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Study Protocol

The COR-School Project: Physical Activity and Cardiometabolic Health in Spanish Youth

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Abstract: In an era where childhood health is increasingly at risk, understanding the role of physical activity in promoting well-being is critical. The COR-School project investigates the impact of physical activity on cardiometabolic health in over 700 Spanish children and adolescents aged 8 to 16 years. Over three years, the study will conduct three assessments (baseline and two follow-ups) to evaluate peak post-exercise levels of high-sensitivity cardiac troponin T (hs-cTnT), a biomarker for cardiac stress, following a submaximal 20 m shuttle run test. Secondary objectives include examining the influence of maturational status, physical activity, and cardiorespiratory fitness on hs-cTnT. Participants will complete fitness tests, questionnaires on health habits, sleep, and diet, as well as anthropometric and body composition measurements. Blood samples collected at baseline and three hours post-exercise will measure cardiac biomarkers and lipid profiles. Cardiovascular responses will be tracked using heart rate monitors. Normal ranges of hs-cTnT will be determined using data distribution (percentiles or mean ± SD), stratified by age, sex, and maturational stage. Statistical analyses, including repeated measures ANOVA and Pearson correlation, will explore trends across time, sex, developmental stages, and other health-related outcomes. Beyond providing clinical insights by establishing reference values for hs-cTnT in healthy youth after exercise, findings will inform educational policies to promote physical activity in schools, emphasizing its role in improving fitness, health behaviors, and overall development.

Keywords: high-sensitivity cardiac troponin T; cTnT; health; youth; physical activity; protocol

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1. Introduction

In an era where physical inactivity is rising among youth, regular physical activity is not just an extracurricular activity—it is a cornerstone of long-term health and well-being. The benefits of physical activity during childhood and adolescence extend far beyond physical fitness, laying the foundation for healthier adulthood (Högström et al., 2014). It improves cardiovascular health and enhances physical fitness (Lloyd et al., 2016; Tremblay et al., 2011). Regular activity also strengthens musculoskeletal systems, supports psychological well-being (Poitras et al., 2016; Rodriguez-Ayllon et al., 2019), and reduces the risk of chronic conditions such as obesity, hypertension, and type 2 diabetes later in life (Bull et al., 2020; Ekelund et al., 2016). However, current global trends reveal that many children and adolescents fail to meet the recommended levels of physical activity

(Aubert et al., 2022; van Sluijs et al., 2021), with sedentary lifestyles becoming increasingly prevalent (Di Cesare et al., 2019; Ding et al., 2016; Guthold et al., 2020). This alarming trend emphasizes the need for structured interventions, particularly in schools, where physical activity can be seamlessly integrated into daily routines and positively shape health behaviors (Messing et al., 2019).

Understanding the physiological responses to exercise, including the release of cardiac biomarkers such as high-sensitivity cardiac troponin T (hs-cTnT), is crucial for ensuring the safety and effectiveness of physical activities in young populations. Cardiac troponin (cTn), a highly specific biomarker for myocardial injury, is widely used to diagnose acute coronary syndromes and other heart-related conditions (Collinson et al., 2001; Reichlin et al., 2009). High-sensitivity assays for cTn (hs-cTn) allow the detection of low-level elevations (Reichlin et al., 2009), even in healthy individuals post-exercise, revealing a physiological rather than pathological response (Gresslien & Agewall, 2016). This has spurred interest in hs-cTnT as a potential early indicator of cardiovascular strain in youth, offering valuable insights into the interplay between exercise and cardiac health (Clerico et al., 2022). The COR-School project represents a novel effort to address this gap, aiming to establish pediatric-specific reference values for hs-cTnT and provide insights into how exercise affects cardiovascular health during development.

Most research on post-exercise troponin elevation has focused on adults, given their higher cardiovascular disease risk and ease of recruitment for studies involving intensive exercise and repeated blood sampling (Gresslien & Agewall, 2016). These studies generally report that exercise intensity and duration correlate with elevated troponin levels (Cirer-Sastre et al., 2021), although there is no consensus on the type or intensity of exercise that most influences this response (Legaz-Arrese et al., 2015a). In contrast, limited research in youth has left critical gaps in understanding whether these mechanisms and reference values apply to younger populations (Conesa-Milian et al., 2023). Adolescents, for example, may exhibit higher troponin elevations than adults following similar exercise, potentially due to the immature myocardium's increased susceptibility to exercise-induced stress (Kannankeril et al., 2002; Tian et al., 2012). Recent studies have demonstrated that exercise intensity and duration are critical determinants of hs-cTnT release (Cirer-Sastre et al., 2019; Legaz-Arrese et al., 2024), while individual responses vary based on factors such as gender, maturational status, and training level (Conesa-Milian et al., 2023). However, these findings remain inconsistent (Papamichail et al., 2023). Despite these findings, the lack of consistent data highlights the need to establish pediatric-specific reference values for hs-cTnT, which are critical for distinguishing normal physiological responses from potential pathological conditions in youth (Wagner et al., 2016). Furthermore, among the few studies conducted in children and adolescents, the majority have focused exclusively on boys, neglecting potential sex-related differences in hs-cTnT responses. This highlights the need for a more inclusive approach that considers both sexes to better understand the cardiovascular adaptations in youth.

The clinical significance of post-exercise troponin elevation in youth remains uncertain. Some researchers propose that frequent, subclinical hs-cTnT elevation in youth could serve as an early marker for potential cardiac issues (Law et al., 2021), while others view it as a benign, transient response to physical exertion without long-term effects (Janssen et al., 2023). Furthermore, variations in methodologies, cutoff values, and analyzers used across studies complicate the interpretation of troponin responses in young populations (Conesa-Milian et al., 2023). This inconsistency highlights the need for longitudinal studies examining variables such as maturational status, age, and physical activity levels to better understand how hs-cTnT responds to exercise in younger populations.

The COR-School project addresses these gaps by systematically examining hs-cTnT responses in children and adolescents aged 8 to 16 years, measured before and after a submaximal 20 m shuttle run test. The study aims to establish normative reference values for hs-cTnT after submaximal exercise, using data from three assessments conducted over three years and stratified by sex, maturational stage, and age. Furthermore, it seeks to provide valuable insights into the physiological responses of youth to physical activity, supporting the development of clinical guidelines and evidence-based educational strategies to improve cardiovascular health and promote physical activity among children and adolescents.

2. Materials and Methods

2.1. The COR-School Program

This program investigates the relationship between physical activity and cardiometabolic health in children and adolescents, focusing on how submaximal exercise influences the release of hs-cTnT, a key biomarker for cardiac strain. By targeting youths aged 8 to 16, it combines rigorous assessments with longitudinal follow-ups to explore critical factors shaping cardiovascular health. The program includes structured physical fitness assessments, aiming to promote long-term cardiovascular health by examining the interplay between physical fitness, health-related habits, maturation, and body composition.

The primary objective of this study is to assess cardiometabolic risk by evaluating the effects of submaximal exercise on hs-cTnT and cholesterol levels, aiming to identify early signs of cardiac strain. Through a multidisciplinary approach, the COR-School program integrates various components to provide a comprehensive assessment of youth health. The COR-School program's logic model (Figure 1) integrates fitness testing, blood sampling for biomarker analysis, and health questionnaires to provide a comprehensive assessment. Physical fitness is measured using the 20 m SRT for cardiorespiratory endurance and the horizontal jump test for explosive strength. Health-related factors, such as physical activity, sleep quality, and diet, are also analyzed to offer a holistic view of cardiovascular health. Maturation is assessed through Tanner stages and maturity offset (time to/from peak height velocity (PHV)) to understand how developmental stages influence both physical fitness and cardiac biomarker responses. Body composition metrics, including height, weight, BMI, and muscle and fat mass, are tracked to examine their impact on health outcomes.

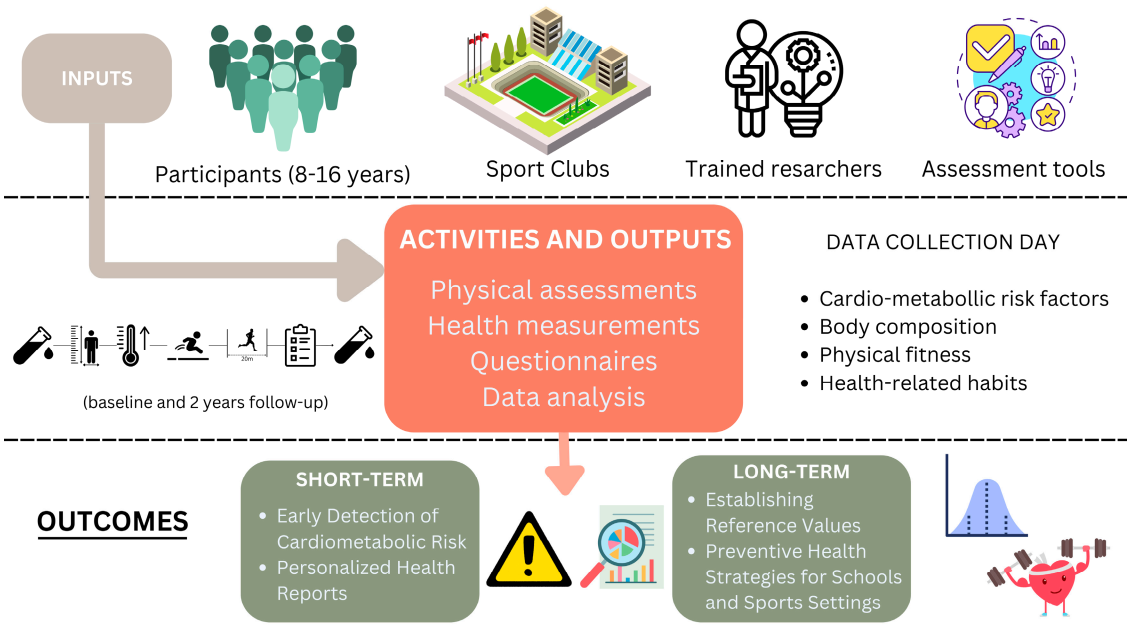

Figure 1. The COR-School program logic model.

The short-term goal is to detect potential early cardiometabolic risk factors in children and adolescents. In the long term, the program aims to establish reference values for hs-cTnT in the youth population and to inform preventive strategies that enhance cardiovascular health through improved school-based physical activity programs. By translating findings into actionable recommendations for schools and healthcare providers, the COR-School program aspires to foster sustainable changes in youth health policies and interventions.

2.2. Study Design

This evaluation employs a quantitative, longitudinal cohort design to assess the physiological and self-reported data of participants over a three-year period, with three assessments conducted: baseline and two follow-up assessments over the subsequent two years. Each assessment includes blood sample collection (immediately before and 3 h after a submaximal 20 m SRT), anthropometric measurements, cardiorespiratory fitness tests, and self-reported questionnaires on health behaviors, sleep, and diet. This design enables the analysis of longitudinal changes in hs-cTnT levels, cardiovascular risk factors, and physical fitness in relation to physical activity levels and maturational status.

2.3. Participants

The study will include boys and girls aged between 8–11 years (primary school) and 12–16 years (secondary school). Participants will be recruited from various sports clubs in north-eastern Spain, specifically in southern Catalonia and northern Valencia, covering both rural and urban areas. This regional diversity aims to ensure that the sample is representative of the general population of children and adolescents involved in organized sports.

The inclusion criteria target healthy children and adolescents capable of performing submaximal-effort exercise. Exclusion criteria will include any medical conditions that could impair physical performance or affect hs-cTnT levels, such as cardiovascular diseases, severe anemia, or acute infections. While recruiting participants through sports clubs introduces a potential selection bias—favoring individuals who are more physically active than the general population—this design aligns with the study's objective of assessing troponin responses in youth engaged in regular physical activity.

The researchers aim to recruit over 700 participants, consisting of approximately 400 boys and 300 girls. While most previous studies on post-exercise troponin responses in youth have predominantly included boys, this study ensures a balanced representation of both sexes to explore potential sex-related differences in hs-cTnT responses and cardiovascular health. This sample size ensures sufficient power to detect significant differences in hs-cTnT levels, cholesterol levels, and cardiorespiratory fitness across different maturational stages. Recruitment will occur through different sports clubs, and participants will be selected based on predefined inclusion and exclusion criteria. Club managers and coaches will be contacted to present the study objectives and methodology to potential participants and their families. Informed consent and medical history forms will be provided, and families will have the option to return these forms on the same day, by post, or during the data collection session. Participants will be selected based on predefined inclusion and exclusion criteria, ensuring ethical and scientific rigor.

In comparison to previous studies with sample sizes of around 20–30 participants (Cirer-Sastre et al., 2019; Conesa-Milian et al., 2023; Papamichail et al., 2023), this larger sample of 700 individuals provides a more robust foundation for detecting meaningful differences and enhances the generalizability of the findings. Based on an a priori power analysis conducted using GPower (version 3.1.9.6), a total sample size of 255 participants is sufficient to achieve a power of 0.80 for detecting small effect sizes (f = 0.10) in a repeated-measures ANOVA with five groups and three measurements. Most previous studies did not report their effect sizes; therefore, we selected a conservative effect size (f = 0.10) to ensure the robustness of our calculations. The planned sample size of 700 participants greatly exceeds this requirement, ensuring robust statistical power and generalizability of findings.

2.4. Data Collection Procedures

Participants will be recruited from sports clubs in north-eastern Spain, with club representatives communicating the proposal to members and their legal guardians. After securing authorization, an informative meeting will be held with adolescents and their parents to explain the project's objectives and methods. Those wishing to participate must sign an informed consent form, which provides detailed information about the study procedures, legal aspects, and participant rights. The form also includes sections for participants to provide their contact details for inclusion in the study and communication with the research team to arrange the three planned assessments (Appendix A).

A medical history form and a brief health questionnaire covering personal and family medical history will also be completed (Appendix B). As established by current legislation on data protection, each adolescent will be assigned an alpha-numeric code to which the results obtained in all the assessments carried out will be associated.

Once the sample has been selected, the work team will prepare a schedule of evaluations that will cover the 3-year period. As it is a longitudinal study, during the period each participant will be assessed three times: baseline assessment and a two-year follow-up, one assessment for each academic year at the same time of the year.

The design of the assessment protocol allows the application in a single day using simple assessment instruments and with the help of a minimum of 4 researchers. Said protocol includes the use of the tools with the highest validity and reliability reported in the recent scientific literature. Tools that our group has widely used in the different projects with children and adolescents carried out in recent years (Cirer-Sastre et al., 2020, 2021; Legaz-Arrese et al., 2017).

Researchers from the University of Lleida, along with research assistants and PhD candidates from the institution, will be present during the data collection days, working together with specialized nurses experienced in blood sampling from children and adolescents. Data from the questionnaires will be collected using tablets and automatically uploaded to a centralized database. Validated international questionnaires, which have also been validated in the local language, will be used to ensure clear communication and consistency. Before data collection begins, the PI researcher will explain the purpose of the evaluation to the participants, address any questions, and obtain informed consent.

Depending on availability, a group of 100 participants will be scheduled for evaluation each day. Participants must attend the session accompanied by their parents or legal guardians at the selected local sports club, which must have the necessary infrastructure for the data collection. Participants will be cited, in groups of 20, every 30 min to avoid waiting times and crowding. Once all the data has been collected, a personalized health report summarizing the results of all tests, along with brief comments on the participant's overall health, will be generated and sent to each participant (see Appendix C). If the troponin levels are found to be elevated above the reference limit (URL), participants will be contacted urgently and advised to visit a cardiologist for further evaluation. Additionally, a medical report will be requested from the participant's doctor to confirm that the elevated values do not pose a health risk and to ensure the participant is fit to continue the following year. A comprehensive summary of the entire protocol, including all key components and procedures, is presented in a flowchart diagram in Figure 2 for a clearer understanding and detailed overview of the methodology.

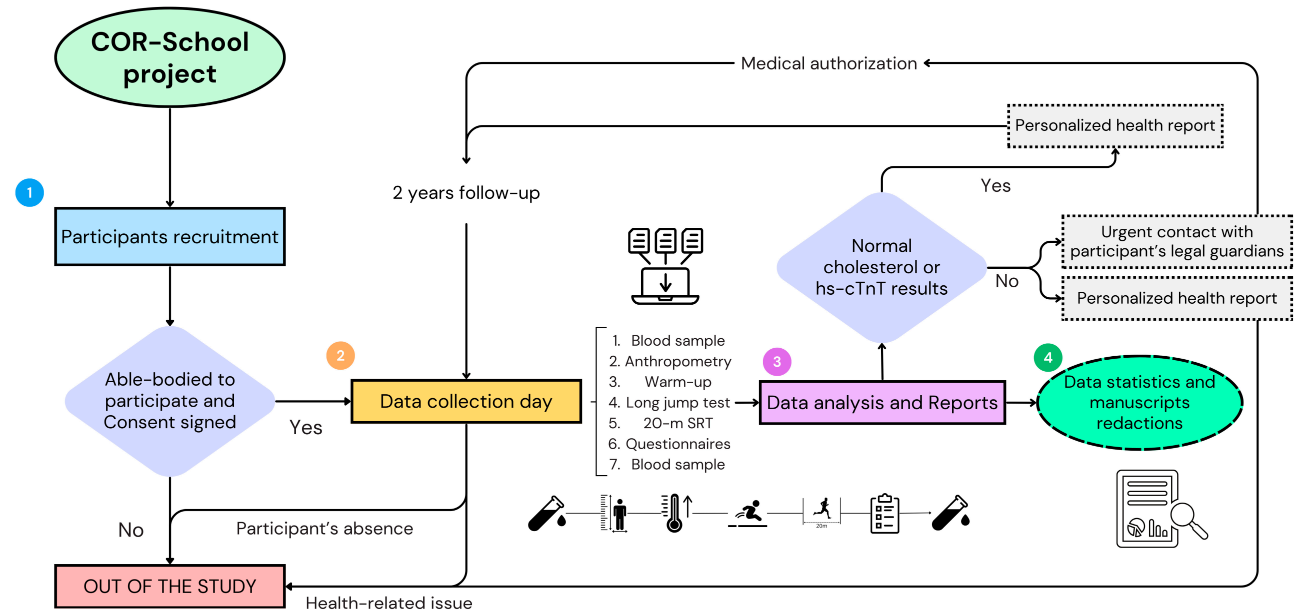

Figure 2. Protocol flowchart. Numbers in the figure represent the four main phases of the research process: (1) Participant recruitment, including consent acquisition; (2) Data collection day, involving physical tests, questionnaires, and biomarker analysis; (3) Data analysis and report preparation; (4) Statistical analysis, manuscript preparation, and dissemination of findings.

In every assessment, data will be collected through a structured 7-step process (Figure 3), which includes: (1) an initial blood sample collection, (2) anthropometric measurements, (3) a standardized 10 min dynamic bodyweight and running warm-up to prepare participants physically and reduce injury risk (Faigenbaum et al., 2022; Richmond et al., 2016), (4) a horizontal jump test, (5) the 20 m SRT, (6) the completion of questionnaires, and (7) a second blood sample collection 3 h after the 20 m SRT, when hs-cTnT levels typically peak (Legaz-Arrese et al., 2017, 2015b). These steps will be repeated in all three assessments. If participants miss one or more follow-ups, their data for those specific assessments will not be included in the corresponding analyses. Missing data will be managed using listwise deletion for most analyses, ensuring consistency across datasets, while pairwise deletion may be applied where appropriate to maximize the use of available data.

2.5. Outcomes and Measures

The study will assess cardiometabolic risk factors, physical fitness, health-related habits, maturity, and body composition. Key measures include hs-cTnT levels before and after the 20 m STR, cholesterol level, and physical fitness. Health-related habits will encompass physical activity levels, fitness levels, sleep quality, quality of life, and dietary habits. Maturity will be evaluated using Tanner stages, while body composition will include various anthropometric measurements.

2.5.1. Cardio-Metabolic Risk Factors Assessment

Blood samples (5 mL) will be taken from one of the antecubital veins by qualified health personnel (a nurse specialized in blood extraction who will be hired specifically for the collection, handling, and transport of blood) from an antecubital vein. Subsequently, the samples obtained will be centrifuged at 3500 min^{-1} in a Sigma 2k-15 centrifuge (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany). Next, the serum will be stored at -80 °C for later analysis of total cholesterol, HDL-cholesterol, and hs-cTnT. The hs-cTnT values will be obtained by means of the Troponin T hs STAT immunoassay on a Cobas E 601 analyzer (Roche Diagnostics, Penzberg, Germany). This assay has a range of 3 to 10,000 ng/L, and the intra-assay coefficient of variation at a mean hs-cTnT of 13.5 ng/L is <10%. The upper limit of reference for hs-cTnT, defined as the 99th percentile in healthy subjects, is 13.5 ng/L (Giannitsis et al., 2010). Concentrations below the detection limit of 3 ng/L will be adjusted to 1.5 ng/L for statistical analysis. Blood samples will be collected before and 3 h post-exercise in alignment with prior research on hs-cTnT kinetics (Legaz-Arrese et al., 2017, 2015b). Also, throughout the 20 m SRT test, participants will wear a heart rate monitor (Polar Team 2; Kempele, Finland) to continuously record their heart rate responses.

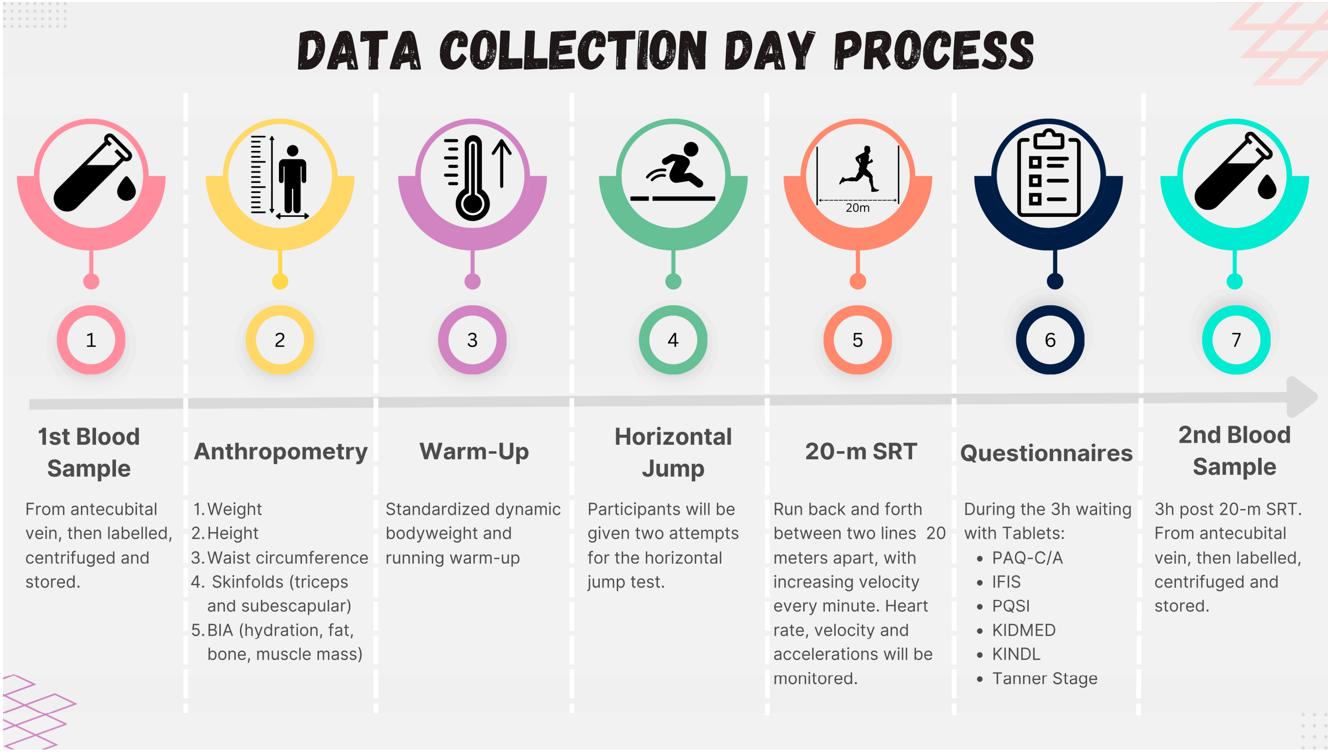

Figure 3. Data collection day process.

2.5.2. Body Composition Assessment

Weight and height will be measured with a scale and wall stadiometer with a precision of 0.1 cm (Year-Sayol, Barcelona, Spain) and weight with a precision of 0.05 kg (SECA 711, Hamburg, Germany), according to the protocol proposed by Nagy et al. (Nagy et al., 2008). The Body Mass Index (BMI) will be calculated by dividing participants weight in kilograms by their height in meters squared (kg/m^2).

Waist circumference will be determined using an approved non-elastic tape measure. The measurement will be carried out at the end of a normal expiration at the narrowest level of the waist, between the edge of the lower costal (10th rib) and the iliac crest (Nagy et al., 2008).

Skinfolds (triceps and subscapular) will be determined using an approved caliper. Two skinfolds will be measured on the left side of the participant's body. The tricipital skinfold will be measured at the mid-upper arm point (between the acromion and the olecranon), and the subscapular skinfold will be measured at a 45° inclined angle to the horizontal plane, just below the lower angle of the scapula (Nagy et al., 2008).

Additional body composition will be assessed using a validated Tanita MC-780MA device (Tanita Corporation, Tokyo, Japan) through bioelectrical impedance analysis (BIA). The device will measure weight, body fat percentage, hydration percentage, muscle percentage, bone mass percentage, and basal metabolic rate. The Tanita MC-780MA has been widely used to assess these parameters in children and adolescents (Azoulay et al., 2021; Rusek et al., 2021; Salton et al., 2022; Verney et al., 2016).

Maturity will be determined using a self-reported questionnaire with brief description and drawings (Carskadon & Acebo, 1993) of the Tanner stages (Tanner & Whitehouse, 1976). Simultaneously, the maturity offset will be calculated to estimate the temporal distance (in years) to/from the PHV (Kozieł & Malina, 2018). The combination of Tanner staging and maturity offset enables a comprehensive assessment of biological age, going beyond chronological age (Syrjälä et al., 2021). Tanner stages provide insight into secondary sexual characteristics, while maturity offset calculations reduce the potential bias of self-reported questionnaires and offer more objective and realistic data. Given the potential variations in hs-cTnT levels, body composition, and physical fitness across maturational stages, this information will be crucial for the study.

2.5.3. Physical Fitness Assessment

Horizontal Jump Test: The standing long jump test is part of the ALPHA-Fitness battery (Ruiz et al., 2011) and will be used to measure lower body explosive strength (Castro-Piñero et al., 2010), which is strictly related to health status in children and adolescents (Tomkinson et al., 2021). Participants will have two attempts to perform the jump. They will start with feet shoulder-width apart, positioned behind a designated line, and must jump forward with both feet simultaneously. The jump should conclude with a controlled landing. The best of the two jumps will be recorded as the final measure of performance.

The 20 m SRT: The 20 m Shuttle Run Test, also known as the Léger Test or Course Navette, is part of the ALPHA-Fitness battery (Ruiz et al., 2011) and will be used to assess cardiorespiratory fitness (Mayorga-Vega et al., 2015). In this test, participants run back and forth between two lines placed 20 m apart, following auditory signals that begin at a speed of 8.5 km/h, with the pace increasing by 0.5 km/h every minute. The test continues until participants are unable to maintain the required pace. Standardization is ensured by following established protocols for the ALPHA-Fitness battery (Ruiz et al., 2011). In addition, participants will be equipped with inertial and positioning devices, WIMU Pro™ (RealTrack Systems, Almería, Spain), to collect data on accelerations, pace changes, average and maximum speeds, as well as other key kinematic variables. Upon completing the 20 m SRT, participants will self-report their perceived exertion level using the Borg CR10 scale, which includes visual aids to assist in identifying their exertion levels (Chen et al., 2017).

2.5.4. Health-Related Habits Assessment

Physical activity will be assessed using the Spanish version of the Physical Activity Questionnaire (PAQ), previously tested and validated for older kids (PAQ-C) (Manchola-González et al., 2017) and adolescents (PAQ-A) (Martínez-Gómez et al., 2009). Those questionnaires include items that ask about activity levels during physical education classes, recess, lunch breaks, after school, evenings, and on weekends, as well as general activity habits. Each question is scored on a 5-point scale, with higher scores indicating more frequent or intense activity. Additionally, simple questions will be included to estimate the time spent on sedentary behaviors such as watching television, using a computer, playing video games, or sleeping.

Fitness Level will be assessed using the Spanish version of the International Fitness Scale (IFIS) questionnaire developed by the PROFITH research group in Spain (Ortega et al., 2013), part of the project funded by the European Union, Study Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA). This questionnaire has been validated in Spanish children (Ayán et al., 2020) and widely used in different countries (Chen et al., 2022; Español-Moya & Ramírez-Vélez, 2014; Vandoni et al., 2021). It also had his reliability and validity tested (De Moraes et al., 2019) as well as used as a standard tool to assess reliability and validity for other questionnaires (Ayán et al., 2020).

Sleep quality will be assessed using the Spanish translation of the Pittsburgh Sleep Quality Index (PQSI), with good reliability in that language (Tomfohr et al., 2013) and validated in young Spanish people (de la Vega et al., 2015). The PSQI assesses various sleep dimensions over the past month, including duration, disturbances, latency, and overall sleep efficiency. It provides a global score (0–21), where higher scores indicate poorer sleep quality, with a score above 5 generally signaling sleep issues.

Quality of life will be evaluated using the Spanish version (Rajmil et al., 2004) of the German Health-related Quality of Life Questionnaire (Kindl) (Ravens-Sieberer & Bullinger, 1998). This questionnaire has already been used in Spanish children (Ávila-García et al., 2021). The KINDL measures multiple dimensions of well-being, including physical, emotional, social, and school-related aspects, providing a comprehensive score that reflects the child's overall quality of life.

Dietary habits, more precisely, the adherence to the Mediterranean diet, will be assessed via the Spanish questionnaire KIDMED, a Mediterranean diet quality index in children and adolescents (Serra-Majem et al., 2004), widely used within the scientific community, demonstrating its reliability and validity in various research settings (García Cabrera et al., 2015). The KIDMED index provides insight into healthy eating patterns by measuring the frequency of specific food choices that align with the Mediterranean diet.

2.6. Data Analysis and Manegement

Statistical analysis will be performed using the IBM Statistical Package for Social Sciences (IBM SPSS Statistics for Windows, version 20.0; IBM Corp, Armonk, NY, USA). Normality of the data will be verified using the Kolmogorov–Smirnov test. Descriptive statistics will be presented as the mean $\pm$ standard deviation (range) or median (interquartile range), depending on the distribution of the data. If normality assumptions are not met, hs-cTnT data will be logarithmically transformed prior to statistical analysis.

For inferential statistics, a repeated-measures ANOVA will be conducted with two within-subject factors (time: pre- and post-exercise; year: baseline, year 1, and year 2) and between-subject factors (maturational stages: Tanner stages 1–5). Homogeneity of variances will be assessed using Levene's test, and corrections (e.g., Greenhouse–Geisser) will be applied if sphericity is violated. A one-way ANOVA will be used to compare peak hs-cTnT values across maturational stages. The association between changes in hs-cTnT and other variables, such as heart rate during exercise and cardiorespiratory fitness, will be assessed using Pearson's correlation coefficient.

Missing data will be handled using listwise deletion, where participants with incomplete data for a specific analysis will be excluded. Pairwise deletion may be applied where appropriate to maximize the use of available data.

Statistical significance will be set at $p < 0.05$.

Data, materials, and protocols will be made available upon request or provided through a public repository upon final manuscript acceptance.

2.7. Ethical Approval

The procedures of this project have been approved by the Ethical Committee for Clinical Research of the Sports Administration of Catalonia (30/CEICGC/2020) and comply with the principles and recommendations of the latest revision of the Declaration of Helsinki (World Medical Association, 2013).

3. Discussion

3.1. Expected Results and Interpretation

The expected outcomes of this study include valuable insights into the variability of hs-cTnT levels post-submaximal exercise in youth, possibly influenced by factors such as maturational status, daily physical activity, and cardiorespiratory fitness (Conesa-Milian et al., 2023). These results could offer a foundational understanding of cardiovascular responses in children and adolescents, filling a critical gap in the existing literature. Establishing reference values for hs-cTnT in this population is another key outcome, providing clinicians and researchers with a crucial benchmark for evaluating cardiovascular risk among youth (Clerico et al., 2021, 2022; Guo et al., 2021). These values will be calculated using mean $\pm$ standard deviation or percentiles, depending on the data distribution, and will be grouped by age, sex, and Tanner stage to ensure clinical applicability. These reference values are not intended to serve as clinical thresholds for diagnosing cardiovascular risk or pathology. Instead, they will provide a baseline for comparing hs-cTnT levels in similar populations and guiding future research.

The study aims to elucidate the complex relationships between physical fitness, health-related habits, maturation, and body composition, offering a more comprehensive understanding of factors affecting cardiovascular health in youth. These insights can inform evidence-based recommendations and early preventive strategies tailored to children and adolescents, particularly in school settings. Participants with elevated hs-cTnT levels will be informed and advised to consult a cardiologist for early detection and follow-up. Tracking these participants over time, in collaboration with medical professionals, may clarify which hs-cTnT levels are linked to cardiovascular risks, contributing to the refinement of future diagnostic thresholds. Although establishing definitive thresholds requires longitudinal studies correlating hs-cTnT levels with actual cardiovascular outcomes, this study provides an essential foundation for understanding the variability of hs-cTnT levels and their relationship with maturational and fitness-related factors.

This research has significant implications across scientific, clinical, and educational domains. Clinically, the establishment of hs-cTnT reference values will provide essential benchmarks to differentiate normal post-exercise responses from potential cardiovascular risks, facilitating early diagnosis and intervention. Personalized health reports generated from the study will further support participants and their physicians in monitoring and maintaining cardiovascular health over time.

Scientifically, this study will contribute to the understanding of post-exercise cardiac biomarker responses in youth, addressing critical gaps in the literature. The reference values and insights into maturational and fitness-related factors will inform future studies and interventions, enabling a more nuanced evaluation of youth cardiovascular health.

Nevertheless, having a predominantly Spanish population can influence physical test results due to cultural factors like diet and popular sports, environmental aspects such as climate and outdoor activity opportunities, and genetic similarities that affect traits like endurance or strength. Socioeconomic factors, including access to sports facilities and education, also play a role. These elements may limit the generalizability of results to other populations. Additionally, the observed trends might differ in regions with distinct dietary patterns, sports preferences, or physical activity opportunities. While these findings provide valuable insights into the Spanish youth population, their broader applicability requires further validation in diverse cultural and geographical settings.

From an educational perspective, findings can support the development of tailored, evidence-based physical activity programs for children and adolescents. Schools could implement routine health assessments informed by these results, fostering healthy habits such as regular exercise, sufficient sleep, and balanced nutrition. For instance, identifying high rates of obesity among students may lead to introducing healthier school meal options, increasing physical education programs, and fostering initiatives like school gardens or nutrition workshops to encourage healthy eating habits. By creating a holistic framework, schools can promote long-term health and well-being in young populations. Those interventions should target the social, economic, environmental, or cultural factors influencing physical activity, addressing barriers that may limit participation. This comprehensive approach ensures that programs are both inclusive and effective, enabling students to adopt sustainable healthy behaviors.

3.2. Strengths and Limitations

The proposed evaluation protocol presents several strengths that can significantly advance current evidence and practice in youth health research. One of its key contributions is its focus on assessing cardiometabolic risk through the measurement of hs-cTnT and cholesterol levels following submaximal exercise, providing valuable insights into the cardiovascular health of children and adolescents. This is particularly relevant as it addresses the need for early detection of cardiac strain, a critical but often overlooked aspect in youth populations. Furthermore, the study aims to establish reference values for hs-cTnT, filling a significant gap in the literature and providing a crucial benchmark for evaluating cardiovascular risk in young individuals.

The inclusion of a large and diverse sample of over 700 children and adolescents enhances the reliability and generalizability of the findings. Unlike previous studies with limited sample sizes (typically 20–30 participants), this study offers a robust foundation for establishing representative hs-cTnT reference values across different maturational stages. Additionally, the longitudinal follow-up over three years allows for the observation of changes in hs-cTnT levels over time, providing unique and previously unavailable data that complement existing cross-sectional studies. These longitudinal insights will significantly contribute to our understanding of cardiovascular development during childhood and adolescence.

By examining the interplay between physical fitness, health-related habits, maturation, and body composition, the evaluation seeks to create a comprehensive understanding of the factors influencing cardiovascular health. These findings will support the development of tailored preventive strategies within educational settings, promoting cardiovascular health and overall well-being in young populations.

Despite its strengths, the evaluation protocol does have limitations that could influence the results. One potential limitation is the reliance on self-reported data for health-related habits, which can be subject to biases and inaccuracies. To mitigate these biases, several strategies have been implemented to improve the accuracy and reliability of the collected data. Firstly, validated questionnaires, widely used in similar populations, have been selected to ensure that the questions are clear, relevant, and comparable with other studies. These instruments help reduce inconsistencies and provide a solid foundation for data collection.

Secondly, questionnaires will be administered digitally using tablets, which allows for structured data entry and minimizes errors in transcription or interpretation of responses. Participants will also receive a detailed explanation on how to complete the surveys before starting, reducing the likelihood of misunderstandings or inaccurate answers. Furthermore, two trained team members will always be present during the questionnaire sessions to clarify doubts and provide support, ensuring that responses are as accurate as possible. For younger participants, parents or legal guardians will be invited to assist in answering questions related to habits like diet or sleep, thereby increasing the precision of the data.

To address potential errors further, consistency checks will be performed during data analysis to identify and address internal inconsistencies or outlier responses, using appropriate statistical techniques or, if necessary, excluding problematic responses. Additionally, participants will be assured that their responses are anonymous and confidential, reducing the pressure to provide socially desirable answers and encouraging honesty.

While these measures aim to minimize inaccuracies, the complex interplay of environmental, social, and individual variables may still not be fully accounted for. Addressing these limitations will be crucial in interpreting the results and ensuring that the evaluation contributes meaningfully to the existing body of knowledge.

3.3. Future Research Directions

The findings from this study will lay the groundwork for future investigations into the cardiovascular health of youth, with implications for both clinical and educational settings. Future research could expand on this study by exploring additional factors such as genetic predisposition, social influences, and broader environmental conditions that may affect cardiometabolic outcomes. Moreover, studies could focus on intervention-based approaches within school environments to test the effectiveness of targeted physical activity programs in reducing cardiovascular risk from a young age. Another avenue for research involves replicating this study with diverse populations across various regions to confirm the generalizability of the findings and to establish global reference values for hs-cTnT in youth.

4. Conclusions

This protocol provides a structured approach to assess cardiometabolic health in children and adolescents, focusing on the impact of submaximal exercise on hs-cTnT and related biomarkers. By examining factors such as maturational status, physical fitness, and health habits, this study aims to establish normative reference values and early indicators of cardiac strain. These reference values are critical for both clinical and educational applications, offering benchmarks to evaluate cardiovascular health and inform tailored preventive strategies.

The insights gained from this research are expected to guide future interventions in educational settings and support long-term health promotion for young populations. Furthermore, integrating these findings into national health policies for youth could enhance the effectiveness of public health initiatives, ensuring that preventive measures are evidence-based and widely applicable.

Author Contributions: Conceptualization, J.R.-M., E.C.-M. and S.A.-R.; methodology, V.H.-G., J.R.-M., A.B.-G. and E.C.-M.; formal analysis, V.H.-G. and S.A.-R.; investigation, E.C.-M., S.A.-R. and J.R.-M.; data curation, V.H.-G. and A.B.-G.; writing—original draft preparation, S.A.-R., E.C.-M., V.H.-G. and J.R.-M.; writing—review and editing, V.H.-G. and J.R.-M.; visualization, S.A.-R., E.C.-M. and A.B.-G.; supervision, J.R.-M. and V.H.-G.; project administration, J.R.-M.; funding acquisition, V.H.-G. and J.R.-M. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The procedures of this project have been approved by the Ethical Committee for Clinical Research of the Sports Administration of Catalonia (30/CEICGC/2020) and comply with the principles and recommendations of the latest revision of the Declaration of Helsinki (World Medical Association, 2013).

Informed Consent Statement: Informed consent will be obtained from all participants and their legal guardians prior to any data collection as part of the protocol procedures.

Data Availability Statement: No data are available at this stage, as this is a study protocol. Data will be collected during the study and, upon completion, will be made available in a publicly accessible repository, subject to privacy and ethical considerations.

Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A Consent Form

Universitat de Lleida

Documento de consentimiento informado

La investigación en curso se titula "Evaluación de diversos parámetros de salud y niveles de actividad física en la escuela primaria y secundaria". Está dirigida por el Dr. Joaquín Reverter y Dr. Francisco Corbi, y realizada por el grupo de investigación consolidado Movimiento Humano (HMRG) de la Generalitat de Catalunya (2021 SGR 01619).
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) Cuantificar los valores pico post-estrés de hs-cTnT tras un test de 20mSRT ejecutado a máxima intensidad, en niños y niñas de diferente estado madurativo, 2) Analizar los principales efectos de la actividad física diaria, la aptitud cardiorrespiratoria y la dosis-respuesta de la MVPA total sobre los valores de hs-cTnT en niños y niñas de diferente estado madurativo y, 3) Analizar longitudinalmente el desarrollo de la FRC (usando los resultados del test 20MSRT) en niñas y niños de 6 a 16 años, y el comportamiento de la aparición de hs-cTnT en diferentes estados madurativos de la misma cohorte de sujetos durante tres años en una fila.

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 multietapa de carrera de ida y vuelta de 20 metros (20mSRT) de la batería ALPHA-Fitness (Assessing Levels of PHysical Activity). Durante la prueba se monitorizará la frecuencia cardíaca del participante. Se realizarán dos extracciones sanguíneas para determinar la concentración de troponina: la primera inmediatamente antes del ejercicio, y la última al cabo de 3h de finalizar la prueba. 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 un cuestionario autoreportado. Los padres y madres del deportista podrán acompañar durante la cumplimentación de los cuestionarios. 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: 30/2020 / 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 lafrecuencia cardíaca y las medidas antropométricas (registro de las medidas y proporciones corporales) que serealizarán 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 Enric Conesa-Milian, núm. Teléfono 973706642 (ext.<6642), correo-e: Enric.conesa@udl.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
- ☐ 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 (2021 SGR 01619) 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) (2021 SGR 01619) 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.

,____ 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 dereferencia normales de un biomarcador de daño cardíaco después de sesiones de actividad física".

Firma:

Appendix B Medical History Form

HISTORIA CLÍNICA

DATOS GENERALES

Nombre:____ Edad:__________ Sexo:___________

Fecha de Nacimiento (Día/mes/año):___________________________Club:_____________________

Dirección:_________________________________Correo electrónico:________________________

ANTECEDENTES CLÍNICOS FAMILIARES

Indique los antecedentes clínicos de interés, sobretodo a nivel cardiovascular:

ANTECEDENTES DEL PACIENTE

Indique los antecedentes o patologías existentes en la persona que forma parte del estudio. (Si necesita realizar alguna observación, realícela en este apartado):

Declaro, bajo mi responsabilidad, que todos los datos anteriormente indicados son verídicos, y para ello firmo a continuación:

FIRMA:

Appendix C Personalized Report

C O R - S C H O O L
2 0 2 4
Estudio sobre la liberación de Troponinas Cardíacas

Destinatario:

Alevín de primer año

Resultados de la valoración

Nombre del deportista	Valoración	Nacimiento	Edad	Talla	Peso	Sexo
--------------------	--------------	--------------	10 años	145 cm	52,6 kg	Mujer

Análisis antropométrico:

Composición corporal del participante

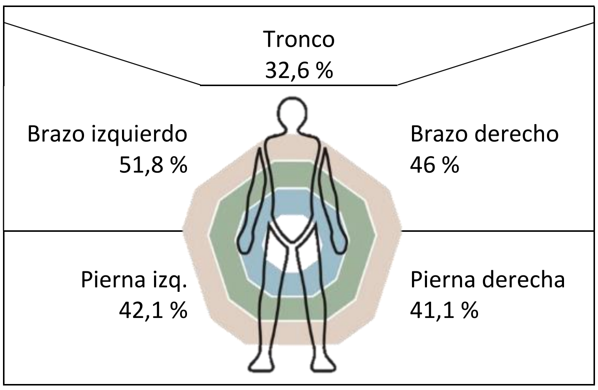
Variable	Valor		Observaciones
Proporción grasa corporal	37,4	%	-
Masa total grasa corporal	19,7	Kg	-
Proporción agua*	45,8	%	-
Masa total agua*	24,1	Kg	-
Proporción masa libre de grasa	32,9	%	-
Masa muscular	31,2	Kg	-
Masa ósea	1,7	Kg	-
Índice de Masa Corporal	25,0		-
Tasa metabólica basal	5540	Kj	-
Tasa metabólica basal relativa	1324	Kcal	-

* La cantidad de agua en el cuerpo humano es altamente variable, y el valor proporcionado refleja únicamente el instante previo a la prueba.

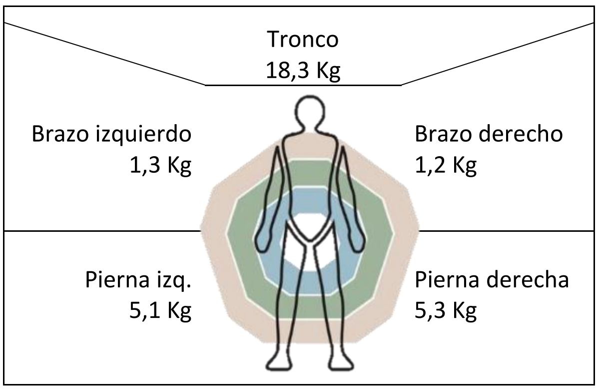
Análisis antropométrico segmental

Composición corporal del participante por segmentos corporales

Proporción de grasa



Masa muscular



INEFC Institut Nacional d'Educació Física de Catalunya Lleida — Generalitat de Catalunya

Universitat de Lleida Grup de Recerca Moviment Humà

Análisis de la adaptación cardíaca al ejercicio

Rendimiento del deportista durante la prueba

Variable	Valor		Valores normales
Resultado de la prueba	580	m	-
Frecuencia cardíaca máxima observada	200,5	bpm	-
Frecuencia cardíaca media observada	178,73	bpm	-
Valor de troponina pre-test	<3	ng/mL	<14
Valor de troponina post-test	<3	ng/mL	<14

Respuesta bioquímica

Los resultados obtenidos indican que el deportista se encuentra dentro de los parámetros normalizados de troponinas cardíacas pre y post esfuerzo

Resultados cuestionarios

Variable	Valor	Observaciones
KINDL	72,5	Escala 1/100
KIDMED	2	
PAQ-C/A	2,60	Escala 1/5

Análisis cuestionarios

El cuestionario KINDL realiza una valoración de la calidad de vida en niños y adolescentes, utiliza una escala del 1 al 100, siendo este último la mejor puntuación. Estudios de referencia muestran que población española de la misma edad presenta valores alrededor de los 76 puntos.

El cuestionario KIDMED evalúa la calidad de la alimentación en niños y adolescentes. La interpretación del resultado es la siguiente: dieta mediterránea pobre (valores menores o iguales a 3), dieta mediterránea media (valores entre 4 y 7) y dieta mediterránea alta (valores mayores de 7).

El cuestionario PAQ-C/A determina los niveles de actividad física realizada los últimos 7 días, utiliza una escala de 1 a 5, siendo este último la mejor puntuación. Los sujetos con valores superiores a 2,75 se consideran físicamente activos.

Interpretación general

El análisis antropométrico refleja unas proporciones corporales del deportista normales para su edad, nivel deportivo y modalidad. El índice de masa corporal del deportista se encuentra dentro del Percentil 95 para su grupo de edad. Esto significa que la relación peso – talla es superior al porcentaje medio de la población de su edad.. La adaptación del ritmo cardíaco al esfuerzo es lineal y con un retorno a los niveles basales rápido durante la recuperación posterior al ejercicio. Los valores bioquímicos no muestran ninguna alteración destacable

*El Grupo de Investigación Movimiento Humano queremos agradecer a ----- su participación en este estudio, y en especial la implicación de los padres y madres que hicisteis posible su realización.

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