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

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Article

1 **Title**

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

3 young swimmers

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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:* Maturational status influences positively absolute pre- and post-exercise cTnT but

24   not its elevation after a bout of 30 min, high-intensity, continuous exercise.

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

2

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-led 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 -80ºC 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 ºC, air temperature 29 ºC,

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/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

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 >

8

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. (X^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 where 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 cTnI or NT-proBNP might be used in future

314 studies to provide a more complete overview of the phenomenon.

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.

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.

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/japplphysiol.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, Piyophirapong 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	*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	**Tanner-II**	**Tanner-III**	**Tanner-IV**	**Tanner-V**
	(n = 14)	**(n = 15)**	**(n = 15)**	**(n = 13)**	**(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.