stages III-IV),[9] or its maximal elevation from baseline (stages III-V),[7] the third found a positive association between maturational stages II-IV and the maximal cTnT elevation from baseline.[12] Discrepancies in these studies could be explained by differences between exercise exposures, or small sample sizes encompassing narrow ranges of maturational stages.

Whilst data addressing the role of participants' maturity is limited and inconclusive, no studies have assessed the exercise-induced cTn release across the entire range of maturational stages.[11] Based on the above paragraphs, further research on the exercise-induced elevation of cTn including wider samples representing all (I to V) maturational stages might reveal new insights about how this phenomenon differs depending on biological maturity. For this reason, the purpose of this study was to determine whether a single bout of 30 min, high-intensity, continuous exercise would induce different elevations of cTnT depending of the maturational stage of the participant. Based on previous research, our hypothesis was that the magnitude of cTnT elevations would be influenced by myocardial development, and result inversely associated with participants maturity, with higher elevations in the less mature athletes.

Methods

This study met the principles of the latest revision of the Declaration of Helsinki,[13] and was approved by the Ethical Committee of Clinical Research of Sports Administration of Catalonia (02/2018/CEICGC). The funders of this research had no role in the design of the study, in the

collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to submit the paper for publication.

Young male swimmers (age range from 6 to 18 years) were invited to participate in this study between April and May, 2018. Six local swimming clubs collaborated by inviting parents to answer an online questionnaire containing the Spanish version of the revised Physical Activity Readiness Questionnaire (PAR-Q), training history, and a self-assessment of pubertal maturation.[15] Inclusion criteria were: favorable PAR-Q, male, aged under 19 years, 3 years of experience in competitive swimming or more, and weekly training volume of 3 days/week or more. Parents of potentially eligible swimmers were then informed of the study and invited to participate. Seventy apparently healthy swimmers and their respective parents agreed to participate, and signed an informed consent form prior to the intervention (Supplementary Table 1). No exclusions were made and all participants completed the study.

Data collection took place during June, 2018. Participants visited our facilities on two occasions separated by two weeks. For logistical purposes, scores from self-assessments of pubertal maturation were used to schedule sessions in groups of ≤ 20 participants. On the first visit, anthropometric measurements were taken, a standard electrocardiogram (ECG) was obtained, participants were classified according to maturational stages, and maximal swimming heart rate was obtained using a specific swimming test. On the second day, participants performed a standardized warmup and then underwent a distance-trial test of 30 min continuous swimming. Participants were asked to avoid moderate or vigorous exercise during the 48h before the swimming test. Venous blood samples were collected before (Pre), immediately after (Post 0h) and 3 h after exercise (Post 3h) for cTnT analysis using a high sensitivity assay. A year after the intervention, parents of participants were interviewed in a telephonic follow-up survey, providing information about the training frequency and volume, and the appearance of cardiac symptoms or events subsequent to the study.

Participants were measured dry and wearing swimming clothes. Body mass was measured with a medical scale (SECA 711, Hamburg, Germany) and height with a wall stadiometer (Año-Sayol, Barcelona, Spain). Standard, 12-led ECG were recorded at the beginning of the study using a digital electrocardiograph (Click ECG BT 12 channel, Milano, Italy). Recordings were obtained with the swimmer in supine position during quiet respiration, after a short period of rest. ECG were assessed in situ by experienced medical personnel, and compared against the international ECG criteria for sports screening.[16] Two experienced pediatricians assessed participants' pubertal status. Genitalia and pubic hair were observed in the presence of parents and swimmers were classified according to the five-stage criteria described by Tanner.[11] Pediatricians were unaware of self-assessment scores, and blinded from each other except in case of disagreements that were resolved by consensus. Pediatricians classification was used in the statistical analysis.

Maximal heart rate (HR max) was obtained by calculating the peak heart rate (HR peak) in a specific swimming protocol.[7] First, swimmers performed a standardized warm up consisting in 100m freestyle, 30 sec recovery, 4 repetitions of 25m with 10 sec recovery between repetitions, 30 sec recovery, and 100m freestyle. Then, participants were asked to perform 6 repeated maximal sprints of 25m with 10 seconds of recovery between repetitions.[7] Measurements were made in a 25m indoor swimming pool, and heart rate during the test was recorded using Polar OH1™ optical heart rate sensors (Polar Electro Oy, Kempele, Finland).[17]

Blood samples were drawn from an antecubital vein by an experienced pediatric nurse at Pre, Post 0h, and Post 3 h. This timing was elected since previous studies reported peak post-exercise cTnT concentrations to occur at 3-4h after exercise.[7,18] Samples were quickly centrifuged and stored at -80ºC for later analysis. Serum cardiac troponin T (cTnT) was analyzed using the Troponin T hs STAT immunoassay in a Cobas E 601 analyzer (Roche Diagnostics, Penzberg, Germany). This assay ranges from 3 to 10000 ng/L, and the intra-assay coefficient of variation at a mean cTnT of 13.5 ng/L is 5.2%. Precision was determined by two cycles daily in duplicate,

each for 21 day. Before the assays were performed, the analyzers were calibrated with standard calibrators according to the manufacturer recommended protocols. The upper reference limit (URL) for cTnT, defined as the 99th percentile of healthy participants was 13.5 ng/L.[19] Concentrations below the limit of detection (LoD) of 3 ng/L were set to 1.5 ng/L for statistical analyses.[20]

The distance-trial test was preceded by a self-paced 5-minute warm-up (<60% of HR max), and consisted in covering the maximum possible distance in 30 minutes at a uniform, continuous pace. The duration for this test was based on previous studies reporting that an exercise bout 30 min can induce an elevation of cTn.[18,21] Furthermore, previous studies reported elevations of cTnT in adolescent swimmers,[7] and demonstrated that cTn concentrations after swimming are comparable with those after running or cycling.[22] For these reasons, and its high participation at early ages, we elected swimming as the exercise mode for this study. Participants had been previously familiarized with distance-trial tests of similar durations, and were instructed as well as verbally encouraged by researchers and their coaches to cover their maximum possible distance during the test. All participants completed the test in the same facilities under standardized, constant conditions (25 m indoor swimming pool, water temperature 28 ºC, air temperature 29 ºC, relative humidity 65%). Heart rate was monitored using Polar OH1™ sensors, and video recordings were used to calculate swimming distances. Participants were allowed to drink water *at libidum* before and after exercise. Immediately after exercise, participants reported their rating of perceived exertion (RPE) in a 0-100 scale.[23]

Dependent variables in this study were cTnT concentrations (ng/L) at Pre, Post 0 h, and Post 3 h, and its derived changes Δ Post 0 h (Post 0 h – Pre), and Δ Post 3 h (Post 3 h – Pre). The main independent variables were maturational stage (five groups from Tanner I to V), cTnT detection (non-detected when cTnT was under the LoD in all three measurements, detected when cTnT was detected in one or more measurements) and responsiveness (non-responders, when cTnT

144 elevations did not exceed the URL in any measurement, responders when one or more cTnT
145 measurements exceeded the URL). Secondary independent variables were participant
146 characteristics [age (years), body height (cm), body mass (kg), body mass index(kg/m^2), training
147 experience (years), frequency (days/week), volume (h/week), and HR max (bpm)], and exercise
148 load during the test [distance (m), mean relative HR (% HR max), peak relative HR (% HR max),
149 RPE (1-100), 1 min recovery HR (bpm) and 3 min recovery HR (bpm)]. Kolgomorov-Srmirnov test
150 was used to verify that all variables were normally distributed except cTnT data, that were right
151 skewed and non-transformable. All variables were presented as mean ± standard deviation, or
152 median [interquartile range], according to the normality of the data.

153 Participant characteristics and exercise load were tested for main differences among maturational
154 stages (Tanner I to V) using one-way analysis of variance. Post-hoc pairwise comparisons
155 between maturational stages when main differences were statistically significant, and differences
156 associated to cTnT detection (detected vs non-detected) and responsiveness (responder vs non-
157 responder) were tested using t-tests for independent samples (Supplementary Tables 1 and 2).
158 Main cTnT differences over time (Pre, Post 0 h, and Post 3 h) were tested using Friedman tests
159 for repeated measures. When these main differences were statistically significant, changes in
160 cTnT (Δ Post 0 h and Δ Post 3 h) were tested using Wilcoxon signed rank tests. Main cTnT
161 differences among maturational stages (Tanner I to V) were tested using Kruskal-Wallis rank sum
162 tests. Post-hoc pairwise comparisons between maturational stages when main differences were
163 statistically significant were made using Wilcoxon rank sum tests (Table 1). The rate of cTnT
164 detection, and responders was compared among maturational groups using generalized mixed
165 effects models for the binomial family. Correlations between basal cTnT (Pre), cTnT changes (Δ
166 Post 0 h and Δ Post 3 h), maturational stage, participant characteristics and exercise load were
167 assessed using Spearman's correlation coefficients (r_s) (Table 2). All statistical analyses were

done using R v3.5.3. Statistical significance was assumed when $p < 0.05$, and Bonferroni corrections were applied when appropriate.

Results

Participant characteristics in terms of age, body height and mass, BMI, training experience, frequency and volume, and HR max in the first visit, and swimming distance during the test were different among maturational stages. However, grouping participants by maturational stage did not reveal differences in % HR peak , % HR mean, RPE, HRR at 1 min, and HRR at 3 min during the distance-trial (Supplementary Table 1).

Fifty-nine participants had detectable cTnT at some measurement (detected) whereas 11 had cTnT < LoD in all measurements (non-detected). Further, cTnT was detected in 35 (50%), 30 (42.9%), and 59 (84.3%) participants at Pre, Post 0 h and Post 3 h, respectively. Age, body height, HR max, % HR peak, and % HR mean were lower in the non-detected (Supplementary Table 2). Peak cTnT concentrations were observed at Post 3 h in all detected cases. Whilst immediate changes in cTnT (∆ Post 0 h) were not conclusive, elevations after 3 h (∆ Post 3 h) were statistically significant. At baseline (Pre), participants in Tanner-V presented higher cTnT than those in I ($p < 0.001$), II ($p < 0.001$) and III ($p = 0.003$), and Tanner-IV higher than those in II ($p = 0.036$). Then, immediately after exercise (Post 0 h) Tanner-V had also higher cTnT than I ($p = 0.002$), II ($p < 0.001$) and III ($p = 0.017$). Peak concentrations (Post 3 h) were only higher in Tanner-V compared with I ($p = 0.024$) (Figure 1). Furthermore, immediate cTnT changes (∆ Post 0 h) were higher in Tanner-V compared with II ($p = 0.016$), but ∆ Post 3 h was comparable among groups ($p = 0.078$). Higher cTnT at Pre was associated to higher cTnT at Post 0 h ($r_s = 0.76$, $p < 0.001$) and Post 3 h ($r_s = 0.34$, $p = 0.004$), but was not associated to any of the changes, namely ∆ Post 0 h ($r_s = 0.16$, $p = 0.19$) and ∆ Post 3 h ($r_s = 0.11$, $p = 0.35$) (Table 1).

< Figure 1 >

< Table 1 >

Higher cTnT at Pre was associated with maturational stage, age, body height and weight, training experience and frequency and HR max in the first visit, and higher distance and % HR mean during the test. Furthermore, higher immediate elevations (Δ Post 0 h) were associated with maturational stage, age, body height and mass and HR max in the first visit, as well as % HR peak and % HR mean during the test. Finally, Δ Post 3 h was positively associated with maturational stage, HR max in the first visit, % HR peak and % HR mean (Supplementary Figure 1), and negatively associated with HRR at 1 min (Table 2).

< Table 2 >

The incidence rate of participants with cTnT > URL (responders) was 25/70 (35.7%), in all cases at Post 3 h, without differences among maturational stages (p = 0.99) (Table 1). Furthermore, responders had higher % HR mean and RPE, and lower HRR at 1 min (Supplementary Table 2). A year after the study, none of the participants reported cardiac symptoms or events subsequent to the study.

Discussion

In this study, we compared the post-exercise concentrations of cTnT in a cohort of 70 young male swimmers stratified by maturational status. Our results support that a distance-trial test of 30 min continuous swimming induces an elevation of cTnT in the following hours. The main findings of this study were that: 1) Basal cTnT (Pre) in apparently healthy, trained, young males is associated to participant characteristics, and might vary among maturational stages (Table 2). 2) Elevations of cTnT from baseline are not conclusive immediately after exercise (Δ Post 0 h), but notable in a period of 3 h (Δ Post 3 h), without significant differences related to maturational stage (Table 1). 3) The incidence rate of participants with cTnT exceeding the URL (responders) following exercise

was ~36%, without differences among maturational status (Table 1). 4) Elevations of cTnT induced by exercise (Δ Post 0 h and Δ Post 3 h) are highly variable among participants, and partially explained by exercise load.

Resting values of cTnT in healthy athletes are normally reported below or close to the assay lower limit of detection.[5,24] It has been reported that these values might vary depending on age and sex,[25] and previously suggested the need to report on age- and sex-specific population cTn values in children and adolescents.[26] In this regard, previous studies found similar baseline concentrations of cTn between adolescent and adult athletes,[7,8,27] and among adolescent athletes at different maturational stages.[7,9,12] However, in the present study we found that swimmers at Tanner-V had higher resting cTnT than those in I -III, and swimmers in Tanner-IV higher than those in II (Table 1), suggesting that normal values might be higher during late-puberty. Furthermore, cTnT at Pre was positively associated with age, body height and mass, training experience and frequency, and HR max (Table 2). On this subject, these results coincide with previous studies suggesting that athletic status may be one of the factors that determine the heterogeneity in baseline cTn values.[18,28] Although speculative, the greater training experience, frequency and HR max of swimmers in late-puberty could explain their higher cTnT at Pre. Furthermore, this is also supported by the higher distances and % HR mean achieved in participants with higher basal values. However, further research is still needed to confirm this hypothesis.

Concentrations of cTnT immediately after exercise were lower than those at 3 h post-exercise. This coincides with the cTn kinetics reported in previous studies,[7,18] and could be compatible with the theory of a transient reduction in cTn clearance during exercise, combined with a transient release of unbound cTn during and after exercise originated from reversible cell wall injury.[29] In the group comparisons, we found that absolute peak cTnT (Post 3 h) was higher in Tanner V compared with I (Table 1). However, besides this absolute difference, and in line with previous

studies,[7,9] maximal cTnT elevations (Δ Post 3 h) were comparable among all stages. This finding, is contrary to our initial hypothesis, and supports that exercise-induced elevations of cTnT might not be influenced by myocardial development. In addition, this result contrasts with a previous study comparing a small cohort of soccer players a Tanner stages II (n = 8), III (n = 8) and IV (n = 4), that reported a positive association between maximal cTnT elevations and maturational stage.[12] To the authors' opinion, this discrepancy might be explained by the small sample size in that study, and other methodological differences derived from participants characteristics and exercise load. Furthermore, previous cross-sectional and longitudinal studies demonstrated that basal cTn is a strong predictor for post-exercise concentrations.[18,28] Likewise, in our study cTnT at Pre was associated with absolute cTnT concentrations (Post 0 h and Post 3 h), however it was not with baseline-normalized changes (Δ Post 0 h and Δ Post 3 h). Accordingly, group differences at Pre could explain the higher values we found at late-puberty in terms of absolute post-exercise cTnT but not in its baseline-normalized changes (Δ Post 0 h and Δ Post 3 h).

Although we could reproduce the exercise-induced elevations of cTnT demonstrated in previous studies, they were highly variable among individuals. On the one hand, 11 (16%) participants in this study had cTnT < LoD in all Pre and Post measurements (non-detected). Pairwise comparisons revealed that these participants were younger, had lower HR max, and achieved lower % HR peak and % HR mean during the distance trial (Supplementary Table 2). On the other hand, 25 participants (incidence rate = 36%) had cTnT > URL at Post 3 h (responders). This incidence rate was lower than the 62% reported by Legaz-Arrese, et al. (X^2 = 8.5, p = 0.003).[7] Even though both studies were conducted in young swimmers, participants in Legaz-Arrese et al. were required to swim twice the duration than ours, and this might explain the difference between incidence rates. In this line, the group of responders where those who achieved higher exercise internal load in terms of % HR mean and RPE, and those with faster cardiac recovery, in terms of HRR at 1 min (Supplementary Table 2). These differences, together with the ones found in the

group of non-detected, coincide with previous findings, suggesting that the highest cTnT elevations (both, Δ Post 0h and Δ Post 3h) occur in better trained athletes (experience, HR max),[18,28] that achieve higher exercise internal loads (% HR peak and % HR mean) during the test.[4,27] This suggests that not only maturation but also other factors affect the magnitude of increase in cTnT after intense exercise. In spite of that, training status and exercise load could only partially explain the high variability in the exercise-induced elevation of cTnT. Thus, to the authors' opinion there might be other, still unknown, individual factors influencing pre- and post-exercise cTnT variability, that might be explored in future research.

Current cut-off values for cTn are taken from adult populations.[30] It has been suggested that reference values of cTn might variate with maturational and training status.[9,31,32] Our results support previous data, however, further research is needed to confirm both hypothesis and provide, if needed, specific population reference values including younger participants and differentiating for fitness level. A year after this study, a telephonic follow-up survey confirmed that all participants continued their training routine after the study, and none of them had cardiac symptoms or events. Previous studies also reported an absence of clinical signs or symptoms during a follow-up period.[32,33] The high incidence of elevated cTn after exercise in healthy athletes, its reproducibility, and the absence of clinical signs or symptoms in a 1-year follow-up period could suggest that cTnT elevations are inherent to exercise, and probably related to a physiological response to exercise. It has been suggested that exercise may induce transient troponin elevations attributable to transient increases in cardiomyocyte membrane permeability,[34] although this requires empirical support. However, our results confirm previous findings showing that there is a high variability between subjects in the release of troponin with exercise that the scientific literature has not been fully able to explain by individual and exercise characteristics,[7,12] and if this could be associated with clinical repercussions in the future is an aspect of interest that is being

289 debated.[35] Our results might be considered in clinical settings when interpreting cTnT values in
290 young, physically active populations, especially in the hours subsequent to exercise.

291 Since previous studies confirmed that peak concentrations occur typically at 3-4h after
292 exercise,[9,20] and with the aim to minimize the number of blood extractions, we did not perform
293 serial measurements during this recovery. However, the limited sampling points in our design
294 imply a potential under-estimation error in the peak cTnT concentrations, as has been previously
295 suggested by others.[31] In addition, cTnT measurements were not performed in duplicate
296 precluding any intra-assay control beyond the calibration and precision calculations recommended
297 by the manufacturer. For this reasons, future research including serial cTnT measurements with
298 duplicates will allow for a more precise time-to-peak comparisons among maturational stages.
299 The distance-trial test performed in this study was partly elected for its similarity with the real effort
300 performed by participants during their trainings and competitions. However, this could have
301 implied differences in relative intensity between swimmers, that could be solved in future studies
302 using other methods to control the relative intensity during exercise. We confirmed that a release
303 of cTnT is inherent to exercise, however, individual variability could only be explained by some
304 variables such as exercise load. Even though we classified participants according to Tanner
305 stages, we did not estimate the age relative to peak height velocity. Further, sample size is a
306 common limitation in studies involving trained athletes from a single sport, concretely when
307 recruiting children and adolescents, and when procedures require venous blood sampling.
308 Although the cohort in our study was similar or larger than the investigated in previous studies,[7,9,12]
309 the authors acknowledge that sample size was small. In this regard, previous research supported
310 that the physiological response to exercise might differ between male and female adolescents.[36]
311 For this reasons, future research should address our limitations by controlling peak height
312 velocity,[37] and including larger samples of both, male and female athletes. Finally, in this study we

only measured cTnT, and other biomarkers such as cTnI or NT-proBNP might be used in future studies to provide a more complete overview of the phenomenon.

Conclusion

A single, distance-trial test of 30 min continuous swimming evoked significant increases of cTnT in young male swimmers at all maturational stages. Baseline values were higher in those at higher maturational stages and better training status. However, whilst differences in post-exercise cTnT values were similar than those at baseline, when considering cTnT changes from baseline the differences among groups disappeared. We observed an incidence rate of 36% presenting cTnT values above the population URL at 3 h post-exercise, that was comparable among maturational stages.

Practical implications

- Clinical decisions should be taken considering that a high-intensity and short duration (30 min) exercise evokes a release of cTnT in a large number of apparently healthy children and adolescents, regardless of their maturational stage.
- From a clinical and technical perspective, our results reject the rationale of contraindicating high-intensity, short-duration efforts in children at lower maturational status based on a higher release of cTnT induced by exercise.
- This study suggests that population values of reference for cTnT might differ among maturational and/or training statuses, and justify further research exploring the individual variability of cTnT at rest.
- Individual variability in the exercise-induced elevation of cTnT is high, and remains incompletely understood even when accounting for exercise load and maturational status.

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Table 1. Summary of cTnT time and group comparisons.

	Time			Time differences			
	Pre	**Post 0 h**	**Post 3 h**	**Δ Post 0 h**	***p* value**	**Δ Post 3 h**	***p* value**
Tanner-I							
cTnT (ng/L)	1.5 [1.5, 5.5] $_{<V}$	1.5 [1.5, 6.5] $_{<V}$	4.7 [1.5, 27] $_{<V}$	0 [-3, 2.7]	0.99	3.2 [0, 22.5]	0.018
Positive Rate (n)	0/14	0/14	2/14				
Tanner-II							
cTnT (ng/L)	1.5 [1.5, 4.5] $_{<IV, V}$	1.5 [1.5, 3.5] $_{<V}$	3.5 [1.5, 27]	0 [-3, 0.3] $_{<V}$	0.99	1 [0, 22.5]	0.027
Positive Rate (n (%))	0/15	0/15	4/15				
Tanner-III							
cTnT (ng/L)	1.5 [1.5, 6.8] $_{<V}$	1.5 [1.5, 6.8] $_{<V}$	7.1 [1.5, 93.5]	0 [-1.7, 2.6]	0.99	5.2 [0, 92]	0.003
Positive Rate (n, %)	0/15	0/15	6/15				
Tanner-IV							
cTnT (ng/L)	3.8 [1.5, 6.3] $_{>II}$	3.9 [1.5, 8.1]	11 [4.6, 29.2]	0 [-4.8, 2.4]	0.99	5 [0.2, 27.7]	< 0.001
Positive Rate (n (%))	0/13	0/13	6/13				
Tanner-V							
cTnT (ng/L)	6.1 [3.8, 8.1] $_{>I, II, III}$	6.5 [1.5, 7.9] $_{>I, II, III}$	14.4 [5.1, 40.8] $_{>I}$	0.4 [-5.7, 1.3] $_{>II}$	0.089	10.2 [-2.1, 34.7]	0.002
Positive Rate (n (%))	0/13	0/13	7/13				
All							
cTnT (ng/L)	2.3 [1.5, 8.1]	1.5 [1.5, 8.1]	8.4 [1.5, 93.5]	0 [-5.7, 2.7]	0.26	5 [-2.1, 92]	< 0.001
Positive Rate (n (%))	0/70	0/70	25/70				

Note. Cardiac Troponin T was expressed as median [range], and rates of positive events as count/total. Subscripts indicate statistically significant differences between groups in each column and their direction. I-V = Tanner stages I-V.

Table 2. Spearman's correlation coefficients between cTnT values and participants' characteristics.

	Pre (ng/L)		Δ Post 0 h (ng/L)		Δ Post 3 h (ng/L)		Maturational Stage (Tanner I - V)	
	r_s	p	r_s	p	r_s	p	r_s	p
Participant characteristics								
Age (years)	0.46	< 0.001	0.32	0.007	0.06	0.63	0.72	< 0.001
Body height (cm)	0.55	< 0.001	0.3	0.012	0.07	0.55	0.78	< 0.001
Body mass (kg)	0.45	< 0.001	0.27	0.023	0.02	0.89	0.7	< 0.001
BMI (kg/m2)	0.13	0.28	0.15	0.20	-0.04	0.72	0.37	0.002
Training experience (years)	0.27	0.023	0.18	0.13	-0.19	0.12	0.45	< 0.001
Training frequency (days/week)	0.25	0.038	-0.04	0.72	0.07	0.58	0.48	< 0.001
Training volume (h/week)	0.16	0.19	0	0.99	-0.08	0.50	0.44	< 0.001
Maximum HR (bpm)	0.39	< 0.001	0.35	0.003	0.37	0.001	0.51	< 0.001
Exercise load								
Distance (m)	0.27	0.025	0.22	0.064	-0.03	0.82	0.47	< 0.001
% Peak HR (% HR max)	0.17	0.16	0.28	0.017	0.34	0.003	0.08	0.53
% Mean HR (% HR max)	0.34	0.004	0.24	0.047	0.28	0.020	0.22	0.066
Rating of Percieved Exertion (0-100)	-0.15	0.23	-0.02	0.87	0.18	0.14	0.14	0.26
1 min Recovery HR (bpm)	0.18	0.13	-0.05	0.67	-0.3	0.013	0.14	0.26
3 min Recovery HR (bpm)	0.2	0.10	0.06	0.62	-0.16	0.19	0.2	0.093

Figure legends

Figure 1. Individual values of cTnT by time and maturational stage.

Note. Gray horizontal line indicates the URL for cTnT. Data above the URL appears in filled dots.

Supplementary material

Supplementary Table 1. Summary of participant characteristics and exercise load data.

	Maturational Stage				
	Tanner-I (n = 14)	Tanner-II (n = 15)	Tanner-III (n = 15)	Tanner-IV (n = 13)	Tanner-V (n = 13)
Participant characteristics					
Age (years)					
	10 +- 2 < IV, V	11 +- 2 < V	12 +- 2 < V	14 +- 3 > I	15 +- 1 > I, II, III
	[7, 12]	[8, 14]	[9, 14]	[5, 18]	[13, 17]
Body Height (cm)					
	139,8 +- 12,2 < III, IV, V	147,8 +- 11,8 < IV, V	155,2 +- 12,2 > I < IV, V	168,8 +- 9 > I, II, III	173,7 +- 6,9 > I, II, III
	[122, 157,5]	[125, 163]	[127, 175]	[151, 182]	[165, 187]
Body Mass (kg)					
	34,4 +- 9,3 < IV, V	41,8 +- 10,8 < IV, V	46,2 +- 11,9 < V	57,8 +- 9,8 > I, II	60,8 +- 8 > I, II, III
	[23,6, 50,4]	[25,1, 56,9]	[23,7, 64,5]	[40,5, 77,8]	[50,1, 77,8]
BMI (kg/m2)					
	17,3 +- 2,2 < IV, V	18,8 +- 3	18,9 +- 3,5	20,2 +- 2,2 > I	20,1 +- 2,1 > I
	[14, 21,2]	[14,2, 24,4]	[14,7, 26]	[16,9, 24,3]	[17,5, 24]
Experience (years)					
	4 +- 2 < IV, V	5 +- 2	4 +- 3	6 +- 2 > I	7 +- 2 > I
	[1, 6]	[2, 10]	[1, 11]	[2, 11]	[2, 11]
Training (days/week)					
	4 +- 1 < IV, V	4 +- 1 < IV	4 +- 1	5 +- 1 > I, II	5 +- 1 > I
	[2, 5]	[3, 5]	[3, 6]	[5, 6]	[3, 6]
Training (h/week)					
	6 +- 5 < IV	7 +- 3 < IV	8 +- 4	12 +- 3 > I II	10 +- 3
	[2, 15]	[3, 15]	[5, 15]	[8, 15]	[5, 15]
HR max (bpm)					
	186 +- 7 < V	189 +- 5 < V	190 +- 9	192 +- 5 < V	198 +- 4 > I, II, IV
	[175, 195]	[179, 203]	[165, 201]	[185, 201]	[188, 201]
Exercise Load					
Distance (m)					
	1446 +- 409 < IV	1533 +- 338 < IV	1583 +- 367	1958 +- 296 > I, II	1874 +- 313
	[650, 2500]	[1000, 2000]	[1000, 2500]	[1250, 2500]	[1300, 2500]
% HR Peak (% HR Max)					
	94 +- 8	94 +- 6	94 +- 8	91 +- 8	97 +- 4
	[77, 105]	[86, 104]	[83, 107]	[78, 110]	[89, 102]
% HR Mean (% HR Max)					
	84 +- 7	87 +- 8	86 +- 9	90 +- 7	88 +- 4
	[71, 98]	[77, 101]	[69, 100]	[75, 99]	[78, 92]
RPE (0-100)					
	76 +- 9	76 +- 18	78 +- 11	74 +- 16	77 +- 17
	[55, 90]	[15, 90]	[45, 90]	[40, 90]	[30, 95]
HRR at 1 min (bpm)					
	-44 +- 15	-46 +- 10	-43 +- 11	-38 +- 13	-42 +- 11
	[-74, -16]	[-62, -28]	[-63, -19]	[-53, -13]	[-60, -28]
HRR at 3 min (bpm)					
	-65 +- 15	-67 +- 12	-62 +- 12	-63 +- 8	-60 +- 7

	[-91, -39]	[-84, -41]	[-77, -38]	[-75, -50]	[-70, -50]

Note. Subscripts indicate statistically significant differences between groups in each column and their direction. I-V = Tanner stages I-V

Supplementary Table 2.

	Detection			Response		
	Non-detected (n = 11)	Detected (n = 59)	T test *p* value	Non-responders (n = 45)	Responders (n = 25)	T test *p* value
Participant characteristics						
Age (years)						
	11 +- 2 < D	12 +- 3 > ND	0.024	12 +- 3	12 +- 3	0.88
	[8, 14]	[5, 18]		[8, 18]	[5, 16]	
Body Height (cm)						
	146,3 +- 12,5 < D	158,4 +- 16,3 > ND	0.013	155,9 +- 16,6	157,5 +- 16	0.69
	[122, 163]	[123, 187]		[122, 187]	[125, 179]	
Body Mass (kg)						
	41,2 +- 11,2	49 +- 14	0.06	47,5 +- 13,6	48,3 +- 14,6	0.81
	[23,6, 56,9]	[23,7, 77,8]		[23,6, 77,8]	[23,7, 77,8]	
BMI (kg/m2)						
	18,9 +- 3,1	19,1 +- 2,8	0.88	19,1 +- 2,6	19 +- 3,2	0.86
	[15,8, 24,4]	[14, 26]		[15,6, 24,4]	[14, 26]	
Experience (years)						
	5 +- 3	5 +- 3	0.84	5 +- 3	5 +- 3	0.13
	[1, 10]	[1, 11]		[1, 11]	[1, 11]	
Training (days/week)						
	4 +- 1	5 +- 1	0.16	4 +- 1	5 +- 1	0.14
	[2, 6]	[2, 6]		[2, 6]	[3, 6]	
Training (h/week)						
	8 +- 5	9 +- 4	0.64	9 +- 5	8 +- 3	0.38
	[2, 15]	[2, 15]		[2, 15]	[2, 15]	
HR max (bpm)						
	184 +- 8 < D	192 +- 6 > ND	0.006	190 +- 7	192 +- 8	0.26
	[175, 203]	[165, 201]		[175, 203]	[165, 201]	
Exercise Load						
Distance (m)						
	1418 +- 501	1715 +- 354	0.085	1665 +- 420	1676 +- 344	0.9
	[650, 2500]	[1000, 2500]		[650, 2500]	[1250, 2500]	
% HR Peak (% HR Max)						
	89 +- 7 < D	95 +- 7 > ND	0.033	93 +- 6	96 +- 7	0.088
	[77, 103]	[78, 110]		[77, 105]	[82, 110]	
% HR Mean (% HR Max)						
	82 +- 8 < D	88 +- 7 > ND	0.045	85 +- 7 < R	90 +- 6 > NR	0.004
	[71, 99]	[69, 101]		[69, 99]	[76, 101]	
RPE (0-100)						
	79 +- 4	76 +- 15	0.17	74 +- 16 < R	81 +- 9 > NR	0.012
	[75, 85]	[15, 95]		[15, 90]	[50, 95]	
HRR at 1 min (bpm)						
	-40 +- 11	-43 +- 12	0.49	-41 +- 12 > R	-47 +- 11 < NR	0.035
	[-53, -19]	[-74, -13]		[-65, -13]	[-74, -25]	

HRR at 3 min (bpm)						
	-58 +- 15	-65 +- 10	0.22	-63 +- 12	-65 +- 10	0.32
	[-80, -38]	[-91, -41]		[-84, -38]	[-91, -49]	

Note. Subscripts indicate statistically significant differences between groups in each column and their direction. ND = Non-detected, D = Detected, NR = Non-responder, R = Responder

Supplementary Figure 1. Associations between post-exercise cTnT and exercise intensity, by maturational status.

Note. Gray horizontal line indicates the URL for cTnT. Data above the URL appears in filled dots.