Level of skeletal troponin I as a marker of skeletal muscle damage after eccentric exercise: a systematic review and meta-analysis

Nivel de troponina I esquelética como marcador de daño muscular esquelético después de ejercicio excéntrico: una revisión sistemática y meta-análisis

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Abstract

Introduction: Eccentric exercise is known to cause muscle damage, referred to as Exercise Induced Muscle damage (EIMD), particularly when performed for the first time. Skeletal troponin I (sTnI) is a highly specific biomarker of skeletal muscle injury, and its release has been demonstrated in several studies following eccentric exercise.
Objective: The aim of this study was to examine the time-course of sTnI release following eccentric exercise.
Methods: Articles were retrieved through searches of Ovid, CINAHL, Scopus, Web of Science, Embase, and PubMed using keywords related to eccentric exercise, muscle damage, and skeletal troponin I. This study adhered to PRISMA guidelines and was registered in PROSPERO (registration number: CRD42022385362).
Results: Out of 6,030 identified studies, three met the inclusion criteria, comprising a total of 36 participants. Significant increases in sTnI levels were observed at 6 hours (SMD = 2.43; 95% CI: 0.69–4.17; P = 0.006) and at 24 hours (SMD = 2.15; 95% CI: 1.32–2.99; P < 0.00001) following eccentric exercise. At 96 hours post-exercise, sTnI levels were not significantly elevated (*P* = 0.07).
Conclusions: sTnI levels were elevated at 6 hours and remained elevated up to 24 hours following eccentric exercise. Given the limited number of included studies, further research is warranted to improve data availability and to provide more detailed evidence on the time course of sTnI changes.

Keywords

Eccentric contraction; eccentric exercise; EIMD; muscle damage; skeletal troponin I.

Resumen

Antecedentes: Se sabe que el ejercicio excéntrico causa daño muscular, llamado daño muscular inducido por el ejercicio (EIMD), especialmente cuando se realiza por primera vez. La sTnI es un biomarcador altamente específico de lesión del músculo esquelético y su liberación se ha demostrado en varios estudios después del ejercicio excéntrico.
Objetivo: El objetivo de este estudio fue explorar las características del curso temporal de la liberación de sTnI después del ejercicio excéntrico.
Métodos: Los artículos fueron obtenidos a través de Ovid, CINAHL, Scopus, Web of Science, Embase y PubMed. Búsquedas utilizando palabras clave relacionadas con ejercicio excéntrico, daño muscular y troponina I esquelética. Este estudio se adhirió a las pautas PRISMA y registrada en PROSPERO (CRD 42022385362).
Resultados: Se incluyeron tres de los 6,030 estudios identificados con un total de 27 participantes. Se encontraron aumentos significativos en los niveles de sTnI a las 6 horas (SMD = 2.43, 95% CI = 0.69 to 4.17, P = 0.006) y a las 24 horas (SMD = 2.15, 95% CI = 1.32 to 2.99, P < 0.00001) después del ejercicio excéntrico. A las 96 horas después del ejercicio excéntrico, los niveles de sTnI no aumentaron significativamente (P=0,07).
Conclusiones: El nivel de sTnI se elevó a las 6 horas y permaneció elevado hasta 24 horas después del ejercicio excéntrico. Considerando el número limitado de estudios incluidos, se deberían realizar más investigaciones en el futuro para mejorar la disponibilidad de datos y proporcionar evidencia sobre una evolución temporal más detallada de los cambios en sTnI.

Palabras clave

Contracción excéntrica, ejercicio excéntrico, EIMD, daño muscular, troponina I esquelética.

Introduction

Eccentric exercise is characterized by the forced lengthening of contracted muscle due to the high mechanical forces involved. As a result, it is widely used to maintain and enhance muscle strength (Hody et al., 2019) and to reduce the risk of injury in athletes (Avila-Quintero et al., 2024). However, despite its benefits, eccentric exercise can also lead to Exercise-Induced Muscle Damage (EIMD), particularly when performed for the first time (Hody et al., 2019; Stožer et al., 2020). Compared to other types of exercise, eccentric contractions cause more severe muscle damage (Hody et al., 2019). This is attributed to the nature of eccentric exercise, which recruits fewer motor units while generating greater force compared to concentric exercise, thereby imposing a higher load per motor unit (Douglas et al., 2017; Hody et al., 2019; Stožer et al., 2020).

The underlying mechanism of EIMD during eccentric contractions involves overstretched sarcomeres resulting from the forced lengthening of contracted muscle fibers (Hody et al., 2019). These conditions precipitate membrane damage, the opening of stretch-activated channels, dysfunction of the excitation-contraction (EC) coupling system, and prolonged force decline due to the degradation of EC coupling proteins (e.g., junctophilins [JPs]) and contractile proteins (e.g., desmin) mediated by calcium-activated calpains. Furthermore, as a result of membrane damage, skeletal muscle proteins such as Creatine Kinase (CK), myoglobin, Myosin Heavy Chain (MHC), and Skeletal troponin I (sTnI) are released from the muscle cytosol into the blood plasma (Stožer et al., 2020).

Based on this mechanism, skeletal muscle protein levels are commonly used as biomarkers of EIMD (Hody et al., 2019). Conventional biomarkers of skeletal muscle damage—such as CK, myoglobin, MHC, lactate dehydrogenase, and aspartate aminotransferase—are frequently measured. However, these markers lack specificity for skeletal muscle damage, as they are present in the cytosol and mitochondria of all high-energy-demanding tissues (Bogomolova & Katrukha, 2024; Goldstein, 2017). In addition to skeletal muscle, CK is also found in cardiac muscle and brain cells. Each tissue contains varying proportions of three CK subunits: MM, MB, and BB. Skeletal muscle comprises approximately 98% CK-MM and 2% CK-MB subunits. Cardiac muscle contains 70–80% MM and 20–30% MB subunits, whereas the brain predominantly expresses BB subunits (Aujla et al., 2024). Myoglobin is a hemoprotein that reversibly binds oxygen and is located in the cytoplasm of cardiac and skeletal muscle cells (Zafar Gondal et al., 2022). Myosin heavy chain (MHC), a contractile protein found in the thick filaments of both cardiac and skeletal muscle, functions as an ATPase, converting chemical energy from ATP into mechanical energy (Sweeney & Hammers, 2018).

Due to the limited specificity of classical biomarkers, novel alternatives—such as sTnI, Myosin Light Chain 3 (Myl3), and Fatty Acid-Binding Protein 3 (FABP3)—have been investigated. Although these novel biomarkers are more sensitive than conventional ones, sTnI remains the only biomarker considered specific to EIMD, as it is exclusively expressed in skeletal muscle (Bogomolova & Katrukha, 2024; Goldstein, 2017;De Matteis et al., 2019). Due to its high specificity, sTnI is not only used as a biomarker for EIMD but also serves as a diagnostic and monitoring tool for conditions such as rhabdomyolysis, orthopedic and soft tissue injuries, inflammatory myopathies, muscular dystrophies, and drug induced myotoxicity (Bogomolova & Katrukha, 2024).

sTnI has two isoforms: TNII1 or slow skeletal troponin I (ssTnI), which is found in slow-twitch (type I) muscle fibers, and TNII2 or fast skeletal troponin I (fsTnI), which is found in fast-twitch (type II) muscle fibers in adults (De Matteis et al., 2019). In EIMD resulting from eccentric contractions, a significant increase is observed only in fsTnI, while sTnI levels remain unchanged. This is because eccentric contractions primarily target and damage fast-twitch skeletal muscle fibers (Bogomolova & Katrukha, 2024).

Numerous studies have investigated the effects of eccentric exercise on sTnI levels and reported significant increase. However, findings regarding the time-course of sTnI elevation remain inconsistent, particularly in identifying the peak concentration. Some studies have reported peak increases in sTnI levels at 24 hours following eccentric exercise (Sorichter, Mair, Koller, Gebert, et al., 1997a; Sorichter, Mair, Koller, Müller, et al., 1997; Willoughby, McFarlin, et al., 2003; Willoughby, Taylor, et al., 2003), whereas others observed peak levels at 6 hours post-exercise (Sorichter et al., 2001; Sorichter, Mair, Koller, Gebert, et al., 1997a). These differences are likely related to variations in the study populations, muscle

groups involved, and the type and intensity of eccentric exercise. Notably, a comprehensive and systematic synthesis using a quantitative approach has not yet been conducted.

Integrating all relevant data from studies examining sTnI release following eccentric exercise into a meta-analysis would provide a larger overall sample size, allowing for more robust and accurate statistical conclusions. Additionally, categorizing individual studies by assessment time points may offer a more precise understanding of the temporal profile of sTnI release. Therefore, we conducted a systematic review and meta-analysis to analyze the time-course characteristics of sTnI levels as a specific marker of muscle damage following eccentric exercise. This analysis will help identify the optimal timing for assessing sTnI levels following eccentric exercise. Furthermore, it holds clinical significance, as sTnI serves as a diagnostic marker in various skeletal muscle disorders, as previously noted.

Method

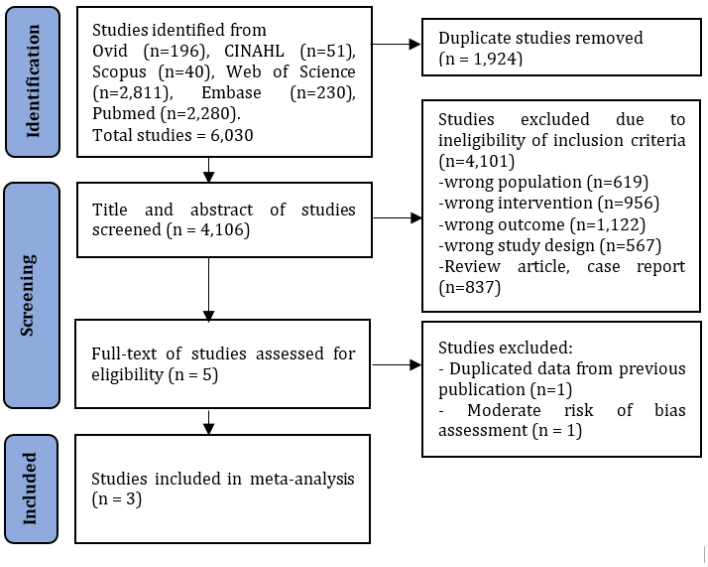
The systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines (see Table S1, Supplemental Digital Content). The protocol was registered in PROSPERO under registration number CRD42022385362.

Identification and Selection of studies

Search Strategies

A literature search was conducted in January 2023 across six databases—Ovid, CINAHL, Scopus, Web of Science, Embase, and PubMed—using keywords related to eccentric exercise, muscle damage, and skeletal troponin I. The search was limited to English language studies involving human subjects. The complete search strategy is detailed in Table S2 of the Supplemental Digital Content. This search yielded 6,030 potential studies, as illustrated in **¡Error! No se encuentra el origen de la referencia.**.

Figure 1 PRISMA flowchart of studies selection



Eligibility Criteria and Selection of Studies

Interventional studies involving human participants were included in this study if they met the following criteria based on the PICO model (Amir-Behghadami & Janati, 2020):

(a) Population: healthy adults aged 18 years or older);

(b) Intervention: eccentric contractions or exercise;

(c) Comparison: pre-exercise sTnI level;

(d) Outcome: sTnI level following eccentric exercise.

The exclusion criteria for this study were as follows:

(a) conference proceedings, seminars, and non-interventional studies;

(b) studies involving non-human subjects;

(c) studies published in languages other than English.

Study selection was conducted independently by two reviewers (IRN and PPI) using Rayyan application (Ouzzani et al., 2016) to identify studies meeting the inclusion criteria. After removing duplicate records, titles and abstracts were screened for eligibility. Any disagreement between the two reviewers were resolved through discussion with a third reviewer (RA). Interrater reliability was assessed using Cohen's Kappa coefficient (Li et al., 2023). Kappa values were interpreted as follows: ≤0 indicated no agreement; 0.01–0.20, none to slight; 0.21–0.40, fair; 0.41– 0.60, moderate; 0.61–0.80, substantial; and 0.81–1.00, almost perfect agreement (Warrens, 2015).

Data Extraction

Data were extracted by the first author (IRN) and cross-checked by the second author (PPI). The extracted information included: first author, year of publication, the number of participants, training status, sex, age, type and intensity of eccentric exercise, and sTnI levels before and after the intervention at all available time points. When data in the included studies were presented in graphical form, values were extracted using ImageJ software (Schneider et al., 2012). This software has demonstrated high test-retest reliability for distance measurements (Suzuki et al., 2021). If the mean and standard deviation (SD) values were not available, they were estimated from available data (Q1, median, and Q3) using the formula proposed by Wan *et al*. (2014). This method provides a slightly imprecise estimate, with a relative error of approximately 5% compared to the true sample SD. When data were incomplete, the corresponding authors of the included studies were contacted.

Quality Analysis and Risk of Bias Assessment

The quality of the eligible studies was independently assessed by two review authors (IRN and PPI) using the JBI Critical Appraisal Tool for Quasi-Experimental Studies (Joanna Briggs Institute, 2017). The JBI tool consists of nine question items, which evaluate: the cause-effect relationship; the similarity of participants characteristics between the comparison groups; the similarity of treatments received by groups aside from the manipulated "cause"; the presence of a control group; whether multiple outcome assessments were conducted before and after the intervention; the completeness of outcome follow-up, with adequate description and analysis of any missing data; whether outcomes were assessed in the same way; whether outcomes were assessed reliably; and the appropriateness of the statistical analysis. Studies with JBI scores above 70% were categorized as high-quality, those scoring between 50% and 70% as medium-quality, and those scoring below 50% as low-quality. Only high-quality studies were included in this review. Any disagreement regarding the quality assessment of the studies was resolved through discussion with a third author (RA). Interrater reliability among the authors' judgments was analyzed using Cohen's Kappa score (Li et al., 2023).

Data Analysis

This study compared sTnI level at multiple time points with baseline values in response to eccentric exercise. The analysis was clustered according to corresponding measurements time points. Quantitative analysis (meta-analysis) was conducted using Review Manager 5.4.1 (Review Manager (RevMan), 2020).

Standardized Mean differences (SMD), also known as effect size, and the confidence interval of sTnI level were calculated to compare the magnitude of sTnI changes between studies with similar units of measurement. Thus, the measurement units for sTnI in the included studies were standardized to a common unit, i.e. µg/L. The calculation of SMD based on the mean and Standard deviation (SD) values between baseline and end point measurements. If the included studies presented sTnI values in two bouts, only the first bout was analyzed.

SMD was considered significant if the p-value was < 0.05 or if the 95% confidence interval did not cross zero. A positive SMD indicates an increase in sTnI after exercise, and vice versa. The magnitude of the

effect size was determined using Cohen's criteria: an SMD less than 0.5 was considered a small effect size; 0.5-0.8, a medium effect size; and greater than 0.8, a large effect size (Cohen, 1988).

A random-effects model was applied due to the potential variation in effects based on the exercise type, intensity, setting, and other factors. Heterogeneity was evaluated using the standard chi-square (χ^2) test. The magnitude of heterogeneity was determined using the I2 statistic. I2 values of 25%, 50%, and 75% indicate low, moderate, and high heterogeneity, respectively (Sterne et al., 2019).

Secondary outcomes of this study included other markers of EIMD. However, only CK could be analyzed quantitatively. MHC and myoglobin were considered for qualitative analysis, as these biomarkers were presented in only one study.

Analysis of publication bias using egger's test and sensitivity analysis could not be performed in this study due to the limited number of included studies in this systematic review.

Results

Search Results

Using the full search strategy described in Table S2 of the Supplemental Digital Content, 6,030 potential studies were retrieved. After removing 1,924 duplicate studies, the titles and abstracts of 4,106 studies were independently assessed for eligibility by two review authors (IR and PPI). The degree of agreement between two authors was 0.66, indicating substantial agreement (McHugh, 2012). Both reviewers included five studies and excluded the same 4,094 studies. The remaining 7 studies were subject to debate and subsequently resolved through discussion with a third reviewer (RA). As a result, five studies met the eligibility criteria. The reasons for study exclusion are detailed in the PRISMA flow chart (**¡Error! No se encuentra el origen de la referencia.**).

Of the five included studies, one study (Willoughby, Taylor, et al., 2003) was excluded due to duplicated data from a previous study (Willoughby, McFarlin, et al., 2003), and another study (Sorichter, Mair, Koller, Gebert, et al., 1997a) was excluded due to moderate quality based on the JBI critical appraisal checklist (Joanna Briggs Institute, 2017). As a result, three high quality studies (Sorichter et al., 2001; Sorichter, Mair, Koller, Müller, et al., 1997; Willoughby, McFarlin, et al., 2003) were included in the analysis (**¡Error! No se encuentra el origen de la referencia.**).

Data were presented as Mean ± SD. Data from two included studies (Sorichter et al., 2001; Willoughby, McFarlin, et al., 2003) were extrapolated from graphics as Mean ± SD using ImageJ Software (Schneider et al., 2012). Mean ± SD from two studies (Sorichter et al., 2001; Sorichter, Mair, Koller, Müller, et al., 1997) were calculated from available data, i.e. median and interquartile range, using an equation proposed by Wan *et al.* (Wan et al., 2014).

Risk of Bias Assessment

The risk of bias assessment of the included studies is summarized in **¡Error! No se encuentra el origen de la referencia.**. Based on the JBI critical appraisal checklist, one study (Sorichter, Mair, Koller, Gebert, et al., 1997a) was rated as moderate quality due to the presence of a group with participants lost to follow-up and the absence of corresponding analysis in the results.

Table 1. Risk of Bias Assessment of the Included Studies

Author	JBI Critical Appraisal Checklist									JBI Score	Risk
	1	2	3	4	5	6	7	8	9		
Willoughby et al., 2003a (Willoughby, McFarlin, et al., 2003)	√	√	√	-	√	√	√	Unknown	√	77.8	Low
Sorichter et al., 2001 (Sorichter et al., 2001)	√	√	√	-	√	√	√	Unknown	√	77.8	Low
Sorichter et al., 1997a (Sorichter, Mair, Koller, Gebert, et al., 1997a)	√	√	√	-	√	-	√	Unknown	√	66.7	Moderate
Sorichter et al., 1997b (Sorichter, Mair, Koller, Müller, et al., 1997)	√	√	√	-	√	√	√	Unknown	√	77.8	Low

Table 1. Risk of Bias Assessment of the Included Studies

Author	JBI Critical Appraisal Checklist									JBI Score	Risk
	1	2	3	4	5	6	7	8	9		

1 = availability of cause-effect relationship; 2 = similarity of participants characteristics between the compared groups; 3 = similarity of the treatment received between groups, aside from the manipulated "cause"; 4 = availability of control group; 5 = Presence of multiple outcome assessments conducted before and after the intervention; 6 = completeness of outcome follow up and if incomplete it should be described and analyzed adequately; 7 = outcomes assessment in same way; 8 = outcomes assessment in a reliable way; 9 = appropriateness of statistical analysis; JBI = Joanna Briggs Institute

Characteristics of Included Studies and Eccentric Exercise Intervention

The characteristics of included studies are provided in **¡Error! No se encuentra el origen de la referencia.**:

Table 2. Overview of included studies

Author	Subjects	Intervention		Comparison	Outcome	
		Type	Intensity		Primary	Secondary
Sorichter (1997b) (Sorichter, Mair, Koller, Müller, et al., 1997)	Ten male healthy physical education teacher trainees, not involved in strength training, aged 21-27 years.	ECC of quadriceps femoris muscle group.	7 sets, 10 repetitions, 150% MVC.	Before vs after ECC	Significantly increased sTnI peak values were observed at 24 h after ECC.	CK peaked simultaneously with sTnI. MRI signal intensity changes are associated with an increase of sTnI and CK. No cTnI was detected.
Sorichter (2001) (Sorichter et al., 2001)	9 male, 9 female. Not involved in any specific training. Male: 21 ± 1.7 years old, 75±3.5 kg. Female: 22 ± 2.1 years, 58±4.7 kg.	20 min of downhill treadmill running with 16% decline.	70% of each subject's VO2peak	Male vs female	In the male and female groups, sTnI was increasing at 6 hours after exercise.	In both groups, Mb and CK significantly increased at 6 hours after exercise, whereas MHC increased at 24 hours after exercise. No differences in the relative muscle protein release between females and males before and after exercise.
Willoughby (2003a) (Willoughby, McFarlin, et al., 2003)	Eight male, untrained, 21.0±2.6 years old, 81.2±12 kg	ECC of the dominant knee extensors	7 sets, 10 repetitions at 150% 1-RM	Bout 1 vs Bout 2	At the first bout, sTnI significantly increased at 2, 4, 6, 24 and 48 h-post ECC. sTnI at the first bout was greater than the 2nd bout.	Both 1st and 2nd bout, IL-6 increased at 4 and 6 hours after ECC. At 24 and 48 hours after the first bout of ECC, muscle soreness increased, whereas MDS and MSS decreased.

ECC = Eccentric Contraction; MVC = Maximal Voluntary Contraction; MDS = Maximum Dynamic Strength; MSS = Maximum Static Strength; CK = Creatine Kinase; Mb = Myoglobin.

Three studies (Sorichter et al., 2001; Sorichter, Mair, Koller, Müller, et al., 1997; Willoughby, McFarlin, et al., 2003) were included in the meta-analysis, involving a small number of participants ranging from 8 to 18, with a total of 36 participants. Two studies (Sorichter, Mair, Koller, Müller, et al., 1997; Willoughby, McFarlin, et al., 2003) included only male participants, while one study (Sorichter et al., 2001) included an equal number of male and female participants. All participants were healthy and not involved in any training program. In two of the studies (Sorichter, Mair, Koller, Müller, et al., 1997; Willoughby, McFarlin, et al., 2003), participants performed eccentric contraction of lower extremities (50%), including the quadriceps femoris muscle group, whereas the other studies (Sorichter et al., 2001) used a downhill treadmill (50%).

Main Outcome

Time-Course Analysis of sTnI Level Following Eccentric Exercise

Meta-analysis of sTnI levels was performed at 6, 24, and 96 hours post-eccentric exercise. At 6 hours post-eccentric exercise, sTnI levels significantly increased (Figure 2, SMD = 2.43, 95% CI = 0.69 to 4.17, P = 0.006) with high and significant heterogeneity (Figure 2, df = 2; P = 0.008; I^2 = 79%). A significant increase in sTnI level was also observed at 24 hours post-eccentric exercise (Figure 3, SMD = 2.15, 95% CI = 1.32 to 2.99, P < 0.00001) with moderate, non-significant heterogeneity (Figure 3, df = 3; P = 0.14; I^2 = 43%). At 96 hours post-eccentric exercise, sTnI was not significantly different from baseline (P = 0.07), and no significant heterogeneity was observed (df = 1, P = 0.94; I^2 = 0%), as shown in Figure .

sTnI levels at 48 hours post eccentric exercise could not be analyzed quantitatively, as they were reported in only one included study (Willoughby, McFarlin, et al., 2003). Willoughby et al. (2003) reported a decrease in sTnI levels at 48 hours post-eccentric exercise compared to the 24-hour level, with no significant difference from the pre-exercise level.

Peak increases of sTnI levels were observed at 6 hours following eccentric exercise (**¡Error! No se encuentra el origen de la referencia.**). However, no statistically significant difference was found between the increases in sTnI levels at 6 and 24 hours post-eccentric exercise (P = 0.78) (Figure 6).

Figure 2. sTnI level changes at 6 hours following eccentric exercise

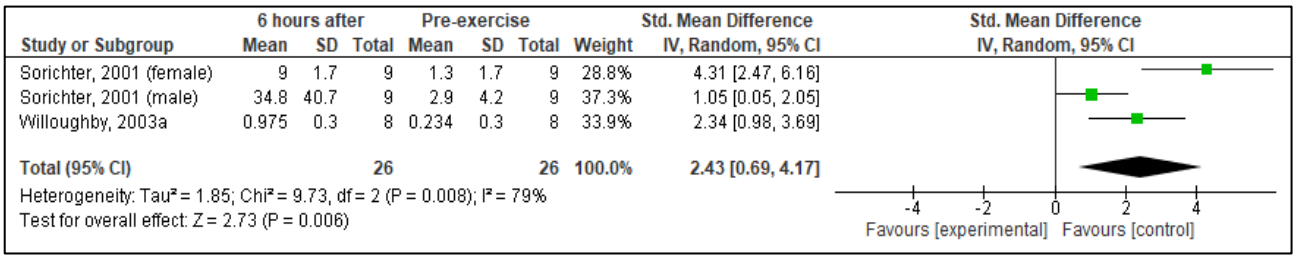


Figure 3. sTnI level changes at 24 hours following eccentric exercise

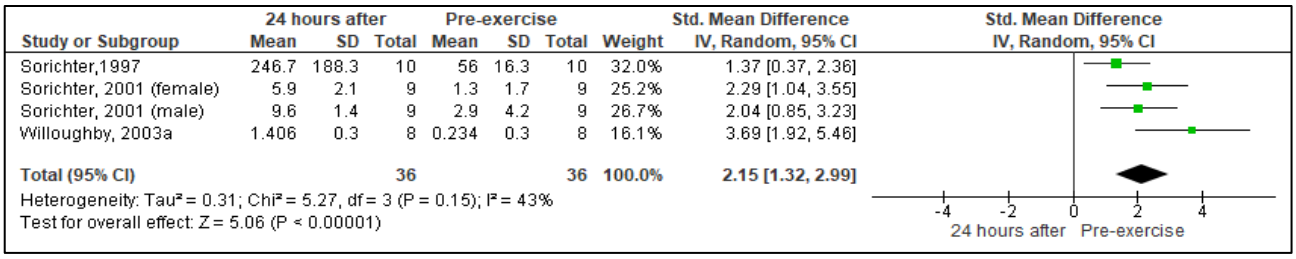
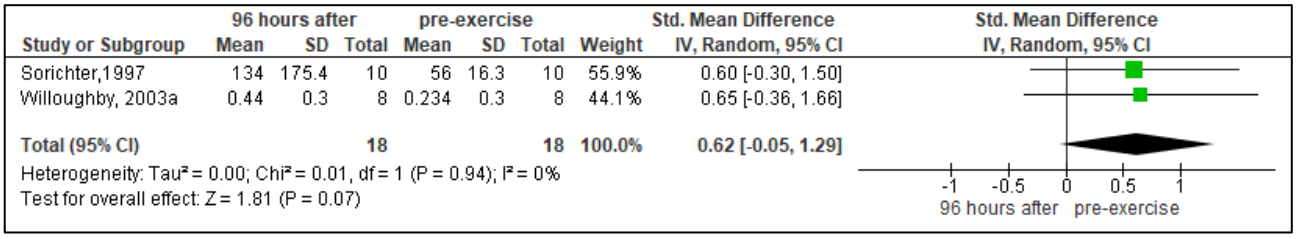


Figure 4. sTnI level changes at 96 hours following eccentric exercise



Secondary Outcomes

In this study, meta-analysis of secondary outcomes was performed only on CK activity due to the limited number of studies reporting other markers, including MHC and myoglobin.

Meta-analysis of CK activity was performed using data from two studies (Sorichter et al., 2001; Sorichter, Mair, Koller, Müller, et al., 1997) at 6 and 24 hours post-eccentric exercise. CK level increased significantly at 6 hours (Figure 4, SMD = 1.49, 95% CI = 0.73 to 2.26, P = 0.0001) and 24 hours (Figure 5, SMD = 2.34, 95% CI = 0.61 to 4.06, *P* = 0.008) post-eccentric exercise. Heterogeneity at 24 hours was significantly high (Figure 5, df = 2; P = 0.005; I^2 = 81%). No included studies reported CK activity at 48 hours, and only the studies of Sorichter et al (1997) reported CK activity at 96 hours, showing no significant increases compared to baseline.

Peak increases of CK levels were observed at 24 hours post-eccentric exercise; however, no significant differences were observed between increases at 6 and 24 hours (P = 0.38) (Figure 6).

Figure 4. Creatine Kinase level changes at 6 hours following eccentric exercise

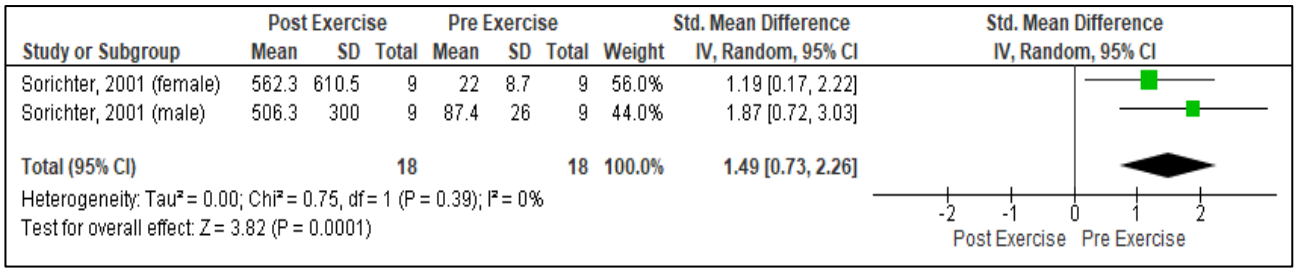


Figure 5. Creatine Kinase level changes at 24 hours following eccentric exercise

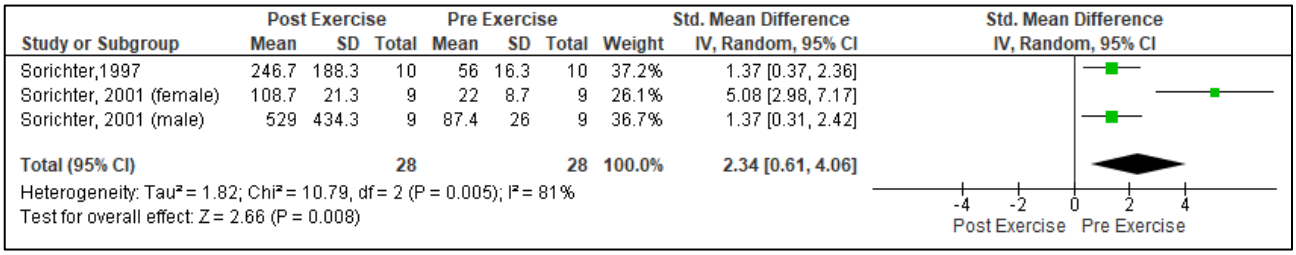
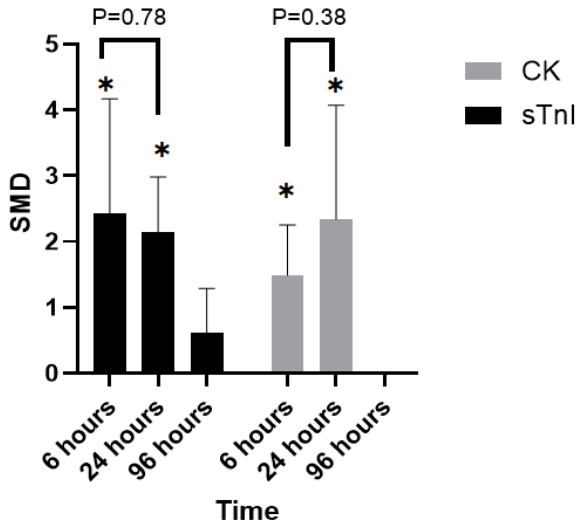


Figure 6. Time-course change of sTnI and Creatine Kinase level after eccentric exercise



SMD = Standard Med Difference.
*Significant differences from baseline level (P < 0.05).

Myoglobin and MHC activity post-eccentric exercise were reported in only one study (Sorichter et al., 2001). The peak increase in Myoglobin was observed at 6 hours post-eccentric exercise, while MHC peaked at 24 hours post-eccentric exercise (Sorichter et al., 2001).

Discussion

sTnI is a specific indicator of skeletal muscle damage because it is specifically located at thin filament of skeletal muscle fibers (Hall, 2016; Sherwood, 2018).

Our study showed significant increases of sTnI levels at 6 hours, persisting until 24 hours following eccentric exercise. Although a greater increase was observed at 6 hours compared to 24 hours, the difference between these time points was not statistically significant. This finding may be explained by ultrastructural changes in skeletal muscle after a single eccentric exercise bout. The most severe skeletal muscle damage occurs at 24 hours post-eccentric exercise. At 48 hours following eccentric exercise, the

degree of ultrastructural damage was less severe than at 24 hours (Ying et al., 2021). Unfortunately, sTnI increases at 48 hours could not be analyzed quantitatively in this study, as only one included study reported this (Willoughby, McFarlin, et al., 2003). Willoughby et al. (2003) reported that sTnI levels decreased at 48 hours post-exercise compared to the 24-hour level, with no significant difference from the pre-exercise baseline. This finding aligns with the ultrastructural changes of skeletal muscle after eccentric exercise reported by Ying et al. (2021).

Our study also found that sTnI levels at 96 hours post-eccentric exercise were not significantly different from baseline. This finding indicates that skeletal muscle injury had returned to normal by this time point. It is consistent with ultrastructural changes reported by Ying et al (2021), which showed a significant decrease in muscle damage beginning at 72 hours post-eccentric exercise. At 72 hours, myofibril arrangement was nearly normal, with clear identification of bands and lines (Ying et al., 2021).

The onset of a significant increase in sTnI at 6 hours post eccentric exercise, as observed in this study, confirms that this marker is suitable as an initial marker for EIMD, as reported in previous studies (Sorichter et al., 2001; Sorichter, Mair, Koller, Gebert, et al., 1997a). One underlying mechanism contributing to its early release is the availability of a soluble TnI precursor pool in the sarcoplasm of skeletal muscle fibers, although it represents only 3-4% of the total sTnI in these fibers. Another mechanism is the susceptibility of sTnI to calpain degradation (Sorichter, Mair, Koller, Gebert, et al., 1997a). Calpain activation is a consequence of eccentric EIMD. Eccentric contraction forcibly over-stretching sarcomers, disrupting myofibril's structure. Because myofibrils and the membrane are connected via T-tubules, over-stretching sarcomers can damage T-tubules and the sarcolemma, increasing membrane permeability. Sarcolemma damage promotes uncontrolled calcium influx into the sarcoplasma, which in turn induces calpain activation (Stožer et al., 2020).

Besides sTnI, our meta-analysis showed significant increases in CK activity beginning at 6 hours post-exercise. CK activity at 24 hours was also significantly increased compared to baseline, with no significant difference between the two time points. CK activity at 48 hours could not be analyzed quantitatively due to the lack of data. Only Sorichter et al. (1997) reported CK activity at 96 hours, showing no significant increases at that time. In contrast, Chapman et al. (2013) documented significant elevation in CK levels from days 1 through 4 post-eccentric exercise. This difference may result from differences in exercise protocols, muscle groups involved, and the degree of muscle damage (Chapman et al., 2013).

Myoglobin is also considered an early marker of EIMD. Sorichter et al. (2001) reported that Myoglobin activity peaked at 6 hours post-eccentric exercise. The early increase in myoglobin is attributed to its small molecular weight (18 kDa) and its direct release into capillaries following muscle fibers damage (Chen et al., 2020). Unfortunately, myoglobin could not be analyzed quantitatively in this study, as only one included study (Sorichter et al., 2001) reported its level.

In contrast to these early biomarkers, MHC shows a more delayed elevation. Sorichter et al. (2001) observed peak increases in MHC at 24 hours following eccentric exercise.

CK, myoglobin, and MHC are classical or conventional biomarkers of skeletal muscle damage commonly used in the clinical settings. However, they are not specific to skeletal muscle, as they are located in the cytosol and mitochondria of all high-energy demanding tissues (Bogomolova & Katrukha, 2024; Goldstein, 2017). For instance, CK is also present in cardiac muscle and brain cells. (Aujla et al., 2024). Similarly, both Myoglobin and MHC are found in cardiac as well as skeletal muscle tissues (Sweeney & Hammers, 2018; Zafar Gondal et al., 2022). Recently, novel biomarkers of skeletal muscle damage have been investigated, including sTnI, myosin light chain 3 (Myl3), and Fatty Acid-Binding Protein 3 (FABP3). Although these novel biomarkers demonstrate greater sensitivity than classical markers, sTnI remains the only biomarker considered specific for EIMD (Bogomolova & Katrukha, 2024; Goldstein, 2017), due to its exclusive expression in skeletal muscle (De Matteis et al., 2019). Because of its specificity, is not only used as a biomarker for EIMD but also serves as a diagnostic tools and therapeutic monitoring marker in conditions such as rhabdomyolysis, orthopedic and soft tissue injuries, inflammatory myopathies, muscular dystrophies, and drug-induced myotoxicity (Bogomolova & Katrukha, 2024).

This study has several limitations that should be addressed in future research. First, only three studies were included in the quantitative analysis. Second, tracking the temporal changes of biomarkers was challenging, as sTnI and CK were not consistently measured at all time points across studies. Specifically, changes in sTnI and CK at 48 and 72 hours post-exercise could not be quantitatively analyzed due to

insufficient data. Third, the secondary outcome focused solely on conventional biomarkers, despite increasing research interest in novel biomarkers of skeletal muscle damage.

Furthermore, the results at several time points in this study showed significantly moderate to high heterogeneity. This variability may be attributed to these heterogeneity, including the small number of included studies, differences in the types of eccentric exercise, and variations in outcome measurement. Only three eligible studies were included, with males comprising 75% of the participants. Sorichter et al. (2001) reported significantly higher levels of sTnI levels following downhill running in males compared to females. However, the magnitude of increase from baseline was not significantly different between sexes (Sorichter et al., 2001). In two of the three included studies (Sorichter, Mair, Koller, Müller, et al., 1997; Willoughby, McFarlin, et al., 2003), participants performed maximal eccentric contraction of the lower extremities, whereas the other study (Sorichter et al., 2001) involved downhill running. Unfortunately, subgroup analysis based on the type of eccentric exercise could not be performed due to the limited number of studies. Sorichter, Mair, Koller, Gebert, et al. (1997b) reported a significant increase in sTnI following both downhill running and eccentric contraction; however, it remains unclear whether differences exist in the time-course characteristics between these exercise modalities. Additionally, the methods used to measure sTnI varied among the included studies. Two studies (Sorichter et al., 2001; Sorichter, Mair, Koller, Müller, et al., 1997) employed IEMA with an intra-assay coefficient of variation (CV) below 6%, while the other study (Willoughby, McFarlin, et al., 2003) used ELISA with an intra-assay CV of 3.84%. Despite methodological differences, both assays are considered highly sensitive, as their coefficient of variations are below the 10% threshold recommended for troponin immunoassays (Clerico et al., 2019; Wesselowski et al., 2023).

Considering the limitations mentioned above, more studies are necessary to enhance data availability and strengthen the existing evidence base. Future research should strive to include a more diverse sample population, particularly by involving more female participants, and incorporate additional time-point measurements to provide a more detailed temporal profile of sTnI changes. Furthermore, direct comparisons between downhill running and eccentric contractions are needed to clarify their respective effects on muscle damage, recovery, and adaptation. It is also important to compare sTnI with other novel biomarkers, such as myosin light chain 3 (Myl3) and Fatty Acid-Binding Protein 3 (FABP3). These improvements will enable more comprehensive and statistically powerful evaluations in future meta-analyses.

Conclusions

In summary, the findings of this meta-analysis indicate that sTnI is a suitable early marker for EIMD, as its level increases significantly as early as 6 hours following eccentric exercise. sTnI offers an extended diagnostic window, remaining elevated up to 24 hours post-eccentric exercise, before returning to baseline by 96 hours. The time-course of sTnI elevation aligns closely with ultrastructural changes observed in skeletal muscle after eccentric exercise. Considering the limited number of included studies, further research is needed to expand data availability and clarify the detailed temporal profile of sTnI changes. Such information will be valuable for assessing the safety of eccentric exercise and monitoring athlete recovery.

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Conflict of Interest

All authors have declared that they have no potential conflict of interest concerning this article.

Data Availability Statement

All obtained and analyzed study results can be found within the article and supplementary materials. The corresponding author provides any other inquired data upon justified demand.

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