Universitat de Lleida

Troponin, Exercise and Growth

Exercise-Induced Release of Cardiac Troponin in Healthy Trained Prepubertal and Pubertal Males

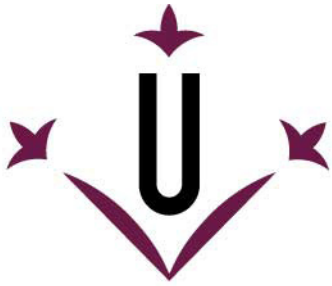
Rafel Cirer-Sastre

Doctoral Thesis

TROPONIN, EXERCISE AND GROWTH

Exercise-induced release of cardiac troponin in healthy, trained, prepubertal and pubertal males

Cirer-Sastre, Rafel



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TESI DOCTORAL

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Exercise-Induced Release of Cardiac Troponin in Healthy Trained Prepubertal and Pubertal Males

Rafel Cirer-Sastre

Memòria presentada per optar al grau de Doctor Internacional per la Universitat de Lleida
Programa de Doctorat en Activitat Física i Esport

Directors
Francisco Corbi
Joaquin Reverter-Masia

Tutor
Antoni Planas Anzano

2020

Cover: Aina Rafart Costa

Design and layout: Aina Rafart Costa

Printing: www.laimprentacg.com

The work in this thesis was conducted at the National Institute of Physical Education of Catalonia (INEFC), Universitat de Lleida (UdL), Lleida, Spain, and was supported by a grant from INEFC (Grant Number 2016PINEF00007).

The following organizations are gratefully acknowledged for the financial support in the publications derived from this thesis: National Institute of Physical Education of Catalonia (INEFC); Section of Physical Education, Universitat de Lleida (UdL); Section of Physical Education and Sports, University of Zaragoza

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- Pediatric Exercise Science, 2019, 31 (1): 28-36, https://doi.org/10.1123/pes.2018-0058. ©Human Kinetics, Inc.
- International Journal of Environmental Research and Public Health, 2019, 16 (23): 4853, https://doi.org/10.3390/ijerph16234853. ©MDPI AG.
- International Journal of Environmental Research and Public Health, 2020, 17 (3): 956, https://doi.org/10.3390/ijerph17030956. ©MDPI AG.
- Journal of Science and Medicine in Sport, 2020, In Press. ©Elsevier BV.

Supervisors

Co-supervisor
Joaquin Reverter-Masia, PhD, Professor Catedràtic
Departament de Didàctiques Específiques
Universitat de Lleida (UdL), Lleida, Spain

Co-supervisor
Francisco Corbi, PhD, Professor Titular
Institut Nacional d'Educació Física de Catalunya (INEFC)
Universitat de Lleida (UdL), Lleida, Spain

Tutor
Antoni Planas Anzano, PhD, Professor Catedràtic
Institut Nacional d'Educació Física de Catalunya (INEFC)
Universitat de Lleida (UdL), Lleida, Spain

Abstract

Cardiac troponin (cTn) is a specific biomarker for heart damage, and the leading criteria for diagnosing myocardial injury. However, solid evidence proved that exercise induces as well transient elevations of cTn in the following hours. Additionally, although the physiological mechanisms underlying the exercise-induced release of cTn remain unclear, its kinetics has been determined in adults allowing its differentiation from myocardial injury, and related too with individual and exercise characteristics. Despite its characterization in adults, studies describing this phenomenon in children and adolescents are still scarce and inconclusive.

Therefore, the objectives of this thesis were: (1) To describe the changes in serum cardiac troponin T (cTnT) related to exercise in healthy trained prepubertal and pubertal males; (2) To assess the effect of biological maturity on the changes in serum cTnT related to exercise in healthy trained males, and (3) To relate exercise load with the subsequent changes in serum cTnT in healthy trained prepubertal and pubertal males. Accordingly, this thesis is presented in a compendium of four publications, using different designs and methodologies: the first of them presents a systematic review and meta-analysis on the cardiac biomarker release after exercise in healthy children and adolescents; while the remaining, offer a series of three quasi-experimental, cross-sectional studies analyzing the influence of exercise load and/or maturational stage in the exercise-induced elevations of cTnT.

Results of this research suggest that serum cTnT respond to exercise in healthy trained prepubertal and pubertal males with an elevation during the subsequent 3 h. Furthermore, basal cTnT, is positively associated to maturational stage, with higher concentrations in the late-puberty. In spite of that, its elevation induced by exercise is independent from maturational stage. Similarly, the rate of athletes exceeding the upper reference limits (URL) for cTn after exercise does not vary among maturational stages. Furthermore, the elevations of cTnT subsequent to exercise are associated to internal and external exercise load. Concretely, the best internal predictor for the peak cTnT concentrations was the time spent at highest cardiac intensity zone, whereas the best external predictors were the peak and average speed during exercise.

The main contributions of this work were that cTnT measurements within the 3 h following exercise in healthy trained prepubertal and pubertal males might be suggestive of a rise in cTn that meets the criteria for acute myocardial injury. Additionally, the prevalence of cTn at rest is higher in the late-puberty. Furthermore, the incidence of healthy trained prepubertal and pubertal males exceeding the URL for cTn at 3 h post-exercise is high. Contrary to prevalence, however, incidence seems not to be associated to maturational stage, suggesting that maturational differences occur in low concentrations. Finally, duration of elevated heart rate as well as peak and average speed during exercise seem to be the best predictors for the exercise-induced elevation of cTnT.

Resum

La troponina cardíaca (cTn) és un biomarcador específic del dany cardíac, i el criteri preferent per al diagnòstic de lesió miocàrdica. No obstant això, existeixen evidències sòlides demostrant que l'exercici físic també indueix elevacions transitòries de cTn durant les hores posteriors. D'altra banda, malgrat que els mecanismes fisiològics subjacents a l'alliberament de cTn induït per l'exercici són incerts, s'ha determinat la seva cinètica en adults permetent així la seva diferenciació de la lesió miocàrdica, i relacionat també amb característiques de l'individu i l'exercici. Tot i la seva caracterització en adults, els estudis que descriuen aquest fenomen en nens i adolescents són encara escassos i inconclusius.

Així doncs, els objectius d'aquesta tesi van ser: (1) Descriure els canvis en la troponina cardíaca T (cTnT) relacionats amb l'exercici en homes sans entrenats prepuberals i puberals; (2) Avaluar l'efecte de la maduració biològica sobre els canvis en la cTnT relacionats amb l'exercici en homes sans i entrenats; i (3) Relacionar la càrrega de l'exercici amb els canvis posteriors en la cTnT en homes sans entrenats prepuberals i puberals. D'acord amb això, aquesta tesi està presentada en un compendi de quatre publicacions, que utilitzen dissenys i metodologies diferents: la primera d'elles, presenta una revisió sistemàtica i meta-anàlisi sobre l'alliberament de biomarcadors cardíacs després de l'exercici en nens i adolescents sans; mentre que els restants, ofereixen una sèrie de tres estudis transversals quasi-experimentals analitzant la influència de la càrrega de l'exercici i/o l'estadi maduratiu sobre les elevacions de cTnT induïdes per l'exercici.

Els resultats d'aquesta recerca suggereixen que la cTnT respon a l'exercici en homes sans entrenats prepuberals i puberals amb una elevació durant les següents 3 h. Així mateix, la cTnT està positivament associada a l'estadi maduratiu, amb concentracions més altes al final de la pubertat. Malgrat això, la seva elevació induïda per l'exercici és independent de l'estadi maduratiu. De forma similar, la taxa d'atletes excedint els límits superiors de referència (LSR) per cTn després de l'exercici no varia entre estadis maduratius. Altrament, les elevacions de cTnT després de l'exercici estan associades a la càrrega interna i externa de l'exercici. Concretament, el millor predictor intern per la concentració pic de cTnT va ser el temps transcorregut a la zona d'intensitat cardíaca màxima, mentre que els millors predictors externs van ser la velocitat mitjana i pic durant l'exercici.

Les contribucions principals d'aquest treball són que els amidaments de cTnT dins les 3 h posteriors a l'exercici en homes sans entrenats prepuberals i puberals podrien suggerir un increment de cTn que compleix amb els criteris per la lesió miocàrdica aguda. Així mateix, la prevalença de cTn en repòs és més alta al final de la pubertat. Addicionalment, la incidència d'homes sans entrenats prepuberals i puberals que excedeix el LSR per cTn a les 3 h post exercici és alta. Tanmateix, en contraposició a la prevalença, la incidència sembla no estar associada a l'estadi maduratiu, suggerint així que les diferències entre estadis maduratius succeeixen a baixes concentracions. Finalment, la durada a elevades freqüències cardíaques i les velocitats pic i mitjana durant l'exercici, semblen ser els millors predictors per l'elevació de cTnT induïda per l'exercici.

Resumen

La troponina cardíaca (cTn) es un biomarcador especifico para daño cardíaco, y el criterio preferente para el diagnóstico de lesión miocárdica. Sin embargo, existen evidencias sólidas demostrando que el ejercicio físico induce también elevaciones transitorias de cTn en las horas posteriores. Adicionalmente, aunque los mecanismos fisiológicos subyacentes a la liberación de cTn inducida por el ejercicio permanecen inciertos, se ha determinado su cinética en adultos permitiendo así su diferenciación de la lesión miocárdica, y se ha relacionado también con características individuales y del ejercicio. A pesar de su caracterización en adultos, los estudios describiendo este fenómeno en niños y adolescentes son todavía escasos e inconcluyentes.

Por consiguiente, los objetivos de esta tesis fueron: (1) Describir los cambios en la troponina cardíaca T (cTnT) relacionados con el ejercicio en varones sanos, entrenados, prepuberales y puberales; (2) Evaluar el efecto de la madurez biológica en los cambios de la cTnT relacionados con el ejercicio en varones sanos y entrenados; y (3) Relacionar la carga del ejercicio con los cambios posteriores en la cTnT en varones sanos, entrenados, prepuberales y puberales. En consecuencia, esta tesis está presentada en un compendio de cuatro publicaciones, que usan diseños y metodologías diferentes: la primera de ellas presenta una revisión sistemática y meta-análisis sobre la liberación de biomarcadores cardíacos después del ejercicio en niños y adolescentes sanos; mientras que las siguientes, ofrecen una serie de tres estudios transversales cuasi-experimentales analizando la influencia de la carga de ejercicio y/o el estadio madurativo sobre las elevaciones de cTnT inducidas por el ejercicio.

Los resultados de esta investigación sugieren que la cTnT responde al ejercicio en varones sanos, entrenados, prepuberales y puberales con una elevación durante las siguientes 3h. Asimismo, la cTnT basal está positivamente asociada al estadio madurativo, con mayores concentraciones en el final de la pubertad. A pesar de ello, su elevación inducida por el ejercicio es independiente del estadio madurativo. De forma similar, la tasa de atletas excediendo el limite superior de referencia (LSR) para cTn después del ejercicio no varía entre estadios madurativos. Además, las elevaciones de cTnT posteriores al ejercicio están asociadas a la carga interna y externa del ejercicio. Concretamente, el mejor predictor interno para la concentración pico de cTnT fue el tiempo transcurrido en la zona de intensidad máxima de la frecuencia cardíaca, mientras que los mejores predictores externos fueron la velocidad media y pico durante el ejercicio.

Las contribuciones principales de este trabajo fueron que las mediciones de cTnT dentro de las 3 h siguientes al ejercicio en varones sanos, entrenados, prepuberales y puberales podrían sugerir un incremento de cTn que cumple con los criterios para la lesión aguda miocárdica. Asimismo, la prevalencia de cTnT en reposo es más altas al final de la pubertad. Además, la incidencia de varones sanos, entrenados, prepuberales y puberales excediendo el LSR para cTn a las 3 h post ejercicio es alta. Al contrario de la prevalencia, sin embargo, la incidencia parece no estar asociada al estadio madurativo, sugiriendo así que las diferencias entre estadios suceden en concentraciones bajas. Finalmente, la duración en elevadas frecuencias cardíacas, así como las velocidades media y pico durante el ejercicio parecen ser los mejores predictores para las elevaciones de cTnT inducidas por el ejercicio.

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Table Abreviatures

AMI	Acute Myocardial Infarction
AU	Arbitrary Units
BMI	Body Mass Index
CI	Confidence Interval
cTn	Cardiac Troponin
cTnI	Cardiac Troponin I
cTnT	Cardiac Troponin T
CV	Coefficient of Variation
CVD	Cardiovascular disease
ECG	Echocardiogram
ED	Emergency Department
eTL	External Training Load
GPS	Global Positioning System
HR	Heart Rate
HRmax	Maximum Heart Rate
HRmean	Average heart rate
HRpeak	Peak Heart Rate
HRV	Heart Rate Variability
ICC	Intraclass Correlation Coefficient
IR	Incidence Rate
iTL	Internal Training Load
LoB	Limit of Blank
LoD	Limit of Detection
LoQ	Limit of Quantification
MI	Myocardial Infarction
NT-proBNP	N-terminal pro B-type natriuretic peptide
PAR-Q	Physical Activity Readiness Questionnaire
PHV	Peak Height Velocity
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
rDistance	Relative distance
rHRav	Average relative heart rate
rHRpeak	Peak relative heart rate
RPE	Rating of Perceived Exertion
SD	Standard Deviation
sTn	Skeletal Troponin
TL	Training Load
TRIMP	Training impulse
URL	Upper Reference Limit

PART 1

Chapter 1 - General Introdudcion and Outline of the Thesis

Chapter 2 - Systematic Review and Meta-analysis

CHAPTER 1

Theoretical Framework and Scientific Background

General Introduction and Outline of the Thesis

This thesis aims to describe how serum cardiac troponin (cTn) respond to exercise in healthy trained prepubertal and pubertal males. The main chapters of this work, address the answers to (1) how does internal and external exercise load affect the post-exercise kinetics of cTn in prepubertal and pubertal athletes? and (2) how pre and post-exercise cTn differ depending on biological maturity in healthy trained prepubertal and pubertal males? With the answers to these questions, this work contributes to the field of sport sciences, by quantifying exercise impact on the cardiac tissue, and to the field of clinical diagnosis, by facilitating the identification of physiological, exercise-related pediatric elevations of cTn. To this end, the main objectives of this research were: (1) To describe the changes in serum cTnT related to exercise in healthy trained prepubertal and pubertal males; (2) To assess the effect of biological maturity on the changes in serum cTnT related to exercise in healthy trained males, and (3) To relate exercise load with the subsequent changes in serum cTnT in healthy trained prepubertal and pubertal males.

1.1 Overarching Topic

1.1.1 Troponin

Troponin (Tn) is a regulatory protein for the contraction of striated muscles, namely cardiac and skeletal.[1] This protein is located in the thin filament and consists of three subunits denominated C, T and I according to their functions: Troponin C (TnC) is the subunit that binds to Ca^{2+}, troponin T (TnT) attaches to tropomyosin, and troponin I that decreases TnC affinity to Ca^{2+} inhibiting myofilaments contraction.[2] In addition, two of these subunits, TnT and TnI, are expressed differently between skeletal (sTn) and cardiac (cTn) cells, and can be detected in specific assays based on high-affinity antibodies.[3,4] This thesis will elaborate on the cTn release into the bloodstream induced by exercise.

Cardiac troponin I (cTnI) and T (cTnT) are mostly bound to myofilaments, but also present in smaller quantities freely within cytosol (3% to 8% of the total amount).[3] Its release from myocytes to the bloodstream has been related to cardiomyocyte necrosis, cell membrane damage, apoptosis, cell wounds, assays' cross-reactivity with sTn, or decreased cTn clearance.[5] As a consequence, serum elevations of cTn are typical in patients with acute and chronic myocardial injury.[4] The American Heart Association and European Society of Cardiology (ESC) recommended cTnT and cTnI as biomarkers for cardiac damage in a clinical context for the first time in 2000.[6] Two decades after, cTn continues being the leading criteria for diagnosing myocardial injury.[7]

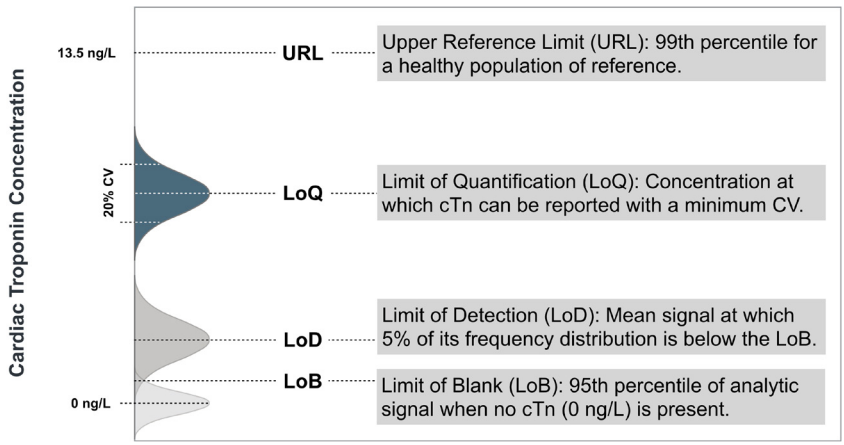

Figure 1 | Analytic terminology for cTn assays, adapted from Januzzi et al., 2019.[8] Limit of Quantification was set to 20% for the sake of the example. Upper Reference Limit was set to 13.5 ng/L using the population values of reference for a Troponin T hs STAT immunoassay in a Cobas E 601 analyzer (Roche Diagnostics, Penzberg, Germany).

The analytical performance of cTn assays could be summarized in three main concepts that will be referenced through this thesis: (1) sensibility, the ability of the assay to detect very low concentrations; (2) specificity, the ability to identify myocardial injury; and (3) cut-off values, that are illustrated in **Figure 1** and will be explained below.[8]

There are four cut-off values of an assay that will be considered at some point of this work: (1) the Limit of Blank (LoB) refers to the concentration measured by the assay in the absence of cTn, and could be understood as the background noise expected in the measurements; (2) the Limit of Detection (LoD) is the lowest concentration of cTn detectable in 95% of the measurements; (3) the Limit of Quantification (LoQ) refers to the lowest troponin concentration that can be reported with a specified certainty, normally a minimum coefficient of variation (CV); and (4) the Upper Reference Limit (URL) that refers to the 99th percentile of cTn measured in a healthy population of reference.[8]

The Fourth Universal Definition of Myocardial Infarction (2018), defines myocardial injury when blood levels of cTn are increased above the URL.[9] In addition, myocardial injury might be considered acute, if there is a rise and/or fall of cTn values, or chronic, in the case of constant elevated values. According to the American College of Cardiology, in the case of myocardial damage, cTn increases peaking after 12 to 48 h, and returns to basal levels within the following 3 to 10 days.[10] Additionally, with the development of cTn immunoassays, their sensitivity has improved drastically in the last decade. In this regard, the current standard of quality for cTn immunoassays requires them to allow the establishment of sex-specific cutoffs with a CV $< 10\%$ at the URL, and possess a low LoD being capable of measuring values in $> 50\%$ of both healthy female and male populations with values greater than the LoD. Assays that meet these criteria are termed "high-sensitivity" assays.[8,11,12]

1.1.2 Exercise

The greater sensitivity and overall improved analytical performance of cTn assays, involves a clear improvement in the early identification of heart damage in the emergency departments (EDs).[13,14] However, it also implies a decrease in its specificity since many additional patients are identified with abnormal cTn values due to other reasons than myocardial injury, leading to potential misdiagnosis and unnecessary further testing.[15] In this context, since the introduction of cTn assays, a vast body of evidence demonstrated that physical exercise can also induce transient elevations of cTn.[16–20] Furthermore, the reported incidence of healthy athletes who exceed the URL for cTn in the hours after exercise is high, and can reach the 100%.[16]

The occurrence of exercise-induced elevations of cTn is potentially important for the triage of athletes who develop chest pain that mimics cardiac injury after exercise, and who might have serum cTn drawn in the EDs. Furthermore, this led to the formulation of two controversial hypothesis: On the one hand, it was postulated that exercise-induced

elevations of cTn indicate irreversible cardiac damage and might be malign.[21,22] This hypothesis was supported in a recent, longitudinal study associating exercise-induced elevations of cTn to higher mortality and cardiovascular events.[23] On the other hand, since these elevations were commonly observed in apparently healthy athletes, were transient, and followed unique, different kinetics than the observed in clinical situations, it was postulated that cTn might be released as a benign, acute physiological response to exercise.[24]

Prior works, characterized the release of cTn induced by exercise in adult athletes with a different serum kinetics than the observed after myocardial injury (**Figure 2**).[25] Concretely, the elevations of cTn observed in healthy athletes subsequent to exercise, normally peak earlier, within 2 to 6 h after exercise cessation and then decrease, returning to basal levels within the subsequent 24 h.[16,26] However, for reasons that are still not completely understood, the magnitude of these exercise-induced elevations of cTn, is highly variable among individuals. The main variables associated to this variability will be summarized later on, in **Section 1.2**.

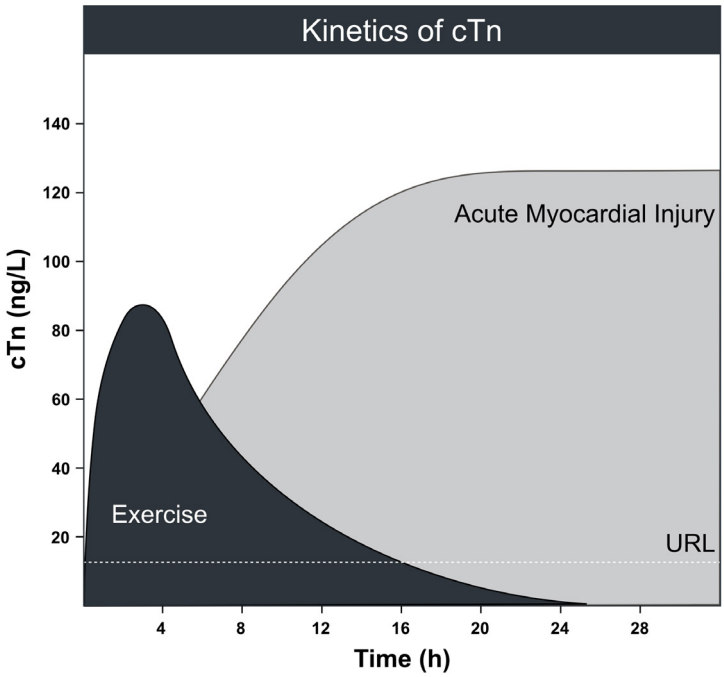

Figure 2 | Scheme of the kinetics of cardiac troponin T after exercise as opposed to after acute myocardial injury

The mechanisms underlying the release of cTn following exercise are still not completely understood. The most accepted hypothesis is that an increased membrane permeability of cardiomyocytes during exercise

would allow unbound cTn from cytosol to diffuse outside the cells.[25] Additionally, mechanical stress in the cardiac tissue during exercise might cause transient disruptions (wounding) in the membrane increasing further its permeability. There are other hypotheses however with strong rationale, that could be simultaneously compatible, among them: normal turnover of myocardial cells, cTn degradation products cellular release, membranous blebs, myocyte apoptosis/necrosis resulting in genuine cardiac injury or skeletal muscle origin of cTn.[27]

1.1.3 Growth

Population reference values of cTn as well as its expected elevation following exercise have been thoroughly investigated and characterized in the last years.[20] Furthermore, special attention has been put in how data fluctuates depending on age. Concretely, baseline and post-exercise cTn, seem to increase with age and this has been repeatedly linked to a higher prevalence of cardiovascular diseases (CVD) in older athletes.[25,28,29] Despite this fact, all existing research, with very few exceptions addressed in **Chapter 2**, was performed in adult or elder participants.[16,30,31]

Existing data about the prevalence of cTn and specially its expected elevation subsequent to exercise in children and/or adolescents is scarce. To the authors' opinion, there are two main reasons for this tendency to investigate the exercise-induced release of cTn in adults rather than children. First, cTn assays in the EDs are mostly performed in adult patients, whom are known to have higher prevalence and incidence of CVD.[32,33] And second, adults are generally a more accessible to recruit population, specially for studies involving high-intensity and/or long-duration exercise interventions that involve repeated blood samplings.

There are several reasons though, that make basal and exercise-induced cTn elevations in young athletes particularly interesting. First, since prevalence of CVD increases with age,[33] healthy young athletes are the most suitable population group to investigate the effect of exercise on the cTn release in the absence of underlying cardiac pathology.[34] This would provide relevant information and help understanding the true physiological mechanisms of exercise-induced release. Second, current URLs for cTn were calculated from adult cohorts,[35] and there are at present no pediatric population values of reference for cTn.[36,37] Establishing specific values of reference would undoubtedly contribute to clinical practice. Finally, children's immature heart experiences higher myocardial work during exercise than the adult heart.[38] Under this rationale, the expected values of cTn following exercise might be higher in children and adolescents when compared with adults.

Chapter 2 will present a comprehensive review of the existing studies addressing the exercise-related serum changes of cTn in young populations. Briefly stated, current evidence is limited but coincides that the exercise-induced elevations of cTn are more variable in young athletes when

compared with adults.[39–41] In spite of the higher variability, studies were controversial when comparing the magnitude of this exercise-induced cTn elevations between adolescents and adults. In some cases, although adolescents' elevations were more variable than adults', group comparisons discarded mean differences related to age.[39,40] Other studies, however, reported significant differences between groups, with higher cTn elevations following exercise in the adolescents.[41]

1.2 Explanatory Variables

There are three main factors that might explain the individual variability in the exercise-induced elevation of cTn: exercise characteristics, athlete characteristics, and the study design (**Figure 3**). In the following sections, we will address briefly the current state of the literature regarding each of these variables. Then, a more comprehensive review and meta-analysis will be presented, in **Chapter 2**.

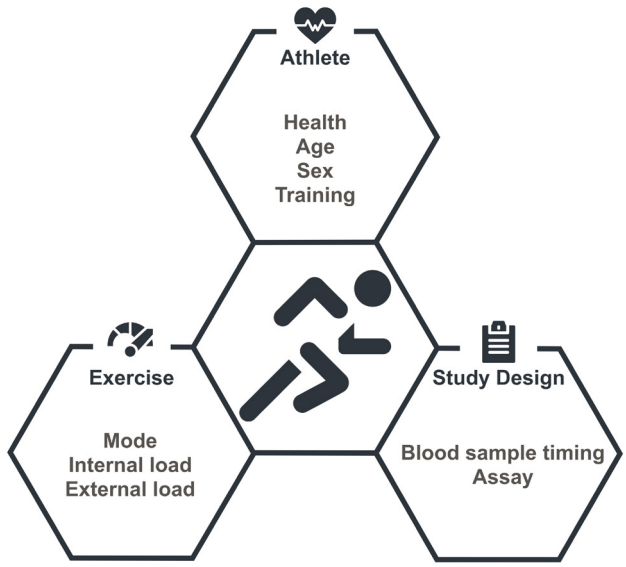

Figure 3 | Suggested predictors for the exercise-induced elevations of cTn

1.2.1 Exercise

The release of cTn has been mostly investigated in sports of high participation such as running,[42,43] cycling,[44–47] swimming,[39,48] soccer[49–51] or basketball[38,40,52]; and other modes such as rowing,[53] table tennis[54] or multi-adventure races[55] have received less attention. Early studies, hypothesized that differences among the metabolic, cardiovascular and muscular demands of different exercise modes might elicit differences in the exercise-induced elevation of cTn. In 2007, Shave et al. published a meta-analysis comparing studies obtaining cTn concentrations in healthy athletes after exercise.[17]

In this study, authors reported a statistically significant mediation of exercise mode in the subsequent elevation of cTn. However, in the years following this study, the analytical sensitivity of the immunoassays for cTn improved drastically, with the appearance of the high-sensitivity immunoassays, further explained in **Sections 1.1.1** and **1.2.3**.[11,56] In 2015, Legaz-Arrese et al. conducted a randomized, cross-over study comparing the release of cTn in a cohort of triathletes after swimming, running and cycling for 60 min at the maximal sustainable intensity.[48] On this occasion, cTn was measured using a high-sensitivity immunoassay and, when controlling the effect of exercise duration and intensity, results were contrary to earlier hypothesis,[17] discarding exercise mode as a potential mediator of the cTn elevation. Finally, in a recent meta-analysis based on 32 studies, Donaldson et al. confirmed that exercise mode does not influence the subsequent elevation of cTn.[20]

Internal exercise load is a construct that encompasses objective measures of the physiological and psychological acute responses that an exercise evokes in the participant.[57] Traditionally, internal load in studies aiming to investigate the release of cTn after exercise was reported in terms of heart rate (HR), as an indicator of exercise intensity. Surprisingly, other relevant indicators of internal load such as recovery heart rate (HRR),[58] heart rate variability (HRV),[42] time spent at each zone of relative heart rate,[59] the rating of perceived exertion (RPE),[60] oxygen uptake (VO2) or blood lactate,[61] have been scarcely reported in this body of research. In spite of that, internal load in terms of mean absolute and relative heart rate (HRmean and rHRmean, respectively) has been mentioned repeatedly as a good predictor for the cTn release induced by exercise.[62–67] In addition, most of the available meta-analyses, correlated positively exercise HR with the magnitude of the subsequent elevations of cTn.[16–20]

External exercise load, on the other hand, is a construct that encompasses externally quantifiable aspects of an athlete performance during exercise. In the specific literature, it has been mostly measured in terms of exercise volume such as duration and distance. Most of the studies investigating the release of cTn were made in running, cycling or swimming time trials, and as a consequence, shorter exercise durations were normally associated to higher exercise intensities.[17] This collinearity between intensity and duration was mentioned as a confounder in early studies reporting that exercise duration was negatively associated to the subsequent release of cTn.[68,66] As the number of studies and sensitivity of the immunoassays grew, it was found that, while internal load was a good predictor for the elevations of cTn,[66] the effect of external load in terms of exercise duration was negligible.[16,20] Notwithstanding that, it is still under debate whether other variables of external load, beyond exercise duration, might be used to predict post-exercise cTn.

1.2.2 Participants

Since cTn is a biomarker for cardiac damage, the main expected cause of elevated cTn is the presence of CVD.[69] In spite of this fact, other lifestyle factors such as smoking, alcohol consumption, sedentary behavior, diet or physical activity, might also influence basal concentrations of cTn.[70] Some studies reported that athletes with the presence of CVD respond to exercise with higher elevations of cTn.[71] However, the interaction between exercise and other lifestyle factors such as those mentioned earlier has not been stablished.

The URL for cTn are higher in males.[35] Simultaneously, research coincides that the elevations of cTn after exercise are highly dependent on basal concentrations.[53,72] Accordingly, it has been reported that the exercise-induced elevations of cTn are also higher in male athletes.[39,73] The mechanisms explaining why males respond to exercise with higher cTn elevations, however, are still under debate. Whilst some authors related this phenomenon to sex hormones,[73,74] others mentioned that sex-differences might be a consequence of larger mean heart size in the men.[37,39] In spite of the mechanism, however, men also present higher risk of cardiovascular disease than women. For these reasons, male athletes are more likely to present at emergency departments with elevated cTn.

As introduced in **Section 1.1.3**, age might also influence the elevations of cTn after exercise. In this regard, and similar to sex differences, previous research reported that basal values for cTn might be different based on age ranges.[2,29,32] Thus, post-exercise concentrations should, theoretically, differ depending on age. However, the real influence of age is still controversial, since previous research focused on adult participants,[16,17,30,31] and studies in young athletes are still scarce. Studies comparing adults with older adults coincide that cTn response to exercise is positively associated to age.[20,36] On the contrary, studies comparing adults with adolescents reported that the response of cTn to exercise might be more variable in the younger.[39–41] Furthermore, some of them found higher values in the adolescents,[41] whereas others did not find differences when compared to adults.[41,42] It has been hypothesized that, the higher elevations of cTn in young athletes might be related to maturation, since the immature myocardium might be more vulnerable to injury in clinical situations.[75] However, studies addressing maturational differences in the exercise-induced release of cTn are still scarce.

Previous research suggested that athletic status might also influence post exercise cTn. In this regard, it was found that participants who had been training for longer periods, where those who achieved higher cTn elevations.[53,72] The main hypothesis here, is that as an adaptive response to training, the capability of maintaining higher intensities during exercise also increases. As a consequence, since the elevations of cTn are positively associated to internal load, post-exercise cTn concentrations in the more trained would also be higher. However, studies addressing the interaction between internal load and training status are still scarce, and more research is needed to determine the real influence of athletic status in the elevations of cTn following exercise.

1.2.3 Study Design

The post-exercise kinetics of cTn is consistent among studies (**Figure 2**). Serum cTn early increases after exercise cessation, peaks within 3 to 6 h (cTnI) and 2 to 5 h (cTnT), and then normalizes in approximately a day.[16] From a methodological point of view, results of previous research should be interpreted according to this kinetics. In this regard, a large portion of the available studies used repeated measures designs with only two measurements, performed before and immediately after exercise.[19,76] Whilst cTn data immediately after exercise is still clinically relevant, at that timing, cTn is still at the outset of its rise. Consequently, true elevations of the biomarker in those studies could be much higher than the reported.[34] Furthermore, similar considerations should be taken for the clinical practice since, repeated cTn measurements in a short period following exercise may reflect a rise, while the same repeated measurements after a longer period would indicate a decrease of the same protein.[34]

The analytical performance of cTn assays, previously introduced in **Section 1.1.1**, is also determinant for the discrepancies in previous research.[27] The lower LoD in the high-sensitivity assays increased drastically the rate of cTn detection in healthy athletes. Accordingly, whilst early studies using conventional immunoassays reported low rates of detection, recent research using high-sensitivity immunoassays present higher rates detection, often above the 50% at baseline.[77] For this reason, a methodological concern in previous studies was the dealing of non-detected cTn data. Since its omission for statistical analyses would lead to overestimated results, the mostly accepted procedure was setting non-detected values to a half of the LoD of the assay.[41,54,73] Additionally analytical performance of cTn assays might differ among assays' manufacturers.[35] Although differences among the main manufacturers are often subtle, this should be considered when comparing the results of different studies, specially those preceding the introduction of high-sensitivity immunoassays.

1.3 Scope and Delimitations

This thesis will elaborate on the exercise-induced elevation of cTnT. Furthermore, it is within the scope of this document to describe how cTnT respond to exercise in a target population of apparently healthy, trained, male, children and adolescent athletes. Concretely, apparently healthy athletes were elected to develop this thesis, since the main differential element to investigate the physiological rather than pathological elevation of cTn is its occurrence in asymptomatic, apparently healthy, and common athletes. Additionally, the target population was further delimited to trained athletes, who could undertake the exercise loads required in the experimental procedures of **Chapters 3, 4** and **5**. Furthermore, studies focused in male athletes since, as mentioned in **Section 1.2.2**, it is the gender with higher prevalence of elevated cTn, and incidence of its exercise-induced elevations exceeding the URL. Finally, given the discrepancies and scarcity of previous studies, this thesis focuses in young athletes, under the age of 18, with a special interest on biological maturity and its influence on the exercise-induced elevation of cTn.

Consequently, studying this phenomenon in clinical populations, female, untrained or adult athletes falls beyond the delimitations of this work and will undoubtedly be addressed in separate, future projects.

According to the last published survey of sporting habits in Spain, football (11 and 7) and swimming are the first and second most practiced, individual and team sports.[78] Additionally, in both exercise modalities children and adolescents normally undertake weekly training frequencies equal or higher than 3 days per week, and endure training volumes often equal or higher than 1h per session. Moreover, from a logistic point of view for this research, both modalities are practiced in regular facilities that facilitate the control and monitoring of athletes' exercise, namely the football pitch and the swimming pool. Finally, according to previous studies,[20] exercise mode at comparable internal and external loads seems not to influence the subsequent elevation of cTn. For these reasons, we elected to perform **Chapters 3** and **4** in cohorts of football players, and **Chapter 5** in a larger cohort of swimmers.

In addition, this compendium focuses on providing a description of how cTnT elevates after exercise and the determinants it might associated with. Since the comparability between cTnT and cTnI has been demonstrated in other studies,[79] we elected cTnT rather than cTnI given its higher presence in previous, related literature, that will be demonstrated in **Chapter 2**. Additionally, the experimental studies in this work were performed using high-sensitivity assays. It is the purpose of this thesis to contribute from a descriptive perspective of the phenomenon. Accordingly, it falls beyond the delimitations of this work to prove or discard any of the suggested physiological mechanisms underlying this exercise-induced release of cTn.

1.4 Research Questions and Hypothesis

Although the causality between exercise and posterior elevations of cTn has been repeatedly demonstrated, the concrete aspects of exercise that lead to these elevations are still poorly characterized, specially in young athletes. As a response, the first question that this thesis aims to answer is how internal and external exercise load does affect the post-exercise kinetics of cTn in healthy trained prepubertal and pubertal males. Since cTn molecules originate from myocardium, we first hypothesize that metrics of internal exercise load will result the strongest predictors for the elevation of the biomarker. Notwithstanding that, depending on age, maturity, training status, or other environmental factors, the physiological response to similar external exercise loads might vary. For these reasons, we also hypothesize that, external load will be also associated to the elevation of cTn.

Apart from the cTn association with exercise load, previous research was inconclusive when establishing the relationship between chronological or biological age and the magnitude of the exercise-induced elevations of cTn. Moreover, studies involving adolescent athletes observed that their cTn response is more variable and might be higher than in the adults.

For this reason, it was suggested that myocardial immaturity might be a determinant for higher cTn elevations.[75] Consequently, the second research question for this thesis was how pre and post-exercise cTn do differ depending on biological maturity in healthy trained prepubertal and pubertal males. According to previous studies, we hypothesize that cTn elevations will be inversely associated to maturation, being higher in children at earlier maturational stages than in adolescents at late-puberty.

1.5 Objectives

1 Describe the changes in serum cTnT related to exercise in healthy trained prepubertal and pubertal males

1.1 Estimate the prevalence of cTnT in healthy trained prepubertal and pubertal males.

1.2 Measure the elevation of cTnT induced by exercise in healthy trained prepubertal and pubertal males.

1.3 Estimate the incidence of healthy trained prepubertal and pubertal males with cTnT above the URL in the hours subsequent to exercise.

2. Assess the effect of biological maturity on the changes in serum cTnT related to exercise in healthy trained males.

2.1 Compare the prevalence of cTnT in healthy trained prepubertal and pubertal males at different maturational stages.

2.2 Compare the changes in serum cTnT related to exercise among healthy trained males at different maturational stages.

2.3 Compare the incidence of healthy trained males with cTnT above the URL in the hours subsequent to exercise among maturational stages.

3. Relate exercise load with the subsequent changes in serum cTnT in healthy trained prepubertal and pubertal males.

3.1 Determine the strength and direction of the association between external exercise load and the subsequent elevation of cTnT in healthy trained pubertal and pubertal males.

3.2 Determine the strength and direction of the association between internal exercise load and the subsequent elevation of cTnT in healthy trained pubertal and pubertal males.

1.6 Outline of the Thesis

In this thesis, we address different aspects of the aforementioned issues and limitations in the knowledge about exercise-induced elevations of cTnT in healthy trained young athletes.

Part I preludes the experimental research by offering a theoretical framework and scientific background. To this end, **Chapter 1** provides a brief introduction of the topic, research questions and objectives of this thesis, and **Chapter 2** addresses Objective 1, developing a systematic review and meta-analysis on the cardiac biomarkers release following exercise in children and adolescents.

Subsequently, **Part II** holds the experimental research and consists of three studies. First, **Chapter 3** focuses on Objective 3, addressing the influence of exercise load; Second, **Chapter 4**, addresses Objectives 1 and 2 by comparing the exercise-induced elevation of cTnT between two cohorts of children and adults; And finally, **Chapter 5** focuses on Objective 2, further investigating the exercise-induced elevation of cTnT among athletes at different maturational stages.

Part III will conclude this work, providing a brief summary of findings in **Chapter 6**, followed by a general discussion of this thesis in **Chapter 7**, and ending with **Chapter 8** that provide the final conclusions of this work.

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CHAPTER 2

Systematic Review and Meta-analysis

Cardiac Biomarker Release After Exercise in Healthy Children and Adolescents: A Systematic Review and Meta-analysis

Rafel Cirer-Sastre, Alejandro Legaz-Arrese, Francisco Corbi, Keith George, Jinlei Nie, Luis Enrique Carranza-García & Joaquim Reverter-Masià

Purpose: We evaluated the impact of acute exercise and 24 h recovery on serum concentration of cardiac troponins (cTnT; cTnI) and NT-proBNP in healthy children and adolescents. We also determined the proportion of participants exceeding the upper reference limits (URL) and acute myocardial infarction (AMI) cut-off for each assay.

Method: Web of Science, SPORTDiscus, MEDLINE, ScienceDirect and Scopus databases were systematically searched up to November 2017. Studies were screened, quality-assessed and data was systematically extracted and analyzed.

Results: From 751 studies initially identified, 14 met the inclusion criteria for data extraction. All three biomarkers were increased significantly after exercise. A decrease from post-exercise to 24 h was noted in cTnT and cTnI, although this decrease was only statistically significant for cTnT. The URL was exceeded by a 76% of participants for cTnT, a 51% for cTnI and a 13% for NT-proBNP. Furthermore, the cut-off value for AMI was exceeded by 39% for cTnT and a 11% for cTnI. Post exercise peak values of cTnT were associated with duration and intensity ($Q(3) = 28.3$, $P < .001$) while NT-proBNP peak values were associated with duration ($Q(2) = 11.9$, $P = .003$).

Conclusion: Exercise results in the appearance of elevated levels of cTnT, cTnI and NT-proBNP in children and adolescents. Post-exercise elevations of cTnT and NT-proBNP are associated with exercise duration and intensity.

Pediatric Exercise Science. 2019;31(1):28-36. doi:10.1123/pes.2018-0058

2.1 Introduction

Cardiac troponin T and I (cTnT and cTnI) are accepted indicators of myocyte necrosis and are considered sensitive markers of acute myocardial injury (MI) and infarction (AMI).[1] Serum cTnT and cTnI are elevated after irreversible heart muscle damage and levels peak during the subsequent days.[2,3] The N-terminal fragment of the prohormone brain natriuretic peptide (NT-proBNP) is a marker accepted to reflect myocardial stretch,[4] which is currently used to detect heart failure and asymptomatic left ventricular dysfunction,[5,6] with the magnitude and duration of release dependent on the severity of stretch and stress.[7]

The lower detection limits of cTnT and cTnI assays have been greatly reduced in recent years[8] with new high sensitivity assays available for both biomarkers. These assays can detect the 99th percentile with a CV $< 10\%$ and measure cTn concentrations in at least a 50% of a healthy population at rest.[8] Although the higher sensitivity of these assays enables better rates of true positive detection,[9] a decline in specificity has been reported such that cTn appearance might be related to etiologies other than AMI.[3,9,10] This can include physical exercise as a known non-pathological cause of cTn increase.[3]

Numerous investigations have described the serological release of cTnT, cTnI and NT-proBNP after physical exercise and its kinetics.[11–13] Contrary to an AMI-related release, cTn values normally peak within 2-5 h (cTnT) and 3-6 h (cTnI) post-exercise and then decrease returning to basal levels after 24 h of recovery in most participants.[12,14] The differences between cTnT and cTnI peaks might be related to differences in their molecular weights.[15] NT-proBNP release normally peaks immediately after exercise and remains elevated during the subsequent 72 h; and its clearance, that seems to take longer than cTn, has been related to a temporary reduction in kidney function subsequent to exercise.[15,16] These observations have important clinical implications, since the elevation of these cardiac biomarkers for several hours after physical exercise might be misinterpreted in physically active patients, admitted to the emergency department for chest pain of origins other than acute coronary syndrome and heart failure.

The 99th percentile of a normal reference population, considered the upper reference limit (URL), is designated as the decision level for the diagnosis of MI for both general and paediatrics populations.[1,17] In this respect, the reported 99th percentiles for children are lower than in adults for cTn and NT-proBNP,[18–20] and both are used for clinical diagnostic.[21]

The magnitude of cTn and NT-proBNP post-exercise release, as well as the prevalence of data above clinical cut-offs have been extensively studied in healthy adults. Only a limited number of studies addressing the cardiac biomarker response to exercise in children and adolescents are currently available. Moreover, these studies are heterogeneous in terms of exercise exposure and often occur with small sample sizes and thus a limited statistical power. As a result, the association of cTn and NT-proBNP with exercise is currently controversial[22–29] and might be confounded with either individual as well as exercise characteristics.

Based on studies with adult participants other individual characteristics, other than age, might influence cardiac biomarkers release. Sex differences in cTn and NT-proBNP are controversial.[30–37] Previous exercise experience has been negatively associated with cTn release,[30,38–40] while training load might be not associated with biomarker appearance.[38,41–45] NT-proBNP is not associated with previous exercise experience either,[45–47] while its association with training load remains controversial.[28,29,42,44,45,47–49] Finally, fitness condition has not been associated with cTn or NT-proBNP data.[45,50] Exercise characteristics have also been studied as to their influence on cardiac biomarker release.[12,51] Exercise intensity was mentioned as a predictor for cTn release while exercise duration has been correlated with both cTn and NT-proBNP data.[16,45,48,52–54] Exercise mode and type have not been fully evaluated and any associations remain controversial.[55–57]

Previous systematic reviews and meta-analyses related to cardiac biomarker release after exercise have been focused on adult participants.[12,51,58,59] To the best our knowledge no systematic review or meta-analysis has been published addressing the cardiac biomarkers response to exercise in children and adolescents. Considering that children and adolescents have a low cardiovascular risk,[60] we selected this special group in order to get a "clean" background and preclude the potential effects of concealed cardiovascular diseases and get "pure" effect of exercise on cardiac biomarkers. Due to variations in sample size and the diversity of participant and exercise characteristics a systematic review with a meta-analysis could contribute to the current knowledge by synthesizing available data into single, more powerful estimates of effect. Moreover, secondary analysis might help to identify possible associations with individual and exercise characteristics that could explain a certain degree of heterogeneity between the current findings.

In accordance with the PRISMA statement[61] the main objective of this study was to systematically review studies whose participants were healthy children and adolescents that were exposed to physical exercise and whose resting and post-exercise measures of cTnT, cTnI and NT-proBNP were described. A secondary objective was to analyse the moderator effects of a) age, b) pubertal status, c) sex, d) previous training (years), e) current training (h/week or km/week), f) exercise duration (minutes), g) exercise intensity (average HR), h) maximum oxygen uptake (VO_2max), and i) exercise mode on the pooled effects determined by the main objective.

2.2 Methods

2.2.1 Search Strategy

We searched Web of Science, SPORTDiscus, MEDLINE, ScienceDirect and Scopus databases between July 1, 2017 and November 30, 2017. A three-component additive search key (#A AND #B AND #C) was used with: #A, measurement; #B, intervention; and #C, population. All searches were restricted to title or abstract, and keywords were stated in English. Measurement was defined with the expression "cardiac biomarker*" OR Troponin OR TnT OR TnI OR cTn* OR hs-cTn* OR "N-terminal prohormone of brain natriuretic peptide" OR "NT-proBNP" OR "NT-pro-BNP". Intervention was specified with: exercise OR sport* OR "physical activity" OR running OR marathon OR soccer OR swim* OR athletes. Finally, population was stated with "children OR adolescent* OR young".

2.2.2 Inclusion and Exclusion Criteria

We selected observational or experimental studies with a repeated measures design. Participants (or a subset of them) must be under the age of 18, not have personal history or clinical evidences of cardiovascular disease, and have a normal 12-lead electrocardiogram and/or echocardiogram at rest.[62] Interventions of interest were those which involved exposure to physical exercise, including sport events and laboratory tests. We searched primarily for studies that reported serum cardiac biomarkers responses to exercise. Specifically, those which reported cTnT and/or cTnI and/or NT-proBNP before and after exercise. Inclusion criteria included the necessity to report some quantitative measure of location and variation (mean with standard deviation (SD); median with range; or median with inter quartile range) of the biomarker's value for a minimum of one time point post-intervention. Studies where participants were exposed to specific pharmacological or nutritional interventions were excluded and the remaining articles were included in our review.

2.2.3 Data Extraction

Studies were inspected to gather the data for (where available): sample size, sex, maturational status, age, training status (years of previous experience, weekly hours of training, weekly km of training), VO_2 max, performed exercise, exposure duration (minutes), average heart rate (surrogate of intensity) and absolute concentration of cTnT, cTnI or NT-proBNP before and after exercise. We also recorded the proportion of participants above the URL for each biomarker, and rate of participants above the cut-off for AMI for cTnT and cTnI. Outcomes reported as median [range] were transformed to mean (SD) using Wan et al.'s formulas. [63] All concentrations were expressed in ng/L,[1] and concentrations of cTn reported as "under limits of detection of 10 ng/L" were represented as 5 ng/L.[54,64]

2.2.4 Quality Assessment

We analysed the methodological quality of studies that met all inclusion criteria in order to detect possible methodological discrepancies that might explain a degree of heterogeneity between studies. In this sense, studies' quality was assessed by two authors independently, filling the Quality Assessment Tool for before-after (Pre-Post) studies with no control group from the National Heart Lung and Blood Institute.[65] This scale considers 12 binary items, which average scores each article from 0 indicating high risk of bias, to 1 indicating low risk of bias (QATi). Discrepancies between assessors were resolved by a third author.

2.2.5 Statistical Analysis

All analyses were performed in R[66] using Viechtbauer's "metafor" package.[67] Random effects meta-analyses were conducted by biomarker (cTnT, cTnI and NT-proBNP) using the following estimates: the baseline concentration, the peak concentration, the concentration at 24 h, the absolute mean difference between baseline and peak concentrations, the absolute mean difference between baseline and concentration after 24 h recovery, the absolute mean difference between peak concentrations and concentrations at 24 h post exercise, the rate of participants whose peak concentration exceeded the assay URL and the rate of participants exceeding the cut-off for AMI. Rates were log-transformed for statistical comparisons and estimates were then back transformed for ease of interpretation. Heterogeneity was measured with Cochrane's Q statistic and I^2 values. We assessed publication bias using Egger's regression test for funnel plot asymmetry.[69,70] Subgroup analyses were conducted when heterogeneity was significant to assess the possible influence of exercise mode, age, intensity and duration on the absolute mean difference between baseline and peak concentrations. In addition, when data was available, we investigated for the possible influence of Tanner stage, sex, VO_2 max, years of previous training, weekly hours of training and weekly km of training, regardless of exercise mode, age, intensity and duration.

Outcome multiplicity from the same groups[54] was controlled introducing a study identification as a random effect.[67,71] Measures are expressed as mean ± 95% confidence intervals (CI) unless otherwise stated and we considered statistically significant differences when $P < .05$.

2.3 Results

The search process appears outlined in **Figure 4**. Fourteen studies met the inclusion/exclusion criteria that included 21 groups covering a total sample of 336 participants (72 females) who had a mean age of 15.1 (2.3) years.[36,39,40,46,54,64,72–79] Two studies provided complete data from more than one subgroups contributing with different estimates by sex[74,77] or Tanner stage,[36] which were treated as different units for the analysis. One study provided four outcome measurements from the same group at different exposures,[54] which were controlled for multiplicity within the models.[67,71] Interventions were based on five different modalities: in nine studies participants ran [three treadmill protocols (45 to 90 min),[46,76,78] five half marathons[39,40,54,64,74] and one full marathon[77]], in two studies basketball was employed,[73,75] in one a soccer match,[79] in one study participants swam for 60 min[36] and one included a set of table tennis exercises.[72] **Table 1** shows the number of groups available for each comparison (k) as well as their respective pooled effect sizes.

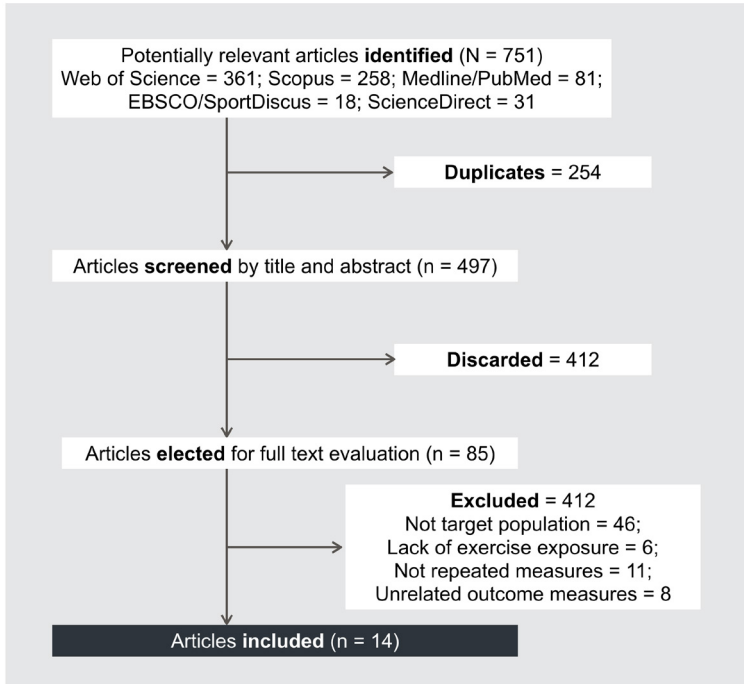

Figure 4 | Flowchart for study inclusion and exclusion stages

Table 1a | Estimated pooled effect sizes (95% CI) by biomarker

	k	Pooled Effect Size	*Z*	$P_{(Z)}$	*Q*	$P_{(Q)}$	I^2
Cardiac Troponin T							
Mean baseline (ng/L)	16	5 (4 to 6)	11.84	< .001	206.47	< .001	98.7%
Mean peak (ng/L)	14	144 (83 to 205)	4.65	< .001	105.78	<.001	96.5%
Mean at 24 h (ng/L)	9	11 (5 to 16)	3.86	< .001	146.52	< .001	98.2%
Dif. Peak - Pre (ng/L)	14	139 (79 to 198)	4.53	< .001	102.72	< .001	96.4%
Dif. 24 h - Peak (ng/L)	7	-89 (-147 to -32)	-3.04	.002	33.85	< .001	93%
Dif. 24 h - Pre (ng/L)	9	7 (1 to 12)	2.5	.012	87.22	< .001	96.3%
MI threshold IR	18	0.76 (0.66 to 0.87)	-3.83	< .001	27.86	.047	13.5%
AMI threshold IR	14	0.39 (0.26 to 0.6)	-4.38	< .001	39.1	< .001	75.4%
Cardiac Troponin I							
Mean baseline (ng/L)	7	16 (10 to 22)	5.15	< .001	89.67	< .001	96.4%
Mean peak (ng/L)	5	248 (17 to 478)	2.1	.036	61.42	< .001	99 %
Mean at 24 h (ng/L)	7	38 (19 to 56)	4.05	< .001	348.01	< .001	97.7%
Dif. Peak - Pre (ng/L)	5	228 (6 to 450)	2.01	.045	54.53	< .001	98.9%
Dif. 24 h - Peak (ng/L)	5	-199 (-404 to 5)	-1.91	.056	42.56	< .001	98.2%
Dif. 24 h - Pre (ng/L)	7	21 (8 to 33)	3.23	.001	100.97	< .001	93.2%
MI threshold IR	7	0.51 (0.32 to 0.81)	-2.85	.004	16.74	.014	60.5%
AMI threshold IR	4	0.11 (0.05 to 0.24)	-5.4	< .001	3.41	.33	24.4%

Table 1b | Estimated pooled effect sizes (95% CI) by biomarker

	k	Pooled Effect Size	*Z*	$P_{(Z)}$	*Q*	$P_{(Q)}$	I^2
			NT-proBNP				
Mean baseline (ng/L)	6	77 (14 to 140)	2.38	.017	217.98	< .001	99.5%
Mean peak (ng/L)	6	106 (17 to 195)	2.34	.019	288.19	< .001	99.5%
Mean at 24 h (ng/L)	4	83 (0* to 182)	1.63	.10	173.89	< .001	99.6%
Dif. Peak - Pre (ng/L)	6	20 (2 to 38)	2.20	.033	13.64	.021	79.2%
Dif. 24 h - Peak (ng/L)	4	-2 (-11 to 7)	-0.48	.63	7.26	.06	0.1%
Dif. 24 h - Pre (ng/L)	4	4 (-8 to 28)	1.55	.44	0.65	.88	0%
MI threshold IR	6	0.13 (0.04 to 0.44)	-3.32	< .001	18.02	.003	74.1%

Note: Estimated effects for Incidence Rates (IR) were back transformed for easier interpretation.
* Mathematically negative and truncated to 0 avoiding values outside the parameter space.

2.3.1 Quality Assessment and Risk of Publication Bias

Studies had a mean quality score of 0.61 (0.07). Pre-specification of sample eligibility criteria, enrollment of all eligible participants and sample size calculation were rated as high risks of bias in all studies. Other concurrent items rated as high risk of bias were blinding of outcome assessors, controlling for confounding variables in statistical analysis, reporting main effect of time with p values, and validity and reliability of outcome measures, in 12, 9, 3 and 1 cases, respectively. On the other hand, Egger's regression test was significant for all three biomarkers cTnT, cTnI and NT-proBNP ($P < .001$), suggesting that current literature was still unrepresentative of the population of completed studies.

2.3.2 Cardiac Troponin T

Participants had an overall cTnT concentration at baseline of 5 ng/L (4 ng/L to 6 ng/L). This concentration was increased ($P < .001$) after 2-5 h, reaching a peak of 144 ng/L (83 to 205 ng/L). Finally, 24 h after exercise cTnT was reduced ($P < .002$) with a pooled concentration of 11 ng/L (5 to 16 ng/L), which was slightly higher than at baseline ($P = .01$) (**Figure 5**). All three pooled concentrations as well as their differences were heterogeneous between studies ($P < .001$ in all comparisons). Overall 76% (66% to 87%, $P < .001$) of participants had a cTnT peak above the assays URL, and a 39% (26% to 60%, $P < .001$) exceeded the cut-off for AMI. Again, both rates, for MI and for AMI, were heterogeneous between studies ($P = .047$ and $P < .001$, respectively).

In the subgroups analyses, cTnT was measured in four exercise modes, namely half marathon, treadmill running, table tennis and swimming. Exercise mode, available in $k = 14$ units with a total of n = 193 participants, had a main effect on cTnT increase-to-peak (Q(3) = 9.98, $P = .02$). Post-hoc analysis revealed that after a half marathon and treadmill run cTnT increases were higher than after intermittent table tennis and swimming ($P < .001$ and $P = .004$, respectively). Multiple regression with exercise mode as a random effect ($k = 11$, n = 138), revealed that age had a negative association ($P < .001$) while intensity and duration were positively associated ($P < .001$ and $P = .003$, respectively) with cTnT increase (Q(3) = 28.3, $P < .001$). Moreover, participants' VO_2 max correlated negatively with cTnT increase ($k = 7$, n = 60, $P = .04$). We did not find associations between cTnT increase and sex ($k = 11$, n = 138, $P = .3$), Tanner stage ($k = 4$, n = 63, $P = .5$), years of previous training ($P = .16$) or weekly km of training ($k = 10$, n = 110, $P = .32$).

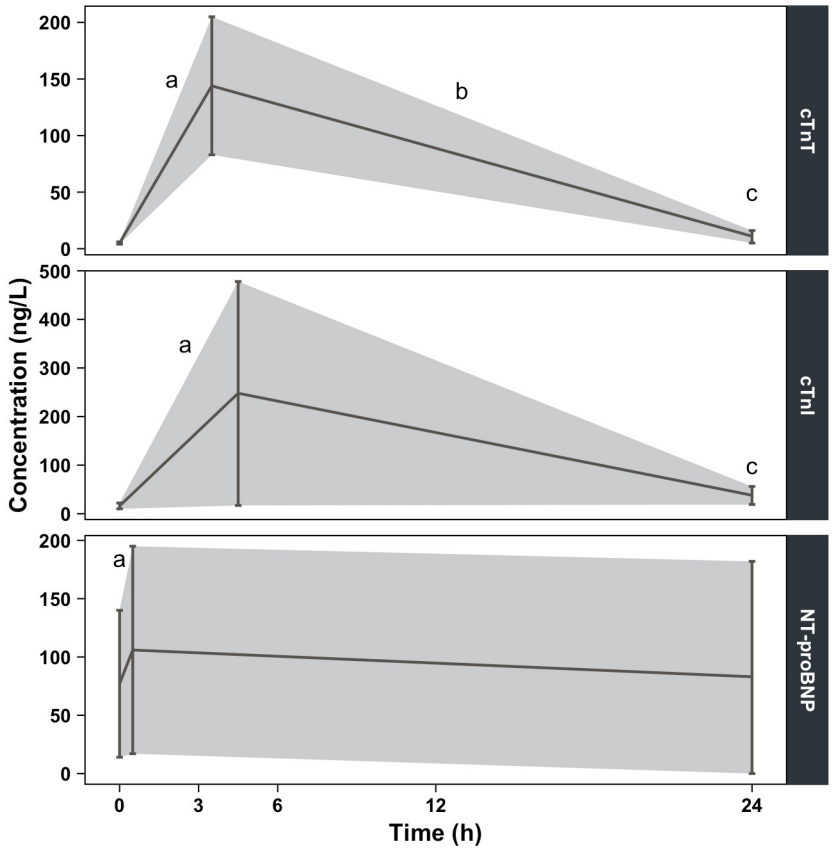

Figure 5 | Estimated kinetics by biomarker before, at peak value and 24 h after exercise, with their respective 95% IC

2.3.3 Cardiac Troponin I

The pooled baseline concentration for cTnI was 16 ng/L (10 to 22 ng/L). After 3-6 h of exercise exposure participants increased this concentration ($P = .04$) up to a peak of 248 ng/L (17 to 478 ng/L). After 24 h recovery, this reduced to 38 ng/L (19 to 56 ng/L) which was not statistically different from the estimated peak concentration ($P = .06$) (**Figure 5**). However, all three pooled concentrations as well as their differences were heterogeneous between studies ($P < .001$ in all comparisons). The proportion of participants with cTnI above the URL was 51% (32% to 81%) and the rate exceeding the cut-off for AMI was 11% (5% to 24%). The rate for MI was heterogeneous ($P = .01$) while the rate for AMI was not ($P = .33$) between individual studies.

In the subgroup analysis, cTnI was measured in four exercise modes, namely half marathon, basketball, table tennis and soccer. The cTnI increase to peak did not differ between exercise modes ($k = 5$, n = 83, $Q(4) = 4.75$, $P = .31$), and did not either in a multiple comparison ($k = 4$, n = 61) at different ages ($P = .33$), intensities ($P = .6$) or durations ($P = .31$). In addition, we did not find differences due to years of training ($k = 3$, n = 33, $P = .37$) or participants' VO_2 max ($k = 3$, n = 33, $P = .54$). Tanner stage and weekly training load data were not available to be modelled.

2.3.4 N-Terminal Prohormone Brain Natriuretic Peptide

The pooled baseline concentration for NT-proBNP corresponded to 77 ng/L (14 to 140 ng/L). This concentration was increased immediately after exercise ($P = .03$) achieving a peak of 106 ng/L (17 to 195 ng/L). Finally, 24 h after exercise NT-proBNP concentration did not differ from its peak ($P = .63$) or baseline ($P = .44$) with an estimate of 83 ng/L (0 to 182 ng/L) (**Figure 5**). All three concentrations were heterogeneous ($P < .001$). The rate of participants with NT-proBNP concentration above the URL was 13% (4% to 44%, $P < .001$), and studies were heterogeneous ($P = .003$).

In the subgroup analysis, NT-proBNP was present in four different exercise modes, namely half marathon, treadmill running, swimming and soccer. Exercise mode, had a main effect on the NT-proBNP post exercise increase ($k = 6$, n = 101, $Q(4) = 25.06$, $P < .001$). Post-hoc comparisons revealed that the higher NT-proBNP increases were related with soccer (estimated increase of 83 ng/L, 95%CI from 34 ng/L to 131 ng/L, $P < .05$) followed by half marathon (estimated increase of 59 ng/L, 95%CI from 12 to 105 ng/L, $P = .01$) and finally followed by swimming (estimated increase of 11 ng/L, 95%CI from 3 to 18 ng/L, $P = .006$), with no differences in the mode of treadmill running ($P = .9$). Moreover, in a multiple regression with exercise mode as a random effect ($k = 4$, n = 62), duration had a positive association with the estimate ($P < .001$) while age ($P = .34$) and intensity ($P = .37$) were not associated with NT-proBNP ($Q(2) = 11.9$, $P = .003$). Finally, we did not find differences in NT-proBNP for sex ($k = 4$, n = 62, $P = .3$), Tanner stage ($k = 3$, n = 50, $P = .6$) and years of previous training ($k = 4$, n = 62, $P = .5$). VO_2 max, and weekly training load data were not available to be modelled.

2.4 Discussion

The main purpose of this systematic review and meta-analysis was to estimate how exercise modulated the blood concentration of cTnT, cTnI and NT-proBNP in children and adolescents. Overall, this review found: (1) all three biomarkers were significantly elevated after exercise; (2) a decrease from peak values after 24 h recovery was only significant for cTnT; (3) the rate of participants exceeding the biomarkers' URL were 76% for cTnT, 51% for cTnI and 13% for NT-proBNP; (4) the rate of participants exceeding the cut-off value for AMI were 39% for cTnT and 11% for cTnI; (5) individual variability was observed between studies; and (6) exercise duration influenced both cTnT and NT-proBNP while intensity influenced only cTnT. Despite these findings, the quality assessment of studies together with the analysis for publication bias revealed that current studies have a fair degree of quality with limited bias.

2.4.1 Cardiac Troponin T and I

Our results indicate that cTn release in children and adolescents is inherent to physical exercise. Data reflect a fast increase of cTnT during the early hours of recovery, with close to complete recovery to baseline at 24 h. Similar results were appreciable for cTnI, although statistical power was limited and lead to only marginally significant differences between peak and 24 h values. Such observations suggest that cTn kinetics in children and adolescents during a 24 h recovery are comparable with the observed in adults.[12,14] Our results coincide with previous research observing the highest cTnT and cTnI concentrations about 2-3 and 3-5 h post exercise, respectively.[12,14] Based upon the foregoing, when repeated blood sampling are not possible, single samples taken within such interval might detect concentrations close to the kinetics peak.

The current data suggest that, as in the case of adults,[41,57] there is a marked individual variability regarding the exercise induced release of cTn, with a high proportion of participants with values exceeding the URL for MI and AMI. As evidenced in controlled studies with adolescents[54] and adults,[45] cTnT variability could be partially explained by exercise intensity and duration, what likely reflects an impact of exercise volume on cardiac work. We also observed a higher cTnT release in the younger participants, and this could explain that the proportion of participants exceeding the URL in our study is higher than the reported by a recent meta-analysis without age restrictions.[58] This would suggest a role for maturity mediating the post exercise cTn release. However, direct comparisons of the release of cTn after exercise in adults and adolescents have disclosed contradictory findings.[36,46,73] Moreover, with the scarce data currently available we did not find any association between cTnT release and pubertal status. At all events, associations with pubertal status require further investigation. Running seems to induce higher cTnT releases than other modes as it was noticed in a previous meta-analysis based on adult participants[51]; nevertheless, such assertion is complex to verify through direct comparisons. Although we observed lower cTnT releases in participants with greater VO_2max, we could not corroborate whether the cTnT increase is mediated by current training or training history. It was not evident whether there were any sex differences in the cTn release. This coincides with previous studies in adults which reported a limited influence of sex and training history on the release of cTn.[35,36,41,73,74,77,80] The scarce number of studies did not allow to explain the between-subjects variability regarding the release of cTnI.

2.4.2 N-Terminal Prohormone Brain Natriuretic Peptide

An increase in NT-proBNP immediately after exercise was confirmed without a significant reduction within the 24 h recovery period that supports past research with adults.[80,81] NT-proBNP may have a longer clearance period that cTn possibly extended to 72 h.[15,16] In this regard, it has been suggested that BNP may play an important role in homeostasis during the transition of the circulation from children to maturity as a marker of myocardial growth.[82] This might reflect an early myocardial adaptation to the intense training stimulus in children and adolescents. In either case, these possibilities require further study.

We noted that NT-proBNP changes with exercise were lower than the observed in cTn. Therefore, the proportion of participants exceeding the URL of NT-proBNP was lower than the reported in studies with adults.[11,15] These differences might be associated with age. However, neither our analysis nor previous studies comparing directly adolescents with adults found NT-proBNP differences for age and pubertal status.[36,46] It is therefore plausible to think that these differences might be related to exercises with less duration in studies conducted with adolescents compared with their equivalents with adults. Our results confirm indeed that in adolescents the release of NT-proBNP is largely associated with exercise duration, as it was reported previously in studies with adults.[27,45] Given the close relationship between pre- and post-exercise values,[41,80] baseline differences between studies might explain part of the differences we observed across NT-proBNP peak values depending on the exercise mode. Our results also confirmed that as in adults[27,35,36,41,45,80] exercise intensity, training, fitness and sex have limited influence on the release of NT-proBNP with exercise.

2.4.3 Clinical Implications

A cardiac biomarker release was observed in most of the participants in all included studies, despite a certain degree of between-study variability. Importantly, this analysis shows that in children and adolescents, the factors mediating cardiac biomarkers after exercise as well as their kinetics, are comparable with the observed in previous studies in adults and differ from the observed after MI and AMI.[1,4] It has been suggested that this reflects a reversible cellular process triggered by a normal physiological response to exercise.[16,47,83,84] Likewise, the increase of cTn might reflect an increased rate and force of cardiac contraction during exercise that causes transient membrane damage and enables cystolic cTn to pass into circulation.[22] On the other hand, a release of NT-proBNP from the ventricular cardiomyocytes might reflect a volume overload and cardiac wall stretch during exercise.[15] Furthermore, some authors suggested that the use of the general population values as a reference might not be appropriate for adult athletes being evaluated for medical conditions using blood indices of cardiac biomarkers. This has prompted the reflection that cardiac biomarkers values might be stratified according to the physical activity of the adult subjects for improving the clinical usefulness of the biomarker.[85] In this sense, our analysis extends this to children and adolescents, and suggests that when evaluating cTnT, cTnI and NT-proBNP in emergency settings, detailed information regarding any recent exercise should be obtained.[73]

2.4.4 Limitations

The main limitation of this systematic review and meta-analysis derives from the incomplete data provided by a range of heterogeneous studies. Moderator analyses were performed with reduced numbers that decreased statistical power. This lack of statistical power might explain some non-significant results such as the inconclusive decrease in cTnI within a 24 h post-exercise recovery. We did not incorporate assay precision to our meta-analysis which could have explained certain degree of the study-to-study heterogeneity.[51] Finally, we found differences between studies regarding when peak concentrations were taken or noted. In conclusion, more research should be conducted with children and adolescents analyzing such covariate parameters.

2.4.5 Conclusion

In conclusion, cardiac biomarkers in children and adolescents are significantly increased form rest to post-exercise with the URL exceeded by a 76% of participants for cTnT, a 51% for cTnI and a 13% for NT-proBNP and the cut-off value for AMI exceeded by 39% for cTnT and a 11% for cTnI. Finally, we confirmed that the cTnT release is mainly associated with exercise duration and intensity, while the NT-proBNP release remains influenced only by exercise duration.

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PART 2

Chapter 3 - Exercise-Induced Release of cTnT and Exercise Load

Chapter 4 - Exercise-Induced Release of cTnT in Children vs Adults

Chapter 5 - Exercise-Induced Release of cTnT and Maturational Stage

CHAPTER 3

Exercise-Induced Release of cTnT and Exercise Load

Effect of Training Load on Post-Exercise Cardiac Troponin T Elevations in Young Soccer Players

Rafel Cirer-Sastre, Alejandro Legaz-Arrese, Francisco Corbi, Isaac López-Laval, Jose Puente-Lanzarote, Vicenç Hernández-González and Joaquín Reverter-Masià

Purpose: Training load (TL) metrics are usually assessed to estimate the individual, physiological and psychological, acute, and adaptive responses to training. Cardiac troponins (cTn) reflect myocardial damage and are routinely analyzed for the clinical diagnosis of myocardial injury. The association between TL and post-exercise cTn elevations is scarcely investigated in young athletes, especially after playing common team sports such as soccer. The objective of this study was to assess the relationship between TL measurements during a small-sided soccer game and the subsequent increase in cTn in young players.

Method: Twenty male soccer players (age 11.9 (SD = 2) years, height 151 (13) cm, weight 43 (13) kg) were monitored during a 5 x 5 small-sided game and had blood samples drawn before, immediately after, and 3 h after exercise for a posterior analysis of cardiac troponin T (cTnT). Internal, external, and mixed metrics of TL were obtained from the rating of perceived exertion (RPE), heart rate (HR), and GPS player tracking.

Results: The results show that the concentration of cTnT peaked at 3 h post-exercise in all participants. The magnitude of cTnT elevations was mainly explained by the exercise duration in the maximal heart rate zone (Maximum Probability of Effect (MPE) = 92.5%), time in the high-speed zone (MPE = 90.4 %), and distance in the high-speed zone (MPE = 90.45%).

Conclusion: Our results support the idea that common metrics of TL in soccer, easily obtained using player tracking systems, are strongly associated with the release of cTnT in children and adolescents.

International Journal of Environmental Research and Public Health. 2019;16(23):4853. doi:10.3390/ijerph16234853

3.1 Introduction

Soccer is the most popular sport around the world and involves more than 270 million people, including players, referees, and coaches.[1] Monitoring training load (TL) has become essential for prescribing and quantifying training stimuli. Strength and conditioning coaches, researchers, and sports analysts establish and routinely supervise players' TL to prevent injuries, prescribe training, and improve performance.[2–4] To this end, TL has been broadly summarized under two main constructs, namely external (eTL) and internal (iTL) training loads.[3] The former refers to externally measurable aspects of the work performed by a player during training, such as repetitions, distance, duration, or speed. The latter, on the other hand, refers to measures of physiological and psychological responses of the player during training, such as oxygen consumption, heart rate (HR), or the rating of perceived exertion (RPE).[2,3] Internal TL is conceptually restricted to measures that can be used to prescribe training and then monitored during exercise. However, there are other measures taken a posteriori, such as the recovery heart rate, post-exercise heart rate variability, or post-exercise lactate concentration, which may also reflect a player's response to the training session. These measures might be effective as surrogate metrics of iTL.[3] In addition, previous authors have proposed comprehensive metrics that combine external and internal measurements of TL, such as session RPE or Edwards' TL.[5,6] These might be applicable as mixed metrics of TL.

Metrics of TL are diverse in terms of measurement error, practical convenience, required equipment, or the time needed to analyze data.[2,3] Such heterogeneity may complicate the selection (e.g., by coaches and analysts) of a set of metrics that meets each team's needs. Coaches commonly prescribe training using external measures of TL to elicit internal responses in the player. Therefore, both constructs remain strongly associated since eTL appears to be the main determinant of iTL.[3] Although iTL correlates with eTL, the responses (iTL) of different players to a common exercise (eTL) might differ depending on their training status, nutrition, health, psychological status, or genetics.[3,7] This variability in the relationship between external and internal TL among different players might be considered to be a player's responsiveness to a training stimulus.[8]

Cardiac troponins (cTn) are heart contractile proteins, and their increased blood concentration reflects myocardial damage.[9] Moreover, when exceeding the upper reference limit (URL) determined for a healthy reference population, they are the preferred biochemical indicator for the diagnosis of myocardial infarction (MI) and acute myocardial infarction (AMI).[10] Numerous studies have reported that healthy populations of all ages have an elevated blood concentration of cTn in the hours after intense exercise.[11,12] Thus, it has been suggested that exercise-induced releases of cTn might be related to a physiological rather than a pathological response to exercise.[13,14]

Exercise-induced release of cardiac troponin T (cTnT) has been associated with the intensity and volume of exercise.[13] However, previous analyses of exercise-induced cTnT have not accounted for most of the TL metrics that are routinely used to assess sports performance.[4,15] In this regard, it is worth noting that intensity and volume do not necessarily imply internal and external TL, respectively. Exercise intensity, for instance, might be reported in terms of relative heart rate (iTL) or relative running speed (eTL).

If cTn increases are related to a physiological response to exercise, then cardiac stress estimated from its release after exercise might be a suitable surrogate metric of iTL, together with recovery heart rate (HR), post-exercise heart rate variability (HRV), or post-exercise lactate. Immunoassays for cTn are becoming increasingly more affordable, less invasive, and quicker. Therefore, cTn measurements might be applicable to sports performance as an objective marker of athletes' cardiac response to exercise, as well as their tolerance to selected TLs. Furthermore, incorporating TL metrics into the investigation of post-exercise cTn kinetics could be used in clinical research for a better understanding of the phenomenon itself, as well as the potential clinical implications. For these reasons, the main purpose of this investigation was to assess the association between exercise-induced increases in cTnT and a set of TL metrics. Since cTnT is a specific marker from myocardial tissue, our main hypothesis was that its exercise-induced release is explained fairly well by internal TL metrics that are based on heart rate activity during the exposure. Furthermore, since internal and external TL metrics are linearly associated, our secondary hypothesis was that cTnT is also explained by the metrics related to external TL.

3.2 Methods

3.2.1 Participants

The subjects in this study were a convenience sample of 20 participants, whose demographic characteristics are reported in **Table 2.** Subjects were recruited through an open invitation to all players participating in training at a soccer technical training campus (Campus de Futbol Formativo Pichi Alonso, Peñíscola, Spain). Parents were invited to fill in a digital form that included the Spanish version of the revised Physical Activity Readiness Questionnaire (PAR-Q),[16] as well as questions related to participants' medical and training histories. Answers were used to screen volunteers who met the following criteria: favorable PAR-Q, male, aged under 19 years, at least 3 years of experience in competitive soccer, and a weekly training volume of at least 3 days/week. Parents were informed of the procedures involved and gave their prior consent. The procedures of this study were approved by the Ethical Committee of Clinical Research of Sports Administration of Catalonia (02/2018/CEICGC) and met the principles of the latest revision of the Declaration of Helsinki.[17]

Table 2 | Participant characteristics

Variable	Mean (SD)
Age (years)	11.9 (2)
Tanner stage (n)	II = 8, III = 8, IV = 4
Hight (cm)	151.2 (13.1)
Weight (kg)	43.1 (13)
BMI (kg/m^2)	18.4 (2.62)
Experience (years)	5.9 (1.7)
HR max (bpm)	208 (2)

Note: Tanner stage, was summarized using its frequency distribution. BMI, body mass index; HR max, maximum heart rate.

3.2.2 Design

This was an observational study with a repeated measures design.

3.2.3 Instruments

Body weight was measured with a medical scale (SECA 711, Hamburg, Germany), and height was measured with a wall stadiometer (Año-Sayol, Barcelona, Spain). Participants were equipped with a WIMU Pro™ (RealTrack Systems, Almería, Spain) GPS tracker (sampling rate = 10 Hz), which was synced with a Garmin™ heart rate band (Garmin, Ltd., Olathe, Kansas, US). This setup has been validated for both heart rate (R^2 = 0.96) and player geospatial tracking (ICC = 0.98) in previous studies.[18,19] For cTnT, blood samples were analyzed using a Troponin T high-sensitivity STAT immunoassay in a Cobas E 601 analyzer (Roche Diagnostics, Penzberg, Germany). The detection range of this assay is 3 to 10,000 ng/L, and the intra-assay CV at a mean cTnT of 13.5 ng/L is <10%. The upper reference limit (URL) for cTnT, defined as the 99th percentile of healthy participants, is 13.5 ng/L.[20] Concentrations below the limit of detection of 3 ng/L were set to 1.5 ng/L for statistical analyses. The rating of perceived exertion (RPE) was obtained immediately after exercise using the CR100 scale, and all participants were already familiar with RPE reporting.[21]

3.2.4 Variables

The response variable was the cTnT concentration before (Pre cTnT), immediately after (Post0h cTnT), and 3 h after exercise (Post3h cTnT). Sampling times were chosen in accordance with previous research, which suggested that the peak cTnT values occur between 2 and 5 h after exercise.[11,12] Blood samples were drawn from an antecubital vein by a nurse and quickly centrifuged. Then, serum and plasma were drawn off and stored at -80 °C for further analysis. Participants were asked to refrain from exercise for 48 h before the intervention, as well as after exercise until the third blood sample was taken.

We grouped predictors for cTnT changes over time into four categories: athlete profile, internal TL, external TL, and mixed metrics of TL. Each athlete profile comprised age (years), Tanner stage (II, III, or IV), training experience (years), and Fox's maximum heart rate (bpm).[22] Internal TL metrics were the average and peak HR, in both absolute (bpm) and relative (% HR max) terms, and the RPE (Arbitrary Units, AU).[21] The maximum heart rates for relative intensities were calculated using the formula of 208 - (0.7 × Age). The external metrics of TL were the total distance covered during exercise (m), the relative distance covered per minute of exercise ($m \cdot min^{-1}$), the average speed during exercise ($km \cdot h^{-1}$), the maximum speed during exercise ($km \cdot h^{-1}$), the distance covered in the high-intensity speed zone (m at >18 $km \cdot h^{-1}$), and the time spent at a high-intensity speed (min at >18 $km \cdot h^{-1}$).[15] Finally, two mixed metrics of TL were calculated, namely, the session RPE (AU) and Edwards' training load (AU).[5,6]

3.2.5 Experimental Setup and Protocol

Measurements were taken at the participants' training facilities on two separate occasions. On the first visit, anthropometric measurements were taken, and a single experienced pediatrician classified participants according to their maturational stage.[23] After these measurements, participants were equipped with the tracking system and performed a familiarization session consisting of the standardized "11 + Kids" warm-up[24] and a simulation of the small-sided game to be performed on the second day.

On the second day, participants attended our facilities in two groups of 10, in a randomized order. On arrival, we extracted baseline blood samples (30 (5) min prior to exercise), and participants were equipped with the tracking system and then warmed up for 15 min by performing the "11 + Kids" exercises. Then, the cohort was randomly assigned to two teams of five to play a small-sided game of 5 × 5. Teams were consistent across bouts. The exercise consisted of 16 min of effort (four efforts of 4 min) alternated with 9 min of passive rest (three rest intervals of 3 min).[25] The game was played on a pitch of 20 m × 30 m and consisted of passing the end lines with the ball. The offside rule was adopted. Researchers and coaches were placed on all four lines, and when a team scored or exceeded the demarcation limits, a new ball was quickly delivered to the other team.[25] Participants were asked to play at maximal intensity, and strong and standardized verbal encouragement was given during the match. Immediately after exercise, participants reported their RPE, and the second blood sample was taken (5 (5) min after exercise). Participants stayed in a resting room and avoided physical exercise until 180 (5) min after exercise when the third blood sample was taken. Players were allowed to drink water ad libitum. The timing of procedures is outlined in **Figure 6.**

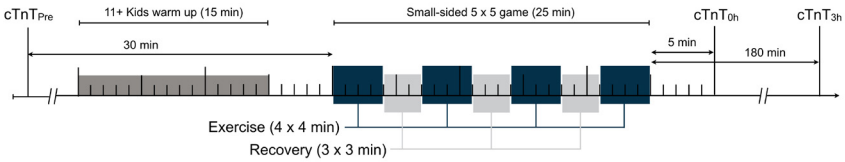

Figure 6 | Flowchart for study inclusion and exclusion stages

3.2.6 Statistical analysis

Tracking data were downloaded onto a computer and extracted to a spreadsheet using S Pro ™ software (RealTrack Systems, Almería, Spain). All statistical analyses were then performed using R version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria). Bayesian models were created in the Stan computational framework[26] accessed with the brms package.[27] Concentrations of cTnT were log-transformed prior to model fitting to achieve a residual normal distribution. Descriptive statistics are presented as the mean (SD) or median (interquartile range). Statistical analyses were performed by fitting Bayesian linear mixed-effects models using Markov Chain Monte Carlo. Since variables were registered on very different scales, data were standardized prior to model fitting to facilitate model convergence and their posterior comparison. After verifying differences in cTnT between baseline and peak values (3 h post), we proceeded by fitting separate models for each variable. The response variable was the cTnT concentration (log-transformed). The reference values for the intercept (β_0) were set to time = baseline and each variable at $Z = 0$. The fixed effects were time = Post 3 h (β_1), each variable separately (β_2), and their interaction (β_3). A random effect for each participant was always included to account for individual variation. The goodness of fit of the models was assessed by the proportion of variance explained (R^2). All model parameters (β_i) are reported as the median (90% credibility interval), and the likelihood of $\beta \neq 0$ is expressed as the Maximum Probability of the Effect (MPE). Effects were considered highly likely when the MPE was above 90%.

3.3 Results

Descriptive statistics of training load metrics are summarized in **Table 3.** Troponin concentrations were under the limit of detection (LoD) in 17 (85%), 15 (75%), and 3 (15%) participants before, immediately after, and 3 h after exercise, respectively. Four participants (20%) exceeded the URL for AMI 3 h after exercise.

Table 3 | Summary of training load metrics

Variable	Mean (SD
Internal Training Load	
HR av (bpm)	181.17 (8.97)
rHR av (% HR max)	87.15 (4.46)
HR peak (bpm)	198.5 (7.32)
rHR peak (% HR max)	95.48 (3.46)
Time HR Zone 5 (min: s)	13:23 (1:15)
ΔHR 1 min recovery (bpm)	-49.86 (7.61)
RPE (AU)	17.25 (0.91)
External Training Load	
Distance (m)	2101.22 (217.31)
rDistance (m min^{-1})	131.33 (13.58)
Speed av (km h^{-1})	7.34 (0.79)
Speed peak (km h^{-1})	22.15 (1.86)
Time Speed Zone 4 (min)	6.48 (5.02)
Distance Speed Zone 4 (min)	40.04 (31.42)
Mixed Metrics of Training Load	
Session RPE (AU)	431.25 (22.76)
Edwards' TRIMP (AU)	6013 (381.64)
cTnT	
Pre (ng/L)	1.5 (IQR = 1.5–1.5)
0 h (ng/L)	1.5 (IQR = 1.5–1.94)
3 h (ng/L)	7.46 (IQR = 4.17–12.57)

Note: Cardiac troponin T (cTnT) is expressed as the mean [interquartile range]. HRav, average heart rate; rHRav, average relative heart rate; HRpeak, peak heart rate; rHRpeak, peak relative heart rate; Time HR Zone 5, time spent in heart rate zone 5; ΔHR 1 min recovery, heart rate decrease in one minute of recovery; RPE, rating of perceived exertion; rDistance, relative distance; Time Speed Zone 4, time spent in maximal speed zone; Distance Speed Zone 4, distance covered at maximal speed; TRIMP, Training impulse.

The results of Bayesian models are presented in **Table 4**. There were differences in cTnT over time R^2 = 64.4% (90% CI 52.5% to 74.7%). Immediate changes in cTnT after exercise were shown to be unlikely (MPE = 84.4%). However, there was a high likelihood of an increase in cTnT between baseline and 3 h after exercise (MPE = 100%). Subsequent models confirmed a highly likely effect of time (β_1), regardless of the analyzed covariate (MPE = 100%). Moreover, any covariate by itself (β_2) appeared to explain the hs-cTnT concentration. **Figure 7** shows the interaction effect (β_3) of each explanatory variable, as described below.

Table 4a | Model coefficients

Coefficient of determination	Parameter	Coefficient	MPE
	Time		
R^2 = 64.4 (52.5–74.7)	β_0 (Intercept)	0.48 (0.26–0.72)	
	β_1 = Post0h cTnT	0.17 (−0.11 to 0.44)	84.4%
	β_2 = Post3h cTnT	1.4 (1.12–1.68)	100% *
	Age (years)		
R^2 = 68.7 (59.4–78.5)	β_0 (Intercept)	0.48 (0.26–0.7)	
	β_1 = Post	1.4 (1.11–1.68)	100% *
	β_2 = Age	0.06 (−0.18 to 0.27)	65.6%
	β_3 = Post × Age	0.42 (0.13–0.74)	98.7% *
	Tanner stage		
R^2 = 63.8 (52.1–74.7)	β_0 (Intercept)	0.48 (0.25–0.73)	
	β_1 = Post	1.39 (1.08–1.74)	100% *
	β_2 = Tanner	0.07 (−0.16 to 0.34)	67.9%
	β_3 = Post × Tanner	0.28 (−0.04 to 0.62)	91.8% *
	Experience (years)		
R^2 = 72.9 (64.3–81.5)	β_0 (Intercept)	0.48 (0.27–0.68)	
	β_1 = Post	1.4 (1.14–1.67)	100% *
	β_2 = Experience	0.05 (−0.13 to 0.28)	66.8%
	β_3 = Post × Experience	0.5 (0.23–0.78)	99.8% *
	BMI (kg/m2)		
R^2 = 59.8 (46.2–72)	β_0 (Intercept)	0.48 (0.22–0.73)	
	β_1 = Post	1.39 (1.08–1.74)	100% *
	β_2 = BMI	0.09 (−0.17 to 0.35)	73%
	β_3 = Post × BMI	0.03 (−0.3 to 0.37)	56.2%
	HR max (bpm)		
R^2 = 69.1 (59.3–79)	β_0 (Intercept)	0.48 (0.26–0.7)	
	β_1 = Post	1.4 (1.13–1.7)	100% *
	β_2 = HR max	−0.05 (−0.28 to 0.17)	64.3%
	β_3 = Post × HR max	−0.43 (−0.71 to −0.11)	99% *
	HR aver (bpm)		
R^2 = 57.6 (42.2–71)	β_0 (Intercept)	0.49 (0.21–0.78)	
	β_1 = Post	1.34 (0.95–1.69)	100% *
	β_2= HR aver	0.07 (−0.2 to 0.36)	65.6%
	β_3 = Post × HR aver	0.09 (−0.31 to 0.45)	66.4%

Table 4b | Model coefficients

Coefficient of determination	Parameter	Coefficient	MPE
	rHR aver (%)		
R^2 = 58.9 (44.4–72.3)	β_0 (Intercept)	0.49 (0.23–0.76)	
	β_1 = Post	1.34 (0.98–1.68)	100% *
	β_2= rHR aver	0.07 (−0.19 to 0.36)	66.6%
	β_3 = Post × rHR aver	0.17 (−0.2 to 0.52)	78.4%
	HR peak (bpm)		
R^2 = 56.6 (41.7–70.5)	β_0 (Intercept)	0.49 (0.2–0.76)	
	β_1 = Post	1.34 (0.99–1.71)	100% *
	β_2 = HR peak	0.05 (−0.24 to 0.35)	60.4%
	β_3 = Post × HR peak	−0.08 (−0.47 to 0.3)	64.8%
	rHR peak (%)		
R^2 = 57 (42–71.6)	β_0 (Intercept)	0.49 (0.22–0.77)	
	β_1 = Post	1.34 (0.98–1.7)	100% *
	β_2 = rHR peak	0.06 (−0.22 to 0.37)	63%
	β_3 = Post × rHR peak	0.03 (−0.38 to 0.4)	54%
	Time HR-Z5 (min)		
R^2 = 60.7 (45.6–73.7)	β_0 (Intercept)	0.5 (0.23–0.78)	
	β_1 = Post	1.3 (0.97–1.68)	100% *
	β_2= Time HR-Z5	0.04 (−0.24 to 0.33)	60%
	β_3= Post × Time HR-Z5	0.31 (−0.04 to 0.67)	92.5% *
	RPE (UA)		
R^2 = 66 (53.8–76)	β_0 (Intercept)	0.48 (0.27–0.72)	
	β_1 = Post	1.39 (1.1–1.72)	100% *
	β_2 = RPE	0.07 (−0.17 to 0.3)	67.1%
	β_3 = Post × RPE	0.34 (0.02–0.66)	96.8% *
	Dist abs (m)		
R^2 = 70.1 (58.8–80.8)	β_0 (Intercept)	0.49 (0.24–0.72)	
	β_1 = Post	1.34 (1.01–1.63)	100% *
	β_2 = Dist abs	0.01 (−0.24 to 0.24)	53%
	β_3 = Post × Dist abs	0.52 (0.22–0.85)	99.4% *
	Dist rel (m/min)		
R^2 = 69.8 (58.7–80.1)	β_0 (Intercept)	0.49 (0.26–0.72)	
	β_1 = Post	1.34 (1.06–1.67)	100% *
	β_2 = Dist rel	0.01 (−0.23 to 0.24)	54%
	β_3 = Post × Dist rel	0.52 (0.21–0.83)	99.6% *

Table 4c | Model coefficients

Coefficient of determination	Parameter	Coefficient	MPE
	Time Speed-Z4 (min)		
R^2 = 60.2 (45–73.1)	β_0 (Intercept)	0.49 (0.22–0.77)	
	β_1 = Post	1.34 (1–1.68)	100% *
	β_2 = Time SP-Z4	−0.04 (−0.32 to 0.23)	59.6%
	β_3 = Post × Time SP-Z4	0.29 (−0.07 to 0.64)	90.4% *
	Dist Speed-Z4 (m)		
R^2 = 60.4 (46.6–74.3)	β_0 (Intercept)	0.49 (0.21–0.74)	
	β_1 = Post	1.34 (1.02–1.71)	100% *
	β_2 = Dist SP-Z4	−0.04 (−0.32 to 0.24)	59%
	β_3 = Post × Dist SP-Z4	0.3 (−0.06 to 0.64)	90.5% *
	Edwards' TL (UA)		
R^2 = 58.1 (42.9–71)	β_0 (Intercept)	0.49 (0.22–0.78)	
	$\beta_1$1 = Post	1.34 (1–1.71)	100% *
	β_2 = TL	0.06 (−0.21 to 0.35)	63.7%
	β_3 = Post × TL	0.13 (−0.25 to 0.49)	72%
	Session RPE (UA)		
R^2 = 65.8 (54.8–76.7)	β_0 (Intercept)	0.48 (0.25–0.71)	
	β_1 = Post	1.39 (1.09–1.7)	100% *
	β_2 = s-RPE	0.07 (−0.18 to 0.31)	68.2%
	β_3 = Post × s-RPE	0.34 (0.02–0.67)	96.6% *
	Speed aver (km/h)		
R^2 = 71.7 (61.3–81.5)	β_0 (Intercept)	0.49 (0.24–0.71)	
	β_1 = Post	1.34 (1.05–1.63)	100% *
	β_2 = Speed aver	0.02 (−0.23 to 0.25)	54.6%
	β_3 = Post × Speed aver	0.55 (0.24–0.85)	99.8% *
	Speed peak (km/h)		
R^2 = 60 (45.6–73.7)	β_0 (Intercept)	0.5 (0.22–0.77)	
	β_1 = Post	1.34 (0.98–1.66)	100% *
	β_2 = Speed peak	−0.05 (−0.32 to 0.24)	60.3%
	β_3 = Post × Speed peak	0.29 (−0.06 to 0.66)	90.3% *

For the athlete profile, the increase in cTnT could be explained by adding an interaction between time and age (R^2 = 68.7%, MPE = 99.1%), Tanner's maturational stage (R^2 = 63.8%, MPE = 91.2%), years of training experience (R^2 = 72.9%, MPE = 99.7%), and HR max (R^2 = 69.1%, MPE = 98.8%). In contrast, a cTnT increase was unlikely to be explained by participants' BMI (R^2 = 59.8%, MPE = 56%). Only two metrics of internal TL could explain the increase in cTnT over time with a high likelihood, namely time spent in the high-intensity HR zone (R^2 = 60.7%, MPE = 92.7%) and RPE (R^2 = 66%, MPE = 96.1%). On the contrary, neither the average (R^2 = 57.6%, MPE = 64.5%) and peak HR (R^2 = 56.6%, MPE = 64.5%) nor the average (R^2 = 58.9%, MPE = 78.4%) and peak relative HR (R^2 = 57%, MPE = 55.3%) appeared to interact with the increase in post-exercise cTnT. All external metrics of TL could explain the increase in cTnT with a high likelihood: absolute (R^2 = 70.1%, MPE = 99.4%) and relative distance (R^2 = 69.8%, MPE = 99.5%); average (R^2 = 71.7%, MPE = 99.7%) and peak speed (R^2 = 60%, MPE = 90.8%); distance covered at high speed (R^2 = 60.4%, MPE = 91.1%); and time spent at high speed (R^2 = 60.2%, MPE = 91.2%). Finally, of the mixed metrics of TL, only session RPE interacted with the increase in cTnT with a high likelihood (R^2 = 65.8%, MPE = 96%), whereas Edwards' TL (R^2 = 58.1%, MPE = 71.6%) did not.

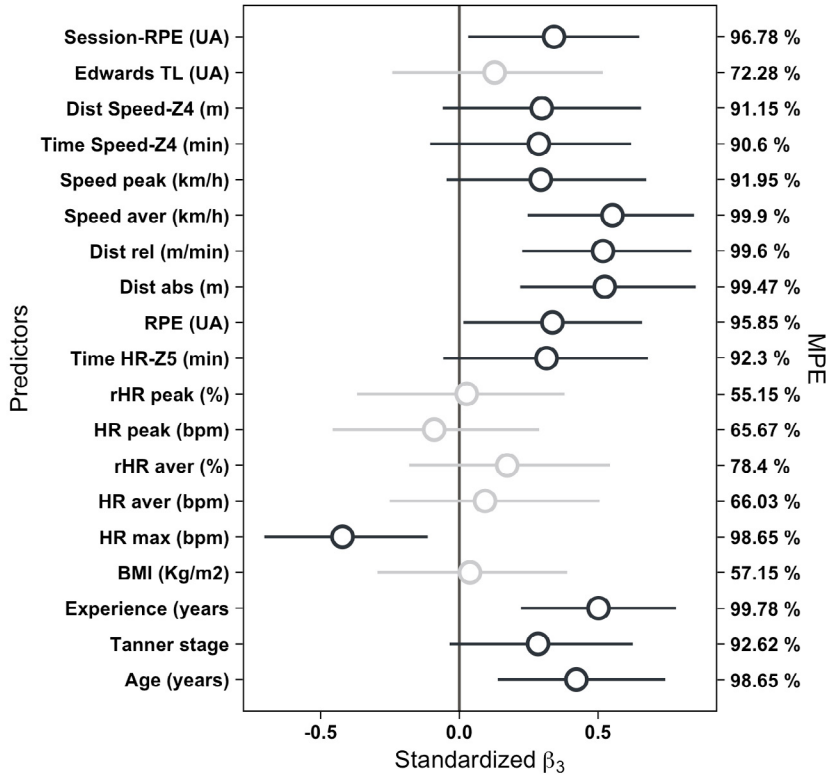

Figure 7 | Standardized interaction coefficients (β_3) by variable. Dark intervals denote variables with Maximum Probability of Effect (MPE) values over 90%

3.4 Discussion

The aim of this study was to assess whether practical, field-based metrics of TL in soccer could be used to predict the exercise-induced release of cTnT in healthy young players. Although previous studies have investigated exercise-induced cTnT release in pubertal participants,[12] studies involving team sports,[28–30] and/or pre-pubertal participants are scarce.[31] Among these studies, only one, to our knowledge, reported hs-cTnT concentrations in subjects after playing soccer.[30] The novel finding of the present study is that common metrics of TL in soccer, easily obtained using player tracking systems, are strongly associated with the release of cTnT in children and adolescents.

Our results coincide with previous research that observed an increase in cTnT in apparently healthy young participants.[12] This increase was highly variable between participants (median increase = 5.96, IQR = 2.67–11.07) and supports previous observations of relatively high variability in cTnT release at early ages.[32] However, our finding that older players (in both chronological and biological terms) are more likely to have higher cTnT concentrations is not in line with the results of Tian et al.,[33] who previously suggested that basketball players at Tanner stage II might reach higher peaks of cTnT than those at Tanner stage III. This discrepancy might be related to a possible interaction between age and TL since more experienced players, from a technical and tactical perspective, might resolve real game situations more efficiently, requiring less intensity than a novice. However, the limited sample size in this study did not allow for multiple modeling to assess this potential interaction. In addition, cTnT associations with age seem to differ depending on the age range observed. Studies that compared adolescents with adults found higher peaks in the younger participants,[28,33] although this association remained inconclusive when comparing adolescents with children.[33–35] Fu et al. hypothesized that the higher variability in adolescents' post-exercise hs-cTnT might be attributable to the immaturity of their myocardium since it would experience greater stress in response to an increased myocardial workload compared with that of adults.[36]

Our main hypothesis was that cTnT release is explained by iTL. Contrary to this hypothesis, only one HR metric (time spent in HR zone 5) explained the hs-cTnT increase, whereas the mean and peak HR, in both absolute and relative terms, did not. To our knowledge, this is the first study to include time spent in the maximal HR zone, which is a metric that provides information about not only the average and peak demands of an exercise (commonly reported as peak and mean HR) but also the distribution of intensities throughout a training session. The overall relevance of HR metrics during exercise in explaining the subsequent change in cTnT is still under debate, especially in young participants. Previous research in adult populations found that cTnT release after exercise was associated with the peak and average heart rate during exercise.[11] However, our results coincide with recent

studies that did not find such an association when investigating the phenomenon in younger participants.[33,37] The acute cardiovascular response to exercise in children is more variable than that in adults; this variability is probably a consequence of anatomical, physiological, and psychological changes that occur during growth and maturation.[38] This high individual variation in exercise-induced HR might confound coaches when they estimate the actual TL from HR-based metrics. Consequently, other available metrics, such as RPE, speed, or distance, might be preferable when quantifying load in young players.

We also hypothesized that the external metrics of TL partially explain the variability of cTnT over time. Our findings confirm that geospatial metrics during exercise are associated with the post-activity increase in cTnT. These results concur with those of previous authors who associated the increase in cardiac biomarkers with indicators of exercise intensity, such as the time needed to cover a certain distance or the distance covered in a fixed time.[11,36] Confirming that cTnT increases are proportional to the physical demands of an exercise would be compatible with the main hypothetical mechanisms for a reversible, physiological release of cTn induced by exercise.[13,14] On the other hand, to our knowledge, this is the first study to analyze the specific effects of exercise volume (time and distance) at maximal intensity. Accordingly, a novel finding of the present study is the likely positive association between the time or distance covered in the high-speed zone and the subsequent increase in cTnT. This association implies that the cTnT release depends not only on the overall intensity of the session but also on its distribution. Furthermore, the possible interaction between exercise duration and intensity was previously investigated in adolescents by Fu et al., who found that, although both parameters were associated with biomarker release, exercise intensity could better explain the increase in cTnT.[36]

In the present study, we assessed two mixed metrics of TL. When modeling the cTnT variability using session RPE, we found a likely interaction with time (MPE = 96.6%), but, in contrast, the interaction between time and Edwards' TL was uncertain (MPE = 72%). In both cases, these results coincide with the likelihood of the main variables in the calculations since RPE was shown to have a likely interaction with time, but heart rate indicators did not show an interaction. To the authors' knowledge, this is the first study to report cTnT associations with both eTL and iTL metrics in young soccer players. However, the association between external and internal TL was not addressed in this study and could be further explored in future research. In this regard, Akubat, Barrett, and Abt proposed the use of an external-internal TL ratio, which combines the individualized training impulse, high-intensity distance, and total distance.[39] This ratio has been previously associated with players' total quality of recovery and muscle soreness, but its potential association with the release of myocardial markers is currently unknown.[8]

From a clinical point of view, the strengths of the present study include the incorporation of practical, field-based TL metrics based on player tracking that are largely missing in clinical literature on cTnT kinetics associated with exercise. In addition, from a practical perspective, our results raise the possibility of using post-exercise cTnT elevations as a surrogate metric of TL. Both approaches might guide future research to evaluate the potential use of novel clinical markers, such as cTnT, when quantifying players' TL. However, several limitations of our study should be considered. First, we only studied a small sample of male soccer players at maturational stages from II to IV. Such a restriction prevents our results from being generalized to both sexes, other sports, or a wider range of maturational stages. For these reasons, future studies should address our limitations by encompassing larger and wider samples in terms of the number of participants, sex, maturational status, and sports modality. In addition, we only assessed a proportion of the available metrics for sports performance assessment. Therefore, future research might consider assessing other variables from both physiological (e.g., ventilatory thresholds or lactate accumulations) and analytical perspectives (e.g., accelerations and decelerations or number of impacts).

Our findings support the notion that children might present elevations of cTnT related to exercise. This evidence could be considered in a clinical setting when interpreting cTnT results in young athletes. In addition, we found that exercise duration or distance in high-intensity zones for both rHR and speed might be associated with the subsequent release of cTnT. Since these measurements are commonly registered by smartwatches worn by athletes to monitor their sessions, our results might also be applicable to clinicians who consult the training history in their patients' smartwatches, when available. Furthermore, the findings of the present study also apply in reverse since we demonstrated that sessions with a higher high-intensity density might be associated with a higher elevation of a cardiac-damage biomarker. Because the mechanisms of exercise-induced cTnT are not completely understood, coaches who work with children should be aware that training sessions that require players to perform in high-intensity zones for long periods might induce the release of cardiac-damage markers such as cTnT.

3.5 Conclusions

Our results confirm that healthy children have elevated blood concentrations of cTnT after an intermittent small-sided soccer game. Players' ages, maturational stages, and training experience were positively associated with the release of cTnT. Internal TL metrics did not explain the elevations of the biomarker, with the exception of time spent in the maximal HR zone. The external metrics of TL were directly associated with the increase in cTnT. A novel finding is that the time that young soccer players spend in the maximal heart rate or speed zones during a training session might be associated with an increase in cTnT in the following 3 h.

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CHAPTER 4

Exercise-Induced Release of cTnT in Children vs Adults

Cardiac Troponin T Release After Football 7 in Healthy Children and Adults

Rafel Cirer-Sastre, Alejandro Legaz-Arrese, Francisco Corbi, Isaac López-Laval, Jose Puente-Lanzarote, Vicenç Hernández-González, and Joaquín Reverter-Masià

Purpose: The objective of this study was to compare the release of cardiac troponin T (cTnT) after a football 7 match between two cohorts of children and adult players.

Method: Thirty-six male football players (children = 24, adult = 12) played a football 7 match, and cTnT was measured before, and 3 h after exercise.

Results: Concentrations of cTnT were compared between groups and time, and correlated with participants' characteristics, as well as internal and external exercise load. Cardiac troponin T was elevated in all participants ($P < .001$), and exceeded the upper reference limit for myocardial infarction in 25 (~70%) of them. Baseline concentrations were higher in adults ($P < .001$), but the elevation of cTnT was comparable between the groups ($P = .37$). Age ($P < .001$), body mass ($P = .001$) and height ($P < .001$), and training experience ($P = .001$) were associated to baseline cTnT values, while distance ($P < .001$), mean speed ($P < .001$), and peak ($P = .013$) and mean ($P = .016$) heart rate were associated to the elevation of cTnT.

Conclusion: The present study suggests that a football 7 match evoked elevations of cTnT during the subsequent hours in healthy players regardless of their age. However, adults might present higher resting values of cTnT than children. In addition, results suggest that the exercise-induced elevations of cTnT might be mediated by exercise load but not participant characteristics.

International Journal of Environmental Research and Public Health. 2020;17(3):956. doi:10.3390/ijerph17030956

4.1 Introduction

The release of cardiac troponins (cTn), typically observed in patients with acute and chronic myocardial injury (MI), is also common in healthy athletes of all ages and training statuses after exercise.[1,2] This subject is potentially important for the triage of athletes who develop chest pain that mimics cardiac injury after exercise and who might have serum cardiac troponins drawn in the emergency room. The relationship between age and the magnitude of exercise-induced elevations of cTn, however, is still controversial, especially when comparing adolescent with adult athletes.[3–5] Whilst some previous studies reported higher peak cTn values in adolescents when compared with the adults,[5] others found no age differences.[3,4] Despite this, studies focusing on this phenomenon in younger populations are still scarce.[6] It has been hypothesized that, the higher elevations of cTn in young athletes might be related to maturation, since the immature myocardium might be more vulnerable to injury in clinical situations.[3,5] Since knowledge about the expected blood concentration of cTn in healthy active children after exercise is still limited, this makes it difficult to differentiate physiological and pathological elevations of cardiac troponin T (cTnT) in young patients involved in exercise in the hours previous to a blood analysis.[7]

This release has been described in a variety of sports, and was related to exercise load.[8] However, although football is the most practiced sport in the world,[9] only two studies, to the best of our knowledge, provided data about cTn elevations after its practice in young players.[10,11] Hosseini et al. investigated the elevations of cTn after a football 11 match in a cohort of 22 adolescents,[11] while Cirer-Sastre et al., described the associations between exercise load in a small-sided 5-by-5 game and the subsequent elevations of cTn in a cohort of 20 adolescents.[10] Despite this, football matches in early age categories are normally played in smaller teams and pitches,[12] usually under regional adaptations of the international rules of football 7, where football matches are played in teams of seven players.[13] To date, no evidence has been reported about cTnT elevations after a football 7 match in young players.

For these reasons, the purpose of this study was to compare the release of cTnT after a football 7 match in two cohorts of children and adult players. Our main hypothesis was that cTnT would be elevated over time and this elevation might vary between age groups and be associated with participant characteristics and exercise load.

4.2 Methods

4.2.1 Participants

Twenty-four children and 12 adults volunteered to participate in this study after an open invitation at the beginning of the season. All players were male, trained 3 days per week in the same local football club (Peñiscola, Spain), were non-smokers and were not under medical, pharmacologic

or dietary treatment. The participants were informed of the purpose, procedures and risks of this study, and gave their prior personal (and parental for those under the age of 18) written informed consent to participate in this study. The inclusion criteria were male, at least 3 years of experience in competitive football, no previous history of cardiovascular disease and favorable readiness according to the revised Physical Activity Readiness Questionnaire (PAR-Q).[14] No exclusions were made. The procedures of this study were approved by the Ethical Committee of Clinical Research of the Sports Administration of Catalonia (02/2018/CEICGC) and met the principles of the latest revision of the Declaration of Helsinki.[15]

4.2.2 Procedures

The participants were required to avoid exercise during the 24 h before the study. On arrival, players underwent a resting 12-lead electrocardiogram (ECG; Click ECG BT 12 channel, Milano, Italy). Then, body mass and height were measured with a medical scale and stadiometer (SECA 711, Hamburg, Germany; and Año-Sayol, Barcelona, Spain; respectively). Subsequently, participants were equipped with a heart rate (HR) chest band (Garmin, Ltd., Olathe, Kansas, US) synced with a global positioning system (GPS) tracker (RealTrack Systems, Almería, Spain). This set up has been previously validated for both heart rate[16] and geospatial tracking.[17] Participants performed a standardized "11+ Kids" warm-up[18] and then played a 7×7 match (2 children's matches, 1 adults' match) following the rules of the International Federation of Football 7.[13] The match time was divided in two parts separated by half time of 10 min, and each part consisted of two quarters of 15 min with a 2 min break between quarters. All participants played the complete match, no player replacements were made and goalkeepers were excluded from data analysis.

Blood samples were taken from an antecubital vein before exercise (pre) and at 3 h after exercise (post). The election of these sampling times was based on previous research reporting an approximate time-to-peak for cTnT of between 2 and 5 h after exercise.[6] Blood samples were quickly centrifuged and stored at -80 °C for further analysis. Cardiac Troponin T was determined using a Troponin T hs STAT immunoassay in a Cobas E 601 analyzer (Roche Diagnostics, Penzberg, Germany). The detection range of this assay is 3 to 10,000 ng/L, and the intra-assay coefficient of variation at a mean cTnT of 13.5 ng/L is $< 10\%$. The upper reference limit (URL) for cTnT, defined as the 99th percentile of healthy participants, is 13.5 ng/L.[19]

The response variable in this study was the participants' blood concentration of cTnT, and the main predictors were time (pre; post) and group (children; adults). A variable called delta (Δ cTnT) was calculated by subtracting individual pre to post cTnT values. The secondary predictors were participant characteristic data and exercise load.

Participant characteristic predictors were age (years), body height (cm) and mass (kg), previous training (years), training frequency (days/week) and training volume (hours/week). The exercise load covariables, obtained during the match, were distance (m), peak speed (SPpeak, km/h), mean speed (SPmean, km/h), peak HR (HRpeak, bpm), peak relative HR (rHRpeak, % HRmax), mean HR (HRmean, bpm), and mean relative HR (rHRmean, % HRmax). The maximum HR (HRmax) for relative calculations was age-predicted using the formula 208.609 – 0.716 × age.[20]

4.2.3 Statistical Analysis

All statistical analyses were performed using R v3.6.1 (R Foundation for Statistical Computing, Vienna, Austria). A Shapiro–Wilk test and data visualization confirmed that data distribution for cTnT was right-skewed and non-transformable. Homogeneity of variances was assessed using a Flinger–Killeen test (X^2). The data were described using mean (SD) and median (range) as appropriate. Between-group comparisons for participant characteristics and performance data were made using an independent-samples t-test (t). Between-group differences for cTnT were tested using an independent 2-group Wilcoxon rank sum test with continuity correction (W). Within-subject differences over time were assessed using dependent 2-group Wilcoxon signed rank tests with continuity correction (V). The rates of positive detection for participants with cTnT above the URL were compared using a 2-sample test for equality of proportions with continuity correction (r^2). Associations between cTnT and participant characteristics or exercise load variables were assessed using Spearman's correlation (r_s). Statistical significance for all hypothesis tests was assumed when $P < .05$.

4.3 Results

4.3.1 Participants

The participant characteristics and exercise load are summarized in **Table 5**. Although there were group differences for age ($P < .001$), body mass ($P < .001$), height ($P < .001$), and years of training experience ($P = .007$), the participants underwent comparable training frequency ($P = .73$) and volume ($P = .55$) at the time of the study. During the match, both groups covered similar distances ($P = .17$); however, adults performed at a higher average and peak speed than children ($P < .001$ and $P = .04$, respectively). By contrast, the absolute peak heart rate (HRpeak) was higher in the children ($P < .001$). Besides that, the relative peak heart rate (rHRpeak), absolute average heart rate (HRaver) and relative average heart rate (rHRaver) were comparable between groups ($P = .14$, $P = .5$ and $P = .084$, respectively).

Table 5 | Summary of participants' characteristics and exercise load

Variable	Children (n = 24)	Adults (n = 12)	All (n = 36)	Between-Groups
Participant characteristics				
Age (years)	10.7 (1.6)	37.5 (12.7)	19.6 (14.7)	*P* < .001
Body height (cm)	146 (14.8)	177 (5.72)	157 (19.1)	*P* < .001
Body mass (kg)	41.3 (15.4)	79.5 (7)	54.0 (22.4)	*P* < .001
Training experience (years)	4.6 (1.7)	23.6 (14.5)	9.4 (10.9)	*P* = .007
Training frequency (days/week)	2.9 (1.2)	3.2 (0.8)	3 (1.1)	*P* = .73
Training volume (hours/week)	4.6 (2.6)	4.9 (2.5)	4.7 (2.5)	*P* = .55
Exercise load				
Distance (m)	5970 (722)	5490 (540)	5810 (697)	*P* = .17
SPpeak (km/h)	23.5 (2.2)	27.1 (1.9)	24.7 (2.7)	*P* < .001
SPmean (km/h)	5.6 (0.7)	6 (0.7)	5.7 (0.7)	*P* = .04
HRpeak (bpm)	202 (6)	188 (7)	197 (9)	*P* < .001
rHRpeak (% HRmax)	100 (3)	105 (6)	102 (5)	*P* = .14
HRmean (bpm)	161 (19)	158 (12)	160 (16)	*P* = .5
rHRmean (% HRmax)	80 (9)	88 (8)	83 (10)	*P* = .084

Note: Values are expressed as mean (SD). SPpeak, peak speed; SPmean, mean speed; HRpeak, peak heart rate; rHRpeak, peak relative heart rate; HRmean, mean heart rate; rHRmean, mean relative heart rate.

4.3.2 Cardiac Troponins

Children had lower resting values of cTnT than adults ($P < .001$). Both groups had elevated cTnT 3 h after the match (children: $P < .001$, adults: $P = .001$). Nevertheless, both the elevation and the absolute post-match concentration were comparable between cohorts ($P = .37$ and $P = .65$, respectively) (**Table 6**). Although data visualization suggested that children's cTnT was more variable than adults (Figure 1), both variances were statistically comparable ($X^2 = 2.53$, $P = .11$). None of the participants exceeded the URL at baseline.

However, 17 (70.83%) children and 8 (66.67%) adults exceeded the cut-off value for MI of 13.5 ng/L at 3 h post exercise. There were no differences between the rates of positive detection ($P = .99$).

Table 6 | Values of cardiac troponin T (cTnT) (ng/L)

	Pre	Post	Delta	Within-Subjects
All (n = 36)	5.20 (3.65, 12.7)	21.1 (4.67, 200)	13.7 (0.45, 194)	*V* = 666 *P* < .001
Children (n = 24)	4.64 (3.65, 12.7)	22.9 (4.67, 200)	16.4 (0.45, 194)	*V* = 300 *P* < .001
Adults (n = 12)	6.54 (4.88, 11.7)	18.6 (8.31, 60.2)	12.4 (0.50, 53.7)	*V* = 78 *P* = .001
Between-groups	*W* = 36 *P* < .001	*W* = 158 *P* = .65	*W* = 17 *P* = .37	

Note: Values are expressed as median (range) (ng/L).

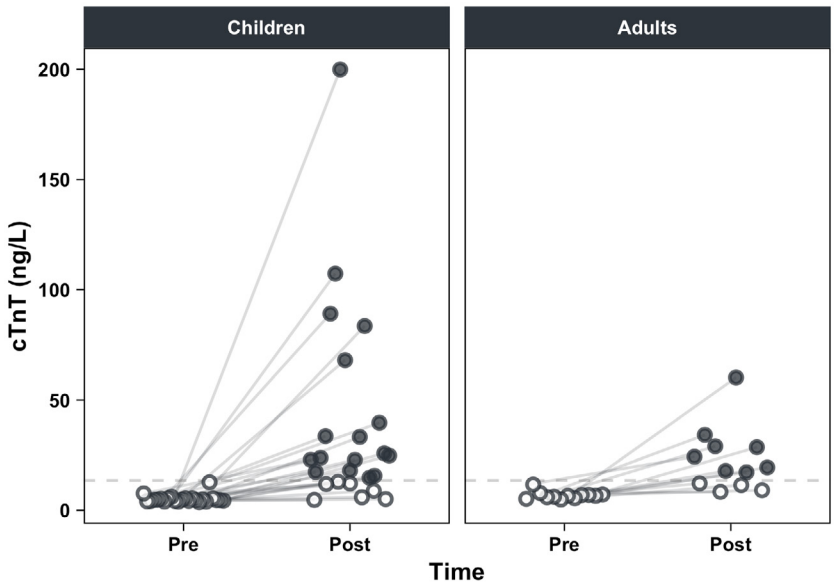

Figure 8 | Individual values of cTnT before and after the match, by group

Correlations between basal values of cTnT, delta cTnT changes, participant characteristics and performance are detailed in **Table 7**. Baseline concentrations correlated positively with age ($P < .001$), body mass ($P = .001$), body height ($P < .001$), and years of previous experience ($P = .001$). Post-exercise elevations were associated with distance ($P < .001$), mean speed ($P < .001$), peak ($P = .013$) and mean ($P = .016$) heart rate.

Table 7 | Table of correlations

Variable	cTnT Pre (ng/L)	Δ cTnT (ng/L)
Troponins		
Δ cTnT (ng/L)	r_s = -0.12, P = .523	
Participant characteristics		
Age (years)	r_s = 0.67, P < .001	r_s = -0.23, P = .21
Body height (cm)	r_s = 0.64, P < .001	r_s = -0.23, P = .2
Body mass (kg)	r_s = 0.55, P = .001	r_s = -0.35, P = .052
Training experience (years)	r_s = 0.56, P = .001	r_s = -0.16, P = .37
Training frequency (days/week)	r_s = 0.32, P = .072	r_s = -0.09, P = .64
Training volume (hours/week)	r_s = 0.2, P = .27	r_s = -0.2, P = .29
Exercise load		
Distance (m)	r_s = -0.133, P = .465	r_s = 0.59, P < .001
SPpeak (km/h)	r_s = 0.32, P = .074	r_s = 0.17, P = .37
SPmean (km/h)	r_s = 0.2, P = .28	r_s = 0.59, P < .001
HRpeak (bpm)	r_s = -0.18, P = .33	r_s = 0.43, P = .013
rHRpeak (% HRmax)	r_s = 0.36, P = .044	r_s = 0.21, P = .25
HRmean (bpm)	r_s = 0.29, P = .11	r_s = 0.42, P = .016
rHRmean (% HRmax)	r_s = 0.52, P = .002	r_s = 0.29, P = .11

Note: cTnT Pre, cTnT concentration at baseline; Δ cTnT, elevation of cTn from baseline to 3h post exercise; SPpeak, peak speed; SPmean, mean speed; HRpeak, peak heart rate; rHRpeak, peak relative heart rate; HRmean, mean heart rate; rHRmean, mean relative heart rate.

4.4 Discussion

This is the first study comparing the release of cTnT after a football 7 match between two cohorts of children and adult players. Our main findings were that (1) basal cTnT might be higher in the adults, (2) exercise induces elevations of different magnitude in all players, regardless of their age, (3) both the absolute post values and the relative increase (Δ cTnT) are comparable between children and adults, (4) participant characteristics were associated with baseline cTnT, but not with its elevation, and (5) exercise load was positively associated with the subsequent cTnT elevation.

Our results are confirmatory of the acute elevation of cTnT in the hours after exercise.[2] As previously noticed in other studies,[3,4] participants had normal ECG and reported no history of personal nor familiar cardiovascular disease. However, the overall rate of positive detection was substantial (25/36, 69.44%), and similar to that in a recent meta-analysis including studies with participants under the age of 18 (166/219, 75.8%, $\chi^2 = 0.37$, $P = .54$).[6]

These findings reinforce the hypothesis that transient elevations of cTn after practicing sport might be a physiological acute response to exercise rather than a pathological sign of cardiovascular disease. Therewith, this implies that exercise might confound clinical cTn assessments of patients attending emergency departments, and should be considered during the anamnesis.[21]

Another finding in this study was that adult players presented higher basal values of cTnT than children, despite having normal ECG and being apparently healthy. Such a difference coincides with previous studies reporting higher population values in the older cohort.[22] This difference might be explained by age in itself, since it is one of the main risk factors for cardiovascular disease (CVD).[23] Moreover, older individuals are also more likely to have concealed CVD that might not be detectable by only assessing a resting ECG, as we did in this study.[22,24] Nevertheless, this difference at baseline was not maintained through time, but it disappeared when comparing post cTnT concentrations as well as Δ cTnT.

Yet another relevant finding in this study is the negative result when testing for between-group differences in Δ cTnT. Although some previous studies found higher cTn elevations in adolescents when compared with adults,[5] ours is not the first study that failed to find this association.[3,4] These previous studies, however, used adolescent players while ours included children. A higher and more variable release of the biomarker during puberty might explain why some differences were found in adolescent–adult comparisons but not in children–adult studies. This hypothesis goes in line with our recent study in which we found that in a cohort of 20 participants of 11.9 (2) years, the elevation of cTnT after exercise was positively associated with the maturational stage of participants obtained using a Tanner scale.[10] In this regard, previous authors have already highlighted the need for age- and sex-specific population values for cTnT concentrations in pediatrics.[25] The present results, therefore, contribute to a better description of how normal and exercise-induced values might vary through age, and evidence the need to stratify population reference values by age.

In this study, no associations between participant characteristics and the cTn response to exercise were found (**Table 7**). Several preceding studies reported that Δ cTnT might be partially explained by age,[5,10,26] anthropometrics[11] and previous training experience,[26,27] while others obtained non-conclusive associations.[11,28–30] These discrepancies might be explained by a variety of methodological differences between studies, such as the sample age range, athletic status, exercise mode or the intensity and duration in the interventions. In this regard, although our results support the assertion that the exercise-induced elevation of cTn might be independent of age, height, weight or experience, larger studies including wider age ranges are still needed to clarify this association. Weekly training frequency and volume at the time of the study, on the other hand, were not associated with Δ cTnT either, coinciding with previous reports.[31]

Exercise load has previously been correlated with Δ cTnT.[8,10] Similarly, players in this study who covered larger distances, ran at a higher speed, or performed at higher HR were those who presented a higher Δ cTnT (**Table 7**). Out of interest, while Δ cTnT was not different between groups, adults played at a higher speed than children and, curiously, speed was indeed positively associated with Δ cTnT (see **Table 5**). In this regard, although adults are expected to run faster given their anthropometric and structural advantages, this inconsistency suggests a potential interaction between participants' age and exercise load that should be further investigated in future studies. We also found that the players with higher baseline cTnT concentrations were those who, during the match, performed at a higher relative HR (% HRmax); however, this association was not true for absolute HR (bpm). By contrast, the exercise-induced elevations of cTnT correlated with absolute, but not relative, HR (see **Table 7**). On the one hand, since HRmax was age-calculated, the baseline differences might be originate from the positive correlation found between basal cTnT and age. On the other hand, although previous research was inconclusive regarding the association between exercise HR and Δ cTnT,[3–5,28,31] we found higher releases in participants achieving higher absolute heart rates. This finding adds to previous knowledge, supporting the idea that exercise load metrics, such as those recorded in training watches or smartphone apps, might be consulted to identify physiological elevations of cTnT related to exercise.

The strengths of the present study were that it is the first study to report values of cTnT after a football 7 match in both healthy children and adults. To the best of our knowledge, this is also the first study comparing adults with children instead of adolescents. In this regard, our results show the need for future studies assessing whether the cTnT elevation after exercise differs between children and adults. In this study, we included a convenience sample of male football players, and this implied a small sample size, particularly in the adult group. Additionally, other factors such as dietary intake or body composition were not assessed in this study.[32] Although the sample size in this study allowed us to demonstrate the elevation of cTnT after a football 7 match in children and adult players, larger numbers would have permitted us to investigate the interaction and partial correlations between cTnT elevations and other characteristics, including performance and lifestyle variables. Another limitation was that we could not incorporate a third blood sample at 24 h after exercise and, hence, we had no data to confirm whether all participants returned to baseline concentrations during the subsequent day, as has been reported in previous studies.[3,4] For these reasons, future studies should address our limitations by encompassing larger samples, wider ranges of age, and a more exhaustive set of blood measurements. Finally, in this study we only measured cTnT, and other biomarkers such as cTnI or NT-proBNP might be used in future studies to provide a more complete overview of the phenomenon.

4.5 Conclusions

In conclusion, this is the first study proving that a football 7 match is enough stimulus to induce elevations of cTnT, exceeding the URL in ~70% of a healthy cohort of participants. Furthermore, adults had higher basal concentrations, although the magnitude of cTnT elevations was comparable between children and adults. Finally, it seems that that the elevations of cTnT could be associated to exercise load but not to participant characteristics.

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CHAPTER 5

Exercise-Induced Release of cTnT and Maturational Stage

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

Rafel Cirer-Sastre, Alejandro Legaz-Arrese, Francisco Corbi, Isaac López-Laval, Keith George, and Joaquín Reverter-Masià

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

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

Results: Basal cTnT was higher in Tanner-V (3.8–8.1 ng/L) compared with I (1.5–5.5 ng/L, $P < .001$), II (1.5–4.5 ng/L, $P < .001$) and III (1.5–6.8 ng/L, $P = .003$), and in IV (1.5–6.3 ng/L) compared with II ($P = .036$). Maximal elevations of cTnT from baseline were notable ($P < .001$) and comparable among maturational stages ($P = .078$). The upper reference limit for myocardial injury was exceeded in 35.7% of the participants, without differences among groups ($P = .18$). Baseline cTnT correlated with participant characteristics, and maximal cTnT elevations from baseline with exercise internal load (%HRpeak, $r_s = 0.34$, $P = .003$; %HRmean, $r_s = 0.28$, $P = .02$).

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

5.1 Introduction

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

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

It has been hypothesized that the higher elevations of cTn in young athletes might be related to maturation, since the myocardium might be more vulnerable to injury in clinical situations during its development.[9,10] In this regard, Tanner stages are a commonly used criteria based on genitalia assessment that allows participant classification in five maturational categories (I to V).[11] To the best of our knowledge, only three studies compared cTnT in children and/or adolescents with indicators of maturational status.[7,9,12] Further, when comparing baseline cTnT, none of these studies found significant group differences among maturational stages III-IV, II-IV and III-V.[7,9,12] In addition, all three coincided that exercise induces significant elevations of cTnT. However, whilst two of them did not report group differences among maturational stages in terms of peak post-exercise concentrations (stages III-IV),[9] or its maximal elevation from baseline (stages III-V),[7] the third found a positive association between maturational stages II-IV and the maximal cTnT elevation from baseline.[12] Discrepancies in these studies could be explained by differences between exercise exposures, or small sample sizes encompassing narrow ranges of maturational stages.

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

our hypothesis was that the magnitude of cTnT elevations would be influenced by myocardial development, and result inversely associated with participants maturity, with higher elevations in the less mature athletes.

5.2 Methods

This study met the principles of the latest revision of the Declaration of Helsinki,[13] and was approved by the Ethical Committee of Clinical Research of Sports Administration of Catalonia (02/2018/CEICGC). The funders of this research had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to submit the paper for publication.

Young male swimmers (age range from 6 to 18 years) were invited to participate in this study between April and May, 2018. Six local swimming clubs collaborated by inviting parents to answer an online questionnaire containing the Spanish version of the revised Physical Activity Readiness Questionnaire (PAR-Q), training history, and a self-assessment of pubertal maturation.[15] Inclusion criteria were: favorable PAR-Q, male, aged under 19 years, 3 years of experience in competitive swimming or more, and weekly training volume of 3 days/week or more. Parents of potentially eligible swimmers were then informed of the study and invited to participate. Seventy apparently healthy swimmers and their respective parents agreed to participate, and signed an informed consent form prior to the intervention (**Table 8**). No exclusions were made and all participants completed the study.

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

Participants were measured dry and wearing swimming clothes. Body mass was measured with a medical scale (SECA 711, Hamburg, Germany) and height with a wall stadiometer (Año-Sayol, Barcelona, Spain). Standard, 12-led ECG were recorded at the beginning of the study using a digital electrocardiograph (Click ECG BT 12 channel, Milano, Italy).

Table 8a | Summary of participant characteristics by 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 [7,12]	<IV, V	11 ± 2 [8,14]	<V	12 ± 2 [9,14]	<V	14 ± 3 [5,18]	>I	15 ± 1 [13,17]	>I, II,III
Body Height (cm)	139,8 ± 12,2 [122, 157,5]	< III, IV, V	147,8 ± 11,8 [125, 163]	< IV, V	155,2 ± 12,2 [127, 175]	> I < IV, V	168,8 ± 9 [151, 182]	> I, II, III	173,7 ± 6,9 [165, 187]	> I, II, III
Body mass (kg)	34,4 ± 9,3 [23,6, 50,4]	< IV, V	41,8 ± 10,8 [25,1, 56,9]	< IV, V	46,2 ± 11,9 [23,7, 64,5]	< V	57,8 ± 9,8 [40,5, 77,8]	> I, II	60,8 ± 8 [50,1, 77,8]	> I, II, III
BMI (kg/m^2)	17,3 ± 2,2 [14, 21,2]	< IV, V	18,8 ± 3 [14,2, 24,4]		18,9 ± 3,5 [14,7, 26]		20,2 ± 2,2 [16,9, 24,3]	> I	20,1 ± 2,1 [17,5, 24]	> I
Experience (years)	4 ± 2 [1, 6]	< IV, V	5 ± 2 [2, 10]		4 ± 3 [1, 11]		6 ± 2 [2, 11]	> I	7 ± 2 [2, 11]	> I
Training (days/week)	4 ± 1 [2, 5]	< IV, V	4 ± 1 [3, 5]	< IV	4 ± 1 [3, 6]		5 ± 1 [5, 6]	> I, II	5 ± 1 [3, 6]	> I
Training (h/week)	6 ± 5 [2, 15]	< IV	7 ± 3 [3, 15]	< IV	8 ± 4 [5, 15]		12 ± 3 [8, 15]	> I II	10 ± 3 [5, 15]	
HR max (bpm)	186 ± 7 [175, 195]	< V	189 ± 5 [179, 203]	< V	190 ± 9 [165, 201]		192 ± 5 [185, 201]	< V	198 ± 4 [188, 201]	> I, II, IV

Table 8b | Summary of exercise load data by maturational stage.

	Tanner - I (n = 14)		Tanner - II (n = 15)		Tanner - III (n = 15)	Tanner - IV (n = 13)		Tanner - V (n = 13)
Exercise load								
Distance (m)	1446 ± 409 [650, 2500]	< IV	1533 ± 338 [1000, 2000]	< IV	1583 ± 367 [1000, 2500]	1958 ± 296 [1250, 2500]	> 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 [-91, -39]		-67 ± 12 [-84, -41]		-62 ± 12 [-77, -38]	-63 ± 8 [-75, -50]		-60 ± 7 [-70, -50]

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

Recordings were obtained with the swimmer in supine position during quiet respiration, after a short period of rest. ECG were assessed in situ by experienced medical personnel, and compared against the international ECG criteria for sports screening.[16] Two experienced pediatricians assessed participants' pubertal status. Genitalia and pubic hair were observed in the presence of parents and swimmers were classified according to the five-stage criteria described by Tanner.[11] Pediatricians were unaware of self-assessment scores, and blinded from each other except in case of disagreements that were resolved by consensus. Pediatricians classification was used in the statistical analysis.

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

Blood samples were drawn from an antecubital vein by an experienced pediatric nurse at Pre, Post 0h, and Post 3 h. This timing was elected since previous studies reported peak post-exercise cTnT concentrations to occur at 3-4h after exercise.[7,18] Samples were quickly centrifuged and stored at -80^{o}C for later analysis. Serum cardiac troponin T (cTnT) was analyzed using the Troponin T hs STAT immunoassay in a Cobas E 601 analyzer (Roche Diagnostics, Penzberg, Germany). This assay ranges from 3 to 10000 ng/L, and the intra-assay coefficient of variation at a mean cTnT of 13.5 ng/L is 5.2%. Precision was determined by two cycles daily in duplicate, each for 21 day. Before the assays were performed, the analyzers were calibrated with standard calibrators according to the manufacturer recommended protocols. The upper reference limit (URL) for cTnT, defined as the 99th percentile of healthy participants was 13.5 ng/L.[19] Concentrations below the limit of detection (LoD) of 3 ng/L were set to 1.5 ng/L for statistical analyses.[20]

The distance-trial test was preceded by a self-paced 5-minute warm-up (<60% of HR max), and consisted in covering the maximum possible distance in 30 minutes at a uniform, continuous pace. The duration for this test was based on previous studies reporting that an exercise bout 30 min can induce an elevation of cTn.[18,21] Furthermore, previous studies reported elevations of cTnT in adolescent swimmers,[7] and demonstrated that cTn concentrations after swimming are comparable with those after running or cycling.[22] For these reasons, and its high participation at early ages, we elected swimming as the exercise mode

for this study. Participants had been previously familiarized with distance-trial tests of similar durations, and were instructed as well as verbally encouraged by researchers and their coaches to cover their maximum possible distance during the test. All participants completed the test in the same facilities under standardized, constant conditions (25 m indoor swimming pool, water temperature 28 ºC, air temperature 29 ºC, relative humidity 65%). Heart rate was monitored using Polar OH1™ sensors, and video recordings were used to calculate swimming distances. Participants were allowed to drink water at libidum before and after exercise. Immediately after exercise, participants reported their rating of perceived exertion (RPE) in a 0-100 scale.[23]

Dependent variables in this study were cTnT concentrations (ng/L) at Pre, Post 0 h, and Post 3 h, and its derived changes Δ Post 0 h (Post 0 h – Pre), and Δ Post 3 h (Post 3 h – Pre). The main independent variables were maturational stage (five groups from Tanner I to V), cTnT detection (non-detected when cTnT was under the LoD in all three measurements, detected when cTnT was detected in one or more measurements) and responsiveness (non-responders, when cTnT elevations did not exceed the URL in any measurement, responders when one or more cTnT measurements exceeded the URL). Secondary independent variables were participant characteristics [age (years), body height (cm), body mass (kg), body mass index(kg/m^2), training experience (years), frequency (days/week), volume (h/week), and HR max (bpm)], and exercise load during the test [distance (m), mean relative HR (% HR max), peak relative HR (% HR max), RPE (1-100), 1 min recovery HR (bpm) and 3 min recovery HR (bpm)]. Kolgomorov-Srmirnov test was used to verify that all variables were normally distributed except cTnT data, that were right skewed and non-transformable. All variables were presented as mean ± standard deviation, or median [interquartile range], according to the normality of the data.

Participant characteristics and exercise load were tested for main differences among maturational stages (Tanner I to V) using one-way analysis of variance. Post-hoc pairwise comparisons between maturational stages when main differences were statistically significant, and differences associated to cTnT detection (detected vs non-detected) and responsiveness (responder vs non-responder) were tested using t-tests for independent samples (**Tables 8** and **9**). Main cTnT differences over time (Pre, Post 0 h, and Post 3 h) were tested using Friedman tests for repeated measures. When these main differences were statistically significant, changes in cTnT (Δ Post 0 h and Δ Post 3 h) were tested using Wilcoxon signed rank tests. Main cTnT differences among maturational stages (Tanner I to V) were tested using Kruskal-Wallis rank sum tests. Post-hoc pairwise comparisons between maturational stages when main differences were statistically significant were made using Wilcoxon rank sum tests (**Table 10**). The rate of cTnT detection, and responders was compared among maturational groups using generalized mixed effects models for the binomial family.

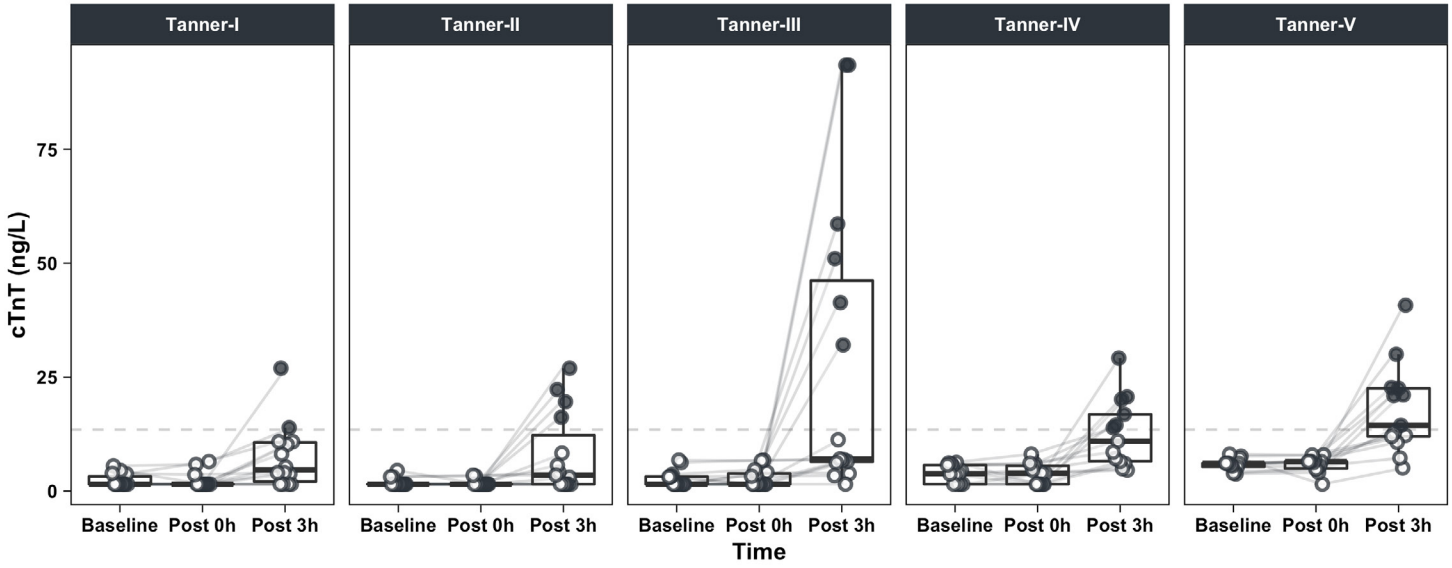
Correlations between basal cTnT (Pre), cTnT changes (Δ Post 0 h and Δ Post 3 h), maturational stage, participant characteristics and exercise load were assessed using Spearman's correlation coefficients (r_s) (**Table 11**). All statistical analyses were done using R v3.5.3. Statistical significance was assumed when $P < .05$, and Bonferroni corrections were applied when appropriate.

5.3 Results

Participant characteristics in terms of age, body height and mass, BMI, training experience, frequency and volume, and HR max in the first visit, and swimming distance during the test were different among maturational stages. However, grouping participants by maturational stage did not reveal differences in % HR peak , % HR mean, RPE, HRR at 1 min, and HRR at 3 min during the distance-trial (**Table 8**).

Fifty-nine participants had detectable cTnT at some measurement (detected) whereas 11 had cTnT < LoD in all measurements (non-detected). Further, cTnT was detected in 35 (50%), 30 (42.9%), and 59 (84.3%) participants at Pre, Post 0 h and Post 3 h, respectively. Age, body height, HR max, % HR peak, and % HR mean were lower in the non-detected (**Table 9**). Peak cTnT concentrations were observed at Post 3 h in all detected cases. Whilst immediate changes in cTnT (Δ Post 0 h) were not conclusive, elevations after 3 h (Δ Post 3 h) were statistically significant. At baseline (Pre), participants in Tanner-V presented higher cTnT than those in I ($P < .001$), II ($P < .001$) and III ($P = .003$), and Tanner-IV higher than those in II ($P = .036$). Then, immediately after exercise (Post 0 h) Tanner-V had also higher cTnT than I ($P = .002$), II ($P < .001$) and III ($P = .017$). Peak concentrations (Post 3 h) were only higher in Tanner-V compared with I ($P = .024$) (**Figure 9**). Furthermore, immediate cTnT changes (Δ Post 0 h) were higher in Tanner-V compared with II ($P = .016$), but Δ Post 3 h was comparable among groups ($P = .078$). Higher cTnT at Pre was associated to higher cTnT at Post 0 h ($r_s = 0.76$, $P < .001$) and Post 3 h ($r_s = 0.34$, $P = .004$), but was not associated to any of the changes, namely Δ Post 0 h ($r_s = 0.16$, $P = .19$) and Δ Post 3 h ($r_s = 0.11$, $P = .35$) (**Table 10**).

Figure 9 | 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.

Table 9a | Summary of participant characteristics by detection and responsiveness.

	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 [8, 14]	< D	12 ± 3 [5, 18]	> ND .024	12 ± 3 [8, 18]	12 ± 3 [5, 16]	.88
Body Height (cm)	146,3 ± 12,5 [122, 163]	< D	158,4 ± 16,3 [123, 187]	> ND .013	155,9 ± 16,6 [122, 187]	157,5 ± 16 [125, 179]	.69
Body mass (kg)	41,2 ± 11,2 [23,6, 56,9]		49 ± 14 [23,7, 77,8]	.06	47,5 ± 13,6 [23,6, 77,8]	48,3 ± 14,6 [23,7, 77,8]	.81
BMI (kg/m^2)	18,9 ± 3,1 [15,8, 24,4]		19,1 ± 2,8 [14, 26]	.88	19,1 ± 2,6 [15,6, 24,4]	19 ± 3,2 [14, 26]	.86
Experience (years)	5 ± 3 [1, 10]		5 ± 3 [1, 11]	.84	5 ± 3 [1, 11]	5 ± 3 [1, 11]	.13
Training (days/week)	4 ± 1 [2, 6]		5 ± 1 [2, 6]	.16	4 ± 1 [2, 6]	5 ± 1 [3, 6]	.14
Training (h/week)	8 ± 5 [2, 15]		9 ± 4 [2, 15]	.64	9 ± 5 [2, 15]	8 ± 3 [2, 15]	.38
HR max (bpm)	184 ± 8 [175, 203]	< D	192 ± 6 [165, 201]	> ND .006	190 ± 7 [175, 203]	192 ± 8 [165, 201]	.26

Table 9b | Summary of exercise load data by detection and responsiveness.

	Detection					Response				
	Non-detected (n=11)		Detected (n=59)		t Test *P* value	Non-responders (n=45)		Responders (n=25)		t Test *P* value
Exercise load										
Distance (m)	1418 ± 501 [650, 2500]		1715 ± 354 [1000, 2500]		.085	1665 ± 420 [650, 2500]		1676 ± 344 [1250, 2500]		.9
% HR Peak (% HR Max)	89 ± 7 [77, 103]	< D	95 ± 7 [78, 110]	> ND	.033	93 ± 6 [77, 105]		96 ± 7 [82, 110]		.088
% HR Mean (% HR Max	82 ± 8 [71, 99]	< D	88 ± 7 [69, 101]	> ND	.045	85 ± 7 [69, 99]	< R	90 ± 6 [76, 101]	> NR	.004
RPE (0-100)	79 ± 4 [75, 85]		76 ± 15 [15, 95]		.17	74 ± 16 [15, 90]	< R	81 ± 9 [50, 95]	> NR	.012
HRR at 1 min (bpm)	-40 ± 11 [-53, -19]		-43 ± 12 [-74, -13]		.49	-41 ± 12 [-65, -13]	> R	-47 ± 11 [-74, -25]	< NR	.035
HRR at 3 min (bpm)	-58 ± 15 [-80, -38]		-65 ± 10 [-91, -41]		.22	-63 ± 12 [-84, -38]		-65 ± 10 [-91, -49]		.32

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

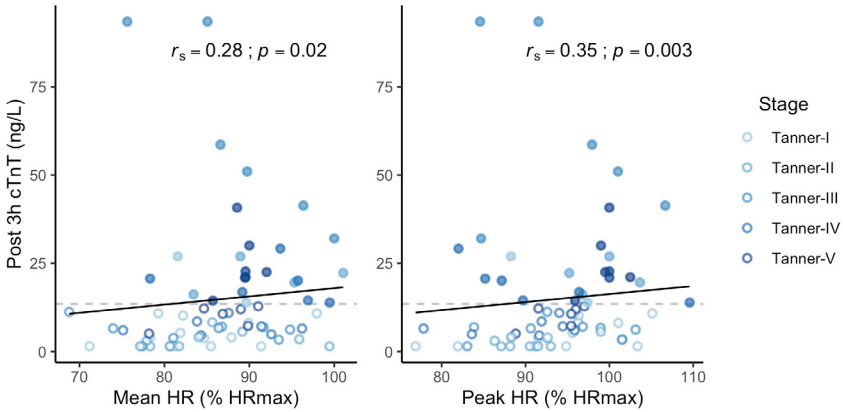
Table 10 | Summary of cTnT time and group comparisons.

	Time			Time differences			
	Pre	Post 0 h	Post 3 h	Δ Post 0 h	*P*	Δ Post 3 h	*P*
			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]	.99	3.2 [0, 22.5]	.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] $^{<V}$	.99	1 [0, 22.5]	.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]	.99	5.2 [0, 92]	.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]	.99	5 [0.2, 27.7]	< .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] $^{>II}$	.089	10.2 [-2.1, 34.7]	.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]	.26	5 [-2.1, 92]	< .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.

Higher cTnT at Pre was associated with maturational stage, age, body height and weight, training experience and frequency and HR max in the first visit, and higher distance and % HR mean during the test. Furthermore, higher immediate elevations (Δ Post 0 h) were associated with maturational stage, age, body height and mass and HR max in the first visit, as well as % HR peak and % HR mean during the test. Finally, Δ Post 3 h was positively associated with maturational stage, HR max in the first visit, % HR peak and % HR mean (**Figure 10**), and negatively associated with HRR at 1 min (**Table 11**).

Figure 10 | 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.

The incidence rate of participants with cTnT > URL (responders) was 25/70 (35.7%), in all cases at Post 3 h, without differences among maturational stages ($P = .99$) (**Table 10**). Furthermore, responders had higher % HR mean and RPE, and lower HRR at 1 min (**Table 9**). A year after the study, none of the participants reported cardiac symptoms or events subsequent to the study.

Table 11 | 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	< .001	0.32	.007	0.06	.63	0.72	< .001
Body height (cm)	0.55	< .001	0.3	.012	0.07	.55	0.78	< .001
Body mass (kg)	0.45	< .001	0.27	.023	0.02	.89	0.7	< .001
BMI (kg/m^2)	0.13	.28	0.15	.20	-0.04	.72	0.37	.002
Training experience (years)	0.27	.023	0.18	.13	-0.19	.12	0.45	< .001
Training frequency (days/week)	0.25	.038	-0.04	.72	0.07	.58	0.48	< .001
Training volume (h/week)	0.16	.19	0	.99	-0.08	.50	0.44	< .001
Maximum HR (bpm)	0.39	< .001	0.35	.003	0.37	.001	0.51	< .001
Exercise load								
Distance (m)	0.27	.025	0.22	0.064	-0.03	0.82	0.47	< .001
% Peak HR (% HR max)	0.17	.16	0.28	0.017	0.34	0.003	0.08	.53
% Mean HR (% HR max)	0.34	.004	0.24	0.047	0.28	0.020	0.22	.066
Rating of Percieved Exertion (0-100)	-0.15	.23	-0.02	0.87	0.18	0.14	0.14	.26
1 min Recovery HR (bpm)	0.18	.13	-0.05	0.67	-0.3	0.013	0.14	.26
3 min Recovery HR (bpm)	0.2	.10	0.06	0.62	-0.16	0.19	0.2	.093

5.4 Discussion

In this study, we compared the post-exercise concentrations of cTnT in a cohort of 70 young male swimmers stratified by maturational status. Our results support that a distance-trial test of 30 min continuous swimming induces an elevation of cTnT in the following hours. The main findings of this study were that: (1) Basal cTnT (Pre) in apparently healthy, trained, young males is associated to participant characteristics, and might vary among maturational stages (**Table 11**), (2) Elevations of cTnT from baseline are not conclusive immediately after exercise (Δ Post 0 h), but notable in a period of 3 h (Δ Post 3 h), without significant differences related to maturational stage (**Table 10**), (3) The incidence rate of participants with cTnT exceeding the URL (responders) following exercise was ~36%, without differences among maturational status (**Table 10**), and (4) Elevations of cTnT induced by exercise (Δ Post 0 h and Δ Post 3 h) are highly variable among participants, and partially explained by exercise load.

Resting values of cTnT in healthy athletes are normally reported below or close to the assay lower limit of detection.[5,24] It has been reported that these values might vary depending on age and sex,[25] and previously suggested the need to report on age- and sex-specific population cTn values in children and adolescents.[26] In this regard, previous studies found similar baseline concentrations of cTn between adolescent and adult athletes,[7,8,27] and among adolescent athletes at different maturational stages.[7,9,12] However, in the present study we found that swimmers at Tanner-V had higher resting cTnT than those in I -III, and swimmers in Tanner-IV higher than those in II (**Table 10**), suggesting that normal values might be higher during late-puberty. Furthermore, cTnT at Pre was positively associated with age, body height and mass, training experience and frequency, and HR max (**Table 11**). On this subject, these results coincide with previous studies suggesting that athletic status may be one of the factors that determine the heterogeneity in baseline cTn values.[18,28] Although speculative, the greater training experience, frequency and HR max of swimmers in late-puberty could explain their higher cTnT at Pre. Furthermore, this is also supported by the higher distances and % HR mean achieved in participants with higher basal values. However, further research is still needed to confirm this hypothesis.

Concentrations of cTnT immediately after exercise were lower than those at 3 h post-exercise. This coincides with the cTn kinetics reported in previous studies,[7,18] and could be compatible with the theory of a transient reduction in cTn clearance during exercise, combined with a transient release of unbound cTn during and after exercise originated from reversible cell wall injury.[29] In the group comparisons, we found that absolute peak cTnT (Post 3 h) was higher in Tanner V compared with I (**Table 8**). However, besides this absolute difference, and in line with previous studies,[7,9] maximal cTnT elevations (Δ Post 3 h) were comparable among all stages. This finding, is contrary to our initial hypothesis, and supports that exercise-induced elevations of cTnT might not be influenced by myocardial development.

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

Although we could reproduce the exercise-induced elevations of cTnT demonstrated in previous studies, they were highly variable among individuals. On the one hand, 11 (16%) participants in this study had cTnT < LoD in all Pre and Post measurements (non-detected). Pairwise comparisons revealed that these participants were younger, had lower HR max, and achieved lower % HR peak and % HR mean during the distance trial (**Table 9**). On the other hand, 25 participants (incidence rate = 36%) had cTnT > URL at Post 3 h (responders). This incidence rate was lower than the ~62% reported by Legaz-Arrese, et al. ($X^2 = 8.5$, $P = .003$).[7] Even though both studies were conducted in young swimmers, participants in Legaz-Arrese et al. were required to swim twice the duration than ours, and this might explain the difference between incidence rates. In this line, the group of responders where those who achieved higher exercise internal load in terms of % HR mean and RPE, and those with faster cardiac recovery, in terms of HRR at 1 min (**Table 9**). These differences, together with the ones found in the group of non-detected, coincide with previous findings, suggesting that the highest cTnT elevations (both, Δ Post 0h and Δ Post 3h) occur in better trained athletes (experience, HR max),[18,28] that achieve higher exercise internal loads (% HR peak and % HR mean) during the test.[4,27] This suggests that not only maturation but also other factors affect the magnitude of increase in cTnT after intense exercise. In spite of that, training status and exercise load could only partially explain the high variability in the exercise-induced elevation of cTnT. Thus, to the authors' opinion there might be other, still unknown, individual factors influencing pre- and post-exercise cTnT variability, that might be explored in future research.

Current cut-off values for cTn are taken from adult populations.[30] It has been suggested that reference values of cTn might variate with maturational and training status.[9,31,32] Our results support previous data, however, further research is needed to confirm both hypothesis and provide, if needed, specific population reference values including younger participants and differentiating for fitness level. A year after this study, a telephonic follow-up survey confirmed that all participants continued their training routine after the study, and none of them had cardiac symptoms or events. Previous studies also reported an absence of clinical

signs or symptoms during a follow-up period.[32,33] The high incidence of elevated cTn after exercise in healthy athletes, its reproducibility, and the absence of clinical signs or symptoms in a 1-year follow-up period could suggest that cTnT elevations are inherent to exercise, and probably related to a physiological response to exercise. It has been suggested that exercise may induce transient troponin elevations attributable to transient increases in cardiomyocyte membrane permeability,[34] although this requires empirical support. However, our results confirm previous findings showing that there is a high variability between subjects in the release of troponin with exercise that the scientific literature has not been fully able to explain by individual and exercise characteristics,[7,12] and if this could be associated with clinical repercussions in the future is an aspect of interest that is being debated.[35] Our results might be considered in clinical settings when interpreting cTnT values in young, physically active populations, especially in the hours subsequent to exercise.

Since previous studies confirmed that peak concentrations occur typically at 3-4h after exercise,[9,20] and with the aim to minimize the number of blood extractions, we did not perform serial measurements during this recovery. However, the limited sampling points in our design imply a potential under-estimation error in the peak cTnT concentrations, as has been previously suggested by others.[31] In addition, cTnT measurements were not performed in duplicate precluding any intra-assay control beyond the calibration and precision calculations recommended by the manufacturer. For this reasons, future research including serial cTnT measurements with duplicates will allow for a more precise time-to-peak comparisons among maturational stages. The distance-trial test performed in this study was partly elected for its similarity with the real effort performed by participants during their trainings and competitions. However, this could have implied differences in relative intensity between swimmers, that could be solved in future studies using other methods to control the relative intensity during exercise. We confirmed that a release of cTnT is inherent to exercise, however, individual variability could only be explained by some variables such as exercise load. 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, concretely 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,[7,9,12] the authors acknowledge that sample size was small. In this regard, previous research supported that the physiological response to exercise might differ between male and female adolescents.[36] For this reasons, future research should address our limitations by controlling peak height velocity,[37] and including larger samples of both, male and female athletes. Finally, in this study we only measured cTnT, and other biomarkers such as cTnI or NT-proBNP might be used in future studies to provide a more complete overview of the phenomenon.

5.5 Conclusion

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

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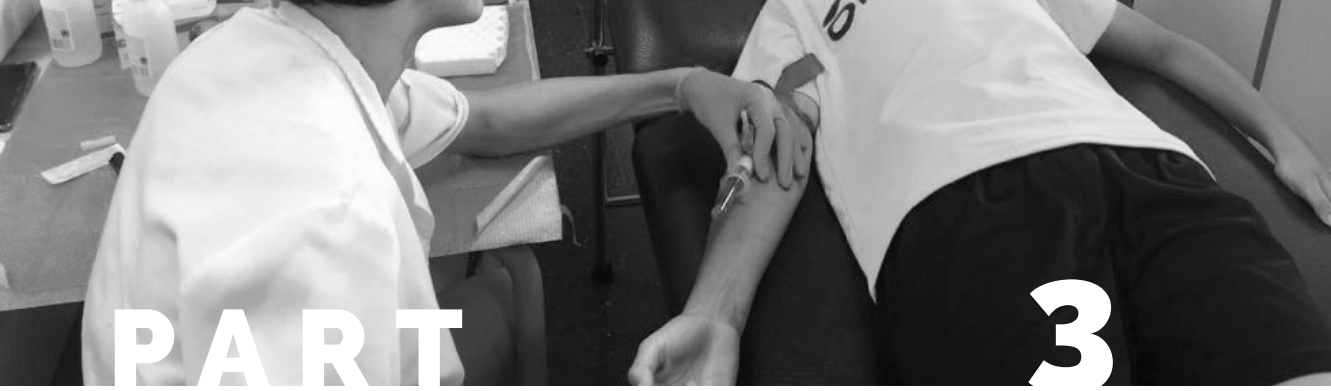

PART 3

Chapter 6 - Summary of Findings

Chapter 7 - General Discussion

Chapter 8 - Conclusions

CHAPTER 6

Summary of Findings

Summary of Findings

Part I described the state of the art in pediatric, exercise-induced elevations of cardiac biomarkers, and discussed the main points of discrepancy within the existing research on cardiac troponins.

Chapter 1, introduced this thesis and presented a brief review of the literature, highlighting the main questions and variables of interest for this work. Concretely, previous studies had observed a more variable cTn response to exercise in adolescent athletes when compared with adults that might be related to maturity. However, data on adolescents and specially children were still scarce and comparisons accounting for participants' maturational stage had not been conclusive. This led to the establishment of **Objective 1**: to describe the changes in serum cTnT related to exercise in healthy trained prepubertal and pubertal males, followed by **Objective 2**: to assess the effect of biological maturity on the changes in serum cTnT related to exercise in healthy trained males. Additionally, it was unclear whether the associations between exercise load and the subsequent elevations of cTn in young athletes were the same than those previously described in adult athletes. Again, it was hypothesized that myocardial immaturity might interact with exercise load when explaining the subsequent elevation of cTn. This led then to the formulation of **Objective 3**: to relate exercise load with the subsequent changes in serum cTnT in healthy trained prepubertal and pubertal males.

Chapter 2 was a systematic review and meta-analysis on the cardiac biomarker release after exercise in healthy children and adolescents. Fourteen studies with a pooled sample of 336 participants (21% girls) that comprised 21 experimental groups were included, providing data on cTnT (18 groups), cTnI (7 groups) and NT-proBNP (6 groups) concentrations in athletes under the age of 18 after exercise. The main results of this work were: (1) The estimated pooled incidence rates for athletes exceeding the URL for myocardial injury in the hours after exercise were 76%, 51% and 13% for cTnT, cTnI and NT-proBNP, respectively; (2) Biomarker elevations were heterogeneous among exercise modes for cTnT and NT-proBNP but n"ot for cTnI; (3) Multiple regression showed that cTnT heterogeneity was mediated by a negative effect of age and VO2 max, and a positive effect of exercise duration and intensity; (4) Heterogeneity for cTnI could not be explained by any variable; and (5) Heterogeneity of NT-proBNP was only explained by exercise duration.

Part II presented a series of three quasi-experimental, cross-sectional studies to analyze the influence of exercise load and maturational stage in the exercise-induced elevations of cTnT.

Chapter 3, aimed to establish the associations between metrics of exercise internal and external load (IL and EL, respectively) and the subsequent elevation of cTnT. To this end, a cohort of 20 young male soccer players (age = 11.9 (2) years) was monitored during a 5 x 5 small-sided game, and cTnT serum concentrations were determined before,

immediately after and 3 h after the exercise. The main results in this study were: (1) The URL for myocardial injury was exceeded by 4/20 (20%) players at 3h post-exercise; (2) Elevations of cTnT were positively associated with age and maturational stage; (3) The only metric of exercise internal load that was associated with cTnT elevations was time at maximal heart rate zone (Time HR-Z5), but coefficients for average heart rate (HR av), average relative heart rate (rHR av), peak heart rate (HR peak), peak relative heart rate (rHR peak), heart rate decrease in one minute of recovery (ΔHR 1 min recovery) and rating of perceived exertion (RPE) were improbable; (4) Elevations of cTnT were associated with external load in terms of absolute and relative distance (Dist abs and Dist rel, respectively), and average and peak speed (Speed aver and Speed peak, respectively) but not with time and distance at maximal speed zone (Time Speed-Z4 and Dist Speed-Z4, respectively).

Chapter 4 compared the elevation of cTnT after a football 7 match between two cohorts of children (n = 24; age =10.7 (1.6) years) and adult (n = 12; age = 37.5 (12.7) years) male players. Participants played a 7 × 7 game of two parts separated by a half time of 10 min, consisting each of two quarters each of 15 min with a 2 min break. Blood samples were obtained before and at 3 hours post exercise for posterior cTnT determination. The main findings of this study were: (1) Seventeen out of twenty-four (71%) children and 8/12 (67%) adults exceeded the URL for cTnT at 3 h post exercise; (2) Basal concentrations of cTnT were slightly lower in the children group than in the adults, and correlated positively with age; (3) Post-exercise cTnT was comparable between children and adults; (4) Whilst adults run at higher mean and peak speeds (SPmean and SPpeak, respectively), children reached higher peak heart rates (HRpeak), however, there were no group differences in distance, peak relative heart rate (rHRpeak), mean heart rate (HRmean) and mean relative heart rate (rHRmean); and (5) Although elevations of cTnT correlated positively with distance, SPmean, HRpeak and HRmean, we found no associations with SPpeak, rHRpeak and rHRmean.

Chapter 5 aimed to compare the exercise-induced elevations of cTnT among 70 male young swimmers (age range 7-18 years), that were classified according to pubertal Tanners' stages (I = 14, II = 15, III = 15, IV = 13, and V = 13). Participants underwent a distance-trial of 30 min continuous swimming, and cTnT was measured before, immediately after and 3 h after exercise. (1) Basal cTnT (Pre) in apparently healthy, trained, young males was associated to participant characteristics, and might vary among maturational stages, (2) Elevations of cTnT from baseline were not conclusive immediately after exercise (Δ Post 0 h), but notable in a period of 3 h (Δ Post 3 h), without significant differences related to maturational stage, (3) The incidence rate of participants with cTnT exceeding the URL (responders) following exercise was 36%, without differences among maturational status, and (4) Elevations of cTnT induced by exercise (Δ Post 0 h and Δ Post 3 h) were highly variable among participants, and partially explained by exercise load.

CHAPTER 7

General Discussion

General Discussion

7.1 Changes in serum cTnT related to exercise

7.1.1 Prevalence of cTnT

Most of the current population reference values for cTnT have been established for adult Caucasians and are sex-specific.[1] However, although differences related to age have been reported, the current reference values are still not age-specific. In addition, the specific effects of age and sex in pediatric populations are still under research.[2,3] In this regard, the studies conducted in this thesis might contribute to highlighting the relevance of specific pediatric reference values of cTn and, consequently, the future establishment of pediatric values of reference will improve the diagnosis and monitoring of pediatric heart disease.[2]

On the one hand, in the experimental studies (**Chapters 3, 4,** and **5**), an overall count of 52/114 (54%) prepubertal and pubertal participants had baseline cTnT concentrations below the LoD of the assay. This data is relevant since, a priori, high-sensitivity assays should be able to detect cTn concentrations in at least a 50% of a healthy population, and in this thesis, it was not fulfilled. In the three experimental studies, we used the same Troponin T hs STAT immunoassay in a Cobas E 601 analyzer (Roche Diagnostics, Penzberg, Germany). According to the manufacturer specifications, it has a LoD of 3 ng/L, and met the high-sensitivity criteria based in a population of 533 adults aged from 20 to 71 years.[4] Notwithstanding that, ours are not the first studies reporting similar, low rates of detection that suggest a lower sensitivity of cTnT assays when used for pediatric populations.[5,6]

On the other hand, in **Chapter 2** and based on a pooled sample of 256 participants proceeding from 16 meta-analytic groups, we estimated a 95% CI for the population baseline concentration of cTnT from 4 ng/L to 6 ng/L. This interval was relatively accurate and low, close to the typical LoD for most of the assays.[4] In the experimental studies, the median cTnT concentrations at baseline were 1.5 ng/L, 4.64 ng/L, and 1.5 ng/L, for **Chapters 3, 4**, and **5**, respectively. This data coincides with the low rate of detection at baseline, and suggest that pediatric populations might present lower resting values. Notwithstanding that, according to the IFCC Task Force on Clinical Applications of Cardiac Biomarkers, a minimum sample size of 300 healthy individuals would be needed to establish population reference values.[7] Moreover, in this thesis we only focused on male athletes and pediatric population reference values are thought to vary between male and female.[1] So, as a consequence, this minimum sample size to establish population reference values would double, with a minimum of 300 per sex.[8,9] For these reasons, our results should not be accounted as reference values, but might justify and preclude larger, future studies aiming to establish these pediatric population reference values, achieving the minimum sample size needed.

7.1.2 Elevation of cTnT induced by exercise

The results of this thesis confirm that common bouts of exercise, such as a soccer match or swimming at high intensity for 30 min, are enough stimulus to elicit elevations of serum cTnT. In the three experimental studies, peak concentrations were found at 3 hours post exercise, as predicted in **Chapter 2** and previously reported in earlier studies.[10] Additionally, in **Chapter 2** we estimated a 95% CI for the exercise-induced elevations of cTnT from 79 ng/L to 198 ng/L. By contrast, however, the median elevations of cTnT found in **Chapters 3, 4**, and **5** were 5.89 ng/L, 16.4 ng/L, and 5 ng/L, respectively. These elevations were lower than the predicted in **Chapter 2** and this might be related to methodological differences among participants and interventions. Moreover, we observed a high individual variability in the experimental chapters that coincides with previous studies,[11] suggesting that other variables such as participants characteristics or exercise load might influence the elevation.

The results of this thesis confirm that serum cTnT elevations are inherent to physical exercise, not only in adults but also in prepubertal and pubertal athletes. In addition, **Chapter 3** proved that cTnT post-exercise kinetics in prepubertal athletes might be comparable with the observed in adults.[11,12] These results, have an important clinical implication since they prove that healthy young athletes might present cTnT elevations in the hours subsequent to exercise. Concretely, according to our results, repeated blood samples within a window of 3 hours after exercise might reflect a raise in cTnT, often exceeding the clinical cut-off values, that if combined with chest pain might mimic cardiac injury. However, according to results in **Chapter 2** and also previous studies,[11,12] after a peak concentration at approximately 3h post exercise, serum cTnT decreases returning to baseline values in a period of approximately 24 hours. This implies that cTnT elevations related to exercise might be easily discarded by measuring its concentration after a relatively short clearance period.

7.1.3 Incidence of cTnT above the URL

One of the aspects that make exercise-induced elevations of cTnT relevant is that concentrations often exceed the URL for myocardial injury. Concretely, in **Chapter 2** we estimated a 95% CI for the pooled incidence rate from 66% to 87%. Then, in the experimental studies, we found rates of positive cTnT of 20%, 71% and 36%, for **Chapters 3, 4**, and **5**, respectively. Besides that, in the experimental studies we only included apparently healthy participants reporting no history of personal nor familiar CVD and at the beginning of the interventions, none of them had ECG abnormalities. In addition, a year after the interventions, participants' parents were interviewed and none of them reported cardiac complications in that period. This, together with the consistent return to basal cTnT values within the subsequent 24 h, reinforces previous hypothesis that cTnT elevations are a transient and physiological response to exercise.

7.2 Effect of biological maturity

7.2.1 Prevalence of cTnT among maturational stages

Previous studies reported that pediatric population values for cTn might increase with age.[3,13] Accordingly, in **Chapter 4** we also calculated a positive association between age and baseline cTnT ($r_s = 0.67$). Furthermore, in **Chapter 5**, this association ($r_s = 0.46$) was not only confirmed, but we also found that swimmers at Tanner-V had higher resting cTnT than those in I -III, and swimmers in Tanner-IV higher than those in II. These findings contribute to a better description of how basal values might vary through age and, more importantly, evidence the need to stratify population reference values by age or maturation. Moreover, this aligns with previous authors who already discussed the need for pediatric-specific population values of reference.[2,3] Besides that, according to results in **Chapter 4**, adults might present higher basal values than children. As suggested previously, this difference might be attributed to a higher risk of concealed CVD that increases with age.[14,15]

Additionally, the results of this thesis also support the advantages of using physiological maturity instead of chronological age as a variable of reference for growth in prepubertal and pubertal athletes.[16] In this regard, we acknowledge that, from a practical point of view, age might be a variable easier and faster to measure. However, from a physiological perspective it might be inaccurate, specially in pediatric populations. By contrast, indicators of maturity such as the Tanner scale used in this thesis, might better reflect the biological growth of the athlete, and specifically the growth of the heart.[16]

As mentioned earlier, in **Chapter 5** we found that basal concentrations of cTnT were higher in Tanner stages IV and V, and correlated positively with age, body mass and height, suggesting that normal values might be higher during late puberty. In this regard, previous research suggested that athletic status might partially determine the heterogeneity in baseline cTn values.[17,18] Thus, although speculative, the greater training experience in late puberty athletes could explain their higher baseline values of cTnT. Notwithstanding that, the interaction between training experience and pubertal stage when explaining normal cTnT values has yet to be modeled. In this regard, further research is still needed to confirm this hypothesis and provide, if needed, specific population reference values including younger participants and differentiating for levels of physical activity.

7.2.2 Changes in serum cTnT related to exercise among maturational stages

Earlier studies were controversial regarding the influence of maturation on the exercise-induced elevations of cTnT. Whilst Tian et al. suggested that basketball players at Tanner stage II might reach higher peaks of cTnT than those at Tanner stage III,[19] Legaz-Arrese et al. found no cTnT elevation differences among swimmers at Tanner stages III, IV and V.[20] A repeated hypothesis for the higher elevations in adolescents when compared with adults was a role of maturity mediating the post-exercise release of cTnT, with the less mature myocardium releasing higher quantities of cTnT as a consequence of exercise. Results of **Chapter 2**, were based on previous research and favored this hypothesis, revealing a negative association ($P < .001$) between age and the exercise-induced elevations of cTnT. In **Chapter 3**, by contrast, we found that both, age and maturational stage, were positively associated with the elevation of cTnT. As a consequence, **Chapter 5** aimed explicitly to better describe this association. Interestingly, we found that absolute post-exercise cTnT (Post 3 h) was higher in the late-puberty, however, and in line with some previous studies,[19,21] maximal cTnT elevations (Δ Post 3 h) were comparable among all stages.

Findings in **Chapter 5**, then, coincide with those in **Chapter 4**, supporting that exercise-induced elevations of cTnT might not be influenced by myocardial development. Thus, discrepancies among previous studies, might be explained by small sample sizes and other methodological differences derived from participants characteristics and exercise load. In this regard, previous cross-sectional and longitudinal studies demonstrated that basal cTn is a strong predictor for post-exercise concentrations.[17,18] Likewise, in **Chapter 5** cTnT at Pre was associated with absolute cTnT concentrations (Post 0 h and Post 3 h), however it was not with baseline-normalized changes (Δ Post 0 h and Δ Post 3 h). Accordingly, group differences at Pre could explain the higher values at late-puberty in terms of absolute post-exercise cTnT but not in its baseline-normalized changes (Δ Post 0 h and Δ Post 3 h). Simultaneously, as mentioned earlier, this might discard the hypothesis that young athletes release more cTnT after exercise as a consequence of myocardial immaturity.[22]

Additionally, in all experimental studies we found that the highest cTnT elevations (both, Δ Post 0h and Δ Post 3h) occur in better trained athletes (experience, HR max), that achieve higher exercise internal loads (% HR peak and % HR mean) during the test. This suggests that other factors beyond maturation might affect the magnitude of increase in cTnT after intense exercise. Particularly, discussed in **Section 7.2.1**, the role of training experience and fitness deserves further investigation, that might be addressed in future studies.

The results of this thesis also prove that the age-differences observed in previous studies might be dependent on the age range in the cohorts. In this regard, most of these previous studies reporting higher post-exercise cTnT variability in young athletes, compared adults with adolescents,[19,21,23] however, when we compared adults with children

instead of adolescents in **Chapter 4**, the group differences disappeared. This was confirmed later on in **Chapter 5** and explains, partially, some of the discrepancies in the previous literature. Consequently, a note of caution should be taken by future researchers attempting to investigate de effect of age in the exercise-induced elevations of cTnT. Although from a logistical point of view it might be challenging, it is still to be determined how these elevations truly differ across the entire, exhaustive range of age in the population, from children to elder.

Finally, since the individual variability in the release of cTn could not be explained by maturation itself, there might exist other variables influencing this variability. The main predictors might derive from exercise load and will be addressed in sections 7.3.1 and 7.3.2. However, we believe there are other factors, some of them unknow, that deserve further investigation and might reveal a relevant effect in the exercise-induced release of cTnT.

7.2.3 Incidence of cTnT above the URL among maturational stages

In **Chapters 4** and **5** we found that the incidence rate of athletes exceeding the URL for cTnT was independent of age and/or maturation. Furthermore, in **Chapter 5** we identified that participants exceeding the clinical cut-off values after exercise where those who achieved higher exercise internal load in terms of % HR mean and RPE, and those with faster cardiac recovery, in terms of HRR at 1 min. This suggests that high exercise internal loads, further discussed in **Section 7.3.2**, might partly explain the incidence of participants exceeding the clinical cut-off values for cTnT.

7.3 Exercise Load Changes in Serum cTnT

7.3.1 External Exercise Load and Elevation of cTnT

In **Chapter 2**, we confirmed a role of external exercise load in terms of duration, for the subsequent elevation of cTnT. However, as introduced in **Section 1.2.1**, most of the studies consisted of time trials, where shorter durations where inherently linked to higher speeds.[19,24] This proves that exercise duration, in some cases, might reflect intensity rather than volume. However, even though both, speed and heart rate, could be used to assess intensity, research is consistent in that individuals might respond with different internal loads (e.g. heart rate) to exercises with the same external load (for the sake of the example, running speed).[25,26] For these reasons, in this thesis we aimed to analyze the effect of external exercise load, since from a practical point of view, it refers to the group of metrics that coaches routinely use to prescribe training exercises.

In **Chapter 3**, we investigated the effect of external load by analyzing geospacial data, including absolute and relative distances, average and peak speed, distance covered at high-speed zone, and time spent at high-speed zone. Interestingly, these variables were the best predictors for post-exercise cTnT elevations, even ahead of exercise heart rate. Further, later on, in **Chapter 4**, we confirmed the association between external exercise load and the subsequent elevations of cTnT. In this regard, the associations found between cTnT elevations and the physical demands of an exercise would be compatible with the main hypothetical mechanisms for a reversible, physiological release of cTnT induced by exercise.[27,28]

7.3.2 Internal Exercise Load and Elevation of cTnT

The acute cardiovascular response to exercise in children is more variable than that in adults, due to anatomical, physiological, and psychological changes that occur during growth and maturation.[29] In addition, it has been suggested that immature myocardium might experience greater stress in response to exercise,[30] as a consequence of higher myocardial work load during exercise when compared with adults.[31] Furthermore, in clinical cases of myocardial injury, serum cTnT elevations are negatively associated with age.[30] For these reasons, in this thesis we hypothesized that, the cTnT response to exercise in young athletes would be strongly associated to internal exercise load.

In **Chapter 2**, we calculated a positive association between cTn elevations and average heart rate during exercise. Later on, in the experimental chapters we confirmed this association. Furthermore, **Chapter 3** revealed that the best internal load predictor for cTnT elevations was not average heart rate per se, but how it was distributed among intensity zones. Concretely, we found that the time spent in high-intensity zone was particularly relevant in explaining individual cTnT variabilities. This finding has been also confirmed in a posterior, recent study by Bjorkavoll-Bergseth, et al.[32] This is particularly relevant in the field of sport sciences since it suggests that it is not cardiac intensity but how it is distributed over time what develops myocardial stress during exercise. Moreover, from a clinical point of view, it has been suggested that there might be a cardiac intensity threshold needed to generate an exercise-induced troponin elevation.[32] Our results complement this hypothesis by incorporating a temporal determinant: it might be not the cardiac intensity an athlete achieves, but how long he/she endured that intensity.

Our results were not consistent regarding the influence of absolute and relative, peak and average heart rate during exercise. Whilst in **Chapter 3** we found unlikely associations with absolute or relative, peak or average HR, later on in **Chapter 4** absolute data for peak and average HR was positively associated with the elevations of cTnT. Furthermore, in **Chapter 5** all four forms of cardiac intensity resulted positively associated with the elevations of cTnT. Those inconsistencies might be related primarily to

the method for the establishment of maximum HR since, in **Chapters 3** and 4 HRmax was estimated from theoretical formulas whereas later on, in **Chapter 5**, it was determined in a field test. If this explanation was true, and relative metrics were truly associated to cTnT, it would support our hypothesis about a potential interaction between maturation and level of fitness, with athletes at late-puberty achieving higher relative cardiac intensities and, consequently, releasing higher quantities of cTnT.

7.4 Strengths and contributions

The main strength of this work was its contribution on exercise-induced elevations of cTnT in a target population typically difficult to access and investigate. In this regard, in **Chapter 2** we identified 10 studies providing data of cTnT, from a pooled sample of 180 male prepubertal and pubertal athletes. Excluding the Adults group in **Chapter 4** (n = 12), the three experimental studies of this thesis provided data of post-exercise cTnT from a pooled sample of 114 new male prepubertal and pubertal athletes, that is equivalent to a ~60% of the individuals studied before this thesis. Therefore, from the total, this work contributed to the knowledge with data from ~40% of the currently known cases of exercise-induced changes of cTnT in male prepubertal and pubertal athletes.

From a clinical perspective, this work was not intended to provide clinical reference values of cTnT for the targeted population. However, we provided non-existent data that confirmed the need, under debate until the moment, for specific values of reference in pediatric population, differentiated for pubertal stages and accounting for the fitness level of the individual. Moreover, our results prove that cTnT measurements in the hours after exercise might be confounded, specially in late-pubertal athletes, by a transient elevation of the biomarker as a consequence of exercise. Additionally, this elevation of cTnT seems to be closely linked to external and internal exercise load and determined by prolonged periods at high cardiac intensity.

7.5 Methodological considerations

7.5.1 Participants definition and inclusion

The experimental studies had small (**Chapter 3**, n = 20; and **Chapter 4**, n = 36) and medium (**Chapter 5**, n = 70) sample sizes, and participants were all Spanish Caucasians. Although those limitations were frequent in the studies analyzed in **Chapter 2**, we assume that this implies two main inferential restrictions: first, statistical power could not achieve the clinical standards for establishing reference values; and second, our results are geographically restricted and can only be extrapolated to other culturally similar, Mediterranean regions. Additionally, participants were limited to trained males. Whilst this helped to set a concrete target population, we assume that larger studies, including mixed samples and different levels of fitness might have led to different conclusions.

7.5.2 Procedures and comparisons

We assessed maturation based on pubic stages, but not skeletal maturity nor estimates of peak height velocity. This election was based on practical advantages of the Tanner scale in terms of time and resources needed for each assessment. However, although all three methods are valid, we acknowledge that the gold standard in the assessment of biological age is skeletal maturity based on radiographic assessments. Moreover, previous research agrees that cTnT elevations decrease during the subsequent 24 h. This was confirmed in **Chapter 2**, and consequently, a third/fourth blood drown at 24h post-exercise was omitted in the three experimental studies. Whilst this minimized the number of invasive procedures and trimmed a day the duration of the interventions, it should be assumed that the normal return to baseline was not controlled. The only post-intervention data accounted for in the experimental studies was the follow-up survey carried out a year after each intervention. As a consequence, we acknowledge that this thesis addresses only the baseline and peak elevations of cTnT but not how long these elevations took to return to baseline.

7.6 Future Perspectives and Lines of Research

In this thesis, we assessed how exercise-induced elevations of cTnT differ depending on the maturational stage of the athlete but, since all participants were well-trained and were training similar weekly training frequencies and volumes, we could not assess the potential interaction between maturation and level of training. According to our results, we hypothesized that cTnT elevations could be indeed associated with fitness and training. Future studies will address this question by including a wider range of fitness and training levels and assessing its interaction with biological maturation. Additionally, this thesis focused on male athletes. A current, parallel line of research aims to determine the effect of growth and exercise load in female athletes. Future research will include mixed samples and compare whether sex influences the exercise-induced elevations and, particularly, its associations with maturational stage and exercise load.

Additionally, most of the existing studies used longer interventions than ours, with durations often beyond 60 min. It is currently unknown though, whether shorter high-intensity exercise durations, under 30 min, induce comparable elevations of cTn.[33] Furthermore, it is still to be determined whether there exists a minimum exercise load needed to induce cTn elevations.[34] Future research might address this topic by comparing post-exercise concentrations after high-intensity but short duration bouts of exercise.

For reasons unknown, the incidence rate of positive cTnT found in **Chapter 4** was higher than the one in **Chapters 3** and **5**. This difference cannot be attributed to exercise mode since **Chapters 3** and **4** had football interventions. However, the intervention in **Chapter 4** was

competitive whereas in **Chapters 3** and **5** it was not. One explanation then might be that competition increases internal exercise load and therewith, the subsequent elevation of cTnT. Additionally, this thesis focused only on two exercise modes, namely swimming and football. According to these reasons, future studies might provide data from other, different sports, addressing the potential effect of competition or type of exercise.

In this thesis, a follow-up of the participants was made a year after the interventions. We did not identify any case of clinical complication nor cardiac symptoms in that period. However, it has been suggested that some athletes have higher risk of developing CVD at long term,[35] and that the exercise-induced elevations of cTn in adults are positively associated to future unfavorable acute cardiac injuries.[36–38] These reasons, justify future longitudinal studies to follow the cohorts of athletes participating in this thesis for a longer period. To this end, participants our studies might be followed-up in the future, in interviews at 3, 5 and 10 years.

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CHAPTER 8

Conclusions

Conclusions

Objective 1: To describe the changes in serum cTnT related to exercise in healthy trained prepubertal and pubertal males.

Subobjective 1.1: To estimate the prevalence of cTnT in healthy trained prepubertal and pubertal males.

More than a half of healthy trained prepubertal and pubertal males presented resting values of cTnT under the LoD of the assay. As a consequence, current assays might be not sensible enough to achieve the high-sensitivity criteria when used in pediatric populations. Prevalence of cTnT in healthy trained prepubertal and pubertal males was low, close to the LoD, within the 95% confidence interval of 4 ng/L to 6 ng/L.

Subobjective 1.2: To measure the elevation of cTnT induced by exercise in healthy trained prepubertal and pubertal males.

Common bouts of exercise elicit elevations of serum cTnT in healthy trained prepubertal and pubertal males. These elevations peak approximately at 3 h after exercise cessation and return to baseline values in a period of approximately 24 hours. Peak post-exercise concentrations are highly variable among individuals.

Subobjective 1.3: To estimate the incidence of healthy trained prepubertal and pubertal males with cTnT above the URL in the hours subsequent to exercise.

The incidence of healthy trained prepubertal and pubertal males exceeding the URL of cTnT at 3 h post-exercise is high. The 95% CI for this incidence in previous literature was of 66% to 87%, and the incidence rates found in the experimental studies were 20%, 71% and 36%. These results were found in apparently healthy individuals reporting ho history of personal nor familiar CVD, with normal baseline ECG who did not report cardiac complications during a 1-year follow-up period after the interventions.

Objective 2: To assess the effect of biological maturity on the changes in serum cTnT related to exercise in healthy trained males.

Subobjective 2.1: To compare the prevalence of cTnT in healthy trained prepubertal and pubertal males at different maturational stages.

Healthy trained prepubertal and pubertal males have lower prevalence of cTnT than adults. This prevalence is positively associated to maturational stage, with the highest values observed in individuals at late-puberty. Besides that, the interaction between maturational stage and athletic status is still unclear.

Subobjective 2.2: To compare the changes in serum cTnT related to exercise among healthy trained males at different maturational stages.

Exercise-induced elevations of cTnT in healthy trained prepubertal and pubertal males are not associated with maturational stage. Concretely, absolute post-exercise cTnT appears to be higher in late-pubertal healthy trained males, however, base-normalized elevations were not related to maturation. This finding discards a recurrent hypothesis that young athletes release more cTnT after exercise as a consequence of myocardial immaturity.

Subobjective 2.3: To compare the incidence of healthy trained males with cTnT above the URL in the hours subsequent to exercise among maturational stages

The incidence of healthy trained prepubertal and pubertal males exceeding the URL of cTnT at 3 h post-exercise does not depend on maturational stage.

Objective 3: To relate exercise load with the subsequent changes in serum cTnT in healthy trained prepubertal and pubertal males.

Subobjective 3.1: To determine the strength and direction of the association between external exercise load and the subsequent elevation of cTnT in healthy trained pubertal and pubertal males.

External exercise load is positively and strongly associated with the subsequent elevation of cTnT in healthy trained prepubertal and pubertal males.

Subobjective 3.2: To determine the strength and direction of the association between internal exercise load and the subsequent elevation of cTnT in healthy trained pubertal and pubertal males.

Internal exercise load is positively and strongly associated with the subsequent elevation of cTnT in healthy trained prepubertal and pubertal males. Time spent in high-intensity cardiac zone was particularly relevant in explaining individual cTnT variabilities.

APPENDIX

A1 - Coauthors

In alphabetic order

Luis Enrique Carranza-García, PhD
Facultad de Organización Deportiva
Universidad Autónoma de Nuevo León

Francisco Corbi, PhD
Institut Nacional d'Educació Física de Catalunya (INEFC)
Universitat de Lleida

Keith Phillip George, PhD
Research Institute for Sport and Exercise Sciences
Liverpool John Moores University

Vicenç Hernández-González, PhD
Departament de Didàctiques Específiques
Universitat de Lleida

Ray Jinlei Nie, PhD
School of Physical Education and Sports
Macao Polytechnic Institute

Alejandro Legaz-Arrese, PhD
Sección de Educación Física y Deporte
Universidad de Zaragoza

Isaac López-Laval, PhD
Sección de Educación Física y Deporte
Universidad de Zaragoza

Joaquin Reverter-Masia, PhD
Departament de Didàctiques Específiques
Universitat de Lleida

Juan José Puente-Lanzarote, MD
Unidad de Bioquímica Clínica
Hospital Universitario Lozano Blesa

A2 - Advisors Report About the Impact Factor of the Publications

Publication 1

Title: Cardiac Biomarker Release After Exercise in Healthy Children and Adolescents: A Systematic Review and Meta-Analysis

Authors: Rafel Cirer-Sastre, Alejandro Legaz-Arrese, Francisco Corbi, Keith George, Jinlei Nie, Luis Enrique Carranza-García, Joaquin Reverter-Masia

Journal: Pediatric Exercise Science

ISSN: 0899-8493

Reference: Cirer-Sastre R, Legaz-Arrese A, Corbi F, et al. Cardiac Biomarker Release After Exercise in Healthy Children and Adolescents: A Systematic Review and Meta-Analysis. *Ped Exer Sci.* 2019;31(1):28-36. doi:10.1123/pes.2018-0058

Contribution of the candidate: Rafel Cirer-Sastre is the main author of the article, and was responsible for the conceptualization of the research, development of the search strategy, search process, data extraction, statistical analysis, and first draft of the manuscript. Additionally, he contributed in the establishment of inclusion and exclusion criteria, the quality assessment of the studies and the validation of the manuscript.

Journal Citations Report Impact Factor (2018): **1.707**

Journal Citations Report Impact Factor percentile (2018): **58th (#29/69-Q2)**

SCImago Journal Rank (2018): **0.791**

CiteScore (2018): **1.37**

CiteScore percentile (2018): **60th (#109/276)**

Source Normalized Impact per Paper (2018): **0.790**

Publication 2

Title: Effect of Training Load on Post-Exercise Cardiac Troponin T Elevations in Young Soccer Players

Authors: Rafel Cirer-Sastre, Alejandro Legaz-Arrese, Francisco Corbi, Isaac López-Laval, Juan José Puente Lanzarote, Vicenç Hernández-González and Joaquin Reverter-Masia

Journal: International Journal of Environmental Research and Public Health

ISSN: 1660-4601

Reference: Cirer-Sastre R, Legaz-Arrese A, Corbi F, et al. Effect of Training Load on Post-Exercise Cardiac Troponin T Elevations in Young Soccer Players. *Int J Environ Res Public Health.* 2019;16(23):4853. doi:10.3390/ijerph16234853

Contribution of the candidate: Rafel Cirer-Sastre is the main author of the article, and was responsible for the conceptualization, data acquisition, project administration, data curation and statistical analysis, and first draft of the manuscript. Additionally, he contributed in the design of the study and validation of the manuscript.

Journal Citations Report Impact Factor (2018): **2.468**

Journal Citations Report Impact Factor percentile (2018): **91th (#33/370-Q1)**

SCImago Journal Rank (2018): **0.818**

CiteScore (2018): **2.81**

CiteScore percentile (2018): **84th (#74/489)**

Source Normalized Impact per Paper (2018): **1.129**

Publication 3

Title: Cardiac Troponin T Release after Football 7 in Healthy Children and Adults

Authors: Rafel Cirer-Sastre, Alejandro Legaz-Arrese, Francisco Corbi, Isaac López-Laval, Juan José Puente Lanzarote, Vicenç Hernández-González and Joaquin Reverter-Masia

Journal: International Journal of Environmental Research and Public Health

ISSN: 1660-4601

Reference: Cirer-Sastre R, Legaz-Arrese A, Corbi F, et al. Cardiac Troponin T Release after Football 7 in Healthy Children and Adults. *Int J Environ Res Public Health.* 2020;17(3):956. doi:10.3390/ijerph17030956

Contribution of the candidate: Rafel Cirer-Sastre is the main author of the article, and was responsible for the conceptualization, data acquisition, project administration, data curation and statistical analysis, and first draft of the manuscript. Additionally, he contributed in the design of the study and validation of the manuscript.

Journal Citations Report Impact Factor (2018): **2.468**

Journal Citations Report Impact Factor percentile (2018): **91th (#33/370-Q1)**

SCImago Journal Rank (2018): **0.818**

CiteScore (2018): **2.81**

CiteScore percentile (2018): **84th (#74/489)**

Source Normalized Impact per Paper (2018): **1.129**

Publication 4

Title: Influence of maturational status in the exercise-induced release of cardiac troponins in healthy young swimmers

Authors: Rafel Cirer-Sastre, Alejandro Legaz-Arrese, Francisco Corbi, Isaac López-Laval, Keith George and Joaquin Reverter-Masia

Journal: Journal of Science and Medicine in Sport

ISSN: 1440-2440

Reference: Cirer-Sastre R, Legaz-Arrese A, Corbi F, López-Laval I, George K, Reverter-Masia J. Influence of maturational status in the exercise-induced release of cardiac troponins in healthy young swimmers. *J Sci Med Sport*. 2020. In Press

Contribution of the candidate: Rafel Cirer-Sastre is the main author of the article, and was responsible for the conceptualization, data acquisition, project administration, data curation and statistical analysis, and first draft of the manuscript. Additionally, he contributed in the design of the study and validation of the manuscript.

Journal Citations Report Impact Factor (2018): **3.623**

Journal Citations Report Impact Factor percentile (2018): **86th (#12/83-Q1)**

SCImago Journal Rank (2018): **1.665**

CiteScore (2018): **3.84**

CiteScore percentile (2018): **95th (#12/244)**

Source Normalized Impact per Paper (2018): **1.494**

A3 - Participation in Other Research Publications

1. Robert P, **Cirer-Sastre R**, López-Laval I, et al. Relationship between the jump capacity and the best brand in different BMX riders level. *Apunts Educació Física i Esport*. 2020;140. 37-43. dio: 10.5672/apunts.2014-0983.es.(2020/2).140.06.

2. Julià-Sánchez S, Álvarez-Herms J, **Cirer-Sastre R**, Corbi F, Burtscher M. The Influence of Dental Occlusion on Dynamic Balance and Muscular Tone. *Front Physiol*. 2020;10. doi:10.3389/fphys.2019.01626.

3. Sitko S, López laval I, **Cirer-sastre R**, Corbi F, Calleja-gonzález J. Physiological demands and characteristics of the participants in a cycling sportive event. *J Sports Med Phys Fitness*. 2020;60(3):367-373. doi:10.23736/S0022-4707.19.10196-X.

4. López-laval I, Sitko S, Muñiz-pardos B, **Cirer-sastre R**, Calleja-gonzález J. Relationship Between Bench Press Strength and Punch Performance in Male Professional Boxers. *J Strength Cond Res.* 2020;34(2):308-312. doi:10.1519/jsc.0000000000003362.

5. Arnau-Salvador R, Hernández-González V, **Cirer-Sastre R**, Corbi-Soler F, Reverter-Masià J. Physical activity from cycling and effects on the mediterranean diet. Project: ASISA. *Acta Medica Mediterranea.* 2019;(4):1893-1896. doi:10.19193/0393-6384_2019_4_295.

6. Sitko S, **Cirer-sastre R**, López laval I. Effects of a low-carbohydrate diet on performance and body composition in trained cyclists. *Nutr Hosp*. 2019;36(6):1384-1388. doi:10.20960/nh.02762.

7. Sitko S, **Cirer-sastre R**, López laval I. Effects of high altitude mountaineering on body composition: a systematic review. *Nutr Hosp*. 2019;36(5):1189-1195. doi:10.20960/nh.02582.

8. **Cirer-Sastre R**, Beltrán-Garrido JV, Corbi F. Contralateral Effects after Unilateral Strength Training: A Meta-Analysis Comparing Training Loads [Response]. *J Sports Sci Med*. 2018;17(1):163-166.

9. **Cirer-Sastre R**, Beltrán-Garrido JV, Corbi F. Contralateral Effects After Unilateral Strength Training: A Meta-Analysis Comparing Training Loads. *J Sports Sci Med.* 2017;16(2):180-186.

A4 - Participation in Other Research Projects

1. **EUPAP – A European Model for Physical Activity on Prescription.** Principal Investigator: Friberg, M; Period: 2014 – 2022; Entity: European Comission. CHAFEA (Consumers, Health, Agriculture and Food Executive Agency); Identificator: Grant, HP-PJ-2018; Project, 847174

2. **Millora de l'objectivitat en les mesures per calcular l'edat de condició física funcional** Principal Investigator: Planas Anzano, A; Period: 2017 – 2021; Entity: Institut Nacional d'Educació Física de Catalunya; Identificator: ECFF

3. **EUPAP-Lleida. Exercici físic i salut en àmbit sanitari i comunitari** Principal Investigator: Mas Alòs, S; Period: 2019 – 2020; Entity: Diputació de Lleida; Identificator: Decret num. 2863; Exp. 201900745

4. **Buscando los valores de referencia normales de biomarcadores de daño cardíaco después de sesiones de actividad física.** Principal Investigator: Reverter-Masia, J; Period: 2018 – 2020; Entity: Institut de Desenvolupament Social i Territorial (INDEST); Identificator: 2018CRINDESTABC

5. **Prevenció de la lesió del lligament encreuat anterior en jugadores d'handbol adolescents.** Principal Investigator: Peirau Teres, J M; Period: 2018 – 2020; Entity: Institut Nacional d'Educació Física de Catalunya; Identificator: 2018PINEF00009

6. **Práctica de actividad física en bicicleta: efectos sobre la salud y calidad de vida.** Principal Investigator: Reverter-Masia, J; Period: 2017 – 2019; Entity: Universidad de Lleida; Identificator: Cátedra ASISA

7. **Envelliment actiu, qualitat de vida i relacions intergeneracionals.** Principal Investigator: Reverter-Masia, J; Period: 2016 – 2017; Entity: Institut de Desenvolupament Social i Territorial (INDEST); Identificator: INDEST2016ww

A5 - PhD Portfolio

Candidate: Rafel Cirer-Sastre
Doctorate school: Universitat de Lleida
Doctorate program: Physical Activity and Sport
PhD period: 2016 - 2020

PhD Training	Year	Workload
Search and management of documentary sources (15-50 h)		
Herramientas de investigación I: Técnicas de procesamiento de datos y de documentos	2015	25 h
Scopus – Nivel Básico	2016	1 h
Scopus – Nivel Avanzado	2016	1 h
El gestor de referencias EndNote Web	2016	1.5 h
Novedades en la Web of Science (JCR y ESI)	2016	1.5 h
Buscar la producción científica de autores y la evaluación con métricos de citas	2016	1.5 h
Evaluación de revistas con métricos en JCR (Nueva versión)	2016	1.5 h
Identificar lo más citado de WoS con ESI (Nueva versión)	2016	1.5 h
Les patents, eines Claus per a la investigación	2018	2 h
Accés obert a la producción científica	2018	4 h
Indicadors i mètodes per avaluar la producción científica	2018	2 h
Analysis and discussion of scientific articles and works (32-47 h)		
Herramientas de investigación II: Comunicación científica	2015	25 h
Scientific debate – Grup de Recerca Moviment Humà	2017	10 h
Specific training courses (30-60 h)		
Research		
Introduction to R Programming	2015	16 h
Intermediate R Programming	2015	6 h
Intro to Statistics with R: Introduction	2015	4 h
Intro to Statistics with R: Student's T-test	2015	3 h
El paper dels tutors i directors de les tesis doctorals	2015	2 h
Noves directrius establertes per a acreditar trams d'investigació (sexennis)	2015	1 h
Els identificadors digitals de la recerca	2015	2 h
Intro to Statistics with R: Analysis of Variance (ANOVA)	2016	4 h
Regresión I con JMP12Pro	2016	12 h
Regresión II con JMP12Pro	2016	12 h
Data Manipulation in R with dplyr	2016	4 h
Intro to Statistics with R: Correlation and Linear Regression	2016	4 h
Projectes competitius: preparació, finançament i seguiment. Pla nacional	2016	9 h
Intro to Statistics with R: Repeated measures ANOVA	2016	4 h
Reporting with R Markdown	2016	3 h

PhD Training	Year	Workload
Curs d'introducció als models mixtes amb R	2016	24 h
Introducció al software Atlas Ti per l'anàlisi de dades qualitatives	2016	2 h
Modelatge amb equacions estructurals (SEM) en educació psicologia i ciències socials	2016	9 h
Sistema de registre electrocardiográfica KAUNAS Load: Anàlisi de dades i aplicación a l'estudi de les adaptacions a l'esforç i a l'entrenament esportiu	2016	4 h
Intro to Statistics with R: Correlation and Linear regression course	2017	4 h
Statistical Modeling in R I	2017	4 h
Data visualization with ggplot2	2017	7 h
Real Track Systems, monitorització I anàlisi de l'activitat física mitjançant els dispositius WIMU	2017	3 h
Fiabilitat i validesa en amidaments, test i qüestionaris	2017	6 h
Hierarchical and Mixed Effects Models	2018	4 h
Impacto del ejercicio extremo sobre el Sistema cardiovascular	2019	3 h
Teaching		
Ús de la plataforma Moodle - Avançat	2016	12 h
Gestió de l'estrès laboral a la Universitat	2017	8 h
Competències i formació pedagógica del professorat universitari	2017	3 h
Impulsar els equips de treball	2017	8 h
Avaluació continuada en grups grans	2017	8 h
Aprenentatges actius en grups nombrosos	2017	4 h
La docència universitària centrada en l'alumne a través de l'aula invertida	2018	4.5 h
La docència centrada en el docent: anàlisi i millora de la sessió expositiva	2018	4 h
Direcció, correcció i avaluació de treballs finals de grau (TFG) i treballs finals de màster (TFM)	2018	8 h
Estratègies de feedback per a l'avaluació formativa	2018	6 h
L'aprenentatge centrat en l'alumnat: estratègies participatives en l'aula universitària	2018	4 h
L'aprenentatge i servei (APS) a la universitat: una nova manera d'interrelacionar la docència, la recerca i la transferencia de coneixement	2018	8 h
Flipped classroom, flipped learning, gamificació, metodologies educatives innovadores i models de desenvolupament profesional	2018	18 h
Utilització del campus virtual en la docència. Aplicació de methodologies I creació de recursos online	2018	20 h
Els procesos d'acreditació del professorat davant d'AQU Catalunya: lector, agregat i catedràtic	2018	4 h

PhD Training	Year	Workload
Presentation in laboratories and research groups (30-60 h)		
Activitat física, salut i benestar 360º	2015	4 h
Creativitat i generació d'idees per la docència i la recerca	2016	3 h
Kineantropometry I – The International Society for the Advancement of Kineanthropometry	2017	24 h
Funcions de l'estudiantat, de la tutorització i de la dirección de la tesi doctoral	2017	2 h
Jornada Anual de Benvinguda de Doctorands	2018	6.5 h
PhD Yearly assessment (35 h)		
Research plan – APTE	2016	5 h
Jornada Annual de Seguiment – APTE	2017	5 h
Jornada Annual de Seguiment – APTE	2018	5 h
Jornada Annual de Seguiment – APTE	2019	5 h
Jornada Annual de Seguiment – APTE	2020	5 h
Assistence and participation in scientific forums (3)		
Assistence – V Jornades Científiques INEFC, campus de Lleida	2015	5 h
Communication "Efectes de l'entrenament de força unilateral sobre l'extremitat contralateral: revisió sistemática i meta-anàlisi" - V Jornades Científiques INEFC, campus de Lleida	2015	5 h
Asssistence - IX Congreso Internacional de la Asociación Española de Ciencias del Deporte	2016	30 h
Communication "Relación entre los niveles de condición física y el rendimiento en crossfit" - IX Congreso Internacional de la Asociación Española de Ciencias del Deporte	2016	2 h
Communication "El entrenamiento cruzado de la fuerza: ¿Cómo deberíamos entrenar?" - IX Congreso Internacional de la Asociación Española de Ciencias del Deporte	2016	2 h
Assistence – VI Jornades Científiques INEFC, campus de Lleida	2016	5 h
Assistence – VII Jornades Científiques INEFC, campus de Lleida	2017	5 h
Communication "Biomarcadores de daño cardíaco después de la práctica de actividad física" – VII Jornades Científiques INEFC, campus de Lleida	2017	5 h
Assistence – Jornada d'activitat docent universitària i TIC #ADUTIC18: Situació actual I propostes de millora	2018	4 h
Communication "El process de tesi doctoral a l'INEFC (Workshop" – Formació continuada INEFC	2019	5 h
Assistence – Seminari Sports Analytics: una visió general de l'estadística, la bioestadística I l'anàlisi de dades en el món de l'esport professional	2019	5 h
Research stage (21 d)		
Facultad de Organización Deportiva, Universidad Autónoma de Nuevo León, México	2020	92 dies

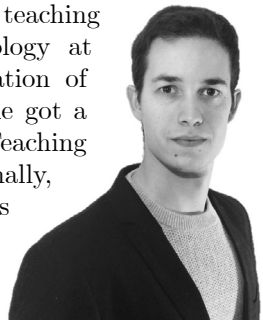
PhD Training	Year	Workload
Elaboration and publication of scientific articles (1)		
Cirer-Sastre R, Legaz-Arrese A, Corbi F, et al. Cardiac Biomarker Release After Exercise in Healthy Children and Adolescents: A Systematic Review and Meta-Analysis. Ped Exer Sci. 2019;31(1):28-36. doi:10.1123/pes.2018-0058	2019	-
Cirer-Sastre R, Legaz-Arrese A, Corbi F, et al. Effect of Training Load on Post-Exercise Cardiac Troponin T Elevations in Young Soccer Players. Int J Environ Res Public Health. 2019;16(23):4853. doi:10.3390/ijerph16234853	2019	-
Cirer-Sastre R, Legaz-Arrese A, Corbi F, et al. Cardiac Troponin T Release after Football 7 in Healthy Children and Adults. Int J Environ Res Public Health. 2020;17(3):956. doi:10.3390/ijerph17030956	2020	-

A6 - Acknowledgements

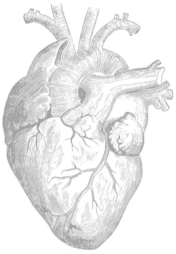
I would like to express my gratitude and appreciation for my advisors, partner and coauthors, whose guidance, support and encouragement has been invaluable thorought this thesis. I also wish to thank INEFC Lleida team who have been a great source of support.

A7 - About the Author

Rafel Cirer-Sastre was born on April 30th 1988, in Bunyola, Illes Balears. He entered the University of Lleida in 2010. Four years after, and following an internship in the High-Performance Sports Centre of Catalonia (CAR), he obtained his Bachelor of Sciences in Physical Activity and Sport Sciences in 2014, at the University of Lleida. A year later, in 2015, he got his Master of Sciences degree in Physical Activity and Health with a major in sports rehabilitation, readaptation and research at the University of Barcelona. Simultaneously, in 2015 he obtained a Postgraduate of Expert in Exercise Prescription for Health at the University of Lleida. In 2016 he started his PhD under the tutoring of Dr. Antoni Planas (National Institute of Physical Education of Catalonia, Lleida campus) and supervision of Dr. Francisco Corbi (National Institute of Physical Education of Catalonia, Lleida campus) and Professor Joaquin Reverter (University of Lleida) in the Doctorate School of the University of Lleida. In 2017 he got a 3-year research grant by the National Institute of Physical Education of Catalonia. His scientific efforts describing the exercise-induced elevation of cardiac troponins in healthy trained prepuberal and pubertal males are summarized in this PhD thesis. During his PhD, he has been teaching Sports Kinesiology and Exercise Technology at the National Institute of Physical Education of Catalonia, University of Lleida. In 2018 he got a Postgraduate of Specialist in University Teaching at the University of Lleida. Additionally, he cohauthored several research papers and participated in research projects, investigating health promotion, exercise physiology and sports performance.



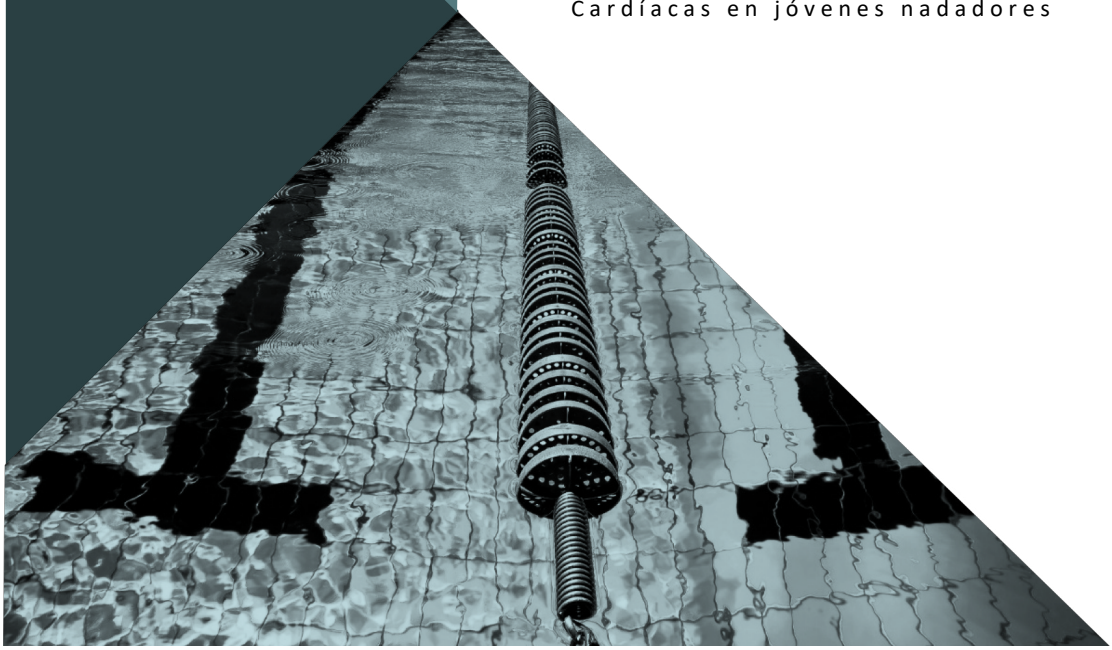
B1- Information Leaflet



SWIMT

2019

Liberación de Troponinas
Cardíacas en jóvenes nadadores



PROYECTO SWIMT

SWIMT es un proyecto de investigación internacional, compartido entre la Universitat de Lleida, SP; la Universidad de Zaragoza, SP; la Universidad John Moores de Liverpool, UK; y el Instituto Politécnico de Macao, CHN. El objetivo de SWIMT es estudiar la respuesta cardíaca al ejercicio en edades infantiles, para identificar los factores asociados a los accidentes cardiovasculares en jóvenes deportistas.

DAÑO CARDÍACO

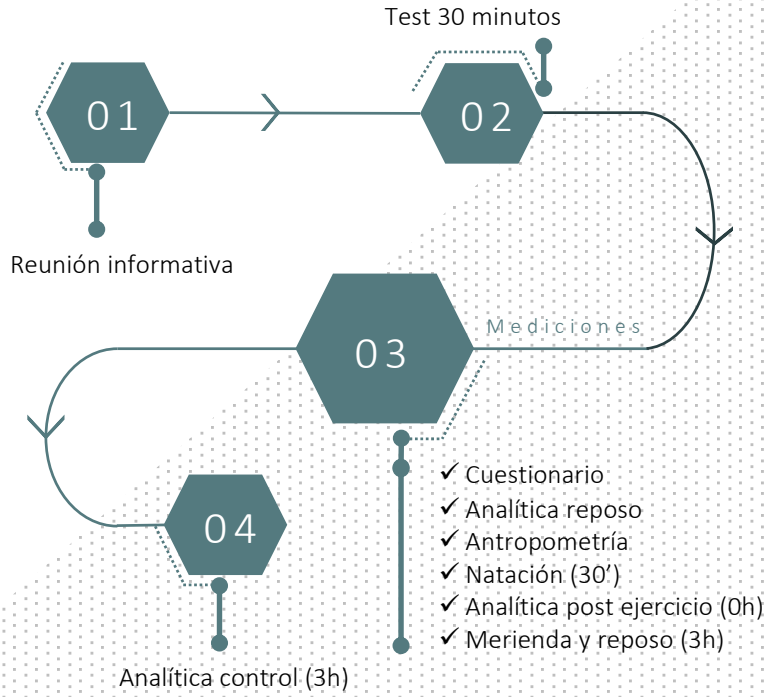
De cada vez son más frecuentes los accidentes cardiovasculares en población deportista. Los más conocidos son la angina de pecho, el infarto agudo de miocardio o la muerte súbita. Para poder diagnosticar clínicamente estas patologías y conocer el impacto del ejercicio físico sobre el corazón, es necesario el estudio de proteínas sanguíneas que reflejan el daño cardíaco, como son las troponinas cardíacas.

Estudios previos describen como la práctica de ejercicio con baja condición física, así como el deporte de elevadas duraciones e intensidades están asociados a la liberación de troponinas. Otro factor relacionado es la edad, ya que los deportistas adolescentes liberan este marcador con más magnitud y de forma más variable. Lamentablemente, y esta es la razón de nuestro proyecto, actualmente se desconoce como es la respuesta antes de la adolescencia.

QUE NECESITAMOS?

Buscamos jóvenes nadadores de categorías benjamín, alevín e infantil masculinas (11-16 años), que entrenen con regularidad y participen en pruebas competitivas.

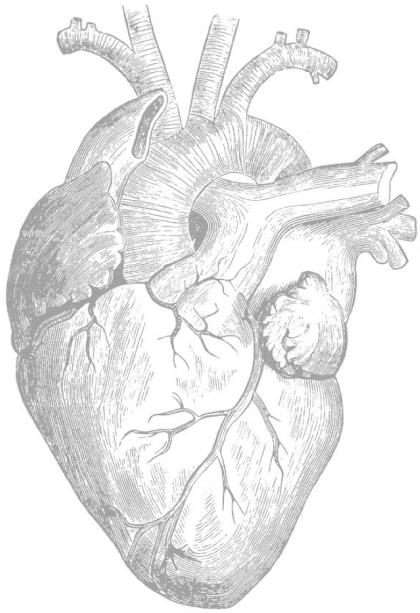
EN QUE CONSISTIRÁ?



Actualmente, se desconoce la respuesta de los marcadores de daño cardíaco después del ejercicio durante el proceso de maduración del miocardio. El estudio **SWIMT** pretende comprender como el corazón en desarrollo responde, tolera y se adapta al entrenamiento.

A demás, los resultados servirán para identificar y prevenir situaciones de riesgo cardiovascular en jóvenes deportistas, hecho que en el futuro, se reflejará con nuevos métodos para individualizar el entrenamiento y nuevas estrategias para el diagnóstico clínico de patologías cardíacas infantiles.

projswimt.inefc@gencat.cat



B2 - Informed Consent Form

Documento de consentimiento informado

La investigación en curso se titula "Buscando los valores de referencia normales de un biomarcador de daño cardíaco después de sesiones de actividad física". Está dirigida por el Dr. Francisco Corbi y Dr. Joaquín Reverter, y realizada por el grupo de investigación consolidado Movimiento Humano (HMRG) de la Generalitat de Catalunya (2017SGR1463).
La troponina es una de las proteínas detectables en la sangre, y su concentración sirve para interpretar el estado del corazón. La concentración de troponinas se eleva tanto después de un accidente cardíaco, como después de haber practicado ejercicio. En este sentido, esta investigación tiene los siguientes dos objetivos: 1) Describir la influencia de la práctica de deportiva sobre la liberación de troponinas cardíacas, y 2) Determinar si esta liberación de troponinas cardíacas está asociada a la edad del participante, desarrollo, sexo, experiencia previa o características del ejercicio.

Protocolo

La participación en este estudio es voluntaria y no comportará ningún tipo de incentivo monetario, ni en especie. Así mismo, tampoco supondrá ningún coste económico. El estudio consistirá en dos pruebas. La prueba principal consistirá en un test de nado continuado con una duración de 30 minutos. Durante la prueba se monitorizará la frecuencia cardíaca del participante. Se realizarán tres extracciones sanguíneas para determinar la concentración de troponina: la primera inmediatamente antes del ejercicio, la segunda inmediatamente después de finalizar la tarea y la ultima pasada una recuperación de 3h. Cada extracción sanguínea tendrá un volumen de 5ml y se tomará de la vena antecubital. Estas extracciones serán siempre realizadas por enfermeros/as con experiencia pediátrica y en condiciones de asepsia rigurosa. Los padres y madres del deportista podrán acompañarle durante las extracciones. El estadio de desarrollo del deportista se comprobará mediante la observación genital por parte de un/a especialista en pediatría. La segunda prueba, que se realizará en un día distinto, consistirá en monitorizar la frecuencia cardíaca del deportista durante una sesión de entrenamiento ordinaria. En esta segunda prueba no se realizará ninguna extracción sanguínea. Los procedimientos que se seguirán en este estudio han sido aprobados previamente por el Comité de Ética de Investigaciones Clínicas de la Administración Deportiva de Cataluña (número de expediente: 02/2018/CEICGC).

Riesgo de la valoración

Las extracciones de sangre pueden provocar un pequeño hematoma en la zona de punción, por ello se recomendará al participante que después realice presión sobre el punto durante unos minutos. La participación en el estudio comporta también los riesgos derivados de la práctica deportiva ordinaria, razón por la cual será dirigida y supervisado por personal especialista en entrenamiento, rendimiento y salud deportiva. La monitorización de la frecuencia cardíaca y las medidas antropométricas (registro de las medidas y proporciones corporales) que se realizarán no comportan ningún riesgo para la salud del participante.

Beneficios del estudio

Con esta investigación se pretende ampliar el conocimiento actual sobre la respuesta cardíaca al ejercicio físico y también sobre los efectos de la práctica deportiva sobre el tejido cardíaco. Los resultados de este trabajo aportarán nueva información sobre la respuesta normal al ejercicio, y permitirán diferenciarla de los indicadores de patología cardíaca basados en el mismo biomarcador.

Responsabilidad del estudio

La identidad del participante será enmascarada a través de un código único. Solamente los investigadores principales del estudio tendrán acceso a los datos personales del participante y a este documento de consentimiento. En **cualquier momento se podrá abandonar** el estudio sin ningún tipo de prejuicio moral ni económico, y también se puede negar la respuesta a cualquiera de las preguntas que se formulen.

Solicitud de información

Podrán realizar cualquier consulta sobre este proyecto, poniéndose en contacto con Rafel Cirer-Sastre, núm. Teléfono 973 27 20 22 (ext. 271), correo-e: *projswimt.inefc@gencat.cat*.

Responsabilidad del participante

Yo, _____ con DNI núm. _____, teléfono de contacto _____ , correo-e _____ ,
Padre/madre/tutor legal de _____
He decidido participar de forma voluntaria en la investigación "Buscando los valores de referencia normales de un biomarcador de daño cardíaco después de sesiones de actividad física" y declaro:

☐ Haber leído detenidamente este documento.
☐ Haber preguntado cualquier duda sobre el mismo al personal investigador.
☐ Entender los posibles daños, molestias y complicaciones derivados de la participación en el estudio.
☐ Doy mi consentimiento a Dr. Francisco Corbi y Dr. Joaquin Reverter para realizar las pruebas descritas.
☐ Autorizo al grupo de investigación consolidado Movimiento Humano (HMRG) de la Generalitat de Catalunya (2017SGR1463) a difundir la información y las imágenes que se deriven de estas pruebas, siempre con interés sanitario, docente y científico, i nunca económico.
☐ He sido informado/da del derecho a renunciar en cualquier momento a finalizar las pruebas descritas.

Responsabilidad del investigador

El grupo de investigación consolidado Movimiento Humano (HMRG) (2017SGR1463) declara,

☒ Haber discutido el contenido de este documento explicando verbalmente los riesgos y beneficios directamente relacionados con la participación en el mismo y aclarando todas las posibles dudas planteadas por la persona que firma.
☒ Se compromete a proteger la identidad e intimidad del participante en todo momento de acuerdo con la Ley Orgánica 15/1999, de 13 de diciembre, de Protección de Datos de Carácter Personal.
☒ Conservará todos los registros realizados por medios mecánicos, electrónicos, magnéticos, grabaciones o cualquier otra información que se derive de los mismos en los términos legalmente previstos.

Fraga, _____ d_____ de _____.

Responsable del estudio: _____ Nombre del tutor legal: _____

Firma: Firma:

Renuncia

(rellenar solo en caso de renuncia)

☐ Yo _____, **revoco** mi participación en la investigación "Buscando los valores de referencia normales de un biomarcador de daño cardíaco después de sesiones de actividad física".

Firma:

A rellenar por el personal investigador

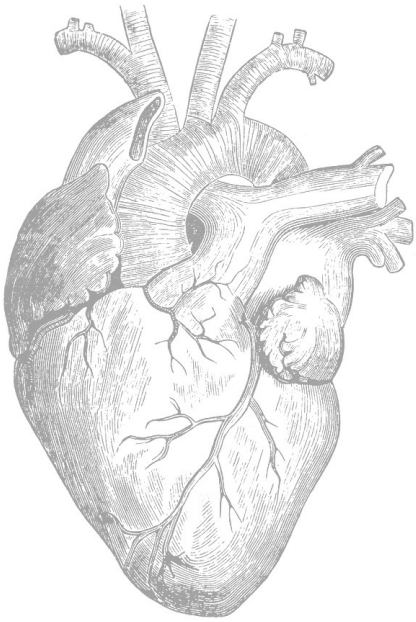
Turno	Id Tanita	Id Polar

Id Pre	Id 0h	Id 3h

Alergia / intolerancia alimentaria

Información pendiente

Información adicional



B3 - Ethical Commite Approval

Generalitat de Catalunya
Comitè d'Ètica d'Investigacions Clíniques
de l'Administració Esportiva de Catalunya

Dr. RAMON BALIUS MATAS, DE LA UNITAT DE MEDICINA DE L'ESPORT I SALUT DEL CONSELL CATALÀ DE L'ESPORT, ACTUANT COM SECRETARI DEL COMITÈ D'ÈTICA D'INVESTIGACIONS CLÍNIQUES DE L'ADMINISTRACIÓ ESPORTIVA DE CATALUNYA

CERTIFICA

Que en la reunió realitzada el dia 7 de febrer de 2018, aquest Comitè d'Ètica acordà avaluar favorablement el projecte presentat pels Srs. Joaquin Reverter Masià i Francesc Corbi Soler, titulat "Buscando los valores de referencia normales de un biomarcador de daño cardíaco después de sesiones de actividad física" (número d'expedient: 02/2018/CEICGC).

Faig constar aquesta avaluació favorable als efectes oportuns.

Esplugues de Llobregat, 24 d'abril de 2018

Generalitat de Catalunya
Consell Català de l'Esport
Av. Països Catalans,40-48
08950 Esplugues de Llobregat

Dr. Ramon Balius i Matas
Metge especialista en Medicina de l'Esport
Col·legiat 23.684 (Barcelona)
Centre de Medicina de l'Esport
Consell Català de l'Esport

Dr. Ramon Balius Matas
Secretari CEICGC

INEFC Institut Nacional d'Educació Física de Catalunya Lleida

Universitat de Lleida
Grup de Recerca
Moviment Humà

Don Joaquim Reverter Masià, investigador principal del Grup de Recerca Consolidat Moviment Humà & Don Francisco Corbi Soler, codirector del projecte i en representació de l'Institut Nacional d'Educació Física de Catalunya,

CERTIFIQUEN / CERTIFICAN

*El **Club** va col·laborar en el projecte d'investigació "Buscant els valors de referència normals dels biomarcador de dany cardíac després de realitzar activitat física" que se va celebrar el de de 2018 a les instal·lacions de l'Institut Nacional d'Educació Física de Catalunya.*

*El **Club** colaboró en el proyecto de investigación "Buscando los valores de referencia normales de los biomarcadores de daño cardíaco después de realizar actividad física" que se celebró el de de 2018 en las instalaciones del Instituto Nacional de Educación Física de Cataluña.*

Lleida, de 2018

Grup de Recerca Consolidat Moviment Humà

Institut Nacional d'Educació Física de Catalunya

Dr. Joaquim Reverter Masià

Dr. Francisco Corbi Soler