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A comparison of modelled serum cTnT and cTnI kinetics after 60 min swimming

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ABSTRACT

Post-exercise elevations of cardiac troponin T (cTnT) and I (cTnI) are often used in isolation but interpreted interchangeably. Research suggests, however, that post-exercise cTn kinetic might differ with each isoform. In this cross-sectional observational study, we collected blood samples before, immediately after (5 minutes), and at 1-, 3-, 6-, 12-, and 24-hours post-exercise in a mixed cohort of 56 participants after a 60-min (age range from 14 to 22, 57.1% female). swimming trial. Cardiac troponin kinetics were modeled using Bayesian mixed-effects models to estimate time to peak (TTP) and peak concentration (PC) for each isoform, while controlling for participants sex, tanner stage and average relative heart rate during the test. Exercise induced an elevation of cTnT and cTnI in 93% and 75% of the participants, respectively. Cardiac troponin T

peaked earlier, at 2.9 h (CI: 2.6 – 3.2 h) post-exercise, whereas cTnI peaked later, at 4.5 h (CI: 4.2 – 4.9 h). Peak concentrations for cTnT and cTnI were 2.5 ng/L, CI: 0 – 11.2 ng/L and 2.16 ng/L, CI: 0 – 22.7 ng/L, respectively. Additionally, we did not observe a systematic effect of sex and maturational status mediating cTn responses.

INTRODUCTION

The elevation of serum of cardiac troponins (cTn), either T (cTnT) or I (cTnI), is common after exercise (Kaleta-Duss et al., 2020; Lippi & Sanchis-Gomar, 2020), often exceeding the upper reference limits for myocardial injury (MI) (Thygesen et al., 2018). Post-exercise elevations of both isoforms are often used and interpreted interchangeably. Some research, however, has suggested that post-exercise concentrations of cTnT reach a peak earlier than cTnI (Gresslien & Agewall, 2016; Klinkenberg et al., 2016). Despite the evidence showing that both isoforms are elevated in healthy individuals after exercise (Bjørkavoll-Bergseth et al., 2020; Klinkenberg et al., 2016), relatively little attention has been given to a direct, within-person, comparison of the timing and magnitude of kinetics (Hammarsten et al., 2018; Lippi & Cervellin, 2020; Starnberg et al., 2020). In clinical scenarios, cTnT has been observed to peak earlier and take longer to return to baseline values than cTnI (Laugaudin et al., 2016; Solecki et al., 2015).

One limitation of exercise-based studies is that the kinetics of both isoforms are dependent on a much smaller range of sample times and treated as a categorical variable (i.e. baseline, immediately after, 3 h after). The application of a non-linear, continuous, approach to kinetic assessment has not been completed (Klinkenberg et al., 2016; Rifai et al., 1999). In addition, some observations of cTn elevation induced by exercise have been associated to biological and/or chronological age, sex, exercise duration and

relative intensity (Cirer-Sastre, Legaz-Arrese, Corbi, López-Laval, George, et al., 2020; Donaldson et al., 2019; Fu et al., 2009; Legaz-Arrese et al., 2017; Tian et al., 2012). The current evidence is heterogeneous in terms of study design and statistical control of these confounding variables, making it difficult to extract conclusions regarding the comparability between isoforms (Cirer-Sastre, Legaz-Arrese, Corbi, López-Laval, George, et al., 2020; Cirer-Sastre et al., 2021). Data interpretation is also affected by the sensitivity of the assays used in each study, since the differences between isoforms as well as the effect of each covariable on the post-exercise kinetics might only be detectable when measuring cTn using high-sensitivity immunoassays (Boeddinghaus et al., 2019; Lackner, 2015; Vaz et al., 2019).

In this line, a reasonable starting point would be to characterize each isoform kinetics in terms of time-to-peak (TTP) and peak concentration (PC) (Nie et al., 2011; Solecki et al., 2015). The purpose of this study was to model the post-exercise kinetics of cTnT and cTnI in a healthy cohort of young swimmers, using a non-linear estimation of the individual kinetics and controlling the confounding effects of sex, maturational status, relative intensity, and exercise duration.

METHODS

Participants

Fifty-six swimmers were included in this study (mean age = 16 [range 14 – 22] years; mean (SD) height = 164 (8) cm; mean (SD) body mass 62.1 (12.2) kg), representing a mixed pubertal population (32 (57.1%) females; Tanner stage, III = 13 (23.2%), IV = 17 (30.4%), and V = 26 (46.4%)) of trained swimmers (previous training = 32 [21 – 61] months). A complete detail of participants characteristics can be found in Table 1. None

of the particpants had any clinical evidence or personal/family history of cardiac disease or arterial hypertension. All had a normal 12-lead electrocardiogram at rest at the beginning of the study. All swimmers provided written informed consent (and parental consent for adolescents). This study followed the ethical guidelines of the Declaration of Helsinki and was approved by the Research Ethics Committee of the Universidad Autónoma de Nuevo León (Autonomous University of Nuevo León).

Table 1.
Descriptive statistics of participants and performance data.

Variable	Summary data (n = 56)
Participant characteristics	
Sex	Female = 32 (57.1%), Male = 24 (42.9%)
Age	15 [14 - 22] years
Tanner stage	III = 13 (23.2%), IV = 17 (30.4%), V = 26 (46.4%)
HR max.[1]	190 (11) bpm
Body height	164 (8) cm
Body mass	62.1 (12.2) kg
BMI[3]	22.6 [20.5 – 24.8]
Fat body mass	19.5 [10.9 – 24.3] % Body mass
Training experience	32 [21 – 61] months
Training frequency	6 [6 – 6] days/week
Training volume	18 [18 – 18] hours/week
Performance data	
HR peak[1]	187 [176 - 191] bpm
rHR peak[2]	98 [96 – 100] % HR max.
HR mean[1]	165 [155 – 175] bpm
rHR mean[2]	87 [81 – 92] % HR max.
Distance	3201 (372) m
Velocity mean	3.2 (0.4) km/h
RPE[4]	18 [18 – 18.2]

[1]HR = Heart Rate; [2]rHR = Relative Heart Rate; [3]BMI = Body Mass Index; [4]RPE = Rating of Perceived Exertion. Categories are described as frequency (%), normally distributed continuous variables as mean (standard deviation), non-normally distributed continuous variables as median [interquartile range]

Procedures

This was a cross-sectional observational study. At the beginning of the study, participants were measured dry and wearing swimming clothes. Body mass was measured with a medical scale (SECA 861, Hamburg, Germany) and height with a

stadiometer (SECA 225, Hamburg, Germany). Standard, 12-led ECG were recorded using a digital electrocardiograph (Quark T12x, Cosmed, Italy). Recordings were obtained with the swimmer in supine position during quiet respiration, after a short period of rest. ECG were assessed in situ by experienced medical personnel and compared against the international ECG criteria for sports screening (Drezner et al., 2017). Two experienced pediatricians assessed participants' pubertal status. Female participants were assessed by a female pediatrician, whereas males were assessed by a male pediatrician. Genitalia and pubic hair were observed in the presence of parents and swimmers were classified according to the five-stage criteria described by Tanner (Tanner, 1981).

Maximal heart rate (HR max) was obtained by calculating the peak heart rate in a specific swimming protocol (Legaz-Arrese et al., 2017). First, swimmers performed a standardized warm up consisting in 100 m freestyle, 30 sec recovery, 4 repetitions of 25 m with 10 sec. recovery between repetitions, 30 sec recovery, and 100 m freestyle. Participants were asked to perform 6 repeated maximal sprints of 25 m with 10 sec of recovery between repetitions (Legaz-Arrese et al., 2017). Measurements were made in a 25 m indoor swimming pool, and heart rate during the test was recorded using a Polar HR monitor (Polar Team 2; Kempele, Finland).

The intervention was performed a week after the maximal heart rate test. Swimmers completed a self-paced 5-minute warm-up (<60% of %HRmax) followed by a 60-minute distance-trial swimming test. All participants were accustomed to the 60-minute distance-trial protocol and were asked to abstain from strenuous exercise for 48 hours before the exercise test. During the exercise test, swimmers made a continuous effort without periods of rest time to complete the maximum possible distance at self-paced velocity. The swimming test took place at 8:00 a.m. in a 50-m indoor pool (water

temperature 26°C, air temperature 29°C, relative humidity 75%). Pairs of subjects competed side-by-side to provide motivation and competition, and strong verbal encouragement was provided during the test. Subjects were constantly aware of the time and distance covered. Water intake was allowed ad libitum. HR was recorded continuously during the tests via a Polar HR monitor (Polar Team 2; Kempele, Finland). Immediately after the test, participants reported the rating of perceived exertion (RPE) Using a Borg 1 – 20 scale.

Repeated venous blood samples were taken before, immediately after (5 minutes), and at 1, 3, 6, 12, and 24 hours after exercise to assess serum cTnT and cTnI levels. Blood samples were drawn from the antecubital vein using venous puncture. The blood was allowed to clot at room temperature and then centrifuged. Serum was drawn off and stored at -80°C for later analysis. Cardiac troponin T was measured via electrochemiluminescence technology using a Cobas 6000 analyzer (Roche Diagnostics, Tokyo, Japan). This assay has a range from 3 to 10 000 ng/L with a lower detection limit of 3 ng/L. The coefficient of variation at a mean hs-cTnT level of 13.5 ng/L was 5.2%, and the upper reference limit (URL) for hs-cTnT, defined as the 99th percentile of healthy participants, was 14 ng/L (Giannitsis et al., 2010). Cardiac troponin I was measured using an Access 2 hsTnI immunoassay (Beckman Coulter, Chaska, Minnesota). This hs-cTnI has a 99th percentile of 11.8 ng/L and 19.7 ng/L for female and male, respectively, a limit of blank of 0.08 ng/L, a limit of detection of 0.32 ng/L, and limit of quantification of 0.77 ng/L (Christenson et al., 2020).

Data analysis

Frequency distributions were inspected visually, and normality assumption was verified with the Shapiro-Wilk test. Continuous data were described as mean (standard deviation) when distributions met normality, otherwise as median [interquartile range],

anc categorical variables as frequency (proportion). The primary outcomes in this study were time to peak (TTP) and peak concentration (PC) for each isoform, cTnT and cTnI. To this end, we fit three Bayesian mixed-effects models explained below.

The first model (M1) was used to estimate the individual kinetics of cTn. More formally, in this model, cTn concentrations were modeled as a response of natural cubic splines of time, isoform (cTnT or cTnI), and their interaction (time x isoform). Natural cubic splines are a flexible curve fitting method where the range of values of the predictor (in this case, time) are subdivided using a set of predefined marks called knots, and separate polynomic curves are fit between each pair of knots. Natural cubic splines have been previously used successfully to model cTn kinetics (Solecki et al., 2015). Knots in this model were set to 0 (immediately after exercise), 3 (maximal observed concentrations in most of the participants) and 12 hours. Posterior distributions of this model were used to estimate individual TTP for each isoform. The second (M2) and third (M3) models compared our estimations of TTP and the observed PC, respectively. Both models (M2 and M3) assessed the fixed effects for isoform, and accounted for the effect of sex, tanner stage and average relative heart rate (rHR) as covariates, according to previous evidence (Cirer-Sastre, Legaz-Arrese, Corbi, López-Laval, George, et al., 2020; Cirer-Sastre et al., 2021; Legaz-Arrese et al., 2017). Medians of posterior distributions were used as point estimates and credible intervals (CI) around each estimate were set to the 95 % highest density interval (HDI).

All models were fit for the gaussian family, log link functions were applied when the response were troponin concentrations (M1 and M3), and identity links were used for time in M2. Each model was run with four independent Markov chains of 5000 iterations, with the first 2000 iterations used only for calibration and then discarded, thus resulting in 12000 post-warm-up samples. Convergence of the chains and sufficient

sampling of posterior distributions were confirmed by ensuring a potential scale reduction factor Rhat < 0.01 and an effective sample size of at least 20% of the number of iterations. Data cleaning, manipulation, and analyses were performed in R v4.0.4 (R Core Team, 2021). The main statistical packages used in the analysis were *brms* (Bürkner, 2017), *splines2* (Wang & Yan, 2021) and *emmeans* (Lenth, 2019).

RESULTS

During the 60 min time trial, swimmers, on average, covered 3201 (±372) m and performed at a mean and peak intensity of 87 [range 81- 92] % HRmax and 187 [range 80 - 92] bpm, respectively. A complete detail of exercise performance data can be found in Table 1.

Individual kinetics

Serum cTn increased above baseline values in 52 (93%) and 42 (75%) participants for cTnT and cTnI, respectively. Additionally, 35 (63%) and 28 (50%) participants exceeded the URL for MI for cTnT and cTnI, respectively. At 24 h post-exercise, 4 (7%) and 8 (14%) participants still exceeded the URL for MI for cTnT and cTnI, respectively. Individual cTn kinetics for each isoform were modeled in a satisfactory way (Conditional $R^2 = 0.994$) and are presented in Figure 1. A summary of M1 coefficients can be found in Supplementary File 1.

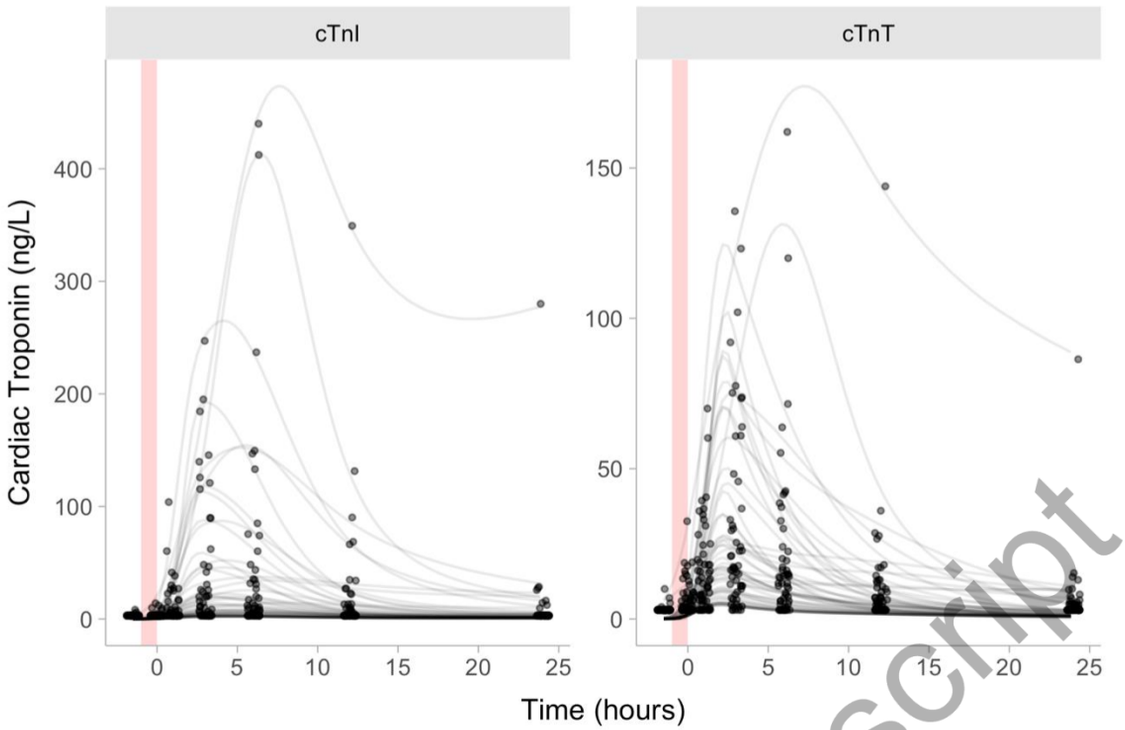

Figure 1. *Individual kinetics of cTn data for both isoforms in whole cohort*

Red bands indicate the 60 min exercise exposure. Dots represent observed concentrations of cTn. Grey lines indicate the estimated individual kinetics after cubic splines regression.

Time to peak (TTP)

Cardiac troponin T peaked earlier, at 2.9 h (CI, 2.6 – 3.2 h) post-exercise, whereas cTnI peaked later, at 4.5 h (CI, 4.2 – 4.9 h). The estimated difference between cTnT and cTnI TTP when controlling the effect of covariates was 1.6 h (CI, 1.3 – 2.0 h). Additionally, the covariate effects of sex, tanner stage and exercise intensity in the TTP estimations were negligible. A summary of M2 coefficients is provided in Supplementary File 2.

Peak concentration (PC)

Even though the estimated PC for cTnI was 1.3 ng/L (CI, 0.00 – 5.82) ng/L higher than for cTnT, prediction intervals for the adjusted PC in both isoforms overlapped and included 0 (cTnT = 2.5 ng/L, CI 0 – 11.2 ng/L; cTnI = 2.16 ng/L, CI 0 – 22.7 ng/L). Additionally, sex appeared to influence PC, even though zero concentrations of cTn

were equally probable in women and men, the center of the probability distribution was systematically higher in men than in women.

We did not find a systematic effect of sex and tanner stage. Detail of predicted marginal distributions for peak cTn concentrations in each isoform, sex, and tanner stage at a fixed median intensity of 95.9% HR max is provided in Table 2.

Table 2.
Estimated marginal distributions for cTn peak concentrations, extracted from M3 coefficients.

	Median	**95% CI**
cTnI	**5.16**	**00 – 22.7**
Female	2.07	00 – 13.17
T-III	0.82	00 – 13.1
T-IV	6.53	00 – 28.9
T-V	4.30	00 – 20.3
Male	12.25	00 – 55.30
T-III	4.60	00 – 75.0
T-IV	32.73	00 – 136.1
T-V	21.17	00 – 61.1
cTnT	**2.50**	**00 – 11.2**
Female	1.00	00 – 6.52
T-III	0.40	00 – 6.5
T-IV	3.16	00 – 14.1
T-V	2.07	00 – 10.1
Male	5.89	00 – 27.37
T-III	2.24	00 – 37.1
T-IV	15.83	00 – 66.9
T-V	10.29	00 – 30.2

All values are expressed in ng/L. T = Tanner stages from III to V.

DISCUSSION

In this study we compared the post-60 min swimming time trial kinetics of cTnT and cTnI using high-sensitivity immunoassays, over a 24-h sampling profile and with the application of novel modelling to determine peak cTn concentration and time to peak values. The key findings were; 1) we confirmed that exercise induces elevations of both isoforms in a high percentage of the population, often exceeding the URL; 2) we confirmed that time to peak concentration of cTnT occurs earlier than cTnI, and 3) we noted that peak cTn concentrations could be higher in males, and that maturational status did not explain cTn variability.

Overall presentation

In our study, more than a half of participants exceeded the URL for cTnT and/or cTnI, all having normal 12-lead ECG and reporting no cardiac symptoms at the time of the study as well as in the following year after the intervention. This would support the contention of previous research that this represents a benign and transient elevation of cTn (Baker et al., 2019; Lippi & Sanchis-Gomar, 2020). Consequently, two main hypotheses have been previously postulated. This doesn't support the hypothesis that such troponin release is directly associated to permanent myocardial damage (Hammarsten et al., 2018; Lippi & Sanchis-Gomar, 2020). This is despite the fact that individual data exceeds the URL for MI in a number of participants (Stavroulakis & George, 2020). Secondly, the transient nature, with rapid decline in the subsequent hours, and the lack of any other sign or symptom of MI could reflect this phenomenon as an adaptive response to cardiovascular exercise and are consequence of mechanical and oxidative stress during exercise (Stavroulakis & George, 2020).

Time to peak

In our data cTnT peaked between 2:36 h and 3:12 h after 60 min continuous swimming, whereas the cTnI peak occurs later, between 4:12 h and 4:54 h after the same exercise. The fact that cTnT peaks earlier than cTnI was previously noted by Gresslien & Agewall (2016) and Klinkenberg et al. (2016). Previous evidence was based on a smaller number of post-exercise blood samples (i.e. pre, 0, 2 and 5 h post-exercise) (Klinkenberg et al., 2016). This allowed for categorical-only comparisons and lead to relatively wide and overlapped intervals of reference for TTP, of 2 to 5 h and 3 to 6 h post-exercise for cTnT and cTnI, respectively (Gresslien & Agewall, 2016). In this regard, we obtained more sensitive intervals of predictions for each isoform, that were narrower and did not overlap. Interestingly though, in clinical scenarios when cTn is released into the bloodstream after MI, cTnT and cTnI TTP seems to be comparable. Laugaudin et al. (2016) noted that TTP of cTnT and cTnI in patients with ST-segment elevation myocardial infarction was comparable regardless of the assay manufacturer. Similarly, Solecki et al., (2015) found no differences between isoforms TTP in patients with revascularized acute myocardial infarction. The fact that cTn isoforms peak at the same time after MI but at different moments after exercise could strengthen the hypothesis of a different and physiological mechanisms of release after exercise.

Peak concentration

Prediction intervals for both isoforms overlapped and included zero. This reflects the fact that the isoforms did not elevate in some athletes. Additionally, our results confirm that the absence of cTn elevations is probable within a 95% CI, not only in both cTn isoforms, but also in both sexes and in all included maturational stages. We confirmed a

recurrent topic in this line of research, that is the high inter-individual variability in the magnitude of the exercise-induced elevations of cTn (Cirer-Sastre, Legaz-Arrese, Corbi, López-Laval, Puente-Lanzarote, et al., 2020; Legaz-Arrese et al., 2015). In a previous study, Cirer-Sastre et al. found that age, body height, HR max, % HR peak, and % HR mean were lower in the non-responders (Cirer-Sastre, Legaz-Arrese, Corbi, López-Laval, George, et al., 2020). If this were true, it is plausible to think that there could exist an individual threshold of minimum exercise intensity needed to "unleash" cTn elevations, that should be further explored in future research. Finally, our results coincide with Cirer-Sastre, et al. (2020) and Legaz-Arrese, et al. (2017) who observed that maturational stage does not influence the exercise-induced release of cTn, but sex, on the other hand, could be a mediator with PC being systematically higher in males than in females (Cirer-Sastre, Legaz-Arrese, Corbi, López-Laval, George, et al., 2020; Legaz-Arrese et al., 2017).

Strengths and limitations

This is the first study that modeled the non-linear kinetics of cTn post-exercise, facilitating a potentially more accurate assessment of TTP and PC data for both cTnI and cTnT. We combined an exhaustive blood sampling protocol, 7 repeated measurements (Mingels et al., 2008), with a non-linear statistical modeling (Laugaudin et al., 2016; Solecki et al., 2015), that allowed more sesneitive TTP intervals for each isoform. Additionally, models controlled the effect of exercise intensity, maturational stage and sex, that are the main covariates identified in the literature (Cirer-Sastre et al., 2021; Cirer-Sastre, Legaz-Arrese, Corbi, López-Laval, George, et al., 2020; Legaz-Arrese et al., 2017). Inherently some limitations persist. Modelled data for PC and TTP would be even more accurate with a greater number of samples in the 24 h period post exercise, but we had to balance sample number with the invasive nature of assessment

in an adolescent population. In addition, cTn measurements were not performed in duplicate precluding any intra-assay control beyond the calibration and precision calculations recommended by the manufacturer. Even though we classified participants according to Tanner stages, we did not estimate the age relative to peak height velocity. Further, sample size is a common limitation in studies involving trained athletes from a single sport, when recruiting children and adolescents, and when procedures require venous blood sampling. Although the cohort in our study was similar or larger than the investigated in previous studies (Cirer-Sastre et al., 2019; Legaz-Arrese et al., 2017; Tian et al., 2012), the authors acknowledge that sample size was moderate. Finally, we were unable to report cTn concentration after corrections for potential post-exercise hemoconcentration (Tian et al., 2012). This does not invalidate the kinetics estimations performed in the study but should be taken into consideration when comparing cTn concentrations between studies in a post-exercise setting.

CONCLUSION

A 60-min swimming time trial in adolescent athletes resulted in a significant increase in both serum cTnI and cTnT. New modelling of the cTn data post-exercise revealed that peak concentrations of cTnT appear earlier than cTnI. Our study could not confirm whether peak concentrations are higher in cTnI compared with cTnT. There is some evidence to suggest that peak concentrations may be higher in male swimmers but maturational phase did not mediate cTn responses.

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SUPPLEMENTARY FILES

Supplementary File 1.
M1 table of coefficients.

Predictors	**Estimates**	**CI (95%)**
Fixed Effects		
Intercept	-5.71	-7.38 – -4.24
Time: Spline 1	19.65	15.02 – 24.52
Time: Spline 2	9.91	7.77 – 12.20
Time: Spline 3	0.43	-0.43 – 1.26
Time: Spline 4	8.68	7.25 – 10.20
Isoform: cTnT	1.71	0.94 – 2.50
Time: Spline 1 × Isoform: cTnT	-6.00	-8.17 – -3.82
Time: Spline 2 × Isoform: cTnT	-0.69	-1.68 – 0.30
Time: Spline 3 × Isoform: cTnT	0.69	0.44 – 0.92
Time: Spline 4 × Isoform: cTnT	-2.13	-2.81 – -1.45
Random Effects		
σ^2	20.84	
$\tau 00_{id}$	11.95	
Adjusted ICC	0.357	
Conditional ICC	0.301	
N_{id}	56	
Observations	784	
Marginal R^2	0.010	0.004 – 0.019
Conditional R^2	0.994	0.994 – 0.995

Intercept was set to Isoform = cTnI; Time = baseline. Model coefficients indicate cTn (ng/L) variations in the log scale.

Supplementary File 2.
M2 table of coefficients.

Predictors	**Estimates**	**CI (95%)**
Fixed Effects		
Intercept	7.19	3.90 – 10.30
Isoform: cTnT	-2.33	-2.84 – 1.85
Sex: Male	0.08	-0.53 – 0.71
Tanner: IV	-0.47	-1.25 – 0.35
Tanner: V	0.41	-0.35 – 1.23
rHR mean	-0.03	-0.07 – 0.01
Random Effects		
σ^2	1.83	

$\tau 00_{id}$	0.21	
Adjusted ICC	0.101	
Conditional ICC	0.057	
N_{id}	56	
Observations	112	
Marginal R^2	0.454	0.349 – 0.536
Conditional R^2	0.510	0.383 – 0.630

Intercept was set to Isoform = cTnI; Sex = Female; Taner = III; rHR mean = 86% HRmax. Model coefficients indicate TTP (h) variations.

Supplementary File 3.
M3 table of coefficients.

Predictors	**Estimates**	**CI (95%)**
Fixed Effects		
Intercept	17.74	-34.51 – 72.50
Isoform: cTnT	-17.78	-48.57 – 11.50
Sex: Male	61.94	7.31 – 115.84
Tanner: IV	31.80	-38.35 – 100.93
Tanner: V	0.83	-69.18 – 69.16
rHR mean	8.40	-16.42 – 34.08
Random Effects		
σ^2	6341.9	
$\tau 00_{id}$	5722.1	
Adjusted ICC	0.474	
Conditional ICC	0.431	
N_{id}	56	
Observations	112	
Marginal R^2	0.529	0.341, 0.666
Conditional R^2	0.125	0.024, 0.229

Intercept was set to Isoform = cTnI; Sex = Female; Taner = III; rHR mean = 86% HRmax. Model coefficients indicate peak cTn (ng/L) variations in the log scale.