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MARKERS OF PHYSICAL FUNCTIONING AND NEUROMUSCULAR FATIGUE FOR THE POST-DISCHARGE FOLLOW-UP OF SUBJECTS ALREADY ASSISTED IN INTENSIVE CARE FOR COVID-19 AND NON-COMMUNICABLE DISEASES.

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Riassunto

L'insorgenza di malattie non trasmissibili con incidenza neuromuscolare e il prolungato ricovero in terapia intensiva comportano profonde implicazioni negative sulla capacità funzionale e sull'autonomia dei pazienti. Queste alterazioni sono dovute all'allettamento prolungato, all'infiammazione sistemica, alle alterazioni neuromuscolari e all'impatto diretto della patologia. A ciò consegue la comparsa di fatica e debolezza muscolare che contribuiscono al significativo declino della prestazione motoria, ostacolando notevolmente il processo di recupero e peggiorando la qualità di vita dei soggetti che ne soffrono.

Le principali disfunzioni motorie osservabili in questi pazienti si manifestano principalmente con la difficoltà di deambulare correttamente, limitando significativamente la loro autonomia nelle attività quotidiane. La compromissione dell'eccitabilità delle vie motorie discendenti del sistema nervoso che causa tali disfunzioni, insorge già durante la fase acuta della malattia e si intensifica man mano che la condizione patologica si cronicizza. Tale quadro clinico è aggravato ulteriormente dalla conseguente inattività fisica, favorita dalle condizioni psicofisiche dei singoli. Di conseguenza, tutto ciò incide notevolmente sulle proprietà contrattili dei muscoli, portando ad alterazioni critiche che influenzano la capacità del sistema nervoso centrale e periferico di reclutare e modulare l'attività delle unità motorie, le unità funzionali fondamentali per la pianificazione, esecuzione e mantenimento dei gesti motori.

Date le complessità associate a queste considerazioni, diventa fondamentale identificare marcatori in grado di quantificare e caratterizzare l'alterazione della capacità funzionale e l'insorgenza della debolezza muscolare e della fatica. Ciò faciliterebbe una diagnosi precoce e, in modo cruciale, il monitoraggio continuo di tali problematiche. Pertanto, l'obiettivo primario di questa ricerca di Dottorato è identificare e analizzare marcatori in grado di supportare efficacemente gli operatori sanitari nella progettazione e nell'implementazione di approcci terapeutici personalizzati per accelerare il recupero di questi individui. Nonostante, infatti, la pratica clinica attualmente in uso negli ospedali offra continui miglioramenti, questa presenta ancora delle limitazioni. Sebbene le valutazioni attualmente impiegate riescano ad identificare la presenza di fatica e debolezza muscolare nei pazienti post-ricovero in terapia intensiva o in soggetti affetti da malattie non trasmissibili, non riescono tuttavia ad indagare a fondo su quali siano le effettive cause che innescano la perdita della forza muscolare o ad esaminare in modo esaustivo i fattori centrali e/o periferici che contribuiscono all'insorgenza della fatica.

Per colmare in modo esaustivo questa lacuna, lo studio ha condotto un'ampia ricerca combinando l'elettromiografia di superficie con la capacità di generare forza muscolare in diverse condizioni patologiche, includendo attivazioni muscolari sia volontarie che indotte elettricamente. Il muscolo oggetto di studio è stato il tibiale anteriore, scelto per il suo ruolo cruciale nella biomeccanica della deambulazione e quindi fondamentale per il mantenimento dell'autonomia motoria.

I risultati presentati sono stati ottenuti attraverso la decomposizione dei segnali elettromiografici registrati utilizzando la tecnica dell'elettromiografia di superficie ad alta densità. Questa tecnica ha consentito l'estrazione e l'analisi delle singole unità motorie reclutate durante i diversi compiti motori richiesti ai pazienti nelle diverse popolazioni studiate, sia nei domini di tempo che di spazio. Il processo di raccolta e analisi dei dati ha rivelato che le variazioni nei valori della forza e nei parametri delle unità motorie possono essere considerati indicatori delle alterazioni del sistema neuromuscolare e del progressivo recupero. Questi fattori sono quindi fondamentali per il follow-up a breve e lungo termine per questi pazienti.

Stabilendo infatti un solido quadro di marcatori, è possibile contribuire allo sviluppo di protocolli basati su evidenze oggettive che migliorino il recupero post-dimissione di questi individui. Questo studio è di fondamentale importanza non solo per le popolazioni coinvolte, ma ha anche il potenziale di suggerire strategie più ampie per la gestione dell'alterazione neuromuscolare nei diversi ambiti clinici. In particolare, lo studio sottolinea che il ricovero in terapia intensiva, così come l'insorgenza di patologie non trasmissibili con un forte impatto neuromuscolare, comportano specifiche alterazioni sia a livello centrale che periferico. Inoltre, queste alterazioni presentano comportamenti e modifiche differenti nei soggetti che accedono al follow-up, differenziate soprattutto per età e genere, sottolineando l'importanza di sviluppare programmi di riabilitazione personalizzati e soggettivati alle esigenze specifiche di ciascun individuo.

Abstract

The onset of non-communicable neuromuscular diseases and prolonged stays in the intensive care unit have deep implications for physical functioning and neuromuscular health. These repercussions arise from muscle deconditioning, systemic inflammation, and the direct impact of the pathology. Moreover, resulting fatigue and acquired muscle weakness contribute to reduced muscular performance, significantly hampering recovery and diminishing overall quality of life.

The predominant motor impairments observed in these patients primarily manifest in their ability to perform correct walking, substantially limiting their independent execution of daily activities. This compromised excitability in descending motor pathways becomes evident during the acute phase of the disease and intensifies as the condition progresses chronically, exacerbated by prolonged physical inactivity. Consequently, this significantly affects the muscle's contractile properties, leading to critical alterations that influence the nervous systems' capacity to recruit and modulate the activity of motor units, the fundamental functional units responsible for planning, executing, and maintaining motor gestures.

Given the complexities entailed by these considerations, it becomes crucial to identify markers that enable the quantification and characterization of physical functioning impairment, muscle weakness and fatigue. This would facilitate early diagnosis and, crucially, the ongoing monitoring of these issues. Thus, the primary goal of this PhD research is to identify and analyze markers that can effectively support healthcare practitioners in devising and delivering personalized therapeutic approaches to expedite the recovery of these individuals.

The overarching objective is to optimize the current clinical practice commonly employed in hospitals. Despite ongoing refinements, these practices still exhibit limitations. While standard assessments succeed in identifying the presence of fatigue and muscle weakness in ICU patients or those afflicted by non-communicable diseases, they fall short of investigating the root causes of muscle strength deterioration or thoroughly probing the central and/or peripheral factors contributing to the emergence of pathological fatigue.

To comprehensively bridge this existing gap, the study undertook an extensive exploration by measuring concurrent joint torques and surface electromyography across various pathological conditions, encompassing both voluntary and electrically induced muscle activations. The focal point was the tibialis anterior muscle, chosen for its pivotal role in gait patterns and consequential influence on individual autonomy.

The presented results were mainly achieved through the decomposition of signals recorded using the High-Density Surface EMG technique. This technique enabled the analysis of individual motor units recruited during motor tasks administered to patients within the studied populations, in both the temporal and spatial domains. The process of data collection and analysis revealed that variations in muscle

strength values and motor unit parameters can serve as indicators of neuromuscular system alterations and progressive recovery. These factors are pivotal for subsequent follow-up procedures.

Indeed, by establishing a robust framework of markers, is possible to contribute to the development of evidence-based protocols that enhance the post-discharge care of these individuals. This study is not only pivotal for these specific cohorts but also holds the potential to inform broader strategies for managing physical impairment and neuromuscular challenges in diverse clinical settings. Notably, the study highlights that hospitalization in intensive care, as well as the onset of non-communicable pathologies with high motor impact, leads to specific alterations in parameters of both central and peripheral neuromuscular pathways. Moreover, these alterations exhibit distinct trajectories during the follow-up period, differentiated by gender. This underscores the imperative for devising personalized rehabilitation regimens tailored to each patient's needs.

List of abbreviations

½RLT ..... Half-relaxation time
6MWT ..... Si-minute walking test
ARDS ..... Acute respiratory distress syndrome
B ..... Bradykinesia
CBS ..... Corticobasal syndrome
CMAP ..... Compound Muscle Action Potential
CNS ..... Central Nervous System
CO ..... Cross-over
CoV ..... Covariation
CT ..... Contraction Time
D ..... Delay
DB ..... Double-Blind
DDR ..... Discharge Rate at derecruitment
DLB ..... Dementia with Lewy Bodies
DLPFC ..... Dorsolateral Prefrontal Corte
D_{MMG} ..... Delay of mechanomyogram
DR ..... Discharge Rate
D_T ..... Delay of torque
DT ..... Derecruitment Threshold
F ..... Falls
FC ..... Frontal Corte
FSS ..... Fatigue Severity Scale
G ..... Gait
GL ..... Gastrocnemius Lateralis
GM ..... Gastrocnemius Medialis
GMF ..... Global Motor Function
HADS ..... Hospital Anxiety and Depression Scale
HDsEMG ..... High Density surface Electromyography
HF ..... High Frequency
HGS ..... Handgrip strength
ICU ..... Intensive Care Unit
ICUAW ..... Intensive Care Acquired Weakness
iEMG ..... intramuscular EMG
ISI ..... Interspike Interval Covariation

LF ..... Low Frequency
LMM ..... Linear mied-effect models
M1 ..... Primary Motor Cortex
MC ..... Muscle compliance
MEP ..... Motor evoked potentials
MMG ..... Mechanomyogram
MMG_{0T} ..... MMG at the end of torque reduction
MoCA ..... Montreal Cognitive Assessment
MRC ..... Medical Research council
MSA ..... Multiple system atrophy
MU ..... Motor Unit
MUAP ..... Motor Unit Action Potential
N ..... Normalized
OC ..... Occipital
OL ..... Open-Label
P-P ..... Peak-to-peak
PD ..... Parkinson's Disease
PENT ..... Peroneal Nerve Test
PG ..... Parallel Group;
PICS ..... Post Intensive Care Syndrome
PNS ..... Peripheral Nervous System
PSP ..... Progressive supranuclear palsy
RDR ..... Discharge Rate at recruitment
RR ..... Rate of reduction
RR_{MMG} ..... Reduction of mechanomyogram signal
RR_{T} ..... Reduction for torque signal
RT ..... Recruitment threshold
SB ..... Single-Blind
sEMG ..... surface Electromyography
SMA ..... Supplementary motor area
SOL ..... Soleus
T ..... Tremor
TA ..... Tibialis Anterior
TB ..... Theta Burst
TMS ..... Transcranial magnetic stimulation

TR.....Time of reduction

TRMMG.....Time of reduction of mechanomyogram

TRT ..... Time of reduction of torque

US.....Ultrasound

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1. Chapter One: Introduction

The present project was carried out at the Department of Clinical and Experimental Sciences - University of Brescia, Brescia, Italy, in collaboration with the Teresa Camplani Foundation at Domus Salutis, Brescia, Italy and ASST Spedali Civili University Hospital, Brescia, Italy.

1.1 Problem Statement

The ability to independently perform activities of daily living is determined by the efficiency with which the neuromuscular system can recruit and harmoniously coordinate various muscle groups involved in movement. The collaborative interplay between the central (CNS) and peripheral nervous systems (PNS) facilitates real-time programming, execution, and dynamic adjustments of movements, driven by continuous feedback from the surrounding environment.

The physiological aging process, as well as the onset of non-communicable pathologies affecting the neuromuscular system and the consequences of intensive care unit (ICU) stays, can compromise this tight interaction, resulting in the individual's inability to maintain efficient neural control and, consequently, a significant limitation in their autonomy.

One of the primary factors contributing to this inability to generate and sustain adequate muscle contractions for the required tasks is fatigue. Its occurrence is a common consequence of aging (Vestergaard et al., 2009), stroke (Wang et al., 2020), Parkinson's disease (Friedman et al., 2007) or ICU stays (Vanhorebeek et al., 2020).

In addition to age-related increases (Vestergaard et al., 2009), in the context of stroke, the aftermath of an acute cerebrovascular event manifests a spectrum of deficits on the affected side. These include hemiparesis, paresthesia, sensory loss, muscle co-activation (Kitatani et al., 2016), and cognitive impairment (Paciaroni & Acciarresi, 2019), all of which contribute to the onset of fatigue (Wang et al., 2020). Likewise, subsequent to hospitalization, complications like polyneuropathy and myopathy can arise, both of which are linked to muscle weakness and fatigue (Latronico & Bolton, 2011). Lastly, fatigue is one of the most debilitating symptoms in patients with Parkinson's Disease (PD) (Lin et al., 2021). Despite its high prevalence, the underlying causes of fatigue are still unknown. It is believed that fatigue can occur independently or in conjunction with other symptoms such as depression and sleep disorders (Kluger et al., 2016) and may be linked to cognitive impairment (Herlofson & Kluger, 2017). While fatigue in PD is rarely associated with CNS alterations, the precise central causes of fatigue remain unclear (Chaudhuri & Behan, 2004).

The onset of fatigue varies significantly depending on the underlying medical condition and can have pronounced short-term and long-term consequences (Vanhorebeek et al., 2020). Indeed, its effects can persist for several months after hospitalization (Joli et al., 2022), promoting physical inactivity and the

subsequent loss of muscle mass. This creates a cycle that exacerbates functional impairment over time and affects motor control and stability. This has significant implications for the lower limbs, negatively affecting balance, coordination and stability during walking or maintaining equilibrium, and thereby impacting the daily lives of discharged patients.

1.2 Research Goal

The work presented in this thesis aims to tackle the functional limitations resulting from aging, non-communicable diseases and extended ICU hospitalization. Its primary goal is to optimize clinical assessments during the follow-ups through innovative techniques to enhance the planning of therapeutic interventions, facilitating the regaining of autonomy in daily activities for individuals after hospital discharge. To achieve this objective, it is crucial to establish a precise and effective method for assessing neuromuscular damage, muscle weakness, and fatigue and to formulate an efficient rehabilitation strategy aimed at preserving existing motor skills and recovering those that have been lost to the greatest possible extent.

To investigate these factors, four different populations were studied (Figure 1): PD patients (study 1), elderly individuals (study 2), subjects in the acute and chronic phases of stroke (study 3), and patients discharged from intensive care units (studies 4, 5 and 6).

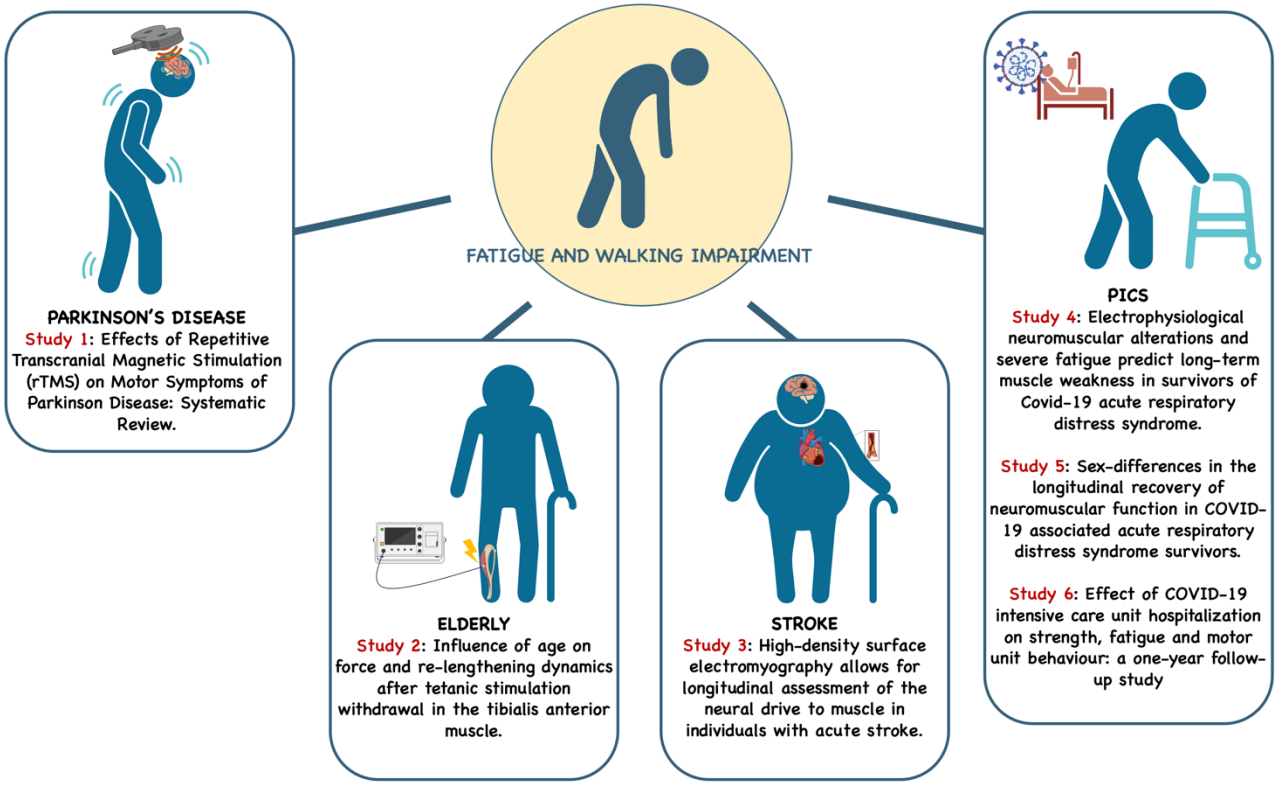

Figure 1: Illustrative representation of the four topics of the Ph.D. project with relative studies (listed below each topic).

The impairment that primarily combines these populations is gait deficit. In patients with PD, gait patterns are profoundly altered by the characteristic symptoms of the condition, such as bradykinesia, rigidity, and reduced amplitude and automaticity of movement. As the disease advances, gait problems worsen, greatly impacting independence and quality of life (Mirelman et al., 2019). In the elderly, gait is significantly impaired due to the slowing of the antagonist muscle's relaxation, which opposes the work of the agonist muscle in rapid and alternating movements (Hunter et al., 2016). Following a stroke, the acute event alters the activity of agonist and antagonist muscles involved in plantar and dorsal flexion of the foot, preventing proper gait (Silva et al., 2015). In individuals discharged from the ICU, gait deficit is associated with muscle atrophy and the onset of myopathies that interfere with proper muscle activation (Latronico & Bolton, 2011). For this reason, the muscle studied in this thesis is the tibialis anterior muscle (TA). Given its significant role in gait, it holds a pivotal position in daily activities and, consequently, in an individual's autonomy. Furthermore, the TA is one of the most extensively studied muscles in motor unit (MU) literature among healthy adults. Hence, it was chosen due to its suitability for comparing our results to prior studies (Arene & Hidler, 2009; Bagnato et al., 2020; Chou et al., 2013; Connelly et al., 1999; McNeil et al., 2005; Trojaborg et al., 2002). Subjects with gait impairment, irrespective of the underlying causes, are predisposed to a decrease in their daily physical activity levels (Nimwegen et al., 2011; Sandroff et al., 2015). This reduction in physical activity can contribute to muscle weakness and fatigue, which is a multifaceted phenomenon characterized by diminished muscle force output, thereby influencing motor control and stability (Boyas & Guével, 2011). Fatigue can stem from various factors, including alterations in CNS control and impaired peripheral factors.

Therefore, investigating these mechanisms that contribute to its onset in the lower limbs is of primary importance, as it has a profound impact on balance and stability during everyday activities such as standing, walking, or running, subsequently affecting efficiency and safety. Consequently, a better understanding of how the CNS and PNS deal with fatigue could provide valuable insights for planning interventions aimed at the functional recovery of these individuals.

In this context, the analysis of the electrical signals generated by muscle activation through electromyography (EMG) serves as an excellent tool for studying the central and peripheral factors that affect motor control. While traditional EMG techniques are commonly used in clinical practice to assess peripheral factors like nerve conduction and compound muscle action potential (CMAP) (Agergaard et al., 2021; Cabañes-Martínez et al., 2020; Daia et al., 2021; Jolley et al., 2016; Labarre-Vila, 2006; Latronico et al., 2021; Villa et al., 2021), they have limitations in deducing CNS changes.

To overcome these limitations, the Ph.D. work employs high-density surface EMG (HDsEMG) instead of the classical bipolar EMG. HDsEMG involves the use of multiple electrodes to target muscles during voluntary contractions, providing a promising approach to investigate both central and peripheral mechanisms related to functional changes in ICU patients (Bazzucchi et al., 2004; Pradhan et al., 2020)

or after stroke (Gallina et al., 2016; Miller et al., 2014). Indeed, this non-invasive approach enables the analysis of individual MU discharge patterns, where factors such as mean discharge rate (DR) and recruitment (RT) or de-recruitment threshold (DT) are primarily influenced by central mechanisms (Castronovo et al., 2015; Duchateau & Baudry, 2014; Martinez-Valdes et al., 2020) and the quantification of MU action potential (MUAP) amplitude, which provides insights into the PNS (Kallenberg & Hermens, 2006; McPherson et al., 2016).

Therefore, the overarching research objectives are to utilize these objective and quantitative measures as valuable tools for identifying neuromarkers of physical functioning and neuromuscular fatigue in COVID-19 patients who have been discharged from the ICU and subjects affected by non-communicable disease.

1.3 Research Aims

This Ph.D. work aims to identify neuromarkers for detecting neuromuscular system alterations in the acute and chronic phase of the pathological event and for objectively monitoring patients' progressive recovery during various follow-up assessments. The goals previously discussed were explored through the following research aims:

1. To compare, during the aging process, the dynamics of both the ankle torque decline and muscle re-lengthening, in the tibialis anterior.
2. To assess the neuromuscular function of patients undergoing rehabilitation therapy in the acute phase post-stroke, combining high-density sEMG (HDsEMG) decomposition with isometric force recording to quantify changes in force production and MU discharge rates.
3. To assess the markers of muscle weakness in patients admitted to the ICU for severe acute respiratory syndrome 2 (SARS-CoV-2) infection, evaluated 6 and 12 months after discharge with in-person visits.
4. To assess sex differences in MU properties and muscle weakness in patients admitted to the ICU for severe acute respiratory syndrome 2 (SARS-CoV-2) infection up to one year or more following ICU discharge.
5. To assess central and peripheral neuromuscular parameters underlying physical functioning recovery of COVID-19 patients up to 1 year following ICU discharge, and to offer a solid and objective method to monitor the progression of pathology in these patients.
6. To explore the therapeutic impact of rTMS on motor function in individuals diagnosed with Parkinson's disease or atypical parkinsonism, identifying the most effective protocol to relieve symptoms both in the short and long term.

2. Chapter Two: Background

2.1 Fatigue

As Mosso (La Fatica, 1891) suggested, fatigue is a debilitating symptom resulting from the combination of energy depletion due to physical effort and the psychological perception of the effort itself. This duality implies both a physical and a cognitive component, the interaction of which is crucial for the ability to perform and, more importantly, sustain a motor task over time. Indeed, this interconnection appears to be the cause of a reduction in force output, changes in electromyographic activity, alterations in mental processes, and the onset of motor deficits (Enoka & Duchateau, 2008; Gandevia, 2001; Scholey & Apps, 2022; Whittaker et al., 2019; Wiehler et al., 2022) (Figure 2). In the scientific literature, there has been a longstanding inquiry into the factors contributing to the emergence of this complex multifactorial phenomenon that influences motor control and stability (Boyas & Guével, 2011). One of the more recent contributions in this field comes from Enoka & Duchateau (Enoka & Duchateau, 2016), who have introduced a taxonomy aimed at elucidating how fatigue indeed stems from two interdependent attributes, namely, performance fatigability and perceived fatigability. This is aimed at bridging the gap in the correct usage of terminology and the conceptualization of fatigue, which refers to an exercise-induced impairment of motor performance and, therefore, to psychophysical exhaustion following strenuous exercise or after prolonged activity. It also includes the sensation of tiredness and weakness that arises concurrently with or because of adverse clinical conditions.

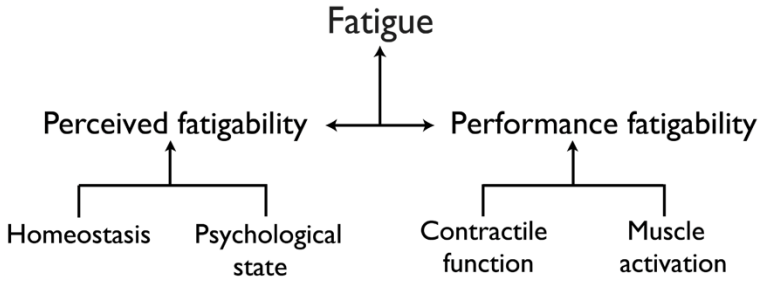

Figure 2: Taxonomy of fatigue (Enoka and Duchateau, 2016)

Given these considerations, an in-depth examination of lower limb fatigue assumes paramount significance, owing to its direct correlation with falls (He et al., 2022; Morrison et al., 2016; Renner et al., 2021) resulting from compromised balance and posture control (Blake et al., n.d.; Granacher et al., 2010). Notably, falls afflict approximately 35% of the population aged 65 and above (Granacher et al., 2010) both to accidental events and, to a large extent, to decrements in postural control and balance (Blake et al., n.d.). Consequently, is crucial to identify the causes that lead to perceived or performance fatigability to counteract the loss of autonomy in gait and the hospitalization due to fall injury. Hospitalization for fall-related fractures is indeed associated with the death of approximately 25% of patients admitted for severe fractures and 4% of those without (Coutinho et al., 2012) within a one-year

timeframe from the traumatic event (Hartholt et al., 2011). Among these cases, the risk of death for elderly hospitalized individuals increases during the first 3 to 6 months after the injury (Joosten et al., 2014), to the extent that two-thirds of deaths due to fractures occur within the initial 3 months of hospitalization (Abrahamsen et al., 2009; Haentjens et al., 2010).

Therefore, fatigue can significantly impact individuals' balance and stability during standing, walking, or running, especially when compounded by the physiological decline associated with aging or neuromuscular comorbidities such as PD, Stroke, or post-intensive care syndrome (PICS). This leads to processes that disrupt both central factors, including the gradual decrease in muscle activation by the CNS, and peripheral factors, stemming from alterations at and beyond the neuromuscular junction (Gandevia, 2001; Taylor et al., 2016).

One of the key joints involved in walking movements is the ankle. As emphasized by Vuillerme et al. (Vuillerme et al., 2002), it has been observed that ankle fatigue leads to an increased demand for attention during the regulation of static postural control. This issue can be initially compensated for by proprioceptive abilities, but as performance fatigability gives way to perceived fatigability, the ability to sustain concentration for prolonged motor tasks becomes compromised. Consequently, this leads to an increased risk of falls.

This Ph.D. study primarily focused on the investigation of the muscles involved in ankle activation to provide support and enhanced objectivity to the well-established clinical practice in assessing muscle weakness and fatigue correlated with potential gait disturbances.

2.2 Aging

The current global population aged 65 and older represents approximately 9% of the world's population. The continuously improving quality of life and life expectancy suggest that this percentage is inevitably set to increase in the coming years, accompanied by a growing need to address all the psychophysiological issues associated with aging.

From a motor control perspective, aging is characterized by multifaceted alterations affecting both central and peripheral components. These changes encompass anatomical and physiological transformations that result in deficits within the CNS, sensory function modifications, alterations in peripheral nerve conduction (Seidler et al., 2010), decrease in muscle fibers cross-section, change in connective tissue (Buckwalter et al., 1985), hypotrophy of rapid fibers, along with the loss of MUs. Specifically, the latter progressively leads to a reduction in precision and an increase in the complexity and fatigue associated with performing motor tasks as individuals age (Voelcker-Rehage & Alberts, 2005).

During the initial stages of aging, the process of re-innervation becomes active, enabling adjacent motor neurons to reinnervate muscle fibers that have become isolated, thanks to axonal sprouting.

However, a critical phase emerges around the age of 80, when this process can no longer keep up with the depletion of MUs. This results in a diminishing ability to control strength and a parallel, gradual decrease in maximal force output (Aagaard et al., 2010; Orizio et al., 2016).

These neuromuscular system alterations have a profound impact on the autonomy of older individuals, manifesting as difficulties in coordination, heightened movement variability, slowed motion, and challenges in maintaining balance and gait (Seidler et al., 2010). These issues, particularly concerning lower limb control, inevitably increase the risk of falls, and possible related bone fractures, which represents one of the main causes leading to a substantial reduction in daily physical activity in favor of a sedentary lifestyle and muscle weakness (Roubenoff & Hughes, 2000). Consequently, there is a pronounced decline in overall quality of life.

Fatigue in elderly

The reduction in the number of MUs, with a particular impact on type II MUs, and changes in contractile properties are the primary causes of decreased strength in older individuals (Bendall et al., 1989). During aging, the process of reinnervation, which helps limit the progression of neuromuscular deficits, involves the formation and increase of slow type I fibers due to axonal sprouting. This results in lower force accuracy (Roubenoff & Hughes, 2000) and a significant reduction in maximal force generation (Rubinstein & Kamen, 2005a). Several studies (Chan et al., 2000; Kent-Braun et al., 2002; Lanza et al., 2004; Narici et al., 1991; Rubinstein & Kamen, 2005b) indicate that this complex physiological process characteristic of aging leads to greater resistance to fatigue in older adults compared to young individuals. This is primarily due to the higher proportion of fatigue-resistant slow type I fibers as opposed to fast type II fibers. Consequently, older individuals may exhibit reduced strength and slower performance compared to younger individuals but are more resistant to the onset of fatigue.

The search for the causes underlying the onset of fatigue in older individuals should not be strictly attributed to the properties of MUs but rather to physical inactivity and the multifactorial nature of cognitive components. In the elderly, there is a predominant correlation between levels of physical inactivity and an increase in both muscle fatigue and perceived fatigue (Torossian & Jacelon, 2020), primarily caused by muscle mass loss. Moreover, from a cognitive standpoint, fatigue in elderly is closely linked to reduced sleep, anxiety, and depression (Moreh et al., 2010).

It is therefore crucial to design appropriate physical activity programs aimed at fall prevention and the preservation of motor efficiency in these individuals. It is important to emphasize that the innervation process becomes less effective in countering the loss of MUs as age advances (McNeil et al., 2005), resulting in further reductions in muscle strength, decreased movement precision, a higher likelihood of falls, and early fatigability. The latter serves as an independent predictor of mortality in this population (Moreh et al., 2010; Torossian & Jacelon, 2020).

2.3 Parkinson's Disease

Parkinson's disease is a chronic neurodegenerative disorder primarily diagnosed after the age of 50 (Lees et al., 2009). PD predominantly affects the extrapyramidal system, responsible for precise movement control, and distinguishes itself from other forms of Parkinsonism. The principal cause of PD is the degeneration and death of dopamine-synthesizing cells in the substantia nigra, a component of the basal ganglia. While the exact causes remain unknown, environmental and genetic factors, alongside aging, mitochondrial dysfunction, and immune responses, are believed to contribute (Kline et al., 2021)

PD symptoms initially affect one side of the body and later spread bilaterally, although the initial side's involvement remains prominent. These symptoms encompass various motor aspects and become apparent when approximately 80% of dopaminergic cells have degenerated (Sveinbjornsdottir, 2016). Resting tremors occur in about 70% of PD patients, predominantly in the upper limbs, often described as "coin-counting" tremors. Rigidity, due to increased muscle tone, initiates in the neck and shoulders, subsequently spreading to the trunk and extremities, making movement challenging. Bradykinesia, the most influential factor in PD-related disabilities, results in slow, limited movement initiation and modification. Walking disturbances appear as the disease progresses, including hesitancy, shuffling steps, and the characteristic camptocormia. Balance issues manifest late in the disease course, heightening the risk of falls (Kim et al., 2018). Developing tailored rehabilitation programs that consider individual capacity and addressing the motivational aspects of daily life are essential steps in effectively managing this complex condition.

Fatigue in PD patients

Fatigue is a prevalent and often underestimated symptom in PD, affecting around 44% of patients (Friedman et al., 2007). Interestingly, it appears to be independent of other PD symptoms and can significantly impact patients' daily lives. As the disease progresses, fatigue can become chronic, contributing to worsened physical disability and complications like cardiovascular disease, diabetes, cognitive decline, osteoporosis, depression, insomnia, and constipation (Friedman et al., 2016). Recognizing and managing fatigue is crucial in improving the overall well-being and quality of life of individuals living with PD.

2.4 Stroke

Stroke can be categorized into two main types: ischemic stroke, which accounts for most cases, and hemorrhagic stroke. Ischemic strokes result from thrombotic or embolic occlusions of blood vessels, while hemorrhagic strokes stem from the rupture of weakened vessels (Andersen et al., 2009). The pathophysiology of stroke involves complex cellular events triggered by reduced blood flow, leading to

hypoxia or anoxia. Energy failure within neurons sets off a cascade of destructive processes, including ion gradient disruption, oxidative damage, inflammation, and cell death (Su et al., 2022).
The consequences of stroke are critical, with millions of deaths and permanent disabilities attributed to its occurrence. Notably, individuals aged 60 and above are most susceptible to stroke, constituting the most affected individuals.

Stroke impairs various aspects of an individual's life, most notably affecting motor function. Upper limb dysfunction remains a prevalent impairment among stroke survivors, with a mere 5% experiencing complete motor function recovery (Hatem et al., 2016). Another compelling problem concerns the ability to walk safely and efficiently. Lower limb motor deficits are common and often result in hemiplegia, impacting walking, balance, and coordination. Furthermore, stroke survivors frequently exhibit altered muscle co-activation patterns, prolonged contractions of agonist and antagonist muscles, and asymmetries during walking, resulting in gait abnormalities (Souissi et al., 2018). This impaired walking autonomy stems not only from neurological deficits resulting directly from the stroke but also from physical deconditioning due to post-stroke inactivity (Botö et al., 2021). Over time, these deficits lead to a decline in aerobic capacity and sensorimotor control, posing a severe threat to the health of stroke survivors and increasing the likelihood of relapse. Consequently, a substantial portion of stroke survivors cannot fully return to work or engage independently in daily activities, often necessitating support devices or assistance from caregivers.

Rehabilitation therapy plays a crucial role in the progressive recovery of limb function, enabling patients to regain muscle activation patterns and adapt to new motor strategies. Understanding the complex interplay of neurological deficits, compensatory mechanisms, and rehabilitation outcomes is crucial in addressing the diverse needs of this population.

Fatigue in stroke patients

Post-stroke fatigue is a prevalent concern affecting a variable percentage of survivors, ranging from 39% to 72% (Puchta, 2008). It encompasses both physical and mental components and represents one of the most disabling symptoms, exhibiting diverse characteristics depending on its onset relative to the acute phase (Paciaroni & Acciarresi, 2019).

During the immediate post-acute phase, fatigue may manifest after intense physical exertion or mental effort, characterized by rapid onset, short duration, and swift recovery. In this context, performance fatigability predominates, manifesting as motor disorders leading to a decrease in output torque and impaired motor control (Kang & Cauraugh, 2015; Patten et al., 2004). These manifestations can be quantified as a reduction in maximal torque or an inability to sustain sub-maximal torque during contractions (Enoka & Duchateau, 2008).

Conversely, chronic fatigue emerges in the late post-stroke phase and is characterized by a significant perceived fatigability component, encompassing mental and psychological symptoms. These symptoms

can evolve into depression, anxiety, sleep disturbances, and overall tiredness, initiating a cycle that often results in reduced physical activity and a subsequent exacerbation of issues.

Given the substantial impact of post-stroke fatigue on rehabilitation, survivors' quality of life, and mortality, a multidisciplinary approach becomes imperative. Such an approach should integrate pharmacological and non-pharmacological treatments specifically tailored to address both physical and psycho-behavioral factors.

2.5 Post Intensive Care Syndrome

Millions of patients are admitted to ICUs annually, with approximately one-third requiring mechanical ventilation. A substantial proportion of individuals admitted to intensive care for acute respiratory distress syndrome (ARDS) subsequently develop PICS, characterized by prolonged physical, mental, and cognitive impairments. Indeed, PICS patients exhibit psychosocial symptoms, including impaired concentration, short-term memory deficits, anxiety, depression, reduced motivation, and post-traumatic stress disorders (Hiser et al., 2023; Lee et al., 2020; Rawal et al., 2017), as well as physical manifestations such as muscle weakness and fatigue (Parry et al., 2020; Puthucheary et al., 2013; Rawal et al., 2017; Vanhorebeek et al., 2020). The degree of neuromuscular impairment, closely linked to the manifestation of fatigue, develops during the hospitalization period and is strongly influenced by the duration of mechanical ventilation, age, gender, and length of hospital stay (Vanhorebeek et al., 2020). Prolonged bed rest and the development of neuropathies or myopathies during hospitalization result in physical inactivity and, consequently, muscle atrophy, significantly reducing the ability to generate and maintain strength over time (Latronico & Bolton, 2011).

Muscle mass loss begins in both limbs as early as the first week of the pathology onset and may persist for several months or years (Parry et al., 2020; Puthucheary et al., 2013). This weakness negatively impacts common daily movements involving the lower limbs, such as walking, maintaining balance, rising and sitting down, and climbing stairs, resulting in a loss of autonomy and significantly increasing the risk of falls (Parry et al., 2020). This results in the need for a caregiver's constant presence. The study conducted by Parry et al. (Parry et al., 2020), despite a limited sample size, demonstrates that approximately 50% of ICU survivors experience at least one fall within the subsequent 6 months following the acute phase of critical illness. This consequently leads to a substantial risk of fractures and further hospitalizations, which, in turn, are destined to increase the mortality risk (Hermans et al., 2014a; Hill et al., 2002), already correlated with ICUAW (Hermans et al., 2014b).

All of this is exacerbated by cognitive impairment. Patients, in fact, exhibit difficulties in sustaining concentration, planning and organizing tasks, or executing complex motor activities that require a cognitive component. This occurs in nearly three-quarters of ICU survivors (Rawal et al., 2017). This

cognitive impairment, coupled with the fear of falling and the insecurity in performing even simple movements independently, considerably hinders the return to normal daily life.

Fatigue in PICS patients

Fatigue, reported by about two-thirds of ARDS survivors, emerges as one of the predominant symptoms, surpassing other symptoms such as physical dysfunction, cognitive decline, anxiety, and depression (Latronico et al., 2017; Neufeld et al., 2020). Both central and peripheral factors can contribute to its onset, even in the acute phase of the disease, persisting and evolving in the months following discharge, negatively impacting the quality of life of survivors (Joli et al., 2022).

Fatigue, accompanied by a decline in an individual's physical functioning, can be attributed to the onset of muscle atrophy and weakness, as well as other early-initiated pathophysiological mechanisms during hospitalization and the onset of anxiety, depression, pain, and other cognitive impairments after discharge (Latronico et al., 2017). These effects may endure for up to 6 months following discharge from the intensive care unit (Dos Santos et al., 2016). Notably, in conjunction with ICU-acquired weakness (ICUAW), patients may also demonstrate reduced fatigue resistance, which is characterized by an objective decline in physical performance during the execution of a motor task. Unlike muscle weakness, which signifies a muscle's diminished capacity to generate maximal force at rest, reduced fatigue resistance indicates an individual's inability to sustain prolonged effort due to neuromuscular and functional alterations.

Fatigue in patients with PICS is a symptom that can persist for varying durations, ranging from a few days to several months after discharge from the ICU, potentially progressing to a chronic condition (Morel et al., 2022; Wintermann et al., 2018b, 2018a). This fatigue serves as both a barrier to engaging in physical exercises and a cause and consequence in a cyclical relationship. The lack of physical activity primarily results in muscle weakness and wasting, which, in turn, are closely linked to the onset of fatigue itself.

It becomes imperative, therefore, to optimize the assessment and implementation of a therapeutic plan that enables these patients to maintain an adequate level of physical activity, which, in turn, helps keep fatigue levels within tolerable ranges. As suggested by Morel *et al.* (Morel et al., 2022), this approach would facilitate the reinforcement of neuromuscular deconditioning and enhance overall quality of life.

2.6 Electromyography

The generation of action potentials in muscle fibers plays a fundamental role in enabling muscle contraction and force production. This allows for the execution of daily life activities, ranging from the simplest and most common ones such as walking and standing, to more complex ones involving fine motor skills such as writing or sewing. The motor gesture, depending on its complexity, demands the

modulation of force output, which is influenced by various factors such as the involved muscle group, the required force, the subject's motor skills, and the presence of conditions that may disrupt its planning and execution (Christou, 2011). Nevertheless, the muscle contraction process remains common to any motor activity.

Muscle contraction begins with the generation of biopotentials, which result from changes in the resting membrane potential of a cell. An external stimulus, such as a neurotransmitter, must be sufficiently strong and sustained to exceed the excitation threshold, thereby initiating the process of depolarization, followed by repolarization involving sodium (Na+) and potassium (K+) ions. This change in ion conductance gives rise to the action potential, which occurs in an all-or-none manner and simultaneously in all muscle fibers. It also triggers the release of calcium (Ca2+) from the sarcoplasmic reticulum within the fibers themselves. This allows for the interaction between the actin and myosin filaments, which alters the length of the sarcomere, leading to the occurrence of the power stroke, or muscle contraction. This entire process of action potential generation occurs rapidly, typically within milliseconds, and is characterized by a cascade of events that extend from the activation of the primary motor cortex to the activation of the muscle fibers (Figure 3).

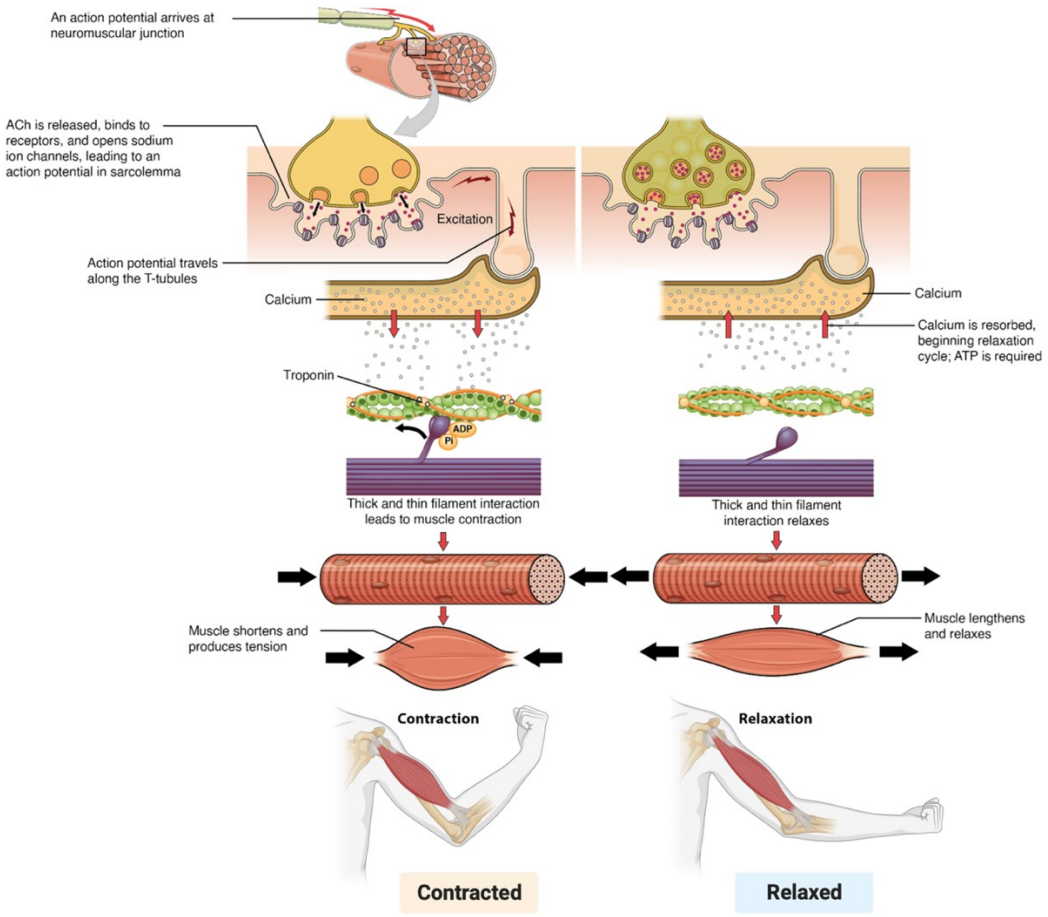

Figure 3: illustration of muscle contraction process. From the primary motor cortex, the action potential arrives at the neuromuscular junction and the influx of sodium (Na+) into the cell starts. This is followed by the release of calcium (Ca2+) from the sarcoplasmic reticulum. Calcium activates actin-myosin coupling, leading to muscle contraction. At the end of the action potential, calcium returns to the sarcoplasmic reticulum, the actin-myosin cross-bridges relax, and the muscle prepares for the next cycle (Biga M. et al., 2019).

Muscle fibers are organized into functional units known as MUs, each consisting of a motor neuron and the muscle fibers it innervates. These motor neurons are located within the spinal cord or brainstem and establish exclusive connections with specific groups of muscle fibers. Upon receiving a motor command, motor neurons transmit action potentials, leading to synchronized contractions of the innervated muscle fibers. The CNS regulates the intensity and speed of muscle contractions through excitatory or inhibitory influences on MUs. Meanwhile, the PNS is characterized by the number and dimensions of the fibers composing the MU itself, which in turn influence the maximum achievable force.

The action potential generated by each active muscle fiber during muscle contraction concur to the global electrical activity that can be detected by electrodes as a biological signal named electromyography (EMG). EMG is a valuable tool for studying muscle activity in various conditions, such as during maximal or submaximal contractions, precision tasks, and fatigue. Indeed, by studying MUAP for medical-rehabilitative purposes, along with torque output measurements, it is possible to assess strength deficiencies, muscle fiber alterations, motor neuron diseases, or nerve conduction issues.

In clinical practice, two common types of electromyography (EMG) are employed: surface EMG (sEMG) and intramuscular EMG (iEMG).

Surface EMG, the first method, offers a less invasive and rapidly applicable approach, facilitating the investigation of a wide area within the target muscle. However, electrode placement on the skin, at a distance from muscle fibers, can potentially affect the amplitude of MUAPs, particularly in cases involving subcutaneous fat or hair. Additionally, the simultaneous activation of multiple muscles (crosstalk) may result in overlapping MUAPs, presenting a challenge in discerning individual MU discharges or isolating the specific muscle of interest.

iEMG, is an invasive method that could be performed by a highly trained specialist. Its advantage lies in the ability to perform an in-depth analysis of the muscle, bypassing the limitations associated with the surface technique. However, iEMG provides limited information restricted to the specific area where the needle is inserted. To obtain a comprehensive view of the muscle, multiple needle insertions would be necessary, potentially causing discomfort to the patient.

To overcome these limitations, studies 3, 5, and 6 have been conducted using the HDsEMG technique. The hypothesis is that this approach, in conjunction with torque output measurements, enables an objective assessment of neuromuscular damage in the various populations under investigation (PD, stroke, and PICS). Furthermore, these data will yield sensitive biomarkers with clinical applications for the restoration of neuromuscular function. Indeed, the use of a 64-electrodes matrix allows to cover a large area of multiple muscles simultaneously, analyzing the MUAPs of the activated MUs in the domains of space and time.

Indeed, once recorded the EMG signals from the different muscles involved in the motor tasks required to the patients, the application of the convolutive blind source separation algorithm (Negro et al., 2016) enables the extraction of individual MUs. This extraction allows for the analysis of their characteristics, including the total number of MUs engaged and their discharge patterns.

3. Chapter Three: Summary

Study 1: Effects of Repetitive Transcranial Magnetic Stimulation (rTMS) on Motor Symptoms of Parkinson's Disease: Systematic Review.

Parkinsonism, including Parkinson's Disease (PD), is a group of chronic progressive neurodegenerative disorders primarily affecting movement control and balance. Diagnosing PD can be challenging due to its similarity to atypical Parkinsonian disorders such as multiple system atrophy, dementia with Lewy Bodies, progressive supranuclear palsy, and corticobasal syndrome, all of which share similar symptoms. These symptoms include resting tremor, muscle rigidity, bradykinesia and balance issues, often leading to gait difficulties, camptocormia, and freezing of gait. New rehabilitation therapies such as repetitive Transcranial Magnetic Stimulation (rTMS), play a crucial role in improving patients' well-being. rTMS is a non-invasive technique that utilizes electromagnetic stimulation of specific brain areas to induce changes in cortical plasticity and is a potential treatment for neurodegenerative diseases. On the other hand, there remains some inconsistency regarding the optimal stimulation parameters, including target areas, frequency, intensity, total number of pulses, and pulse interval. Indeed, this review aimed to explore the existing literature to determine the most effective rTMS settings for addressing motor symptoms in PD. Therefore, a systematic review of the PubMed and Clinicaltraisl.gov databases was performed to identify published and unpublished interventional trials using repetitive TMS for movement disorders in patients with PD or Parkinsonism. Symptoms were categorized into five areas of assessment: UPDRS (Unified Parkinson's Disease Rating Scale), bradykinesia, freezing of gait, falls, and tremor and the efficacy of the different rTMS protocols was evaluated for each specific area. The results display that high-frequency stimulation of the primary motor cortex and supplementary motor area showed the most consistent benefits. However, there's a need to explore new stimulation areas and intensities, especially for tremor and fall prevention.

Pilotto Andrea and Benedini Marco, Negro Francesco, Orizio Claudio, Borroni Barbara, Rizzeti Maria Cristina, Padovani Alessandro. (In submission)

Study 2: Influence of age on force and re-lengthening dynamics after tetanic stimulation withdrawal in the tibialis anterior muscle

The coordinated action of flexor and extensor muscles of ankle, knee, and elbow, classified as agonists and antagonists respectively, plays a pivotal role in facilitating joint movements. Notably, the roles of agonist and antagonist muscles switch each time the direction of joint rotation changes.

Two critical factors influence this changeover and the subsequent re-lengthening phase of the previously active agonist muscle. Firstly, it's influenced by the reduction in tension within the previously active muscle group. Secondly, the compliance of this muscle group to elongate when the new agonist takes over also matters significantly. Understanding these biomechanical parameters during these transitions offers valuable insights into the sequential activity of agonist and antagonist muscles. Indeed, this alternating joint movement is particularly relevant in the context of gait, which can be seen as a comprehensive series of alternating flexion and extension movements across multiple joints. Factors such as aging, imply a delayed relaxation of antagonist muscles that can impede rapid, alternating movements, a phenomenon of great consequence during normal locomotion. This has been associated with a higher risk of falls and hospitalization, making the study of these dynamics crucial for understanding health and mobility in the elderly population. The study aims to comprehensively evaluate the dynamics of muscle tension reduction and re-lengthening after activation, particularly in young and old individuals. Indeed, understanding these processes, especially in the TA due to its significant role in the gait cycle, is crucial.

Twenty young individuals aged between 21 and 33 years, as well as twenty older individuals aged between 65 and 80 years. All participants were recreational and physically active, without any orthopedic or neurological disorders. In the study, their legs were positioned in an ergometer equipped with a load cell to measure the torque produced during electrically stimulated contractions of the TA.

The hip and knee joints were maintained at fixed angles of 90° and 180°, respectively, while the ankle was set at 110° in a neutral position. The participants' feet were securely fastened to a wooden plate connected to the load cell, and the force signal was carefully filtered and amplified for subsequent analysis. To measure the displacement of the TA muscle surface during contraction, an optical laser distance sensor was employed. Electrical stimulation was applied to the TA using skin electrodes positioned over the muscle's primary motor point. sEMG was used to monitor muscle activity throughout the study. The primary focus of the analysis was on the relaxation phase that followed the electrically stimulated contractions. Various parameters, including the relaxation phase duration of the stimulated tetanic contraction, the rate at which torque and MMG signals decreased, and the time it took for both normalized torque and MMG to decrease, were calculated and analyzed.

The key findings indicate that age-related changes in muscle mechanics during the relaxation phase can explain some of the distinctive characteristics observed in the walking patterns of elderly individuals.

Specifically, the time delay between the end of electrical activity and the onset of torque and MMG decay is consistently longer for MMG than for torque. This suggests that force declines before muscle re-lengthening during relaxation, with this complex process influenced by factors such as tendon shortening and other biomechanical elements. Furthermore, it was observed that in both torque and MMG signals, the onset of decay and the reduction velocity occur later and are slower in older individuals compared to their younger counterparts. This difference may be attributed to changes in SERCA characteristics, myosin cross-bridge kinetics, and alterations in muscle and tendon stiffness associated with aging. Indeed, the study's findings suggest that age-related differences in muscle relaxation and re-lengthening processes can contribute to the distinct characteristics of gait observed in elderly individuals, negatively affecting the safe walking in these subjects and increasing their risk of falls.

Cogliati Marta, Cudicio Alessandro, Benedini Marco, Cabral V. Hélio, Negro Francesco, Reggiani Carlo, Orizio Claudio. (European Journal of Applied Physiology, 123(8), 1825–1836)

Study 3: High-density surface electromyography allows for longitudinal assessment of the neural drive to muscle in individuals with acute stroke.

Acute stroke leads to motor impairments that vary based on the affected area of the CNS. These impairments hinder daily activities, exacerbated by inactivity and physical deconditioning following the hospitalization. Early rehabilitation can enhance mobility and independence, yet many patients still have persistent motor deficits. Current knowledge of neuromuscular changes post-stroke primarily stems from chronic-phase studies, leaving the acute phase relatively unexplored but evidence suggests that post-stroke force production issues result from impaired excitability in motor pathways, affecting MU behaviour and recruitment. Bipolar surface EMG (sEMG) is commonly used in clinical and research settings to assess these neurological changes. However, sEMG has limitations due to non-physiological factors. HDsEMG offers instead a more precise evaluation, allowing for non-invasive assessment of MUs discharge patterns. Indeed, this study's main objective was to combine HDsEMG with isometric force measurements to quantify changes in force production and MU DR during different phases of inpatient rehabilitation in acute stroke patients. Additionally, the study aimed to compare two approaches, bipolar sEMG and HDsEMG signal decomposition, for assessing neuromuscular changes in the acute phase of stroke.

To achieve these objectives, seven patients in the acute phase of stroke (less than 12 weeks post-event) were recruited. All patients had severe lower limb impairment. The experimental protocol was repeated in three sessions: one when detectable dorsi- and plantarflexion movements were regained after stroke, another 15 days later, and a third 30 days later. An additional session at the initial time point also assessed the non-affected side. During these 30 days, patients underwent acute rehabilitation therapy, including various exercises and the use of robotic devices. In each protocol session, participants had their legs positioned on an ergometer, and ankle force was recorded using a load cell. The patients were asked to perform different isometric contractions in plantar and dorsiflexion of the foot at two force levels (10% and 30% of MVC), with visual feedback provided. HDsEMG signals were recorded from TA, Gastrocnemius Medialis (GM), Gastrocnemius Lateralis (GL) and Soleus (SOL). Two approaches were used to assess neuromuscular alterations: classical bipolar sEMG and decomposition of HDsEMG signals.

The findings displayed that RMS amplitude from single bipolar sEMG signals did not show significant differences over time at 10% MVC. However, at 30% MVC, there was a reduction in normalized RMS amplitude over time for TA and GL but not for SOL and GM, suggesting potential changes in neural drive input to perform submaximal tasks at the acute phase. However, the subcutaneous layer thickness changing over time can impact the RMS amplitude calculated from bipolar sEMG signals. Moreover, the use of global sEMG makes it difficult to distinguish between changes in MUs' central and peripheral patterns. On the other hand, the HDsEMG signal decomposition approach indicated a decrease in MU

DR on the paretic side during the acute phase of stroke. Notably, significant increases in mean DR were observed during one month of rehabilitation, particularly in the TA and SOL muscles during the 10% MVC task. These changes were not captured by the classical bipolar sEMG approach, highlighting the advantages of HDsEMG analysis in providing more detailed insights into neuromuscular adaptations.
In conclusion, this study underscores the importance of understanding neural adaptations post-stroke and optimizing rehabilitation interventions. Combining force recordings with HDsEMG analysis appears promising for assessing muscle activation patterns and force production capabilities. Such an approach could aid in tailoring rehabilitation programs to individual patient needs and ultimately lead to more effective, personalized rehabilitation strategies to enhance motor function recovery in stroke patients.

Benedini Marco, Cabral V. Hélio, Cogliati Marta, Falciati Luca, Bissolotti Luciano, Orizio Claudio, McPherson M. Laura, Negro Francesco. (Submitted)

Study 4: Sex-differences in the longitudinal recovery of neuromuscular function in COVID-19 associated acute respiratory distress syndrome survivors.

Severe Acute Respiratory Syndrome 2 (SARS-CoV-2) has affected millions worldwide, leading to neuromuscular issues, particularly in ICU patients. Muscle weakness is one of the main symptoms that persist for over a year after ICU discharge, with females experiencing more significant weakness and fatigue than males. The reasons behind this difference remain unclear, possibly involving neuromuscular dysfunction. Indeed, it is necessary to gap the lack of information on sex-specific neuromuscular function post-SARS-CoV-2 infection in ICU-discharged patients, critical for tailored rehabilitation. Since the increase in muscle force is modulated through the progressive recruitment of MUs and an increase in the MU firing rate, dysfunction in MU properties may lead to muscle weakness. MU properties vary between healthy males and females in various muscles. Healthy older females typically have lower MU firing rates in the TA, while males exhibit greater overall leg strength. Given the observed greater muscle weakness in females post-ICU discharge, it's likely that this weakness is related to neuromuscular dysfunction, possibly involving MU firing rate patterns. This study aimed to assess sex differences in physical functioning following ICU discharge for SARS-CoV-2 infection, with hypotheses suggesting greater muscle weakness and more significant MU impairment in females compared to males. Therefore, critically ill adult patients with confirmed SARS-CoV-2 infection in the ICU were involved in the study. They underwent various assessments, including FSS, 6MWT and peroneal nerve test (PENT). After these, the Compound Muscle Action Potential (CMAP) was recorded to assess peroneal nerve function. Surface electrodes were placed on the TA, and stimulation was applied to the common peroneal nerve to obtain CMAP responses. Following CMAP acquisition, patients performed three trials of maximal isometric ankle dorsiflexion to measure the MVC. HD-sEMG was then applied to the TA using a 64-electrode matrix. Submaximal isometric ramp-and-hold trials were performed at 30%, 50%, and 70% of MVC levels with real-time visual feedback provided to the patients. These trials involved generating torque through ankle dorsiflexion following a specific trajectory. Lastly, the recorded signals went through the decomposition process to extract the single MUs recruited during the tasks. The findings showed that there were no sex differences in physical functioning, maximal torque, fatigue, CMAP amplitude, or MU properties at the 3-to-6-month follow-up. However, at the 6-to-12-month follow-up, sex differences emerged, with females exhibiting greater impairments in strength and motor unit firing rate.

This suggests that males recover better than females in the first year following ICU discharge for SARS-CoV-2. Females continued to experience muscle weakness and neuromuscular dysfunction up to one year after discharge. These findings emphasize the importance of considering sex-specific factors in post-COVID neurorehabilitation.

Lulic-Kuryllo Tea, Benedini Marco, Cogliati Marta, Cudicio Alessandro, Guarneri Bruno, Gazzina Stefano, Piva Simone, Latronico Nicola, Orizio Claudio, Negro Francesco. (Frontiers in Medicine, 10, 1185479, 2023).

Study 5: Electrophysiological neuromuscular alterations and severe fatigue predict long-term muscle weakness in survivors of Covid-19 acute respiratory distress syndrome

ICU patients face long-lasting challenges in the form of long-term impairments. Therefore, it is crucial to explore the persistent physical, cognitive, and mental health alterations collectively referred to as PICS. These impairments significantly impact physical functioning, including aspects such as limb strength, range of motion, and balance. Notably, PICS is a common syndrome experienced by ICU survivors, including those who have struggled with the aftermath of COVID-19. Therefore, the study aims to identify predictors of long-term muscle weakness in these patients. Additionally, it seeks to conduct a comparative analysis of various clinical instruments employed in evaluating neuromuscular deterioration, utilizing both functional and electrophysiological objective parameters.

This study consists of an observational longitudinal investigation of 52 adult ARDS survivors with confirmed SARS-CoV-2 infection, who were treated in the ICU. Their physical, cognitive, and mental health status was assessed at 6 and 12 months after ICU discharge. Muscle strength was measured using MVC for lower limb muscles and handgrip strength (HGS) test for upper limb muscles. The study employed the Medical Research Council (MRC) sum score to comprehensively evaluate muscle strength. Electrophysiological and electroneurographical evaluations were conducted using the CMAP and PENT techniques specifically targeting the TA muscle. Additionally, factors such as fatigue, mental health, and cognitive abilities, were assessed with the FSS, the Hospital Anxiety and Depression Scale (HADS) and the Montreal Cognitive Assessment (MoCA), given their potential influence on muscle strength and overall recovery. The findings unveil significant insights into the long-term impact of the disease. Notably, the study identifies a positive trend indicating improved muscle weakness among survivors after 12 months, suggesting substantial recovery potential over time. Furthermore, HGS demonstrates a robust correlation with MVC, while an independent association emerges between MVC and both CMAP amplitudes and severe fatigue. Initially, abnormal CMAP indicate neuromuscular impairments in generating MVC, but improvement is evident after a year. The study also underscores the intricate relationship between physical and cognitive aspects of post-COVID fatigue, potentially serving as a reliable predictor of muscle weakness in critically ill survivors. Therefore, CMAP and FSS independently emerge as valuable tools for predicting the risk of long-term muscle weakness in [C]ARDS survivors.

Benedini Marco, Cogliati Marta, Lulic-Kuryllo Tea, Peli Elena, Stefano Mombelli Stefano, Calza, Stefano, Guarneri Bruno, Cudicio Alessandro, Rizzardi Andrea, Bertoni Michele, Gazzina Stefano, Renzi Stefania, Gitti Nicola, Rasulo A. Frank, Goffi Alberto, Pozzi Matteo, Orizio Claudio, Negro Francesco, Latronico Nicola, Piva Simone. (Frontiers in Neurology, 14:1235734, 2023)

Study 6: Effect of COVID-19 intensive care unit hospitalization on strength, fatigue and motor unit behaviour: a one-year follow-up study.

Critical illness and prolonged ICU stays have severe consequences on COVID-19 patients' physical functioning. To restore the autonomy of these subjects, is crucial to highlight the prevalence of ICUAW in these subjects and the potential factors contributing to muscle atrophy and functional impairments post-ICU discharge. COVID-19 patients may experience in fact various neuromuscular impairments during the acute phase of the disease and the subsequent hospitalization due to changes in both the CNS and PNS. To address the limitations of current clinical tests such as the MRC and the FSS, which rely on subjective assessments and may not pinpoint the exact cause of weakness or fatigue, this study provides objective evaluations. Indeed, the study explores the use of HDsEMG, along with torque output measurement and voluntary or electrically evoked contractions, to distinguish central and peripheral mechanisms underlying alterations in the MUs patterns. Additionally, the correlation between functional variables (FSS, MVC and 6MWT) and central (Mean DR, RT, DT, interspike interval covariation – ISI COV, Mean DR at recruitment, Mean DR at derecruitment) and/or peripheral variables (peak—to-peak MUAP, twitch contraction time, twitch peak, half relaxation time) was analyzed, shedding light on the impact of COVID-19, hospitalization and subsequent recovery on physical functioning.

Sixty adult participants, who had survived ARDS caused by SARS-CoV-2 and had been discharged from the ICU, were divided into two groups based on their assessment time points following ICU discharge: one group was evaluated at 3 and 6 months post-discharge, while the other group was assessed at 6 and 12 months post-discharge. The experimental protocol comprised three main procedures conducted sequentially during each evaluation session: (I) an assessment of physical functional capacity, (II) transcutaneous maximal electrical stimulations (evoked potentials) applied to the peroneal nerve to assess force output, and (III) the recording of TA contractions using the HDsEMG technique during both maximal (MVC) and submaximal voluntary isometric dorsiflexion of the foot. Perceived fatigue was assessed using the FSS while functional capacity was measured through the 6MWT. Additionally, the evaluation of central variables involved the analysis of MU discharge behavior, which was examined at different contraction levels (30%, 50%, and 70% of MVC). Peripheral variables included the analysis of MUAP amplitude and twitch characteristics.

The study's key findings included significant improvements in functional parameters, such as the 6MWT improved walked distance from 3 to 6 months follow-up, MVC increased in its value in both 3-6 months and 6-12 months groups, and FSS reduced its score in the 6-12 months group. These improvements were accompanied by changes in both CNS and PNS, enabling the restoration of optimal MU activation strategies. Notably, there were direct associations between functional improvements and central modifications. Central changes were most prominent during the initial 3-6 months, focusing on the

restoration of force-generating capacity, while improvements in fatigue were more evident during the last 6-12 months post-ICU discharge.

Therefore, the study provided valuable insights into the complex neuromuscular recovery process following ICU discharge in COVID-19 patients. It emphasized the importance of continuous monitoring and tailored interventions to optimize functional outcomes and reduce fatigue during the recovery journey.

Benedini Marco, Cogliati Marta, Cabral V. Hélio, Inglis J. Greig, Lulic-Kuryllo Tea, Piva Simone, Latronico Nicola, Orizio Claudio, Negro Francesco. (Submitted)

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5. Chapter Five: Effects of Repetitive Transcranial Magnetic Stimulation (rTMS) on Motor Symptoms of Parkinson Disease: Systematic Review.

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In Submission

5.1 Abstract

Introduction: Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive technique of electromagnetic stimulation of brain tissue that could induce changes in cortical plasticity. rTMS is currently used to evaluate and treat various movement disorders and related adaptive/compensatory responses of the brain. The purpose of this review is to investigate the therapeutic effects of rTMS on motor function in patients with Parkinson's disease or with atypical parkinsonism, in order to define which protocol allows to obtain the greatest benefits (short and long term) on the symptoms classified into 5 macro-areas: global motor function, gait, bradykinesia, tremor, fall. **Material and methods:** PubMed was used as the database for published studies since 1995 and ClinicalTrials.gov for ongoing studies. The outcomes, protocols, follow-up and results of the included clinical trials were analyzed. The effectiveness of rTMS was studied according to the main stimulation sites (M1, SMA, DLPFC) and the different stimulation intensities and frequencies (low, high, theta-burst) used. **Results:** Sixty-five studies met full eligibility criteria and were included in the review. High-rate (14 out of 15, 93%) M1 stimulation, followed by Teta-Burst (2 out of 2, 100%), low-rate (5 out of 6, 83%), high-rate (4 out of 5, 80%) SMA stimulation is effective in achieving benefit on global motor function. The use of both low and high frequency on M1 led to improvements in bradykinesia and gait (19 out of 20, 95%). The improvement in gait is also favored by the stimulation of the SMA at all the studied frequencies (8 out of 8, 100%) and by high-frequency stimulation of DLPFC (3 out of 4, 75%). Regarding tremor and the risk of falls, the stimulation of the same three areas did not lead to any significant benefit (4 out of 14, 29%). **Conclusions:** Due to the high heterogeneity of the outcomes is difficult to determine a defined protocol that has the greatest benefits on motor symptoms. The Unified Parkinson's Disease Rating Scale - Section III was the primary outcome measure used to assess global motor function, gait, and bradykinesia. The greatest effects on these symptoms were obtained by stimulating M1, SMA and DLPFC based on their predominant role in planning voluntary and complex movements. Conversely,

unsatisfying results were found in the treatment of tremor and the risk of falls. On-going studies show a growing interest in defining new areas of stimulation to improve these symptoms and a marked focus on the application of rTMS on atypical parkinsonism. New devices and assessment methods are being developed to obtain an objective assessment of gait and falls during daily life activities.

5.2 Introduction

Parkinsonism and more specifically Parkinson's Disease (PD) are chronic progressive neurodegenerative disorders that primarily involve movement control and balance. They are the second most common neurodegenerative disorder that affects approximately seven million people worldwide with an incidence of 4% in people over the 80s (Tysnes & Storstein, 2017).

The clinical picture of PD is often superimposable to that of atypical parkinsonian disorders, which is why its diagnosis is difficult. Parkinsonism – multiple system atrophy (MSA), dementia with Lewy Bodies (DLB), progressive supranuclear palsy (PSP), and corticobasal syndrome (CBS) – differs from PD for the different causes of onset but the symptomatology remains almost unchanged (Keener & Bordelon, 2016). The main motor symptoms are resting tremor, which affects about 70% of patients; rigidity, caused by the increase of muscle tone; bradykinesia, which makes gait difficult; walking and balance disorders, associated with camptocormia and freezing of gait. A constant feature of PD is the asymmetric onset: initially symptoms and signs affect one side of the body and subsequently, with the progressive development of the disease, a bilateral diffusion of the clinical manifestation is observed. Parkinsonism is on the other hand a clinical syndrome in which the onset of symptoms varies considerably according to the different diseases.

Having a negative impact on daily life activities and social interaction, PD and parkinsonism both affect patients' quality of life.

The pathophysiology of these motor deficits and compensation mechanisms in PD is not yet fully clarified by current research. The available drug therapies can control the early stages of the disease, but they are not always able to improve the motor complications of the advanced stages, with a profound impact on the patient's quality of life (Ferreira et al., 2013)(Schaeffer et al., 2014). Therefore, rehabilitation therapy (Tomlinson et al., 2007) and non-pharmacological interventions assume a very important role (Ekker et al., 2016).

Parkinsonism, on the other hand, has a different etiology if compared to Parkinson's, as it involves vascular causes or toxic agents which makes dopaminergic treatment ineffective. This leads to a more severe prognosis and the need for a new therapy that is effective in counteracting its symptoms.

Today, it is possible to induce changes in cortical plasticity through the use of repetitive Transcranial Magnetic Stimulation (rTMS). rTMS is a non-invasive technique based on electromagnetic stimulation of the brain tissue that could induce changes in cortical plasticity (by applying repetitive TMS impulses)

through the repetitive application of TMS impulses in specific areas of the brain. The electromagnetic induction is given by a coil arranged over the scalp and its number of pulses, intensity, frequency and the interval between the trains of pulses are set up.

The passage of the current flow inside the coil generates an electromagnetic field capable of generating a corresponding current flow which bypasses the scalp placed inside the aforementioned electromagnetic field. This current flow reaches the brain tissue and produces motor evoked potentials (MEP) in the excitable neurons of the cerebral cortex. By analyzing MEPs through the induced muscle response, corticospinal excitability can be studied. The effects of the technique do not depend directly on the magnetic field but on the induced electric field which causes neuronal depolarization.

Initially used as a therapy for depression, drug abuse and obsessive-compulsive behavior, in the last years rTMS has been strongly considered a valid treatment for neurodegenerative diseases such as PD and parkinsonism.

Several studies confirm the beneficial effects of this therapy, but there is still a discrepancy regarding the parameters of the supplied pulses and the precise area of the brain to stimulate.

The review aims at exploring the literature, to find what is the setting of the rTMS (target, frequency, intensity, total number of pulses, interval between the train of pulses) able to obtain the best benefits for the motor symptoms in PD. There are already numerous studies in the literature that highlight the efficacy of rTMS on motor symptoms. What this review wants to reach is the subdivision of the results of the included studies into five macro-areas of assessment - UPDRS, Bradykinesia, Freezing of Gait, Falls, Tremor - finding the rTMS set-up that allows getting the biggest improvements in each of them. This would make it possible to find in the literature the ideal stimulation parameters to be adopted for a new study depending on the objective to be achieved.

Relating the rTMS parameters with the relative effects in each of the five areas can be crucial for new studies in establishing which protocol to adopt to obtain the desired effects. Attention was also focused on the stimulation areas subject to future studies and on the stimulation intensities and frequencies to understand if substantial changes are expected, compared to the direction of the studies already published. Further classification was made regarding the study design (single, double or triple-blind, observational, open-label) and short or long-term follow-up.

5.3 Methods

5.3.1 Search methods

A systematic review of the *PubMed* and Clinicaltraisl.gov database was performed to identify published and unpublished interventional trials using repetitive TMS for movement disorders. This review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009).

5.3.2 Participants and interventions

All the patients in the selected studies were included with no restriction on age, gender, year of onset of the disease, severity of the pathology and symptoms, type of assessment and control group therapy.

5.3.3 Data collection and analysis

Data extraction.

We searched PubMed for human studies published between 1995 and September 2023 using combinations of the following terms: "repetitive transcranial magnetic stimulation", “transcranial stimulation”, "Parkinson’s disease", “progressive supranuclear palsy”, “multiple system atrophy”, “Lewy bodies dementia” and “corticobasal syndrome”.

The analysis of the articles was independently performed by two authors (A.P, M.B.) in an unblinded fashion and disagreements between reviewers were discussed with senior authors (A.P., M.C.R, F.N).

The inclusion criteria for the articles and trials included in the systematic review were:

- Use of rTMS as a treatment strategy for motor symptoms in patients with parkinsonism (thus including PD, PSP, MSA, CBS and DLB).
- Details about the rTMS full protocol and set-up (number of subjects, design, stimulated brain area, stimulation frequency and intensity, follow-up).
- Inclusion of original data (i.e. review or partial dataset were excluded).

Selection of studies.

All search results were screened by the authors to identify studies meeting the inclusion criteria. The first screening of abstracts was performed to exclude duplicates and incomplete works. A more detailed analysis was carried out by reading the full text of the article; studies with unavailable details about stimulation protocol were thus excluded from the systematic review (Figure 1).

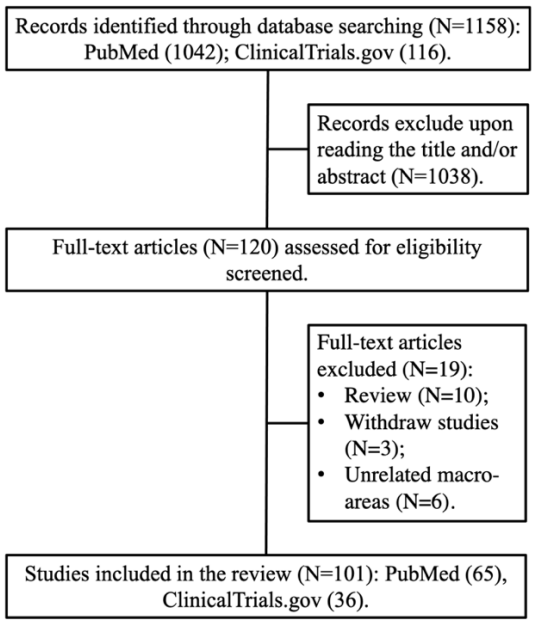

Figure 1: Flow chart of the included studies.

Data extraction and analysis

The information extracted for each available published trial were: (1) total number of subjects included in the study who received real and/or sham stimulation; (2) average age of patients; (3) design of the study (cross-over, parallel-group, open-label, single or double-blind); (6) length of follow-up (immediate after stimulation and/or long term); (7) site of stimulation (for example primary motor cortex, dorsolateral prefrontal cortex, supplementary motor area, cerebellum,…); (8) frequency of stimuli (Hertz); (9) intensity of stimuli (percentage of resting or active motor threshold); (10) total number of pulses and interval time between them; (11) clinical outcome measures (see next paragraph 2.4).

5.3.4 Outcome measures for published studies.

Parkinsonian features were clustered in five main areas, namely total motor function, bradykinesia, gait, falls and tremor. The efficacy of the different rTMS protocols was thus re-evaluated for specific features indicated by subitems of UPDRS-III. Freezing of gait was considered a specific subtype of gait. The efficacy of specific protocol (including target areas and protocol type) was evaluated with the Chi-square test, significance set at p=0.05.

5.3.5 Outcome measures for the unpublished ongoing clinical trials.

For ongoing trials recorded on Clinicaltrials.gov, attention was paid to the same outcome measures evaluated by already published articles. Thus, specific protocols, inclusion/exclusion criteria and primary/secondary endpoints were recorded to evaluate the new results and trials with upcoming results.

5.4 Results

5.4.1 Systematic review of published studies

Out of the 1042 records identified 77 studies met full eligibility criteria and underwent data extraction (Figure 1). Sixty-five studies focused on PD patients, whereas 12 studies were focused on atypical parkinsonism, 5 studies treat the PSP, 6 the MSA and only one the CBS (specified in session 3.3)

Target population and outcomes of PD studies.

All the PD studies included patients with a mean age of 63.9 ± 4.2 years. The mean sample size was 25.9 ± 21.3 participants (starting from a minimum of 6 up to a maximum of 106 subjects included).

Out of the 65 studies included, 29 had a parallel design with a single or double-blind, placebo-controlled study whereas 27 had a crossover design with a single or double-blind, placebo-controlled study. The remaining 9 studies had an open-label design.

The majority of studies included global motor function as the main outcome (n=54, 83%); additionally, 24 (37%) focused their attention on gait, 11 (17%) on bradykinesia, 10 (15%) on tremor and two studies (3%) analyzed the risk of falls. Forty-two out of 65 studies included a short-term follow-up only (immediately after the stimulation or < 4 weeks). The remaining 23 studies included a long-term follow-up (≥ 4 weeks) with a mean duration of 67 days (31 to 186 days).

Efficacy

Out of the 65 studies screened, 24 did not show any benefits induced by the rTMS (efficacy rate 63%) in general, whereas the efficacy was different for the main categories (Figure 2).

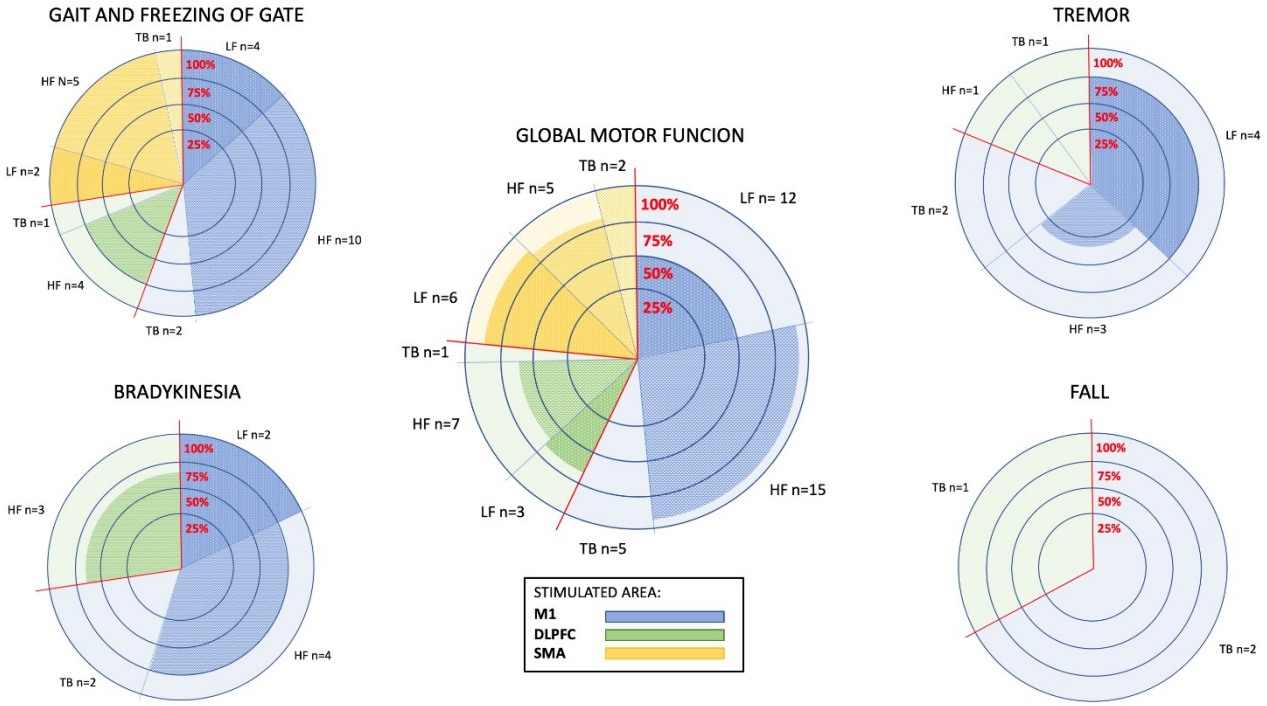

Figure 2: Efficacy of different rTMS protocols on specific motor macro-areas. (LF: Low-Frequencies; HF: High-Frequencies; TB: Theta-Burst; M1: Primary Motor Cortex; DLPFC: Dorsolateral Prefrontal Cortex; SMA: supplementary motor Area).

Global motor function

Fifty-four studies focused their attention on the effects induced by the rTMS on global motor function, assessed as the total score of the UPDRS-III. Thirty-two (59%) of these studies showed improvements induced by the stimulation. The main stimulated brain sites were the primary motor cortex (M1) (n=28), the supplementary motor area (SMA) (n=13) and the dorsolateral prefrontal cortex (DLPFC) (n=11).

Low-frequency stimulation (0.2-0.5-1-5 Hz) was used in 28 Studies, high frequency (10-25 Hz) in 21 and theta-burst stimulation in 8.

The efficacy of rTMS on the UPDRS-III score differ according to the frequency of stimulation used: 81% of high stimulation protocol, 62% of low-frequency protocol and 25% of theta burst stimulation (Figure 2).

The stimuli were delivered at an intensity varying between 60 and 150% of the resting motor threshold (RMT) with a total number of stimuli unfortunately not available for all studies (Table 1).

As highlighted in Figure 2, the efficacy was higher in M1 HF (14 out of 15, 93%), followed by SMA TB (2 out 2, 100%), SMA LF (5 out 6, 83%), SMA HF (4 out 5, 80%), DLPFC LF (2 out 3, 67%), DLPFC HF (4 out 7, 57%), M1 LF (6 out 12, 50%). (p<0.00001).

Thirty-four studies included a short-term follow-up while 20 included a long-term follow-up with a mean duration of 69 days.

Bradykinesia

Eleven studies analyzed the effects induced by the rTMS on bradykinesia. Six studies (55%) showed improvements induced by the stimulation and the main stimulated brain sites were M1 (73% of the selected studies; n=8) and DLPFC (27% of the selected studies; n=3).

Four studies used low frequency (0.5-1-5 Hz), Five used high frequency (10-25 Hz) and two used theta-burst stimulation.

As highlighted in Figure 2, the efficacy was higher in M1 LF (2 out of 2, 100%), followed by M1 HF (3 out 4, 75%), and DLPFC HF (2 out 3, 67%). (p=0.407).

Seven studies included a short-term follow-up while 4 included a long-term follow-up with a mean duration of 85 days.

Tremor

Ten studies evaluated the effects induced by the stimulation on tremor. Only four studies (40%) showed improvement and in all of these, the stimulated brain area was the M1. Of these four studies, three stimulated at 90% of RMT (1, 5 and 50 Hz) and three had a long-term follow-up.

Five studies used low frequency (0.2-1-5 Hz), 3 used high frequency (10 Hz) and 2 used theta-burst stimulation.

Five studies included a short-term follow-up while 4 included a long-term follow-up with a mean duration of 70 days.

Gait and Freezing of gait

Twenty-four studies analyzed the effects induced by the rTMS on the freezing of gait. Twenty (83%) of these studies showed improvements induced by the stimulation and the main stimulated brain sites were M1 (58% of the selected studies; n=14), SMA (33% of the selected studies; n=8) and DLPFC (21% of the selected studies; n=5).

LF stimulation was used in seven studies (0.5-1-5 Hz), HF (10-25 Hz) in 15 and TB in 4.

As highlighted in figure 2, the efficacy was higher in M1 HF (10 out of 10, 100%), followed by SMA HF (5 out 5, 100%), M1 LF (4 out 4, 100%), SMA LF (2 out 2, 100%), SMA TB (1 out 1, 100%) and DLPFC HF (3 out 4, 75%). (p<0.00001).

Thirteen studies included a short-term follow-up only, while 11 included a long-term follow-up with a mean duration of 62 days.

Falls

Two studies analyzed the effects induced by the rTMS on the average number of falls but none of them showed any benefits induced by the stimulation. Both studies adopt a protocol with iTBS stimulation

(50 Hz). The first one stimulates only M1 (80% AMT) while the second one also stimulates DLPFC (80% RMT), both with follow-up immediately and one month after the last stimulation.

5.4.2 Systematic review of ClinicalTrials.com.

Forty-two interventional clinical trials focused on rTMS are listed on ClinicalTrails.com. Three studies were excluded due to the lack of information regarding the study protocol, resulting in 39 studies. Of them, 6 studies were *recruiting*, 21 were *completed*, 1 was *not yet recruited*, 8 were *unknown* and 3 were *withdrawn* and were eliminated.

Target population and outcomes

The target number of participants is very different from one study to the other, with an average number of 45.64 ± 48 participants, with a range of 4-252 subjects.

As highlighted in Figure 3, twenty-seven (75%) of the 36 included studies used UPDRS as outcomes for the rTMS assessment, 22 (61%) focused their attention on freezing of gait, 6 focused the protocol on tremor, 6 on bradykinesia and 5 on falls.

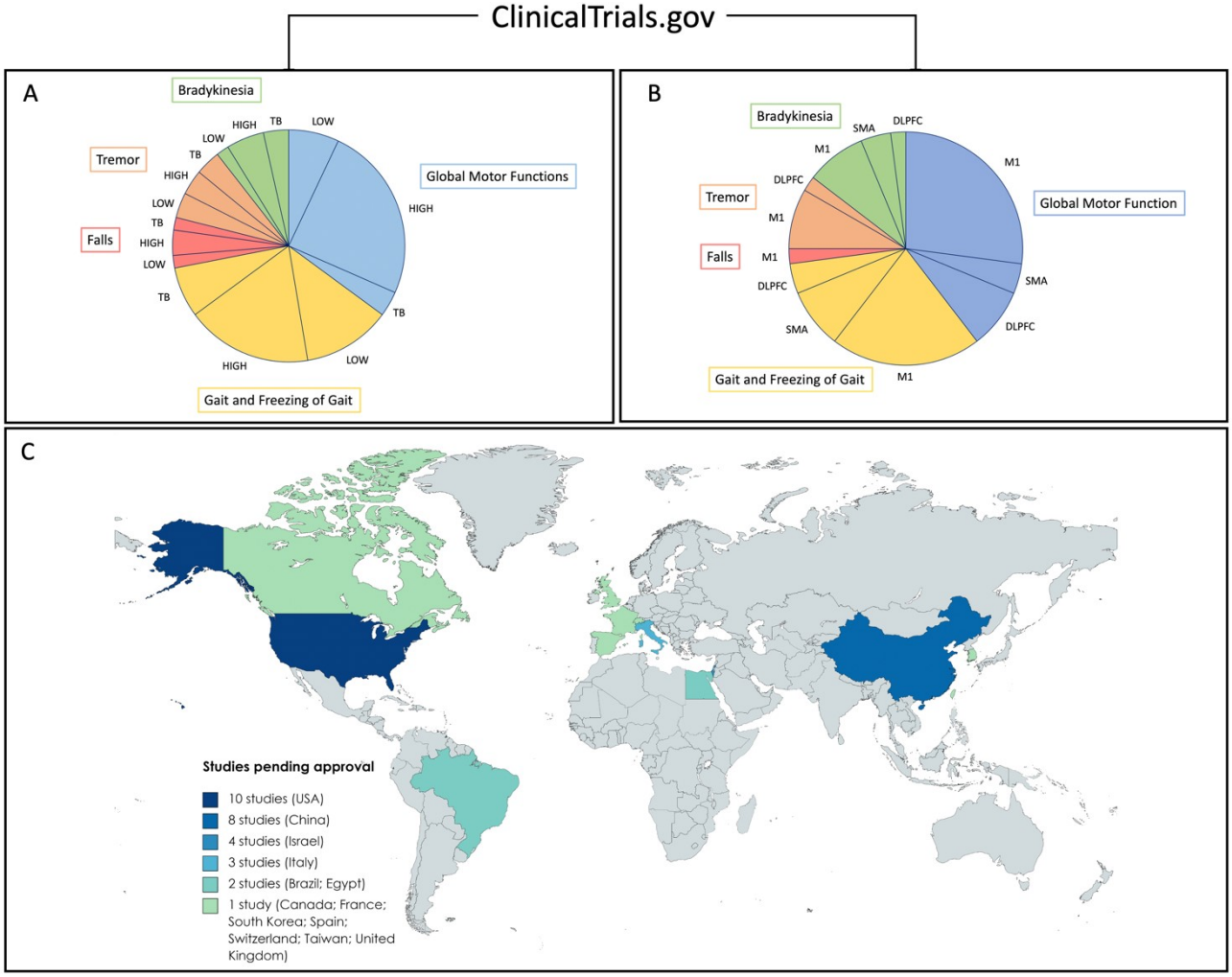

Figure 3: (A) stimulation areas and (B) stimulation frequencies used by the studies included in ClinicalTrials.gov; (C) site of the ongoing studies (M1: Primary Motor Cortex; DLPFC: Dorsolateral Prefrontal Cortex; SMA: supplementary motor Area).

Out of the 36 studies included, 14 (39%) have a double-blind design, 6 (17%) Quadruple-blind (Participant, Care Provider, Investigator, Outcomes Assessor), 5 (14%) are triple-blind (Participant, Investigator/care provider, Outcomes Assessor), and 3 single-blind.

Nineteen studies (53%) involve a short and long-term follow-up (immediately and after 1, 2, 3 or 6 months from the last stimulation).

The stimulation areas included M1, DLPFC and SMA with a frequency of 1, 10, 20, 25 and 50 Hz and intensity between 80 and 120% of the RMT.

5.4.3 Data available on atypical parkinsonism

The analysis of the literature shows that TMS has been used exclusively for diagnostic purposes in atypical parkinsonism – MSA, DLB, PSP and CBS - except for five studies on PSP (Santens et al., 2009; Brusa et al., 2014; Dale et al., 2019; Major et al., 2019; Pilotto et al., 2021), six studies on MSA (Chou et al., 2015; Wang et al., 2016; Hana et al., 2017; Liu et al., 2018; Song et al., 2020; Pan et al., 2022) and one study on CBS (Shehata et al., 2015). About PSP, Dale et al. highlighted the positive effect on balance but took only two subjects into the analysis. Brusa et al. and Santens et al. obtained positive effects on gait, stimulating cerebellum and M1 respectively while Pilotto et al. focused their attention on gait and falls following theta-burst stimulation on the cerebellum, using an innovative mobile health technology as the evaluation system. Song et al. provide evidence that the iTBS over the cerebellum has a positive effect on motor symptoms in people with MSA while Liu et al., Chou et al. and Wang et al. explored the effects of M1 stimulation on the global motor functions. Only Shehata et al. studied the effect of the M1 stimulation at low frequencies on CBS. The research on Clincaltrials.gov has identified 3 studies (NCT02734485, NCT04468932 and NCT04222218) on the effects of rTMS on PSP, two studies on MSA (NCT04313530 and NCT04595578), one study on PSP and CBS (NCT01174771) and one on DLB (NCT05138588). About the PSP, the studies have a crossover design and the stimulated areas are the motor cortex and the cerebellum. The study that analyzes both PSP and CBS, foresees a stimulation at 5Hz for the first and 1Hz for the second, both in M1 and DLPFC. Two studies are focused on MSA (NCT04313530, NCT04595578), analyzing changes in Fatigue Severity Scale (FSS) and improvement in gait, induced by rTMS. Lastly, the crossover study on the DLB focused its attention on the stimulation of the insular cortex.

5.5 Discussion and conclusion

The review analyzed the published and pending approval studies by relating the stimulation areas and intensities with the relative effects on the 5 macro-areas covered by the study. The setting of rTMS used by the individual studies was analyzed to define the protocol that guarantees the greatest benefits in the treatment of symptoms related to Parkinson's disease and atypical parkinsonism. An important aspect

concerns the fact that many articles report results highlighted by short-term follow-up and therefore, the need to explore the benefits of the adopted protocol in long-term follow-up.

Following the analysis, it emerged that it was impossible to determine a defined protocol due to the high heterogeneity of the outcomes used by the studies included in this review. Only the UPDRS was the common outcome used in almost all the studies that analyze the global motor function.

Regarding the global motor functions, the high-frequency stimulation confirms the best benefits on the primary motor cortex as it is responsible for the planning and control of voluntary movements. Positive effects are also confirmed in the stimulation of SMA at every stimulation frequency. The results of the rTMS protocols adopted for Gait and Bradykinesia go in the same direction. M1 is the most stimulated area with positive effects at low and high frequencies. Moreover, the stimulation of SMA is found to be effective in reducing gait deficits by all the stimulation frequencies, in relation to its predominant role in execution, coordination and control of sequences of complex movements. Regarding tremor and risk of falls, published studies indicate the need to explore new areas and intensities of stimulation as no article shows significant positive effects in the stimulation of M1 and DLPFC except for 3 studies showing positive effects on tremor due to low-frequency stimulation of M1.

Especially in the risk of falls, ongoing studies show a growing interest in the stimulation of the cerebellum given its crucial role in modulating coordination and precision of movement. Being a problem closely linked to gait and turning, it will be crucial to study the effects of stimulation of this brain area in both macro-areas. Furthermore, the ongoing studies aim to explore the effects of stimulation with a long-term follow-up, in relation to the areas already explored by published studies and to the new ones.

Regarding atypical parkinsonism, the ongoing studies confirm what has already been reported by the published studies. Five published studies have analyzed the effects of rTMS on PSP, six on MSA and one on CBS while three ongoing studies will explore the benefits for PSP, two for MSA and one for CBS. The only difference is due to a study that will explore the effects of rTMS on the disease.

A further not negligible problem is the fact of not having an objective and standardized method for assessing falls and tremor.

The UPDRS is a solid outcome for the evaluation of global motor functions, but it is lacking for an objective evaluation of falls and tremor disorders. Inertial sensors are proposed as new motion analysis systems by integrating accelerometers, magnetometers and gyroscopes. The wearable motion sensors allow to record and provide a dynamic three-dimensional analysis of movement during daily-life activities. Therefore, it will be possible to analyze both the walking phase and the turning phase and quantify any episodes or potential falls. Finally, in addition to being relatively cheap and reliable, the sensors are easily manageable with a video interface on a personal computer. The exploration of new

areas of stimulation such as the cerebellum and the use of new technologies represent the new frontier for the evaluation and treatment of the symptoms of Parkinson's disease and atypical parkinsonism.

Table 1: Characteristics of the selected studies (PG: Parallel Group; DB: Double-Blind; SB: Single-Blind; OL: Open-Label; CO: Cross-over; GMF: Global Motor Function; B: Bradykinesia; T: Tremor; G: Gait; F: Falls; MC: Motor Cortex; OC: Occipital; FC: Frontal Cortex; M1: Primary Motor Cortex; SMA: Supplementary Motor Area; DLPFC: Dorsolateral Pre-Frontal Cortex; PMd: Left Dorsal Pre-Motor Cortex; PFC: Pre-Frontal cortex; RMT: Resting Motor Threshold; AMT: Active Motor Threshold; MT: Motor Threshold).

STUDY	GMF	B	T	G	F	Nº SUBJECTS	DESIGN	FOLLOW-UP	TMS-PROTOCOL		
									Area	Fq (Hz)	Intensity (RMT)
LOW FREQUENCIES											
(Okabe et al., 2003)	-					85	PG DB	IA + 1m + 2m	MC + OC	0.2	110% AMT
(Ikeguchi et al., 2003)	+					12	OL	IA	OC + FC	0.2	n.a
(Shimamoto et al., 2001)	+					18	PG DB	2m	FC	0.2	n.a
(Kimura et al., 2011)	+		+			12	CO DB	IA	M1	0.2	n.a
(Brusa et al., 2006)	+					10	CO	IA	SMA	1	90%
(Arias et al., 2010)	-					18	PG DB	IA	vertex of the skull	1	90%
(Filipović et al., 2009)	-					10	CO SB	IA	M1	1	90%
(Sayin et al., 2014)	-					17	PG DB	IA	SMA	1	90%
(Filipović et al., 2010a)	-	-	-			10	CO SB	IA	cerebral cortex	1	n.a.
(Buhmann et al., 2004)	-					10	OL	IA	PMd	1	80% AMT
(Filipović et al., 2010b)	-					9	CO DB	IA	M1	1	110% + 120% + 130% + 150%
(Málly et al., 2017)	+					66	OL	6m	DLPFC	1	n.a.
(Fricke et al., 2019)	-		-			20	CO DB	IA	M1 + PMd	1	95%
(Taib et al., 2019)			+			18	PG DB	1m	M1	1	90%
(Shirota et al., 2013)	+			+		106	PG DB	5m	SMA	1	n.a.
(Khedr et al., 2019)	+					52	OL	1m	M1	1	100% + 90%
(Chung et al., 2020)				+		51	PG DB	3m	M1	1	80%
(Zhuang et al., 2020)	+					33	PG SB	IA + 1m + 3m + 6m	DLPFC	1	110%
(Flamez et al., 2016)	-					15	CO DB	IA	M1	1	90%

(Cohen et al., 2018)	-					48	PG DB	IA	M1	1	110% MT
(Siebner et al., 2000)	+	+	+			10	CO SB	IA	M1	5	90%
(Khedr et al., 2003)	+			+		36	PG DB	IA + 1m	M1	5	120%
(Hamada et al., 2008)	+					98	PG DB	IA	SMA	5	110%
(Mak, 2013)				+		22	PG DB	IA	M1	5	n.a.
(Siebner et al., 1999)		+				12	CO	IA	M1	5	90%
(Mir et al., 2005)	-					10	OL	IA	PMd	5	90% AMT
(Pal et al., 2010)	-					22	PG DB	IA + 1m	DLPFC	5	90%
(Yang et al., 2013)				+		20	PG DB	IA	M1	5	100%
(Rothkegel et al., 2009)	-					22	CO	IA	M1	0.5	80%
(Lefaucheur et al., 2004)	+	+		+		12	CO DB	IA	left motor cortex	0.5	80%
(Makkos et al., 2016)	+					44	PG DB	IA + 1m	M1	5	90%
(Hanoğlu et al., 2020)	+					16	OL	IA	M1 + SMA	5	n.a.
(Saricaoglu et al., 2021)						20	OL	IA	SMA	5	n.a.
HIGH FREQUENCIES											
(Brys et al., 2016)	+	+	-	+		61	PG DB	IA + 1m + 3m + 6m	M1 + DLPFC	10	n.a.
(Maruo et al., 2013)	+	-	-			21	CO DB	IA	M1	10	100%
(Boylan et al., 2001)	-					8	CO DB	IA	SMA	10	110%
(Börnke et al., 2004)	+					12	CO DB	IA	M1	10	110%
(del Olmo et al., 2007)	-					13	PG DB	IA	DLPFC	10	90%
(Sedláčková et al., 2009)	-	-				10	CO DB	IA	PMd + DLPFC	10	100%
(Rothkegel et al., 2009)	-					22	CO	IA	M1	10	80%
(Kim et al., 2015)	+			+		17	CO DB	IA	M1	10	90%
(Yokoe et al., 2018)	+					19	CO DB	IA	M1 + SMA + DLPFC	10	100%
(Chang et al., 2016)	+			+		8	CO SB	IA	M1	10	90%
(Chang et al., 2017)				+		32	CO DB	IA	M1	10	90%

(Dagan et al., 2017)	+			+		7	CO DB	IA	medial PFC	10	100%
(Lee et al., 2014)	+			+		20	CO DB	IA	M1 + SMA + DLPFC	10	120%
(Mi et al., 2019)	+			+		30	PG DB	IA + 1m	SMA	10	90%
(Ma et al., 2019)	+			+		28	PG DB	IA + 1m	SMA	10	90%
(Rektorova et al., 2007)	-			-		6	CO DB	IA	MC + DLPFC	10	90%
(Spagnolo et al., 2021)	+		+			59	PG DB	1m	M1 + PFC	10	90% + 100%
(Mi et al., 2020)				+		30	PG DB	1m	SMA	10	n.a.
(Lomarev et al., 2006)	+	+		+		18	PG DB	IA + 1m	M1 + DLPFC	25	100%
(González-García et al., 2011)	+	+				17	PG DB	IA +3m	M1	25	80%
(Khedr et al., 2006)	+			+		55	OL	IA + 1m	M1	25 + 10	100%
(Khedr et al., 2007)	+					20	OL	IA	M1	25	100%
(Khedr et al., 2019)	+					52	OL	1m	M1	20	100% + 90%
(Chung et al., 2020)				+		51	PG DB	3m	M1	25	80%
(Kim et al., 2018)				+		12	PG DB	IA	SMA + M1	25	100%
THETA BURST											
(Benninger et al., 2012)	-	-		-	-	26	CO DB	IA + 1m	M1	50	80% AMT
(Benninger et al., 2011)	-		-	-	-	26	PG DB	IA + 1m	M1 + DLPFC	50	80%
(Rothkegel et al., 2009)	-					22	CO	IA	M1	50	80%
(Koch et al., 2009)	-	-				20	CO + PG	IA + 1m	lateral cerebellum	50	80% AMT
(Benninger et al., 2010)	-	-				10	OL	IA	M1	50	60-90%
(Bologna et al., 2015)			-			13	PG DB	IA	M1 + cerebellum	50	100% + 120% + 140%
(Ji et al., 2021)	+					42	PG DB	IA + 2m	SMA	50	80%
(Eggers et al., 2015)	+					26	CO DB	IA	SMA	50	90% AMT
(Brugger et al., 2021)				+		12	CO DB	IA	SMA	50	100% AMT
(Zamir et al., 2012)	-					22	PG DB	IA	M1	50	80% AMT
(Tard et al., 2016)				-		15	CO SB	IA	left premotor cortex	50	120%

Table 2: Characteristics of the ongoing studies (CO: Cross-over; PG: Parallel Group; IN: Interventional; OB: Observational; OL: Open-Label; SB: Single-Blind; DB: Double-Blind; TB: Triple-Blind; QB: Quadruple-Blind; GMF: Global Motor Function; B: Bradykinesia; T: Tremor; G: Gait; F: Falls; SMA: Supplementary Motor Area; M1: Primary Motor Cortex; DLPFC: Dorsolateral Pre-frontal Cortex; MC: Motor Cortex).

STUDY	GMF	B	T	G	F	Nº SUBJECTS	DESIGN	FOLLOW-UP	TMS-PROTOCOL		
									Area	Fq (Hz)	Intensity (RMT)
NCT03273270				x		20	CO TB	IA	SMA	1	100%
NCT04238000				x	x	20	CO DB	IA	cerebellum	50	80%
NCT03552861	x			x		252	PG QB	IA + 1m + 3m	DLPFC	1	100%
NCT02741765				x		24	IN DB	IA	M1	n.a.	n.a.
NCT00977184	x	x	x	x		26	IN DB	1d + 1m	M1	50	n.a.
NCT02701647	x	x	x	x		51	IN DB	IA + 1m + 3m	M1	25 + 1	80%
NCT00753519	x	x	x	x		28	PG DB	IA + 1m	M1 + DLPFC	50	n.a.
NCT00029666				x		40	IN	IA	cerebral cortex	25	n.a.
NCT02969941	x			x		46	PG TB	2m	n.a.	n.a.	n.a.
NCT00001665		x				12	OB	n.a.	SMA	n.a.	n.a.
NCT02221544	x			x	x	20	CO TB	IA	frontal lobe areas	10	n.a.
NCT02012647	x					4	OL	IA + 4m	n.a.	n.a.	n.a.
NCT04116216	x	x	x	x	x	30	CO TB	IA	M1	10	100%
NCT00023062	x					80	OB	n.a.	MC	n.a.	n.a.
NCT03645538	x		x	x	x	20	OB	IA	MC	n.a.	n.a.
NCT02734485				x		20	CO DB	n.a.	MC + PFC	1 + 10	100 + 110%
NCT04017481	x					50	IN DB	IA	M1	10	80%
NCT03217110	x					200	PG SB	IA + 2m	cerebellum	n.a.	n.a.
NCT01275573	x					30	CO QB	IA	M1	20	95%
NCT04638777	x					60	PG QB	IA + 1m + 2m	M1 + DLPFC	n.a.	n.a.
NCT03879551	x					30	PG SB	3m	MC	25	80%
NCT03350464	x					10	OL	IA	n.a.	n.a.	n.a.
NCT04048265	x					52	PG DB	1m	n.a.	n.a.	n.a.
NCT04707378	x					42	PG QB	1m	DLPFC	10	100%
NCT04699149	x					36	PG SB	IA	n.a.	n.a.	n.a.
NCT04431570	x			x		66	PG QB	IA	M1 + SMA	10	100%
NCT04695496				x		30	OL	1m	SMA	50	n.a.
NCT02387346			x	x	x	50	PG DB	IA	cerebellum	1	120%
NCT00625300	x					20	PG DB	3m	M1 + PFC	20	n.a.
NCT01367782	x			x		51	PG TB	IA + 1m + 2m + 3m	M1	1 + 10	100% + 110%

NCT00858546	x			x		10	OL	IA + 1m + 2m + 3m	PFC	1 + 20	n.a.
NCT03219892	x	x		x		30	PG DB	IA + 1m	SMA	10	90%
NCT05174299	x			x		40	PG DB	IA + 1m	M1	n.a.	n.a.
NCT05271513	x			x		50	PG QB	2m	M1	10 + 20	80%
NCT01080794	x					61	PG DB	IA + 1m + 3m + 6m	M1 + PFC	10	90%
NCT02850159				x		32	PG DB	IA	M1	n.a.	n.a.

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6. Chapter Six: Influence of age on force and re-lengthening dynamics after tetanic stimulation withdrawal in the tibialis anterior muscle.

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6.1 Abstract

Purpose: During alternate movements across a joint the changeover from one direction of rotation to the opposite may be influenced by the delay and rate of tension reduction and the compliance to re-lengthening of the previously active muscle group. Given the ageing process may affect the above mentioned factors, this work aimed to compare the dynamics of both the ankle torque decline and muscle re-lengthening, mirrored by mechanomyogram (MMG), in tibialis anterior because its important role in gait. **Methods:** During the relaxation phase, after a supramaximal 35 Hz stimulation applied at the superficial motor point, in 20 young (Y) and 20 old (O) subjects the torque (T) and MMG dynamics characteristics were measured. **Results:** The T and MMG analysis provided: I) the beginning of the decay after cessation of stimulation (T: 22.51 ± 5.92 ms [Y] and 51.35 ± 15.21 ms [O]; MMG: 27.38 ± 6.93 ms [Y] and 61.41 ± 18.42 ms [O]); II) the maximum rate of reduction (T: -110.4 ± 45.56 Nm/s [Y] and -52.72 ± 32.12 Nm/s [O]; MMG: -24.47 ± 10.95 mm/s [Y] and -13.76 ± 6.54 mm/s [O]); III) the muscle compliance, as the MMG reduction every 10% reduction of torque (bin 20-10%: 15.69±7.5[Y] and 10.8±3.3 [O]; bin 10-0%: 22.12±10.3 [Y] and 17.58±5.6 [O]). **Conclusion:** The muscle relaxation results different in Y and O and can be monitored by a non-invasive method measuring physiological variables of torque and re-lengthening dynamics at the end of the electromechanical coupling previously induced by the neuromuscular stimulation.

6.2 Introduction

The alternated movement of a joint, such as the ankle, knee and elbow, is evidently related to the coordinated, alternated activation of the flexors and extensors muscles acting across the hinge. While the muscles providing an angular momentum towards the joint rotation direction are referred as agonist, the muscle group generating an opposite angular momentum is defined as antagonist. Every time the joint rotation is reversed, there is a swap between the roles of antagonist and agonist muscles. The changeover from one direction of rotation to the opposite as well as the re-lengthening phase of the past agonist is influenced by: a) the tension reduction of the previously active muscle group, b) the compliance of this last to re-elongation by the new active agonist. Consequently, the assessment of biomechanical parameters during these two aforementioned processes could provide functional data to characterize the muscular features affecting the agonist-antagonist sequential activity.

Gait can be considered as a global alternating movement resulting by the combination of several joints alternating flex-extension sequences. According to Westerblad et al. (1997) “slowed relaxation of antagonist muscle might counteract the desired movement during rapid, alternating movements”. Thus, during normal locomotion the slowing of relaxation of the previously active muscle group may greatly affect the dynamic of the joint transition from a rotational direction to the following. This may influence the locomotion parameters particularly in aged subjects. Indeed, data about gait analysis suggest that age influences the gait in length stride and phases duration (Mulas et al. 2021, Fukuchi et al. 2019). Changes in gait in elderly have been also associated with an increased risk of institutionalization and death. For instance, reduction of the walking speed has been demonstrated to be predictive of life expectancy (Studenski et al. 2011). Furthermore, disturbances in balance and gait have been implicated in an increased risk of falls (Osoba et al. 2019).

On this base it seems important to evaluate the dynamics of muscle tension reduction and re-lengthening after activation in young and old subjects. The functional parameters describing the force and re-lengthening process during the relaxation phase are not well comparatively described in the above-mentioned populations. The force decrement onset delay, from the myoelectric activity cessation, or its velocity of decay in different conditions such as pre and post stretching maneuver (Longo et al. 2017; Longo et al. 2014) or before and after fatigue (Cè et al. 2014a; Cè et al. 2014b) have been investigated in the literature. Only few studies report the behavior of tension and surface mechanomyogram (MMG) signal detected by an accelerometer, monitoring the muscle re-elongation, in the relaxation phase when simultaneously recorded (Cè et al. 2014a; Cè et al. 2013b; Esposito et al. 2016; Longo et al. 2014). Indeed, they used the MMG as an indicator of re-lengthening onset but not of its time dynamics.

In order to assess the torque and muscle length behavior at the end of muscle contraction it is possible to use an experimental setup previously described by our group (Cogliati et al. 2020), in which the isometric torque of ankle dorsiflexion and the tibialis anterior length were simultaneously measured by

a load cell and surface mechanomyography, respectively. The rationale can be summarized as follow. Since the muscle is a constant volume system, each shortening during a contraction provides an increase in the transverse diameter of the muscle. This dimensional variation can be picked-up by a laser sensor. By analogy, during muscle relaxation after activity the laser distance signal can be considered as an index of the muscle re-elongation process. The study of muscle length changes by surface MMG has already been implemented in detail (Orizio et al. 1996; Yoshitake et al. 2005; Beck et al. 2005). The rationale for adopting MMG as an indirect measure of the muscle length changes, instead of the collection of the ultrasound (US) images from the active muscle, is based on the following considerations: a) at present the US technique unlikely provides more than 30 - 60 frames per second with a time resolution of 30 – 15 ms (too low for a good tracking of the time behavior of re-elongation after cessation of the muscle activity), b) the experimental set-up is quite complex asking for a robotic arm sustaining the US probe immersed in a pool able to accommodate the distal leg, c) the post-processing of each images to extract the length change is complex and time consuming, d) the cost of a US system is much greater that a simple laser distance sensor. Finally, the choice of the MMG signal make easy the replication of the study.

An experimental design that can neatly provide basic data about the tension reduction and re-lengthening processes relationship of an active muscle once the activity is withdrawn must be based on stimulated contractions. In this way it is possible to minimize the uncertainty of the individual fading pattern of the central nervous system drive suspension that may influence the outcome of the changes in muscle contractile status during the relaxation phase.

Given the possible meaning in determining the mechanical efficiency of alternating movements, the aim of this work was to compare in tibialis anterior of young and old subjects the dynamics of:

a) the torque reduction at the ankle

b) the muscle re-lengthening during the relaxation phase

It is worth to underline how important is the study of tibialis anterior mechanics given its major role in the gait cycle for both stabilization of the ankle joint during the early phase of stance and for elevation of the foot during the early phase of swing (Lacquaniti et al. 2012). As a consequence, its relaxation dynamics may deeply influence the timing of transition to the following phases of the gait.

6.3 Materials and Methods

6.3.1 Subjects and measurements

Twenty recreationally active young participants (10 males and 10 females; age 21-33 years old) and twenty recreationally active older participants (10 males and 10 females; age 65-80 years old) with no orthopedic or neurological disorders were recruited to participate in this study. After receiving a full explanation of the experiments, they provide their written informed consent. The subjects were asked to

refrain from caffeine intake and intense physical activity in the 24 hours preceding the test. This study was conducted in accordance with the latest version of the Declaration of Helsinki and approved by the local Ethical Committee. The participants' dominant lower limb was positioned on a specific ergometer equipped with a load cell (Figure 1), which measured the torque generated during the electrically stimulated contractions of the tibialis anterior muscle (Cogliati et al. 2020).

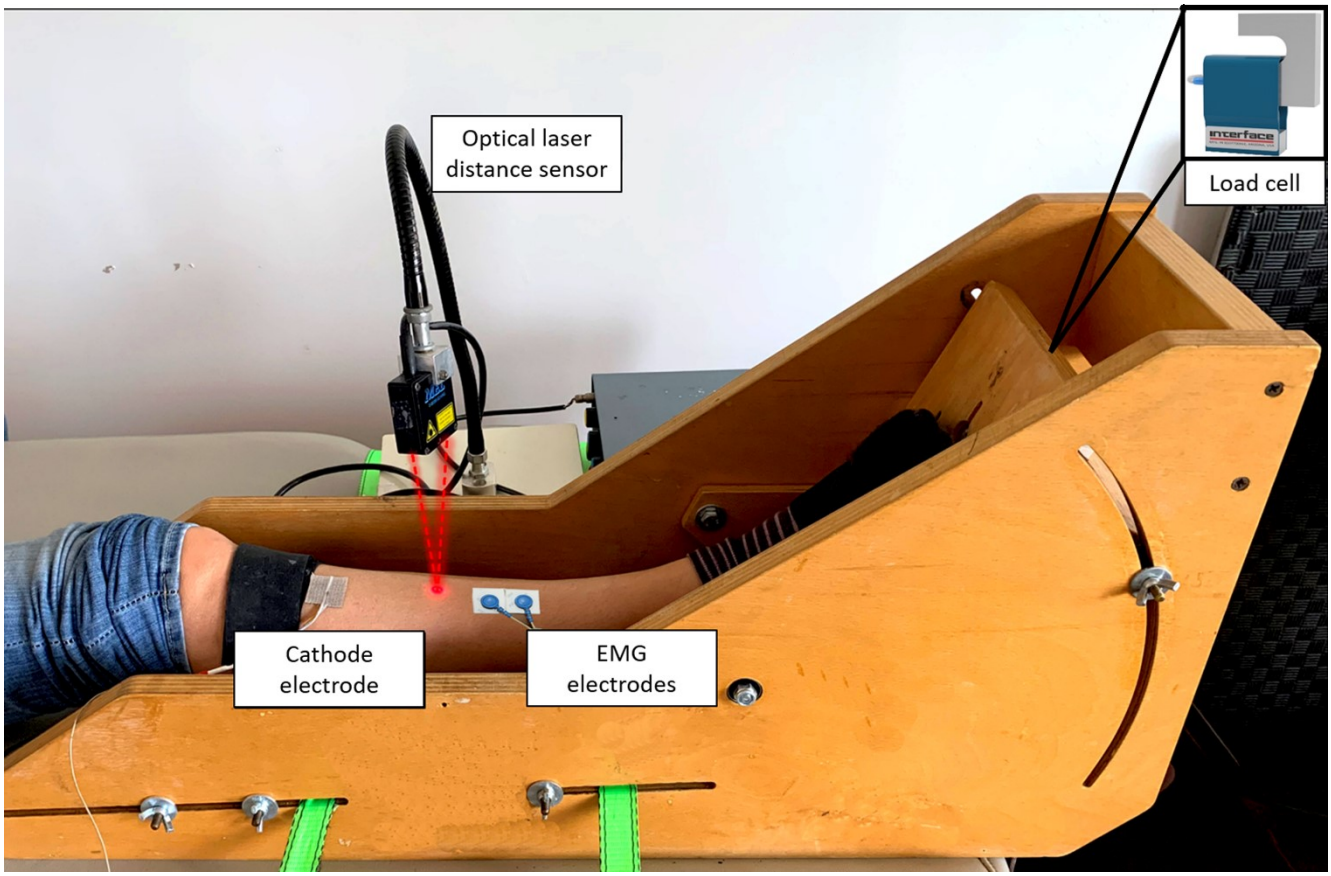

Figure 1: Schematic representation of the experimental set-up. The custom-made wooden ergometer for static contraction of the tibialis anterior muscle with the load cell for torque measurement is represented. The cathode at the tibialis anterior main motor point, the optical laser distance sensor pointing at the muscle belly (MMG detection) and two electrodes for the detection of the EMG in differential mode are reported.

While the hip and the knee were, respectively, fixed at 90° and 180°, the ankle was positioned in a neutral position at 110°. The foot was strapped to the wood plate connected to the load cell (model SM-100 N, by Interface Inc., Scottsdale, US-AZ). The force signal acquired by the load cell was band-pass filtered at 0-64 Hz and amplified (MISO- OT Bioelettronica; Turin–Italy). In order to get the dorsiflexion torque produced by each subject, the distance between the ankle fulcrum and the load cell at the foot plate was measured and used to convert the force signal in torque [T = F (N) x d (m)]. According to Orizio et al. (Orizio et al. 1999, 2008) the displacement of the tibialis anterior muscle surface was transduced as a mechanomyographic signal using an optical laser distance sensor (M5L/20, MEL Mikroelektronik, Germany). The instrument has the following features: range of measurement ±10 mm, sensitivity 1 V/mm, linearity 0.6%, resolution <6 μm, bandwidth 0–10 kHz. The laser beam was pointed

to the tibialis anterior muscle belly presenting the largest displacement during the tetanic stimulation. The common position was at about 1 cm from the tibial crest as reported in figure 1. The device provided an output DC voltage proportional to the distance between the laser-beam head emitter and the reflecting muscle surface. The measure of the distance of the reflecting surface from the laser source was not affected by surface rotation within ±15° and ±30° with respect to the short and long axis of the laser head, respectively. The force and MMG were digitized at a frequency of 1024 samples/s (CED-1401 of Cambridge Electronic Design of Cambridge).

An electrical stimulator was used to deliver biphasic rectangular stimuli (100 μs duration of each phase) on the tibialis anterior muscle. The cathode electrode (5 x 5 cm) was placed at the skin region over the main motor point of the tibialis anterior (Figure 1), which was identified according to Gobbo et al. (2011). The anode electrode (15 x 10 cm) was positioned on the gastrocnemius muscle. By increasing the amplitude of a 1 Hz stimulation train (10 pulses per each 0.1 V amplitude level), from the minimum value of 0.5 V, the maximum stimulation pulse was identified as the stimulus amplitude eliciting the largest single twitch. Three trains of 35 Hz pulses lasting 3 seconds were administered to the motor point of the muscle with a 1-minute pause between stimulations. The surface EMG evoked by the stimulation train was detected by means of two self-adhesive pre-gelled silver electrodes (1 cm in diameter; inter-electrode distance 30mm). EMG was conditioned using a third order Butterworth band-pass filter (10-512 Hz). After A/D conversion by CED-1401 (Cambridge Electronic Design, Cambridge, UK) the digitized signals were stored on a PC and sampled at 1024 samples/s.

6.3.2 Signal processing: Analyzed parameters during the relaxation phase

To achieve the purpose of the work, the analysis described here below concerns the relaxation phase of the stimulated tetanic contraction which has been partly already considered by several studies (Cè et al. 2013b; Cè et al. 2014a; Esposito et al. 2016; Longo et al. 2016; Esposito et al. 2011; Cè et al. 2013c).

Out of the three stimulation trains, the one with the greatest torque value in the 100 ms time interval before the last stimulus was selected for each subject. The torque and MMG were digitally low-pass filtered at 50 Hz and subsequently normalized to their 100% referred to the average values in 100 ms time interval. The EMG signal was used to identify the end of the electrical activity due to the tetanic stimulation train. The time at which the electrical activity was finished, after the last stimulus, was the time mark at which the evoked EMG reached its average value ± 3 SD calculated from 1 s signal sample before the tetanic stimulation (see Figure 2).

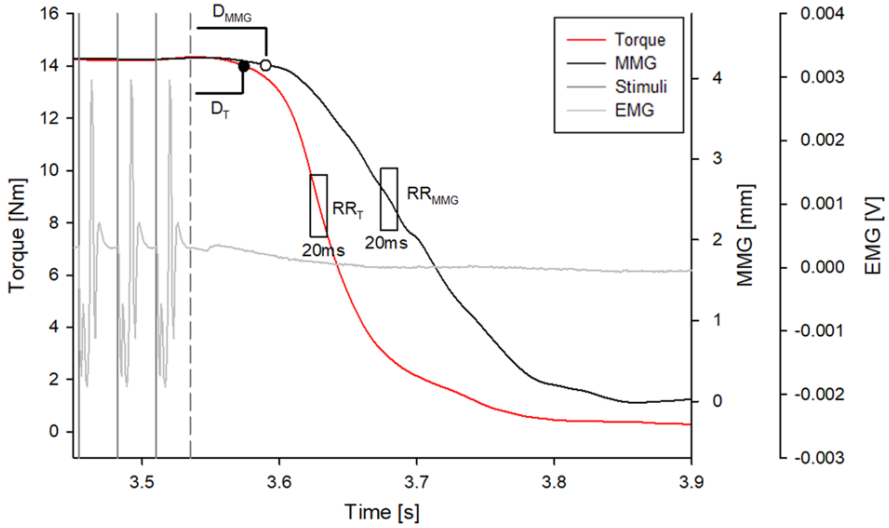

Figure 2: Torque and MMG during the relaxation phase in a representative subject. The 20 ms moving windows on the two signals, used for the maximum rate of reduction identification, are reported. The torque and MMG delays correspond to the time intervals between the end of the evoked EMG activity (dashed grey line) and the beginning of the signal decays (open and filled circles). The EMG signal and the last three stimuli of the 35 Hz train are reported in light and dark grey, respectively.

6.3.3 Relaxation electromechanical delay

During the relaxation phase, a delay (D) can be observed between the end of electrical activity and the beginning of torque and MMG decay. D was calculated as the time instant when the signals decrease 3 standard deviations of their average value during stimulation, both for torque (D_T) and MMG (D_{MMG}) (Figure 2).

6.3.4 Rate of torque reduction and rate of MMG reduction

The rate of reduction for torque signal (RR_T) and MMG signal (RR_{MMG}) were calculated as the ratio between the Δtorque or ΔMMG and Δtime (Figure 2). Specifically, a 20 ms moving window with a step of 1 ms was used across the two signals to identify the maximum rate of reduction (Cogliati et al. 2020; Haff et al. 2015). The same calculation was performed on the normalized signals to obtain NRR_T and NRR_{MMG}

6.3.5 *Time interval for 80%-20% signals reduction*

Additionally, to the discrete information provided by RR or NRR, the time of reduction of both the normalized torque and MMG (TR_T and TR_{MMG}) in the range of 80–20% of their reductions were calculated to further characterize the dynamics of signals decay. The selected range allows to compare the time behavior of the two signals from young and old subjects when both are dynamically changing out of the initial and final transients.

6.3.6 Muscle compliance

In order to have a detailed description of the time relationship between the torque decrement and muscle re-lengthening, the amount of relative MMG variation for each of the ten bins of relative torque decrease

(from 100 to 0%: 100-90%, 90-80%, 80-70%, …, 10-0%) was calculated. This value mirrors the bin-by-bin muscle compliance (MC) to re-elongation throughout the relaxation process.

6.3.7 MMG at the end of torque reduction (MMG_{0T})

The %MMG, amount of re-lengthening left, when torque reduction process finished and reached 0% was quantified for each subject. The parameter was identified as MMG_{0T} and will provide a measure of the whole re-lengthening process efficiency compared to the tension reduction. In other words, how much the re-lengthening is incomplete once the force felt to 0.

6.3.8 Statistical analysis

The data were analyzed using a statistical software (Sigmaplot 11). A Two-Way Analysis of Variance (ANOVA) was used to examine the main and interaction effect age (young and old) and signals (torque and MMG) on the D, NRR, TR. When ANOVA was significant, pairwise comparisons were made with Tukey post-hoc test. For muscle compliance the two factors for ANOVA were the age and the relative torque decrement bin. Furthermore, independent t-test was used to investigate differences between the groups (young and old) for maximal torque during stimulated contraction, RR_T, RR_{MMG} and MMG_{0T} (statistical significance $p<0.05$).
In the graphs the number of asterisks (*) indicates statistically significant differences as follows: $p<0.05$ (*); $p<0.01$ (**); $p<0.001$ (***).

The data reported in this work deal with signals detected during the tibialis anterior tetanic response used to compare muscle mechanics at the onset of voluntary and stimulated contractions in young and old subjects already published (Cogliati et al. 2020).

6.4 Results

An example of the normalized torque (red line) and MMG (black line) signals from representative young and old subject, from which the parameters listed in the previous section have been calculated, can be found in Figure 3. It is evident the difference in the beginning of the decay of the two signals between young and old subjects as well as the different slopes of the two signals through the relaxation process.

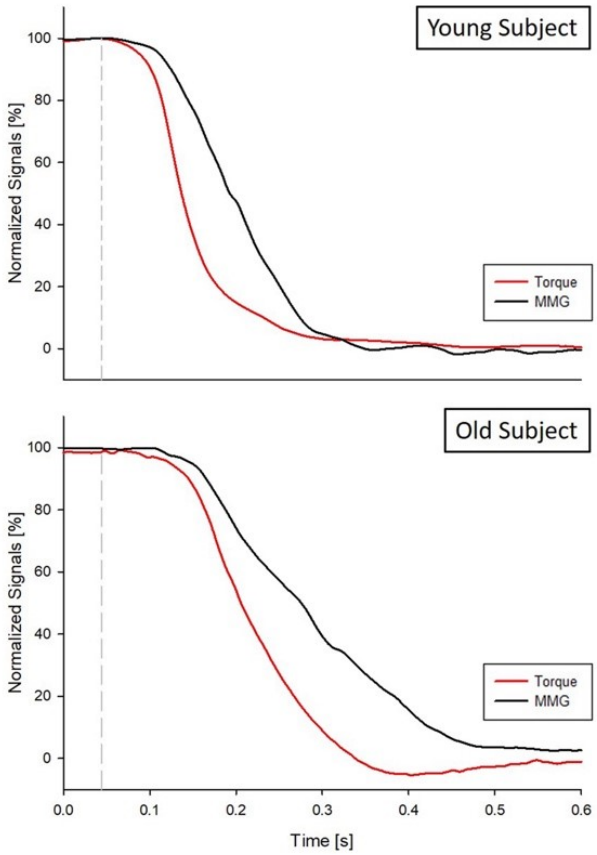

Figure 3: Normalized mechanical transients in two representative young (upper panel) and old (lower panel) subjects. The dashed grey lines mark the end of the electrical evoked activity. The influence of age on the beginning of the transient phase and its time behaviour can be appreciated by comparison of the signals in the upper and lower panel.

6.4.1 Maximal stimulated contraction

The maximal torque in stimulated contraction was significantly different between young and old adults (4.9 ± 2.5 Nm for young and 2.6 ± 1.7 Nm for older; $p<0.001$). A t-test revealed a significant difference ($p=0.002$) between young (3.01 ± 1.17 mm) and old (2.01 ± 0.73 mm) subjects for the maximal surface displacement transduced as MMG.

6.4.2 Relaxation electromechanical delay (D_T and D_{MMG})

The Two-way ANOVA revealed a significant effect of age ($p<0.001$) and signal ($p=0.009$) on the D, but without an interaction between these factors ($p = 0.354$). Specifically, the older subjects had a longer delay compared to young. Moreover, the beginning of relaxation for the MMG started after the torque signals.

Torque. At the beginning of the relaxation phase, the D_T was significantly different between young and older individuals (22.51 ± 5.92 ms for young and 51.35 ± 15.21 ms for older; $p<0.001$) (Fig 4).

MMG. D_{MMG} showed the same behavior of D_T, with a significant difference being observed between young and older individuals (27.38 ± 6.93 ms for young and 61.41 ± 18.42 ms for older; $p<0.001$) (Figure 4).

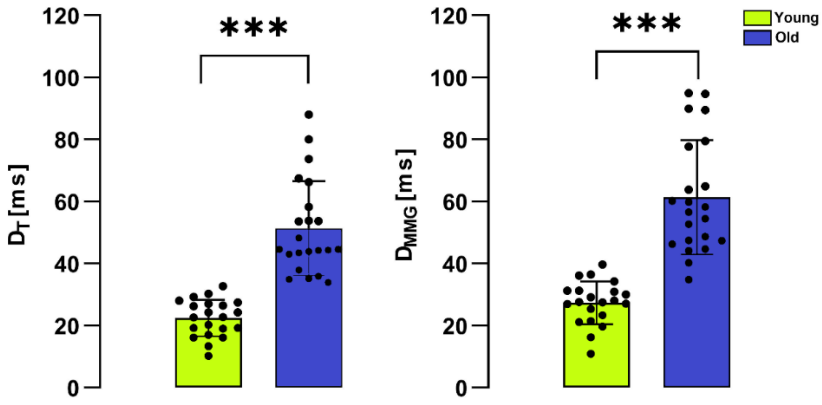

Figure 4: Delay on torque (left panel) and MMG (right panel) in the two groups. The differences in DT and DMMG are statistically different (p<0.001) between young (grey bar plot) and old (white bar plot) subjects.

6.4.3 Rate of torque reduction and rate of MMG reduction (RR_T and RR_{MMG})

During the decay phase after the stimulated contraction withdrawal the maximal RR_T in young and old was -110.4 ± 45.56 Nm/s and -52.72 ± 32.12 Nm/s, respectively, showing a statistical difference between groups (independent t-test; p<0.001). Accordingly, the maximal RR_{MMG} in young (-24.47 ± 10.95 mm/s) was significantly higher than in old (-13.76 ± 6.54 mm/s) subjects (independent t-test, P<0.001). When considering the normalized signals, the results were similar. The Two-way ANOVA revealed a significant effect of age (p<0.001) and signal (p<0.001) on the NRR, but without an interaction between these factors (p=0.508). Specifically, the NRR was higher for young subjects compared to old and the decrease of the MMG signal was slower than the torque signal (Figure 5). Moreover, the maximal NRR_T in young and old was -1256.16 ± 333.36 %/s and -1026.26 ± 267.76 %/s, respectively, showing a statistical difference (p=0.004). Similarly, NRR_{MMG} was statistically different between young (-867.79 %/s ± 148.6%/s) and older (-710.35 ± 178.84 %/s) subjects (p=0.044).

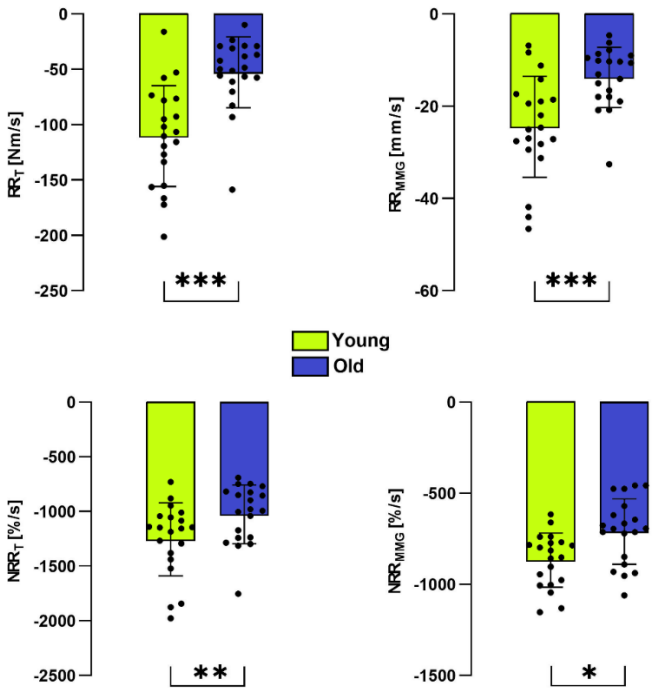

Figure 5: Rate of torque (left panel) and MMG (right panel) reduction in the two groups for the absolute (upper panel) and normalized (lower panel) signals. The differences in the values of the calculated RR are statistically different when Y and O data are compared (RRT and RRMMG $p<0.001$; NRRT $p<0.01$; NRRMMG $p<0.05$).

6.4.4 Time interval for 80%-20% signals reduction (TR_T and TR_{MMG})

The Two-way ANOVA showed a significant effect of age ($p<0.001$) and signal ($p<0.001$) on the TR, but without an interaction between these factors ($p=0.468$).

The TR was lower for young subjects compared to old and the decrease of the MMG signal was slower than the force signal. The TR_T was significantly lower for young (62.7 ± 17.08 ms) than old (81.65 ± 22.36 ms) ($p=0.022$). Similar results were observed for TR_{MMG} in which a statistical difference was observed between young (104.7 ± 28.9 ms) and older (132 ± 31.48 ms) subjects ($p=0.001$).

6.4.5 Muscle Compliance (MC)

The Two-way ANOVA revealed a significant effect of age ($p = 0.016$) and a significant effect of relative torque bin ($p<0.001$) on the relative MMG changes. In addition, interaction was found between these factors ($p = 0.013$). As shown in Figure 6, within bin analysis indicated significant differences between groups for 20-10% bin (15.69±7.5 [young] and 10.8±3.3 [old], $p<0.001$) and 10-0% bin (22.12±10.3 [young] and 17.58±5.6 [old], $p=0.001$). The two groups did not show any other significant difference for the rest of the bins ($p> 0.05$).

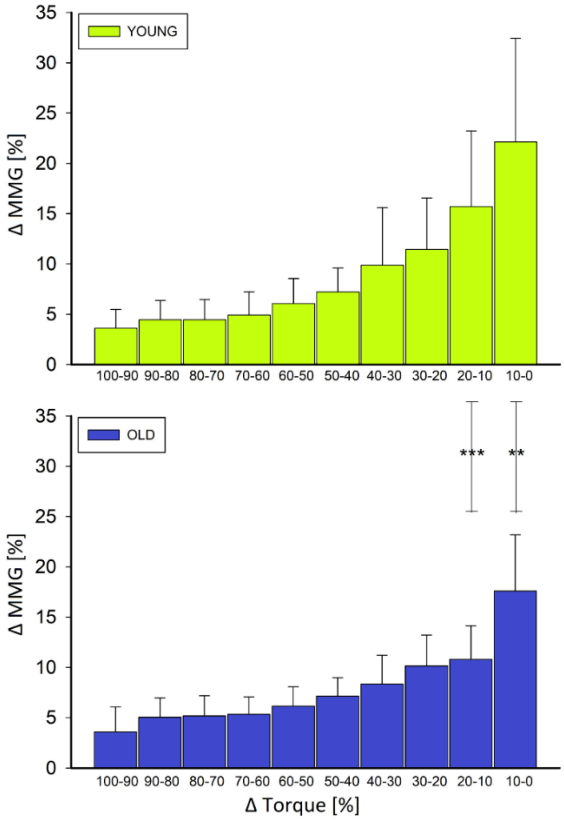

Figure 6: Muscle compliance to the re-elongation throughout the relaxation process. The amount of relative MMG variation for each of the ten bins of relative torque decrease (from 100 to 0%: 100-90%, 90-80%, 80-70%, …, 10-0%) is reported. Statistical significant differences between Y and O MMG variations can be found for 20-10% ($p<0.001$) and 10-0% ($p=0.001$) bins.

6.4.6 MMG at the end of torque reduction (MMG_{0T})

The MMG_{0T} was significantly lower for young (5.41 ± 6.92 %) than old (19.79 ± 11.95 %) (independent t-test, $p<0.001$).

6.5 Discussion

In this work we assessed the torque and MMG reduction at the end of electrically stimulated contraction of tibialis anterior in young and old subjects. Our main findings suggest that the age-dependent changes in muscle mechanics during relaxation process with age are well described by the specific parameters obtained through the analysis of the dynamics of these two signals. These alterations could partially explain the specific features of alternating movements of walking in elderly.

6.5.1 Time course of torque and MMG

The comparative analysis of the time course of muscle length changes, indirectly measured using the MMG, and the force in human muscles has been already reported in the literature (Celichowski et al. 1998; Yoshitake et al. 2005; Yoshitake et al. 2008; Orizio et al. 2008; Cogliati et al. 2020; Cè et al. 2017; Longo et al. 2017; Longo et al. 2016; Shinohara and Søgaard 2006; Jaskólska et al. 2003; Esposito et al. 2011; Esposito et al. 2016; Cè et al. 2013a; Cè et al. 2013c). Only few papers, however, investigate the

phenomena during constant frequency tetanic stimulation in order to isolate the mechanical muscle response from the features of the neural control involved in voluntary contraction. In particular, several authors investigated the torque and MMG changes at the onset (Cè et al. 2017; Cogliati et al. 2020; Esposito et al. 2011; Cè et al. 2013c; Cè et al. 2013a; Esposito et al. 2016) or at the end of evoked activity (Longo et al. 2016; Esposito et al. 2011; Cè et al. 2013c; Esposito et al. 2016, Cè et al. 2014a; Cè et al. 2013b). The MMG detection techniques used the accelerometers (Longo et al. 2016; Esposito et al. 2011; Cè et al. 2013b; Cè et al. 2017; Esposito et al. 2016) or the laser distance sensor (Cogliati et al. 2020; Orizio et al. 2013; Yoshitake et al. 2008). Here, the experimental design based on tetanic stimulation allowed, for the first time in humans, to describe the time relationship between the tension decrease and the muscle re-lengthening throughout the entire relaxation process. These results are in substantial agreement with the data reported in cats (Orizio et al. 2003).

We identified the end of electrical activity by analogy and mirroring the criteria generally accepted for the calculation of the time spent for electromechanical coupling during evoked contraction, in which the time interval between the first applied stimulus (onset of EMG signal) and the detectable tension increase is measured. As a consequence, the delay (i.e., the time interval spent before starting the relaxation process) was measured from the end of electrical activity time instant and the beginning of torque and MMG decay (see Methods for its identification procedure). The comparison of our results with relaxation data from the literature is difficult given the scarce number of papers on this topic. Moreover, the delay of torque and MMG reported in some papers (Booth et al. 1997; Hespel et al. 2002; Cè et al. 2014a, Cè et al. 2013b, Esposito et al. 2011) have been obtained using different techniques of signal detection (e.g., the MMG was transduced by an accelerometer) and/or included the whole duration of the electrical activity evoked by the last stimulus of the tetanic train. On the contrary in our procedure this interval was not considered. For this reason, the absolute values of D_T and D_{MMG} reported are not directly comparable with those determined by the cited authors. Nonetheless, the literature data and ours agree that the D_{MMG} is always greater than D_T. These differences, in the signals decay onset, as well as in the absolute (RR_T and RR_{MMG}) and relative (NRR_T and NRR_{MMG}) decay rates consistently indicate that force declines before muscle re-lengthening. This last complex process is influenced by several determinants such as the tendon shortening after fall of tension, and other possible factors (elastic energy storage restitution, changes in fluid distribution, variation in intramuscular pressure, etc.) as discussed by (Orizio et al. 2003) and partly described by the model suggested by Uchiyama and Hashimoto (2011).

6.5.2 Influence of age on muscle relaxation process monitored by torque and MMG

As above mentioned, the D_T and D_{MMG} measured in this study are not directly comparable with those determined in several studies about the mechanical process of relaxation (Cè et al. 2013b; Cè et al. 2014a; Esposito et al. 2016; Longo et al. 2016; Longo et al. 2017) when the young population is considered. On the contrary the TR_T (reflecting the time spent from 80 % to 20 % force decay) is in line

with those reported for the investigated subjects below 30 years old when the different muscle mass and the interval along the tension reduction period are taken into account (Cè et al. 2014a; Booth et al. 1997; Hespel et al. 2002). No data are reported in the literature about the muscle surface dynamics (MMG) during the relaxation phase after tetanic stimulation.

When the aged population is considered, no data about all the above mechanical considered parameters after tetanic stimulation can be found in the literature.

Our data indicate that in both torque and MMG the beginning of decay and their reduction velocity (absolute and relative) occur later and are slower in old than young, respectively. To discuss this influence of age on the relaxation process some basic consideration on its cellular mechanism can be useful. However, we have to keep in mind that the data retrieved from single fibers or isolated myofibrils cannot be directly used to explain experimental data obtained from the whole muscle tendon unit in humans. The cellular data can only highlight some factors that cannot be disregarded but their action can be "filtered" by the much more complex situation of the "in-vivo" experimental set-up.

Muscle contraction is a consequence of the electromechanical coupling. Basically, it is determined by the increase of the cytosolic [Ca^{++}] that removes the thin filament inhibitory state and allows the acto-myosin interaction or cross-bridge cycle. Vice versa, during muscle relaxation, the muscle active tension decreases for the decline of cytosolic [Ca^{++}] restores the inhibitory state of the thin filament so that myosins which end the cross-bridges cycle cannot start a new one. The sarcoendoplasmic reticulum calcium transport ATPase (SERCA) pump plays a key role given its function of removing calcium from the cytoplasm and moving it in the reticulum (Periasamy and Kalyanasundaram 2007). It is important to recall that when [Ca^{++}] decreases below the threshold for thin filament activation, the number of attached active cross bridges initially slowly decays and then undergoes to a fast collapse with a sudden increased rate of cross-bridges detachment (Cleworth and Edman 1972; Tesi et al. 2002). Thus, at the end of an isometric contraction, the tension decline takes place in two phases: in the first phase, the sarcomeres are kept in an isometric condition (slow phase) while in the following one the sarcomeres either shorten or elongate with a dramatic force fall (fast or chaotic phase) (for a review see Poggesi et al. 2005; Hill et al. 2021). Even if it is not known the extent to which the described phenomena can contribute to the time course of relaxation in intact mammalian muscle at physiological temperature (Hill et al. 2021) the body of knowledge on muscular relaxation process must drive our interpretation on the differences in torque and MMG parameters in young and old population found in this study.

Following the interpretation outlined above, the rate of myoplasm Ca^{++} removal likely determines the time at which the threshold for tension decay is reached and probably is monitored by D_T and D_{MMG} parameters. The difference of these parameters in young and old may be due to differences in their SERCA characteristics. Indeed, Lamboley et al. (2014) reported that two different SERCA isoforms are expressed in fast and slow fibers. They appear to have different rates of calcium re-uptake. The slow

fibers, with SERCA2 isoform, have a slower Ca^{++} re-uptake than fast fibers with SERCA1 pump. Given the prevalence of slow fibers in aged tibialis anterior (Orizio et al. 2016; von Haehling et al. 2010; Miljkovic et al. 2015) this can explain the longer delay of mechanical decay in old vs young. Moreover, Xu and Van Remmen (2021) reported a lower functionality of SERCA pump in aged subjects.

The steeper portion of torque and MMG decay, described by RR and TR, is likely and strongly influenced by the myosin cross-bridge kinetics. As shown by Belus et al. (2003) and by Stehle et al. (2002), the chaotic and fast phase of tension decline is markedly dependent of myosin isoforms. Therefore, the above-mentioned prevalence of slow fibers in tibialis anterior of elderly subjects can well explain the lower RR and NRR.

When the whole muscle tendon unit during relaxation after activity is considered, our data showed that aged tibialis anterior re-elongates at a lesser extent for the same tension reduction compared to young tibialis anterior (for muscle compliance in the 20-0% tension reduction). This is in agreement with the statistically significant greater value of MMG_{0T}. The explanation can be found in the changes of muscle and tendon stiffness with aging. While tendon stiffness decreases (Magnusson et al. 2008), muscle stiffness increases with aging. The factors influencing the increase in muscle stiffness, passive resistance, in aged mammals are well described in Gajdosik et al. (2005) and can be summarized as: a. a substitution process of the contractile tissue with fat and connective tissue; b. a larger collagen amount in muscles dominated by slow twitch muscle fibers as those of old subjects. By analogy the compliance of the muscle to re-elongation (tracked by MMG measure) due to the passive restitution of the elastic energy stored during tetanic stimulation may be influenced by the changes of the muscle viscoelastic properties with ageing.

6.6 Conclusion

Our data suggest that the degree of age-dependent impairment, during the different phases of gait cycle, can be partly related to the delay of the implied muscles to relax and re-elongate after their activity. This experimental set-up measures the individual, internal muscle capacity to re-lengthening as part of the whole resistance to ankle joint angle changes when the calf muscles are activated. Eventually, our results contribute to the estimation of muscle mechanics properties alteration that may play a key role in the elongation of the gait phases in aged persons.

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7. Chapter Seven: High-density surface electromyography allows for longitudinal assessment of the neural drive to muscle in individuals with acute stroke.

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7.1 Abstract

Previous work on neuromuscular impairments following stroke has mainly focused on the chronic phase of recovery, and relatively little is known regarding the acute phase. Studies demonstrating impairments in muscle activation have typically used single bipolar surface electromyography (sEMG) recordings, which may lead to a mischaracterization of muscle excitation. In this study, we assessed neuromuscular function of patients undergoing rehabilitation therapy in the acute phase post-stroke, combining high-density sEMG (HDsEMG) decomposition with isometric force recording to quantify changes in force production and motor unit discharge rates in comparison with global amplitude of a single bipolar sEMG signal. Seven patients with acute hemiparetic stroke were tested, beginning when a detectable dorsi- and plantarflexion movement could be observed (T0) and then again 15 and 30 days later (T15 and T30). The isometric maximal voluntary contraction (MVC) in dorsi- and plantarflexion were measured at these time points. HDsEMG signals recorded from tibialis anterior, gastrocnemius lateralis and medialis, and soleus muscles during isometric contractions at 10% and 30% MVC were decomposed into motor unit discharge offline. Our main results revealed significant impairments in maximal force production at T0, which improved over the 30 days of inpatient rehabilitation therapy. There were also increases in mean motor unit discharge rate for TA and SOL muscles at 10% MVC. These neuromuscular changes could not be captured by using the classical, bipolar sEMG approach. Our results suggest that the combination of force recordings with HDsEMG may provide useful information in the acute phase of stroke and, longitudinally, during inpatient rehabilitation therapy.

7.2 Introduction

It is well established that hemiparetic stroke results in motor impairments that vary according to the area of the central nervous system affected by the ischemic or hemorrhagic event [1]. These impairments can

significantly affect the ability of stroke survivors to perform daily activities and are compounded by subsequent inactivity and physical deconditioning [2]. Early rehabilitation therapy can improve a patient's functional mobility and independence in daily life [3-6]; however, for many patients, motor recovery is suboptimal and substantial motor deficits persist. The vast majority of our knowledge about neuromuscular alterations following a stroke has come from studies examining patients in the chronic phase (> 6-12 months post-stroke), but little is known about how they evolve in the acute phase [7]. A more complete understanding of early neuromuscular changes and their time course during acute rehabilitation therapy would help the development of novel, more effective rehabilitation interventions.

Compelling evidence suggests that post-stroke dysfunction in force production is likely caused by impaired excitability in descending motor pathways [8] and resulting changes in motor unit frequency coding and recruitment [9]. In both clinical and research settings, bipolar surface electromyography (sEMG) has typically been used to infer these neurological alterations and the following recovery. For instance, sEMG has been applied for detecting fibrillation potentials in the hemiparetic side [10], and as a biofeedback intervention, for targeting motor impairments in stroke patients [11]. However, there are several non-physiological factors affecting the acquisition and interpretation of bipolar sEMG, which may result in a mischaracterization of muscle excitation during specific motor tasks [12]. One possibility for minimizing these factors to apply several electrodes over the muscle of interest (i.e., high-density surface electromyography; HDsEMG), which provides a more accurate assessment of muscle excitation than single bipolar sEMG recordings [13]. Additionally, when combined with convolutive blind source separation techniques, HDsEMG allows for the non-invasive assessment of the discharge patterns of many individual motor units [14]. Considering the motor units are the final common pathway of the neuromuscular system, their assessment provides a direct window to the neural control of human movement. Indeed, HDsEMG signals decomposition combined with isometric strength measurements has provided insights into the motor unit neural drive from multiple muscles in chronic stroke patients [15, 16]. However, there is still a lack of this information in the acute phase of the disease and the initial stages of rehabilitation and recovery of function.

The primary objective of this study was to combine HDsEMG signals decomposition with isometric force recordings to quantify changes in force production and motor unit discharge rates in acute stroke patients during different time points of inpatient rehabilitation therapy. Additionally, we aimed to compare results from two different approaches, bipolar sEMG and HDsEMG signals decomposition, for assessing neuromuscular alterations during the acute phase of stroke. By comparing the results obtained from each method, we sought to provide insights that can guide researchers in selecting the most appropriate approach for evaluating stroke patients in cross-sectional neuromechanistic studies as well as when assessing the efficacy of rehabilitation interventions.

7.3 Methods

7.3.1 Participants

Seven patients in the acute phase of stroke (mean ± SD age 52 ± 19.98 years; time post-stroke < 12 weeks; 4 females) were recruited at Fondazione Teresa Camplani - Casa di Cura Domus Salutis (Brescia, Italy). All patients had severe lower limb impairment.

The inclusion criteria required that the participants have suffered from either ischemic or hemorrhagic stroke and be in the acute phase of the disease (< 12 weeks post-stroke). Being able to perform plantarflexion and dorsiflexion of the ankle and understanding and following the protocol were also inclusion criteria. This ensured that only patients who were cognitively and physically able to perform the tasks were included in the study. All participants signed a written informed consent before starting the experiments. This study was conducted in accordance with the latest version of the Declaration of Helsinki and approved by the local Ethics Committee (NP2490).

7.3.2 Experimental protocol

The study consisted of three experimental sessions during hospitalization and rehabilitation therapy of the patients: when they recovered detectable dorsi- and plantarflexion movements following stroke (T0), 15 days after T0 (T15), and 30 days after T0 (T30). An additional session, only at T0, was conducted to measure the maximal ankle force production of the non-affected side. During these 30 days, they followed subjective acute rehabilitation therapy consisting of passive mobilization, gait re-education, strengthening, proprioceptive and coordination exercises and motor rehabilitation exercises with the support of robotic devices. In each experimental session, patients had their legs comfortably positioned on a wooden design ergometer with their knee fully extended [17]. The foot of the tested leg was fixed with straps to an adjustable footplate that held the ankle at specific angles. This footplate was connected to a load cell (SM-500 N, Interface, Arizona, USA) to record the dorsi- and plantarflexion isometric forces produced by the ankle (Figure 1A).

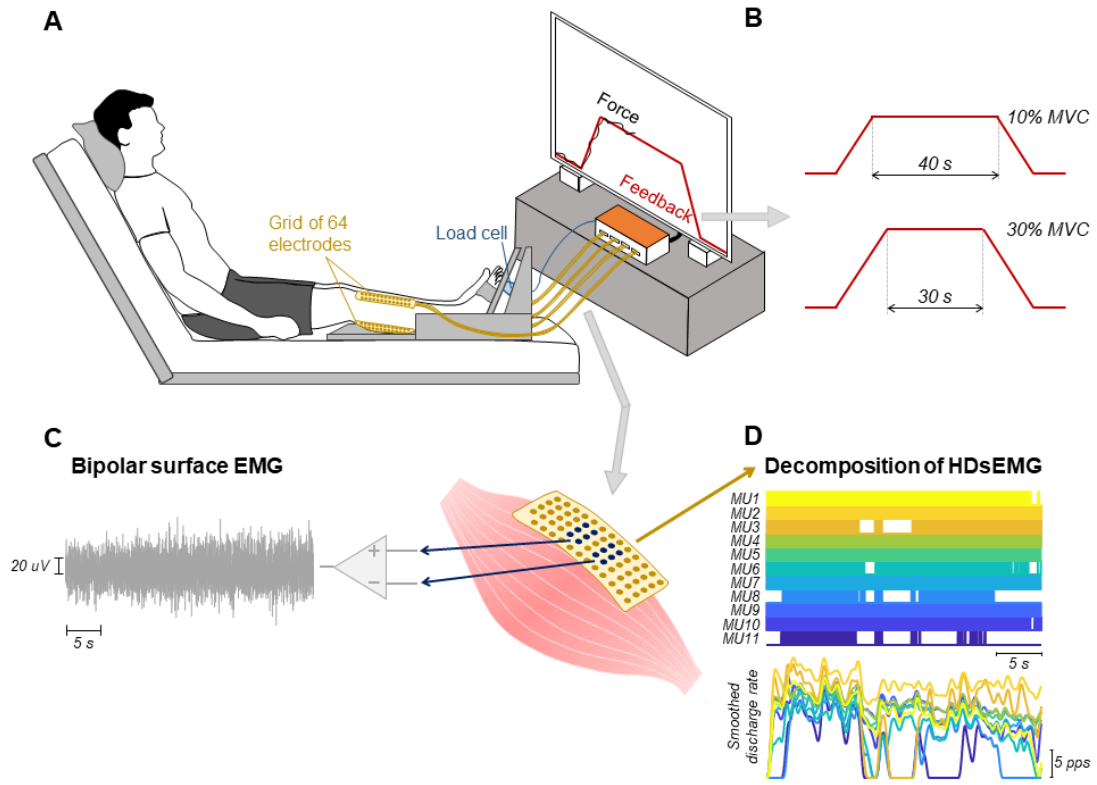

Figure 1: (A) Schematic representation of the position of the participants, the electrodes position for TA and GL, and the screen used for visual feedback. (B) Trapezoidal profiles provided for the patients during the submaximal tasks, with the plateau region reaching 10% and 30% MVC. (C) and (D) shows the two approaches used to assess the neuromuscular alterations in acute stroke patients: simulated bipolar surface EMG from the grid (C) and decomposition of HDsEMG signals (D).

Before starting the data collection, participants underwent a brief training session to familiarize themselves with the procedures. After that, they were asked to perform three isometric maximum voluntary contractions (MVC) in which they were instructed to achieve their MVC within three seconds and hold it for five seconds [16]. The highest force value achieved across the three trials was then set as the reference value for the submaximal tasks. At least 2 min after MVCs, participants were asked to follow trapezoidal profiles at two force levels, 10%, and 30% MVC. Specifically, they had to isometrically increase the force from 0% MVC to the target in 5 s, hold it for 40 s (10% MVC) or 30 s (30% MVC), and decrease it to 0% MVC in 5 s (Figure 1B). This trapezoidal profile was repeated two times for each of two force levels, with a rest-in period of 2 min. During all submaximal trials, participants were provided with visual feedback of the ankle force on a screen (Figure 1A). The protocol was repeated for both dorsi- and plantarflexion contractions in a randomized order. The ankle joint was positioned at 110º and 90º for the dorsiflexion and plantarflexion tasks, respectively [18].

7.3.3 Data collection

In addition to the acquisition of isometric ankle forces, HDsEMG signals were recorded using four 64-electrode matrices (Figure 1A; 8 mm inter-electrode distance; OT Bioelettronica, Turin, Italy). The matrices were positioned according to [19], longitudinally on the belly of the tibialis anterior (TA),

gastrocnemius lateralis (GL), soleus (SOL), and gastrocnemius medialis (GM) muscles. Prior to electrode placement, the skin was cleaned with abrasive paste (EVERI, Spes Medica, Genova, Italy), and shaved when necessary. The reference electrode was positioned on the ankle.

HDsEMG signals were recorded in monopolar derivation and amplified by a variable factor across subjects (from 2,000 to 5,000) using a 12-bit A/D converter (20-500 Hz; EMG-USB2+, OT Bioelettronica, Turin, Italy). The force signal provided by the load cell and HDsEMG signals were sampled synchronously at a frequency of 2048 Hz.

7.3.4 Data analysis

Force and HDsEMG signals were analyzed offline using Matlab (The MathWorks Inc., Natick, Massachusetts, USA). First, raw force signals acquired during MVCs were low-pass filtered at 15 Hz using a third-order Butterworth filter. To quantify changes in dorsi- and plantarflexion force production, the peak force was computed as the average force value over a 100 ms window centered at the peak. The values obtained at T0, T15, and T30 for the affected leg were then normalized with respect to the non-affected side, which was recorded only at T0.

Monopolar HDsEMG signals were filtered with a third-order Butterworth filter (20-500 Hz cut-off frequencies). After visual inspection, channels with low signal-to-noise ratio or artifacts were discarded (average per subject: 6 ± 9 for TA; 6 ± 8 for GM; 5 ± 7 for GL; 4 ± 3 for SOL). We then used two approaches to assess neuromuscular alterations in acute stroke patients during different time points of inpatient rehabilitation therapy: classical bipolar sEMG (Figure 1C) and decomposition of HDsEMG signals (Figure 1D).

1) Approach 1: classical bipolar sEMG

For this approach, we simulated one bipolar EMG detection site over the muscle belly (size 16 x 8 mm; 24 mm inter-electrode distance). Considering the electrode matrices were positioned as described in [19], the simulated bipolar sEMG was out of the innervation zone region. We specifically calculated the difference between the average monopolar signals from two groups of 6 channels within the electrode grid (dark blue electrodes in Figure 1). Then the root-mean-square (RMS) amplitude was calculated to estimate the degree of TA, GL, SOL, and GM activation. For the MVCs, a time window of 250 ms before the peak force was used. For the submaximal tasks, the RMS was calculated over the steady part of the contraction (30 or 40 s) using non-overlapping windows of 250 ms, and the average was considered for further analyses. The averaged RMS amplitude obtained was then normalized with respect to the RMS of MVC, separately for each time (T0, T15 and T30)

2) Approach 2: decomposition of HDsEMG signals

HDsEMG signals were decomposed into motor unit spike trains using a convolutive blind-source separation algorithm [14]. Briefly, after extending and whitening HDsEMG signals, a fixed-point algorithm that seeks sources that maximize a measure of sparsity was applied to identify the motor unit pulse trains (see raster plot in Figure 1D). The spikes were then separated from the noise and other potential sources using K-means and, while iteratively updating the motor unit separation vectors, the discharge times estimation was further refined by minimizing the coefficient of variation of the inter-spike intervals. This decomposition method has been previously validated in simulated and experimental signals [14]. After the automatic identification of motor units, all the motor unit spike trains were visually inspected. Missing pulses or incorrectly assigned pulses producing non-physiological discharge rates were manually and iteratively edited by an experienced operator [18]. Subsequently, the instantaneous discharge rate of individual motor units was calculated as the multiplicative inverse of the inter-spike interval (bottom part of Figure 1D). The mean discharge rate was then obtained by averaging discharge rate values during the steady part of the submaximal tasks.

7.3.5 Statistical analysis

All statistical analyses were performed in R (version 4.2.2), using RStudio environment. Linear mixed-effect models (LMM) were applied for all statistical analysis, as they account for the non-independence of data points within each participant. For all variables (i.e., Peak MVC, RMS amplitude, and mean discharge rate), random intercept models with time (T0, T15 and T30) as fixed effect and participant as random effect were applied. LMMs were implemented using the package *lmerTest* [20] with the Kenward-Roger method to approximate the degrees of freedom and estimate the p-values. The *emmeans* package was used, when necessary, for multiple comparisons and to determine estimated marginal means with 95% confidence intervals.

7.4 Results

Figure 2A shows a representative example of the dorsiflexion isometric MVCs acquired for each condition. It is possible to see that there was a reduction of ~40% in the dorsiflexion MVC at T0 (orange line) compared to the non-affected side (dark blue line). Conversely, the dorsiflexion peak MVC at T15 (green line) and T30 (yellow line) was much closer to the non-affected side (reduction of less than 10%). Similarly, for the group data, there was a significant effect of time on the peak MVC values for both dorsiflexion (Figure 2A; LMM; $F = 14.82$; $P = 0.002$) and plantarflexion (Figure 2B; LMM; $F = 7.54$; $P = 0.010$). Specifically, for dorsiflexion (Figure 2B), the median peak MVC significantly increased from 51.4 [22.2, 80.7] % at T0 to 94.8 [65.5, 124] % and 78.8 [49.5, 108] % at T15 and T30, respectively ($P < 0.028$ for both), with no significant differences between T15 and T30 ($P = 0.246$). For the plantarflexion (Figure 2C), there was also a significant increase from 62.2 [26.3, 110] % at T0 to 95.7

[53.8, 137] % at T30 (P = 0.011), but without significant differences between T0 and T15 (P = 0.069), as well as between T15 and T30 (P = 0.895).

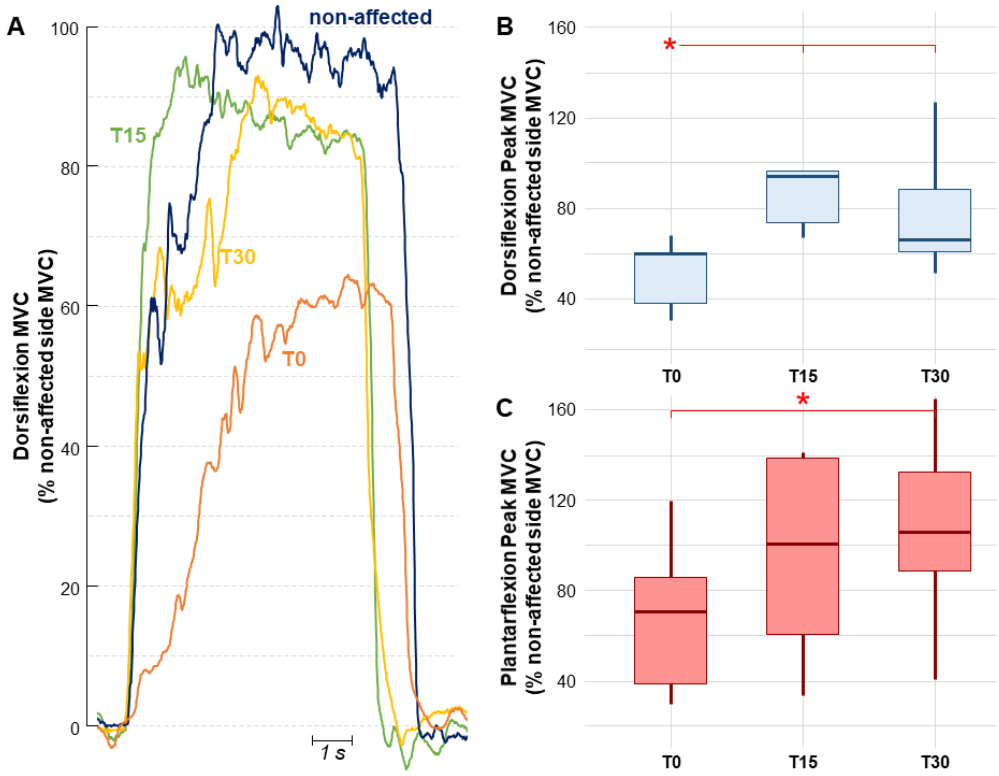

Figure 2: (A) Example of dorsiflexion isometric MVCs acquired at T0 (orange), T15 (green), T30 (yellow), and for the non-affected side (dark blue) of a representative participant. (B) and (C) shows the group results of dorsi- and plantarflexion peak MVCs. Values are normalized with respect to non-affected side. Horizontal traces, boxes, and whiskers, respectively, denote median value, interquartile interval, and distribution range.

In order to assess the neuromuscular alterations in acute stroke patients as classically done in the literature, we estimated the degree of TA, GL, SOL and GM activation through the calculation of the RMS amplitude of a simulated bipolar signal from the grid of electrodes (normalized by RMS at MVC). Figure 3 shows the RMS amplitude results for all muscles and for both force levels.

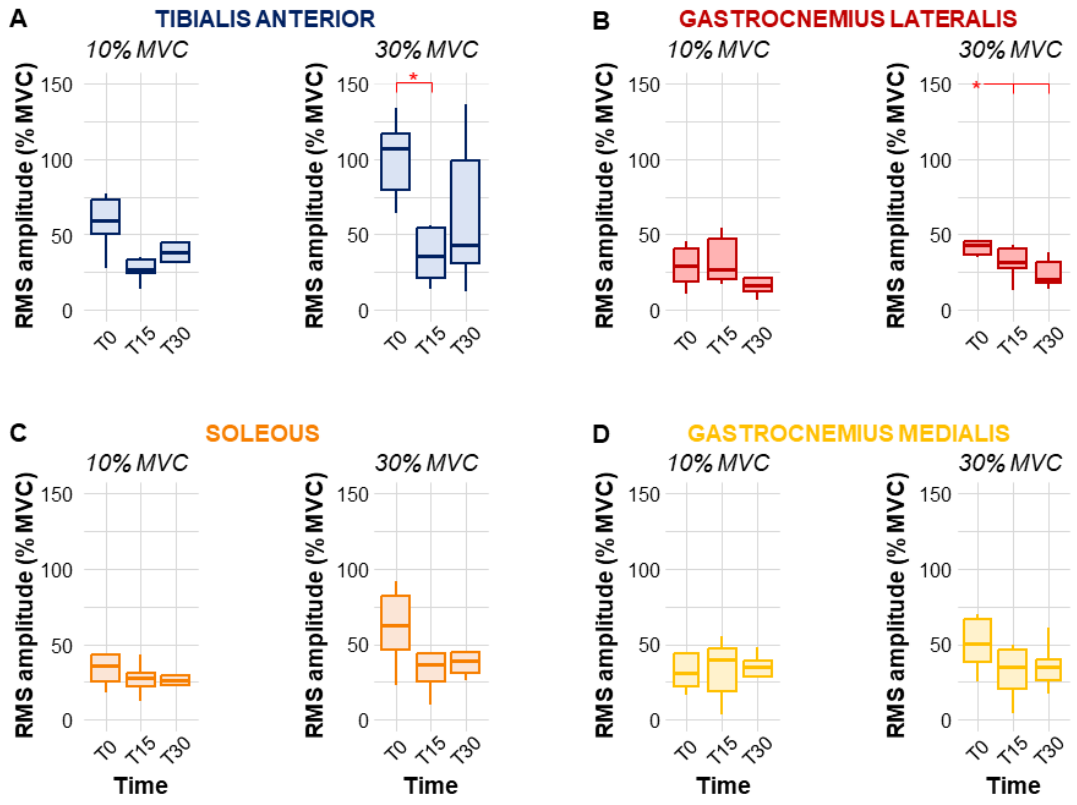

Figure 3: Group results of RMS amplitude for tibialis anterior (A), gastrocnemius lateralis (B), soleous (C), and gastrocnemius medialis (D) muscles. Values are normalized with respect the RMS value obtained at MVC. Horizontal traces, boxes, and whiskers, respectively, denote median value, interquartile interval, and distribution range.

At 10% MVC, there was no significant effect of time on RMS amplitude for any of the investigated muscles (LMM; $F < 2.55$ and $P > 0.128$ for all cases). The SOL and GM activation also did not differ over time at 30% MVC (LMM; $F < 2.35$ and $P > 0.146$ for both). Conversely, the TA and GL activation significantly changed over time at 30% MVC (LMM; $F > 4.98$ and $P < 0.032$ for both). For the TA, there was a significant reduction in RMS amplitude between T0 and T15 ($P = 0.032$), with no changes between T0 and T30 ($P = 0.285$) or between T15 and T30 ($P = 0.672$). For the GL, the RMS amplitude significantly decreased at T15 and T30 when compared to T0 ($P < 0.047$ for both), with no significant differences between T15 and T30 ($P = 1.000$).

The other approach we used to investigate the neuromuscular changes during the acute phase of stroke and subsequent days of rehabilitation was to assess the mean discharge rate of individual motor units using the decomposition of HDsEMG signals into motor unit spike trains. The number of identified motor units varied according to the participants, time of assessment, and muscle. Overall, a greater number of motor units were decomposed from the TA muscles than from the GL, GM, and SOL muscles. Within the triceps surae, the SOL muscle yielded the largest number of identified motor units. All details regarding the number of motor units identified for each participant are provided in Table 1 (10% MVC) and Table 2 (30% MVC).

Mean discharge rate results for all muscles and both force levels are shown in Figure 4.

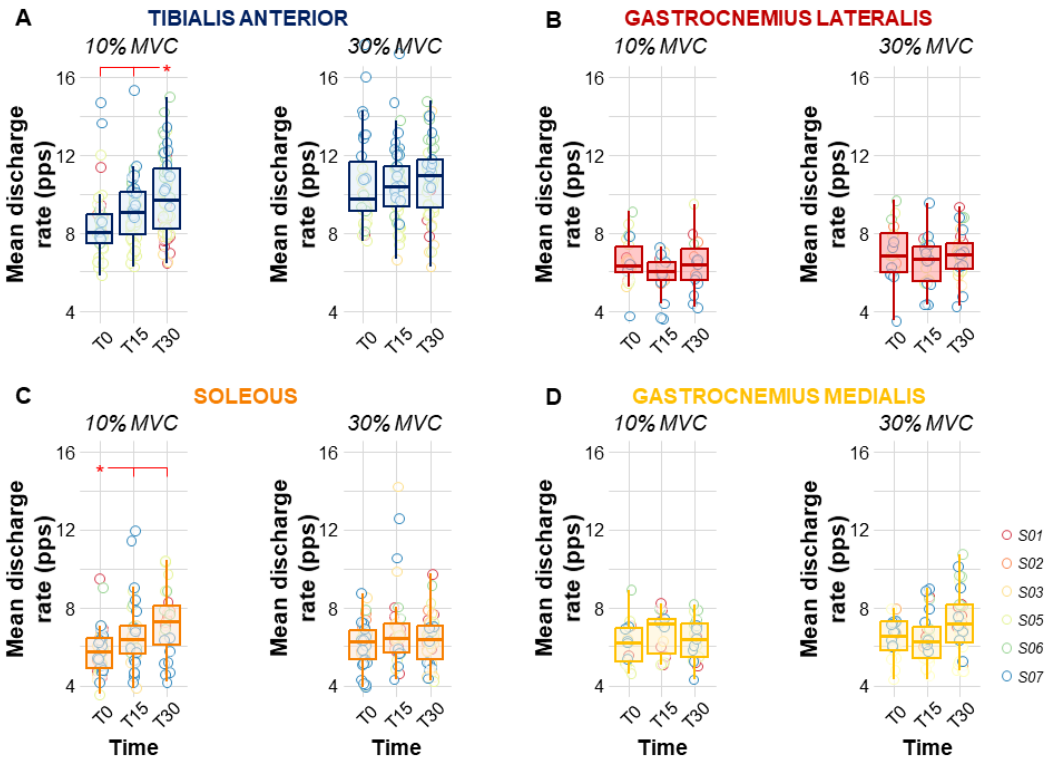

Figure 4: Group results of mean discharge rate for tibialis anterior (A), gastrocnemius lateralis (B), soleous (C), and gastrocnemius medialis (D) muscles. Horizontal traces, boxes, and whiskers, respectively, denote median value, interquartile interval, and distribution range.

For TA and SOL muscles, there was a significant effect of time on mean discharge at 10% MVC (LMM; $F > 6.72$ and $P < 0.002$ for both), but not at 30% MVC (LMM; $F < 2.46$ and $P > 0.090$ for both). Specifically, the TA mean discharge rate significantly increased from 8.47 [6.78, 10.2] pps at T0 and 8.47 [7.08, 10.5] pps at T15 to 9.98 [8.28, 11.7] pps at T30 (Figure 4A; $P < 0.001$ for both). Moreover, as displayed in Figure 4C, the SOL mean discharge rate significantly changed from 5.88 [5.28, 6.49] pps at T0 to 6.80 [6.12, 7.42] pps at T15 ($P = 0.027$) and 7.20 [6.48, 7.92] pps at T30 ($P = 0.004$). Conversely, for GL and GM muscles (Figure 4B and 4D), the mean discharge rate did not significantly change over time neither at 10% MVC (LMM; $F < 1.45$ and $P > 0.246$ for both) or 30% MVC (LMM; $F < 2.95$ and $P > 0.059$ for both).

Table 1: Number of motor units decomposed for 10% MVC

Muscle	Condition	Participants						
		S01	*S02*	*S03*	*S04*	*S05*	*S06*	*S07*
TA	T0	4	1	-	0	22	5	6
	T15	0	1	-	0	28	7	15
	T30	12	0	-	6	26	19	9
	Non-paretic	15	0	0	7	18	15	4
GL	T0	3	1	7	-	5	2	4
	T15	2	4	5	-	0	1	10
	T30	1	3	0	-	1	1	9
	Non-paretic	2	1	0	4	3	3	8
SOL	T0	7	6	13	-	6	1	15
	T15	2	1	11	-	2	4	15
	T30	3	3	0	-	5	4	8
	Non-paretic	7	1	0	10	11	5	7
GM	T0	1	1	2	-	5	1	5
	T15	6	0	1	-	3	1	3
	T30	3	0	0	-	5	4	7
	Non-paretic	0	0	0	2	5	4	9

*A dash indicates data was not collected for the given condition.

Table 2: Number of motor units decomposed for 30% MVC

Muscle	Condition	Participants						
		S01	*S02*	*S03*	*S04*	*S05*	*S06*	*S07*
TA	T0	3	0	-	2	15	4	11
	T15	0	1	-	1	29	9	17
	T30	4	0	-	7	20	13	9
	Non-paretic	19	0	21	4	2	19	12
GL	T0	0	2	1	-	7	2	7
	T15	1	1	1	-	7	2	12
	T30	3	0	11	-	6	3	8
	Non-paretic	5	0	3	6	7	2	14
SOL	T0	0	5	11	-	6	1	19
	T15	12	2	10	-	4	2	7
	T30	6	6	14	-	9	3	8
	Non-paretic	8	1	7	9	18	5	7
GM	T0	0	0	2	-	7	0	9
	T15	5	0	1	-	9	3	7
	T30	6	0	3	-	12	6	7
	Non-paretic	6	0	4	1	3	4	6

*A dash indicates data was not collected for the given condition.

7.5 Discussion

In this paper, we combined isometric force recordings with HDsEMG signals decomposition to longitudinally assess changes in force production and motor unit discharge rates during the initial stages of rehabilitation of individuals with stroke. Moreover, we simulated a single bipolar sEMG signal from the grid of electrodes to investigate changes in global muscle activation, as typically done in the literature. As previously discussed, our main findings revealed that the HDsEMG signals decomposition approach combined with isometric force recordings, which has been previously applied in chronic stroke

patients, may provide rich information in the acute phase of stroke and, longitudinally, during the first 30 days of inpatient rehabilitation therapy.

Results from seven individuals with acute stroke showed significant decreases of ~50% and ~40% on average of dorsiflexion and plantarflexion MVCs, respectively, when compared to the non-paretic side at T0 (i.e., time point when they recovered detectable dorsi- and plantarflexion movements following stroke). These results are in line with the well-established loss in force production capability following stroke [21, 22]. Interestingly, our study found that dorsiflexion appears to be more affected than plantarflexion at T0, as indicated by the interquartile ranges of peak MVC in Fig. 2A and Fig. 2B. These findings are consistent with previous research on stroke-related impairments in dorsiflexor function in the chronic phase, which can lead to an inability to effectively lift the toes during the swing phase of walking, increasing the risk of falls and reducing mobility and independence [23]. Another interesting finding from our results is that these impairments identified at T0 progressively improved during 30 days of inpatient rehabilitation therapy. As further discussed below, we adopted two approaches to examine underlying mechanisms of changes in force control in acute stroke individuals: a classical bipolar sEMG approach and HDsEMG signals decomposition.

When assessing changes in muscle excitation using the RMS amplitude of single bipolar sEMG signal, we did not find differences over time for any muscle investigated at 10% MVC. In contrast, at 30% MVC, there was a significant reduction in normalized RMS amplitude over time for TA and GL but not for SOL and GM. The decrease in amplitude in T15 and T30 relative to T0 is most notable in TA (10% and 30% MVC; Fig. 3). The comparability high level of activation at T0 may indicate that acute stroke patients require a larger neural drive input to perform submaximal tasks at the acute phase. It could also indicate that isometrically contractions for a duration of 40 s (10% MVC) or 30 s (30% MVC) induced fatigue, which is typically accompanied by marked increases in sEMG signal amplitude [24]. However, there are several confounding factors affecting the acquisition and interpretation of bipolar sEMG that, when disregarded, may potentially lead to equivocal conclusions [25, 26]. For instance, if the subcutaneous layer thickness changed over time, the RMS amplitude calculated from bipolar sEMG signal can be affected. In addition, global sEMG limits the possibility to separate central and peripheral motor unit changes. Therefore, the results provided by this approach need to be interpreted carefully.

Our data suggest that post-stroke individuals exhibit decreased discharge rates of motor units on the paretic side during the acute phase, as previously demonstrated in the literature during the chronic phase [16, 27]. In addition, we demonstrated that there were significant increases in the mean discharge rate during one month of inpatient rehabilitation therapy. These modifications were specific to the TA and SOL muscles, and for the 10% MVC task (see Fig. 4A and Fig. 4C). This may indicate that changes in force control in acute stroke patients are muscle- and force-level dependent, which is in line with muscle-specific adaptations in motor unit behavior in chronic stroke individuals [16]. Notably, these changes over

time in TA and SOL muscles at 10% MVC were not revealed using the classical, bipolar sEMG approach, indicating that this approach may not completely capture the changes underlying the improvements in force control during the 30 days following the stroke event. Although some of these changes could be inferred from global sEMG, the confounding variables are fewer when interpreting results from individual motor units, enhancing the robustness of the obtained inferences. A final consideration concerns the number of motor units identified. Although the number of units decomposed varied across patients (see Tables 1 and 2), we showed a substantial increase in motor unit yield compared with typical studies in the literature, which vary widely but often include 20-40 motor units in total. In addition, we do not believe that the difference in the number of decomposed units between participants affected our results, as the statistical analysis considered within subject changes over time (LMM with participant as random effect).

In conclusion, gaining a deeper understanding of neural adaptations following stroke and determining how to optimize physical rehabilitation interventions are crucial goals for preventing long-term motor impairment in people with hemiparetic stroke. As one step toward these goals, it can be helpful to analyze the activity of multiple muscles simultaneously [16] and objectively evaluate modified muscle control strategies in the early stages of rehabilitation [28]. Our results suggest that combining force recordings with HDsEMG analysis may be a useful approach for achieving these objectives. By providing a more comprehensive understanding of muscle activation patterns and force production capabilities, such approach could help clinicians tailor rehabilitation programs to individual patients' needs and improve their overall outcomes. Ultimately, future research in this area may lead to the development of more effective, personalized rehabilitation strategies that can help stroke patients recover their motor function and improve their quality of life.

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8. Chapter Eight: Electrophysiological neuromuscular alterations and severe fatigue predict long-term muscle weakness in survivors of Covid-19 acute respiratory distress syndrome.

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Submitted

8.1 Abstract

Introduction: Long-term weakness is common in survivors of COVID-19–associated acute respiratory distress syndrome ([C]ARDS). We longitudinally assessed the predictors of muscle weakness in patients evaluated 6 and 12 months after intensive care unit discharge with in-person visits. **Methods:** Muscle

strength was measured by isometric maximal voluntary contraction (MVC) of the tibialis anterior muscle. Candidate predictors of muscle weakness were follow-up time, sex, age, mechanical ventilation duration, use of steroids in the intensive care unit, the compound muscle action potential of the tibialis anterior muscle (CMAP-TA-S100), six-minute walk test, severe fatigue, depression and anxiety, post-traumatic stress disorder, cognitive assessment, and body mass index. We also compared the clinical tools currently available for the evaluation of muscle strength (handgrip strength, Medical Research Council sum score) and electrical neuromuscular function (simplified peroneal nerve test, PENT) with more objective and robust measures of force (MVC) and electrophysiological evaluation of the neuromuscular function of the tibialis anterior muscle (CMAP-TA-S100) for its essential role in ankle control. **Results:** MVC improved at 12 months compared with 6 months. CMAP-TA-S100 (P = 0.016) and the presence of severe fatigue (P = 0.036) were independent predictors of MVC. MVC was strongly associated with handgrip strength, whereas CMAP-TA-S100 was strongly associated with PENT. **Discussion:** Electrical neuromuscular abnormalities and severe fatigue are independently associated with reduced MVC and can be used to predict the risk of long-term muscle weakness in [C]ARDS survivors.

8.2 Introduction

Intensive care unit (ICU) patients surviving critical illness may suffer prolonged physical, cognitive, and mental health impairments, collectively known as "post-intensive care syndrome" (1–4). Physical function is affected in 20% to 80% of ICU survivors and significantly impacts the quality of life, independence in activities of daily living, and return to work. (4–6) Physical function impairments manifest with reduced limb strength and range of motion, modified proprioception and balance, pain(7,8), fatigue,(9) activity limitations, and restrictions on participation in social contexts (10) and are common in COVID-19 ICU survivors(11–22). ICU-acquired muscle weakness is described in 43% (interquartile range 25%–75%) of critically ill patients (23) and is a major predictor of long-term weakness(24). Post-hospital predictors of long-term weakness have not been explored.

In a previous study of COVID-19–associated acute respiratory distress syndrome ([C]ARDS) survivors(11), we found that handgrip strength (HGS) assessed with dynamometry was 70% of the predicted normal value at 3 months and significantly improved over time. Simplified electroneurography of the peroneal nerve (PENT)(25) showed a critical illness polyneuromyopathy in 23 of 59 patients (39%). However, global muscle strength assessed using the Medical Research Council (MRC) sum score (MRCss) found significant weakness (MRCss <48) in only three patients at 3 months and in one patient at 6 and 12 months. The MRCss, despite its ability to quickly identify muscle weakness, is influenced by subjective judgment, leading to variability and potential bias, particularly in follow-up assessments conducted by different operators. MRCss has other limitations, including a ceiling effect that prevents

the detection of milder forms of muscle weakness, and it does not account for factors such as muscle length and shortening velocity, which can significantly impact muscle force generation capacity. To overcome these limitations, it is essential to use objective measures of force in muscle groups relevant to daily life activities, such as standing and walking.

Maximum Voluntary Contraction (MVC) serves as an excellent alternative in this regard; MVC is based on an objective value that remains uninfluenced by the operator's subjective perception, ensuring measurement reproducibility over time, and enabling accurate monitoring of changes in muscle strength. Furthermore, MVC can detect even subtle variations in strength, increasing its sensitivity.

Similarly, PENT focuses on the evaluation of nerve function in specific muscles of the foot that may not be associated with the ankle movements that are more relevant for individual and functional independence. Robust electrophysiological measures (e.g., compound muscle action potential, CMAP) of targeted muscles that are important for daily life activities may be more appropriate for evaluating the rate of neural impairment of specific muscle groups.

The aims of this study were 1) to identify the post-hospital predictors of long-term muscle strength as measured by isometric MVC of the tibialis anterior (TA) muscle, and 2) to compare the clinical tools currently available for evaluating neuromuscular function (HGS, MRCss, PENT) using objective and robust measures of force (MVC) and electrophysiological evaluation of nerve function (CMAP) on the TA muscle for its essential role in ankle control.

8.3 Methods

We conducted an observational longitudinal study of adult (≥18 years old) ARDS survivors with confirmed SARS-CoV-2 infection, admitted to the ICUs of the ASST Spedali Civili University Hospital of Brescia, Italy, from February 25, 2020, to November 17, 2021. In this study, we used data from our follow-up clinic, founded in 2014 and partnered in 2020 by a research center on LOng-Term Outcomes (called LOTO) in critical illness survivors (26). The LOTO database contains data from 2014 and continuously records data on patients visited at the follow-up clinic.

ARDS was diagnosed according to the Berlin criteria, and all patients received invasive mechanical ventilation. The Ethics Committee of Brescia approved this study (study title: The PIC syndrome: follow-up of the intensive care patient – approval number: NP3369 – approval date: December 11, 2018) and written informed consent was obtained from all participants (or substitute decision-makers) before data collection. The study was carried out according to the declaration of Helsinki of 1975 and the EU GCP-ICH Guidelines. Patient demographic and clinical characteristics at ICU admission were obtained from hospital records. We adhere to the STROBE reporting guidelines(27). The present study was registered at ClinicalTrial.gov (NCT: NCT04608994).

8.3.1 Follow-up protocol

Patients were invited to attend a post-ICU clinic, where a standardized assessment of physical, cognitive, and mental health status was performed for each patient at 6 and 12 months after ICU discharge. A detailed presentation of the protocol has been published elsewhere. (see appendix of 11)

Neuromuscular function was assessed with MVC, HGS, MRCss, CMAP of the TA (CMAP-TA-S100), and PENT. We also assessed fatigue and mental and cognitive variables because we hypothesized that they could influence muscle strength.

Muscle strength was primarily assessed with the measurement of the MVC (lower limb dynamometry; Figure 1). Briefly, patients were asked to perform maximal isometric ankle dorsiflexion with their dominant leg. The foot was strapped to the plate of a custom-made carbon dynamometer equipped with a load cell (model SM-100N) to measure the applied tension during ankle dorsiflexion. The knee was fully extended (180°), with the ankle placed in a neutral position (110°)(28). Patients performed three MVCs in dorsiflexion of the foot with a 1.5-minute rest between each trial. Each trial allowed 3 seconds to reach the maximal contraction, which was maintained for another 3 seconds. During the trial, researchers verbally encouraged participants, and the maximal force recorded was used as a measure of MVC.

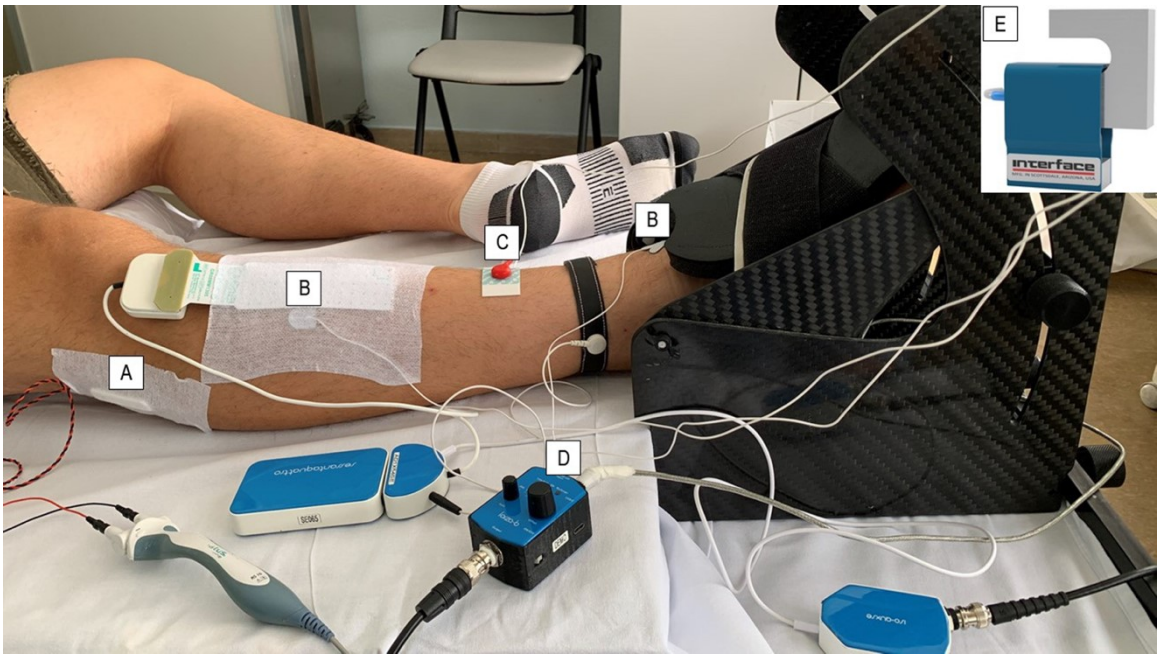

Figure 1: Follow-up protocol set-up. The patients were asked to position their dominant leg in a carbon ankle ergometer, and the foot was supported with Velcro straps. The common peroneal nerve was stimulated under the peroneal head with a (A) bar stimulator using the CMAP scan technique. (B) Surface electrodes were placed on the belly and distally on the tendon of the TA muscle to identify the CMAP-TA-S100 value. The (C) ground electrode was placed at the ankle of the same leg to prevent interference with biopotential signals. (D) The force amplifier amplified the force signal detected by (E) the load cell during both stimulated contraction and maximal voluntary contraction.

HGS was measured using a dynamometer (upper limb dynamometry). Three repetitions were performed, and the maximal value was used as a measure of HGS. We also reported the HGS as a percentage of the predicted normal value standardized per age and sex (29).

In addition, we assessed the MRCss, which provides a global measure of muscle strength. MRCss <48 indicated significant weakness (30). We also considered MRCss ≤55 to indicate mild weakness (31).

Electrical neuromuscular function was assessed by measuring the CMAP of the deep peroneal nerve at the level of the TA muscle (CMAP-TA) and of the extensor digitorum brevis muscle (PENT). The PENT, obtained by the minimum stimulation amplitude that evokes the maximal activation of the extensor digitorum brevis muscle, was considered abnormal if the amplitude was <5.26 mV in both legs(25,32). The CMAP-TA, which was obtained at the level of the TA muscle to better correlate the strength performance measured with the isometric ankle dorsiflexion, was recorded using a novel CMAP scan application on a Viking Select EMG system (CareFusion, San Diego, CA) (Figure 1). Briefly, CMAP-TA was obtained from the TA of the dominant leg using surface electrodes. The negative and positive electrodes were placed on the TA belly and distally on the tendon, respectively, and the ground electrode was placed at the ankle of the same leg. The leg positioning was the same as for the previously described MVC. The common peroneal nerve was stimulated under the peroneal head with a bar stimulator (Spes Medica Srl, Genoa, Italy) with an interelectrode distance of 2.5 cm while patients remained fully relaxed. The stimulation was started at 0 mA and then increased by 0.1 mV in intensity. Two thresholds were recorded: 1) the minimum stimulation intensity needed to obtain a visible action potential (S0), and 2) the minimum stimulation intensity needed to elicit a maximal response from the TA (S100). The detection of these two threshold values allowed identification of the interval within which 500 stimuli (frequency 2 Hz and duration 0.2 ms) were applied in decrements from S100 to S0. The action potential amplitude in millivolts obtained at S100 (CMAP-TA-S100) was used as the main measure of neuromuscular function. PENT is a simplified neurophysiological technique with high sensitivity (100%) and good specificity (85%) that has been validated as a screening test for critical illness polyneuropathy and myopathy(25,32).

Activity limitation was evaluated by 6-minute walk test (6MWT), as a performance-based measure, and fatigue severity score (FSS). For 6MWT predicted values were calculated according to Enright PL et al.(34) Self-reported fatigue was assessed using the Fatigue Severity Score (FSS), a 9-item scale with questions about how fatigue has affected the person's activities and lifestyle during the past 2 weeks. An FSS score ≥36 indicated severe fatigue(35).

Mental health assessment was performed by administering 1) the Hospital Anxiety and Depression Scale (HADS) questionnaire, on which a score ≥8 for both subdomains of depression and anxiety was considered abnormal (36); Cognition was assessed using the Montreal Cognitive Assessment (MoCA), a short cognitive screening tool that has been validated as a general cognitive screening test(22,37).

8.3.2 Statistical analyses

Quantitative variables were described with means ± standard deviations (SD) or median ± interquartile range (IQR), while categorical variables were summarized with counts and percentages. We check the normality of the variables using the Shapiro-Wilk test. The relationship between the measured physical performance variables and the follow-up times (6 and 12 months) was modeled using linear mixed models (LMMs) or generalized LMMs, as appropriate. All models were fit assuming participants as the random intercept and follow-up visit time as a fixed effect.

MVC was modeled using LMM with random intercepts (participants). The final models were defined using a backward variable selection based on the Akaike Information Criterion (AIC), starting from a full model that included the following variables as candidate predictors: follow-up time, sex, age, body mass index (BMI), mechanical ventilation duration, use of steroids in ICU, SAPS II, 6MWT, CMAP-TA-S100, severe fatigue using the FSS, HADS scores for depression and anxiety, presence of cognitive impairment using the MoCA scale, and all pairwise interaction terms between follow-up time and all variables included in the model. The formula for the model was as follows:

$$\text{MVC} \sim 1 + \text{follow up time} + \text{variables} + (1 \mid \text{record\_id})$$

To analyze the correlations between MVC, HGS, and MRCss and between CMAP-TA-S100 and PENT, we used LMM with random intercept (participant). All tests were two-sided, and $P < 0.05$ was considered statistically significant. No data imputation was performed, and all analyses were conducted using R (version 4.1.1).

8.4 Results

A total of 52 patients, 38 (73.1%) male, were enrolled in the study and visited at 6 and 12 months. Demographic and clinical characteristics of patients during their ICU stay are presented in Table 1.

Table 1: Demographic characteristics and ICU variables of patients enrolled in the study.

	6 Months (N=52)
Sex, Male N° (%)	38 (73.1%)
Age (year), Mean (SD)	61.3 (8.75)
BMI at ICU Admission (kg/m^2), Mean (SD)	28.3 (3.58)
SAPS II, Mean (SD)	30.3 (9.37)
Use of NIV pre-ICU, N° (%)	36 (69.2%)
Duration of mechanical ventilation (days), Mean (SD)	11.7 (15.1)
Pronation, N° (%)	25 (48.1%)
Tracheostomy, N° (%)	17 (32.7%)
Use of Steroids, N° (%)	37 (71.2%)
Catecholamines, N° (%)	21 (40.4%)

	6 Months (N=52)
Comorbidities	
No Comorbidity	5 (9.6%)
1 Comorbidity	18 (34.6%)
2 Comorbidities	11 (21.2%)
3 Comorbidities	1 (1.9%)
≥4 Comorbidities	5 (9.6%)
ICU LOS (days), Mean (SD)	16.2 (17.0)
H LOS (days), Mean (SD)	37.8 (29.0)

List of abbreviations: SD, standard deviation. BMI, Body Mass Index. SAPS II, Simplified Acute Physiology Score. NIV, Non-Invasive Ventilation. iNO. ICU, Intensive Care Unit. H, Hospital. LOS, Length of Stay.
Missing values [number of patients (percentage)]: SAPS II: 12 (23.1%); Use of pre-ICU admission NIV: 3 (5.8%); Mechanical ventilation duration: 3 (5.8%); Pronation:3 (5.8%); Tracheostomy: 6 (11.5%); Steroids: 13 (25.0%); Catecholamine: 4 (7.7%); Comorbidities: 12 (23.1%).

Muscle strength improved over time. MVC improved at 12 months (estimate difference [ED] = 3.43 kg when compared with 6 months, P adjusted = 0.003). HGS improved at 12 months as both absolute values in kilograms and percentage predicted value (ED =5.39%, p-adjusted <0.001). MRCss was ≥48 in all patients. CMAP-TA-S100 (ED 0.4 mV, P-adjusted = 0.106) did not improve at 12 months, whereas PENT (ED 1.19 mV, P-adjusted = 0.003) improved at 12 months (Table 2, Figure 2). Severe fatigue was reported by 30.8% at 6 months, and 21.2% at 1 year, without significant improvement over time. Cognitive impairment was present in a significant proportion of patients (21.2% at 6 months and 15.4% at 12 months).

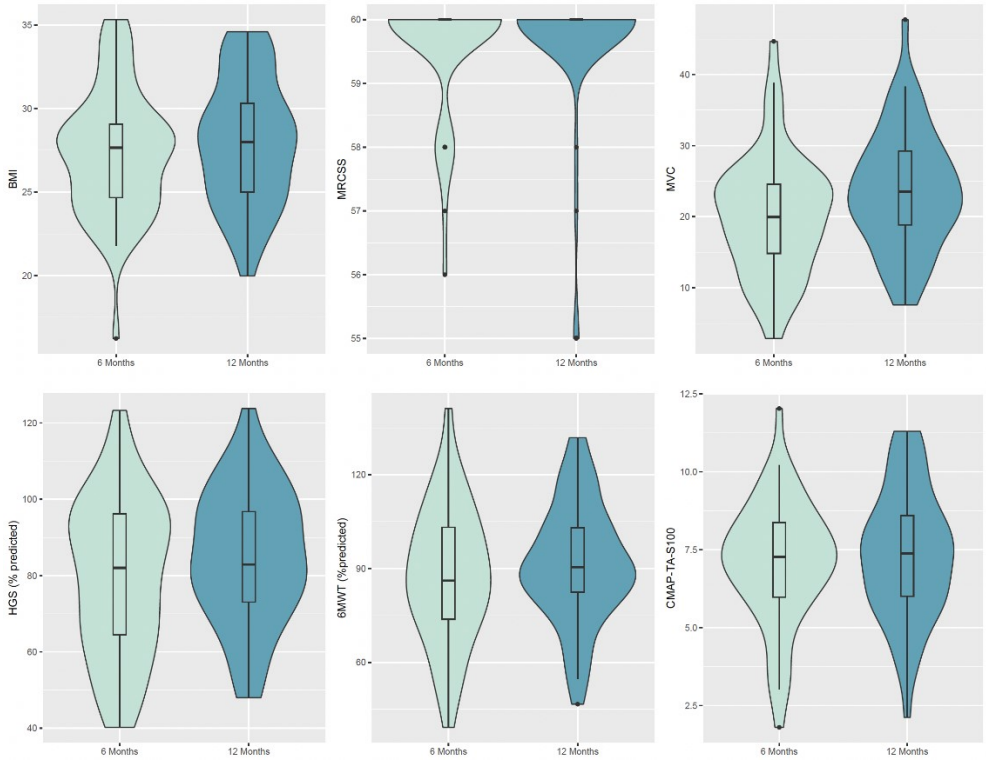

Figure 2: Violin plot for the considered variables at 6 and 12 months. Boxes indicate the first and third quartiles. Black thick lines in the bar graphs denote the median value. The whiskers extending from the box show the range of the data, excluding outliers. Patients exhibited significant changes from 6 to 12 months in BMI (p-adjusted <0.001), MVC (p-adjusted = 0.003) and HGS (% predicted, p-adjusted <0.001). List of Abbreviations: BMI, body mass index. MRCss, Medical Research Council Sum Score. MVC, Maximal Voluntary Contraction. HGS, Handgrip Dynamometry. 6MWT, 6-minute walk test. CMAP, the Compound Muscle Action Potential. TA, Tibialis Anterior.

Table 2: Summary of physical, mental health, and cognitive function variables and neuromuscular electrophysiological measurements at 6 and 12 Months.

	6 Months (N=52)	**12 Months (N=52)**	**Estimate Difference (95%C.I.)**	**P**	**P adjusted [d]**
BMI (kg/m2), Mean (SD)	27.4 (3.79)	28.0 (3.74)	0.61 (0.27– 0.96)	0.001	<0.001
MRCss, [a] Median (IQR)	60 (60-60)	60 (60-60)	0.11 (-0.24 – 0.56)	0.533	0.630
MVC (Kg), Mean (SD)	20.2 (8.55)	23.6 (8.41)	3.43 (2.05 – 4.82)	<0.001	0.003
Dominant hand grip strength (kg), Mean (SD)	30.5 (10.4)	32.4 (9.72)	1.95 (0.928 – 2.96)	<0.001	<0.001
Dominant hand grip strength (% predicted) [b], Mean (SD)	79.7 (20.5)	85.0 (17.6)	5.39 (2.71 – 8.07)	<0.001	<0.001
6-minute walk test, Mean (SD)	450 (108)	474 (96.1)	24.0 (-4.89 – 52.8)	0.096	0.156
6-minute walk test (%predicted) [c], Mean (SD)	87.9 (21.8)	91.2 (19.0)	4.41 (-1.46 – 10.3)	0.132	0.190

	6 Months (N=52)	12 Months (N=52)	Estimate Difference (95%C.I.)	P	P adjusted [d]
CMAP-TA-S100 (mV), Mean (SD)	7.09 (1.99)	7.36 (2.07)	0.4 (0.02 – 0.8)	0.049	0.106
PENT (mV), Mean (SD)	6.21 (4.80)	6.82 (5.21)	1.19 (0.52 – 1.86)	0.001	0.003
Fatigue (Fatigue Severity Score ≥36), N. (%)	16 (30.8%)	11 (21.2%)	-3.0 (-0.31 – -6)	0.076	0.141
Presence of depression (HADS>7), N. (%)	10 (19.2%)	6 (11.5%)	-15.2 (-24.1 – -1.18)	0.781	0.831
Presence of anxiety (HADS>7), N. (%)	7 (13.5%)	10 (19.2%)	0.138 (-1.13 – 1.4)	0.831	0.831
Cognitive impairment (MoCA<26), N. (%)	11 (21.2%)	8 (15.4%)	-1.45 (-3.59 – 0.69)	0.185	0.241

List of Abbreviations: BMI, body mass index. SD, standard deviation. MVC, Maximal Voluntary Contraction. MRCss, Medical Research Council Sum Score. CMAP, the Compound Muscle Action Potential. TA, Tibialis Anterior. PENT, PEroneal Nerve Test. HADS, Hospital Anxiety and Depression Scale. MoCA, Montreal Cognitive Assessment.
Missing value (6 months-12months): MRCss: 9 (17.3%)- 2 (3.8%); HADS: 12(23.1%)-0(0%); PTSD: 0 (0%) – 2(3.8%); MoCA: 3 (5.8%)-0 (0%).
[a] None of the patients had an MRCss<55.
[b] Calculated using established reference values provided by Gilbertson L et al. [14]
[c] Predicted value for the six-minute walk test was calculated according to Enright PL et al. [18]
[d] P adjusted using Benjamini and Hochberg (BH) correction.

Multivariable analysis showed that MVC was independently associated with CMAP-TA-S100 (ED 1.4 kg for each millivolt increase in CMAP-TA-S100, P =0.016), and fatigue (ED -4.88kg in patients with fatigue, p=0.036). There was no interaction between follow-up time and the selected variables (Table 3, Figure 3).

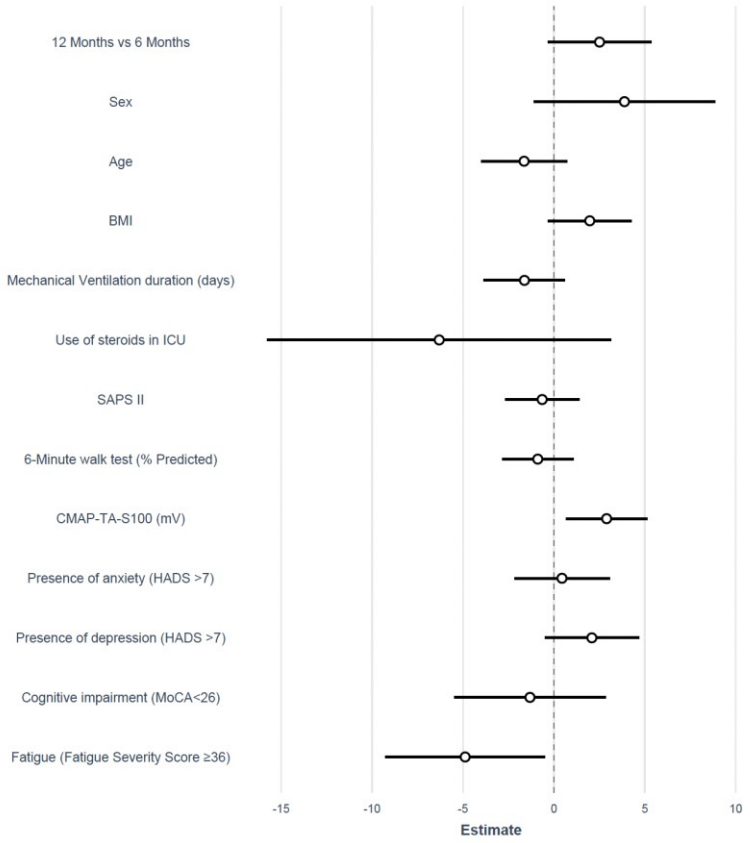

Figure 3: Forest plot of estimate difference for the adjusted mixed model on MVC. List of Abbreviations: BMI, body mass index. ICU, Intensive care unit. SAPS II, Simplified Acute Physiology Score. CMAP, the Compound Muscle Action Potential. TA, Tibialis Anterior. HADS, Hospital Anxiety and Depression Scale. MoCA, Montreal Cognitive Assessment.

Table 3: Effect estimates and corresponding 95% confidence interval (C.I.) for muscle strength prediction using maximal voluntary contraction (MVC in kg) computed using a linear mixed model (LMM) with random intercept. The variable included in the final model were selected using a backward procedure using AIC criterion.

	Model MVC(Kg)		
Coefficient	*Estimates*	*95% C.I.*	*P*
12 Months vs 6 Months	2.51	-0.43 – 5.45	0.092
Sex (Male)	3.88	-1.29 – 9.05	0.137
Age (years)	-0.18	-0.45 – 0.09	0.184
Body Mass Index (Kg/m^2)	0.54	-0.11 – 1.19	0.103
Mechanical Ventilation duration (days)	-0.09	-0.21 – 0.04	0.164
Use of steroids in ICU	-6.31	-16.09 – 3.48	0.200
SAPS II	-0.06	-0.27 – 0.14	0.538
6-Minute walk test (% Predicted)	-0.05	-0.15 – 0.06	0.382
CMAP-TA-S100 (mV)	1.40	0.28 – 2.52	**0.016**
Presence of anxiety (HADS >7)	0.12	-0.63 – 0.87	0.743
Presence of depression (HADS >7)	0.52	-0.15 – 1.19	0.122

Cognitive impairment (MoCA<26)	-1.32	-5.63 – 2.99	0.540
Fatigue (Fatigue Severity Score ≥36)	-4.88	-9.43 – -0.34	**0.036**
Marginal R^2 / Conditional R^2	0.579 / 0.778		

List of Abbreviations: CI, Confidence Interval. SAPS II, Simplified Acute Physiology Score. CMAP, Compound Muscle Action Potential amplitude. TA, Tibialis Anterior. HADS, Hospital Anxiety and Depression Scale. MoCA, Montreal Cognitive Assessment.

MVC was strongly associated with HGS (ED 0.41 kg [95% confidence interval: 0.26–0.56] increase in HGS for each 1-kg increase in MVC, P < 0.001), but not with MRCss (ED= 0.25, P = 0.712). CMAP-TA-S100 was strongly associated with PENT (ED 0.21 mV increase in PENT for each millivolt increase in CMAP-TA-S100, P < 0.001). Patients with abnormal PENT (<5.26 mV) had a mean (SD) CMAP-TA-S100 of 6.87 (1.94) mV, whereas patients with normal PENT had a mean (SD) CMAP-TA-S100 of 7.95 (1.83) mV.

8.5 Discussion

In this longitudinal, 1-year study of [C]ARDS survivors, we found that muscle weakness in COVID-19 patients improved at 12 months and was associated with electrical neuromuscular dysfunction (measured using CMAP-TA-S100) and severe fatigue. Moreover, we found a strong correlation between HGS and lower limb dynamometry (MVC), and between CMAP-TA-S100 and PENT.

MVC was independently associated with CMAP amplitudes measured from the TA muscle: strength increased by 1.4 kg for each millivolt increase in CMPA-TA-S100 amplitude. These findings indicate that [C]ARDS patients have abnormal CMAP-TA-S100 and thus impairments in MVC generation, with improvements observed at 1-year post-hospital discharge. Reduced MVC has previously been reported in quadriceps and biceps brachii in COVID-19 survivors(11).

MVC was independently associated with severe fatigue (patients with fatigue had a mean MVC of 5 kg lower than patients without fatigue). Fatigue was present in 30% of our patients at 6 months without a significant improvement over time, in line with our previous result(38,39) and two recent meta-analysis on post-COVID fatigue(40). To our knowledge, this is the first study to demonstrate that severe fatigue predicts muscle weakness in critically ill survivors. Post-COVID fatigue, defined as an overwhelming and sustained subjective sense of physical, emotional, and/or cognitive exhaustion that is not related to recent physical activity(41), has been associated to a distinct pattern of pathological brain changes involving the thalamus and the basal ganglia(42) which support important cognitive functions such as memory, motivation, and reward-guided behavior a wide range of functions in addition to motor control. Our findings that the persistent subjective experience of fatigue is related to peripheral measures of

physical performance may have implications for future treatments, such as self-guided or health professional-guided physical and cognitive interventions(25,32).

The strong association between CMAP-TA-S100 and PENT amplitudes is important for two reasons. First, it confirms that electrical neuromuscular alterations are diffuse so that the recording site when a peroneal nerve is stimulated (i.e., TA versus extensor digitorum brevis) does not lead to differences in diagnostic findings. Second, CMAP-TA-S100 requires specialized personnel and instruments, and values in a normal population are not available. In contrast, the PENT is a rapid screening test that has been validated in multi-center studies(43) and can be quickly administered. Despite a strong association between a risk factor (i.e., altered electrical neuromuscular activity assessed with CMAP-TA-S100 or PENT) and the disease outcome (muscle weakness), not every predictor is a cause(44), and validation studies are needed before the altered electrical neuromuscular function can be considered causally related to muscle weakness in [C]ARDS survivors.

HGS was strongly associated with MVC measured from the TA muscle (for each 0.5-kg increase in HGS, there was a 1-kg increase in MVC, $P < 0.001$), suggesting that HGS is representative of global muscle strength in [C]ARDS survivors and might serve as a quick screening tool for repeated muscle strength assessment during follow-up(44). However, further studies are needed to validate the diagnostic accuracy of HGS compared with MVC in a new cohort of patients.

MRCss was mostly normal, regardless of the timing of assessment and despite abnormalities in MVC, HGS, and electrophysiological parameters. This result confirms that the MRCss misses an important group of [C]ARDS patients with milder weakness at long-term follow-up.

Some study limitations should be considered in the interpretation of our results. This work was conducted at a single center, and the findings need to be externally validated in an independent cohort. Patients were followed up for 1 year, but assessment at all time points in all patients was not possible because of restricted hospital access and patient unwillingness to continue participation in the study. Moreover, the study was conducted in patients with [C]ARDS, and the generalization to patients with classic ARDS is not possible although plausible. Finally, the associations we found do not imply causality.

8.6 Conclusions

In conclusion, electrical neuromuscular abnormalities (CMAP-TA-S100), and the presence of severe fatigue were independently associated with reduced MVC and can be used to predict the risk of long-term muscle weakness in [C]ARDS survivors.

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9. Chapter Nine: Sex-differences in the longitudinal recovery of neuromuscular function in COVID-19 associated acute respiratory distress syndrome survivors.

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9.1 Abstract

Introduction: Patients admitted to the intensive care unit (ICU) following severe acute respiratory syndrome 2 (SARS-CoV-2) infection may have muscle weakness up to one year or more following ICU discharge. However, females show greater muscle weakness than males, indicating greater neuromuscular impairment. The objective of this work was to assess sex differences in longitudinal physical functioning following ICU discharge for SARS-CoV-2 infection. **Methods:** We performed longitudinal assessment of physical functioning in two groups (fourteen participants (7 males, 7 females) in the 3-to-6 month and twenty-eight participants (14 males, 14 females) in the 6-to-12 month group) following ICU discharge and assessed differences between the sexes. We examined self-reported fatigue, physical functioning, compound muscle action potential (CMAP) amplitude, maximal strength, and the neural drive to the tibialis anterior muscle. **Results:** We found no sex differences in the assessed parameters in the 3-to-6-month follow-up, indicating significant weakness in both sexes.

Sex differences emerged instead in the 6-to-12-month follow-up. Specifically, females exhibited greater impairments in physical functioning, including lower strength, walking lower distances, and high neural input even one year following ICU-discharge. **Discussion:** Females infected by SARS-

CoV-2 display significant impairments in functional recovery up to 1 year following ICU discharge. The effects of sex should be considered in post-COVID neurorehabilitation.

9.2 Introduction

Severe Acute Respiratory Syndrome 2 (SARS-CoV-2) has infected millions of individuals worldwide. SARS-CoV-2 infection negatively affects multiple organ systems, including the neuromuscular system(1,2), especially in patients admitted to the intensive care unit (ICU). Patients admitted to the ICU due to SARS-CoV-2 infection present with muscle weakness for up to one year or more following ICU discharge(3–6), regardless of the disease severity. Sex differences in recovery of muscle function and fatigability were observed following ICU discharge in these patients. Specifically, females exhibit greater muscle weakness and fatigue up to one year following hospital discharge compared to males(3,4). These findings suggest that the progression of recovery between the sexes following ICU discharge may be different, and rehabilitation protocols may need to be sex specific. The cause and mechanisms leading to greater muscle weakness in females are unclear, although muscle weakness may be a consequence of greater neuromuscular dysfunction. No studies to date have examined sex differences in neuromuscular function longitudinally following SARS-CoV-2 infection in patients discharged from the ICU. This knowledge is critical for post-COVID rehabilitation considering sex differences in disease outcome, and progression were already established.

Patients infected with SARS-CoV-2 frequently present with neuromuscular dysfunction following infection. Other than muscle weakness, the neurophysiological tests commonly show low compound muscle action potential (CMAP) amplitude, polyphasic motor unit potentials, and spontaneous fibrillations(1). These findings suggest that motor unit neural control may be impaired, and only limited data are currently available(7). Since the increase in muscle force is modulated through the progressive recruitment of motor units and an increase in the motor unit firing rate, dysfunction in motor unit properties may lead to muscle weakness. Moreover, motor unit properties are different between healthy males and females in several leg muscles(8). For the tibialis anterior, a primary muscle involved in ankle dorsiflexion, healthy older females typically have lower motor unit firing rates than age-matched healthy males(9,10), while overall leg strength is greater in males compared to females(10). Considering greater muscle weakness was demonstrated in females following ICU discharge for SARS-CoV-2 infection, it is likely that females have greater neuromuscular dysfunction than males following ICU discharge, possibly related to alteration in motor unit firing rate modulation. Furthermore, the literature lacks studies on the use of EMG signal decomposition to monitor the progress of patients over time.

Therefore, the present study aimed to assess sex differences in longitudinal physical functioning following ICU discharge for SARS-CoV-2 infection. We hypothesized that females would display lower motor unit firing rates than males if no abnormalities in motor unit firing properties were present following ICU discharge. Further, we hypothesized that females would have greater muscle weakness than males post-ICU discharge. Lastly, we hypothesized that females would have lower CMAP amplitudes and greater physical impairment than males.

9.3 Material and methods

9.3.1 Participants

This study was conducted on critically ill adult patients with confirmed SARS-CoV-2 infection admitted to the ICU at the Spedali Civili University Hospital in Brescia, Italy, from February 2020 to December 2021. All patients admitted to the ICU tested positive for SARS-CoV-2 infection on a C-reactive Protein test. The data presented in this study is part of a larger longitudinal study. The sample consisted of two groups of patients who were assessed longitudinally at either three- and six-months or six- and twelve months following ICU discharge. Specifically, seven females (64 ± 9.4 years) and seven males (64 ± 8.5 years) were assessed 3- and 6- months following ICU discharge, while fourteen females (62 ± 8.8 years) and fourteen males (62 ± 8.4 years) were assessed 6- and 12-months following ICU discharge. Patients were diagnosed with acute respiratory distress syndrome (ARDS) according to the Berlin criteria. Patients were invited to attend a post-ICU clinic, where the assessment was performed. Demographic information, including age, weight, and height of the participant, was collected. This study was reviewed and received ethics approval from the Brescia Ethics Committee (NP3369) and conformed to the Declaration of Helsinki. Written informed consent was obtained from each participant prior to data collection.

9.3.2 Experimental Design

Patients visited the post-ICU clinic on three occasions, where they performed several tests to examine their physical functioning, fatigue, peripheral nerve and muscle function, strength, and muscle activation. The experimental session started with an assessment of fatigue. Patients were asked to self-report activity limitations by filling out a short questionnaire that required patients to rate their fatigue level. This questionnaire provided a fatigue severity scale (FSS), with a higher score indicating greater fatigue. Following this, physical functioning was examined using the six-minute walking test (6MWT). The 6MWT is a standardized, objective assessment of physical performance, which tests both cardiopulmonary and skeletal muscle function. 6MWT was performed in accordance with the American Thoracic Society recommendations. The absolute distance walked in 6 minutes was measured.

Following 6MWT, patients were lying in bed, and the CMAP was used to assess the peroneal nerve function. CMAP was recorded using a novel technique with Nicolet Viking EDX (Natus Medical incorporated Middleton, WI). CMAP was obtained from the tibialis anterior muscle of the dominant leg using surface electrodes (Figure 1A).

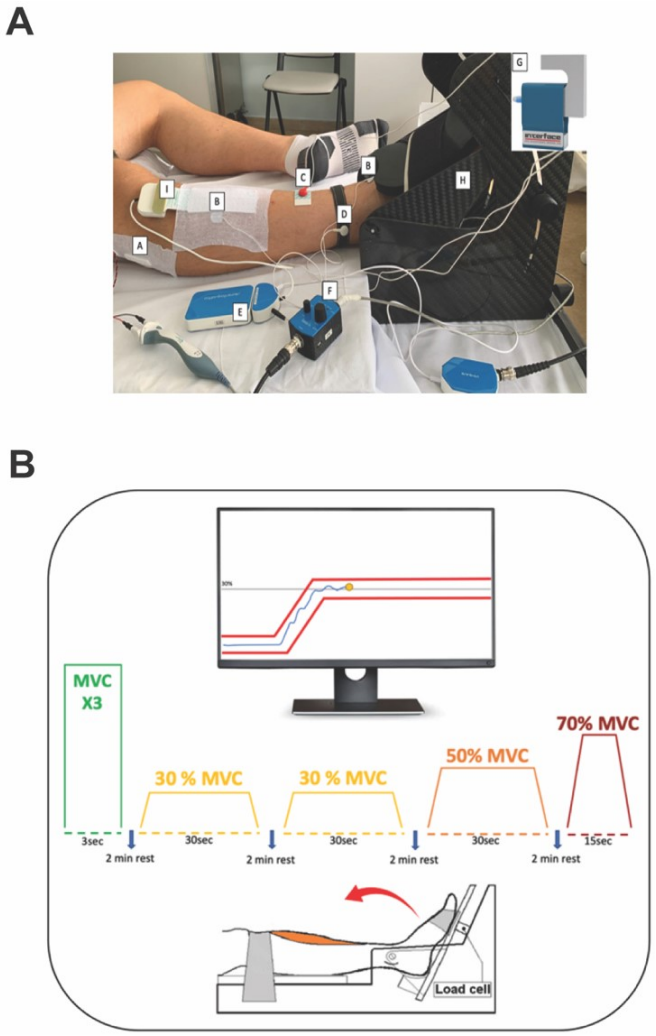

Figure 1: ***(A)*** *Protocol set-up: (A) Stimulator bar placed over the common peroneal nerve. (B) Surface electrodes for the identification of the CMAP-TA. (C) Stimulator ground electrode and (D) Ground electrode for the (E) EMG amplifier to prevent interference with biopotential signals. (F) Analog force amplifier to amplify the force signal detected by the (G) Load cell. The foot of the patient was strapped in a (H) carbon ankle-ergometer. (I) 64 Electrode Matrix was placed over the belly of the tibialis anterior muscle.* ***(B)*** *Experimental protocol: With the help of the visual feedback, patients were asked to perform three maximal voluntary contractions (MVC) involving ankle dorsiflexion followed by submaximal trials at 30%, 50% and 70% MVC. During the tasks at different MVC levels, the patients were required to keep the yellow dot between the red lines through ankle dorsiflexion.*

The negative and positive electrodes were placed on the tibialis anterior muscle belly and distally on the tendon, respectively, with the ground electrode placed on the ankle of the same leg. The foot was strapped to a custom-made dynamometer equipped with a load cell (model SM-500N). The knee was fully extended (180°), with the ankle in neutral position (110°) (11,12). The common peroneal nerve was stimulated under the peroneal head with a bar electrode (BARR0026 - Spes Medica) with an interelectrode distance of 2.5 centimeters while patients remained fully relaxed. The stimulation

started at 0 and automatically increased its intensity until the minimum stimulation intensity needed to elicit a maximal response from the tibialis anterior was identified. CMAP was defined as the action potential obtained at the maximal stimulation amplitude.

Following CMAP acquisition, patients performed maximal isometric ankle dorsiflexion with their dominant leg. Maximal voluntary contractions (MVC) were performed three times and involved maximal foot dorsiflexion with a 1.5-minute rest between each trial. Each trial lasted 3 seconds to reach the MVC and 3 seconds of isometric contraction. Patients were verbally encouraged by the researchers throughout MVC performance. The peak force of three trials was used as a measure of MVC (11,12). The load cell signals were amplified with an analog force amplifier (OT Bioelecttronica, Turin, Italy) connected to a portable bioelectrical signal amplifier (Sessantaquattro; OT Bioelettronica, Turin, Italy).

Following MVC performance, high-density surface electromyography (HD-sEMG) was applied to the tibialis anterior muscle (Figure 1A). HD-sEMG signals were recorded in monopolar mode with an adhesive 64 electrodes matrix (GR08MM1305:13 rows by 5 columns, 8mm IED; OT Bioelettronica, Turin, Italy). The matrix was placed following the guidelines of Barbero et al.(13) over the muscle belly thanks to a double-sided foam covered with conductive paste (NEURGEL250V – Spes Medica). Prior to applying the matrix, the skin was shaved and cleaned with abrasive paste (EVERI160SPE – Spes Medica) and water. Reference electrodes were positioned proximally over malleoli of the dominant leg. HD-sEMG signals were band-pass filtered (10-500 Hz), and sampled at 2000 Hz with a 16-bit A/D resolution.

Following rest, patients performed several submaximal isometric ramp-and-hold trials (Figure 1B). This task involved following a trapezoidal trajectory displayed on a computer monitor by contracting the tibialis anterior to generate torque using ankle dorsiflexion. Real-time visual feedback was provided to the patients throughout the trial. Patients performed submaximal trials at three levels: 30%, 50%, and 70% MVC randomly, with a 2-minute rest between each trial. The 30% MVC trapezoid was repeated twice, and it was 36 seconds in duration, consisting of a 3-second ramp-up phase from baseline, a 30 second hold phase at 30% MVC, and a 3 second ramp-down phase. The 50% MVC trapezoid was 40 seconds in duration, consisting of a 5 second ramp-up phase from baseline, 30 second hold phase at 50% MVC, and a 5 second ramp-down phase. Lastly, the 70% MVC trapezoid was 29 seconds in duration, consisting of a 7 second ramp-up phase from baseline, 15 second hold phase at 70% MVC, and a 7 second ramp-down phase. All participants were familiarized with the experimental protocol prior to data collection.

9.3.3 Data analysis: Motor unit decomposition

Monopolar HD-sEMG signals were decomposed into individual motor unit spike trains using a convolutive blind source separation algorithm previously validated(14). The EMG signals were band-pass filtered (3rd order Butterworth, 20-500 Hz). The decomposition outputs were inspected by highly trained operators, and erroneous discharges were corrected. Only motor units with a silhouette value greater than 0.90 were used in further analyses(14).

9.3.4 Data analysis: Motor unit properties

Several motor unit properties were quantified from the decomposed data. First, mean firing rates and coefficient of variation for inter-spike-intervals were quantified from the hold phase of the trapezoidal submaximal contractions. For the 30% MVC, we analyzed the motor unit properties of low-threshold motor units, while for the 50% and 70% MVC, we examined only the high-threshold motor units. Recruitment and derecruitment thresholds (%MVC) were estimated from the generated torque of the first and last firing of the identified motor units. Firing rates at recruitment and derecruitment were quantified as the average of the first and last six firings of the identified motor units.

9.3.5 Statistical analyses

All statistical analyses were performed in SPSS (IBM, version 21). Data were checked for normality using the Shapiro-Wilk test. An independent two-sample t-test was used to assess age, weight, and height differences between groups. Most of the variables included in this study satisfied this condition. For the data that were normally distributed, a two-way repeated measures ANOVA was performed with a within-subject factor follow-up visit (Group 1: 3 and 6 months; Group 2: 6 and 12 months) and between-subject factor sex (Male, Female). Non-parametric statistical tests were used if data were not normally distributed. Specifically, to test group differences, the Mann-Whitney U test was used, while within-subject factor differences were tested using a Wilcoxon Signed Rank test. Significance was set to $p < 0.05$, and post-hoc tests were Bonferroni corrected.

9.4 Results

Thirty-three patients were selected from a large number of subjects who took part in this study. Nine patients (4 females and 5 males) were assessed at 3-, 6- and 12- months and were allocated to both groups analyzed by the study, five patients (3 females and 2 males) were evaluated only at 3- and 6-months while nineteen patients (10 females and 9 males) were only at 6- and 12- months. It was not possible to assess all these patients in all three follow-ups due to the lockdown, which forcibly interrupted research activity in hospitals.

9.4.1 Patient demographics and physical functioning

Patient demographics and treatment details are presented in Table I. There were no age differences between the sexes in patients assessed at 3- and 6-months (two samples t-test, p = 0.88) or 6- and 12-month follow-ups (two samples t-test, p = 0.82). There were no sex differences in the duration of hospitalization in our 3-to-6-month group (p = 0.93) nor 6-to-12-month group (p = 0.41). Similarly, no sex differences existed in the duration of mechanical ventilation in our 3-to-6-month group (p = 0.18) or 6-to-12-month group (p = 0.14). Additional data regarding corticosteroids and other drugs administered in ICU can be found in Table I.

Table I: Participant demographics with standard deviations.

	Group 1 (3-6 months)		**Group 2 (6-12 months)**	
	Female (N = 7)	**Male (N = 7)**	**Female (N = 14)**	**Male (N = 14)**
Age (years)	64 ± 9.4	64 ± 8.5	62 ± 8.8	62 ± 8.4
Height (cm)	163.1 ± 7	174.1 ± 9.3	161 ± 6.7	174.9 ± 8.1
Weight (kg)	84 ± 12.3	82.8 ± 13.4	77.7 ± 14.8	83.9 ± 12.3
Duration of Hospitalization Stay, days – Mean (SD)	28.7 ± 15.2	28 ± 16.1	34.1 ± 16.4	29.1 ± 15.4
Duration of ICU Stay, days – Mean (SD)	8.1 ± 5.7	9.4 ± 6.3	13.4 ± 10	9.9 ± 6.5
Intubation – N (%)	7 (100%)	5 (71.4%)	14 (100%)	13 (92.8%)
Duration of Intubation, days – Mean (SD)	8.4 ± 9	3.4 ± 2.7	12.4 ± 10.2	7.2 ± 5.9
ICU Catecholamine – N (%)	4 (57%)	1 (14.2%)	6 (42.9%)	6 (42.9%)
ICU Tocilizumab – N (%)	1 (14.2%)	0 (0%)	1 (7.1%)	3 (21.4%)
Steroids in ICU – N (%)	7 (100%)	6 (71.4%)	12 (86%)	13 (92.8%)
ECMO – N (%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Tracheosotomy - N	1 (14.2%)	1 (14.2%)	4 (28.6%)	4 (28.6%)

List of abbreviations: ICU, Intensive Care Unit. ECMO, Extracorporeal Membrane Oxygenation.

9.4.2 Fatigue

No sex differences existed in fatigue scores at 3 or 6 months (3 months: $p = 0.62$; 6 months: $p = 0.90$; Table II), nor at 6 or 12 months (6 months: $p = 0.54$; 12 months: $p = 0.76$; Table III). However, in 3-to-6-month follow-up group, 2 out of 7 females and 3 out of 7 males had fatigue at 3 months, while 4 out of 7 females and 2 out of 7 males had fatigue at 6 months. Further, in 6-to-12-month follow-up group, 8 out of 14 females and 4 out of 14 males had fatigue at 6 months, while 6 out of 14 females and 3 out of 14 males had fatigue at 12 months.

9.4.3 Physical functioning

Patients assessed at 3- and 6-month follow-ups walked a 30% greater distance at 6 months ($F1,12= 14.2$, $p = 0.003$, $\eta p^2= 0.5$; Table II). No sex differences ($p = 0.17$) and no interaction effects between sex and follow-up month ($p = 0.90$) were observed in this group. Males walked a greater distance than females in the 6- to 12-month follow-up group ($F_{1,24}= 6.8$, $p = 0.01$, $\eta_p^2= 0.22$; Table III). No differences in follow-up month ($p = 0.55$), and no interaction between sex and follow-up month ($p = 0.98$) existed.

Table 2: Summary of fatigue severity scale score, six-minute walking test score, and CMAP amplitude with standard deviations for participants tested at 3- and 6-months following ICU discharge.

	3 months		6 months	
	Female	**Male**	**Female**	**Male**
FSS	26.7 ± 18.1	30.1 ± 17.8	32.6 ± 20.6	30.5 ± 17.2
6MWT (m)	318.5 ± 95.9	381.4 ± 128.1	420 ± 99.4	490 ± 72.8
CMAP	6.3 ± 2.2	5.6 ± 1.3	7.4 ± 1.9	6.6 ± 1.3

List of abbreviations: FSS, fatigue severity scale. 6MWT, six-minute walking test. CMAP, compound muscle action potential.

Table 3: Summary of fatigue severity scale score, six-minute walking test score, and CMAP amplitude with standard deviations for participants tested at 6- and 12-months following ICU discharge.

	6 months		12 months	
	Female	**Male**	**Female**	**Male**
FSS	35.2 ± 18	30.5 ± 18.8	31.6 ± 20.1	27 ± 19.7
6MWT (m)	418.4 ± 132.7	506.4 ± 74.99	418.5 ± 115.6	510 ± 84.8
CMAP	7.3 ± 1.8	6.8 ± 1.4	7.9 ± 2	7.2 ± 1.9

List of abbreviations: FSS, fatigue severity scale. 6MWT, six-minute walking test. CMAP, compound muscle action potential.

9.4.4 Maximal torque

Maximal torque produced by the patients was similar between the sexes and follow-up visits in patients assessed at 3-to-6-month follow-up ($p = 0.54$; Figure 2A). In contrast, a significant interaction existed between follow-up month and sex for patients assessed at 6-to-12-month follow-up ($F_{1,26} = 4.7$, $p = 0.03$, $\eta_p^2 = 0.15$; Figure 2B). Specifically, at 12-months males had greater maximal torque than females ($p = 0.013$), but not at 6-months ($p = 0.11$). Maximal torque did not change in females ($p = 0.059$) but increased in males ($p < 0.001$) between 6 and 12-month follow-up visits.

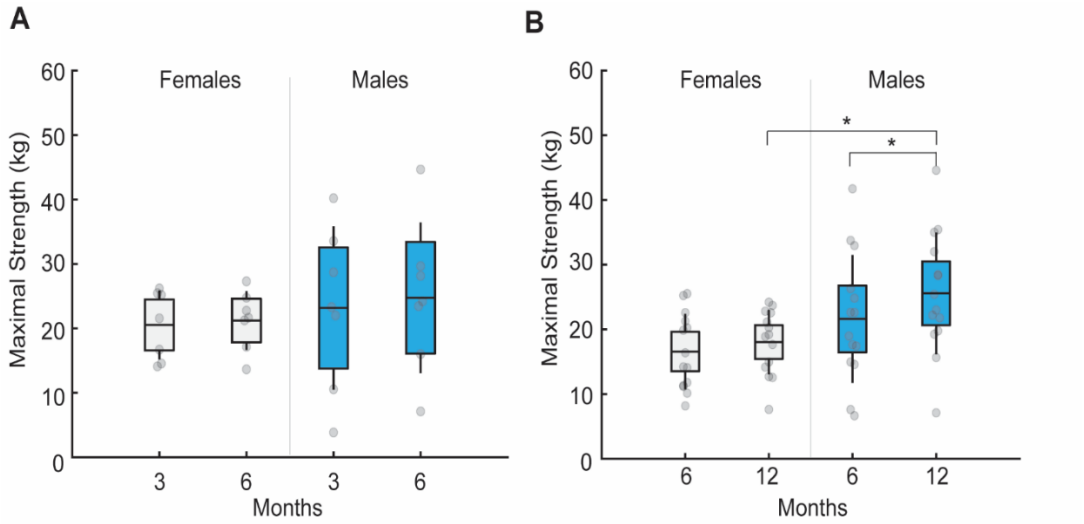

Figure 2: Mean maximal strength in males and females across different follow-up months. Black lines in the bar graphs denote means. Colored bars with lines denote 95% confidence intervals with standard errors. Scatter dots represent individual participan

9.4.5 CMAP amplitude

CMAP amplitude was 17% greater at 6- compared to 3-months ($F_{1,12} = 11.2$, $p = 0.006$, $\eta_p^2 = 0.48$; Table II) and 7% greater at 12- compared to 6-months ($F_{1,26} = 5.06$, $p = 0.03$, $\eta_p^2 = 0.16$; Table III)

irrespective of the sex. No sex differences existed in CMAP amplitude in 3-to-6-month follow-up ($p = 0.41$) or 6-to-12-month follow-up group ($p = 0.42$), and no interaction effects between sex and follow-up month were observed in either group (3-to-6-month follow-up: $p = 0.76$; 6-to-12-month follow-up: $p = 0.61$).

9.4.6 Motor unit properties

Due to technical difficulties, we were unable to decompose HD-sEMG signals in one female at 50% MVC and 70% MVC in our 3-to-6-month group and at 70% MVC in our 6-to-12-month group. Therefore, this data and their age-matched male data were not included in the analyses.

9.4.7 Number of motor units decomposed

In our 3-to-6-month group, we decomposed a total of 202 and 209 motor units in females and 279 and 302 motor units in males at 30% MVC at 3 and 6 months, respectively. At 50% MVC, we decomposed a total of 31 and 43 motor units in females and 52 and 40 motor units in males at 3 and 6 months, respectively. Lastly, at 70% MVC, we decomposed a total of 44 and 48 motor units in females and 51 and 63 motor units in males at 3 and 6 months, respectively.

In our 6-to-12-month group, we decomposed 413 and 376 motor units in females and 611 and 532 motor units in males at 30% MVC at 6 and 12 months, respectively. At 50% MVC, we decomposed a total of 86 and 101 motor units in females and 124 and 166 motor units in males at 6 and 12 months, respectively. Lastly, at 70% MVC, we decomposed a total of 91 and 86 motor units in females and 151 and 163 motor units in males at 6 and 12 months, respectively.

9.4.8 Mean motor unit firing rates

In 3-to-6-month follow-up, no interaction effects between sex and follow-up month were observed in mean motor unit firing rate when patients performed isometric ramp-and-hold trials at 30% MVC ($p = 0.79$; Figure 3A), 50% MVC ($p = 0.58$; Figure 3B), and 70% MVC ($p = 0.18$; Figure 3C). Further, no sex differences were observed in the mean motor unit firing rate in this group at 30% MVC ($p = 0.95$), 50% MVC ($p = 0.066$), or 70% MVC ($p = 0.18$).

An interaction effect between sex and follow-up month was observed in mean motor unit firing rates in 6-to-12-month follow-up group. Specifically, at 30% MVC, a significant month by sex interaction existed ($F_{1,26} = 4.70$, $p = 0.03$, $\eta_p^2 = 0.15$; Figure 3D). At 6-month follow-up, no sex differences in low-threshold motor unit firing rates existed ($p = 0.20$), but at a 12-month follow-up, low-threshold motor unit firing rates were 2.01 pps greater in females than males ($p < 0.001$). Further, males had 0.89 pps lower low-threshold motor unit firing rates at 12- compared to 6-months ($p = 0.006$), while low-threshold motor unit firing rates in females did not differ between the two visits ($p = 0.59$). At 50% MVC, females had greater high-threshold motor unit firing rates than males, irrespective of the follow-up month ($F_{1,26} = 9.5$, $p = 0.005$, $\eta_p^2 = 0.26$; Figure 3E). Lastly, no differences in high-threshold

motor unit firing rates between follow-up months and sexes existed at 70% MVC (all $p > 0.05$; Figure 3F).

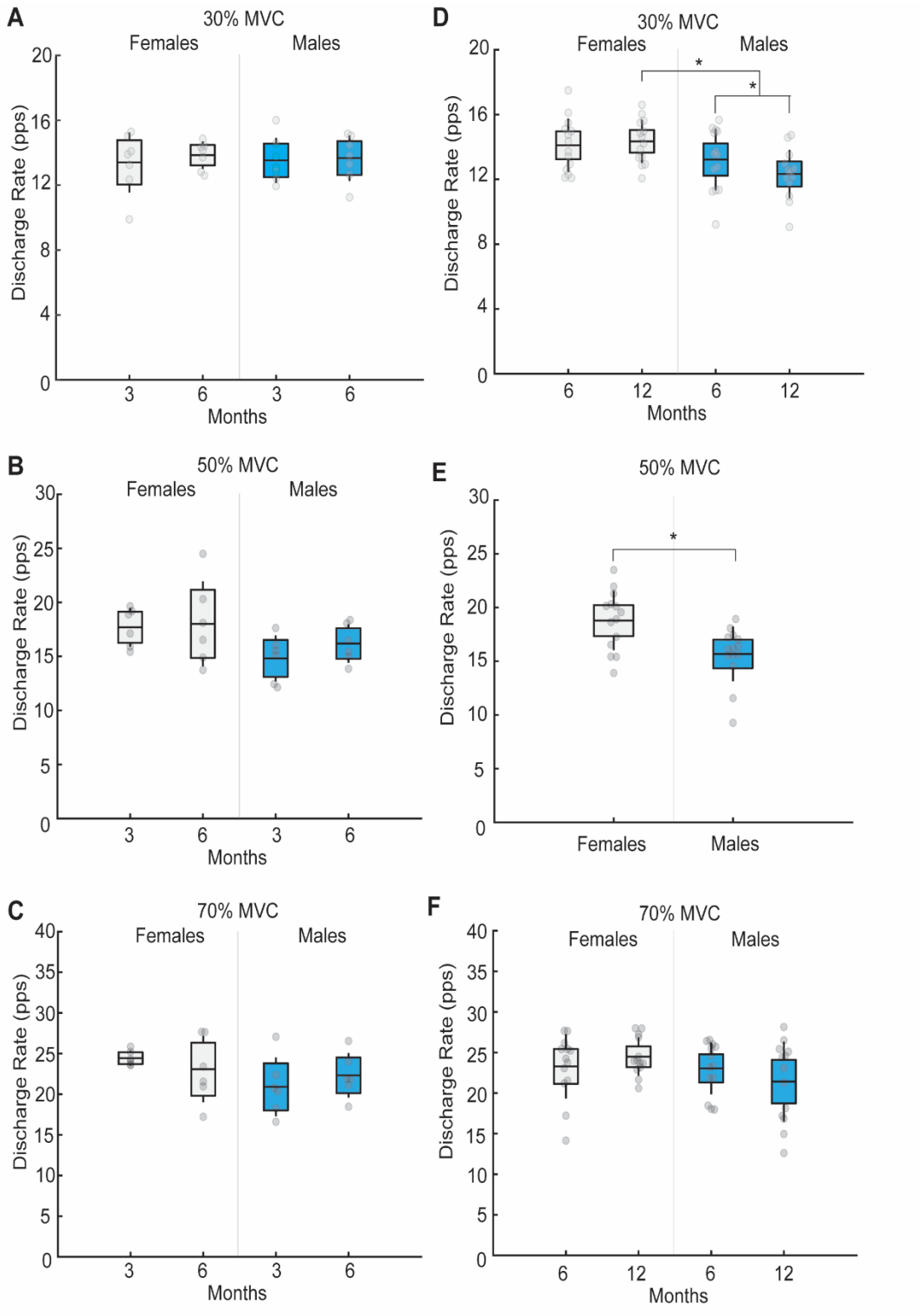

Figure 3: Mean motor unit firing rates in males and females across different follow-up months for 30%, 50% and 70% submaximal tasks. Black lines in the bar graphs denote means. Colored bars with lines denote 95% confidence intervals with standard errors.

9.4.9 Motor unit firing rate at recruitment

No month-by-sex interaction or main effects of sex existed in our 3- to 6-month follow-up or 6- to 12-month follow-up groups in firing rate at recruitment at any submaximal task level (all $p > 0.05$).

9.4.10 Motor unit recruitment threshold

No month-by-sex interaction or main effects of sex existed in our 3-to-6-month follow-up group in the recruitment threshold at any submaximal task level (all $p > 0.05$). In contrast, the recruitment threshold was greater at 12- compared to 6-month follow-up irrespective of the sex at 30% MVC ($F_{1,26} = 5.6$, $p = 0.02$, $\eta_p^2 = 0.17$), 50% MVC ($F_{1,26} = 8.6$, $p = 0.007$, $\eta_p^2 = 0.24$), and 70% MVC (Mann-Whitney U: $p = 0.014$).

9.4.11 Motor unit derecruitment threshold

Derecruitment threshold was not different between the sexes in 3- to 6-month follow-up at 30% MVC (Mann-Whitney U: all $p > 0.05$), 50% MVC ($p = 0.47$), or 70% MVC ($p = 0.28$). Similarly, no interactions between sex and follow-up month existed in the derecruitment threshold in 6- to 12-month follow-up group at 30% MVC ($p = 0.97$), 50% MVC ($p = 0.95$), or 70% MVC ($p = 0.12$).

9.5 Discussion

To our knowledge, this is the first study to objectively investigate sex differences in the longitudinal effects of SARS-CoV-2 hospitalization on physical functioning and neural drive to the muscle. Our findings revealed no sex differences in physical functioning, maximal torque, fatigue, CMAP amplitude, or motor unit properties in the 3-to-6-month follow-up group. In contrast, sex differences existed in physical functioning, maximal torque, and motor unit firing characteristics but not fatigue or CMAP amplitude in our 6-to-12-month follow-up group. Our findings demonstrate that neuromuscular function is impaired in both males and females, up to 6 months following ICU-discharge for SARS-CoV-2 following which recovery at one year is only observed in males. Additionally, to our knowledge, this is also the first study to longitudinally evaluate changes in neural drive to muscles in a disease state.

9.5.1 Impairments in physical functioning

Physical impairment was present in our 3-to-6-month follow-up group in both, males and females. In healthy adults, males typically walk a greater distance than females when not corrected for height(15). However, we did not observe these differences in our data. Both, males and females, had impairments in physical functioning at 3-months following ICU discharge as their mean scores for the 6MWT fell well below the typical scores for healthy older adults(15,16). Moreover, the 6-minute walking distance at 3 months in females was lower compared to patients who had the classic ARDS (females: 318 meters vs. ARDS: ~360 meters)(17) and SARS-CoV-2 patients assessed at 3 months (382

meters) (18). These findings align with previous observations that female ARDS patients typically have lower 6MWT results(17). In contrast, male six-minute walking distance at 3 months was slightly less impaired than that of ARDS patients (males: 381 meters vs. ARDS: ~360 meters), similar to that of SARS-CoV-2 patients tested at 3-months following infection (382 meters) (18), but below those values achieved in healthy older males (healthy older males: ~690 meters) (16). These results indicate that males affected by and hospitalized for SARS-CoV-2 may be less impaired than classic ARDS patients, but still have substantial physical impairments compared to healthy older males. By 6-months, both sexes exhibit substantial increases in six-minute walking distance and have less impairment than classic ARDS patients (for reference values, see Parry et al.(17)) although the values for both sexes still fall below those of healthy older adults.

In our 6-to-12-month follow-up group, males walked a greater distance than females irrespective of the follow-up month, which aligns with what is typically observed in healthy adults(15,16,19). However, female results still fell below those reported in classic ARDS patients(17) and were significantly below those documented in healthy older females(16). In contrast, males showed less impairment than classic ARDS patients, but their results still fell below those observed in healthy older males. Moreover, observed sex differences in healthy older adults are typically between 30 to 62 meters(16,19). However, in our study, this difference was ~88 meters at 6 months and 91 meters at 12 months, indicating that female recovery in physical functioning is lagging. Females admitted to the ICU usually have greater illness severity(20), which may contribute to reductions in peak exercise aerobic capacity(21). This is partly due to the deconditioning and muscular limitation. Therefore, greater impairments in the cardiopulmonary or muscular system may be the contributing factor to the impairments in physical functioning observed in females.

9.5.2 S*trength impairments*

Healthy older males have greater strength than females in ankle dorsiflexion(10). The absence of sex differences in strength in our 3-to-6-month follow-up group and at 6-months in our 6-to-12-month follow-up group indicates the presence of muscle weakness in males, although muscle weakness in females cannot be ruled out. By 12-months, sex differences in maximal strength are observed and the differences can be primarily attributed to the increase in muscle strength in males, which reaches similar values to that reported in healthy older adults(11). These findings indicate that males recover their maximal strength by 12-months following ICU-discharge. Considering that muscle strength does not change in females from 3 to 6 months nor 6 to 12 months, these data suggest that females continue to exhibit muscle weakness up to 1 year following ICU discharge. These findings support those of Huang et al.(4), which found greater muscle weakness in females 6- and 12-months post ICU-discharge compared to males. Patients requiring hospitalization and ICU stay are at greater risk

of muscle atrophy, sensory-motor axonal polyneuropathy (critical illness polyneuropathy) and myopathy(22) due to pathological changes such as increased inflammation, mitochondrial dysfunction, and reduced physical activity. Females have a more than four fold greater risk of developing ICU acquired weakness during the ICU stay than males(23,24) and thus, longitudinal muscle weakness in females may be a consequence of multiple factors including polyneuropathy, myopathy, and/or greater muscle atrophy(22,23). Additionally, corticosteroid therapy during ICU was also shown to be associated with greater muscle weakness at 12 months(4).

9.5.3 Altered motor unit firing

The central nervous system controls muscle force production by modulating the number of recruited motor units and their firing rate. In healthy older adults, sex differences exist in tibialis anterior motor unit firing rates, such that females have lower motor unit firing rates than males when submaximal isometric torques are generated at minimal (25% MVC (10)) to high force levels (100% MVC (9)). Despite this, we found no sex differences in motor unit firing rates in our 3-to-6-month follow-up group. Moreover, we found no sex differences in low-threshold motor unit firing rates at 6 months post-ICU discharge in our 6-to-12-month follow-up group. These findings indicate that patients discharged from the ICU following SARS-CoV-2 infection have impaired low-threshold motor unit firing rates up to 6 months following ICU discharge. Further, sex differences in low-threshold motor unit firing rates were present at 12-months following ICU discharge. These differences were due to a decrease in motor unit firing rate in males from 6 to 12-month follow-up. In contrast, female low-threshold motor unit firing rates remained the same across follow-up months. Moreover, we also observed greater high-threshold motor unit firing rates in females in our 6-to-12-month follow-up group, irrespective of the follow-up month. These findings indicate that motor unit firing rates in males are elevated up to 6 months following ICU discharge and decrease with recovery by one year. Decrease in motor unit firing rates in our patients occurred concurrently with improvements in strength, physical functioning, and CMAP amplitude in males.

These findings demonstrate that patients hospitalized for SARS-CoV-2 infection require increased neural input to optimize muscle force production in the first 6-months following ICU discharge and agree with previous observations in patients with myopathic disease(25). As the muscle recovers, there is less requirement for increased neural input to the muscle. Thus, neuromuscular system becomes more efficient at force production one year following ICU discharge in males, signifying neuromuscular recovery. In contrast, females do not recover in terms of physical functioning and strength, and thus, the neural input to the muscle remains high even one year following ICU-discharge. The exact mechanisms associated with the lack of recovery in motor unit firing rates in females are unclear but may be due to structural changes in the muscle fiber, which consequently

affect motor unit firing rates. Considering that females tend to lose significantly more muscle mass than males in the ICU, it is likely that some motor unit denervation occurs as a consequence of concurrent critical illness polyneuropathy. Due to motor unit loss, motor units must fire at higher rates to produce a desired force at low force levels. Moreover, patients with myopathy commonly exhibit high motor unit firing rates(26), which may explain the current findings in females.

9.5.4 Lack of sex differences in CMAP amplitude, fatigue, and other motor unit characteristics

Our study demonstrates that there is a significant recovery of CMAP amplitudes at one year following ICU discharge for SARS-CoV-2 infection, irrespective of sex. A previous study did not find reductions in peroneal nerve CMAP amplitude in SARS-CoV-2 patients(27). However, none of their patients required ICU treatment, and half did not require hospitalization, which may have resulted in differences between our findings and theirs. Reduced peroneal and tibial nerve CMAP amplitude in ICU admitted SARS-CoV-2 patients were documented previously in case studies(28,29). Reduced CMAP amplitudes are observed in patients who have critical illness myopathy and critical illness polyneuropathy(30), which cannot be discounted in our patient group.

We observed no differences in fatigue scores between the sexes in either group, although a larger proportion of females reported fatigue compared to males. Fatigue is a commonly reported symptom in SARS-CoV-2 patients(31), and it is more prominent in females (32).

Lastly, we observed an increase in recruitment threshold of low and high-threshold motor units between 6- and 12-months following ICU discharge irrespective of the sex while no differences in recruitment threshold between months or sexes were observed in our 3-to-6-month follow-up group. This increase in recruitment threshold force indicates that the identified motor units were recruited later during the isometric contraction. In general, due to the bias of the surface decomposition algorithms towards higher threshold units(33,34), it is not straightforward to link this result to an underlying neurophysiological mechanism. Probably, due to tendency to have higher MVC and, therefore, likely, higher motor unit recruitment after six months, the complexity of the EMG signal was increasing during the follow-up resulting in the tendency for the decomposition algorithm to identify higher threshold units.

9.5.5 Limitations

Our study has several limitations. First, the sample size in our 3-to-6-month follow-up group is small and should be considered when interpreting the findings. In general, females are less likely to be admitted to the ICU(20) and ARDS is more frequent in males than females(35), making the recruitment of female patients challenging. Assessment at all time points for all patients in this study was not possible due to patient's unwillingness to participate longer than 6 months or late recruitment of the patients at 6 months. Second, we did not recruit a control group for this study as the likelihood

of asymptomatic SARS-CoV-2 infection is high in the general population, making it difficult to control for the lack or history of infection. Even mild SARS-CoV-2 infection was shown to negatively affect the neuromuscular system in individuals who were not hospitalized(27). We did not examine structural adaptations to the muscle, including atrophy, which may have contributed to muscle weakness.

9.6 Conclusion

In conclusion, we demonstrated sex differences in longitudinal neuromuscular function in patients infected with SARS-CoV-2 following ICU discharge, specifically in physical functioning, maximal strength, and motor unit firing rates. Our data demonstrated that females display significant impairments in functional recovery up to 1 year following ICU discharge for SARS-CoV-2. Therefore, the effects of sex should be considered in post-COVID neurorehabilitation.

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10. Chapter Ten: Effect of COVID-19 intensive care unit hospitalization on strength, fatigue and motor unit behaviour: a one-year follow-up study.

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10.1 Abstract

Prolonged intensive care unit (ICU) hospitalization from COVID-19 significantly impacts physical function by increasing fatigability and reducing strength. In this study, we combined force measurements and high-density sEMG decomposition, during voluntary contractions, and electrically evoked potentials to investigate central and peripheral mechanisms underlying functional changes and recovery in post-ICU COVID-19 patients. Sixty COVID-19 patients were evaluated at 3, 6 and 12 months post-ICU discharge. Functional capacity was examined by the 'Fatigue Severe Scale' (FSS), six-minute walk test (6MWT) and maximal voluntary contractions (MVC). The tibialis anterior central and peripheral neuromuscular variables were evaluated at 30%, 50% and 70% MVC. Results showed that 6MWT distance increased at 3-6 months, but not at 6-12. FSS revealed no differences between 3-6 but decreased between 6-12 months. MVC increased between 3-6 and 6-12 months. The average motor unit (MU) discharge rate decreased from 6-12 months at all contraction levels, and from 3-6 months at 50% and 70%, but not 30% MVC. MU discharge variability decreased at all contraction levels, except 70% MVC from 3-6 months. MU amplitude, twitch force contraction

time, and peak twitch force increased from 3-6 months, but not 6-12. Significant correlations between central motor unit properties and maximal voluntary force were observed at 3-6 months and between central motor unit properties and fatigue scores at 6-12 months. Our findings suggest a two-step progression underlying the restored force-generating capacity in post-ICU COVID-19 patients. Neural adaptations are primarily associated with the recovery of muscle force capacity during the initial 3-6 months, followed by fatigue recovery in the last 6-12 months.

New & Noteworthy

The present study fills a critical knowledge gap by combining HDsEMG signal decomposition, and voluntary and evoked force outputs to investigate post-intensive care unit (ICU) recovery in individuals with COVID-19 acute respiratory distress syndrome. Our results identified adaptations within the neuromuscular system, highlighted by central and peripheral changes following ICU discharge. We report patients regain optimal force-generating capacity through modifications which restore optimal motor unit activation strategies, normal neuromuscular function and reduced fatigue.

10.2 Introduction

The detrimental effects of critical illness and prolonged intensive care unit (ICU) stays on physical functioning in COVID-19 patients have been extensively documented in the literature (Carenzo et al., 2021; Gervasoni et al., 2022; Heesakkers et al., 2022; Latronico et al., 2022; Lulic-Kuryllo et al., 2023; Medrinal et al., 2021; Paneroni et al., 2021; Rahiminezhad et al., 2022). Notably, a significant percentage of these patients experience prolonged ICU-acquired weakness (ICUAW), characterized by increased muscle fatigability and a reduction in strength (Latronico et al., 2022; Vanhorebeek et al., 2020; B. Wang et al., 2020; W. Wang et al., 2020). Various pathophysiological factors, such as neuroendocrine alterations, immobilization, hypoxia, neuronal damage, and skeletal muscle dysfunction, are supposed to contribute to the development of muscle atrophy and functional impairments following ICU discharge (Ibrahim et al., 2021; Jara et al., 2021; Mao et al., 2020; Vanhorebeek et al., 2020; W. Wang et al., 2020). COVID-19 patients may exhibit corticospinal and corticobulbar tract impairments, ataxia, extrapyramidal disorders, critical illness myopathy and acute neuroinflammation during the onset of disease and subsequent hospitalization (Guerrero et al., 2021). Consequently, the observed neuromuscular and functional alterations following ICU discharge in these patients, may arise from changes in both the central and peripheral nervous system (Ellul et al., 2020; Leonardi, 2021; Mao et al., 2020; Serrano-Castro et al., 2020; Stefanou et al., 2022).

Currently, the assessment and follow-up from ICUAW rely mainly on clinical tests, such as the Medical Research Council (MRC) sum score and Fatigue Severity Scale (FSS). The MRC sum score provides a global measure of muscle strength, while the FSS provides a score in reference to the severity of fatigue and its effect on an individual's lifestyle and ability to perform activities of daily living (Latronico et al., 2022; Panitz et al., 2015; Roodbol et al., 2014). However, despite their common and well-established use in clinical practice, these clinical tests have limitations. For instance, the FSS scale provides information on pathological fatigue without indicating if the origin of the fatigue is a result of central or peripheral factors. Furthermore, the score obtained in the FSS is based on the patient's responses to the questionnaire. This introduces a significant bias as it relies on

the patient's self-assessment, which may be influenced by their subjective interpretation of fatigue or their current psychosocial state. Additionally, the MRC sum score may be influenced by the subjective judgment of the observer and may lack insights into specific causes of strength impairment. To address these gaps and provide more objective evaluations of neuromuscular disorders, it is crucial to identify potential alterations in specific parameters of central and peripheral neuromuscular pathways which are less influenced by an observer's subjective judgement. In clinical settings, classic electromyographic examination has been widely utilized to determine neuromuscular changes dictated by the progression of a pathology and subsequent follow-up visits. Current clinical practice predominantly uses bipolar surface electromyography (sEMG) myoelectric recordings along with peripheral nerve stimulation, such as nerve conduction velocity and compound muscle action potentials to evaluate the functionality of peripheral nerves (Agergaard et al., 2021; Cabañes-Martínez et al., 2020; Daia et al., 2021; Jolley et al., 2016; Labarre-Vila, 2006; Latronico et al., 2022; Villa et al., 2021). While these approaches allow for an immediate estimation of peripheral changes, they are unable to identify potential central changes.

During a voluntary contraction, recording muscle activity from multiple sEMG signals over the active muscle (i.e., high-density surface electromyography; HDsEMG) and force output (simultaneously) presents a promising avenue to investigate and separate the central and peripheral mechanisms underlying ICU-acquired functional alterations (Bazzucchi et al., 2004; Pradhan et al., 2020). Additionally, integrating the recorded HDsEMG signal with convolutive blind source separation algorithms (Negro et al., 2016), to decompose the surface interference pattern into its individual motor unit (MU) action potential trains (MUAPT), allows for the investigation of MU behavior non-invasively. Further examination of changes in MU behaviour (i.e. MU recruitment, rate coding) allows for the exploration of the neural commands responsible for neuromuscular function (Cogliati et al., 2020; Cudicio et al., 2022; Holobar et al., 2010; Lulic-Kuryllo et al., 2023; Yavuz et al., 2015). From these explorations, MU behaviours are of particular interest, as they are primarily influenced by central mechanisms reflecting the level of corticospinal input to the alpha motor neurons.

Additionally, quantification of the MUAP amplitude reveals information regarding the peripheral nervous system such as the number and size of active muscle fibres within a MU (Kallenberg and Hermens, 2006; McPherson et al., 2016). Therefore, these measures may serve as objective, quantitative tools that can be used to understand both muscle function and dysfunction in COVID-19 patients following ICU discharge.

In this study, we propose a novel approach to assess central and peripheral neuromuscular parameters underlying the recovery of physical function in COVID-19 patients up to one year following ICU discharge. We specifically combined HDsEMG decomposition, during voluntary isometric force output recordings, and electrically evoked contractions (twitch force characteristics assessment) to discriminate between central and peripheral mechanisms that contribute to ICU-acquired functional alterations. Given that ICUAW is a major predictor of long-term weakness potentially impacting the quality of life and autonomy of ICU survivors (Hermans and Van den Berghe, 2015; Jolley et al., 2016; Meyer-Frießem et al., 2021; Needham et al., 2012; Vanhorebeek et al., 2020), our study aimed to offer a valid and reliable objective method to monitor the progression of the pathology in COVID-19 patients discharged from the ICU. Furthermore, this study introduces new tools for improving current clinical practice and developing effective therapeutic strategies to address neuromuscular alterations in these patients. Our hypothesis is that there is a strong correlation between functional variables and central and/or peripheral variables that can provide a clear understanding of how the pathology and subsequent hospitalization have negatively impacted physical functioning. Furthermore, we aim to elucidate how these parameters develop during the follow-up period, allowing patients to regain autonomy in their daily activities.

10.3 Materials and methods

10.3.1 Participants

Sixty adults (≥18 years old) participated in this observational longitudinal study. All participants were admitted to the ICU between February 2020 and December 2021 and survived acute respiratory

distress syndrome caused by coronavirus (SARS-CoV-2), which was diagnosed based on the Berlin criteria. The data analyzed in this study is a subset of a larger longitudinal study that examined patients' physical functioning, fatigue, muscle activation, strength, peripheral nerve and muscle function at various time points following discharge from the ICU (Lulic-Kuryllo et al., 2023). In this study, participants were divided into two groups according to the assessment time points following ICU discharge. The first group included 32 subjects (61 ± 11 years; 7 females) who were evaluated at 3 and 6 months following ICU discharge. The second group consisted of 46 subjects (61 ± 8.3 years; 14 females) who were assessed at 6 and 12 months following ICU discharge. Eighteen subjects were assessed at both 3 to 6 and 6 to 12 months. Demographic information such as age, height (H), mass (M) and body mass index (BMI) are presented in **Table 1**. The study took place at the follow-up clinic of the ASST Spedali Civili di Brescia, Italy.

Table 1: Participant demographics with means (standard deviations).

	Group 1 (3-6 months)	**Group 2 (6-12 months)**
Age (years)	61 (11)	61 (8.3)
Height (cm)	172.13 (8.5)	169.43 (8.5)
Mass (kg)	82.56 (11.8)	79.93 (12)
BMI (kg/m^2)	27.88 (3.6)	27.86 (4)
Duration of Hospitalization Stay (days)	31.13 (14.9)	34.26 (20.2)
Duration of ICU Stay (days)	9.88 (8.4)	13.87 (16.6)

Prior to data collection, the study received ethical approval from the Ethics Committee of Brescia (NP3369). All procedures were conducted in accordance with the latest version of the Declaration of Helsinki. Prior to data collection, written informed consent was obtained from each participant confirming their voluntary participation and understanding of the study objectives and procedures.

10.3.2 Experimental Protocol

In each experimental session (3, 6 and 12 months following ICU discharge), patients underwent three data collection procedures in the following order: (i) evaluation of patients' physical functional capacity; (ii) transcutaneous maximal electrical stimulations (evoked potentials) applied to the peroneal nerve during involuntary dorsiflexion to examine force output; and (iii) recordings of the tibialis anterior myoelectric signal from HDsEMG electrodes and force output while participants performed maximal and submaximal voluntary isometric dorsiflexion contractions. Except for the evaluation of physical functional capacity, these procedures were done with the participants lying supine on a padded table with their dominant leg positioned on a custom-made ankle dynamometer (**Figure 1A**). The knee was fixed with straps in a fully extended position (180°), the ankle was positioned neutrally at 110° of plantar flexion (Cogliati et al., 2023; Cudicio et al., 2022), and the foot was secured to the dynamometer's plate. The dynamometer foot plate was connected to a load cell (model SM-500N, Interface Inc., Scottsdale, USA) and amplified (OT Bioelettronica, Turin, Italy), to record isometric dorsiflexion force outputs.

For the assessment of physical functional capacity, two tests were performed. First, participants rated the severity of perceived fatigue during certain activities of daily living using the 9-item FSS. The higher the FSS score, the more severe the fatigue is and the greater the impairment on the participants' activities of daily living. The FSS assessment was followed by the six-minute walk test (6MWT) which assesses an individual's velocity based on the distance covered over a six-minute period. The lower the velocity, the greater the chance of becoming infirm or losing the ability to live independently. The 6MWT was conducted in accordance with the American Thoracic Society (Bolliger et al., 2002). A detailed description of the experimental procedures for the electrically evoked and voluntary contractions are reported below.

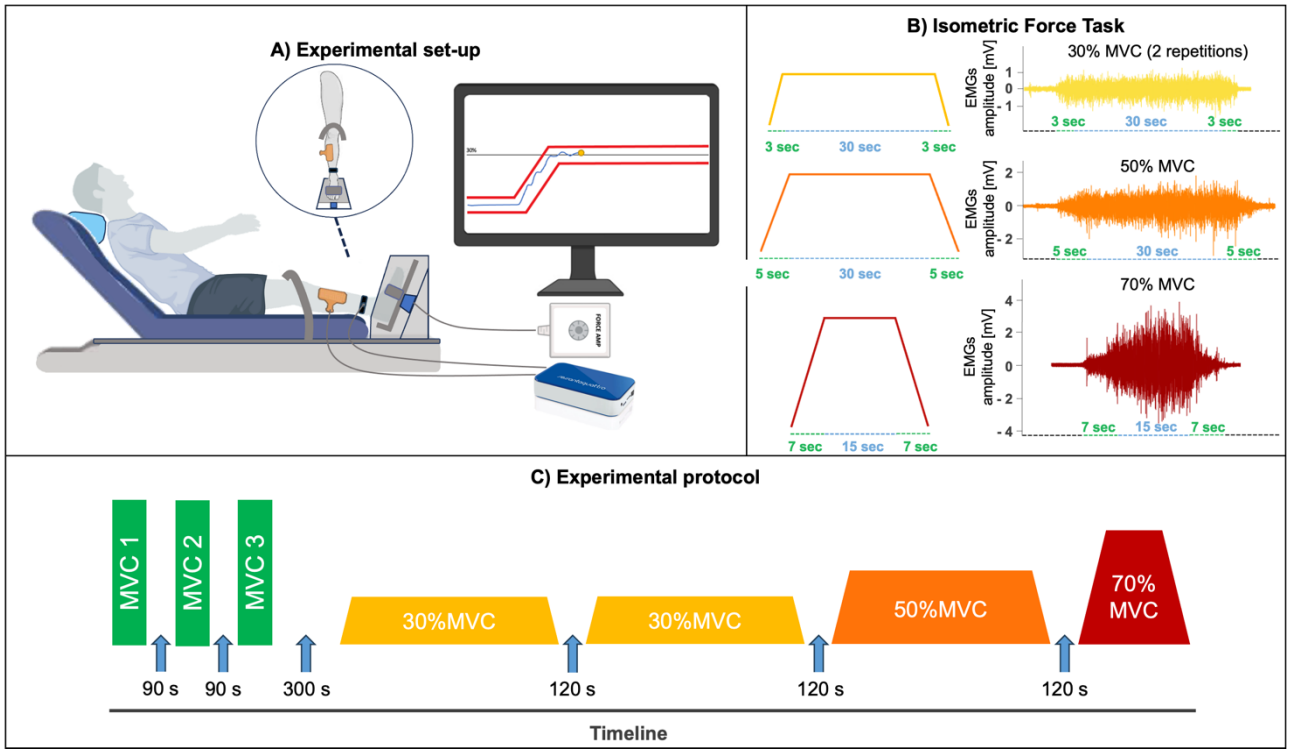

Figure 1: (A) Experimental set-up: The patient lay supine on the bed with the testing leg secured in a custom-made ergometer. The ankle was fastened with a strap to the platform connected to a load cell. Force signals were amplified by an analog force amplifier. EMG signals were recorded by a 64-electrode matrix positioned on the tibialis anterior and amplified by a factor of 200. With the aid of visual feedback, the patient was required to perform voluntary isometric contractions at various force levels, identified by the trajectory bounded by the red lines, within which the yellow marker had to be maintained. (B) Isometric Force Task: The task involved dorsiflexion at 30%, 50%, and 70% of MVC. Each task consisted of a contraction phase lasting 10% of the required force level. Subsequently, an isometric muscle contraction phase of 30 seconds for 30% and 50% MVC, and 15 seconds for 70% MVC. This was followed by a muscle deactivation phase, with the same duration as the preceding ascending phase, to return the muscle to a relaxed state. (C) Experimental protocol: Patients were asked to perform three maximal voluntary contractions (MVC) with a 90-second rest between each trial. After a 5-minute rest period, patients were instructed to execute the submaximal isometric contractions at different force levels, with a 120-second rest between each trial.

10.3.3 Stimulation protocol

The common peroneal nerve was stimulated transcutaneously in a bipolar (anode and cathode) configuration using a Nicolet Viking EDX system stimulator (Natus Medical incorporated Middleton, WI). Initially, the peroneal head of the dominant leg was identified through palpation and marked with indelible ink on the skin surface. The stimulating electrode (BARR0026; Spes Medica; interelectrode distance of 2.5 cm) was then placed over the identified mark on the peroneal head and used to evoke responses from the dorsiflexors. The stimulation intensity began at 0 mA (S0) and was increased gradually until there was no clear increase in dorsiflexion response; this stimulation intensity was defined as the maximal stimulation intensity (S100). For each stimulation at the specific

intensity, a single rectangular pulse (200 μs) was applied. The maximal evoked potentials were recorded to investigate the maximal twitch force characteristics.

10.3.4 Voluntary protocol

Prior to data collection, all participants were familiarized with the maximal voluntary contraction (MVC) procedure and the use of visual feedback for submaximal contractions. Following familiarization, patients performed three maximal voluntary isometric dorsiflexion contractions, with a 90s rest period in between each bout. For each trial, the participants were instructed to reach their maximum force within three seconds and sustain it for a further three seconds (Lulic-Kuryllo et al., 2023). Throughout the MVC, verbal encouragement was provided by the same researcher. The peak force achieved across the three trials was considered as the individual's maximum dorsiflexion force (Cogliati et al., 2020; Cudicio et al., 2022; Lulic-Kuryllo et al., 2023). After a 5-min rest, participants performed submaximal isometric ramp-and-hold dorsiflexion contractions following trapezoidal trajectories displayed on a monitor with real-time visual feedback of the force output (**Figure 1A**). The trapezoidal submaximal contractions (**Figure 1B**) were performed at three levels, 30%, 50%, and 70% of MVC in a randomized order, with a 2-min rest between each bout. The 30% MVC was repeated twice and lasted 36 seconds, consisting of a 3s ramp-up from baseline, a 30s hold and a 3s ramp-down back to baseline. The 50% MVC lasted 40 seconds and involved performing a 5s ramp-up from baseline, a 30s hold, and a 5s ramp-down back to baseline. The 70% MVC was 29s in duration, comprising of a 7s ramp-up phase from baseline, a 15s hold at 70% MVC, and a 7s ramp-down back to baseline (Lulic-Kuryllo et al., 2023). **Figure 1C** provides a summary of the experimental protocol for the voluntary contractions.

10.3.5 High-density surface EMG recordings

HDsEMG myoelectric signals were recorded from the tibialis anterior during the voluntary protocol using a matrix of 64 electrodes (GR08MM1305;13 rows by 5 columns; 8mm inter-electrode distance; OT Bioelettronica, Turin, Italy). The electrode was prepared by filling the holes with conductive gel (NEURGEL250V – Spes Medica). The skin was primed prior to positioning the matrix by shaving,

mildly abrading (EVERI160SPE – Spes Medica) and cleaning with distilled water. Following the guidelines of Barbero and associates (2017), the matrix was placed over the tibialis anterior muscle belly where the electrode was secured to the skin surface using double-sided adhesive foam. To prevent electrode displacement during the contraction, the matrix was further secured using adhesive tape. Subsequently, a reference electrode was positioned over the malleolus of the dominant leg. The HDsEMG myoelectric signals were recorded in a monopolar derivation using a portable bioelectrical signal amplifier (10-500 Hz bandwidth; 16-bit A/D resolution; Sessantaquattro, OT Bioelettronica, Turin, Italy). HDsEMG and force output were recorded simultaneously at a sampling rate of 2000 Hz.

10.3.6 Data analysis

To better understand the potential different mechanisms related to functional and neuromuscular alterations up to 12 months following ICU discharge, the variables that were investigated were divided into: (1) functional, (2) central and (3) peripheral variables. Force output and HDsEMG were analyzed offline using dedicated MATLAB (The MathWorks Inc., Natick, Massachusetts, USA) scripts.

Functional variables.

The FSS total score, 6MWT distance, and peak dorsiflexion force output were recorded at the beginning of the voluntary protocol to assess the functional capacity of the participants.

Central variables.

Force steadiness and MU discharge behaviours were used to assess central mechanisms governing force production. All central variable analyses were conducted separately for each contraction level (30%, 50% and 70% MVC) (**Figure 2**). First, monopolar HDsEMG signals were band-pass filtered using a 3rd-order Butterworth filter with a frequency range of 20-500 Hz. Then, the HDsEMG signals were decomposed into their constituent MUAPTs using a convolutive blind source separation algorithm that has been previously validated (Negro et al., 2016) and applied in several studies

(Cogliati et al., 2020; Cudicio et al., 2022; Lulic-Kuryllo et al., 2023). Briefly, after identifying the sources (i.e., MUAPTs), the algorithm separates the spikes from the noise using K-means (bottom panel of **Figure 2**). Subsequently, the discharge time estimation is further refined by interactively updating the MU separation vectors by minimizing the coefficient-of-variation (CoV) of the inter-spike-intervals (ISI) of the extracted source. The decomposed individual MUAPs were carefully visually inspected by highly trained operators to manually identify missed or misidentified MU discharges that produced non-physiological discharge rates. Only MUs with a silhouette value, which is a metric to assess decomposition accuracy (Negro et al., 2016), greater than 0.90 were selected for further analysis (Lulic-Kuryllo et al., 2023, 2021; Martinez-Valdes et al., 2018).

For each decomposed MU, various parameters were calculated to characterize MU discharge behaviours. First, the mean discharge rate (DR) and CoV of the ISI (CoV-ISI) were calculated from the most stable portion of the force output. In addition, recruitment, and de-recruitment thresholds (RT, DT) were estimated as the force output (%MVC) in which the first and last MU discharges occurred, respectively. The MU discharge rate at recruitment (RDR) and at de-recruitment (DDR) were also calculated as the average discharge rate produced by the first six and last six MU discharges, respectively. **Figure 2** shows examples of decomposed MUs for each contraction level along with their DRs. To examine force steadiness, the CoV of the force output (CoV-Force) was computed as the standard deviation of the force output divided by the mean force output (%MVC) from the most stable portion of the contraction.

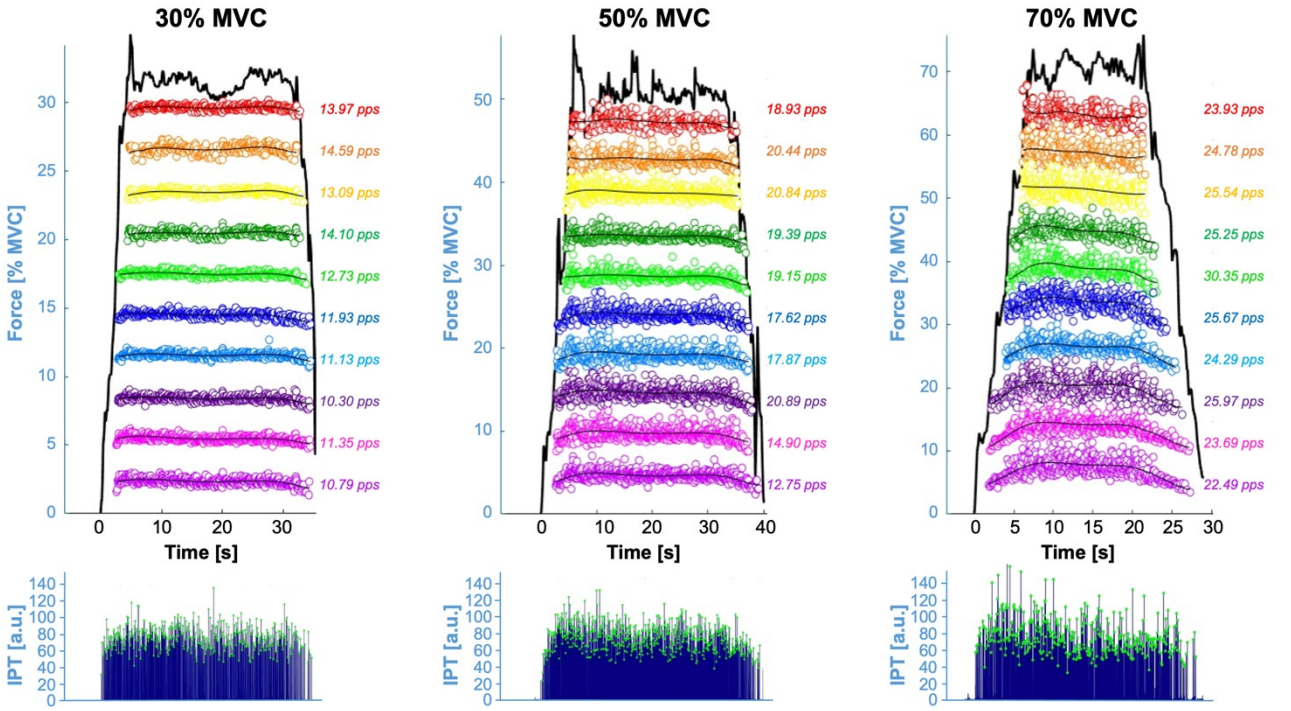

Figure 2: Illustrative MUs' Decomposition Process: Each EMG myoelectric signal was recorded during the isometric contractions and decomposed using the convolutive blind source separation technique. For each force level, MUs were extracted along with their corresponding DRs. The individual instantaneous MUs' discharge rates (colored dots) are illustrated, revealing their innervation pulse trains (IPT, blue vertical lines).

Peripheral variables.

Two variables were considered for the assessment of the peripheral mechanisms: global MUAP amplitude and maximal twitch force characteristics. For MUAP amplitude analysis, the MUAP waveforms across the matrix (MUAP template) were calculated by spike-triggered averaging the HDsEMG signals using the discharge time of each MU. Then, the peak-to-peak amplitude of each MUAP (P-P MUAP) waveform across the matrix was calculated and the average was considered for each MU (Negro et al., 2016). The average P-P MUAP amplitude of 100 random MUs selected from all MUs identified at 30%, 50% and 70% MVC were computed and used for further analysis (global P-P MUAP amplitude). For the maximal twitch force characteristics, the twitch force evoked using S100 (see *Stimulation protocol*) was used for analysis. Specifically, peak twitch force, contraction time (CT) and half-relaxation time (½RLT) were calculated as previously described by Orizio and associates (2016), to evaluate changes in maximal twitch force characteristics following ICU discharge.

10.3.7 Statistical analysis

All statistical analyses were performed in R (version 4.2.2; R Foundation for Statistical Computing, Vienna, Austria), using the RStudio environment. To assess differences between time points (3, 6 and 12 months following ICU discharge) in the functional variables (FSS, 6MWT, MVC), peripheral variables (global MUAP P-P amplitude, maximal twitch force characteristics), and force steadiness (CoV-Force), Wilcoxon signed-rank tests were used (given the data distribution was non-Gaussian; Kolmogorov-Smirnov test).

Linear mixed-effect models (LMM) were used to investigate the effect of follow-up time (3-, 6- and 12 months) on central variables (mean DR, CoV-ISI, RT, DT, RDR and DDR). This statistical model accounts for the repeated measures nature of the data (non-independence of observations), while allowing the inclusion of all detected units and not just the mean value for each participant (Boccia et al., 2019). Random intercept models were applied with follow-up time as the fixed effect and participant as the random effect. LMMs were implemented using the package *lmerTest* (Kuznetsova et al., 2017) with a Kenward-Roger analysis to approximate the degrees of freedom and estimate the p-values. Post-hoc pairwise comparisons were performed using the *emmeans* package, which estimates the marginal means and their differences with 95% confidence intervals. For all central variables, the analysis was performed separately for each contraction level (30%, 50%, and 70% MVC).

Repeated measures correlations were used to determine the common within-individual associations between central and functional variables, and peripheral and functional variables. For this analysis, MU discharge behaviours were averaged within participants to have a single value per participant (Valli et al., 2023). Repeated measures correlations were implemented using the *rmcorr* package with fixed slopes to estimate a single correlation coefficient for all participants (Valli et al., 2023).

10.3.8 Data availability

The data associated with this paper are not publicly available but are available from the corresponding author upon reasonable request.

10.4 Results

10.4.1 Functional variables

The results of the 6MWT showed that the distance walked by the patients significantly increased from 3-6 months (**Figure 3A**; p = 0.012), but not from 6-12 months follow-up (**Figure 3D**; p = 0.292). The FSS analysis did not reveal any significant differences between 3-6 month follow-ups (**Figure 3B**; p = 0.767), but there was a significant decrease between the 6-12 month follow-ups (**Figure 3E**; Wilcoxon signed-rank test, p = 0.013). The maximal dorsiflexion force output (MVC) differed significantly at both 3-6 and 6-12 month follow-ups. Specifically, the MVC significantly increased from 3-6 months (28.78%) (**Figure 3C**; Wilcoxon signed-rank test, p = 0.003), and from 6-12 months (19.67%) (**Figure 3F**; Wilcoxon signed-rank test, p < 0.001).

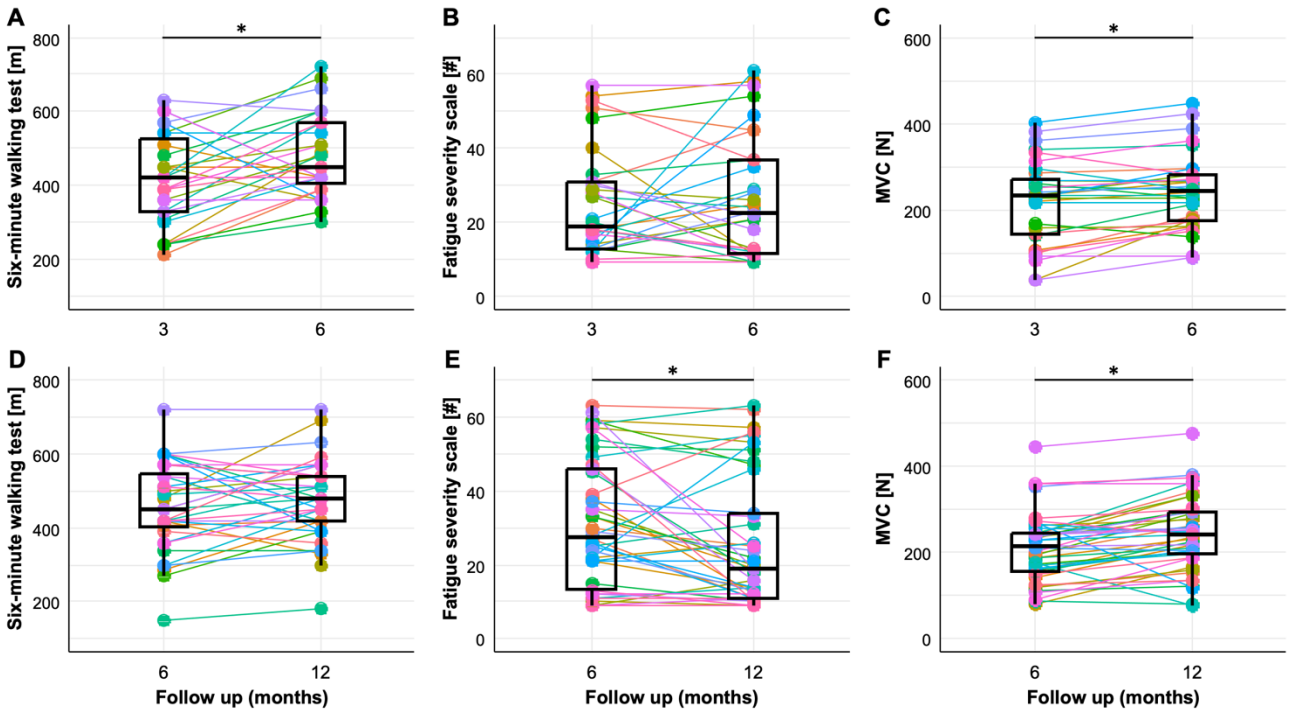

Figure 3: Functional parameters over the 12 month follow-up period in the study cohort. The participants evaluated at 3, 6, and 12 months are represented by different colored dots and lines. Boxes indicate the first and third quartiles. The horizontal line inside the box denotes the median value. The whiskers extending from the box show the range of the data, excluding outliers. Significant differences between the follow-up groups are indicated by black stars. A, D: The meters covered in the 6MWT were measured during various follow-ups. The only statistically significant change observed was an increase in the distance covered by patients from the baseline to the 6 month follow up. B, E: The values obtained by the participants on the FSS showed no significant differences between 3 and 6 months, followed by a noteworthy reduction in fatigue from the 6th to the 12th month. C, F: MVCs were collected between different follow-ups. The maximal force demonstrated a significant ($p \leq 0.05$) and continuous improvement in both groups.

10.4.2 Central variables

Motor unit discharge behaviours

All the results of the MU discharge behaviours are shown in **Figures 4, 5** and **6** for 30%, 50% and 70% MVC, respectively (3-6 months on the top panel and 6-12 months on the bottom panel). The average DR of MUs showed a significant decrease from 6-12 months for all contraction levels ($p < 0.01$ for all), and from 3-6 months at 50% and 70% MVC ($p < 0.001$ for both), but not 30% MVC ($p = 0.836$). However, the DRR significantly increased from 3-6 months only at 30% MVC ($p < 0.001$). Additionally, the DRD significantly increased from 3-6 months at 30% MVC ($p = 0.001$) and decreased from 6-12 months at 30% and 70% MVC ($p < 0.031$ for both). The CoV-ISI significantly decreased for all contraction levels and follow-up times ($p < 0.01$ for all), except for 3-6 months at 70% MVC which did not significantly change ($p = 0.059$). Regarding the RT/DT results, the RT significantly increased from 6-12 months for all contraction levels ($p < 0.01$), while from 3-6 months significantly increased only at 70% MVC ($p = 0.013$). The DT significantly increased only at 30% and 50% MVC for the 3-6 month follow-ups ($p = 0.005$ both).

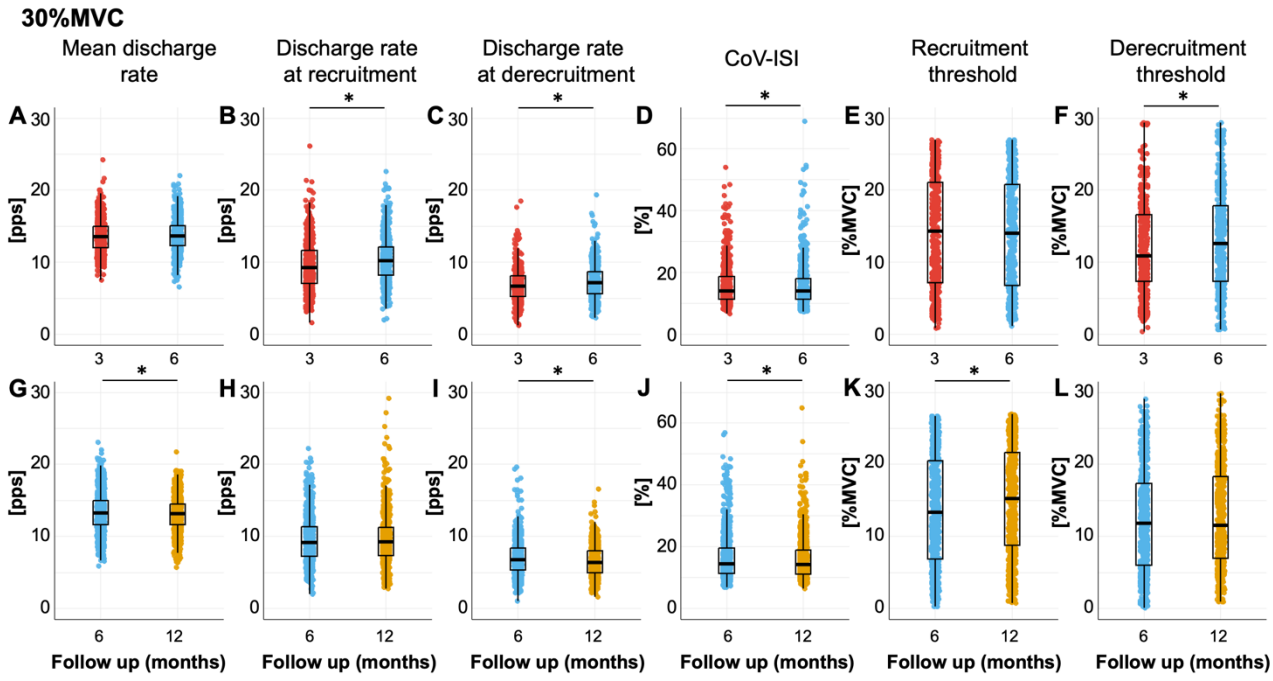

Figure 4: MU discharge behavior at 30% MVC. The participants evaluated at 3 months are represented by red dots, the ones at 6 months by blue dots, and the ones at 12 months by yellow dots. Black thick lines in the bar graphs denote means. Black bars with lines

denote 95% confidence intervals with standard errors. The 3-6 months group displayed significant changes over time in the mean discharge rate at recruitment (B), mean discharge rate at derecruitment (C) inter-spike-intervals covariation (D) and derecruitment threshold (F). The 6-12 months group displayed significant ($p \leq 0.05$) changes over time in the mean discharge rate (G), mean discharge rate at derecruitment (I) inter-spike-intervals covariation (J) and recruitment threshold (K).

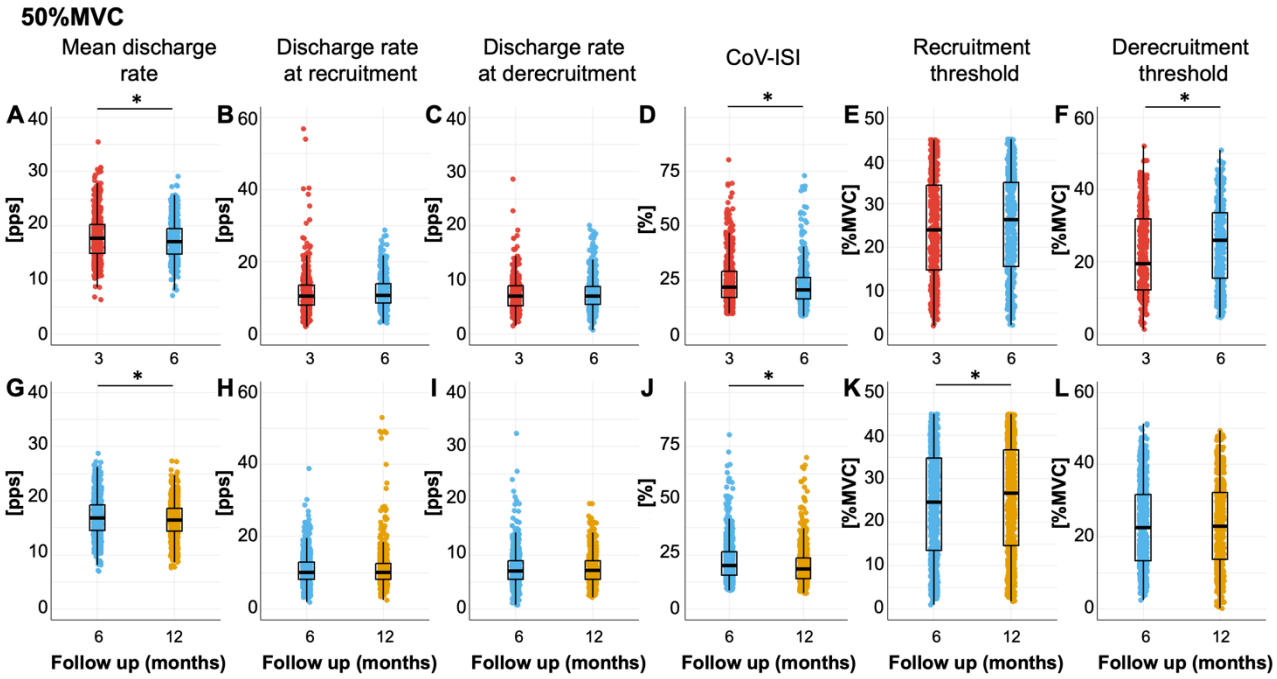

Figure 5: MU discharge behavior at 50% MVC. The participants evaluated at 3 months are represented by red dots, the ones at 6 months by blue dots, and the ones at 12 months by yellow dots. Black thick lines in the bar graphs denote means. Black bars with lines denote 95% confidence intervals with standard errors. The 3-6 months group displayed significant changes over time in the mean discharge rate (A), inter-spike-intervals covariation (D) and derecruitment threshold (F). The 6-12 months group displayed significant ($p \leq 0.05$) changes over time in the mean discharge rate (G), inter-spike-intervals covariation (J) and recruitment threshold (K).

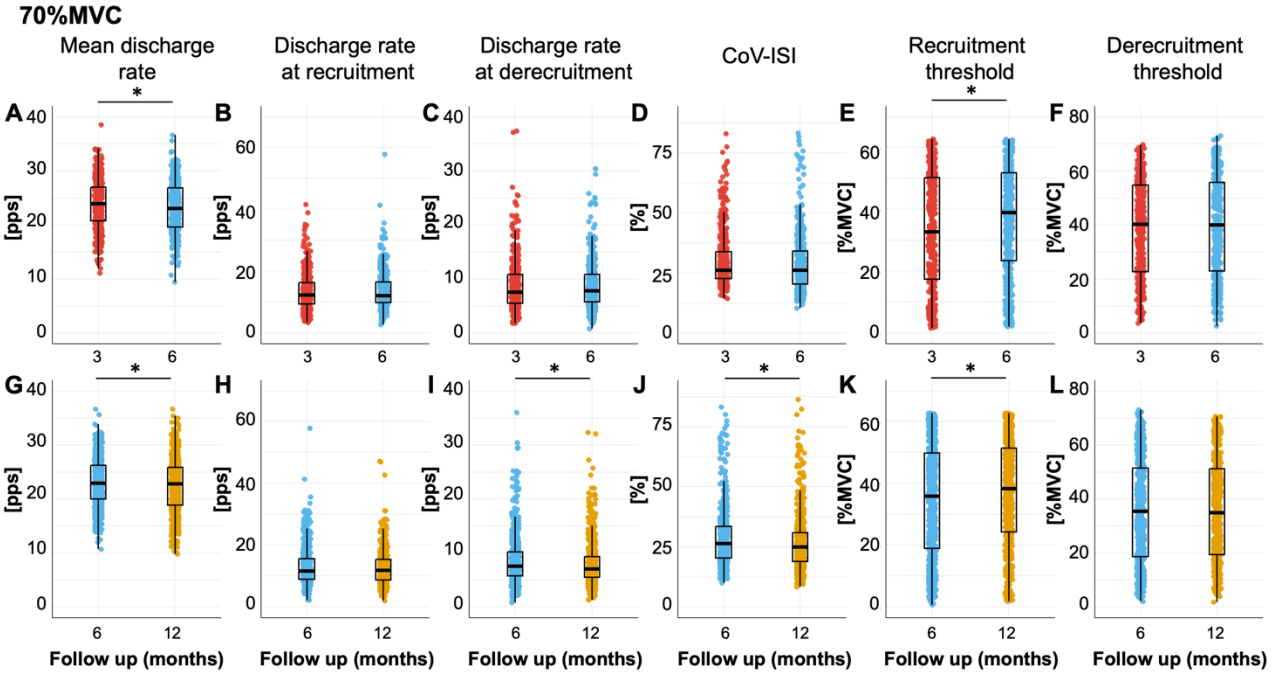

Figure 6: MU discharge behavior at 70% MVC. The participants evaluated at 3 months are represented by red dots, the ones at 6 months by blue dots, and the ones at 12 months by yellow dots. Black thick lines in the bar graphs denote means. Black bars with lines denote 95% confidence intervals with standard errors. The 3-6 months group displayed significant changes over time in the mean

discharge rate (A) and recruitment threshold (E). The 6-12 months group displayed significant ($p \leq 0.05$) changes over time in the mean discharge rate (G), mean discharge rate at derecruitment (I), inter-spike-intervals covariation (J) and recruitment threshold (K).

Coefficient of variation of force.

The CoV-Force was significantly lower at 12 compared to 6 month follow-ups at 70% MVC (**Figure 7F**; Wilcoxon signed-rank test, $p = 0.038$), however it did not change for all of the other conditions (**Figures 7A-E**; Wilcoxon signed-rank test; $p > 0.08$ for all).

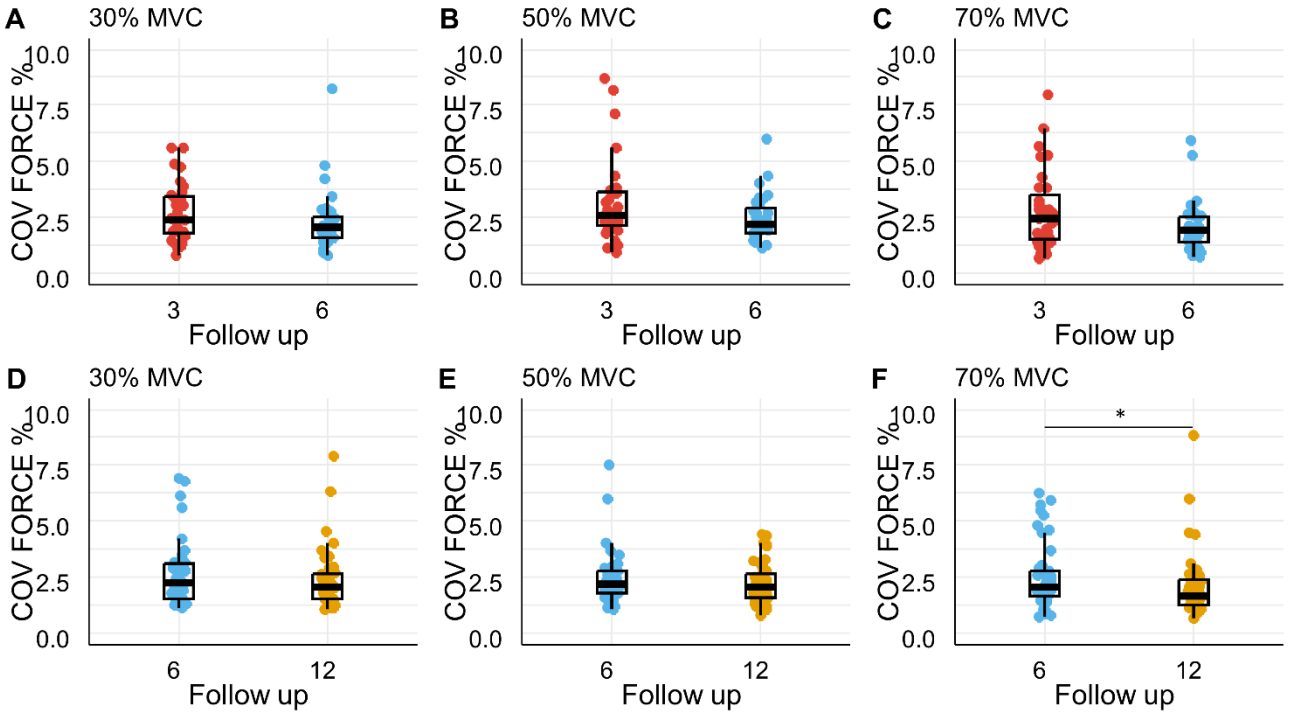

Figure 7: Coefficient of variation of force. The participants evaluated at 3 months are represented by red dots, the ones at 6 months by blue dots, and the ones at 12 months by yellow dots. Black thick lines in the bar graphs denote means. Black bars with lines denote 95% confidence intervals with standard errors. The only significant ($p \leq 0.05$) change over time was shown in the 6-12 months group at 70% MVC (F).

10.4.3 Peripheral variables

Global MUAP amplitude.

The global P-P MUAP amplitude was significantly greater at 6 compared to the 3 month follow-ups (**Figure 8A**; Wilcoxon signed-rank test; $p < 0.001$). Conversely, no statistically significant differences were found between 6 and 12 month follow-ups (**Figure 8E**; Wilcoxon signed-rank test; $p = 0.606$).

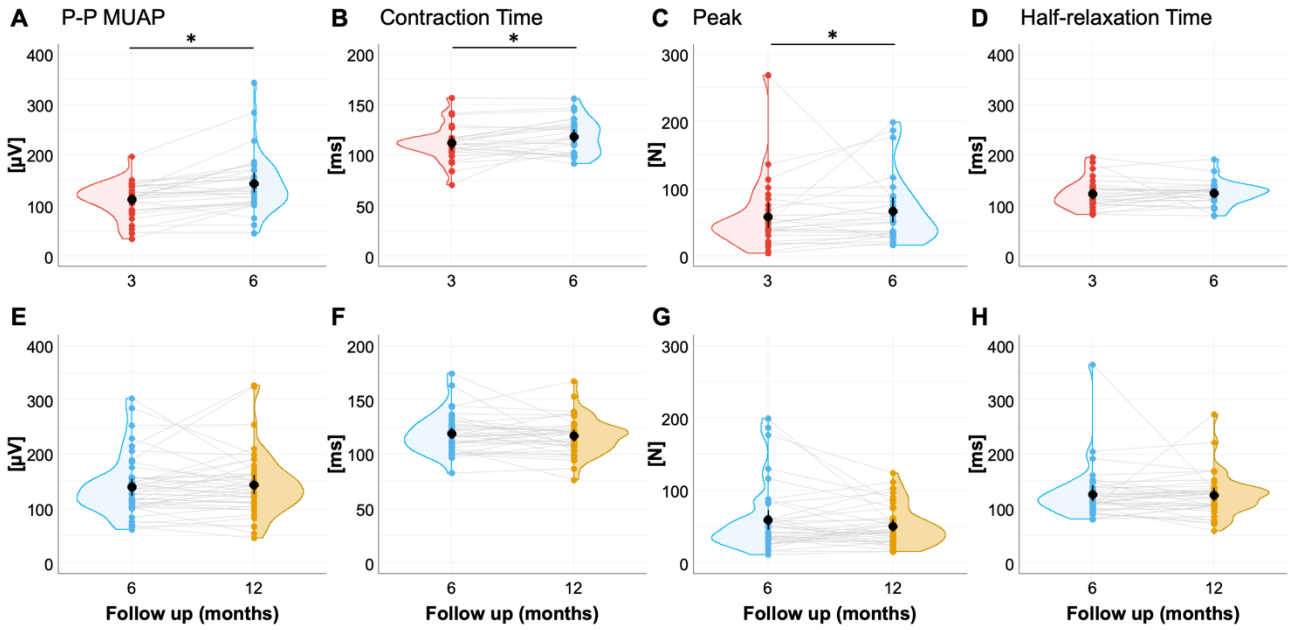

Figure 8: Peripheral variables. The participants evaluated at 3 months are represented by red dots, the ones at 6 months by blue dots, and the ones at 12 months by yellow dots. The same colors were used for the Kernel density estimation. The black dot denotes means with standard errors while grey lines connect the values of the same participants in the different follow-ups. Significance ($p \leq 0.05$) was displayed only in the 3-6 months group for the peak-to-peak MUAP (A), force twitch CT (B) and force twitch peak amplitude (C).

Maximal twitch force characteristics

The CT of the twitch force significantly increased from the 3-6 month follow-ups (**Figure 8B**; Wilcoxon signed-rank test; p = 0.018), while no significant differences were observed during the 6-12 month follow-up times (**Figure 8F**; Wilcoxon signed-rank test; p = 0.485). The peak amplitude of the maximal twitch force also significantly increased from the 3-6 month follow-ups (**Figure 8C**; Wilcoxon signed-rank test; p = 0.04), while no statistically significant changes were observed between 6 and 12 month follow-ups (**Figure 8G**; Wilcoxon signed-rank test; p = 0.685). Conversely, no differences were observed in the ½RLT during the 3-6 or 6-12 month follow-up times (**Figure 8D** and **8H**; Wilcoxon signed-rank tests; p > 0.350 for both).

10.4.4 Correlation analysis

Figure 9 depicts the main significant correlations between central/peripheral and functional variables. For both 3-6 and 6-12 month follow-ups, MVC and FSS were positively correlated with the central variables. Specifically, for the 3-6 month follow-up, the MVC values were significantly correlated with the mean DRR at 30% MVC (**Figure 9A**; R = 0.40; p = 0.02), the mean RT at 70% MVC (**Figure**

9B; R = 0.43; p = 0.02), and the mean DT at 70% MVC (**Figure 9C**; R = 0.40; p = 0.03). For the 6-12 month follow-up, the CoV-Force at 30% MVC (**Figure 9D**; R = 0.36; p = 0.02), 70% MVC (**Figure 9F**; R = 0.33; p = 0.03), and the mean DR at 50% MVC (**Figure 9E**; R = 0.38; p < 0.01) were significantly correlated with the FSS. No other statistically significant correlations were found between the central and functional variables.

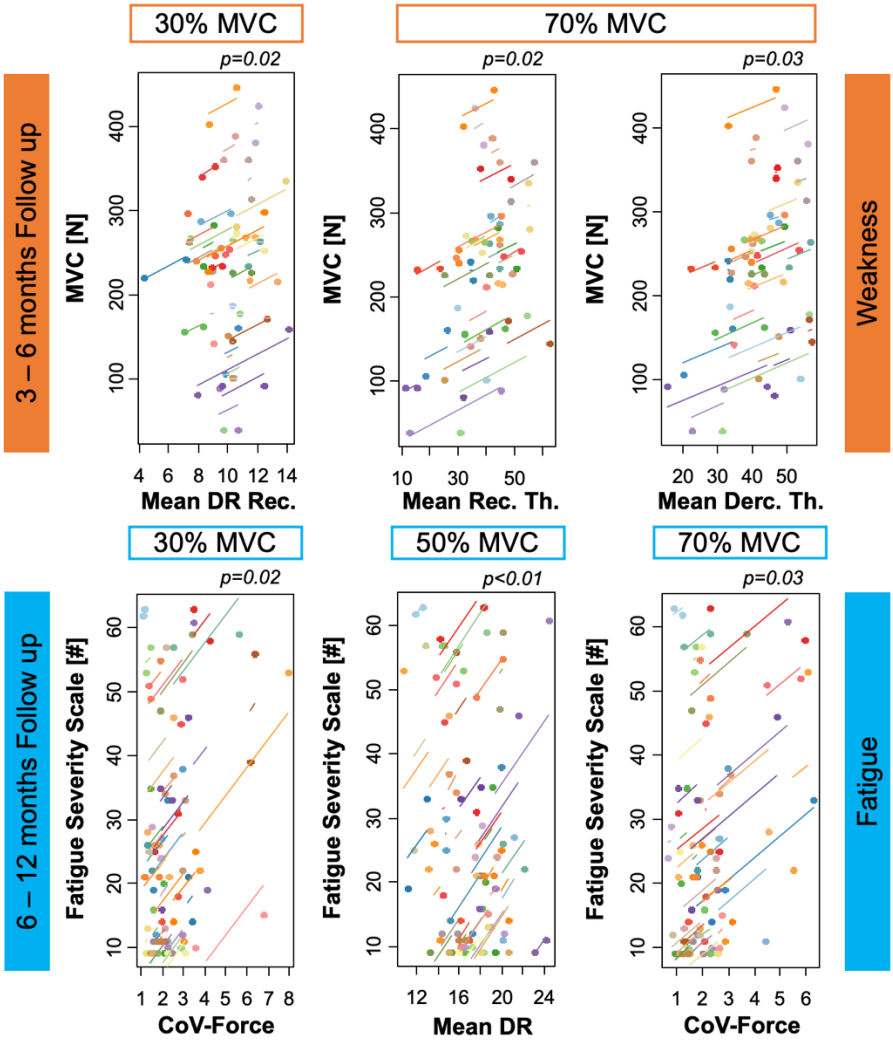

Figure 9: Correlation analysis. The repeated-measures correlation plots in the upper panels describe the common within-individual significant associations between MVC and mean discharge rate at recruitment (A) for 30% MVC contraction, and between MVC and mean recruitment (B) and derecruitment threshold (C) for 70% MVC in the 3-6 months group. Conversely, the 6-12 months group in the lower panels exhibits significance in the association of FSS with force covariation at 30% (D) and 70% (F), and with mean discharge rate at 50% (E). All the other correlations did not show significance. The p-value is reported in the upper right corner of each figure.Additional significant correlations were observed at the 3-6 month follow-ups between the peripheral and functional variables. Specifically, P-P MUAP amplitude was positively correlated with the 6MWT (p = 0.01) and MVC (p = 0.048). No other significant correlations were found between peripheral and functional variables.

10.5 Discussion

In this study, we combined HDsEMG myoelectric signals, isometric force outputs, and electrically evoked potentials to investigate the effects of ICU recovery within the 1-year following discharge in individuals hospitalized for COVID-19 acute respiratory distress syndrome. Our findings revealed improvements in functional parameters (6MWT, MVC, and FSS) both in the 3-6 and 6-12 month post-ICU discharge periods, highlighting the relevance of long-term monitoring of physical functioning within this patient cohort. These functional improvements were accompanied by changes in central and peripheral motor unit behaviour and, more importantly, there were direct associations between functional improvements and central modifications. The central modifications were primarily associated with restoration of the force-generating capacity during the initial 3-6 months and improvements in FSS in the last 6-12 months post-ICU. Additionally, these findings underscore the essential need to conduct an objective assessment which may be used to monitor neurophysiological recovery following ICU discharge.

10.5.1 Functional Variables

As reported in the literature (Lopez-Leon et al., 2021; Morel et al., 2022; Neufeld et al., 2020), fatigue is a prominent symptom reported by two-thirds of patients in the year following the acute phase of acute respiratory distress syndrome. Its prevalence is greater than that of other frequently encountered symptoms, including physical dysfunction, cognitive impairment, anxiety, and depression. In our study, FSS score did not improve between 3 to 6 months but did show substantial improvements between 6 and 12 months. These results agree with the recent review by Joli and colleagues (2022) that indicated the onset of fatigue in ICU patients can commence as early as the acute phase of the pathology, subsequently persisting for a minimum of 6 months post-ICU discharge. This helps to explain the lack of difference in FSS at the 3-6 month follow-up. However, as highlighted by our findings, following this period improvements can be achieved within one year from the beginning of recovery.

Approximately 80% of ICU patients exhibit critical illness, neuropathy and/or myopathy, conditions that are closely connected to the ICUAW (Jolley et al., 2016; Latronico and Bolton, 2011). The degree of neuromuscular impairment developed during hospitalization becomes increasingly pronounced when coupled with extended periods of mechanical ventilation and prolonged hospital stays. The development of neuromuscular impairment may also lead to diminished handgrip strength and compromised physical function leading to a reduction in health-related quality of life (Fan et al., 2014). Moreover, prolonged hospitalization paired with bed rest directly results in a marked increase in physical inactivity and consequently enhanced muscle atrophy (Coker and Wolfe, 2012). The combination of inactivity and reduction in muscle quality may lead to a substantial reduction in an individual's ability to generate and sustain force outputs. Nonetheless, our results revealed the potential for continuous improvement in force production following ICU discharge, with significant increases in MVC over the 12 months following ICU discharge. Notably, one year after discharge from the ICU, the patients reached levels of strength comparable to those of age-matched adults in a healthy population (Cogliati et al., 2020). These findings are in agreement with previous work (Lulic-Kuryllo et al. 2023; Latronico et al. 2022), which reported strength recovery, in acute respiratory distress syndrome survivors, in the year following extended hospitalization.

10.5.2 Central variables

The evaluation of MU behaviour at various levels of voluntary force output revealed notable changes across force levels and over time. Specifically, reductions in mean DR were observed in the 3-6 month follow-ups at mid and high levels of force output, and from the 6-12 month follow-ups at each level of force output. These modifications likely reflect adjustments in MU recruitment and rate coding during recovery, revealing the close connection between restoring adequate neural input and the patients' ability to regain force-generating capacity (Lulic-Kuryllo et al., 2023). In essence, after a one-year follow-up, there is a diminished requirement for an increased neural drive to the muscle (Lulic-Kuryllo et al., 2023). The diminished requirement is due to the improved efficiency of the neuromuscular system (i.e., improvements in force production) one year following ICU discharge. In

contrast, the mean DR remained unaltered at low levels of force output during the 3-6 month follow-up. Instead, a simultaneous increase in the mean DRR and DRD was observed for this time point. These results suggest that at low levels of force output, at the motor unit level, improved efficiency of the neuromuscular system, following ICU discharge, is predominantly utilized during the ascending and descending phases of the voluntary contraction, and not the sustained portion.

MU discharge rate variability (CoV-ISI) decreased at all levels of force output, except for the 3-6 month follow-up at 70% MVC. This outcome may indicate an enhanced neural drive efficiency, as lower discharge rate variability implies a narrower range of the mean MUs ISI (Jones et al., 2002). In accordance with several studies (Enoka and Farina, 2021; Germer et al., 2020; Inglis and Gabriel, 2021; Moritz et al., 2005; Petrović et al., 2023), variations in CoV-ISI may also be linked to force fluctuations. In contrast, our results did not indicate a concomitant reduction in the CoV-Force alongside the decrease in CoV-ISI, except during the isometric contractions at 70% MVC for the 6-12 month follow-up. A plausible explanation for the absence of this relationship might stem from the CoV-Force not being solely contingent upon MU discharge rate variability, but may also be influenced by other neural factors (Enoka and Farina, 2021; Farina and Negro, 2015), such as oscillations in the shared synaptic input across the motor neuron pool (Dideriksen et al., 2012). These other potential factors warrant further investigation.

10.5.3 Peripheral variables

The peripheral variable results showed significant changes only in the 3-6 month follow-up. Specifically, the modifications were observed in both the P-P MUAP amplitude and twitch force characteristics, except for ½RLT. CT, the time difference between the twitch force onset to its time to reach peak tension (Dahmane et al., 2005), is partially influenced by the properties of the involved MUs (Enoka and Farina, 2021; Inglis et al., 2017). Our results showed slower CTs throughout the year following the prolonged ICU stay and subsequent discharge. These values were similar to those reported by Connelly and associates (1999), who compared contractile properties between younger (20-22 yrs) and older males (80-85 yrs), reporting that the older group had a significantly longer CT

and ½RLT compared to the younger group. These slower CTs in our participants could be related to several factors due to extended hospitalization and bed rest which altered neuromuscular control. First, patients affected by COVID-19 acute respiratory distress syndrome exhibit critical illness myopathy, polyneuropathy, and acute inflammatory conditions that can cause delays in nerve signal transmission to the muscle, consequently resulting in prolonged twitch force CTs (Logigian et al., 1990; Milner-Brown et al., 1974). Lastly, it is possible that this elongated CT was caused by the immobilization during the stay in the ICU. Prolonged immobilization, leading to muscle atrophy, results in preferential recruitment of slow MUs at the expense of fast ones. Given that slow MUs exhibit longer CTs than fast ones (Sica and McComas, 1971), this could help explain the temporal increases of the twitch force contractions. The significant increases in CT in the 3-6 month, but not the 6-12 month, follow-ups may be attributed to fatigue as it appeared during the acute phase and evolved within the first 6 months of the pathology (Joli et al., 2022). The increase in CT in the 3-6 month follow-up could therefore be influenced by the incidence of fatigue, as has been previously reported (Behm and St-Pierre, 1997; Hamada et al., 2003; Shields et al., 1997). Indeed, improvements in fatigue were observed after the first 6 months post-hospitalization, which was followed by a non-statistically significant trend toward a decrease in CT.

The observed increase in global P-P MUAP amplitude and peak twitch force amplitude may be related to the observed increases in force output detected during the initial 6 month follow-up. Considering the increase in both global P-P amplitude and peak twitch force, these findings suggest an enhanced muscle force-generating capacity due to adaptations in the muscle fibre contractile properties (Inglis et al., 2017, 2013), recovered muscle hypertrophy (Behm et al., 2002) and/or neural adaptations during the early rehabilitation phase (Gabriel et al., 2006).

10.5.4 Variable correlations

The correlation results between functional and central variables revealed that MVC values were positively correlated with the average DRR (at low force levels) and with the RT and DT (at high force levels) during the 3-6 month follow-up. However, at the 6-12 month follow-up there were

significant correlations between FSS and CoV-Force (at 30% and 70%), and FSS and mean DR (at 50%). These correlations reveal an inverse association between perceived fatigue and both the CoV-Force and mean DR. These results confirm that during the initial months post-hospitalization, the improvements achieved by the patients are exclusively reflected in the recovery of their force-generating capacity and continue during the subsequent months. Our results are in agreement with Aarden and associates (2019) who reported increases in muscle strength are associated with the progression of mobility, with the greatest improvements occurring during the initial months following the acute phase of the disease. Subsequently, changes in the central variables exhibited an increased association with fatigue, as fatigue progression ceased after the initial 6 months post-ICU discharge (Joli et al., 2022). Taken together, these findings suggest a two-step progression leading to the restored autonomy of patients during the first year following ICU discharge. Based on the findings in the current study, we suggest the recovery of muscle force capacity is the primary adaptation during the first 3-6 months, followed by an emphasis on fatigue recovery in the final 6-12 month period post-ICU discharge.

Finally, in the 6-12 month follow-up, fatigue was correlated with the CoV-Force at 30% and 70% MVC, as well as the mean DR at 50%, suggesting that increased stability in MU activation might indicate a reduction in fatigue (Almeida et al., 2008; Lin et al., 2014). This seems particularly relevant for higher force levels, where a significant decrease in the CoV-Force was observed. This in turn may optimize MU recruitment and consequently reduce fatigue caused by their derecruitment, particularly during tasks requiring higher force outputs. Furthermore, as previously reported by Lulic-Kuryllo and associates (2023), a reduction in the mean DR is an indication of an increase in neural drive efficiency, which may lead to a delay in the onset of fatigue.

10.6 Conclusion

The present study fills a critical knowledge gap in the existing literature by combining the identification of MU behaviours (HDsEMG), voluntary isometric force outputs (central factors), and

electrically evoked potentials (peripheral factors) to investigate post-ICU discharge recovery in individuals hospitalized for COVID-19 acute respiratory distress syndrome. Our results outline intricate adaptations and modifications within the neuromuscular system in these patients during the post-ICU discharge follow-up period. The results shed light on neural and physiological changes induced by prolonged ICU hospitalization on both central and peripheral factors. Additionally, the current work provides insights into how patients regain optimal force-generating capacity and further benefit from fatigue reduction. These positive modifications, as evidenced by the significant correlation between functional and central variables, primarily stem from the central nervous system's adaptations, enabling the restoration of optimal MU activation strategies aimed at maximizing energy efficiency, minimizing fatigue, and ultimately restoring normal neuromuscular function.

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11. Chapter Eleven: General conclusions.

The primary objective of the work presented in this thesis was to explore changes in neuromuscular function, the occurrence of muscle weakness, and the onset of fatigue in different pathological conditions. The goal was to identify biomarkers that can facilitate an objective and optimal evaluation of the subject physical capacity and the planning of tailored rehabilitation program. Given the significant impact that aging, intensive care admission, and the development of non-communicable diseases have on an individual's ability to maintain independent and efficient ambulation, the studies presented in chapters 6, 7, 8, 9, and 10 were focused on the TA, given its key role in walking biomechanics. Chapter 5, despite being a review of different setups and effects of rTMS on Parkinson's patients, sought to highlight the primary areas and stimulation methods that lead to improvement, among others, in bradykinesia and freezing of gait. This improvement enhances walking, postural stability, and turning, thereby reducing the risk of falls and associated injuries closely linked to mortality.

In clinical settings, commonly employed techniques to assess muscle function and its relative impairments include iEMG or global sEMG, which suffer from significant limitations – namely, they do not allow for the simultaneous measurement of MU discharge in various muscles or different areas of the same muscle during joint torque detection. In particular, the two detection systems cannot provide detailed spatial information about the different regions of the muscle that are recruited during specific tasks. As highlighted in Chapter 7, the HDsEMG methodology represented a non-invasive important and reliable tool to get deeper in the study of neural control over muscle activation.

Specifically, the work demonstrated that: (I) further research is required to better understand the impact of rTMS on Parkinson's disease through the identification of the optimal stimulation sites for enhancing gait, addressing bradykinesia, mitigating tremor, and reducing the risk of falls. Moreover, there’s a pressing need for standardized methods to assess falls and tremors, with inertial sensors proposed as a reliable solution for objective evaluation. The combined utilization of rTMS, inertial sensors, and HDsEMG could provide crucial information for designing diagnostic and rehabilitative plans for these patients. (II) The age-related gait impairment was linked to delayed muscle relaxation and re-elongation during various phases of the gait cycle. The research measured a muscle's ability to re-lengthen within the resistance to ankle joint angle changes when calf muscles were active. These findings suggest that alterations in muscle mechanics may significantly impact gait phases in older individuals. (III) The HDsEMG, despite the classical bipolar sEMG, provided more nuanced insights into muscle activation patterns and motor unit behavior changes over time in stroke patients. Overall, this approach could inform tailored rehabilitation programs for these patients and improve their outcomes, potentially leading to more effective, personalized strategies for motor function recovery.

(IV) Electrical neuromuscular abnormalities (measured by CMAP-TA-S100) and the presence of severe fatigue were linked independently to decreased muscle strength (MVC) and could serve as indicators for predicting the likelihood of long-term muscle weakness in individuals who have survived [C]ARDS. (V) the HDsEMG technique could provide valuable information about disparities between sexes in the ongoing neuromuscular recovery of individuals who contracted [C]ARDS. These differences were particularly evident in terms of physical functioning, maximum strength, and motor unit firing rates. Study findings revealed that females experience significant challenges in regaining their functional abilities for up to a year after leaving the ICU following SARS-CoV-2 infection. Consequently, it's crucial to take gender into account when planning post-COVID neurorehabilitation strategies. (VI) HDsEMG analysis during voluntary and electrically evoked muscle contractions, addressed a crucial gap in understanding the recovery of individuals hospitalized for [C]ARDS after leaving the ICU. The findings revealed complex adaptations in the neuromuscular system during this period, showing the impact of prolonged ICU stays on both central and peripheral factors. These insights demonstrated how patients regain strength and reduce fatigue, primarily through adaptations in the central nervous system, optimizing muscle activation strategies and restoring normal neuromuscular function.

The advanced HDsEMG technology overcame the constraints of conventional clinical assessment methods by providing a precise means to identify and distinguish individual MUs within a muscle. This capability yields invaluable insights into the recruitment and firing patterns of these units, making it an indispensable tool for both researchers and clinicians involved in the examination of neuromuscular disorders and the monitoring of muscle function during rehabilitation. This technology resulted extremely effective in identifying biomarkers of neuromuscular function efficiency and neuromuscular fatigue during post-discharge follow-ups for individuals previously treated in intensive care for COVID-19 and non-communicable diseases.

12. Chapter Twelve: List of publications.

LIST OF PUBLICATIONS

Cogliati M, Cudicio A, **Benedini M**, Cabral HV, Negro F, Reggiani C, Orizio C. Influence of age on force and re-lengthening dynamics after tetanic stimulation withdrawal in the tibialis anterior muscle. Eur J Appl Physiol. 2023 Aug;123(8):1825-1836. doi: 10.1007/s00421-023-05198-0. Epub 2023 Apr 18. PMID: 37071199; PMCID: PMC10363076.

Lulic-Kuryllo T, **Benedini M**, Cogliati M, Cudicio A, Guarneri B, Gazzina S, Piva S, Latronico N, Orizio C, Negro F. Sex-differences in the longitudinal recovery of neuromuscular function in COVID-19 associated acute respiratory distress syndrome survivors. Front Med (Lausanne). 2023 Jun 26;10:1185479. doi: 10.3389/fmed.2023.1185479. PMID: 37435534; PMCID: PMC10330713.

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PROCEEDINGS

Benedini M, Cabral HV, Cogliati M, Falciati L, Bissolotti L, Orizio C, McPherson LM, Negro F. “High-density surface electromyography allows for longitudinal assessment of the neural drive to muscle in individuals with acute stroke”.

Benedini M, Cogliati M, Cabral HV, Inglis JG, Lulic-Kuryllo T, Piva S, Latronico N, Orizio C, Negro F. “Effect of COVID-19 intensive care unit hospitalization on strength, fatigue and motor unit behaviour: a one-year follow-up study”.

Pilotto A and **Benedini M**, Negro F, Orizio C, Borroni B, Rizzeti MC, Padovani A, “Effects of Repetitive Transcranial Magnetic Stimulation (rTMS) on Motor Symptoms of Parkinson Disease: Systematic Review”.

CONGRESS ABSTRACT and PRESENTATION

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