UNIVERSITAT DE BARCELONA

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Acute and chronic muscle responses of the lower limbs to iso-inertial training

Victor Illera Dominguez

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Víctor Illera Domínguez

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ABSTRACT

In this doctoral thesis, I aim to identify and characterise the acute and chronic responses of the lower limbs to iso-inertial training sessions using the half-squat exercise. The document is composed of four consecutive studies.

Study 1 investigates the validity of a friction encoder against a criterion measure at providing force, velocity and power measures on the iso-inertial resistance training device. The results suggest that the friction encoder provides valid measures of velocity, force and power on flywheel exercise devices. Such a device is a solution for the control of training sessions which can provide athletes with live-augmented feedback on a day by day basis.

Study 2 investigates the acute effects of an iso-inertial training session on different indicators of performance. These indicators include sprint and jump performance, and isometric force generating capacity. The response of the participants was highly heterogeneous, with some showing signs of moderate muscle damage that affected functional performance until 72 hours post exercise. These results highlight the need for specific tests for assessing readiness and recovery after sessions.

Study 3 investigates the chronic effects of a structured 4-week iso-inertial training programme on muscle function and hypertrophy (total volume) measured by magnetic resonance imaging (MRI). Participants showed great increases in quadriceps volume after two (+ 5.5%) and four (+ 8.6%) weeks of training. These results were accompanied by increases in half-squat force (≈ 30% over four weeks) and power (≈ 50% over four weeks). Increases in strength were also seen in knee extensor exercise (+ 28%) after four weeks, but no changes were seen in knee flexor exercise. This study reports the earliest onset of whole-muscle hypertrophy documented to date (5 sessions / 14 days).

Finally, *Study 4* investigates the protective effect conferred by iso-inertial training sessions against muscle damage from intense exercise. The parameters assessed include: isometric force, vertical jump height, muscle soreness, and blood biochemical markers of muscle damage (Creatine kinase, sarcomeric mitochondrial creatine kinase, creatine kinase MB isoform, aspartate aminotransferase, alanine aminotransferase, titin, and cardiac troponin I). Despite performing much higher workloads after the training period (+38.9%, +21.0%, and +65.3% in production of concentric force, velocity, and power, respectively), all markers of muscle damage were attenuated, and the recovery processes were faster (the index of protection ranged from 75.4% to 79.7% for the muscle function parameters and from 52.5% to 85.5% for blood biochemical markers: and was 48.0% for muscle soreness).

RESUMEN

El objetivo de esta tesis doctoral es el de identificar y caracterizar las respuestas agudas y crónicas del tren inferior ante sesiones de entrenamiento iso-inercial utilizando el ejercicio de media sentadilla. El documento está compuesto por cuatro estudios consecutivos.

El *Estudio 1* valora la validez de un codificador de fricción contra una medida criterio para proporcionar valores de fuerza, velocidad y potencia en el dispositivo de entrenamiento iso-inercial. Los resultados sugieren que el codificador de fricción proporciona medidas válidas de velocidad, fuerza y potencia. Este dispositivo es una solución para el control de sesiones de entrenamiento que permite proporcionar retroalimentación en vivo a los atletas día a día.

El estudio 2 investiga los efectos agudos de una sesión de entrenamiento iso-inercial en diferentes indicadores de rendimiento, incluyendo sprint y salto, y la capacidad de generación de fuerza isométrica. La respuesta entre los participantes fue muy heterogénea, y algunos participantes mostraron signos de daño muscular moderado, que afectaron el rendimiento funcional hasta 72 horas después del ejercicio. Estos resultados destacan la necesidad de pruebas específicas para evaluar la disponibilidad y la recuperación tras las sesiones.

El estudio 3 investiga los efectos crónicos de un programa estructurado de entrenamiento iso-inercial de 4 semanas sobre la función muscular y la hipertrofia (volumen total) medido por resonancia magnética. Los participantes mostraron grandes aumentos de volumen en el cuádriceps después de dos (+ 5.5%) y cuatro (+ 8.6%) semanas de entrenamiento. Estos resultados se acompañaron de aumentos en la fuerza (≈ 30% en cuatro semanas) y la potencia (≈ 50% en cuatro semanas) en la media sentadilla. También se observaron aumentos en la fuerza en el ejercicio de extensión de rodilla (+ 28%) después de cuatro semanas, pero no se observaron cambios en el ejercicio de flexión de rodilla. Este estudio reporta del inicio más temprano de hipertrofia de músculo completo documentado hasta la fecha (5 sesiones / 14 días).

Finalmente, el Estudio 4 investiga el efecto protector conferido por las sesiones de entrenamiento iso-inercial contra el daño muscular causado por el ejercicio intenso. Los parámetros evaluados incluyen: fuerza isométrica, altura de salto vertical, dolor muscular y marcadores bioquímicos sanguíneos de daño muscular (p. Ej., Creatina quinasa, creatina quinasa mitocondrial sarcomérica, creatina quinasa isoforma MB, aspartato aminotransferasa, alanina aminotransferasa, titina y troponina cardíaca I). A pesar de realizar cargas de trabajo mucho más altas después del período de entrenamiento (+ 38.9%, + 21.0% y + 65.3% de producción de fuerza concéntrica, velocidad y potencia, respectivamente), todos los marcadores de daño muscular se atenuaron y los procesos de recuperación fueron más rápidos (El índice de protección varió del 75.4% al 79.7% para los parámetros de función muscular, del 52.5% al 85.5% para los marcadores bioquímicos sanguíneos y fue del 48.0% para el dolor muscular).

ABBREVIATIONS

ALT	Alanine aminotransferase
AM	Adductor magnus (muscle)
AST	Aspartate aminotransferase
BFL	Biceps femoris long head (muscle)
BFS	Biceps femoris short head (muscle)
CK	Creatine Kinase
CK-MB	Creatine Kinase -MB isoform
CMJ	Counter-movement jump
CON	Concentric
CSA	Cross sectional area
cTnI	Cardiac Troponin I
DOMS	Delayed onset muscle soreness
ECC	Eccentric
EIMD	Exercise induced muscle damage
EMG	Electromyography
F-V	Force–velocity
GM	Gluteus maximus (muscle)
GR	Gracilis (muscle)
ISO	Isometric
mfMRI	Muscle functional magnetic resonance imaging
MI	Mild responders
MO	Moderate responders
MRI	Magnetic resonance imaging
MVIC	Maximum voluntary isometric contraction
RBE	Repeated bout effect
RCT	Randomized control trial
RF	Rectus femoris (muscle)
RM	Repetition maximum
ROI	Region of interest
ROM	Range of movement
RPE	Rate of perceived exertion
SJ	Squat Jump
SM	Semimembranosus (muscle)
sMtCK	Sarcomeric mitochondrial Creatine Kinase
SSC	Stretch shortening cycle
ST	Semitendinosus (muscle)
T2	Transverse relaxation time
TEE	Typical error of the estimate
VI	Vastus intermedius (muscle)
VL	Vastus lateralis (muscle)
VM	Vastus medialis (muscle)

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INTRODUCTION

1. INTRODUCTION

1.1. ECCENTRIC MUSCLE ACTIONS

Eccentric (ECC) muscle actions are defined as those that occur when an external force (i.e., weight) applied to a muscle exceeds the momentary force generated by that muscle, and therefore the muscle–tendon system "actively elongates" [1]. This mode of muscle action accounts for an important part of most movements performed in sports and also during daily activities (i.e., changing direction, walking downstairs or running downhill).

ECC actions are also referred to as "negative work," because, during the process, the muscle absorbs the mechanical energy developed by the external load [2]. The absorbed energy may be dissipated as heat (i.e., on absorbing shock or landing), or partially stored by the muscle–tendon system as elastic recoil energy and recovered in the subsequent concentric (CON; i.e., muscle shortening) action [1]. The coupling of ECC with CON muscle actions, similarly to a spring, is defined as the stretch shortening cycle (SSC) [3].

While the molecular mechanics of isometric (ISO; i.e., constant length) and CON actions have been well described for over half a century [4,5], ECC muscle actions are still not fully understood [6]. Despite this, there is a consensus that ECC actions imply distinctive molecular and neural features that result in unique muscular adaptations [7–10].

1.1.1. Differential characteristics of eccentric actions.

Compared to ISO or CON actions, greater forces (30%–40%) are generated during ECC muscle actions [11]. Yet the muscle activation (measured as electromyography (EMG) amplitude) is lower during maximal ECC actions than maximal ISO or CON actions [12]. Moreover, for a given muscle tension, the energy and oxygen consumed during ECC actions is some four times less than during CON actions [2].

The unique characteristics of ECC muscle actions described in this section have important implications for acute and chronic responses following bouts of ECC exercise.

Molecular Characteristics

The increased force production of ISO, compared to CON, actions and of slow, compared to fast, CON actions is well explained by the sliding filament theory of muscle contraction and by the force–velocity relationship [4,13]. With increasing speed, the exposure time of myosin heads to actin binding sites decreases [6], and therefore the number of cross-bridges is reduced. Furthermore, myosin molecules are not fully stretched (tensed) during fast CON actions [10]. During ISO actions, cross-bridge turnover (i.e., continuous dissociation and formation of new bridges maintaining net cross-bridge count) still occurs and thus energy expenditure still takes place even in the absence of external work [14]. However, the greater forces generated during ECC actions (i.e., residual force enhancement) at a lower metabolic cost compared to ISO actions are not well explained by previous molecular theories. Therefore, new theories have been put forth in order to explain these observations.

It has been postulated that during ECC actions, the number of cross-bridges might be augmented. While during ISO and CON actions only one myosin head of the molecule is bound, the increased strain on a single myosin head during lengthening may facilitate the activation of the second (i.e., partner) head [7]. This molecular mechanism would potentially duplicate the number of active cross-bridges and could be increasingly utilised with increasing lengthening velocity [7]. Additionally, the myosin S2 complex (proximal part of the tail) is fully stretched and able to pull maximally on the actin filaments [10].

Moreover, the mechanical detachment of active cross-bridges during ECC actions may account for some of the differences reported between different types of muscular actions. During ECC actions, cross-bridges do not complete the full cycle [15]; instead, myosin heads remain in an active state, bound to actin, and are detached by force, followed by a rapid re-attachment [16]. As the detachment is not “actively” produced by adenosine tri-phosphate, less energy is consumed to produce force [7,16].

Finally, it is believed that passive structural factors of the sarcomere (i.e., the cytoskeleton), and specifically the protein titin (also known as connectin), plays a major role in ECC muscle actions [6]. Titin is the largest known polypeptide in the natural world: with a span of 1 µm, each molecule crosses half a sarcomere from the Z-band to the M-line [17]. Overlap of molecules of titin in series, both in Z-disks and M-lines, produces a titin filament system that extends the entire length of the myofibre [18]. Titin acts in two different ways. Firstly, it acts as a passive element. The I-band region of Titin is elastic, acting as a spring. When the sarcomere stretches, even in the absence of muscular activation, titin offers passive muscle force and largely determines the passive elasticity of muscle [18,19]. Secondly, it acts as an active filament in muscle actions (the three-filament model of contraction). In the presence of calcium, the N2A region of titin binds actin filaments in the region of the I-band, which shortens and stiffens the titin spring [18,20,21]. Further to this, it is also believed that titin acts as a “winding filament”: during contraction, cross-bridges pull and rotate actin filaments [22], winding-up titin on actin and thereby stretching and storing elastic energy in the titin “spring” molecule [18,21] (Figure 1).

Several regions of titin responsible for tension (mechanosensors), mainly located in the Z-disc, the elastic I-band and the M-band, have been linked to different signalling pathways. This may partially explain the differential hypertrophic adaptive response to ECC, as compared to CON, resistance training [23].

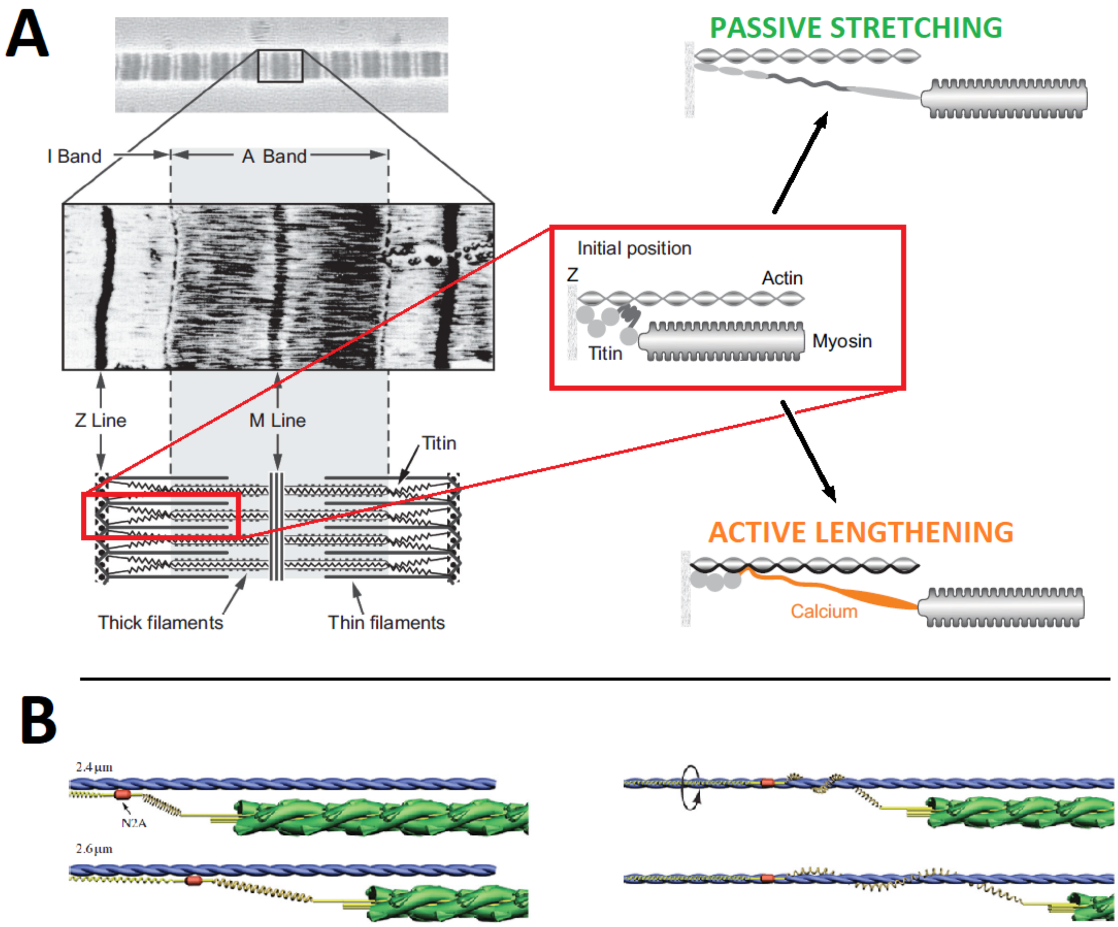


Figure 1. Figure 2. Schematic illustration of the contribution of titin to residual force enhancement during eccentric (ECC) actions. A: the different stiffness of the titin molecule in the presence or absence of calcium (i.e., active or passive lengthening respectively). B: two proposed active mechanisms of ECC force enhancement by titin: binding of the N2A region of titin to actin filaments (Left) and titin winding over actin filaments as a result of cross-bridge cycling (Right).

Modified from Herzog (2014) [6] (A); and from Nishikawa et al. (2012) [21] (B).

Neural Characteristics

ECC actions exhibit unique neural characteristics under both maximal and submaximal conditions [9]. Untrained individuals show a marked inhibited voluntary activation during maximal ECC actions, mainly constrained by spinal inhibition mechanisms (both pre- and post-synaptic) that produce lower and more variable motor unit discharge rates [24]. However, heavy load resistance training attenuates such reflex inhibition, improving the neuromuscular activation and the maximal ECC strength [25]. In submaximal ECC actions, and due to the residual force enhancement, fewer or smaller motor units are required to match the equivalent CON action force demands [26].

Finally, irrespective of the intensity and the lower motor unit activity during ECC actions, cortical excitability is enhanced and there is more brain area involved [27,28]. As efferent motor output depends on central descending pathways and the inflow from sensory reflex pathways, such as Golgi organs, muscle spindles, muscle afferents and Renshaw cells [12], enhanced cortical excitability and descending drive has been postulated as a compensatory response to spinal inhibition [29].

1.1.2. Acute responses to eccentric exercise.

Fatigue

As previously mentioned, ECC actions are more efficient than CON actions, given that for the same power output, ECC exercise is less metabolically demanding and requires the activation of fewer motor units [7]. This results in lower perceived exertion, blood lactate accumulation, energy expenditure, carbohydrate oxidation, and higher fat oxidation during ECC than CON exercise, at a matched mechanical workload [30]. These facts have important practical implications, as it may be possible to achieve higher session workloads with ECC training [7].

Hormonal and molecular responses

The hormonal response after resistance training (testosterone, insulin-like growth factor-I, growth hormone, insulin, cortisol, etc.) is not greatly influenced by the type of muscular action (ECC vs CON). Indeed, the main mediator of the response is the combination of load and time under tension [7]. Nevertheless, the current body of evidence suggests that the influence of acute hormonal post-exercise changes on long-term adaptations is, at most, very modest [31].

Regarding the acute molecular response, maximal ECC exercise induces an earlier rise in protein synthesis and greater myofibrillar protein accretion in the 8.5 hours following exercise, compared to matched-work maximal CON exercise [32]. Currently there is no clear consensus on which myogenic mechanisms regulate the transduction of differential ECC action versus CON action stimuli into different adaptations [10], but it is believed to be mediated by distinct molecular pathways [33]. Some authors have hypothesised that the increased mechanical tension (i.e., stretch) from ECC actions within the Z-line region of titin elicits a specific anabolic signalling response [7,23].

Finally, increases in the concentration of cytokine interleukin-6, a signalling molecule for satellite cell activation, have been reported after ECC exercise [34]. Furthermore, acute satellite cell proliferation has been reported after maximal ECC, but not maximal CON, resistance training [35]. It is believed that the tissue damage associated with ECC actions is the main stimulus for the activation of the satellite cell gene pool [36].

Exercise induced muscle damage

Exercise-induced muscle damage (EIMD) is the physical damage or tearing of the muscle (ultrastructural disorganisation) typically caused by unaccustomed ECC actions or ISO actions at long muscle lengths [8]. When muscle fibres are subjected to very high tensions, the weakest functional units of the chain (sarcomeres or even half sarcomeres) will be stretched beyond the point of filament overlap, resulting in the disruption of the sarcomere (also expressed as "popped" sarcomeres) [37]. Commonly, damaged regions do not extend along the whole length of the fibre; instead, many are located in single half sarcomeres of single fibres [37–39] (See Figure 3).

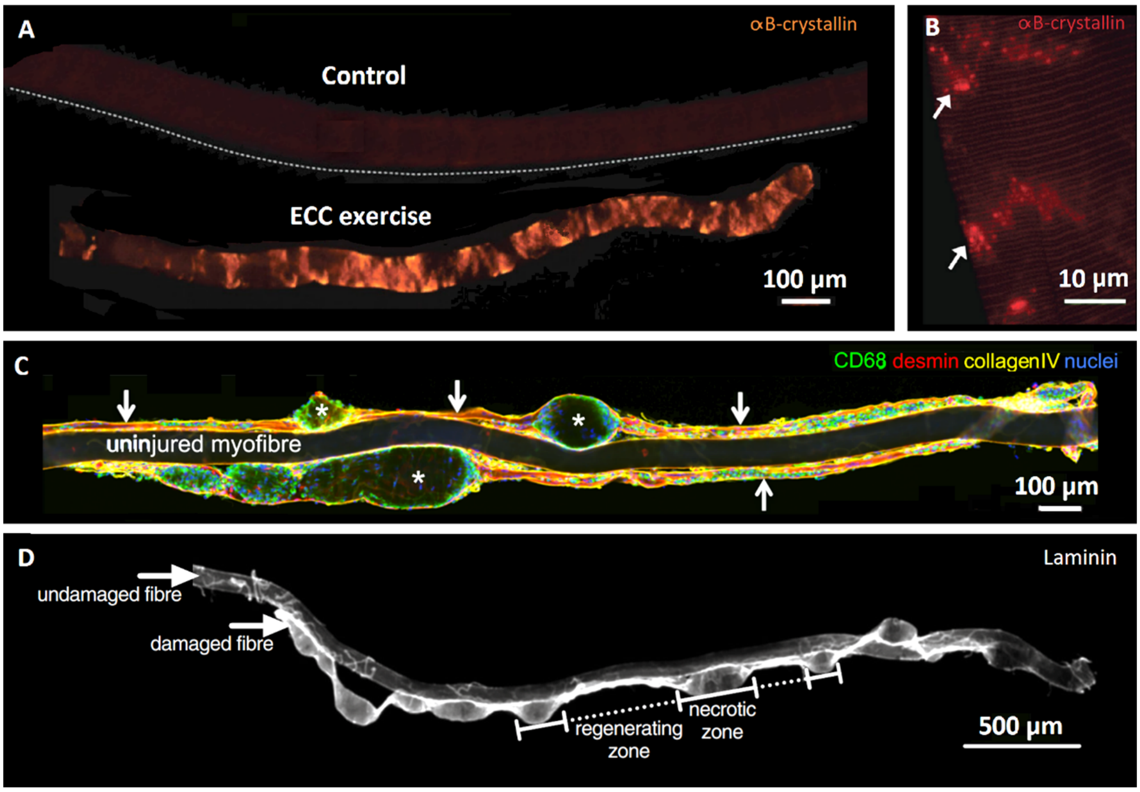


Figure 3. Wide-field microscopy images of immuno-stained human muscle fibres. A) Control vs eccentric (ECC)-exercised fibre, stained for αB-crystallin, a heat shock protein which translocates from the cytoplasm to cytoskeletal or myofibrillar structures during exercise to prevent damage to these structures during ECC actions. B) Amplified detail of the fibre. Arrows point to specific zones of αB-crystallin accumulation. C and D) Fibres 7 days after ECC actions + electrostimulation; regenerating zones are interspersed with necrotic zones. In C, arrows = regenerating zones; asterisks = necrotic zones.

Modified from Paulsen et al. (2009) [39] (A,B); and from Mackey & Kjaer (2017) [38] (C,D).

Microscopic evaluation of the histological abnormalities or ultrastructural disorganisation of the sarcomeres is the only direct method to assess EIMD [40]. However, in order to do this, a muscle biopsy is required, which can be unpleasant for participants and requires medical resources (therefore, this technique is limited in research and not practical in most settings). In addition, the biopsy is taken from a very specific point of the muscle belly, providing a very small quantity of tissue; therefore, it may be unreliable and not representative of whole-muscle changes [40–43]. To overcome this, there are several indirect (proxy) markers of EIMD, including measurements to quantify symptoms (i.e., muscle weakness, swelling, fatigue or stiffness) that typically occur after ECC resistance training, which can offer information about the process of damage and subsequent repair [40,44] (Figure 4). These markers are not necessarily associated in terms of their time courses (i.e., appearance and clearance) or the magnitude of the changes [42].

Some proteins and enzymes also leak into the bloodstream from muscles after damaging exercise. The mechanisms by which these markers appear in blood are still not fully elucidated, but it is believed that features such as their molecular weight and structure, charge, or the cellular compartment in which the marker is originally located influence the pathway the marker follows and the rate of appearance and clearance in blood [45–49]. Additionally, some markers or isoforms are ubiquitously expressed in various tissues (i.e., creatine kinase; CK), while others are tissue specific (i.e., cardiac troponin I; cTnI), or even tissue-type specific (i.e., slow and fast myosin). Therefore, the evaluation of different blood markers in the hours after damaging exercise allows the damage to be profiled (severity and location) [42,49,50]. Due to the relative ease of quantification of these blood markers, compared to muscle biopsies, are commonly used as proxy markers of EIMD.

EIMD is also typically accompanied by the appearance of delayed-onset muscle soreness (DOMS); but this symptom correlates poorly with other indirect EIMD markers and is not considered to be very reliable reflection of the magnitude of muscle damage [51]. By contrast, muscle function (i.e., force generating capacity measured as maximal concentric or ISO strength) is generally considered as one of the most reliable and valid markers for assessing the degree of muscle damage [42].

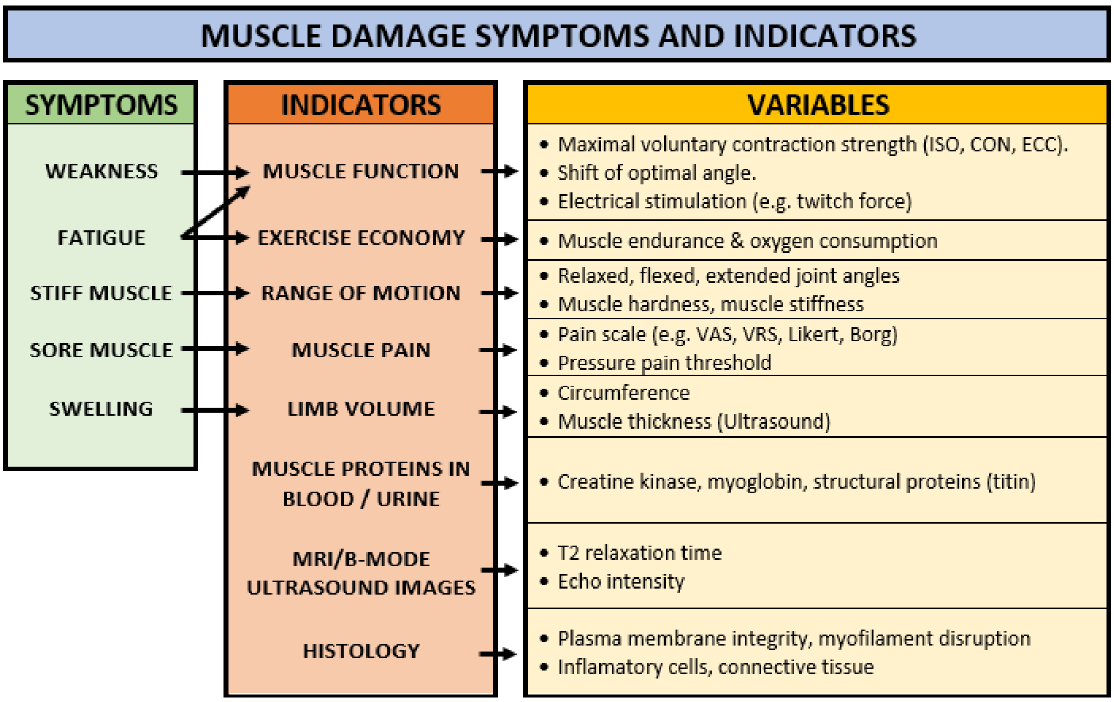


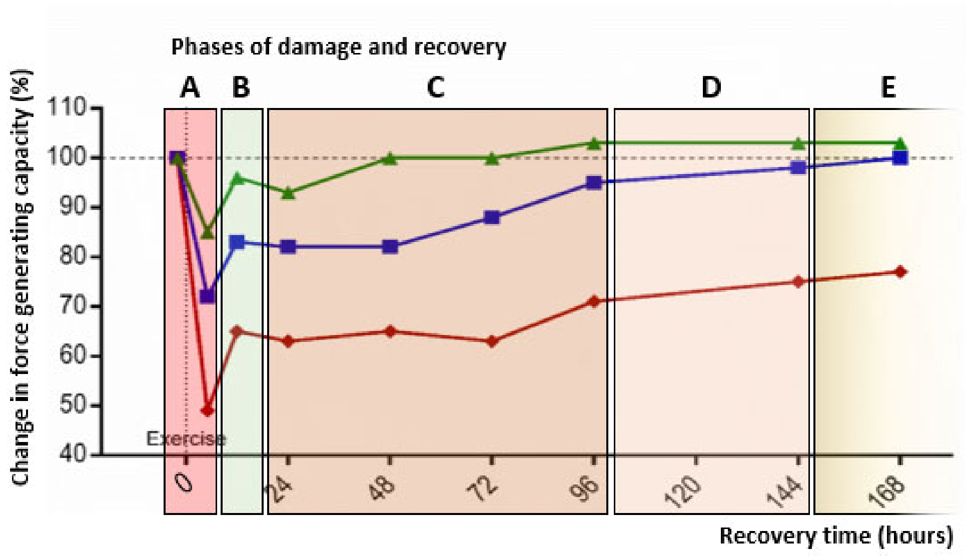
Figure 4. Symptoms, indicators, and measures of exercise-induced muscle damage (EIMD). Modified from Nosaka (2009) [44].

Monitoring one or more of these indicators is imperative in order to ensure that training stimuli have the desired effect and are not excessive. Moreover, they provide information of the exact time course of the recovery process, and therefore allow us to assess if the organism is ready to cope with another training session [52].

Initially, ECC resistance training produces direct damage to the muscle structures; combined with an acute state of fatigue these produce a rapid decrease in muscle function after exercise, with mild progressive recovery in the first hours, as fatigue disappears [42]. However, if the initial stimulus was strenuous enough, the initial mechanical damage triggers a downstream cell signalling cascade leading to more severe secondary damage [9,42,53]. Briefly, the initial mechanical damage, loss of calcium homeostasis, a possible inflammatory reaction and the production of reactive oxygen species, all contribute to the secondary damage phase, degrading the sarcolemma or opening stretch-activated channels and therefore further increasing calcium concentration inside muscle cells [42]. The increase of calcium activates muscle proteases (calpains) which break down structural proteins. This process could also be enhanced by other proteolytic pathways such as the ubiquitin–proteasome system [54]. Depending on several factors, the resulting severity and duration of EIMD can vary drastically and may not be completely recovered until 1-3 weeks after the damaging exercise [42] (Figure 5).

The magnitude and duration of EIMD after performing a given exercise is highly variable. Depending on the percentage loss in the force generating capacity and the time needed for complete recovery after the damaging exercise, EIMD is classified as (i) mild EIMD, when the loss does not exceed 20% and complete recovery is achieved within 48 hours; (ii) moderate EIMD, when loss is between 20% and 50% and is recovered within 2–7 days; and (iii) severe EIMD, when loss exceeds 50% and is not recovered within one week [42].

The response is influenced by several factors, such as the preconditioning status (i.e., previous exposure), the speed of contraction, the muscle length at which the exercise is performed, the muscle group, etc. [8,44]. As shown in Figure 6, fast ECC muscle actions are potentially the most damaging, although the final result is determined by the interrelation of different factors. Another less important factor is age (preadolescent children are less susceptible to EIMD than young adults; but elderly adults are not necessarily more prone to suffer EIMD than young and middle-aged adults) [55,56]. There is also controversy regarding differences in susceptibility to EIMD between men and women; although men seem to be more prone to suffer greater EIMD, this sex difference does not appear to be very large [57].



		SEVERITY OF EXERCISE-INDUCED MUSCLE DAMAGE		
PHASES		MILD	MODERATE	SEVERE
A	During exercise	Transient decreases in muscle force generating capacity < 20% decrease	20 – 50% decrease	> 50% decrease
		Metabolic fatigue Primary damage: Mechanical disruptions to the myofibrillar structures and membranes (including elements of the excitation-contraction coupling) (①) and to the connective tissue		
B	First hours after exercise	Recovery from metabolic fatigue and partial recovery of structural changes Secondary damage: Loss of Ca^{2+} regulation → Increased calpain activity → Increased structural disruptions (①) Formation of a chemo-attractive gradient for leucocyte accumulation		
C	1-4 days after exercise	Complete recovery within 48 hours	Halted recovery Increased accumulation of leucocytes in the extracellular space (②)	
			Recovery of Ca^{2+} regulation and remodelling of myofibrillar structures	Continuous high intracellular Ca^{2+} High calpain & ubiquitin-proteasome activity, increased lysosomal digestion
D	5-7 days after exercise		Normalization of force, proteolytic activity and complete remodelling of myofibrillar structures	Swollen myofibers with segmental necrosis (③). Major release of soluble proteins (e.g. CK). Loss of cytoskeletal proteins. Massive leucocyte accumulation (④) and increased satellite cell/myoblast activity (⑤) Continuous depression of force capacity
E	1-3 weeks after exercise			Regeneration. Satellite cells divide and myoblasts form myotubes.

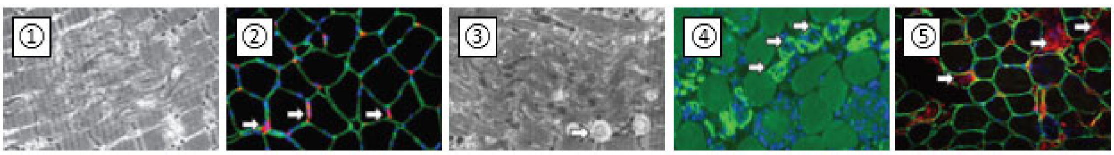


Figure 5. Events during the recovery of the force-generating capacity after mild (green line), moderate (blue line) and severe (red line) exercise induced muscle damage (EIMD). Differences between mild, moderate and severe damage increase over time as secondary damage progresses. In severe EIMD, myofibers must be regenerated (by myoblasts), a process that may take several weeks and is manifested by a long-lasting reduction in the force-generating capacity. ① & ③ Electron Microscopy (longitudinal sections), ②, ④ & ⑤ Fluorescence Microscope (cross-sections): Image ② and ④: Red stain: leucocytes; green stain: basal lamina (laminin); blue stain: nuclei (DAPI). Image ⑤: Green stain: satellite cells/myoblasts (NCAM); blue stain: nuclei (DAPI). Modified from Paulsen et al. (2012) [42].

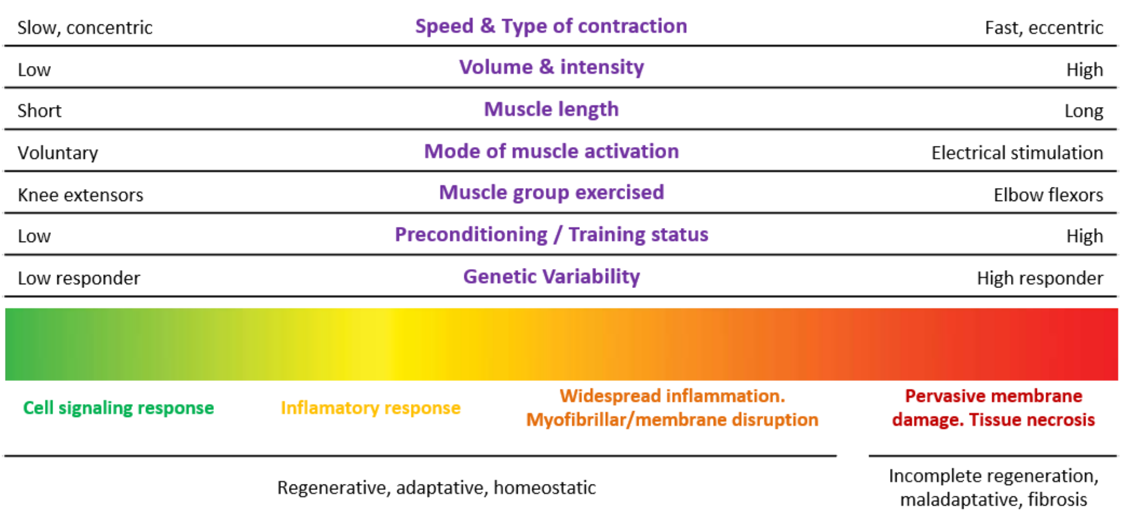


Figure 6. Continuum of the response of muscle fibre to exercise, from an adaptive cell signalling response to maladaptive injury. Response to exercise is dependent on several factors (shown in purple). The lines projecting in both directions under these factors indicate that the response under each condition can occur anywhere along the continuum (left: lower damage; right: higher damage; i.e., fast eccentric actions may result in more severe disruptions and a greater inflammatory response than slow concentric actions).

Modified from Hyldahl & Hubal (2014) [8] *and Nosaka (2009)* [44].

1.1.3. Chronic muscle adaptations to eccentric exercise.

Structural adaptations – Evidence of hypertrophy

Muscle hypertrophy is a biological phenomenon that can be measured at different levels (i.e., ultrastructural level / organ level / whole muscle level), using a wide variety methods (i.e., microscopy, ultrasound, MRI, etc.) and means (i.e., protein or mass quantification, 1-dimensional; muscle thickness, 2-dimensional; muscle / fibre cross-sectional area (CSA), 3-dimensional; muscle volume) [43]. The changes in these different descriptors may not correlate. Currently, MRI is widely considered the “gold standard” for the quantification of whole muscle hypertrophy.

Several studies have indicated a possible superior overall effectivity of ECC resistance training in terms of promoting higher and earlier muscle hypertrophy over CON or conventional strength training methods, although the high variability in the results between studies hinders clear confirmation [58]. A recent systematic review and meta-analysis comparing the effects of ECC and CON actions on muscle growth showed greater muscle mass gain with ECC, but the results did not reach statistical significance [59].

Again, it must be taken into account that higher muscular tensions can be reached with ECC actions [11], and mechanical tension is considered the primary driver of muscle hypertrophy [60,61]. Given that in several comparative studies the ECC training loads were higher than those used in CON training, in another review in the topic [62], the authors concluded that total increases in muscle size are similar between ECC and CON training when matched for load or work. Nevertheless, as higher active and passive maximal tensions can be attained during ECC actions [11,36], and taking into account the energetic demands to produce similar force or work, ECC exercise may be considered more efficient at producing hypertrophic changes [58].

Together with mechanical tension, metabolic stress and muscle damage have been proposed as factors that contribute to muscle hypertrophy via different myogenic pathways [61]. EIMD induces the release of growth factors that regulate satellite cell activation and proliferation, as well as anabolic signalling. Therefore, EIMD has been posited as a possible additive factor with ECC training [36]. However, it must be noted that muscle hypertrophy can occur in the absence of any symptoms of muscle damage [63], and that a high level of EIMD may prolong the recovery period excessively [42] (Figure 5), or may even result in incomplete regeneration or maladaptation [8] (Figure 6. Figure 6).

Regarding muscle composition, greater increases in the type II fibre area compared with type I muscle fibres has been reported after ECC training [36,58,64]. Furthermore, ECC resistance training appears to be even more effective in increasing the type II fibre area when performed at higher intensities or velocities of movement [10]. It has been suggested that this is due to the increased tension generating capacity of type II fibres [36,65]. A shift in the myosin heavy-chain phenotype after ECC training has also been reported, moving from type I and IIx to fast-oxidative hybrids (MHC-I/IIa + MHC-IIa/IIx), overall producing a shift towards a faster muscle phenotype [36,58,66].

Another significant difference between ECC and CON resistance training is contraction-specific regional hypertrophy where ECC is more closely associated with distal growth and CON with more pronounced mid-belly hypertrophy [10,36,67,68].

In addition, the different hypertrophic responses to ECC and CON exercise seem to take place via different structural adaptations mediated by distinct myogenic and molecular responses [10]. While both CON and ECC resistance training increase muscle fascicle length and pennation angle, ECC seems to produce a greater increase in fascicle length, and CON favours greater increases in pennation angle, possibly reflecting the differential addition of sarcomeres in either series or parallel, respectively [10,62,69] (Figure 7). Although this muscle architecture change might be particularly interesting for injury prevention and sports performance [70], the validity of the traditional methods used for the assessment of changes in muscle architecture (narrow field-of-view two-dimensional static image ultrasonography) is hotly debated [71–73].

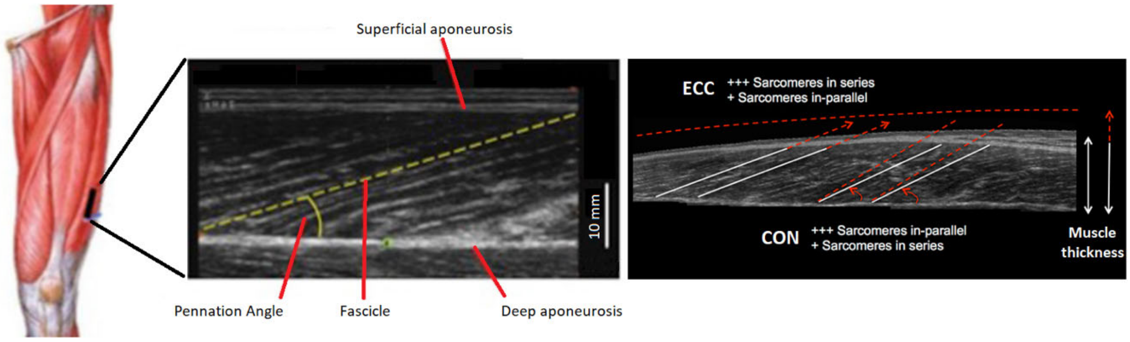


Figure 7. Diagram illustrating muscle architecture changes in response to eccentric (ECC) or concentric (CON) resistance training (assessed by ultrasound images). While similar increases in muscle thickness can be achieved, CON will preferentially add sarcomeres in parallel, with an increase in pennation angle, and ECC will preferentially add sarcomeres in series, with an increase in fascicle length. +++ = preferential addition; + = marginal response; white lines = pre-training; dotted red lines = post-training.

Modified from Franchi et al. (2017) [10].

Functional adaptations

In a systematic review and meta-analysis with clinical trials [74], ECC resistance training was shown to promote significantly greater increases in muscle strength. However, the results suggested that this was mediated by the higher forces developed during maximal training in both modalities, given that when the subgroup for matched ECC and CON intensities was analysed, no statistical differences between the training regimes were found [74].

Adaptations after ECC resistance training have shown to be highly specific to the velocity and type of contraction performed (i.e., showing greater effects in ECC strength and at the velocities trained) [36,74]. Indeed, the increase in ECC strength after ECC training is greater than the gain in CON strength after CON training [9,75]. Although strength improvements after ECC resistance training arise from a combination of neural, morphological and architectural adaptations [36,74], increased agonist volitional drive via disinhibition of excitatory inflow to spinal motor neurons is considered the main contributor to the marked increases in ECC strength observed following ECC training [12,29]. ECC resistance training programmes also appear to improve performance in actions involving SSC training (i.e., vertical jumping) to a greater extent than CON resistance training [36,76].

Finally, it is well established that ECC training is able to shift the optimum zone of the length–tension relationship to longer muscle lengths [70].

The repeated bout effect

After suffering EIMD, skeletal muscle initiates an adaptive response to develop protection against subsequent damaging stimuli. This response is commonly known as the repeated bout effect (RBE). Typically, the RBE is observed when the same ECC exercise is repeated; however, protection against EIMD may also be conferred by a different non-damaging ECC exercise (i.e., lower volume or intensity ECC training), or even by an ISO exercise performed at greater muscle lengths [77]. For most EIMD indicators, the RBE lasts at least 6 months but is lost after between 9 and 12 months [78].

The magnitude of the protective effect (quantified as the % reduction of EIMD indicators from the first to the second bout) is influenced by several first-bout exercise variables, such as intensity, volume (i.e., total number of damaging contractions), velocity, muscle length and muscle group, as well as the time elapsed since the first bout [79]. As summarised in a recent review on the topic by Hyldahl et al. (2017), the effects of the RBE on the different indicators also vary [80]: The strongest RBE effect is seen on CK activity, with almost a complete reduction. DOMS is also strongly reduced (i.e., 40% to 80% reduction). The strength loss immediately after (≤ 1 hour) ECC exercise is similar between bouts, but the recovery rate is greatly increased (i.e., ≥80% faster recovery between immediately after and 1 day post-exercise). Although not completely elucidated, these variations point to different mechanisms underpinning the RBE on the different indicators of EIMD (Figure 8).

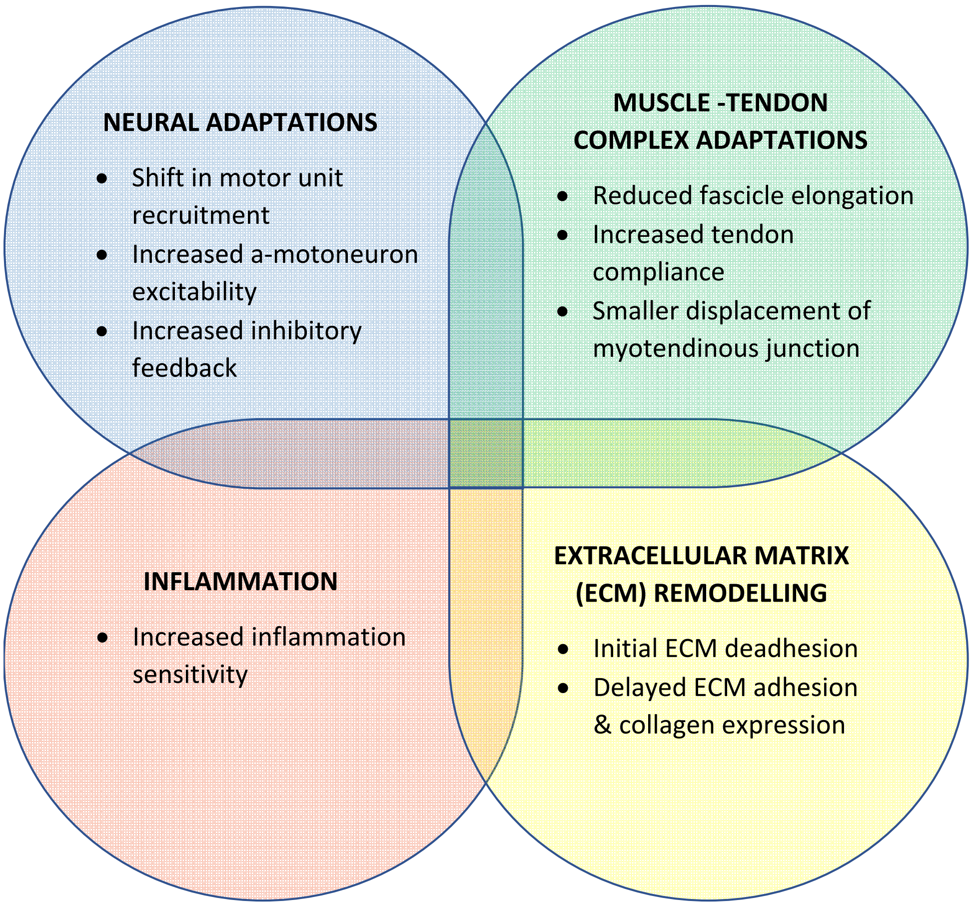


Figure 8. Interaction of the potential mechanisms responsible for the repeated bout effect (RBE). Some mechanisms (i.e., neural and inflammatory) might drive the early (~1 day to 2 weeks) manifestations of the RBE, while others (i.e., extracellular matrix (ECM) remodelling) may be responsible for the longer lasting (2–6 weeks) effects of the RBE.

Modified from Hyldahl et al. (2017) [80].

1.1.4. Eccentric based training modalities and applications.

The main characteristics, special features, and both acute and chronic adaptations to ECC muscular actions have been summarised throughout this first chapter of the introduction. These special characteristics confer ECC actions with the potential to produce beneficial effects, but some considerations have to be taken into account to minimise potential associated risks (Figure 9).

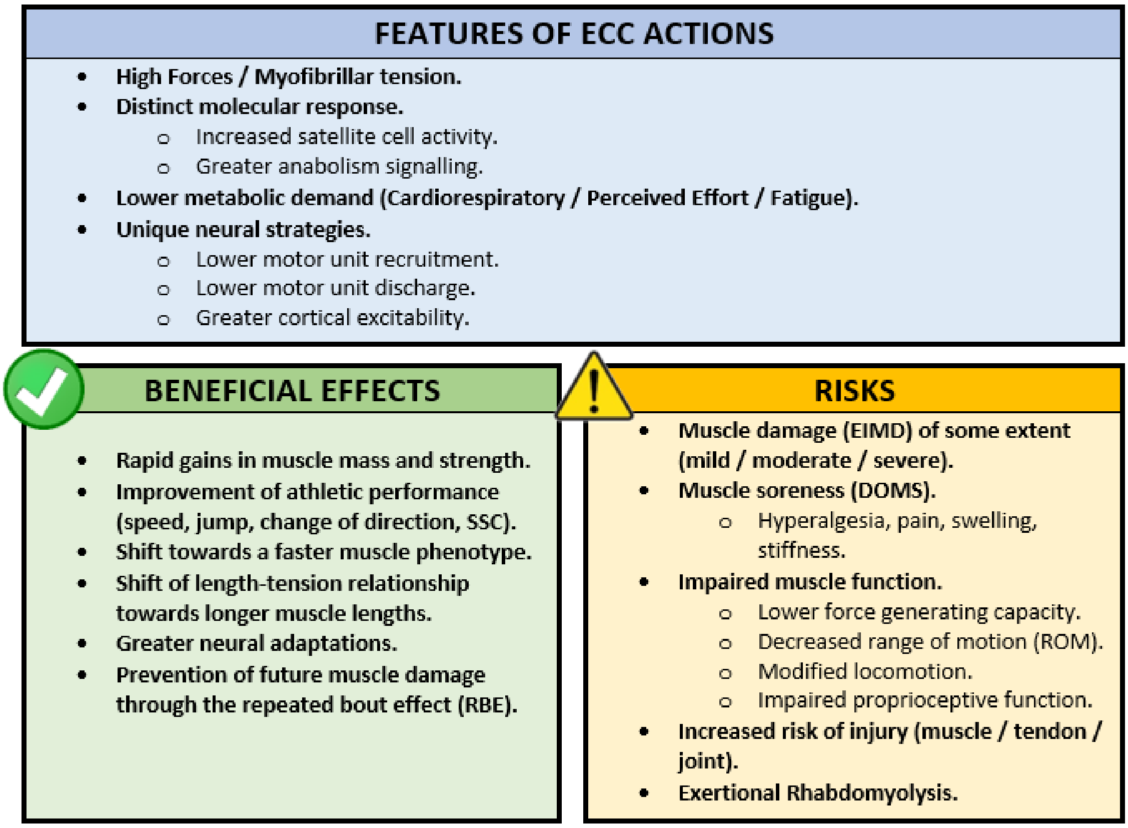


Figure 9. Summary of the main specific features of eccentric (ECC) actions, their beneficial effects and the potential risks associated with unaccustomed or maximal ECC exercise.

Modified from Hody et al. (2019) [9].

Researchers tend to consider ECC training as a single entity, with standard acute responses and chronic adaptions. However, ECC muscle work (with or without a CON phase or an accentuated ECC load), can be performed using different exercise modalities that are commonly classified as constant weight (iso-weight), constant velocity (iso-kinetic) or constant inertia (iso-inertial) training [*]. Each modality provides different biomechanical stimuli, acute responses and chronic adaptions [81–83].

[*] The term "iso-inertial training", if considered literally, would also include iso-weight training, as inertia remains constant. Although this term may not be the most suitable for defining this modality of training, it is commonly used to describe the mode of exercise that uses the state of movement of a mass (usually a rotational movement) to create the ECC resistance.

Iso-weight ECC exercise includes body weight exercises, free weights and most weight-training machines. It is the most accessible and simple ECC training modality, and consequently the most used in a practical setting [81]. The main limitation of this type of exercise is that high intensity ECC actions are sometimes difficult to achieve, requiring either advanced strategies (i.e., combining a bilateral CON phase with a unilateral ECC phase, varying the lever arm length in the ECC phase, etc.), using very heavy loads, an external aid in the CON phase (i.e., training partner), or a combination of these [81,84].

Iso-kinetic ECC training allows ECC actions to be reproduced in standardised conditions with optimal loading along the whole range of movement (ROM) [85]. However, it requires the use of a iso-kinetic dynamometer, which is still an expensive and relatively inaccessible piece of equipment for most users [81]. Moreover, this mode of ECC training has been continuously questioned for consisting on isolated, constant-velocity single-joint actions and therefore having very low functionality [85]. For these reasons, the use of this technology is mainly limited to research settings, and it has provided the vast majority of the evidence regarding ECC training [81].

Finally, as the so-called "iso-inertial training" will be the main focus of this thesis and the topic will be reviewed thoroughly in the next chapter. This modality of training has been increasingly introduced into practical settings due to the ease of producing an overload in the ECC phase of different exercises and complex movements or displacements [86]. One of the main limitations of this modality (for some populations) is that the ECC action cannot be performed alone, and is highly reliant on the CON performance [81,82]. Additionally, the quantification of exercise load is more complex than when using free weights [81,87].

These three modalities can be used to produce different stimuli, varying in mechanical stress (force/load), muscle length or ROM, and velocity of ECC actions, to produce different adaptions [81,83]. Over recent years, ECC-based training methods have become of interest in several fields. Specifically, different forms of ECC training can be used for: (a) rehabilitation of tendon injuries (tendon remodelling); (b) prevention of muscle injuries (increase in ECC strength and shift in optimum length); (c) accentuated / supramaximal ECC loading (for increasing strength, hypertrophy, and performance); and, (d) high-velocity ECC actions for increasing sport performance (SSC optimisation) [83,88]. Additionally, ECC exercise is being increasingly implemented with elderly individuals, overweight and obese people, and in the rehabilitation of some pathological conditions, because high muscle tensions can be achieved at low metabolic cost [58,86,89].

1.2. ISO-INERTIAL TRAINING

1.2.1. Historical context

Iso-inertial flywheel machines have been used for centuries in mechanical applications, to accumulate kinetic energy (i.e., potters wheels, mills, vehicles, industrial machinery, etc.). The first documented iso-inertial machine to be used as a resistance training device was the “Gymnasticon” (France, 1796), invented by Francis Lowndes [90] (Figure 10A). Pioneer scientific studies using iso-inertial training devices were performed at the University of Copenhagen during the early 1920s [91], although the focus of those studies was not the specific responses or adaptions to this type of training (Figure 10B). Notwithstanding these scientific studies, for decades the use of iso-inertial flywheels as a resistance training method was a rarity, with few references documenting it [92] (Figure 10C).

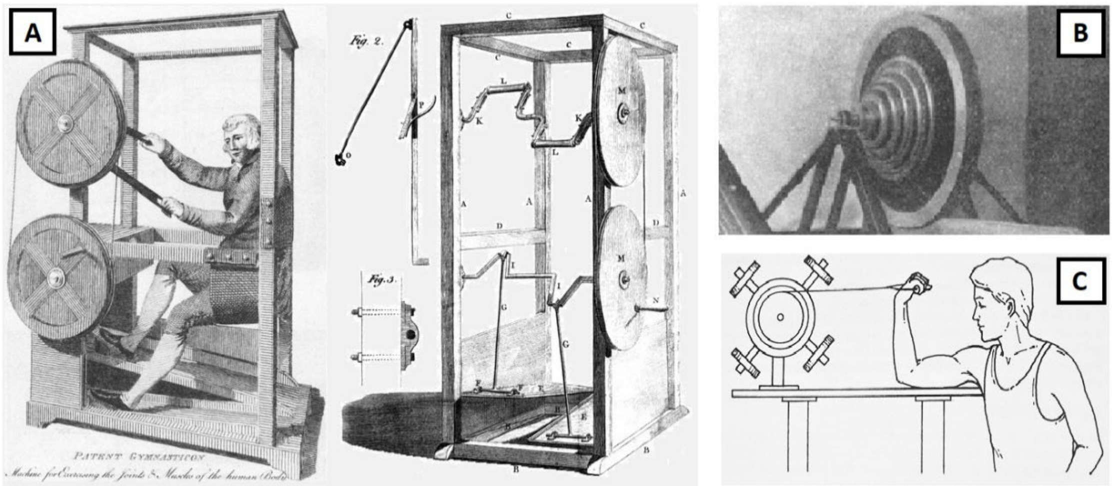


Figure 10. Iso-inertial resistance training devices. (A) Gymnasticon, image from a patent repertory dated 1797 [90]. (B) First iso-inertial resistance training devices used for research, image from Hansen & Lindhard (1923) [91]. (C) Iso-inertial machine used in the Soviet Union during the 1960s, image from Zatsiorsky (1966) [92].

It was not until the early 1900s that iso-inertial training started to be studied specifically, as a distinct training method. Interest grew within the framework of a project shared between the US National Aeronautics and Space Administration (NASA) and the European Space Agency (ESA). Physiologists were looking for a solution to severe muscle atrophy and bone mass loss in astronauts subjected to microgravity for extended periods of time. After trying different solutions, Swedish scientists Hans Berg and Per Tesch successfully designed a gravity-independent iso-inertial training device for use in space [93,94]. At first, interest in this training method focused on its application in astronauts. However, as new research was conducted, interest spread to application in “on-earth” resistance training programmes, and progressively multiple flywheel resistance training devices were developed for different exercises and muscle groups. In recent years, there has been an exponential increase in the use of iso-inertial resistance training devices in sports facilities, accompanied by a parallel increase in research [86].

1.2.2. Principles and characteristics

The term "iso-inertial training", in a literal interpretation, would include several training methods such as iso-weight training, since inertia remains constant. However, the common use of the term, both in the scientific literature and in practice, refers to the use of either (i) a regular-axis flywheel or (ii) a conical pulley (i.e., a flywheel with a cone-shaped axis) to create resistance, through the change in the state of rotational movement of the mass [81]. In both cases, the operating principle is similar to that of a yo-yo, which is based on the conservation of angular momentum (Figure 11).

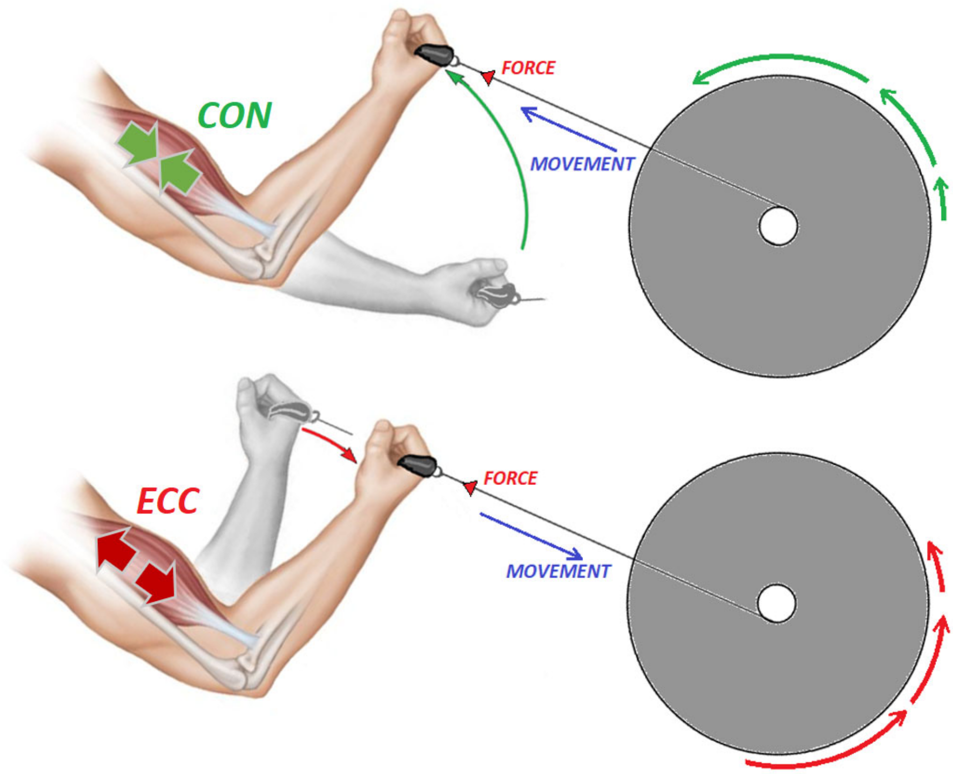


Figure 11. Depiction of the operating principle of iso-inertial training devices. A strap or rope is attached to an axis, to which one or more flywheels are fixed. The other end of the strap or rope is attached to a handgrip, vest, mechanical arm, etc., where the person applies force. Before starting the exercise, the strap has to be rolled up for a few revolutions around the axis, then the concentric (CON) phase of the movement can start. During the CON phase, muscles pull the rope to unwind it from the axis. The resistance against the rotational acceleration applied by the rope is created by the rotational inertia of the discs attached to the axis (they tend to remain static, in opposition to increasing the rotational velocity). At the end of the CON phase, the rope is completely unwound and the axis is rotating at its maximum velocity. During the eccentric (ECC) phase, muscles pull the rope in an attempt to prevent it from rolling up around the axis again. Therefore, the rope applies a rotational deceleration to the axis (braking) which again is resisted by the rotational inertia of the discs attached to the axis (they tend to remain at the same rotational velocity, in opposition to braking) [95].

Unlimited load

The amount of force that has to be applied in order to get a certain velocity in the CON phase depends on the rotational inertia (commonly known as moment of inertia) of the flywheels [87,96]. This parameter is determined by the mass of the flywheels and by the distribution of that mass relative to the axis of rotation (Equation 1). From a practical point of view, this means that the greater the moment of inertia used, the greater the force needed to achieve the same execution velocity. Alternatively, if the same forces are applied under both conditions, the exercise performed with a higher moment of inertia would be slower [87,96,97]. One substantial difference with iso-weight training is that there is no dynamic 1-repetition maximum (1RM), because even with a very high moment of inertia, the force applied would (very slowly) accelerate the flywheel.

Equation 1. Rotational inertia (I, commonly known as moment of inertia) depends on the mass (m) of the rotating object and the radius from the axis of rotation (r). It is measured in kg·m² (SI units).

$$I = m \cdot r^2$$

Accommodated resistance

One of the most differential features of iso-inertial training is that the load is variable, and at any instant proportional to the developed force. With iso-weight training, the load of the set is determined by what can be moved through the sticking region of the ROM of the CON phase of the last repetition, and therefore, by definition, all previous effort of the set will be submaximal [98]. In contrast, iso-inertial systems can offer a near-maximal resistance along the whole ROM and throughout the exercise set, and thus, the mechanical tension developed through one set is higher (Figure 12).

Figure 12. Illustration of the principle of accommodated resistance. With iso-weight training, the exercise ends when the maximum force that can be developed falls below the set load (XRM) due to fatigue. All repetitions, except the last, are by definition sub-maximal. With iso-inertial training, every repetition is performed at the maximum strength that the athlete is able to produce, even if it decreases due to fatigue. RM, repetition maximum.

Eccentric overload

During traditional iso-weight training (consecutive CON-ECC phases), because the same absolute load is displaced during the repetition, motor units are de-recruited during ECC to enable the weight to be lowered. Therefore, muscle activation and relative loading are much lower during ECC than during CON [10].

During iso-inertial training (which is a mode of CON-ECC training that it highly dependent on the CON performance), the same energy transferred to the flywheel during the CON phase, has to be dissipated (throughout the braking action) in the ECC phase [81,86]. Therefore, iso-inertial training has the potential to accentuate force during the ECC phase over that developed during traditional iso-weight training. However, this does not guarantee the so-called “ECC-overload” effect (i.e., increase the ECC force beyond that applied during the CON phase). Rather, brief episodes of ECC overload can be created during the ROM, depending on the exercise technique when receiving the load [49,98–100].

A possible way to overload part of the ECC phase is to delay or concentrate the braking action in the last portion of the ROM. Ignoring friction, as the mechanical impulse (i.e., force · time) has to be equal from the CON to the subsequent ECC phase, concentrating the braking action in a shorter period of time would oblige the application of higher mean forces during that time [99]. Another common way to create an ECC overload is to have assistance during the CON phase (a partner or bilateral CON to unilateral ECC phase).

1.2.3. Quantification of the external load

One of the main drawbacks of iso-inertial training has historically been the quantification of the training dose [81]. As previously explained (in Principles and characteristics), with this mode of training it is not possible to quantify intensity as a percentage of the 1-RM. Given this limitation, the vast majority of research on this training method only reports the moment of inertia used, but no other kinematic parameters such as force, velocity or power [101–103]. Furthermore, some recent studies do not even report the moment of inertia employed [104–106], which can be difficult to calculate if it is not provided by the manufacturer. The vital importance of these variables on the training adaption outcomes limits the potential replicability of those studies.

The *gold standard* for measuring the kinematic variables of exercise on iso-inertial training devices is considered to be the use of a force platform / force transducer, in combination with a linear transducer / video motion analysis system [49,107,108]. However, these measurement systems are not widely accessible and are very unpractical outside a laboratory setting for day-to-day training quantification.

New approaches to the quantification of exercise kinematic variables include the use of fixed axis rotary encoders. These encoders track the rotation of the axis (displacement and angular velocity), to estimate repetition force and power [107]. Despite one manufacturer showing acceptable validity levels of force and power measurements, for the moment these solutions are system-specific, and cannot be adapted to other machines or configurations (i.e., intermediate pulleys, additional masses, etc.) [107]. However, a new prototype friction encoder has been developed which can be attached to different flywheel or conical pulley systems and adapted to a wide variety of configurations [95]. This new tool may allow practitioners to monitor almost any iso-inertial exercise easily. Nevertheless, the validity of data gathered by this means remains to be shown.

Despite the difficulties presented in this chapter, iso-inertial training is increasingly being studied and characterised, in order to provide rigorous and reproducible guidelines for exercise prescription [86,87,96].

1.2.4. Acute responses to iso-inertial exercise

After different protocols of iso-inertial resistance exercise, consistent increases in indirect blood markers of muscle damage have been reported [49,104,109]. For instance, the concentration of CK in blood increases post exercise, and remains elevated in the subsequent hours (for up to 72 hours in resistance-training-naive volunteers [49,104,110], and up to 24 hours in experienced volunteers [111]). Furthermore, significant peak increases in the concentration of fast myosin in blood 144 hours after exercise suggest that muscle structure is affected [49]. Overall, these results indicate that a mild to moderate degree of muscle damage is produced by this type of training, depending on the protocol and the previous experience of the trainees [42].

In agreement with these findings, muscle function impairments can persist for several hours after iso-inertial exercise. For example, Coratella et al. (2016) reported a significant decrease of ISO peak torque of the knee extensors immediately after exercise, which lasted for 72 hours [104]. Piqueras-Sanchiz et al. (2019) also reported changes in hamstring muscle contractile properties measured by tensiomyography, which were dependent on the iso-inertial training load (i.e., moment of inertia) [87]. However, these reductions in muscle performance are highly dependent on the protocol used, and in fact, several studies have investigated a post-activation potentiation effect with high-intensity, low-volume iso-inertial resistance exercise loads [112–114].

Finally, a robust increase in circulating extracellular vesicles has recently been reported; they contain several “myo-miRNAs” (markers of muscle remodelling and adaption) and pro-inflammatory cytokines after a single iso-inertial resistance exercise session in experienced, resistance-trained men. These findings indicate that iso-inertial resistance exercise entails a potent stimulus for adaption processes, which elicits a systemic molecular response, even in resistance-trained volunteers [111].

1.2.5. Chronic adaptations to iso-inertial exercise.

As previously explained, over recent years there has been increasing research interest in the science and application of iso-inertial training programmes. Due to the high volume of research output and the heterogeneity of the intervention designs, two recent reviews by Nuñez & Sáez (2017) [115] and by Petré et al. (2018) [116] aimed to determine the chronic effects of iso-inertial training on healthy people, in terms of muscle hypertrophy and strength. Additionally, the latter review aimed to assess the effects on power, development of horizontal movement, and development of vertical movement [116]. This chapter will focus especially on the meta-analysis performed by Petré et al. (2018), as it is more recent, broader, and contains new studies in addition to those included in the former review.

From their bibliography search performed in August 2018, the authors finally included twenty high-quality studies (354 participants) for the meta-analysis (Table 1).

The results from this meta-analysis in terms of muscle hypertrophy show an overall mean effect size of 0.59, and significant mean increases of 0.20% and 0.19% per day in volume/mass and CSA respectively, over the first 5–10 weeks of flywheel training 2–3 times per week [116]. Specifically, the study performed by Lundberg et al. (2013) reports the highest rate of muscle hypertrophy (0.4% increase in volume per day over the first 5 weeks) in the scientific literature [117]. Additionally, the study conducted by Seynnes et al. (2007) reports the earliest onset of macroscopic muscle hypertrophy (4.2% increase in CSA after only 20 days of training / nine training sessions and a 2.4% increase in fibre length after only 10 days of training / five training sessions) [103]. However, some studies have questioned the validity of large muscle hypertrophy after a short period of time (i.e., only 20 days), claiming a possible confounding effect of muscle oedema, in spite of an actual increase of muscle tissue [118]. Therefore, future studies controlling this parameter and using high-precision methods to quantify whole-muscle hypertrophy are warranted [119].

The highest total effect size of iso-inertial training is reported in terms of maximal strength (1.33), with a mean percentage increase of 0.41% per day in well-trained individuals, and 0.23% per day in untrained individuals [116]. It would be logical to expect greater increases in untrained individuals as the training potential is higher, but the available studies with iso-inertial training do not support this idea. Several confounding factors may have influenced this result, but a possible explanation is that untrained individuals develop maximal strength most effectively with moderate loads (60% of 1RM, four sets, three times per week), while moderately trained individuals also respond better to moderate loads (80% of 1RM, four sets, two times per week), but well-trained individuals seem to need higher relative loads (85% of 1RM, eight sets, two times per week) [120]. As iso-inertial resistance devices offer near-maximal resistance along the whole ROM, this stimulus might be especially effective for developing maximum strength in well-trained individuals.

For power, a very similar total effect size (1.19) is found, as too are average rates of improvement, with percentage increases of 0.60% per day in well-trained individuals, 0.44% in moderately trained individuals, and 0.32% in untrained individuals [116].

Table 1. Characteristics of the studies included in the systematic review and meta-analyses performed by Petré et al. (2018) [116], modified. Level of training is coded as: 0= untrained; 1= moderately trained; 2=highly trained.

Study		Participants				Intervention					Effects of Training (Change in %)					
											Hypertrophy		Specific Function		Movement transference	
Authors & reference	Year	n	sex	age	Level of training*	Exercise	Sets x reps	Rest (s)	Duration (weeks)	Sessions per week	CSA	Volume or mass	Maximal strength	Power	Horizontal (time)	Vertical
Núñez et al. [232]	2018	27	M	23±3	2	Squat and lunges	4 x 7	180	6	2		+6.1		+30.0	+0.03	+5.0
Maroto-Izquierdo et al. [128]	2017	15	M	20±1	2	Knee extensor	4 x 7	180	6	3			+11.8	+15.3	-10.8	+9.8
Sabido et al. [227]	2017	11	M	24±4	2	Squat and lunges	4 x 8 & 2 x 8	120	7	1			+14.2	+38.2	-2.5	+6.0
Gual et al. [233]	2016	27	MF	23±4	2	Squat	4 x 8	120	24	1				+59.2		+3.4
Naczk et al. [122]	2016	37	M	21±1	2	Knee extensor	3 x 15s	120	5	3		+11.0	+25.7	+32.6		+10.8
Naczk et al. [130]	2016	20	M	22±1	2	Elbow flexor & extensor	3 x 15s	120	5	3		+13.7	+14.4	+17.8		
Owerkowicz et al. [106]	2016	17	MF	22±1	0	Knee extensor	4 x 7	300	5	2	+9.9		+16.5			
Bruseghini et al. [234]	2015	12	M	68±4	1	Knee extensor	4 x 7	180	8	3	+4.2	+4.9	+6.5			
De Hoyo et al. [102]	2015	18	M	18±1	2	Squat & Leg curl	3-6 x 6	180	10	1-2					-1.5	+7.3
Fernandez-Gonzalo et al. [109]	2014	32	MF	24±1	1	Leg press	4 x 7	180	6	2-3		+5.0	+22.4	+5.3		+5.8
Lundberg et al. [131]	2014	10	M	26±5	1	Knee extensor	4 x 7	120	5	2-3	+4.1	+4.2	+10.8	+17.1		
Naczk et al. [129]	2014	33	M	21±1	2	Shoulder adduction	3 x 20s	120	4	3			+13.5	+14.8		
Lundberg et al. [117]	2013	10	M	25±4	1	Knee extensor	4 x 7	120	5	2-3	+10.8	+10.7	+28.5	+26.1		
Norrbrand et al. [125]	2010	9	M	39±5	0	Knee extensor	4 x 7	120	5	2-3			+8.2			
Norrbrand et al. [126]	2008	7	M	39±9	0	Knee extensor	4 x 7	120	5	2-3		+6.2	+7.6	+10.5		
Onambele et al. [134]	2008	27	M	23±3	0	Knee extensor	1-4 x 8-12	300	12	3				+27.8		
Seynnes et al. [103]	2007	7	MF	20±2	1	Knee extensor	4 x 7	120	5	3	+6.9		+38.7			
Caruso et al. [136]	2005	10	MF	59±2	0	Leg press	4 x 8	90	10	3		+3.9	+2.5			
Tesch et al. [127]	2004	10	MF	39±8	1	Knee extensor	4 x 7	120	5	2-3		+6.1				
Askling et al. [124]	2003	15	M	26±4	2	Leg curl	4 x 8	60	10	1-2			+17.1		-2.4	

Regarding the effects on horizontal or vertical movement, there is a clear effect on both variables (effect sizes of -1.00 and 0.85 respectively) [116]. As expected, several studies have reported a highly specific adaptive response in terms of the force–application vector. That is, horizontal-type resisted exercises (i.e., conical pulley resisted displacements) tend to improve horizontal-type movements more (i.e., sprint), and vertical-type resisted exercises (i.e., squat) tend to improve vertical-type movements more (i.e., jumps) [102,121]. Additionally, a high-velocity protocol seems to improve the jumping capacity more (0.38% per day over 4 weeks) than others with higher inertias [122]. In a recent study by Sabido (2019) comparing high (0.075 kg·m^2) and low (0.025 kg·m^2) iso-inertial loads in rugby players, those authors showed better results in horizontal tests with lower iso-inertial loads. They concluded that this might be a better option for optimising athletic performance [123].

Overall, the available scientific evidence indicates that iso-inertial resistance exercise is a valid method for producing fast and significant improvements in muscle hypertrophy, strength, power, horizontal movement, and vertical movement [116].

Iso-inertial training and the repeated bout effect

Given the important ECC component of iso-inertial training, protection against a future damaging exercise or sporting event is one of the adaptations that is desired by the strength and conditioning coach. This will ultimately allow better recovery after training sessions and a lower risk of muscle injury. To the best our knowledge, only one study, performed by Coratella et al. (2016), has specifically aimed to determine the adaptive effects of iso-inertial training in terms of the RBE [104].

That study monitored blood CK, peak torque of the quadriceps and muscle soreness as markers of muscle damage in the 72 hours following two training sessions. Each session, separated by 4 weeks of rest, consisted of two similar bouts (10 sets of 10 repetitions of iso-inertial squats, with a non-reported moment of inertia). All muscle damage markers were significantly lower after the second bout than after the first [104]. Although that study does provide interesting information of the protective effect of a iso-inertial exercise, the hypothetical scenario of similar exercise and ECC force stimulus is far removed from real training programmes, because participants typically experience a great increase in force production (i.e., 0.41% increase per day) [116], and therefore, the potentially damaging training stimulus will be greater than that in the first training session.

Another study by Fernandez-Gonzalo et al. (2014) [109] reported that blood CK concentration changes in the 72 hours following the first and the last training sessions of a 6-week iso-inertial training programme (15 sessions). Although they reported a ≈50% increase in power from the first to the last training sessions, CK response following training sessions was highly attenuated. These results provide a basis for the hypothesis that even though the ECC component increases greatly (and thus so does the external load), the exercise tolerance increases at a higher rate, thereby decreasing muscle damage and recovery time. However, it should be noted that blood CK concentration as the sole marker of

EIMD does not provide enough data to evaluate the damage [42,109]. Moreover, the exact kinematic parameters of the exercise were not reported (i.e., applied CON and ECC force). Therefore, a more exhaustive study is needed in order to investigate how training stimulus (i.e., external load) increases due to accommodated resistance, and how muscle reacts to the more demanding training session (i.e., internal load).

Iso-weight training vs iso-inertial training

In addition to the previous reviews and meta-analysis discussed in this chapter, two systematic reviews with meta-analysis have been published. They aimed to evaluate the possible superiority of iso-inertial training over conventional iso-weight training.

The first review, performed by Maroto-Izquierdo et al. (2017), aimed to examine the adaptive effects of iso-inertial training with ECC overload on muscle size and functional capacities, and compare them with those caused by traditional iso-weight resistance training [99]. Nine studies were included in the review (276 participants; athletes or healthy subjects) [102,122,124–130]. Interventions ranged from 4 to 10 weeks with a frequency of 2.33 ± 0.72 sessions per week and a total number of sets and repetitions ranging from 3 to 6 and from 6 to 8 respectively. The most common moment of inertial employed was between 0.07 and 0.145 $kg \cdot m^2$ [125–128]. The muscle groups trained were knee extensors and flexors, shoulder abductors, and elbow flexors and extensors. The authors found subtotal higher gains after iso-inertial ECC overload training compared with other methods, in terms of strength, muscle power, hypertrophy, jump capacity, and speed [99]. Of the variables assessed, muscle power showed the greatest benefit from iso-inertial training. Additionally, the authors found that increases were larger in ECC than CON force after iso-inertial training [124,131], and that studies assessing adaptations in the upper limbs reported the largest effects [129,130].

In a second review, Vicens-Bordas et al. (2017) re-examined the superiority of either iso-inertial or iso-weight resistance training methods on producing strength gains [132]. Three randomised controlled trials (RCTs) were included in the review (76 participants) [105,133,134]. While one study favoured iso-inertial over iso-weight resistance training [105], two studies did not [133,134]. It must be noted that one of these latter studies [133] was performed with individuals with multiple knee injuries, compliance to the training protocols was very low (means of 19 and 22 sessions, out of 37, in the iso-inertial and iso-weight training groups, respectively), and the intended velocity of the exercise in the iso-inertial group was not maximal, but instead was marked by a metronome, set at approximately 3 seconds for CON and 3 seconds for ECC phases (the moment of inertia was not reported). Moreover, in the other study that reported no differences [134], the moment of inertia and other descriptors of the iso-inertial load were not given. Additionally, the two groups of older adults included in the study were significantly different in terms of pre-training ISO maximum voluntary strength, which may have favoured a greater increase in the iso-weight training group. Another stratified analysis in the work of Vicens-Bordas et al. (2017) [132] with non-RCTs included four studies

[125,126,135,136] (71 participants): two studies favoured iso-inertial training over iso-weight training [125,126], and two did not [135,136]. In the latter two studies, again, the moment of inertia and other descriptors of the iso-inertial loads were not given [135,136]. Additionally, the authors mentioned that, due to inherent differences in leg press devices, the iso-weight exercise protocol led to greater strain magnitudes during training (a possible confounding factor) [135,136]. In this review with meta-analysis, Vicens-Bordas and colleagues could not conclude the superiority of either iso-inertial or iso-weight resistance training in terms of strength gains [132].

Finally, two experimental studies covering this topic were published in 2019. One study in soccer players (1 session per week, over 8 weeks, either 48 iso-weight or iso-inertial resisted squats) showed greater improvements in change of direction (COD) drills (20 m + 20 m shuttle test and T-test), and eccentric quadriceps strength in the iso-inertial group [137]. The other study, performed by Lundberg et al. (2019), showed that after training for 8 weeks, 2-3 days/week, one leg with iso-weight and the other with iso-inertial resisted knee extensions, both training systems showed similar results in terms of hypertrophy and strength. However, given that that the iso-inertial protocol consisted of a lower volume (4 × 7 repetitions versus 4 × 8-12 repetitions), the authors concluded that this training method is more efficient in producing the outcomes [138].

In conclusion, although the topic is still hotly debated [139,140], the evidence suggests that iso-inertial resistance training produces robust improvements in muscle mass, strength, power, and horizontal and vertical movements; and that such improvements are generally greater than those attained by iso-weight resistance methods or achieved in a more efficient way (i.e., less training for equivalent results). Therefore, given its versatility, ease of use, and multiple advantages, research on iso-inertial training (i.e., programme variables, training outcomes, application to different populations, etc.) in the coming years is guaranteed.

RESEARCH AIMS AND HYPOTHESES

2. RESEARCH AIMS AND HYPOTHESES

2.1. RESEARCH AIMS

2.1.1. General aims

The general aim of the present thesis was to identify and characterise acute and chronic lower-limb responses to iso-inertial training sessions.

2.1.2. Specific aims

- To validate a tool (friction encoder) for the accurate quantification of the external load of the iso-inertial training sessions on a daily basis (*Study 1*).
- To evaluate the acute changes in lower-limb functional capacity (i.e., sprint and jump performance, and ISO force generating capacity) as a result of an iso-inertial half-squat training session (*Study 2*).
- To evaluate the inter- and intra-muscular activity patterns of the lower limbs (measured by MRI) in response to iso-inertial half squats and their relation with chronic hypertrophic changes (*Study 3*).
- To evaluate the chronic changes in muscle functional capacity in response to a 4-week iso-inertial half-squat training programme (*Study 3*).
- To evaluate the regional and total hypertrophy in lower-limb muscles due to a 4-week iso-inertial half-squat training programme (*Study 3*).
- To quantify the increased demands of exercise sessions (external maximal accommodated loads) as a result of a 4-week iso-inertial half-squat training programme (*Study 3* and *Study 4*).
- To quantify the protective effect of iso-inertial exercise on muscle damage using functional and biochemical markers in response to high-volume maximal-voluntary-intensity iso-inertial training sessions (*Study 4*).

2.2. HYPOTHESES

2.2.1. Study 1.

- The friction encoder provides valid measures of the force, velocity and power produced on an iso-inertial resistance device.

2.2.2. Study 2.

- Lower-limb functional capacity is affected for several hours by an iso-inertial half-squat training session, with high inter-individual variations.
- Lower-limb functional capacity is affected by an iso-inertial half-squat training session to a different extent, depending on the manifestation of force tested.
- The sprint force–velocity spectrum offers information regarding the impact of the training load and the process of recovery.

2.2.3. Study 3.

- Hypertrophy in response to iso-inertial training can be detected early in the process (i.e., 2 weeks).
    - Hypertrophy is greater in the distal portion of muscles as a result of iso-inertial training.
    - Chronic hypertrophic changes correlate with the intra-muscular and inter-muscular differences of activation (measured by MRI) of the first session.
    - Hypertrophy is greater in the quadriceps than the hamstring muscles as a result of iso-inertial half-squat training.
- Functional capacity (force generating capacity) increases more than muscle hypertrophy over the first four weeks of training.
    - Force increases are specific (greater increases in the iso-inertial squat than in isolated knee extension and flexion exercises).
- Training sessions imply greater external loads (i.e., force production) as the volunteers increase their force generating capacity, due to the maximal effort of the protocol and the accommodated resistance.

2.2.4. Study 4.

- Unaccustomed iso-inertial exercise produces different levels of muscle damage, as evaluated using functional and biochemical markers.
    - The different functional and biochemical markers used to evaluate muscle damage are affected to varying degrees by several factors.
    - Titin concentration in serum increases as a result of EIMD and can be used as a novel marker.
- Even though iso-inertial training sessions imply increasing external loads from session to session, the muscle damage response to these increased loads will be similar or lower, not higher.
    - The level of attenuation is different, depending on the marker analysed.
    - Functional capacity is recovered earlier after training sessions, allowing for an increased weekly training frequency.

METHODS

3. METHODS

This thesis compiles four consecutive studies. In this section, I summarise the general and common aspects of the methods used in those studies. For further more specific details, the reader is referred to the corresponding studies.

3.1. RATIONALE OF THE CONSECUTIVE STUDIES

In order to achieve the specific research aims of this thesis, detailed in the previous section, four consecutive studies with different designs were performed.

- *Study 1* investigated the validity of a friction encoder at providing force, velocity and power measures on an iso-inertial resistance training device.
- *Study 2* investigated the acute effects of an iso-inertial training session on different performance indicators.
- *Study 3* investigated the chronic effects of a structured iso-inertial training programme on muscle function and hypertrophy.
- *Study 4* investigated the protective effect conferred by iso-inertial training sessions against muscle damage from intense exercise.

3.2. SUBJECTS

Forty healthy male (15) and female (25) subjects gave written, informed consent to participate in the experiments. All the participants were previously informed of the aims, experimental protocols, procedures, benefits, and risks of the studies.

The main characteristics of the subjects are displayed in Table 2.

Table 2. Main characteristics of the subjects in the studies compiled in this thesis (mean ± standard deviation). (*) Data for Studies 3 and 4 were gathered from the same sample of 12 participants, although due to the impossibility of obtaining all measurements from all the participants, the final n of these studies varied slightly (see the specific Methods section of each study for further details).

Study	Subjects	n	Age (years)	Mass (kg)	Height (cm)
Study 1	Male physical education students	7	29.1 ± 5.2	76.3 ± 8.5	178.4 ± 6.2
	Female physical education students	3	25.0 ± 3.3	57.3 ± 6.4	165.3 ± 3.2
	Total	**10**	**27.9 ± 4.9**	**70.6 ± 11.9**	**174.5 ± 8.3**
Study 2	**Female physical education students**	**18**	**24.8 ± 3.0**	**164.3 ± 6.6**	**58.1 ± 6.8**
Study 3 & *Study 4*	Male physical education students	10	23.2 ± 3.8	72.8 ± 4.8	178.0 ± 6.3
	Female physical education students	2	22.5 ± 5.2	70.7 ± 6.8	165.4 ± 1.6
	Total*	**12**	**23.1 ± 3.8**	**70.9 ± 6.1**	**175.9 ± 7.5**

3.3. ETHICS OF EXPERIMENTATION

All the studies included in this thesis were conducted in accordance with the code of ethics of the World Medical Association (Declaration of Helsinki) and the were approved by the Ethics Committee of the Catalan Sports Council (Generalitat de Catalunya).

3.4. EXERCISE PROTOCOLS

Although the specific doses, in terms of training load, varied from one study to another (details are given in the Methods section of each specific study), all four shared the same iso-inertial training system and exercise biomechanics

Exercise protocols consisted of CON – ECC bilateral half squats performed on a "YoYo squat" inertial flywheel device (YoYo Technology AB, Stockholm, Sweden). The start and end position of each repetition was a 90° knee angle (Figure 13). The participants were verbally encouraged to perform the concentric phase at their fastest voluntary speed and to adjust the braking force to maintain the same ROM in each repetition. All participants were previously introduced to the training system during a familiarisation session.

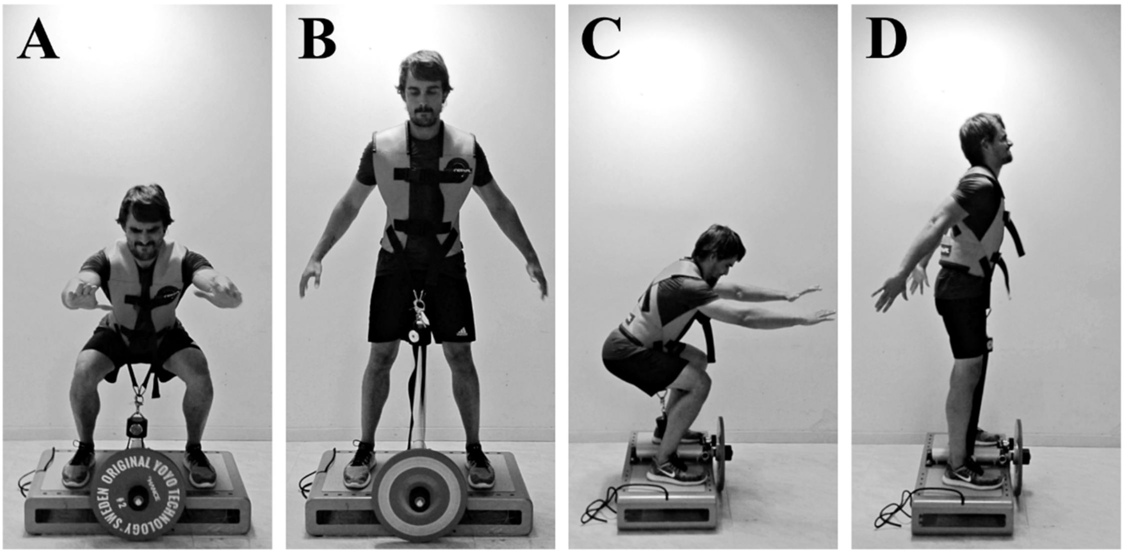


Figure 13. Concentric (CON) – eccentric (ECC) bilateral half squats performed on a "YoYo squat" inertial flywheel device: anterior view (A and B), lateral view (C and D). A and C: bottom position of the exercise and transition from the ECC to the CON phases. B and D: top position of the exercise and transition from the CON to the ECC phases. The vest is attached to a strap wound around the flywheel axis. As resistance is the moment of inertia of the flywheel, the force applied during the CON phase to unwind the strap determines the force needed during the ECC phase (as the strap rewinds) to impede the spin of the axis.

3.5. EXPERIMENTAL DESIGN

3.5.1. Study 1

The design of the first study was an observational assessment of concordance between two methods for computing the kinematic variables of the inertial exercise. The participants performed two sets of 14 maximal squats on the flywheel inertial device using two different resistances. The variables of interest (mean repetition force, velocity and power) were simultaneously measured via a friction encoder (Chronojump, Barcelona, Spain), and a strain gauge combined with a linear encoder (Muscle Lab 6000, Ergotest Technology AS, Porsgrunn, Norway). The statistical analysis included the assessment of mean bias, typical error of estimate and correlation between assessments at the different load configurations.

3.5.2. Study 2

The approach adopted for the second study was a quasi-experimental design of repeated measures (before exercise, immediately after exercise, and 1, 24, 48 and 72 hours after the exercise), to determine the acute effects of a strenuous RT session (10 sets of 10 repetitions) on the dependent variables (ISO force, vertical jump height, and sprint mechanics and performance) throughout the recovery process. The main and interactive effects of time were assessed by comparing the baseline with all other assessments for the dependent variables.

3.5.3. Study 3

In the third study, a quasi-experimental design of repeated measurements was used to determine the effects of a 4-week training intervention on different variables. The training programme consisted on 5 sets of 10 repetitions, 2-3 times per week. The variables assessed for thigh muscles were: muscular activation, muscle hypertrophy and muscle performance. Activation was assessed through a specific MRI technique. Hypertrophy was assessed through increases in total volume and regional CSAs, using MRI. Muscle performance was assessed through ISO force of the knee extensors and knee flexors, as well as by squat dynamic force and power. The assessments were performed before, and 2 and 4 weeks after training; and also during each of the 10 training sessions. The main and interactive effects of time were assessed by comparing the baseline with all other assessments.

3.5.4. Study 4

In the fourth study, repeated measurements were taken (before exercise, and 1, 24, 48, 72, and 144 hours after exercise) to determine the effects of a maximum-effort iso-inertial half-squat training session (ten sets of ten repetitions each) on the following: ISO force, vertical jump height, muscle soreness, and blood biochemical markers of EIMD. The protocol was performed 1 week before starting the 4-week iso-inertial training programme (assessment A) and 1 week after completing the programme (assessment B) to determine the protective effects of the training.

STUDY 1

“Validation of a low-cost friction encoder for measuring velocity, force and power in flywheel exercise devices.”

4. STUDY 1. "Validation of a low-cost friction encoder for measuring velocity, force and power in flywheel exercise devices."

4.1. ABSTRACT

This study aimed to investigate the validity of a low-cost friction encoder against a criterion measure at assessing velocity, force and power in flywheel exercise devices. Ten volunteers performed two sets of 14 squats on a flywheel inertial device. Two different resistances were used (0.075 kg·m^2 for the first set; 0.025 kg·m^2 for the second). Mean velocity (Vrep), force (Frep) and power (Prep) for each repetition were assessed simultaneously via a friction encoder (Chronojump, Barcelona, Spain), and with a strain gauge combined with a linear encoder (MuscleLab 6000, Ergotest, Porsgrunn, Norway). The results are displayed as (mean [CI 90%]). Mean bias for Vrep, Frep and Prep were moderate (-0.95 [-0.99 to -0.92]), small (0.53 [0.50 to 0.56]) and moderate (-0.68 [-0.71 to -0.65]) respectively. The typical error of the estimate (TEE) was small for all three parameters: Vrep (0.23 [0.20 to 0.25]), Frep (0.20 [0.18 to 0.22]) and Prep (0.18 [0.16 to 0.20]). Correlations were nearly perfect for all the measurements in all load configurations. These results suggest that the friction encoder provides valid measures of velocity, force and power in flywheel exercise devices. However, as error did exist between measurements, the same testing protocol should be used when aiming to perform comparisons.

Key words: Iso-inertial, Monitoring, Resistance training, Feedback

4.2. INTRODUCTION

Quantification of training loads is an important consideration for sports scientists and practitioners. Accurate quantification can ensure that training is both appropriate and meets the programme requirements [141]. Furthermore, this information is essential for decision making, so that negative training outcomes (e.g., overtraining, illness, and maladaptation) can be avoided [142]. For this reason, tools that are used to quantify training need to be valid so that accurate training information can be gathered. This is relevant for all training methods, including resistance training.

Traditional resistance training methods (e.g., gravity-dependent loads such as dumbbells and barbells), are often quantified through the mass and number of repetitions completed (e.g., volume load) [52]. However, alternative methods of resistance training, such as flywheel training, can be more challenging to quantify as they do not have an external load and rely on athletes overcoming inertia (i.e., iso-inertial training) [98]. Additionally, the force that is applied to an athlete during the ECC portion of the movement is directly proportional to the force that is applied during the CON portion of the movement [81]. Yet, the popularity of flywheel devices has grown recently, due to their capacity to promote positive adaptations in strength, power, and velocity [116]. Furthermore, their capacity to apply an ECC overload may provide benefits that can be difficult to achieve using traditional training methods [99,116]. Nevertheless, technological progress may enable practitioners to monitor the external load that is applied to athletes as they train accurately.

Recent work by Weakley et al. (2019) [107] has demonstrated that flywheel resistance training can be accurately quantified through the use of a rotary encoder. However, that research was specific to the type of flywheel device that was being used. To overcome that issue, rotary friction encoders have been developed to assess the force, power, and velocity produced by the athlete during training. These variables are calculated in real time from the encoder measurements of rotational velocity, using given configuration parameters such as the moment of inertia of the wheel, additional masses, and diameters of the pieces (i.e., axis, etc.). Therefore, by introducing these parameters into the software, the sensor becomes adapted to different inertial training systems. This is a very important feature, as it allows for the monitoring of existing non-sensorised equipment in a few minutes (other axis rotary sensors require industrial mechanisation of the pieces). Although the practicality and relevance of rotary friction encoders are evident, their criterion validity has not been assessed, so the accuracy of the feedback provided is still unknown. Thus, in this study I aimed to assess the criterion validity of the force, power, and velocity outputs that are calculated at two different iso-inertial loads from a rotary friction encoder.

4.3. MATERIALS AND METHODS

The study was designed to evaluate the criterion validity of the force, velocity and power values measured with a commonly used low-cost friction encoder (Chronojump, Barcelona, Spain). In order to do that, half squats performed on a flywheel device with two different iso-inertial loads (0.075 kg·m^2 and 0.025 kg·m^2) were simultaneously monitored using a friction encoder attached at a known diameter of the flywheel (the practical measure), and a strain gauge combined with a linear encoder attached to the harness (the criterion measure). The level of agreement between the force, velocity and power measurements obtained from the two methods was assessed.

4.3.1. Subjects

Ten physically active male and female volunteers (age 27.9 ± 4.9 years, body mass 70.6 ± 11.9 kg, height 174.5 ± 8.3 cm) were recruited to take part in this study. The subjects were free from injury and illness during data collection. All the subjects had previously trained with the exercise device and were familiar with the testing protocol and iso-inertial loads used. All experimental procedures were approved by the ethics committee of the Catalan Sports Council (Generalitat de Catalunya), and written consent was provided by all the subjects before study initiation.

4.3.2. Procedures

As described in the Methods section (Section 3.4), the exercise protocol consisted of maximal voluntary CON-ECC bilateral half squats performed on a "YoYo squat" flywheel device (YoYo Technology, Stockholm, Sweden). The subjects performed two sets of the exercise, consisting of three sub-maximal repetitions to accelerate the disk, followed by 14 repetitions with maximal intent. In order to test the validity of the friction encoder in different load configurations, two different resistances were used: the moment of inertia was 0.075 kg·m^2 for one set and 0.025 kg·m^2 for the other. The order in which the subjects completed these different iso-inertial loads was randomised via coin toss. Mean velocity, force and power for each repetition were assessed simultaneously using the friction encoder, and the strain gauge combined with a linear encoder.

Criterion measurements of velocity, force and power were assessed by a strain gauge synchronised with a linear encoder using a MuscleLab 6000 (Ergotest Technology, Porsgrunn, Norway). The strain gauge (accuracy: 63 grams, sampling rate: 200 Hz) was attached between the strap of the flywheel and the vest; the linear encoder (accuracy: 0.019 mm; sampling rate: 200 Hz) was placed on the flywheel device and attached to the vest, perpendicular to the floor (Figure 14).

The friction encoder (Chronojump, Barcelona, Spain), (accuracy: 1 mm, sampling rate: 1000 Hz) was tightly attached at a known diameter of the flywheel, sharing the same linear velocity (Figure 14). The set-up parameters such as disk inertia, axis diameter, and the volunteer's body mass were entered into the associated Chronojump software (v1.8.1-95) to compute the practical measurements of velocity, force and power in real time. Chronojump is open-code software, and a complete repository of the code and formulas used can be found online [143].

As mentioned in the previous paragraph, axis diameter is an important parameter for computing kinematic variables from the friction encoder, because it determines the point at which the strap exerts the unwinding force, and therefore modifies the moment arm and torque. In the particular training device used in this study, the diameter varies slightly during the repetition, given that the strap rolls up on itself, thereby increasing the diameter. For this reason, a mean diameter value was used for the estimations.

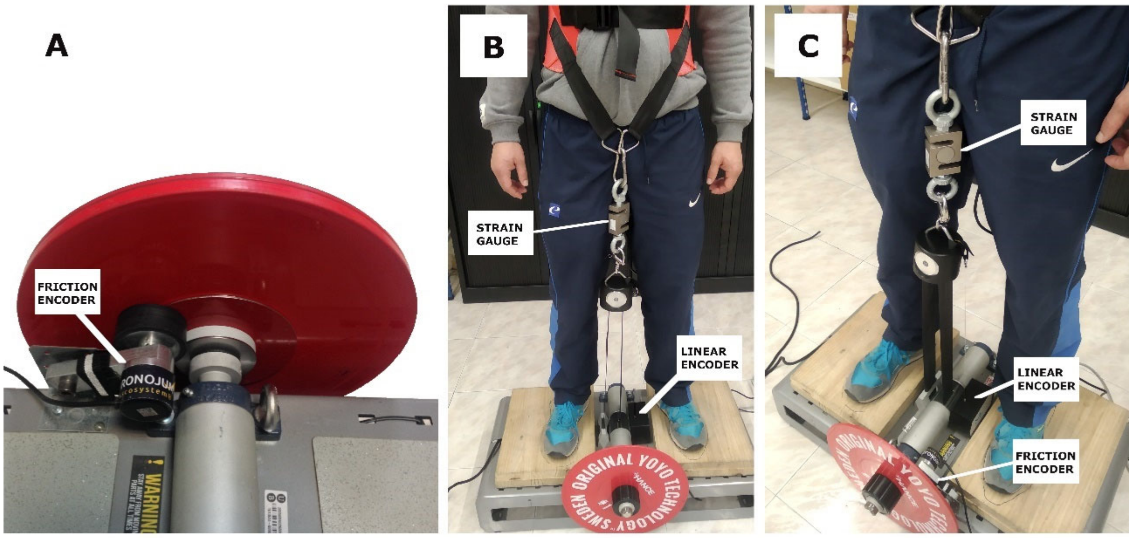


Figure 14. Disposition of the different sensors used in the study. (A) The friction encoder was tightly attached at a known diameter of the flywheel, sharing the same linear velocity. (B) The strain gauge was attached between the flywheel strap and the vest, and the linear encoder was placed on the flywheel device and attached to the vest, perpendicular to the floor. (C) General view of the setup.

Raw data from each of the 14 high-intensity repetitions in each set were averaged to compute repetition mean velocity (Vrep), force (Frep) and power (Prep), using both measuring systems.

4.3.3. Statistical Analysis

Agreement between the criterion measurements (MuscleLab) and the practical measurements (Chronojump) of Vrep, Frep and Prep was assessed using an Excel spreadsheet [144] designed to calculate the mean bias, typical error of the estimate (TEE) and Pearson correlation coefficient, all with 90% confidence intervals. The standardised mean bias was rated as trivial (≤0.19), small (0.2-0.59), moderate (0.6-1.19) or large (1.2-1.99) [144]. The standardised typical error was rated as trivial (<0.1), small (0.1-0.29), moderate (0.3-0.59) or large (>0.59) [144]. The correlation was rated as trivial (<0.1), small (0.1-0.29), moderate (0.3-0.49), large (0.5-0.69), very large (0.7-0.89) or nearly perfect (0.9-0.99) [144].

4.4. RESULTS

When compared to the criterion measurements, the mean bias for the practical measurements of Vrep, Frep and Prep were moderate, small and moderate, respectively. The TEE was small for all three parameters (Table 3). Correlations with MuscleLab were nearly perfect for all measurements in both load configurations (Table 3).

Table 3. Comparison of mean repetition velocity (Vrep), force (Frep) and power (Prep) between MuscleLab and Chronojump in the high (0.075 kg·m^2) and low (0.025 kg·m^2) load configurations, and for the total pooled data. Data are presented as mean values (± standard deviation (SD)) and mean bias, typical error of the estimate and Pearson correlation coefficient, all with 90% confidence limits.

Load	Variable	Criterion Measure	Practical Measure	Bias	TEE	Correlation
High (0.075 kg·m^2)	V_{rep}	MuscleLab 0.37 ± 0.06 m·s^{-1}	Chronojump 0.28 ± 0.04 m·s^{-1}	-1.44 [-1.49 to -1.39] *(large)*	0.26 [0.23 to 0.31] *(small)*	0.97 [0.96 to 0.97] *(nearly perfect)*
	F_{rep}	MuscleLab 1090.0 ± 306.1 N	Chronojump 1294.6 ± 353.2 N	0.67 [0.63 to 0.70] *(moderate)*	0.18 [0.15 to 0.20] *(small)*	0.98 [0.98 to 0.99] *(nearly perfect)*
	P_{rep}	MuscleLab 414.2 ± 160.6 W	Chronojump 312.4 ± 118.2 W	-0.63 [-0.67 to -0.59] *(moderate)*	0.13 [0.12 to 0.15] *(small)*	0.99 [0.99 to 0.99] *(nearly perfect)*
Low (0.025 kg·m^2)	V_{rep}	MuscleLab 0.55 ± 0.10 m·s^{-1}	Chronojump 0.41 ± 0.07 m·s^{-1}	-1.48 [-1.54 to -1.42] *(large)*	0.38 [0.33 to 0.44] *(moderate)*	0.93 [0.91 to 0.95] *(nearly perfect)*
	F_{rep}	MuscleLab 830.1 ± 222.3 N	Chronojump 942.0 ± 243.9 N	0.50 [0.46 to 0.54] *(small)*	0.27 [0.24 to 0.32] *(small)*	0.96 [0.95 to 0.97] *(nearly perfect)*
	P_{rep}	MuscleLab 478.0 ± 177.9 W	Chronojump 344.8 ± 126.5 W	-0.75 [-0.80 to -0.70] *(moderate)*	0.20 [0.17 to 0.23] *(small)*	0.98 [0.97 to 0.99] *(nearly perfect)*
Total	V_{rep}	MuscleLab 0.46 ± 0.13 m·s^{-1}	Chronojump 0.34 ± 0.09 m·s^{-1}	-0.95 [-0.99 to -0.92] *(moderate)*	0.23 [0.20 to 0.25] *(small)*	0.98 [0.97 to 0.98] *(nearly perfect)*
	F_{rep}	MuscleLab 960.1 ± 297.1 N	Chronojump 1118.3 ± 350.7 N	0.53 [0.50 to 0.56] *(small)*	0.20 [0.18 to 0.22] *(small)*	0.98 [0.98 to 0.98] *(nearly perfect)*
	P_{rep}	MuscleLab 446.1 ± 172.2 W	Chronojump 328.6 ± 123.3 W	-0.68 [-0.71 to -0.65] (moderate)	0.18 [0.16 to 0.20] *(small)*	0.98 [0.98 to 0.99] *(nearly perfect)*

The regression equations to estimate the criterion measurements from the practical measurements fitted the data well (Figure 15) and are presented as follows, where Y is the estimated criterion measurement and X is the practical measure:

$$Y = \text{intercept} + (\text{slope} \cdot X)$$

The regression equations were:

- Vrep $\rightarrow Y = -0.026 + (1.4247 \cdot X)$
- Frep $\rightarrow Y = 31.558 + (0.8303 \cdot X)$
- Prep $\rightarrow Y = -5.6196 + (1.3747 \cdot X)$

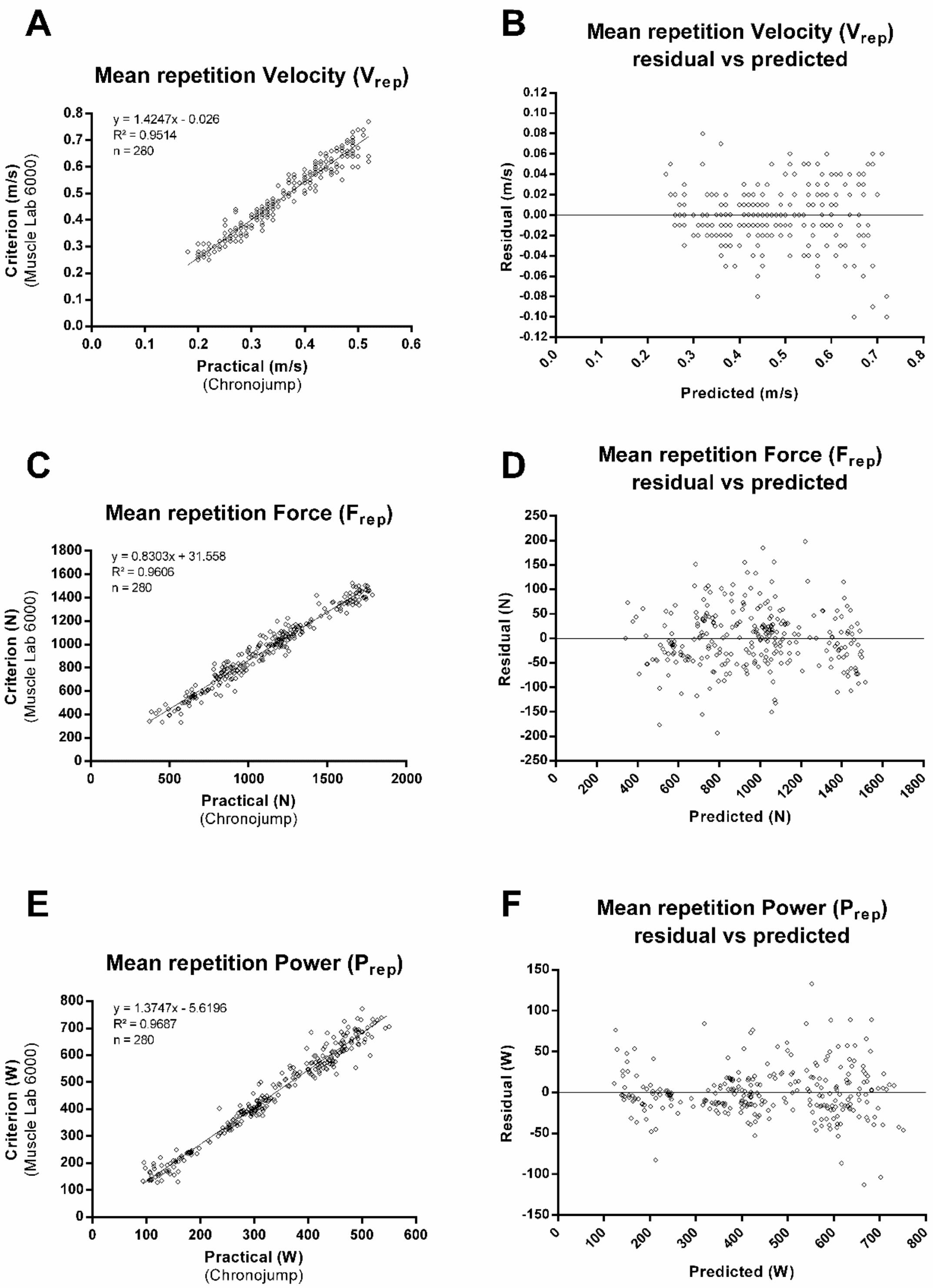


Figure 15. Agreement between the criterion and practical measures Vrep (A), Frep (C) and Prep (E). Residual versus predicted values for Vrep (B), Frep (D) and Prep (F). Mean repetition velocity (Vrep), force (Frep) and power (Prep)

4.5. DISCUSSION

The primary finding of this study demonstrates acceptable levels of agreement between a low-cost, easy and versatile method (friction encoder) and criterion measurement (strain gauge combined with a linear encoder) for the assessment of velocity, force and power in a flywheel exercise device with different iso-inertial loads. The friction encoder demonstrated small to moderate TEE and nearly perfect relationships across all kinetic and kinematic variables at both iso-inertial loads. However, small to large bias was demonstrated which should be acknowledged when implemented in training and future research. Considering these findings, the Chronojump friction encoder is a valid tool for the quantification of training loads when executing flywheel resistance training.

The monitoring of kinetic and kinematic variables is an important consideration for the accurate quantification of resistance training intensity. Furthermore, these external stimuli provide a greater understanding of the internal and fatigue responses that determine muscular adaptations [145]. Small to moderate TEE were found for all, which suggests that the Chronojump device can adequately provide repetition information during exercise. This will not only be of use to practitioners and scientists, but also to athletes, as this device can provide live augmented feedback that may enhance psychological traits during resistance training and subsequent physical adaptations. However, the small to large bias that was present suggests that findings may be smaller or greater depending on the variable assessed (i.e., velocity and power may be consistently lower and force consistently higher than actual values). Thus, it is advised that, due to the lowest TEE and bias observed, power outputs are monitored for the detection of changes in neuromuscular performance.

One limitation of this study is that measurements were recorded exclusively from one iso-inertial training system ("YoYo squat" - YoYo Technology, Stockholm, Sweden). Therefore, extrapolation of the results to substantially different systems (i.e., conical pulleys) is limited. Another aspect to take into account is that the diameter of the axis of the system used varied slightly across the ROM, depending on the winding up of the strap. Therefore, the mean diameter of the axis was used for the calculations. As this may have contributed to increasing the error of measurement, better accuracy is to be expected in other inertial systems in which the axis diameter remains constant throughout the repetition.

Despite the existence of other valid methods to monitor velocity, force and power in flywheel devices, to our knowledge, currently there are no other low-cost tools that work with free, open-code software to monitor a wide variety of flywheel resistance exercises. Given the practicality (e.g. minimal set-up), versatility (e.g. applicable to different systems and configurations) and low cost of this sensor compared with the combination of a linear encoder and a strain gauge, or other rotational encoders, practitioners may prefer this method for assessing Vrep, Frep and Prep in flywheel exercises.

4.6. PRACTICAL APPLICATIONS

In recent years, there has been growing interest in flywheel resistance training. Research in this area has shown higher increases in power and force with flywheel training [96,99,125,132], improvements in changes of directions [121,146] and other kinematics responses [102,147], early hypertrophic adaptations [95] and changes in movement variability with functional resistance training exercises [148]. Nonetheless, only a few papers have studied the accuracy of rotary encoders for monitoring flywheel resistance training [107,149] and none had previously validated the use of a rotary friction encoder which can be adapted to different inertial training systems.

Regarding the importance of the use of feedback [150,151] and the control of velocity loss [152,153] in resistance training using free weights, the validation of this rotary friction encoder and open-code software could help strength and conditioning coaches to confidently monitor force, power and velocity on any flywheel machine, and therefore improve their control of training whenever they are using these resistance training devices, with both low and high training loads.

Coaches and sports scientists may confidently use a Chronojump friction encoder to assess Vrep, Frep and Prep in flywheel exercises, allowing for a simplified, accurate and versatile way of testing and monitoring athletes on a day-to-day basis.

STUDY 2

“Acute changes in sprint force–velocity spectrum as a result of muscle damage in women.”

5. STUDY 2. "Acute changes in sprint force–velocity spectrum as a result of muscle damage in women."

5.1. ABSTRACT

This study aimed to screen for acute changes in the sprint performance and Force-Velocity (F-V) spectrum as a result of lower-limb muscle damage. Eighteen recreationally active young women (age: 24.8 ± 3.0 years) performed 10 sets of 10 half-squats on a flywheel iso-inertial device. Maximal voluntary isometric force of the knee extensors was used to determine the impact of this exercise and to classify two response groups (mild and moderate responders) during the follow-up (comparing pre-exercise to immediately and 1, 24, 48, and 72 hours after). During this period, radar was used to assess sprint performance over 40 m. Mechanical properties of sprint, including maximal force (F0), maximal power (Pmax), maximal ratio of forces (RFmax), and maximal sprinting velocity (V0) were assessed using a validated method applied to the speed–time data. Mechanical sprint properties were altered for several days after the exercise, reducing sprint performance. Sprint V0 decreased as a result of acute fatigue, but showed a progressive recovery during the follow-up. Conversely, the variables related to shorter distances and high demands of force (short-distance times, F0, Pmax and RFmax) were more dependent on the amount of muscle damage (responses clearly differentiated between groups), overall showing small impairments immediately after the exercise, and a tendency towards worsening as the hours passed. These variables had not completely recovered after 72 hours. In conclusion, the sprint F-V spectrum offers complementary information on the neuromuscular dynamics of the lower limbs and might be considered for testing recovery processes.

Keywords: Force–Velocity spectrum, sprint, strength, flywheel, recovery.

5.2. INTRODUCTION

Resistance training for sports usually includes ECC-based exercises with the aim of improving muscle performance and reducing injury risk factors [8,82]. These interventions are commonly periodized, taking into account the progressive overload principle, to minimize EIMD and the corresponding recovery time. Despite allowing for this and other principles, some degree of EIMD may occur after training sessions, as multiple factors influence athlete's responses [154]. EIMD symptoms include transient impairment of muscle force-generating capacity, reduced ROM, swelling, and DOMS, all of which may persist for several days after a strenuous resistance training session [40,155]. The severity of strength impairment is considered one of the most valid and reliable indirect measures of EIMD and correlates with the number of myofibres disrupted [40,42,54].

It is essential to assess the impact of training loads and the time course of recovery to ensure that the stimulus is adequate and the athlete is coping well with the imposed stress. The better understanding of the individual responses to training allows us to minimize the risk of non-functional overreaching, injury, and illness [142]. Effective monitoring strategies should combine a variety of tools to evaluate internal and external loads and provide the coach with meaningful information [141]. Furthermore, due to the time constraints of the training schedules, there is a need for easily and rapidly applicable tests, to provide feedback for decision-making before additional training sessions or competitions [142]. Therefore, simple measurements of strength or strength–time properties (e.g. rate of force development) from single-joint tests or more complex sport-specific movements (e.g., jumping) are commonly used to monitor fatigue, readiness, and the impact of training loads [40,49,156].

Sprinting capacity has been studied widely due to its high relevance in many sports. Among the different parameters that can be studied, in recent years there has been growing interest in the sprint force–velocity (F-V) spectrum, which can be accurately computed from anthropometric (body mass and stature) and spatiotemporal (split times or instantaneous velocity) variables, using a validated computerized method [157]. The F-V spectrum describes the linear inverse relationship between force and velocity, and has shown large inter-individual differences caused by muscle properties, morphological factors, neural mechanisms, and segmental dynamics [157,158]. Consequently, the sprint F-V spectrum (like similar spectra for other movements) can be used by coaches to individualize training programmes and also to assess the adaptive effects to these interventions [159]. Additionally, research has shown that the sprint F-V spectrum is modified under certain conditions, such as sport-specific acute fatigue or post-hamstring-injury return to play [160,161]. Therefore, although it has not yet been studied, it would also be reasonable to expect changes in sprint mechanics after a strenuous lower-body resistance training session. If this expectation is met, the implementation of simple, rapidly applicable field-based sprint tests would also provide coaches with relevant information regarding the impact of training loads and recovery status.

The aim of the present study was to screen for acute changes in lower limbs functional capacity (i.e., sprint performance, F-V spectrum, jump capacity, and isometric strength) as a result of lower-limb muscle damage induced by a single bout of high-intensity CON–ECC iso-inertial half-squat exercise.

5.3. MATERIALS AND METHODS

5.3.1. Experimental design

Repeated measures (pre-exercise (Pre), immediately after (Post), and after 1 (Post1h), 24 (Post24h), 48 (Post48h), and 72 hours (Post72h)) were taken to determine the acute effects of a strenuous resistance training session on isometric force, vertical jump height, muscle soreness and sprint mechanics and performance. A graphical description of the protocol is provided in Figure 16.

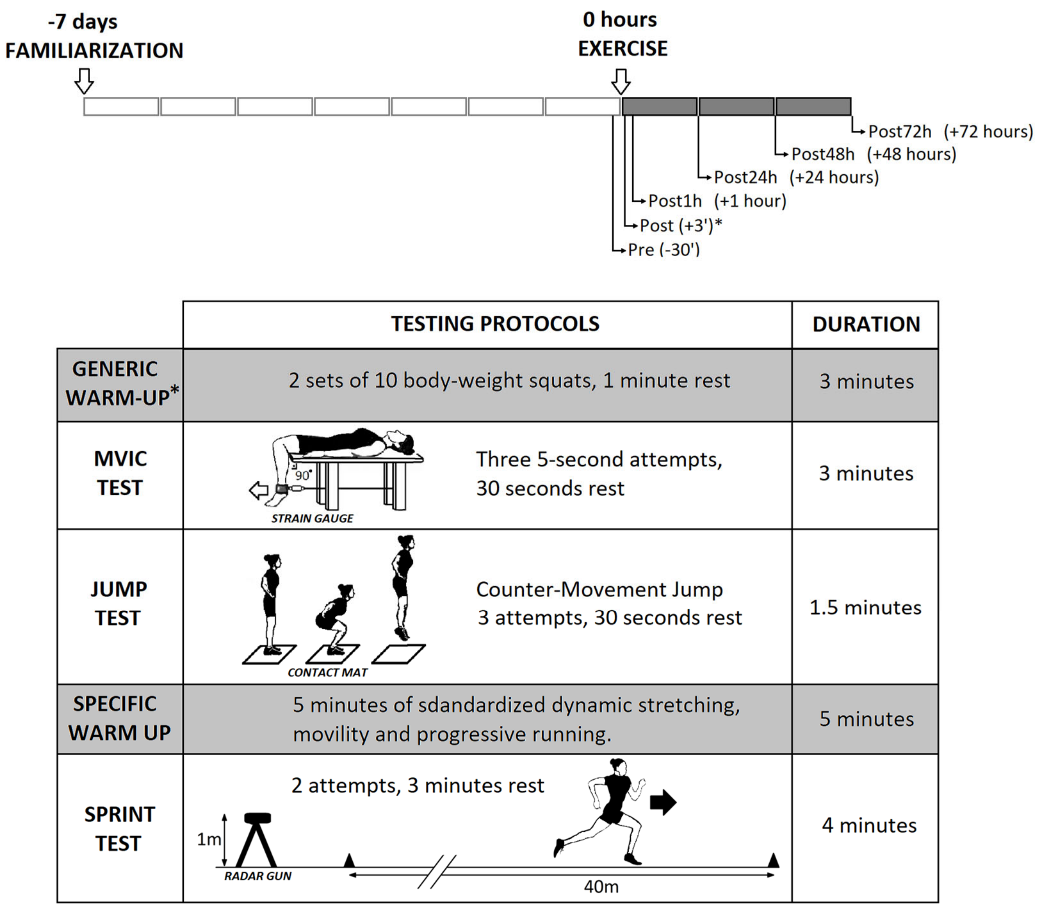


Figure 16. Graphical description of the study timeline. Each rectangle represents a day (24 hours). The time scale "0" refers to the performance of the damaging exercise. Under the time scale, testing sessions are presented in order, with the exact time between curved brackets. All testing sessions were performed as depicted in the box (same tests and order), except the session at post, which did not include the generic warm up (*). Instead, the first 3 minutes after exercise were used to take blood lactate measurements and scores of perceived exertion. Abbreviation: MVIC, maximal voluntary isometric contraction.

5.3.2. Subjects

Eighteen young women (age 24.8 ± 3.0 years; height 164.3 ± 6.6 cm; weight 58.1 ± 6.8 kg) with no history of muscle–tendinous injuries in the previous six months were recruited. All were recreationally active physical education students who had participated in physical activities including sprints, and had previously been introduced to the exercise device and measuring protocols. Ethics approval was granted by the Ethics Committee of the Catalan Sports Council (Generalitat de Catalunya) and written informed consent was acquired from all subjects.

5.3.3. Exercise protocol and measures of external & internal load

As described in the methods section (section 3.4), the exercise protocol consisted of maximal voluntary CON-ECC bilateral half-squats performed on a “YoYo squat” flywheel device (YoYo Technology, Stockholm, Sweden), using a moment of inertia of 0.030 kg·m^2. The participants performed 10 sets of the exercise, consisting of three sub-maximal repetitions to accelerate the disk, followed by 10 high-intensity voluntary repetitions, with no pause. They were given five minutes of rest between sets.

Mean force and power production for each repetition was monitored using a Chronopic friction encoder (Chronojump, Barcelona, Spain) [95]. Visual and verbal feedback on the performance was provided in each repetition using a computer. Other variables such as session rate of perceived exertion (RPE) on a 0-10 scale [162] and lactate from fingertip capillary blood using a Lactate Plus meter (Nova Biomedical, Waltham, Massachusetts) were monitored during the exercise to assess in addition the internal load (RPE: post; Lactate: pre, and 3 min. post).

5.3.4. Maximum voluntary isometric contraction

Maximum voluntary ISO Contraction (MVIC) of knee extensor (knee angle = 90°; hip angle = 180°) [95] was measured with a strain gauge (MuscleLab 6000; Ergotest, Norway. Frequency: 200 Hz) to evaluate muscle performance in ISO conditions, and to indirectly assess muscle damage [42]. The best attempts of 3 unilateral 5-second trials for each limb (30 seconds between trials) were selected at each time point for further analysis. Results are shown as the mean values of both limbs.

Previous research has shown that the acute response to ECC exercise varies greatly between individuals [44,50,80]. Therefore, according to the percentage of reduction of the MVIC and its time course, participants were classified as mild responders (MI, relatively small MVIC decreases <20%, and recovery within 48 h), or moderate responders (MO, notable MVIC decreases 20%–50%, and recovery between 48 h and 7 days), as proposed by Paulsen et al. [42].

5.3.5. Jump testing

The counter-movement jump (CMJ) test [163] was used to measure lower body dynamic strength. Jump height was calculated from flight time using a contact mat digital timer (accuracy ± 0.001 seconds) (Chronojump Bosco system, Barcelona, Spain). The best attempt of three jumps (30 seconds between trials) was selected at each time point for further analysis.

5.3.6. Muscle soreness

Participants were required to rate the severity of soreness in their lower limbs using a rating scale of 0–10 arbitrary units (a.u.), where 0 represented 'no pain' and 10 represented 'intolerably intense pain' [49].

5.3.7. Sprint testing

For sprint testing, the participants were asked to cover 40 metres on the athletics track in the minimum time possible. Instantaneous velocity over that distance was measured by radar (Sampling rate: 48 Hz) (Stalker ATSII, Plano, Texas). The device was placed on a tripod 1.5 m behind the participants at the start of the sprint at a height of 1 m. The starting position was standing, with the torso leaning forward at a 90º angle to the advanced leg (dominant leg). Two attempts per subject were recorded at each time point (3 minutes between trials). The best attempt (i.e., lower 40 m time) was selected for further analysis. Data were analysed post hoc using R Studio software v0.99.489 (RStudio, Boston, Massachusetts) to compute sprint F-V spectrum. The model applied has been validated with force plates [157] and described in detail elsewhere [158]. Briefly, raw values for horizontal velocity (v_h) and time (t) in the acceleration phase were adjusted to an exponential function using least-square regression (

Equation 2A) [164]. After respective integration and derivation of $v_h(t)$, the horizontal position (x_h) and acceleration (a_h) of the body centre of mass (CM) as a function of time can be expressed as shown in

Equation 2B & C. To estimate net horizontal force (F_h) applied, anterior-posterior forces were considered, as shown in

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Equation 2D, where m is the runner's body mass (kg) and F_{aero} the aerodynamic drag, computed from running velocity, an estimation of runner's frontal area, and the drag coefficient [165].

The F–V relationship was modelled using least-square linear regression, and extrapolated to obtain F_0 and V_0 as the intercepts of the F-V line with the force and velocity axis, respectively [166]. F_0 is the maximal theoretical horizontal force produced relative to body-mass and corresponds to the runner's initial push. V_0 is the maximal theoretical running velocity and represents the capability to produce horizontal force at very high running velocities [158]. Maximal power ($Pmax$) values were computed as shown in

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Equation 2E [167].

Once the data were adjusted to the model, the modelled running velocity of each sprint was used to compute 5, 10, 20, 30, and 40 m sprint times, V_0, F_0 and $Pmax$ (maximal power relative to body-mass) and $RFmax$ (maximal mechanical effectiveness, in % of the horizontal force to the corresponding total resultant ground reaction force from the completion of the initial push off (t > 0.3 s) over the entire 40 m). The complete method and formulae can be consulted in previous studies [157,158,166].

Equation 2. Mechanical model for the computation of the force-velocity spectrum from velocity-time raw data of the acceleration phase. v_h horizontal velocity, $v_h\ max$ maximal velocity reached at the end of acceleration, τ acceleration time constant, x_h horizontal position, a_h horizontal acceleration, F_h net horizontal force applied, m runners body mass, F_{aero} aerodynamic drag, F_0 and V_0 the intercepts of the F-V line with the force and velocity axis, respectively, $Pmax$ maximal mechanical power produced by the runner [157,158,166].

$$v_h(t) = v_h max \cdot \left(1 - e^{-\frac{t}{\tau}}\right) \quad [A]$$

$$x_h(t) = v_h max \cdot \left(t + \tau \cdot e^{-\frac{t}{\tau}}\right) - v_h max \cdot \tau \quad [B]$$

$$a_h(t) = \left(\frac{v_h max}{\tau}\right) \cdot e^{-\frac{t}{\tau}} \quad [C]$$

$$F_h(t) = m \cdot a_h(t) + F_{aero}(t) \quad [D]$$

$$Pmax = \frac{F_0 \cdot V_0}{4} \quad [E]$$

5.3.8. Statistical Analyses

Data are presented as mean ± SD. Statistical analysis was performed using SPSS v.21.0.0 (IBM, Armonk, New York). For between-group comparisons, the normal distribution of the data was checked using the Shapiro-Wilk test, and unpaired t-tests were applied. Statistical significance was set at $P < 0.05$. A linear mixed-effects model was used to address the main and interactive effects of time, comparing the baseline (Pre) with all other assessments (Post, Post1h, Post24h, Post48h and Post72h) for the dependent variables. Afterwards, magnitude-based inferences were calculated using the estimates from the linear mixed-effects model (representing percentage differences) and were compared against a smallest worthwhile effect threshold equivalent to 0.2 of the between-subject standard deviations (i.e., small effect) using a dedicated spreadsheet [168]. Effects were classified as unclear if the percentage likelihood that the true effect was positive and negative were both >5%. Otherwise, the effect was deemed clear, and was qualified with a probabilistic term for increase or decrease using the following scale: <0.5%, most unlikely; 0.5%-4.9%, very unlikely; 5%-24.9%, unlikely; 25%-74.9%, possible; 75%-94.9%, likely; 95%-99.5%, very likely; >99.5%, almost certainly [169] . The comparisons were also assessed via standardized mean differences (Cohen’s d) and respective 90% confidence intervals. Thresholds for effect size statistics were <0.20, trivial; 0.20-0.59, small; 0.6-1.19, moderate; 1.20-1.99, large; and >2.0, very large [169].

5.4. RESULTS

As previously described, depending on the largest reduction in the MVIC force-generating capacity and the time needed to recover during the follow-up, the participants were classified in two groups: MI (n = 10; age 24.4 ± 3.4 years; height 165.5 ± 5.4 cm; weight 58.1 ± 6.6 kg); and MO (n = 8; age 25.4 ± 2.6 years; height 162.8 ± 8.1 cm; weight 58.1 ± 7.5 kg).

Regarding the exercise protocol, the participants completed all the repetitions as planned, maintaining the ROM (displacement variability: 7.56% ± 2.39%). No significant differences were found between groups in mean force (MI 1206.8 ± 177.8 N; MO 1213.3 ± 130 N; P = 0.62), or power (MI 378.7 ± 86.2 W; MO 370. ± 71.1 W; P = 0.67) produced on the flywheel. After the exercises, self-reported session RPE scores were slightly higher for MI (8.4 ± 0.6 a.u.) than for MO (7.2 ± 1.4 a.u.), (P = 0.032). Blood lactate levels rose from 2.2 ± 0.9 mM/L to 9.2 ± 3.1 mM/L and from 2.1 ± 0.9 mM/L to 10.0 ± 1.5 mM/L respectively for MI and MO groups 3 minutes after the last set of exercises, without significant differences between groups (P = 0.084).

No baseline differences were observed between groups in MVIC (P = 0.19) or CMJ performance (P = 0.06). MVIC capacity diminished in the hours following the exercises, with MO showing large changes (-31.5% ± 15.1% Post24h, -25.9% ± 20.9% Post48h) with no complete recovery after 72 hours; and MI showing small changes (-9.6% ± 12.4% Post24h, +1.4% ± 11.5% Post48h) and full recovery after 48 hours (Figure 17A & B). Similar dynamics of impairment and recovery were seen in CMJ performance, although the intrinsic variability of this test makes the statistical inferences less clear (Figure 17C & D). Decreases in muscle performance were also accompanied by increases in perceived lower limb muscle soreness (Figure 17E & F).

The instantaneous velocity values recorded in the sprint tests fitted well with the model described by Equation 2 (R^2 = 0.997; P < 0.001). Times to complete the total and partial distances, as well as V_0, F_0, $Pmax$ and $RFmax$, are shown in Table 4. Magnitude-based inference analyses of these variables are shown in Figure 18.

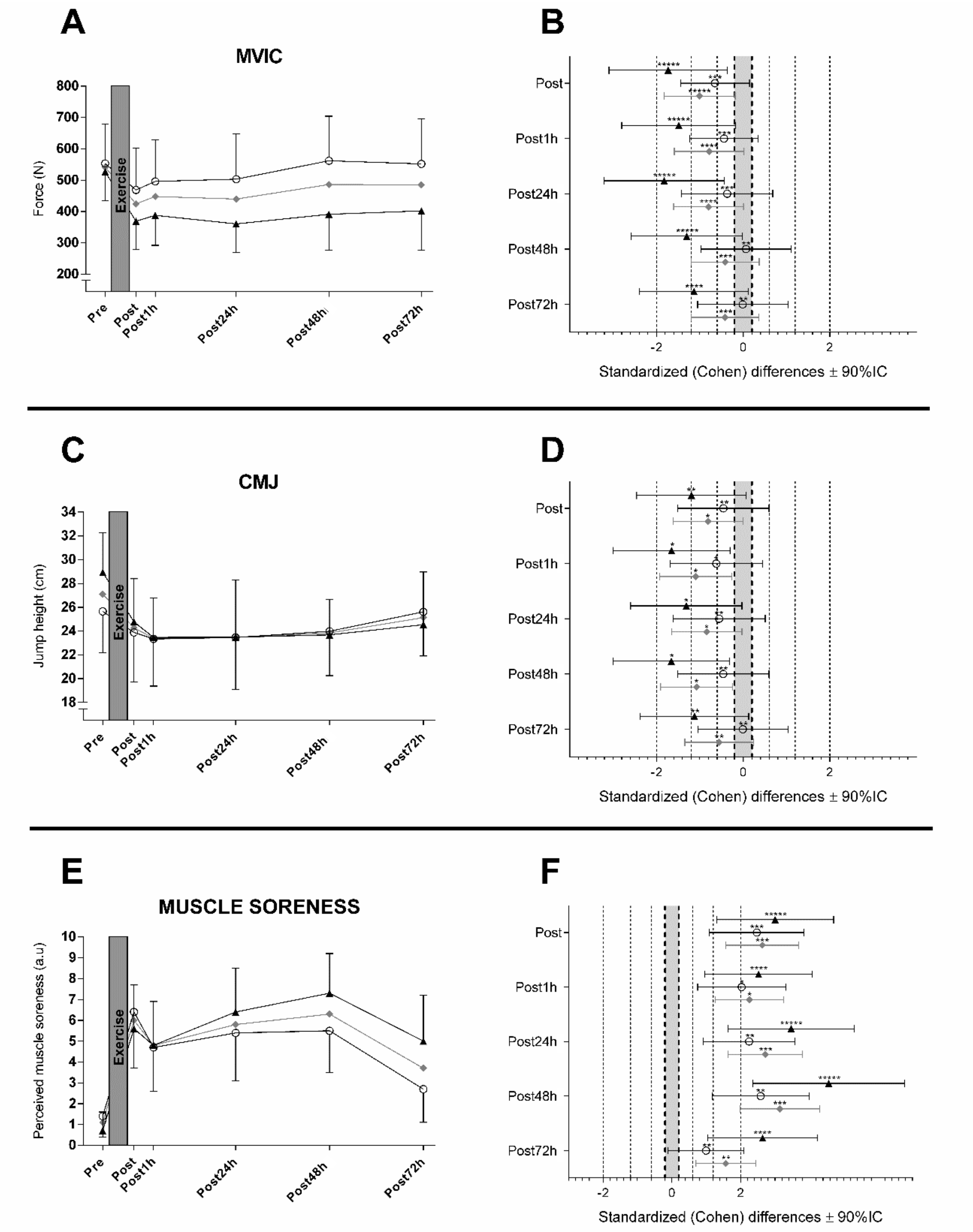


*Figure 17. Maximum voluntary isometric contraction (MVIC) of knee extensors (A), counter-movement jump (CMJ) (C) and perceived muscle soreness of the lower limbs (E) for the different groups during the follow-up. Data is presented as mean ± SD. Standardized differences (Cohen's d) in MVIC (B), CMJ (D) and perceived muscle soreness (F) comparing the baseline (Pre) with all other assessments (Post, Post1h, Post24h, Post48h and Post72h) for the different groups. Error bars indicate uncertainty in the true mean changes with a 90% confidence interval. Asterisks indicate the probability of increase or decrease: * (5%-24.9%, unlikely); ** (25%-74.9%, possible); *** (75%-94.9%, likely); **** (95%-99.5%, very likely); ***** (>99.5%, almost certain).*

Table 4. Sprint mechanical properties and times to complete 40 m, as well as partial distances of 5, 10, 20, and 30 m, during the follow-up. F_0: maximal theoretical horizontal force; V_0: maximal theoretical running velocity; $Pmax$: maximal power; $RFmax$: maximal value of RF, computed for sprint times >0.3 s.

	GROUP	Pre (Baseline)	Post	Post1h	Post24h	Post48h	Post72h
F0 (N/kg)	Moderate	7.38 ± 0.79	7.00 ± 0.80	6.47 ± 0.92	6.70 ± 0.72	6.36 ± 0.98	6.67 ± 0.62
	Mild	7.39 ± 0.69	6.90 ± 1.02	7.15 ± 1.57	7.07 ± 0.93	6.78 ± 0.80	6.74 ± 0.63
	All	**7.38 ± 0.71**	**6.94 ± 0.90**	**6.84 ± 1.33**	**6.90 ± 0.84**	**6.59 ± 0.88**	**6.70 ± 0.61**
V0 (m/s)	Moderate	7.11 ± 0.64	6.50 ± 0.67	6.70 ± 0.51	6.67 ± 0.54	6.86 ± 0.43	6.83 ± 0.46
	Mild	6.69 ± 0.47	6.39 ± 0.47	6.52 ± 0.41	6.48 ± 0.52	6.54 ± 0.52	6.69 ± 0.47
	All	**6.88 ± 0.58**	**6.44 ± 0.55**	**6.60 ± 0.45**	**6.57 ± 0.52**	**6.68 ± 0.49**	**6.75 ± 0.46**
Pmax (W/kg)	Moderate	13.41 ± 1.69	11.68 ± 1.96	11.13 ± 1.95	11.46 ± 1.69	11.11 ± 1.96	11.58 ± 1.41
	Mild	12.93 ± 1.68	11.53 ± 2.13	12.17 ± 2.94	11.96 ± 1.90	11.50 ± 1.88	11.62 ± 1.43
	All	**13.15 ± 1.65**	**11.59 ± 2.00**	**11.70 ± 2.54**	**11.74 ± 1.78**	**11.33 ± 1.87**	**11.60 ± 1.38**
RFmax (%)	Moderate	40.82 ± 2.17	38.54 ± 2.74	37.71 ± 2.76	38.28 ± 2.39	37.48 ± 3.43	38.48 ± 1.95
	Mild	40.31 ± 2.31	38.18 ± 2.18	38.78 ± 2.90	38.90 ± 2.55	38.27 ± 2.60	38.44 ± 2.11
	All	**40.54 ± 2.20**	**38.34 ± 2.80**	**38.30 ± 2.81**	**38.63 ± 2.43**	**37.92 ± 2.93**	**38.46 ± 1.98**
5 m time (s)	Moderate	1.45 ± 0.07	1.51 ± 0.09	1.55 ± 0.09	1.52 ± 0.07	1.56 ± 0.13	1.53 ± 0.06
	Mild	1.45 ± 0.08	1.52 ± 0.12	1.49 ± 0.10	1.49 ± 0.08	1.53 ± 0.09	1.52 ± 0.06
	All	**1.45 ± 0.07**	**1.51 ± 0.10**	**1.52 ± 0.10**	**1.51 ± 0.08**	**1.54 ± 0.11**	**1.52 ± 0.06**
10 m time (s)	Moderate	2.29 ± 0.10	2.40 ± 0.15	2.43 ± 0.14	2.41 ± 0.13	2.45 ± 0.18	2.39 ± 0.10
	Mild	2.31 ± 0.11	2.42 ± 0.15	2.38 ± 0.15	2.38 ± 0.12	2.41 ± 0.13	2.40 ± 0.10
	All	**2.30 ± 0.10**	**2.41 ± 0.14**	**2.40 ± 0.14**	**2.39 ± 0.12**	**2.43 ± 0.15**	**2.40 ± 0.10**
20 m time (s)	Moderate	3.76 ± 0.16	4.00 ± 0.24	4.00 ± 0.21	3.97 ± 0.19	4.00 ± 0.24	3.95 ± 0.17
	Mild	3.84 ± 0.18	4.02 ± 0.23	3.96 ± 0.21	3.96 ± 0.20	3.99 ± 0.22	3.96 ± 0.19
	All	**3.81 ± 0.17**	**4.01 ± 0.23**	**3.98 ± 0.21**	**3.96 ± 0.19**	**3.99 ± 0.22**	**3.95 ± 0.18**
30 m time (s)	Moderate	5.19 ± 0.24	5.55 ± 0.35	5.51 ± 0.27	5.48 ± 0.25	5.49 ± 0.31	5.43 ± 0.24
	Mild	5.31 ± 0.27	5.56 ± 0.31	5.47 ± 0.27	5.48 ± 0.29	5.52 ± 0.31	5.46 ± 0.28
	All	**5.26 ± 0.26**	**5.55 ± 0.32**	**5.48 ± 0.26**	**5.48 ± 0.27**	**5.51 ± 0.30**	**5.45 ± 0.25**
40 m time (s)	Moderate	6.61 ± 0.33	7.09 ± 0.46	7.01 ± 0.35	6.99 ± 0.33	6.98 ± 0.38	6.93 ± 0.33
	Mild	6.78 ± 0.36	7.11 ± 0.40	6.98 ± 0.34	7.00 ± 0.39	7.04 ± 0.42	6.96 ± 0.38
	All	**6.70 ± 0.35**	**7.10 ± 0.42**	**6.99 ± 0.33**	**6.99 ± 0.36**	**7.01 ± 0.39**	**6.95 ± 0.35**

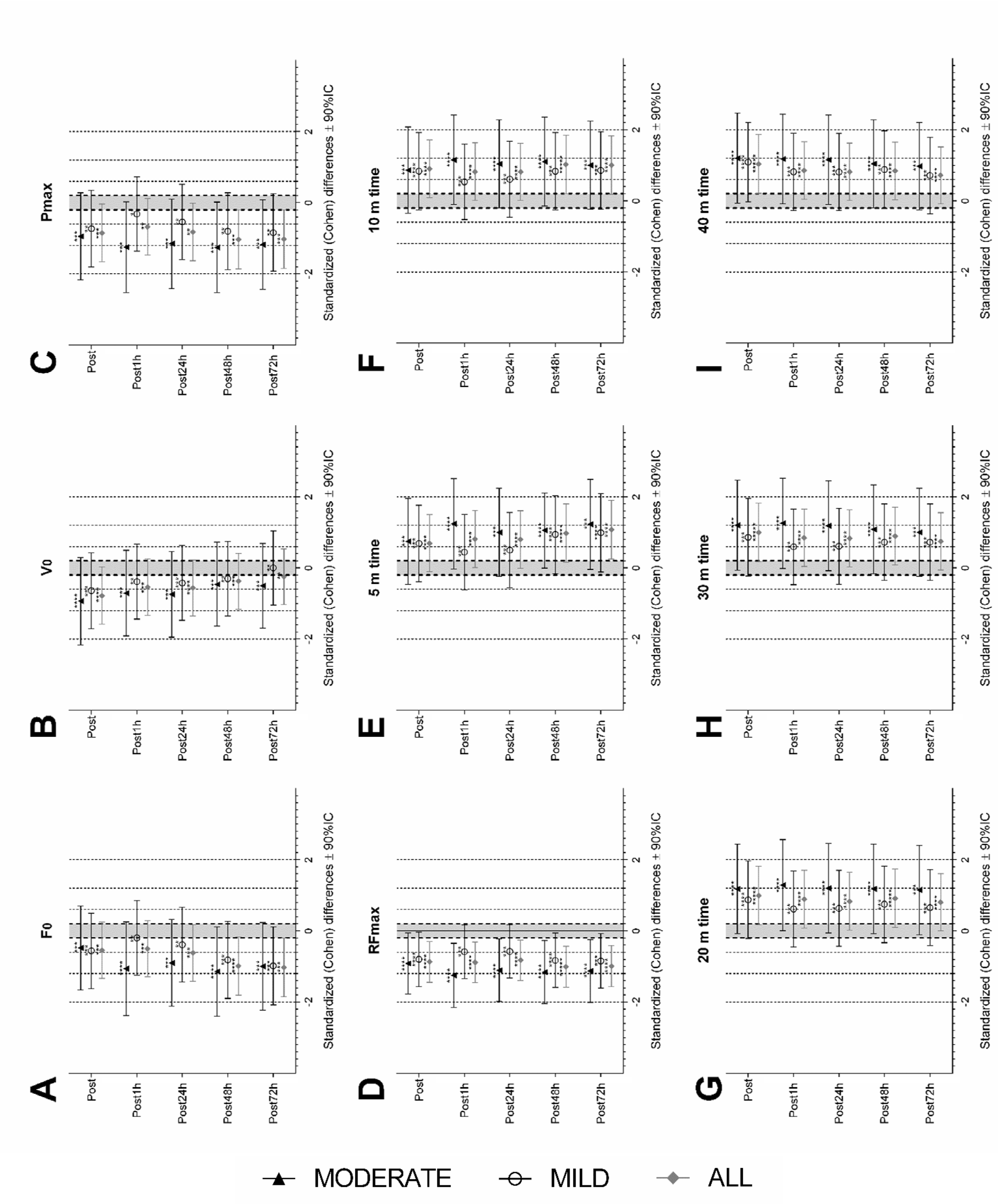


*Figure 18. Standardized differences (Cohen's d) for sprint running performance and mechanical parameters comparing the baseline (Pre) with all other assessments (Post, Post1h, Post24h, Post48h and Post72h) for the different groups. F_0: maximal theoretical horizontal force (A); V_0: maximal theoretical running velocity (B); $Pmax$: maximal power (C); $RFmax$: maximal value of RF (D); 5 m time (E); 10 m time (F); 20 m time (G); 30 m time (H); 40 m time (I). Error bars indicate uncertainty in the true mean changes with a 90% confidence interval. Asterisks indicate the probability of increase or decrease: * (5%-24.9%, unlikely); ** (25%-74.9%, possible); *** (75%-94.9%, likely); **** (95%-99.5%, very likely); ***** (>99.5%, almost certain).*

5.5. DISCUSSION

This is the first study of changes in sprint F-V spectrum as a result of EIMD. First, the extent of muscle damage was characterized using different markers such as strength (MVIC and CMJ) and perceived soreness, in order to classify the participants into two groups of response to EIMD [42]. Based on the decreases in knee extensor MVIC (Figure 17A & B) the exercise produced moderate or mild EIMD (MO and MI groups respectively) [42]. Similar dynamics of impairment and recovery were seen in the CMJ performance (Figure 17B & D). The peak in DOMS at Post24h or Post48h (Figure 17E & F) is consistent with the dynamics of this parameter in previous studies that assess EIMD [155].

After the classification into response groups, changes in sprint performance and F-V spectra were assessed. The main findings of the present study were that: (i) both groups (MI and MO) suffered impaired sprint performance, changes in the F-V spectrum and decrements in mechanical effectiveness during the follow-up as a result of EIMD in the lower limbs (Figure 18); and (ii) the dynamics of impairment and recovery during the follow-up were distinct for the different parameters assessed (V_0 as opposed to F_0 and $Pmax$).

V_0 showed a homogeneous response across both groups, with a temporary reduction (moderate to small) in the first hours after exercise (Post, Post1h, and Post24h), and a progressive recovery during the follow-up. As opposed to this, the changes in the variables related to short distances and high demands of force (5 and 10 m times, F_0 and $Pmax$) showed small impairments immediately after the exercises, and a tendency towards worsening as the hours passed. Additionally, the response was clearly differentiated between groups in these variables, showing a greater decrement in the MO group, especially in the time interval between Post1h and Post24h. These findings are consistent with those of Nagahara et al. who reported significant reductions in V_0 (depending on the volume of high-speed efforts) immediately after a football match accompanied by the absence of changes in F_0 and $Pmax$ [160]. Additionally, Jiménez-Reyes et al. reported that fatigue-induced reductions in performance immediately after a repeated sprint test with rugby sevens players were associated with greater decrements in V_0 than in F_0 [170]. Overall, these results suggest that the impairments in V_0 and maximal velocity capabilities are more influenced by a state of acute fatigue (i.e., due to greater metabolite-induced disturbances [170], or glycogen unavailability [160]), but the situation is overcame fast, as metabolic homeostasis is recovered. In contrast, those variables related to short distances and high demands of force seem to be more influenced by the presence of lower-limb muscle damage, given the differential response between the groups in these parameters (Figure 18A, C-F). In accordance with these results, previous research assessing the effects of EIMD has reported decrements in short-distance sprint performance (5 and 10 m) presumably caused by reduced reflex sensitivity affecting the SSCju in the propulsive phase [171]. Supporting this hypothesis, Horita et al. reported more extended knee angles during the drop jump in the presence of EIMD, which affected the stretch-shortening cycle [172]. Such a change, if present during sprinting, would also be a plausible contributor to the persistent $RFmax$ alterations. Additionally, it has been shown that EIMD and soreness of the quadriceps muscles produce alterations in the agonist–antagonist muscle activities depending on the characteristics of the force task [173]. Therefore, the adjustments in the central motor commands might have a greater impact on the initial steps at relatively slower velocities and higher forces than on the strides at maximum velocity and lower force application. These results also support previous observations that greater strength loss is produced at lower angular velocities of movement [171].

Another notable finding is that while both groups showed moderate decreases in F_0, $Pmax$ and $RFmax$ and moderate increases in 5 and 10 m times that had not recovered 72 h after the exercises; MVIC and CMJ had already recovered in the MI group by that time. These facts illustrate that the testing protocols used are complementary and consequently practitioners should consider the inclusion of multiple testing conditions, in order to holistically assess the process of recovery, which might differ depending on the neuromuscular manifestation tested.

One limitation of this study is that, as in previous EIMD studies, only one specifically located measure of MVIC was taken, focalized on knee extension [171,172]. Given that hip extensors actively participate in the squat [174] and that lower-limb muscle soreness was reported generally (i.e., not specifically located), we should expect that some degree of EIMD was present in the hip extensors, contributing to the reported results.

This study provides further evidence of the benefits of quantifying the macroscopic mechanical properties of the sprint to gain better insight into post-exercise changes. From the results presented in this study and some of the discussed bibliography [160,170] it is to be expected some impairments in the sprint variables related to maximal velocities and performance variables (i.e., times) in longer sprint distances (i.e., 40 m), as a result of acute fatigue after exercise. However, these acute changes might not be reflected in the sprint parameters related to the initial accelerative phase and performance variables (i.e., times) in shorter sprint distances (i.e., 5 or 10 m). On the contrary, the presence of EIMD as a result of a strenuous or unaccustomed training session might result in the impairment of sprint parameters related to the application of high forces in the initial accelerative phase. Therefore, performance (i.e., times) in short sprint distances (i.e., 5 or 10 m) is expected to worsen for several days depending on the amount of EIMD. These impairments seem to last for a longer time than those seen in other tests (i.e., CMJ, MVIC). Consequently, coaches and trainers might take in consideration these outcomes in order to better allocate weekly training loads in relation to competition.

Finally, the authors suggest that the use of a 10 m sprint test might be appropriate for testing EIMD-recovering athletes, since (i) It seems to be sensible to the changes produced by EIMD in the lower limbs and changes last longer than those seen in other tests (i.e., CMJ, MVIC); (ii) It is a very specific test for a wide variety of sports; (iii) It is a fast applicable field test; and (iv) It is less stressful than a full 40 m maximal run (needed for sprint F-V profiling).

5.6. CONCLUSIONS

The mechanics of sprint running were altered for several days after a strenuous session of inertial half-squat exercise, resulting in reduced performance. Sprint V_0, which corresponds to the application of low forces at very high speeds, was altered after the exercises as a result of acute fatigue, but showed a progressive recovery during the follow-up. Conversely, the variables related to shorter distances and high demands of force (5 and 10 m times, F_0 and $Pmax$) were more dependent on the amount of muscle damage, with a clearly differentiated response between groups, overall showing small impairments immediately after the exercises and a tendency towards worsening as the hours passed.

Sprinting offers complementary information of the neuromuscular dynamics of the lower limbs and might be considered for recovery testing purposes.

STUDY 3

“Early functional and morphological muscle adaptations during short-term iso-inertial squat training.”

6. STUDY 3. "Early functional and morphological muscle adaptations during short-term iso-inertial squat training."

6.1. ABSTRACT

The purpose of this study was to assess early changes in muscle function and hypertrophy, measured as increases in muscle cross sectional areas (CSAs) and total volume, over a 4-week inertial resistance training program.

Ten young resistance training naive volunteers (age 23.4 ± 4.1 years) underwent 10 training sessions (2-3 per week) consisting of 5 sets of 10 flywheel squats (moment of inertia 0.090 kg·m^2). Magnetic resonance imaging (MRI) scans of both thighs were performed before (PRE), and after 2 (IN) and 4 (POST) weeks of training to compute individual muscle volumes and regional CSAs. Scans were performed after ≥ 96 hours of recovery after training sessions, to avoid any influence of acute muscle swelling. PRE and POST regional muscle activation was assessed using muscle functional MRI (mfMRI) scans. Concentric (CON) and eccentric (ECC) squat force and power, as well as maximal voluntary isometric contraction force (MVIC) of knee extensors and flexors, were measured in every training session.

Significant quadriceps hypertrophy was detected during (IN: 5.5% ± 1.9%) and after (POST: 8.6% ± 3.6%) the training program. Increases in squat force (CON: 32% ± 15%, ECC: 31 ± 15%) and power (CON: 51% ± 30%, ECC: 48% ± 27%) were observed over the training program. Knee extensor MVIC significantly increased 28% ± 17% after training, but no changes were seen in knee flexor MVIC. No correlation was found between regional muscular activation in the first session and the % of increase in regional CSAs (r = -0.043, P = 0.164).

This study reports the earliest onset of whole-muscle hypertrophy documented to date. The process initiates early and continues in response to resistance training, contributing to initial increases in force. The results call into question the reliability of mfMRI as a tool for predicting the potential hypertrophic effects of a given strengthening exercise.

Keywords: Flywheel, Strength, Resistance training, Hypertrophy, MRI.

6.2. INTRODUCTION

Resistance training has been shown to induce profound and specific changes in virtually all biological systems [175]. Optimizing the stimulus and time invested to produce these changes is one of the goals of strength and conditioning trainers. In an attempt to achieve this, training programs are usually periodized, based on the different dynamics of adaptation for each physiological variable. Muscle hypertrophy induced by resistance training has generally been considered to be a slow process with a delayed onset, with initial strength gains mostly attributed to neural factors [176,177]. Biologically, muscle hypertrophy is the result of a positive balance between the synthesis and breakdown of proteins, which is manifested as microscopic (fiber thickness) and macroscopic (muscle thickness) surrogate variables. It is known that remodeling processes are highly dynamic in skeletal muscle, and that single bouts of resistance training immediately upregulate intramuscular anabolic signaling, amino acid transport and protein synthesis [178–180]. Therefore, if the protein balance remains positive after training sessions [181], it would be theoretically possible to accrete muscle mass early, starting after the very first session. In line with this idea, in a recent RCT in humans, increases in Type II fiber CSA were detected after only two weeks of training [182]. Although this histological evidence suggests a continuous process of adaptation, the macroscopic evidence of adaptation in the early phase of resistance training is less convincing.

Given this background, the time course of macroscopic muscle hypertrophy was revisited in a recent review of studies involving different training protocols and at least 3 muscle size measurements over time [183]. Even though most of the studies analyzed used a similar sample (untrained young volunteers), a lack of agreement between the results was observed. For the lower limbs, the reported results range from no significant changes in muscle size after 5-12 weeks, [177,184] to significant muscle hypertrophy after just 3-4 weeks of resistance training [185–189]. To date, the study conducted by Seynnes et al. [103] reports the earliest onset of macroscopic muscle hypertrophy, showing an increase of quadriceps femoris CSA after only 20 days of training (9 training sessions). The differences in the training stimulus, frequency of the assessments and sensitivity of the measurement methods all contribute to the spread of results in the available literature [183]. It is hoped that new studies using an optimized resistance training stimulus, and more sensitive and frequent assessment will shed light on the topic.

In order to produce fast and significant increases in muscle size, the training stimulus has to meet certain characteristics. Mechanical tension is the primary driver of muscle hypertrophy [61]. Thus, training interventions aiming to increase muscle volume should focus on the magnitude and the length of time producing tension. Several training systems and techniques can be used to achieve an enhanced stimulus. For instance, inertial flywheels allow for accommodated maximal or near maximal actions from the very first repetition of a set in the CON and ECC phases [127], (see section 1.2.2 and Figure 13). To the best of our knowledge, Lundberg et al. [117] reported the fastest rate of whole muscle hypertrophy over a period of 5 weeks (0.4% increase per day), using a combination of flywheel resistance training and intense aerobic exercise. In a recent meta-analysis, flywheel training showed to be more effective than conventional weights in promoting increases in muscle volume, strength and power [99].

A wide range of techniques can be employed to estimate changes in whole-muscle size [183,190]. MRI is regarded as the gold standard for clinical and research imaging of skeletal muscle, and is commonly used to compute 1 (middle), 2 (proximal and distal), or 3 (proximal, middle and distal) CSAs of a given muscle [103]. However, single CSAs may not be representative of whole-muscle changes, given that different patterns of hypertrophy (ventral or distal) have been reported in response to specific CON or ECC loading [191]. New MRI approaches for the assessment of total muscle volume, and not only CSAs of specific muscle sites, provide a better measurement of whole-muscle changes [119].

Other acute physiological processes need to be carefully taken into account when measuring muscle hypertrophy. Resistance training results in a rapid activity-dependent influx of fluid and accumulation of osmolytes (phosphate, lactate and sodium) and a subsequent acute inflammatory response that can last for several hours after exercise [118]. These processes temporally alter muscle volume and may interfere with hypertrophy measurements [192], but they can also provide valuable information. For instance, acute fluid changes in muscles can be assessed indirectly by muscle functional MRI (mfMRI) [193]. This non-invasive technique measures the “T2 shift”, which is the increase in the transverse relaxation time (T2) of muscle water from pre-exercise to post-exercise [194]. The T2 shift is positively correlated with EMG activity, at least when muscle groups are exercised in isolation [195]. In consequence, and although it can only be considered as a proxy marker, mfMRI is commonly used as a tool to determine total or regional muscle metabolic activation in different exercises [196]. Given that swelling is caused by muscular activity, and that cell swelling is a known upregulator of anabolic signaling pathways in a wide variety of cells [197], it would be reasonable to expect that muscles with greater T2 shifts after acute resistance training will display greater hypertrophy if the same exercise is systematically repeated over time. In fact, previous studies have found a correlation between these parameters when an isolated muscle or muscle group is exercised over time [198,199].

The purpose of this study was to assess early changes in thigh muscles function (force and power), hypertrophy (total volume and regional CSAs), and muscle activation (T2 shift), during a 4-week iso-inertial squat resistance training program. We hypothesized that the training program would produce early (≈ 2 weeks) detectable increases in muscle function and size. Additionally, we studied the relationship between muscle hypertrophy and activation in the first session, under the hypothesis that different degrees of hypertrophy of thigh muscles would be directly related to muscle task-specific activity assessed by mfMRI.

6.3. METHODS

6.3.1. Experimental approach

A quasi-experimental design of repeated measurements was used to determine the effects of an intervention on different variables. The independent variable in this study was a 4-week training program consisting of bilateral half-squats performed on an inertial flywheel device. The variables assessed for thigh muscles were: muscular activation, muscle hypertrophy and muscle performance. Activation was assessed through the T2 shift by mfMRI. Hypertrophy was assessed through increases in total volume and regional CSAs using MRI. Muscle performance was assessed through MVIC of the knee extensors and knee flexors, as well as by squat dynamic force and power. The assessments were performed before (PRE), and after 2 (IN) and 4 (POST) weeks of training; and also during each of the 10 training sessions. Figure 19 is a schematic representation of the study protocol.

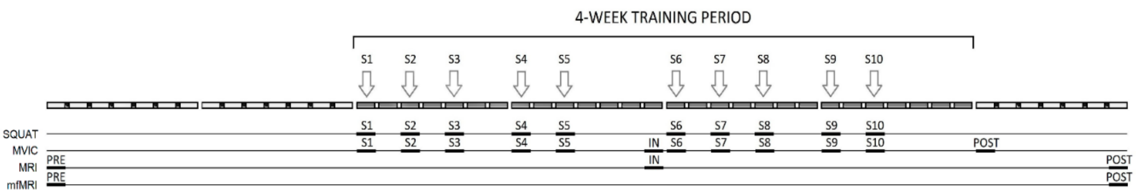


Figure 19. Schematic representation of the study protocol. S1-S10 training sessions, SQUAT performance (kinetics), MVIC maximal voluntary isometric contraction force of knee extensors and knee flexors, MRI muscle volume assessed by magnetic resonance imaging, mfMRI muscle activation assessed by muscle functional magnetic resonance imaging.

6.3.2. Subjects

Ten young volunteers (men = 8; women = 2) (age 23.4 ± 4.1 years; height 174.8 ± 7.7 cm; weight 71.0 ± 6.8 kg; self-reported weekly moderate intensity activity 6.3 ± 3.4 h·week^{-1}) who had not suffered muscle or tendon injuries in the previous 6 months were recruited for the study. All were recreationally active physical education students who had not been involved in any resistance training program during the 6 months preceding the study. 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 of the Catalan Sports Council (Generalitat de Catalunya). A familiarization session was conducted two weeks before starting the study. All the volunteers were informed of the aims, experimental protocol, procedures, benefits, and risks of the study, and their written informed consent was obtained. The volunteers were instructed to maintain their usual level of physical activity throughout the experimental period, and to refrain from moderate or heavy physical activities in the 96 hours before the MRI.

6.3.3. Training protocols

The training period comprised of 10 training sessions distributed over 4 weeks (2 or 3 sessions per week). Each training session consisted of a standardized warm-up (2 sets of 10 body-weight squats, with 1 min of rest between the sets), followed by 5 sets of flywheel exercise. Each set of exercise included 3 sub-maximal repetitions to accelerate the disk, followed by 10 maximal voluntary repetitions. There was three minutes of rest between sets. Exercise consisted of CON – ECC bilateral half-squats performed on a “YoYo squat” inertial flywheel device (YoYo Technology AB, Stockholm, Sweden) (Figure 13). Given the properties of the flywheel, the resulting moment of inertia was 0.090 $kg \cdot m^2$. The start and end position of each repetition was a 90° knee angle. The participants were verbally encouraged to perform the concentric phase at their fastest voluntary speed.

6.3.4. Squat performance

Exercise kinetics were monitored during each session using a Chronopic friction encoder (Chronojump, Barcelona, Spain), (accuracy ± 1 mm, sampling rate 1000 Hz). The sensor was tightly attached at a known diameter of the flywheel, sharing the same linear speed. The set-up parameters such as disk inertia, axis diameter, and the volunteer’s body mass were entered into the associated Chronojump software (v1.6.0.0) to compute the kinematic exercise variables in real time. Visual and acoustic feedback on the performance was provided for each repetition using a computer. The variables calculated for the CON and ECC phase of each repetition were displacement and mean values of force and power. Chronojump is open-code software, and a complete repository of the code and formulas used can be found online [143].

6.3.5. Maximum voluntary isometric contraction

MVIC of the knee extensors and knee flexors was tested. For data collection, a strain gauge and a custom-built bench were used. The signal was recorded at a frequency of 200 Hz, using a Muscle Lab 6000 (Ergotest Technology AS, Porsgrunn, Norway) and real-time results were provided on a computer monitor. For assessment of knee extensors, the volunteers were positioned supine (knee angle = 90°; hip angle = 180°); for knee flexors, lying prone (knee angle = 135°; hip angle = 180°). In both positions, the volunteers were fixed using adjustable straps and the strain gauge was attached at the mid-level of the malleolus tibiae forming a perpendicular angle with the leg. Three unilateral 5-second trials were recorded for the dominant limb, with 30 seconds of recovery between trials. Subjects were instructed and verbally encouraged to perform and maintain maximum force output. Pre-contraction conditions were standardized and attempts with counter movements were rejected. Maximum force values were selected using mean force over a 1-second mobile window once a force plateau had been established [50,127]. The best attempt was selected at each time point for further analysis. MVIC was assessed before each training session, and at IN and POST, after the standard warm-up.

6.3.6. Image acquisition and processing

MRI was used to compute the volumes of the individual muscles of the thigh and mfMRI to assess the acute muscle activation after exercise. The volunteers were placed supine inside a 3-T MRI scanner (Magnetom VERIO, Siemens, Erlangen, Germany), with their heads outside the MR-bore and thighs covered with one 32- and two flexible 4-channel coils, respectively, in the proximal and distal segments. A custom-made foot-restraint device was used to standardize and fix limb position, and to avoid any compression of thigh muscles. To ensure the same anatomical area was assessed each time, the range was centered at the mid-length of the femur, as measured on the coronal plane image.

Muscle Activation

For the assessment of PRE and POST muscle activation, one scan was performed in basal conditions and another 3-5 min after finishing an acute bout of the exercise [194,200] (10 sets of 10 flywheel squats). To minimize the effects of fluid shifts caused by walking, the volunteers remained recumbent for a minimum of 10 min before basal data acquisition [201], and were assisted over the 10 m between the exercise room and the MRI scan. In each scan, twelve contiguous (each 31.5 mm) 3.5 mm cross-sectional images of both thighs were obtained using the following scan sequence: SE MULTIECO, repetition time: 1800 ms, echo times: 20, 40, 60, 80 and 100 ms, field of view: 40 x 40 cm, matrix: 224 x 320, and total acquisition time: 6 min. A parametric image was generated from the T2 mapping sequence using Leonardo workstation (Siemens). The T2 of the muscles in both thighs was measured using OsiriX 8.5.2. (Pixmeo, Geneva, Switzerland). The assessment was performed by the same researcher on all occasions. All the sequences from a given volunteer were processed in parallel, with the researcher blinded. A circular region of interest (ROI) was selected for the muscles gluteus maximus (GM), rectus femoris (RF), vastus intermedius (VI), vastus medialis (VM), vastus lateralis (VL), adductor magnus (AM), gracilis (GR), biceps femoris long head (BFL), biceps femoris short head (BFS), semitendinosus (ST) and semimembranosus (SM) in each of the T2 mapping images where these muscles were visible. ROIs of similar size and anatomical location were placed in the subsequent image sets to ensure positioning identical to that in the first analysis [200]. The intra-class correlation coefficients and coefficient of variation for the intra-rater agreement of the T2 values were: 0.97 ± 0.06 and 1.09% ± 0.54% for the different muscles assessed. The T2 values for each muscle were computed as the mean value of the different ROIs. The T2 shift was then calculated by subtracting T2 basal values from T2 values post-exercise, and expressed as a percentage of the basal value. The results are shown as the average T2 shift of right and left thighs.

Muscle Volume

To assess muscle volume, PRE, IN and POST scans were performed. To minimize the effects of fluid shifts caused by walking [201], the volunteers remained recumbent for a minimum of 10 min before data acquisition. In each scan, 288 contiguous (each 1.5 mm) 1.5 mm cross-sectional images of both thighs were obtained, using the following scan sequence: VIBE 3D Dixon, field of view: 40 x

40 cm, matrix: 320 x 320, and total acquisition time: 2 min. The edges of the RF, VM, VL+VI, adductors, BFL, BFS, ST, and SM muscles were manually outlined, image by image, by the same researcher using OsiriX 8.5.2. (Pixmeo, Geneva, Switzerland) (Figure 20). Because there may appear to be substantial fusion between VL and VI on some slices [202], these muscles were outlined together (VL+VI). The same approach was adopted for the assessment of adductors volume, which includes pectineus, and adductor longus, brevis and magnus. All the sequences from a given volunteer were processed in parallel, with the researcher blinded. The total volume of muscles was computed from all the CSAs of the images where they were visible, in the range within the last image where the ischial tuberosity was visible and the last image where the femoral condyles were visible (Figure 20). The intra-class correlation coefficients and coefficient of variation for the intra-rater agreement of the CSA segmentation were 1.00 ± 0.00 and 1.44% ± 0.85% for the different muscles assessed. The intra-class correlation coefficients and coefficient of variation for the intra-rater agreement of the volumetric values were 1.00 ± 0.00 and 0.57% ± 0.42% for the different muscles assessed, similar to previous estimations of error using this method [119]. Quadriceps, hamstrings and total thigh muscle volume were computed post hoc as shown in Equation 3:

Equation 3. Computation of the combined volumes used in this study. RF rectus femoris, VM vastus medialis, VL vastus lateralis, VI vastus intermedius, BFL biceps femoris long head, BFS biceps femoris short head, ST semitendinosus, SM semimembranosus, TOTAL combined total volume, Adductors volume included pectineus, and adductor longus, brevis and magnus.

$$\boldsymbol{Quadriceps = RF + VM + VL + VI}$$
$$\boldsymbol{Hamstrings = BFL + BFS + ST + SM}$$
$$\boldsymbol{TOTAL = Quadriceps + Hamstrings + Adductors}$$

Volume changes were calculated by subtracting the PRE muscle volume from that IN or POST, expressed as a percentage of the PRE value. The results are shown as the average of right and left thighs.

As depicted in Figure 19, the scans were performed with at least 96 hours of recovery after the previous training session, so as to account only for hypertrophic changes and avoid any influence of acute muscle swelling [118]. To verify the absence of muscle edema at IN and POST, basal T2 values of the assessed muscles were compared with those obtained at PRE [118].

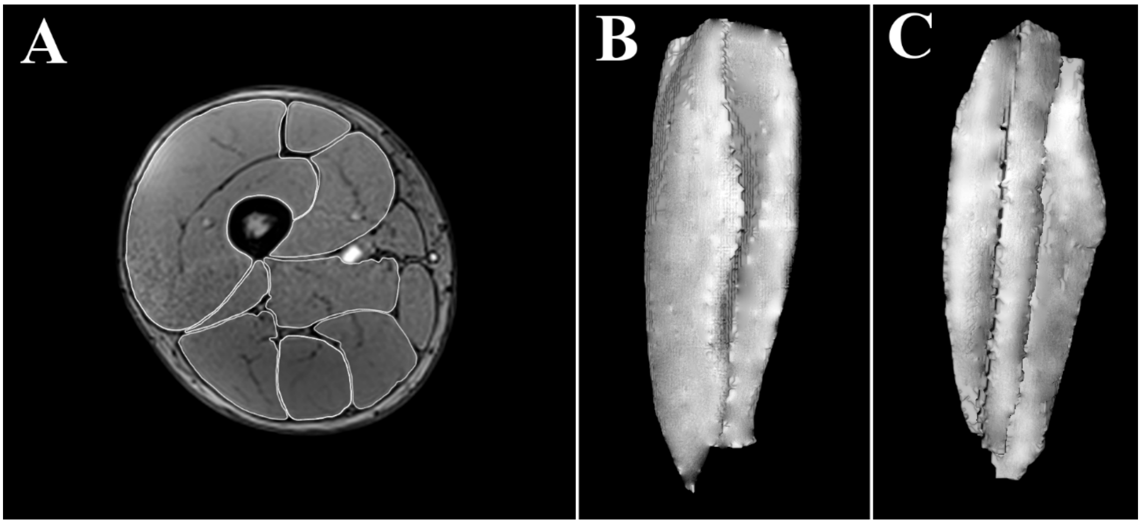


*Figure 20. Manually outlined muscle perimeters in a single cross-sectional image (≈ 50% length) of a right thigh (**A**). Three-dimensional reconstruction of a right vastus intermedius + vastus lateralis from all (≈ 250) the cross sectional areas assessed; anterior view (**B**), posterior view (**C**).*

6.3.7. Regional muscle activation and hypertrophy

Complementary analysis was performed to evaluate regional activation and hypertrophy of the muscles assessed and the relationship between these variables. PRE SE MULTIECO, and both PRE and POST VIBE 3D sequences were spatially synchronized. Thus, PRE regional T2 shifts were matched with PRE and POST regional CSAs. The exact anatomical placement of the cross-sectional images where T2 ROIs were measured was used to assess regional CSAs. Due to the differences in the protocols used for assessing these variables, the regional T2 shift of AM and the mean regional T2 shifts of VL and VI were matched with the adductors and VL+VI regional CSAs, respectively.

6.3.1. Statistical analysis

Data are presented as mean ± SD. All statistical analysis was performed using SPSS v.23.0.0.0 (IBM, Armonk, New York). A normal distribution of the data was checked using the Shapiro-Wilk test and Levene's test for homogeneity of variances was performed at each time level. For normal data, one-way ANOVA for repeated measures (with Mauchly's sphericity test) was used to determine the effects of time (PRE, Sessions 1-5, IN, Sessions 6-10, POST) on the different parameters assessed (see Figure 19). When significant effects were found, post hoc testing was performed by applying paired t-tests with a Bonferroni correction for multiple comparisons. For non-normal data, Friedman's test was applied. For the assessment of within-subject squat displacement variability, coefficients of variation were calculated for all the squat repetitions performed by each volunteer. Differences between CON and ECC force and power were calculated using t-tests for paired samples. The correlation between regional T2 shifts and regional CSAs was assessed by Pearson's product r values. Due to differences in femur lengths and muscles anatomy between participants, only regional T2 shifts and CSAs of anatomical regions for which the sample was $n > 7$ volunteers appear in the results section. Statistical significance was set at $P < 0.05$.

6.4. RESULTS

6.4.1. Squat performance

Mean displacement during the training sessions was 294 ± 24 mm, with low within-subject displacement variability (4.7% ± 1.5%). Higher forces (2.23% ± 0.39%; P = 0.008) and power (2.98% ± 0.69%; P = 0.011) were developed during the ECC than the CON phase. Significant effects of time were found for CON (P < 0.001) and ECC (P = 0.001) force, and for CON (P = 0.001) and ECC (P = 0.002) power. Increases in squat force (CON 32% ± 15%, ECC 31% ± 15%) and power (CON 51% ± 30%, ECC 48% ± 27%) were seen after the training period (Figure 21).

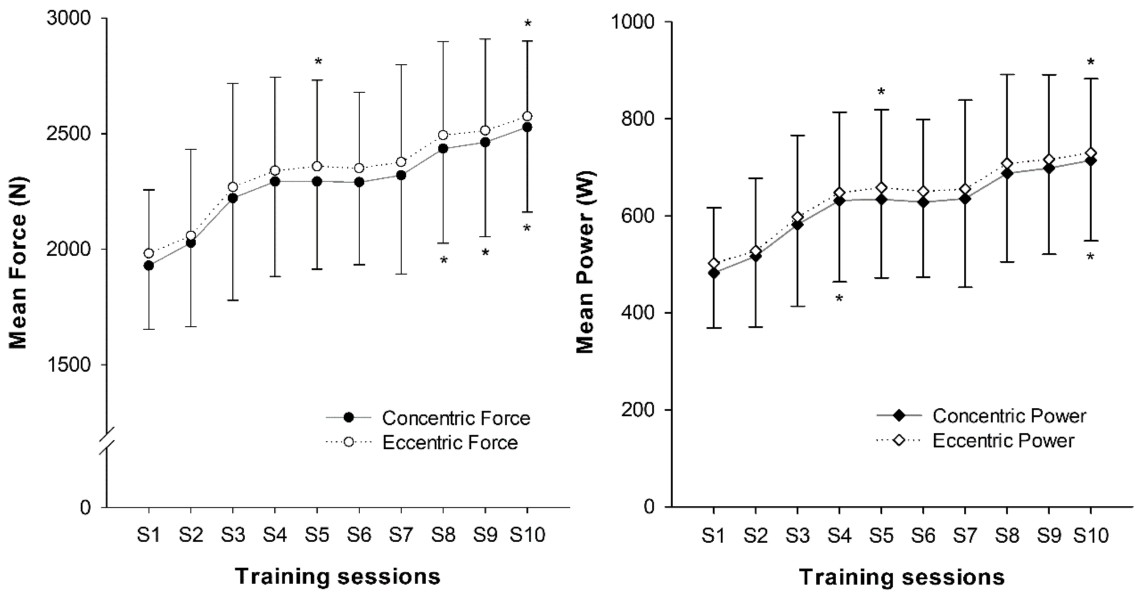


Figure 21. Progression of squat mean values of force and power over the 10 training sessions (S). (*) indicates significant changes from baseline values (P < 0.05).

6.4.2. Maximum voluntary isometric contraction

Significant increases in knee extensor MVIC were seen over the training sessions (P < 0.001), with an overall increase of 28% ± 17% after the training period. No significant changes were seen in knee flexor MVIC (P = 0.368) (Figure 22).

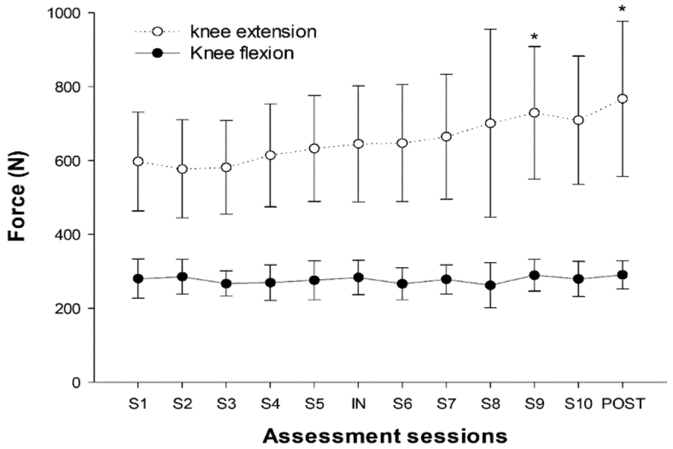


Figure 22. Progression of maximum voluntary isometric contraction force values throughout the training period. (*) indicates significant changes from baseline values (P < 0.05).

6.4.3. Muscle activation

The number of images analyzed per subject for each muscle was GM 3.4 ± 0.5; RF 7.3 ± 0.7; VI 8.4 ± 1.1; VM 8.7 ± 0.7; VL 9.4 ± 0.7; AM 7.0 ± 1.0; GR 8.8 ± 0.8; BFL 7.3 ± 0.7, BFS 5.2 ± 0.8, ST 8.3 ± 0.7, SM 6.4 ± 1.0. Of these, GM, RF, VI, VM, VL, and AM showed a positive T2 shift, whereas GR, BFL, BFS, ST, and SM showed a negative T2 shift (Figure 23). No significant effects of time (PRE vs. POST) were seen in T2 shift in any of the muscles assessed.

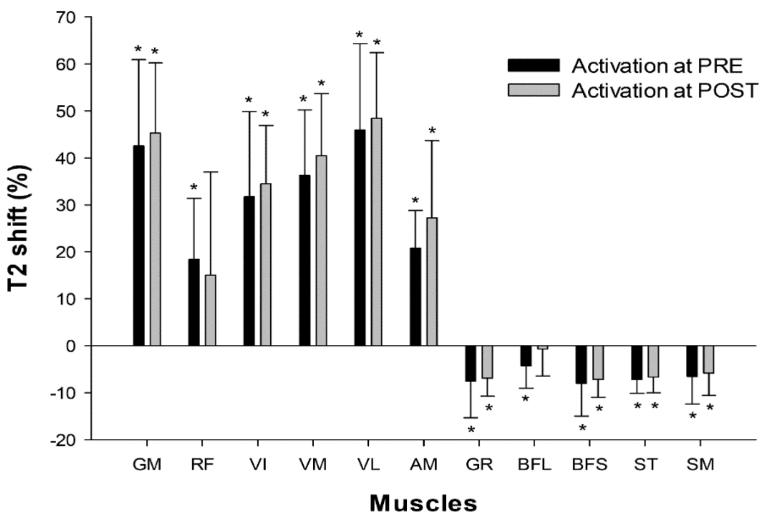


Figure 23. Muscular activation assessed by muscle functional magnetic resonance imaging (T2 shift) after 10 sets of 10 maximal squats on the flywheel device; before (PRE) and after (POST) the training period. GM gluteus maximus, RF rectus femoris, VI vastus intermedius, VM vastus medialis, VL vastus lateralis, AM adductor magnus, GR gracilis, BFL biceps femoris long head, BFS biceps femoris short head, ST semitendinosus, SM semimembranosus. (*) indicates significant post-exercise changes from basal T2 values (P < 0.05). No significant differences were found between PRE and POST activation.

6.4.4. Muscle volume

There was a significant main effect of time for all the muscles analyzed, except for SM (Table 5). Significant increases in RF, VM, VL+VI, Adductors and BFL volume were detected from PRE to IN and from PRE to POST (all $P < 0.05$). A representative case is shown in Figure 7.

Table 5. Muscle volumes at baseline (PRE) and after 5 (IN) and 10 training sessions (POST). RF rectus femoris, VM vastus medialis, VI+VL vastus intermedius + vastus lateralis, BFL biceps femoris long head, BFS biceps femoris short head, ST semitendinosus, SM semimembranosus. (*) indicates significant changes from basal values (P < 0.05).

	VOLUME (cm^3)			VOLUME CHANGE (%)		P-value time effect
	PRE	IN	POST	PRE-IN	PRE-POST	
Individual muscles						
RF	237.0 ± 42.9	244.5 ± 45.2*	248.1 ± 46.9*	3.2*	4.7*	<0.001
VM	472.6 ± 107.6	501.5 ± 110.2*	518.9 ± 125.3*	6.1*	9.8*	<0.001
VI+VL	1250.1 ± 233.0	1320.8 ± 239.9*	1360.7 ± 253.1*	5.7*	8.8*	<0.001
BFL	224.9 ± 28.0	229.6 ± 30.5*	232.8 ± 29.6*	2.1*	3.5*	<0.001
BFS	105.0 ± 23.8	104.5 ± 23.5	108.4 ± 22.5	-0.5	3.2	0.028
ST	231.6 ± 45.2	232.9 ± 44.8	238.4 ± 47.5	0.6	3.0	0.020
SM	246.7 ± 31.0	246.3 ± 32.8	250.9 ± 33.7	-0.2	1.7	0.066
Muscle groups						
QUADRICEPS	1959.8 ± 358.2	2066.8 ± 369.9*	2127.7 ± 399.2*	5.5*	8.6*	<0.001
ADDUCTORS	1037.8 ± 141.5	1065.4 ± 147.1*	1096.2 ± 172.7*	2.7*	5.6*	<0.001
HAMSTRINGS	808.2 ± 108.7	813.3 ± 109.7	830.5 ± 112.0*	0.6	2.8*	<0.001
Σ thigh muscles						
TOTAL	3805.7 ± 583.3	3945.5 ± 600.1*	4054.4 ± 656.8*	3.7*	6.5*	<0.001

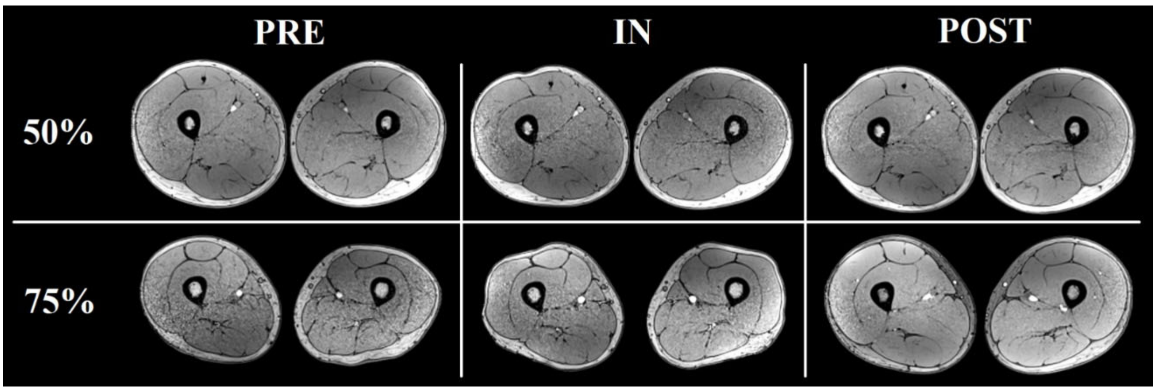
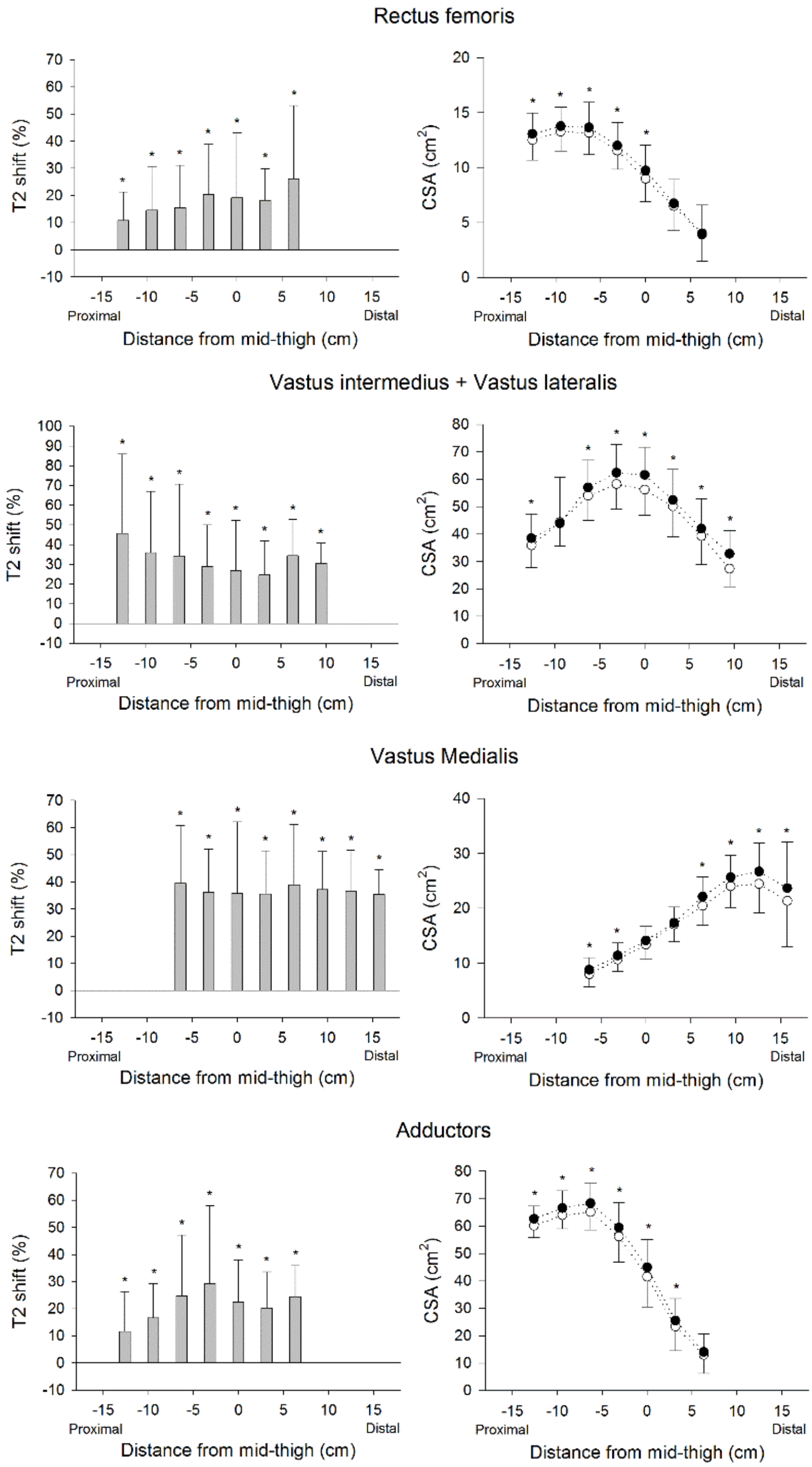


Figure 24. Representative increases in thigh muscles cross sectional areas (at ≈ 50% and ≈ 75% the length of the femur) as assessed by consecutive MRI scans before (PRE), and after 5 (IN) and 10 (POST) training sessions from one of the volunteers.

6.4.5. Regional muscle activation and hypertrophy

Figure 25 shows the PRE regional T2 shifts and regional CSA changes from PRE to POST. No significant correlations were found between the PRE regional T2 shifts and percentage increase in regional CSAs, except for ST (Total: r = -0.043, P = 0.164; AM: r = 0.131, P = 0,142; BFL: r = -0.023, P = 0,792; BFS: r = -0.120, P = 0.250; RF: r = -0.033, P = 0.706; SM: r = 0.135, P = 0.157; ST: r = -0.225, P = 0.007; VM: r = -0.143, P = 0.075; VL+VI: r = -0.051, P = 0.524).



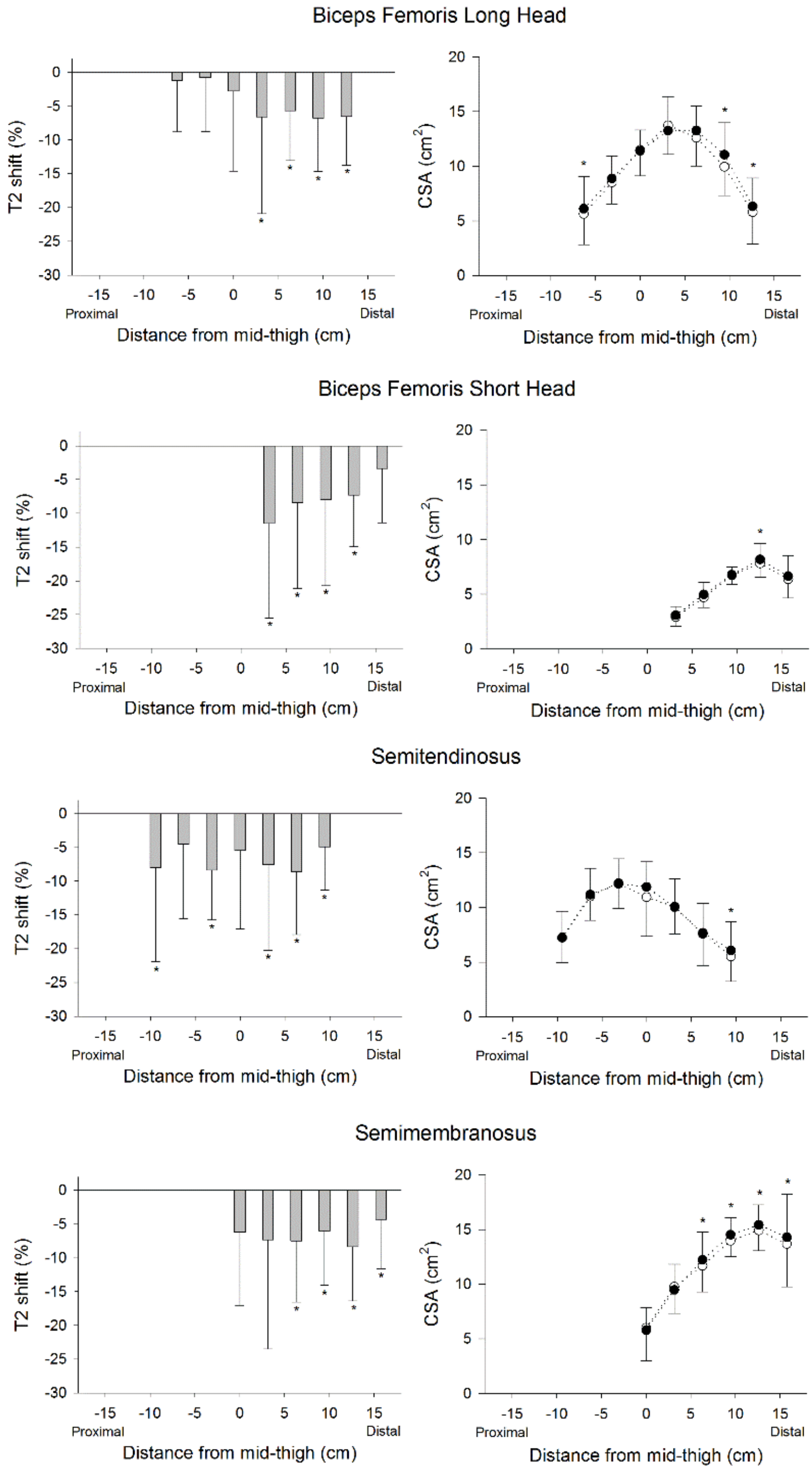


Figure 25. (Divided in two consecutive pages) Regional muscle activation assessed as T2 shift in the first session (PRE) and changes in regional CSAs from baseline (PRE) to the end of the 4-week training intervention (POST). In the activation graphs (left), (*) indicates significant post-exercise changes from basal T2 values ($P < 0.05$). In the CSA graphs (right), (*) indicates significant differences between PRE and POST CSAs ($P < 0.05$).

6.5. DISCUSSION

This study reports significant muscle hypertrophy after only 14 days (5 sessions) of a 4-week training period. To our knowledge, these findings represent the earliest evidence of macroscopic muscle hypertrophy (without the interference of acute muscle edema) to date.

6.5.1. Muscle performance

Fast and notable improvements in muscle performance were seen after the training program (see Figure 21). This outcome was favored by the great window of adaptation of the sample (resistance training naive young volunteers), and the effectiveness of the stimulus applied. The training system used in this study had previously been shown to be more effective in promoting increases in muscle volume, strength and power than conventional weights [99]. The efficacy of the method has been suggested to be mediated by the achievement of higher forces during the ECC phase [127]. A marked ECC-overload was not a deliberate outcome when the exercise protocols used in this study were designed, however, force and power values were significantly higher during the ECC phase (see squat performance results). Similar increases were reported in previous studies involving similar samples and training systems [103,109].

6.5.2. Muscle activation

As shown in Figure 23, the GM and VL muscles were the most active with the squat exercise. Globally, quadriceps muscles increased in T2 intensity, whereas hamstrings muscles decreased in intensity. Of the quadriceps muscles, however, the activation of RF between participants was highly variable. RF is the bi-articular muscle of the quadriceps group, and its activation pattern is highly influenced by the hip position [203]. Biomechanical differences (i.e., femur lengths) between subjects may have caused different hip flexion angles during execution and thus different RF activation patterns. Concerning hamstrings, the T2 shift values reported may appear unexpected, given that previous studies have shown a moderate contribution of hamstrings during squats when assessed by EMG [174]. It must be noted that T2 values are a quantitative index of the amount and distribution of water in skeletal muscle [194]. When assessed on a single active muscle group, T2 values are highly correlated with EMG activity [195]. However, these two assessment tools have different physiological bases and therefore may lack complete agreement in some situations. When muscles of a whole region (i.e., thighs) are assessed after being non-uniformly recruited, the distribution of water is increased in the most active muscles but it is decreased in less active muscles [204]. Thus, acute decreases in the T2 signal in hamstrings muscles after squat exercise indicate relative inferior activation compared to quadriceps muscles, but not a lack of activation [205].

Regarding the adaptive response to training, no significant changes were found in the T2 shift values from PRE to POST in any of the muscles assessed (Figure 23). Therefore, the recruitment pattern did not change over the training period. As exercises for determining T2 shifts were maximal both PRE and POST, it is highly likely that a ceiling was reached over which T2 values did not increase further [194]. However, it is not clear whether the magnitude of the T2 shifts would have been similar if the external load had remained the same in PRE and POST, given that force outputs in the latter were much higher.

6.5.3. Muscle hypertrophy

This study shows a rate of increase in quadriceps volume of 0.39% per day over the first 14 days of training (5 training sessions), which is almost double what Seynnes et al. [103] reported (0.2%) for the increase in CSA over the first 20 days of resistance training (9 training sessions). This difference may be explained by greater exercise volume per session (5 x 10 vs 4 x 7), as the resistance training volume seems to be one of the most important factors affecting muscle hypertrophy [206]. In addition, hypertrophic changes in the study performed by Seynnes et al. [103] were measured as changes in the CSA of two specific MRI slices at 25% and 50% of the femur bone length. Given that flywheel devices increase tension in the ECC phase of the movement and a distal pattern of hypertrophy has been reported in response to ECC loading [191], changes in the CSAs measured may have underestimated whole-muscle changes. Supporting this idea, data displayed at Figure 25 show less increase in the middle region of VL+VI (9.9% at 0 cm) and VM (7.6% at 0 cm) compared to their distal regions (VL+VI 21.3% at +9.45 cm; VM 14.0% at +15.75 cm). Given these non-uniform changes, the present study provides a more representative picture of whole-muscle changes by using total muscle volume [119]. Lundberg et al. [117] used a similar flywheel training volume per session (4 x 7) in combination with intense aerobic exercise, and reported a rate of quadriceps hypertrophy (measured as total volume) of 0.4% per day over the first 5 weeks. It should be noted that the rate of CSA volume increase reported in the present study is similar for the first two weeks (0.39% per day) but tends to decrease as the trainee progresses (0.30% per day over the first 28 days). The faster rate of increase reported by Lundberg et al. [117] could have been influenced by the additional training volume of the intense aerobic training, given the untrained condition of the volunteers.

Prior to this study, DeFreitas et al. [192] reported significant increases in thigh muscle CSA (measured by quantitative computed tomography) after only one week (two sessions) of resistance training. However, testing was performed 48 hours after a high-intensity resistance training protocol performed by unaccustomed subjects. Those authors concluded that early increases in CSA may have been due to edema, and considered that significant skeletal muscle hypertrophy occurred around weeks 3–4. Similarly, Krentz and Farthing [207] also reported significant increases in biceps brachii thickness (measured by ultrasound) after only 8 days (3 sessions) of eccentric resistance training. In that case, the volunteers were tested 48 hours after performing a training session, and there was a concomitant decrease in strength, indirectly indicating that exercise-induced muscle damage was present [42]. Therefore, in both cases, the early increase in muscle size was not considered to be hypertrophy, but edema-induced muscle swelling due to muscle damage [118].

It must be made clear that in the present study, the increases in muscle volume were assessed at least 96 hours after the last training session to avoid acute muscle swelling. The T2 signal under basal conditions of the muscles assessed did not change significantly from PRE to IN (P = 0.101 – 0.934, with differences of -1.5% ± 1.4%) or from PRE to POST (P = 0.137 – 0.849, with differences of -2.4% ± 1.7%), which indicates the absence of muscle edema [118]. Additionally, in all cases, pre-training-session MVIC force levels had recovered when the scans were performed, indirectly indicating the absence of muscle damage [42]. Therefore, we are confident that the changes in muscle volume reported here are accounted for by chronic hypertrophic changes, and not acute processes such as swelling.

6.5.4. Relationship between T2 shift and hypertrophy

Establishing a relationship between T2 shifts and hypertrophy would be useful as a predictive tool for resistance training exercises. In fact, strengthening exercises are commonly classified by the magnitude of activation of certain muscles or muscle regions, in order to allow trainers to focus on a specific target of the training intervention [196,200]. It must be noted that different approaches are used to assess T2 shifts. For instance, it can be assessed as percentage activated area [198,199], or as percentage change of a representative ROI [196,200]. Despite both variables commonly being used to quantify the same physiological phenomena, the relationship between them remains unclear. Therefore, differences between procedures may also have influenced the findings discussed here. In any case, the results presented in this study suggest that the regional percentage of change in the T2 signal after exercise is not a reliable tool for predicting the magnitude of increase in CSA, given that no correlation was found between those two variables.

Previous studies have found correlations between the acute T2 shift of specific muscle regions or muscles after exercise and the acute (swelling) [208], and chronic (hypertrophic) [198,199] morphological response measured by MRI. Given this background, a correlation between regional T2 shifts and the percentage of increase in regional CSAs was expected. A plausible explanation for the lack of correlation in the present study is that the previously mentioned studies used highly analytic exercise protocols, in which a given muscle or muscle group worked in isolation. T2 shifts in analytic (i.e., single joint) exercises are related to other variables that are more relevant for promoting muscle hypertrophy [195] (such as magnitude and time producing mechanical tension [61]). Although metabolic activity and T2 changes might correlate with mechanical tension and morphological changes in some situations, results suggest that this relation is weakened in complex multi-joint movements such as the squat, in which antagonistic and synergistic muscle groups are recruited throughout the movement [205]. For instance, the hamstrings muscle group displays a negative T2 shift in the squat [204], despite being electromyographically activated and under tension [174]. As a result, in the present study hamstrings muscle volume increased significantly after 4 weeks (see Table 5), even though BFL, BFS, SM and ST showed a significant decrease in the T2 signal after exercise (Figure 23).

Therefore, the use of mfMRI as a tool for predicting the potential hypertrophic effects of a given strengthening exercise should be questioned. We encourage future research to consider the physiological basis on which this method relies to correctly interpret mfMRI data from different exercises.

6.6. SUMMARY AND CONCLUSION

Muscle hypertrophy is initiated early and is progressive in response to resistance training, potentially contributing to initial strength gains. In this study, quadriceps muscles increased 5.5% ± 1.9% in volume after only 14 days (5 training sessions) of flywheel iso-inertial training. This represents the earliest onset of whole-muscle hypertrophy without the interference of acute muscle edema, documented to date. After 4 weeks of iso-inertial squat resistance training, great increases in knee extensor MVIC (28% ± 17%) and in quadriceps muscle volume (8.6% ± 3.6%) were observed.

The application of a robust resistance training stimulus in combination with a sensitive and precise evaluation tool such as 3D volumetry by MRI has been decisive for these findings. However, this method is at present much more time consuming than the assessment of single CSAs. Therefore, the level of sensitivity and precision needed, and the time available for assessment must be taken into consideration together to decide the best approach in each case [119].

Regional T2 shifts after the first assessment session were not found to be correlated with the relative increase in CSA after the training program. These results call into question the reliability of mfMRI as a tool for predicting the potential hypertrophic outcomes of a given exercise.

6.7. LIMITATIONS

In this study, the number of volunteers could be regarded as a limitation on the interpretation of the results. However, it should be taken into account that the responses were very similar in all the participants after the training protocol. Moreover, the sample size of the study was adjusted based on previous related research [103,117]. Finally, another limitation is the long time currently needed for the volumetric assessment of each muscle. As this precise analysis becomes more automatized with developing imaging technology, research will become easier in the future.

STUDY 4

“Protective effect on skeletal muscle conferred by a 4-week iso-inertial training protocol.”

7. STUDY 4. "Protective effect on skeletal muscle conferred by a 4-week iso-inertial training protocol."

7.1. ABSTRACT

In this study, we aimed to quantify and characterise exercise-induced muscle damage (EIMD) and to assess the protective effect conferred by a structured, progressive, 4-week, maximum-effort, iso-inertial resistance training programme.

Eleven young volunteers participated (10 men and 1 woman; mean age 23.8 ± 3.8 years). Repeated measures were taken (before exercise, and at 1, 24, 48, 72, and 144 hours after exercise) to determine the effects of a maximum-effort iso-inertial half-squat training session (ten sets of ten repetitions each) on the following: isometric force, vertical jump height, muscle soreness, and blood biochemical markers of EIMD (e.g., creatine kinase, sarcomeric mitochondrial creatine kinase, creatine kinase MB isoform, aspartate aminotransferase, alanine aminotransferase, titin, and cardiac troponin I). The protocol was performed 1 week before starting the 4-week iso-inertial training programme (assessment A) and 1 week after completing the programme (assessment B) to determine the protective effects of the training.

Compared with assessment A, external loads were greater at assessment B (+38.9%, +21.0%, and +65.3% production of concentric force, velocity, and power, respectively), all markers of EIMD were significantly ($P > 0.05$) attenuated, and the recovery processes were faster. Values corresponded to moderate EIMD at assessment A and mild EIMD at assessment B. The index of protection ranged from 75.4% to 79.7% for the muscle function parameters, from 52.5% to 85.5% for blood biochemical markers, and 48.0% for muscle soreness.

Keywords: Flywheel, Repeated bout effect, Muscle strength, resistance exercise.

7.2. INTRODUCTION

In recent years, iso-inertial training has gained momentum in practical and research settings for strength and conditioning thanks to its effectiveness in producing chronic adaptations and its potential for multiple sport- and health-related applications [86,95,99]. The operating principle of this training is based on the conservation of angular momentum, where resistance is created by the rotational inertia of discs. This couples (i) resistance against the rotational acceleration in the CON phase (discs tend to remain static, in opposition to increasing its rotational velocity) and (ii) resistance against the rotational deceleration applied by muscles (braking) in the ECC phase (discs tend to remain with the conferred rotational velocity, in opposition to getting static) [93–95] (Figure 11). This allows for accommodated resistance (i.e., near-maximal resistance along the whole ROM and throughout the repetitions of the set) and for brief episodes of ECC-overload (i.e., ECC outputs surpassing those of the CON phase), depending on the exercise technique when receiving the load [49,98–100].

The differential features of iso-inertial training as compared to traditional resistance training methods (i.e., free weights or weight stack machines) allow for increased total force production (and therefore muscle tension) during workouts, particularly during ECC movement [86]. However, ECC muscle actions are more prone to producing EIMD [8] and iso-inertial training usually enhances muscle tension during the ECC phase. As such, this type of training is commonly associated with some EIMD symptoms and markers [49,104,109].

EIMD is typically characterised by a decrease in muscle function (i.e., force-generating capacity; a highly reliable and valid indirect marker of the degree of muscle damage) [42] and the appearance of muscle soreness [51] that can persist for several days after the exercise. Additionally, certain proteins and enzymes are released into the bloodstream by damaged muscles and can be used as proxy markers of EIMD. The molecular weight and structure, the charge, or the cellular compartment in which these markers are located may be associated with the pathway and the rate of appearance and clearance in blood [45–49]. Therefore, evaluation of different markers in the hours after a damaging exercise can be used to quantify and profile the damage (i.e., its severity and location) [42,49,50]. If EIMD and the associated recovery processes are not monitored and accounted for in the resistance training process, there will be an increased risk of non-functional overreaching, injury, and illness [142].

After an initial damaging exercise, adaptive processes are initiated that help to protect against subsequent damaging stimuli. This effect is commonly known as the RBE and is influenced by variables such as intensity, volume, velocity, muscle length, and the time elapsed since the exercise [79]. The effects of RBE also seem to vary among the different indicators, suggesting that there are different underpinning mechanisms, including adaptations to the nervous system, the mechanical behaviour of musculotendinous structures, remodelling of the muscle extracellular matrix, and the inflammatory response [80]. These adaptations to exercise might be important to improving a person’s ability to cope with the stressors of training and/or competitive sport while taking on progressive loads.

Most studies of EIMD have been performed using ECC actions only (i.e., iso-weight or iso-kinetic resistance) [80]. Although iso-inertial training is typically classified as an ECC training modality because of its enhanced ECC phase, the acute responses and chronic adaptations vary between specific modalities [81]. To the best of our knowledge, only one study has specifically aimed to determine the adaptive effects of iso-inertial training regarding the RBE [104]. Their experimental design comprised two identical bouts of exercise (ten sets of ten repetitions of iso-inertial squats) interspersed by 4 weeks of rest. EIMD markers assessed in the 72 hours following exercise, including blood CK, peak torque of the quadriceps, and DOMS, were significantly decreased after the second identical session [104]. This experimental study provided an invaluable initial insight into the protection from EIMD conferred by an initial iso-inertial load in which exercise and force stimuli are similar between two EIMD-inducing training bouts. However, this is not necessarily transferrable to a real-world progressive training scenario; for example, participants have been reported to experience a great increase in force production over the first few weeks of training (i.e., a 0.41% increase per day over 4 weeks) [116], indicating that the second EIMD-inducing training bout would involve greater force production than the first bout.

In another study, researchers used a 6-week iso-inertial training programme. Despite reporting an approximate 50% increase in applied power, they also reported that there were highly attenuated responses of CK in the 72 hours following the last (i.e., fifteenth) from the first training session [109]. These results provide a basis for our hypothesis that even when the ECC component of a training session increases (external load), exercise tolerance increases at a higher rate and will potentially decrease muscle damage and recovery time. However, it should be acknowledged that blood CK concentrations as a sole marker of EIMD are insufficient to provide data for evaluating EIMD [42,109]. Therefore, a more exhaustive study on the protective effect of iso-inertial training is needed to investigate how muscle tissue reacts and recovers to the increased demands of training sessions.

We had three aims in the present study. First, to characterise the acute responses to unaccustomed iso-inertial resistance training loads. Second, to quantify the protection conferred by a structured, progressive, 4-week, iso-inertial squat training programme on markers of muscle damage. Third, to evaluate the increased demands of exercise sessions due to increased force production (i.e., the external load of the maximal accommodated resistance).

7.3. MATHERIALS AND METHODS

7.3.1. Experimental design

Repeated measures were taken to determine the effects of a “maximum-effort” iso-inertial half-squat training session (i.e., single bouts of exercise) on isometric force, vertical jump height, muscle soreness and blood biochemical markers of muscle damage.

Assessments were made pre-exercise (Pre), and after 1 (Post1h), 24 (Post24h), 48 (Post48h), 72 (Post72h), and 144 hours (Post144h). The entire protocol was performed one week before starting a 4-week training programme (A) and 1 week after completing that programme (B).

An overview of the study design to determine the protective effects of the training programme on EIMD is provided in Figure 26.

7.3.1. Participants

We enrolled 11 volunteers (10 men; 1 woman) with a mean age of 23.8 ± 3.8 years, a mean height of 176.0 ± 7.2 cm, and a mean weight of 71.8 ± 6.2 kg. The participants self-reported that they engaged in weekly activity of moderate intensity for 6.7 ± 3.6 $h \cdot week^{-1}$, that they had not suffered muscle or tendon injuries in the previous 6 months, and that they had not been involved in a specific resistance training programme in the 6 months preceding the study. All volunteers were recreationally active physical education students.

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 of the Catalan Sports Council (Generalitat de Catalunya). All volunteers were informed of the aims, experimental protocol, procedures, benefits, and risks of the study, and their written informed consent was obtained before starting.

A familiarisation session was conducted 1week before starting the study. The volunteers were instructed to maintain their usual level of physical activity throughout the experimental period, and to refrain from moderate or heavy physical activities in the 96 h before the exercise after the baseline session (Bout A) and after the follow-up session (Bout B), or during the 144-hour follow-up period (Figure 26).

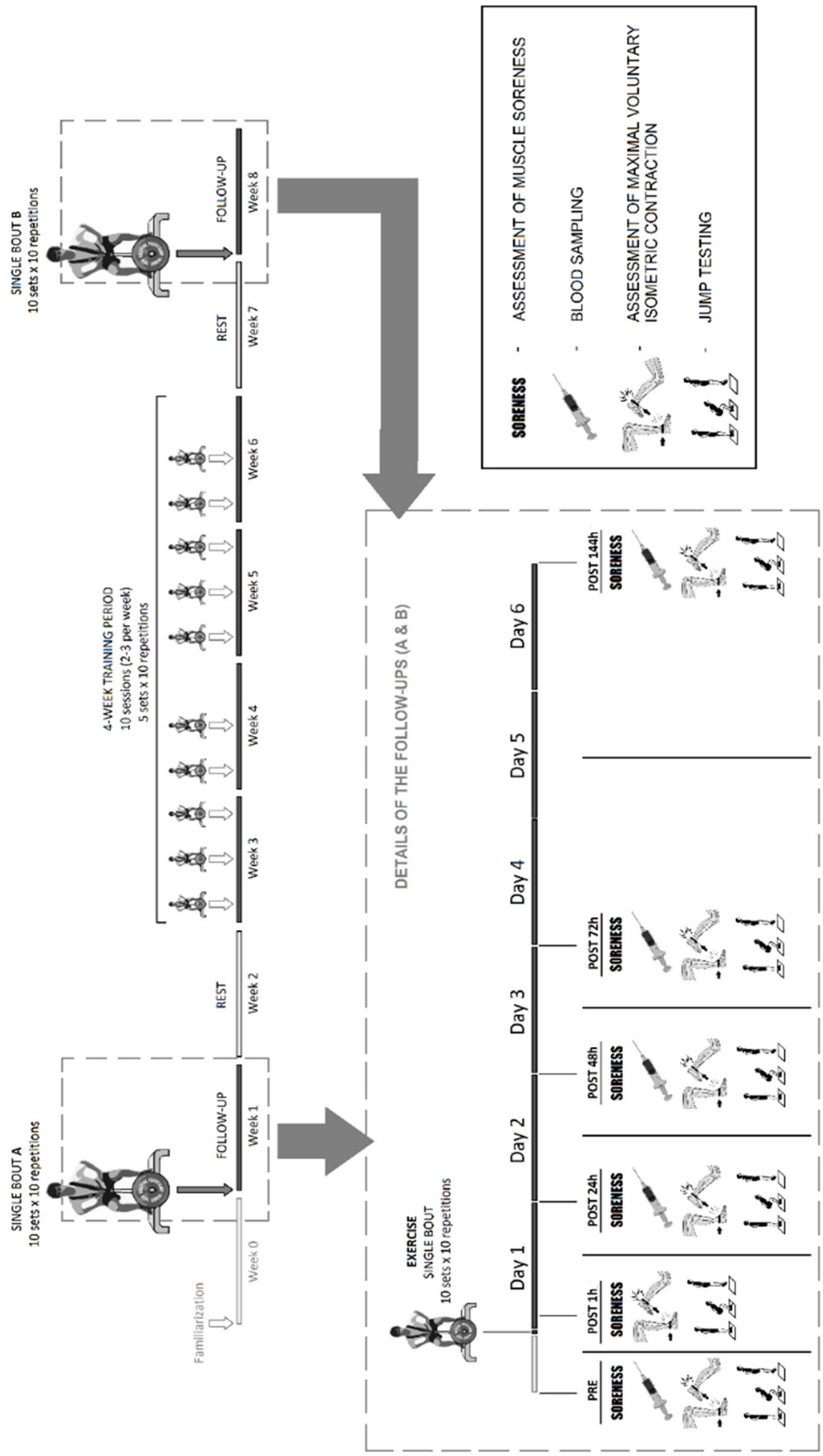


Figure 26. Study design. Single bouts of exercise were performed one week before (A) and one week after (B) a structured 4-week training programme. Outcomes were assessed pre-exercise (Pre) and at 1 (Post1h), 24 (Post24h), 48 (Post48h), 72 (Post72h), and 144 (Post144h) hours after performing the exercise. Ordered as shown in the figure.

7.3.2. Single bouts of inertial damaging exercise A and B

Both exercise protocols (A and B) comprised ten sets of flywheel CON–ECC bilateral half-squats performed on a "YoYo squat" inertial device (YoYo Technology AB, Stockholm, Sweden) (Figure 13) with a moment of inertia of 0.090 $kg \cdot m^2$. Each set of exercises included three sub-maximal repetitions to accelerate the disc, followed by ten maximal voluntary repetitions, with a 3-min rest interval between sets. The start and end position of each repetition was a knee angle of 90°. Exercise Bouts A and B were performed as the maximal voluntary efforts at each session, and participants were continuously encouraged, verbally, to perform the CON phase as fast as they could.

Exercise kinetics were monitored using a Chronojump friction encoder (Chronojump, Barcelona, Spain), (accuracy ± 1 mm, sampling rate 1000 Hz) [95]. Visual and acoustic feedback on the performance was provided for each repetition using a computer. The variables calculated for the CON and ECC phases of each repetition were the mean force, velocity, and power. Chronojump is an open-code software, and a complete repository of the code and formulas we used can be found online [143].

7.3.3. Training protocol

The 4-week training protocol comprised ten training sessions distributed as two or three sessions per week (Figure 26). Each training session included a standardised warm up (two sets of body-weight squats) and five sets of flywheel half-squats (three accelerative repetitions and ten maximal voluntary repetitions per set). Training sessions were similar to Bouts A and B, except for a lower training volume in the former (five versus ten sets, respectively).

7.3.4. Maximum voluntary isometric contraction.

MVIC of the knee extensors and knee flexors was measured on a custom-built bench. For assessments, volunteers were positioned supine for knee extensors (knee angle = 90°; hip angle = 180°) and prone for knee flexors (knee angle = 135°; hip angle = 180°), and fixed with adjustable straps. A strain gauge was attached at the mid-level of the malleolus tibiae forming a perpendicular angle with the leg. Force signal was recorded at a frequency of 200 Hz using a Muscle Lab 6000 (Ergotest Technology AS, Porsgrunn, Norway), and real-time feedback was provided on a computer monitor. Participants were verbally encouraged to perform and maintain maximum force output. Pre-contraction conditions were standardised and attempts with counter-movements were discarded. Three unilateral 5 s trials were recorded for the dominant limb, with 30 s recovery between trials. Maximum force was selected as the mean force over a 1 s mobile window once a force plateau had been established [50,95]. The best attempt at each assessment time was selected for further analysis (Figure 26).

7.3.5. Jump testing

The squat jump (SJ) and CMJ tests were used to assess dynamic and reactive strength of the lower limbs, respectively [163]. Jump height was calculated from the flight time obtained by a contact mat connected to a digital timer (accuracy, ±0.001 s) (Chronojump Bosco system, Barcelona, Spain). Verbal encouragement and immediate feedback on performance were provided. Attempts performed without proper technique were discarded. The best of three attempts (30 s between trials) at each assessment time was used for further analysis (Figure 26).

7.3.6. Muscle soreness

At each time point (Figure 26), participants were asked to rate the level of soreness in their lower limbs using a scale of 0–10 arbitrary units (a.u.), where 0 represented 'no pain' and 10 represented 'intolerably intense pain' [49].

7.3.7. Blood sampling and processing

We obtained 5 mL of blood from volunteers at each follow-up assessment after exercise Bouts A and B (Figure 26). Blood samples were allowed to clot for 30 minutes in a tube (SST II Advance, Becton Dickinson Vacutainer Systems, UK) before being centrifuged at 2000 ×*g* for 10 min at 4°C. Serum was aliquoted and stored at −80°C until needed for analysis of the following: CK, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) as markers of muscle damage [209–211]; Sarcomeric mitochondrial CK (sMtCK), as a potential marker of muscle cell organelle disruption [212]; CK-MB isoform (CK-MB) and titin, as markers of muscle sarcomere structure damage [213,214]; and cTnI concentration as a marker of heart damage [215]. Biochemical analysis of CK, AST, and ALT was performed in an Advia 2400 automatic device (Siemens Healthcare Diagnostics, Tarrytown, New York, USA), following the International Federation of Clinical Chemistry's Committee primary reference procedures [216]. A Dimension Clinical Chemistry System (Siemens Healthcare Diagnostics, Tarrytown, NY, USA) was used to determine CK-MB (range 0.5–300 $ng \cdot mL^{-1}$) and cTnI (0.017–40 $ng \cdot mL^{-1}$) [212]. Commercial enzyme-linked immunosorbent assay (sandwich) kits were used to determine the concentrations of sMtCK (REF: SEC386Hu; Cloud Clone Corp., Houston, TX, USA) and titin (REF: E-EL-H0439; Elabscience Biotechnology Co., Houston, TX, USA), following the manufacturers' protocols.

7.3.8. Statistical analysis

Statistical analysis was performed using IBM SPSS version 23 (IBM Corp., Armonk, NY, USA). The normality of the data distribution was checked using the Shapiro–Wilk test and homogeneity of variances was performed using Levene's test at each time level. For normal data, one-way ANOVA was used for repeated measures (with Mauchly's sphericity test) to determine the effects of time (Pre, Post1h, Post24h, Post48h, Post72h, and Post144h) on dependent variables. When significant effects were found, post-hoc testing was performed by applying paired t-tests with Bonferroni correction for multiple comparisons. Friedman's test was applied on data that did not show a normal distribution (i.e., CK, CK-MB, AST, and cTnI). When significant effects were found, post-hoc testing was performed by applying Wilcoxon signed rank tests. To assess the differences between training Bouts A and B, variables at each time point were transformed to percentages of increase or decrease compared to baseline conditions (i.e., Pre-A and Pre-B). Then, the percent change at Post1h, Post24h, Post48h, Post72h, and Post144h were compared between A and B, using paired t-tests or Wilcoxon signed rank tests (depending on the data distribution). Paired t-tests were used to compare the exercise kinetics from A to B. The significance level was set at $P < 0.05$ and data are presented as means ± standard deviations, unless otherwise stated.

7.3.9. Numerical quantification of the repeated bout effect.

To quantify and compare the protective effect of exercise in this experiment with previous literature on the RBE, the index of protection was calculated according to the formula and procedures proposed by Hyldahl et al. (2017) [80] (Equation 4).

Equation 4. Index of protection, as defined by Hyldahl et. al. (2017) [80].

$$Index\ of\ protection = \frac{(Bout\ A\ - Bout\ B)}{Bout\ A} \cdot 100$$

For muscle function parameters (i.e., MVIC and jump performance), the index was based on the individual magnitude of the decrease from baseline at 1 day post-exercise (i.e., Bout A or Bout B in the formula corresponded to the respective percent decrease at Post24h) [80]. For perceived muscle soreness and serum biomarkers, the index was based on the individual peak during a given participant’s follow-up (i.e., Bout A or Bout B in the formula corresponded to the maximal change from baseline in absolute units) [80]. The index of protection was only calculated for those variables that showed significant changes during the follow-up.

7.4. RESULTS

7.4.1. Exercise kinetics

Bouts A and B were performed as maximal voluntary efforts at each assessment, but due to the 4-week training programme, the mean force, velocity, and power outputs increased significantly from A to B in the CON and ECC phases (Table 6). Although no specific indications were given regarding the braking technique, there was a statistically significant ECC-overload (ECC outputs surpassed those of the concentric phase).

*Table 6. Metrics for single bouts of repetitions before and after a 4-week training programme. Means ± standard deviations are shown for force, velocity and power over the 10 sets × 10 repetitions of iso-inertial half-squats performed before (A) and after (B) the 4-week training programme. * P < 0.05 between concentric and eccentric values (i.e., Eccentric overload). ** P < 0.001 on performance between bouts.*

		Single Bout A		Single Bout B		% increase from A to B
Force	**Concentric**	**1722.0**	± 276.4	**2391.3**	± 323.8	+ 38.9**
	Eccentric	**1771.7**	± 285.8	**2442.5**	± 318.6	+ 37.9**
	% Eccentric Overload	+ 2.9*		+ 2.1*		
Velocity	**Concentric**	**0.25**	± 0.03	**0.30**	± 0.03	+ 21.0**
	Eccentric	**0.27**	± 0.04	**0.32**	± 0.03	+ 17.8**
	% Eccentric Overload	+ 7.1*		+ 4.3*		
Power	**Concentric**	**402.9**	± 104.8	**665.8**	± 142.6	+ 65.3**
	Eccentric	**422.8**	± 110.7	**684.5**	± 143.2	+ 61.9**
	% Eccentric Overload	+ 4.9*		+ 2.8		

7.4.2. Maximal voluntary isometric contractions

The 4-week training programme produced a significant increase in the MVICs of the knee extensors (+27.81%; P < 0.001) from Bout A to Bout B, but there were no significant changes in the MVICs of the knee flexors (+2.30%; P = 0.368) (Table 7). In terms of relative acute decrease after exercise (i.e., due to muscle damage), Bout A produced a significant decrease in the MVICs of knee extensors (peak, -32.90% at Post1h; P = 0.000) that did not recover until Post72h. After Bout B, the decrease at Post1h (-12.70%; P = 0.022) was not statistically significantly different than that after Bout A (P = 0.07); however, the recovery was much faster, being achieve by Post24h. There was also a statistically significant overcompensation in MVIC values at Post144h (+9.21%; P = 0.013) compared with those at the Pre-assessment (Figure 27). No significant changes were seen in the MVICs of the knee flexors during follow-up. The results for the index of protection for the MVIC are shown in Figure 28.

7.4.3. Jump testing

After the training programme, no significant increases were seen in either SJ (+4.80%; P = 0.058) or CMJ (-0.17%; P = 0.767) (Table 7). The patterns of change were comparable for these measures after exercise in Bout A: from baseline, there was a fast impairment immediately after exercise that peaked at Post24h for SJ (-19.6%, P = 0.001) and CMJ (-15.3%, P = 0.001) before progressive posterior improvement without complete recovery by Post144h (Figure 27). The magnitude of impairment was greater for the SJ test. After Bout B, the decreases were attenuated compared with Bout A and also showed an earlier peak at Post1h for SJ (-8.0%, P = 0.038) and CMJ (-3.94%; P = 0.053). Statistically significant changes were only seen for SJ during in the first 2 days after exercise (Post1h and Post48h) (Figure 27). The results for the index of protection for SJ and CMJ are shown in Figure 28.

7.4.4. Muscle soreness

Exercise Bout A produced a fast increase in perceived muscle soreness that peaked at Post48h (7.14 ± 1.82 a.u.) and remained elevated until Post144h. By contrast, the peak soreness presented earlier after Bout B (i.e., Post24h) and was attenuated (3.89 ± 1.29 a.u.) (Table 7). The results of the index of protection for muscle soreness are displayed in Figure 28.

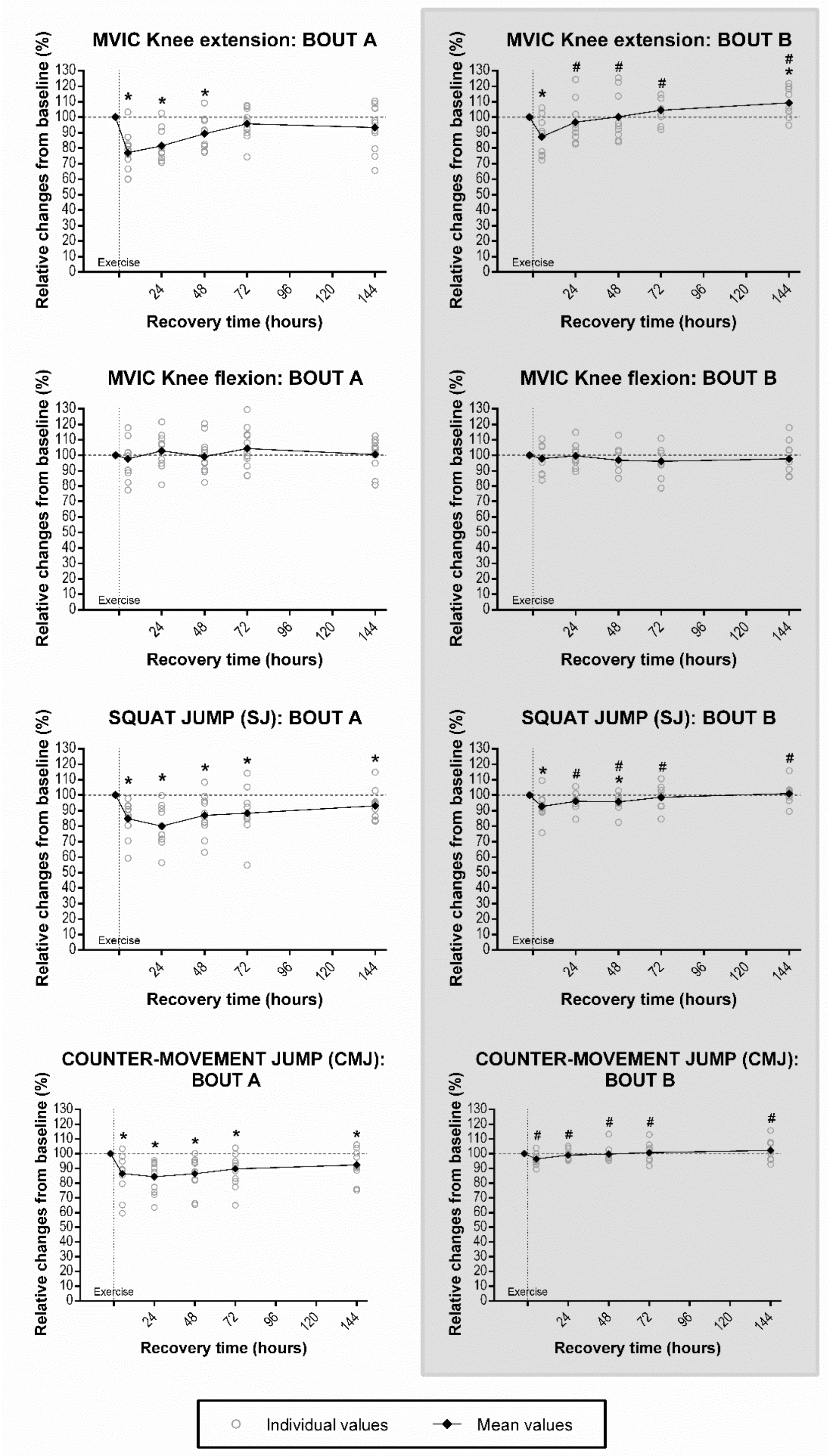


*Figure 27. Percent change in muscle function from baseline to follow-up. The percent changes in muscle function from pre- to post-exercise are shown for Bouts A (white background) and B (grey background). * P < 0.05 from baseline. # P < 0.05 from B compared to A at the same temporal point. Abbreviation: MVIC, maximal voluntary isometric contraction.*

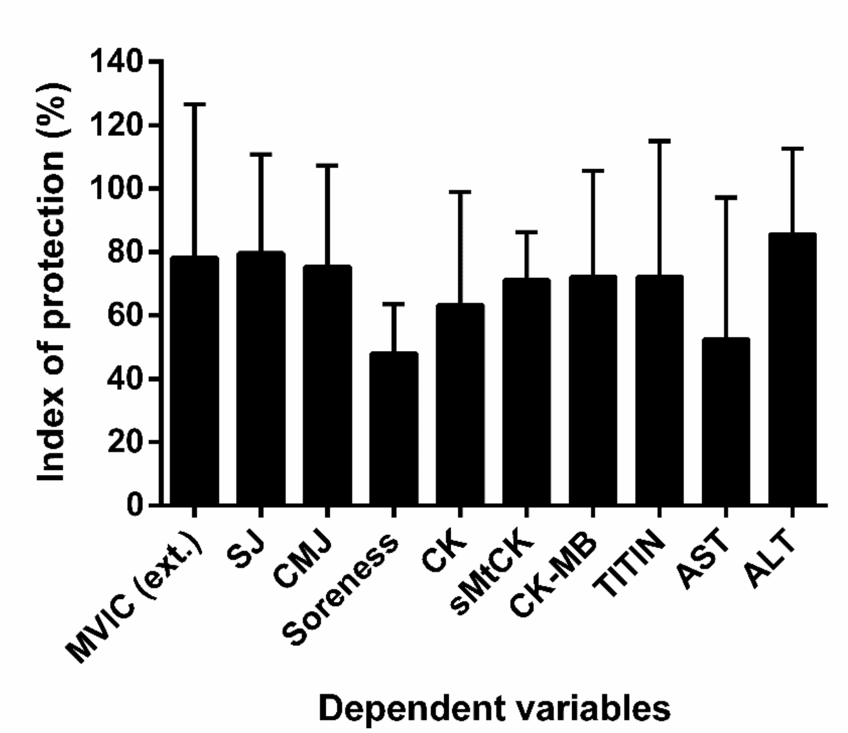


Figure 28. Changes in variables during follow-up. The index of protection [80] for variables that changed significantly during follow-up was calculated as [(Bout A – Bout B) / Bout A] × 100, with muscle function (MVIC, SJ and CMJ) based on the magnitude of the decrease from baseline to 1 day after exercise and muscle soreness/serum biomarkers (CK, sMtCK, CK-MB, titin, AST, and ALT) based on peaks during follow-up. Abbreviations: ALT, alanine aminotransferase; AST, Aspartate aminotransferase; CK, creatine kinase; CK-MB, creatine kinase MB isoform; CMJ, countermovement jump; cTnI, cardiac troponin I; MVIC, (ext.) maximal voluntary isometric contraction of the knee extensors; sMtCK, sarcomeric mitochondrial creatine kinase; SJ, squat jump.

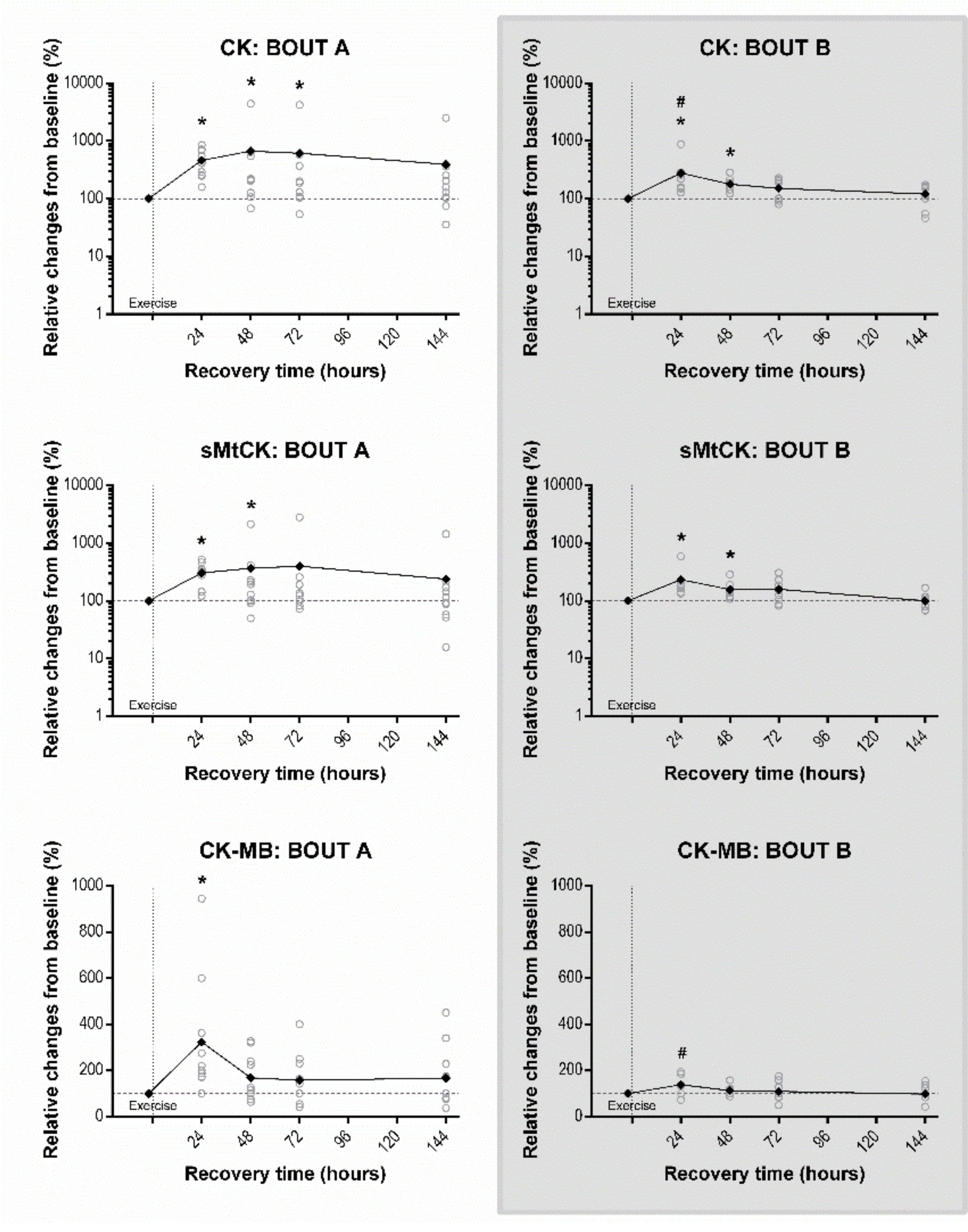
7.4.5. Serum biomarkers

The serum concentrations of the biomarkers analysed during follow-up are displayed in Table 7. No significant differences were found between the baseline concentrations of markers analysed before Bouts A and B (i.e., the training programme had no effect on baseline levels).

After Bout A, all markers of muscle damage (i.e., CK, CK-MB, AST, ALT, sMtCK, and titin) showed significant increases during the follow-up (Figure 29). The cytosolic markers CK, AST, and ALT showed consistent increases during follow-up, with peak increases from baseline of 666.34% ± 1331.69% at Post48h, 197.25% ± 136.59% at Post48h, and 161.03% ± 64.43% at Post144h, respectively. The mitochondrial marker sMtCK showed a very similar timeline to that of CK, but increased less during follow-up, peaking at Post72h (400.61% ± 845.47%). Finally, the sarcomeric markers showed different dynamics during the follow-up: CK-MB showed an early peak increase at Post24h (323.46% ± 259.79%), with almost complete clearance to baseline thereafter; titin showed consistently and significantly elevated levels from Post24h to Post72h, with a peak increase of 126.32% ± 31.84% at Post48h.

After Bout B, only CK and sMtCK showed significant changes during the follow-up (Figure 29). Additionally, peak increases in these markers were lower and appeared earlier than those seen after Bout A. CK peaked by 279.61% ± 250.18% at Post24h whereas sMtCK peaked by 233.08% ± 148.61% at Post24h). Baseline levels recovered for all markers at Post72h.

The indexes of protection for serum CK, CK-MB, AST, ALT, sMtCK, and titin are shown in Figure 3. No significant changes were seen in cTnI during follow-up after both bouts (Table 7).



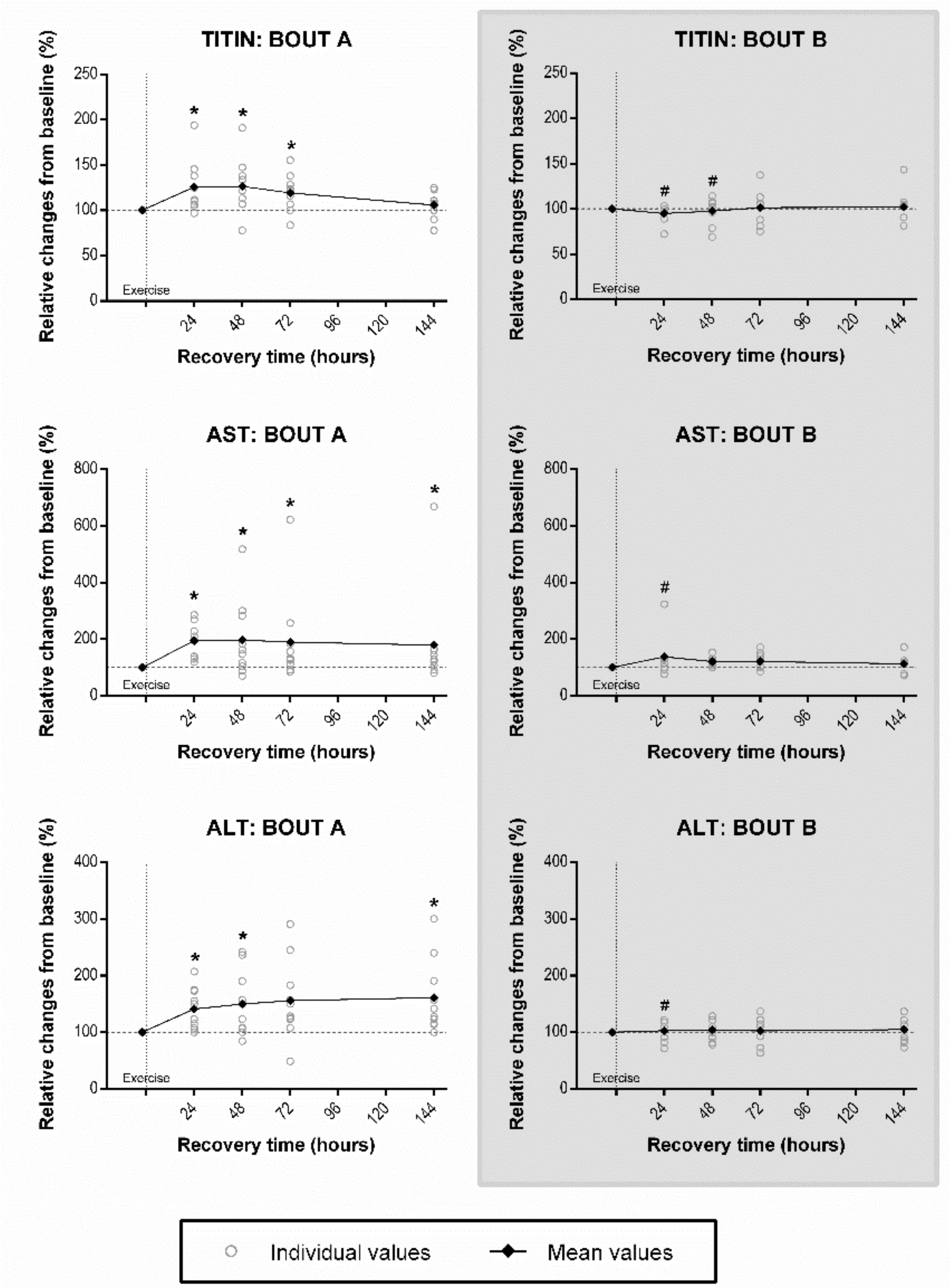


*Figure 29. (Divided in two consecutive pages). Percent change in serum biochemical markers from baseline to follow-up. The percent changes in serum biochemical markers of muscle damage from pre- to post-exercise are shown for bouts A (white background) and B (grey background). * P < 0.05 from baseline. # P < 0.05 from B compared to A at the same temporal point. Abbreviations: ALT, alanine aminotransferase; AST, Aspartate aminotransferase; CK, creatine kinase; CK-MB, creatine kinase MB isoform; sMtCK, sarcomeric mitochondrial creatine kinase.*

*Table 7. Mean outcome data before and after maximum-effort iso-inertial half-squat training sessions before and after a 4-week training programme. Means ± Standard deviations are shown. Measures were taken pre-exercise (Pre), and after 1 (Post1h), 24 (Post24h), 48 (Post48h), 72 (Post72h), and 144 (Post144h) hours of performing maximum-effort 10 × 10 iso-inertial half-squat training. Single Bout A was performed 1 week before and single Bout B was performed 1 week after completing a 4-week iso-inertial training programme. Abbreviations: AST, Aspartate aminotransferase; ALT, alanine aminotransferase; CK, creatine kinase; CK-MB, creatine kinase MB isoform; CMJ, countermovement jump; cTnI, cardiac troponin I; MVIC, maximal voluntary isometric contraction; SJ, squat jump; sMtCK, sarcomeric mitochondrial creatine kinase. * P < 0.05 relative to pre-exercise (Pre) values.*

		SINGLE BOUT A						SINGLE BOUT B					
		Pre	**Post1h**	**Post24h**	**Post48h**	**Post72h**	**Post144h**	**Pre**	**Post1h**	**Post24h**	**Post48h**	**Post72h**	**Post144h**
knee extension MVIC (N)	**mean**	**580,3**	**446,4***	**471,6***	**518,6***	**559,0**	**542,3**	**741,4**	**638,7***	**701,6**	**736,0**	**770,0**	**803,9***
	SD	±105,3	±98,1	±98,2	±111,7	±133,9	±140,5	±202,4	±167,9	±152,9	±217,0	±211,4	±209,1
knee flexion MVIC (N)	**mean**	**282,4**	**273,5**	**292,6**	**276,5**	**289,1**	**280,9**	**288,9**	**282,6**	**288,6**	**280,2**	**278,6**	**282,5**
	SD	±65,8	±57,1	±77,2	±54,8	±46,6	±58,8	±40,0	±48,4	±50,4	±50,8	±50,0	±51,0
SJ height (cm)	**mean**	**27,00**	**23,48***	**21,71***	**23,07***	**23,28***	**24,78***	**28,30**	**26,03***	**27,01**	**26,90**	**27,75**	**28,41**
	SD	±4,4	±4,0	±3,43	±4,97	±4,78	±3,13	±3,92	±2,70	±2,47	±2,33	±2,96	±2,67
CMJ height (cm)	**mean**	**33,63**	**29,39***	**28,46***	**28,61***	**29,41***	**30,34***	**33,57**	**32,25**	**33,22**	**33,39**	**33,66**	**34,12**
	SD	±5,33	±4,42	±4,46	±5,02	±4,22	±4,46	±4,57	±3,89	±4,25	±4,27	±3,91	±3,64
Muscle soreness (a.u)	**mean**	**0,0**	**3,8***	**6,5***	**7,1***	**5,0***	**0,5***	**0,0**	**2,0***	**3,9***	**2,5***	**1,1***	**0,0**
	SD	±0,0	±1,8	±1,8	±1,8	±1,5	±0,4	±0,0	±1,2	±1,3	±1,2	±0,7	±0,0
CK (U/L)	**mean**	**203,70**	**-**	**909,40***	**1030,80***	**947,70***	**610,00**	**138,13**	**-**	**319,38***	**233,50***	**208,94**	**157,38**
	SD	±91,59		±574,70	±1642,90	±1579,67	±926,90	±87,02		±224,61	±151,98	±180,62	±133,40
sMtCK (ng/ml)	**mean**	**17,81**	**-**	**53,18***	**38,44***	**33,61**	**23,36**	**9,58**	**-**	**20,44***	**14,80***	**12,75**	**9,17**
	SD	±15,55		±48,53	±34,28	±30,76	±21,06	±6,22		±13,44	±9,84	±5,30	±5,64
CK-MB (ng/ml)	**mean**	**1,04**	**-**	**3,09***	**1,71**	**1,48**	**1,80**	**1,06**	**-**	**1,64**	**1,28**	**1,16**	**0,98**
	SD	±0,66		±2,90	±1,43	±1,25	±2,57	±0,55		±1,28	±0,89	±0,77	±0,62
Titin (ng/ml)	**mean**	**0,351**	**-**	**0,445***	**0,452***	**0,430***	**0,380**	**0,402**	**-**	**0,383**	**0,404**	**0,419**	**0,414**
	SD	±0,223		±0,330	±0,341	±0,292	±0,261	±0,231		±0,241	±0,261	±0,276	±0,253
AST (U/L)	**mean**	**24,00**	**-**	**46,20***	**48,40***	**47,20***	**45,10***	**20,00**	**-**	**27,00**	**24,25**	**24,25**	**22,00**
	SD	±3,37		±13,93	±37,86	±45,84	±50,33	±7,13		±15,51	±9,19	±10,70	±8,30
ALT (U/L)	**mean**	**17,00**	**-**	**22,70***	**24,20***	**23,60**	**26,40***	**14,25**	**-**	**15,00**	**15,25**	**15,25**	**15,38**
	SD	±9,56		±9,43	±11,43	±10,51	±14,65	±4,50		±6,72	±7,01	±7,80	±7,21
cTnI (ng/ml)	**mean**	**0,020**	**-**	**0,022**	**0,026**	**0,021**	**0,020**	**0,017**	**-**	**0,018**	**0,019**	**0,019**	**0,018**
	SD	±0,007		±0,012	±0,022	±0,011	±0,006	±0,001		±0,004	±0,003	±0,004	±0,002

7.5. DISCUSSION

This study highlights the protective effect of exercise, even when the damaging stimulus is matched to the increased force-generating capacity. The present experimental design tested the effect of two maximal bouts of exercise before (A) and after (B) a 4-week training period. This design simulated a “real-life” training scenario in which loads are constantly adjusted to create a progressive overload. Therefore, training at Bouts A and B are volume-matched, comparable, and under maximal loads, with the effect of training over the 4-week programme resulting in greater external loads performed on B (+38.9%, +21.0%, and +65.3% production of CON force, velocity, and power; See Table 6).

7.5.1. Muscle function

The 4-week training programme not only increased the force-generating capacity of the knee extensors but also conferred a strong protective effect against the damaging stimulus. In terms of functional capacity, the loss of force after the Bout A corresponded to symptoms of moderate muscle damage (i.e., the MVIC of the knee extensors decreased by 20%–50%, and recovery was between 2 and 7 days) [42]. In a practical context, this extended requirement for recovery could interfere with the weekly training schedule, causing days off, lower performance in subsequent training sessions, a state of non-functional overreach, or a combination of these. Conversely, after Bout B, the loss of force corresponded to mild muscle damage (MVIC of the knee extensors decreased by <20% and recovered within 2 days) [42]. In fact, after 1 day, the knee extensor MVIC had recovered back to baseline values with statistical significance, suggesting that participants were ready to undergo another training session by that time. It was interesting to note that almost all participants showed an overcompensation of the MVIC of the knee extensors at 6 days (Post144h), indicating correct adaptation was leading to complete recovery (i.e., functional overreaching).

The index of protection for the MVIC of the knee extensors was higher than reported in previous studies of RBE, and typically have lower indexes than for CK [80]. This may be caused by the differences in the protocols typically used in studies of RBE (i.e., training vs complete rest between bouts), which may trigger different adaptive mechanisms for the RBE [80].

However, the MVIC of the knee flexors showed no changes during the follow-up after either bout (i.e., acute response after Bout A and Bout B) or as a result of the training programme (i.e., chronic response from Bout A to Bout B). These results are likely explained by a much lower activation of the knee flexors compared to the knee extensors when performing the squat [174,204].

An RBE was also evident on the jumping capacities after the training programme. After Bout A, the capacities of both SJ and CMJ significantly diminished at all temporal follow-up points and did not recover by day 6 (Post144h). The impairment was greater on the SJ test, suggesting that the elastic capacity of the muscle–tendon complex was less affected than the contractile capacity during follow-up [217]. After Bout B, an almost complete index of protection was seen on the CMJ and SJ capacity (Figure 28), and only the SJ was significantly affected in the first hours after exercise (Figure 27). Similar to the MVIC of the knee extensors, peak impairment of SJ capacity was seen shortly after the exercise (Post1h), and the decrease was less steep than that seen after Bout A.

These data confirm the results of previous research pointing to a superior protection on muscle contractile function in the days following exercise (i.e., between 1 and 6 days) than on the acute response after exercise (i.e., 1 hour after exercise), which may be linked to processes other than muscle damage (i.e., metabolic fatigue) [80,104].

7.5.2. Perceived muscle soreness

Although not being correlated with the timeline of structural damage to muscle, or to other commonly used proxy markers of muscle damage [51], perceived muscle soreness is a highly relevant outcome. Indeed, it may limit performance or produce changes in movement patterns (i.e., compensatory mechanisms) during training or sportive competition, and in doing so, increase the risk of injury [218]. The present study showed both an attenuation and a speed-up of soreness appearing and disappearing. The index of protection was similar to those reported in previous studies of ECC exercise [80].

7.5.3. Serum biomarkers

The serum markers analysed in this study were selected for their ability to reflect different cellular locations, helping to identify which myofiber compartment was affected by exercise.

CK is mainly located in the sarcoplasm and is released into the bloodstream by membrane disruption or increased permeability [219]. After Bout A, participants showed a great increase in CK values that peaked at 2 days (Post48h) and remained significantly elevated at 3 days (Post72h). After Bout B, the leakage of CK was attenuated and there was only a smaller peak at 1 day (Post24h) with a complete clearance by 3 days (Post72h). The index of protection on CK was comparable to that reported in previous studies of RBE after ECC training. AST and ALT are common markers of muscle damage and liver disfunction due to their location in the cytoplasm of liver, muscle, and other tissues including red blood cells [210,211]. Although not tissue specific, it is accepted that transient increases in these markers originate from muscle cells after strenuous exercise [210]. In the present study, both AST and ALT increased after Bout A, but not after Bout B. These results for CK, AST, and ALT suggest that although much higher forces were applied at Bout B, the muscle was less vulnerable to cellular, chemical, and/or physical stress on the membranes [219].

Located in the mitochondria of skeletal and heart muscle, sMtCK is closely coupled to the electron transport chain [209,219]. We found that sMtCK had dynamics comparable to those of CK after both study bouts, but that the percent increase was lower. To the best of our knowledge, one previous study has include serum sMtCK as a marker of mitochondrial disruption after intense exercise [212]. A possible explanation for the serum increase is that the elevated energy demands of exercise may cause disturbances in Ca^{2+} homeostasis, initiating a mitochondrial Ca^{2+}-tapering process that induces some degree of mitochondrial damage and leakage of its components into the sarcoplasm [209,212,220]. Previous research in rats supports this hypothesis and the observed RBE. For example, these have shown mitochondrial damage induced by prolonged exercise, with training having a protective effect [221] that appears to result from reducing mitochondrial Ca^{2+} uptake [222] and increasing the antioxidant capacity [223].

We could find no other study using titin as a serum marker of EIMD. Titin is a sarcomeric structural giant protein that was only recently used as a novel urinary marker of EIMD [213]. Due to its location in the sarcomere (i.e., crossing half sarcomere from the Z-band to the M-line) [17], it is likely that titin leakage into the blood is related not only to membrane disturbances but also to deeper ultrastructural sarcomere disruptions. Compared with the other serum markers, the increase in this marker after Bout A was lower, presumably due to the greater stress requirements to induce its release. Nonetheless, it is noteworthy that significant serum increases in titin were seen from 1 to 3 days after Bout A (Post24h–Post72h). Serum increases in CK-MB have also been linked to pervasive damage of the myofibril structure [212] because of the location of the M-subunit in the M-band of the sarcomere [224]. After Bout A, the serum concentration of CK-MB increased steeply by 1 day (Posrt24hr) and made a fast recovery thereafter. This early pattern compared with that of titin may be explained by differences in the molecular mass of each marker (i.e., 86–90 and 500–4000 kDa, respectively) [17,219]. During follow-up after Bout B, we found no significant changes in either of these structural markers of muscle damage. Given these results and the high indexes of protection for each marker (Figure 28), the exercise programme was highly effective at protecting muscle against structural damage to sarcomeres.

Finally, the absence of changes in cTnI during follow-up confirms that the reported changes in serum markers were caused by disturbances in skeletal, and not cardiac, muscle [211,215].

In conclusion, unaccustomed iso-inertial exercise is expected to produce moderate EIMD and a loss of muscle strength (20%–50% decrease) that is prolonged (1–7 days for recovery). These functional impairments are accompanied by the leakage of several biochemical markers in the serum that suggest the following are affected, in order of decreasing severity: (i) the myocyte membrane, (ii) the sarcomeric mitochondria, and (iii) the muscle ultrastructure. Our structured iso-inertial resistance training programme generates adaptations that confer a better response to stress in skeletal muscle, allowing it to tolerate much higher workloads with lower damage. Additionally, this highly attenuated damage may be restricted to membrane and mitochondrial disruptions, with no significant changes shown for the markers of ultrastructural damage.

7.6. PERSPECTIVE

This is the first study to have provided detailed quantification and characterisation of the acute responses to training loads, as well as the protection on several markers of EIMD conferred by a structured, progressive, 4-week, iso-inertial training programme. Most prior studies of EIMD focused on ECC activity only (i.e., iso-weight or iso-kinetic resistance), without considering iso-inertial exercise [80]. This is important because these produce different acute and chronic responses [81]. Studies of iso-inertial training have produced three main conclusions. First, that training is typically accompanied by some EIMD synthons [49,104,109]. Second, that some markers of EIMD are attenuated when the exact same bout of exercise is repeated after 4 weeks, with no intervention between bouts. Third, that the CK response was highly attenuated by a 6-week training programme in which loads were increased over time [104]. In the present study, we provide an overview of the different markers of EIMD and quantify the RBE following a 4-week iso-inertial squat training programme. Compared with results before the training programme, and with participants delivering maximal relative intensities of effort at both assessment bouts, participants are better prepared to tolerate exercise and can assume a higher weekly volume/frequency despite marked increases in external loads after the training programme.

GENERAL DISCUSSION

8. GENERAL DISCUSSION

My main aim in the present thesis was to identify and characterise the acute and chronic responses of the lower limbs to iso-inertial training sessions. Throughout this work, I have studied and discussed several aspects of iso-inertial training of the lower limbs. In this section, a final global discussion structured by topics in different sub-sections is provided.

First, the validity and suitability of specific low-cost monitoring systems will be discussed. Then, a brief reflexion regarding the selection of different iso-inertial loads is provided. Another sub-section will cover the muscle activation seen in the iso-inertial half-squat exercise. Finally, the acute and chronic responses to the different experimental protocols performed will be discussed in two consecutive sub-sections.

8.1. VALIDITY OF A SPECIFIC LOW-COST ENCODER

The starting point of my work was the accurate quantification of iso-inertial squat exercises. Because of the inherent differences between iso-inertial loads and gravitational loads, I needed a more sophisticated method for the quantification of training loads. Previously, some studies had used either a combination of a strain gauge and a linear encoder [49] or a combination of video motion analysis and force plates [107] in order to measure force, velocity and power. However, those methods usually require post-hoc treatment of the data and therefore they are not suitable for offering real-time feedback on performance. Research has shown that exercise feedback has a direct impact on the quality of the training sessions, enhancing psychological traits during resistance training and subsequent physical adaptations [107]. To overcome these limitations, I opted for the use of a rotational friction encoder, which registers kinematic variables of the exercise and also provides real-time feedback from the repetitions of the exercise. This method also had an additional interesting feature, as it could easily be installed in other iso-inertial training systems, and therefore we have the possibility of experimenting with other exercises in the future. Despite the advantages of this system over other available solutions, it was necessary to test the validity of the data it provided. In *Study 1*, the validity of the rotational friction encoder was tested against a criterion measure for assessing velocity, force and power in flywheel exercise devices. The friction encoder demonstrated a small to moderate TEE and nearly perfect relationships across all kinetic and kinematic variables, at both high and low iso-inertial loads (overall: small TEE and nearly perfect correlations in velocity, force and power). Therefore, acceptable levels of agreement were observed between the criterion measures and those taken using the rotational encoder for the variables assessed. Therefore, given the aforementioned advantages over other measurement systems, I opted for this approach. However, it must be acknowledged that due to small to moderate bias (i.e., velocity and power may be consistently lower and force consistently higher than actual values), the same measurement system has to be used when performing assessments over time or when aiming to perform inter-subject comparisons. Additionally, due to lower TEE and bias, it is recommended to use power (or alternatively force) values to track exercise performance and to provide feedback to trainees.

Another important consideration found during *Study 1* was that the iso-inertial training system used throughout this thesis ("YoYo squat" - YoYo Technology, Stockholm, Sweden) has a slightly variable diameter of the axis across the ROM, at the point where the strap is fixed (the strap winds up around the axis, increasing the moment arm) and therefore, as recommended by the rotational encoder manufacturer, the mean diameter during a repetition was used for the calculations of the kinematic variables in the computer software (Figure 30). I strongly believe that this explains a great deal of the TEE and bias reported in the validation. For instance, the greatest values of force were produced in the transition between the ECC and the CON action (SSC): a point of the ROM where the strap is totally wound-up and therefore the moment arm is underestimated, causing an overestimation of the force needed to accelerate the disc. Consequently, I hypothesise that more valid results could be attained if the measurements were taken in other flywheels in which the axis diameter remains constant (i.e., with a cable instead of a strap). Conversely, higher TEE and bias are to be expected in systems with more variable diameters (i.e., conical pulleys). However, these hypotheses remain to be tested.

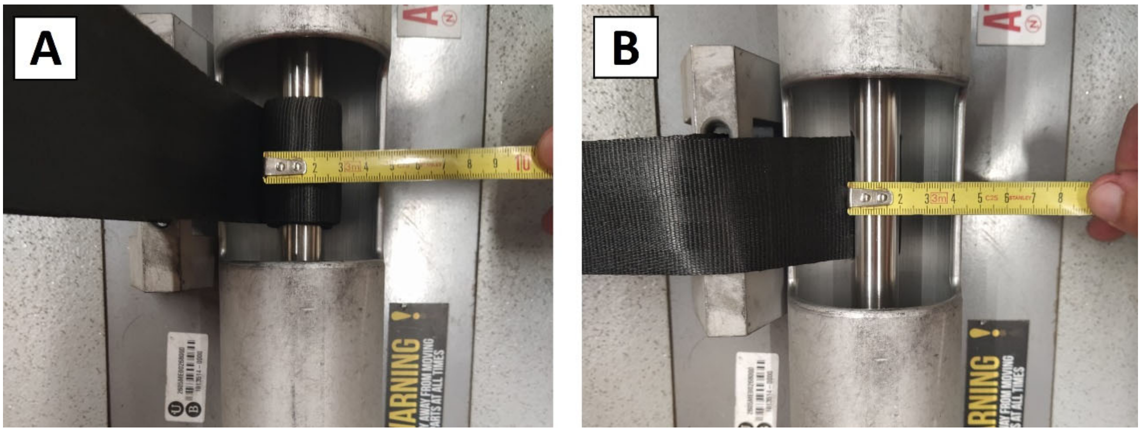


Figure 30. Variation of the axis diameter in the iso-inertial training system used throughout this thesis ("YoYo squat" - YoYo Technology, Stockholm, Sweden). At the end of the eccentric phase, the strap is wound up around the axis, increasing the diameter (and thus, the moment arm) to ≈ 3 cm (A). At the end of the concentric phase, the strap is totally unwound, decreasing the diameter (and thus, the moment arm) to ≈2 cm. As the axis diameter was slightly variable during the range of movement of each repetition (ranging from 2 to 3 cm), the mean diameter was used in the software associated with the friction encoder to perform the calculations.

8.2. KINEMATIC VARIABLES OF EXERCISE WITH DIFFERENT ISO-INERTIAL LOADS

The different iso-inertial loads used in the studies influenced the kinematic variables of the exercise. As seen in Table 3, with a higher moment of inertia the movement velocity was less, and therefore the mean forces applied during the repetition were higher. Generally, mean repetition power was higher with the lower (0.025 kg·m^2) than with higher (0.075 kg·m^2) moment of inertia (Table 3). This was the case for 7 out of the 10 volunteers who participated in *Study 1*, the other 3 developed greater power with higher inertia (0.075 kg·m^2). Given these inter-individual differences, when the aim of the intervention is to develop maximum power, it is advised to perform a progressive-loads test to check which moment of inertia is optimal for each trainee.

Recent research has also shown that individual acute responses differ according to the different external iso-inertial loads that provoke them (0.075 vs 0.1 kg·m^2) [225]. Therefore, in the following sub-sections of the discussion, the reader has to take into account that: the acute and chronic responses reported may have been different if other moments of inertia had been used, and inter-individual differences in the response may be partly attributed to different correspondences of the external load (i.e., moment of inertia) used in the experiments with the individual F-V spectrum of the volunteers.

8.3. MEASUREMENT OF LOWER-LIMB MUSCLE ACTIVATION IN THE ISO-INERTIAL HALF SQUAT EXERCISE

Previous EMG research has shown a higher contribution of quadriceps, together with a moderate contribution of hamstrings, during squats [174]. The GM and VL muscles were the most active in the iso-inertial half-squat exercise (Figure 23). While quadriceps muscles increased in T2 intensity, hamstring muscles tended to decrease in intensity (Figure 31). It must be noted that T2 values are a quantitative index of the amount and distribution of water in skeletal muscle [194]. Before the pilot study, I expected to see slight increases in hamstring T2 intensity, due to their moderate contribution during the squat when measured by EMG [174], and previous single-muscle-group design studies showing T2 values correlated strongly with EMG activity [195]. However, these two assessment tools have different physiological bases. While T2 values are a quantitative index of the amount and distribution of water in skeletal muscle, EMG measures electrical muscle activity, and therefore may lack complete agreement in some situations. As supported by my results and a previous study on T2 activation, when muscles of a whole region (i.e., thighs) are assessed after being non-uniformly recruited, the concentration of water is increased in the most active muscles but it is decreased in less active muscles [204]. Thus, acute decreases in the T2 signal in hamstring muscles after squat exercises indicate relative inferior activation compared to quadriceps muscles, but not a lack of activation [205]. Among the individual quadriceps muscles, high inter-individual differences were seen in the activation of RF: the bi-articular muscle of the quadriceps group. The RF activation pattern is highly influenced by the hip position [203]. Therefore, biomechanical differences (i.e., femur length) between subjects may have caused different hip flexion angles during execution and thus different RF activation patterns.

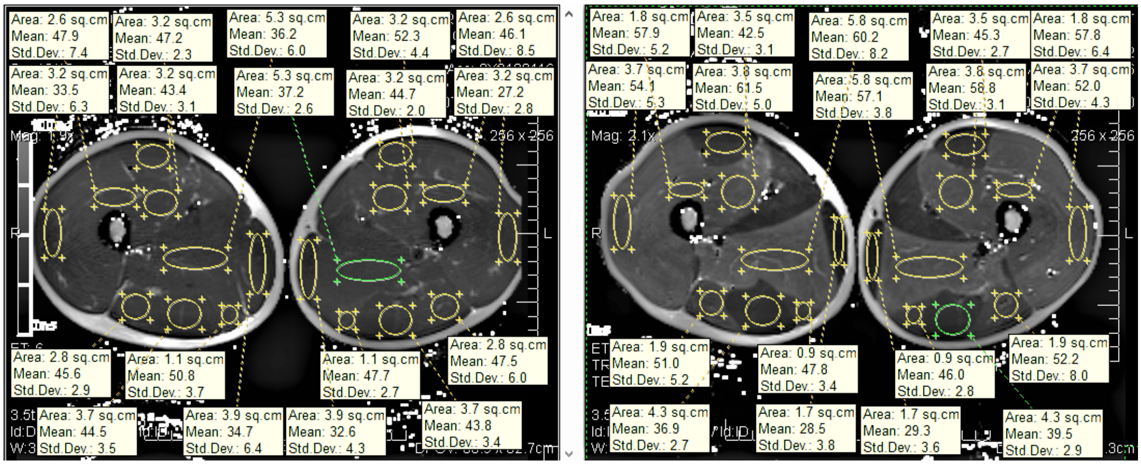


Figure 31. Representative regional changes in transverse relaxation time (T2) (at ≈ 50% the length of the femur) as assessed by consecutive MRI scans before and 3-5 min after finishing an acute bout of the exercise (10 sets of 10 flywheel squats) from one of the volunteers. Lighter areas represent a higher T2 mean value, whereas darker areas represent lower T2 mean value.

8.4. ACUTE RESPONSES TO ISO-INERTIAL EXERCISE

The acute responses to intense resistance training have previously been shown to vary greatly between individuals [42]. Unaccustomed CON-ECC iso-inertial exercise caused impairments in functional capacity, increased concentrations of biochemical markers of muscle damage in blood and increased perception of muscle soreness. The protocols used in this thesis for the evaluations of acute responses entailed 10 sets of 10 exercise repetitions with a moment of inertia of 0.030 kg·m^2 (*Study 2*) and 0.090 kg·m^2 (*Study 4*).

8.4.1. Muscle function

Muscle function impairments were seen both in *Study 2* and *Study 4* (acute effects of iso-inertial exercise bouts). It has been shown, as explained throughout this thesis and suggested by previous research, that the iso-inertial half-squat exercise predominantly implicates the activation of knee extensor muscles, over knee flexor muscles [174]. This probably explains the persistent decrease of knee extensor MVIC in the hours after performing the exercise and the lack of changes in knee flexor MVIC. Overall, the mean duration and magnitude of the impairments in MVIC of the knee extensor were greater with higher inertia (0.090 kg·m^2) than with lower (0.030 kg·m^2). As repetition velocities were lower under the former condition, the time under tension (and total duration) was higher, and so the forces applied could be higher. Therefore, the higher mechanic and metabolic stress placed on active muscles would explain the differences. Although mean responses in MVIC suggest between mild and moderate EIMD after iso-inertial exercise, the individual responses varied greatly. For instance, in *Study 4*, 6 days (144 h) after performing the exercise, 3 volunteers showed a decrease of MVIC of around 20% (Figure 27), which corresponds to severe muscle damage.

Other manifestations of applied lower-limb force, such as jumping or sprinting, were also affected after unaccustomed iso-inertial exercise. In *Study 2*, large decreases were seen in CMJ performance, lasting up to 3 days (72 h) after exercise. In *Study 4*, I tracked changes in CMJ and SJ tests, with results in both tests being significantly diminished at all temporal follow-up points and not having recovered by day 6 post exercise (Figure 27). The impairment was greater on the SJ test, suggesting that the elastic capacity of the muscle–tendon complex was less affected than the contractile capacity during follow-up [217]. Regarding the effects on sprint performance (*Study 2*), the iso-inertial exercise also produced transient increases in sprint times, together with changes in the F-V spectrum and decreases in mechanical effectiveness during the follow-up (Figure 18). An interesting finding of *Study 2* is that the dynamics of impairment and recovery during the follow-up were distinct for the different parameters assessed (V_0 as opposed to F_0 and $Pmax$). My results suggest that the impairments in V_0 and maximal velocity capabilities are influenced more by a state of acute fatigue (i.e., due to greater metabolite-induced disturbances [170], or glycogen unavailability [160]), but the situation is recovered from quickly, as metabolic homeostasis is recovered. This effect was seen in volunteers regardless of the severity of muscle damage. In contrast, those variables related to short distances and high demands of force (such as F_0 or $Pmax$) seem to be influenced more by the presence of lower-limb muscle damage and are likely to show inter-individual differences depending on the EIMD caused by the training load (Figure 18). These results are in accordance with previous research showing decreases in V_0 immediately after performing an exhausting exercise together with smaller or a complete absence of changes in F_0 and $Pmax$ [160,170]. Also, a previous study reported decreases in short-distance sprint performance (5 and 10 m) in volunteers with lower-limb EIMD, which were attributed to reduced reflex sensitivity affecting the SSC in the propulsive phase [171]. I hypothesise that alterations in joint angles [172] and in the agonist–antagonist muscle activities [173] in the presence of DOMS and EIMD may be the cause of the changes in F_0, $Pmax$ and $RFmax$. These adjustments in the central motor commands might have a greater impact on the initial steps at relatively slower velocities and higher forces, than on the strides at maximum velocity and lower force application.

While several muscle-function tests (i.e., MVIC of knee extensors, jump tests and sprint tests) were affected after exercise, the magnitude and progression of the impairments varied between tests. Therefore, in order to assess the process of recovery holistically, practitioners should consider the inclusion of multiple testing conditions. Also, in order to check the readiness of the athlete, it is advisable to include testing protocols with high dynamic correspondence to the performance actions (i.e., sprint tests). An interesting line of research for the future is the loss of function immediately after exercise and its relation with the total time until complete recovery (the relation of fatigue and primary damage to secondary damage). Greater exploration of this field may help the adjustment of optimum training per session.

8.4.2. Serum Biomarkers

Different protocols of iso-inertial resistance exercise have been shown to produce consistent post-exercise increases in indirect blood markers of EIMD [49,104,109]. For instance, some studies reported CK increases in blood which remain elevated for up to 72 hours in resistance-training-naive volunteers [49,104,110], and up to 24 hours in experienced volunteers [111]. Additionally, increases in the concentration of fast myosin in blood peaking 144 hours post exercise provided the basis of the hypothesis that muscle structure is also affected [49].

Given this background, in *Study 4*, I selected different serum biomarkers for their differential cellular locations, in order to identify which myofibre compartment was most affected by exercise. It is important to note that all the markers of muscle damage analysed (CK, CK-MB, AST, ALT, sMtCK, and titin) showed significant increases at the first follow-up (Figure 29). Therefore, an unaccustomed iso-inertial half-squat training session has a great impact on several locations of the myofibre, causing leakage of cellular content from the muscle into the bloodstream.

The cytosolic markers CK, AST, and ALT showed consistent increases during follow-up, with peak increases from baseline of 660% ± 1330% at 2 days (48 h), 200% ± 140% at 2 days (48 h), and 160% ± 60% at 6 days (144 h) post exercise, respectively. CK is mainly located in the sarcoplasm and is released into the bloodstream by membrane disruption or increased permeability [219]. AST and ALT are common markers of muscle damage and liver disfunction due to their location in the cytoplasm of liver, muscle, and other tissues including red blood cells [210,211]. Although not tissue specific, it is accepted that transient increases in these markers originate from muscle cells after strenuous exercise [210]. Together, these findings support the hypothesis that unaccustomed iso-inertial exercise causes disruptions or increased permeability of the cellular membranes of working skeletal muscles [219].

Additionally, the mitochondrial marker sMtCK showed a very similar timeline to that of CK, but increased less during follow-up, peaking at 3 days (72 h) post exercise (400% ± 850%). sMtCK is located in the mitochondria of skeletal and heart muscle and is closely coupled to the electron transport chain [209,219]. One previous study described sMtCK as a marker of mitochondrial disruption after intense exercise [212]. Serum increases of sMtCK are probably indicative of mitochondrial swelling, disruption and decline in muscle respiratory capacity as a result of very intense exercise [221]. A possible explanation for these increases is that the elevated energy demands of exercise may cause disturbances in Ca^{2+} homeostasis, initiating a mitochondrial Ca^{2+}-tapering process that induces some degree of mitochondrial damage and leakage of its components into the sarcoplasm [209,212,220]. The fact that this marker showed a lower increase than that reported for CK may suggest that this type of damage to the mitochondria is produced at a lower magnitude than the damage to the sarcolemma.

Finally, skeletal muscle structural markers showed different dynamics during the follow-up. Serum increases in CK-MB are linked to pervasive damage of the myofibril structure [212] because of the location of the M-subunit in the M-band of the sarcomere [224]. This marker showed an early peak increase at 1 day (24 h) post exercise (320% ± 260%), with almost complete clearance to baseline thereafter, as a result of unaccustomed iso-inertial exercise. Titin is a giant structural protein located in the sarcomere [17]. Although it has recently been used as a novel urinary marker of EIMD [213], *Study 4* is the first work to analyse serum changes in this marker as a result of EIMD. Due to its location in the sarcomere (i.e., crossing half the sarcomere from the Z-band to the M-line) [17], it is likely that titin leakage into the blood is related to deep ultrastructural sarcomere disruptions. After performing an unaccustomed iso-inertial training session, titin showed consistently and significantly elevated levels from 1 day (24 h) to 3 days (72 h) post exercise, with a peak increase of 130% ± 30% at 2 days (48 h). Compared to the markers discussed above, the percentage increase in structural markers after exercise was low, presumably due to the greater stress required to induce their release. When comparing the dynamics of release and clearance of CK-MB and titin, the early pattern of CK-MB is noteworthy (peak after 1 day and clearance thereafter) compared to that of titin (persistent mild increases). These differences may be explained by the molecular mass of each marker (86–90 and 500–4000 kDa, respectively) [17,219].

In conclusion, unaccustomed iso-inertial exercise is expected to produce the leakage of several biochemical markers into serum. These suggest that the following are affected, in order of decreasing severity: (i) the myocyte membrane, (ii) the sarcomeric mitochondria, and (iii) the muscle ultrastructure.

8.5. CHRONIC (ADAPTIVE) RESPONSES TO ISO-INERTIAL EXERCISE

Regular resistance training triggers adaptive mechanisms that ultimately lead to several improvements in the different biological systems. Iso-inertial training, presumably due to the different pattern / increased tension placed on muscles as compared to iso-weight training, has been shown to be particularly effective at triggering some of these adaptions [99]. Along this thesis, I studied muscle function (i.e., squat, jump, sprint, MVIC), whole muscle hypertrophy, and the RBE (i.e., protection against damaging exercise in terms of loss of function and biochemical markers of EIMD), as chronic responses to iso-inertial training.

8.5.1. Muscle function

The structured 4-week iso-inertial half-squat training programme implemented in *Study 3* produced significant increases in squat force (CON 32% ± 15%, ECC 31% ± 15%) and power (CON 51% ± 30%, ECC 48% ± 27%) (Figure 21). These numbers represent a mean percentage increase of 1.13% per day in force and 1.77% per day in power, over the four weeks of the intervention. A previous meta-analysis reported a mean percentage increase in maximum strength of 0.41% per day in well-trained individuals, and 0.23% per day in untrained individuals [116]. Average rates of improvement for power were reported to be 0.60% per day in well-trained individuals, 0.44% in moderately trained individuals, and 0.32% in untrained individuals [116]. The substantial differences between the results presented in *Study 3* and previous research may be attributed to two main aspects. Firstly, increases in strength and power follow an inverse-exponential tendency, where greater improvements are achieved in the first weeks of training and a plateau is reached as the athlete progresses [226]. This phenomena is reflected by the fact that over the first 5 sessions of training increases were 19% in force and 32% in power, compared to an increase of 12% in force and 18% in power over the last 5 sessions (Figure 21). Previous research on the effects of iso-inertial training on muscle strength and power has typically used longer interventions (see Table 1). Therefore, although total increases are comparable with previous studies [103,138,227], the increased duration (in weeks) of these interventions accounts for the plateau effect of the increases in strength and power, and therefore, the rate of increase reported per day is much lower. Secondly, increases in strength and power are lower when the training intervention is comprised of single-joint exercises (i.e., knee extension) as compared to multi-joint exercises (i.e., squat) [228]. As most studies included in the cited meta-analysis [116] are based on single-joint exercises, a lower mean effect is to be expected in these parameters, compared to the results obtained in studies employing multiple-joint exercises, such as the half squat.

Study 3 also showed a significant overall increase of 28% ± 17% in knee extensor MVIC after the training period (i.e., 1% increase per day over 4 weeks). In contrast, no significant changes were seen in knee flexor MVIC (Figure 22). Again, these differences are possibly explained by the lower activation of the hamstrings than of the quadriceps muscles during the squat [174,204]. During squats, hamstrings provide a stabilising force at the knee by producing a posteriorly directed force on the tibia that counteracts the anterior tibial force generated by the quadriceps [229]. Because of this, and the predominant activity of the gluteal and quadriceps muscles [174], the squat is commonly seen as a “complete” lower-body exercise. However, although EMG activity of the hamstrings during squats doubles that seen during leg presses or knee extensions [230], it represents only approximately half of the activation seen during leg curls or deadlifts [231]. This suboptimal stimulus causes the underdevelopment of hamstrings, as compared to quadriceps, and may lead to an undesirable muscle imbalance and increase the likelihood of injury [229]. Consequently, it is highly recommended to include hip hinge and knee flexion exercises when using the squat in the context of an equilibrated resistance training programme to avoid such imbalances.

Despite the great increases in specific (i.e., squat) force and power, and MVIC of the knee extensors after the 4-week training programme, no significant changes were seen in terms of jumping (i.e., CMJ or SJ) or running (i.e., 40-metre time) performance. These results may seem unexpected, as previous research has shown great effects of iso-inertial training on horizontal or vertical movement [116]. The lack of improvement in these tests may be explained by the moment of inertia employed in *Study 3*. Previous research has shown that, in general terms, a high-velocity protocol (i.e., 0.025 kg·m^2) seems to improve jumping and sprinting capacities more than others with higher inertias (0.025 kg·m^2) [122,123]. Furthermore, previous studies have shown that an individualised approach (i.e., profiling the F-V spectrum of sprint and jump actions to address the sub-developed loading zones) may yield better results and achieve improvements in a higher proportion of participants [158,159]. In line with these studies, after the 4-week iso-inertial training intervention (*Study 3*), only some of the volunteers showed improvements (SJ: 6 out of 10, CMJ: 5 out of 10, 40 m sprint: 6 out of 10). This illustrates heterogeneous responses to a similar stimulus.

Another aspect that may have influenced the absence of significant improvements in sprint performance is the different force-application vector of the squat (vertical) as compared with the sprint (horizontal). Previous research has shown that horizontal-type resisted exercises (i.e., conical pulley resisted displacements) tend to improve horizontal-type movements (i.e., sprint) more than vertical-type exercises [102,121].

Therefore, if the aim of the intervention is to improve sport-specific actions such as jumping or sprinting, it is highly recommendable to test the individual sprint and jump F-V spectrum and adjust the training load to specific necessities (i.e., maximum strength deficit: training with a high moment of inertia; maximum velocity deficit: training with a low moment of inertia) [158,159]. If individualised testing is not possible, lower moments of inertia (i.e., 0.025 kg·m^2) have generally shown better results [122,123]. Furthermore, exercises with high dynamic correspondence (e.g., in terms of the force-application vector) with the sport action should be included.

8.5.2. Muscle hypertrophy.

Previous research has highlighted the skeletal-muscle hypertrophic potential of iso-inertial training, with significant average increases of 0.20% and 0.19% per day of volume/mass and CSA respectively, during the first 5–10 weeks of flywheel training 2–3 times per week [116]. Also, the earliest onset of macroscopic muscle hypertrophy was reported during an iso-inertial training programme (4.2% increase in CSA after only 20 days of training / nine training sessions; 2.4% increase in fibre length after only 10 days of training / five training sessions) [103].

As some studies had questioned the validity of large muscle hypertrophy after a short period of time (a possible confounding effect of muscle oedema) [118], one of the objectives of *Study 3* was to quantify muscle hypertrophy in the early phase (i.e., 14 days), controlling the absence of muscle oedema and using a high-resolution method in order to detect small changes in volume (Table 5).

Study 3 shows a rate of increase in quadriceps volume of 0.39% per day over the first 14 days of training (5 training sessions), which almost doubles the averaged increase per day in CSA over the first 20 days of resistance training (9 training sessions) [116]. These differences may be explained by greater exercise volume per session (5 sets x 10 repetitions; see other protocols in Table 2), as the resistance training volume seems to be one of the most important factors that affect muscle hypertrophy [206]. In addition, hypertrophic changes in previous studies were measured with low-resolution methods (i.e., CSAs at specific muscle lengths), instead of a high-resolution volumetric assessment. Lundberg et al. [117] used a similar flywheel training volume per session (4 x 7) in combination with intense aerobic exercise, and reported the highest rate of muscle hypertrophy (0.4% increase in quadriceps volume per day over the first 5 weeks) in the scientific literature (measured as total volume). As with the case of strength gains, the rate of muscle volume increase reported in *Study 3* is higher in the first two weeks (0.39% per day) but tends to decrease as the trainee progresses (0.30% per day over the first 28 days).

It must be stressed that in *Study 3*, the increases in muscle volume were assessed at least 96 hours after training sessions, to avoid acute muscle swelling. The T2 signal under basal conditions of the muscles assessed did not change significantly from PRE to IN ($P = 0.101 – 0.934$, with differences of -1.5% ± 1.4%) or from PRE to POST ($P = 0.137 – 0.849$, with differences of -2.4% ± 1.7%). This indicates the absence of muscle oedema [118]. Additionally, in all cases, pre-training-session MVIC force levels had recovered when the scans were performed, indirectly indicating the absence of muscle damage [42]. Therefore, I am confident that the changes in muscle volume reported were due to chronic hypertrophic changes, and not acute processes such as swelling.

Another important finding of *Study 3* is the lack of correlation between acute regional T2 shifts and the percentage of increase in regional CSAs after the intervention. Previous studies with highly analytic exercise protocols (i.e., a given muscle or muscle group worked in isolation) found correlations between the acute T2 shift of specific muscle regions or muscles after exercise and the acute (swelling) [208], and chronic (hypertrophic) [198,199] response measured by MRI. T2 shifts in analytic (i.e., single-joint) exercises are likely to correlate to other variables that are more relevant for promoting muscle hypertrophy [195] (such as the magnitude and time producing mechanical tension [61]). *Study 3* shows that in complex multi-joint movements such as the squat, in which antagonistic and synergistic muscle groups are recruited throughout the movement [205], the prediction of hypertrophy from T2 values is not possible. For instance, the hamstring muscle group displays a negative T2 shift in the squat [204], despite being electromyographically activated and under tension [174]. As a result, in *Study 3*, hamstring muscle volume increased significantly after 4 weeks (see Table 5), even though BFL, BFS, SM and ST showed significant decreases in the T2 signal after exercise (Figure 23).

8.5.3. Repeated bout effect

Another important aim of the present thesis was to quantify protection against future damaging exercise, as one of the desired adaptations resulting from iso-inertial training. Previous research into this was scarce, and protocols either did not use incremental loads [104] or did not use a wide variety of markers to profile the RBE [109]. *Study 4* thoroughly quantifies the protective effect of iso-inertial exercise in a "real-life" scenario, where the damaging stimulus is matched to the increased force generating capacity after a training programme (Figure 26). Therefore, I compared the effects of two exercise bouts (A and B), which were volume matched, comparable, and under maximal loads, performed before and after the 4-week training programme, respectively. The effect of adaptation resulted in greater external loads performed in B (+39%, +21%, and +65% production of CON force, velocity, and power, respectively; see Table 6). Despite the increased external load performed in B, the 4-week intervention conferred a strong protective effect against damaging stimuli in terms of muscle function, perceived muscle soreness and blood markers of muscle damage.

The loss of force after the Bout A corresponded to symptoms of moderate muscle damage (i.e., the MVIC of the knee extensors decreased by 20%–50%, and recovery was between 2 and 7 days) [42]. Conversely, after Bout B, the loss of force corresponded to mild muscle damage (MVIC of the knee extensors decreased by <20% and was recovered within 2 days) [42]. In a practical context, this adaptation helps to cope with a weekly training schedule (avoiding functional overreaching) or even allows the frequency of training sessions to be increased. The index of protection for the MVIC of the knee extensors was higher than reported in previous studies of RBE [80], probably caused by the differences in the protocols typically used in studies of RBE (i.e., training vs complete rest between bouts), which may trigger different adaptive mechanisms for the RBE [80]. The RBE effect was also seen in jumping capacities. After Bout A, both SJ and CMJ diminished and had not recovered after 6 days. After Bout B, an almost complete index of protection was seen in CMJ and SJ capacity (Figure 28), and only the SJ was significantly affected in the first hours after exercise (Figure 27). Overall, the RBE on functional capacities of muscle were high (around 80% index of protection), showing a superior effect of protection on muscle contractile function in the days following exercise (i.e., between 1 and 6 days) than on the acute response (i.e., 1 hour after exercise), which may be linked to processes other than muscle damage (i.e., metabolic fatigue) [80,104].

Study 4 also showed attenuation and also a speeding up of soreness appearing and disappearing. The index of protection (48%) was similar to those reported in previous studies of ECC exercise [80]. Although DOMS is not correlated with other markers of EIMD [51], it is highly relevant for training and competition, as it may limit performance or produce changes in movement patterns (i.e., compensatory mechanisms), and in doing so, increase the risk of injury [218].

Serum markers of muscle damage also showed significant reductions after the training intervention. The leakage of CK was attenuated (index of protection 63%) and there was only a smaller peak at 1 day, with complete clearance by day 3. AST and ALT did not increase in serum after Bout B. These results for CK, AST, and ALT suggest that although much higher forces were applied in Bout B, the muscle was less vulnerable to cellular, chemical and physical stress on the membranes [219]. sMtCK also showed a lower and earlier peak after Bout B (index of protection 71%), which may reflect lower metabolic stress or better tolerance to it, with the result of lower mitochondrial disruption after intense exercise [212]. During follow-up after Bout B, I found no significant changes in the structural markers of muscle damage, titin and CK-MB. Given these results and the high indexes of protection for each marker (72% in both markers), the exercise programme was highly effective at protecting muscle against structural damage to sarcomeres. Therefore, a 4-week structured iso-inertial resistance training programme generates adaptations that confer a better response to stress in skeletal muscle, allowing it to tolerate much higher workloads while suffering less damage. This highly attenuated damage may be restricted to membrane and mitochondrial disruptions, with no significant changes shown for the markers of ultrastructural damage.

CONCLUSIONS

9. CONCLUSIONS

This thesis provides novel knowledge on the control, and the acute and chronic effects, of iso-inertial training of the lower limbs. The main conclusions of this work are presented in the following list.

- The Chronojump friction encoder represents an accurate and versatile way to assess velocity, force and power in flywheel exercises, which can provide live-augmented feedback to athletes on a day by day basis.
- Unaccustomed iso-inertial half-squat exercise is expected to produce transient acute changes, including:
    - Moderate decreases in knee extensor MVIC, but not in knee flexor MVIC, decrements in jump performance (especially affecting the contractile component) and reduced sprint performance.
        - While sprint maximal velocity decreases as a result of acute fatigue (but shows progressive recovery thereafter), early acceleration is dependent on the amount of EIMD, worsening as the hours pass.
    - Leakage of several biochemical markers into the serum. The combination of different markers provides information on the severity and location of EIMD, and suggests the following are affected, in order of decreasing severity: (i) the myocyte membrane, (ii) the sarcomeric mitochondria, and (iii) the muscle ultrastructure.
        - Serum concentration of titin, a novel marker of muscle structural damage, may be useful in determining the severity of muscle damage.
- A structured iso-inertial half-squat training programme produces early chronic adaptions in several systems, including:
    - Muscle function. Early increases (i.e., after four weeks) in squat force (CON 32% ± 15%, ECC 31% ± 15%) and power (CON 51% ± 30%, ECC 48% ± 27%), and in knee extensor MVIC (28% ± 17%). No significant changes were seen in knee flexor MVIC, jumping (i.e., CMJ or SJ) or running (i.e., 40-metre time) performance.
    - Muscle hypertrophy is initiated early and progressively, potentially contributing to initial strength gains. Quadriceps muscles increased 5.5% ± 1.9% in volume after only 14 days (5 training sessions). This represents the earliest onset of whole-muscle hypertrophy, without the interference of acute muscle oedema, documented to date. Percentage volume increases after 4 weeks were 8.6% for quadriceps, 5.6% for adductors and 2.8% for hamstrings.
        - Regional acute T2 shifts were not found to be correlated with the relative increase in CSA after the training programme.

- RBE. Several adaptations confer a better response to stress in skeletal muscle, allowing it to tolerate much higher workloads with less damage. Even when the damaging stimulus is matched to the increased force capabilities, the EIMD response decreases.
  - Loss of maximal and functional strength is limited and recovered earlier. Overall, the RBE shows a higher attenuation of EIMD (i.e., strength loss between 1 and 6 days) than of acute fatigue response after exercise (i.e., strength loss 1 hour after exercise).
  - Soreness is attenuated and the process of appearance and disappearance is sped up.
  - Serum markers of muscle damage also show significant reductions after the training intervention. This highly attenuated damage may be restricted to membrane and mitochondrial disruptions, with no significant changes shown for the markers of ultrastructural damage.

PRACTICAL APPLICATIONS

10. PRACTICAL APPLICATIONS

Unaccustomed iso-inertial half-squat exercise is expected to produce transient EIMD, with high inter-individual differences in the response. These changes may affect compliance with training sessions, performance in competitions and acutely increase the likelihood of injury. Therefore, it is recommended that the exposure to this type of training stimulus is periodised.

Analytical (i.e., knee extensor) and functional (i.e., sprint, jump) strength tests offer meaningful information on the process of impairment and recovery after training sessions. It is recommended to use a combination of different tests for this purpose.

Iso-inertial half-squat training will effectively develop quadriceps strength and volume, but the effect on hamstrings will be limited or inexistent. To avoid an undesirable muscle imbalance, it is highly recommended to include hip hinge and knee flexion exercises when using the half squat in resistance training programmes.

As the adaptations underpinning the repeated bout effect are achieved in the process of iso-inertial training, trainees will develop higher external power outputs with less muscle damage and shorter times needed for full recovery. However, this effect is not so robust in preventing acute fatigue after exercise, and therefore transient decreases in performance have to be expected in the first hours after strenuous iso-inertial training, even in trained subjects.

Novel muscle damage biochemical markers such as titin may offer increased insight into the processes of adaption and recovery. In the near future, if faster and less invasive procedures for its determination are developed, they may become a useful complement for assessing the impact of training sessions.

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ANNEX

12. ANNEX

12.1. ANNEX 1. Article "Early Functional and Morphological Muscle Adaptations During Short-Term Inertial-Squat Training"

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Early Functional and Morphological Muscle Adaptations During Short-Term Inertial-Squat Training

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Purpose: To assess early changes in muscle function and hypertrophy, measured as increases in muscle cross-sectional areas (CSAs) and total volume, over a 4 weeks inertial resistance training (RT) program.

Methods: Ten young RT-naive volunteers (age 23.4 ± 4.1 years) underwent 10 training sessions (2–3 per week) consisting of five sets of 10 flywheel squats (moment of inertia 900 kg·cm^2). Magnetic resonance imaging (MRI) scans of both thighs were performed before (PRE), and after 2 (IN) and 4 (POST) weeks of training to compute individual muscle volumes and regional CSAs. Scans were performed after ≥96 h of recovery after training sessions, to avoid any influence of acute muscle swelling. PRE and POST regional muscle activation was assessed using muscle functional MRI (mfMRI) scans. Concentric (CON) and eccentric (ECC) squat force and power, as well as maximal voluntary isometric contraction force (MVIC) of knee extensors and flexors, were measured in every training session.

Results: Significant quadriceps hypertrophy was detected during (IN: 5.5% ± 1.9%) and after (POST: 8.6% ± 3.6%) the training program. Increases in squat force (CON: 32% ± 15%, ECC: 31 ± 15%) and power (CON: 51% ± 30%, ECC: 48% ± 27%) were observed over the training program. Knee extensor MVIC significantly increased 28% ± 17% after training, but no changes were seen in knee flexor MVIC. No correlation was found between regional muscular activation in the first session and the % of increase in regional CSAs (r = -0.043, P = 0.164).

Conclusion: This study reports the earliest onset of whole-muscle hypertrophy documented to date. The process initiates early and continues in response to RT, contributing to initial increases in force. The results call into question the reliability of mfMRI as a tool for predicting the potential hypertrophic effects of a given strengthening exercise.

Keywords: flywheel, strength, resistance training, hypertrophy, MRI

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INTRODUCTION

Resistance training (RT) has been shown to induce profound and specific changes in virtually all biological systems (Egan and Zierath, 2013). Optimizing the stimulus and time invested to produce these changes is one of the goals of strength and conditioning trainers. In an attempt to achieve this, RT programs are usually periodized, based on the different dynamics of adaptation for each physiological variable. Muscle hypertrophy induced by RT has generally been considered to be a slow process with a delayed onset, with initial strength gains mostly attributed to neural factors (Moritani and DeVries, 1979; Blazevich et al., 2007). Biologically, muscle hypertrophy is the result of a positive balance between the synthesis and breakdown of proteins, which is manifested as microscopic (fiber thickness) and macroscopic (muscle thickness) surrogate variables. It is known that remodeling processes are highly dynamic in skeletal muscle, and that single bouts of RT immediately upregulate intramuscular anabolic signaling, amino acid transport and protein synthesis (Bickel et al., 2005; Gonzalez et al., 2016; Song et al., 2017). Therefore, if the protein balance remains positive after RT sessions (Reitelseder et al., 2014), it would be theoretically possible to accrete muscle mass early, starting after the very first session. In line with this idea, in a recent clinical trial in humans, increases in Type II fiber cross-sectional area (CSA) were detected after only 2 weeks of RT (Holloway et al., 2017). Although this histological evidence suggests a continuous process of adaptation, the macroscopic evidence of adaptation in the early phase of RT is less convincing.

Given this background, the time course of macroscopic muscle hypertrophy was revisited in a recent review of studies involving different RT protocols and at least three muscle size measurements over time (Counts et al., 2017). Even though most of the studies analyzed used a similar sample (untrained young volunteers), a lack of agreement between the results was observed. For the lower limbs, the reported results range from no significant changes in muscle size after 5–12 weeks (Abe et al., 2000; Blazevich et al., 2007) to significant muscle hypertrophy after just 3–4 weeks of RT (Lüthi et al., 1986; Narici et al., 1989; Kubo et al., 2010; Baroni et al., 2013; Brook et al., 2015). To date, the study conducted by Seynnes et al. (2007) reports the earliest onset of macroscopic muscle hypertrophy, showing an increase of quadriceps femoris (QUAD) CSA after only 20 days of training (nine training sessions). The differences in the training stimulus, frequency of the assessments and sensitivity of the measurement methods all contribute to the spread of results in the available literature (Counts et al., 2017). It is hoped that new studies using an optimized RT stimulus, and more sensitive and frequent assessment will shed light on the topic.

In order to produce fast and significant increases in muscle size, the training stimulus has to meet certain characteristics. Mechanical tension is the primary driver of muscle hypertrophy (Schoenfeld, 2010). Thus, RT interventions aiming to increase muscle volume should focus on the magnitude and the length of time producing tension. Several training systems and techniques can be used to achieve an enhanced stimulus. For instance, inertial flywheels allow for accommodated maximal or near maximal actions from the very first repetition of a set in the concentric (CON) and eccentric (ECC) phases (Tesch et al., 2004; see **Figure 1**). To the best of our knowledge, Lundberg et al. (2013) reported the fastest rate of whole muscle hypertrophy over a period of 5 weeks (0.4% increase per day), using a combination of flywheel RT and intense aerobic exercise. In a recent meta-analysis, flywheel training showed to be more effective than conventional weights in promoting increases in muscle volume, strength and power (Maroto-Izquierdo et al., 2017).

A wide range of techniques can be employed to estimate changes in whole-muscle size (Counts et al., 2017; Franchi et al., 2018). Magnetic resonance imaging (MRI) is regarded as the gold standard for clinical and research imaging of skeletal muscle, and is commonly used to compute 1 (middle), 2 (proximal and distal), or 3 (proximal, middle and distal) CSAs of a given muscle (Seynnes et al., 2007). However, single CSAs may not be representative of whole-muscle changes, given that different patterns of hypertrophy (ventral or distal) have been reported in response to specific CON or ECC loading (Franchi et al., 2017). New MRI approaches for the assessment of total muscle volume, and not only CSAs of specific muscle sites, provide a better measurement of whole-muscle changes (Nordez et al., 2009).

Other acute physiological processes need to be carefully taken into account when measuring muscle hypertrophy. RT results in a rapid activity-dependent influx of fluid and

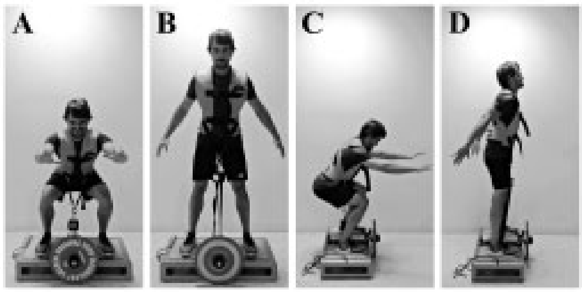

FIGURE 1 | Concentric–eccentric bilateral half-squats performed on a "YoYo squat" inertial flywheel device; anterior view **(A,B)**, lateral view **(C,D)**. **(A,C)** Bottom position of the exercise and transition between the eccentric and the concentric phases. **(B,D)** Top position of the exercise and transition between the concentric and the eccentric phases. The vest is attached to a strap wound on the flywheel axis. As resistance is the moment of inertia of the flywheel, the force applied during the concentric phase to unwind the strap determines the force needed during the eccentric phase (as the strap rewinds) to impede the spin of the axis.

Abbreviations: RT, resistance training; CSA, cross-sectional area; QUAD, quadriceps femoris; CON, concentric; ECC, eccentric; MRI, magnetic resonance imaging; mfMRI, muscle functional magnetic resonance imaging; T2, transverse relaxation time; MVIC, maximal voluntary isometric contraction force; PRE, before the training intervention; IN, after 2 weeks of training intervention; POST, after 4 weeks of training intervention; ROI, region of interest; GM, gluteus maximus; RF, rectus femoris; VI, vastus intermedius; VM, vastus medialis; VL, vastus lateralis; AM, adductor magnus; GR, gracilis; BFL, biceps femoris long head; BFS, biceps femoris short head; ST, semitendinosus; SM, semimembranosus; ADD, adductors; HAMS, hamstrings.

accumulation of osmolytes (phosphate, lactate, and sodium) and a subsequent acute inflammatory response that can last for several hours after exercise (Damas et al., 2016). These processes temporally alter muscle volume and may interfere with hypertrophy measurements (DeFreitas et al., 2011), but they can also provide valuable information. For instance, acute fluid changes in muscles can be assessed indirectly by muscle functional MRI (mfMRI) (Damon et al., 2007). This non-invasive technique measures the "T2 shift," which is the increase in the transverse relaxation time (T2) of muscle water from pre-exercise to post-exercise (Cagnie et al., 2011). The T2 shift is positively correlated with electromyographic activity, at least when muscle groups are exercised in isolation (Adams et al., 1992). In consequence, and although it can only be considered as a proxy marker, mfMRI is commonly used as a tool to determine total or regional muscle metabolic activation in different exercises (Fernandez-Gonzalo et al., 2016). Given that swelling is caused by muscular activity, and that cell swelling is a known upregulator of anabolic signaling pathways in a wide variety of cells (Lang, 2007), it would be reasonable to expect that muscles with greater T2 shifts after acute RT will display greater hypertrophy if the same exercise is systematically repeated over time. In fact, previous studies have found a correlation between these parameters when an isolated muscle or muscle group is exercised over time (Wakahara et al., 2012, 2013).

The purpose of this study was to assess early changes in thigh muscles function (force and power), hypertrophy (total volume and regional CSAs), and muscle activation (T2 shift), during a 4 weeks inertial-squat RT program. We hypothesized that inertial RT would produce early (≈2 weeks) detectable increases in muscle function and size. Additionally, we studied the relationship between muscle hypertrophy and activation in the first session, under the hypothesis that different degrees of hypertrophy of thigh muscles would be directly related to muscle task-specific activity assessed by mfMRI.

MATERIALS AND METHODS

Experimental Approach

A quasi-experimental design of repeated measurements was used to determine the effects of an intervention on different variables. The independent variable in this study was a 4 weeks training program consisting of bilateral half-squats performed on an inertial flywheel device. The variables assessed for thigh muscles were: muscular activation, muscle hypertrophy and muscle performance. Activation was assessed through the T2 shift by mfMRI. Hypertrophy was assessed through increases in total volume and regional CSAs using MRI. Muscle performance was assessed through maximal voluntary isometric contraction force (MVIC) of the knee extensors and knee flexors, as well as by squat dynamic force and power. The assessments were performed before (PRE), and after 2 (IN) and 4 (POST) weeks of training; and also during each of the 10 training sessions. **Figure 2** is a schematic representation of the study protocol.

Subjects

Ten young volunteers (men = 8; women = 2) (age 23.4 ± 4.1 years; height 174.8 ± 7.7 cm; weight 71.0 ± 6.8 kg; self-reported weekly moderate intensity activity 6.3 ± 3.4 $h \cdot week^{-1}$) who had not suffered muscle or tendon injuries in the previous 6 months were recruited for the study. All were recreationally active physical education students who had not been involved in any RT program during the 6 months preceding the study. 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 of the Catalan Sports Council (Generalitat de Catalunya). A familiarization session was conducted 2 weeks before starting the study. All the volunteers were informed of the aims, experimental protocol, procedures, benefits, and risks of the study, and their written informed consent was obtained. The volunteers were instructed to maintain their usual level of physical activity throughout the experimental period, and to refrain from moderate or heavy physical activities in the 96 h before the MRI.

Procedures

Training Protocols

The training period comprised of 10 training sessions distributed over 4 weeks (two or three sessions per week). Each training session consisted of a standardized warm-up (two sets of 10 body-weight squats, with 1 min of rest between the sets), followed by five sets of flywheel exercise. Each set of exercise included three sub-maximal repetitions to accelerate the disk, followed by 10 maximal voluntary repetitions. There was 3 min of rest between sets. Exercise consisted of CON – ECC bilateral half-squats performed on a "YoYo squat" inertial flywheel device (YoYo Technology AB, Stockholm, Sweden) (**Figure 1**). Given the properties of the flywheel, the resulting moment of inertia was 900 $kg \cdot cm^2$. The start and end position of each repetition was a 90° knee angle. The participants were verbally encouraged to perform the concentric phase at their fastest voluntary speed.

Squat Performance

Exercise kinetics were monitored during each session using a Chronopic friction encoder (Chronojump, Barcelona, Spain), (accuracy ± 1 mm, sampling rate 1000 Hz). The sensor was tightly attached at a known diameter of the flywheel, sharing the same linear speed. The set-up parameters such as disk inertia, axis diameter, and the volunteer's body mass were entered into the associated Chronojump software (v1.6.0.0) to compute the kinematic exercise variables in real time. Visual and acoustic feedback on the performance was provided for each repetition using a computer. The variables calculated for the CON and ECC phase of each repetition were displacement and mean values of force and power. Chronojump is open-code software, and a complete repository of the code and formulas used can be found online (Chronojump, 2018).

MVIC

Maximal voluntary force production of the knee extensors and knee flexors was tested in isometric conditions. For data collection, a strain gauge and a custom-built bench were used.

The signal was recorded at a frequency of 200 Hz, using a Muscle Lab 6000 (Ergotest Technology AS, Porsgrunn, Norway) and real-time results were provided on a computer monitor. For assessment of knee extensors, the volunteers were positioned supine (knee angle = 90°; hip angle = 180°); for knee flexors, lying prone (knee angle = 135°; hip angle = 180°). In both positions, the volunteers were fixed using adjustable straps and the strain gauge was attached at the mid-level of the malleolus tibiae forming a perpendicular angle with the leg. Three unilateral 5 s trials were recorded for the dominant limb, with 30 s of recovery between trials. Subjects were instructed and verbally encouraged to perform and maintain maximum force output. Pre-contraction conditions were standardized and attempts with counter movements were rejected. Maximum force values were selected using mean force over a 1 s mobile window once a force plateau had been established (Tesch et al., 2004; Carmona et al., 2018). The best attempt was selected at each time point for further analysis. MVIC was assessed before each training session, and at IN and POST, after the standard warm-up.

Image Acquisition and Processing

MRI was used to compute the volumes of the individual muscles of the thigh and mfMRI to assess the acute muscle activation after exercise. The volunteers were placed supine inside a 3-T MRI scanner (Magnetom VERIO, Siemens, Erlangen, Germany), with their heads outside the MR-bore and thighs covered with one 32- and two flexible 4-channel coils, respectively, in the proximal and distal segments. A custom-made foot-restraint device was used to standardize and fix limb position, and to avoid any compression of thigh muscles. To ensure the same anatomical area was assessed each time, the range was centered at the mid-length of the femur, as measured on the coronal plane image.

Muscle activation

For the assessment of PRE and POST muscle activation, one scan was performed in basal conditions and another 3–5 min after finishing an acute bout of the exercise (Cagnie et al., 2011; Mendez-Villanueva et al., 2016) (10 sets of 10 flywheel squats). To minimize the effects of fluid shifts caused by walking, the volunteers remained recumbent for a minimum of 10 min before basal data acquisition (LeBlanc et al., 2000), and were assisted over the 10 m between the exercise room and the MRI scan. In each scan, 12 contiguous (each 31.5 mm) 3.5 mm cross-sectional images of both thighs were obtained using the following scan sequence: SE MULTIECO, repetition time: 1800 ms, echo times: 20, 40, 60, 80, and 100 ms, field of view: 40 × 40 cm, matrix: 224 × 320, and total acquisition time: 6 min. A parametric image was generated from the T2 mapping sequence using Leonardo workstation (Siemens). The T2 of the muscles in both thighs was measured using OsiriX 8.5.2. (Pixmeo, Geneva, Switzerland). The assessment was performed by the same researcher on all occasions. All the sequences from a given volunteer were processed in parallel, with the researcher blinded. A circular region of interest (ROI) was selected for the muscles gluteus maximus (GM), rectus femoris (RF), vastus intermedius (VI), vastus medialis (VM), vastus lateralis (VL), adductor magnus (AM), gracilis (GR), biceps femoris long head (BFL), biceps femoris short head (BFS), semitendinosus (ST), and semimembranosus (SM) in each of the T2 mapping images where these muscles were visible. ROIs of similar size and anatomical location were placed in the subsequent image sets to ensure positioning identical to that in the first analysis (Mendez-Villanueva et al., 2016). The intra-class correlation coefficients and coefficient of variation for the intra-rater agreement of the T2 values were: 0.97 ± 0.06 and 1.09% ± 0.54% for the different muscles assessed. The T2 values for each muscle were computed as the mean value of the different ROIs. The T2 shift was then calculated by subtracting T2 basal values from T2 values post-exercise, and expressed as a percentage of the basal value. The results are shown as the average T2 shift of right and left thighs.

Muscle volume

To assess muscle volume, PRE, IN, and POST scans were performed. To minimize the effects of fluid shifts caused by walking (LeBlanc et al., 2000), the volunteers remained recumbent for a minimum of 10 min before data acquisition. In each scan, 288 contiguous (each 1.5 mm) 1.5 mm cross-sectional images of both thighs were obtained, using the following scan sequence: VIBE 3D Dixon, field of view: 40 cm × 40 cm, matrix: 320 × 320, and total acquisition time: 2 min. The edges of the RF, VM, VL+VI, adductors (ADD), BFL, BFS, ST, and SM muscles were manually outlined, image by image, by the same researcher using OsiriX 8.5.2. (Pixmeo, Geneva, Switzerland) (**Figure 3**). Because there may appear to be substantial fusion between VL and VI on some slices (Pareja-Blanco et al., 2016), these muscles were outlined together (VL+VI). The same approach was adopted for the assessment of ADD volume, which includes pectineus, and adductor longus, brevis and magnus. All the sequences from a given volunteer were processed

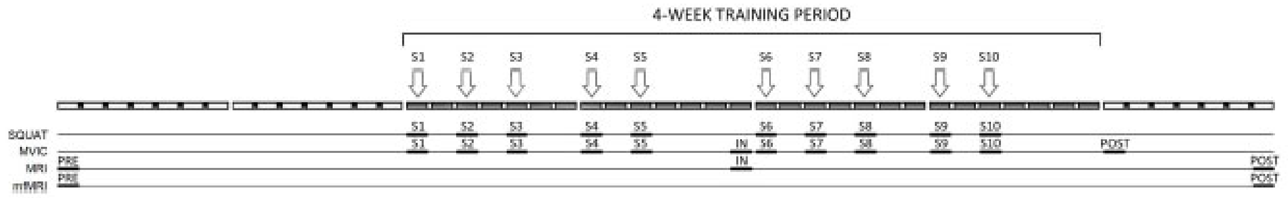

FIGURE 2 | Schematic representation of the study protocol. S1–S10 training sessions, SQUAT squat performance (kinetics), MVIC maximal voluntary isometric contraction force of knee extensors and knee flexors, MRI muscle volume assessed by magnetic resonance imaging, mfMRI muscle activation assessed by muscle functional magnetic resonance imaging.

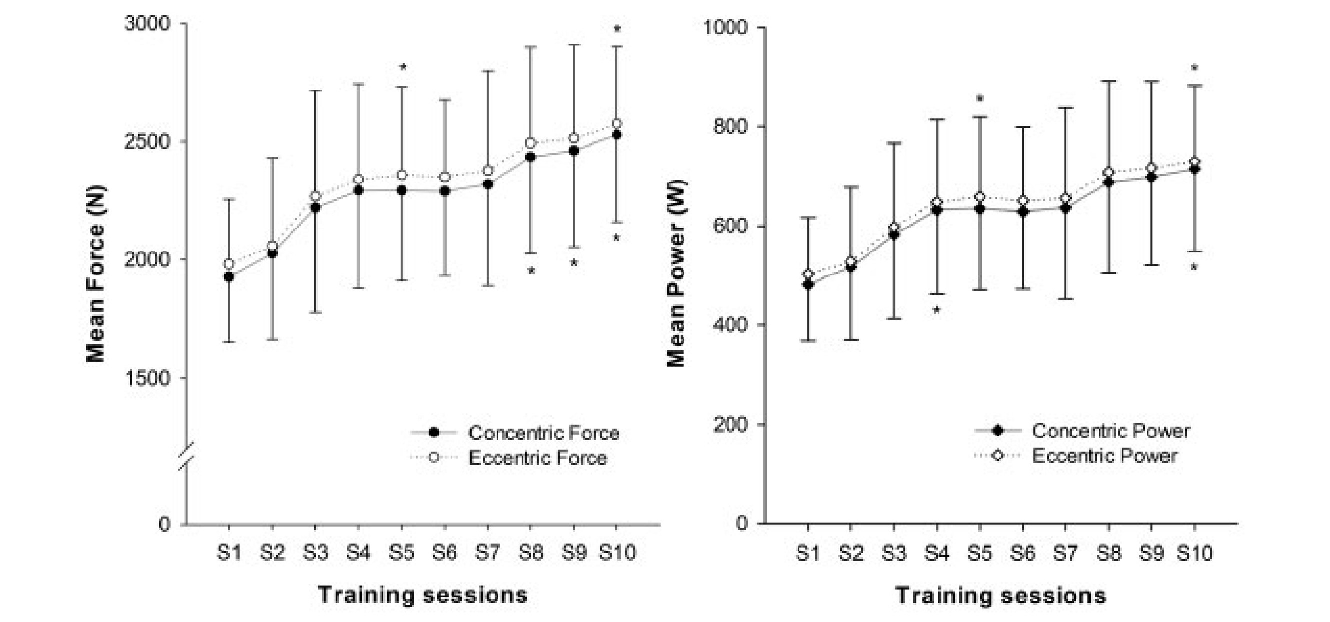

FIGURE 4 | Progression of squat mean values of force and power over the 10 training sessions (S). (*) indicates significant changes from baseline values ($P < 0.05$).

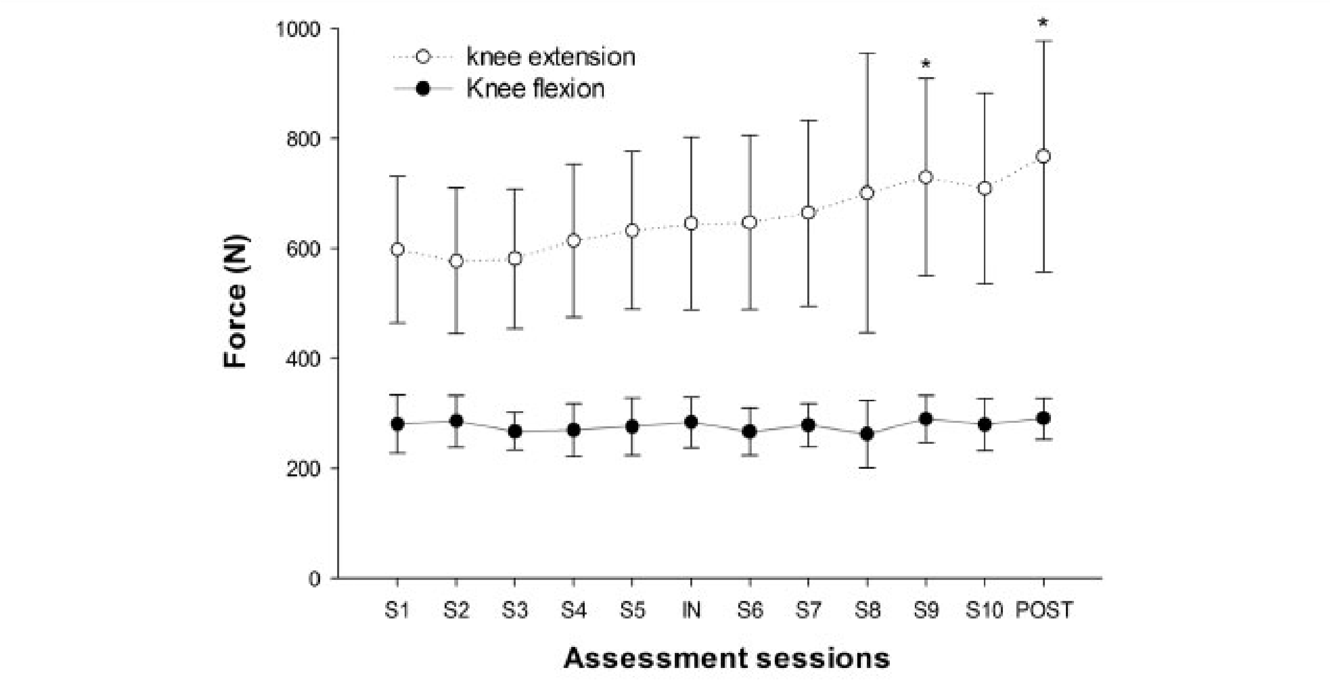

FIGURE 5 | Progression of MVIC force values throughout the training period. (*) indicates significant changes from baseline values ($P < 0.05$).

effects of time (PRE vs. POST) were seen in T2 shift in any of the muscles assessed.

Muscle Volume

There was a significant main effect of time for all the muscles analyzed, except for SM (**Table 1**). Significant increases in RF, VM, VL+VI, ADD, and BFL volume were detected from PRE to IN and from PRE to POST (all $P < 0.05$). A representative case is shown in **Figure 7**.

Regional Muscle Activation and Hypertrophy

Figure 8 shows the PRE regional T2 shifts and regional CSA changes from PRE to POST. No significant correlations were

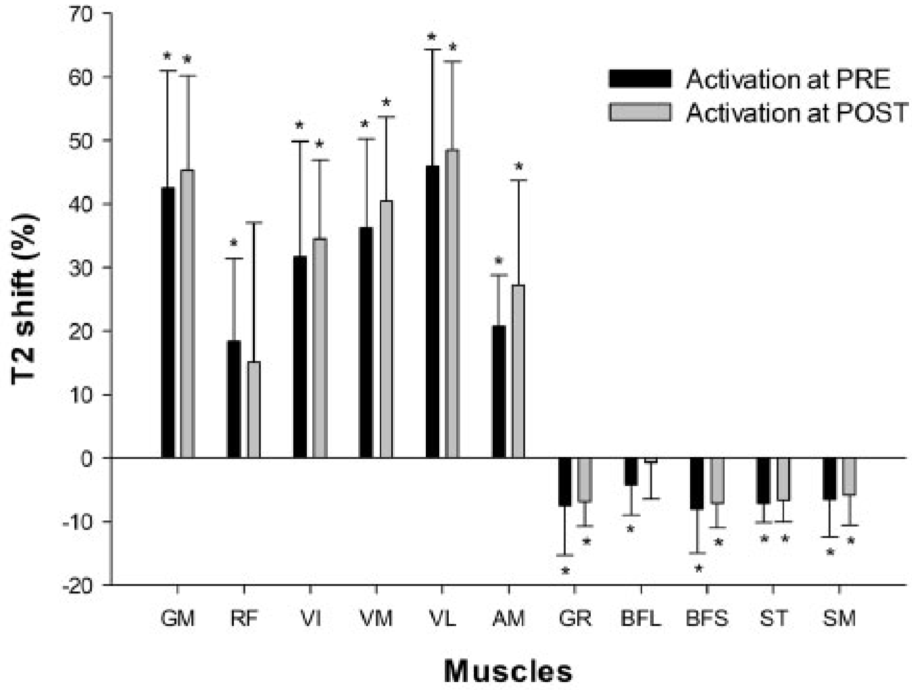

FIGURE 6 | Muscular activation assessed by mfMRI (T2 shift) after 10 sets of 10 maximal squats on the flywheel device; before (PRE) and after (POST) the training period. GM, gluteus maximus; RF, rectus femoris; VI, vastus intermedius; VM, vastus medialis; VL, vastus lateralis; AM, adductor magnus; GR, gracilis; BFL, biceps femoris long head; BFS, biceps femoris short head; ST, semitendinosus; SM, semimembranosus. (*) indicates significant post-exercise changes from basal T2 values ($P < 0.05$). No significant differences were found between PRE and POST activation.

TABLE 1 | Muscle volumes at baseline (PRE) and after 5 (IN) and 10 training sessions (POST).

	Volume (cm^3)			Volume change (%)		*P*-value time effect
	PRE	IN	POST	PRE-IN	PRE–POST	
Individual Muscles						
RF	237.0 ± 42.9	244.5 ± 45.2*	248.1 ± 46.9*	3.2*	4.7*	<0.001
VM	472.6 ± 107.6	501.5 ± 110.2*	518.9 ± 125.3*	6.1*	9.8*	<0.001
VI+VL	1250.1 ± 233.0	1320.8 ± 239.9*	1360.7 ± 253.1*	5.7*	8.8*	<0.001
BFL	224.9 ± 28.0	229.6 ± 30.5*	232.8 ± 29.6*	2.1*	3.5*	<0.001
BFS	105.0 ± 23.8	104.5 ± 23.5	108.4 ± 22.5	−0.5	3.2	0.028
ST	231.6 ± 45.2	232.9 ± 44.8	238.4 ± 47.5	0.6	3.0	0.020
SM	246.7 ± 31.0	246.3 ± 32.8	250.9 ± 33.7	−0.2	1.7	0.066
Muscle Groups						
QUAD	1959.8 ± 358.2	2066.8 ± 369.9*	2127.7 ± 399.2*	5.5*	8.6*	<0.001
ADD	1037.8 ± 141.5	1065.4 ± 147.1*	1096.2 ± 172.7*	2.7*	5.6*	<0.001
HAMS	808.2 ± 108.7	813.3 ± 109.7	830.5 ± 112.0*	0.6	2.8*	<0.001
Σ Thigh Muscles						
Total	3805.7 ± 583.3	3945.5 ± 600.1*	4054.4 ± 656.8*	3.7*	6.5*	<0.001

RF, rectus femoris; VM, vastus medialis; VI+VL, vastus intermedius + vastus lateralis; BFL, biceps femoris long head; BFS, biceps femoris short head; ST, semitendinosus; SM, semimembranosus; QUAD, quadriceps; ADD, adductors; HAMS, hamstrings. (*) indicates significant changes from basal values ($P < 0.05$).

found between the PRE regional T2 shifts and percentage increase in regional CSAs, except for ST (Total: $r = -0.043$, $P = 0.164$; AM: $r = 0.131$, $P = 0.142$; BFL: $r = -0.023$, $P = 0.792$; BFS: $r = -0.120$, $P = 0.250$; RF: $r = -0.033$, $P = 0.706$; SM: $r = 0.135$, $P = 0.157$; ST: $r = -0.225$, $P = 0.007$; VM: $r = -0.143$, $P = 0.075$; VL+VI: $r = -0.051$, $P = 0.524$).

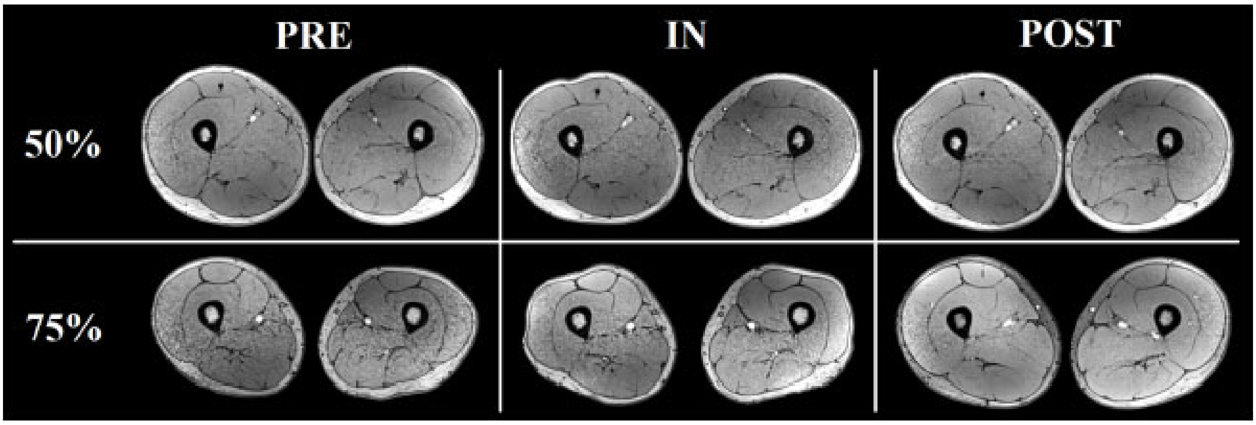

FIGURE 7 | Representative increases in thigh muscles CSA (at ≈50% and ≈75% the length of the femur) as assessed by consecutive MRI scans before (PRE), and after 5 (IN) and 10 (POST) training sessions from one of the volunteers.

DISCUSSION

This study reports significant muscle hypertrophy after only 14 days (five sessions) of a 4 weeks training period. To our knowledge, these findings represent the earliest evidence of macroscopic muscle hypertrophy (without the interference of acute muscle edema) to date.

Muscle Performance

Fast and notable improvements in muscle performance were seen after the training program (see **Figure 4**). This outcome was favored by the great window of adaptation of the sample (RT naive young volunteers), and the effectiveness of the stimulus applied. The training system used in this study had previously been shown to be more effective in promoting increases in muscle volume, strength and power than conventional weights (Maroto-Izquierdo et al., 2017). The efficacy of the method has been suggested to be mediated by the achievement of higher forces during the ECC phase (Tesch et al., 2004). A marked eccentric overload was not a deliberate outcome when the exercise protocols used in this study were designed, however, force and power values were significantly higher during the eccentric phase (see squat performance results). Similar increases were reported in previous studies involving similar samples and training systems (Seynnes et al., 2007; Fernandez-Gonzalo et al., 2014).

Muscle Activation

As shown in **Figure 6**, the GM and VL muscles were the most active with the squat exercise. Globally, QUAD muscles increased in T2 intensity, whereas HAMS muscles decreased in intensity. Of the QUAD muscles, however, the activation of RF between participants was highly variable. RF is the bi-articular muscle of the QUAD group, and its activation pattern is highly influenced by the hip position (Miyamoto et al., 2012). Biomechanical differences (i.e., femur lengths) between subjects may have caused different hip flexion angles during execution and thus different RF activation patterns. Concerning HAMS, the T2 shift values reported may appear unexpected, given that previous studies have shown a moderate contribution of hamstrings during squats when assessed by electromyography (Caterisano et al., 2002). It must be noted that T2 values are a quantitative index of the amount and distribution of water in skeletal muscle (Cagnie et al., 2011). When assessed on a single active muscle group, T2 values are highly correlated with electromyographic activity (Adams et al., 1992). However, these two assessment tools have different physiological bases and therefore may lack complete agreement in some situations. When muscles of a whole region (i.e., thighs) are assessed after being non-uniformly recruited, the distribution of water is increased in the most active muscles but it is decreased in less active muscles (Norrbrand et al., 2011). Thus, acute decreases in the T2 signal in HAMS muscles after squat exercise indicate relative inferior activation compared to QUAD muscles, but not a lack of activation (Slater and Hart, 2017).

Regarding the adaptive response to training, no significant changes were found in the T2 shift values from PRE to POST in any of the muscles assessed (**Figure 5**). Therefore, the recruitment pattern did not change over the training period. As exercises for determining T2 shifts were maximal both PRE and POST, it is highly likely that a ceiling was reached over which T2 values did not increase further (Cagnie et al., 2011). However, it is not clear whether the magnitude of the T2 shifts would have been similar if the external load had remained the same in PRE and POST, given that force outputs in the latter were much higher.

Muscle Hypertrophy

This study shows a rate of increase in QUAD volume of 0.39% per day over the first 14 days of training (5 training sessions), which is almost double what Seynnes et al. (Seynnes et al., 2007) reported (0.2%) for the increase in CSA over the first 20 days of RT (9 training sessions). This difference may be explained by greater exercise volume per session (5 × 10 vs. 4 × 7), as the RT volume seems to be one of the most important factors affecting muscle hypertrophy (Figueiredo et al., 2017). In addition, hypertrophic changes in the study performed by Seynnes et al. (2007) were measured as changes in the CSA

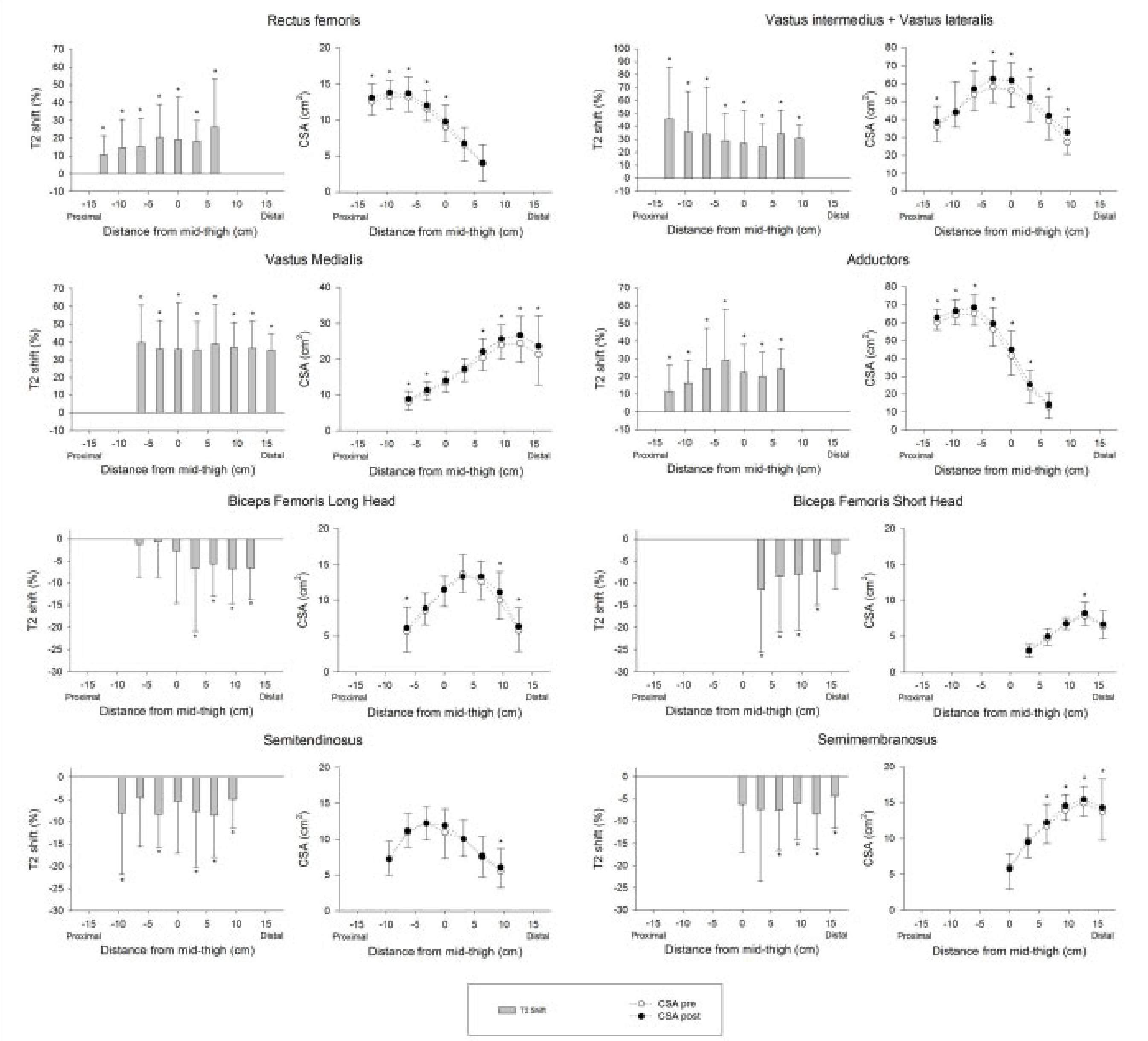

FIGURE 8 | Regional muscle activation assessed as T2 shift in the first session (PRE) and changes in regional CSAs from baseline (PRE) to the end of the 4 weeks training intervention (POST). In the activation graphs (left), (*) indicates significant post-exercise changes from basal T2 values ($P < 0.05$). In the CSA graphs (right), (*) indicates significant differences between PRE and POST CSAs ($P < 0.05$).

of two specific MRI slices at 25 and 50% of the femur bone length. Given that flywheel devices increase tension in the ECC phase of the movement and a distal pattern of hypertrophy has been reported in response to ECC loading (Franchi et al., 2017), changes in the CSAs measured may have underestimated whole-muscle changes. Supporting this idea, data displayed at **Figure 8** show less increase in the middle region of VL+VI (9.9% at 0 cm) and VM (7.6% at 0 cm) compared to their distal regions (VL+VI 21.3% at +9.45 cm; VM 14.0% at +15.75 cm). Given these non-uniform changes, the present study provides a more representative picture of whole-muscle changes by using total muscle volume (Nordez et al., 2009). Lundberg et al. (2013) used a similar flywheel RT volume per session (4 × 7) in combination with intense aerobic exercise, and reported a rate of QUAD hypertrophy (measured as total volume) of 0.4% per day over the first 5 weeks. It should be noted that the rate of CSA volume increase reported in the present study is similar for the first 2 weeks (0.39% per day) but tends to decrease as the trainee progresses (0.30% per day over the first 28 days). The faster rate of increase reported by Lundberg et al. (2013) could have been influenced by the additional training volume of the intense aerobic training, given the untrained condition of the volunteers.

Prior to this study, DeFreitas et al. (2011) reported significant increases in thigh muscle CSA (measured by quantitative computed tomography) after only 1 week (two sessions) of RT. However, testing was performed 48 h after a high-intensity RT protocol performed by unaccustomed subjects. Those authors concluded that early increases in CSA may have been due to edema, and considered that significant skeletal muscle hypertrophy occurred around weeks 3–4. Similarly, Krentz and Farthing (Krentz and Farthing, 2010) also reported significant increases in biceps brachii thickness (measured by ultrasound) after only 8 days (three sessions) of eccentric RT. In that case, the volunteers were tested 48 h after performing a RT session, and there was a concomitant decrease in strength, indirectly indicating that exercise-induced muscle damage was present (Paulsen et al., 2012). Therefore, in both cases, the early increase in muscle size was not considered to be hypertrophy, but edema-induced muscle swelling due to muscle damage (Damas et al., 2016).

It must be made clear that in the present study, the increases in muscle volume were assessed at least 96 h after the last training session to avoid acute muscle swelling. The T2 signal under basal conditions of the muscles assessed did not change significantly from PRE to IN (P = 0.101–0.934, with differences of -1.5% ± 1.4%) or from PRE to POST (P = 0.137–0.849, with differences of -2.4% ± 1.7%), which indicates the absence of muscle edema (Damas et al., 2016). Additionally, in all cases, pre-RT-session MVIC force levels had recovered when the scans were performed, indirectly indicating the absence of muscle damage (Paulsen et al., 2012). Therefore, we are confident that the changes in muscle volume reported here are accounted for by chronic hypertrophic changes, and not acute processes such as swelling.

Relationship Between T2 Shift and Hypertrophy

Establishing a relationship between T2 shifts and hypertrophy would be useful as a predictive tool for RT exercises. In fact, strengthening exercises are commonly classified by the magnitude of activation of certain muscles or muscle regions, in order to allow trainers to focus on a specific target of the RT intervention (Fernandez-Gonzalo et al., 2016; Mendez-Villanueva et al., 2016). It must be noted that different approaches are used to assess T2 shifts. For instance, it can be assessed as percentage activated area (Wakahara et al., 2012, 2013), or as percentage change of a representative ROI (Fernandez-Gonzalo et al., 2016; Mendez-Villanueva et al., 2016). Despite both variables commonly being used to quantify the same physiological phenomena, the relationship between them remains unclear. Therefore, differences between procedures may also have influenced the findings discussed here. In any case, the results presented in this study suggest that the regional percentage of change in the T2 signal after exercise is not a reliable tool for predicting the magnitude of increase in CSA, given that no correlation was found between those two variables.

Previous studies have found correlations between the acute T2 shift of specific muscle regions or muscles after exercise and the acute (swelling) (Kubota et al., 2007), and chronic (hypertrophic) (Wakahara et al., 2012, 2013) morphological response measured by MRI. Given this background, a correlation between regional T2 shifts and the percentage of increase in regional CSAs was expected. A plausible explanation for the lack of correlation in the present study is that the previously mentioned studies used highly analytic exercise protocols, in which a given muscle or muscle group worked in isolation. T2 shifts in analytic (i.e., single joint) exercises are related to other variables that are more relevant for promoting muscle hypertrophy (Adams et al., 1992) (such as magnitude and time producing mechanical tension, Schoenfeld, 2010). Although metabolic activity and T2 changes might correlate with mechanical tension and morphological changes in some situations, results suggest that this relation is weakened in complex multi-joint movements such as the squat, in which antagonistic and synergistic muscle groups are recruited throughout the movement (Slater and Hart, 2017). For instance, the HAMS muscle group displays a negative T2 shift in the squat (Norrbrand et al., 2011), despite being electromyographically activated and under tension (Caterisano et al., 2002). As a result, in the present study HAMS muscle volume increased significantly after 4 weeks (see **Table 1**), even though BFL, BFS, SM, and ST showed a significant decrease in the T2 signal after exercise (**Figure 6**).

Therefore, the use of mfMRI as a tool for predicting the potential hypertrophic effects of a given strengthening exercise should be questioned. We encourage future research to consider the physiological basis on which this method relies to correctly interpret mfMRI data from different exercises.

CONCLUSION

Muscle hypertrophy is initiated early and is progressive in response to RT, potentially contributing to initial strength gains. In this study, QUAD muscles increased 5.5% ± 1.9% in volume after only 14 days (fivetraining sessions) of flywheel inertial training. This represents the earliest onset of whole-muscle hypertrophy without the interference of acute muscle edema, documented to date. After 4 weeks of inertial squat RT, great increases in knee extensor MVIC (28% ± 17%) and in QUAD muscle volume (8.6% ± 3.6%) were observed.

The application of a robust RT stimulus in combination with a sensitive and precise evaluation tool such as 3D volumetry by MRI has been decisive for these findings. However, this method is at present much more time consuming than the assessment of single CSAs. Therefore, the level of sensitivity and precision needed, and the time available for assessment must be taken into consideration together to decide the best approach in each case (Nordez et al., 2009).

Regional T2 shifts after the first assessment session were not found to be correlated with the relative increase in CSA after the training program. These results call into question the reliability of mfMRI as a tool for predicting the potential hypertrophic outcomes of a given exercise.

LIMITATIONS

In this study, the number of volunteers could be regarded as a limitation on the interpretation of the results. However, it should be taken into account that the responses were very similar in all the participants after the training protocol. Moreover, the sample size of the study was adjusted based on previous related research (Seynnes et al., 2007; Lundberg et al., 2013). Finally, another limitation is the long time currently needed for the volumetric assessment of each muscle. As this precise analysis becomes more automatized with developing imaging technology, research will become easier in the future.

AUTHOR CONTRIBUTIONS

VI-D, GC, JP, and JC contributed to the conception and design of the study. VI-D organized the database. VI-D, GC, SN, JP, ML, XA, XP, and JC performed the experiments. VI-D, GC, SN, and JC wrote the first draft of the manuscript. VI-D, SN, GC, JP, XP, ML, RC, XA, and JC contributed to manuscript revision, and also read and approved the submitted version of the manuscript.

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