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Biochemical and neuromuscular biomarkers to monitor hamstrings muscle damage and recovery

Pedro Luis Cosío Fernández



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BIOCHEMICAL AND NEUROMUSCULAR BIOMARKERS TO MONITOR HAMSTRINGS MUSCLE DAMAGE AND RECOVERY

*Biomarcadores bioquímicos y neuromusculares para
monitorizar el daño muscular y la recuperación de los
músculos isquiosurales*

Universitat de Barcelona
Institut Nacional d'Educació Física de Catalunya

Programa de Doctorado EEES (HDK02)

Actividad Física, Educación Física y Deporte

Barcelona, 2024

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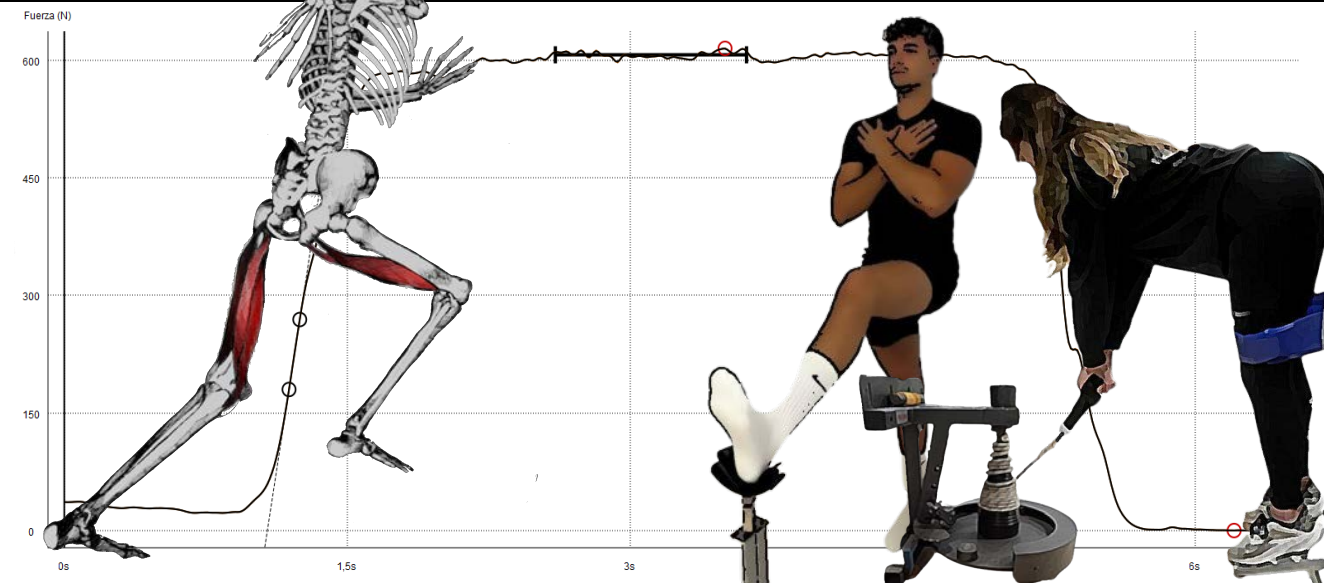
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Pedro Luis Cosío Fernández
TESIS DOCTORAL

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*Biomarcadores bioquímicos y neuromusculares para monitorizar el daño muscular y
la recuperación de los músculos isquiosurales*

Tesis doctoral presentada por:

Pedro Luis Cosio Fernández

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Dr. Andreu Farrán Codina

Tutorizada por:

Dr. Joan Aureli Cadefau Surroca

Para optar al título de doctor por la Universitat de Barcelona

Barcelona, 2024

A mis padres, por darme la oportunidad de desarrollar mi carrera profesional, y por su incombustible apoyo.

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JOAN AURELI CADEFAU SURROCA, catedrático de universidad del departamento de Salud y Ciencias aplicadas del *Institut Nacional d'Educació Física de Catalunya (INEFC)*

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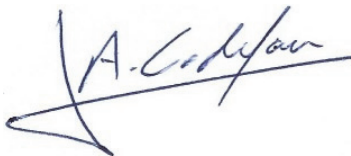
ANDREU FARRAN CODINA, profesor agregado del departamento Nutrición, Ciencias de los Alimentos y Gastronomía de la Facultad de Farmacia y Ciencias de la Alimentación de la *Universitat de Barcelona (UB)*

HACEN CONSTAR:

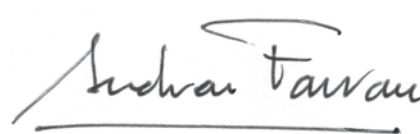
Que **Pedro Luis Cosío Fernández** ha ejecutado la tesis doctoral titulada "*Biochemical and neuromuscular biomarkers to monitor hamstrings muscle damage and recovery*" bajo su dirección en el departamento de Salud y Ciencias aplicadas del *Institut Nacional d'Educació Física de Catalunya*.

Que consideran que la tesis doctoral está finalizada, y es apta para ser presentada y defendida para obtener el grado de doctor por la *Universitat de Barcelona*.

Y para que conste, firmamos la presente en Barcelona (Barcelona), a 08 de julio de 2024



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Declaración

Esta tesis doctoral ha sido realizada gracias al apoyo del *Institut Nacional d'Educació Física de Catalunya (INEFC)* y del *Grup de recerca en Activitat Física, Alimentació i Salut (GRAFAiS)*, financiado por la *Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR, 2021SGR/01190)*, dentro del marco del proyecto financiado *FATIGA2 (AEI-010500-2022b-111)*. Pedro Luis Cosio Fernández ha recibido el apoyo de un contrato predoctoral dentro del marco del programa de ayudas para la *Formación de Profesorado Universitario* del *Ministerio de Universidades (FPU20/02747)*.

La tesis doctoral se compone de 2 artículos publicados y 2 artículos pendientes de publicar. La serie de manuscritos se precede de una introducción general (Capítulo 1) y de los objetivos e hipótesis de la tesis doctoral (Capítulo 2). A continuación, se describe la metodología general y común a los artículos (Capítulo 3). Después, en los Capítulos 4 a 7 se incluye la serie de manuscritos que componen la tesis doctoral. Finalmente, se integra el cuerpo de conocimiento derivado de los hallazgos de la tesis doctoral en una discusión general (Capítulo 8). Todos los capítulos de la tesis doctoral presentan un formato, sistema de numeración y de referenciación común. En los anexos, se encuentra el artículo 1 con su formato original de publicación (numeración, estilo de referencias, figuras, tablas), y la carta de aceptación del artículo 2.

Declaro que el trabajo contenido en esta tesis doctoral es de mi autoría. Los manuscritos publicados y/o pendientes de publicar que han sido escritos en coautoría están claramente identificados y mi contribución individual al trabajo en coautoría está claramente indicada como primer autor. Esta es una obra bajo licencia *Creative Commons Reconocimiento-NoComercial-SinObraDerivada 4.0 Internacional (CC BY-NC-ND 4.0)*.

Pedro L. Cosio Fernández
Julio, 2024

Declarations

This thesis has been conducted under the support of the *Institut Nacional d'Educació Física de Catalunya (INEFC)* and the *Grup de recerca en Activitat Física, Alimentació i Salut (GRAFAiS)*, funded by the *Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR, 2021SGR/01190)*, within the framework of the *FATIGA2* funded project (AEI-010500-2022b-111). Pedro Luis Cosio Fernández has received the support of a predoctoral contract within the program *Formación de Profesorado Universitario* by the *Ministerio de Universidades (FPU20/02747)*.

The thesis consists of 2 published articles and 2 articles pending publication. The sequence of manuscripts is preceded by a general introduction (Chapter 1) and the objectives and hypotheses of the dissertation (Chapter 2). Subsequently, there is a description of the general methodology (Chapter 3). Then, Chapters 4 to 7 include the sequence of manuscripts that constitute the thesis. Finally, the body of knowledge derived from the findings of the dissertation is integrated into a general discussion (Chapter 8). All the chapters of the thesis present a common format, numbering and referencing system. The annexes include article 1 with its original publication format (numbering, reference style, figures, tables), and the letter of acceptance of article 2.

I declare that the work contained in this thesis is of my authorship. Manuscripts published and/or pending publication that have been co-authored are clearly identified and my individual contribution to the co-authored work is clearly indicated as first author. This work is licensed under *Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)*.

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Cosio, P. L., Moreno-Simonet, L., Mechó, S., Padulles, X., Padulles, J. M., & Cadefau, J. A. (2024). **Individual and region-specific hamstring muscles use during a novel Flywheel Russian belt Deadlift exercise.** *Apunts Sports Medicine*, In press (accepted on June 10th, pending DOI).

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Cosio, P. L., Moreno-Simonet, L., Lloret, M., Padulles, X., Padulles, J. M., Farran-Codina, A., & Cadefau, J. A. **Repeated sprints training damages muscle mitochondria and increases oxidative stress status.** *28th Annual Congress of the European College of Sport Science (ECSS 2023)*, July 2023, Paris (France).

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Resumen

Los músculos isquiosurales se ven significativamente afectados por ejercicios de alto volumen o intensidad, desacostumbrados o de naturaleza excéntrica, como resultado del daño inducido por la tensión muscular durante movimientos comunes en deportes de equipo (p.e., repetidas fases de balanceo tardío del ciclo de carrera) o métodos de entrenamiento (p.e., entrenamiento con dispositivos de resistencia inercial). Este proceso de daño muscular inducido por ejercicios excéntricos conduce a una pérdida de rendimiento y, lo que es más importante, a un mayor riesgo de lesiones miotendinosas en los isquiosurales. En consecuencia, es necesario identificar el estado de recuperación muscular de los músculos isquiosurales durante el período de recuperación posterior al ejercicio, utilizando pruebas rápidas y asequibles. De este modo, se podrían adaptar las cargas de entrenamiento en cada microciclo para equilibrar la optimización del entrenamiento con la recuperación muscular, mejorando así la calidad de vida y disminuyendo el riesgo de lesiones miotendinosas. Por tanto, el objetivo de la tesis fue evaluar las diferentes fases del continuo daño muscular-recuperación de los isquiosurales tras diferentes modelos de ejercicio, mediante pruebas neuromusculares específicas de isquiosurales y biomarcadores asociados de daño estructural y oxidativo.

En el **Estudio I**, se evaluaron métricas neuromusculares, mediante pruebas específicas de isquiosurales, y biomarcadores bioquímicos, a través de analíticas sanguíneas, en 29 adultos jóvenes físicamente activos y sanos (15 hombres, 14 mujeres) durante 72 horas después de un entrenamiento de sprints repetidos específico de deportes de equipo (10x40m, recuperación: 3'). Los resultados revelaron una alta variabilidad interindividual en la respuesta al entrenamiento de sprints repetidos, identificable mediante la evaluación del momento torsional de la contracción isométrica voluntaria máxima (MVIC torque) por medio del $90^{\circ}_{hip}:20^{\circ}_{knee}$ test. Además, los participantes que perdieron MVIC torque durante el periodo de recuperación de 72 horas también mostraron un aumento del dolor percibido en los isquiosurales, una pérdida de la amplitud de movimiento (ROM) de la cadena posterior por medio del *Jordan test* (sólo en los participantes masculinos), así como un aumento de biomarcadores de daño estructural (creatina quinasa [CK], CK mitocondrial sarcomérica [sMtCK]) y oxidativo (malondialdehído [MDA], índice de estrés oxidativo [OSI]). Por el contrario, los participantes que no perdieron MVIC torque de los isquiosurales tampoco mostraron cambios en los demás biomarcadores neuromusculares y bioquímicos.

Posteriormente, en el **Estudio II**, los biomarcadores neuromusculares y bioquímicos se aplicaron a la práctica deportiva real y fueron monitorizados durante el periodo de recuperación de 72 horas

tras un partido de fútbol en 19 jugadores de fútbol de primera división regional masculina. Los resultados demostraron que la pérdida de MVIC torque mediante el $90^{\circ}_{hip}:20^{\circ}_{knee}$ test se recuperó antes de las 72 horas, mientras que las métricas tempranas de la tasa de desarrollo de fuerza (RFD) necesitaron más de 72 horas para recuperarse después de un partido de fútbol. Asimismo, las disminuciones de la capacidad de generar fuerza rápida y máxima se acompañaron de una disminución del ROM de la cadena posterior mediante el *Jurdan test*, y de un aumento del dolor percibido en los isquiosurales, así como de biomarcadores de daño estructural (CK, sMtCK) y oxidativo (MDA, OSI) durante el periodo de recuperación.

Por último, con el fin de ampliar la aplicación de las métricas neuromusculares y los marcadores bioquímicos al entrenamiento de fuerza, se diseñó un nuevo ejercicio dirigido específicamente a los músculos isquiosurales (**Estudio III**). Los resultados indicaron que el nuevo ejercicio diseñado produce una activación significativa de los 4 músculos isquiosurales, siendo los músculos de posicionamiento lateral (cabeza larga y corta del bíceps femoral) los más activados. A continuación, en el **Estudio IV**, se monitorizaron los biomarcadores neuromusculares y bioquímicos durante el periodo de recuperación posterior a una sesión de entrenamiento (5 series x 10 repeticiones) en 30 adultos jóvenes sanos y físicamente activos (15 hombres, 15 mujeres). Los resultados indicaron que el ejercicio provocó una elevada sobrecarga excéntrica ($18,3 \pm 3,2\%$), así como fatiga inmediata en la capacidad de generar fuerza rápida y máxima de los músculos isquiosurales. En cuanto al periodo de recuperación, el MVIC torque y el RFD mediante el $90^{\circ}_{hip}:20^{\circ}_{knee}$ test no se recuperaron en 72 horas, así como el ROM de la cadena posterior por medio del *Jurdan test* (sólo en los participantes masculinos), y el dolor percibido de los isquiosurales. Por último, la disminución de las capacidades neuromusculares de los isquiosurales también se acompañó de un aumento de los biomarcadores de daño estructural (CK, sMtCK) y oxidativo (MDA, OSI).

De acuerdo con los hallazgos de los estudios que componen esta tesis, un periodo de recuperación estándar de 72 horas no es adecuado para recuperar los músculos isquiosurales tras ejercicios basados en sprints o entrenamientos con dispositivos de resistencia inercial. El estado de recuperación de los músculos isquiosurales puede controlarse mediante el $90^{\circ}_{hip}:20^{\circ}_{knee}$ test utilizando el MVIC torque y, especialmente, las métricas de RFD tempranas. La evaluación puede acompañarse del *Jurdan test* para el ROM de la cadena posterior en hombres, y de biomarcadores sanguíneos de daño estructural (CK, sMtCK) y oxidativo (MDA, OSI). Finalmente, la irisina circulante se ve incrementada durante los periodos de recuperación post-ejercicio, por lo que podría estar involucrada en procesos de regeneración muscular, y en consecuencia, podría servir como biomarcador del estado de recuperación muscular.

Resum

Els músculs isquiosurals es veuen significativament afectats per exercicis d'alt volum o intensitat, no habituats o de naturalesa excèntrica, com a resultat del dany induït per la tensió muscular durant moviments comuns en esports d'equip (p.e., repetides fases de balanceig tardà del cicle de carrera) o mètodes d'entrenament (p.e., entrenament amb dispositius de resistència inercial). Aquest procés de dany muscular induït per exercicis excèntrics condueix a una pèrdua de rendiment i, cosa que és més important, a un major risc de lesions miotendinoses en els isquiosurals. En conseqüència, és necessari identificar l'estat de recuperació muscular dels músculs isquiosurals durant el període de recuperació posterior a l'exercici, utilitzant proves ràpides i assequibles. D'aquesta manera, es podrien adaptar les càrregues d'entrenament en cada microcicle per a equilibrar l'optimització de l'entrenament amb la recuperació muscular, millorant així la qualitat de vida i disminuint el risc de lesions miotendinoses. Per tant, l'objectiu de la tesi va ser avaluar les diferents fases del procés de dany muscular-recuperació dels isquiosurals després de diferents models d'exercici, mitjançant proves neuromusculars específiques d'isquiosurals i biomarcadors associats al dany estructural i oxidatiu.

En l'**Estudi I**, es van avaluar mètriques neuromusculars, mitjançant proves específiques d'isquiosurals, i biomarcadors bioquímics, a través d'analítiques sanguínies, en 29 adults joves físicament actius i sans (15 homes, 14 dones) fins a les 72 hores després d'un entrenament d'esprints repetits específic d'esports d'equip (10x40m, recuperació: 3'). Els resultats van revelar una alta variabilitat interindividual en la resposta a l'entrenament d'esprints repetits, identificable mitjançant l'avaluació del moment torsional de la contracció isomètrica voluntària màxima (MVIC torque) per mitjà del $90^{\circ}_{hip}:20^{\circ}_{knee}$ test. A més, els participants que van perdre MVIC torque durant el període de recuperació de 72 hores també van mostrar un augment del dolor percebut en els isquiosurals, una pèrdua de l'amplitud de moviment (ROM) de la cadena posterior per mitjà del *Jurdan test* (només en els participants masculins), així com un augment dels biomarcadors de dany estructural (creatina quinasa [CK], CK mitocondrial sarcomèrica [sMtCK]) i oxidatiu (malondialdehid [MDA], índex d'estrès oxidatiu [OSI]). Per contra, els participants que no van perdre MVIC torque dels isquiosurals tampoc van mostrar canvis en els altres biomarcadors neuromusculars i bioquímics.

Posteriorment, en l'**Estudi II**, els biomarcadors neuromusculars i bioquímics es van aplicar a la pràctica esportiva real i van ser monitoritzats durant el període de recuperació de 72 hores després d'un partit de futbol en 19 jugadors de futbol de primera divisió regional masculina. Els resultats van demostrar que la pèrdua de MVIC torque mitjançant el $90^{\circ}_{hip}:20^{\circ}_{knee}$ test es va recuperar abans

de les 72 hores, mentre que les mètriques inicials de la taxa de desenvolupament de força (RFD) van necessitar més de 72 hores per a recuperar-se després d'un partit de futbol. Així mateix, les disminucions de la capacitat de generar força ràpida i màxima es van acompanyar d'una disminució del ROM de la cadena posterior mitjançant el *Jurdan test*, i d'un augment del dolor percebut en els isquiosurals, així com dels biomarcadors de dany estructural (CK, sMtCK) i oxidatiu (MDA, OSI) durant el període de recuperació.

Finalment, amb la finalitat d'ampliar l'aplicació de les mètriques neuromusculars i els marcadors bioquímics a l'entrenament de força, es va dissenyar un nou exercici dirigit específicament als músculs isquiosurals (**Estudi III**). Els resultats van indicar que el nou exercici dissenyat produeix una activació significativa dels 4 músculs isquiosurals, sent els músculs de posicionament lateral (cap llarga i curta del bíceps femoral) els més activats. A continuació, en l'**Estudi IV**, es van monitoritzar els biomarcadors neuromusculars i bioquímics durant el període de recuperació posterior a una sessió d'entrenament (5 sèries x 10 repeticions) en 30 adults joves sans i físicament actius (15 homes, 15 dones). Els resultats van indicar que l'exercici va provocar una elevada sobrecàrrega excèntrica ($18,3 \pm 3,2\%$), així com fatiga immediata en la capacitat de generar força ràpida i màxima dels músculs isquiosurals. Quant al període de recuperació, el MVIC torque i el RFD mitjançant el $90^{\circ}_{hip};20^{\circ}_{knee}$ test no es van recuperar en 72 hores, així com el ROM de la cadena posterior per mitjà del *Jurdan test* (només en els participants masculins) i el dolor percebut dels isquiosurals. Finalment, la disminució de les capacitats neuromusculars dels isquiosurals també es va acompanyar d'un augment dels biomarcadors de dany estructural (CK, sMtCK) i oxidatiu (MDA, OSI).

D'acord amb els resultats dels estudis que componen aquesta tesi, un període de recuperació estàndard de 72 hores no és adequat per a recuperar els músculs isquiosurals després d'exercicis basats en esprints o entrenaments amb dispositius de resistència inercial. L'estat de recuperació dels músculs isquiosurals pot controlar-se mitjançant el $90^{\circ}_{hip};20^{\circ}_{knee}$ test utilitzant el MVIC torque i, especialment, les mètriques inicials de RFD. L'avaluació pot acompanyar-se del *Jurdan test* per al ROM de la cadena posterior en homes, i de biomarcadors sanguinis de dany estructural (CK, sMtCK) i oxidatiu (MDA, OSI). Finalment, la irisina circulant es veu incrementada durant els períodes de recuperació post-exercici, per la qual cosa podria estar involucrada en processos de regeneració muscular, i en conseqüència, podria servir com biomarcador de l'estat de recuperació muscular.

Abstract

Hamstring muscles are significantly affected by high volume or intensity, unaccustomed or eccentric-based exercises, as a result of muscle strain-induced damage during common movements in team sports (e.g., repeated late swing phases of the running gait cycle) or training methods (e.g., flywheel training). This process of eccentric exercise-induced muscle damage leads to a loss of performance, and most importantly, an increased risk of hamstrings myotendinous injuries. Consequently, it is necessary to identify the recovery status of the hamstring muscles during the post-exercise recovery period, using rapid and cost-effective testing procedures. Thus, training workloads could be adapted in each microcycle in order to balance optimizing training with muscle recovery, thus improving the quality of life and decreasing the risk of myotendinous injuries. Therefore, the purpose of the thesis was to assess the different phases of the hamstrings muscle damage-recovery continuum after different exercise models through hamstring-specific neuromuscular tests and associated structural and oxidative damage biomarkers.

In **Study I**, neuromuscular metrics, through hamstring-specific tests, and biochemical biomarkers, through blood analytics, were evaluated in 29 healthy, physically active, young adults (15 males, 14 females) during 72 hours after a team sport-specific repeated-sprint training (10x40m, recovery: 3'). The results revealed a high inter-individual variability in response to repeated-sprint training, identifiable through the assessment of maximal voluntary isometric contraction (MVIC) torque by means of the $90^{\circ}_{hip}:20^{\circ}_{knee}$ test. In addition, participants who lost MVIC torque during the 72-hour recovery period also showed an increase in perceived hamstrings soreness, posterior chain range of motion (ROM) loss by the *Jurdan test* (only male participants), as well as an upregulation of structural (creatine kinase [CK], sarcomeric mitochondrial CK [sMtCK]) and oxidative damage (malondialdehyde [MDA], oxidative stress index [OSI]) biomarkers. In contrast, participants who did not lose hamstrings MVIC torque also showed no changes in the other neuromuscular and biochemical biomarkers.

Subsequently, in **Study II**, the neuromuscular and biochemical biomarkers were applied to real sports practice and were monitored during the 72-hour recovery period after a soccer match in 19 male, regional first-division soccer players. The results demonstrated that the loss of MVIC torque by the $90^{\circ}_{hip}:20^{\circ}_{knee}$ test was recovered before 72 hours, while early rate of force development (RFD) metrics required more than 72 hours to be recovered after a soccer match. Likewise, rapid and maximal force-generating capacity decrements were accompanied by a decrease in posterior

chain ROM by the *Jurdan test*, and an increase in perceived hamstrings soreness and structural (CK, sMtCK) and oxidative damage (MDA, OSI) biomarkers during the recovery period.

Finally, in order to further apply the neuromuscular metrics and biochemical markers to resistance-based hamstrings training, a new exercise was designed to specifically target the hamstring muscles (**Study III**). The results indicated that the novel exercise designed triggers a significant activation of all 4 hamstring muscles, being the lateral positioning muscles (long and short head of the *biceps femoris*) the most activated hamstring muscles. Then, in **Study IV**, the neuromuscular and biochemical biomarkers were monitored during the recovery period after a training session (5 sets x 10 repetitions) in 30 healthy, physically active, young adults (15 males, 15 females). The results indicated that the exercise elicited a high eccentric overload ($18.3 \pm 3.2\%$), as well as immediate fatigue in the capacity to generate rapid and maximal force of the hamstring muscles. Regarding the recovery period, MVIC torque and RFD through the $90^{\circ}_{hip}:20^{\circ}_{knee}$ test were not recovered within 72 hours, as well as posterior chain ROM by the *Jurdan test* (only male participants) and perceived hamstrings soreness. Finally, the decrease in hamstrings neuromuscular capacities was also accompanied by an increase in biomarkers of structural (CK, sMtCK) and oxidative damage (MDA, OSI) to the myocyte during the 72-hour recovery period.

Based on the findings of the studies comprising this thesis, a standard 72-hour recovery period is not adequate to recover the hamstring muscles after sprint-based exercises or flywheel training. The recovery status of the hamstring muscles can be monitored through the $90^{\circ}_{hip}:20^{\circ}_{knee}$ test using MVIC torque, and especially, early RFD metrics. The assessment screening can be accompanied by the *Jurdan test* for posterior chain ROM in males, and by blood biomarkers of structural (CK, sMtCK) and oxidative (MDA, OSI) damage. Finally, circulating irisin is upregulated during post-exercise recovery periods, thus it might be involved in muscle regeneration processes, and therefore, it could serve as a biomarker of muscle recovery status.

Abbreviations, in alphabetical order

25OHD	25-hydroxyvitamin D	NLRP3	nucleotide-binding oligomerization domain-like receptor family pyrin domain containing 3
Akt	protein kinase B	Nrf2	nuclear factor erythroid 2-related factor 2
ALT	alanine aminotransferase	NRS	numerical rating scale
A_{max}	peak acceleration	OSI	oxidative stress index
AMPK	AMP-activated protein kinase	PCSA	physiological cross-sectional area
AST	aspartate aminotransferase	PGC-1α	peroxisome proliferator-activated receptor γ co-activator 1 α
BF_{LH}	biceps femoris long head	PI3K	phosphoinositide-3-kinase
BF_{SH}	biceps femoris short head	P_{max,rel}	relative peak power
Ca²⁺	calcium ion	PPAR-γ	peroxisome proliferator-activated receptor γ
CK	creatine kinase	RBE	repeated bout effect
EO	eccentric overload	RFD	rate of force development
EIMD	exercise-induced muscle damage	ROI	region of interest
eNOS	endothelial nitric oxide synthase	ROM	range of motion
ERK	extracellular signal-regulated kinase	ROS	reactive oxygen species
F0_{rel}	theoretical relative horizontal maximal force	RPE	rate of perceived exertion
FNDC5	fibronectin type III domain-containing protein 5	SD	standard deviation
F-V	force-velocity spectra	SM	semimembranosus
HO-1	hemoxygenase-1	sMtCK	sarcomeric mitochondrial CK
HR	high responders	SOD	superoxide dismutase
HSI	hamstrings strain injuries	ST	semitendinosus
HSRd	high-speed running distance	STAT6	signal transducer and activator of transcription 6
ICC	intraclass correlation coefficient	TAS	total antioxidant status
IGF-1	insulin-like growth factor 1	TD	total distance covered
IL	interleukin	Time₁₀	time to 10 meters
IL-1ra	IL-1 β receptor antagonist	Time₄₀	time to 40 meters
IPC	isometric posterior chain	Time_{P,max}	time to peak power
JAK2	janus kinase 2	TNF-α	tumor necrosis factor alpha
K_{fitted}	rate of force development normalized by peak force	TLR4	toll-like receptor 4
LR	low responders	TOS	total oxidant status
MAPK	mitogen-activated protein kinase	UCP-1	uncoupling protein-1
MD	match day	uMtCK	ubiquitous mitochondrial CK
MDA	malondialdehyde	V₀	theoretical maximal horizontal velocity
mfMRI	muscle functional magnetic resonance imaging	VAS	visual analogue scale
MVIC	maximal voluntary isometric contraction	Vit D	vitamin D
MyD88	myeloid differentiation primary response 88		
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells		

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INTRODUCTION

1. INTRODUCTION

Skeletal muscle tissue provides several functions in the human body, including joint movement generation, heat production, tissue and organ protection from the external environment, and stabilizing and shaping the body [1,2]. The above-mentioned functions of skeletal muscle tissue are possible because muscle cells, which are named myocytes, are capable of transforming chemical energy into mechanical energy, in addition to comprising a complex myofibrillar structure that enables muscle contraction [2]. The proper maintenance of the skeletal muscle tissue structure and function is essential for the preservation of health. Such common phenomena as decreased skeletal muscle contractile activity as a result of a sedentary lifestyle promotes rapid muscle atrophy and changes in contractile properties [3]. Furthermore, the atrophy of skeletal muscle tissue in response to physical inactivity is accompanied by epigenetic modifications associated with alterations in gene expression. However, these epigenetic alterations are reversible once the skeletal muscle tissue returns to normal contractile activity [4].

During high intensity or high volume, eccentric-based or unaccustomed exercises, the contractile demands overcome the contractile capacity of the muscle tissue, leading to the so-called "exercise-induced muscle damage (EIMD)" [5,6]. The series of events that contribute to the development of EIMD are classified as (i) the initial phase or primary damage during the exercise bout (i.e., mechanical and metabolic stress), and (ii) the secondary damage propagated after the exercise bout (i.e., sarcolemma permeability-derived proteolytic, phospholipolytic and oxidative processes) [7]. The EIMD phenomenon is characterized by systemic changes (e.g., the appearance of muscle-specific enzymes and proteins in the bloodstream), accompanied by symptomatic processes (e.g., acute and residual fatigue, muscle soreness), and histologic alterations (e.g., myofibrillar disruptions) [6]. These EIMD symptoms are used as biomarkers (i.e., biological markers), defined by the National Institute of Health as a "defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes or responses to an exposure or intervention" [8], which usually show their own extent and time course during the days after exercise, causing the athlete to be unable to perform his or her exercise activity with the intended frequency or intensity.

Subsequently, a recovery process involving multiple cellular and molecular processes is initiated immediately after EIMD in order to regenerate skeletal muscle. The molecular signaling processes during muscle regeneration are redox-mediated [9]. Exercise-induced free radicals production, under optimal concentrations, serve as local autocrine signaling molecules [10], thus facilitating an adequate contractile activity [11] and regulating muscle remodeling processes (e.g., removal

of necrotized muscle fibers, promotion of myogenesis) [9,12]. However, excessive EIMD causes free radicals accumulation, triggering a process of oxidative damage (e.g., lipid peroxidation, mitochondrial damage, sarcolemma permeability) [13], and a further decrease in neuromuscular capacities [11].

The significance of EIMD arises from the fact that symptomatic processes (e.g., acute and residual fatigue) during muscular recovery are risk factors for developing myotendinous injuries [14]. Field team sports are one of the sprint-based sports modalities with the highest hamstrings strain injuries (HSI) incidence in both female and male athletes [15], because high-speed running is recurrent [16,17] and decisive in match-winning actions [18,19]. During high-speed running or sprinting, the hamstring muscles undergo an active, eccentric lengthening contraction in the late swing phase of the running gait cycle [20], representing the main HSI-related mechanism of injury [21–23]. Thus, high-intensity and repeated eccentric lengthening contractions during repeated sprints result in ultrastructural damage to the hamstring muscles tissue, and subsequently, long-lasting reductions in hamstring muscles force-generating capacity owing to myofibrillar disruptions and/or excitation-contraction uncoupling [24].

To monitor hamstrings muscle recovery is therefore a key concern for practitioners in order to balance the optimization of training with muscle recovery and reduce injury risk, as it has been shown that re-exercising before full recovery aggravates impairments in neuromuscular function [25], which increases HSI risk [14]. Therefore, the understanding of EIMD, its etiology, mechanisms pathways, local and systemic responses, and modulator factors, is a key element to establish a strategy for muscle recovery assessment [26].

Overall, a major problem needs to be solved. Given that the hamstring muscles are very susceptible to being damaged during sprint-based field team sports, there is the need to identify the recovery status of the hamstring muscles during the post-exercise recovery period using rapid and cost-effective methods. In this way, workloads and training programs can be better adjusted to the individual hamstrings condition of each athlete, thereby improving the health and performance of the muscle tissue, and reducing HSI risk.

1.1. Exercise-induced muscle damage (EIMD)

Despite being one of the most important research topics in the field of exercise physiology and sport sciences, no definition has been established for the symptoms of muscle weakness and soreness in the hours and days following a high intensity or volume, eccentric-based, or unaccustomed exercise that exceeds the contractile capacity of muscle tissue. In the scientific community, these symptoms have been referred to as "muscular soreness", "delayed onset muscular soreness" or "stretch-induced muscle damage". Currently, these symptoms are attributed to a tear of the sarcolemma and/or the complex myofibrillar structure and is collectively referred to as "exercise-induced muscle damage" (EIMD) [24]. However, there is a well-documented consensus that EIMD is characterized by a set of physiological signs and symptoms, such as the appearance of muscle-specific proteins in the blood, accompanied by long-lasting decreases in force-generating capacity and range of motion (ROM), and perceived muscle soreness [5,6].

1.1.1. EIMD mechanisms

The underlying mechanisms responsible for the damage to the myocyte structure and subsequent remodeling processes are not fully understood. The first mechanisms described by *Armstrong* [27] included physical damage to structural components, metabolic waste products accumulation, increased muscle temperature and/or spastic contractions derived from altered neuromuscular control. Nevertheless, the most comprehensive models classify the events that contribute to EIMD into two major areas: (i) the initial phase or primary damage during the exercise bout, and (ii) the secondary damage propagated after the exercise bout [7].

Primary damage

The initial phase or primary damage is a direct consequence of exercising at high volume or intensity, through eccentric-based or unaccustomed exercises, and its source can be subdivided into two possible pathways: mechanical stress pathway and metabolic stress pathway [28–30].

The mechanical stress pathway proposes that muscle damage is the result of an excessive mechanical load to the muscle structures [29–31]. This hypothesis is based on the assumption that during muscle contractions, if the strain in a structural component of the fiber exceeds the yield strength of that component (i.e., maximum normal stress theory), the structural components will rupture [30]. Human studies have shown structural alterations in the sarcomeres, membranes, and connective tissue of muscle fibers after eccentric exercises [24]. *Fridén et al.*, [32] proved that after eccentric-based cycling exercise, the sarcomeres are deformed, demonstrating exacerbated Z-line streaming until 2 to 3 days post-exercise. The cause of this physical damage to the sarcomeres is attributed to the fact that sarcomeres are stretched beyond the optimum actin-

myosin overlap point [33], and therefore, the passive structures assume additional tension disrupting the Z-line. Considering that the myofibrillar structure is attached to the sarcolemma through the dystrophin complex [34], overstretching of sarcomeres is potentially disrupting the anchoring structures and sarcolemma [33]. The sarcomeres and sarcolemma are also connected to the extracellular matrix through the dystrophin-associated glycoprotein complex and $\alpha 7\beta 1$ integrin [35], enabling the muscle connective tissue to serve as a structural support of the skeletal muscle tissue, while simultaneously allowing for the transmission of forces laterally [24]. However, such connection between the connective tissue and the myofibrillar structure and the sarcolemma also leads to damage in the connective tissue of the skeletal muscle (i.e., extracellular matrix) after eccentric exercises, as reflected by areas where the extracellular matrix is detached from the muscle fibers [36] and by the increased and synchronized degradation and subsequent synthesis of growth factors and collagen [37,38].

High mechanical loading-derived damage is also evident in the T-tubule system, negatively affecting the excitation-contraction coupling process [33]. Eventually, sarcomeres overstretching and structural components disruptions lead to an increased sarcolemma permeability and failures in contractile mechanics, and consequently, a reduction in force-generating capacity [33,39]. On the other hand, the metabolic stress pathway proposes that muscle damage is the result of ischemia or hypoxia processes during exercise, in addition to increased temperature, decreased pH, insufficient mitochondrial respiration and free radicals production [29,30].

Secondary damage: loss of Ca^{2+} homeostasis

During the initial phase of EIMD (i.e., primary damage), the sarcolemma is permeabilized, so there is an uncontrolled inflow and outflow of both intra- and extracellular elements (e.g., ions, proteins). The source of the sarcolemma permeability was believed to be direct damage and rupture of the membrane. However, it has been found that the use of stretch-activated channels blockers decreases the influx of extracellular ions, including calcium ions (Ca^{2+}), and consequently, attenuates muscle damage processes [40]. Therefore, recent hypotheses suggest the activation of stretch-activated channels as the cause of membrane permeability [41,42]. The critical process resulting from the sarcolemma permeability is an influx of Ca^{2+} from extracellular sources, resulting in an alteration of the sarcoplasmic Ca^{2+} homeostasis [42]. The disruption of sarcoplasmic Ca^{2+} homeostasis initiates a sequence of proteolytic and phospholipolytic damaging events to the myocyte, through further alterations in the cytoskeleton and myofibrils, together with disruption of the mitochondria and free radicals production [31,43,44].

First, elevated intracellular Ca^{2+} activates phospholipase A2, promoting further damage to the sarcolemma [44], and upregulating Ca^{2+} -dependent calpains [45]. Calpains are predominantly

localized in the Z-line and I-band regions, and have been shown to play a critical role in muscle damage, as they are disruptive to the Z-line and myofibrillar protein integrity [46]. *Raastad et al.*, [47] reported for the first time increases in calpain activity after an eccentric exercise, and this increases in calpain activity were coupled with disruptions in myofibrillar structure and Z-line streaming. Recently, it has been shown that calpain activity is elevated after exercising independently of the training status [48]. In particular, the proteolytic pathway of Ca^{2+} -activated calpains (e.g., Calpain 3) impair contractile proteins (e.g., desmin), and/or excitation-contraction coupling proteins (e.g., junctophilins) [46,49]. The increased sarcoplasmic Ca^{2+} as a consequence of exercise-induced mechanical and metabolic stress is modulated by mitochondrial Ca^{2+} uptake [50], via the mitochondrial Ca^{2+} uniporter [51]. The proximity of mitochondria to Ca^{2+} stores, together with the presence of specific membrane contact with the sarcoplasmic reticulum, may explain the large amounts of Ca^{2+} entering the mitochondria [52]. Mitochondria are key organelles for myocytes, as they are responsible for energy production through oxidative phosphorylation, modulated by mitochondrial Ca^{2+} [50,53]. However, excessive Ca^{2+} accumulation in the mitochondrial matrix triggers the formation of the mitochondrial permeability transition pore [54]. As a result, there is an uncontrolled release of Ca^{2+} and free radicals (e.g., superoxide anion radical, hydrogen peroxide) to the sarcoplasm and bloodstream [55].

Free radicals are chemical species with at least one unpaired electron, which makes them extremely reactive [28]. Under homeostatic conditions, free radical production by mitochondria, which is considered a by-product of oxidative metabolism during adenosine triphosphate (ATP) production, is regulated by antioxidant defense mechanisms [55]. Free radicals not only serve as paracrine/endocrine molecules, but also as local autocrine signaling molecules that can provide beneficial effects locally in muscle [10,56]. Exercise-induced free radical production acts as an intracellular signaling and upregulates key transcription factors for mitochondrial biogenesis, such as peroxisome proliferator-activated receptor γ co-activator 1 α (PGC-1 α), and for the antioxidant response, such as nuclear factor erythroid 2-related factor 2 (Nrf2) [10,57]. In addition, optimal free radical concentrations provide proper force-generating capacity and contractile activity, as well as protection against damage [11]. Similarly, molecular signaling processes during muscle damage and regeneration are also redox-mediated [9]. Initially, free radicals generated during muscle recovery are essential for the removal of necrotized fibers, whereas subsequently, the balance of free radicals upregulates the proliferation, differentiation, and maturation of muscle progenitor cells (i.e., satellite cells) [12]. Specifically, each step of the myogenesis continuum during muscle regeneration is regulated by redox balance, including antioxidant enzymes during proliferation, such as superoxide dismutase (SOD), or redox-mediated signaling pathways (e.g., Nrf2) during skeletal muscle satellite cell differentiation [9].

However, when the production of free radicals exceeds the capacity of the antioxidant system to reduce them, a condition termed oxidative stress arises [58]. The cellular antioxidant system is a key component for regulating oxidation/reduction (i.e., redox) balance. Antioxidants are defined as a substance that delays or prevents oxidation of a substrate and are divided into enzymatic and non-enzymatic antioxidants that work as a complex regulatory network to control free radicals [13]. Therefore, oxidative stress is defined as “an imbalance between oxidant and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control, and/or molecular damage” [59]. In the event of muscle damage and recovery cycle, free radical production is a common process derived from impaired muscle mitochondria and immune system in order to remove and regenerate damaged muscle tissues [9,12,33,43]. However, excessive free radicals accumulation as a result of the loss of Ca^{2+} homeostasis trigger a process of oxidative damage, marked by protein oxidation and lipid peroxidation in cell membranes, including mitochondrial membranes and sarcolemma [13]. In this regard, an excessive concentration of free radicals leads to mitochondrial swelling and DNA damage, thereby causing a loss of mitochondrial integrity and a further decrease in neuromuscular function [11,13,57]. In addition, free radical overproduction is associated with an increased sarcolemma permeability [28,55,60] and stimulated stretch-activated channels [61], which leads to exacerbated secondary muscle damage [12].

Overall, free radicals play a critical role in signaling muscle regeneration after EIMD. However, the increase in free radicals as a consequence of mitochondria Ca^{2+} overload following exercise-induced mechanical and metabolic excessive stress results in lipid peroxidation of membranes. Such lipid peroxidation further increases sarcolemma permeability and disruption of intracellular homeostasis, resulting in the efflux of both structural and cytosolic enzymes and proteins into the bloodstream and oxidation by-products, which can be used as EIMD biomarkers [62,63]. Therefore, exercise-induced free radicals production promotes positive physiological adaptations in skeletal muscle (e.g., removal of damaged tissues). However, when the production of free radicals is excessive due to secondary muscle damage, the macromolecular structures (i.e., membranes, proteins) are further damaged [13,64].

To summarize, the loss of sarcoplasmic Ca^{2+} homeostasis is a self-feeding cycle (**Figure 1.1**). Gissel [44] described this cycle as an initial damaging event (e.g., eccentric exercise) that produces permeability of the sarcolemma. Sarcolemma permeability results in extracellular Ca^{2+} influx and consequently altered sarcoplasmic Ca^{2+} homeostasis. The increase in sarcoplasmic Ca^{2+} upregulates calpains, phospholipase A2 and free radicals, which further impair sarcolemma, and consequently, increased sarcolemma permeability, reinitiating the self-feeding cycle [44].

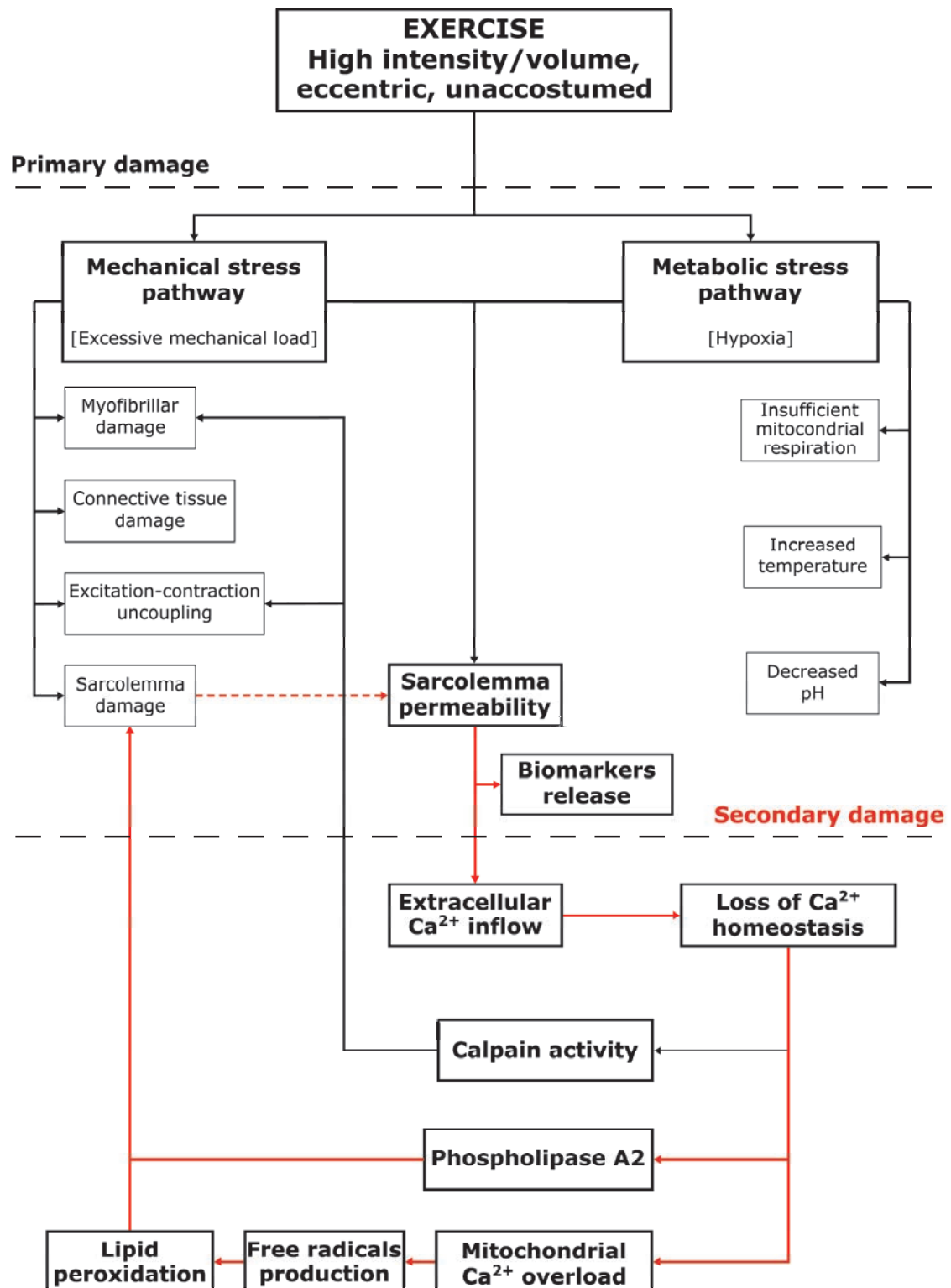


Figure 1.1. Comprehensive model of hypothetical events underlying the mechanistic of exercise-induced muscle damage. Red lines indicate the self-feeding cycle of secondary damage. Ca^{2+} , calcium ion.

Remodeling phase

Multiple cellular and molecular processes are activated immediately after EIMD in order to regenerate skeletal muscle and connective tissues [65–69]. First, a local inflammatory response is initiated to clear waste products [65,66]. Leukocytes (mainly neutrophils) and macrophages (M1 phenotype) are attracted to the damaged site and contribute to the degradation of damaged muscle tissue by releasing free radicals and producing pro-inflammatory cytokines [65–67]. The principal pro-inflammatory cytokines are tumor necrosis factor alpha (TNF- α), followed by interleukin (IL)-1 β . TNF- α stimulates acute inflammatory processes via M1 macrophages in addition to inducing IL-1 β synthesis, while IL-1 β attracts M1 macrophages to the damaged site, whereby both pro-inflammatory cytokines feedback on each other [69]. Under these inflammatory conditions, IL-6 would mediate and appear to trigger the initiation of an anti-inflammatory response to restore homeostasis [69]. Therefore, rather than a marker of inflammation, IL-6 is considered an "inflammation-responsive" cytokine acting as a mediator and turn-point towards the anti-inflammatory effects of exercise [70]. Thus, it has been proven that IL-6 inhibits TNF- α production [71] and upregulates anti-inflammatory cytokines (e.g., IL-10) and biological inhibitor IL-1 receptor antagonist (IL-1ra) [72].

Subsequently, there is a systemic response which is potentially anti-inflammatory, through the release of anti-inflammatory cytokines [65,66]. Anti-inflammatory cytokines (e.g., IL-4 and IL-10) and biological inhibitors of pro-inflammatory cytokines (e.g., IL-1ra) attenuate the initial inflammatory response, regulate muscle cell proliferation and differentiation, and promote muscle regeneration [69]. The main anti-inflammatory role provided by IL-4 is the activation of M2a macrophages that repair damaged tissues, while IL-10 activates M2c macrophages that inhibit M1 macrophages and TNF- α and IL-1 β pro-inflammatory cytokines, reducing secondary muscle damage and promoting myogenesis [69]. M1 macrophages polarization into M2 macrophages is therefore a key event for the normal progression of muscle regeneration [68,69].

The final resolution of inflammation induced by muscle damage is influenced by specific interactions between pro-inflammatory cytokines, and anti-inflammatory cytokines, which act as regulators to modulate inflammatory and muscle repair processes [65,66,69]. An uncontrolled inflammatory reaction driven by pro-inflammatory cytokines (i.e., TNF- α and IL-1 β), affect satellite cell differentiation and fusion, and promote further damage, abnormal extracellular matrix deposition and fibrosis of muscle tissue [69]. However, a balance between pro-inflammatory and anti-inflammatory cytokines is crucial to attenuate an excessive inflammatory reaction, preventing the development of muscle fibrosis and promoting an adequate regeneration process [69].

Considering the fact that balancing pro-inflammatory and anti-inflammatory processes is crucial to attenuate an excessive inflammatory reaction, to prevent the development of muscle fibrosis, and to promote an adequate regeneration process [69], anti-inflammatory myokines (e.g., IL-6, IL-10, irisin) play a key role in providing a balanced oxidative-inflammatory environment [69].

In summary, high volume or high intensity, eccentric-based or unaccustomed exercise causes mechanical and metabolic damage to skeletal muscle tissue, leading to a disruption of intracellular homeostasis. The sequence of local damaging events includes damage to myocyte components (i.e., myofibrillar complex, sarcolemma, mitochondria, connective tissue), excitation-contraction uncoupling, and Ca^{2+} accumulation. Consequently, systemic responses are triggered to repair the damaged tissue, driven by increased oxidative stress and an acute inflammatory response. Both local and systemic responses are manifested by the appearance of muscle-specific proteins in the blood, accompanied by loss of force-generating capacity and ROM, and/or muscle soreness.

1.1.2. Factors affecting EIMD

The underlying mechanisms of EIMD are multifactorial and can affect different cellular structures and organelles through different pathways. In addition to the multifactorial nature of EIMD mechanisms, there are several factors that affect the extent and time course of EIMD, and that explain the large inter-individual variability in EIMD responses [73]. Among these modifiers of the EIMD response are sex and age, genetics, mode, intensity and duration of exercise, fitness level and/or nutrition [74,75].

Sex, age, and genetics

Sexual dimorphism is one of the most debated factors affecting EIMD in the scientific community [76–78]. The primary reason for the controversy is a potential protective effect of estrogens. In fact, a protective effect of estrogens on muscle membranes has been demonstrated in female animal models, mainly acting as antioxidants to counteract lipid peroxidation and therefore limiting the EIMD-associated symptomatic processes [79]. Other hypotheses in animal models suggest that females have a greater capacity to preserve Ca^{2+} homeostasis, thereby attenuating secondary muscle damage [80]. However, findings in human studies have shown no between-sex differences in the EIMD response [81]. More specifically, the magnitude of force-generating capacity loss and perceived muscle soreness, as well as the ultrastructural alterations after eccentric exercises are similar between women and men [76]. These results have been supported by a recent meta-analysis by *Morawetz et al.*, [82] which reported that there is no significant differences in force-generating capacity loss and perceived muscle soreness after eccentric exercises in the upper and lower extremities. Therefore, sexual dimorphism is not expected to have an impact on the symptomatic processes and histological alterations resulting from primary

and secondary muscle damage. Aging is another factor that might influence the EIMD response. Similar to sexual dimorphism, an increased age-dependent susceptibility to muscle damage is found in animal models [83]. However, human studies have found inconsistent results, showing increased [84], decreased [85], and no differences [86] in the EIMD response as the skeletal muscle tissue becomes older.

Genetic variability is an EIMD moderator that has been of increasing interest during the last years. There are genes of muscle proteins that show polymorphisms which modify gene expression, protein formation, and the extent and time course of EIMD [87]. One of the most studied polymorphisms is a single nucleotide polymorphism in the *ACTN3* gene coding for α -actinin-3, which anchors actin filaments to the Z-line in the sarcomere. A α -actinin-3 deficiency would make the Z-lines more elastic and less susceptible to stretch-induced damage [87]. On the other hand, individuals with rare alleles of the *myosin light chain kinase* exhibit increased efflux of muscle-specific proteins into the blood (e.g., CK and myoglobin) [88], as well as increased risk of exertional rhabdomyolysis [89]. Single nucleotide polymorphism in the gene coding for *collagen-I* increases perceived soreness and affects recovery after EIMD through a dysregulation of the collagen network that negatively affects the mechanical properties of the muscle-tendon unit [90]. Polymorphisms in the *TRIM63* gene increase the resistance of muscle fibers to EIMD, owing to a greater stiffness of the titin protein that allows a higher transmission of longitudinal and transverse forces [91].

Finally, polymorphisms have also been found in genes related to the antioxidant and/or anti-inflammatory capacity. A single nucleotide polymorphism in SOD-2 reduces the efficiency of the SOD antioxidant enzyme, resulting in increased susceptibility to oxidative damage during the self-feeding secondary phase of EIMD [33,92]. On the other hand, rare alleles of *chemokine ligand 2* and its receptor are associated with exacerbated force-generating capacity loss, perceived soreness and creatine kinase (CK) activity [93].

Recently, *Baumert et al.*, [94] examined the interaction between EIMD responses in males and females with single nucleotide polymorphisms. The findings showed that perceived soreness was associated with *collagen-I*, *collagen-V*, and *TTN* gene polymorphisms, while ROM was associated with *collagen-V*, *IGF-2*, *TRIM63* and *VDR* gene polymorphisms. However, isometric torque was only associated with the *MMP3* gene polymorphism. Furthermore, these 7 genes polymorphisms that interact with EIMD responses were analyzed together using a total genotype score, whereby individuals were subdivided into groups according to their score. Interestingly, the group of individuals with lower genotype score showed lower force-generating capacity and ROM, together with higher perceived soreness after eccentric-based exercises [94].

Mode, duration, and intensity of exercise

The type of physical exercise conducted also affects EIMD. It is believed that the metabolic stress pathway strongly influences muscle damage after high-volume exercises, whereas the mechanical stress pathway seems to produce muscle damage after high-intensity or eccentric-based exercises [31]. Eccentric (lengthening) muscle contractions arise when a force applied to the muscle exceeds the force produced by that muscle, resulting in a forced lengthening of the muscle-tendon unit while contracting [75]. Such muscle contractions are recurrent in daily activities (e.g., walking downstairs) or in sport-specific tasks (e.g., high-speed running or sprinting) [75]. During eccentric contractions greater forces are generated and fewer motor units are recruited, and consequently, a greater force is absorbed per motor unit (i.e., greater mechanical stress) [33,75,95]. Furthermore, there is a larger increase in intracellular Ca^{2+} after eccentric exercises, due to a greater stimulation of stretch-activated channels [42]. Briefly, eccentric contractions exhibit distinctive molecular and neural characteristics, which make them potentially damaging owing to greater exercise-induced mechanical stress [75].

The extent of EIMD after eccentric-based exercises is further exacerbated under particular conditions of exercise [7]. First, it has been proven that the muscle group exercised can influence EIMD. Specifically, the hamstrings are more susceptible to be damaged than the quadriceps, possibly owing to a lower pennation angle and greater muscle length [96]. Also, EIMD is greater after exercises conducted at faster angular velocities [97], longer muscle lengths [98] and greater forces [99].

Repeated bout effect

Previous exposure to eccentric exercise is the most robust EIMD moderator, providing a protective effect. This protective effect reduces the susceptibility of a muscle to be damaged in the following exercises bouts and is collectively referred to as “repeated bout effect” (RBE) [100]. Specifically, EIMD proxy markers (e.g., muscle soreness, blood biomarkers) are downregulated after an eccentric exercise within 4 to 12 weeks of the original stimulus [101].

The underlying mechanisms for the protective effect of RBE are controversial. Changes in neural factors produced by the original eccentric stimulus, such as motor unit recruitment patterns or synchronization, were thought to play a key role in the protective effect of RBE. However, the protective effect of RBE is also found after contractions evoked by electrical stimulation (no neural control), thus neural changes appear not to be the protective mechanisms of the RBE [24]. On the other hand, restructuring of muscular components appears to be an important mechanism contributing to the protective effect of RBE. Muscle factors include the addition of serial sarcomeres, remodeling of intermediate filaments, changes in the mechanical properties of the

muscle (e.g., stiffness), or increased tendon compliance that reduces mechanical strains to muscle fascicles [24,100,102].

Finally, alterations in the muscle connective tissue (i.e., extracellular matrix) also contribute to the RBE protective effect, via upregulated expressions of collagen, laminins, and matrix growth factors after the original stimulus [37,38]. It might be likely that the above-mentioned mechanisms work coordinately to manage the protective effect of the RBE, so that immediate protection (i.e., 1-2 days) is mediated by neural mechanisms, while prolonged protection (i.e., until 12 weeks) is mediated by muscular mechanisms and the anabolic response of the extracellular matrix [100].

Dietary intake

Several approaches (e.g., pharmacological strategies, manual therapies) have been proposed to attenuate EIMD symptoms, or to accelerate neuromuscular function recovery [31]. However, given the skeletal muscle is a particularly plastic tissue, which can be responsive and adaptable to different stimuli such as physical exercise or nutrient bioavailability, it is important to consider dietary intake and supplements during recovery after EIMD [7,74,103]. The nutritional strategies include fruits and fruit-derived supplements (e.g., cherries), vegetables and vegetable-derived supplements (e.g., beetroot), herbs and herbal supplements (e.g., curcumin), antioxidants supplements (i.e., vitamin C and E), amino acid and protein supplements, or vitamin D (Vit D) supplementation [104].

The consumption of antioxidants (i.e., vitamin C and E) is based on the fact that free radicals are produced, and an oxidative stress state is imposed during secondary muscle damage. Thus, it is suggested that the consumption of antioxidants (i.e., vitamin C and E) may reduce the symptoms of EIMD through the reduction of free radicals-induced damage. However, the scientific literature is contradictory regarding the effectiveness of dietary antioxidants [7,31,105].

Vit D intake and/or 25-hydroxyvitamin D (25OHD) serum levels play a protective role on muscle mitochondria [106]. Vit D acts through binding to the Vit D receptor, which is expressed in skeletal muscle, and has been shown to play an important role in facilitating Ca^{2+} transport in the sarcoplasmic reticulum that improves muscle contraction and modulates 25OHD uptake in myofibers [107]. Vit D deficiency turns into mitochondrial disruption and subsequently, increased free radical production caused by suppressed expression of Vit D receptor [108] and finally, skeletal muscle weakness [109]. Therefore, adequate Vit D levels contribute to the control of oxidative stress, inflammatory processes, and mitochondrial function [110,111]. Accordingly, Vit D concentrations are inversely correlated with pro-oxidant components (e.g., nitric oxide), and directly correlated with antioxidant defenses (e.g., SOD) [112]. Vit D concentration is not only

related to mitochondrial health, but also to post-exercise muscle regeneration by enhancing mitochondrial biogenesis and mitigating free radical production [113]. Higher Vit D levels have been shown to have a significant effect on muscle force-generating capacity and reduced injury incidence [114]. Likewise, exercise-induced force-generating capacity loss is inversely correlated with serum Vit D concentration [109].

On the other hand, Vit D supplementation has been studied in muscle damage and recovery processes. It has been proved that raising 25OHD levels through Vit D supplementation ranging from 100,000 to 200,000 IU per month improves oxidative stress status by reducing lipid peroxidation and improving antioxidant defenses [115]. In addition, increased 25OHD levels in Vit D-deficient individuals improved mitochondrial health (i.e., oxidative phosphorylation rate) after exercise [116]. Force-generating capacity is also favorably influenced by Vit D supplementation, especially Vit D₃ [117–119]. Further, Vit D supplementation decreased the presence of muscle damage (e.g., CK, myoglobin), oxidative stress (i.e., lipid peroxidation) and inflammation (i.e., TNF- α and IL-1 β) biomarkers after exercise [120–122]. Overall, Vit D supplementation improves muscle recovery from 48 hours to 7 days after eccentric exercise [123]. Therefore, increased Vit D intake, particularly Vit D₃ (i.e., cholecalciferol), ameliorates EIMD symptoms [104] and plays an active role in muscle repair processes at the cellular level [7].

Finally, protein intake and/or supplementation is one of the most widespread strategies during the muscle recovery process. Protein intake stimulates intracellular mechanisms such as mitochondrial biogenesis, hypertrophy or muscle fiber-type switching [124]. Physical exercise together with protein intake modulates muscle protein synthesis through the mechanistic target of rapamycin complex 1 (mTORC1) signaling pathway, as a result of increased bioavailability of exogenous essential amino acids [125,126], finally resulting in a positive muscle protein balance and net protein accretion [103]. In fact, a higher protein intake triggers a greater and more prolonged anabolic response, increasing whole-body protein net balance, as well as myofibrillar and muscle connective tissue synthesis rates [127]. Consequently, increased muscle protein synthesis during the recovery period after acute exercise promotes muscle adaptation and structural protein repair, facilitating force-generating capacity recovery [128]. Also, protein intake during post-exercise recovery is not only related to muscle protein synthesis, but also to an increased mitochondrial biogenesis and enhanced muscle performance [129].

Recent applied studies have shown that increasing protein intake enhances functional recovery after sprint training [130], while muscle recovery after a soccer match has also been shown to be enhanced by animal-based protein intake [131], leading to a greater reduction of EIMD markers than plant-based protein intake [132]. In addition, it is important to consider the timing of protein

intake, as amino acids intake during the 4-day recovery period was more effective than pre- and immediately post-exercise intake in reducing EIMD symptoms (e.g., CK, muscle soreness) [133]. Therefore, protein or amino acids intake alleviate EIMD symptoms and enhance muscle recovery [134–136], in addition to accelerating the replenishment of glycogen deposits [74].

To conclude, EIMD is a multifactorial phenomenon with different etiologies, underlying mechanisms or pathways, and different symptomatic (e.g., force-generating capacity loss), systemic (e.g., appearance of muscle-specific proteins and enzymes in the blood) and histological (e.g., myofibrillar disruptions) responses, modulated by factors such as genetics, fitness level or nutritional intake. On this point, it is noteworthy to highlight the fact that symptomatic processes (e.g., acute and residual fatigue) during muscular recovery after primary and secondary muscle damage are risk factors for developing hamstrings myotendinous injuries [14].

1.2. Hamstring muscles

1.2.1. Anatomy and morphology

The hamstring muscles are located in the posterior region of the thigh, forming a muscle group comprising the *biceps femoris* (short head, BF_{SH}; and long head, BF_{LH}), *semitendinosus* (ST) and *semimembranosus* (SM) muscles. The hamstring muscles are distributed in both a superficial and a deep plane. Specifically, the BF_{LH} and ST are located in the superficial plane, while the BF_{SH} and SM are in the deep plane (**Figure 1.2**). Further, they are characteristically biarticular, crossing the hip and knee joint, and therefore, their main functions are hip extension and knee flexion [1,137]. The morphology of the hamstring muscles differs between them, showing differences in the origin, insertion, muscle size and shape, irrigation and/or innervation. However, the anatomical structural characteristics of the hamstring muscles are similar between male and female athletes [138].

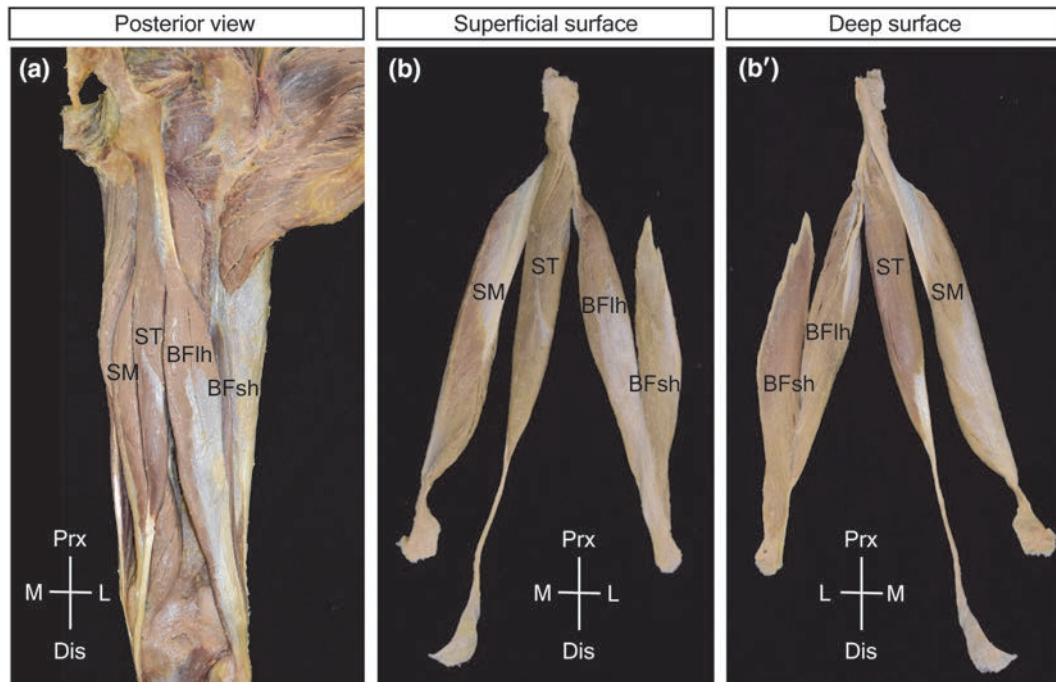


Figure 1.2. "The four hamstrings in situ and in isolated specimens" by *Takeda et al.*, [140] (Licensed under CC BY 4.0). (a) In situ posterior view of the hamstrings in the posterior thigh. (b, b') Isolated specimens of the whole hamstrings in the superficial surface (b) and deep surface (b'). **BF_{LH}**, biceps femoris long head; **BF_{SH}**, biceps femoris short head; **SM**, semimembranosus; **ST**, semitendinosus.

The *biceps femoris* is positioned in the postero-lateral region of the thigh. The quadrilateral short head (i.e., BF_{SH}) is originated from the lateral lip of the *linea aspera* of the femur, exhibiting a physiological cross-sectional area (PCSA) from 2.04 cm² to 5.03 cm² and a pennation angle of 13.17° to 19.16° [139,140]. The fusiform long head (i.e., BF_{LH}), originated from an infero-medial impression of the ischial tuberosity, displays a PCSA from 8.04 cm² to 12.68 cm² and a pennation

angle of 10.31° to 13.46° [139,140]. The BF_{LH} is constituted by a muscle belly that descends laterally across the sciatic nerve and ends in an aponeurosis that receives BF_{SH} muscle fibers to completely merge into a common tendon. The common tendon insertion is located at both the head of the fibula and the lateral condyle of the tibia. The BF_{LH} and BF_{SH} are mainly irrigated by the first to third perforating arteries, and innervated by the sciatic nerve (L5, S1, S2), derived from the tibial and common peroneal branches, respectively [1,137].

The ST muscle is positioned in the postero-medial region of the thigh, exhibiting a PCSA from 9.14 cm^2 to 9.20 cm^2 and a pennation angle of 3.14° to 5.39° [139,140]. It is originated from an infero-medial impression of the ischial tuberosity and formed by a fusiform muscle belly and a long tendon in the distal region (56% length, as a proportion of total muscle length) [141], inserted in the superior region of the medial condyle of the tibia. The ST muscle is irrigated superiorly by the medial circumflex femoral artery and the first perforating artery, while inferiorly by the first perforating artery. The tibial branch of the sciatic nerve (L5, S1, S2) innervates the ST muscle [1,137].

Similarly, the SM muscle is positioned in the postero-medial region of the thigh, displaying a PCSA from 13.22 cm^2 to 18.21 cm^2 and a pennation angle of 13.82° to 15.95° [139,140]. It is originated from a supero-lateral impression of the ischial tuberosity and formed by a fusiform muscle belly and a long tendon in the proximal region (63% length, as a proportion of total muscle length) [141]. The insertion is placed in the posterior aspect of the medial condyle of the tibia. The SM is irrigated by the perforating arteries, principally the first one, and innervated by the tibial branch of the sciatic nerve (L5, S1, S2) [1,137].

1.2.2. Functional biomechanics

The hamstring muscles cover the posterior region of the thigh, crossing the hip and knee joints, accelerating concentrically the movements of hip extension and knee flexion, while eccentrically decelerating the movements of hip flexion and knee extension [1]. In addition, the hamstrings can also provide additional functions under specific hip and/or knee joint positioning. When the hip joint is extended to 0° , the BF_{LH} is a lateral rotator of the hip, while the ST and SM are medial rotators of the hip. On the other hand, when the knee joint is semi-flexed, the BF_{LH} and BF_{SH} are lateral rotators of the knee, while the ST and SM are medial rotators of the knee [137]. In view of the anatomical and morphological differences of each hamstring muscle, it is important to highlight that the contribution of the hamstring muscles can be different depending on the joints positioning and/or hip or knee dominance of the movement.

The biomechanical response of each hamstring muscle is heterogeneous, given that they display a variety of force-length properties and angle-specific joint torques [142]. Lower limb positioning near to hip flexion (i.e., 90°) with the knee extended (i.e., 0°) selectively activates the BF_{LH} [143]. Specifically, the BF_{LH} is more sensitive to hip extension movements than knee flexion movements because the peak in the length-tension relationship of the BF_{LH} occurs with the hip joint at 90° and the knee joint at 0° , the point at which the greatest BF_{LH} function is reached [144]. Furthermore, the BF_{LH} displays a smaller knee moment arm compared to the ST and SM muscles [20]. It has been demonstrated that hip-dominant movements cause a greater activation of the lateral hamstrings than the medial hamstrings, representing a 4-times greater activation of the BF_{LH} compared to the ST muscle [145]. In this regard, the product of the PCSA and the hip moment arm of the BF_{LH} is greater than the ST muscle [142]. Thus, during hip-dominant movements, the hamstrings are responsible for driving the movement, with a strong participation of the BF_{LH} [145,146]. Therefore, the actions that shift the center of gravity forward of the transverse axis of the hip joints (e.g., forward bending of the trunk with hip flexion), cause a significant contraction of the lateral hamstring muscles, particularly the BF_{LH} [137,147].

Conversely, hip movement is locked during knee-dominant movements, restricting the BF_{LH} involvement, and increasing ST muscle activity [148–153]. During knee-dominant movements, the SM muscle also shows a greater knee flexion torque and moment arm compared to the BF_{LH} [20,142]. However, positioning the knee joint near full extension (i.e., $< 30^\circ$), maximizes the activation of the BF_{LH} [142].

On the other hand, in movements such as sprinting, the hip and knee joint angles change simultaneously, so the hamstring muscles are repeatedly operating concentrically and eccentrically from one combination of the hip and knee joints angles to another combination [142]. According to *Schache et al.*, [154], the hamstring muscles undergo a fast stretch-shortening cycle during sprinting, reaching the peak lengthening and biomechanical load during the late swing phase of the running gait cycle (i.e., extensive hip flexion with reduced knee flexion). Likewise, it has been proven that as the running speed approaches the maximum, the hamstrings extremely increase their workload due to high muscle activations [155]. Thus, the hamstrings muscle function during the late swing phase is based on an eccentrically lengthening, energy-absorbing, brake-driving model [156]. Under these conditions (i.e., lengthening contractions), the BF_{LH} displays a higher force output compared to the other hamstring muscles [142].

Overall, there is a selective recruitment of the hamstring muscles depending on the movement pattern, with the BF_{LH} being preferentially and eccentrically activated when the hip flexes and/or the knee extends in order to decelerate the movement [142].

1.2.3. Hamstrings strain injuries (HSI)

HSI are the most prevalent myotendinous injuries in male and female team sports players [157,158], including soccer [159], field hockey [160], or rugby [161], decreasing the performance and quality of life of athletes, and increasing the burden and financial cost [15]. It has been estimated that the time lost due to HSI is 14 to 35 days [158] and the economic cost in a professional player can reach 500,000 euros [162].

HSI incidence has remained unchanged over the last 30 years in field team sports [15]. However, in highly competitive congested team sports (e.g., soccer), HSI showed a two-fold increase over the last 20 years [163]. HSI account for 9% of all injuries in field team sports, more frequent during matches (3.57 per 1000 hours) than training sessions (0.43 per 1000 hours) [15]. Specifically, HSI account for 24% of all injuries in soccer (4.99 and 0.52 per 1000 hours of matches and training, respectively) [163] and 19% of all injuries in field hockey [160]. The location of the HSI is variable, covering different hamstring muscles and different muscle regions. Nevertheless, the *biceps femoris* is the most affected hamstring muscle, representing 45.8% of the HSI in field team sports in both males and female athletes [158], 4 out of every 5 HSI located in the BF_{LH} in soccer [163] and rugby [164].

The most affected anatomical structures by HSI in field team sport players are the myofascial tissue [158,164] and the myotendinous junction [165], whereas the predominant movement pattern during HSI is sprinting or high-speed running (4 out of every 5 HSI) [158,163,164]. High-speed running is recurrent during field team sports (e.g., soccer, field hockey) [16,17], and a key determinant in match-winning (scoring) actions [18,19]. Depending on the playing position, sprint distance in soccer matches ranges from 190 to 420 meters [166], in field hockey matches from 418 to 513 meters [167], and in rugby matches from 65 to 392 meters [168]. Furthermore, high-intensity efforts, such as high-speed running distance (HSRd) or the number of high-intensity sprints, have been significantly increasing during the last seasons of the soccer *Spanish LaLiga* in all playing positions by 6.1% and 11.3%, respectively [169].

During sprinting, acceleration capacity is directly determined by the production of horizontal ground reaction force [170], predicted by both the hamstrings electromyographic activity and eccentric torque during the late swing phase of the running gait cycle [171]. The late swing phase is a key point of the sprint, involving two simultaneous damaging events. First, there is an extensive hip flexion with reduced knee flexion leading to a maximum elongation of the hamstrings muscle-tendon unit. Concurrently, the hamstrings undergo an active, eccentric lengthening contraction [20]. Therefore, it has been shown that the peak force of the BF_{LH} is synchronized with the moment of maximum elongation [172].

Overall, repeated eccentric lengthening contractions during repeated late swing phases of the running gait cycle are the main mechanisms contributing to HSI [21–23]. In particular, the BF_{LH} has been found to be the most important hamstring muscle during the late swing phase, in order to decelerate the shaft before ground contact [155,173]. The reason is that the lower limbs positioning during the late swing phase causes a greater BF_{LH} stretching and moment arm [20], electromyographic activity [155] and myotendinous strains [154], compared to the ST or SM muscles, and explains why BF_{LH} strain injuries are the most common HSI in sprint-based team sports in both males and female athletes [158]. In addition, loss of hamstrings force-generating capacity after repeated sprints is inversely correlated with the PCSA of the BF_{LH} [173]. Therefore, high-intensity and repeated eccentric lengthening contractions during repeated sprints result in ultrastructural damage to the hamstring muscles tissue, and subsequently, long-lasting reductions in hamstring muscles force-generating capacity owing to contractile mechanics malfunction and excitation-contraction uncoupling [24,173].

In summary, sprint-based team sports male and female players are at high HSI risk, mainly located in the myofascial tissue or myotendinous junction of the BF_{LH}, and caused by repeated eccentric lengthening contractions during sprinting or high-speed running. Therefore, regular assessment of hamstrings muscle damage and recovery is a key concern for practitioners in order to both reduce the HSI risk and improve performance, especially during periods of congested schedules.

1.3. Assessment of hamstrings muscle recovery

Muscle recovery is defined as the return to baseline performance values, so if the muscular performance of an athlete is not recovered to baseline levels, the athlete is not fully recovered [74]. Assessment of hamstrings muscle recovery is extremely important because (i) it has been shown that re-exercising while not fully recovered aggravates impairments in neuromuscular function [25] and (ii) the scar tissue resulting from a HSI is mechanically weaker [174] and more susceptible to being reinjured [14]. In addition, athletes with HSI history play fewer minutes and exhibit decreased physical performance (e.g. total distance covered, lower average speed) after recovery from the injury [175]. Hence, it is therefore required to repeatedly assess hamstrings muscle recovery in order to reduce HSI risk and increase performance, by balancing the training workload with the muscle recovery status.

Direct assessment of muscle damage requires examination by histological techniques, via muscle biopsies [33,176]. However, the use of muscle biopsies has been discontinued due to its underlying concerns: (i) the invasiveness of collecting the muscle tissue sample, (ii) the damage and/or inflammation generated by the technique itself, and (iii) the evaluation of an entire muscle through a microscopic sample [6,24]. In addition, in order to accurately determine the extent and time course of muscle damage and recovery, multiple biopsies should be obtained at the same location [177]. Instead, proxy markers such as force-generating capacity, ROM, and/or perceived soreness, accompanied by the appearance of muscle-specific proteins and enzymes in the bloodstream are complementarily used to monitor the extent and time course of muscle damage and recovery [6,24,33,176,178].

Considering that muscle recovery is multifactorial in nature [179], the process of hamstrings muscle recovery should be evaluated by a panel of markers [180,181]. Performance measures are the most sport-specific outcomes, while other physiological measures provide comprehensive information about the recovery and biophysical balance of the athlete [182]. The most commonly used proxy markers (e.g., force-generating capacity, perceived soreness, CK) are associated with different genes and molecular pathways, with less than 1% (16/9087) overlapping between them, meaning that each marker represents specific and unique mechanistic processes [183].

Therefore, it is essential to consider that during muscle damage and recovery, different symptomatic processes may or may not concur, and consequently, each symptomatic process must be evaluated to fully monitor the muscle damage and recovery process. The panel of markers ideally assesses at least the following processes: (i) loss of myofibrillar integrity or excitation-contraction uncoupling, related to reduced maximal (i.e., MVIC torque) and rapid (i.e., rate of

force development [RFD]) force-generating capacity [24,33,47,73]; (ii) connective tissue damage, related to increases in perceived muscle soreness [184–188] and/or ROM decreases [189–193]; and (iii) sarcolemma permeability and/or secondary damage, related to muscle damage and oxidative stress blood biomarkers [33,62].

1.3.1. Maximal voluntary isometric contraction (MVIC)

Mechanisms

Loss of rapid and maximal force-generating capacity in the hours and days following exercise is considered the most reliable indicator of EIMD [6,33,73,176,194]. While other indirect markers do not correlate temporally with direct markers of muscle damage, loss of force-generating capacity is representative of the severity of histological damage [24,73]. In this regard, the magnitude of peak voluntary isokinetic force loss is highly correlated with the number of muscle fibers with myofibrillar disruptions ($r = 0.89$) [47]. Therefore, force-generating capacity loss is key marker that must be included in hamstrings muscle recovery screening.

There are different hypotheses describing the mechanisms by which there might be a loss of muscle force after damaging exercises. First, the hypothesis of *Morgan & Proske* [195] on "popped sarcomeres" states that the nonuniformity of the half-sarcomeres during eccentric lengthening contractions leads to the weaker half-sarcomere assuming the majority of the sarcomere length change. Accordingly, when the filaments are stretched beyond the overlap point, passive structures (desmin, synemin and titin) absorb additional tension due to sarcomere heterogeneity, resulting in Z-line streaming [31]. Repeated eccentric lengthening contractions over time would result in increased number of non-overlapping sarcomeres, leading to myofibrillar damage and membrane permeability [195]. Other hypotheses attribute the loss of muscle force to a failure in the excitation-contraction coupling process [196,197], although the combination of the two hypothesis is believed to play a key role in eccentric exercise-induced force loss. In this regard, nonuniform sarcomere elongation causes greater forces across the sarcolemma, notably the T-tubule system, leading to excitation-contraction uncoupling [195]. Furthermore, an increase in sarcoplasmic Ca^{2+} content and the subsequent activation of Ca^{2+} -dependent proteases (i.e., calpains) might result in a degradation of contractile proteins and, consequently, in force-generating capacity loss [24].

Assessment tests

Isometric, isokinetic and dynamic measurements of strength and power have been extensively used in the assessment of force-generating capacity recovery after damaging exercises [74]. Isokinetic assessment is the standard method for assessing force-generating capacity, using linear or angular dynamometers. However, assessment by isokinetic devices has limitations that should

be considered. Firstly, the measurements are velocity-dependent, so they require to be adjusted to the sport movement under evaluation [74]; second, isokinetic devices are expensive and limited to a few movement patterns [198–200]; and finally, isokinetic testing is not correlated to HSI risk [14]. On the other hand, although isometric assessment appears to represent different neuromuscular domains compared to dynamic assessments [201], there is a moderate to strong correlation between isometric force and dynamic performance in high-intensity tasks [202]. Thus, isometric assessment can be a substitute for the standard isokinetic assessment, providing a fast, simple and cost-effective method with similar longitudinal construct validity [203] and high test-retest reliability [204] in both single- and multi-joint testing protocols, in addition to be associated with HSI risk [14]. Overall, isometric assessment allows more frequent testing to identify muscle recovery and evaluate return to play markers [205]. Therefore, the use of maximal voluntary isometric contraction (MVIC) torque is recommended under isometric testing procedures [73,176].

Isometric assessment is based on a MVIC against a non-yielding resistance in series to a transducer device (e.g., strain gauge, force platform) which measures the applied force or torque (e.g., MVIC torque) [204]. To ensure that the isometric assessment is valid and reliable, the following methodological considerations must be considered [204]: (i) conduct at least one familiarization session, (ii) minimal levels of muscular pretension, and (iii) the testing position must be as specific as possible to the sport task or movement pattern of interest.

Hamstring muscles force-generating capacity has been commonly assessed using isometric posterior chain (IPC) supine tests [137] at 90° or 30° of knee flexion [206,207]. IPC tests for the posterior thigh muscles (i.e. hamstring muscles) have been shown to be valid and reliable for monitoring hamstrings force-generating capacity [199]. For example, IPC supine tests are valid for assessing hamstrings force generating capacity and monitoring changes in performance and/or residual fatigue in soccer players [208,209]. However, as both the largest decrease of isokinetic peak torque [210] and peak electromyographical activity [143] in the hamstring muscles appears in the most lengthened positions (e.g., <30° of knee flexion), *Matinlauri et al.*, [211] recently designed a new, reliable, and sensitive standing test to monitor hamstrings force-generating capacity, the 90°_{hip}:20°_{knee} IPC test. The design of the test causes a lengthening of the hamstrings, and specifically, a greater *biceps femoris* hip extension than knee flexion moment arm, compared to the ST and SM muscles, in a similar pattern to the late swing phase of the running gait cycle [20]. In addition, lower limb positioning with high hip flexion angle and the knee close to full extension selectively activate the BF_{LH} [143]. Consequently, the hamstrings (and specially the BF_{LH}) are biomechanically exposed during the 90°_{hip}:20°_{knee} IPC test, thus facilitating hamstring-

related acute and residual fatigue monitoring [211]. In this sense, it has been shown that a greater HSRd in male soccer players is associated with a decrease in force-generating capacity as measured through the $90^{\circ}_{\text{hip}}:20^{\circ}_{\text{knee}}$ IPC test [212]. However, it has not been proven that the $90^{\circ}_{\text{hip}}:20^{\circ}_{\text{knee}}$ IPC test can effectively monitor hamstrings residual fatigue and recovery induced by sprint-based exercises or resistance-based exercises.

1.3.2. Rate of force development (RFD)

Mechanisms

The rate of force development (RFD) is defined as the slope of the force-time curve and is an expression of the ability of a muscle or muscle group to rapidly develop force at the onset of contraction [213,214]. The assessment of RFD provides valuable and reliable information on the ability to generate rapid force for individuals of different training status and is easily performed by executing either single or multi-joint movements through isometric muscle actions [215,216]. During isometric contractions of muscle groups crossing a given joint, RFD is calculated as the slope of the moment-time curve (i.e., $\Delta\text{moment}/\Delta\text{time}$), which can be determined from the onset of the contraction to a specific point of the curve, or between two specific points of the curve [216]. RFD is subdivided into early and late RFD according to the contraction elapse time from contraction onset, so that the contraction in the first 100 milliseconds is referred to as early RFD, and the contraction beyond the first 100 milliseconds is referred to as late RFD [217].

RFD is considered an important function of skeletal muscle tissue. In fact, RFD is a key factor in fast movements and sport-specific tasks where a short contraction time may not allow maximum force to be reached [213–216]. An interesting example is sprinting, because it is a fast stretch-shortening cycle exercise (< 150 ms of ground contact during sprinting) [171]. More specifically, hamstrings are required to apply rapid force (i.e., < 100 ms) to decelerate the shaft during the late swing phase of the running gait cycle [218], which is the stage where the injury-related mechanism of HSI occurs [21–23]. Therefore, it is recommended to monitor rapid force production (i.e., RFD) during post-exercise recovery, in order to identify hamstrings neuromuscular function deficits that increases HSI risk [218].

RFD is a multifactorial phenomenon, influenced by both neural (e.g., motor unit recruitment) and muscular factors (e.g., muscle size and architecture) [215,216]. The determinants of the force rise time during an isometric contraction are the magnitude and velocity of muscle shortening (**Figure 1.3**), which depends on two simultaneous muscle physiological processes and properties [219]. First, 60% of the time to reach 50% MVIC (i.e., early RFD), is mediated by activation processes, including both Ca^{2+} release, diffusion, and binding, as well as changes in filament organization to allow cross-bridge binding, while the remaining 40% of the time is influenced by the compliance

of muscle elastic components [219]. The key determinants of early RFD activation processes are the motor unit discharge rate and muscle fiber type [215,216]. Specifically, fast-twitch (type-II) fibers are correlated to early RFD, as a consequence of greater Ca^{2+} release per action potential allowing faster cross-bridge contraction cycles [215,216], but are also more susceptible to be damaged after eccentric training [220]. On the other hand, the main determinant of force rise beyond 50% MVIC (i.e., late RFD) are the potential contractile muscle properties [219], including muscle size and architecture, and peak MVIC torque [215].

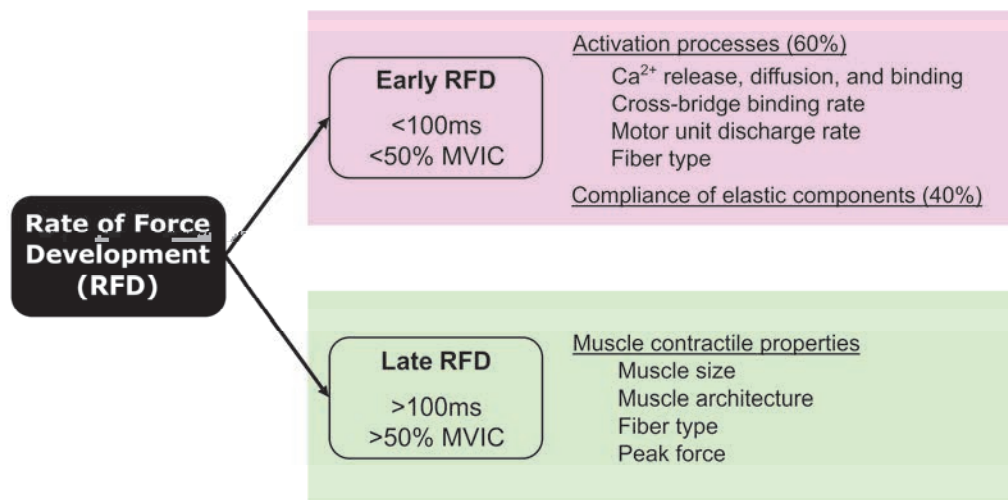


Figure 1.3. Determinants of the force rise time during an isometric contraction. Ca^{2+} , calcium ion; ms, milliseconds; MVIC, maximal voluntary isometric contraction.

Besides, RFD is a useful complement in order to evaluate neuromuscular residual fatigue and muscle damage [217]. Particularly, early RFD (i.e., <100 ms) has been demonstrated to provide unique and non-invasive evidence about neuromuscular recovery kinetics [221], while late RFD (i.e., >100 ms) was also found to be a hamstring-specific muscle damage and recovery predictor [222]. Usually, there are longer-lasting decreases in RFD compared to MVIC torque assessment after damaging exercises, in addition to greater decreases in early RFD than late RFD [217]. Therefore, considering that RFD is recognized as specific and sensitive marker of neuromuscular damage and recovery [221–223], it has been recommended to monitor RFD metrics during post-exercise recovery period [217].

Assessment tests

The assessment of hamstrings RFD metrics is simple, valid, and reliable through single- or multi-joint isometric conditions [209,224,225]. RFD metrics are numerous, including average RFD (i.e., peak force divided by time to peak force), time-intervals RFD (e.g., 0-50 ms, 0-100 ms, 100-200 ms) or peak RFD (i.e., steepest part of the curve over a specific epoch) [215]. The recommended

RFD measures to monitor muscle damage and recovery are time-intervals RFD, both during the early and late phases [226]. Time-interval RFD from 0 to 10 milliseconds (RFD_{0-10}) and from 0 to 50 milliseconds (RFD_{0-50}) are decreased after eccentric exercising of the forearm flexors, providing unique information about the time course of muscle recovery [221], whereas late RFD metrics (e.g., RFD_{0-200}) have also been shown to be reduced after eccentric exercises [223]. Particularly, time-interval RFD from 100 to 200 ms ($RFD_{100-200}$) is a promising measure of muscle recovery, as it is reflective of muscle contractile potential [223]. It is important to highlight that regardless of the measurement, multiple time points or periods provide a more comprehensive description of the complete ascending curve than single measurements [215].

Finally, the choice of the isometric task to be evaluated is determinant for the purpose of the evaluation. For example, hamstrings RFD has been evaluated through knee flexor exercises in supine [209,224] or seated position [225], showing moderate to good reliability RFD measurements. However, assessment of hamstrings RFD through the $90^{\circ}_{hip}:20^{\circ}_{knee}$ *IPC test* might provide specific information on the rapid force-generating capacity during standing movements (e.g., late swing phase of the running gait cycle).

1.3.3. Range of motion (ROM)

Mechanisms

ROM is defined as “the arc over which a joint can operate” and is determined by the properties of the tissues involved in the movement (e.g., connective tissue, tendon, joint capsule, muscle fibers) [176]. After damaging exercises, the stiffness of the damaged tissue is increased, and consequently, there is a decrease in the ROM of the joint in which the damaged muscle is involved [227,228], leading to the inability to fully flex or extend the joint [33]. Specifically, hamstrings muscle stiffness has been found to be a negative predictor of posterior chain ROM [191–193]. Increased muscle stiffness might be derived from myofibrillar and structural myocyte damage, although the connective tissue of the muscle is considered to be a major contributor to passive muscle stiffness [189,190]. Therefore, ROM assessment is recommended for human studies on muscle damage and recovery [176,229], primarily reflecting connective tissue (i.e., extracellular matrix) properties [189].

Assessment tests

ROM assessment is conducted by measuring the angle between two body segments, using passive or active methods, and single- or multi-joint tasks, by means of several instruments (e.g., goniometer, videorecording) [230]. The most commonly used posterior chain ROM screening tests are the *modified Thomas test*, *passive straight leg raise*, *active knee extension*, and *sit and reach*, showing good to excellent test-retest and intra-rater reliability [231]. However, considering

that the hip flexors activity during the late swing phase of the running gait cycle induce an increase of contralateral BF_{LH} stretch [232], measuring the active interaction between the hamstrings and hip flexors provides substantial information when assessing posterior chain ROM after sprint-based exercises.

The *Jurdan test*, in contrast to other ROM screening tests, is designed closely related to running biomechanics [233]. The *Jurdan test* evaluates the interaction between hip flexor and hamstrings flexibility, measured as the differential angle between the actively lengthened leg shin angle and the opposite leg passive thigh angle, indicating increased passive tension derived from damage to the iliopsoas and/or hamstring muscles [233]. Thus, potential ROM losses through the *Jurdan test* are likely derived from increased stiffness of the iliopsoas and/or hamstring muscles. Consequently, assessment of ROM through the *Jurdan test* might provide specific information on the hamstring muscles active extensibility, in a similar way to the damaging late swing phase of the running gait cycle.

1.3.4. Perceived soreness

Mechanisms

Delayed onset muscle soreness is defined as "the sensation of discomfort or pain in the skeletal muscles that occurs following unaccustomed muscular exertion" [27]. The source of the perceived muscle soreness derives from a combination of factors, including (i) physical damage to structural components; (ii) disruption of Ca^{2+} homeostasis resulting from stretch-activated Ca^{2+} channels; and (iii) nerve ending sensitization (i.e., type-III and type-IV nerve afferents) derived from inflammatory by-products (e.g., prostaglandins, histamine, bradykinins) of noxious chemicals and invading inflammatory cells [6,24,27], including two pathways. One of the pathways concerns the activation of *B2-bradykinin receptors*, while the other pathway related to *cyclooxygenase-2 glial cell line-derived neurotrophic factor* [234].

However, perceived soreness does not correlate with histological damage as consistently as loss of force-generating capacity [235], indicating that these classical pathogenic models focused on muscle tissue fail to completely explain the perceived muscle soreness derived from EIMD [184]. Accordingly, considering the significant sensory innervation (e.g., free nerve endings, nociceptors) of the connective tissue [236], the actual mechanism describing the onset of perceived soreness is related to morphological changes in the connective tissue [185–188]. In particular, eccentric hamstring exercises have been shown to increase the stiffness of the muscle connective tissue, which is correlated with the sensation of post-exercise muscle soreness [185]. Therefore, the most recent investigations indicate that the pathogenesis of perceived soreness after EIMD derives from the muscle connective tissue [187,188].

Assessment tests

Typically, the assessment of muscle perceived soreness is conducted using either objective or subjective approaches. The main objective method is the pressure pain threshold, which measures the force applied to the muscle until the pain threshold. However, scientific consensus concludes that pain intensity should be assessed using self-report based unidimensional scales [237]. The most commonly used subjective methods are the visual analogue scale (VAS) and the numerical rating scale (NRS) [33,176]. The NRS is the preferred assessment approach since it shows higher compliance and ease of use in comparison with the VAS [237]. In addition, the NRS also has a strong correlation with the VAS, providing a valid and easy-to-use tool for monitoring perceived soreness [237–240]. The NRS is an 11-point scale (graphically or verbally administered) supported by pain descriptors, ranging from "0" for "no pain" to "10" for "unbearable pain" [240], and has been shown to be sensitive to eccentric exercise-induced pain in hamstring muscles [241].

1.3.5. Membrane permeability: muscle-specific enzymes and proteins

The analysis of blood biomarkers is a complementary, rapid, and accurate indicator of muscle damage and recovery [63]. The use of blood biomarkers, in combination with neuromuscular biomarkers, provides a more in-depth understanding of the physiological response to exercise, along with the extent of muscle damage and recovery status [63]. The National Institute of Health defined a biomarker (i.e., biological marker) as a "defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes or responses to an exposure or intervention" [8]. Specifically, blood biomarkers are used as an indicator of a biological state to objectively measure physiological or pathogenic processes in the body that occur during health, disease, or in response to pharmacological treatment [242].

The underlying mechanism explaining the outflow of blood biomarkers is the rupture and/or permeability of the sarcolemma during primary and self-feeding secondary muscle damage [24,33,62]. Changes in blood biomarkers are dependent on both the amount of protein released from the damaged tissue (and the volume of damaged tissue), and the blood clearance rate [33], which explains why blood biomarkers poorly correlates with neuromuscular markers or histological signs of damaged muscle tissue [6,73,176]. For example, greater histological signs of muscle damage in a low-volume muscle might result in lower muscle-specific enzymes and proteins leakage, compared to a lower damage in a muscle with higher volume.

Therefore, the presence of muscle-specific enzymes and proteins in the blood is an indicator of (i) cellular structures and organelles that have been damaged, and (ii) activation of muscle remodeling and recovery processes, rather than reflecting the extent of muscle damage [176]. Nevertheless, it should be highlighted that there is no single blood biomarker that accounts for

the complete process of muscle damage and recovery [179,242], and therefore, a panel of biomarkers should be reported to simultaneously assessing different physiological processes during post-exercise recovery period [243].

Creatine kinase (CK)

CK is a dimeric globular protein composed of two 43-45 kDa polypeptide subunits: the M subunit (i.e., muscle type) and the B subunit (i.e., brain type). The resulting combinations of these subunits constitute three cytosolic CK isoenzymes. First, in the cytosol there are three isoenzymes. CK-MM is the cytosolic CK isoenzyme located in skeletal muscle tissue, CK-BB is the cytosolic CK isoenzyme located in brain tissue, and CK-MB is the cytosolic CK isoenzyme located in both muscle and brain tissue [244]. On the other hand, there are two mitochondrial CK isoenzymes. The sarcomeric mitochondrial CK (sMtCK) located exclusively in skeletal muscle tissue, and non-sarcomeric (i.e. ubiquitous) mitochondrial CK (uMtCK) located in several tissues such as brain and smooth muscle [245]. Therefore, among the five CK isoenzymes, three are located in the cytosol (i.e., CK-MM, CK-BB and CK-MB), and two in the mitochondria (i.e., sMtCK and uMtCK), providing tissue- and organelle-specific information given their distribution.

Although the list of muscle-specific proteins and enzymes is extensive, total CK activity is the most commonly used muscle-specific biomarker [24,33,63,74]. The upper reference limit of the catalytic activity concentration of CK is $145 \text{ IU}\cdot\text{L}^{-1}$ for females and $171 \text{ IU}\cdot\text{L}^{-1}$ for males [246]. Damaging exercises that cause sarcolemma disruption and permeability result in an increase in total CK activity [62], showing a peak in the 24-48 hours following damaging exercise [74]. Peak CK values are observed during recovery period after hamstrings resistance-based eccentric exercises ($4,150 \pm 4,611 \text{ IU}\cdot\text{L}^{-1}$) [247], or after repeated sprinting exercises ($776 \pm 312 \text{ IU}\cdot\text{L}^{-1}$) [248]. Thus, CK activity determination provides information on the presence of structural damage and/or permeability of the sarcolemma.

Sarcomeric mitochondrial CK (sMtCK)

Given the involvement of mitochondria in modulating sarcolemma permeability-derived intracellular Ca^{2+} accumulation, muscle mitochondria can be damaged as a consequence of self-feeding secondary muscle damage [50–54], resulting in a rupture of the mitochondrial matrix and intermembrane proteins efflux (i.e., sMtCK, free radicals) [55]. However, the structural integrity of the muscle mitochondria can be monitored by measuring striated muscle mitochondrial-specific CK isoenzyme (i.e., sMtCK). sMtCK is an octameric CK isoenzyme mainly localized in both the peripheral intermembrane space and the cristae space [245,249], and it is extremely susceptible to oxidative damage [245]. Considering the aforementioned localization of sMtCK, and that the necrosis of striated muscle mitochondria determined by histological evidence resulted

in increased sMtCK in plasma, the efflux of sMtCK into the bloodstream reflects muscle mitochondria disruptions [250]. Furthermore, it is believed that it could be indicative of the number of myofibrils that have died by apoptosis and/or necrosis [241,251].

The normal resting values of serum sMtCK are below $25 \text{ ng}\cdot\text{mL}^{-1}$ [241,251]. However, peak sMtCK values are shown during the 72-hour recovery period after hamstrings resistance-based eccentric exercise ($547 \pm 115 \text{ ng}\cdot\text{mL}^{-1}$) [241] or running exercises ($132 \pm 32 \text{ ng}\cdot\text{mL}^{-1}$) [251]. Thus, sMtCK assessment provides information on damage to muscle mitochondria and the development of secondary muscle damage.

Transaminases

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are 90 kDa enzymes mainly located in striated muscle tissue and liver and are considered one of the most important markers of liver damage [62,252,253]. Long-lasting increases in ALT and AST activity after damaging exercises have been shown to be accompanied by other muscle damage markers (e.g., CK), which is an indicative of muscle fiber injury and/or exertional rhabdomyolysis [254,255]. In addition, CK/ALT and CK/AST ratios can be used to discriminate exercise-induced rhabdomyolysis (i.e., high values) against liver damage (i.e., low values) [252,253]. Therefore, the combined increase of serum intracellular markers (CK, ALT, AST), together with an increase in the transaminases ratios, is indicative of the degree of cellular integrity (e.g., sarcomeres) and permeability of the sarcolemma [24,62,252,253].

The upper reference limit of the catalytic activity concentration of ALT is $34 \text{ IU}\cdot\text{L}^{-1}$ for females and $45 \text{ IU}\cdot\text{L}^{-1}$ for males, while the upper reference limit of the catalytic activity concentration of AST is $31 \text{ IU}\cdot\text{L}^{-1}$ for females and $35 \text{ IU}\cdot\text{L}^{-1}$ for males [246]. ALT has been shown to increase after hamstrings resistance-based eccentric exercises ($34.9 \pm 31.3 \text{ IU}\cdot\text{L}^{-1}$) [247], while AST increases after hamstrings resistance-based eccentric exercises ($143.7 \pm 135.4 \text{ IU}\cdot\text{L}^{-1}$) [247] or running exercises ($63 \pm 7 \text{ IU}\cdot\text{L}^{-1}$) [251]. In relation to the CK to transaminase ratios, a cutoff in the CK/ALT or CK/AST ratio of 15 units is useful to differentiate muscle damage from hepatic damage [252]. In this regard, when exercise causes muscle damage, both CK and transaminases appear in the blood through increased sarcolemma permeability.

1.3.6. Oxidative stress markers

Free radical production is a common process in the muscle damage and recovery cycle [43], derived from impaired muscle mitochondria and immune system in order to remove damaged muscle tissues [33]. It has been documented that high intensity exercise bouts results in increased oxidative stress markers during a short period [256], while muscle-damaging exercise increases

oxidative stress markers over 96 hours following exercise [257]. Similar to muscle damage biomarkers, there is no single biomarker that accounts for the complete process of exercise-induced oxidative stress, and it is recommended to monitor more than one oxidative stress biomarker [62]. Given the infeasibility of directly measuring oxidative stress in muscle tissue (i.e., need for biopsy), measurement of oxidative stress in blood has been shown to be valid to reflect changes in oxidative stress in other tissues (e.g., skeletal muscle tissue) [258].

Oxidative stress is monitored by blood determination of free radicals, such as superoxide anion radical, free radicals-induced damage in lipids, such as malondialdehyde (MDA), and damage in proteins, such as protein carbonyls, as well as by measurement of activity and/or concentration of antioxidant molecules (e.g., SOD) [62,64,259]. Quantification of free radicals is problematic due to high reactivity and short biological half-life [260]. However, the production of free radicals can be inferred by their induced effect on protein or lipid structures, generating specific compounds that cannot be formed by other mechanisms, and which are used as biomarkers of oxidative damage [260].

MDA is a low-molecular weight aldehyde widely used as free radicals-induced lipid peroxidation biomarker after damaging exercises [261]. Gold standard MDA monitoring requires the use of liquid chromatography, which is very expensive. However, the use of immunoassay-based methods has been validated to determine serum MDA, which reduces the cost of MDA measurement [262]. MDA monitoring after eccentric exercise results in an increase during the post-exercise recovery period until 72 hours [263].

Nevertheless, since oxidative stress is a complex dynamic process involving a large number of pro-oxidant components (e.g., total hydroperoxides, MDA) and antioxidant components (e.g., SOD, glutathione peroxidase), integrative-based quantification methods such as oxidative stress index (OSI) are recommended to fully understand the complex interaction between EIMD and oxidative stress [264]. Separate measurement of different oxidant and antioxidant molecules is complex, time-consuming, and the oxidant or antioxidant effects are additive [265,266]. However, OSI considers the measurement of both oxidant and antioxidant activity produced by the action of multiple components of oxidative stress measured as one [259].

The advantages of these automated methods are that they are simple, fast, and affordable, but their results indicate the overall oxidative stress status and do not provide information on specific oxidative and antioxidant compounds [267,268]. OSI may therefore be a powerful tool for monitoring exercise-induced oxidative stress in a cost-effective way [269].

1.3.7. Irisin

Irisin, a 112 amino acid protein hormone-like myokine, is a fibronectin III domain of the protein sequence of fibronectin type III domain-containing protein 5 (FNDC5) and dependent on PGC-1 α , which after being N-glycosylated [270] and cleaved by proteins of the disintegrin and metallopeptidase domain family (e.g., ADAM10) [271,272], is secreted into the blood in response to several stimulus (e.g., exercise) [273,274]. Circulating irisin binds to α V β 5 integrin receptor through a two-step process involving extracellular heat shock protein-90 α (eHsp90 α), in order to mediate its autocrine and endocrine effects in target tissues (e.g., myocytes, adipocytes, osteocytes) [274–276].

The myokine irisin has been extensively studied regarding its potential therapeutic effects since its discovery by *Boström et al.*, [273]. Irisin mainly promotes browning of white adipose tissue into brite/beige adipocytes increasing mitochondrial uncoupling protein-1 (UCP-1) [273], mediated via phosphorylation of the p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) [270,277]. Thus, it has been suggested that irisin may contribute to a healthy systemic metabolism through increased total energy expenditure [273], glucose homeostasis and insulin sensitivity [270,278,279], induced thermogenesis [273] and modulated and lipid metabolism [278]. Likewise, irisin appears to increase glycerol release and to reduce lipid accumulation in adipocytes [280]. Therefore, muscle-derived irisin has been suggested to play an important role in both adverse metabolic conditions, such as obesity or type-II diabetes mellitus [281].

Irisin also provides potential protection against inflammation and oxidative processes [282]. First, the anti-inflammatory properties of irisin are associated with the inhibition of Toll-like receptor 4/myeloid differentiation primary response 88 downstream signaling pathway (TLR4/MyD88) in macrophages, which is related to the suppression of MAPK phosphorylation, leading to reduced activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) [283]. Additionally, irisin upregulates the Nrf2/hemoxygenase-1 (HO-1) pathway [284], thereby further reducing NF- κ B downstream activation [285]. As a result of depressed NF- κ B, irisin downregulates the expression of pro-inflammatory cytokines (i.e., TNF- α , IL-1 β , IL-6) [283,286,287], as well as stimulates macrophage polarization from the pro-inflammatory M1 response to the anti-inflammatory M2 response [288–290]. M1 macrophages polarization into M2 macrophages is a key event for the normal progression of muscle regeneration [68,69]. Irisin-induced macrophage polarization is mediated through the Janus kinase 2 (JAK2) - signal transducer and activator of transcription 6 (STAT6) - dependent transcriptional activation of

peroxisome proliferator-activated receptor gamma (PPAR- γ) - related anti-inflammatory system and Nrf2-related antioxidant genes [290].

Also, irisin has been shown to be involved in counteracting oxidative processes related to free radical production [291], via AMP-activated protein kinase (AMPK) - phosphoinositide-3-kinase (PI3K) - protein kinase B (Akt) - endothelial nitric oxide synthase (eNOS) signaling pathway [282]. Irisin-induced upregulation of the Nrf2/HO-1 pathway also enhances antioxidant mechanisms in macrophages, resulting in a decrease of free radicals in cells [284,290]. For example, irisin stimulates antioxidative enzymes such as SOD-1 and SOD-2, glutathione peroxidase, or catalase-9, decreasing the production of free radicals (e.g., hydrogen peroxide) by macrophages [292,293].

The above-mentioned irisin-mediated effects together with the irisin-induced inhibition of reactive oxygen species (ROS)-nucleotide-binding oligomerization domain-like receptor family pyrin domain containing 3 (NLRP3) inflammasome pathway led to a depressed oxido-inflammatory response [294–296]. Overall, irisin exerts significant anti-inflammatory and antioxidant effects [297], suggesting that irisin can be an important contributor to a protective effect under exercise conditions (**Figure 1.4**) [292].

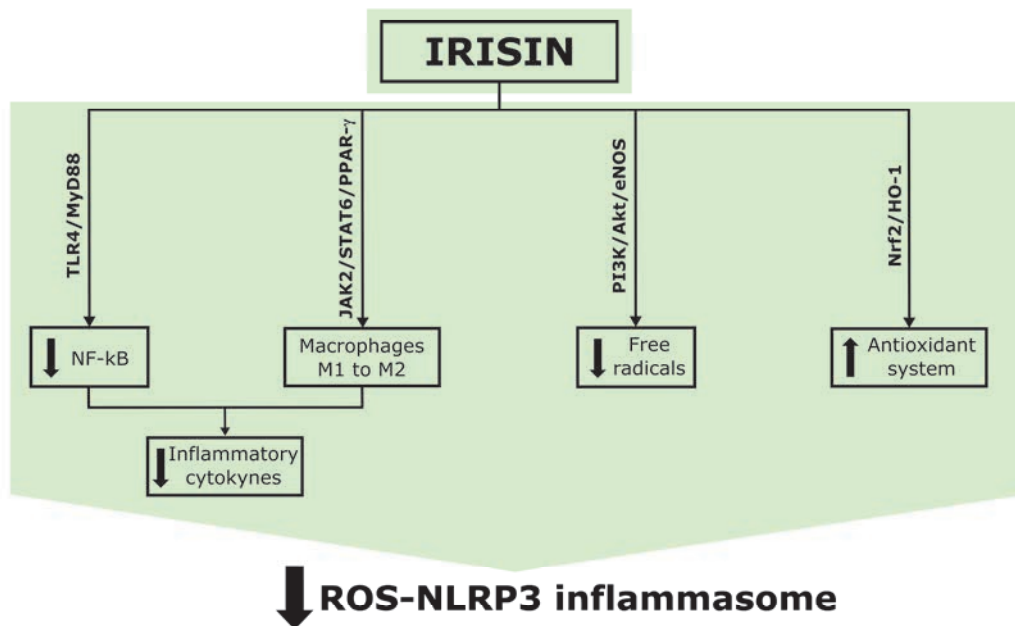


Figure 1.4. Schematic overview depicting the irisin-induced underlying anti-inflammatory/antioxidant mechanisms during the post-exercise recovery period. **Akt**, protein kinase B; **eNOS**, endothelial nitric oxide synthase; **ERK**, extracellular signal-regulated kinase; **HO-1**, hemoxygenase-1; **JAK2**, janus kinase 2; **MyD88**, myeloid differentiation primary response 88; **NF-kB**, nuclear factor kappa-light-chain-enhancer of activated B cells; **NLRP3**, nucleotide-binding oligomerization domain-like receptor family pyrin domain containing 3; **Nrf2**, nuclear factor erythroid 2-related factor 2; **PI3K**, phosphoinositide-3-kinase; **PPAR- γ** , peroxisome proliferator-activated receptor gamma; **ROS**, reactive oxygen species; **STAT6**, signal transducer and activator of transcription 6; **TLR4**, toll-like receptor 4.

However, a new emerging role of irisin has been recently discovered in musculoskeletal disorders, by slowing skeletal muscle loss and atrophy [274], as well as ageing-associated sarcopenia [298] and muscle regeneration [299]. Irisin is secreted during myocyte differentiation, increasing skeletal muscle cell growth through upregulated insulin-like growth factor 1 (IGF-1), and decreasing myostatin through upregulation of the ERK1/2 pathway [300]. Thus, irisin prevents C2C12 myoblasts atrophy through IGF-1-mediated signaling [301]. Further, irisin downregulates negative modulators of myogenesis (e.g., *sox8*) and upregulates pro-myogenic modulators (e.g., *sema3f*) [302]. Accordingly, irisin stimulates the expression of genes involved in both early (e.g., *Pax7*) and late (e.g., *MyoD*) phases of myogenesis [303]. On the other hand, irisin reduces skeletal muscle fibrosis and extracellular matrix deposition by activating PI3K/Akt-mediated amelioration of redox imbalance [304] and by increasing *caveolin-1* expression [305]. Furthermore, irisin protect against sarcolemma instability in damage myofibers [306]. Sphingolipids (e.g., S1P), which are components of the cell membrane, in addition to their structural function, trigger signaling responses in skeletal muscle cells (e.g., muscle regeneration in injured myocytes) [307]. Interestingly, it has been found that S1P increases irisin formation, demonstrating the autocrine effects of irisin on myoblast proliferation and myogenic differentiation [308]. Consequently, irisin stimulates satellite cell activation and reducing protein degradation in injured skeletal muscle tissues (**Figure 1.5**) [302].

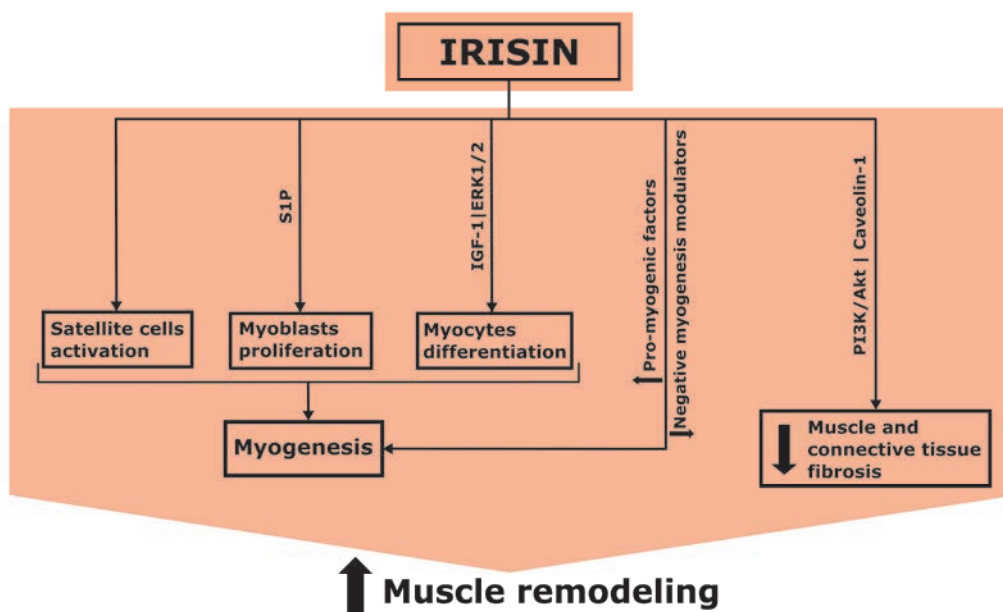


Figure 1.5. Schematic overview depicting the irisin-induced underlying muscle regenerative mechanisms during the post-exercise recovery period. **Akt**, protein kinase B; **ERK**, extracellular signal-regulated kinase; **IGF-1**, insulin-like growth factor 1; **PI3K**, phosphoinositide-3-kinase; **S1P**, sphingolipids.

Overall, irisin plays a pleiotropic role in skeletal muscle cells, promoting muscle growth and regeneration. Irisin-induced muscle regeneration is driven by increased myogenesis and satellite cells, myogenic differentiation, protein synthesis and sarcolemma protection during recovery [299]. Hence, irisin is a potential candidate for regulating signaling processes during the remodeling phase of EIMD.

Actually, irisin existence has been questioned [309] ascribing its existence to a low specificity detection method by antibodies [310]. Nevertheless, it has been demonstrated by tandem mass spectrometry that human irisin exists, circulates, and has similar or identical structure to mice irisin [311,312]. Although the main source of FNDC5/irisin is skeletal muscle tissue [273,313], it is also secreted by subcutaneous adipose tissue [314], but in a 100-to-200-fold lower expression [315]. Accordingly, it is assumed that 72% of the circulating FNDC5/irisin is attributed to muscle secretion [273].

The gold standard for irisin detection is mass spectrometry [316], although immunoassays are the most frequently used technique [317]. ELISA kits (i.e., #EK-067-52, #EK-067-29) have been validated by Western blot and mass spectrometry and correctly detect irisin under physiological conditions [318,319]. Accordingly, *Perakakis et al.*, [320] concluded that the #EK-067-29 model (Phoenix Pharmaceuticals Inc., Burlingame, CA, USA) is the preferred available option for the determination of circulating irisin in humans.

Reference values for circulating irisin range between 4 to 8 ng·mL⁻¹ [311,321–323], that increases both after acute [324] and chronic [325] physical exercise. Moreover, it has been suggested that damaged myocytes could lead to the release of irisin into the circulation in both humans [326] and murine models [327], indicating that the rupture of the muscle fibers might trigger an increase of irisin [328]. It is believed that the exercise-induced irisin response may be associated with both muscle damage, correlated with myoglobin levels, and inflammation, correlated with IL-6 levels [329]. The underlying mechanism explaining the production of irisin during exercise and recovery is repeated muscle contractions and the mechanical and oxidative damage to cellular and mitochondrial integrity (i.e., Ca²⁺ influx), leading to an upregulation of the AMPK - PGC-1 α - FNDC5 signaling axis pathway, and subsequent irisin cleavage [273,274] .

In summary, given both its potential autocrine and endocrine anti-inflammatory and antioxidant effects, as well as its involvement in muscle remodeling processes, irisin is suggested to be a promising myokine that might (i) balance the acute inflammatory/oxidative response and (ii) promote an adequate post-EIMD muscle remodeling process.

1.3.8. Interindividual variability

The multifactorial nature of the underlying mechanisms of EIMD, together with the several moderators that affect the extent and time course of EIMD (e.g., genetics, nutrition), explain the large inter-individual variability in EIMD responses [73,330]. Classifying individuals into different susceptibility levels to EIMD can reduce interindividual variability and provide a better interpretation of EIMD responses. An approach to classify individuals into different levels of EIMD susceptibility is to cluster individuals according to a EIMD marker responsiveness, which reduces intra-group variance. Accordingly, it is important to identify the EIMD marker that better reflects the extent of the EIMD [331]. Loss of rapid and maximal force-generating capacity are considered the most reliable indicator of EIMD [6,33,73,176,194]. In contrast to other EIMD markers (e.g., CK, perceived soreness), loss of force-generating capacity is representative of the severity of histological damage [24,73]. In this regard, the magnitude of force-generating capacity loss is highly correlated with the number of muscle fibers with myofibrillar disruptions ($r = 0.89$) [47].

Paulsen et al., [73] suggested to group individuals according to the extent of the decrease in force-generating capacity after damaging exercises. A small reduction (i.e., <20%) in force-generating capacity and complete recovery within 48 hours would be considered as mild damage; notable decreases (i.e., 20-50%) in force-generating capacity and complete recovery between 48 hours and 7 days would be considered as moderate damage; and large decreases (i.e., >50%) in force-generating capacity and long-lasting recovery after 7 days would be considered as severe damage [73]. Clustering individuals according to decreases in force-generating capacity leads to consistency between the stratification and the extent and time course of other surrogate markers (i.e., CK activity, ROM, perceived soreness) [194]. Therefore, force-generating capacity loss is the preferred EIMD single marker to stratify individuals into different susceptibility levels in order to reduce inter-individual variability and provide a better interpretation of EIMD responses [73,194].

RESEARCH AIMS AND HYPOTHESES

2. RESEARCH AIMS AND HYPOTHESES

2.1. Main purpose

The main purpose of the thesis was to assess the different phases of the hamstrings muscle damage-recovery continuum after different exercise models through hamstring-specific neuromuscular tests and associated structural and oxidative damage biomarkers.

2.1.1. Specific purposes

1. To design exercise models based on both analytical and global structures capable of inducing muscle damage and residual fatigue in the hamstrings (**Studies I, II, III and IV**).
2. To monitor the effect of different damaging exercise models on hamstring-specific neuromuscular tests (i.e., $90^{\circ}_{hip}:20^{\circ}_{knee}$ *IPC test* and *Jurdan test*) and new serum biomarkers (i.e., OSI and irisin), in both women and men (**Studies I, II and IV**).
3. To evaluate the use of hamstring-specific neuromuscular tests (i.e., $90^{\circ}_{hip}:20^{\circ}_{knee}$ *IPC test* and *Jurdan test*) and derived metrics (i.e., MVIC torque, RFD, ROM, perceived soreness) as markers of primary muscle damage (**Studies I, II and IV**).
4. To examine the use of new serum biomarkers (i.e., OSI and irisin) as secondary muscle damage biomarkers (**Studies I, II and IV**).
5. To analyze the suitability of RFD metrics as markers of residual fatigue induced by both analytical and global-based structure exercises (**Studies II and IV**).
6. To build a new analytical structure-based exercise capable of activating the hamstring muscles, and specifically, the BF_{LH} (**Study III**).

2.2. Hypotheses

General hypotheses

- A 72-hour recovery period is not adequate to recover the hamstring muscles after sprint-based exercises or flywheel training, in both women and men.
- The $90^{\circ}_{hip}:20^{\circ}_{knee}$ *IPC test* is able to monitor hamstrings primary muscle damage, notably through RFD metrics, thus providing an easy, rapid, low demanding, and cost-effective tool for assessing hamstrings muscle recovery, in both women and men.
- Hamstring-damaging exercises trigger a systemic response in which OSI and circulating irisin are increased, thereby potentially serving as secondary muscle damage and recovery biomarkers.

Hypotheses of study I

- There is inter-individual heterogeneity (i.e., high and low responders) in hamstrings muscle recovery after a sport-specific sprint training, identifiable through MVIC torque monitoring by the $90^{\circ}_{hip}:20^{\circ}_{knee}$ IPC test.
- The loss of hamstrings neuromuscular capacity in both males and females is associated with damage to muscle mitochondria, which increases OSI and triggers an upregulation of circulating irisin.
- Individuals showing larger decreases in neuromuscular capacities (i.e., high responders) are not recovered within 72 hours after the exercise.

Hypotheses of study II

- Football (soccer) match-induced hamstrings residual fatigue can be monitored through RFD metrics by the $90^{\circ}_{hip}:20^{\circ}_{knee}$ IPC test.
- Decreases in RFD metrics are accompanied by an increase in serum levels of structural and oxidative damage biomarkers.
- A usual 3-day recovery period after a soccer match is not adequate to completely recover the hamstrings neuromuscular capacities.

Hypotheses of study III

- The new analytical structure-based exercise (i.e., *Flywheel Russian belt Deadlift*) is effective in producing significant hamstring muscles activation.
- Hamstring muscles activity during the exercise is greater in the postero-lateral thigh muscles compared to the postero-medial thigh muscles, as well as greater in the proximal regions of the hamstring muscles compared to the distal regions.

Hypotheses of study IV

- The *Flywheel Russian belt Deadlift* exercise triggers homogeneous decreases in hamstrings neuromuscular capacities and increases in serum biomarkers of structural and oxidative damage for 72 hours, in both women and men.
- RFD metrics are effective for monitoring hamstrings muscle recovery after the *Flywheel Russian belt Deadlift*, reflecting distinctive sources of primary and secondary muscle damage.

EXPERIMENTAL APPROACH

3. EXPERIMENTAL APPROACH

The thesis is composed of data from three experimental studies and one case study. New biochemical and neuromuscular biomarkers of hamstrings muscle recovery were monitored by a global structure-based laboratory protocol (**Study I**) and a field protocol (**Study II**). The case study analyzed the use of the hamstring muscles after a new designed analytical structure-based hamstring-specific exercise (**Study III**). Finally, hamstrings neuromuscular capacity and biochemical markers were assessed after the designed analytical structure-based laboratory protocol (**Study IV**). The shared methodology of the studies is presented below. Detailed information for each individual study can be found in the corresponding articles.

3.1. Participants

A total of 79 individuals (50 males and 29 females) voluntarily participated in the studies. Potentially eligible volunteers were required to meet the following inclusion criteria: (i) be young adults, (ii) with no pathologies or unfavorable conditions for exercise practice, and (iii) be physically active, but not enrolled in regular resistance training programs. Participants were excluded if they had suffered any myotendinous injury to the lower limbs within the last 6 months prior to the experiments. Also, participants were strongly encouraged to avoid any type of physical exercise during the 3 days prior to the experiments, and to maintain their daily diet and lifestyle. The anthropometric characteristics and habitual physical activity of the participants [332] are shown in **Table 3.1**.

Table 3.1. Summary description of the participants for each study in the thesis.

Study	Subjects	n	Sex	Age (years)	Mass (Kg)	Height (cm)	METS·min·wk ⁻¹
I	Healthy and physically active youngs	15	Male	22.5 ± 3.2	74.2 ± 7.4	175.9 ± 6.8	6544 ± 4795
		14	Female	22.2 ± 3.1	60.3 ± 7.1	165.2 ± 4.8	5263 ± 2960
II	Healthy amateur soccer players	19	Male	20.9 ± 2.0	72.6 ± 11.9	175.9 ± 6.9	5639 ± 4104
III	International-level field hockey player	1	Male	22.7	67.0	1.72	-
IV	Healthy and physically active youngs	15	Male	21.4 ± 1.9	74.3 ± 8.9	178.2 ± 7.2	5913 ± 4651
		15	Female	21.5 ± 3.4	60.5 ± 8.8	163.4 ± 5.1	6564 ± 3153

Data are presented as mean ± standard deviation.

All participants were previously informed and gave their written consent that they received complete and specific information about the purpose of the studies, experimental tests, and associated risks. The experiments were conducted in accordance with the code of ethics of the World Medical Association (Declaration of Helsinki) and were approved by the Ethics Committee for Clinical Research of the Catalan Sports Council (Generalitat de Catalunya) under reference: 037/CEICGC/2021.

3.2. Experimental designs

3.2.1. Study I

Study I monitored hamstrings muscle recovery through a single-group repeated measures design. Briefly, participants completed baseline measurements, followed by the global structure-based laboratory exercising protocol, and finally, a 72-hour follow-up monitoring of hamstrings neuromuscular metrics and biochemical markers (**Table 3.2**). The exercising protocol consisted of a sport-specific repeated-sprint training, formed by 10 repeated 40-meter sprints, with a 3-minute rest between repetitions [333].

Table 3.2. Schematic overview showing the experimental approach of the Study I.

	Day -7	Day -1	Day 0		Day 1	Day 2	Day 3
PARAMETER	-168h	-24h	-0.5h	EXERCISE	+24h	+48h	+72h
Familiarization	x			Repeated-sprint training Number: 10 Distance: 40m Rest: 3'			
Blood samples			x		x	x	x
Force-generating capacity			x		x	x	x
Range of motion			x		x	x	x
Perceived soreness			x		x	x	x
Sprint performance			x		x	x	x
Dietary intake		x	x			x	

3.2.2. Study II

Study II monitored hamstrings muscle recovery through a single-group repeated measures design. Briefly, participants completed baseline measurements, followed by the global structure-based field exercising protocol, and finally, a 72-hour follow-up monitoring of hamstrings neuromuscular metrics and biochemical markers (**Table 3.3**). The exercising protocol consisted of a 90-minute football (soccer) match, in which players were required to cover at least a total distance (TD) of 10,000 meters, a minimum HSRd (i.e., $> 21 \text{ km} \cdot \text{h}^{-1}$) of 400 meters, and at least 30 accelerations and 30 decelerations over $3 \text{ m} \cdot \text{s}^{-2}$ [169,334–336].

Table 3.3. Schematic overview showing the experimental approach of the Study II.

	Day -7	Day -1	Day 0		Day 1	Day 2	Day 3
PARAMETER	-168h	-24h	-0.5h	EXERCISE	+24h	+48h	+72h
Familiarization	x			Soccer match TD > 10,000m HSRd > 400m Acc > 30 Dec > 30			
Blood samples			x		x	x	x
Force-generating capacity			x		x	x	x
Sprint performance			x		x	x	x
Dietary intake		x	x			x	

3.2.3. Study III

Study III assessed individual- region- and limb-specific hamstring muscles use during a new designed analytical structure-based hamstring-specific exercise (i.e., *Flywheel Russian belt Deadlift*), through an observational descriptive repeated measures design (pre-post). In brief, the evaluation was conducted immediately before the exercise, followed by the *Flywheel Russian belt Deadlift* training session, and finally, testing immediately after the exercise (**Table 3.4**). The *Flywheel Russian belt Deadlift* training session consisted of 10 sets of 10 repetitions with an inertia momentum of $0.124 \text{ kg}\cdot\text{m}^2$ [337], and a 3-minute rest between sets.

Table 3.4. Schematic overview showing the experimental approach of the Study III.

Experimental day			
PARAMETER	Pre	EXERCISE	Post
Muscle functional Magnetic Resonance Imaging	x	<i>Flywheel Russian belt Deadlift</i> Inertia = $0.124 \text{ kg}\cdot\text{m}^2$ Series: 10 Repetitions: 10 Rest: 3'	x

3.2.4. Study IV

Study IV monitored hamstrings muscle recovery through a single-group repeated measures design. Briefly, participants completed baseline measurements, followed by the analytic structure-based laboratory exercising protocol, immediately post-exercise measurements and finally, a 72-hour follow-up monitoring of hamstrings neuromuscular metrics and biochemical markers (**Table 3.5**). The exercising protocol consisted of 5 sets of 10 repetitions [147,148] of the new designed *Flywheel Russian belt Deadlift* exercise, with an inertia momentum of $0.159 \text{ kg}\cdot\text{m}^2$ [337], and a 3-minute rest between sets.

Table 3.5. Schematic overview showing the experimental approach of the Study IV.

	Day -7	Day -1	Day 0			Day 1	Day 2	Day 3
PARAMETER	-168h	-24h	-0.5h	EXERCISE	+0.5h	+24h	+48h	+72h
Familiarization	x			<i>Flywheel Russian belt Deadlift</i>				
Blood samples			x			x	x	x
Force-generating capacity			x	Inertia = 0.159 kg·m ²	x	x	x	x
Range of motion			x		x	x	x	x
Perceived soreness			x	Series: 5 Repetitions: 10 Rest: 3'	x	x	x	x
Dietary intake		x	x				x	

3.3. Data collection and analysis procedures

3.3.1. Force-generating capacity

Maximal voluntary isometric contraction (MVIC) torque

MVIC torque of the hamstring muscles was measured unilaterally (i.e., right and left lower limb) by the $90^{\circ}_{hip}:20^{\circ}_{knee}$ IPC test [211]. Briefly, participants were strongly verbally encouraged to apply force “as fast and hard as possible” to a rigid custom-built support attached to a previously validated 160-Hz sampling frequency strain gauge (Chronojump, Barcelona, Spain) [338].

The participants completed three trials of the test with each lower limb (3 seconds MVIC), with a 30-seconds rest between trials [205]. Then, MVIC force was registered as the average force in a 1-s window once a force plateau had been established [339], and the residual force resulting from the mass of the tested leg at rest was then subtracted. Finally, the MVIC torque was computed to facilitate comparisons of data sets, as the product of the MVIC force and the distance between the hip joint and the force application point (d), multiplied by the sine of the angle [MVIC torque = $MVIC\ force \times d \times \sin(\alpha)$].

Additional trials were conducted if any countermovement or joint balancing was noted. The best of the three trials from each limb was recorded for further analyses [205]. The positioning (confirmed by goniometry) during the $90^{\circ}_{hip}:20^{\circ}_{knee}$ IPC test is shown in **Figure 3.1A (Studies I, II and IV)**.

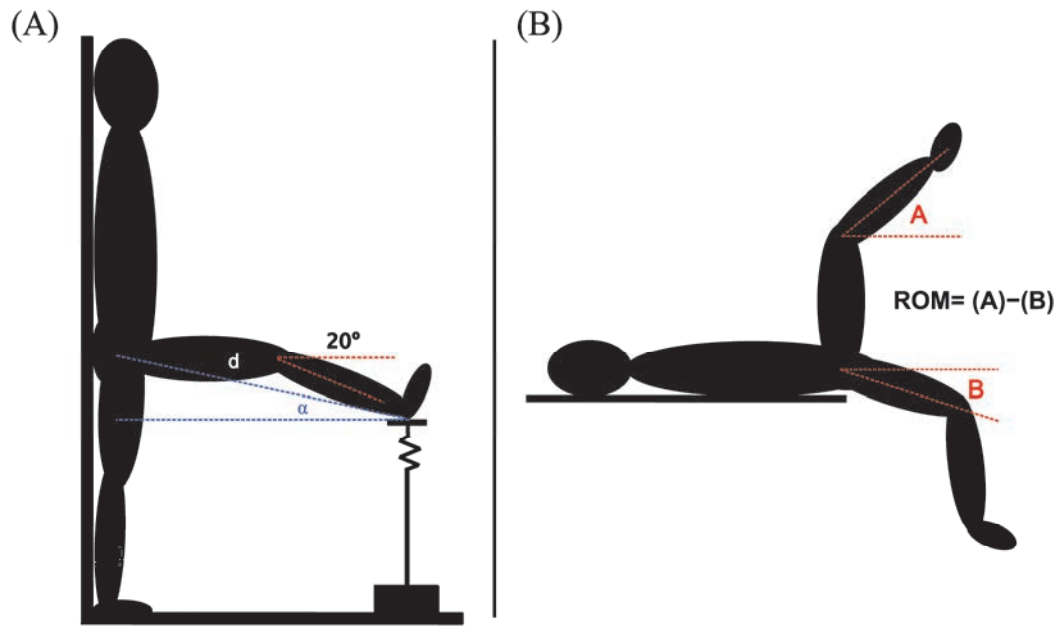


Figure 3.1. Positioning of the participants during the execution of (A) the $90^\circ_{\text{hip}}:20^\circ_{\text{knee}}$ IPC test and (B) the Jurdan test.

Rate of force development (RFD)

Time-interval RFD to 50 milliseconds (RFD_{0-50}), to 100 milliseconds (RFD_{0-100}), to 250 milliseconds (RFD_{0-250}), from 100 to 200 milliseconds ($RFD_{100-200}$) and peak RFD, that are the steepest part of the curve and the steepest part of the curve in a 25-millisecond epoch, (RFD_{max} and $pRFD_{25}$, respectively), were calculated by means of the $90^\circ_{\text{hip}}:20^\circ_{\text{knee}}$ IPC test [211], using a recording rigid custom-built strain gauge (Chronojump, Barcelona, Spain) which provides a low baseline noise amplitude (0.096% MVIC Torque). The strain gauge has been proven to be reliable for RFD metrics assessment [338], as it has been demonstrated that lowering the sampling frequency (e.g., 1,000 to 100 Hz) during unilateral IPC test increases the absolute reliability and reduces the coefficient of variability [218]. According to the recommendations of Maffiuletti *et al.*, [215], (i) the contraction onset was determined through a systematic manual-based method at the moment of “the last trough before force deflects above the range of the baseline noise”; (ii) no filtering or signal smoothing was applied; and (iii) average RFD of the three best trials was retained for further analysis (**Studies II and IV**).

It is important to notice that although the used terminology is rate of force development (RFD) for a better understanding and interpretation of the findings given that the majority of the existing scientific literature is using this term, the metrics were calculated based on the torque of the participants to facilitate comparison between studies and/or individuals.

3.3.2. Range of motion (ROM)

The *Jurdan test* was used to evaluate the interaction between hip flexor and hamstring flexibility, measured as the differential angle between the actively lengthened leg shin angle and the opposite leg passive thigh angle [233]. The test was photographically registered in duplicate by a *SONY DSC-RX100M5A* placed on a tripod located at the height of the table and three meters from the center of the hip of the participant. 2D shin and thigh angles were measured, relative to the horizontal, as the best of two trials on each lateral approach using the validated sports kinematics analysis software *Kinovea v0.9.3* (Chartman and contributors) [340]. Then, resulting angles were used to compute a composite angle defined as the difference between the actively lengthened leg shin angle and the opposite leg passive thigh angle (**Studies I, II and IV**). The positioning and analysis technique of the *Jurdan test* is shown in **Figure 3.1B**.

3.3.3. Sprint performance and mechanical properties

The performance and mechanical properties during 40-meter sprints was evaluated through the analysis of the force-velocity (F-V) spectra (**Studies I and II**). The linear velocity sensor *RaceAnalyzer* (Chronojump, Barcelona, Spain) was located on a 1-meter-high tripod behind the participants. The device operates with a distance accuracy of 3 centimeters and temporal accuracy of 4 microseconds. Participants were equipped with a belt attached to the linear velocity sensor and were strongly verbally encouraged during the test (**Figure 3.2**).



Figure 3.2. Representative illustration showing the sprint performance and mechanical properties assessment using the linear velocity sensor *RaceAnalyzer* (Chronojump, Barcelona, Spain).

Then, instantaneous velocity over the 40-meter sprints was recorded and post hoc analyzed using *Chronojump software* v2.1.2-2 (Chronojump, Barcelona, Spain) to compute sprint F-V spectra using the validated method by *Samozino et al.*, [341]. Individual linear F-V relationships were then extrapolated to calculate theoretical relative (to body mass) horizontal maximal force ($F_{0,rel}$) and theoretical maximal horizontal velocity (V_0). Subsequently, the time to 10 meters ($Time_{10}$) and total time to 40 meters ($Time_{40}$) were calculated, together with peak acceleration (A_{max}), relative peak power ($P_{max,rel}$), time to peak power ($Time.P_{max}$) and RFD normalized by peak force (K_{fitted}).

3.3.4. Perceived soreness

Perceived soreness of the hamstring muscles was recorded using an 11-point NRS, in the relaxed position of the $90^{\circ}_{hip}:20^{\circ}_{knee}$ IPC test (**Studies I, II and IV**). The scale was supported by pain descriptors, ranging from "0" for "no pain" to "10" for "unbearable pain" [240], and has been shown to be sensitive to eccentric exercise-induced pain in hamstring muscles [241].

3.3.5. Blood sampling and processing

Samples of an 8-mL blood volume were collected from an antecubital vein and then were clotted at room temperature for 30 minutes in *SST II Advance tubes* (Becton Dickinson Vacutainer Systems, Plymouth, UK). Subsequently, samples were centrifuged at 4,000 g for 8 minutes at 4°C. Finally, as many 500 µl aliquots of serum as possible were immediately pipetted into *Eppendorf safe-lock tubes* (Eppendorf SE, Hamburg, Germany) and stored at -80°C until analysis (**Studies I, II and IV**).

Measurement of CK (**Studies I, II and IV**), ALT (**Study II**) and AST (**Study II**) enzyme activities were conducted on an *Advia 2400 automated device* (Siemens™ Medical Solutions Diagnostics, Tarrytown, NY, USA), following the method of the International Federation of Clinical Chemistry Reference Committee for the measurement of enzyme catalytic activity [342]. Serum sMtCK (**Studies I, II and IV**) was measured by ELISA kit #SEC386Hu (Cloud Clone Corp., Houston, TX, USA) according to the instructions of the manufacturer (intra-assay coefficient of variability [CV] < 10%; inter-assay CV < 12%; minimum detectable value of 1.56 ng·mL⁻¹).

Serum MDA (**Studies I, II and IV**) was measured by kit #E-EL-0060 (Elabscience, Wuhan, China), with an intra-assay CV < 10%, inter-assay CV < 10% and a minimum detectable value of 18.75 ng·mL⁻¹. Further, the Erel approach [266] was used to quantify the oxidizing material in the samples (TOS), based on the oxidation of ferrous iron to ferric iron, with an intra-assay CV of 2.3%, inter-assay CV of 3.5% and a minimum detectable value of 2.5 µmol H₂O₂ Equiv.·mL⁻¹ (Elabscience #E-BC-K802-M, Wuhan, China). Similarly, the Erel 2,2'-azino-di-3-

ethylbenzthiazoline sulfonate+ approach [265] with an intra-assay CV of 4.6%, inter-assay CV of 7.0% and a minimum detectable value of 0.23 mmol Trolox Equiv.·mL⁻¹ (Elabscience #E-BC-K801-M, Wuhan, China) was conducted to quantify total antioxidant status (TAS). Then, OSI was calculated as the TOS to TAS ratio. To calculate OSI, the result unit of TAS, mmol Trolox equivalent·L⁻¹, was converted to μmol Trolox equivalent·L⁻¹. Then, OSI value was calculated using the following formula: OSI (arbitrary units, AU) = [(TOS, μmol H₂O₂ equivalent·L⁻¹)/(TAS, μmol Trolox equivalent·L⁻¹) x 100]. Circulating irisin (**Studies I, II and IV**) was quantified using the previously validated kit against mass spectrometry [319] #EK-067-29 (Phoenix Pharmaceuticals Inc, Burlingame, CA, USA), according to the instructions of the manufacturer (intra-assay CV < 10%; inter-assay CV < 15%; minimum detectable value of 1.29 ng·mL⁻¹).

Fasting blood was drawn to determine 25OHD concentrations (**Study I**) measured by chemiluminescence immunoassay procedures conducted on a *Advia Centaur Vitamin D Total Assay* (Siemens™ Healthcare Diagnostics, Tarrytown, NY, USA). The method is certified by the Centers for Disease Control and Prevention and adhered to de Vit D Standardization-Certification Program [343]. Vit D deficiency has been defined at <20 ng·mL⁻¹ (< 50 nmol·L⁻¹) of 25OHD, while appropriate range is 20-50 ng·mL⁻¹ (50-125 nmol·L⁻¹) [344].

3.3.6. Dietary intake

In order to verify that nutritional intake was homogeneous and within normal ranges for each macro- and micronutrient, the volunteers were instructed to record their diet for 3 days (-24h, 0h and +48h) [345], and were given detailed verbal and written instructions describing procedures for recording the daily diet, together with a list of commonly used household and measurements quantities (**Studies I, II and IV**). The participants were strongly instructed to maintain their usual diet, and to be accurate in recording the amounts and types of food and drinks intake. The diet of each participant was calculated as the 3-day average by using the dietary analysis software *PCN Pro v1*. (Universitat de Barcelona, Barcelona, Spain) [346], which is based on and updated with a Spanish food composition database [347].

3.4. Statistics

First, sample size was independently and a priori estimated using *G*Power software* v3.1.9.6 for each study in the thesis, considering the experimental design, the target effect size, the required statistical power, and a 5% alpha error. The normality of the data set was assessed by visual inspection of the histograms, combined with the *Shapiro-Wilk test*. Asymmetrically distributed variables were log- or square-transformed prior to analysis. If transformed variables remained non-conformed to the *Normal distribution*, non-parametric statistical procedures were conducted.

Repeated measures ANOVAs were performed to identify the effect of the different exercising models on hamstrings neuromuscular metrics and serum biomarkers, together with between-time points, between-sex, and/or between-limbs interactions (**Studies I, II and IV**). In case of violation of *Mauchly's* test for sphericity, the *Greenhouse-Geisser* ($\epsilon < 0.75$) or *Huynh-Feldt* ($\epsilon > 0.75$) correction were applied. A four-way mixed model [time (pre, post) x muscle (BF_{SH}, BF_{LH}, ST, SM) x limb (dominant, non-dominant) x region (proximal, middle, distal)] was used to determine exercise-induced hamstring muscles activation (T2 values) after the *Flywheel Russian belt Deadlift* exercise. Subsequently, a three-way mixed model [muscle (BF_{SH}, BF_{LH}, ST, SM) x limb (dominant, non-dominant) x region (proximal, middle, distal)] was used to determine differences in hamstring muscles T2 shift immediately after the exercise (**Study III**).

In order to reduce family-wise error rate, post-hoc Bonferroni-corrected *paired t-tests* were conducted to identify significant differences from baseline values. The alternative statistical approach if the variables were nonparametric was the *Friedman test*, together with the *Conover test* for post-hoc Bonferroni-corrected comparisons. Effect sizes were calculated using Cohen's *d* to assess the difference between means when statistically significant differences were found. Thresholds for standardized differences statistics were (< 0.20), trivial; ($0.20 < 0.59$), small; ($0.60 < 1.19$), moderate; ($1.20 < 1.99$), large; and (> 2.0), very large [348].

Associations between variables of interest were analyzed using Pearson's or Spearman's correlation coefficients (according to normality assumption), considering the following thresholds: (< 0.30), small; ($0.30 < 0.51$), moderate; ($0.51 < 0.70$), large; ($0.71 < 0.90$), very large; and (> 0.90), extremely large [348]. The intraclass correlation coefficient (ICC) was used to assess the reliability of a single data collector that examines the degree of concordance of scores between repetitions or sessions. The ICC was calculated for average measures using a 2-way random effect model of absolute agreement. Thresholds for ICC were ($0.00 < 0.50$), poor; ($0.51 < 0.75$), moderate; ($0.76 < 0.90$), good; and ($0.91 < 1.00$), excellent [349].

Data are presented as mean $\pm$ standard deviation (SD), and the level of significance was set at $p < 0.05$. Statistical analysis was performed with SPSS v29.0.0.0 (SPSS Statistics, IBM Corp., Armonk, NY, USA).

RESULTS

4. STUDY I. Assessment of inter-individual variability in hamstring muscle recovery after a sport-specific sprint training in women and men

Assessment of inter-individual variability in hamstring muscle recovery after a sport-specific sprint training in women and men

ABSTRACT

Background: Hamstring muscles are most affected by multiple sprint-based sports as a result of muscle strains during sprinting, leading to reduced performance and increased hamstring strain injury (HSI) risk. Therefore, the purpose of the study was to assess inter-individual variability in hamstrings recovery after a sport-specific repeated-sprint training, through sprint-specific markers of muscle recovery ($90^{\circ}_{hip}:20^{\circ}_{knee}$ test and *Jurdan test*) and associated muscle damage biomarkers in women and men.

Methods: Healthy females ($n = 14$) and males ($n = 15$) underwent 10 repeated 40-meter sprints with a 3-minute rest pause between each repetition. Maximal voluntary isometric contraction (MVIC) torque by the $90^{\circ}_{hip}:20^{\circ}_{knee}$ test and range of motion (ROM) *Jurdan test*, together with serum biomarkers (sMtCK, oxidative stress, irisin) were tested at baseline and 24-, 48- and 72-hours post-exercise through a repeated measures design. Participants were classified according to MVIC torque loss into high responders (HR) and low responders (LR).

Results: 21 individuals (10 females, 11 males) were classified as HR (MVIC torque loss $> 20\%$ and recovery $> 48h$), while 8 individuals (4 females, 4 males) were classified as LR. HR individuals showed unrecovered MVIC torque until 72 hours post-training ($p = 0.003$, $n_p^2 = 0.170$), whereas only HR males showed decreased ROM ($p = 0.026$, $n_p^2 = 0.116$). HR individuals also showed increased sMtCK ($p = 0.016$, $n_p^2 = 0.128$), oxidative stress ($p = 0.038$, $n_p^2 = 0.106$) and irisin ($p = 0.019$, $n_p^2 = 0.123$).

Conclusion: There is inter-individual variability in the muscular response to a sport-specific repeated-sprint training, identifiable by MVIC torque assessment. The findings support that the $90^{\circ}_{hip}:20^{\circ}_{knee}$ test is a powerful indirect test to screen hamstrings recovery in both women and men, in a cost-effective way. However, the *Jurdan test* might not be able to monitor hamstrings recovery in sportswomen after a repeated-sprint training. Decreases in muscle capacity are linked to damage to muscle sarcolemma and mitochondria until 72 hours post-exercise. Overall, 72 hours will not be adequate time to restore hamstrings structure and function after a sport-specific repeated-sprint training in both female and male responders.

Keywords: exercise-induced muscle damage, fatigue, force-generating capacity, muscle recovery, repeated sprint ability.

INTRODUCTION

Repeated sprints, defined as periods of high-intensity running interspersed with lower intensity jogging and walking, are recurrent in multiple-sprint team sports (e.g., soccer, field hockey) [16]. During sprinting, the muscle-tendon unit of the hamstring muscles reaches its maximum length in the late swing phase, while simultaneously undergoing an active lengthening eccentric contraction [20]. Thus, repeated high intensity eccentric contractions employed in repeated-sprint training result in loss of hamstring muscles function [173]. Muscle recovery is multifactorial in nature, as there are a wide range of intrinsic (e.g., training history) and extrinsic factors (e.g., seasonal moment) which influence the internal load suffered by athletes [350]. The primary approach for assessing muscle recovery is muscle histology. However, this method is not used by professionals and practitioners due to its invasiveness and intrinsic errors of the approach (i.e., evaluation of the entire muscle by means of a microscopic muscle sample) [6]. Instead, there is a high correlation ($r = 0.89$) between the reduction in force-generating capacity and the fraction of muscle fibers presenting ultrastructural alterations [47]. Consequently, force-generating capacity is the preferred indirect marker of muscle damage accounting for myofibrillar alterations, inflammation, and necrosis [73]. Assessment of hamstring force-generating capacity has been commonly conducted using supine tests [206]. However, as the largest decrease of isokinetic peak torque in the hamstring muscles appears in the most lengthened positions (i.e., 10° of knee flexion), *Matinlauri et al.*, [211] recently designed a new, reliable, and sensitive standing test to monitor hamstring-related acute and residual fatigue after sprint-based exercises, the $90^\circ_{hip}:20^\circ_{knee}$ IPC test. The design of the test causes a lengthening of the hamstrings, and specifically, a greater *biceps femoris* hip extension than knee flexion moment arm, compared to the ST and SM muscles, in a similar pattern to the late swing phase of the running gait cycle [20]. Consequently, the hamstrings are biomechanically exposed during the test, thus facilitating hamstring-related acute and residual fatigue monitoring [211].

Repeated high intensity eccentric contractions also result in changes of biochemical markers of muscle damage and oxidative stress, which indicate the status of myocyte structure [24,62]. Even there is no single gold-standard biomarker, a panel of biomarkers might be reported to simultaneously assessing different physiological processes and provide a more comprehensive understanding of muscle recovery. Mechanical-induced physical damage to membrane has been the underlying hypothesis explaining membrane permeability following eccentric actions. However, it has been found that increased intracellular Ca^{2+} over time as a consequence of stretch-activated Ca^{2+} channels plays a key role in the permeability of the sarcolemma [61]. Considering a perspective applicable to the day-to-day sports practice, transient sarcoplasmic oxidation by-

products and Ca^{2+} upregulation derived from damaging exercises results in a mild force-generating capacity loss as a consequence of failures in the excitation-contraction coupling process [42]. Given that the mitochondria modulate Ca^{2+} overload through the mitochondrial Ca^{2+} uniporter located in the inner mitochondrial membrane [50], mild decreases in muscle function might be preserved by amino acids and/or micro-nutrients intake (e.g., Vit D) owing to the maintenance of the muscle mitochondria structural integrity and function [106,125]. However, persistent Ca^{2+} intracellular elevation activates the mitochondrial permeability transition pore [54] and upregulates Ca^{2+} -dependent calpains [45]. Calpains are predominantly localized in the Z-disk and I band regions, and have been shown to play a critical role in EIMD, as they are disruptive to the Z-disk and myofibrillar protein integrity [46]. In particular, Ca^{2+} -activated calpains impair contractile proteins (e.g., desmin) and/or excitation contraction coupling proteins (e.g., junctophilins) [46]. As a result, there is an uncontrolled release of Ca^{2+} and oxidation by-products to the sarcoplasm and bloodstream, finally resulting in rupture of the mitochondrial outer membrane and intermembrane proteins efflux [55].

However, the structural integrity of the muscle mitochondria can be monitored by measuring striated muscle mitochondrial-specific isoenzyme, sMtCK [241]. sMtCK is mainly localized in both the peripheral intermembrane space and the cristae space [245,249]. Considering the aforementioned localization of sMtCK, and that striated muscle mitochondria necrosis determined by histological evidence resulted in increased sMtCK in plasma, the efflux of sMtCK into the bloodstream reflects mitochondrial disruptions [250]. The afore-mentioned damage to cellular and mitochondrial integrity also leads to an upregulation of the signaling pathway for irisin cleavage [274]. The myokine irisin plays a significant role in muscle remodeling by ameliorating protein degradation [302]. Recently, irisin has also been shown to be involved in counteracting oxidative processes and pro-inflammatory cytokines [284]. Considering the aforementioned irisin-mediated effects together with the fact that a recent study revealed that aerobic exercise-induced irisin partly mediates amelioration of oxidative stress and cell apoptosis in mice skeletal muscle [351], the myokine irisin is suggested to be increased during muscle recovery.

Finally, sarcomeres and mitochondria impairment caused by eccentric contractions during sprinting results in long-lasting decreases in force-generating capacity [24,42], which is a fundamental source for the loss of performance described in both sportswomen [352] and men [248] after a repeated-sprint training. Accordingly, there is a need for practitioners to monitor force-generating capacity and ROM using simple, time and cost-effective sprint-specific muscle function tests associated with biochemical markers using an integrative-based approach. To our

knowledge, the use of the $90^{\circ}_{hip}:20^{\circ}_{knee}$ *IPC test* to monitor hamstrings force-generating capacity during the recovery period after repeated-sprint training is described for the first time. Therefore, the purpose of the research was to assess inter-individual variability in hamstrings recovery after a sport-specific repeated-sprint training, through sprint-specific markers of muscle recovery and associated muscle damage biomarkers in women and men.

MATERIALS AND METHODS

Participants

Fifteen healthy males (age = 22.5 ± 3.2 years, mass = 74.2 ± 7.4 kg, height = 175.9 ± 6.8 cm [mean $\pm$ SD]) and fourteen females (age = 22.2 ± 3.1 years, mass = 60.3 ± 7.1 kg, height = 165.2 ± 4.8 cm [mean $\pm$ SD]) voluntarily attended the study. Participants were required to be physically active according to the World Health Organization (at least 150 minutes of moderate-intensity exercise per week), but not enrolled in a regular specialized training program. Exclusion criteria were non-healthy participants and lower limb muscle injuries within 6 months prior to the experiment. Participants were strongly encouraged to (i) avoid any type of physical exercise during the 3 days prior to the experiment and during the 72-hour follow-up, and (ii) to maintain their daily diet and lifestyle. All participants were previously informed and gave their written consent that they received complete and specific information about the purpose of the study, experimental tests, and associated risks. The experiment was conducted in accordance with the code of ethics of the World Medical Association (Declaration of Helsinki) and was approved by the Ethics Committee for Clinical Research of the Catalan Sports Council (Generalitat de Catalunya) (037/CEICGC/2021).

Experimental Design

A repeated-measures design was used to examine the extent and time course of serum biomarkers and muscle function before (pre) and at 24 (+24h), 48 (+48h) and 72 hours (+72h) after a repeated-sprint training, consisting of 10 repeated 40-meter sprints, with a 3-minute rest between each repetition. Depending on the playing position, sprint distance in soccer matches ranges from 190 to 420 meters [166], in field hockey matches from 418 to 513 meters [167], and in rugby matches from 65 to 392 meters [168]. In addition, similar repeated-sprint training protocols (e.g., 3 sets x 6 repetitions x 20 meters = 360 meters, rest: 1.5 minutes between repetitions and 4 minutes between sets) have shown decreases in hamstrings electrical activity and knee flexors torque [353]. Accordingly, the total training distance (i.e., 400 meters) was determined based on the aforementioned sprint demands of outfield team sports, and the distance of each sprint (i.e., 40 meters) was selected as it allows reaching the maximum sprint velocity, which is the main injury

mechanism of HSI [23]. Long rest periods between repetitions were selected to reduce intra-session fatigue and maintain the maximum intensity in each 40-meter repetition. The designed protocol is further supported by the findings of a recent systematic review and meta-analysis on the demands of repeated sprints in team sports [333], which include that (i) a distance of 40 meters (or 6-second sprints), commonly used in team sports, allows the maximum velocity to be reached; and (ii) longer rest intervals are recommended in order to maintain the quality of the repetitions when the total volume is greater.

Then, blood samples were collected to determine changes in serum biomarkers, and muscle function was assessed by posterior chain force-generating capacity, lower limbs ROM and perceived soreness, and sprint performance and mechanical properties test. Participants completed the familiarization one week before the start of the experimental procedure. All neuromuscular tests were repeated by following the instructions of the researcher until successful completion. A schematic overview of the experimental design is shown in **Figure 4.1**.

			Training Day				
	-168h	-24h	Pre	Repeated sprints	+24h	+48h	+72h
Neuromuscular tests (MVIC torque, ROM, PS, SMP)	Familiarization		↓	Repetitions: 10 Distance: 40m recovery: 3'	↓	↓	↓
Serum markers (EIMD, OS, Irisin)			↓		↓	↓	
Dietary intake		↓	↓			↓	

Figure 4.1. Schematic overview showing the experimental design. **EIMD**, exercise-induced muscle damage; **MVIC**, maximal voluntary isometric contraction; **OS**, oxidative stress; **PS**, perceived soreness; **ROM**, range of motion; **SMP**, sprint mechanical properties.

To further explain the findings given the inter-individual variations in response to EIMD, participants were categorized according to the percentage of reduction of the force-generating capacity during the 3-day follow up [73]. Then, participants were grouped into HR (i.e., participants who showed notable decreases in force-generating capacity of 20-50% of baseline values and recovery after more than 48 hours) and LR (i.e., participants who showed force-generating capacity decreases of <20% and complete recovery within 48 hours) [73].

Hamstring-specific neuromuscular tests

Force-Generating Capacity

Force-generating capacity of hamstring muscles was measured unilaterally as the MVIC torque by the $90^{\circ}_{\text{hip}}:20^{\circ}_{\text{knee}}$ IPC test [211], using a 160 Hz sampling frequency strain gauge (Chronojump, Barcelona, Spain). Participants were verbally encouraged to exert their maximum effort and completed three trials (3-5s MVIC) of the test with each lower limb, with a 30-seconds rest between trials. The MVIC torque was registered as the average force in a 1-s window once a force plateau had been established [339] and the residual force resulting from the mass of the tested leg at rest was then subtracted. The best of the three trials from each limb was recorded for further analyses. Additional trials were conducted if any countermovement or joint balancing was noted.

Range of Motion

The *Jurdan test* was used to evaluate the interaction between hip flexor and hamstring flexibility, measured as the differential angle between the thigh and contralateral shin [233]. The test was photographically registered by a SONY DSC-RX100M5A placed on a tripod located at the height of the table and 3 meters from the center of the hip of the participant. 2D shin and thigh angles were measured, relative to the horizontal, as the best of two trials on each lateral approach using the sports kinematics analysis software *Kinovea* v0.9.3. Then, resulting angles were used to compute a composite angle defined as the difference between the actively lengthened leg shin angle and the opposite leg passive thigh angle.

Perceived soreness

Perceived soreness of the hamstring muscles was recorded using an 11-point NRS, in the relaxed position of the $90^{\circ}_{\text{hip}}:20^{\circ}_{\text{knee}}$ IPC test. The scale was supported by pain descriptors, ranging from "0" for "no pain" to "10" for "unbearable pain" [240], and has been shown to be sensitive to eccentric exercise-induced pain in hamstring muscles [241].

Sprint performance and mechanical properties

Instantaneous velocity over 40 meter-sprints was measured by a friction encoder *RaceAnalyzer* (Chronojump, Barcelona, Spain), with a distance accuracy of 3 centimeters and temporal accuracy of 4 microseconds. The device was located on a 1-meter-high tripod placed behind the participants. Then, participants were positioned into a belt attached to the friction encoder and were strongly verbally encouraged during the test to ensure maximal effort. Data were post hoc analyzed using Chronojump software v2.1.2-2 (Chronojump, Barcelona, Spain) to compute sprint F-V spectra using the validated method described in detail elsewhere [341]. Individual linear F-V relationships were then extrapolated to calculate V_0 , $F_{0\text{rel}}$, A_{max} , $P_{\text{max,rel}}$, $\text{Time}_{P.\text{max}}$ and K_{fitted} .

Blood sampling and processing

An 8-mL blood sample was drawn from an antecubital vein. Samples were allowed to clot for 30 minutes at room temperature in an *SST II Advance tube* (Becton Dickinson Vacutainer Systems, UK) and were then centrifuged at 4,000 g for 8 minutes at 4°C. After separation of the serum, aliquots were stored at -80 °C until analysis.

Measurement of CK activity was conducted on an *Advia 2400 automated device* (Siemens™ Medical Solutions Diagnostics, Tarrytown, NY, USA) and serum sMtCK was measured by ELISA kit #SEC386Hu (Cloud Clone Corp., Houston, TX, USA) according to manufacturer's instructions. The Erel approach [266] was used to quantify the oxidizing material in the samples, based on the oxidation of ferrous iron to ferric iron (Elabscience #E-BC-K802-M, Wuhan, China). Similarly, the Erel 2,2'-azino-di-3-ethylbenzthiazoline sulfonate (ABTS)+ approach [265] (Elabscience #E-BC-K801-M, Wuhan, China) was conducted to quantify TAS. Then, OSI was calculated as the TOS to TAS ratio. In order to control the OSI results, serum MDA was measured by kit #E-EL-0060 (Elabscience, Wuhan, China). Circulating irisin was quantified using the previously validated kit against mass spectrometry [319] #EK-067-29 (Phoenix Pharmaceuticals Inc, Burlingame, CA, USA), according to manufacturer's instructions.

25OHD concentration was measured by chemiluminescence immunoassay procedures conducted on a *Advia Centaur Vitamin D Total Assay* (Siemens™ Healthcare Diagnostics, Tarrytown, NY, USA). The method is certified by the Centers for Disease Control and Prevention and adhered to de Vitamin D Standardization-Certification Program [343].

Dietary Intake Record

Participants were instructed to record their diet for 3 days (-24h, 0h and +48h) and were given detailed verbal and written instructions describing procedures for recording the daily diet, together with a list of commonly used household measurements (e.g., dessert spoon, glass, cup) and measurements quantities (e.g., grams, milliliters). The participants were strongly instructed to maintain their usual diet, and to be accurate in recording the amounts and types of food and drinks intake. The diet of each participant was calculated as the 3-day average by using the dietary analysis software *PCN Pro v1*. (Universitat de Barcelona, Barcelona, Spain) [346], which is based on and updated with a Spanish food composition database [347].

Statistical analysis

First, sample size was estimated using G*Power software (version 3.1) based on MVIC change ($90^{\circ}_{hip}; 20^{\circ}_{knee}$ IPC test) of the study by *Matinlauri et al.*, [211]. A total of 25 participants were

calculated to be included given an effect size of 0.68, a power of 90% and a 5% alpha error. Asymmetrically distributed variables were log-transformed prior to analysis.

A three-way repeated measures ANOVA (time [baseline, 24h, 48h, 72h] x sex [male, female] x group [HR, LR]) was used to identify the effect of time on MVIC torque, ROM, perceived soreness, sprint performance and mechanical properties and serum biomarkers, together with interactions between sex and groups. Post-hoc Bonferroni-corrected paired t-test were conducted to identify significant differences from baseline values. Effect sizes were calculated using Cohen's *d* to assess the difference between means when statistically significant differences were found. Thresholds for standardized differences statistics were (< 0.20), trivial; ($0.20 < 0.59$), small; ($0.60 < 1.19$), moderate; ($1.20 < 1.99$), large; and (> 2.0), very large [348]. Associations between variables of interest were analyzed using Spearman's Rho correlation coefficient, considering the following thresholds: (< 0.30), small; ($0.30 < 0.51$), moderate; ($0.51 < 0.70$), large; ($0.71 < 0.90$), very large; and (> 0.90), extremely large [348]. The ICC was used to assess the reliability of a single data collector that examines the degree of concordance of scores between repetitions or sessions. The ICC was calculated for average measures using a 2-way random effect model of absolute agreement. Thresholds for ICC were ($0.00 < 0.50$), poor; ($0.51 < 0.75$), moderate; ($0.76 < 0.90$), good; and ($0.91 < 1.00$), excellent [349]. Independent samples t-test were used to assess between-groups differences in dietary intakes. Data are presented as mean $\pm$ SD and the level of significance was set at $p < 0.05$. Statistical analysis was performed with SPSS version 27.0.1.0 (SPSS Statistics, IBM Corp., Armonk, NY, USA).

RESULTS

According to the percentage of reduction in force-generating capacity [73], 21 individuals (10 females, 11 males) were classified under HR conditions, as they presented notable decreases in MVIC torque of 20-50% of baseline values and recovery after more than 48 hours. Otherwise, 8 individuals (4 females, 4 males) showed MVIC torque decreases of $< 20\%$ and complete recovery within 48 hours and were therefore classified as LR.

Neuromuscular biomarkers panel

Maximal voluntary isometric contraction (MVIC) torque. The ICC was excellent for the dominant leg with a value of 0.973 (95% IC 0.941 to 0.988) and non-dominant leg with a value of 0.969 (95% IC 0.937 to 0.986). As illustrated in **Figure 4.2A**, hamstrings MVIC torque was decreased and unrecovered in HR individuals during the 72-hours recovery period. There was no sex-related time course difference in MVIC torque.

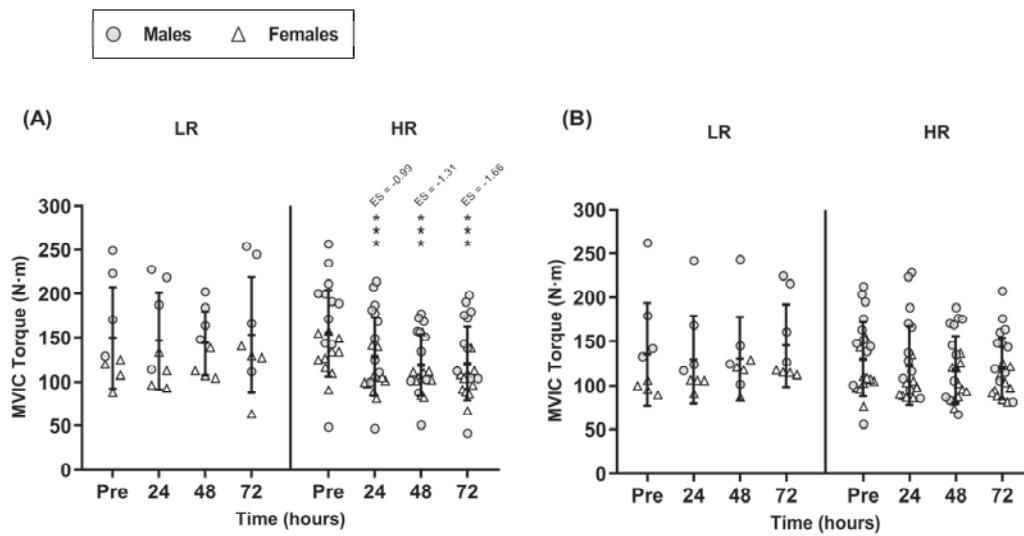


Figure 4.2. Maximal voluntary isometric contraction (MVIC) torque of the lower limb showing (A) the larger and (B) the smaller reduction of MVIC torque during recovery period. Data are expressed as mean $\pm$ SD. (*), (**), and (***) indicate a significant difference from the baseline value at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. **ES**, effect size; **HR**, high responders; **LR**, low responders; **N·m**, newtons-meter.

Range of Motion (ROM). There was a significant time-group-sex interaction in the posterior chain ROM following the repeated-sprint training [$F(3,75) = 3.277$, $p = 0.026$, $n^2_p = 0.116$]. Post-hoc Bonferroni-corrected paired t-test showed unchanged ROM in LR group, while ROM was only decreased in males HR group during the 72-hours recovery period (**Figure 4.3A**).

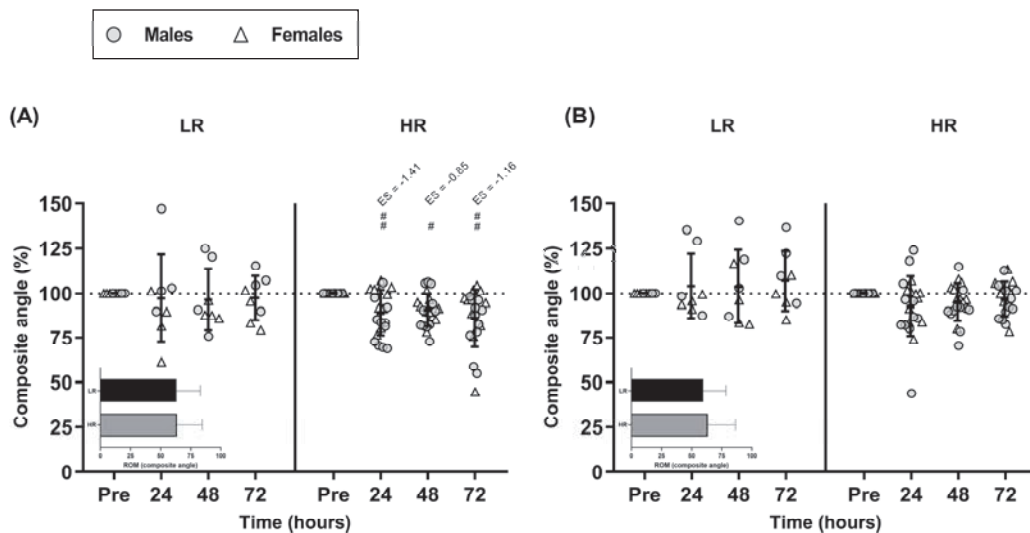


Figure 4.3. Percentages of posterior chain range of motion (ROM) of (A) the most damaged and (B) the least damage lower limb during recovery period. Data are expressed as mean $\pm$ SD. Baseline values of ROM composite angle are shown in the inset graphs. (#), (##), and (###) indicate a significant difference from the baseline value in males at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. **ES**, effect size; **HR**, high responders; **LR**, low responders.

Perceived soreness. As shown in **Figure 4.4A and 4.4B**, perceived hamstrings soreness was increased in HR individuals until +48h in both lower limbs [$X^2(3) = 21.620$, $p < 0.001$], [$X^2(3) = 25.036$, $p < 0.001$]. However, LR individuals showed no perceived hamstrings soreness in both lower limbs [$X^2(3) = 6.684$, $p = 0.083$], [$X^2(3) = 5.706$, $p = 0.127$].

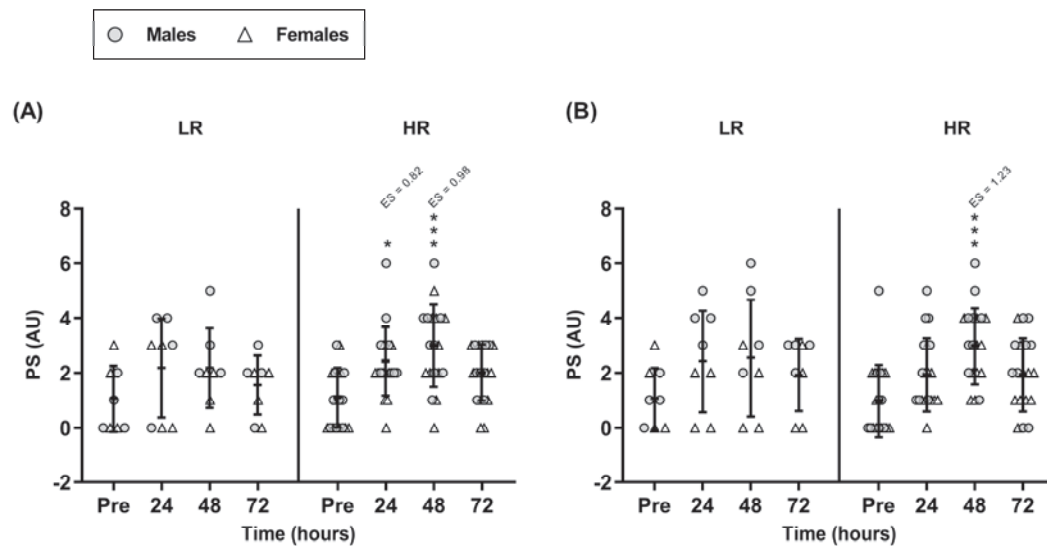


Figure 4.4. Hamstrings perceived soreness of (A) the most damaged and (B) the least damage lower limb during recovery period. Data are expressed as mean $\pm$ SD. (*), (**), and (***) indicate a significant difference from the baseline value at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. AU, arbitrary units; ES, effect size; HR, high responders; LR, low responders; PS, perceived soreness.

Sprint performance and mechanical properties

The repeated-sprint training increased split time to 10 meters and total time to 40 meters during recovery period. Also, Both the LR and HR individuals showed decreased $F0_{rel}$, A_{max} , $P_{max,rel}$, K_{fitted} and increased $Time_{P,max}$ during the post-training recovery period. V_0 was only decreased in HR individuals. There were no group and sex-related time course differences (**Table 4.1**).

Table 4.1. Sprint performance and mechanical properties during post-exercise recovery period.

Parameter	Group	Pre	+24h	+48h	+72h
Time to 10m (s)	LR	2.185 ± 0.085	2.297 ± 0.184	2.415 ± 0.209***	2.407 ± 0.222**
	HR	2.171 ± 0.114	2.316 ± 0.173***	2.288 ± 0.126**	2.289 ± 0.149**
	ALL	2.175 ± 0.106	2.311 ± 0.173***	2.323 ± 0.160***	2.321 ± 0.176***
Time to 40m (s)	LR	6.178 ± 0.387	6.242 ± 0.473	6.465 ± 0.472**	6.530 ± 0.471**
	HR	6.217 ± 0.569	6.416 ± 0.548**	6.402 ± 0.513***	6.417 ± 0.549**
	ALL	6.207 ± 0.519	6.368 ± 0.526*	6.420 ± 0.494***	6.448 ± 0.523***
V₀ (m·s⁻¹)	LR	8.341 ± 0.801	8.314 ± 0.758	8.216 ± 1.029	8.034 ± 0.693
	HR	8.256 ± 1.117	7.992 ± 0.943**	7.943 ± 0.988**	7.926 ± 0.984**
	ALL	8.280 ± 1.026	8.081 ± 0.895	8.018 ± 0.989*	7.956 ± 0.903**
F_{0rel} (N·Kg⁻¹)	LR	7.608 ± 0.582	6.732 ± 0.995	6.268 ± 1.118***	6.313 ± 1.660**
	HR	7.790 ± 0.740	6.717 ± 0.922***	7.042 ± 0.845***	6.953 ± 1.004**
	ALL	7.740 ± 0.695	6.721 ± 0.924***	6.829 ± 0.973***	6.776 ± 1.222***
A_{max} (m·s⁻²)	LR	7.677 ± 0.580	6.806 ± 1.002	6.339 ± 1.118***	6.382 ± 1.663**
	HR	7.859 ± 0.745	6.787 ± 0.929***	7.112 ± 0.849***	7.022 ± 1.008**
	ALL	7.809 ± 0.698	6.792 ± 0.931***	6.898 ± 0.975***	6.845 ± 1.226***
P_{max,rel} (W·Kg⁻¹)	LR	15.097 ± 1.668	13.972 ± 2.919	12.735 ± 2.577***	12.587 ± 3.568**
	HR	15.287 ± 2.663	13.407 ± 2.869***	13.897 ± 2.501**	13.723 ± 2.769**
	ALL	15.235 ± 2.402	13.563 ± 2.842**	13.577 ± 2.531***	13.410 ± 2.988***
Time_{P,max} (s)	LR	0.721 ± 0.112	0.863 ± 0.128*	0.931 ± 0.238***	0.926 ± 0.245***
	HR	0.698 ± 0.100	0.832 ± 0.140***	0.791 ± 0.139**	0.800 ± 0.132**
	ALL	0.704 ± 0.102	0.840 ± 0.135***	0.830 ± 0.179***	0.835 ± 0.175***
K_{fitted} (s⁻¹)	LR	1.011 ± 0.144	0.850 ± 0.112**	0.812 ± 0.165***	0.829 ± 0.217**
	HR	1.047 ± 0.155	0.885 ± 0.123***	0.938 ± 0.162**	0.927 ± 0.172**
	ALL	1.037 ± 0.150	0.876 ± 0.120***	0.903 ± 0.170***	0.900 ± 0.187***

Data are expressed as mean ± SD. †Significantly different between groups at baseline. (*), (**), and (***) indicate a significant difference from the baseline value at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. **A_{max}**, peak acceleration; **F_{0rel}**, relative theoretical maximal horizontal force production; **HR**, high responders; **K_{fitted}**, rate of force development normalized by peak force; **LR**, low responders; **P_{max,rel}**, relative peak power; **Time_{P,max}**, time to peak power; **V₀**, theoretical maximal running velocity.

Biochemical measures

CK activity remained unchanged in the LR group, while CK was peaked in HR group at +24h ($p < 0.001$; ES = 1.77) and remained increased at +48h ($p < 0.001$; ES = 1.50) and at +72h ($p < 0.001$; ES = 1.02). Also, there was a significant time-group interaction in serum sMtCK values [$F(3,75) = 3.665$, $p = 0.016$, $n^2_p = 0.128$] following the repeated-sprint training (**Figure 4.5A**). Similarly, MDA and OSI were only increased in HR individuals and remained elevated during recovery period (**Figure 4.5B and Figure 4.5C**).

Finally, there was a significant time-group interaction in circulating irisin values following the repeated-sprint training [$F(3,75) = 3.505$, $p = 0.019$, $n^2_p = 0.123$]. Post-hoc Bonferroni-corrected paired t-test showed unchanged irisin in LR group, while irisin was increased in HR group at +24h, peaked at +48h, and remained increased at +72h (**Figure 4.5D**). There were no between-sex differences in the interaction of time and group for CK, sMtCK MDA, OSI, and irisin.

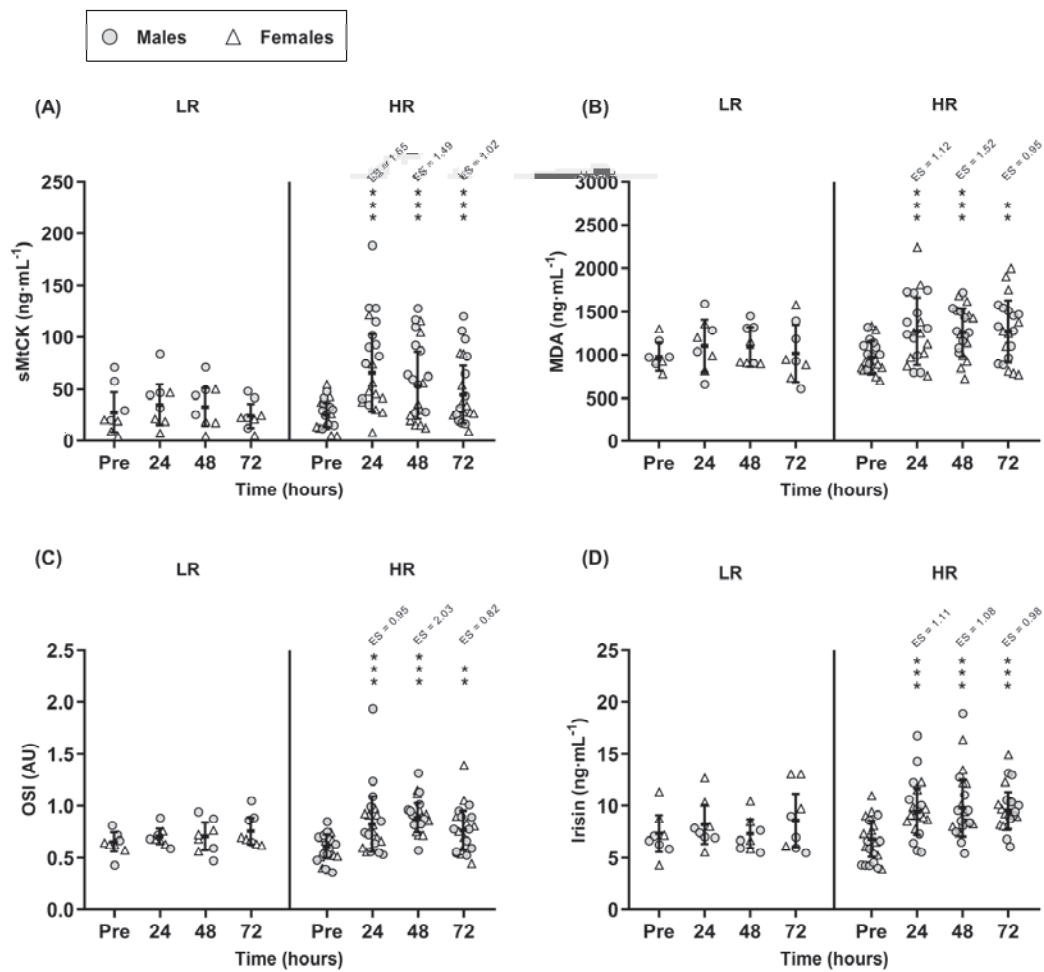


Figure 4.5. Mean ($\pm$ SD) values of (A) sarcomeric mitochondrial creatine kinase (sMtCK); (B) malondialdehyde (MDA); (C) oxidative stress index (OSI); and (D) irisin. (*), (**), and (***) indicate a significant difference from the baseline value at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. AU, arbitrary units; ES, effect size; HR, high responders; LR, low responders.

The correlation matrix between changes in serum biomarkers and daily dietary intakes is shown in **Table 4.2**. CK and sMtCK increases were negatively and moderately correlated with daily total protein intake ($p = 0.028$ and $p = 0.007$; respectively). In addition, there was a strong correlation between the extent of damage to muscle mitochondria, oxidative stress and irisin.

Table 4.2. Spearman's rank correlation coefficient matrix of peak fold-changes in serum biomarkers and daily dietary intakes (n = 29).

	CK	sMtCK	MDA	OSI	Irisin	TE	TC	TPI	API
CK	--								
sMtCK	0.663*	--							
MDA	0.197	0.201	--						
OSI	0.383*	0.595*	0.238	--					
Irisin	0.262	0.554*	0.123	0.618*	--				
TE	0.074	-0.130	-0.115	-0.185	-0.174	--			
TC	0.099	0.011	-0.015	-0.260	-0.021	0.753*	--		
TPI	-0.423*	-0.511*	-0.150	-0.261	-0.503*	0.673	0.404	--	
API	-0.350	-0.460*	-0.202	-0.146	-0.360	0.667	0.277	0.940*	--
PPI	-0.048	-0.033	0.238	-0.068	-0.348	0.200	0.405*	0.267	0.029

(*) indicates a significant correlation at $p < 0.05$. **API**, animal-based protein intake; **CK**, creatine kinase; **MDA**, malondialdehyde; **OSI**, oxidative stress index; **PPI**, plant-based protein intake; **sMtCK**, sarcomeric mitochondrial creatine kinase; **TC**, total carbohydrates intake; **TE**, total energy intake; **TPI**, total protein intake.

Dietary intake

There were no differences between HR and LR individuals in energy intake, neither in macronutrient nor micronutrient intake (**Table 4.3**). Also, there were no statistically significant differences between HR group ($29.4 \pm 9.0 \text{ ng} \cdot \text{mL}^{-1}$) and LR group ($26.7 \pm 9.0 \text{ ng} \cdot \text{mL}^{-1}$) in serum 25OHD status during the experimental protocol ($\text{MD} = 2.77 \text{ ng} \cdot \text{mL}^{-1}$; $t = 0.737$; $p = 0.468$).

Table 4.3. Summary of macro- and micronutrient intakes.

	HR (n = 21)	LR (n = 8)
Total energy (kcal·day ⁻¹)	2164 ± 539	2282 ± 594
Total Protein Intake (g·Kg ⁻¹ ·day ⁻¹)	1.67 ± 0.30	1.91 ± 0.66
Animal-based Protein Intake (g·Kg ⁻¹ ·day ⁻¹)	1.22 ± 0.27	1.39 ± 0.53
Plant-based Protein Intake (g·Kg ⁻¹ ·day ⁻¹)	0.42 ± 0.10	0.41 ± 0.07
Total carbohydrates (g·day ⁻¹)	210 ± 56	215 ± 38
Total lipids (g·day ⁻¹)	97 ± 35	95 ± 31
Water (g·day ⁻¹)	2389 ± 773	2437 ± 723
Vit C (μg·day ⁻¹)	117.5 ± 60.8	116.0 ± 55.6
Vit D (μg·day ⁻¹)	4.31 ± 4.74	4.06 ± 3.85
Vit E (mg·day ⁻¹)	8.87 ± 3.00	8.96 ± 3.99

Data are expressed as mean ± SD. *Significantly different between groups, independent samples t-test, $p < 0.05$. **HR**, high responders; **LR**, low responders.

DISCUSSION

Novel sprint and hamstring-specific markers of muscle recovery were monitored during 72 hours after a sport-specific repeated-sprint training in women and men. The findings of the research highlight that (i) there is heterogeneity in the muscular response to a repeated-sprint training, identifiable by MVIC torque assessment; (ii) the $90^{\circ}_{hip}:20^{\circ}_{knee}$ *IPC test* for males and females, and the *Jurdan test* for males, could be powerful indirect tests to screen hamstrings muscle recovery after a sport-specific repeated-sprint training; (iii) there is a release of sMtCK into the bloodstream during recovery period, potentially related to mitochondrial dysfunction, or mitochondrial membrane permeability/rupture; and (iv) oxidative stress and circulating irisin are upregulated during the 72-hours recovery period in both HR females and males.

Hamstring muscles capacity is impaired during recovery within high responders

It has been widely reported that high-velocity eccentric contractions employed in repeated-sprint training leads to increased muscle-specific proteins leakage into the bloodstream, muscle soreness, as well as reduced performance during 48 to 72 hours in both collegiate team-sportswomen [352] and men [248]. However, to further explain the results given the within-human heterogeneity in response to exercise training and adaptation, individuals were categorized according to the percentage of force-generating capacity loss [73]. HR participants ($n = 21$) showed an average hamstring MVIC torque decrease of 21.7% at +72h, while the LR individuals ($n = 8$) showed non-significant changes in hamstrings MVIC torque. Given the well-established correlation between ultrastructurally damaged myocytes and force-generating capacity [47], hamstrings-specific muscle function tests were examined to determine muscle recovery. Previous results after repeated-sprint training showed decreases in quadriceps MVIC until +48h in men [248], while there were no decreases in women [352]. However, the $90^{\circ}_{hip}:20^{\circ}_{knee}$ *IPC test* reported decreases in hamstrings MVIC torque at +24h, +48 hours and +72 hours post-training in HR individuals, whereas LR participants showed no significant differences during the recovery period. Loss of performance in the $90^{\circ}_{hip}:20^{\circ}_{knee}$ *IPC test* represents a reduced force-generating capacity in the late swing phase derived from disrupted sarcomeres [211], which is the phase in which most hamstring injuries occur as it is biomechanically exposed [22]. Then, our data suggest that the $90^{\circ}_{hip}:20^{\circ}_{knee}$ *IPC test* can be used to monitor hamstrings muscle recovery in both women and men.

On the other hand, the *Jurdan test* showed decreases in the ROM of HR males until 72 hours post-training. LR individuals, as well as HR females, showed no differences in ROM. Anatomical differences between females and males (e.g., muscle mass size, joint geometry) and physiological

differences (e.g., greater hamstring extensibility, lower passive stiffness, better stretch tolerance) result in women usually exhibiting a greater ROM [354]. Also, considering that the *Jurdan test* is an indicator of increased passive tension derived from damage to the sarcolemma and connective tissue of the iliopsoas and/or hamstring muscles [233], and the aforementioned between-sex differences, females might be less likely to experience ROM loss. Therefore, although HR females showed impaired muscle structure and function, the *Jurdan test* may not show such impairment after a repeated-sprint training. Also, damage to the connective tissue might be reflected by an increase in perceived soreness, because connective tissue displays significant sensory innervation (e.g., free nerve endings) [186]. Accordingly, our results showed moderate perceived soreness increases until +48h in HR individuals, further supporting exercise-induced connective tissue damage.

Finally, considering the characteristics of the damaging model, sprint testing is the most specific test to determine performance recovery. There was a degradation of sprint times and mechanical properties in both HR and LR participants. Only V_0 was decreased in HR compared to LR individuals during the post-training recovery period, accounting for a horizontal force-generating capacity loss at high velocities. This finding may be attributed to the fact that the hamstring muscles are the key mediators of the forward orientation of the ground reaction force during the sprint acceleration phase [170,171]. Overall, HR individuals, as determined by MVIC torque loss, also showed decreased ROM and horizontal force-generating capacity during the sprint acceleration phase until +72h, with no between-sex differences.

Is loss of hamstring muscles capacity linked to serum biomarkers?

Blood biomarkers further support the analysis of exercise-induced damage and muscle recovery [63], since the structural status of myocytes can be assessed by changes in biochemical markers [62]. Peak CK activity observed in this study ($723 \pm 526 \text{ IU} \cdot \text{L}^{-1}$) is consistent with other similar repeated-sprint training studies ($776 \pm 312 \text{ IU} \cdot \text{L}^{-1}$) [248], indicating a permeability and/or breakage of the sarcolemma [24,62]. The lipid peroxidation marker MDA was also moderately increased during the recovery period ($ES = 0.95$), further supporting oxidative damage to the sarcolemma. Sarcolemma impairment-derived sarcoplasmic Ca^{2+} concentration is modulated by mitochondrial Ca^{2+} uptake [50]. Excessive Ca^{2+} accumulation in the mitochondrial matrix leads to a rupture of the mitochondrial outer membrane and intermembrane content efflux (i.e., sMtCK) [55]. In this way, the muscle mitochondria were moderately damaged in HR individuals until +72h ($ES = 1.02$), while no changes were observed in LR individuals. Muscle mitochondria play a critical role during skeletal muscle repair, as it requires a substantial energy supply, and

mitochondria are the primary source of energy production. In this sense, satellite cell signaling, which is a key factor in muscle repair, can be regulated by the mitochondria [355]. Mitochondria have also been shown to be essential in the processes of myoblast differentiation and migration into damaged fibers since mitochondrial dynamics disorders are an impediment to correct myogenic differentiation [356]. Further, transplantation of healthy mitochondria into damaged skeletal muscle of murine models improved myocyte repair and recovery of muscle function [357]. Thus, given that skeletal muscle repair is limited by mitochondrial dysfunction, repeated-sprint training-derived damage to muscle mitochondria is a key aspect to monitor during hamstring muscles recovery through sMtCK assessment. There is also an outflow of ROS to the myocyte and bloodstream [55] given that both the sarcolemma and mitochondria of the HR individuals were affected by the sport-specific repeated-sprint training. Since oxidative stress is a dynamic process involving large number of pro-oxidant components (e.g., total hydroperoxides, MDA) and antioxidant components (e.g., SOD, glutathione peroxidase), integrative-based quantification methods such as OSI should be used to fully understand the complex interaction between exercise and oxidative stress [269]. Separate measurement of different oxidant and antioxidant molecules is complex, time-consuming, and the oxidant or antioxidant effects are additive. However, OSI considers the measurement of both oxidant and antioxidant activity produced by the action of multiple components measured as one [259]. The repeated-sprint training led to a moderate oxidative stress state ($ES = 0.82$) until +72h post-training. There was also a notable correlation between oxidative stress and damage to muscle mitochondria, explained by the significant implication of mitochondria in the efflux of ROS into the bloodstream after damaging exercises [55].

Repeated muscle contractions during the repeated-sprint training and the afore-mentioned mechanical and oxidative damage to cellular and mitochondrial integrity leads to an upregulation of the AMPK- PGC-1 α - FNDC5 signaling axis pathway, and subsequent irisin cleavage [273,274]. The myokine irisin plays an important role in muscle remodeling by stimulating satellite cell activation and ameliorating protein degradation [302]. Also, irisin has been shown to be involved in counteracting oxidative processes related to free radical production [291]. Irisin plays a role in inhibiting the TLR4/MyD88 downstream signaling pathway, resulting in reduced activation of NF- κ B [283]. As a result of depressed NF- κ B, irisin downregulates the expression of tumor necrosis factor- α , IL-6 and IL-1 β pro-inflammatory cytokines [283] and promotes the polarization of pro-inflammatory M1 into anti-inflammatory M2 macrophages [288]. The above-mentioned irisin-mediated effects together with the irisin-induced inhibition of ROS-NLRP3 inflammasome pathway led to an attenuated oxido-inflammatory response [294]. Furthermore, muscle-damaging exercise leads to the release of irisin into the bloodstream in

humans immediately after a single bout of exercise [326]. Accordingly, the findings of the present study revealed a moderate increase in circulating irisin over 50.0% (ES = 0.98) during the 72-hour recovery period. Considering that irisin reduces the activity of ROS-producing processes [291], downregulating ROS-NLRP3 inflammasome signaling [294], and that aerobic exercise-induced irisin partly mediates amelioration of oxidative stress and cell apoptosis in mice skeletal muscle [351], we suggest that increased circulating irisin during recovery period is a counteracting mechanism to reduce EIMD and oxidative stress. Further, the findings showed statistically large and positive correlations between irisin, and both OSI and sMtCK. Hence, EIMD and oxidative processes might be a trigger for irisin synthesis to counteract muscle cell damage.

Inter-individual heterogeneity in hamstring muscle recovery

The LR individuals showed no changes in blood markers of muscle damage/oxidative stress, nor in muscle function during recovery period. This between-group heterogeneity might be related to endogenous factors (e.g., age, training status) and/or exogenous factors (e.g., volume, intensity, and type of exercise) [73]. In this study, all the individuals were young, healthy, physically active, and with no previous experience in eccentric exercising, in addition to undergoing the same standardized training. Therefore, it is assumed that the response heterogeneity is not attributable to these factors. However, inter-individual variations in response to exercise are in most cases due to genetics and/or dietary intake [330].

Physical exercise together with protein intake modulates muscle protein synthesis, as a result of increased bioavailability of exogenous essential amino acids [125]. As a result, upregulated muscle protein synthesis during the recovery period after acute endurance exercise promotes muscle adaptation and structural protein repair, facilitating force-generating capacity recovery [128]. Other recent applied studies have shown that increasing protein intake enhances functional recovery after sprinting training [130]. In this way, our findings showed that both total protein intake and animal-based protein intake were moderately and inversely correlated with EIMD markers. Thus, we suggest that increased daily protein intake during the peri-exercise days might protect LR individuals. Finally, Vit D intake and/or 25OHD serum levels also play a protective role on mitochondrial membranes [106]. However, our results indicated no association between muscle recovery and Vit D intake or 25OHD levels. This may be attributable to the fact that the majority of individuals presented Vit D levels above the insufficiency threshold ($>20.0 \text{ ng}\cdot\text{mL}^{-1}$), so it would hardly be possible to detect potential associations even if they existed.

Limitations

The study also presented some limitations to highlight when interpreting the results. First, while a priori computed sample size was sufficient to achieve the target statistical power, the number of participants was lowered when splitting into groups according to the post-hoc analysis criteria of *Paulsen et al.*, [73]. However, the use of this classification accounts for inter-individual variations, and provides a better presentation and interpretation of the response to exercise-induced residual fatigue [73]. Finally, neuromuscular and biochemical markers of residual fatigue were affected during the 72-hour recovery period after the repeated-sprint training in HR individuals. Consequently, further research conducting additional blood and muscle function measurements (e.g., +96 hours, until complete recovery) are required to better understand the potential implications of repeated-sprint training on hamstring muscles recovery.

CONCLUSION

There is inter-individual variability (i.e., high and low responders), in the hamstring muscles response to a sport-specific repeated-sprint training, identifiable by MVIC torque assessment through the $90^{\circ}_{hip}:20^{\circ}_{knee}$ *IPC test*, which is a powerful indirect test to monitor hamstring muscle recovery in both women and men, in a cost-effective way. However, although female responders showed impaired muscle structure and function, the *Jurdan test* may not show such impairment in sportswomen after a sport-specific repeated-sprint training. Loss of muscle function is associated with impaired mitochondria, triggering an increase in oxidative stress status and circulating irisin in both HR females and males. Finally, muscle function remains unrecovered during the 72-hour recovery period, revealing that 3 days will not be adequate time to restore hamstrings structure and function after a sport-specific repeated-sprint training in both female and male responders.

5. STUDY II. Football (soccer) match-derived hamstring muscles residual fatigue can be monitored using early rate of force development

Football (soccer) match-derived hamstring muscles residual fatigue can be monitored using early rate of force development

ABSTRACT

Background: Hamstring muscles play a critical role in multiple sprint-based sports, given their significant involvement in horizontal ground reaction force production during sprint biomechanics. Therefore, the research objective was to determine football (soccer) match-induced hamstrings residual fatigue and recovery through maximal voluntary isometric contraction (MVIC) torque, rate of force development (RFD) and associated biochemical parameters.

Methods: The recovery kinetics of MVIC torque and RFD, together with structural and oxidative damage biomarkers, were assessed in 19 male, regional first-division soccer players, before the soccer match (MD) and post-24 hours (MD₊₁), post-48 hours (MD₊₂) and post-72 hours (MD₊₃) through a repeated measures design.

Results: Early RFD to 50 milliseconds ($p < 0.001$) and to 100 milliseconds ($p < 0.001$) remained unrecovered on MD₊₃ in both lower limbs. However, hamstrings MVIC torque of the dominant and non-dominant lower limb was unrecovered on MD₊₂ ($p = 0.004$ and $p = 0.002$, respectively), and quasi-significant unrecovered on MD₊₃ ($p = 0.057$ and $p = 0.070$, respectively). Further, neuromuscular deficits were coupled with structural and oxidative damage biomarkers.

Conclusion: Early RFD₀₋₅₀ and RFD₀₋₁₀₀ monitoring, through the $90^{\circ}_{hip} \cdot 20^{\circ}_{knee}$ IPC test, is a cost-effective method for assessing soccer match-induced hamstring muscles residual fatigue and recovery, in order to comprehensively balance muscle recovery with optimizing training. Based in the findings, soccer match-induced hamstrings residual fatigue is not recovered within a three-day recovery period.

Keywords: exercise-induced muscle damage, force-generating capacity, hamstring injury, muscle recovery, sprint-based sports.

INTRODUCTION

Soccer is a team sport in which different motor behaviors are interspersed (e.g., repeated sprints, changes of direction) [17]. GPS-based devices have provided information that the TD covered by soccer players exceeds 10,000 meters [169] and HSRd exceeds 400 meters [334]. Furthermore, high-intensity efforts (e.g., HSRd, number of high-intensity sprints) have been significantly increasing during the last seasons of the *Spanish LaLiga* in all playing positions by 6.1% and 11.3%, respectively [169]. Consequently, HSI showed a two-fold increase over the last 20 years, predominantly located in the *biceps femoris* (4 out of every 5 HSI), due to repeated sprinting in soccer matches every 72-96 hours [163].

High-speed running is recurrent during field team sports (e.g., soccer) [16,17], and a key determinant in match-winning (scoring) actions [18,19]. Hamstring muscles play a key role during high-speed running or sprinting, as it has been shown that there is a relationship between horizontal ground reaction force production and both hamstrings eccentric torque and electromyographic activity during the late swing phase of the running gait cycle [171]. During the late swing phase, there is an extensive hip flexion with reduced knee flexion leading to a maximum elongation of the hamstring muscle-tendon unit, while simultaneously undergoing an active lengthening eccentric hamstrings contraction [20]. Eccentric contraction-derived muscle strains during repeated late swing phases of the running gait cycle are the main mechanisms contributing to HSI [21–23]. In particular, the BF_{LH} has been found to be the most important hamstring muscle during the late swing phase, in order to decelerate the shaft before ground contact [155,173]. The reason is that the lower limbs positioning during the late swing phase causes a greater BF_{LH} stretching and moment arm [20], electromyographic activity [155] and musculotendinous strains [154], compared to the ST or SM muscles, and explains why *biceps femoris* strains are the most common HSI in sprint-based team sports in both male and female athletes [158]. Hence, high-intensity eccentric contractions during repeated sprints result in ultrastructural damage to hamstring muscles tissue, and subsequently, long-lasting reductions in hamstring muscles force-generating capacity owing to myofibrillar disruptions and/or excitation-contraction uncoupling [24,173].

To monitor hamstring muscles recovery is a key concern for practitioners in order to both decrease HSI risk and improve performance, as it has been shown that re-exercising while not fully recovered aggravates impairments in neuromuscular function [25], which significantly increases HSI risk [14]. It has been estimated that the time lost due to HSI is 14 to 35 days [158] and the economic cost in a professional player can reach 500,000 euros [162]. The primary approach for assessing muscle recovery is muscle histology and immunohistochemistry. Nevertheless, the use

of muscle biopsies has been discontinued because of its underlying concerns: (i) the invasiveness of collecting the muscle tissue sample, (ii) the damage and/or inflammation generated by the technique itself, and (iii) the evaluation of an entire muscle through a microscopic sample [6,24]. Instead, force-generating capacity loss is strongly correlated ($r = 0.89$) with the fraction of muscle fibers presenting ultrastructural alterations [47], and it is considered the most reliable indicator of muscle damage and recovery [6,73,176,194]. Hamstring muscles force-generating capacity has been commonly assessed using IPC supine tests [206,207], which has been shown to be valid and reliable for monitoring hamstrings force-generating capacity [209]. In fact, there are studies showing both residual fatigue [358] and no changes [208] in hamstrings MVIC after a soccer match. However, considering that lower limb positioning near to hip flexion (i.e., 90°) and knee extension (i.e., $<30^\circ$) selectively activates the *biceps femoris* [143], triggering the peak in the length-tension relationship [144], and the peak electromyographical activity [143], *Matinlauri et al.*, [211] designed a new standing IPC test ($90^\circ_{hip}:20^\circ_{knee}$ IPC test) which has been found to be effective for monitoring hamstring-related residual fatigue after sprint-based exercises [**Study I**].

Sprinting is a fast stretch-shortening cycle exercise (<150 milliseconds of ground contact during sprinting) [171]. More specifically, hamstring muscles are required to apply rapid force (i.e., <100 milliseconds) to decelerate the shaft during the late swing phase of the running gait cycle [155,173,218], which is the phase where the injury-related mechanism of HSI occurs [21–23]. Therefore, it has been recommended to monitor the RFD during post-exercise recovery period [217], in order to identify hamstrings neuromuscular function deficits that increases HSI risk [218]. Interestingly, *Bettariga et al.*, [359] recently demonstrated that there is a significant decrease in hamstrings RFD immediately after a repeated sprint ability protocol with changes of direction, while *Grazioli et al.*, [360] proved that RFD decrements immediately after a professional soccer match are greater than MVIC torque decrements of the hamstring muscles. However, to the best of our knowledge, no prior studies have monitored hamstrings RFD during the post-soccer match 72-hour recovery period.

Accordingly, monitoring hamstring muscles until 72 hours post-soccer match is a key concern for practitioners in order to balance optimizing training with muscle recovery and decreasing HSI risk, requiring time- and cost-effective hamstring-specific tests. Therefore, the purpose of the research was to monitor soccer match-induced hamstrings residual fatigue and recovery through RFD using a hamstring-specific neuromuscular test (i.e., $90^\circ_{hip}:20^\circ_{knee}$ IPC test), together with associated serum biomarkers recovery kinetics.

MATERIALS AND METHODS

Participants

Nineteen male, regional first-division soccer players (age = 20.9 ± 2.0 years, mass = 72.6 ± 11.9 kg, height = 175.9 ± 6.9 cm [mean $\pm$ SD]) accepted to voluntarily participate in the study. Exclusion criteria were lower limb myotendinous injuries within 6 months prior to the experiment. In addition, players were required to avoid any type of physical exercise and/or training in the three days prior to and during the experiment. All participants gave their written consent to participate in the study and confirmed that they received information on the experimental approach and associated risks. The experiment was conducted in accordance with the code of ethics of the World Medical Association (Declaration of Helsinki) and was approved by the Ethics Committee for Clinical Research of the Catalan Sports Council (Generalitat de Catalunya) (037/CEICGC/2021).

Experimental approach

The extent and time course of hamstring-specific neuromuscular function and serum biomarkers were assessed before a soccer match (MD) and post-24 hours (MD₊₁), post-48 hours (MD₊₂) and post-72 hours (MD₊₃) through a single-group repeated measures design (**Figure 5.1**).

	MD-7	MD-1	Match Day		MD+1	MD+2	MD+3
	-168h	-24h	Pre	Soccer Match	+24h	+48h	+72h
Neuromuscular tests (MVIC torque, RFD, ROM, PS, SMP)	Familiarization		↓	GPS-based device TD > 10,000 m HSRd > 400 m Acc > 30 Dec > 30	↓	↓	↓
Serum markers (EIMD, OS, Irisin)			↓		↓	↓	↓
Dietary intake		↓	↓			↓	

Figure 5.1. Schematic overview showing the experimental approach. **Acc**, accelerations; **Dec**, decelerations; **EIMD**, exercise-induced muscle damage; **GPS**, global positioning system; **HSRd**, high-speed running distance; **MD**, match day; **MVIC**, maximal voluntary isometric contraction; **OS**, oxidative stress; **PS**, perceived soreness; **RFD**, rate of force development; **ROM**, range of motion; **SMP**, sprint mechanical properties; **TD**, total distance.

Participants were real-time monitored during an outfield soccer match (under current *FIFA laws of the game*) by means of validated *WIMUTM* inertial devices (RealTrack Systems, Almeria, Spain) [361]. The following thresholds were simultaneously monitored during the soccer match [169,334,335]: TD > 10,000 meters; HSRd > 400 meters running over 21 km·h⁻¹; Acc > 30 over

$3 \text{ m}\cdot\text{s}^{-2}$; Dec > 30 over $3 \text{ m}\cdot\text{s}^{-2}$. The external load thresholds are similar between professional and amateur soccer players [336]. In the event of any volunteer failing to reach the target thresholds, extra loading was immediately conducted at the end of the soccer match. Additionally, the players reported their 0 to 10-point Borg rate of perceived exertion (RPE) after 30 minutes of the end of the soccer match. Participants completed the familiarization one week before the start of the experimental procedure, by repeating the neuromuscular tests until they were technically correct.

Hamstring-specific neuromuscular tests

Force-generating capacity

Hamstrings MVIC torque was measured unilaterally by the $90^\circ_{\text{hip}}:20^\circ_{\text{knee}}$ IPC test [211]. Briefly, participants were asked to apply force “as fast and hard as possible” to a rigid custom-built support attached to a strain gauge (Chronojump, Barcelona, Spain), and completed three trials of the test (3 seconds MVIC), with a 30-seconds rest between trials. The used strain gauge is valid and reliable for MVIC and RFD assessment [338]. Then, MVIC was recorded as the 1-second window with the highest average force in the plateau of the signal, and the residual force resulting from the mass of the tested limb at rest was then subtracted. The best of the three trials was recorded for further analyses.

In addition, time-interval RFD to 50 milliseconds (RFD_{0-50}), to 100 milliseconds (RFD_{0-100}), to 250 milliseconds (RFD_{0-250}), and peak RFD (RFD_{max}), that is the steepest part of the curve, were calculated. According to the recommendations of *Maffiuletti et al.*, [215], (i) the recording rigid custom-built strain gauge provides a low baseline noise amplitude (i.e., 0.148% MVIC torque); (ii) the contraction onset was determined through a systematic manual-based method at the moment of “the last trough before force deflects above the range of the baseline noise”; (iii) no filtering or signal smoothing was applied; and (iv) average RFD of the three best trials was retained for further analysis.

Range of motion

The interaction between hip flexor and hamstring flexibility, measured as the differential angle between the actively lengthened leg shin angle and the opposite leg passive thigh angle was measured by the *Jurdan test* [233]. The test was photographically registered in duplicate by a *SONY DSC-RX100M5A* placed on a tripod located at the height of the table and three meters from the center of the hip of the participant. The sports kinematics analysis software *Kinovea v0.8.24* was used to compute the composite angle derived from 2D shin and thigh angles (relative to the horizontal).

Perceived soreness

Perceived soreness of the hamstring muscles was recorded using an 11-point NRS, in the relaxed position of the $90^{\circ}_{\text{hip}}:20^{\circ}_{\text{knee}}$ *IPC* test. The scale was supported by pain descriptors, ranging from "0" for "no pain" to "10" for "unbearable pain" [240].

Sprint performance and mechanical properties

The linear velocity sensor *RaceAnalyzer* (Chronojump, Barcelona, Spain) was located on a 1-meter-high tripod behind the participants. Participants were equipped with a belt attached to the linear velocity sensor and were strongly verbally encouraged during the test. Then, instantaneous velocity over 40 meter-sprints was recorded and post hoc analyzed using Chronojump software v2.1.2-2 (Chronojump, Barcelona, Spain) to compute sprint F-V spectra using the validated method described in detail elsewhere [341]. Individual linear F-V relationships were then extrapolated to calculate $F_{0\text{rel}}$ and V_0 .

Blood sampling and processing

Blood samples were collected from an antecubital vein and then were clotted at room temperature for 30 minutes in an *SST II Advance tube* (Becton Dickinson Vacutainer Systems, UK). Subsequently, samples were centrifuged at 4,000 g for 8 minutes at 4°C. After separation of the serum, aliquots were stored at -80 °C until analysis. Measurement of CK, ALT and AST activity were conducted on an *Advia 2400 automated device* (Siemens™ Medical Solutions Diagnostics, Tarrytown, NY, USA) following the International Federation of Clinical Chemistry's reference procedures [342]. sMtCK was measured by ELISA kit #*SEC386Hu* (Cloud Clone Corp., Houston, TX, USA) according to manufacturer's instructions. MDA was measured by kit #*E-EL-0060* (Elabscience, Wuhan, China). Further, the oxidizing material in the samples was quantified using the Erel approach [266], based on the oxidation of ferrous iron to ferric iron #*E-BC-K802-M* (Elabscience, Wuhan, China). TAS #*E-BC-K801-M* (Elabscience, Wuhan, China) was measured using the 2,2'-azino-di-3-ethylbenzthiazoline sulfonate (ABTS)+ method [265]. Then, OSI was then calculated as the TOS to TAS ratio. The previously validated kit against mass spectrometry #*EK-067-29* (Phoenix Pharmaceuticals Inc., Burlingame, CA, USA) was used to quantify circulating irisin [319].

Dietary Intake Record

The players were instructed on the procedures for recording their daily dietary intakes for three days (MD_{-1} , MD, and MD_{+2}), and were also provided with descriptors of commonly used household measurements (e.g., dessert spoon) and quantity measures (e.g., grams, milliliters), in order to verify that nutritional intake was homogeneous and within normal ranges for each macro-

and micronutrient. Then, the diet of each participant was calculated as the 3-day average by using the dietary analysis software *PCN Pro v1*. (Universitat de Barcelona, Barcelona, Spain) [346], which is based on and updated with a Spanish food composition database [347]. The volunteers were also provided with a standardized meal (200 grams of pasta and 150 grams of roasted chicken) two hours before the soccer match.

Statistical analysis

First, sample size was computed a priori using G*Power software v3.1.9.6 based on MVIC change ($90^{\circ}_{hip}; 20^{\circ}_{knee}$ IPC test) of the study by *Matinlauri et al.*, [211]. A total of 19 participants were calculated to be included given an effect size of 0.68, a power of 80% and a 5% alpha error.

Asymmetrically distributed variables were log- or square-transformed prior to analysis. A two-way repeated measures ANOVA (time [MD, MD₊₁, MD₊₂, MD₊₃] x limb [dominant, non-dominant]) was used to identify the effect of the soccer match on hamstrings MVIC torque, RFD ROM, and perceived soreness, while a one-way repeated measures ANOVA (time [MD, MD₊₁, MD₊₂, MD₊₃]) was used to identify soccer match-induced changes in sprint mechanical properties and serum biomarkers. Post-hoc Bonferroni-corrected paired t-tests were conducted to identify significant differences from baseline values. Effect sizes were calculated using Cohen's *d*, considering the following thresholds: (< 0.20), trivial; (0.20 < 0.59), small; (0.60 < 1.19), moderate; (1.20 < 1.99), large; and (> 2.0), very large [348]. The area under the curve was calculated by the trapezoidal method. Associations between areas under the curve were analyzed using Pearson's or Spearman correlation coefficient (according to normality) considering the following thresholds: (< 0.30), small; (0.30 < 0.51), moderate; (0.51 < 0.70), large; (0.71 < 0.90), very large; and (> 0.90), extremely large [348]. The ICC was used to assess the reliability of a single data collector that examines the degree of concordance of scores between repetitions or sessions. The ICC was calculated for average measures using a 2-way random effect model of absolute agreement. Thresholds for ICC are (0.00 < 0.50), poor; (0.51 < 0.75), moderate; (0.76 < 0.90), good; and (0.91 < 1.00), excellent [349]. Data are presented as mean $\pm$ SD and the level of significance was set at $p < 0.05$. Statistical analysis was performed with SPSS v.27.0.1.0 (SPSS Statistics, IBM Corp., Armonk, NY, USA).

RESULTS

Soccer match loading

The players reached the following load parameters during the soccer match: TD = $10,463 \pm 623$ meters; HSRd = 466 ± 257 meters; 45 ± 21 accelerations over $3m \cdot s^{-2}$ and 59 ± 24 decelerations over $3m \cdot s^{-2}$. The average 0 to 10-point Borg RPE was 8.7 ± 0.7 arbitrary units.

Neuromuscular biomarkers panel

Maximal voluntary isometric contraction (MVIC) torque. The ICC was excellent for both dominant and non-dominant lower limb with a value of 0.963 (95% CI 0.922 to 0.985) and 0.973 (95% CI 0.941 to 0.989), respectively. As shown in **Figure 5.2A**, there was MVIC torque loss in both dominant and non-dominant hamstrings until MD₊₂ [$F(3,54) = 8.891$, $p < 0.001$, $\eta_p^2 = 0.331$], and were quasi-significantly unrecovered on MD₊₃ ($p = 0.057$ and $p = 0.070$, respectively). There were no between-limbs differences in hamstrings MVIC torque loss during post-match recovery period [$F(3,54) = 0.350$, $p = 0.789$].

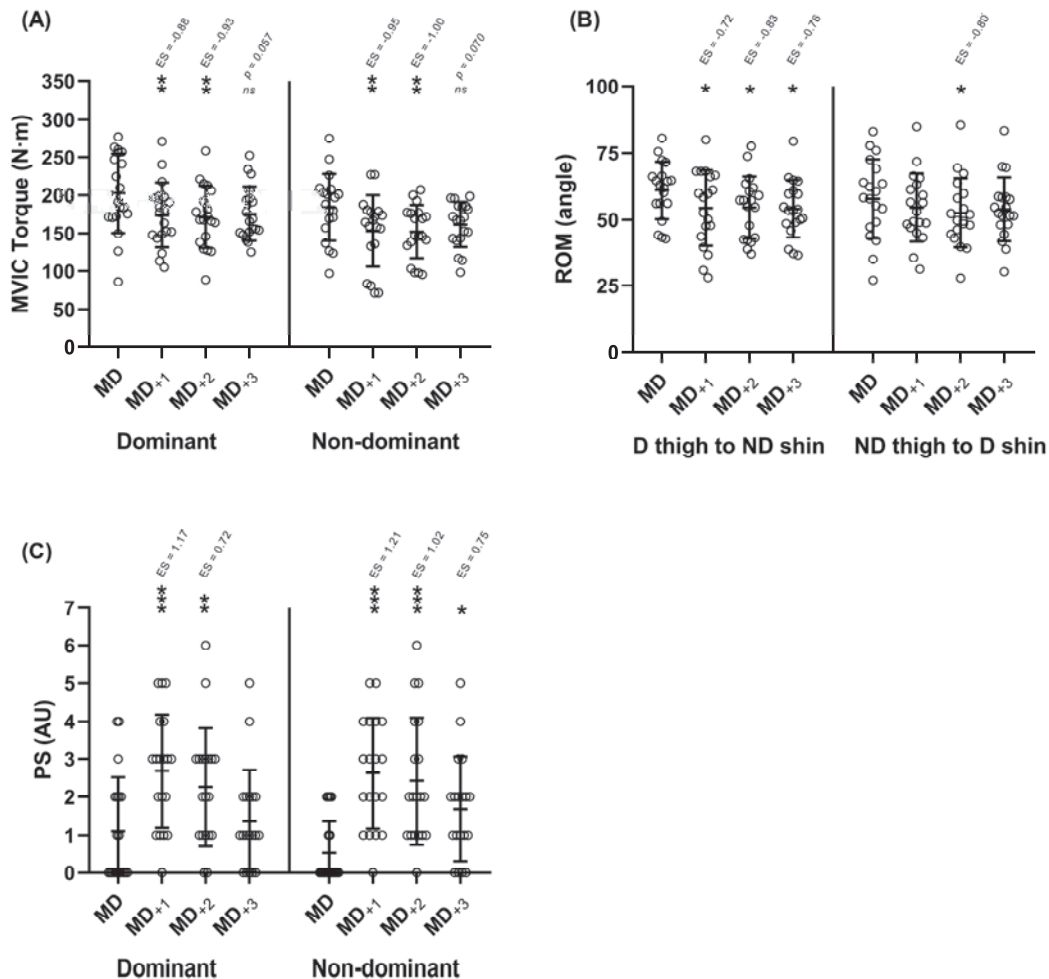


Figure 5.2. Individual, mean $\pm$ SD values of (A) maximal voluntary isometric contraction torque, (B) posterior chain range of motion, and (C) perceived soreness during post-soccer match recovery period. (*), (**), and (***) indicate a significant difference from the baseline value at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. **D**, dominant; **ES**, effect size; **MVIC**, maximal voluntary isometric contraction; **ND**, non-dominant; **ns**, non-significant; **PS**, perceived soreness; **ROM**, range of motion.

Rate of force development (RFD). Early RFD_{0-50} [$F(3,54) = 33.224$, $p < 0.001$, $\eta_p^2 = 0.649$] and RFD_{0-100} [$F(3,54) = 17.754$, $p < 0.001$, $\eta_p^2 = 0.497$] were decreased in both dominant and non-dominant hamstring muscles until MD_{+3} (**Figure 5.3**), while late RFD_{0-250} was decreased until MD_{+2} [$F(3,54) = 7.894$, $p < 0.001$, $\eta_p^2 = 0.305$]. RFD_{max} remained unchanged during the recovery period. There were no between-limbs differences in RFD_{0-50} , RFD_{0-100} , RFD_{0-250} and RFD_{max} during post-match recovery period.

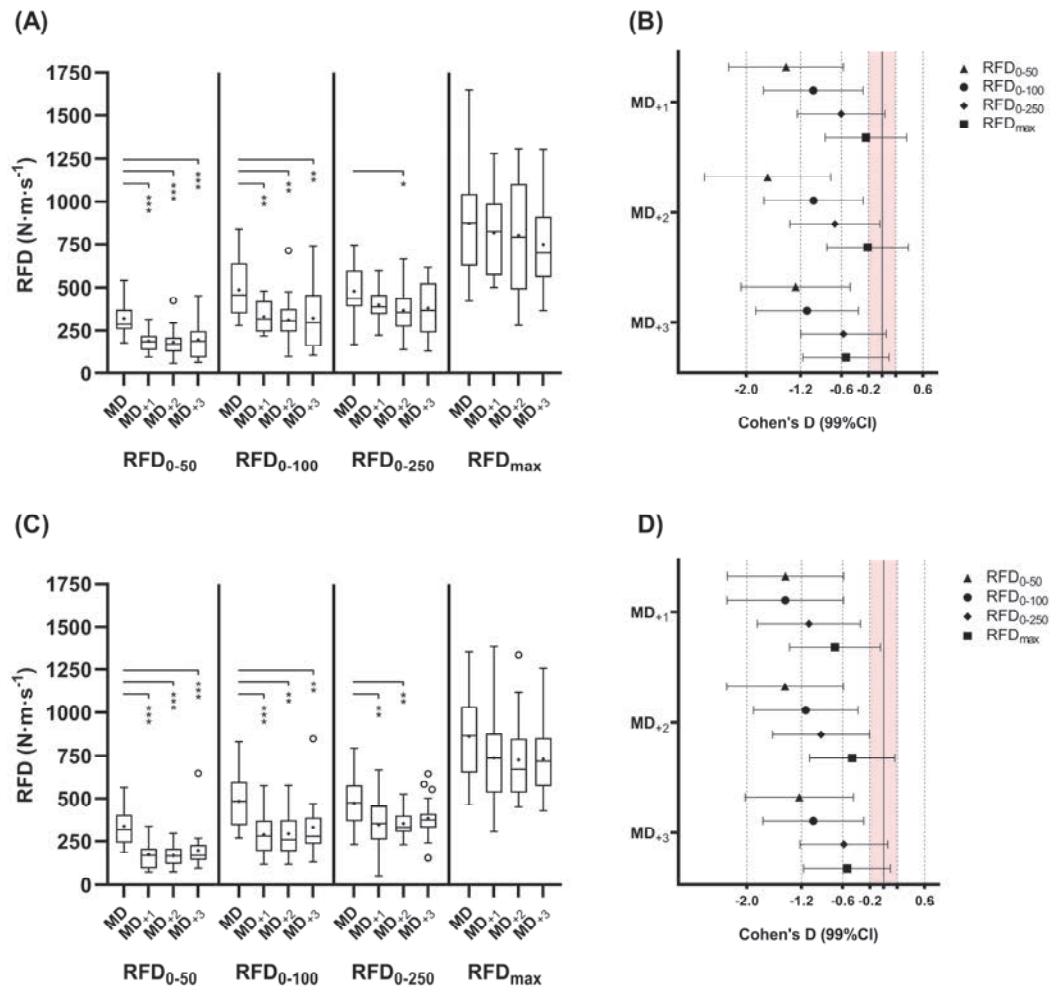


Figure 5.3. Tukey boxplot showing the rate of force development (RFD) during post-soccer match recovery period in (A) dominant and (C) non-dominant hamstring muscle. The upper to lower edge of the box contains the interquartile range of the data set, while “whiskers” indicate the minimum and maximum data values (outliers are illustrated as open dots). Rate of force development standardized differences comparing baseline values with post-24 hours (MD_{+1}), post-48 hours (MD_{+2}) and post-72 hours (MD_{+3}) in (B) dominant and (D) non-dominant hamstring muscle. Error bars indicate uncertainty in the true mean changes with a 99% confidence interval. (*), (**), and (***) indicate a significant difference from the baseline value at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. **MD**, match day; **RFD_{0-50}** , rate of force development (0-50ms); **RFD_{0-100}** , rate of force development (0-100ms); **RFD_{0-250}** , rate of force development (0-250ms); **RFD_{max}** , peak rate of force development.

An example of MVIC torque on MD and MD₊₃ is shown in **Figure 5.4A**, while **Figure 5.4B** shows baseline-torque recordings prior to contraction onset.

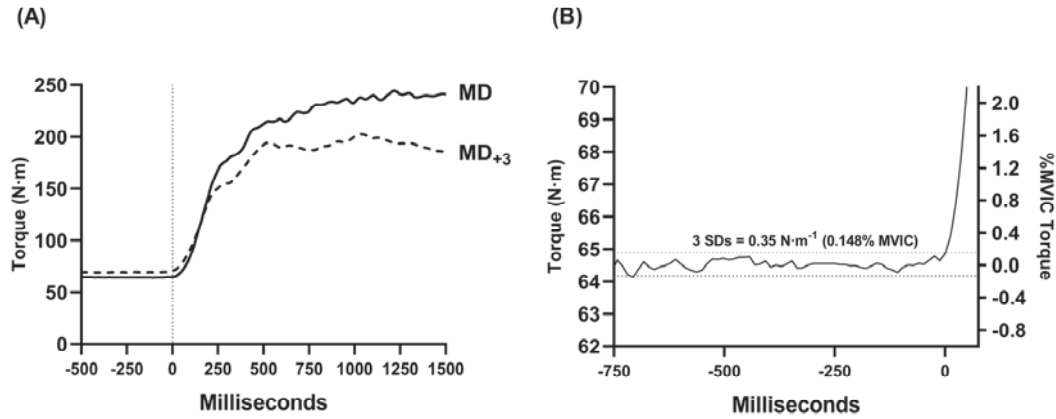


Figure 5.4. Panel A exemplifies the isometric torque output of volunteer 17, on MD and MD₊₃, while Panel B shows baseline-torque recordings prior to contraction onset. **MD**, match day; **SD**, standard deviation.

Range of motion (ROM). As shown in **Figure 5.2B**, dominant thigh to non-dominant shin ROM was decreased until MD₊₃, while non-dominant thigh to dominant shin ROM was only decreased on MD₊₂ [$F(3,54) = 6756$, $p = 0.001$, $\eta_p^2 = 0.273$].

Perceived soreness. As shown in **Figure 5.2C**, perceived soreness in the dominant hamstring was increased until MD₊₂ [$X^2(3) = 25.441$, $p < 0.001$], while perceived soreness in the non-dominant hamstring was further increased to MD₊₃ [$X^2(3) = 28.459$, $p < 0.001$].

Sprint performance and mechanical properties. Split time to 10 meters and total time to 40 meters were increased until MD₊₃ while V_0 , F_{0rel} , A_{max} and $P_{max,rel}$ were decreased until MD₊₃ (**Table 5.1**). Also, early RFD₀₋₅₀ loss is significantly correlated with loss of sprint performance, measured as total time to 40 meters ($r = 0.653$, $p = 0.002$; $R^2 = 0.426$, $p = 0.003$).

Table 5.1. Sprint performance and mechanical properties during the 72-hour recovery period.

Parameter		MD	MD ₊₁	MD ₊₂	MD ₊₃
Time to 10 m (s)	Mean $\pm$ SD	2.197 $\pm$ 0.100	2.295 $\pm$ 0.125*	2.291 $\pm$ 0.120	2.350 $\pm$ 0.148**
	% Δ (95% CI)		4.5 (1.7 to 7.2)	4.3 (1.6 to 6.9)	7.0 (3.7 to 10.2)
	ES (95% CI)		0.70 (0.19 to 1.20)	0.65 (0.14 to 1.13)	1.03 (0.46 to 1.58)
Time to 40 m (s)	Mean $\pm$ SD	5.945 $\pm$ 0.271	6.237 $\pm$ 0.286***	6.247 $\pm$ 0.282***	6.310 $\pm$ 0.353***
	% Δ (95% CI)		4.9 (2.6 to 7.2)	5.1 (2.8 to 7.4)	6.1 (3.3 to 9.0)
	ES (95% CI)		1.80 (1.05 to 2.53)	1.83 (1.08 to 2.57)	2.12 (1.29 to 2.93)
V ₀ (m·s ⁻¹)	Mean $\pm$ SD	8.647 $\pm$ 0.579	8.197 $\pm$ 0.522**	8.151 $\pm$ 0.528***	8.170 $\pm$ 0.665***
	% Δ (95% CI)		-5.2 (-8.1 to -2.3)	-5.7 (-8.7 to -2.8)	-5.5 (-9.2 to -1.8)
	ES (95% CI)		-1.07 (-0.49 to -1.63)	-1.47 (-0.80 to -2.11)	-1.63 (-0.93 to -2.32)
F _{0rel} (N·kg ⁻¹)	Mean $\pm$ SD	7.740 $\pm$ 0.718	7.133 $\pm$ 0.669*	7.215 $\pm$ 0.604*	7.087 $\pm$ 0.672**
	% Δ (95% CI)		-7.8 (-12.0 to -3.7)	-6.8 (-10.5 to -3.0)	-8.4 (-12.6 to -4.2)
	ES (95% CI)		-0.78 (-0.25 to -1.28)	-0.68 (-0.17 to -1.17)	-0.88 (-0.34 to -1.40)
A _{max} (m·s ⁻²)	Mean $\pm$ SD	7.820 $\pm$ 0.723	7.205 $\pm$ 0.673*	7.286 $\pm$ 0.608*	7.159 $\pm$ 0.674**
	% Δ (95% CI)		-7.9 (-12.0 to -3.7)	-6.8 (-10.6 to -3.1)	-8.5 (-12.6 to -4.3)
	ES (95% CI)		-0.79 (-0.26 to -1.30)	-0.69 (-0.18 to -1.19)	-0.89 (-0.35 to -1.42)
P _{max,rel} (w·kg ⁻¹)	Mean $\pm$ SD	16.654 $\pm$ 2.118	14.553 $\pm$ 1.877***	14.633 $\pm$ 1.654***	14.402 $\pm$ 1.839***
	% Δ (95% CI)		-12.6 (-18.0 to -7.2)	-12.1 (-16.9 to -7.3)	-13.5 (-18.8 to -8.2)
	ES (95% CI)		-1.53 (-0.85 to -2.19)	-1.33 (0.70 to 1.94)	-1.63 (-0.93 to 2.32)

Data are expressed as mean $\pm$ SD. (*), (**), and (***) indicate a significant difference from the baseline value at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. **95% CI**, 95% confidence interval; % Δ , delta percentage change; **A_{max}**, peak acceleration; **ES**, effect size; **F_{0rel}**, relative theoretical maximal horizontal force production; **MD**, match day; **P_{max,rel}**, relative peak power; **SD**, standard deviation; **V₀**, theoretical maximal running velocity.

Biochemical markers panel

CK activity [$F(3,48) = 22.673$, $p < 0.001$, $\eta_p^2 = 0.586$], CK/ALT ratio [$F(3,51) = 35.786$, $p < 0.001$, $\eta_p^2 = 0.678$], CK/AST ratio [$F(3,51) = 47.686$, $p < 0.001$, $\eta_p^2 = 0.737$] and sMtCK [$F(3,54) = 39.814$, $p < 0.001$, $\eta_p^2 = 0.689$] were increased until MD₊₃. Similarly, MDA [$F(3,54) = 15.139$, $p < 0.001$, $\eta_p^2 = 0.457$], OSI [$F(3,54) = 14.451$, $p < 0.001$, $\eta_p^2 = 0.445$] and circulating irisin [$F(3,54) = 16.300$, $p < 0.001$, $\eta_p^2 = 0.475$] were increased after the soccer match until MD₊₃ (**Table 5.2**).

Table 5.2. Soccer match-induced biochemical alterations during the 72-hour recovery period.

Marker		MD	MD ₊₁	MD ₊₂	MD ₊₃
CK (IU·L ⁻¹)	Mean ± SD	203.6 ± 100.3	1220.2 ± 799.2***	741.5 ± 488.1**	456.8 ± 218.6**
	%Δ (95% CI)		499.3 (297.5 to 701.2)	264.2 (141.0 to 387.5)	124.4 (69.2 to 179.6)
	ES (95% CI)		1.29 (0.63 to 1.93)	1.16 (0.53 to 1.77)	1.14 (0.51 to 1.74)
CK/ALT (AU)	Mean ± SD	26.2 ± 25.8	59.2 ± 33.5***	43.7 ± 21.1***	33.5 ± 15.9**
	%Δ (95% CI)		126.3 (62.6 to 190.0)	66.8 (26.8 to 106.8)	27.9 (-2.3 to 58.2)
	ES (95% CI)		1.61 (0.90 to 2.31)	1.41 (0.74 to 2.06)	0.96 (0.39 to 1.51)
CK/AST (AU)	Mean ± SD	12.4 ± 10.5	48.4 ± 34.0***	43.7 ± 21.1***	33.5 ± 15.9**
	%Δ (95% CI)		289.3 (153.4 to 425.2)	113.8 (53.1 to 174.5)	65.6 (20.9 to 110.4)
	ES (95% CI)		1.82 (1.05 to 2.57)	1.45 (0.77 to 2.11)	1.05 (0.46 to 1.62)
sMtCK (ng·ml ⁻¹)	Mean ± SD	18.5 ± 15.2	100.4 ± 48.9***	71.9 ± 32.4***	46.6 ± 26.5**
	%Δ (95% CI)		443.6 (316.0 to 571.2)	289.3 (204.8 to 373.9)	151.9 (82.7 to 221.1)
	ES (95% CI)		1.69 (0.97 to 2.39)	1.62 (0.92 to 2.30)	0.94 (0.39 to 1.48)
MDA (ng·ml ⁻¹)	Mean ± SD	836.8 ± 105.7	1197.7 ± 360.4**	1358.5 ± 364.5***	1384.8 ± 479.5**
	%Δ (95% CI)		43.1 (22.4 to 63.9)	62.3 (41.3 to 83.3)	65.5 (37.9 to 93.1)
	ES (95% CI)		1.06 (0.9 to 1.62)	1.40 (0.75 to 2.03)	1.15 (0.56 to 1.73)
OSI (AU)	Mean ± SD	0.656 ± 0.233	1.459 ± 0.696***	1.552 ± 0.471***	1.172 ± 0.281***
	%Δ (95% CI)		122.4 (71.3 to 173.5)	136.6 (102.0 to 171.2)	78.7 (58.0 to 99.3)
	ES (95% CI)		1.20 (0.59 to 1.78)	1.72 (0.99 to 2.42)	1.34 (0.70 to 1.95)
Irisin (ng·ml ⁻¹)	Mean ± SD	7.198 ± 1.223	9.410 ± 2.560**	10.186 ± 2.532***	10.505 ± 2.062***
	%Δ (95% CI)		30.7 (13.6 to 47.9)	41.5 (24.6 to 58.5)	45.9 (32.1 to 59.7)
	ES (95% CI)		0.88 (0.34 to 1.40)	1.39 (0.74 to 2.01)	1.61 (0.94 to 2.29)

Data are expressed as mean ± SD. (*), (**), and (***) indicate a significant difference from the baseline value at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. **95% CI**, 95% confidence interval; **%Δ**, delta percentage change; **AU**, arbitrary units; **CK**, creatine kinase; **CK/ALT**, CK to alanine aminotransferase (ALT) ratio; **CK/AST**, CK to aspartate aminotransferase (AST) ratio; **ES**, effect size; **IU**, international units; **MD**, match day; **MDA**, malondialdehyde; **OSI**, oxidative stress index; **SD**, standard deviation; **sMtCK**, sarcomeric mitochondrial creatine kinase.

Correlations between serum enzyme levels and maximal and long-lasting reductions in neuromuscular markers are shown **Table 5.3**.

Table 5.3. Correlation coefficient matrix between long-lasting reductions in neuromuscular markers, serum enzyme levels and soccer match-induced load.

	CK	sMCK	CK/ ALT	CK/ AST	MDA	OSI	IR	MVIC (D)	MVIC (ND)	RFD ₅₀ (D)	RFD ₅₀ (ND)	RFD ₁₀₀ (D)	RFD ₁₀₀ (ND)	RFD ₂₅₀ (D)	RFD ₂₅₀ (ND)	RFD _{max} (D)	RFD _{max} (ND)	ROM (D)	ROM (ND)	PS (D)	PS (ND)	TD	HSRd	
CK	–																							
sMCK	0.907*	–																						
CK/ ALT	0.882*	0.826*	–																					
CK/ AST	0.735*	0.672*	0.600*	–																				
MDA	-0.074	0.082	-0.090	-0.207	–																			
OSI	0.422	0.613*	0.526*	0.288	-0.389	–																		
IR	0.316	0.533*	0.279	0.725*	-0.351	0.397	–																	
MVIC (D)	-0.032	0.151	-0.281	0.241	-0.170	0.124	0.486*	–																
MVIC (ND)	-0.113	-0.155	-0.083	-0.026	-0.053	0.035	0.296	0.454	–															
RFD ₅₀ (D)	-0.081	-0.045	-0.110	0.195	-0.161	-0.140	0.484*	0.507*	0.200	–														
RFD ₅₀ (ND)	0.284	0.262	0.485*	0.238	-0.004	0.313	0.285	0.030	-0.090	0.290	–													
RFD ₁₀₀ (D)	0.213	0.294	0.258	0.377	-0.037	0.150	0.519*	0.564*	0.197	0.729*	0.448	–												
RFD ₁₀₀ (ND)	0.304	0.335	0.363	0.418	-0.065	0.258	0.467*	0.381	-0.178	0.736*	0.716*	0.716*	–											
RFD ₂₅₀ (D)	0.378	0.367	0.269	0.315	-0.032	0.210	0.275	0.581*	0.325	0.348	0.086	0.578*	0.519*	–										
RFD ₂₅₀ (ND)	0.096	0.268	0.199	0.179	0.119	0.222	0.385	0.565*	0.256	0.346	0.459*	0.607*	0.723*	0.736*	–									
RFD _{max} (D)	-0.086	0.053	0.074	0.176	0.059	0.187	0.225	0.597*	0.398	0.378	0.305	0.591*	0.533*	0.690*	0.821*	–								
RFD _{max} (ND)	0.444	0.329	0.205	0.365	-0.142	0.234	0.281	0.640*	0.155	0.448	0.000	0.603*	0.507*	0.802*	0.527*	0.571*	–							
ROM (D)	0.204	0.302	0.092	0.146	0.028	0.499*	0.422	0.171	0.297	-0.042	0.070	-0.035	-0.220	0.152	-0.130	-0.076	-0.063	–						
ROM (ND)	-0.069	0.176	-0.152	-0.024	0.083	0.274	0.322	0.293	0.327	-0.142	0.040	-0.159	-0.069	0.377	0.282	0.245	0.065	0.681*	–					
PS (D)	0.546*	0.278	0.485*	0.600**	-0.356	0.163	0.400	0.079	-0.061	0.331	0.418	0.199	0.327	0.185	0.046	-0.100	0.090	0.205	0.164	–				
PS (ND)	0.462	0.262	0.487*	0.467	-0.295	0.227	0.376	0.029	0.028	0.276	0.525*	0.194	0.320	0.176	0.152	-0.013	-0.023	0.257	0.212	0.885*	–			
TD	-0.025	-0.072	-0.067	-0.038	0.265	-0.330	0.193	0.010	0.370	0.233	0.016	0.196	0.077	0.393	0.352	0.122	0.148	0.037	0.328	0.238	0.318	–		
HSRd	0.110	0.177	0.236	0.009	0.042	0.188	-0.042	-0.046	0.086	-0.189	0.079	-0.037	0.000	0.335	0.270	0.143	-0.137	0.482*	0.549*	0.274	0.383	0.189	–	
Borg RPE	0.555*	0.367	0.432	0.265	0.048	0.054	-0.081	-0.157	0.080	0.123	0.213	0.150	0.040	0.316	0.052	-0.074	0.146	0.237	0.042	0.404	0.313	0.303	0.373	

(*) indicates a significant correlation at $p < 0.05$, **ALT**, alanine aminotransferase; **AST**, aspartate aminotransferase; **CK**, creatine kinase; **D**, dominant; **HSRd**, high-speed running distance; **IR**, irisin; **MDA**, malondialdehyde; **MVIC**, maximal voluntary isometric contraction; **ND**, non-dominant; **OSI**, oxidative stress index; **PS**, perceived soreness; **RFD₅₀**, rate of force development (0-50ms); **RFD₁₀₀**, rate of force development (0-100ms); **RFD₂₅₀**, rate of force development (0-250ms); **RFD_{max}**, peak rate of force development; **ROM**, range of motion; **RPE**, rate of perceived exertion; **sMCK**, sarcomeric mitochondrial CK; **TD**, total distance.

Dietary Intake

Macronutrient and micronutrient intakes during the peri-exercise days are summarized in **Table 5.4**.

Table 5.4. Descriptive summary of macro- and micronutrient intakes.

Parameter	Value
Total energy (kcal·day ⁻¹)	2261 ± 787
Total Protein Intake (g·Kg ⁻¹ ·day ⁻¹)	1.65 ± 0.69
Animal-based Protein Intake (g·Kg ⁻¹ ·day ⁻¹)	1.16 ± 0.53
Plant-based Protein Intake (g·Kg ⁻¹ ·day ⁻¹)	0.46 ± 0.19
Total carbohydrates (g·day ⁻¹)	219 ± 83
Total lipids (g·day ⁻¹)	101 ± 44
Water (g·day ⁻¹)	2456 ± 1167
Vit C (µg·day ⁻¹)	97.5 ± 87.9
Vit D (µg·day ⁻¹)	4.81 ± 3.73
Vit E (mg·day ⁻¹)	9.53 ± 5.71

Data are expressed as mean ± SD.

DISCUSSION

The recovery kinetics of hamstring-specific neuromuscular markers were monitored during 72 hours after a soccer match. The findings of the research highlight that (i) early RFD₀₋₅₀ and RFD₀₋₁₀₀ monitoring, through the 90°_{hip}:20°_{knee} IPC test, is a cost-effective method for assessing soccer match-induced hamstring muscles residual fatigue and recovery; (ii) loss of rapid force-generating capacity (i.e., early RFD) is accompanied by biomarkers of muscle damage and recovery; and (iii) 72 hours is not enough time to recover hamstring muscles after a soccer match.

Decreased hamstrings capacity to generate rapid and maximal force

Available scientific evidence is limited and contradictory on soccer match-derived hamstring muscles residual fatigue. So far, *Nedelec et al.*, [358] and *Constantine et al.*, [208] conducted supine IPC at 90° and 30° of knee flexion and found moderate decreases and complete recovery of hamstrings force-generating capacity at 72 hours post-soccer match, respectively. Similarly, *Wollin et al.*, [362] found recovered hamstrings force-generating capacity at 72 hours post-soccer match, evaluated through a prone knee flexion test. We report changes of hamstrings MVIC torque during the post-match recovery period, using the 90°_{hip}:20°_{knee} IPC test. Interestingly, a

significant decrease in both dominant and non-dominant hamstring MVIC torque was reported until MD₊₂ (ES = -0.93 and ES = -1.00, respectively), while quasi-significant MVIC torque loss was shown on MD₊₃ (p = 0.057 and p = 0.070, respectively). Reduced performance in the 90°_{hip}:20°_{knee} IPC test accounts for a loss of hamstrings force-generating capacity, owing to repeated eccentric lengthening contractions-induced non-overlapping sarcomeres and failures in the excitation-contraction coupling process, leading to myofibrillar damage and membrane permeability [24,47,211]. Specifically, since the 90°_{hip}:20°_{knee} IPC test design is similar to the late swing phase of the running gait cycle, the hamstrings are in a lengthened position (large hip flexion and small knee flexion), so the *biceps femoris* displays a greater hip extension than knee flexion moment arm, compared to ST and SM [20,211]. This positioning of the lower limb during the 90°_{hip}:20°_{knee} IPC test selectively activates the *biceps femoris* [143], triggering the peak in the length-tension relationship [144], and the peak electromyographical activity [143]. Therefore, loss of performance in the 90°_{hip}:20°_{knee} IPC test is suggestive of hamstrings (and most likely the *biceps femoris*) residual fatigue.

Besides, RFD is a useful complement to the classic MVIC assessment to evaluate neuromuscular residual fatigue and muscle damage [217,223]. However, to our knowledge, the use of hamstrings RFD monitoring during the 72-hour recovery period after a soccer match is described for the first time. Interestingly, both dominant and non-dominant hamstring muscles showed significant decreases in early RFD₀₋₅₀ (ES = -1.27 and ES = -1.21, respectively) and RFD₀₋₁₀₀ (ES = -1.08 and ES = -1.01, respectively) until MD₊₃, whereas RFD_{max} remained unchanged. Compared to MVIC torque assessment, there were longer-lasting decreases in early RFD. In addition, there were greater decreases in early RFD than late RFD, supporting the findings of a recent comprehensive review [217]. The determinant of the force rise time during an isometric contraction are the magnitude and shortening velocity of the muscle, which depends on two simultaneous muscle physiological processes and properties [219]. First, 60% of the time to reach 50% MVIC (i.e., early RFD), is mediated by activation processes, including both Ca²⁺ release, diffusion, and binding, as well as changes in filament organization to allow cross-bridge binding, while the remaining 40% of the time is influenced by the compliance of muscle elastic components [219]. Fast-twitch (type-II) fibers are correlated to early RFD, as a consequence of increased motor unit discharge rates and greater Ca²⁺ release per action potential, which allows faster cross-bridge contraction cycles [215]. However, type-II fibers are also more susceptible to be damaged after eccentric exercising [220]. Eccentric contraction-induced muscle strains during repeated late swing phases of the running gait cycle lead to the disruption of elastic components (e.g., muscle membranes), causing sarcolemma permeability affecting Ca²⁺ homeostasis and, consequently, delays in muscle activation processes (e.g., excitation-contraction coupling process

failures) [24]. Thus, soccer match-induced long-lasting early RFD reduction in the hamstring muscles likely result from (i) excitation-contraction uncoupling as a result of loss of Ca^{2+} homeostasis and (ii) reduced stiffness of cellular force transmission associated with impaired myocyte integrity.

Accordingly, elastic components and structural damage in the volunteers were evidenced by an increase in CK activity until MD₊₃ (ES = 1.14). In addition, elevated CK/ALT (ES = 0.96) and CK/AST ratios (ES = 1.05) until MD₊₃, together with CK activity, is an indicative of a disruption in cellular and sarcomeres integrity, as well as permeability of the sarcolemma [24,253]. Sarcolemma impairment-derived Ca^{2+} accumulation disrupts the mitochondrial matrix, finally resulting in rupture of the mitochondrial outer membrane and intermembrane proteins efflux (i.e., sMtCK) [55]. In this sense, sMtCK, a muscle mitochondria damage marker that is increased after sprint-based exercises [**Study I**], was also increased in the volunteers until MD₊₃ (ES = 0.94). Impaired mitochondria-derived free radicals release to the myocyte and bloodstream increases oxidative by-products (e.g., MDA). In this regard, there were moderate increases in MDA until MD₊₃ (ES = 1.15). Coupled with increases in CK activity, MDA further supports sarcolemma impairment and protein leakage into the bloodstream [363]. Therefore, oxidative stress was significantly upregulated during the post-soccer match recovery period until MD₊₃ (ES = 1.34). Furthermore, oxidative stress was correlated with damage to muscle mitochondria ($r = 0.61$), likely explained given the significant implication of the muscle mitochondria in the efflux of free radicals into the bloodstream after damaging exercises [55]. The aforementioned mechanical and oxidative damage to myocyte and mitochondria integrity leads to an upregulation of the signaling pathway for irisin cleavage [274]. The myokine irisin provides protection against inflammation, oxidative and apoptosis processes [282], but also plays a key role in musculoskeletal disorders by upregulating the expression of pro-myogenic factors, stimulating satellite cell activation and ameliorating protein degradation [302]. Accordingly, the findings of the present study revealed a large increase ranging from 32.1% to 59.7% in circulating irisin on MD₊₃ (ES = 1.65), supporting the previous results after sprint-based exercises [**Study I**]. Indeed, the findings showed significant moderate-to-strong correlations between irisin and CK/AST ratio ($r = 0.73$), damage to muscle mitochondria ($r = 0.53$), MVIC torque loss ($r = 0.49$), RFD₀₋₅₀ loss ($r = 0.48$) and RFD₀₋₁₀₀ loss ($r = 0.52$). Thereby, EIMD might trigger irisin synthesis to regulate signaling processes during muscle recovery.

Taken together, serum biomarkers upregulation support the loss of cellular integrity, and consequently, a reduced stiffness of cellular force transmission which is likely might explain the long-lasting early RFD decrements of the hamstring muscles [223].

Early RFD is also reported to be correlated with sprint times [364]. In this sense, our results revealed increases in sprint times to 10 and 40 meters until MD₊₃ (ES = 1.03 and ES = 2.12, respectively). This decreased performance is attributed to a reduced horizontal force-generating capacity at low (F_{0rel}) and high velocities (V_0) until MD₊₃ (ES = -0.88 and ES = -1.63, respectively). During sprinting, forward orienting the ground reaction force is a critical determinant during the acceleration phase, mainly mediated by the hamstring muscles [171]. Accordingly, extrapolation of individual linear F-V relationships showed unrecovered A_{max} on MD₊₃ (ES = -0.89). Furthermore, in agreement with *Ishøi et al.*, [365], our results also showed a significant association between decrements in early RFD and sprint performance. Based on these findings, both sprinting horizontal force production (i.e., F_{0rel}) and isolated hamstring rapid force-generating capacity (i.e., RFD₀₋₅₀ and RFD₀₋₁₀₀) are long-lastingly reduced after a soccer match during at least three days, leading to reduced performance and increased HSI risk [14,366,367]. We therefore recommend the use of the $90^{\circ}_{hip}:20^{\circ}_{knee}$ IPC test, which is easy, fast, and cost-sensitive to monitor the recovery kinetics of MVIC torque and, especially, early RFD during soccer match-induced hamstring muscles residual fatigue.

Finally, there is limited evidence of hip and knee ROM response to a soccer match apart from *Wollin et al.*, [362], who found no transient changes in lower limb ROM, when measured by the modified *Thomas test*. In this study we found a significant moderate decrease of the composite angle by the interaction between hip flexor and contralateral hamstring flexibility by the *Jurdan test* during the 72 hours of recovery. Considering that the *Jurdan test* is an indicator of increased passive tension, and in contrast to other ROM screens, the *Jurdan test* is designed closely related to running biomechanics [233], ROM loss reported in this study is likely derived from damage to the connective tissue of the iliopsoas and/or hamstring muscles. *Wollin et al.*, [362] have also reported increases in perceived soreness until MD₊₁, while our results showed moderate increases in perceived soreness until MD₊₃ in the $90^{\circ}_{hip}:20^{\circ}_{knee}$ IPC test position, indicating damage to muscle connective tissue.

Are hamstring muscles recovered within 72 hours after a soccer match?

Silva et al., [368] reviewed the recovery kinetics of biochemical and functional markers combining both real soccer matches and soccer match-simulation protocols. Soccer match-simulation protocols are under criticism for not accurately replicating the neuro-mechanical loading and physiological alterations of a real soccer match. In this sense, the authors found that several markers were less affected by soccer match-simulation protocols, although it was concluded that muscle function is not recovered within 72 hours after a real soccer match [368]. These findings were based on the combination of studies that assessed several muscle groups and

tests, and furthermore, few references to 72-hour post-soccer match monitoring. Particularly, compared to immediately after the match, the frequency of biochemical markers evaluated at 24-, 48- and 72-hours post-soccer match were low (58.6%, 35.4% and 19.6%, respectively), whereas less than 5% of the included studies monitored hamstring muscles force-generating capacity until 72 hours [368]. While there is an International Olympic Committee consensus to intersperse soccer matches by at least 96 hours [369], the governing sports authorities and professional leagues continue to schedule games every 72 hours [370]. Overall, combining the findings hereby presented on decreased hamstring muscles capacity to generate rapid and maximal force (early RFD, MVIC torque, and sprint horizontal force production), together with sustained biochemical responses until MD₊₃, we postulate that a soccer match impairs hamstring muscles for at least 72 hours. Based in the findings, 72 hours is not adequate time to recover soccer match-induced hamstring muscles residual fatigue. Instead, we recommend the use of the $90^{\circ}_{hip}:20^{\circ}_{knee}$ IPC test, which is easy, fast, and cost-sensitive to monitor the recovery kinetics of force-generating capacity and, especially, early RFD of the hamstring muscles after multiple sprints-based sports, in order to balance optimizing training and muscle recovery to decrease the HSI risk.

Limitations

The study also presented some limitations to highlight when interpreting the results. First, although the used strain gauge provides a low baseline noise amplitude and is reliable for RFD recording and analysis [338], the load cell used did not provide a 1,000 Hz sampling frequency, as recommended by *Maffiuletti et al.*, [215]. However, it has been recently demonstrated that lowering the sampling frequency (e.g., from 1,000 Hz to 100 Hz) increases the absolute reliability and reduces the coefficient of variability during unilateral IPC tests (i.e., $90^{\circ}_{hip}:20^{\circ}_{knee}$ IPC test) [218]. In addition, given that early RFD together with biochemical assessments were affected during the 72-hour recovery period after the soccer match, further research conducting additional biochemical and RFD measurements (e.g., +96h, until complete recovery) are warranted to comprehensively understand soccer match-induced hamstrings residual fatigue and recovery.

CONCLUSION

The assessment of early RFD₀₋₅₀ and RFD₀₋₁₀₀ through the $90^{\circ}_{hip}:20^{\circ}_{knee}$ IPC test is a cost-effective screening tool to monitor muscle recovery during soccer match-induced hamstring muscles residual fatigue. Overall, hamstring muscles recovery is not fully completed within three days after a soccer match, owing to neuromuscular alterations associated with structural and oxidative damage biomarkers.

6. STUDY III. Individual and region-specific hamstring muscles use during a novel *Flywheel Russian belt Deadlift* exercise

Individual and region-specific hamstring muscles use during a novel *Flywheel Russian belt Deadlift* exercise

ABSTRACT

Background: Hamstring strain injuries (HSI) are prevalent in sprint-based team sports. However, despite the implementation of specific exercises to reduce HSI risk, the injury incidence has remained unchanged over the past 30 years. Therefore, the purpose of the study was to analyze hamstrings muscle activation induced by a novel *Flywheel Russian belt Deadlift* exercise, together with individual muscle-, region- and limb-specific differences.

Methods: The activation of hamstring muscles before and immediately after a 10x10 *Flywheel Russian belt Deadlift* training session was assessed by the T2 shift technique through muscle functional magnetic resonance imaging (mfMRI), in one international-level field hockey male player. The individual use of the *biceps femoris* long head (BF_{LH}) and short head (BF_{SH}), semitendinosus (ST), and semimembranosus (SM) were analyzed, together with the limb- and region-specific activation for each muscle, computed as the areas at 0-30% (proximal), 30-70% (middle) and 70-100% (distal) of thigh length.

Results: T2 values significantly increased immediately after exercise in all regions of all the hamstring muscles in both dominant and non-dominant lower limb. However, a significant main effect for muscle were observed for T2 shift [$F(3,106) = 9.557$, $p < 0.001$], showing a lesser SM activation, compared to BF_{LH}, BF_{SH} and ST. Overall, the most activated muscle by the *Flywheel Russian belt Deadlift* training session was the *biceps femoris* (short head +13.8%, long head, +12.7%), followed by the ST (+11.3%), and lastly the SM (+6.9%).

Conclusion: The findings of the study highlight that a homogeneous and consistent hamstring muscles activation can be triggered by the novel *Flywheel Russian belt Deadlift* exercise. Particularly, the lateral positioning muscles (i.e., BF_{LH} and BF_{SH}) were more activated than the medial positioning muscles (i.e., ST and SM). Therefore, the *Flywheel Russian belt Deadlift* exercise could enhance training programs to strengthen and activate the hamstring muscles, and specifically, the BF_{LH}, in order to reduce HSI risk.

Keywords: eccentric overload; functional magnetic resonance imaging; inertial training; hamstring strains; sprint-based team sports.

INTRODUCTION

Hamstring strain injuries (HSI) are among the most common musculotendinous injuries in team sports in both males and females, by increasing the burden and financial cost, and decreasing the performance and quality of life of athletes [15]. Predominantly, repeated sprints are one of the most frequently high-intensity actions in team sports, leading to a higher HSI risk [158]. During sprinting, acceleration is directly determined by the production of horizontal ground reaction force [170]. This horizontal ground reaction force is predicted by the hamstrings electromyographic activity and eccentric torque during the late swing phase of the running gait cycle [171]. The late swing phase is a key point of the sprint, involving two simultaneous damaging events. First, the maximum length of the hamstrings muscle-tendon unit is reached. Concurrently, the hamstrings undergo an active, eccentric lengthening contraction [20]. Overall, repeated hamstrings eccentric contractions during repeated late swing phases of the running gait cycle are the main mechanism contributing to hamstring muscles strains [23]. In particular, the BF_{LH} has been found to be most important hamstring muscle to decelerate the shaft during the late swing phase [155,173]. The reason is that the lower limbs positioning during the late swing phase causes a greater BF_{LH} stretching, and moment arm, compared to the ST or SM muscles [20], and explains why *biceps femoris* strains are the most common HSI in sprint-based team sports in both males and female athletes [158].

Focusing on prepare the hamstrings for the repeated eccentric contraction-derived muscle strains during repeated late swing phases of the running gait cycle is therefore essential to both reduce the HSI risk and improve performance. Although resistance-based training is the most popular method, different approaches to hamstrings training have been explored, including eccentric training, inertial training, knee-dominant or hip-dominant exercises. The *Nordic hamstring exercise* has become one of the most widely used exercises to train the hamstrings since it provides a large eccentric workload. However, mfMRI-based studies have demonstrated that this knee-dominant exercise causes great activations of the ST and BF_{SH} [149,151–153]. On the other hand, flywheel inertial training is a recognized training method to reach eccentric overload (EO) [371], allowing to prepare the eccentric phase of a movement. The most commonly used flywheel exercise to train the hamstrings is the *flywheel prone leg curl* which, similar to the *Nordic hamstring exercise*, generates a high activation of the ST and BF_{SH}, but not BF_{LH} [151,152].

Contrary to the above-mentioned knee-dominant exercises, hip-dominant exercises, such as the *Russian belt deadlift* or the *unilateral hip extension conic-pulley*, seem to provide the most stimulation to the BF_{LH}, compared to the other hamstrings muscles, although modest T2 shift were shown (i.e., 7% and 6% respectively) [151]. The reason is that hip-dominant exercises activate

the lateral hamstrings more than the medial hamstrings, representing a 4-times greater activation of the BF_{LH} compared to the ST [145]. Specifically, the product of the PCSA and the hip moment arm is higher for the BF_{LH} compared to the ST muscle [142], so during hip-dominant movements, there is a strong participation of the BF_{LH} [145,146]. Therefore, the actions that shift the center of gravity forward of the transverse axis of the hip joints (e.g., forward bending of the trunk with hip flexion) cause a significant contraction of the lateral hamstring muscles, particularly the BF_{LH} [137,147]. In short, depending on the selected exercise and technique, there is a selective recruitment of the hamstrings, with the BF_{LH} being preferentially and eccentrically activated when the hip flexes and/or the knee extends in order to decelerate the movement [142].

Despite the above-mentioned approaches for hamstrings training, the injury incidence has remained unchanged during the last 30 years [15]. Considering that (i) loading exercises during extensive lengthening reduce the time to return to play, and (ii) active trunk stabilization during exercise decreases reinjury rates [372], it is reasonable that hamstrings training, particularly BF_{LH}, should be based on a hip-dominant exercise with special emphasis on working active eccentric lengthening contractions conducted with high loads and long musculotendon lengths [21,154]. Consequently, it is proposed to combine the *Russian belt deadlift* with flywheel training to build a new *Flywheel Russian belt Deadlift* exercise. Given that the selection of exercises targeting specific muscles in injury prevention or rehabilitation programs can be conducted by mfMRI [146], the purpose of the study was to determine exercise-induced hamstring muscles activation (T2 shift) immediately after a *Flywheel Russian belt Deadlift* training session, together with individual muscle-, region- and limb-specific differences.

MATERIALS AND METHODS

Experimental approach

One international-level field hockey male player (age = 22.7 years, mass = 67.0 kg, height = 1.72 cm) voluntarily accepted to participate in the study, in order to assess hamstring muscles activation before and immediately after the *Flywheel Russian belt Deadlift* training session. The volunteer gave their written consent that received information about the purpose of the study and associated risks. The experiment was conducted in accordance with the code of ethics of the World Medical Association (Declaration of Helsinki) and was approved by the Ethics Committee for Clinical Research of the Catalan Sports Council (Generalitat de Catalunya) (037/CEICGC/2021).

Hip-dominant flywheel deadlift exercise

The training session started with a standardized warm-up, consisting of dynamic joint mobilization and 2 sets of 8 repetitions of the *Flywheel Russian belt Deadlift* exercise under progressively increasing and submaximal effort. Then, the *Flywheel Russian belt Deadlift* training session consisted of 10 sets of 10 repetitions conducted on a conical flywheel device *Proinertial pulley pro C2* (Inertial systems S.L., Barcelona, Spain), with a 3-minute rest period between sets. The inertia load was set at $0.124 \text{ kg}\cdot\text{m}^2$, as greater moments of inertia lead to greater EO [337]. Given that the first repetitions of inertial training are used to increase the speed of the flywheel and are considered not to be effective repetitions, the first three repetitions of each set were used to “increase momentum” and were excluded from the data analysis [373].

The standardized starting position during the novel *Flywheel Russian belt Deadlift* exercise was with the feet on a 30-degree sloping wedge, the lower limbs and the body weight held by a *Russian belt* attached to the wall, self-selected knee flexion between 0 and 10° , hip flexion down to 90° , and abdominal bracing together with scapular retraction (**Figure 6.1A**). Then, the exercise started conducting a hip extension to 0° (i.e., concentric phase) (**Figure 6.1B**), and back to hip flexion to the bottom of the ROM (i.e., eccentric phase). The volunteer was verbally encouraged to perform the concentric phase as fast as possible, and to brake as hard as possible during the last part of the eccentric phase in order to increase EO [374].

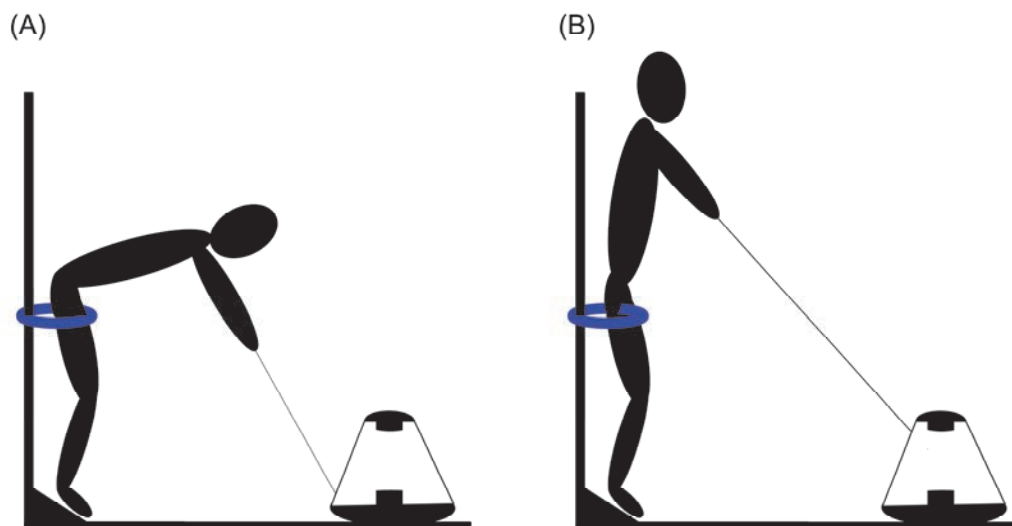


Figure 6.1. Standardized (A) starting and (B) finishing position of the novel *Flywheel Russian belt Deadlift* exercise.

Image Acquisition and Processing

The activation of hamstring muscles before and immediately after the *Flywheel Russian belt Deadlift* training session was assessed by the T2 shift technique through mfMRI [375]. To minimize the effects of fluid shifts caused by walking, the individual remained recumbent for a minimum of 10 minutes before basal condition image acquisition, while immediately after the exercise the individual was wheelchair-assisted between the exercise room and the MRI scanner [375,376]. Then, the individual was placed on the magnetic resonance scan *Vantage Galan 3T* (Cannon Medical Systems, Tochigi, Japan), in supine position with his thighs covered with two overlapped 16-channel Atlas SPEEDER body coils. A custom-made foot-restraint device was used to maximize the repeatability of limb placement in the MRI scanner.

T2-weighted imaging was acquired with the following parameters: TE 20, 60, 100 and 140 ms; TR 3200 ms; in-plane resolution 0.25 x 0.25 mm; slice thickness 3 mm; gap 18 mm. Total acquisition time: 6 min. Pre-exercise acquisitions also included axial fat-suppressed proton density-weighted imaging with TE 33 ms; TR 2954 ms; in-plane resolution 0.49 x 0.49 mm; slice thickness 3 mm; gap 18 mm. The imaging protocol was conducted in two blocks to cover the extension of hamstring muscles from the distal margin of the ischial tuberosity to the tibial plateau.

Subsequently, a parametric image was generated from the T2 mapping sequence using *parametric MRI (pMRI) software v.1.3.3-b* (Philadelphia, PA, USA). The MRI data were then evaluated for T2 relaxation time (T2 value) of the hamstring muscles. A circular region of interest (ROI) was defined for the BF_{LH}, BF_{SH}, ST, and SM muscles in each of the T2 mapping images where these muscles were visible. Intramuscular vascular structures, connective tissue and the boundaries of the muscles were avoided as the T2 values could be affected. ROIs of similar size and anatomical location were placed in the subsequent image sets to ensure positioning identical to that in the basal analysis (**Figure 6.2**) [152]. A multi-echo 2DFSE T2-weighted was applied to measure the mean T2 value. Images taken at different TEs were fit to a monoexponential time curve to extract the T2 values [377]. Region-specific muscle activation for each muscle were computed as the mean T2 absolute value of the different ROIs containing of the areas at 0-30% (proximal), 30-70% (middle) and 70-100% (distal) of thigh length, from the lower border of the ischial tuberosity (0%) to the upper border of the tibial plateau (100%) [377]. T2 shift was finally calculated by subtracting T2 baseline values from T2 post-exercise values and expressed as a percentage of the difference [378]. Two independent researchers conducted the MRI scanning and the T2 shift analysis.

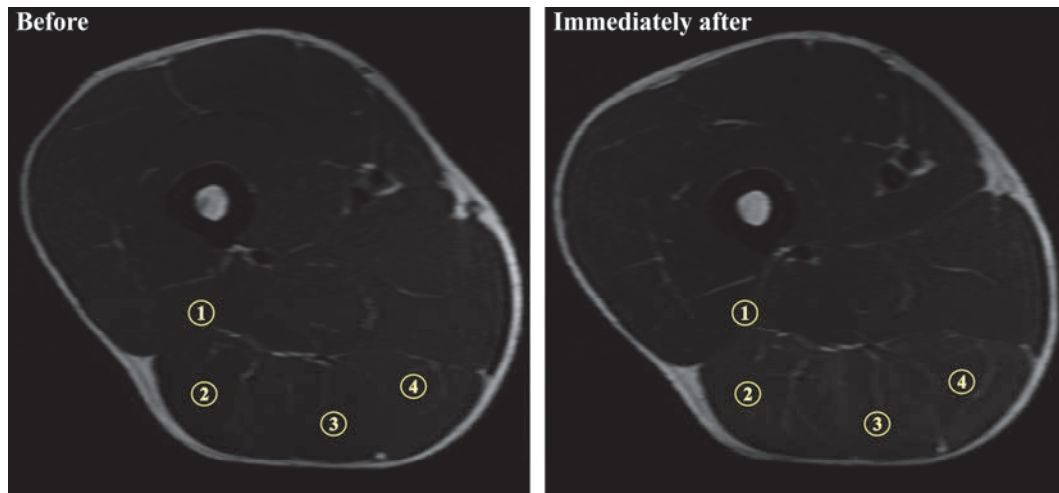


Figure 6.2. Selected magnetic resonance images (50% of thigh length) acquired before and immediately after the *Flywheel Russian belt Deadlift* training session, depicting regions of interest (ROIs). 1, m. biceps femoris short head (**BF_{SH}**); 2, m. biceps femoris long head (**BF_{LH}**); 3, m. semitendinosus (**ST**); 4, m. semimembranosus (**SM**).

Statistical analysis

A four-way mixed model (time [pre, post] x muscle [BF_{SH}, BF_{LH}, ST, SM] x limb [dominant, non-dominant] x region [proximal, middle, distal]) was used to determine exercise-induced hamstring muscles activation (T2 values). Subsequently, a three-way mixed model (muscle [BF_{SH}, BF_{LH}, ST, SM] x limb [dominant, non-dominant] x region [proximal, middle, distal]) was used to determine differences in hamstring muscles T2 shift immediately after the *Flywheel Russian belt Deadlift* exercise. Bonferroni's corrected post hoc analysis was conducted if the model showed statistically significant main effects or interaction effects. Data are presented as mean $\pm$ SD and the level of significance was set at $p < 0.05$. Statistical analysis was performed with SPSS v.27.0.1.0 (SPSS Statistics, IBM Corp., Armonk, NY, USA).

RESULTS

Representative region-specific T2-weighted magnetic resonance images before and immediately after 10x10 *Flywheel Russian belt Deadlift* training session are presented in **Figure 6.3**.

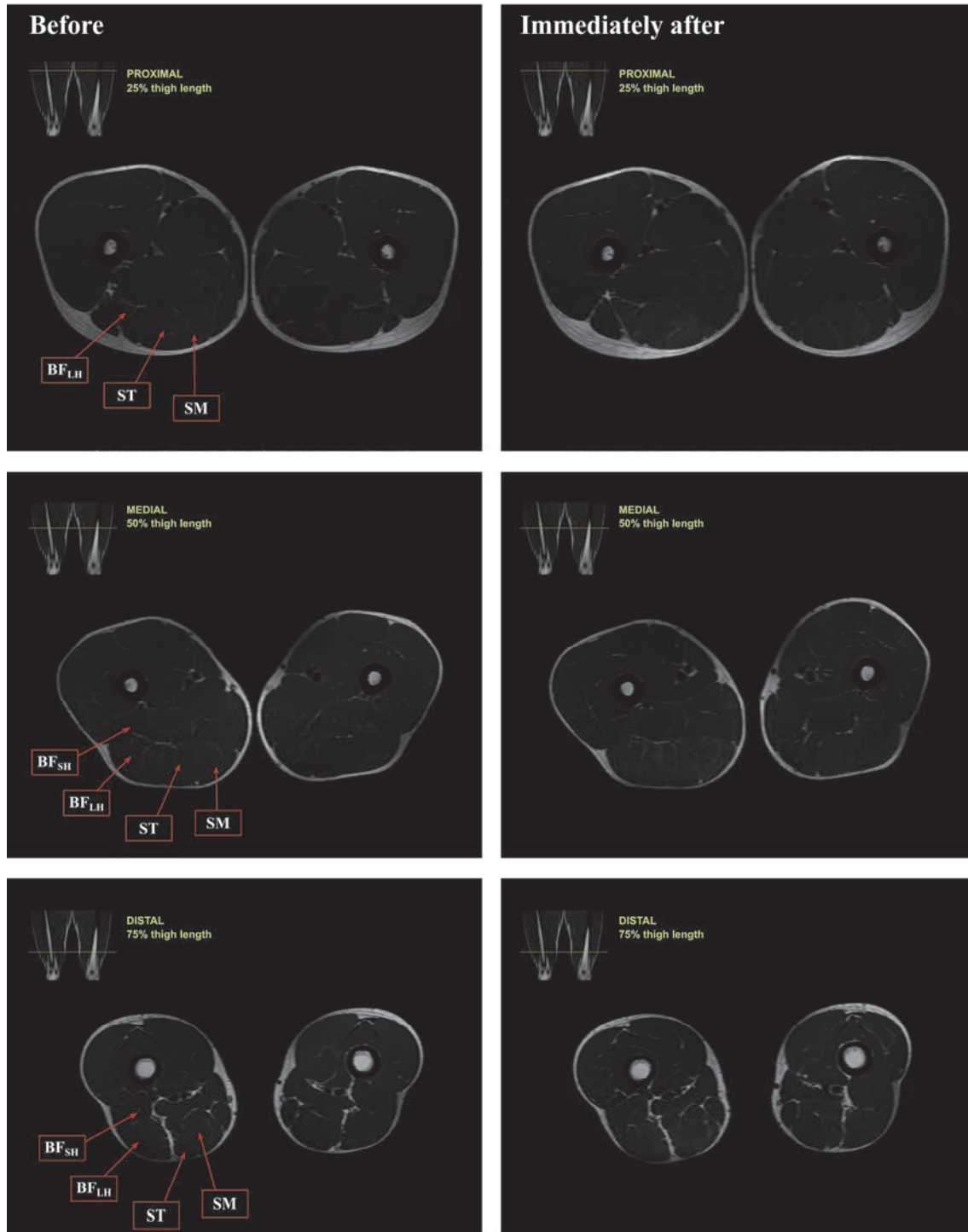


Figure 6.3. Representative T2- weighted magnetic resonance images of the proximal region (25% of thigh length), middle region (50% of thigh length) and distal region (75% of the thigh length) before and immediately after the *Flywheel Russian belt Deadlift* training session. **BF_{SH}**, m. biceps femoris short head; **BF_{LH}**, m. biceps femoris long head; **ST**, m. semitendinosus; **SM**, m. semimembranosus.

A significant main effect for time were observed for the T2 values [$F(1,106) = 51838.462$, $p < 0.001$]. T2 values substantially increased from the basal condition to immediately after exercise in all regions of all the hamstring muscles in both dominant and non-dominant lower limb (**Table 6.1**).

Table 6.1. Region-specific T2 values, mean difference (MD) and percentage of the difference (% Δ) of hamstring muscles before and immediately after the *Flywheel Russian belt Deadlift* training session.

Region-specific	BF _{SH}		BF _{LH}		ST		SM	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Dominant								
Proximal	Not applicable		35.2 $\pm$ 0.8 MD = 4.1, % Δ = 11.8*	39.4 $\pm$ 2.3 MD = 3.7, % Δ = 10.1*	37.0 $\pm$ 1.4 MD = 3.7, % Δ = 10.1*	40.7 $\pm$ 1.4 MD = 1.9, % Δ = 4.5*	41.0 $\pm$ 1.8 MD = 1.9, % Δ = 4.5*	42.8 $\pm$ 2.5 MD = 3.6, % Δ = 9.2*
Middle	33.3 $\pm$ 1.0 MD = 3.7, % Δ = 11.1*	37.0 $\pm$ 1.5 MD = 3.7, % Δ = 11.1*	35.1 $\pm$ 1.4 MD = 4.2, % Δ = 12.1*	39.3 $\pm$ 1.4 MD = 4.2, % Δ = 12.1*	35.1 $\pm$ 1.8 MD = 4.0, % Δ = 11.5*	39.0 $\pm$ 0.9 MD = 4.0, % Δ = 11.5*	39.1 $\pm$ 2.8 MD = 3.6, % Δ = 9.2*	42.7 $\pm$ 3.4 MD = 3.6, % Δ = 9.2*
Distal	35.1 $\pm$ 0.9 MD = 6.1, % Δ = 17.6*	41.3 $\pm$ 1.4 MD = 6.1, % Δ = 17.6*	35.3 $\pm$ 0.9 MD = 5.1, % Δ = 14.3*	40.3 $\pm$ 1.8 MD = 5.1, % Δ = 14.3*	35.0 $\pm$ 0.2 MD = 5.6, % Δ = 15.9*	40.5 $\pm$ 0.4 MD = 5.6, % Δ = 15.9*	38.8 $\pm$ 2.8 MD = 2.9, % Δ = 7.5*	41.7 $\pm$ 2.5 MD = 2.9, % Δ = 7.5*
Total	34.2 $\pm$ 1.3 MD = 4.9, % Δ = 14.4*	39.1 $\pm$ 2.6 MD = 4.9, % Δ = 14.4*	35.2 $\pm$ 1.1 MD = 4.5, % Δ = 12.7*	39.6 $\pm$ 1.8 MD = 4.5, % Δ = 12.7*	35.8 $\pm$ 1.8 MD = 4.1, % Δ = 11.5*	39.9 $\pm$ 1.3 MD = 4.1, % Δ = 11.5*	39.3 $\pm$ 2.6 MD = 3.0, % Δ = 7.8*	42.4 $\pm$ 2.8 MD = 3.0, % Δ = 7.8*
Non-dominant								
Proximal	Not applicable		35.6 $\pm$ 3.1 MD = 4.0, % Δ = 11.6*	39.6 $\pm$ 3.5 MD = 3.5, % Δ = 10.5*	35.3 $\pm$ 3.6 MD = 3.5, % Δ = 10.5*	38.8 $\pm$ 1.6 MD = 3.2, % Δ = 8.7*	37.4 $\pm$ 0.5 MD = 3.2, % Δ = 8.7*	40.6 $\pm$ 0.4 MD = 3.2, % Δ = 8.7*
Middle	38.1 $\pm$ 2.7 MD = 4.3, % Δ = 11.6*	42.4 $\pm$ 2.3 MD = 4.3, % Δ = 11.6*	37.8 $\pm$ 1.5 MD = 5.2, % Δ = 13.5*	43.0 $\pm$ 3.8 MD = 5.2, % Δ = 13.5*	35.4 $\pm$ 1.5 MD = 4.1, % Δ = 11.6*	39.5 $\pm$ 1.0 MD = 4.1, % Δ = 11.6*	35.9 $\pm$ 0.3 MD = 1.7, % Δ = 4.9*	37.6 $\pm$ 0.8 MD = 1.7, % Δ = 4.9*
Distal	37.9 $\pm$ 1.6 MD = 5.5, % Δ = 14.7*	43.4 $\pm$ 1.9 MD = 5.5, % Δ = 14.7*	36.3 $\pm$ 1.4 MD = 4.5, % Δ = 12.6*	40.9 $\pm$ 1.2 MD = 4.5, % Δ = 12.6*	34.4 $\pm$ 0.7 MD = 3.6, % Δ = 10.6*	38.0 $\pm$ 2.0 MD = 3.6, % Δ = 10.6*	35.6 $\pm$ 0.6 MD = 2.3, % Δ = 6.5*	37.9 $\pm$ 1.1 MD = 2.3, % Δ = 6.5*
Total	38.0 $\pm$ 2.1 MD = 4.9, % Δ = 13.2*	42.9 $\pm$ 2.0 MD = 4.9, % Δ = 13.2*	36.8 $\pm$ 2.1 MD = 4.7, % Δ = 12.7*	41.4 $\pm$ 3.3 MD = 4.7, % Δ = 12.7*	35.2 $\pm$ 2.3 MD = 3.8, % Δ = 11.1*	39.0 $\pm$ 1.4 MD = 3.8, % Δ = 11.1*	36.0 $\pm$ 0.8 MD = 2.2, % Δ = 6.1*	38.2 $\pm$ 1.4 MD = 2.2, % Δ = 6.1*
Combined								
Proximal	Not applicable		35.4 $\pm$ 2.1 MD = 4.1, % Δ = 11.7*	39.5 $\pm$ 2.8 MD = 3.6, % Δ = 10.3*	36.1 $\pm$ 2.8 MD = 3.6, % Δ = 10.3*	39.7 $\pm$ 1.8 MD = 2.6, % Δ = 6.6*	39.2 $\pm$ 2.3 MD = 2.6, % Δ = 6.6*	41.7 $\pm$ 2.0 MD = 2.6, % Δ = 6.6*
Middle	35.7 $\pm$ 3.2 MD = 4.0, % Δ = 11.4*	39.7 $\pm$ 3.4 MD = 4.0, % Δ = 11.4*	36.4 $\pm$ 2.0 MD = 4.7, % Δ = 12.8*	41.1 $\pm$ 3.4 MD = 4.7, % Δ = 12.8*	35.2 $\pm$ 1.6 MD = 4.0, % Δ = 11.5*	39.3 $\pm$ 0.9 MD = 4.0, % Δ = 11.5*	37.5 $\pm$ 2.6 MD = 2.7, % Δ = 7.0*	40.2 $\pm$ 3.6 MD = 2.7, % Δ = 7.0*
Distal	36.5 $\pm$ 1.9 MD = 5.8, % Δ = 16.1*	42.3 $\pm$ 1.9 MD = 5.8, % Δ = 16.1*	35.8 $\pm$ 1.3 MD = 4.8, % Δ = 13.4*	40.6 $\pm$ 1.5 MD = 4.8, % Δ = 13.4*	34.7 $\pm$ 0.5 MD = 4.6, % Δ = 13.3*	39.3 $\pm$ 1.9 MD = 4.6, % Δ = 13.3*	37.2 $\pm$ 2.6 MD = 2.6, % Δ = 7.0*	39.8 $\pm$ 2.7 MD = 2.6, % Δ = 7.0*
Total	36.1 $\pm$ 2.6 MD = 4.9, % Δ = 13.8*	41.0 $\pm$ 3.0 MD = 4.9, % Δ = 13.8*	36.0 $\pm$ 1.8 MD = 4.6, % Δ = 12.7*	40.5 $\pm$ 2.8 MD = 4.6, % Δ = 12.7*	35.5 $\pm$ 2.1 MD = 3.9, % Δ = 11.3*	39.4 $\pm$ 1.4 MD = 3.9, % Δ = 11.3*	37.7 $\pm$ 2.5 MD = 2.6, % Δ = 6.9*	40.3 $\pm$ 3.0 MD = 2.6, % Δ = 6.9*

Data are expressed as mean $\pm$ SD. (*) indicates significant post-exercise change from baseline T2 value. % Δ , percentage of delta change BF_{SH}, m. biceps femoris short head; BF_{LH}, m. biceps femoris long head; MD, mean difference; ST, m. semitendinosus; SM, m. semimembranosus.

A significant main effect for muscle were observed for T2 shift [$F(3,106) = 9.557$, $p < 0.001$]. T2 shift of the SM was significantly smaller after the exercise in both dominant and non-dominant limb, compared to BF_{LH}, BF_{SH} and ST (**Figure 6.4**). There were no between-limbs [$F(1,106) = 0.403$, $p = 0.527$] nor between-regions [$F(2,106) = 1.564$, $p = 0.214$] differences in hamstring muscles T2 shift. There were also no significant muscle-limb [$F(3,106) = 0.096$, $p = 0.962$], muscle-region [$F(5,106) = 0.670$, $p = 0.647$], limb-region [$F(2,106) = 1.152$, $p = 0.320$], nor muscle-limb-region [$F(5,106) = 0.766$, $p = 0.576$] interactions. Therefore, the most activated muscles by the *Flywheel Russian belt Deadlift* training session, marked by T2 shift, were the *biceps femoris* (short head +13.8%, long head, +12.7%), followed by the ST (+11.3%), and lastly the SM (+6.9%).

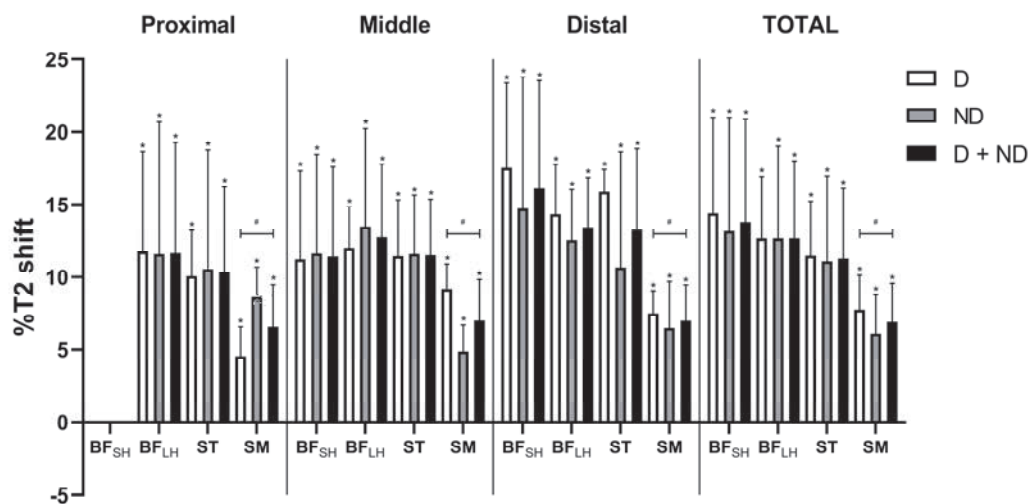


Figure 6.4. Mean ($\pm$ SD) of changes in the transverse relaxation time (%T2 shift) of the 30% (proximal), 50% (middle) and 70% (distal) regions of thigh length in m. biceps femoris short head (BF_{SH}), m biceps femoris long head (BF_{LH}), m. semitendinosus (ST), and m. semimembranosus (SM), immediately after the *Flywheel Russian belt Deadlift* training session. All values are given as a percentage of the pre-value. (*) indicates significant T2 shift and (#) indicates substantial differences in SM, compared to BF_{SH}, BF_{LH} and ST. **D**, dominant limb; **ND**, non-dominant limb.

DISCUSSION

Individual muscle-, region- and limb-specific differences in hamstring muscles activation (T2 shift) were assessed immediately after a novel *Flywheel Russian belt Deadlift* exercise. The findings of the study highlight that all hamstring muscles were activated during the *Flywheel Russian belt Deadlift* training session. Particularly, the *biceps femoris* (i.e., BF_{LH} and BF_{SH}) was the most activated hamstring muscle, followed by the ST, while the SM was activated to a lesser extent. Finally, there were no between-region nor between-limb differences in hamstring muscles activation after the *Flywheel Russian belt Deadlift* training session.

Flywheel Russian Belt Deadlift-induced hamstring muscle activity

The transverse relaxation time (T2 value) of hydrogen protons in skeletal muscle can be quantified by mfMRI. Changes in the T2 value between before and after a single exercise (T2 shift) have been described as a highly reliable and non-invasive indicator of muscle activation during exercise [375]. The proposed *Flywheel Russian belt Deadlift* exercise significantly increased the T2 shift of all the hamstring muscles, ranging from 6.9% for the SM to 13.2% for the *biceps femoris*. To our knowledge, no previous MRI-based study has reported activations of all hamstring muscles after a single exercise. In fact, a comparative study of four frequently used hamstring exercises (i.e., *Nordic hamstring exercise*, *flywheel leg curl*, *Russian belt deadlift* and *hamstrings kick coney-pulley*) found that neither exercise was able to significantly target all hamstring muscles [151]. As examples, a 4x8 *flywheel leg curl* training session with a moment of inertia of 0.072 kg·m² or body-weighted *Nordic hamstring exercise* provided large T2 shifts of the ST and BF_{SH} [151], and are recommended when the goal is to target knee-dominant hamstring muscles [152]. Conversely, hip-dominant exercises such as the *Russian belt deadlift* or the *hamstrings kick coney-pulley* were effective in triggering the activation of the BF_{LH} (7% and 6%, respectively), although the remaining hamstring muscles were not activated [151]. Using a *Russian belt* to hold the body weight and bend the trunk forward together with small knee flexion (between 0° and 10°), the *Flywheel Russian belt Deadlift* exercise allowed to achieve a more stretched positioning of the hamstrings. Therefore, the stretching of the hamstrings, together with the large EO workload provided by the flywheel inertial device, leads to focusing the exercise on working active eccentric lengthening contractions conducted with high loads and long musculotendon lengths [21,154], which are HSI risk factors during the late swing phase of the running gait cycle [23].

The biomechanical response of each hamstring muscle is heterogeneous, given that they display a variety of force-length properties and angle-specific joint torques [142]. The hereby proposed *Flywheel Russian belt Deadlift* exercise elicited the greatest activation of the *biceps femoris*. Specifically, the BF_{LH} is more sensitive to hip extension than knee flexion because the peak in the length-tension relationship of the BF_{LH} occurs with the hip at 90° and the knee at 0°, the point at which the greatest BF_{LH} function is produced [144]. Furthermore, lower-limb positioning near to hip flexion (i.e., 90°) with the knee extended (i.e., 0°) selectively activates the BF_{LH} [143]. Thus, during hip-dominant exercises (e.g., forward bending of the trunk with hip flexion), the hamstrings are responsible for driving the movement, with a strong participation of the BF_{LH} [145]. Conversely, the BF_{LH} involvement is restricted in knee-dominant exercises since hip movement is locked, and explains the large ST and BF_{SH} activations during knee-dominant exercises [148–153,377].

Region-specific (i.e., proximal, middle, distal) differences in hamstring muscles activity during hamstring exercises has been investigated before. The hamstrings *kick conic-pulley* has shown greater BF_{LH} proximal and middle activation compared to the other hamstring muscles, albeit to a small extent [152]. In contrast, the *Nordic hamstring exercise* and the *flywheel leg curl* have shown the same ST and BF_{SH} activation in all muscle regions, while the *Russian belt deadlift* seemed to be the unique exercise capable of activate the SM across muscle regions [152]. Given the *Russian belt* use during the proposed *Flywheel Russian belt Deadlift* exercise, SM muscle activation was also effective in all regions of the thigh. Similarly, the *Flywheel Russian belt Deadlift* exercise resulted in no region-specific differences in BF_{LH} , BF_{SH} and ST activation. Overall, the *Flywheel Russian belt Deadlift* exercise appears to provide a significant and homogenous (i.e., all muscle regions) hamstring muscles activation. The present study proposes the combination of the *Russian belt deadlift* with flywheel training to build a novel *Flywheel Russian belt Deadlift* exercise which significantly increases the activation of the hamstrings, with special focus on the BF_{LH} due to the injury incidence in sprint-based team sports. It has been found that the designed *Flywheel Russian belt Deadlift* exercise is a promising training method to produce significant active eccentric lengthening contractions conducted with high loads and long musculotendon lengths in the *biceps femoris*, followed by the ST, and lastly the SM.

Limitations

The study presents limitations to consider when interpreting the results. First, the design of the study provides limited sample power, and therefore generalizing the findings should be cautious. In addition, post-exercise muscle recovery indicators (i.e., force-generating capacity, perceived soreness) were not monitored, thus the short-term effect of the *Flywheel Russian belt Deadlift* exercise on hamstrings neuromuscular capacities is still unknown. Given the limitations of the study, future studies increasing the sample size to achieve greater statistical power, and monitoring hamstrings muscle recovery after a *Flywheel Russian belt Deadlift* training session are warranted. In addition, studies on the effects of a long-term *Flywheel Russian belt Deadlift* exercise-based training program on HSI incidence and/or performance would be meaningful.

CONCLUSION

The *Flywheel Russian belt Deadlift* exercise is able to trigger a homogeneous and consistent activation of all the hamstring muscles (i.e., BF_{LH} , BF_{SH} , ST and SM) throughout the hamstring regions and both dominant and non-dominant limbs. More specifically, the BF_{LH} and BF_{SH} were the most activated hamstring muscles, followed by the ST, and lastly the SM. Therefore, training programs in order to strengthen and activate the hamstring muscles, and particularly the BF_{LH} , could be improved by the inclusion of the *Flywheel Russian belt Deadlift* exercise.

**7. STUDY IV. Hamstring-specific neuromuscular and
biochemical responses to a *Flywheel Russian belt*
Deadlift in women and men**

Hamstring-specific neuromuscular and biochemical responses to a *Flywheel Russian belt Deadlift* in women and men

ABSTRACT

Background: The $90^{\circ}_{hip}:20^{\circ}_{knee}$ test and the *Jurdan test* have been proven to be suitable for monitoring hamstrings muscle recovery after a sprint-based exercises in males and females, and in males, respectively. Therefore, the purpose of the study was to analyze hamstrings muscle damage and recovery after a novel *Flywheel Russian belt Deadlift* exercise using hamstring-specific neuromuscular tests and associated biochemical markers of structural damage in males and females.

Methods: Healthy females (n = 15) and males (n = 15) underwent 5 sets of 10 repetitions of the *Flywheel Russian belt Deadlift* exercise, with a 3-minute rest pause between sets. Hamstrings maximal voluntary isometric contraction (MVIC) torque and rate of force development (RFD) over several time-intervals by the $90^{\circ}_{hip}:20^{\circ}_{knee}$ test and range of motion (ROM) *Jurdan test*, together with serum biomarkers of muscle damage and oxidative stress, were tested at baseline (Pre) and post-24 hours (+24h), post-48 hours (+48h) and post-72 hours (+72h) through a repeated measures design.

Results: *Flywheel Russian belt Deadlift*-induced residual fatigue was observed as a reduction in dominant and non-dominant hamstrings MVIC torque and RFD at all time-intervals until +72h in both males and females. Also, hamstrings neuromuscular capacity reductions were accompanied with serum biomarkers of structural and oxidative damage to the myocytes. However, although males decreased ROM until +72h, females showed no ROM changes.

Conclusion: Overall, the *Flywheel Russian belt Deadlift* triggered a decrease in hamstrings neuromuscular capacities, and an upregulation of biochemical markers of muscle damage and oxidative stress until 72 hours after the training session, suggesting that *Flywheel Russian belt Deadlift* might be a promising exercise for *biceps femoris* strengthening. The $90^{\circ}_{hip}:20^{\circ}_{knee}$ test is a powerful test to screen hamstrings recovery in both women and men after flywheel training, through MVIC torque and both early and late RFD metrics. However, the *Jurdan test* might not be able to monitor hamstrings ROM recovery in females after flywheel eccentric training.

Keywords: exercise-induced muscle damage, force-generating-capacity, flywheel training, hamstring muscles, muscle recovery, residual fatigue.

INTRODUCTION

Hamstring strain injuries (HSI) are the most prevalent musculotendinous injuries in field team sports players [158]. It has been estimated that the time lost due to HSI is 14 to 35 days [158] and the economic cost in a professional player can reach 500,000 euros [162]. HSI incidence has remained unchanged over the last 30 years in field team sports [15]. In fact, in highly competitive congested team sports, HSI showed a two-fold increase over the last 20 years [163]. Although the location of the HSI is variable, the BF_{LH} is the most affected hamstring muscle, representing 4 out of every 5 HSI [163]. Therefore, given that the hamstrings are very susceptible to being injured, it is essential to train the hamstrings for the repeated eccentric contraction-derived muscle strains during repeated late swing phases of the running gait cycle, which is the injury-related mechanism of HSI [23].

Hamstrings training has been popularly based on the combination of eccentric and knee-dominant exercises (e.g., *Nordic hamstring*). However, it has been proven that knee-dominant exercises trigger large activations of knee-dominant muscles (i.e., ST and BF_{SH}), but not hip-dominant muscles (i.e., BF_{LH}) [151–153]. On the other hand, flywheel training is one of the most widely used training methods to prepare the eccentric phase of a movement, as it allows to reach EO [371]. Thus, the most commonly used flywheel exercise to train the hamstrings is the *flywheel prone leg curl*, which also generates a high activation of knee-dominant muscles (i.e., ST and BF_{SH}), but not hip-dominant muscles (i.e., BF_{LH}) [151,152]. Contrary, hip-dominant exercises, such as the *Russian belt deadlift*, seem to provide the most activity to the BF_{LH}, although modest T2 shift was shown (i.e., 7%) [151]. The reason is that hip-dominant exercises activate the lateral hamstrings more than the medial hamstrings, representing a 4-times greater activation of the BF_{LH} compared to the ST [145]. Therefore, the actions that shift the center of gravity forward of the transverse axis of the hip joints (e.g., forward bending of the trunk with hip flexion) cause a significant activity of the lateral hamstring muscles, particularly the BF_{LH} [147]. In short, there is a selective recruitment of the hamstrings depending on the exercise and technique employed. Accordingly, the *Russian belt deadlift* has been combined with flywheel training to build a new hip-dominant *Flywheel Russian belt Deadlift* exercise, which provides a greater activation (T2 shift) to the lateral positioning hamstring muscles (i.e., *biceps femoris*) compared to the medial positioning hamstring muscles (i.e., ST and SM) [**Study III**].

On the other hand, to monitor hamstrings muscle recovery is a key concern for practitioners in order to balance the optimization of training with recovery and reduce HSI risk. Thus, the rationale for assessing a panel of markers is that muscle damage and recovery is a multifactorial phenomenon derived from a variety of physiological processes [179]. A panel of recovery

markers ideally assesses at least the following processes: (i) loss of myofibrillar integrity or excitation-contraction uncoupling, related to reduced rapid and maximal force-generating capacity [24,33,73]; (ii) connective tissue damage, related to increases in muscle perceived soreness [185] and/or ROM loss [191,192]; and (iii) sarcolemma permeability and/or secondary damage, related to muscle damage and oxidative stress blood biomarkers [62]. Recently, it has been reported that there are hamstring-specific neuromuscular tests that are suitable for monitoring exercise-induced residual fatigue after sprint-based exercises [**Studies I and II**]. Specifically, the $90^{\circ}_{hip}:20^{\circ}_{knee}$ IPC test by *Matinlauri et al.*, [211] can be used to monitor hamstrings MVIC torque in both males and females [**Studies I and II**] and RFD in males [**Study II**], whereas the *Jurdan test* [233] was capable of identifying ROM losses in only males [**Study I**]. However, there is no evidence whether (i) these neuromuscular testing procedures (i.e., $90^{\circ}_{hip}:20^{\circ}_{knee}$ IPC test and *Jurdan test*) can effectively monitor flywheel training-induced hamstrings residual fatigue and recovery, and (ii) the associated biochemical response to the novel *Flywheel Russian belt Deadlift* exercise. Therefore, the purpose of the research was to monitor *Flywheel Russian belt Deadlift* training-induced residual fatigue through hamstring-specific and cost-effective neuromuscular tests and associated biochemical markers of impaired structures in women and men.

MATERIALS AND METHODS

Participants

Fifteen physically active and healthy males (age = 21.4 ± 1.9 years, mass = 74.3 ± 8.9 kg, height = 178.2 ± 7.2 cm [mean $\pm$ SD]) and fifteen females (age = 21.5 ± 3.4 years, mass = 60.5 ± 8.8 kg, height = 163.4 ± 5.1 cm [mean $\pm$ SD]) voluntarily attended the study. Exclusion criteria were lower limb myotendinous injuries during the 6 months before the experiment. The volunteers were also required to avoid exercising during the 72 hours before and during the experiment. All participants gave their written consent that they received information about the purpose of the study and associated risks. The experiment was conducted in accordance with the code of ethics of the Declaration of Helsinki and was approved by the Ethics Committee for Clinical Research of the Catalan Sports Council (Generalitat de Catalunya) (037/CEICGC/2021).

Experimental approach

The extent and time course of hamstring-specific neuromuscular and biochemical responses were examined during 72 hours through a repeated measures design. All tests were conducted at baseline prior to the *Flywheel Russian belt Deadlift* training day (Pre) and at 24 (+24h), 48 (+48h) and 72 (+72h) hours after the *Flywheel Russian belt Deadlift* training session. Participants were

familiarized with the *Flywheel Russian belt Deadlift* and the neuromuscular assessments 7 days prior to the experiment (**Figure 7.1**)

			Training Day					
	-168h	-24h	Pre	FRD	Post	+24h	+48h	+72h
Neuromuscular tests (MVIC torque, RFD, ROM, PS)	Familiarization		↓	Sets: 5 Reps: 10	↓	↓	↓	↓
Serum markers (EIMD, OS, Irisin)			↓	Inertia 0.159 kg·m ²		↓	↓	↓
Dietary intake		↓	↓				↓	

Figure 7.1. Schematic view of the experimental approach. **EIMD**, exercise-induced muscle damage; **FRD**, *Flywheel Russian belt Deadlift*; **MVIC**, maximal voluntary isometric contraction; **OS**, oxidative stress; **PS**, perceived soreness; **RFD**, rate of force development; **ROM**, range of motion.

The standardized starting position during the *Flywheel Russian belt Deadlift* was with the feet on a 30-degree sloping wedge, the lower limbs held by a *Russian belt* attached to the wall, self-selected knee flexion between 0 and 10°, hip flexion down to 90°, and scapular retraction (**Figure 7.2A**). Then, the exercise started conducting a hip extension to 0° (i.e., concentric phase) (**Figure 7.2B**), and back to hip flexion to the bottom of the ROM (i.e., eccentric phase).

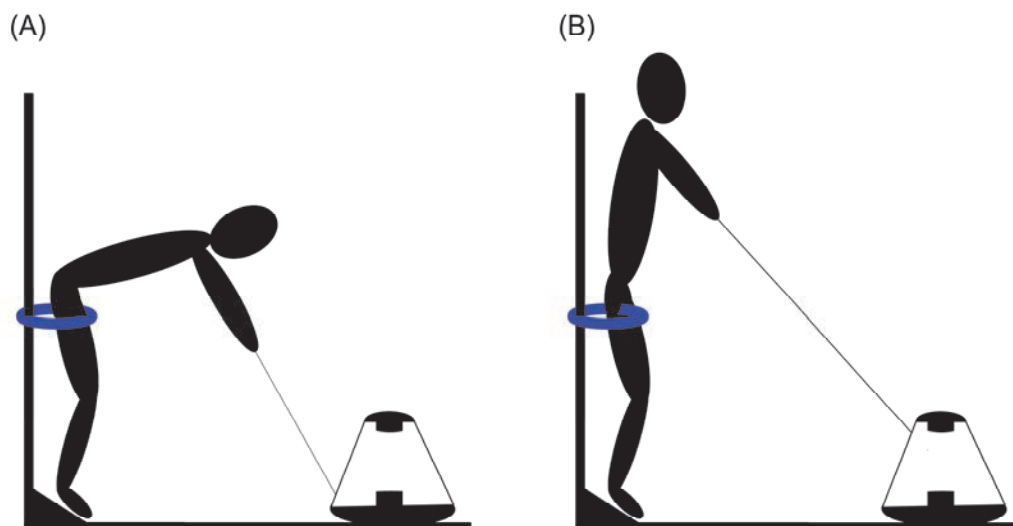


Figure 7.2. Standardized (A) starting and (B) finishing position of the *Flywheel Russian belt Deadlift* exercise.

The intervention commenced with a standardized warm-up consisting of dynamic joint mobilization and 5 sets of 10 repetitions of the *Flywheel Russian belt Deadlift* exercise under progressively increasing and submaximal effort. The *Flywheel Russian belt Deadlift* training session consisted of 5 sets of 10 repetitions at maximal intensity [147,148] conducted on the conical flywheel device *Procami Conical* (Procami SL, Barcelona, Spain), with a 3-minute rest period between sets. Given that the first repetitions of inertial training are used to increase the speed of the flywheel and are considered not to be effective repetitions, the first three repetitions of each set were used to “increase momentum” and were excluded from the data analysis [373]. The inertia load was set at $0.159 \text{ kg}\cdot\text{m}^2$, as greater moments of inertia lead to greater EO during flywheel hamstrings training [337]. Participants were verbally encouraged to perform the concentric phase as fast as possible, and to brake as hard as possible during the last part of the eccentric phase in order to increase EO [374]. During each repetition, concentric and eccentric power, together with EO, were recorded by a 1,000 Hz rotary encoder and transmitter *Chronopic* (Chronojump, Barcelona, Spain) connected to the flywheel device [374]. concentric and eccentric power was transmitted in real-time to *Chronojump v2.3.0-12* (Chronojump, Barcelona, Spain) and simultaneously displayed to the participants [374]. The variables recorded for data analysis were peak concentric power, peak eccentric power, and the ratio between eccentric peak power and concentric peak power [379], reported as the %EO ((eccentric peak power - concentric peak power / concentric peak power) * 100) [337].

Hamstring-specific neuromuscular tests

Force-generating capacity

MVIC torque of hamstring muscles was measured unilaterally (dominant and non-dominant lower limb) using the $90^\circ_{\text{hip}}:20^\circ_{\text{knee}}$ *IPC test* [211]. Briefly, participants were verbally encouraged to apply force “as fast and hard as possible” to a rigid custom-built support attached to a strain gauge (Chronojump, Barcelona, Spain). The used strain gauge has been proven to be reliable for MVIC and RFD assessment [338], and provides a low baseline noise amplitude (0.096% MVIC Torque). Participants completed three trials of the test (3 seconds MVIC), with a 30-second rest between trials. The 1-second window with the highest average force in the plateau of the signal was recorded as MVIC, and the residual force resulting from the mass of the tested limb at rest was then subtracted. The best of the three trials was recorded for further analyses. In addition, time-interval RFD to 50 milliseconds (RFD_{0-50}), to 100 milliseconds (RFD_{0-100}), from 100 to 200 milliseconds ($\text{RFD}_{100-200}$) and peak RFD (pRFD_{25}), that is the steepest part of the curve in a 25-millisecond epoch, were calculated as the average of the three best trials. According to the recommendations of *Maffiuletti et al.*, [215], (i) the contraction onset was determined through a

systematic manual-based method at the moment of “the last trough before force deflects above the range of the baseline noise”, and (ii) no filtering or signal smoothing was applied.

Range of motion

The differential angle between the actively lengthened leg shin angle and the opposite leg passive thigh angle was measured by the *Jurdan test* [233]. The test was photographically registered in duplicate by a *SONY DSC-RX100M5A* placed on a tripod located at the height of the table and three meters from the center of the hip of the participant. The sports kinematics analysis software *Kinovea v0.8.24* was used to compute the composite angle derived from 2D shin and thigh angles.

Perceived soreness

Perceived soreness of the hamstring muscles was recorded using an 11-point NRS, in the relaxed position of the $90^{\circ}_{hip}:20^{\circ}_{knee}$ *IPC test*. The scale was supported by pain descriptors, ranging from "0" for “no pain” to "10" for “unbearable pain” [240], and has been shown to be sensitive to eccentric exercise-induced pain in hamstring muscles [241].

Blood sampling and processing

Blood samples were drawn from an antecubital vein and were allowed to clot for 30 minutes at room temperature in an *SST II Advance tube* (Becton Dickinson Vacutainer Systems, UK). After centrifugation at 4,000g and at 4°C for 8 minutes, serum was collected and stored in aliquots at -80°C until analysis. CK activity was measured on an *Advia 2400 automated device* (Siemens™ Medical Solutions Diagnostics, Tarrytown, NY, USA) and sMtCK was measured by ELISA kit #SEC386Hu (Cloud Clone Corp., Houston, TX, USA) according to manufacturer’s instructions. MDA was measured by kit #E-EL-0060 (Elabscience, Wuhan, China). Also, the Erel approach, based on the oxidation of ferrous iron to ferric iron, was used to quantify the oxidizing material in the samples. The 2,2'-azino-di-3-ethylbenzthiazoline sulfonate (ABTS)+ method was used to measure TAS. Then, OSI was computed as the TOS to TAS ratio. The previously validated kit against mass spectrometry #EK-067-29 (Phoenix Pharmaceuticals Inc., Burlingame, CA, USA) was used to quantify circulating irisin [319].

Dietary Intake Record

Participants were given detailed verbal and written instructions describing procedures for recording the daily diet for 3 days (-24h, 0h and +48h), together with a list of commonly used household measurements (e.g., glass, cup) and measurements quantities (e.g., grams, milliliters). the diet of each participant was calculated as the 3-day average by using the dietary analysis software *PCN Pro v1*. (Universitat de Barcelona, Barcelona, Spain) [346], which is based on and updated with a Spanish food composition database [347].

Statistical analysis

First, sample size was a priori estimated using G*Power software v3.1.9.6 for a repeated measures design, within (four measurement time points) - between (males, females) interaction. A total sample size of 28 participants was calculated to be included given a computed effect size of 0.454 based on MVIC torque change ($90^{\circ}_{hip} \cdot 20^{\circ}_{knee}$ IPC test) in the **Study I of the thesis**, a power of 90%, and a 5% alpha error. Asymmetrically distributed variables were transformed prior to analysis. If transformed variables remained non-conformed to the Normal distribution, non-parametric statistical procedures were conducted.

A three-way repeated measures ANOVA (Time [Pre, +24h, +48h, +72h] x sex [male, female] x limb [dominant, non-dominant]) was used to identify the effect of the *Flywheel Russian belt Deadlift* training session on hamstrings neuromuscular tests, while *Flywheel Russian belt Deadlift*-induced changes in serum biomarkers were identified using a two-way repeated measures ANOVA (time [Pre, +24h, +48h, +72h] x sex [male, females]). Post-hoc Bonferroni-corrected paired t-tests were conducted to identify significant differences from baseline values. Effect sizes were calculated using Cohen's *d*, considering the following thresholds: (< 0.20), trivial; (0.20 < 0.59), small; (0.60 < 1.19), moderate; (1.20 < 1.99), large; and (> 2.0), very large [348].

The ICC was used to assess the reliability of a single data collector that examines the degree of concordance of scores between repetitions or sessions. The ICC was calculated for average measures using a 2-way random effect model of absolute agreement. Thresholds for ICC are (0.00 < 0.50), poor; (0.51 < 0.75), moderate; (0.76 < 0.90), good; and (0.91 < 1.00), excellent [349]. Data are presented as mean $\pm$ SD and the level of significance was set at $p < 0.05$. Statistical analysis was performed with SPSS v.29.0.0.0 (SPSS Statistics, IBM Corp., Armonk, NY, USA).

RESULTS

Mechanical output

Average peak concentric power was 321.1 ± 8.8 W (males: 404.5 ± 11.1 W, females: 238.0 ± 12.3 W), while average peak eccentric power was 379.7 ± 9.2 W (males: 476.5 ± 28.2 W, females: 283.2 ± 14.4 W), accounting for an average %EO of $18.3 \pm 3.2\%$ (males: $17.8 \pm 5.3\%$, females: $19.0 \pm 4.0\%$).

Post-*Flywheel Russian belt Deadlift* fatigue produced an immediate decrease in MVIC torque and RFD in both dominant and non-dominant hamstrings (**Table 7.1**).

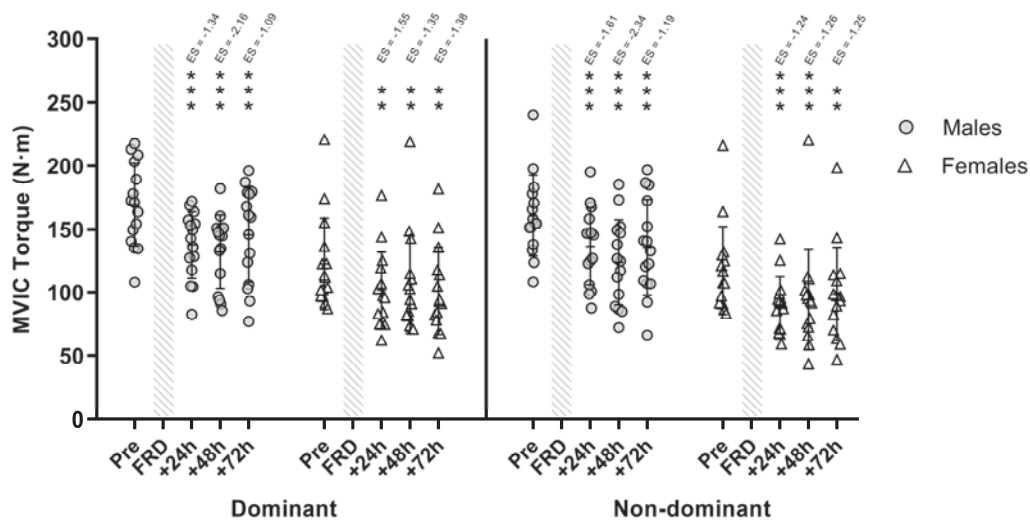
Table 7.1. Maximal voluntary isometric contraction (MVIC) torque and rate of force development (RFD) values at baseline and immediately after the training session.

		Males			Females		
		Pre-FRD	Post-FRD	ES	Pre-FRD	Post-FRD	ES
MVIC torque (N·m)	D	169.2 ± 32.7	140.1 ± 27.2	-1.54*	122.5 ± 36.3	105.0 ± 32.7	-1.51*
	ND	160.6 ± 31.8	132.8 ± 25.3	-1.40*	117.3 ± 34.9	97.4 ± 37.1	-1.35*
RFD₀₋₅₀ (N·m·s⁻¹)	D	219.5 ± 123.6	106.1 ± 45.4	-1.36*	106.6 ± 57.9	48.5 ± 18.5	-2.00*
	ND	212.8 ± 102.9	108.1 ± 37.7	-1.72*	97.1 ± 46.7	54.4 ± 20.8	-1.52*
RFD₀₋₁₀₀ (N·m·s⁻¹)	D	344.7 ± 151.3	167.8 ± 85.3	-1.78*	160.1 ± 97.4	78.6 ± 42.8	-1.69*
	ND	355.5 ± 151.8	175.3 ± 78.1	-1.86*	144.1 ± 54.7	83.2 ± 35.5	-1.63*
RFD₁₀₀₋₂₀₀ (N·m·s⁻¹)	D	484.9 ± 216.9	292.2 ± 94.6	-1.52*	249.6 ± 91.0	165.9 ± 82.8	-1.28*
	ND	455.8 ± 141.7	307.8 ± 134.1	-1.32*	218.8 ± 89.6	162.0 ± 95.4	-1.05*
pRFD₂₅ (N·m·s⁻¹)	D	755.5 ± 305.5	563.5 ± 111.7	-0.64	390.6 ± 134.1	322.5 ± 97.7	-0.69
	ND	720.4 ± 213.6	528.6 ± 137.8	-0.71*	360.2 ± 89.5	302.4 ± 95.3	-0.69*

Data are expressed as mean ± SD. (*) indicates a significant difference from the baseline value at $p < 0.05$. **D**, dominant hamstring; **ES**, effect size; **FRD**, Flywheel Russian belt Deadlift; **ND**, non-dominant hamstring; **pRFD₂₅**, the steepest part of the curve in a 25-millisecond epoch; **RFD₀₋₅₀**, RFD to 50 milliseconds; **RFD₀₋₁₀₀**, RFD to 100 milliseconds; **RFD₁₀₀₋₂₀₀**, RFD from 100 to 200 milliseconds.

Neuromuscular biomarkers panel

Maximal voluntary isometric contraction (MVIC) torque. The ICC was excellent for both dominant and non-dominant hamstrings with a value of 0.951 (95% CI 0.902 to 0.977) and 0.955 (95% CI 0.908 to 0.978), respectively. As shown in **Figure 7.3**, there was MVIC torque loss in both dominant and non-dominant hamstrings until +72h [$F(4,112) = 28.982$, $p < 0.001$, $\eta_p^2 = 0.509$]. There were nor between-limb nor between-sex differences in hamstrings MVIC torque loss [$F(4,112) = 1.471$, $p = 0.216$].

**Figure 7.3.** Mean ± SD values of maximal voluntary isometric contraction (MVIC) torque during recovery period. (*), (**), and (***) indicate a significant difference from the baseline value at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. **ES**, effect size; **FRD**, Flywheel Russian belt Deadlift.

Range of motion (ROM). There was a significant effect of time on ROM [$F(4,112) = 13.040$, $p < 0.001$, $\eta^2_p = 0.318$]. However, as shown in **Figure 7.4**, post-hoc Bonferroni corrected paired t-tests revealed that only males decreased the dominant thigh to non-dominant shin ROM approach until +48h, and the non-dominant thigh to dominant shin ROM approach until +72h.

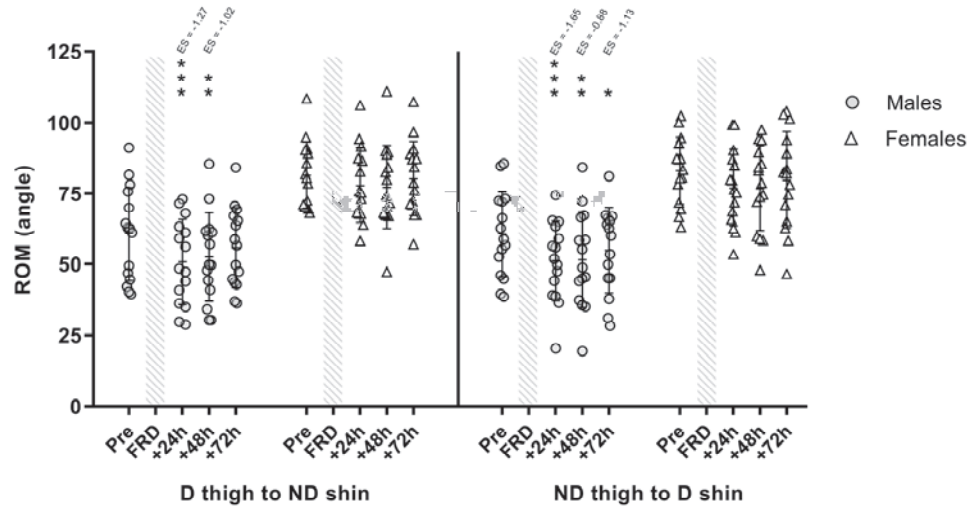


Figure 7.4. Mean $\pm$ SD values of posterior chain range of motion (ROM) during recovery period. (*), (**), and (***) indicate a significant difference from the baseline value at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. **D**, dominant; **ES**, effect size; **FRD**, Flywheel Russian belt Deadlift; **ND**, non-dominant.

Perceived soreness. As shown in **Figure 7.5**, perceived soreness was increased in both the dominant hamstring until +48h in males [$X^2(4) = 39.208$, $p < 0.001$] and females [$X^2(4) = 31.062$, $p < 0.001$], and the non-dominant hamstring until +48h in males [$X^2(4) = 39.752$, $p < 0.001$] and females [$X^2(4) = 39.854$, $p < 0.001$].

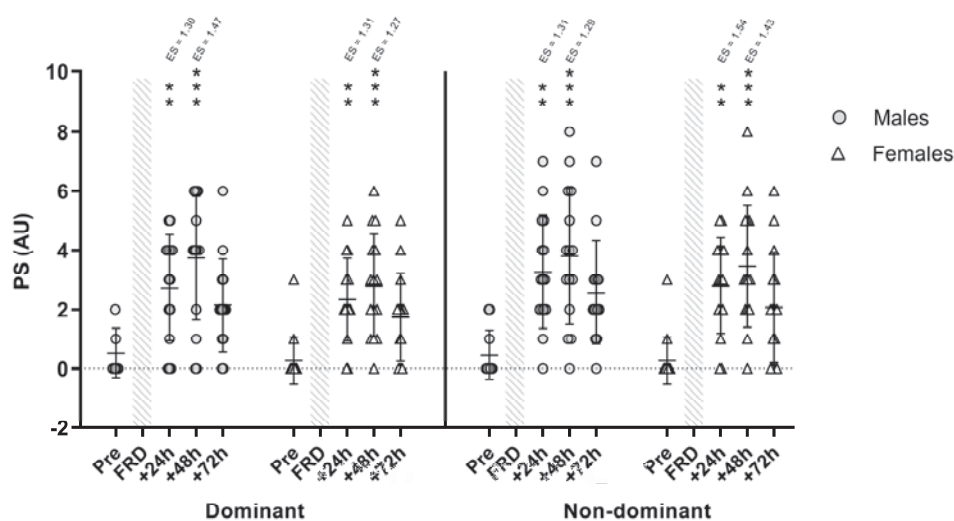


Figure 7.5. Mean $\pm$ SD values of perceived hamstrings soreness (PS) during recovery period. (*), (**), and (***) indicate a significant difference from the baseline value at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. **AU**, arbitrary units; **ES**, effect size; **FRD**, Flywheel Russian belt Deadlift.

Rate of force development (RFD). Early RFD_{0-50} [$F(4,112) = 49.217$, $p < 0.001$, $\eta_p^2 = 0.637$] and RFD_{0-100} [$F(4,112) = 45.892$, $p < 0.001$, $\eta_p^2 = 0.621$], and late $RFD_{100-200}$ [$F(4,112) = 26.325$, $p < 0.001$, $\eta_p^2 = 0.485$] were decreased in both dominant and non-dominant hamstring muscles until +72h (**Figure 7.6**), while $pRFD_{25}$ was decreased until +48h [$F(3,54) = 7.894$, $p < 0.001$, $\eta_p^2 = 0.305$]. There were no between-sex differences in RFD_{0-50} , RFD_{0-100} , $RFD_{100-200}$ and $pRFD_{25}$ during post-Flywheel Russian belt Deadlift recovery period.

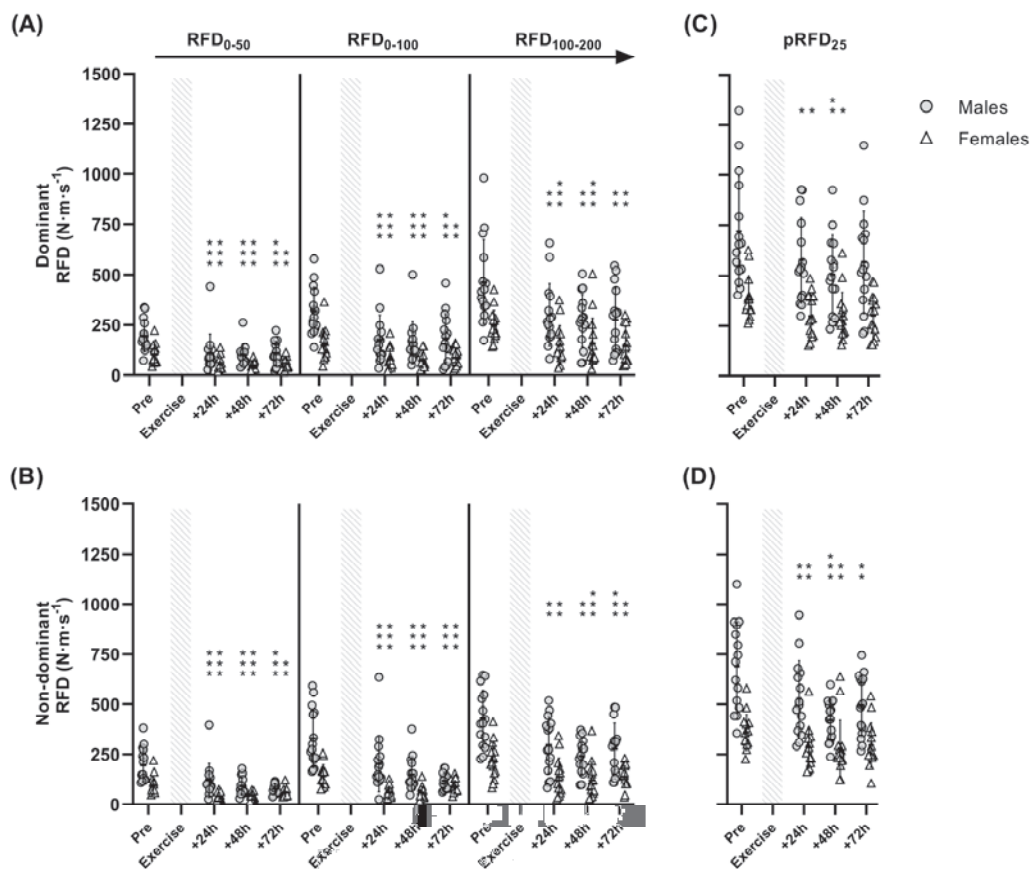


Figure 7.6. Mean $\pm$ SD values of time-intervals rate of force development (RFD) in (A) dominant and (B) non-dominant hamstring muscle, and peak RFD in (C) dominant and (D) non-dominant hamstring muscle. (*), (**), and (***) indicate a significant difference from the baseline value at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. $pRFD_{25}$, peak RFD; RFD_{0-50} , RFD to 50 milliseconds; RFD_{0-100} , RFD to 100 milliseconds; $RFD_{100-200}$, RFD from 100 to 200 milliseconds.

The standardized differences between time points and for each RFD metric in males and females are shown in **Figure 7.7**.

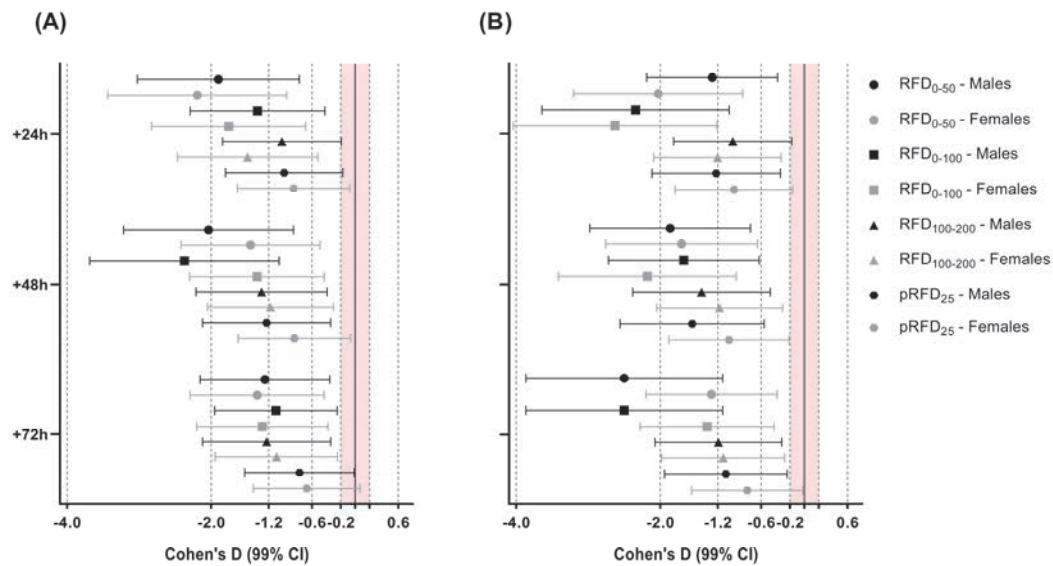


Figure 7.7. Rate of force development (RFD) standardized differences (Cohen's *d*) comparing baseline values with post-24 hours (+24h), post-48 hours (+48h) and post-72 hours (+72h) in (A) dominant and (B) non-dominant hamstring muscle. Error bars indicate uncertainty in the true mean changes with a 99% confidence interval. **CI**, confidence interval; **pRFD₂₅**, peak RFD; **RFD₀₋₅₀**, RFD to 50 milliseconds; **RFD₀₋₁₀₀**, RFD to 100 milliseconds; **RFD₁₀₀₋₂₀₀**, RFD from 100 to 200 milliseconds.

Biochemical markers panel

The extent and time course of the serum biomarkers are shown in **Table 7.2**. CK activity [$F(3,84) = 17.821$, $p < 0.001$, $\eta_p^2 = 0.389$] was increased until +48h and +72h in males and females, respectively. Similarly, sMtCK [$F(3,84) = 40.377$, $p < 0.001$, $\eta_p^2 = 0.591$], MDA [$F(3,84) = 33.482$, $p < 0.001$, $\eta_p^2 = 0.554$], OSI [$F(3,84) = 27.125$, $p < 0.001$, $\eta_p^2 = 0.492$] and circulating irisin [$F(3,84) = 47.196$, $p < 0.001$, $\eta_p^2 = 0.628$] were increased in both males and females until +72h.

Table 7.2. Flywheel Russian belt Deadlift-induced biochemical alterations during the 72-hour recovery period

Marker		Pre	+24h	+48h	+72h	
CK (IU·L ⁻¹)	M	Mean ± SD	201.3 ± 125.8	462.5 ± 323.1**	416.0 ± 537.8*	460.7 ± 857.6
		%Δ (95% CI)		59.5 (46.5 to 72.6)	39.7 (22.4 to 56.9)	29.5 (6.9 to 52.1)
		ES (95% CI)		1.08 (0.43 to 1.71)	0.66 (0.09 to 1.22)	0.51 (-0.04 to 1.04)
	F	Mean ± SD	129.1 ± 41.0	243.1 ± 182.6**	207.4 ± 107.2*	336.4 ± 370.8*
		%Δ (95% CI)		40.2 (29.6 to 50.8)	33.4 (19.4 to 47.4)	38.0 (19.7 to 56.3)
		ES (95% CI)		0.98 (0.34 to 1.58)	0.90 (0.29 to 1.49)	0.86 (0.25 to 1.44)
sMtCK (ng·ml ⁻¹)	M	Mean ± SD	15.6 ± 5.6	68.6 ± 44.6***	56.2 ± 42.6***	49.8 ± 45.0***
		%Δ (95% CI)		51.5 (38.6 to 64.4)	42.4 (29.0 to 55.8)	35.4 (19.6 to 51.2)
		ES (95% CI)		2.29 (1.30 to 3.25)	1.56 (0.79 to 2.32)	1.30 (0.59 to 1.98)
	F	Mean ± SD	10.7 ± 6.0	23.2 ± 20.5**	20.5 ± 14.3*	29.4 ± 23.5**
		%Δ (95% CI)		27.2 (21.1 to 42.4)	23.7 (7.9 to 39.5)	35.8 (17.2 to 54.4)
		ES (95% CI)		1.05 (0.41 to 1.68)	1.06 (0.41 to 1.68)	1.12 (0.46 to 1.76)
MDA (ng·ml ⁻¹)	M	Mean ± SD	1017.0 ± 249.3	1394.4 ± 620.7***	1414.7 ± 577.3***	1380.2 ± 619.1***
		%Δ (95% CI)		3.7 (1.2 to 6.3)	4.3 (1.9 to 6.7)	3.8 (1.5 to 6.1)
		ES (95% CI)		1.05 (0.40 to 1.67)	1.35 (0.63 to 2.04)	1.13 (0.46 to 1.77)
	F	Mean ± SD	910.5 ± 161.9	1163.1 ± 250.9**	1270.5 ± 375.5***	1175.5 ± 279.3**
		%Δ (95% CI)		3.5 (0.8 to 6.2)	4.6 (2.1 to 7.1)	3.6 (1.2 to 6.1)
		ES (95% CI)		1.58 (0.77 to 2.36)	1.72 (0.87 to 2.55)	1.35 (0.60 to 2.07)
OSI (AU)	M	Mean ± SD	0.487 ± 0.078	0.954 ± 0.307***	0.942 ± 0.196***	0.815 ± 0.264**
		%Δ (95% CI)		95.8 (65.5 to 126.0)	93.2 (73.8 to 112.6)	67.3 (38.8 to 95.9)
		ES (95% CI)		1.45 (0.70 to 2.17)	1.94 (1.05 to 2.79)	1.12 (0.45 to 1.76)
	F	Mean ± SD	0.519 ± 0.105	0.869 ± 0.247***	0.826 ± 0.159***	0.767 ± 0.262*
		%Δ (95% CI)		67.4 (39.0 to 95.8)	59.2 (41.0 to 77.4)	47.7 (20.9 to 74.5)
		ES (95% CI)		1.53 (0.76 to 2.28)	2.23 (1.26 to 3.18)	0.93 (0.31 to 1.53)
Irisin (ng·ml ⁻¹)	M	Mean ± SD	7.659 ± 1.173	10.991 ± 1.962**	11.266 ± 2.260***	11.475 ± 2.838***
		%Δ (95% CI)		17.8 (13.5 to 22.1)	18.8 (14.1 to 23.5)	19.3 (14.1 to 24.5)
		ES (95% CI)		1.52 (0.75 to 2.26)	1.53 (0.76 to 2.27)	1.29 (0.59 to 1.97)
	F	Mean ± SD	7.984 ± 1.049	10.101 ± 1.630**	10.220 ± 1.725**	10.347 ± 1.721**
		%Δ (95% CI)		11.2 (7.0 to 15.4)	11.7 (7.1 to 16.3)	12.3 (7.2 to 17.5)
		ES (95% CI)		1.46 (0.71 to 2.18)	1.53 (0.76 to 2.28)	1.62 (0.83 to 2.39)

Data are expressed as mean ± SD. (*), (**), and (***) indicate a significant difference from the baseline value at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. **95% CI**, 95% confidence interval; **%Δ**, delta percentage change; **AU**, arbitrary units; **CK**, creatine kinase; **ES**, effect size; **F**, females; **IU**, international units; **M**, males; **MDA**, malondialdehyde; **OSI**, oxidative stress index; **SD**, standard deviation; **sMtCK**, sarcomeric mitochondrial CK.

Dietary Intake

Macronutrient and micronutrient intakes during the peri-exercise days are summarized in **Table 7.3**.

Table 7.3. Descriptive summary of macro- and micronutrient intakes.

	Males (n = 15)	Females (n = 15)
Total energy (kcal·day ⁻¹)	2679 ± 935	2042 ± 395
Total Protein Intake (g·Kg ⁻¹ ·day ⁻¹)	1.93 ± 0.79	1.79 ± 0.65
Animal-based Protein Intake (g·Kg ⁻¹ ·day ⁻¹)	1.48 ± 0.72	1.27 ± 0.57
Plant-based Protein Intake (g·Kg ⁻¹ ·day ⁻¹)	0.45 ± 0.11	0.52 ± 0.19
Total carbohydrates (g·day ⁻¹)	245 ± 66	196 ± 32
Total lipids (g·day ⁻¹)	121 ± 53	88 ± 27
Water (g·day ⁻¹)	2933 ± 1190	2031 ± 609
Vit C (µg·day ⁻¹)	142.8 ± 91.9	95.4 ± 78.8
Vit D (µg·day ⁻¹)	4.20 ± 3.00	3.35 ± 2.66
Vit E (mg·day ⁻¹)	12.30 ± 6.62	8.59 ± 3.59

Data are expressed as mean ± SD.

DISCUSSION

The recovery kinetics of hamstring-specific neuromuscular metrics and associated biochemical markers was monitored during 72 hours after a *Flywheel Russian belt Deadlift* training session. The main findings of the study are that (i) the exercise caused a decrease in hamstrings MVIC torque, early and late RFD, and ROM, as well as an increase in PS and associated biochemical markers of muscle damage and oxidative stress during the 72-hour recovery period after the training session, and (ii) the $90^{\circ}_{hip}:20^{\circ}_{knee}$ *IPC test* can be used to monitor *Flywheel Russian belt Deadlift*-induced MVIC torque and RFD loss after flywheel training in both males and females, while the *Jurdan test* can be used to monitor ROM loss in males.

The hamstring-specific $90^{\circ}_{hip}:20^{\circ}_{knee}$ *IPC test* showed that MVIC torque was moderately to largely reduced in both dominant (males, ES = -1.09 ; females, ES = -1.38) and non-dominant (males, ES = -1.19; females, ES = -1.25) hamstrings until +72h. All male and female participants experienced MVIC torque decreases between 20% and 50%, and recovery to baseline values later than 48 hours, indicating moderate *Flywheel Russian belt Deadlift*-induced damage according to

the criteria of *Paulsen et al.*, [73]. These findings are comparable to the results reported after a sprint-based exercise [**Study I**] or after a soccer match [**Study II**] using the same test. The reduction in the $90^{\circ}_{hip}:20^{\circ}_{knee}$ IPC test output is explained by a loss of hamstrings force-generating capacity as a result of repeated eccentric lengthening contractions that induce both non-overlapping sarcomeres and excitation-contraction coupling process failures, finally leading to myofibrillar damage and membrane permeability [24,73,211]. Specifically, the positioning of the lower limbs during the $90^{\circ}_{hip}:20^{\circ}_{knee}$ IPC test selectively activates the *biceps femoris*, triggering the peak electromyographical activity [143]. Therefore, loss of performance in the $90^{\circ}_{hip}:20^{\circ}_{knee}$ IPC test is indicative of hamstrings (and most likely *biceps femoris*) residual fatigue.

Early rate of force development loss and associated sources

RFD assessment is recognized as a specific and sensitive marker of neuromuscular damage and recovery [217]. In particular, early RFD (i.e., <100 ms) has been demonstrated to provide unique and non-invasive evidence about neuromuscular recovery kinetics [221]. Interestingly, hamstring muscles showed decreases in both early RFD₀₋₅₀ and RFD₀₋₁₀₀ until +72h in male and female volunteers through the $90^{\circ}_{hip}:20^{\circ}_{knee}$ IPC test. The key determinants of early RFD are (i) activation processes, including Ca^{2+} release, diffusion, and binding, and (ii) the compliance of muscle elastic components [219]. Eccentric contraction-induced muscle strains during the *Flywheel Russian belt Deadlift* training session cause an impairment of elastic components (e.g., muscle membranes) leading to delays in muscle activation processes (e.g., excitation-contraction uncoupling) as a result of sarcolemma permeability-induced loss of Ca^{2+} homeostasis [24]. Sarcolemma permeability was confirmed in this study by the increase of blood CK activity [24]. As a consequence of sarcolemma permeability-derived Ca^{2+} inflow, there was also a rupture of the mitochondrial matrix and intermembrane proteins efflux (i.e., sMtCK) [55], leading to an increase in oxidative by-products (e.g., MDA) until +72h, which further supports sarcolemma damage.

The aforementioned mechanical and oxidative damage to myocyte integrity leads to an upregulation of the signaling pathway for irisin cleavage [274]. The myokine irisin provides protection against inflammation, oxidative and apoptosis processes [282], but also plays a key role in musculoskeletal disorders by upregulating the expression of pro-myogenic factors, stimulating satellite cell activation and ameliorating protein degradation [302]. Similar to previous results after a sprint-based exercise [**Study I**] or after a soccer match [**Study II**], irisin was increased during recovery period until +72h. We therefore suggest an involvement of irisin in muscle recovery that should be further investigated. Taken together, serum biomarkers support the loss of cellular integrity, and consequently, sarcolemma permeability-induced loss of Ca^{2+}

homeostasis, which likely affected muscle activation processes (e.g., excitation-contraction uncoupling), leading to long-lasting decrements of early RFD after the *Flywheel Russian belt Deadlift* training session.

Late rate of force development loss and associated sources

On the other hand, late RFD (i.e., >100 ms), is also a hamstring-specific muscle damage and recovery predictor [222,223]. After the *Flywheel Russian belt Deadlift* training session, hamstring muscles showed decreases in late RFD₁₀₀₋₂₀₀. Particularly, RFD₁₀₀₋₂₀₀ is a promising measure of muscle recovery, as it is reflective of muscle contractile potential [223]. The key determinants of force rise beyond 50% MVIC (i.e., late RFD) are (i) the contractile capacity of the myofibrillar complex (e.g., muscle size and architecture, peak force-generating capacity) and (ii) the capacity to transmit such contractile potential longitudinally and laterally through the muscle belly (e.g., stiffness) [215,219]. During the *Flywheel Russian belt Deadlift* training session, repeated hamstrings eccentric lengthening contractions induced non-overlapping and disrupted sarcomeres, finally leading to myofibrillar damage and loss of peak force-generating capacity [24], as demonstrated by CK increments and MVIC torque loss.

On the other hand, the connective tissue allows the transmission of forces through the muscle belly [24]. Damage to the connective tissue can be reflected by ROM loss, because the connective tissue bears the passive muscular stiffness [189]. There is limited evidence on lower limb ROM response to eccentric hamstrings training, except for *Brusco et al.*, [222] who found a decrease in hamstrings ROM (i.e., *passive straight-leg raise test*) until +72h. However, in **Study I of the thesis**, the *Jurdan test* was conducted after a sprint-based exercise and found significant ROM loss until +72h in male, but not in female participants. Similarly, we found a significant moderate decrease of the composite angle by the interaction between hip flexor and contralateral hamstring extensibility by the *Jurdan test* during the 72 hours of recovery after the *Flywheel Russian belt Deadlift* training session in male volunteers. Interestingly, although females showed a decrease in hamstrings neuromuscular capacity (i.e., MVIC torque and RFD), together with alterations in biochemical markers of muscle damage and oxidative stress, the *Jurdan test* did not detect changes in lower limb ROM after the *Flywheel Russian belt Deadlift* training session. Between-sex differences in ROM loss are likely explained by anatomical differences (e.g., muscle mass size, joint geometry) and physiological differences (e.g., greater hamstring extensibility, lower passive stiffness, better stretch tolerance) [354]. Considering that the *Jurdan test* is an indicator of increased passive stiffness derived from damage to the sarcolemma and connective tissue of the iliopsoas and/or hamstring muscles [233], and the aforementioned between-sex differences,

females might be less likely to experience ROM loss. Therefore, although females showed impaired neuromuscular function and biochemical alterations, the *Jurda test* may not show such impairment after a *Flywheel Russian belt Deadlift* training session. Also, damage to the connective tissue might be reflected by an increase in perceived soreness, because connective tissue displays significant sensory innervation (e.g., free nerve endings) [186]. *Brusco et al.*, [222] reported increases in perceived soreness until +72h, while our results showed moderate increases until +48h in the $90^{\circ}_{hip}:20^{\circ}_{knee}$ *IPC test* position, further supporting *Flywheel Russian belt Deadlift*-induced connective tissue damage. Taken together, myofibrillar complex and sarcolemma damage (evidenced by decreased MVIC torque and increased CK), together with connective tissue damage (evidenced by decreased ROM and increased perceived soreness) result in a decreased stiffness of serial/lateral force transmission through the muscle belly [223], which explains the long-lasting late RFD decrements after the *Flywheel Russian belt Deadlift* training session.

The *Flywheel Russian belt Deadlift* exercise is a promising training method to produce significant active eccentric lengthening contractions conducted with high loads and long musculotendon hamstrings lengths, which has been demonstrated to reduce the time to return to play after HSI [372]. Therefore, training programs, in order to strengthen the hamstring muscles, and particularly the BF_{LH} , might be improved by the inclusion of the *Flywheel Russian belt Deadlift* exercise. Furthermore, using the $90^{\circ}_{hip}:20^{\circ}_{knee}$ *IPC test*, regular assessment of hamstrings muscle damage through MVIC torque and RFD is an easy and cost-effective screening tool for adapting workloads and training programs to the individual hamstrings muscle tissue condition of each athlete, thereby reducing HSI risk and improving performance, especially during periods of congested schedules.

Limitations

The study also presented some limitations that must be highlighted when interpreting the results. First, there is no direct evidence of *Flywheel Russian belt Deadlift*-induced hamstrings ultrastructural damage. Future research focused on assessing direct damage to myocyte structure by histological examination would be meaningful. Second, although the used strain gauge provides a low baseline noise amplitude and is reliable for RFD recording and analysis [338], the load cell used did not provide a 1,000 Hz sampling frequency, as recommended by *Maffiuletti et al.*, [215]. However, *Ripley et al.*, [218] recently demonstrated that lowering the sampling frequency (i.e., from 1,000 to 100 Hz), increases the absolute reliability and reduces the coefficient of variability during unilateral *IPC tests* (i.e., $90^{\circ}_{hip}:20^{\circ}_{knee}$ *IPC test*). Nevertheless, the results might be verified using a measurement tool with a higher sampling frequency.

CONCLUSIONS

The *Flywheel Russian belt Deadlift* training session triggers hamstrings neuromuscular capacity decrements until 72 hours post-exercise, accompanied by biochemical indicators of muscle damage and oxidative stress in both males and females, indicating that the novel *Flywheel Russian belt Deadlift* exercise targets the hamstrings, and could be a promising hamstring-strengthening exercise. Regarding hamstrings muscle recovery, the $90^{\circ}_{hip}:20^{\circ}_{knee}$ *IPC test* can be used to monitor exercise-induced loss of MVIC torque and RFD after flywheel training in both males and females, while the *Jurdan test* allows to monitor ROM loss in males.

GENERAL DISCUSSION

8. GENERAL DISCUSSION

The main purpose of the thesis was to assess the different phases of the hamstrings muscle damage-recovery continuum after different exercise models through hamstring-specific neuromuscular tests and associated structural and oxidative damage biomarkers. For this purpose, neuromuscular metrics, through hamstring-specific tests, and biochemical markers, through blood analytics, were monitored during the recovery period after a repeated-sprint training specific to sprint-based team sports (**Study I**). Thereafter, the neuromuscular and biochemical biomarkers were applied to real sports practice and were monitored during the recovery period after a soccer match consisting of a professional-level external load (**Study II**). Finally, in order to extend the application of the neuromuscular metrics and biochemical markers to resistance training, a new flywheel exercise was specifically developed for the hamstring muscles (**Study III**), and then the biochemical and neuromuscular markers were monitored during the recovery period after such exercise (**Study IV**).

Based on the findings of the studies included in the thesis, it is proposed to establish a screening model to monitor the hamstring muscles through the muscle damage-recovery continuum, using hamstring-specific neuromuscular tests which may be sensitive to structural and oxidative impairments during primary and/or secondary muscle damage, in both women and men (**Table 8.1**).

Table 8.1. Proposed screening model comprising neuromuscular and biochemical markers for monitoring hamstrings muscle damage and recovery.

ASSESSMENT OF HAMSTRINGS MUSCLE DAMAGE AND RECOVERY			
	Physiological process	Marker	Assessment test
Primary damage	Myofibrillar damage and excitation-contraction uncoupling	MVIC Torque RFD	$90^{\circ}_{hip}:20^{\circ}_{knee}$ test
	Connective tissue damage	ROM	<i>Jurdan test</i>
		Perceived soreness	<i>NRS</i>
	Structural damage and sarcolemma permeability	Muscle-specific enzymes and proteins	<i>CK</i> <i>sMtCK</i>
Secondary damage	Oxidative damage	Oxidative stress	<i>MDA</i> <i>OSI</i>
	Inflammation/remodeling		<i>Irisin</i>

CK, creatine kinase; **MDA**, malondialdehyde; **MVIC**, maximal voluntary isometric contraction; **NRS**, numerical rating score; **OSI**, oxidative stress index; **RFD**, rate of force development; **ROM**, range of motion; **sMtCK**, sarcomeric mitochondrial CK.

8.1. Assessment of hamstring muscles primary damage and recovery

The series of events that contribute to the development of primary muscle damage are based on excessive mechanical load to the skeletal muscle tissues [7,29–31], which include (i) the myofibrillar complex and the sarcolemma, affecting the contractile potential of the muscle and increasing the permeability of the sarcolemma [24], (ii) the T-tubule system, leading to failures in muscle activation processes (e.g., excitation-contraction uncoupling) [33,39], and (iii) the muscle connective tissue, causing a worse transmission of forces through the muscle belly [24].

8.1.1. Hamstrings capacity to generate maximal force

By the use of the $90^{\circ}_{hip}:20^{\circ}_{knee}$ IPC test, the ICC was excellent for both dominant and non-dominant MVIC torque of the hamstring muscles across the studies, with a value of 0.965 (95% CI 0.942 to 0.979) and 0.977 (95% CI 0.965 to 0.986), respectively. Accordingly, the $90^{\circ}_{hip}:20^{\circ}_{knee}$ IPC test provides an excellent reliability in the measurement of the hamstring muscles MVIC torque. As shown in **Figure 8.1**, we reported moderate decreases in hamstrings MVIC torque during the recovery period after a sport-specific repeated-sprint training (**Study I**) and after a soccer match (**Study II**), compared to large losses during the recovery period after the *Flywheel Russian belt Deadlift* training session (**Study IV**).

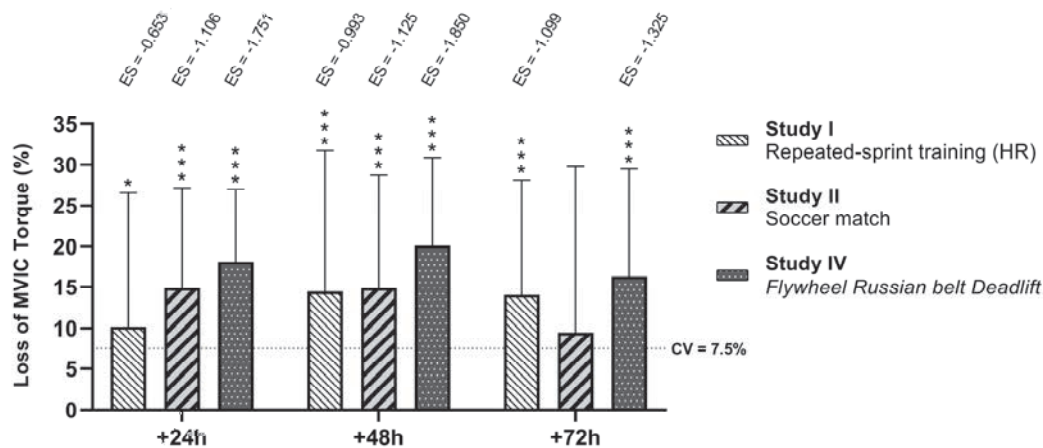


Figure 8.1. Mean $\pm$ SD values of maximal voluntary isometric contraction (MVIC) torque loss during the post-exercise recovery period in each study. (*), (**), and (***) indicate a significant difference from the baseline value at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. CV, coefficient of variability; ES, effect size, HR, high responders.

The differences between studies in the loss of hamstrings MVIC torque might be caused by different factors.

First, a repeated-sprint training (**Study I**) caused the lowest MVIC torque loss, owing to a high inter-individual variability in the response of the participants. Accordingly, the cohort of

individuals was stratified according to susceptibility (i.e., responsiveness) to primary muscle damage on the basis of the magnitude of MVIC torque loss in the hamstrings after the repeated-sprint training. As a consequence, the variability of the results was diminished, showing that low responders (LR) participants did not vary their MVIC torque during the post-exercise recovery period, whereas high responders (HR) individuals lost MVIC torque in the hamstring muscles to a similar magnitude to the loss after the soccer match (**Study II**).

Second, the recovery of hamstrings MVIC torque within 72 hours after the soccer match, compared to the other two exercise models, is attributable to the fact that the participants in **Study II** were regional first-division soccer players, and might be presumably more adapted to the soccer match loading. The difference in force-generating capacity loss between the sprint-based exercises (**Studies I and II**) and the *Flywheel Russian belt Deadlift* (**Study IV**) might arise from the type of exercise conducted, as it is a potent modifier of EIMD. Although both sprint-based exercises involved a large amount of HSRd (> 400 m), the *Flywheel Russian belt Deadlift* is an analytical structure-based exercise that has been proved to trigger a significant activation of the hamstrings, especially the *biceps femoris* (**Study III**). Finally, between-studies differences are also attributable to the fact that external loads were not matched between protocols for sprint-based exercises (**Studies I and II**) and flywheel training (**Study IV**).

Similar to our results, *Baumert et al.*, [173] and *Chen et al.*, [380] found a 20% loss of hamstrings strength at 48 hours after a repeated-sprint training session. In the case of the impact of a soccer match, small decreases in hamstrings force-generating capacity were found until 24 hours (-5%) using the *prone leg curl test* [362], and until 48 hours (-6%) using the *Nordic hamstring exercise* [381]. In contrast, the use of the $90^{\circ}_{hip}:20^{\circ}_{knee}$ IPC test resulted in a reduction of hamstrings MVIC torque greater than 15% during recovery after a soccer match. Considering that the output between the *Nordic hamstring exercise*, the *isometric 15° knee flexion test* and the $90^{\circ}_{hip}:20^{\circ}_{knee}$ IPC test are not correlated with each other, the mechanical properties and/or hamstring muscle evaluated in each test are different [205]. Therefore, the $90^{\circ}_{hip}:20^{\circ}_{knee}$ IPC test might be more sensitive to team sports-induced hamstrings primary damage.

In any case, the reduction in MVIC torque measured by the $90^{\circ}_{hip}:20^{\circ}_{knee}$ IPC test is explained by a loss in force-generating capacity of the hamstring muscles due to repeated eccentric lengthening contractions during repeated late swing phases of the running gait cycle (**Studies I and II**) or repetitions of the *Flywheel Russian belt Deadlift* exercise (**Study IV**). This results in non-overlapping sarcomeres and excitation-contraction uncoupling, leading to myofibrillar complex damage and sarcolemma permeability [24,33]. Accordingly, given that the design of the test causes a lengthening of the hamstrings and triggers a selective activation and peak electromyographic activity of the *biceps femoris* [143], the loss of MVIC torque output measured

by the $90^{\circ}_{\text{hip}}:20^{\circ}_{\text{knee}}$ IPC test is indicative of hamstrings (and likely the *biceps femoris*) primary muscle damage, which represents a risk factor for developing HSI [14]. Therefore, MVIC torque monitoring through the $90^{\circ}_{\text{hip}}:20^{\circ}_{\text{knee}}$ IPC test is an easy and cost-effective marker of primary muscle damage and hamstrings muscle recovery.

8.1.2. Rate of force development: sensitive metrics for monitoring primary muscle damage

Complementary to MVIC torque, RFD provides valuable information on the ability of a muscle or muscle group to rapidly develop force at the onset of contraction [213,214]. Therefore, it has been recommended to monitor rapid force production (i.e., RFD) during post-exercise recovery, in order to identify hamstrings neuromuscular deficits that increase HSI risk [218]. However, the assessment of RFD metrics during the recovery period is a research topic that has been rarely studied to date. To the best of our knowledge, RFD metrics during the recovery period after a soccer match or after a flywheel hamstrings training have never been studied before.

First, the assessment of early RFD metrics (i.e., < 100 ms) to monitor primary muscle damage in the hamstring muscles was conducted during the recovery period after a soccer match (**Study II**) and after the *Flywheel Russian belt Deadlift* training session (**Study IV**). The $90^{\circ}_{\text{hip}}:20^{\circ}_{\text{knee}}$ IPC test provided a good-to-excellent ICC for both dominant and non-dominant RFD metrics of the hamstring muscles across the studies. Accordingly, the $90^{\circ}_{\text{hip}}:20^{\circ}_{\text{knee}}$ IPC test provides an adequate reliability in the measurement of the hamstring muscles RFD metrics during post-exercise recovery period (**Table 8.2**).

Table 8.2. Reliability of maximum voluntary isometric contraction (MVIC) torque and rate of force development (RFD) metrics across the studies.

	Dominant		Non-dominant	
	ICC	95% CI	ICC	95% CI
MVIC Torque	0.965	0.942 to 0.979	0.977	0.965 to 0.986
RFD₀₋₅₀	0.885	0.809 to 0.933	0.858	0.770 to 0.916
RFD₀₋₁₀₀	0.901	0.836 to 0.943	0.871	0.791 to 0.924
RFD₀₋₂₅₀	0.912	0.848 to 0.951	0.911	0.854 to 0.947
RFD₁₀₀₋₂₀₀	0.903	0.815 to 0.953	0.898	0.809 to 0.949
pRFD₂₅	0.905	0.815 to 0.955	0.848	0.717 to 0.924

ICC, intraclass correlation coefficient; **pRFD₂₅**, peak RFD; **RFD₀₋₅₀**, RFD to 50 milliseconds; **RFD₀₋₁₀₀**, RFD to 100 milliseconds; **RFD₀₋₂₅₀**, RFD to 250 milliseconds; **RFD₁₀₀₋₂₀₀**, RFD from 100 to 200 milliseconds.

We reported large decreases of hamstrings early RFD metrics (i.e., RFD_{0-50} and RFD_{0-100}) during the recovery period after a soccer match, and after the *Flywheel Russian belt Deadlift* training session (**Figure 8.2**). Interestingly, although MVIC torque after the soccer match was recovered at 72 hours, early RFD metrics remained unrecovered. Moreover, and similarly to the loss of MVIC torque, early RFD losses are higher during recovery period after the *Flywheel Russian belt Deadlift* training session (**Study IV**), in comparison to after the soccer match (**Study II**).

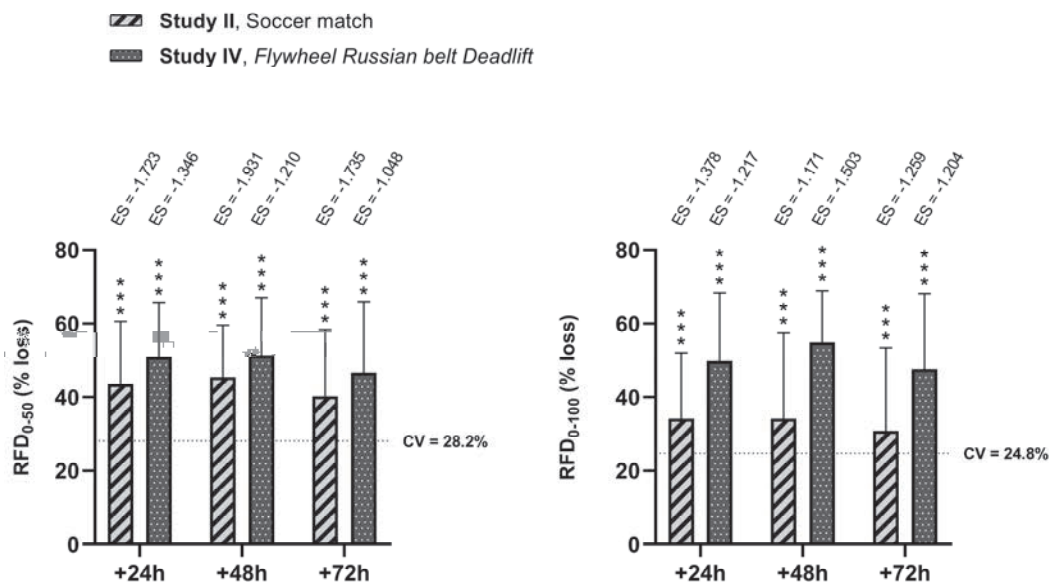


Figure 8.2. Mean $\pm$ SD values of early rate of force development (RFD) loss during the post-exercise recovery period in each study. (*), (**), and (***) indicate a significant difference from the baseline value at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. CV, coefficient of variability; ES, effect size.

To date, only the study by *Brusco et al.*, [222] focused on hamstrings RFD during the recovery period after an eccentric knee-dominant exercise (i.e., *seated isokinetic leg curl*). Similarly to our findings, *Brusco et al.*, [222] found that RFD_{0-100} were decreased (-49%) during the 72-hour recovery period after the hamstring-damaging exercise model. However, RFD_{0-50} was recovered in 48 hours. Contrary to our findings, these conflicting results in RFD_{0-50} might be caused by (i) the differences in the hamstring-damaging exercise model (knee-dominant *seated isokinetic leg curl* vs hip dominant *Flywheel Russian belt Deadlift*) that may have affected different hamstring muscles, (ii) the knee-dominant hamstrings protocol conducted did not generate acute RFD_{0-50} fatigue, whereas the hip-dominant *Flywheel Russian belt Deadlift* exercise generated significant immediate fatigue (-44.0% to -54.5%), and/or (iii) the assessment test used in **Studies II and IV** (i.e., $90^{\circ}_{hip}:20^{\circ}_{knee}$ IPC test) might be more sensitive to changes in hamstrings early RFD metrics.

Early RFD is influenced by activation processes and the compliance of muscle elastic elements [219]. During repeated late swing phases of the running gait cycle (**Study II**) or repetitions of the *Flywheel Russian belt Deadlift* exercise (**Study IV**), there are eccentric lengthening contractions-

induced muscle strains, leading to a disruption of the muscle elastic elements (e.g., sarcolemma). Damage to the sarcolemma was confirmed in volunteers of both studies by an increase in CK activity, and oxidative damage by an increase in MDA [24]. Thus, upregulation of CK activity, together with MDA increments, provide evidence on the presence of structural damage and permeability of the sarcolemma. Taken together, the loss of myocyte integrity and the resulting sarcolemma permeability-induced loss of Ca^{2+} homeostasis, likely affected muscle activation processes (e.g., excitation-contraction uncoupling) [24], leading to long-lasting decrements of early RFD after the soccer match (**Study II**) and the *Flywheel Russian belt Deadlift* training session (**Study IV**).

Therefore, considering that (i) the $90^{\circ}_{\text{hip}}:20^{\circ}_{\text{knee}}$ IPC test provides an adequate reliability in the measurement of hamstrings early RFD metrics, and that (ii) early RFD decrements are greater than MVIC torque decrements, early RFD monitoring is a more sensitive indicator of primary muscle damage recovery of the hamstring muscles, compared to MVIC torque.

Early RFD of the hamstring muscles, in addition to determining the capacity to generate rapid force, is a performance determinant during a sprint [365]. In the studies where the exercising model was based on repeated sprints (**Studies I and II**), the performance and mechanical properties of the ability to sprint 40 meters were monitored during the post-exercise recovery period. Interestingly, the sprinting performance was significantly reduced during the recovery period in both studies, showing an increase in the split time to reach 10 meters, and the total time to reach 40 meters. Decreases in sprint performance are caused by a lower capacity to apply anteroposterior ground reaction forces. During sprinting, forward orienting the ground reaction force is a critical determinant during the acceleration phase, mainly mediated by the hamstring muscles [170,171], as strong correlations have been found between hamstrings muscle volume and sprint performance [382,383]. By relating performance to the mechanical properties of the sprint, the increase in sprint times is attributable to a decrease in V_0 (i.e., high-speed horizontal force-generating capacity) and F_0 (i.e., low-speed horizontal force-generating capacity). Accordingly, extrapolation of individual linear F-V relationships also showed unrecovered A_{max} during the recovery period in both studies. Hence, a lower F_0 (i.e., peak horizontal force production), as measured by a 40-meter maximal linear sprint test, is associated with an increased HSI risk [366,367].

Furthermore, our results also showed a significant association between losses of early RFD and decrements in sprint performance (**Figure 8.3**). This is attributed to the fact that ground contact time during sprinting is less than 150 milliseconds [171].

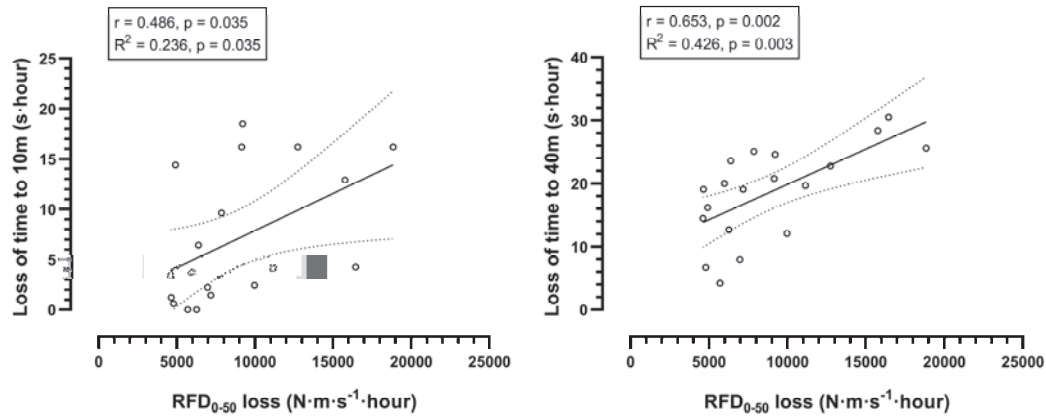


Figure 8.3. Correlations between long-lasting reductions in sprint performance and early RFD₀₋₅₀. Dotted lines represent 95% confidence intervals.

On the other hand, the assessment of late RFD metrics (i.e., > 100 ms) to monitor primary muscle damage and recovery of the hamstring muscles was also conducted after a soccer match (**Study II**) and after the *Flywheel Russian belt Deadlift* training session (**Study IV**). In addition, a new late metric (i.e., RFD₁₀₀₋₂₀₀) was incorporated in **Study IV**, as it has recently been shown to be a strong predictor of the contractile potential of skeletal muscle tissue [222]. Interestingly, we reported decreases of hamstrings RFD₀₋₂₅₀ until 48 hours of recovery after a soccer match (**Study II**), whereas after the *Flywheel Russian belt Deadlift* training session (**Study IV**), hamstrings RFD₀₋₂₅₀ remained unrecovered during the 72-hour period. Similarly, hamstrings RFD₁₀₀₋₂₀₀ failed to recover within 72 hours after the *Flywheel Russian belt Deadlift* training session (**Figure 8.4**).

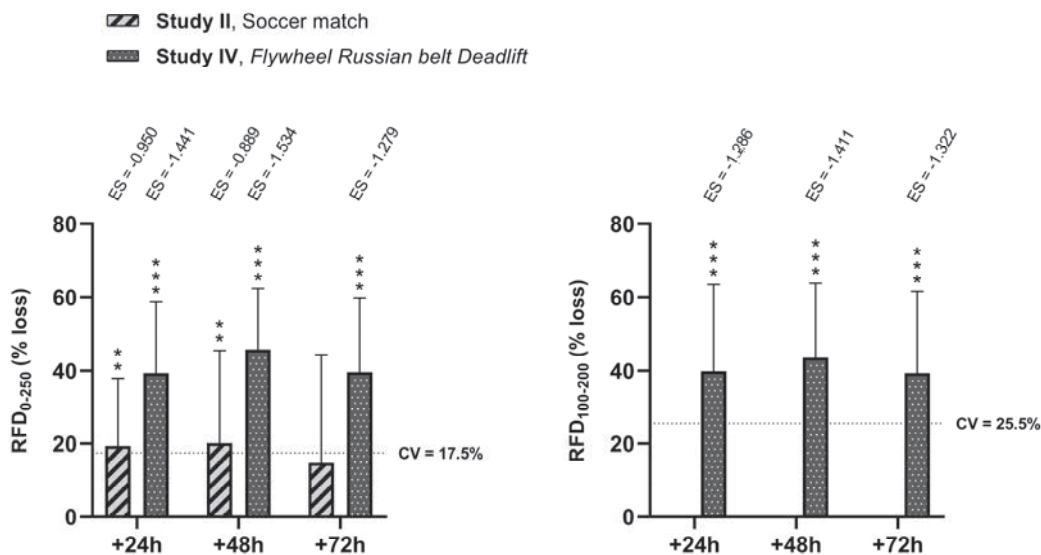


Figure 8.4. Mean $\pm$ SD values of late rate of force development (RFD) loss during the recovery period in each study. (*), (**), and (***) indicate a significant difference from the baseline value at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. CV, coefficient of variability; ES, effect size.

The main determinants of force rise beyond 50% MVIC (i.e., late RFD) are (i) the contractile capacity of the myofibrillar complex (e.g., muscle size and architecture, MVIC torque) and (ii) the capacity to transmit such contractile potential longitudinally and laterally through the muscle belly (e.g., stiffness) [215,219].

First, during repeated late swing phases of the running gait cycle (**Study II**) or repetitions of the *Flywheel Russian belt Deadlift* training session (**Study IV**), there are eccentric lengthening contractions that induce non-overlapping and disrupted sarcomeres, finally leading to myofibrillar complex damage and loss of peak force-generating capacity [24], as previously demonstrated by loss of hamstrings MVIC torque. Therefore, since MVIC torque is a robust predictor of late RFD [215], the recovery of RFD₀₋₂₅₀ prior to 72 hours post-soccer match might be explained by the fact that MVIC torque was also recovered prior to 72 hours during the recovery period.

Secondly, the capacity to transmit the muscle contractile potential longitudinally and laterally through the muscle belly, which is also a predictor of late RFD, is mediated by the connective tissue [24]. Thus, damaging the connective tissue would decrease the transmission of forces through the muscle belly, affecting late RFD metrics. In this regard, damage to the connective tissue could be reflected by both loss of joint ROM and/or increased perceived muscle soreness [186,189]. Accordingly, damage to muscle connective tissue was reported in volunteers of both studies, reflected by a loss of posterior chain ROM until 72 hours in both studies, and increased perceived hamstrings soreness until 48 hours after a soccer match and until 72 hours after the *Flywheel Russian belt Deadlift* training session. Hence, the recovery of late RFD within 72 hours after a soccer match, in addition to being explained by the recovery of hamstrings MVIC torque, might also be attributed to a lesser damage to the muscle connective tissue, enabling a better transmission of forces through the muscle belly.

Taken together, myofibrillar complex and sarcolemma damage (evidenced by decreased MVIC torque and increased CK activity and MDA), together with connective tissue damage (evidenced by decreased ROM and increased perceived soreness), result in a decreased stiffness of serial/lateral force transmission through the muscle belly, which explains the long-lasting late RFD decrements after the soccer match (**Study II**) and after the *Flywheel Russian belt Deadlift* training session (**Study IV**).

Combining all the results on the capacity of the hamstring muscles to generate rapid force (i.e., early and late RFD), and for the same reasons as the loss of MVIC torque, the reduction in hamstrings RFD is greater after the *Flywheel Russian belt Deadlift* training session (**Study IV**), compared to after the soccer match (**Study II**). In addition, early RFD metrics decrements are greater compared to MVIC torque or late RFD metrics decrements in both studies. Finally, none of the studies found significant between-sex differences regarding the magnitude and recovery

kinetics of maximal (i.e., MVIC torque) and rapid (i.e., RFD) force-generating capacity loss. These results confirm previous evidence indicating that the magnitude of force-generating capacity loss after muscle-damaging exercises is similar between women and men [76,82].

Therefore, considering that (i) the assessment of early RFD metrics through the $90^{\circ}_{hip}:20^{\circ}_{knee}$ *IPC test* provides an adequate reliability, (ii) early RFD metrics decrements are greater compared to MVIC torque or late RFD metrics decrements, and (iii) early RFD decrements are correlated with sprinting performance loss, early RFD metrics monitoring through the $90^{\circ}_{hip}:20^{\circ}_{knee}$ *IPC test* is a simple, cost-effective, low demanding, and more sensitive indicator of primary muscle damage and recovery, in both women and men.

8.1.3. Range of motion and perceived soreness: markers of damage to the connective tissue

The assessment of posterior chain ROM also provides information on the state of connective muscle tissue [189,191–193]. By the use of the *Jurdan test*, we reported moderate decreases of posterior chain ROM in male participants during the 72-hour recovery period (**Figure 8.5**).

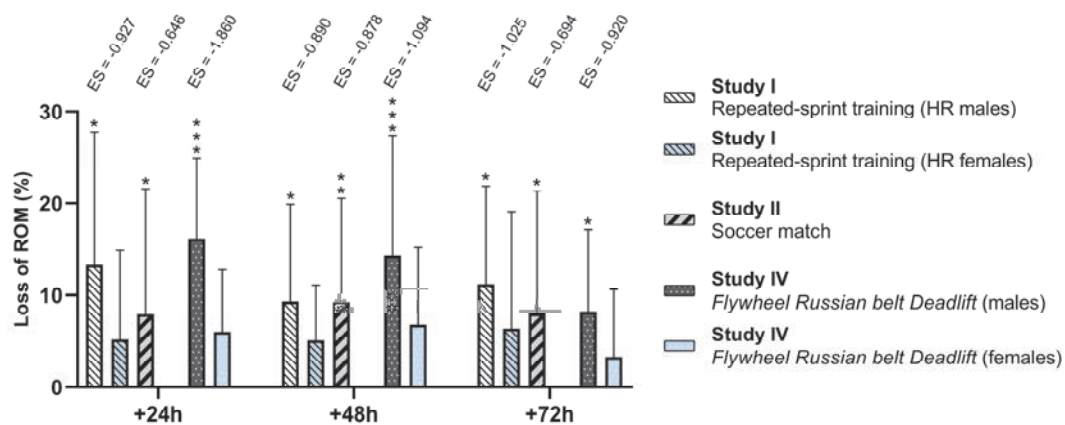


Figure 8.5. Mean $\pm$ SD values of posterior chain range of motion (ROM) loss during the post-exercise recovery period in each study. (*), (**), and (***) indicate a significant difference from the baseline value at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. ES, effect size, HR, high responders.

Surprisingly, female participants showed no significant variations in posterior chain ROM during the recovery period after a repeated-sprint training (**Study I**) or after the *Flywheel Russian belt Deadlift* training session (**Study IV**). Similar to our results, *Minahan et al.*, [384] also reported no significant changes in the lower limb ROM of collegiate team-sportswomen after repeated-sprint training. This results are attributable to female-specific anatomical (e.g., joint geometry) and physiological characteristics (e.g., lower passive stiffness) [190,354]. In particular, it has been proven that hamstrings muscle stiffness in females is approximately 70-80% compared to that of males [385]. Therefore, although females showed a decrease in hamstrings neuromuscular capacities (i.e., MVIC torque, RFD and sprint performance), together with alterations in

biochemical markers of structural and oxidative damage, the *Jurdan test* detected no changes in posterior chain ROM after the repeated-sprint training (**Study I**) or after the *Flywheel Russian belt Deadlift* training session (**Study IV**). Consequently, the *Jurdan test* seems to be ineffective for monitoring posterior chain ROM losses after muscle-damaging exercises in females.

Other research after repeated-sprint training found posterior chain ROM loss (-10%) until 24 hours, measured by the *passive straight leg raise test* [380], while after a soccer match, no variations in posterior chain ROM have been reported measured by the *Thomas test* [362]. Accordingly, the assessment of posterior chain ROM by the *Jurdan test* might be more sensitive to variations caused by sprint-based exercises, compared to the *passive straight leg raise test* or the *Thomas test*. Overall, the loss of differential angle between the actively lengthened leg shin angle and the opposite leg passive thigh angle, as reported by the *Jurdan test*, is an indicator of increased passive stiffness derived from primary damage to the sarcolemma and connective tissue of the iliopsoas and/or hamstring muscles [233].

On the other hand, considering the significant sensory innervation of the muscle connective tissue [236], assessment of perceived muscle soreness can be used, in combination with joint ROM, as a marker of damage to muscle connective tissue [184–188]. Using the relaxed positioning of the lower limb during the $90^{\circ}_{\text{hip}}:20^{\circ}_{\text{knee}}$ *IPC test*, we reported moderate-to-large increments of perceived hamstrings soreness during the recovery period after a sport-specific repeated-sprint training (**Study I**), after a soccer match (**Study II**), and after the *Flywheel Russian belt Deadlift* training session (**Study IV**). However, as shown in **Figure 8.6**, the perceived soreness in the hamstring muscles decreased to baseline values at 72 hours after the soccer match.

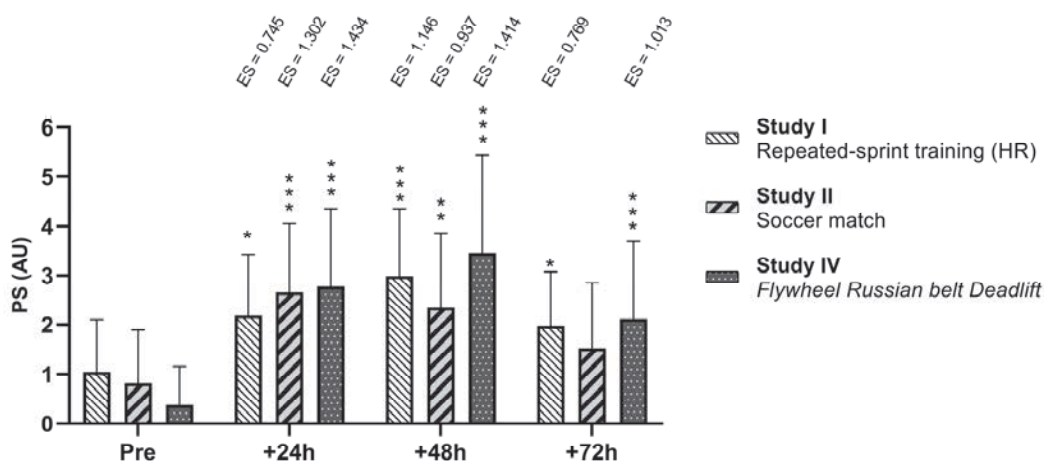


Figure 8.6. Mean $\pm$ SD values of perceived soreness (PS) in the hamstrings during the post-exercise recovery period in each study. (*), (**), and (***) indicate a significant difference from the baseline value at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. AU, arbitrary units; ES, effect size, HR, high responders.

Similarly, other research after repeated-sprint training have found increases in self-report based unidimensional scales of perceived soreness until 24 hours (+1.3 arbitrary units) [384] and 48 hours (+2.9 arbitrary units) [173], while after a soccer match, increases in perceived hamstrings soreness have been reported until 24 hours (+1.5 arbitrary units) [362] and 72 hours (+1.4 arbitrary units) [358]. These conflicting results are likely because perceived soreness is a subjective measure depending on the analyzed position and caused by a combination of factors (e.g., swelling, noxious chemicals, by-products of inflammation) [6,27]. To conclude, combining the results on the posterior chain ROM loss and increased perceived hamstrings soreness, the muscle connective tissue of the volunteers in ***Studies I, II and IV*** was affected during the 72-hour post-exercise recovery period, which deteriorated the transmission of forces through the muscle belly.

8.2. Blood biomarkers provide insights into the underlying mechanism of hamstrings residual fatigue

The use of serum biomarkers, in combination with neuromuscular metrics, provides a more in-depth understanding of secondary muscle damage [63]. The event that initiates the process of self-feeding secondary muscle damage is the permeability of the sarcolemma, and consequent inflow and outflow of elements to and from the sarcoplasm, leading to an alteration of intracellular homeostasis [24,33,62].

However, such phenomena reported in the studies of this thesis as sustained sarcolemma permeability over time, increases in the concentration of the sarcomeric mitochondrial isoform of CK (i.e., sMtCK) and associated oxidative processes need to be highlighted and discussed in detail.

8.2.1. Sarcolemma permeability

CK activity is the most consistent and frequently used biomarker that indicates sarcolemma permeability among the scientific literature [24,33,63,74]. Accordingly, we reported increases in CK activity, peaking at 24 hours post-exercise in the 3 studies, and progressively recovering to baseline values.

However, the most remarkable point to note is the magnitude of the increases. Interestingly, the extent of CK activity was smaller after the *Flywheel Russian belt Deadlift (Study IV)* compared to repeated-sprint training (*Study I*), or soccer match (*Study II*), which induced the largest increases in CK activity (*Figure 8.7*).

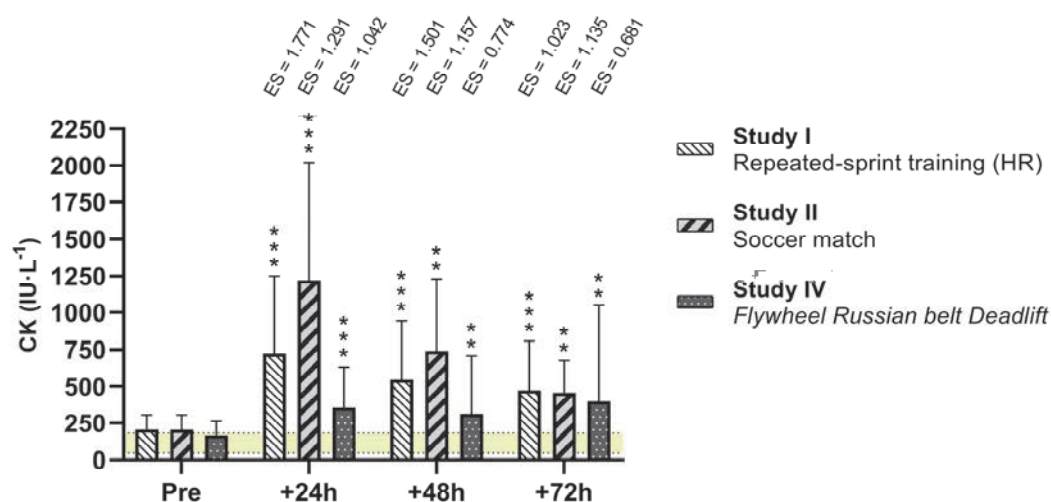


Figure 8.7. Mean $\pm$ SD values of creatine kinase (CK) activity during the post-exercise recovery period in each study. The yellow range represents the normal reference values. (*), (**), and (***) indicate a significant difference from the baseline value at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. ES, effect size, HR, high responders.

The larger increase in CK activity after global structure-based exercises, compared to after the *Flywheel Russian belt Deadlift*, is based on the fact that serum biomarkers are dependent on the volume of affected muscle tissue [33]. The muscles that are involved in sprint-based exercises are not only the hamstring muscles, but also other muscle groups are involved (e.g., gastrocnemius, gluteus maximus). However, the *Flywheel Russian belt Deadlift*, given its analytical structure, focuses the training workload on the hamstrings muscles, particularly on the lateral positioning muscles, that is the *biceps femoris* (**Study III**). Therefore, the lower magnitude of CK activity after the *Flywheel Russian belt Deadlift* compared to after a repeated-sprint training or a soccer match is attributed to a lower quantity of damaged muscles.

An increase in blood CK activity is indicative of sarcolemma permeabilization that perturb the intracellular homeostasis. Specifically, loss of sarcoplasmic Ca^{2+} homeostasis is critical in the development of self-feeding secondary muscle damage, triggering a sequence of proteolytic and phospholipolytic phenomena, which further damages the myofibrillar complex and the myocyte cytoskeleton [44]. As a result, the primary muscle damage and associated symptomatic processes (e.g., force-generating capacity loss) are further exacerbated.

8.2.2. Secondary damage: mitochondria and oxidative stress

The increase in sarcoplasmic Ca^{2+} concentration induced by sarcolemma permeability is the triggering phenomenon for self-feeding secondary muscle damage and subsequent oxidative processes [44], causing a loss of mitochondrial integrity [11,57]. Accordingly, we reported sMtCK increases during the 72-hour recovery period (**Figure 8.8**).

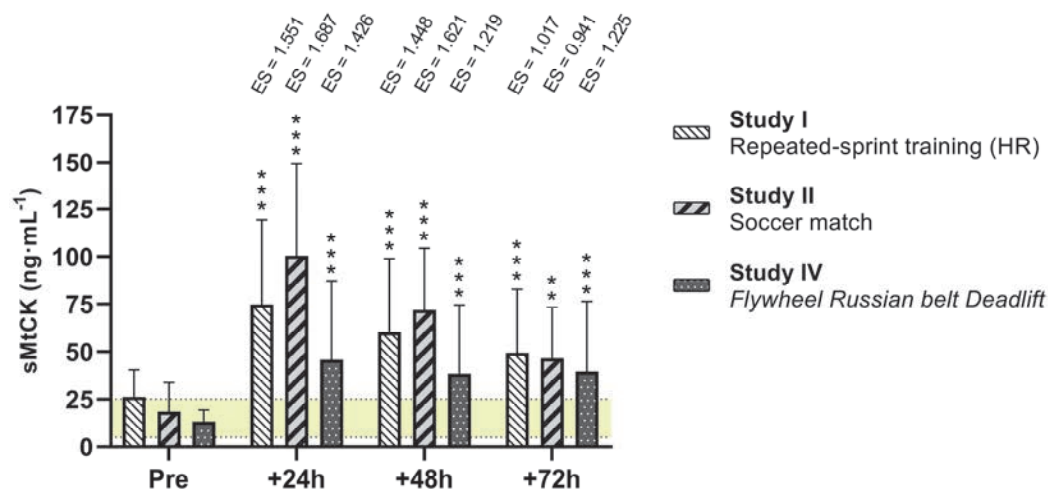


Figure 8.8. Mean $\pm$ SD values of sarcomeric mitochondrial creatine kinase (sMtCK) during the post-exercise recovery period in each study. The yellow range represents the normal reference values. (*), (**), and (***) indicate a significant difference from the baseline value at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. ES, effect size, HR, high responders.

The extent and recovery kinetics of sMtCK were also identical to those of CK activity, peaking at 24 hours post-exercise in the 3 studies, and progressively recovering to baseline values. In addition, higher sMtCK values were found after global structure-based exercises (**Studies I and II**) compared to the analytical structure-based exercise (**Study IV**). In this sense, a strong correlation was observed between the increase in CK activity and sMtCK values (**Figure 8.9**).

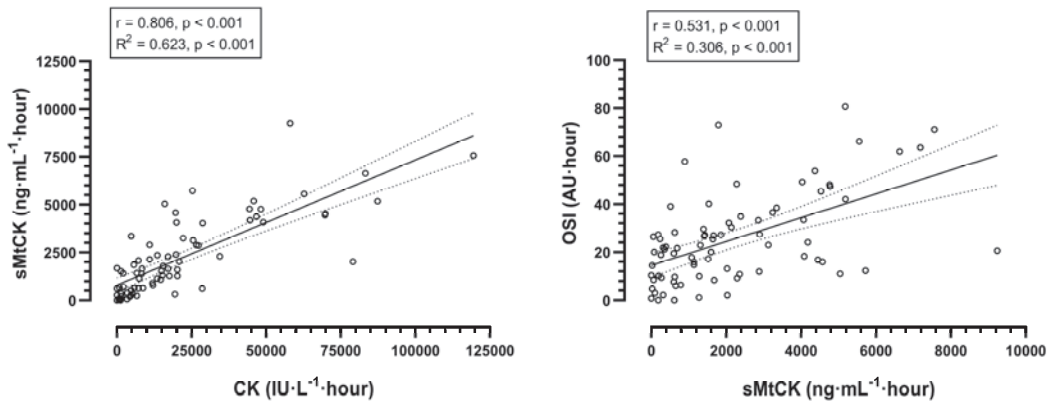


Figure 8.9. Correlations between increments of creatine kinase (CK) activity with sarcomeric mitochondrial CK (sMtCK), and sMtCK with oxidative stress index (OSI). Dotted lines represent 95% confidence intervals.

The rationale for these correlations might be based on the permeability of the sarcolemma, which leads to an increase in sarcoplasmic Ca^{2+} concentration. Consequently, mitochondria absorb Ca^{2+} in order to restore intracellular homeostasis [50], until the moment that excessive Ca^{2+} is absorbed and mitochondria disruptions are produced, resulting in the release of intermembrane elements (i.e., sMtCK) [54].

Likewise, the correlation between damage to muscle mitochondria and the increase in oxidative stress values was moderate. This finding can be attributed to the muscle mitochondria disruptions, leading to uncontrolled release of free radicals and increased oxidative stress [55].

The reference biomarker of oxidative damage in the studies included in the thesis was MDA. In complement to MDA, the OSI has also been quantified, because integrative-based quantification methods such as OSI has been recommended to fully understand the complex interaction between muscle damage and oxidative stress in the area of sport sciences [264,269].

Interestingly, we reported moderate MDA and OSI increases during the 72-hour post-exercise recovery period in the three studies (**Figure 8.10**).

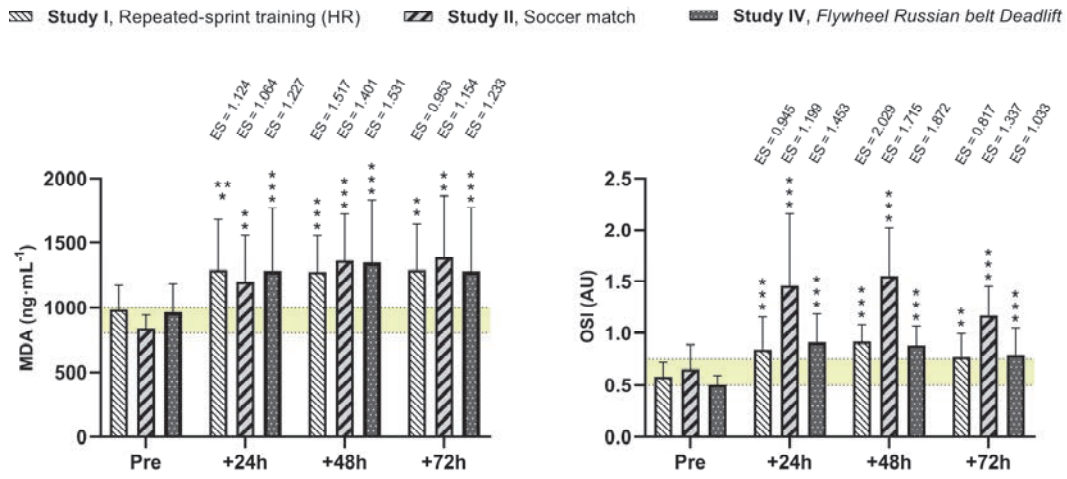


Figure 8.10. Mean $\pm$ SD values of malondialdehyde (MDA) and oxidative stress index (OSI) during the post-exercise recovery period in each study. The yellow range represents the normal reference values. (*), (**), and (***) indicate a significant difference from the baseline value at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. AU, arbitrary units; ES, effect size, HR, high responders.

MDA increases have also been found during the recovery period after a soccer match [363] and after eccentric resistance training of the lower extremities, in both trained and untrained volunteers [263]. In this sense, increases in MDA are the result of lipid peroxidation by free radicals [260], further supporting structural damage to the myocyte, as indicated by increased CK activity. However, the correlation between MDA increases and CK activity was significant but weak ($r = 0.392$, $p = 0.001$). This is attributed to the fact that sarcolemma permeability is not only a consequence of direct damage to the membrane, but also of other mechanisms such as the stretch-activated channels [41,42]. Moreover, the correlation between MDA increases and OSI was also significant but weak ($r = 0.329$, $p = 0.003$). In this case, such weak correlation is attributed to the fact that OSI indicates a global oxidative state beyond the lipid peroxidation evidenced by the increase in MDA.

Other integrative-based methods such as the ratio of reduced/oxidized glutathione (GSH/GSSG) have shown decreases after a soccer match [363] or a hamstrings eccentric-based exercise [257], indicating an increase in oxidative stress. Overall, while appropriate oxidative stress is necessary to mediate beneficial responses, such as mitochondrial biogenesis [10] or remodeling processes [9], excessive free radicals accumulation triggers a process of lipid peroxidation in cell membranes [13], including mitochondrial membranes and sarcolemma [11], which exacerbates secondary muscle damage [12].

Finally, the findings regarding serum biomarkers after hamstrings muscle-damaging exercises reported in this thesis did not detect statistically significant between-sex differences in the magnitude and recovery of structural and oxidative damage biomarkers. However, it should be highlighted that individualized analysis of the participants according to sex showed that female volunteers tend to experience lower peaks of muscle damage biomarkers (i.e., CK, sMtCK), which might be the result of estrogen production-derived membrane stabilization [79].

8.2.3. Irisin: a promising post-exercise muscle remodeling biomarker?

The myokine irisin has been suggested to be connected to the secondary muscle damage processes. For example, it has been suggested that greater muscle damage could increase the outflow of irisin into the bloodstream immediately after exercise [326]. Moreover, irisin is associated with biomarkers of muscle damage (i.e., myoglobin) and inflammation (i.e., IL-6) [329]. Therefore, it is hypothesized that rupture of the muscle fibers might trigger an increase of circulating irisin [328].

Research on circulating irisin during the recovery period after predominantly concentric exercises, in which there is no evidence of muscle damage, found no increases in irisin [386,387]. Also, it has been found that doubling the intensity of a running training session triggers an increase in circulating irisin until the subsequent day [388]. However, similar to the above-mentioned studies, there is no evidence of muscle damage. Therefore, circulating irisin levels during the 72-hour post-exercise recovery period after damaging exercises have not been previously analyzed. Interestingly, we reported moderate increases of circulating irisin during the 72-hour recovery period in the three studies (**Figure 8.11**).

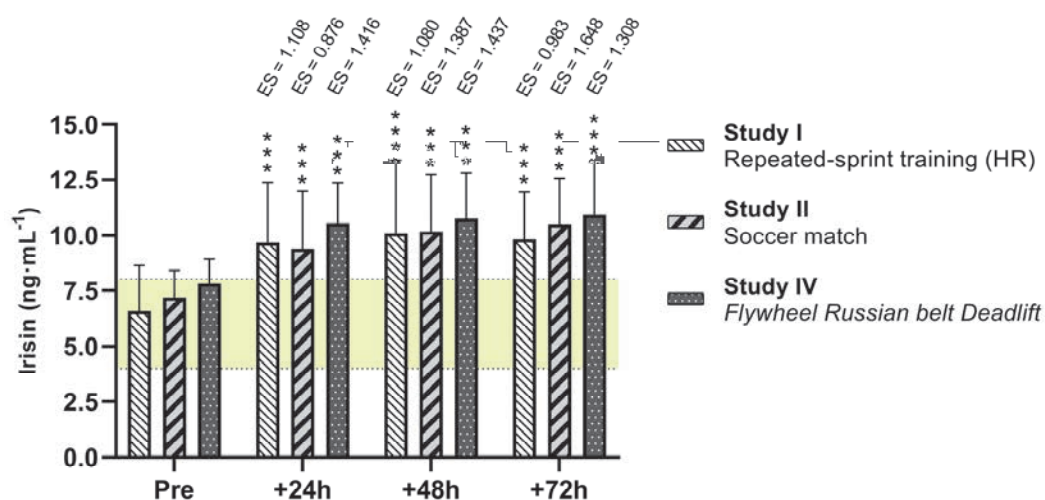


Figure 8.11. Mean $\pm$ SD values of irisin during the post-exercise recovery period in each study. The yellow range represents the normal reference values. (*), (**), and (***) indicate a significant difference from the baseline value at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. ES, effect size, HR, high responders.

The rationale for an increase in circulating irisin during the post-exercise recovery period is based on repeated eccentric muscle contractions that induce mechanical and oxidative damage to myocyte structures, leading to an increase in sarcoplasmic Ca^{2+} . As a result, there is an upregulation of the AMPK - PGC-1 α - FNDC5 signaling axis pathway, and subsequent irisin cleavage [273,274]. Accordingly, a moderate correlation was observed between the increase in sMtCK and circulating irisin. Likewise, the correlation between the increase in oxidative stress values and circulating irisin was moderate (**Figure 8.12**).

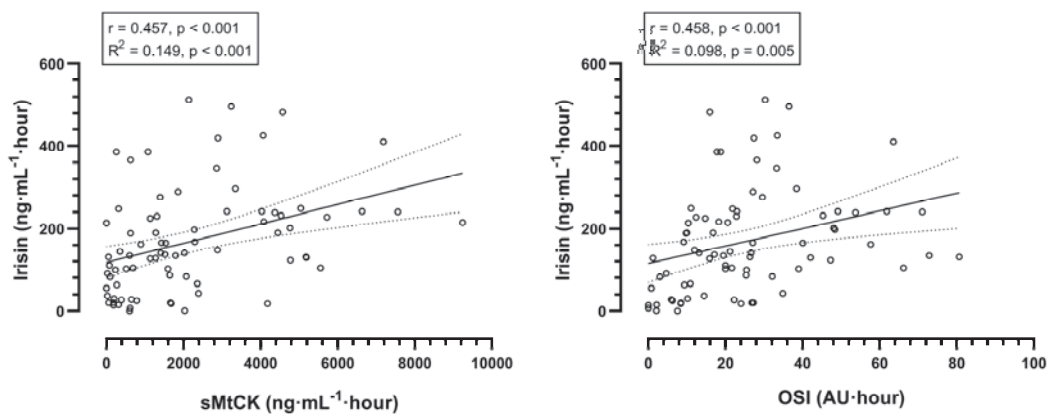


Figure 8.12. Correlations between increments of circulating irisin with sarcomeric mitochondrial creatine kinase (sMtCK), and oxidative stress index (OSI). Dotted lines represent 95% confidence intervals.

Irisin upregulates the expression of regulatory genes for mitochondrial fusion (i.e., *DPL1* and *Mfn*) [293]. In this sense, the upregulation of circulating irisin during the post-exercise recovery period might be an attempt to protect mitochondria by promoting mitochondrial fusion. Likewise, it has been found in murine models that physical exercise increases irisin in skeletal muscle tissue, reducing oxidative stress (i.e., OSI) [389]. Moreover, aerobic exercise-induced irisin mediates the reduction of oxidative stress and cell apoptosis in skeletal muscle [351]. Thus, irisin induces the polarization of pro-inflammatory M1 to anti-inflammatory M2 macrophages [290], which is a key event in the continuum of muscle regeneration [68,69].

Surprisingly, and in contrast to the other analyzed serum biomarkers in the thesis, the extent of circulating irisin was higher after the *Flywheel Russian belt Deadlift* training session (**Study IV**), compared to after the repeated-sprint training (**Study I**), or after the soccer match (**Study II**). Combining this finding with the greater force-generating capacity loss in the hamstring muscles after the *Flywheel Russian belt Deadlift* training session, correlation analysis showed that the increase in circulating irisin during the post-exercise recovery period was associated with losses in rapid force-generating capacity (**Figure 8.13**).

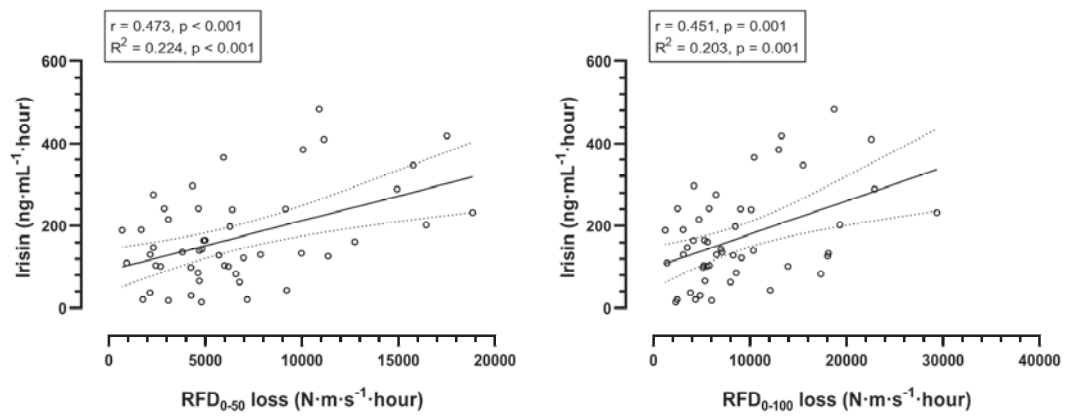


Figure 8.13. Correlations between increments of circulating irisin with early rate of force development (RFD) metrics. Dotted lines represent 95% confidence intervals.

This remarkable finding might be attributable to the relationship between circulating irisin levels and force-generating capacity which have also been found in other studies. Specifically, it has been demonstrated that increases in circulating irisin protect against loss of force generating capacity [390], through a lower expression of *MuRF1* (i.e., protein degradation marker) and *p21 mRNA* (i.e., senescence marker) [391].

Therefore, considering that a balanced oxido-inflammatory environment is required to avoid muscle fibrosis and promote an adequate regeneration process [9,12,69], and the previously mentioned effects of irisin on muscle protection and remodeling, we suggest that the increased circulating irisin during the post-exercise recovery period might be a mechanism to counteract underlying processes associated with primary and secondary muscle damage.

8.3. Is a 72-hour recovery period sufficient for the hamstrings to be recovered?

The combined discussion of the thesis findings after different hamstring-damaging exercises clearly indicates that 72 hours is not enough time to completely recover the neuromuscular capacity of the hamstring muscles. However, there are differences between studies that should be highlighted and discussed.

First, the sport-specific repeated-sprint training (**Study I**) was the least damaging exercise of the studies composing the thesis. In fact, two separate groups were detected on the basis of the reduction in force-generating capacity (i.e., MVIC torque). Accordingly, LR participants showed no alterations in the neuromuscular and biochemical response to a repeated-sprint training, whereas HR individuals decreased their neuromuscular capacity and upregulated biomarkers of structural and oxidative damage during the 72-hour post-exercise recovery period. Thus, a repeated-sprint training causes heterogeneous responses among participants, whether male or female.

Secondly, muscular recovery after a soccer match is a widely studied research topic. An interesting study compared the differences in muscle recovery by resting either 72 or 96 hours between soccer matches, revealing that muscle recovery was lower and physiological stress was higher when resting between games for 72 hours compared to 96 hours [392]. Conversely, a recent study reported that even metabolic stress biomarkers were still elevated at 72 hours post-soccer match, physical performance was recovered and similar to pre-soccer match values [393]. However, in the above-mentioned study, the measurement of physical performance did not include the assessment of hamstring muscles, which are the most important muscles involved during sprinting [383], nor their force-generating capacity, which is the preferred marker of muscle recovery [33,47,73,194].

Our findings after a soccer match (**Study II**) indicated that MVIC torque and perceived soreness in the hamstring muscles were recovered and similar to pre-match values after 48 hours of recovery. However, early RFD metrics and sprint performance, together with serum biomarkers of structural and oxidative damage did not recover even at 72 hours after the soccer match, indicating HSI risk. Therefore, 72 hours is not adequate time to recover soccer match-induced hamstring muscles residual fatigue, and thus, it is required to rest for at least 96 hours to completely recover the hamstring muscles.

Finally, the *Flywheel Russian belt Deadlift* was designed to trigger a significant activation of the hamstring muscles, particularly the BF_{LH}, in order to be used in programs for injury prevention or performance enhancement (**Study III**). In addition, the recovery kinetics of the hamstring muscles after the *Flywheel Russian belt Deadlift* were analyzed in **Study IV**, showing a large

decrease in the neuromuscular capacities of the hamstring muscles (i.e., MVIC torque RFD, ROM and perceived soreness), and an increase in biochemical indicators of structural and oxidative damage for more than 72 hours. Therefore, hamstrings muscle recovery after a training session using the analytical structure-based exercise (i.e., *Flywheel Russian belt Deadlift*) also requires more than 72 hours (**Figure 8.14**).

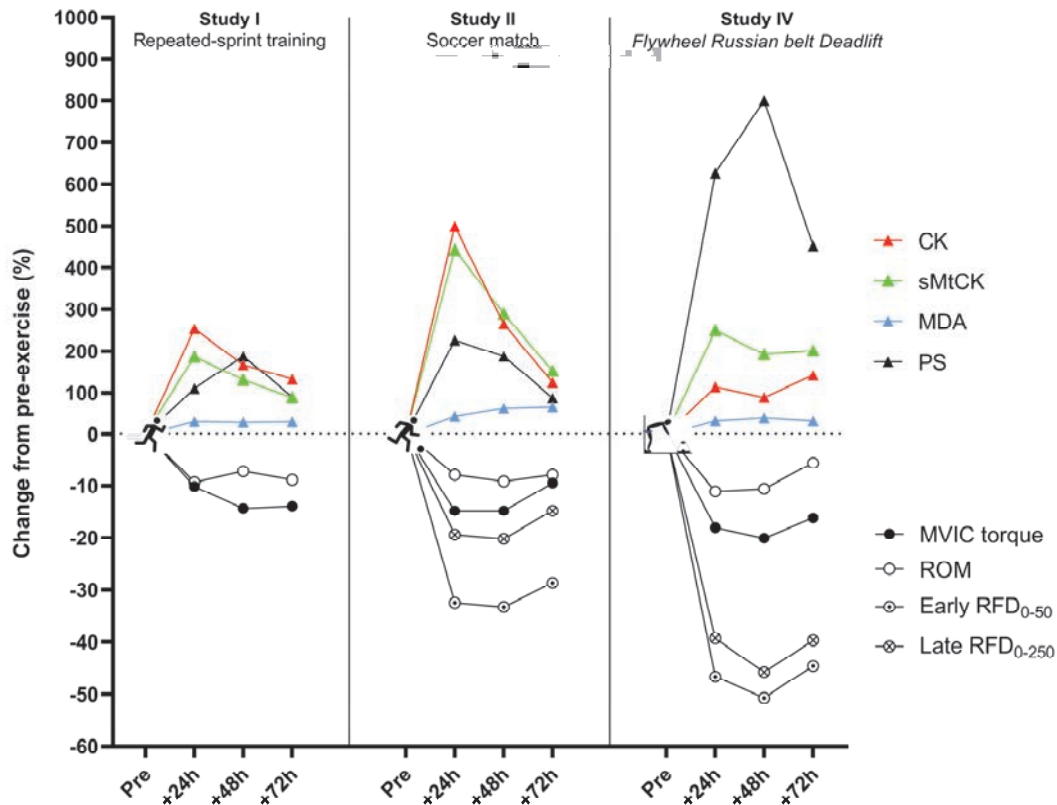


Figure 8.14. Schematic illustration displaying the panel of biochemical and neuromuscular biomarkers (% change) during the post-exercise recovery period in each study. **CK**, creatine kinase; **MDA**, malondialdehyde; **MVIC**, maximal voluntary isometric contraction; **PS**, perceived soreness; **RFD**, rate of force development; **ROM**, range of motion; **sMtCK**, sarcomeric mitochondrial CK.

In summary, the findings of the thesis indicate that *Flywheel Russian belt Deadlift* exercise (**Study IV**), compared to a repeated-sprint training (**Study I**) or a soccer match (**Study II**), produces the greatest extent of damage to the hamstring muscles, characterized by a decrease in neuromuscular capacities combined with an increase in structural and oxidative damage biomarkers for more than 72 hours. However, repeated measurements during the post-exercise recovery period of hamstrings MVIC torque, early RFD, and perceived soreness through the $90^{\circ}_{hip}:20^{\circ}_{knee}$ IPC test, provide valuable information on the recovery status of the hamstring muscles in a simple, rapid and non-demanding approach.

8.4. Limitations

This thesis also presented limitations that should be highlighted when interpreting the findings. The specific limitations of each individual study are reported in the respective chapters. However, the global limitations of the thesis are discussed in this section.

The first limitation is that the biochemical and neuromuscular biomarkers of muscle damage and recovery were not recovered to baseline values during the analyzed 72 hours of post-exercise recovery. Therefore, future research conducting additional measurements (e.g., +96h, until complete recovery) is required to better understand the implication of a repeated-sprint training, a soccer match or the *Flywheel Russian belt Deadlift* on the recovery kinetics of the hamstring muscles. It is also imperative to replicate and validate the findings after the soccer match with female players.

Second, the analytical structure-based exercise model, despite triggering the greatest loss of force-generating capacity, also induced the smallest increases in serum biomarkers. Thus, there were no significant correlations between the extent and time course of muscle damage/oxidative stress biomarkers and neuromuscular metrics. However, serum biomarkers are dependent on the volume of affected muscle tissue (i.e., the more muscle volume affected, the more outflow of biomarkers into the bloodstream) [33]. Therefore, quantification of the total muscle volume affected by each exercise model would have provided valuable information to normalize the increase of serum biomarkers by affected muscle volume.

Another limitation was that inflammatory biomarkers (i.e., IL-6, white blood cells count), which could provide additional information on the muscle recovery process, were not monitored. On the other hand, OSI does not provide information on specific compounds [267,268], so it is only indicative of the global oxidative stress status [259]. However, OSI has been included considering that (i) the quantification of free radicals is challenging due to their high reactivity and short biological half-life [260], (ii) the operability due to the large number of samples to be analyzed, and (iii) the potential significance as a global oxidative stress biomarker in sport sciences [264,269]. Finally, circulating irisin quantification was conducted by immunoassays. Despite antibody-based methods of irisin quantification were believed to provide low specificity [310], the used ELISA kit (i.e., #EK-067-29, *Phoenix Pharmaceuticals Inc.*) has been validated against both Western blot and mass spectrometry, and is therefore capable of correctly detecting circulating irisin [318,319]. Consequently, immunoassays were used to quantify circulating irisin due to the validity of the method and the operability due to the large number of samples to be analyzed. However, quantification of irisin by the reference method (i.e., mass spectrometry) [316] is required to validate the findings.

CONCLUSIONS

9. CONCLUSIONS

The thesis provides new knowledge about the monitoring of the different phases of the hamstrings muscle damage-recovery continuum. The following are the main conclusions derived from the findings of the studies included in the thesis.

- A sport-specific repeated-sprint training, a soccer match or a *Flywheel Russian belt Deadlift* training session trigger primary and secondary muscle damage to the hamstring muscles.
 - A recovery period of more than 72 hours is required to completely recover the hamstring muscles. There is no evidence that the duration of the recovery period differs between sexes.
- The $90^{\circ}_{hip}:20^{\circ}_{knee}$ *IPC test* is an easy, fast, low demanding, and cost-effective method to monitor hamstrings muscle damage and recovery, in both women and men.
 - MVIC torque and RFD metrics provide an adequate reliability.
 - Early RFD metrics (i.e., RFD_{0-50} and RFD_{0-100}) are the most decreased after hamstrings-damaging exercise models and are correlated with loss of sprint performance, thereby providing a more sensitive measure of muscle damage and recovery, compared to MVIC torque and late RFD metrics.
 - The assessment of perceived hamstring soreness in the $90^{\circ}_{hip}:20^{\circ}_{knee}$ *IPC test* position is a rapid complement that provides valuable evidence of the extent of primary muscle damage.
- The *Jurdan test* has been demonstrated to be an effective method for monitoring posterior chain ROM in male subjects after sprint-based exercises or flywheel hamstrings training. However, the assessment of posterior chain ROM in female subjects using the *Jurdan test* does not provide specific information on hamstrings muscle recovery.
- The loss of hamstrings neuromuscular capacities in both males and females is associated with a systemic biomarker response that is characterized by:
 - Disruptions in myocyte structure, including sarcolemma (i.e., CK activity) and muscle mitochondria (i.e., sMtCK).
 - Increases in oxidative stress biomarkers (i.e., MDA and OSI), exacerbating the self-feeding secondary muscle damage.
 - Upregulation of circulating irisin, which might be used as a post-exercise muscle remodeling biomarker.

9.1. Practical application and perspectives

Regular assessment of the hamstring muscles using the screening presented in this thesis, particularly early RFD metrics through the $90^{\circ}_{hip}:20^{\circ}_{knee}$ IPC test, provides an easy, fast, low demanding and cost-effective monitoring of the hamstring muscles recovery status. Accordingly, health professionals and practitioners can accurately individualize training programs by balancing optimizing training with muscle recovery, thereby reducing HSI risk, especially during periods of congested schedules.

The designed *Flywheel Russian belt Deadlift* exercise is a promising training method to produce significant active eccentric lengthening contractions in the hamstring muscles, which have been demonstrated to reduce the time to return to play after HSI [372]. The advantage of flywheel training compared to isokinetic training is the applicability to the specific task of the sport and lower cost, while the advantage compared to free-weight exercises is the ease of providing eccentric overload [394]. In this regard, short-term flywheel hamstrings training programs (i.e., 6 weeks) have been shown to increase hip extension concentric and eccentric peak power [395]. Therefore, training programs, in order to strengthen the hamstring muscles, and particularly the BF_{LH}, might be improved by the inclusion of the *Flywheel Russian belt Deadlift* exercise. Nevertheless, it remains to be elucidated whether a training program based on the *Flywheel Russian belt Deadlift* exercise can effectively improve performance or reduce HSI risk in the long term, and thus longitudinal studies would be meaningful. In addition, it has recently been demonstrated that muscle preactivation before the onset of eccentric exercises increases the workload on muscle fascicles [396], thereby offering a promising strategy for enhancing muscle adaptations to flywheel training (e.g., improving performance, preventing injuries).

Furthermore, it will be appropriate to analyze direct evidence of ultrastructural damage (i.e., muscle histology and immunohistochemistry) to the hamstring muscles after the designed *Flywheel Russian belt Deadlift*. Notwithstanding the intrinsic limitations of muscle biopsies, future research assessing direct damage to the hamstring myocytes would be meaningful. It could therefore be demonstrated whether, in addition to the significant activation of the BF_{LH}, the *Flywheel Russian belt Deadlift* is capable of triggering BF_{LH} adaptations.

Additionally, the hamstrings recovery screening presented in this thesis could be improved in the future by including (i) subjective measures of recovery (i.e., short recovery and stress scale), since it has been proven to be associated with muscle recovery markers [397], and (ii) the sprint mechanics assessment score, since it is associated with biomechanical patterns related to HSI mechanisms [398].

Finally, the findings addressed new areas of applied research, using an integrative-based and cost-effective method to monitor exercise-induced oxidative stress, and suggesting circulating irisin as a promising post-exercise muscle remodeling biomarker. Therefore, the application of new biomarkers provides a better understanding of the underlying processes of primary and secondary muscle damage. For example, a new research area has recently demonstrated that skeletal muscle tissue releases extracellular vesicles into the bloodstream after damaging exercises [399], and that these extracellular vesicles (e.g., Annexin A5) activate intra- and extracellular signaling pathways to coordinate muscle repair and remodeling [400]. Consequently, the monitoring of circulating extracellular vesicles could be a new area of research on biomarkers of primary and secondary muscle damage.

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10. REFERENCES

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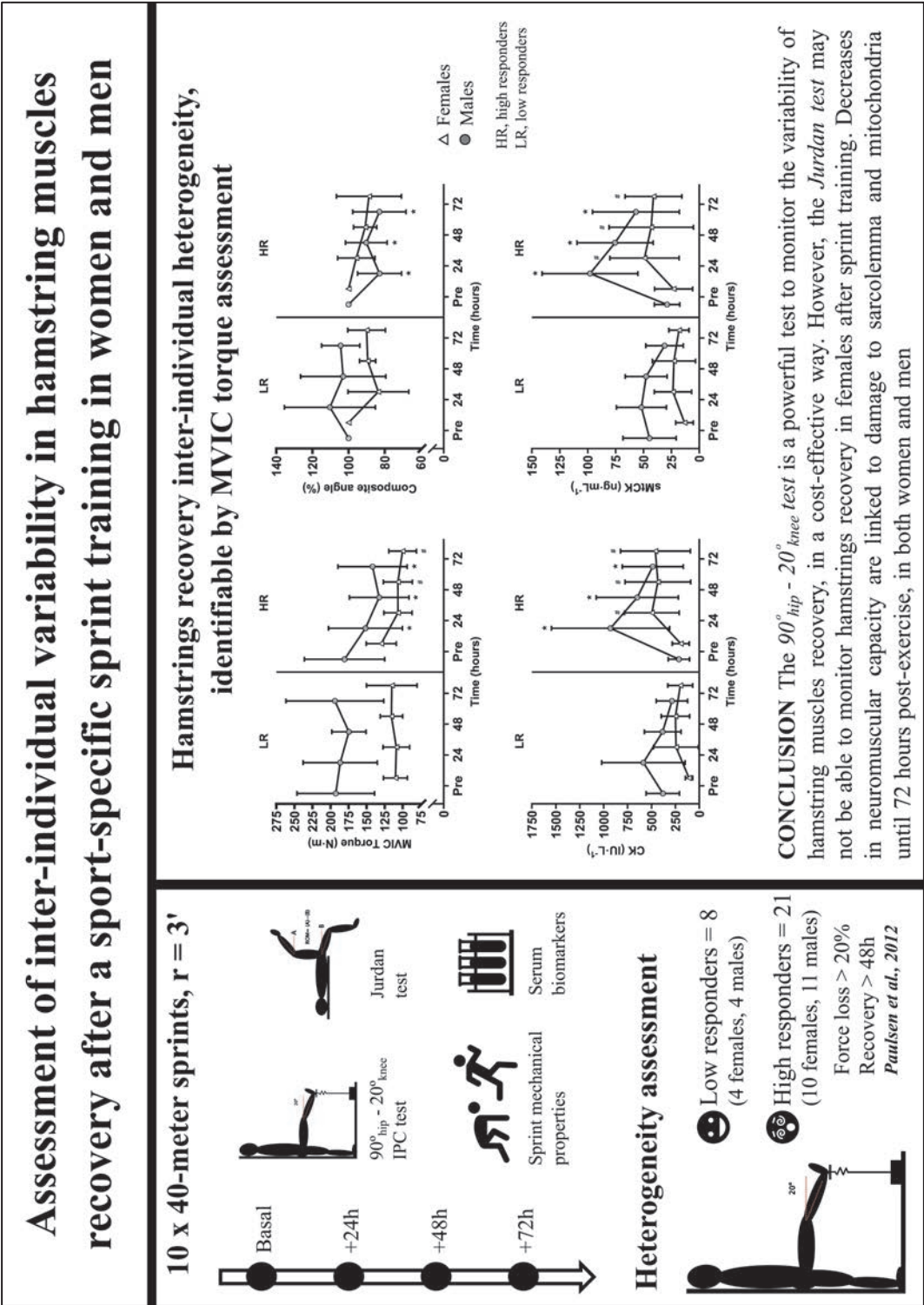
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ANNEXES

11. ANNEXES

11.1. Appendix 1: Graphical abstract of study 1



Football (soccer) match-derived hamstring muscles residual fatigue can be monitored using early rate of force development

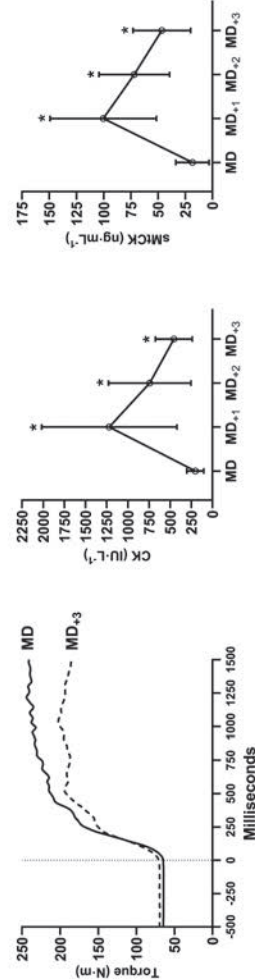
TD > 10,000 m
HSRd > 400 m
Acc > 30
Dec > 30

The diagram illustrates the study design timeline and associated tests. A horizontal timeline at the bottom shows four points: Basal, MD₊₁, MD₊₂, and MD₊₃. Above the timeline, two tests are detailed:

- Juridan test:** Shown with an icon of a person in a starting position (labeled A and B) and a text box stating "90° hip - 20° knee IPC test".
- Sprint mechanical properties:** Shown with an icon of a person in a starting position.

At the top, a bar chart icon is labeled "Serum biomarkers".

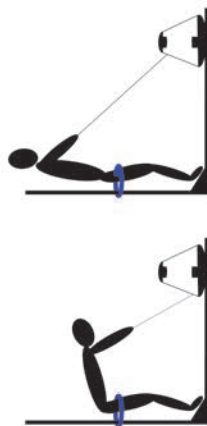
Figure 1 consists of two bar charts. The left chart shows RFD (N·m·s⁻¹) for MD and MD+1 conditions across three time points (MD-1, MD-2, MD-3). The right chart shows RFDmax (N·m·s⁻¹) for MD and MD+1 conditions across three time points (MD-1, MD-2, MD-3). Both charts include error bars representing standard error of the mean (SEM). Significance levels are indicated by asterisks (*, **, ***) and brackets. A legend indicates that grey bars represent MD and white bars represent MD+1. A red shaded area at the top of the RFDmax chart indicates a significant difference between MD and MD+1 conditions.



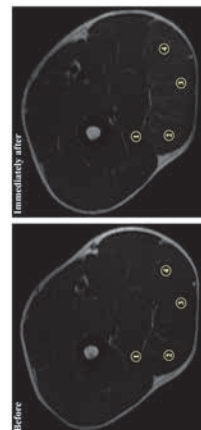
CONCLUSION Based in the findings, 72 hours is not adequate time to recover soccer match-induced hamstring muscles residual fatigue. Instead, we recommend the use of the $90^{\circ}_{hip} - 20^{\circ}_{knee}$ test, which is easy, fast, and cost-sensitive to monitor the recovery kinetics of force-generating capacity and, especially, early RFD of the hamstring muscles in multiple sprints-based sports

Individual and region-specific hamstring muscles use during a novel Flywheel Russian belt Deadlift exercise

Flywheel Russian belt Deadlift
10 sets x 10 repetitions, r: 3'



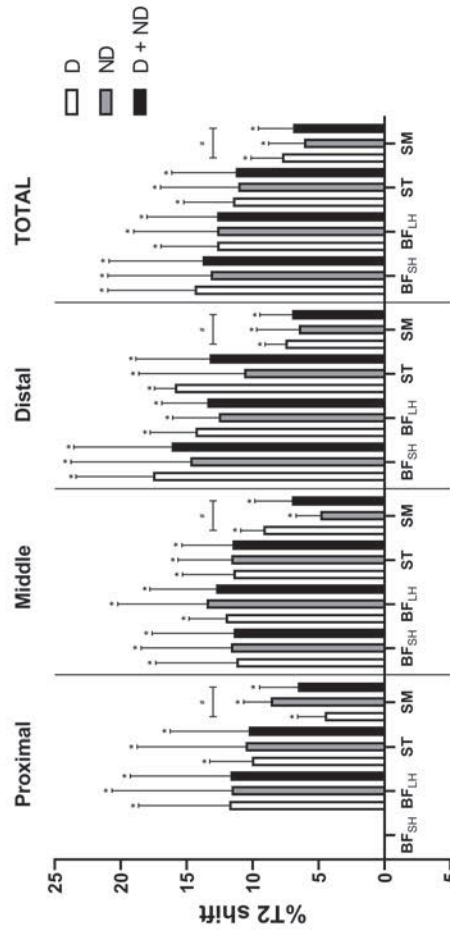
Muscle functional magnetic resonance imaging (mfMRI)
Baseline to immediately post-exercise



Regions of interest (ROIs)

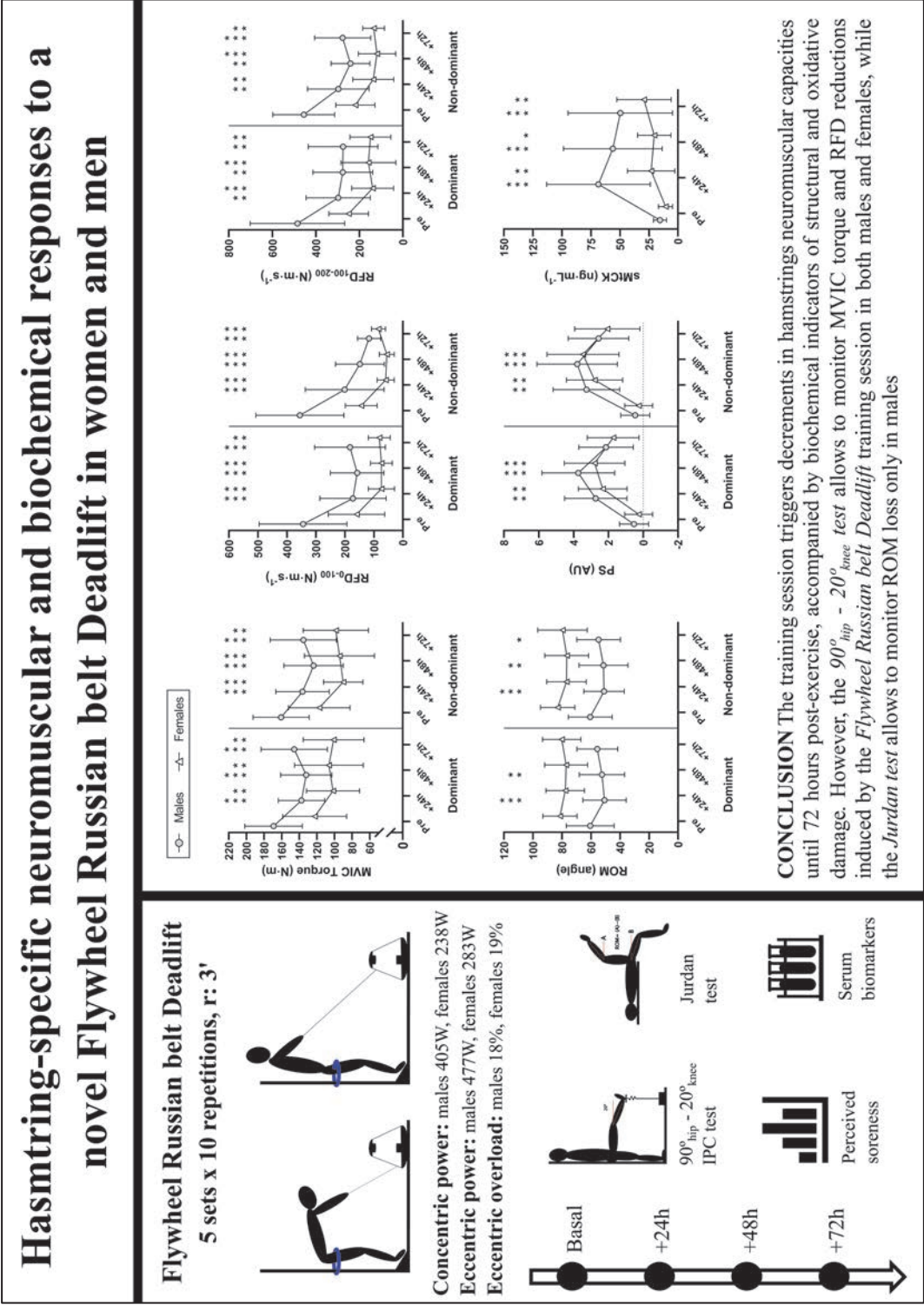
- 1 m. Biceps femoris, short head
- 2 m. Biceps femoris, long head
- 3 m. Semitendinosus
- 4 m. Semimebranosus

%T2 shift of the 30% (proximal), 50% (middle), and 70% (distal) regions of thigh length immediately after the 10x10 Flywheel Russian belt Deadlift exercise



(*) indicates significant T2 shift and (#) indicates substantial differences in SM, compared to BF_{SH}, BF_{LH} and ST.

CONCLUSION The findings of the study highlight that a homogeneous and consistent hamstring muscles activation can be triggered by the exercise. Particularly, the lateral positioning muscles (i.e., BF_{LH} and BF_{SH}) were more activated than the medial positioning muscles (i.e., ST and SM). Therefore, the *Flywheel Russian belt Deadlift* exercise could enhance training programs to strengthen and activate the hamstring muscles, and specifically, the BF_{LH}, in order to reduce injury risk.



11.5. Appendix 5: “Assessment of inter-individual variability in hamstring muscle recovery after a sport-specific sprint training in women and men”



OPEN ACCESS

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Assessment of inter-individual variability in hamstring muscle recovery after a sport-specific sprint training in women and men

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Background: Hamstring muscles are most affected by multiple sprint-based sports as a result of muscle strain during sprinting, leading to reduced performance and increased risk of injury. Therefore, the purpose of the study was to assess inter-individual variability in hamstrings recovery after a sport-specific repeated-sprint training (RST), through sprint-specific markers of muscle recovery and associated muscle damage biomarkers in women and men.

Methods: Healthy females ($n = 14$) and males ($n = 15$) underwent 10 repeated 40-m sprints with a 3-min rest pause between each repetition. Force-generating capacity (FGC) by the 90°_{hip} – 20°_{knee} test and range of motion *Jordan* test, together with serum biomarkers [sarcomeric mitochondrial creatine kinase (sMtCK), oxidative stress, irisin] were tested at baseline and 24-, 48- and 72-h post-exercise through a repeated measures design. Participants were classified according to FGC loss into high responders (HR) and low responders (LR).

Results: 21 individuals (10 females, 11 males) were classified as HR (FGC loss >20% and recovery >48 h), while 8 individuals (4 females, 4 males) were classified as LR. HR individuals showed unrecovered maximal voluntary isometric contraction (MVIC) torque until 72 h post-training ($p = 0.003$, $\eta_p^2 = 0.170$), whereas only HR males showed decreased range of motion ($p = 0.026$, $\eta_p^2 = 0.116$). HR individuals also showed increased sMtCK ($p = 0.016$, $\eta_p^2 = 0.128$), oxidative stress ($p = 0.038$, $\eta_p^2 = 0.106$) and irisin ($p = 0.019$, $\eta_p^2 = 0.123$).

Conclusion: There is inter-individual variability in the muscular response to a sport-specific RST, identifiable by MVIC torque assessment. The findings support

Abbreviations: 25OHD, 25-hydroxyvitamin D; A.max, peak acceleration; AMPK, AMP-activated protein kinase; Ca^{2+} , calcium ion; CK, creatine kinase; CV, coefficient of variability; F0.rel, relative (to body mass) theoretical maximal horizontal force production; FGC, force-generating capacity; FNDC5, fibronectin domain-containing 5 protein; HR, high-responders; IL, interleukin; K.fitted, rate of force development normalized by peak force; LR, low-responders; MD, muscle damage; MDA, malondialdehyde; MVIC, maximal voluntary isometric contraction; NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B cells; OS, oxidative stress; OSI, oxidative stress index; PGC-1 α , peroxisome proliferator-activated receptor γ co-activator 1 α ; P.max.rel, relative peak power; ROM, range of motion; ROS, reactive oxygen species; RST, repeated-sprint training; sMtCK, sarcomeric mitochondrial CK; TAS, total antioxidant status; Time.P.max, time to peak power; TOS, total oxidant status; V0, theoretical maximal running velocity.

that the $90^{\circ}_{hip}:20^{\circ}_{knee}$ test is a powerful indirect test to screen hamstrings recovery in both women and men, in a cost-effective way. However, the *Jordan* test might not be able to monitor hamstrings recovery in sportswomen after RST. Decreases in muscle capacity are linked to damage to muscle sarcolemma and mitochondria until 72 h post-exercise. Overall, 72 h will not be adequate time to restore hamstrings structure and function after a sport-specific RST in both female and male responders.

KEYWORDS

exercise-induced muscle damage, fatigue, force-generating capacity, muscle recovery, repeated sprint ability

1 Introduction

Repeated sprints, defined as periods of high-intensity running interspersed with lower intensity jogging and walking, are recurrent in multiple-sprint team sports, e.g., football, field hockey (Thompson et al., 1999). During sprinting, the muscle-tendon unit of the hamstring muscles reaches its maximum length in the late swing phase, while simultaneously undergoing an active lengthening eccentric contraction (Thelen et al., 2005). Thus, repeated high intensity eccentric contractions employed in repeated-sprint training (RST) result in loss of hamstring muscle function (Baumert et al., 2021). Muscle recovery is multifactorial in nature, as there are a wide range of intrinsic, e.g., training history, and extrinsic factors, e.g., seasonal moment, which influence the internal load suffered by athletes (Paul et al., 2015). The primary approach for assessing muscle recovery is muscle histology. However, this method is not used by professionals and practitioners due to its invasiveness and intrinsic errors of the approach (evaluation of the entire muscle by means of a microscopic muscle sample) (Clarkson and Hubal, 2002). Instead, there is a high correlation ($r = 0.89$) between the reduction in force-generating capacity (FGC) and the fraction of muscle fibers presenting ultrastructural alterations (Raastad et al., 2010). Consequently, FGC is the preferred indirect marker of muscle damage (MD) accounting for myofibrillar alterations, inflammation, and necrosis (Paulsen et al., 2012). Assessment of hamstring FGC has been commonly conducted using isometric posterior chain tests, such as the $90:90$ or $90:30$ supine test (Schache et al., 2011). However, as the largest decrease of isokinetic peak torque in the hamstring muscles appears in the most lengthened positions (10° of knee flexion), Matinlauri et al., 2018 recently designed a new, reliable, and sensitive test to monitor hamstring-related acute and residual fatigue after sprint-based exercises, the $90^{\circ}_{hip}:20^{\circ}_{knee}$ test. The design of the test causes a lengthening of the hamstrings, and specifically, a greater biceps femoris hip extension than knee flexion moment arm, compared to the semitendinosus and semimembranosus muscles, in a similar pattern to the late swing phase of the running gait cycle (Thelen et al., 2005). Consequently, the hamstrings are biomechanically exposed during the test, thus facilitating hamstring-related acute and residual fatigue monitoring (Matinlauri et al., 2018).

Repeated high intensity eccentric contractions also result in changes of biochemical markers of MD and oxidative stress (OS), which indicate the status of myocyte structure (Brancaccio et al., 2010; Hyldahl and Hubal, 2014). Even there is no single gold-standard

biomarker, a panel of biomarkers might be reported to simultaneously assessing different physiological processes and provide a more comprehensive understanding of muscle recovery. Mechanical-induced physical damage to membrane has been the underlying hypothesis explaining membrane permeability following eccentric actions. However, it has been found that increased intracellular calcium ion (Ca^{2+}) over time as a consequence of stretch-activated Ca^{2+} channels plays a key role in the permeability of the sarcolemma (Allen et al., 2010). Considering a perspective applicable to the day-to-day sports practice, transient sarcoplasmic oxidation by-products and Ca^{2+} upregulation derived from damaging exercises results in a mild FGC loss as a consequence of failures in the excitation-contraction coupling process (Kano et al., 2012). Given that the mitochondria modulate Ca^{2+} overload through the mitochondrial calcium uniporter located in the inner mitochondrial membrane (Rossi et al., 2019), mild decreases in muscle function might be preserved by amino acids and/or micro-nutrients intake, e.g., Vitamin D, owing to the maintenance of the muscle mitochondria structural integrity and function (Drummond et al., 2009; Salles et al., 2022). However, persistent Ca^{2+} intracellular elevation activates the mitochondrial permeability transition pore (Bauer and Murphy, 2020) and upregulates calcium-dependent calpains (Belcastro et al., 1998). Calpains are predominantly localized in the Z-disk and I band regions, and have been shown to play a critical role in exercise-induced damage, as they are disruptive to the Z-disk and myofibrillar protein integrity (Hyatt and Powers, 2020). In particular, calcium-activated calpains impair contractile proteins, e.g., desmin, and/or excitation contraction coupling proteins, e.g., junctophilins (Hyatt and Powers, 2020). As a result, there is an uncontrolled release of Ca^{2+} and oxidation by-products to the sarcoplasm and bloodstream, finally resulting in rupture of the mitochondrial outer membrane and intermembrane proteins efflux (Görlach et al., 2015). However, the structural integrity of the muscle mitochondria can be monitored by measuring striated muscle mitochondrial-specific isoenzyme, sarcomeric mitochondrial creatine kinase (sMtCK) (Carmona et al., 2018). sMtCK is mainly localized in both the peripheral intermembrane space and the cristae space (Kottke et al., 1994; Schlattner et al., 2006). Considering the aforementioned localization of sMtCK, and that striated muscle mitochondria necrosis determined by histological evidence resulted in increased sMtCK in plasma, the efflux of sMtCK into the bloodstream reflects mitochondrial disruptions (Ishikawa et al., 1997). The aforementioned damage to cellular and mitochondrial integrity also leads to an upregulation of the signaling pathway for irisin cleavage (Liu et al., 2022). The myokine irisin plays a significant

role in muscle remodeling by ameliorating protein degradation (Reza et al., 2017). Recently, irisin has also been shown to be involved in counteracting oxidative processes and proinflammatory cytokines (Mazur-Bialy and Pocheć, 2021). Considering the aforementioned irisin-mediated effects together with the fact that a recent study revealed that aerobic exercise-induced irisin partly mediates amelioration of oxidative stress and cell apoptosis in mice skeletal muscle (Ren et al., 2022), the myokine irisin is suggested to be increased during muscle recovery.

Finally, sarcomeres and mitochondria impairment caused by eccentric contractions during sprinting results in long-lasting decreases in FGC (Kano et al., 2012; Hyldahl and Hubal, 2014), which is a fundamental source for the loss of performance described in both sportswomen (Keane et al., 2015) and men (Howatson and Milak, 2009) after a RST. Accordingly, there is a need for practitioners to monitor FGC and range of motion (ROM) using simple, time and cost-effective sprint-specific muscle function tests associated with biochemical markers using an integrative-based approach. To our knowledge, the use of the 90°_{hip} - 20°_{knee} test to monitor hamstrings FGC during the recovery period after RST is described for the first time. Therefore, the purpose of the research was to assess inter-individual variability in hamstrings recovery after a sport-specific RST, through sprint-specific markers of muscle recovery and associated muscle damage biomarkers in women and men.

2 Materials and methods

2.1 Participants

Fifteen healthy males [age = 22.5 ± 3.2 years, mass = 74.2 ± 7.4 kg, height = 175.9 ± 6.8 cm (mean $\pm$ SD)] and fourteen females [age = 22.2 ± 3.1 years, mass = 60.3 ± 7.1 kg, height = 165.2 ± 4.8 cm (mean $\pm$ SD)] voluntarily attended the study during 2021 and 2022 spring. Participants were required to be physically active according to the World Health Organization (at least 150 min of moderate-intensity exercise per week), but not enrolled in a regular specialized training program. Exclusion criteria were non-healthy participants and lower limb muscle injuries within 6 months prior to the experiment. Participants were strongly encouraged to (i) avoid any type of physical exercise during the 3 days prior to the experiment and during the 72-h follow-up, and (ii) to maintain their daily diet and lifestyle. All participants were previously informed and gave their written consent that they received complete and specific information about the purpose of the study, experimental tests, and associated risks. The experiment was conducted in accordance with the code of ethics of the World Medical Association (Declaration of Helsinki) and was approved by the Ethics Committee for Clinical Research of the Catalan Sports Council (Generalitat de Catalunya) (037/CEICGC/2021).

2.2 Experimental design

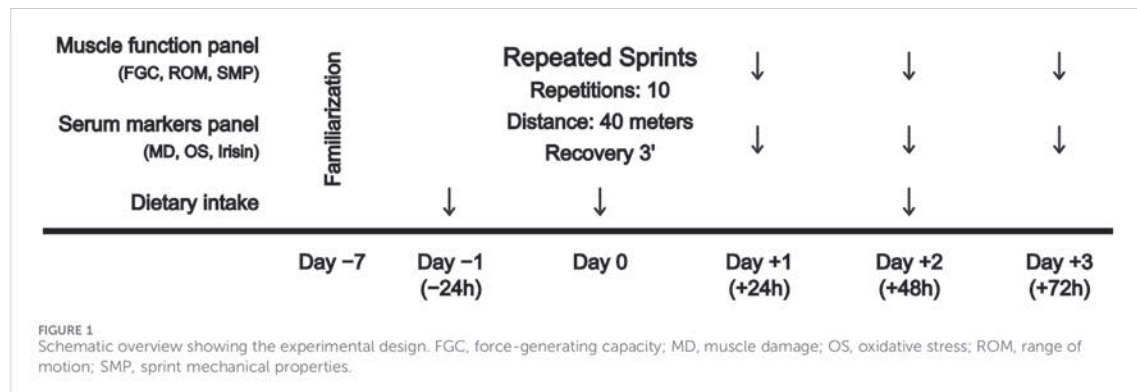
A repeated-measures design was used to examine the extent and time course of serum biomarkers and muscle function before (pre)

and at 24 (+24 h), 48 (+48 h) and 72 h (+72 h) after a RST, consisting of 10 repeated 40-m sprints, with a 3-min rest between each repetition. Depending on the playing position, sprint distance in football matches ranges from 190 to 420 m (Altmann et al., 2021), in field hockey matches from 418 to 513 m (Sunderland and Edwards, 2017), and in rugby matches from 65 to 392 m (Jones et al., 2015). In addition, similar RST protocols (3 sets $\times$ 6 repetitions $\times$ 20 m = 360 m, rest: 1.5 min between repetitions and 4 min between sets) have shown decreases in hamstrings electrical activity and knee flexors torque (Timmins et al., 2014). Accordingly, the total training distance (400 m) was determined based on the aforementioned sprint demands of outfield team sports, and the distance of each sprint (40 m) was selected as it allows reaching the maximum sprint velocity, which is the main injury mechanism of hamstrings strains (Danielsson et al., 2020). Long rest periods between repetitions were selected to reduce intra-session fatigue and maintain the maximum intensity in each 40-m repetition. The designed protocol is further supported by the findings of a recent systematic review and meta-analysis on the demands of RST in team sports (Thurlow et al., 2023), which include that (i) a distance of 40 m (or 6-s sprints), commonly used in team sports, allows the maximum velocity to be reached; and (ii) longer rest intervals are recommended in order to maintain the quality of the repetitions when the total volume is greater. Then, blood samples were collected to determine changes in serum biomarkers, and muscle function was assessed by posterior chain FGC, lower limbs ROM and sprint performance and mechanical properties test. Participants completed the familiarization 1 week before the start of the experimental procedure. All functional tests were repeated by following the instructions of the researcher until successful completion. A schematic overview of the experimental design is shown in Figure 1.

To further explain the findings given the inter-individual variations in response to exercise-induced MD, participants were categorized according to the percentage of reduction of the FGC during the 3-day follow up (Paulsen et al., 2012). Then, participants were grouped into “high-responders” (HR), participants who showed notable decreases in FGC of 20%–50% of baseline values and recovery after more than 48 h, and “low-responders” (LR), participants who showed FGC decreases of <20% and complete recovery within 48 h (Paulsen et al., 2012).

2.3 Force-generating capacity (FGC)

FGC of hamstring muscles was measured unilaterally as the maximal voluntary isometric contraction (MVIC) torque by the 90°_{hip} - 20°_{knee} test (Matinlahti et al., 2018), using a 160 Hz sampling frequency strain gauge (Chronojump, Barcelona, Spain). Participants were verbally encouraged to exert their maximum effort and completed three trials (3–5 s MVIC) of the test with each lower limb, with a 30-s rest between trials. The maximum MVIC torque was registered as the average force in a 1-s window once a force plateau had been established (Tesch et al., 2004) and the residual force resulting from the mass of the tested leg at rest was then subtracted. The best of the three trials from each limb was recorded for further analyses. Additional trials were conducted if any countermovement or joint balancing was noted.



2.4 Range of motion (ROM)

The *Juridan test* was used to evaluate the interaction between hip flexor and hamstring flexibility, measured as the differential angle between the thigh and contralateral shin (Lahti et al., 2020). The test was photographically registered by a SONY DSC-RX100M5A placed on a tripod located at the height of the table and 3 m from the center of the hip of the participant. 2D shin and thigh angles were measured, relative to the horizontal, as the best of two trials on each lateral approach using the sports kinematics analysis software *Kinovea* v0.9.3. Then, resulting angles were used to compute a composite angle defined as the difference between the actively lengthened leg shin angle and the opposite leg passive thigh angle.

2.5 Sprint performance and mechanical properties

Instantaneous velocity over 40 m-sprints was measured by a friction encoder *RaceAnalyzer* (Chronojump, Barcelona, Spain), with a distance accuracy of 3 cm and temporal accuracy of 4 microseconds. The device was located on a 1-m-high tripod placed behind the participants. Then, participants were positioned into a belt attached to the friction encoder and were strongly verbally encouraged during the test to ensure maximal effort. Data were *post hoc* analyzed using Chronojump software v2.1.2-2 (Chronojump, Barcelona, Spain) to compute sprint F-V spectra using the validated method described in detail elsewhere (Samozino et al., 2016). Individual linear F-V relationships were then extrapolated to calculate theoretical maximal running velocity (V_0), relative (to body mass) theoretical maximal horizontal force production (F_0 .rel), peak acceleration (A .max), relative peak power (P .max.rel), time to peak power ($Time.P$.max) and rate of force development normalized by peak force (K .fitted).

2.6 Blood sampling and processing

An 8-mL blood sample was drawn from an antecubital vein. Samples were allowed to clot for 30 min at room temperature in an SST II Advance tube (Becton Dickinson Vacutainer Systems, UK) and were then centrifuged at 4,000 g for 8 min at 4°C. After

separation of the serum, aliquots were stored at -80°C until analysis. Measurement of creatine kinase (CK) activity was conducted on an Advia 2400 automated device (SiemensTM Medical Solutions Diagnostics, Tarrytown, NY, United States) and serum sMtCK was measured by ELISA kit #SEC386Hu (Cloud Clone Corp., Houston, TX, United States) according to manufacturer's instructions (intra-assay coefficient of variability (CV) < 10%; inter-assay CV < 12%; minimum detectable value of 1.56 ng mL^{-1}). The Erel approach (Erel, 2005) was used to quantify the oxidizing material in the samples, based on the oxidation of ferrous iron to ferric iron with an intra-assay CV of 2.3%, inter-assay CV of 3.5% and a minimum detectable value of $2.5\text{ }\mu\text{mol H}_2\text{O}_2\text{ Equiv. mL}^{-1}$ (Elabscience #E-BC-K802-M, Wuhan, China). Similarly, the Erel 2,2'-azino-di-3-ethylbenzthiazoline sulfonate (ABTS)+ approach (Erel, 2004) with an intra-assay CV of 4.6%, inter-assay CV of 7.0% and a minimum detectable value of $0.23\text{ mmol Trolox Equiv. mL}^{-1}$ (Elabscience #E-BC-K801-M, Wuhan, China) was conducted to quantify total antioxidant status (TAS). Then, OS index (OSI) was calculated as the oxidizing material (TOS) to TAS ratio. In order to control the OSI results, serum malondialdehyde (MDA) was measured by kit #E-EL-0060 (Elabscience, Wuhan, China) with an intra-assay CV < 10%, inter-assay CV < 10% and a minimum detectable value of 18.75 ng mL^{-1} . Circulating irisin was quantified using the previously validated kit against mass spectrometry (Ruan et al., 2019) #EK-067-29 (Phoenix Pharmaceuticals Inc, Burlingame, CA, United States), according to manufacturer's instructions (intra-assay CV < 10%; inter-assay CV < 15%; minimum detectable value of 1.29 ng mL^{-1}). 25-hydroxyvitamin D (25OHD) concentration was measured by chemiluminescence immunoassay procedures conducted on a *Advia Centaur Vitamin D Total Assay* (SiemensTM Healthcare Diagnostics, Tarrytown, NY, United States). The method is certified by the Centers for Disease Control and Prevention and adhered to de Vitamin D Standardization-Certification Program (Binkley and Sempos, 2014).

2.7 Dietary intake record

Participants were instructed to record their diet for 3 days (-24 h , 0 h and $+48\text{ h}$) and were given detailed verbal and written instructions describing procedures for recording the daily

diet, together with a list of commonly used household measurements (e.g., dessert spoon, glass, cup) and measurements quantities (e.g., grams, milliliters). The participants were strongly instructed to maintain their usual diet, and to be accurate in recording the amounts and types of food and drinks intake. The diet of each participant was calculated as the 3-day average by using the dietary analysis software *PCN Pro v1*. (Universitat de Barcelona, Barcelona, Spain).

2.8 Statistical analysis

First, sample size was estimated using G*Power software (version 3.1) based on MVIC change ($90^{\circ}_{hip}; 20^{\circ}_{knee}$ test) of the study by Matinlauri et al. (2018). A total of 25 participants were calculated to be included given an effect size of 0.68, a power of 90% and a 5% alpha error. Asymmetrically distributed variables were log-transformed prior to analysis. A three-way repeated measures ANOVA [Time (baseline, 24h, 48h, and 72 h) x Sex (male, female) x Group (HR, LR)] was used to identify the effect of time on MVIC torque, ROM, sprint performance and mechanical properties and serum biomarkers, together with interactions between sex and groups. Post-hoc Bonferroni-corrected paired *t*-test were conducted to identify significant differences from baseline values. Effect sizes (ES) were calculated using Cohen's *d* to assess the difference between means when statistically significant differences were found. Thresholds for standardized differences statistics were (<0.20), trivial; (0.20 < 0.59), small; (0.60 < 1.19), moderate; (1.20 < 1.99), large; and (>2.0), very large (Hopkins et al., 2009). Associations between variables of interest were analyzed using Spearman's Rho correlation coefficient, considering the following thresholds (<0.30), small; (0.30 < 0.51), moderate; (0.51 < 0.70), large; (0.71 < 0.90), very large; and (>0.90), extremely large (Hopkins et al., 2009). The intraclass correlation coefficient (ICC) was used to assess the reliability of a single data collector that examines the degree of concordance of scores between repetitions or sessions. The ICC was calculated for average measures using a 2-way random effect model of absolute agreement. Thresholds for ICC were (0.00 < 0.50), poor; (0.51 < 0.75), moderate; (0.76 < 0.90), good; and (0.91 < 1.00), excellent (Koo and Li, 2016). Independent samples *t*-test were used to assess between-groups differences in dietary intakes. Data are presented as mean $\pm$ standard deviation (SD) and the level of significance was set at $p < 0.05$. Statistical analysis was performed with SPSS version 27.0.1.0 (SPSS Statistics, IBM Corp., Armonk, NY, United States).

3 Results

According to the percentage of reduction in FGC (Paulsen et al., 2012), 21 individuals (10 females, 11 males) were classified under HR conditions, as they presented notable decreases in FGC of 20%–50% of baseline values and recovery after more than 48 h. Otherwise, 8 individuals (4 females, 4 males) showed FGC decreases of <20% and complete recovery within 48 h and were therefore classified as LR.

3.1 Muscle function

3.1.1 MVIC torque

The ICC was excellent for the dominant leg with a value of 0.973 (95% IC 0.941–0.988) and non-dominant leg with a value of 0.969 (95% IC 0.937–0.986). As illustrated in Figure 2A and Figure 2B, hamstring MVIC torque was decreased and unrecovered in HR individuals during the 72-h recovery period. There was no sex-related time course difference in MVIC torque.

3.1.2 Range of motion

There was a significant time-group-sex interaction in the composite ROM following RST [$F(3,75) = 3.277, p = 0.026, n^2_p = 0.116$]. Post-hoc Bonferroni-corrected paired *t*-test showed unchanged ROM in LR group, while ROM was only decreased in males HR group during the 72-h recovery period (Figure 2C and Figure 2D).

3.1.3 Sprint performance and mechanical properties

The RST increased split time to 10 m and total time to 40 m during recovery period. Also, Both the LR and HR individuals showed decreased F0.rel, A.max, P.max.rel, K.fitted and increased Time.P.max during the post-RST recovery period. V0 was only decreased in HR individuals. There were no group and sex-related time course differences (Table 1).

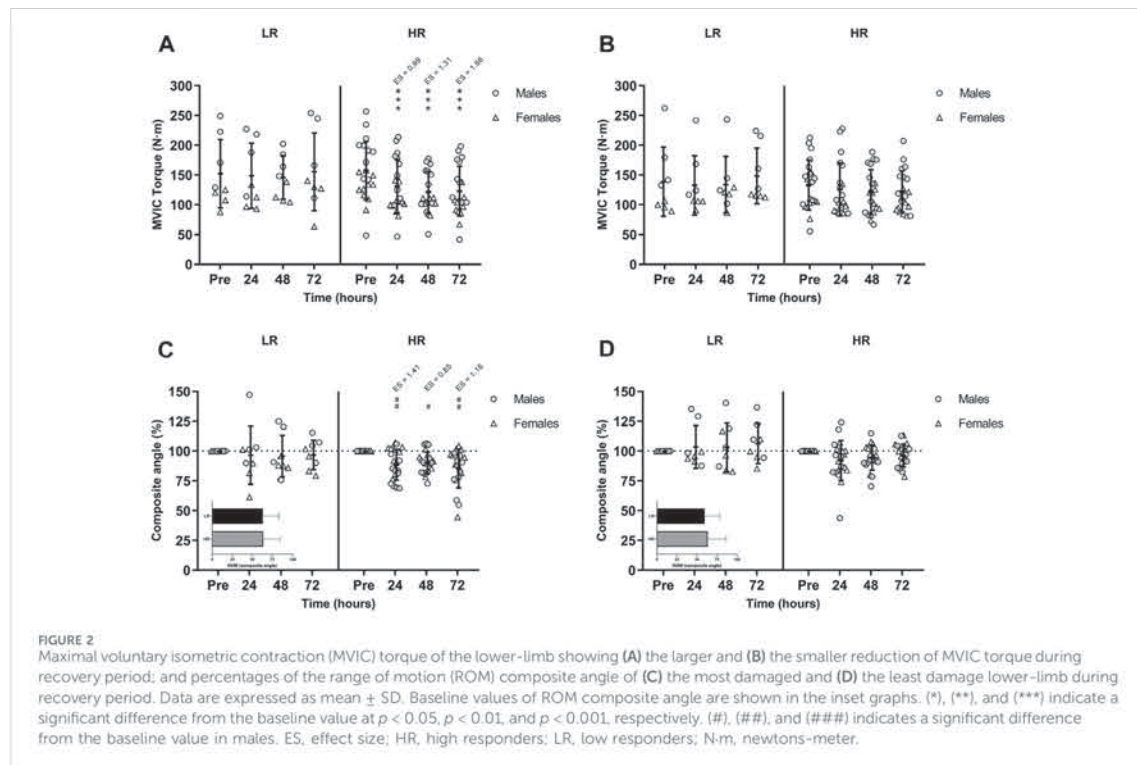
3.2 Biochemical measures

CK activity remained unchanged in the LR group, while CK was peaked in HR group at +24 h ($p < 0.001$; ES = 1.77) and remained increased at +48 h ($p < 0.001$; ES = 1.50) and at +72 h ($p < 0.001$; ES = 1.02). Also, there was a significant time-group interaction in serum sMtCK values [$F(3,75) = 3.665, p = 0.016, n^2_p = 0.128$] following RST (Figure 3A). MDA and OSI were only increased in HR individuals and remained elevated during recovery period (Figures 3B, C). Similarly, HR participants showed increased TOS at +24 h ($p = 0.002$; ES = 0.91), +48 h ($p < 0.001$; ES = 1.83) and +72 h ($p = 0.002$; ES = 0.83). However, TAS recovered basal values at +72 h. Finally, there was a significant time-group interaction in circulating irisin values following RST [$F(3,75) = 3.505, p = 0.019, n^2_p = 0.123$]. Post-hoc Bonferroni-corrected paired *t*-test showed unchanged irisin in LR group, while irisin was increased in HR group at +24 h, peaked at +48 h, and remained increased at +72 h (Figure 3D). There were no between-sex differences in the interaction of time and group for CK, sMtCK, MDA, OSI, TOS, TAS and irisin.

Table 2 shows the correlation matrix between changes in serum biomarkers and daily dietary intakes. CK and sMtCK increases were negatively and moderately correlated with daily total protein intake ($p = 0.028$ and $p = 0.007$; respectively). In addition, there was a strong correlation between the extent of damage to muscle mitochondria, oxidative stress and irisin.

3.3 Dietary intake

There were no differences between HR and LR individuals in energy intake, neither in macronutrient nor micronutrient intake



(Table 3). Also, there were no statistically significant differences between HR group ($29.4 \pm 9.0 \text{ ng mL}^{-1}$) and LR group ($26.7 \pm 9.0 \text{ ng mL}^{-1}$) in serum 25OHD status during the experimental protocol ($MD = 2.77 \text{ ng mL}^{-1}$; $t = 0.737$; $p = 0.468$).

4 Discussion

Novel sprint and hamstring-specific markers of muscle recovery were monitored during 72 h after a sport-specific RST in women and men. The findings of the research highlight that (i) there is heterogeneity in the muscular response to an RST, identifiable by MVIC torque assessment; (ii) the $90^\circ_{\text{hip}}:20^\circ_{\text{knee}}$ test for males and females, and the *Jurdan test* for males, could be powerful indirect tests to screen hamstring muscle recovery after a sport-specific RST; (iii) there is a release of sMtCK into the bloodstream during recovery period, potentially related to mitochondrial dysfunction, or mitochondrial membrane permeability/rupture; and (iv) oxidative stress and circulating irisin are upregulated during the 72-h recovery period in both HR females and males.

4.1 Hamstring muscle capacity is impaired during recovery within high responders

It has been widely reported that high-velocity eccentric contractions employed in RST leads to increased muscle-specific proteins leakage into the bloodstream, muscle soreness, as well as

reduced performance during 48–72 h in both collegiate team-sportswomen (Keane et al., 2015) and men (Howatson and Milak, 2009). However, to further explain the results given the within-human heterogeneity in response to exercise training and adaptation, individuals were categorized according to the percentage of FGC loss (Paulsen et al., 2012). HR participants ($n = 21$) showed an average hamstring FGC decrease of 21.7% at +72 h, while the LR individuals ($n = 8$) showed non-significant changes in hamstring FGC. Given the well-established correlation between ultrastructurally damaged myocytes and FGC (Raastad et al., 2010), sprint-specific muscle function tests were examined to determine muscle recovery. Previous results after RST showed decreases in quadriceps MVIC until +48 h in men (Howatson and Milak, 2009), while there were no decreases in women (Keane et al., 2015). However, the $90^\circ_{\text{hip}}:20^\circ_{\text{knee}}$ test reported decreases in hamstring MVIC torque at +24 h, +48 h and +72 h post-RST in HR individuals, whereas LR participants showed no significant differences during the recovery period. Loss of performance in the $90^\circ_{\text{hip}}:20^\circ_{\text{knee}}$ test represents a reduced FGC in the late swing phase derived from disrupted sarcomeres (Matinlauri et al., 2018), which is the phase in which most hamstring injuries occur as it is biomechanically exposed (Chumanov et al., 2012). Then, our data suggest that the $90^\circ_{\text{hip}}:20^\circ_{\text{knee}}$ test can be used to monitor hamstring muscle recovery in both women and men.

On the other hand, the *Jurdan test* showed decreases in the ROM of HR males until 72 h post-RST. LR individuals, as well as HR females, showed no differences in ROM. Anatomical differences between females and males, e.g., muscle mass size, joint geometry,

TABLE 1 Sprint performance and mechanical properties during post-exercise recovery period.

Parameter	Group	Pre	+24 h	+48 h	+72 h
Time to 10 m (s)	LR	2.185 ± 0.085	2.297 ± 0.184	2.415 ± 0.209***	2.407 ± 0.222**
	HR	2.171 ± 0.114	2.316 ± 0.173***	2.288 ± 0.126**	2.289 ± 0.149**
	ALL	2.175 ± 0.106	2.311 ± 0.173***	2.323 ± 0.160***	2.321 ± 0.176***
Time to 40 m (s)	LR	6.178 ± 0.387	6.242 ± 0.473	6.465 ± 0.472**	6.530 ± 0.471**
	HR	6.217 ± 0.569	6.416 ± 0.548**	6.402 ± 0.513***	6.417 ± 0.549**
	ALL	6.207 ± 0.519	6.368 ± 0.526*	6.420 ± 0.494***	6.448 ± 0.523***
V0 (m·s ⁻¹)	LR	8.341 ± 0.801	8.314 ± 0.758	8.216 ± 1.029	8.034 ± 0.693
	HR	8.256 ± 1.117	7.992 ± 0.943**	7.943 ± 0.988**	7.926 ± 0.984**
	ALL	8.280 ± 1.026	8.081 ± 0.895	8.018 ± 0.989*	7.956 ± 0.903**
F0.rel (N·Kg ⁻¹)	LR	7.608 ± 0.582	6.732 ± 0.995	6.268 ± 1.118***	6.313 ± 1.660**
	HR	7.790 ± 0.740	6.717 ± 0.922***	7.042 ± 0.845***	6.953 ± 1.004**
	ALL	7.740 ± 0.695	6.721 ± 0.924***	6.829 ± 0.973***	6.776 ± 1.222***
A.max (m·s ⁻²)	LR	7.677 ± 0.580	6.806 ± 1.002	6.339 ± 1.118***	6.382 ± 1.663**
	HR	7.859 ± 0.745	6.787 ± 0.929***	7.112 ± 0.849***	7.022 ± 1.008**
	ALL	7.809 ± 0.698	6.792 ± 0.931***	6.898 ± 0.975***	6.845 ± 1.226***
P.max.rel (W·Kg ⁻¹)	LR	15.097 ± 1.668	13.972 ± 2.919	12.735 ± 2.577***	12.587 ± 3.568**
	HR	15.287 ± 2.663	13.407 ± 2.869***	13.897 ± 2.501**	13.723 ± 2.769**
	ALL	15.235 ± 2.402	13.563 ± 2.842**	13.577 ± 2.531***	13.410 ± 2.988***
Time.P.max (s)	LR	0.721 ± 0.112	0.863 ± 0.128*	0.931 ± 0.238***	0.926 ± 0.245***
	HR	0.698 ± 0.100	0.832 ± 0.140***	0.791 ± 0.139**	0.800 ± 0.132**
	ALL	0.704 ± 0.102	0.840 ± 0.135***	0.830 ± 0.179***	0.835 ± 0.175***
K.fitted (s ⁻¹)	LR	1.011 ± 0.144	0.850 ± 0.112**	0.812 ± 0.165***	0.829 ± 0.217**
	HR	1.047 ± 0.155	0.885 ± 0.123***	0.938 ± 0.162***	0.927 ± 0.172**
	ALL	1.037 ± 0.150	0.876 ± 0.120***	0.903 ± 0.170***	0.900 ± 0.187***

Data are expressed as mean ± SD. *Significantly different between groups at baseline. (*), (**), and (***) indicate a significant difference from the baseline value at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Bold values represent the data for the complete sample, considering high responders and low responders together. A.max, peak acceleration; F0.rel, relative theoretical maximal horizontal force production; HR, high responders; K.fitted, rate of force development normalized by peak force; Kg, kilograms; LR, low responders; m, meters; N, newtons; P.max.rel, relative peak power; s, seconds; Time.P.max, time to peak power; V0, theoretical maximal running velocity; W, watts.

and physiological differences, e.g., greater hamstring extensibility, lower passive stiffness, better stretch tolerance, result in women usually exhibiting a greater ROM (Marshall and Siegler, 2014). Also, considering that the *Jurdan test* is an indicator of increased passive tension derived from damage to the sarcolemma of the iliopsoas and/or hamstring muscles (Lahti et al., 2020), and the aforementioned between-sex differences, females might be less likely to experience ROM loss. Therefore, although HR females showed impaired muscle structure and function, the *Jurdan test* may not show such impairment after RST. Finally, considering the characteristics of the damaging model, sprint testing is the most specific test to determine performance recovery. There was a degradation of sprint times and mechanical properties in both HR and LR participants. Only V0 was decreased in HR compared to LR individuals during the post-RST recovery period, accounting for a horizontal FGC loss at high velocities. This finding may be attributed to the fact that the hamstring muscles are the key

mediators of the forward orientation of the ground reaction force during the sprint acceleration phase (Morin et al., 2012; Morin et al., 2015). Overall, HR individuals, as determined by MVIC torque loss, also showed decreased ROM and horizontal FGC during the sprint acceleration phase until +72 h, with no differences between sex.

4.2 Is loss of hamstring muscle capacity linked to serum biomarkers?

Blood biomarkers further support the analysis of exercise-induced damage and muscle recovery (Bessa et al., 2016), since the structural status of myocytes can be assessed by changes in biochemical markers (Brancaccio et al., 2010). Peak CK activity observed in this study (723 ± 526 IU L⁻¹) is consistent with other similar RST studies (776 ± 312 IU·L⁻¹) (Howatson and Milak, 2009), indicating a permeability and/or breakage of the sarcolemma

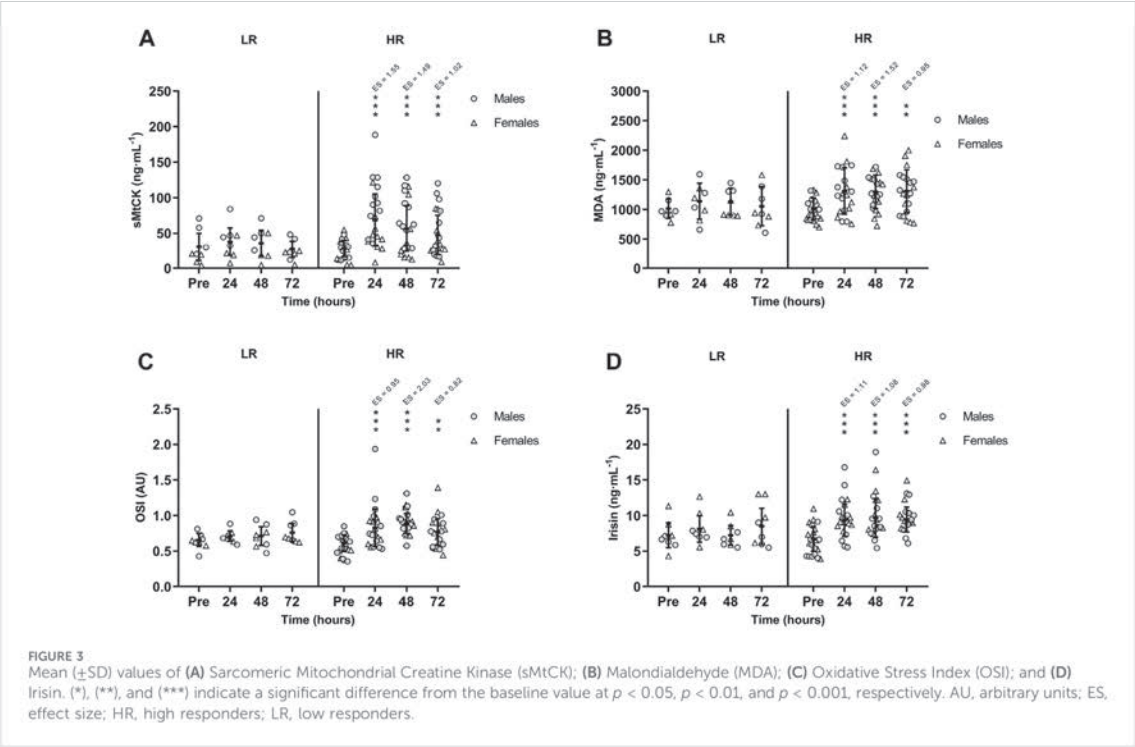


TABLE 2 Spearman's rank correlation coefficient matrix of peak fold-changes in serum biomarkers and daily dietary intakes ($n = 29$).

	CK	sMtCK	MDA	OSI	TOS	TAS	Irisin	TE	TC	TPI	API	PPI
CK	—											
sMtCK	0.663*	—										
MDA	0.197	0.201	—									
OSI	0.383*	0.595*	0.238	—								
TOS	0.375*	0.382*	0.230	0.796*	—							
TAS	−0.277	−0.442*	−0.185	−0.626*	−0.159	—						
Irisin	0.262	0.554*	0.123	0.618*	0.390*	−0.372*	—					
TE	0.074	−0.130	−0.115	−0.185	−0.163	0.134	−0.174	—				
TC	0.099	0.011	−0.015	−0.260	−0.171	0.165	−0.021	0.753*	—			
TPI	−0.423*	−0.511*	−0.150	−0.261	−0.213	0.234	−0.503*	0.673	0.404	—		
API	−0.350	−0.460*	−0.202	−0.146	−0.200	0.150	−0.360	0.667	0.277	0.940*	—	
PPI	−0.048	−0.033	0.238	−0.068	−0.013	−0.255	−0.348	0.200	0.405*	0.267	0.029	—

(*) indicates a significant correlation at $p < 0.05$. API, animal-based protein intake; CK, creatine kinase; MDA, malondialdehyde; OSI, oxidative stress index; PPI, plant-based protein intake; sMtCK, sarcomeric mitochondrial creatine kinase; TAS, total antioxidant status; TC, total carbohydrates intake; TE, total energy intake; TOS, total oxidant status; TPI, total protein intake. The significant correlations are in bold type for a clearer visualization of the data set.

(Brancaccio et al., 2010; Hyldahl and Hubal, 2014). The lipid peroxidation marker MDA was also moderately increased during the recovery period (ES = 0.95), further supporting oxidative damage to the sarcolemma. Sarcolemma impairment-derived sarcoplasmic Ca^{2+} concentration is modulated by mitochondrial Ca^{2+} uptake (Rossi et al., 2019). Excessive Ca^{2+} accumulation in the mitochondrial matrix leads to a rupture of the mitochondrial outer membrane and intermembrane content efflux, i.e., sMtCK, (Görlach et al., 2015). In this way, the muscle mitochondria were moderately damaged in HR individuals until +72 h (ES = 1.02), while no changes were observed in LR individuals. Muscle mitochondria play a critical role during skeletal muscle repair, as it requires a substantial energy

TABLE 3 Descriptive summary of macro- and micronutrient intakes.

	HR (<i>n</i> = 21)	LR (<i>n</i> = 8)
Total energy (kcal·day ⁻¹)	2163.6 ± 538.6	2281.8 ± 594.1
Total Protein Intake (g·Kg ⁻¹ ·day ⁻¹)	1.67 ± 0.30	1.91 ± 0.66
Animal-based Protein Intake (g·Kg ⁻¹ ·day ⁻¹)	1.22 ± 0.27	1.39 ± 0.53
Plant-based Protein Intake (g·Kg ⁻¹ ·day ⁻¹)	0.42 ± 0.10	0.41 ± 0.07
Total carbohydrates (g·day ⁻¹)	210.0 ± 56.3	215.3 ± 37.7
Total lipids (g·day ⁻¹)	96.8 ± 34.8	94.5 ± 30.7
Water (g·day ⁻¹)	2388.6 ± 773.4	2436.8 ± 722.7
Vitamin C (μg·day ⁻¹)	117.46 ± 60.75	116.02 ± 55.58
Vitamin D (μg·day ⁻¹)	4.31 ± 4.74	4.06 ± 3.85
Vitamin E (mg·day ⁻¹)	8.87 ± 3.00	8.96 ± 3.99

Data are expressed as mean ± SD. *Significantly different between groups, independent samples *t*-test, *p* < 0.05. HR, high responders; LR, low responders.

supply, and mitochondria are the primary source of energy production. In this sense, satellite cell signaling, which is a key factor in muscle repair, can be regulated by the mitochondria (Alway et al., 2023a). Mitochondria have also been shown to be essential in the processes of myoblast differentiation and migration into damaged fibers since mitochondrial dynamics disorders are an impediment to correct myogenic differentiation (Kim et al., 2013). Further, transplantation of healthy mitochondria into damaged skeletal muscle of murine models improved myocyte repair and recovery of muscle function (Alway et al., 2023b). Thus, given that skeletal muscle repair is limited by mitochondrial dysfunction, RST-derived damage to muscle mitochondria is a key aspect to monitor during hamstring muscle recovery through sMtCK assessment. There is also an outflow of reactive oxygen species (ROS) to the myocyte and bloodstream (Görlach et al., 2015) given that both the sarcolemma and mitochondria of the HR individuals were affected by the sport-specific RST. Since OS is a dynamic process involving large number of pro-oxidant components, e.g., total hydroperoxides, MDA, and anti-oxidant components, e.g., superoxide dismutase, glutathione peroxidase, integrative-based quantification methods such as OSI should be used to fully understand the complex interaction between exercise and OS (Thirupathi et al., 2021). Separate measurement of different oxidant and anti-oxidant molecules is complex, time-consuming, and the oxidant or antioxidant effects are additive. However, OSI considers the measurement of both oxidant and antioxidant activity produced by the action of multiple components measured as one (Sánchez-Rodríguez and Mendoza-Núñez, 2019). RST led to a moderate OS state (ES = 0.82) until +72 h post-RST. There was also a notable correlation between OS and damage to muscle mitochondria, explained by the significant implication of mitochondria in the efflux of ROS into the bloodstream after damaging exercises (Görlach et al., 2015).

Repeated muscle contractions during the RST and the aforementioned mechanical and oxidative damage to cellular and mitochondrial integrity leads to an upregulation of the AMP-activated protein kinase (AMPK) - peroxisome proliferator-activated receptor γ co-activator 1α (PGC-1α) - fibronectin domain-containing

5 protein (FNDC5) signaling axis pathway, and subsequent irisin cleavage (Boström et al., 2012; Liu et al., 2022). The myokine irisin plays an important role in muscle remodeling by stimulating satellite cell activation and ameliorating protein degradation (Reza et al., 2017). Also, irisin has been shown to be involved in counteracting oxidative processes related to free radical production (Mazur-Bialy, 2017). Irisin plays a role in inhibiting the Toll-like receptor 4/Myeloid differentiation factor 88 downstream signaling pathway, resulting in reduced activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (Mazur-Bialy et al., 2017). As a result of depressed NF-κB, irisin downregulates the expression of tumor necrosis factor-α, interleukin (IL)-6 and IL-1β proinflammatory cytokines (Mazur-Bialy et al., 2017) and promotes the polarization of pro-inflammatory M1 into anti-inflammatory M2 macrophages (Dong et al., 2016). The above-mentioned irisin-mediated effects together with the irisin-induced inhibition of ROS-NLRP3 inflammasome pathway led to an attenuated oxido-inflammatory response (Deng et al., 2018). Furthermore, muscle-damaging exercise leads to the release of irisin into the bloodstream in humans immediately after a single bout of exercise (Huh et al., 2015). Accordingly, the findings of the present study revealed a moderate increase in circulating irisin over 50.0% (ES = 0.98) during the 72-h recovery period. Considering that irisin reduces the activity of ROS-producing processes (Mazur-Bialy, 2017), downregulating ROS-NLRP3 inflammasome signaling (Deng et al., 2018), and that aerobic exercise-induced irisin partly mediates amelioration of OS and cell apoptosis in mice skeletal muscle (Ren et al., 2022), we suggest that increased circulating irisin during recovery period is a counteracting mechanism to reduce exercise-induced MD and OS. Further, the findings showed statistically large and positive correlations between irisin, and both OSI and sMtCK. Hence, exercise-induced MD and oxidative processes might be a trigger for irisin synthesis to counteract muscle cell damage.

4.3 Inter-individual heterogeneity in hamstring muscle recovery

The LR individuals showed no changes in blood markers of MD/OS, nor in muscle function during recovery period.

This between-group heterogeneity might be related to endogenous factors, e.g., age, training status, and/or exogenous factors, e.g., volume, intensity, and type of exercise (Paulsen et al., 2012). In this study, all the individuals were young, healthy, physically active, and with no previous experience in eccentric exercising, in addition to undergoing the same standardized training. Therefore, it is assumed that the response heterogeneity is not attributable to these factors. However, inter-individual variations in response to exercise are in most cases due to genetics and/or dietary intake (Mann et al., 2014). Physical exercise together with protein intake modulates muscle protein synthesis, as a result of increased bioavailability of exogenous essential amino acids (Drummond et al., 2009). As a result, upregulated muscle protein synthesis during the recovery period after acute endurance exercise promotes muscle adaptation and structural protein repair, facilitating FGC recovery (Howarth et al., 2009). Other recent applied studies have shown that increasing protein intake enhances functional recovery after sprinting training (Kritikos et al., 2021). In this way, our findings showed that both total protein intake and animal-based protein intake were moderately and inversely correlated with MD markers. Thus, we suggest that increased daily protein intake during the peri-exercise days might protect LR individuals. Finally, vitamin D intake and/or 25OHD serum levels also play a protective role on mitochondrial membranes (Salles et al., 2022). However, our results indicated no association between muscle recovery and vitamin D status or 25OHD levels. This may be attributable to the fact that the majority of individuals presented vitamin D levels above the insufficiency threshold ($>20.0 \text{ ng mL}^{-1}$), so it would hardly be possible to detect potential associations even if they existed.

5 Conclusion

There is inter-individual variability, i.e., high and low responders, in the hamstring muscles response to a sport-specific RST, identifiable by MVIC torque assessment through the 90°_{hip} ; 20°_{knee} test, which is a powerful indirect test to monitor hamstring muscle recovery in both women and men, in a cost-effective way. However, although female responders showed impaired muscle structure and function, the *Jurdan test* may not show such impairment in sportswomen after a sport-specific RST. Loss of muscle function is associated with impaired mitochondria, triggering an increase in oxidative stress status and circulating irisin in both HR females and males. Finally, muscle function remains unrecovered during the 72-h recovery period, revealing that 3 days will not be adequate time to restore hamstring structure and function after a sport-specific RST in both female and male responders.

5.1 Limitations

The study also presented some limitations to highlight when interpreting the results. First, while *a priori* computed sample size was sufficient to achieve the target statistical power, the number of participants was lowered when splitting into groups according to the *post hoc* analysis criteria of Paulsen et al. (2012). However, the use of

this classification accounts for inter-individual variations, and provides a better presentation and interpretation of the response to exercise-induced residual fatigue (Paulsen et al., 2012). Finally, muscular and biochemical markers of residual fatigue were affected during the 72-h recovery period after the RST in HR individuals. Consequently, further research conducting additional blood and muscle function measurements, e.g., +96 h, until complete recovery are required to better understand the potential implications of RST on hamstring muscles recovery.

5.2 Perspectives

Two sprint and hamstring-specific muscle function tests (90°_{hip} ; 20°_{knee} test and *Jurdan test*) were examined to determine muscle recovery after sport-specific RST in women and men. Using this screening, health professionals and practitioners can better determine the hamstring recovery status of sportswomen and men, and thus more accurately individualize training programs in multiple sprint-based sports in order to reduce injury risk. Furthermore, the findings addressed new areas of applied research, using an integrative-based and cost-effective method to monitor exercise-induced OS, and suggesting irisin as a key link between exercised-induced MD/OS and muscle recovery. Nevertheless, it remains unclear whether (i) other types of exercise training, e.g., resistance training, or (ii) the sport practice *per se*, e.g., football match, influence the relationship between hamstring-specific muscle function and the recovery kinematics of MD, OS and/or irisin.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Ethics Committee for Clinical Research of the Catalan Sports Council, Generalitat de Catalunya (037/CEICGC/2021). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

PC: Conceptualization, Methodology, Investigation, Data curation, Formal Analysis, Writing—original draft, Writing—review and editing. LM-S: Data curation, Formal Analysis, Investigation, Writing—review and editing. AP: Investigation, Writing—review and editing. ML: Investigation, Writing—review and editing. XP: Investigation, Writing—review and editing. JP: Investigation, Writing—review and editing. AF-C: Investigation, Methodology, Writing—review and editing. JC: Conceptualization, Investigation, Methodology, Project administration, Writing—original draft, Writing—review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer JT declared a shared affiliation with the authors to the handling editor at the time of review.

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11.6. Appendix 6: “Individual and region-specific hamstring muscles use during a novel Flywheel Russian belt Deadlift exercise”

Fecha: 10/06/2024
A: "Joan Aureli Aureli Cadefau" jcadefau@gencat.cat;jcadefau@ub.edu
De: "Apunts Sports Medicine" apunts@elsevier.com
Asunto: APUNTS-D-24-00036R1: decisión de los editores / editorial decision

Apreciado Dr. Cadefau:

Le comunicamos que su manuscrito "Individual and region-specific hamstring muscles use during a novel Flywheel Russian belt Deadlift exercise" (Ref. APUNTS-D-24-00036R1) ha sido aceptado para su publicación en Apunts Sports Medicine.

Recuerde que en su momento le remitiremos las pruebas de autor en formato pdf a esta misma dirección electrónica.

Reciba un cordial saludo,

Apunts Sports Medicine

Dear Dr. Cadefau,

We are glad to inform you that your article "Individual and region-specific hamstring muscles use during a novel Flywheel Russian belt Deadlift exercise" (Ref. APUNTS-D-24-00036R1) has been accepted for its publication in Apunts Sports Medicine.

Please remember that, before publication, you will receive an e-mail with the galley proofs of your article in pdf format.

Thank you for your contribution to the journal.

Yours sincerely,

Apunts Sports Medicine

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11.7. Appendix 7: Favorable certificate from the Ethics Committee



DR. RAMON BALIUS MATAS,
ACTING AS SECRETARY OF THE ETHICS COMMITTEE
FOR CLINICAL RESEARCH
OF THE CATALAN SPORTS COUNCIL.

CERTIFIES

At the meeting on November 05, 2021, the Ethics Committee agreed to favorably assess the project presented by Joan Aureli Cadefau (PhD), Gerard Carmona (PhD) & Gil Rodas (PhD), number 037/CEICGC/2021, entitled "Análisis de parámetros de calidad muscular y respuesta al ejercicio físico"

We note this favorable assessment for the appropriate purposes.



Dr. Ramon Balius Matas
Esplugues de Llobregat, November 5, 2021

11.8. Appendix 8: Informed consent written form



Formulario de consentimiento informado

Análisis de parámetros de calidad muscular y respuesta al ejercicio físico

Fecha:

Nombre:

DNI:

Teléfono:

Por favor contesta a las siguientes preguntas:

1. ¿Has obtenido información precisa referida al proyecto? Si____No____
2. ¿Has tenido oportunidad de plantear preguntas y discutir sobre el estudio con los investigadores? Si____No____
3. ¿Has recibido contestaciones adecuadas a tus preguntas? Si____No____
4. ¿Has recibido suficiente información acerca del estudio? Si____No____
5. ¿Con qué investigadores has hablado?

6. ¿Has entendido que eres libre de retirarte del estudio sin dar explicaciones? Si____No____
7. ¿Estás de acuerdo en la publicación de los resultados del estudio en foros científicos y revistas especializadas? Si____No____

Habiendo sido informado de las condiciones de participación, manifiesto que comprendo los procedimientos y sus posibles riesgos, que participo bajo mi propia responsabilidad y que consiento, libre y voluntariamente, participar en este estudio para analizar la calidad muscular mediante técnicas de análisis sanguíneo, tisular y funcional

Firma:

Los Investigadores responsables del proyecto manifiestan que la persona participante tiene la posibilidad de abandonar su participación en el estudio, en cualquier momento y sin la obligación de dar explicaciones de su voluntad.

Firma:

Investigadores responsables y datos de contacto

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