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1 **Title**

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

4 **Authors**

5 Rafel Cirer-Sastre, MSc ^{a,b*}, Alejandro Legaz-Arrese, PhD ^c, Francisco Corbi, PhD ^{a,b}, Isaac López-
6 Laval, PhD ^c, Keith George, PhD ^d, Joaquín Reverter-Masia, PhD ^{b,e}

7 **Abstract**

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

10 *Design:* Quasi-experimental, cross-sectional study.

11 *Methods:* Seventy male, young, well trained swimmers (age range 7–18 years, training experience
12 1–11 years) were classified by maturational stages: Tanner stage I (n = 14), II (n = 15), III (n =
13 15), IV (n = 13), and V (n = 13). Participants underwent a distance-trial of 30 min continuous
14 swimming, and cTnT was measured before, immediately after and 3 h after exercise. Changes in
15 cTnT over time were compared among groups, and associated with exercise load.

16 *Results:* Basal cTnT was higher in Tanner-V (3.8–8.1 ng/L) compared with I (1.5–5.5 ng/L, $p <$
17 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)
18 compared with II ($p =$ 0.036). Maximal elevations of cTnT from baseline were notable ($p <$ 0.001)
19 and comparable among maturational stages ($p =$ 0.078). The upper reference limit for myocardial
20 injury was exceeded in 35.7% of the participants, without differences among groups ($p =$ 0.18).
21 Baseline cTnT correlated with participant characteristics, and maximal cTnT elevations from
22 baseline with exercise internal load (%HRpeak, $r_s =$ 0.34, $p =$ 0.003; %HRmean, $r_s =$ 0.28, $p =$ 0.02).

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

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

27 **Introduction**

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

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

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

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

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

65 **Methods**

66 This study met the principles of the latest revision of the Declaration of Helsinki,¹³ and was
67 approved by the Ethical Committee of Clinical Research of Sports Administration of Catalonia
68 (02/2018/CEICGC). The funders of this research had no role in the design of the study, in the

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

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

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

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

105 Maximal heart rate (HR max) was obtained by calculating the peak heart rate (HR peak) in a
106 specific swimming protocol.⁷ First, swimmers performed a standardized warm up consisting in
107 100m freestyle, 30 sec recovery, 4 repetitions of 25m with 10 sec recovery between repetitions,
108 30 sec recovery, and 100m freestyle. Then, participants were asked to perform 6 repeated
109 maximal sprints of 25m with 10 seconds of recovery between repetitions.⁷ Measurements were
110 made in a 25m indoor swimming pool, and heart rate during the test was recorded using Polar
111 OH1™ optical heart rate sensors (Polar Electro Oy, Kempele, Finland).¹⁷

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

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

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

139 Dependent variables in this study were cTnT concentrations (ng/L) at Pre, Post 0 h, and Post 3 h,
140 and its derived changes Δ Post 0 h (Post 0 h – Pre), and Δ Post 3 h (Post 3 h – Pre). The main
141 independent variables were maturational stage (five groups from Tanner I to V), cTnT detection
142 (non-detected when cTnT was under the LoD in all three measurements, detected when cTnT
143 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/m²), 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-Smirnov 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 \pm 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

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

170 **Results**

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

176 Fifty-nine participants had detectable cTnT at some measurement (detected) whereas 11 had
177 cTnT < LoD in all measurements (non-detected). Further, cTnT was detected in 35 (50%), 30
178 (42.9%), and 59 (84.3%) participants at Pre, Post 0 h and Post 3 h, respectively. Age, body height,
179 HR max, % HR peak, and % HR mean were lower in the non-detected (Supplementary Table 2).
180 Peak cTnT concentrations were observed at Post 3 h in all detected cases. Whilst immediate
181 changes in cTnT (Δ Post 0 h) were not conclusive, elevations after 3 h (Δ Post 3 h) were statistically
182 significant. At baseline (Pre), participants in Tanner-V presented higher cTnT than those in I ($p <$
183 0.001), II ($p < 0.001$) and III ($p = 0.003$), and Tanner-IV higher than those in II ($p = 0.036$). Then,
184 immediately after exercise (Post 0 h) Tanner-V had also higher cTnT than I ($p = 0.002$), II ($p <$
185 0.001) and III ($p = 0.017$). Peak concentrations (Post 3 h) were only higher in Tanner-V compared
186 with I ($p = 0.024$) (Figure 1). Furthermore, immediate cTnT changes (Δ Post 0 h) were higher in
187 Tanner-V compared with II ($p = 0.016$), but Δ Post 3 h was comparable among groups ($p = 0.078$).
188 Higher cTnT at Pre was associated to higher cTnT at Post 0 h ($r_s = 0.76$, $p < 0.001$) and Post 3 h
189 ($r_s = 0.34$, $p = 0.004$), but was not associated to any of the changes, namely Δ Post 0 h ($r_s = 0.16$,
190 $p = 0.19$) and Δ Post 3 h ($r_s = 0.11$, $p = 0.35$) (Table 1).

191 < Figure 1 >

192

< Table 1 >

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

200

< Table 2 >

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

206 **Discussion**

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

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

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

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

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

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

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

273 Current cut-off values for cTn are taken from adult populations.³⁰ It has been suggested that
274 reference values of cTn might variate with maturational and training status.^{9,31,32} Our results
275 support previous data, however, further research is needed to confirm both hypothesis and
276 provide, if needed, specific population reference values including younger participants and
277 differentiating for fitness level. A year after this study, a telephonic follow-up survey confirmed that
278 all participants continued their training routine after the study, and none of them had cardiac
279 symptoms or events. Previous studies also reported an absence of clinical signs or symptoms
280 during a follow-up period.^{32,33} The high incidence of elevated cTn after exercise in healthy athletes,
281 its reproducibility, and the absence of clinical signs or symptoms in a 1-year follow-up period could
282 suggest that cTnT elevations are inherent to exercise, and probably related to a physiological
283 response to exercise. It has been suggested that exercise may induce transient troponin
284 elevations attributable to transient increases in cardiomyocyte membrane permeability,³⁴ although
285 this requires empirical support. However, our results confirm previous findings showing that there
286 is a high variability between subjects in the release of troponin with exercise that the scientific
287 literature has not been fully able to explain by individual and exercise characteristics,^{7,12} and if this
288 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

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

315 **Conclusion**

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

323 **Practical implications**

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

335 **References**

- 336 1. Cirer-Sastre R, Legaz-Arrese A, Corbi F, et al. Cardiac Biomarker Release After Exercise in Healthy
337 Children and Adolescents: A Systematic Review and Meta-Analysis. *Pediatr Exerc Sci.*
338 2019;31(1):28-36. doi:10.1123/pes.2018-0058
- 339 2. Thygesen K, Alpert JS, Jaffe AS, et al. Fourth Universal Definition of Myocardial Infarction (2018). *J*
340 *Am Coll Cardiol.* 2018;33(20):2551-2567. doi:10.1016/j.jacc.2018.08.1038
- 341 3. Baker P, Leckie T, Harrington D, Richardson A. Exercise-induced cardiac troponin elevation: An update
342 on the evidence, mechanism and implications. *Int J Cardiol Heart Vasc.* 2019;22:181-186.
343 doi:10.1016/j.ijcha.2019.03.001
- 344 4. Fu F, Nie J, Tong T. Serum Cardiac Troponin T in Adolescent Runners: Effects of Exercise Intensity
345 and Duration. *Int J Sports Med.* 2009;30(3):168-172. doi:10.1055/s-0028-1104586
- 346 5. Mehta R, Gaze D, Mohan S, et al. Post-Exercise Cardiac Troponin Release is Related to Exercise
347 Training History. *Int J Sports Med.* 2012;33(05):333-337. doi:10.1055/s-0031-1301322
- 348 6. Donaldson JA, Wiles JD, Coleman DA, Papadakis M, Sharma R, O'Driscoll JM. Left Ventricular
349 Function and Cardiac Biomarker Release—The Influence of Exercise Intensity, Duration and Mode:
350 A Systematic Review and Meta-Analysis. *Sports Med.* 2019;49:1275–1289. doi:10.1007/s40279-019-
351 01142-5
- 352 7. Legaz-Arrese A, Carranza-García LE, Navarro-Orocio R, et al. Cardiac Biomarker Release after
353 Endurance Exercise in Male and Female Adults and Adolescents. *J Pediatr.* 2017;191:96-102.
354 doi:10.1016/j.jpeds.2017.08.061
- 355 8. López-Laval I, Legaz-Arrese A, George K, et al. Cardiac troponin I release after a basketball match in
356 elite, amateur and junior players. *Clin Chem Lab Med CCLM.* 2016;54(2):333-338. doi:10.1515/cclm-
357 2015-0304
- 358 9. Tian Y, Nie J, Huang C, George KP. The kinetics of highly sensitive cardiac troponin T release after
359 prolonged treadmill exercise in adolescent and adult athletes. *J Appl Physiol.* 2012;113(3):418-425.
360 doi:10.1152/jappphysiol.00247.2012
- 361 10. Kannankeril PJ, Pahl E, Wax DF. Usefulness of troponin I as a marker of myocardial injury after
362 pediatric cardiac catheterization. *Am J Cardiol.* 2002;90(10):1128-1132. doi:10.1016/S0002-
363 9149(02)02781-9
- 364 11. Tanner JM. Growth and maturation during adolescence. [Review] [14 refs]. *Nutr Rev.* 1981;39(2):43-
365 55. doi:10.1111/j.1753-4887.1981.tb06734.x/epdf
- 366 12. Cirer-Sastre R, Legaz-Arrese A, Corbi F, et al. Effect of Training Load on Post-Exercise Cardiac
367 Troponin T Elevations in Young Soccer Players. *Int J Environ Res Public Health.* 2019;16(23):4853.
368 doi:10.3390/ijerph16234853
- 369 13. World Medical Association. Declaration of Helsinki: ethical principles for medical research involving
370 human subjects. *JAMA.* 2013;310(20):2191-2194. doi:10.1001/jama.2013.281053

- 371 14. Rodríguez FA. Spanish version of the Physical Activity Readiness Questionnaire (C-AAF/rPAR-Q).
372 *Apunts*. 1994;31:301-310.
- 373 15. Rasmussen AR, Wohlfahrt-Veje C, Tefre de Renzy-Martin K, et al. Validity of Self-Assessment of
374 Pubertal Maturation. *Pediatrics*. 2015;135(1):86-93. doi:10.1542/peds.2014-0793
- 375 16. Drezner JA, Sharma S, Baggish A, et al. International criteria for electrocardiographic interpretation
376 in athletes: Consensus statement. *Br J Sports Med*. 2017;51(9):704-731. doi:10.1136/bjsports-2016-
377 097331
- 378 17. Schubert M, Clark A, De La Rosa A. The Polar® OH1 Optical Heart Rate Sensor is Valid during
379 Moderate-Vigorous Exercise. *Sports Med Int Open*. 2018;02(03):E67-E70. doi:10.1055/a-0631-0920
- 380 18. Legaz-Arrese A, López-Laval I, George K, et al. Individual variability in cardiac biomarker release
381 after 30 min of high-intensity rowing in elite and amateur athletes. *Appl Physiol Nutr Metab*.
382 2015;40(9):951-958. doi:10.1139/apnm-2015-0055
- 383 19. Giannitsis E, Kurz K, Hallermayer K, Jarausch J, Jaffe AS, Katus HA. Analytical validation of a high-
384 sensitivity cardiac troponin T assay. *Clin Chem*. 2010;56(2):254-261.
385 doi:10.1373/clinchem.2009.132654
- 386 20. Ma G, Liu Y, Liu K, et al. Influence of repeated bouts of table tennis training on cardiac biomarkers
387 in children. *Pediatr Cardiol*. 2014;35(4):711-718. doi:10.1007/s00246-013-0842-x
- 388 21. Shave R, Ross P, Low D, George K, Gaze D. Cardiac troponin I is released following high-intensity
389 short-duration exercise in healthy humans. *Int J Cardiol*. 2010;145(2):337-339.
390 doi:10.1016/j.ijcard.2009.12.001
- 391 22. Legaz-Arrese A, López-Laval I, George K, et al. Individual variability of high-sensitivity cardiac
392 troponin levels after aerobic exercise is not mediated by exercise mode. *Biomarkers*. 2015;20(4):219-
393 224. doi:10.3109/1354750X.2015.1068851
- 394 23. Borg E, Borg G. A comparison of AME and CR100 for scaling perceived exertion. *Acta Psychol*
395 *(Amst)*. 2002;109(2):157-175. doi:10.1016/S0001-6918(01)00055-5
- 396 24. Mingels A, Jacobs L, Michielsen E, Swaanenburg J, Wodzig W, van Dieijen-Visser M. Reference
397 Population and Marathon Runner Sera Assessed by Highly Sensitive Cardiac Troponin T and
398 Commercial Cardiac Troponin T and I Assays. *Clin Chem*. 2008;55(1):101-108. doi:10/b5fxrj
- 399 25. Gore MO, Seliger SL, DeFilippi CR, et al. Age- and Sex-Dependent Upper Reference Limits for the
400 High-Sensitivity Cardiac Troponin T Assay. *J Am Coll Cardiol*. 2014;63(14):1441-1448.
401 doi:10.1016/j.jacc.2013.12.032
- 402 26. Bohn MK, Higgins V, Kavsak P, Hoffman B, Adeli K. High-Sensitivity Generation 5 Cardiac
403 Troponin T Sex- and Age-Specific 99th Percentiles in the CALIPER Cohort of Healthy Children and
404 Adolescents. *Clin Chem*. 2019;65(4):589-591. doi:10.1373/clinchem.2018.299156
- 405 27. Cirer-Sastre R, Legaz-Arrese A, Corbi F, et al. Cardiac Troponin T Release after Football 7 in Healthy
406 Children and Adults. *Int J Environ Res Public Health*. 2020;17(3):956. doi:10.3390/ijerph17030956

- 407 28. Legaz-Arrese A, López-Laval I, George K, et al. Impact of an endurance training program on exercise-
408 induced cardiac biomarker release. *Am J Physiol-Heart Circ Physiol.* 2015;308(8):H913-H920.
409 doi:10.1152/ajpheart.00914.2014
- 410 29. Omland T, Aakre KM. Cardiac Troponin Increase After Endurance Exercise: A New Marker of
411 Cardiovascular Risk? *Circulation.* 2019;140(10):815-818.
412 doi:10.1161/CIRCULATIONAHA.119.042131
- 413 30. Sribhen K, Piyophipong S, Wannasilp N. Cardiac troponin T concentrations in healthy adolescents.
414 *Clin Chim Acta.* 2010;411(19-20):1542-1543. doi:10/ds9z33
- 415 31. Nie J, P. George K, K. Tong T, et al. The Influence of a Half-Marathon Race Upon Cardiac Troponin
416 T Release in Adolescent Runners. *Curr Med Chem.* 2011;18(23):3452-3456.
417 doi:10.2174/092986711796642625
- 418 32. Kong Z, Nie J, Lin H, et al. Sex differences in release of cardiac troponin T after endurance exercise.
419 *Biomarkers.* 2016;22(3-4):345-350. doi:10.1080/1354750X.2016.1265007
- 420 33. Nie J, Tong TK, George K, Fu FH, Lin H, Shi Q. Resting and post-exercise serum biomarkers of
421 cardiac and skeletal muscle damage in adolescent runners. *Scand J Med Sci Sports.* 2010;21(5):625-
422 629. doi:10.1111/j.1600-0838.2010.01096.x
- 423 34. Shave RE, Oxborough D. Exercise-Induced Cardiac Injury: Evidence From Novel Imaging
424 Techniques and Highly Sensitive Cardiac Troponin Assays. *Prog Cardiovasc Dis.* 2012;54(5):407-
425 415. doi:10.1016/j.pcad.2012.01.007
- 426 35. Aengevaeren VL, Hopman MTE, Thompson PD, et al. Exercise-Induced Cardiac Troponin I Increase
427 and Incident Mortality and Cardiovascular Events. *Circulation.* 2019;140(10):804-814.
428 doi:10.1161/CIRCULATIONAHA.119.041627
- 429 36. Armstrong N, McManus AM. Physiology of elite young male athletes. *Med Sport Sci.* 2011;56:1-22.
430 doi:10.1159/000320618
- 431 37. Malina RM. Skeletal Age and Age Verification in Youth Sport. *Sports Med.* 2011;41(11):925-947.
432 doi:10.2165/11590300-000000000-00000

433

Table 1. Summary of cTnT time and group comparisons.

	Time			Time differences			
	Pre	Post 0 h	Post 3 h	Δ Post 0 h	<i>p</i> value	Δ Post 3 h	<i>p</i> 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		Δ Post 0 h		Δ Post 3 h		Maturational Stage	
	(ng/L)		(ng/L)		(ng/L)		(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/m ²)	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 Perceived 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 [7, 12]	11 +- 2 <V	12 +- 2 <V	14 +- 3 >I	15 +- 1 >I, II, III
Body Height (cm)	139,8 +- 12,2 <III, IV, V [122, 157,5]	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
Body Mass (kg)	34,4 +- 9,3 <IV, V [23,6, 50,4]	41,8 +- 10,8 <IV, V	46,2 +- 11,9 <V	57,8 +- 9,8 >I, II	60,8 +- 8 >I, II, III
BMI (kg/m²)	17,3 +- 2,2 <IV, V [14, 21,2]	18,8 +- 3	18,9 +- 3,5	20,2 +- 2,2 >I	20,1 +- 2,1 >I
Experience (years)	4 +- 2 <IV, V [1, 6]	5 +- 2 [2, 10]	4 +- 3 [1, 11]	6 +- 2 >I	7 +- 2 >I
Training (days/week)	4 +- 1 <IV, V [2, 5]	4 +- 1 <IV	4 +- 1 [3, 6]	5 +- 1 >I, II	5 +- 1 >I
Training (h/week)	6 +- 5 <IV [2, 15]	7 +- 3 <IV	8 +- 4 [5, 15]	12 +- 3 >III	10 +- 3
HR max (bpm)	186 +- 7 <V [175, 195]	189 +- 5 <V	190 +- 9 [165, 201]	192 +- 5 <V	198 +- 4 >I, II, IV
Exercise Load					
Distance (m)	1446 +- 409 <IV [650, 2500]	1533 +- 338 <IV	1583 +- 367 [1000, 2500]	1958 +- 296 >I, II	1874 +- 313 [1300, 2500]
% HR Peak (% HR Max)	94 +- 8 [77, 105]	94 +- 6 [86, 104]	94 +- 8 [83, 107]	91 +- 8 [78, 110]	97 +- 4 [89, 102]
% HR Mean (% HR Max)	84 +- 7 [71, 98]	87 +- 8 [77, 101]	86 +- 9 [69, 100]	90 +- 7 [75, 99]	88 +- 4 [78, 92]
RPE (0-100)	76 +- 9 [55, 90]	76 +- 18 [15, 90]	78 +- 11 [45, 90]	74 +- 16 [40, 90]	77 +- 17 [30, 95]
HRR at 1 min (bpm)	-44 +- 15 [-74, -16]	-46 +- 10 [-62, -28]	-43 +- 11 [-63, -19]	-38 +- 13 [-53, -13]	-42 +- 11 [-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		T test <i>p</i> value	Response		T test <i>p</i> value
	Non-detected (n = 11)	Detected (n = 59)		Non-responders (n = 45)	Responders (n = 25)	
Participant characteristics						
Age (years)	11 +- 2 < _D [8, 14]	12 +- 3 > _{ND} [5, 18]	0.024	12 +- 3 [8, 18]	12 +- 3 [5, 16]	0.88
Body Height (cm)	146,3 +- 12,5 < _D [122, 163]	158,4 +- 16,3 > _{ND} [123, 187]	0.013	155,9 +- 16,6 [122, 187]	157,5 +- 16 [125, 179]	0.69
Body Mass (kg)	41,2 +- 11,2 [23,6, 56,9]	49 +- 14 [23,7, 77,8]	0.06	47,5 +- 13,6 [23,6, 77,8]	48,3 +- 14,6 [23,7, 77,8]	0.81
BMI (kg/m²)	18,9 +- 3,1 [15,8, 24,4]	19,1 +- 2,8 [14, 26]	0.88	19,1 +- 2,6 [15,6, 24,4]	19 +- 3,2 [14, 26]	0.86
Experience (years)	5 +- 3 [1, 10]	5 +- 3 [1, 11]	0.84	5 +- 3 [1, 11]	5 +- 3 [1, 11]	0.13
Training (days/week)	4 +- 1 [2, 6]	5 +- 1 [2, 6]	0.16	4 +- 1 [2, 6]	5 +- 1 [3, 6]	0.14
Training (h/week)	8 +- 5 [2, 15]	9 +- 4 [2, 15]	0.64	9 +- 5 [2, 15]	8 +- 3 [2, 15]	0.38
HR max (bpm)	184 +- 8 < _D [175, 203]	192 +- 6 > _{ND} [165, 201]	0.006	190 +- 7 [175, 203]	192 +- 8 [165, 201]	0.26
Exercise Load						
Distance (m)	1418 +- 501 [650, 2500]	1715 +- 354 [1000, 2500]	0.085	1665 +- 420 [650, 2500]	1676 +- 344 [1250, 2500]	0.9
% HR Peak (% HR Max)	89 +- 7 < _D [77, 103]	95 +- 7 > _{ND} [78, 110]	0.033	93 +- 6 [77, 105]	96 +- 7 [82, 110]	0.088
% HR Mean (% HR Max)	82 +- 8 < _D [71, 99]	88 +- 7 > _{ND} [69, 101]	0.045	85 +- 7 < _R [69, 99]	90 +- 6 > _{NR} [76, 101]	0.004
RPE (0-100)	79 +- 4 [75, 85]	76 +- 15 [15, 95]	0.17	74 +- 16 < _R [15, 90]	81 +- 9 > _{NR} [50, 95]	0.012
HRR at 1 min (bpm)	-40 +- 11 [-53, -19]	-43 +- 12 [-74, -13]	0.49	-41 +- 12 > _R [-65, -13]	-47 +- 11 < _{NR} [-74, -25]	0.035

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.