Promoting brain plasticity during subacute stroke: the interactive role of exercise and genotype

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List of Abbreviations

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
BBB	Blood-brain barrier
BBT	Box and blocks test
BDNF	Brain-Derived Neurotropic Factor
BMI	Body-mass index
CCI	Commodity Channel Index
CE	Cardiovascular exercise
CNS	Central nervous system
CRF	Cardiorespiratory fitness
CSE	Corticospinal excitability
CSP	Cortical silent period
CST	Corticospinal tract
EEG	Electroencephalography
FMA	Fugl-Meyer
fMRI	Functional magnetic resonance imaging
fNIRS	Functional near-infrared spectroscopy
GABA	Gamma-aminobutyric acid
GXT	Graded exercise test
HIIT	High-intensity interval training
HR	Heart rate
ICF	Intracortical facilitation
IGF-1	Insulin-like growth factor-1
iTBS	Intermittent theta-burst stimulation
LMM	Linear Mixed Model
M1	Primary motor cortex
MEP	Motor-evoked potential
Met	Methionine
MICT	Moderate-intensity interval training
MoCA	Montreal Cognitive Assessment
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
MVC	Maximal voluntary contraction
NIHSS	National Institutes of Health Stroke Scale
NMDA	N-methyl-D-aspartate
PASIPD	Physical activity scale for individuals with physical disabilities
PPO	Peak power output

RMT	Resting motor threshold
RPE	Rate of perceived exertion
SICI	Short intracortical inhibition
SNP	Single nucleotide polymorphism
TDCS	Transcranial direct current stimulation
TMS	Transcranial magnetic stimulation
trkB	Tropomyosin receptor kinase B
Val	Valine
VEGF	Vascular endothelial growth factor
VIF	Variance inflation factor

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Abstract

Along with the lesion's location and size, the fast-remodeling processes that the brain experiences during the first weeks following a stroke dictate the long-term functional recovery of the patient. Interventions that increase the capacity of the brain to reorganize itself during these initial phases are critical to maximize functional recovery. Animal studies demonstrate that introducing cardiovascular activities as early as a few days after stroke constitutes a simple yet effective strategy to promote recovery through neuroplasticity and neural repair changes. However, the effects of cardiovascular exercise on the brain in people with stroke remain largely unexplored, particularly during the early phases of recovery when the brain may be more responsive to treatments. Additionally, the potential influence of genetic makeup on an individual's responsiveness to such exercise is currently unknown. In this thesis, we addressed these gaps by first conducting a comprehensive review on the effects of cardiovascular exercise on neuroplasticity biomarkers after stroke. We subsequently conducted a randomized control trial to investigate the effects of cardiovascular exercise on two key neuroplasticity biomarkers—corticospinal excitability and brain-derived neurotrophic factor — in individuals during the subacute recovery phase, addressing one of the major identified gaps in the literature. Our findings revealed that 8 weeks of progressive cardiovascular exercise significantly improved cardiorespiratory fitness in individuals with subacute stroke, yet had minimal impact on biomarkers related to neuroplasticity, suggesting limited brain reparative effects. Additionally, the BDNF Val66Met polymorphism, a genetic variant linked to neuroplasticity processes, showed little modulatory impact on biomarker response to exercise. We explore and discuss several factors that may have contributed to these results. These findings suggest that cardiovascular exercise may not promote neuroplasticity in the early post-stroke stages, warranting a revaluation of how this intervention is applied and assessed as a neuroplasticity-promoting intervention during this critical period.

Résumé

Outre la localisation et la taille de la lésion, les processus de remodelage rapide du cerveau au cours des premières semaines suivant un accident vasculaire cérébral déterminent la récupération fonctionnelle à long terme du patient. Les interventions qui augmentent la capacité du cerveau à se réorganiser pendant ces phases initiales sont essentielles pour maximiser la récupération fonctionnelle. Des études animales montrent que l'introduction d'activités cardiovasculaires dès les premiers jours suivant un AVC constitue une stratégie simple mais efficace pour promouvoir la récupération par le biais de la neuroplasticité et des changements dans la réparation neuronale. Cependant, les effets de l'exercice cardiovasculaire sur le cerveau des personnes ayant subi un AVC restent largement inexplorés, en particulier durant les premières phases de la récupération, lorsque le cerveau peut être plus réceptif aux traitements. En outre, l'influence potentielle de la constitution génétique sur la réactivité d'un individu à ce type d'exercice est actuellement inconnue. Dans cette thèse, nous avons comblé ces lacunes en procédant tout d'abord à un examen complet des effets de l'exercice cardiovasculaire sur les biomarqueurs de la neuroplasticité après un AVC. Nous avons ensuite mené un essai contrôlé randomisé pour étudier les effets de l'exercice cardiovasculaire sur deux biomarqueurs clés de la neuroplasticité - l'excitabilité corticospinale et le facteur neurotrophique dérivé du cerveau - chez des individus au cours de la phase de récupération subaiguë, comblant ainsi l'une des principales lacunes identifiées dans la littérature. Nos résultats ont révélé que 8 semaines d'exercices cardiovasculaires progressifs amélioraient de manière significative la condition physique cardiorespiratoire chez les personnes ayant subi un AVC subaigu, mais n'avaient qu'un impact minime sur les biomarqueurs liés à la neuroplasticité, ce qui suggère des effets réparateurs limités sur le cerveau. En outre, le polymorphisme BDNF Val66Met, une variante génétique liée aux processus de neuroplasticité, n'a eu qu'un faible impact modulateur sur la réponse des biomarqueurs à l'exercice. Nous examinons et discutons plusieurs facteurs susceptibles d'avoir contribué à ces résultats. Ces résultats suggèrent que l'exercice cardiovasculaire ne favorise pas la neuroplasticité dans les premiers stades post-AVC, ce qui justifie une réévaluation de la manière dont cette intervention est appliquée et évaluée en tant qu'intervention favorisant la neuroplasticité au cours de cette période critique.

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Contribution to Original Knowledge

This thesis presents original material that has not been published elsewhere, except where specific references are made. The manuscripts in Chapters 1, 2, and 3 provide significant contributions to the field of post-stroke rehabilitation and recovery, particularly focusing on the effects of cardiovascular exercise on biomarkers of brain plasticity.

This work is the first to combine non-invasive brain stimulation, lab assay, and genotyping techniques to explore the interaction between cardiovascular exercise, brain plasticity, and genotype in subacute stroke patients, a subgroup that has been understudied despite having great recovery potential.

We used central and peripheral brain plasticity biomarkers to examine the potential reparative effects of cardiovascular exercise on the injured brain during the time-limited therapeutic window that opens in the subacute phase post-stroke, and investigated whether a specific polymorphism associated with altered brain processes influenced these responses. This complex yet unique approach allowed us to investigate the potential therapeutic effect of cardiovascular exercise and the variability in individual patient responses.

Additionally, by measuring these biomarkers after a progressive yet vigorous cardiovascular exercise training, we demonstrated the effectiveness and safety of a treatment that is often underutilized in the early stages of recovery, which could positively impact clinical practice.

All data presented in this thesis were collected at the Feil & Oberfeld Research Center of the Jewish Rehabilitation Hospital, affiliated to McGill University. All studies have been approved by the Ethics Board of the Center for Interdisciplinary Research in Rehabilitation of Greater Montreal (CRIR) (CRIR-1265-0817)

Contribution of Authors

This thesis is presented in manuscript format and comprises three manuscripts: one published and two pending submissions. I, Bernat de las Heras de Miguel, am the principal contributor and lead author for all the manuscripts included in this thesis. My contributions encompass data collection and analysis, interpretation of findings, preparation of figures, tables, and supplementary materials, manuscript submissions and revisions following peer review, and the writing of the dissertation.

In the manuscripts presented in Chapters 2 and 3, Dr. Marc Roig (supervisor) was responsible for the conception and design of the study, while I, Bernat de las Heras de Miguel, contributed to the design, conducted data collection and analyses, and prepared the manuscripts.

Co-authors Lynden Rodrigues, Jacopo Cristini, Roya Khalili, and Dr. Marc Roig assisted with data acquisition and analysis, and provided critical reviews and improvements to the manuscripts. Drs. Eric Yu and Ziv Gan-Or contributed to the genetic analysis portion in Chapters 2 and 3 and offered critical reviews and enhancements to the manuscripts. Nathalie Arbour supported blood analysis assays in Chapter 3 and provided critical reviews and improvements in Chapter 3. Kevin Moncion and Michelle Ploughman critically reviewed and improved the manuscript in Chapter 1. Additionally, Dr. Ada Tang, Dr. Joyce Fung, Dr. Alexander Thiel, and Dr. Janice J. Eng contributed to the study design of this thesis and provided critical reviews and improvements. This dissertation was also read and approved by Dr. Marc Roig (supervisor).

General Introduction

The Museum of your Mind

Densely packed, and intricately patterned, the human brain serves as the museum of our minds, the medium in which we store memories, the gateway to experience and impact the world, and conform the very essence of our self-identity. For all these reasons, efforts aimed at protecting the brain should not be perceived merely as a pursuit of health but also as to safeguard a part of the world's heritage.

Throughout history, humanity has embarked on a quest to unravel the enigmatic workings of the brain. Most of the pivotal breakthroughs and advances in the field of neuroscience have emerged from the study of the brain during states of disease and injury. Investigating neurological diseases has played a pivotal role in deepening our understanding of brain function across various levels, including anatomy, cellular and molecular processes, and behavior. This research has helped unravel the complexity of the human brain, shedding light on the mechanisms that govern its function while, at the same time, offering the foundation for identifying and treating biological targets through therapeutic applications.

Nevertheless, despite remarkable progress over the last century, the study of the brain remains in its infancy. We are still far from completely preventing and curing brain-related diseases, a reality reflected in the significant number of individuals who continue suffering the consequences of these conditions and the incapacity of the healthcare system to deliver effective solutions. However, with each passing year, we are witnessing unprecedented breakthroughs in science that are rapidly advancing our understanding of the underlying mechanisms causally involved in the development, slowdown, and repair of most common neurological diseases. This exciting juncture anticipates a bright future for the field of neuroscience - a future where restoring the brain from injury and disease may no longer be a mystery but a tangible reality. In the words of Erik Kandel, Nobel Laureate, and pioneer in behavioral neuroscience and memory research: "Our generation of scientists has come to believe that the biology of the mind will be as scientifically important to this century as the biology of the gene has been to the 20th century". Solving these mysteries, especially in the context of illness, holds great potential to provide

innovative means to alleviate the suffering for many individuals, improve their quality of life, and enable them to continue making meaningful contributions to the world.

The Brain: An Essential yet Vulnerable Organ

The human brain, a vital yet delicate organ composed of millions of interconnected neurons, plays a pivotal role in processing information, controlling bodily functions, and generating thoughts and emotions. Its optimal function depends on a complex vascular system comprising large and small blood vessels, which provide essential nutrients and oxygen to various brain regions, sustaining its essential functions. However, when this intricate vascular system is compromised due to an interruption in blood flow, be it from a blood clot or hemorrhagic transformation, even if it lasts only a few seconds, the consequences can be catastrophic. In just one minute, 1.9 million neurons, 14 billion synapses, and 12 km of myelinated nerve fibers can be irreversibly damaged [1]. This damage affects neural connections within the affected and remote areas, leading to cognitive, sensory, motor, or speech impairments depending on the lesion location and, in severe cases, even death [1].

This is what happens after suffering a stroke, a condition that affects 12.2 million people annually worldwide, or in other words, one every three seconds. It has emerged as the second leading cause of death and the first cause of disability-adjusted life years among adults worldwide [2]. Stroke poses a major global healthcare problem with serious social and economic consequences. More than 100 million people live with the long-term consequences of stroke, a number that has nearly doubled over the last 30 years, resulting in a global annual economic burden of US$721 billion, equivalent to 0.66% of the global GDP [3].

Regarding its incidence, one in four adults will experience a stroke at some point in their life, a statistic that has increased by 50% over the past 17 years. Additionally, due to the progressive aging of the population, this number is projected to rise even further, with studies estimating an 80% increase over the next two decades [4,5]. For instance, in countries like Spain, where an inverted demographic pyramid indicates an aging population, there has been an increase of 444% in the incidence of strokes from 2011 to 2023 **(Figure 1)** and is expected to rise further in the coming years. Similar patterns are occurring in other countries with comparable demographic characteristics. Strikingly however, in 2019, 63% of reported strokes occurred in

individuals under the age of 70, revealing that stroke is no longer exclusively a disease of the elderly population.

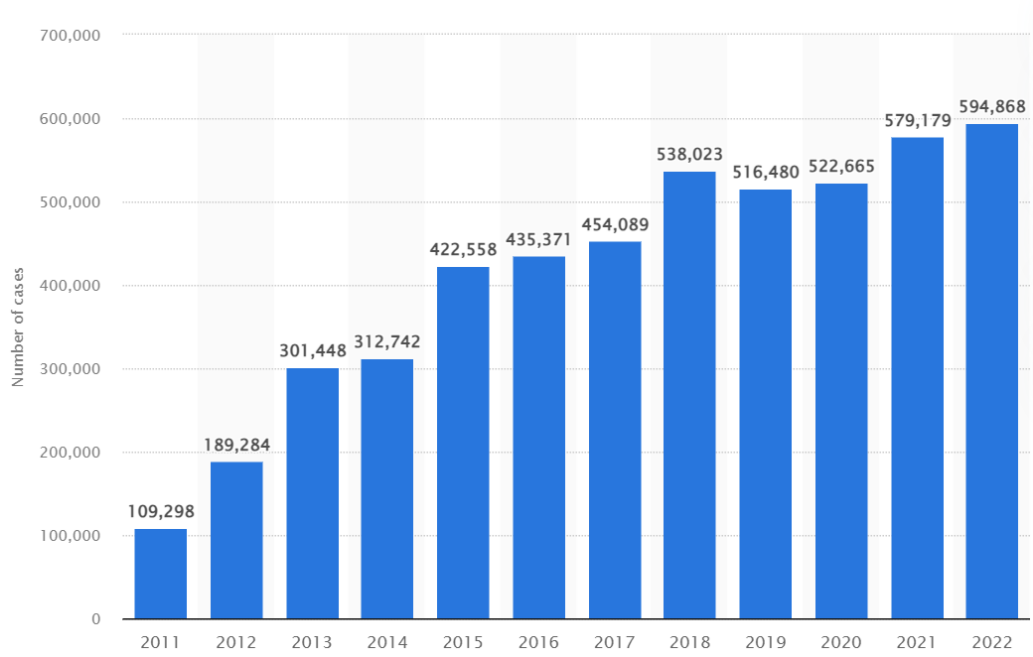

Figure 1. Number of stroke cases in Spain from 2011 to 2023. Extracted from the Spanish Ministry of Health.

Despite these alarming trends, mortality rates attributable to strokes have exhibited a steady decline over the last few decades [6]. Technological advances, evidence-based practices in acute stroke care, together with public awareness campaigns have contributed to this positive trend. Thrombolytic therapies (clot-busting drugs), such as tissue plasminogen activator (tPA) and endovascular mechanical thrombectomy, administered within the critical early hours post-stroke, have significantly reduced both mortality rates and long-term impairment in ischemic stroke, which represents nearly 87% of all cases compared to hemorrhagic strokes [7]. These interventions have revolutionized stroke management, preserving brain function by successfully clearing blood clots obstructing regular brain flow mitigating acute and long-term deficits for stroke survivors [8,9].

However, despite advancements in reducing mortality and impairment, we are still far from a comprehensive "cure" for stroke. This is evident from the substantial proportion of survivors - up to 80% at acute stages and approximately 50% six months post-stroke- who continue to experience disabilities after the injury [10-12]. These deficits often lead to significant challenges in

performing essential daily activities [13,14], profoundly impacting functional independence and severely disrupting the quality of life, not only of those affected but also for their caregivers [15].

Taken together, this body of evidence emphasizes that while stroke may no longer be predominantly fatal, a substantial proportion of survivors continue to suffer its aftereffects. Until more effective treatments become readily available, there is no doubt that rehabilitation and recovery interventions are the inevitable next frontier for minimizing functional disability among stroke survivors [14,16]. To achieve this, however, a comprehensive understanding of how the injured brain operates and its underlying mechanisms is necessary.

Damage, Plasticity, and Stability: The Dynamic Neurobiology of Stroke

The initial impairment and subsequent recovery following stroke result from a complex interplay of pathophysiological, reparative, and adaptive processes triggered by the injury. These processes operate at molecular, cellular, and systems levels, evolving dynamically over time and varying based on factors such as the type, size, and location of the injury. While these mechanisms constitute a continuous process, consensus has identified distinct critical timepoints **(Figure 2)**, each associated with unique neurobiological processes related to recovery of function or lack thereof [17].

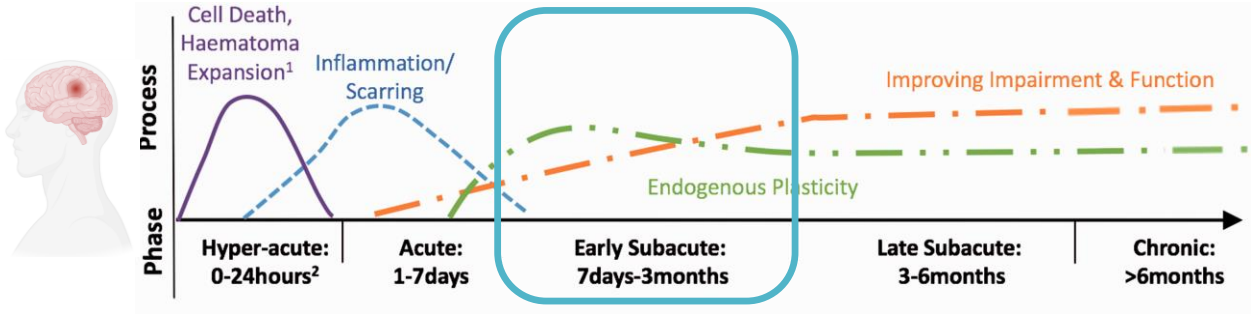

Figure 2. Post-stroke phases. Framework outlining key post-stroke timepoints and their connections to the known biological processes of recovery.

Although the impact of stroke depends on several factors (e.g., size lesion, location, age of the individual) and heterogeneity is a classical feature of stroke research, the first 24 hours to one week post-stroke, known as the hyperacute and acute phases, tend to be characterized by a sudden decrease in function on motor, somatosensory, cognitive or language domains depending on

factors such as lesion size and location. These result from initial neuronal damage caused by disruption in blood flow, and a subsequent cascade of molecular and cellular events overflowing the injured brain within minutes after injury [1]. These processes include excitotoxicity, ionic imbalance, inflammation, and the generation of free radicals, all contributing to progressive cell death [18,19]. These pathophysiological events lead to permanent loss of neurons, microglia, astrocytes, and endothelial cells, affecting the connections and excitability of relevant functionally working neural networks in peri-infarct regions and connected areas [17]. During these acute phases, the brain becomes highly sensitive to further stresses, including systemic infection, elevated temperatures, intense physical activity and/or alterations in neural excitability, any of which can exacerbate excitotoxic responses and worsen the infarct lesion and behavioral outcomes [20-23].

Remarkably, despite the initial severe neurological damage, the human brain possesses an exceptional capacity for self-recovery by transitioning to a repair and adaptation phase. In the subacute phase, which extends from about 7 to 90 days after injury, the brain enters a state of heightened neuroplasticity. During this phase, which is when most functional recovery occurs, surviving neural systems become highly malleable and responsive to experiences [24]. This period of heightened neural malleability is characterized by the upregulation of growth-promoting genes, increased levels of growth factors, dendritic spine turnover, axonal sprouting, alterations in the excitability of neuronal circuits, and reorganization of functional neural networks [25-27]. These transient endogenous repair processes create a "window of neuroplasticity" allowing spontaneous yet incomplete recovery.

After this period, the brain enters into a chronic phase, marked by the stabilization of neuroplasticity and a reduced capacity for spontaneous recovery [17]. Functional improvements during the chronic phase require intense neurorehabilitation therapy and substantial commitment from the patient [28]. Even with these efforts, however, the extent of recovery during this phase appears to be around 10% of what is observed in the early subacute phases [29,30], and it is argued that most of these improvements are mediated by compensatory strategies rather than true recovery [31]. Collectively, this evidence underscores that despite severe neural damage, stroke can open a unique yet short-lived window of plasticity in the adult brain, providing new opportunities for recovery and treatment [32].

“Time is Recovery”

Harnessing the brain's innate neuroplasticity is the basis of poststroke neurorehabilitation. The premise of stroke rehabilitation is built upon the idea that the brain's inherent neuroplasticity can be deliberately manipulated to achieve greater recovery outcomes than those typically observed spontaneously [33]. Animal studies have confirmed that treatment-induced neuroplastic changes are the primary driver of functional recovery gains after stroke [34]. This is promising news for rehabilitation, as it demonstrates that external stimuli in the form of treatment can amplify the spontaneous stroke-induced neuroplastic processes, potentially leading to larger gains in recovery [47]. The main challenge, however, is to identify which type of therapies, dosages and, perhaps most importantly, precise timings by which these interventions can yield the most benefits.

Time is brain [1]. Facial drooping, Arm weakness, Speech difficulties and Time, "F.A.S.T". This widely recognized acronym, utilized both in research and for public awareness campaigns for stroke, emphasizes the rapid and irretrievable loss of human nervous tissue that occurs if not acting fast during stroke, underscoring the urgent need for acute therapeutic treatments. Similarly, once the first phase of damage gradually subsides and reparative processes begin, time remains a critical factor in the recovery process. Indeed, "time is recovery".

The human brain has shown remarkable plasticity in response to experiences, including stroke. However, while neuroplasticity evolves throughout the lifespan, there are specific time periods during which it is particularly active [35]. A series of pioneering studies in animals introduced the concept of critical periods of heightened neuroplasticity by studying how the brain responds to temporary sensory stimuli [36,37]. These studies found that depriving animals of visual stimuli only during developmental periods results in permanent changes in the responsiveness of cortical connections. This is particularly interesting as parallel mechanistic processes have been identified during subacute stages after stroke when more significant improvements from impairment have been attributed to heightened plasticity states [38]. These two contrasting yet mechanistically similar phases in life, growth and injury, ironically illustrate the dynamic nature of the nervous system, highlighting that periods of profound adaptability exist but have limited lifespans. Implementing treatments that modulate neuroplasticity during these sensitive periods could represent a unique opportunity to enhance recovery.

Animal studies have confirmed the existence of a sensitive period within a month after stroke, during which most recovery from impairment occurs, and motor interventions yield larger behavioral gains compared to later, chronic stages [39,40]. This heightened responsiveness to training has been attributed to structural and functional neuroplasticity characterized by the upregulation of growth factors, structural neuronal changes, and background alterations in corticospinal excitability [41]. One of the most relevant studies, led by Corbett and colleagues, demonstrated that exposure to an enriched environment, which encouraged physical and social engagement, resulted in larger gains when provided earlier rather than later after stroke. Animals receiving enriched rehabilitation 5 or 14 but not 30 days after stroke exhibited larger motor recovery gains and enhanced structural plasticity [39]. In humans, while evidence regarding critical recovery periods is limited, a few studies suggest a similar time-sensitive period spanning from the first week to three months post-injury, a critical timepoint termed early-subacute, when almost all functional recovery occurs [42,43] and greater improvements can be achieved in response to rehabilitation treatments [29,44]. It is important to note, however, that this window of recovery, anchored to stroke's onset and location, varies across neural systems and functional domains, leading to different outcome trajectories and response periods for various interventions [17]

Surprisingly, despite the overwhelming evidence from animal models indicating that poststroke neuroplasticity declines as a function of time and that a time-sensitive period exists in the early phases with increased responsiveness to training, most clinical research studies have focused on individuals in the chronic stages. This emphasis on chronic-stage patients mainly results from pragmatic considerations [16]. Patients at chronic stages are typically easier to recruit, while including patients from the acute and subacute stages can disrupt the standard care provided in rehabilitation clinics, requiring close coordination with the stroke medical units. Additionally, conducting additional research experiments and interventions during these early phases can pose challenges, as patients can still be medically unstable and often experience high fatigue levels. Furthermore, individuals at chronic phases tend to have more stable baselines, making it easier to attribute changes in performance to experimental treatments.

Unfortunately, this lack of evidence in individuals during the early subacute periods has translated into clinical practice, where the critical period of spontaneous biological recovery is largely ignored. It has been stated: "In rehabilitation medicine, spontaneous recovery is perceived

as one of the most neglected features of the clinical course of stroke" [45]. This incongruity between experimental evidence and clinical research can have negative repercussions in both research and clinical fields. In the absence of concrete evidence on early stages of recovery, it becomes practically impossible to identify critical periods of recovery in individuals with stroke and, likewise, to determine whether rehabilitative interventions can improve recovery beyond what is observed spontaneously. Consequently, this knowledge gap can significantly impact evidence-based practices within the medical system, potentially leading to suboptimal patient care and outcomes. Investigating treatments with capacity to induce neuroplastic changes during these critical periods is therefore critical to determine the full extent of recovery capacity following stroke [17].

A Promising Intervention for Stroke Recovery?

"The brain is the source of behavior, but in turn, it is modified by the behaviors it produces" [46]. In the context of stroke recovery, a wide range of restorative therapies have been implemented, including motor training, non-invasive brain stimulation, and pharmacological treatments, all aiming to promote neuroplasticity in ways to enhance recovery [47,48]. Motor training interventions have been one of the most used treatments due to their potential to impact both neuroplastic and behavioral recovery outcomes [49]. Abundant evidence demonstrates motor training interventions like constraint-induced movement therapy (CIMT) [50], robot-assisted therapy [51], and intensive upper-limb extremity training [52] result in positive yet somewhat limited (standardized mean difference, SMD: 0.15-0.36) improvements in recovery, likely due to treatment-induced neuroplasticity and cortical reorganization of the central nervous system. Critically, in order to capitalize on the critical period of recovery, it remains an open question what the optimal timing, intensity, amount, and type of motor intervention should be for maximizing recovery [53].

Much of our mechanistic understanding of how the brain responds to motor training originates from animal experimentation in enriched environments. Enriched environments are experimental paradigms designed to enhance sensory, motor, and cognitive stimulation by providing equipment and spaces where animals are continuously challenged. Exposure to enriched environments has been shown to elicit behavioral, cellular, and molecular changes, shedding light on the mechanisms of experience-dependent neuroplasticity [54]. In stroke animal models, exposure

to enriched environments combined with intensive task-specific skill training has led to enhanced motor function recovery [55]. These improvements are accompanied by neuroplasticity changes, including increased dendritic spine density [56] and elevated trophic factors such as brain-derived neurotrophic factor (BDNF) [57], a pivotal protein due to its role in neuronal growth, survival, and plasticity. Beyond sensory and cognitive stimuli, a common denominator of enriched environments is a significant increase in the levels of physical activity. This focus prompted research into the exclusive enhancement of motor activity, an activity that can be more easily controlled and that, at the same time, is analogous to what humans practice and call "exercise."

Physical exercise has gained attention as a promising treatment for stroke recovery. In healthy animals, access to running wheels or forced running on treadmills has been shown to improve memory and motor learning processes while inducing plasticity-like events such as increased synaptic plasticity, excitability, systems of neurogenesis and angiogenesis and upregulation of neurotrophic factors such as BDNF [58,59]. These positive effects on the brain have also been observed in stroke models, where exercise regimes have led to improvements in functional outcomes, such as enhanced coordination, movement integration, and skilled reaching ability, together with underlying neurobiological changes, including reduced lesion size, protection from oxidative damage and inflammation processes, as well as increases in growth factors, cellular metabolism, synaptic and dendritic plasticity, angiogenesis, and neurogenesis [60-62]. In summary, considering the preclinical evidence derived from animal models, exercise appears to be a promising intervention for stroke recovery.

In humans, mounting evidence has established cardiovascular exercise (CE) as one of the most effective "medicines" to reduce cardiovascular disease, all-cause mortality, and cancer-related mortality [63]. Importantly, these benefits can also extend to the nervous system, where CE can protect, maintain, and repair brain function. Regular CE participation and high levels of physical activity can protect the brain against age-related atrophy and memory loss [64], counter degenerative neurological diseases like dementia and Alzheimer's [65], and lower the risk of suffering stroke [66] while also potentially reducing its severity upon admission [67].

For stroke survivors, once medically stable, CE is a recommended practice and core component of stroke rehabilitation [68]. Following stroke, CE has been demonstrated to be a cost-effective and safe intervention with positive effects at multiple levels. Stroke survivors often

experience a significant reduction in cardiorespiratory fitness (CRF), measured by peak oxygen consumption (VO_2peak), falling by about 50% compared to age-matched sedentary individuals [69]. Critically, these values often fall below the threshold necessary for independent living (stroke-adjusted ≈ 19 $mL.Kg^{-1}.min^{-1}$) [70]. Additionally, secondary symptoms, such as fatigue and depression, are prevalent among patients, with prevalence rates ranging from 35% to 90%, contributing to a sedentary lifestyle [71,72]. Cardiovascular training, performed on either cycle ergometers, steppers or treadmill training, has been shown to enhance cardiovascular fitness, walking capacity, reduce symptoms of post-stroke depression [73] and address cognitive and perceptual impairments [74,75], which can affect up to 80% of stroke patients [76]. Notably, CE training could protect the brain from subsequent strokes by reducing cardiovascular disease risk factors, including hypertension, arterial function, and insulin response [68,77,78]. This holds clinical importance, as recurrent strokes occur in approximately ~30% of patients, with 18% of these being fatal [79].

The reality, however, is that despite the myriad of benefits, CE remains underutilized as a therapeutic intervention in the general stroke population, often with intensities below what is required for optimal training effects [80]. This underutilization can be especially detrimental in individuals in subacute stages when severe deconditioning and impairment can hinder optimal participation in rehabilitation activities [81]. Moreover, although there is still much debate about the best timing to introduce CE interventions [82], recent evidence suggests that larger functional gains can be achieved when this is implemented during the subacute phases compared to later stages [83].

In summary, CE has consistently demonstrated positive effects on stroke survivors' functional, cardiovascular, and metabolic outcomes, becoming a core component in most rehabilitation guidelines [84]. Nevertheless, unlike animal models, where molecular and cellular neuronal processes can be invasively studied, our understanding of the effects of CE on the brain in people after stroke is limited [85]. Considering that most spontaneous and treatment-induced recovery is driven by neuroplastic and reparative events occurring within the nervous system, particularly during the early-subacute stages of recovery, it is essential to access the neurobiological responses to CE in people with stroke to quantify its therapeutic potential [86].

Biomarkers: Accessing the Brain's Neuroplasticity

While our understanding of the molecular and cellular mechanisms underlying spontaneous and treatment-induced recovery following stroke has been elucidated through invasive techniques in rodents and non-human primates [87], a critical challenge in the field of stroke recovery and rehabilitation is to gain a deeper understanding of these neurobiology processes in humans [17].Recent advancements in technology and science have brought us closer than ever to the molecular and cellular mechanisms that govern the nervous system in humans. Biomarkers are indicators of disease states that reflect molecular and cellular changes [86,88]. Generally, biomarkers have been categorized into neurophysiological, neuroimaging, and blood biomarkers **(Figure 3)** [86,89,90], each one providing unique insights into molecular, cellular, or system-level events related to the nervous system.

While biomarkers have not yet achieved the level of precision in capturing neuroplastic changes that is attainable in animal studies, their emergence has revolutionized the entire field of neuroscience, as they provide the only means of indirectly accessing neurobiological events in humans through the utilization of less invasive techniques. These recent advancements have marked a turning point in the field of stroke recovery and rehabilitation, where biomarkers have been increasingly integrated to quantify the extent of neurological damage, predict part of initial and long-term recovery outcomes, and measure the neuroplastic responses to rehabilitative therapies [91]. The emergence of biomarkers has enabled researchers to start investigating mechanisms underlying treatments in individuals after stroke, facilitating the measurement of their capacity to induce brain changes that may support recovery [92].

Neurophysiological and blood biomarkers are among the most used biomarkers in stroke research. These measures and their respective techniques have been increasingly used due to their potential to serve as valid surrogates of neural states and neuroplastic changes in individuals after stroke, while at the same time, offering a cost-effective alternative to neuroimaging techniques such as functional magnetic resonance imaging (fMRI) or MRI. In the work presented in this thesis, our focus was on two specific biomarkers, corticospinal excitability (CSE) and BDNF, which respectively reflect central and peripheral biological events associated with neuroplastic changes and post-stroke recovery. We selected these specific biomarkers for two primary reasons.

Firstly, both measures, changes in excitability and BDNF expression, have been associated with recovery improvements in animal models due to their capacity to promote neuroplasticity. And secondly, both biomarkers appear to respond to exercise interventions, potentially reflecting neuroplastic and reparative effects. Therefore, examining how these two biomarkers respond to CE in individuals at subacute stages post-stroke holds the potential to provide valuable mechanistic insights into the potential to promote neuroplasticity and enhance recovery through this intervention.

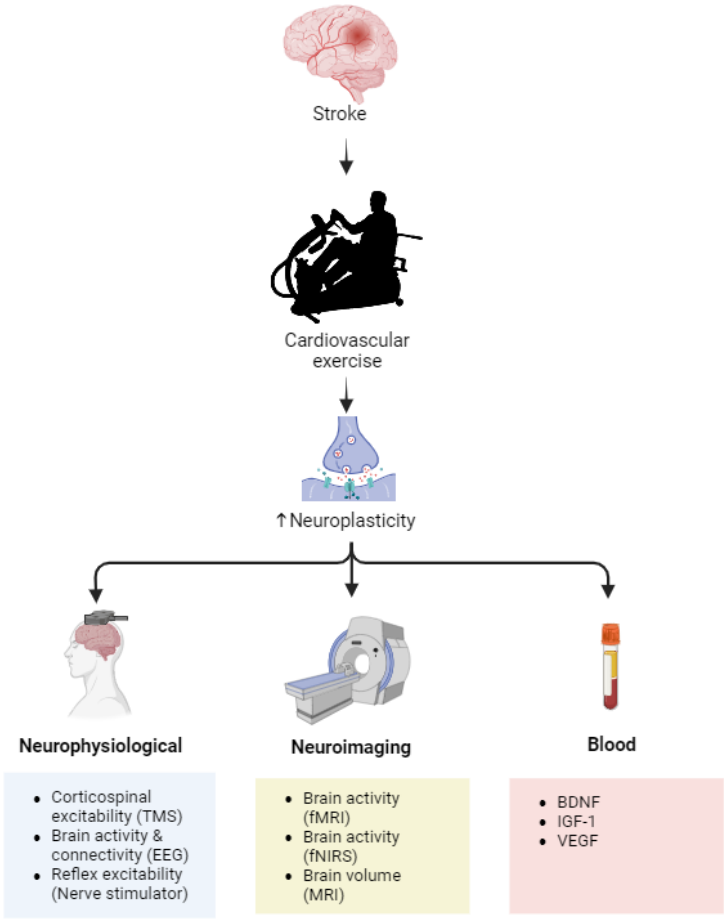

Figure 3. Biomarkers of neuroplasticity in stroke. BDNF, brain-derived neurotrophic factor; EEG, electroencephalography; fMRI, functional magnetic resonance imaging; fNIRS, functional near-infrared spectroscopy; IGF-1, insulin-like growth factor 1; MRI, magnetic resonance imaging; TMS, transcranial magnetic stimulation; VEGF, vascular endothelial growth factor.

The Modulating Role of Genotype

Stroke recovery and the adaptive response to rehabilitation are highly variable among individuals. While factors such as stroke type, location, and the type and intensity of rehabilitation play a significant role in determining recovery outcomes [93,94], the substantial heterogeneity observed in long-term outcomes, even among patients with similar initial stroke severities, suggests the existence of individual mechanisms related to neural repair and neuroplasticity processes influencing spontaneous and treatment-induced recovery trajectories.

Genetic factors have been receiving increased attention in stroke recovery due to their ability to modulate brain function, repair mechanisms, and influence the overall recovery process [95]. Molecular, cellular, and physiological events following brain ischemia, particularly those amplified during the acute and subacute periods, are mediated by genetic factors [26]. Several of these genes regulate growth-associated processes crucial for recovery, such as axonal sprouting, dendritic spine formation, and the formation of new connections within the brain [38]. Some genetic variants, known as single nucleotide polymorphisms (SNP), have been shown to influence these programs, thereby affecting neuroplastic processes and accounting for previously unexplained variations in recovery [96].

Emerging evidence, particularly concerning candidate SNPs related to BDNF, dopamine, and apolipoprotein E, suggests differential effects on the behavioral outcomes of stroke recovery, most likely through their influence on underlying neuroplasticity mechanisms [97]. One of the most studied SNPs in stroke recovery is the Val66Met variant in the BDNF gene, known for its role in regulating the activity-dependent secretion of BDNF protein [98]. Individuals carrying this SNP have shown altered TMS-measured excitability and brain activity patterns, often resulting in reduced motor recovery outcomes [99-102]. Similarly, other SNPs in genes like catechol-o-methyltransferase (COMT Met/Met) and APOE (ApoE ε4) have also been shown to impact neural repair and neuroplasticity, typically associated with diminished stroke recovery [101,103,104]. Various other genetic polymorphisms have demonstrated indirect involvement in stroke recovery by influencing neuroplasticity processes [105]. Notably, the emergence of genome-wide association studies (GWAS), which allow testing hundreds of thousands of SNPs simultaneously in an unbiased and

agnostic fashion, have been very successful and offer a promising approach to providing new genetic variants associated with neuroplasticity and functional recovery [106].

Environmental experiences, such as motor training and physical exercise, have been shown to alter genetic expression following ischemic events. In parallel, specific genetic variants can impact the neurobiological systems underlying the response to treatment [49,107]. Since rehabilitation aims to target molecular and cellular processes post-stroke, genetic variations could contribute to differential responses to treatment by altering these underlying mechanisms [108]. While the current evidence is somewhat limited and findings are equivocal [109-111], it has been proposed that genetic variants associated with neuroplasticity and repair, such as BDNF Val66Met or ApoE ε4, may influence individual responses to motor training after stroke [112].

In neurotypical populations, genetic variants, such as the BDNF polymorphism, have been examined as potential modulators of cognitive function in response to CE and physical activity, yielding inconsistent results [113]. However, despite the suggestive evidence [114], no studies have yet explored the role of this genotype in modulating neuroplasticity mechanisms in response to CE in stroke individuals. Given the considerable variability observed in recovery and response to treatments, investigating the role of genetics on the effects of CE on neuroplasticity biomarkers could pave the way for identifying those patients more likely to benefit from such an intervention, and in turn, facilitate the design of more individualized rehabilitation strategies.

The Rationale of this Thesis

Given its capacity to modulate neuroplasticity-like mechanisms that parallel recovery from ischemic damage, CE holds promise as a rehabilitative treatment following stroke. Growing evidence from experimental studies highlights the presence of a critical window for neuroplasticity and recovery after stroke. However, there remains a gap in understanding the neuroplastic effects of CE when implemented in individuals during the early-subacute stages of recovery. Additionally, it is still unclear whether the physiological and neurophysiological adaptations potentially induced through non-task-specific CE alone could translate to recovery gains in humans. Since early subacute stages are characterized by heightened endogenous neuroplasticity and increased responsiveness to interventions, investigating the impact of CE during this critical period would be crucial to determine its potential reparative capacity.

Despite the robust body of evidence linking CSE and BDNF biomarkers with neuroplasticity, stroke recovery, and their responsiveness to CE interventions, no studies have yet investigated their response to CE in subacute stroke populations. Additionally, some patients appear to respond more favorably to rehabilitation than others, suggesting a possible influence of genetic factors on modulating neuroplastic changes in response to CE. The studies of this thesis are the first to combine non-invasive brain stimulation, laboratory assay, and genotyping techniques to investigate the interplay between CE, neuroplasticity, and genotype in subacute stroke. By determining how CE influences neuroplasticity during the early poststroke phases and whether genotype impacts the response to this intervention, this study could provide valuable insights into determining its reparative capacity. Specifically, the following thesis attempts to answer the following three research questions presented as three manuscript chapters:

- What is the current evidence for the use of neuroplasticity biomarkers in response to CE in individuals after stroke?
- Among adults with subacute stroke, what are the effects of CE on central and peripheral biomarkers of neuroplasticity, namely CSE and BDNF?
- Does the presence of the BDNF Val66Met polymorphism modulate the response of these biomarkers to CE in these individuals?

Addressing these three key questions will allow us to determine the efficacy of biomarkers in capturing neuroplastic changes in individuals with stroke following CE and whether this intervention can effectively promote neuroplasticity during the critical therapeutic subacute window. Additionally, investigating the potential impact of genotype will help us begin to unravel the heterogeneous responses to CE in relation to neuroplasticity and recovery outcomes.

Chapter 1: Measuring Neuroplasticity in Response to Cardiovascular Exercise in People with Stroke: A Critical Perspective

Running title: Biomarkers of Exercise in Stroke

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Abstract

Background: Rehabilitative treatments that promote neuroplasticity are believed to improve recovery after stroke. Animal studies have shown that cardiovascular exercise promotes neuroplasticity but the effects of this intervention on the human brain and its implications for the functional recovery of patients remain unclear. The use of biomarkers has enabled the assessment of cellular and molecular events that occur in the central nervous system after brain injury. Some of these biomarkers have proven to be particularly valuable for the diagnosis of severity, prognosis of recovery, as well as for measuring the neuroplastic response to different treatments after stroke. **Objectives**: To provide a critical analysis on the current evidence supporting the use of neurophysiological, neuroimaging, and blood biomarkers to assess the neuroplastic response to cardiovascular exercise in individuals poststroke. **Results**: Most biomarkers used are responsive to the effects of acute and chronic cardiovascular exercise interventions, but the response appears to be variable and is not consistently associated with functional improvements. Small sample sizes, methodological variability, incomplete information regarding patient's characteristics, inadequate standardization of training parameters, and lack of reporting of associations with functional outcomes preclude the quantification of the neuroplastic effects of cardiovascular exercise poststroke using biomarkers. **Conclusion**: Consensus on the optimal biomarkers to monitor the neuroplastic response to cardiovascular exercise is currently lacking. By addressing critical methodological issues, future studies could advance our understanding of the use of biomarkers to measure the impact of cardiovascular exercise on neuroplasticity and functional recovery in patients with stroke.

Keywords: stroke, cardiovascular exercise, neuroplasticity, biomarkers, recovery

Introduction

Neuroplasticity can be broadly defined as the capacity of the nervous system to adapt and, more specifically, as the functional and structural changes that occur in the nervous system in response to intrinsic and extrinsic stimuli. Following stroke, the adult brain demonstrates a remarkable capacity to repair itself by undergoing plasticity of the surviving neural systems, a state of neural malleability that contributes to recovery and forms the basis for rehabilitation [1]. Potentiating this neuroplastic capacity of the nervous system to adapt is a primary goal of poststroke neurorehabilitation.

Therapeutic interventions that facilitate neuroplasticity are thought to improve functional recovery after stroke. Neurorehabilitation and pharmacological interventions amplify neuroplasticity beyond spontaneous neurological recovery, resulting in larger improvements across multiple functional domains, including motor, somatosensory, cognitive, and language recovery [2]. However, unlike animal experiments, where molecular and cellular processes can be studied invasively, in humans, we are still far from understanding the specific neurobiological mechanisms that underpin stroke recovery [3].

Biomarkers, which are indicators of disease state that reflect molecular and cellular changes [4], can be categorized as neurophysiological, neuroimaging, and blood biomarkers (**Figures 1-3; Box 1-3**) [4]. The study of biomarkers is an emerging area in the field of stroke recovery and rehabilitation because they can quantify not only the extent of the neurological damage and thus help predict long-term recovery outcomes but also enable the measurement of neurobiological events in response to rehabilitative therapies, providing, in turn, mechanistic insights about their potential to promote neuroplasticity and brain repair [5].

Cardiovascular exercise (CE), defined as any physical rhythmic activity maintained continuously that involves large muscle groups and targets the cardiorespiratory system, has demonstrated to be a safe and effective intervention to improve walking capacity, cardiovascular health, and quality of life in people with stroke [6]. Given its potential to also mitigate the risk of stroke recurrence, and simultaneously improve cardiorespiratory, metabolic, and neural recovery targets, CE is recommended as a core component of stroke rehabilitation [7]. Evidence from animal studies supports the beneficial effects of CE on brain recovery via multiple mechanisms, including

reductions in the size of the lesion, oxidative damage, inflammation, as well as increases in growth factors, cellular metabolism, synaptic and dendritic plasticity, angiogenesis, and neurogenesis [8].

Cardiovascular exercise can protect and maintain brain function by promoting changes in the nervous system [9]. However, its effects on neuroplasticity and brain recovery in individuals after stroke remain largely unknown [8]. This could be, in part, because of the inadequate use of biomarkers capturing the neuroplastic response to this intervention. Without a better knowledge of the effects of CE on neuroplasticity, there is little chance of objectively quantifying the potential restorative capacity of this intervention, limiting, in turn, its evidence-based implementation to maximize stroke recovery [4].

The aim of this point of view manuscript is to present a critical analysis of the biomarkers currently utilized to assess neuroplasticity in CE studies in people after stroke. Additionally, we provide context to these findings within the current state-of-the-art use of biomarkers in stroke recovery, identify gaps in knowledge and propose directions to guide future studies. A systematic search in accordance with PRISMA guidelines was conducted to identify relevant studies. Details on the methodology used and the main results of the search are provided as supplementary material.

Neurophysiological Biomarkers

- **Corticospinal Excitability**

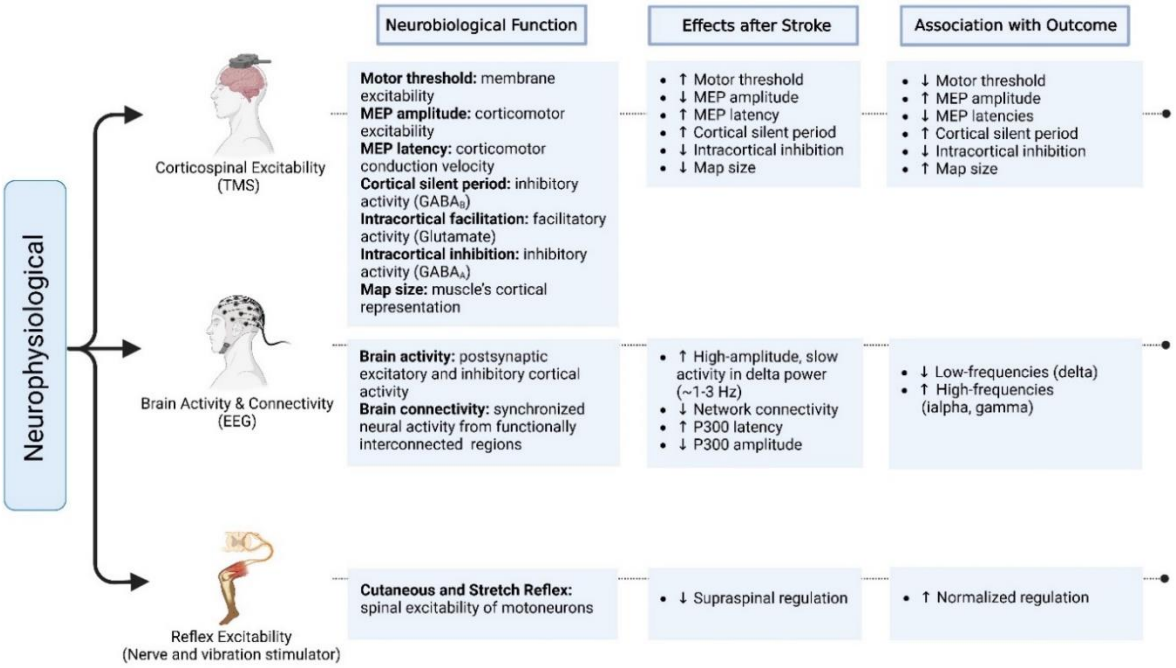

Figure 1. Neurophysiological biomarkers in stroke recovery

Box 1 | **Neurophysiological biomarkers in stroke recovery**

Corticospinal excitability: Transcranial magnetic stimulation (TMS) applied over the primary motor cortex provides different measures of corticospinal excitability (CSE) such as motor thresholds, amplitudes, and latencies of motor evoked potentials (MEP). TMS can also provide measures of facilitation and inhibition such as intracortical facilitation, intracortical inhibition, and the cortical silent period (CSP), which have been associated with glutamate, $GABA_A$, and $GABA_B$ neurotransmitter systems, respectively. In addition, TMS allows for the assessment of dynamic changes in the representations of peripheral muscles on the cortex (i.e., cortical maps). Although CSE evolves with stroke recovery, and inter-individual variability can be significant, CSE alterations, especially in the ipsilesional hemisphere have been consistently reported early after stroke. Abnormal CSE is usually manifested as increased motor thresholds, reduced MEP amplitudes as well as increased MEP latencies and longer CSPs. In the ipsilesional hemisphere, reductions in intracortical inhibition and cortical map sizes have also been observed. Functional improvements have been associated with the restoration of interhemispheric balance via increased CSE in the ipsilesional hemisphere. Reductions in motor thresholds, increased MEP amplitudes, shorter MEP latencies, longer CSPs, decreased intracortical inhibition, as well as increases in the cortical map size of the ipsilesional hemisphere, have been related to improved functional outcome.

Brain activity and connectivity: Electroencephalography (EEG) can record continuous cortical oscillations reflecting postsynaptic excitatory and inhibitory activity. EEG biomarkers include parameters (e.g., latency, amplitude) of event related potentials (ERPs) marking time-locked changes in brain activity associated with sensory and cognitive processes. EEG can also capture patterns of neural communication and synchronization between functionally interconnected regions (e.g., brain connectivity). Early poststroke, there is a rapid emergence of high amplitude, slow activity in the delta power range (1-3 Hz) within the affected territories, along with diffused network connectivity and disruptions in P300 latency and amplitude. Decreases of power in low-frequencies and increases in high-frequencies have been associated with improved recovery outcomes.

Reflex excitability: Peripheral nerve and vibration stimulation can be used to study the characteristics of reflex excitability in spinal motoneurons. A stroke can interrupt the normal connectivity between supraspinal and spinal centers, reducing motor input and altering reflex excitability. Normalized modulation of reflex excitability between paretic and non-paretic sides has been reported alongside improvements in motor function.

Obtained via single or paired-pulse transcranial magnetic stimulation (TMS) protocols, different corticospinal excitability (CSE) measures can capture multiple excitability and connectivity alterations in cortico-cortical and cortico-spinal pathways after a stroke (**Figure 1; Box 1**). The acute and chronic responses to CE in terms of changes in CSE are examined with a single and multiple training sessions, respectively. Eight acute [10-17] and three chronic [18-20] studies used TMS to assess different markers of CSE in response to CE (**Table 1**). Seven out of eight acute studies reported significant changes following a single bout of CE. One study showed that five minutes of exercise at vigorous intensity increased the amplitude of the resting motor evoked potential (MEP) on the ipsilesional hemisphere [10]. Two studies investigated acute CSE changes following a graded exercise test [11,16], with only one study showing significant changes via decreases in short-interval intracortical inhibition on the ipsilesional hemisphere leading to a reduction of excitability imbalances between hemispheres [16].

Two acute studies investigated the impact of CE intensity on CSE by comparing the effects of a single bout of moderate-intensity continuous training (MICT) to high-intensity interval training (HIIT) [11,12]. In both studies, HIIT evoked larger changes in CSE than MICT on the ipsilesional hemisphere that manifested in reductions of motor threshold during a low force isometric muscle contraction (i.e., active motor threshold) [11] and increases in MEP latency [12]. In one of the studies, the association between the acute CSE responses to MICT and HIIT with upper limb function measured with the box and blocks test and handgrip maximal voluntary contraction was examined, with no significant results [12]. This result reinforces the complex functional relationship between CSE and motor function, and the importance of interpreting the information provided by MEPs with much caution [21].

Four acute studies explored the priming effects of non-invasive brain stimulation facilitatory protocols in combination with a single bout of CE on CSE [13-15,17]. While no significant effects were observed following exercise alone, one study showed significant increases in the amplitudes of active MEPs on the ipsilesional side and reductions on the contralesional side when anodal transcranial direct current stimulation (tDCS) was applied prior to HIIT [13]. A second study showed that, compared to anodal tDCS applied alone or during exercise, a single bout of light-intensity cycling exercise alone exhibited greater reductions in the amplitudes of active MEPs on the ipsilesional hemisphere [14]. Finally, two studies applied intermittent theta burst stimulation

(iTBS) delivered through TMS following CE [15,17], with only one showing significant priming effects on CSE on the contralesional hemisphere [17].

Two chronic studies found increases in CSE following four weeks of treadmill training via reductions in the resting motor threshold on the ipsilesional [18] and contralesional [19] hemisphere. The same studies demonstrated increases in the size of the cortical map bilaterally [19] and ipsilesionally [18]. One of these studies showed a significant association between reductions in the resting motor threshold and increases in the map size on the contralesional hemisphere with improvements in balance and step length, respectively [19]. Another study failed to show any significant effect on CSE after four weeks of MICT treadmill provided alone or following other interventions including movement-based priming and anodal tDCS [20].

In summary, CE appears to modulate some aspects of CSE in people with stroke, but the effects vary substantially among studies. Changes in CSE, which are more often detected in the ipsilesional hemisphere, include increases in the size of the cortical map, longer MEP latencies and greater amplitudes, as well as reductions in resting motor thresholds and interhemispheric imbalances. In neurotypical individuals, a single vigorous bout of CE has shown to elicit acute increases in CSE [22]. In individuals after stroke, exercise intensity could modulate the CSE in response to acute CE, with higher intensities evoking more pronounced CSE changes on the ipsilesional hemisphere. Whether both acute and chronic CE can augment the priming effects of tDCS or iTBS on CSE is unclear. Furthermore, while most CSE changes reported here have been previously associated with recovery improvements (**Figure 1; Box 1**), whether CE can modulate CSE in patients at earlier stages of recovery and to what extent these changes are associated with functional improvement is yet to be determined [21].

- **Brain Activity and Connectivity**

Brain activity and connectivity recorded with electroencephalography (EEG) can capture brain oscillations reflecting postsynaptic excitation and inhibition, both of which have been used in the diagnosis of cerebral ischemia, outcome prediction, and treatment response after stroke (**Figure 1; Box 1**). Three studies used EEG to examine the effects of CE on brain activity and connectivity poststroke [23-25] (**Table 1**). In one study, acute changes in brain activity were examined while performing a modified Eriksen Flanker executive function task following a single bout of

stepping exercise performed at moderate intensity [23]. Exercise evoked shorter latencies and increased amplitudes in the P300 wave measured from the ipsilesional hemisphere. Increases in amplitude and reduced latencies in P300 have been associated with better cognitive performance. Given that cognitive and perceptual impairments along with disruptions in the P300 wave are prevalent after stroke [26], this waveform could provide insights into exercise-induced cognitive improvements in this population. The chronic changes in brain activity were examined in a similar study using the same stimulus-evoked paradigm to study cortical inhibition (N200) and facilitation (P300) over the frontal cortical region following four weeks of treadmill and overground walking [25]. While no significant changes in cortical activity were evoked with CE, a positive association between cortical (N200 latency) and behavioural (response inhibition in Flanker task) inhibitory indices was found after the intervention, suggesting a link between cortical processing and cognitive inhibitory responses.

The other study that employed EEG investigated chronic changes in cortico-cortical and cortico-muscular connectivity (i.e., coherence) by comparing regular treadmill with turning-based treadmill training, which requires patients to walk along a rotating circular belt, making them continually turn rather than walk straight [24]. Compared to a conventional treadmill walking, four weeks of a turning-based treadmill intervention led to significant increases in cortico-cortical and cortico-muscular coherence in gamma power frequencies (23-40 Hz) over the frontal-central-parietal areas of the brain. Importantly, increases in brain connectivity correlated with improvements in gait symmetry only after turning-based training, suggesting that adding more cognitively challenging motor actions during walking may enhance functionally relevant brain connectivity. These findings align well with the hypothesis that increased connectivity in high-band frequencies may signal motor improvements following rehabilitation [27].

Taken together, the results of these three EEG studies reinforce the potential use of this technique to measure brain activity and connectivity as biomarkers to detect changes in cortical function in response to CE. Cardiovascular exercise has been shown to induce acute increases in brain activity among neurotypical individuals, increasing the amplitude in the P300 waveform [28] and enhancing power in both low and high-band frequencies [29]. Nevertheless, in stroke, more studies are needed to identify which specific EEG patterns provide the most sensitive measures to

monitor the effects of CE on brain activity and connectivity and their associations with functional recovery.

- **Reflex Excitability**

Reflexes evoked through both nerve and vibration stimulation can characterize mechanisms associated with neurophysiological integrity and neuroplasticity in both healthy individuals and neurological patients (**Figure 1; Box 1**). Following stroke, spinal networks tend to be relatively preserved, however, disruption in descending motor commands from the brain to the spine can negatively impact rhythmic motor limb activities such as walking or cycling [30]. Normalized modulation of reflex excitability accompanies improvements in motor function following motor training interventions in individuals after stroke [31].

Three studies examined the chronic effects of CE on cutaneous [32-34] and stretch [32,33] reflex excitability (**Table 1**). Reflexes were measured by capturing the response to nerve and vibration stimulation before and after five weeks of light-intensity CE while performing rhythmic motor tasks such as walking or arm and leg cycling. Upper-limb ergometry training induced a significant inhibition of cutaneous reflexes on the paretic side, resulting in more normalized amplitudes between the paretic and non-paretic sides [32]. Similarly, following five weeks of upper and lower limb ergometry, two studies reported enhanced bilateral symmetry of cutaneous reflexes that were driven by increases in excitability in the paretic side and decreases in the non-paretic side [33,34]. Significant training-induced effects were also observed in stretch reflexes, where reductions on the paretic side led to more normalized patterns of excitability [32,33].

In summary, these results provide preliminary evidence supporting the use of reflex excitability as a potential biomarker to assess neurophysiological changes following CE training in individuals poststroke. In non-neurological populations, changes in reflex excitability, measured with the H-reflex, have been reported following acute bouts of CE [35]. The studies reviewed revealed that CE training modulates both cutaneous and stretch reflexes, suggesting that this type of exercise can help restore the imbalances in reflex excitability typically observed between affected and unaffected limbs after stroke and promote neural activity patterns similar to those observed in neurotypical individuals [31]. However, whether these changes in reflex excitability can be used to predict improvements in functional recovery and thus guide clinical practice has yet to be demonstrated.

Table 1. Main characteristics of the studies including neurophysiological biomarkers.

First author, Year (Design)	Demographics	Study Arms	Intervention	Technique	Biomarkers (outcomes)	Main Findings
Abraha, 2018 (Within-subjects study)	**N=** 12 **Sex (M/F)=** 10/2 **Age=** 62.5±9 **Stroke Type (I/H)=** 11/1 **Time poststroke=** Chronic	HIIT MICT	**Mode:** Recumbent stepper **Duration:** Acute **Intensity:** MICT=Moderate; HIIT=Vigorous **Time:** 25 min	TMS (IH, CH)	CSE (Resting motor threshold, Resting MEP amplitude, MEP latency, Intracortical facilitation, Intracortical inhibition)	Compared to MICT condition, the HIIT condition exhibited significant lengthening of MEP latencies on the IH.
Boyne, 2019 (Within-subjects study)	**N=** 16 **Sex (M/F)=** 9/7 **Age=** 57.4±9.7 **Stroke Type (I/H)=** 12/4 **Time poststroke=** Chronic	GXT-treadmill HIIT-treadmill HIIT- recumbent stepper MICT-treadmill	**Mode:** Treadmill; Recumbent stepper **Duration:** Acute **Intensity:** MICT treadmill=Moderate; HIIT treadmill=Vigorous; HIIT stepper=Vigorous **Time:** 25 min	TMS (IH)	CSE (Active motor threshold, Cortical silent period)	Following GXT, no significant CSE changes were observed. Compared to MICT-treadmill, the HIIT-treadmill condition showed significant decreases in active motor threshold on the IH.
Hill, 2023 (Randomized trial)	**N=** 33 **Sex (M/F)=** 20/13 **Age=** 63.82±10.2 **Stroke Type (I/H)=** NR **Time poststroke=** Chronic	Exercise+iTBS Rest+iTBS	**Mode:** Recumbent cycle ergometer **Duration:** Acute **Intensity:** Moderate **Time:** 20 min	TMS (CH)	CSE (Resting MEP Amplitude)	Compared to Rest+iTBS, exercise+iTBS showed significant increases in resting MEP amplitude on the CH.
Li, 2019 (Within-subjects study)	**N=** 13 **Sex (M/F)=** 11/2 **Age=** 65.77±7.2 **Stroke Type (I/H)=** 12/1 **Time poststroke=** Chronic	Exercise Rest	**Mode:** Treadmill **Duration:** Acute **Intensity:** Vigorous **Time:** 5 min	TMS (IH, CH)	CSE (Resting MEP amplitude, Intracortical inhibition)	Compared to the rest condition, exercise showed significant increases in resting MEP amplitude on the IH.
Madhavan, 2016 (Within-subjects study)	**N=** 11 **Sex (M/F)=** 4/7 **Age=** 58±3.31 **Stroke Type (I/H)=** 7/4 **Time poststroke=** Chronic	HIIT HIIT+tDCS	**Mode:** Treadmill **Duration:** Acute **Intensity:** Moderate **Time:** 40 min	TMS (IH, CH)	CSE (Active MEP amplitude)	No changes in active MEP amplitude were reported following HIIT alone, while HIIT+tDCS showed significant pre-post increases in the IH and decreases on the CH.
Murdoch, 2016 (Within-subjects study)	**N=** 12 **Sex (M/F)=** 8/4 **Age=** 65.3±7.8 **Stroke Type (I/H)=** NR **Time poststroke=** Chronic	Exercise Exercise+iTBS Rest+iTBS	**Mode:** Recumbent cycle ergometer **Duration:** Acute **Intensity:** Moderate **Time:** 30 min	TMS (IH)	CSE (Resting MEP amplitude, Intracortical inhibition)	Compared to Rest+iTBS, no significant changes in any CSE markers were reported following exercise alone or in combination with iTBS.
Nepveu, 2017 (Pre-Post)	**N=** 22 **Sex (M/F)=** 16/6 **Age=** 64.85±11.45 **Stroke Type (I/H)=** 15/7 **Time poststroke=** Chronic	GXT	**Mode:** Recumbent stepper **Duration:** Acute **Intensity:** Vigorous **Time:** 9.85 ± 4.75 min	TMS (IH, CH)	CSE (Resting and active MEP amplitude, Intracortical facilitation, Intracortical inhibition, Cortical silent period)	Following a GXT, pre-post reductions of intracortical inhibition were exhibited on the IH, resulting in significant improvements in interhemispheric balance.
Sivaramakrishnan, 2020	**N=** 26 **Sex (M/F)=** 21/5	Exercise	**Mode:** Recumbent cycle ergometer	TMS (IH, CH)	CSE (Active MEP amplitude, Intracortical	Compared to tDCS alone, exercise+tDCS did not exhibit

(Within-subjects study)	**Age=** 60.2±7 **Stroke Type (I/H)=** 18/8 **Time poststroke=** Chronic	Exercise+tDCS tDCS	**Duration:** Acute **Intensity:** Light **Time:** 25 min		inhibition, Cortical silent period)	significant CSE changes. Exercise alone showed significant reductions in active MEP amplitude on the IH compared to both tDCS and exercise+tDCS conditions.
Madhavan, 2020 (Randomized trial)	**N=** 81 **Sex (M/F)=** 55/26 **Age=** 58.75±9.75 **Stroke Type (I/H)=** 53/28 **Time poststroke=** Chronic	HIIT HIIT+movement priming HIIT+tDCS HIIT+tDCS+movement priming	**Mode:** Treadmill **Duration:** 4 weeks **Frequency:** 3/week **Intensity:** Moderate **Time:** 40 min	TMS (IH, CH)	CSE (Active motor threshold, Active MEP amplitude)	No significant changes were shown in CSE following any exercise conditions.
Yen, 2008 (Randomized trial)	**N=** 14 **Sex (M/F)=** 9/5 **Age=** 56.67±14.56 **Stroke Type (I/H)=** 9/5 **Time poststroke=** Chronic	Exercise+standard care Standard care	**Mode:** BWSTT **Duration:** 4 weeks **Frequency:** 3/week **Intensity:** BWS and speed increased according to patient's improvement **Time:** 30 min	TMS (IH, CH)	CSE (Resting motor threshold, map size)	Compared to standard care, the exercise group showed significant decreases in resting motor threshold on the CH. Significant pre-post increases in map size were observed bilaterally in the exercise group, while no changes were observed in the standard care group.
Yang, 2010 (Randomized trial)	**N=** 18 **Sex (M/F)=** 10/8 **Age=** 56.05±3.72 **Stroke Type (I/H)=** 9/9 **Time poststroke=** Chronic, late subacute	Exercise+standard care Standard care	**Mode:** BWSTT **Duration:** 4 weeks **Frequency:** 3/week **Intensity:** BWS and speed increased according to patient's improvement **Time:** 30 min	TMS (IH)	CSE (Resting motor threshold, map size)	Compared to standard care, the late sub-acute group exhibited significant decreases in resting motor threshold on the IH following exercise. Both chronic and late subacute groups showed significant increases in map size on the IH following exercise compared to standard care.
Swatridge, 2017 (Within-subjects study)	**N=** 9 **Sex (M/F)=** 6/3 **Age=** 57.8±11.4 **Stroke Type (I/H)=** 6/1/2NR **Time poststroke=** Chronic	Exercise Rest	**Mode:** Recumbent stepper **Duration:** Acute **Intensity:** Moderate **Time:** 20 min	EEG	Brain Activity (P300 event-related potential: latency and amplitude)	Compared to the resting condition, exercise exhibited significant shorter P300 latencies and greater amplitudes 20 and 40 minutes post-exercise, respectively.
Chen, 2019 (Randomized trial)	**N=** 18 **Sex (M/F)=** 17/1 **Age=** 52.5±9.63 **Stroke Type (I/H)=** 9/9 **Time poststroke=** Chronic	Turning-based treadmill Regular treadmill	**Mode:** Turning-based treadmill; regular treadmill **Duration:** 4 weeks **Frequency:** 3/week **Intensity:** speed increased 0.05 m/s every 5 min **Time:** 30 min	EEG	Brain Activity (Cortico-cortical and cortico-muscular connectivity)	Compared to regular treadmill, turning-based treadmill showed significant increases in cortico-cortical and cortico-muscular connectivity in gamma power frequencies.
Palmer, 2023 (Pre-Post)	**N=** 12 **Sex (M/F)=** 8/4 **Age=** 61 ± 11 **Stroke Type (I/H)=** 7/5 **Time poststroke=** Chronic	Exercise	**Mode:** Treadmill and overground walking **Duration:** 4 weeks	EEG	Brain Activity (N200 & P300 event-related potential: latency and amplitude)	No significant changes were shown in N200 or P300 following exercise training.

			Intensity: MICT=Moderate; HIIT=Vigorous **Time:** 40 min			
Kaupp, 2018 (Time series)	**N=** 19 **Sex (M/F)=** 11/8 **Age=** 73±8.89 **Stroke Type (I/H)=** NR **Time poststroke=** Chronic	Exercise Multiple baseline control	**Mode:** Upper limb ergometer **Duration:** 5 weeks **Frequency:** 3/week **Intensity:** Light **Time:** 30 min	Nerve and vibration stimulator	Reflex excitability (Cutaneous and stretch reflex)	Compared to an initial control phase, exercise exhibited significant reductions in cutaneous and stretch reflexes on the paretic side, resulting in improved bilateral normalization.
Klarner, 2016 (Time series)	**N=** 19 **Sex (M/F)=** 14/5 **Age=** 67.6±11.21 **Stroke Type (I/H)=** NR **Time poststroke=** Chronic	Exercise Multiple baseline control	**Mode:** Upper&Lower limb cycle ergometer **Duration:** 5 weeks **Frequency:** 3/week **Intensity:** Light **Time:** 30 min	Nerve and vibration stimulator	Reflex excitability (Cutaneous and stretch reflex)	Compared to an initial control phase, exercise exhibited significant increases and decreases in cutaneous reflexes on paretic and non-paretic sides, respectively, resulting in improved bilateral normalization. Reductions of stretch reflexes on the paretic side were observed following training.
Klarner, 2016 (Time series)	**N=** 19 **Sex (M/F)=** 14/5 **Age=** 67.6±11.21 **Stroke Type (I/H)=** NR **Time poststroke=** Chronic	Exercise Multiple baseline control	**Mode:** Upper&Lower limb cycle ergometer **Duration:** 5 weeks **Frequency:** 3/week **Intensity:** Light **Time:** 30 min	Nerve stimulator	Reflex excitability (Cutaneous reflex)	Compared to an initial control phase, exercise exhibited significant increases and decreases in cutaneous reflexes on paretic and non-paretic sides, respectively, resulting in improved bilateral normalization.

Abbreviations: BWSTT, body-weight supported treadmill training; CH, contralesional hemisphere; CSE, corticospinal excitability; EEG, electroencephalography; F, female; GXT, graded exercise test; H, hemorrhagic; HIIT, highintensity interval training; I, ischemic; IH, ipsilesional hemisphere; iTBS, intermittent theta burst stimulation; M, male; MEP, motor evoked potential; MICT, moderate-intensity continuous training; N, number of subjects; NR, not reported; tDCS, transcranial direct-current stimulation; TMS, transcranial magnetic stimulation.

Neuroimaging Biomarkers

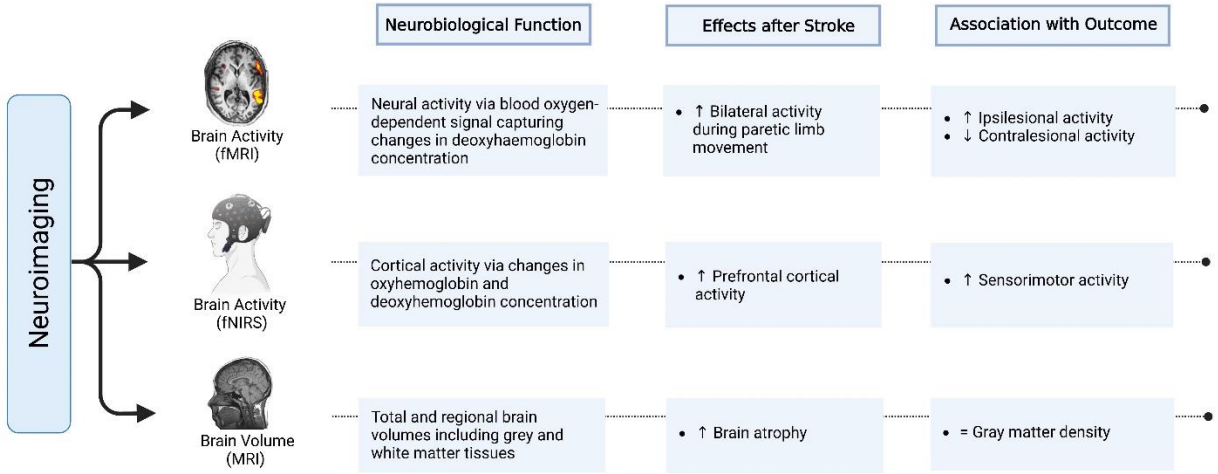

Figure 2. Neuroimaging biomarkers in stroke recovery.

Box 2 | **Neuroimaging biomarkers in stroke recovery**

Brain activity: Functional magnetic resonance imaging (fMRI) infers changes in neural activity through changes in blood oxygen-level-dependent (BOLD) signal. A stroke can lead to abnormal fMRI-captured brain activity patterns such as enhanced bilateral activity during paretic arm movement. Improved post-stroke outcome has been associated with normalized patters of fMRI-measured brain activity via ipsilesional increases and contralesional decreases.

Functional near-infrared spectroscopy: (fNIRS) can also be used to assess brain activity. fNIRS captures alterations in oxyhemoglobin and deoxyhemoglobin concentration at a cortical level. After stroke, overactivation of prefrontal areas has been reported while performing motor tasks and activity increases in sensorimotor regions has been observed in conjunction with improvements in functional recovery.

Brain volume: Structural magnetic resonance imaging (MRI) can be used to assess the volume of the brain and its different structures. Structural MRI can also provide detailed information about the density of gray and white matter areas of the brain and spinal cord. Accelerated rates of brain atrophy have been reported after stroke. Preserved gray matter density has been associated with reduced motor deficits and greater response to rehabilitation.

- **Brain Activity**

Besides EEG, brain activity can also be assessed using functional neuroimaging techniques that can monitor hemodynamic responses associated with changes in neural activity (e.g., neurovascular coupling) (**Figure 2; Box 2**). These neuroimaging techniques characterize recovery and can measure neuroplastic changes following different treatments poststroke. Functional magnetic resonance imaging (fMRI) based applications such as blood oxygenation-dependent (BOLD) signal estimate changes in brain activity from fluctuations in the ratio of oxyhemoglobin and deoxyhemoglobin. Despite the idiosyncratic heterogeneity common in stroke, disproportionate increases in bilateral activity during the paretic hand movement have been

reported in fMRI studies[36]. Normalized brain activity patterns via ipsilesional increases and contralesional decreases have been shown to be associated with better recovery after different rehabilitative motor interventions [37].

Three of the four studies [38-41] examining the effect of chronic CE on brain activity measured with fMRI reported significant changes after training (**Table 2**). One study with sub-acute ischemic patients undergoing three weeks of virtual reality-enhanced treadmill training reported augmented brain activity during paretic limb movement in the ipsilesional primary sensorimotor cortex and bilateral supplementary motor areas [38]. Furthermore, this increased activity on the ipsilesional sensorimotor cortex was positively associated with improvements in walking speed.

In another fMRI study, despite significant improvements in walking speed and endurance after four weeks of light-intensity treadmill training, no significant brain activity changes were observed [39]. However, improvements in walking endurance were positively associated with increases in brain activity in bilateral sensorimotor cortex, cingulate motor areas, caudate nuclei, and the ipsilesional thalamus. A third study reported significant increases in brain activity during paretic limb movement in cerebellar and midbrain regions that were correlated with walking speed gains after six months of vigorous-intensity treadmill training [40]. Finally, the most recent study showed significant decreases in resting-state functional connectivity between the right dorsolateral prefrontal cortex and the sensoriomotor network following five consecutive days of HIIT paired with motor training compared to a control group [41]. Regardless of the group, decreases in functional connectivity correlated to improved processing speed in a cognitive-motor task, suggesting a reduced dependence on cognitive resources to complete a demanding motor task.

Another neuroimaging technique that can estimate brain activity is functional near-infrared spectroscopy (fNIRS) (**Figure 2; Box 2**). With fNIRS, brain activity is inferred from changes in oxyhemoglobin and deoxyhemoglobin concentration in superficial areas of the cortex. Studies using fNIRS have shown that stroke individuals performing motor tasks such as walking tend to over-activate the prefrontal cortex to compensate for motor deficits [42]. Furthermore, rehabilitative interventions such as intensive physical therapy have been shown to heighten fNIRS-measured cortical activity in brain regions responsible for the planning and acquisition of complex movements such as the supplementary motor areas [43].

The three studies that examined changes in brain activity using fNIRS revealed significant effects after CE [44-46] (**Table 2**). One acute study comparing a single bout of 15-minutes light-intensity cycling to a resting control condition showed significant post-exercise increases in oxyhemoglobin on the right prefrontal cortex while performing a working memory task [44]. The remaining two studies examined the chronic effects of CE. In one study, when comparing 12 weeks of accurate adaptability walking to a steady state walking, both at vigorous intensities, the two groups exhibited significant decreases in prefrontal cortex oxyhemoglobin during walking, with the accurate adaptability group showing larger reductions [45]. However, no significant correlation was observed between brain activity changes and walking function post-intervention. Finally, another study examined intensity-dependent effects by comparing 12 weeks of cycle ergometry HIIT vs. MICT [46], with the HIIT group exhibiting larger increases in deoxyhemoglobin and total hemoglobin on the ipsilesional prefrontal cortex during a graded exercise test.

In summary, fMRI studies have provided inconsistent results in terms of changes in brain activity after chronic CE interventions although associations with changes in function were observed in all the studies analyzed. Taken together, the results of the fNIRS studies showed that a single bout of light-intensity CE elevated oxyhemoglobin in the prefrontal cortex while chronic interventions resulted in significant reductions in oxyhemoglobin and increases in deoxyhemoglobin and total hemoglobin, suggesting increased and/or more efficient brain oxygen utilization. In neurotypical populations, CE interventions have been shown to increase brain connectivity, as measured by fMRI, in regions subserving motor and cognitive processes [47]. Additionally, in fNIRS studies, a single bout of CE has shown significant increases in prefrontal cortex activity [48], while chronic interventions resulted in reduced activity [49]. Despite these promising findings, to validate fMRI and fNIRS as potential biomarkers in response to CE, further studies should investigate whether such changes in neural activity are associated with improvements in behavioral outcomes.

- **Brain Volume**

Structural neuroimaging techniques such as MRI provide relevant information regarding the structural integrity of the CNS, improving our capacity to predict recovery outcomes poststroke and monitor treatment response following rehabilitative interventions (**Figure 2; Box 2**). In stroke, functional impairment is directly associated with the extent of structural damage in the brain [50].

Total and regional brain volumes measured with MRI tend to show accelerated atrophy following brain ischemia. Indeed, accelerated brain atrophy is a common hallmark in stroke that correlates with residual motor and cognitive deficits as well as reduced improvements in motor function in response to rehabilitation [51].

Structural MRI studies in neurologically intact older populations have shown that CE can protect against aging-related brain atrophy by preserving or even increasing brain volumes [52]. In agreement with this evidence, two studies also showed that chronic CE can preserve or increase brain volumes in stroke survivors [53,54] (**Table 2**). The first study compared changes in brain volume after four weeks of walking HIIT and a control phase with no intervention [53]. Following HIIT, the supratentorial volume of both ipsilesional and contralesional hemispheres exhibited significant increases compared to the control phase. The other study compared a 19-week multimodal exercise program, which included 15 minutes of CE per session, to a stretching control group [54]. Despite no differences between groups, the control group was the only one to exhibit significant pre-post bilateral atrophy of the medial temporal lobe, while in the exercise group, brain volumes remained preserved. The reason why one study showed volume increase [53] and the other preservation [54] is not clear, but could be due to differences in the CE interventions (e.g., 4 v. 19 weeks of training) or the areas of the brain investigated (supratentorial v. medial temporal lobe). Regardless, these findings suggest that CE may potentially confer protection from the rapid atrophy that the brain experiences poststroke and emphasize the potential use of structural MRI biomarkers such as brain volume to detect neuroplastic changes in response to CE.

Table 2. Main characteristics of the studies including neuroimaging biomarkers.

First author, Year (Design)	Demographics	Study Arms	Intervention	Technique	Biomarkers (outcomes)	Main Findings
Andrushko, 2023 (Randomized trial)	**N=** 25 **Sex (M/F)=** 19/6 **Age=** 67±9.5 **Stroke Type (I/H)=** NA **Time poststroke=** Chronic	HIIT+motor training Rest+motor training	**Mode:** Recumbent cycle ergometer **Duration:** 5 days **Frequency:** 5/week **Intensity:** Vigorous **Time:** 23 min	fMRI	Functional connectivity	Compared to control, HIIT exhibited pre-post decreases in functional connectivity between the right dorsolateral prefrontal cortex and the sensoriomotor network.
Enzinger, 2009 (Time Series)	**N=** 18 **Sex (M/F)=** 10/8 **Age=** 59.8±13.5 **Stroke Type (I/H)=** 18/0 **Time poststroke=** Chronic	Exercise Multiple baseline control	**Mode:** Treadmill **Duration:** 4 weeks **Frequency:** 3/week **Intensity:** Light **Time:** 20 min	fMRI	Brain activity	Compared to an initial control phase, exercise did not show significant changes in brain activity after training.
Luft, 2008 (Randomized trial)	**N=** 32 **Sex (M/F)=** 11/21 **Age=** 63.75±9.6 **Stroke Type (I/H)=** 32/0 **Time poststroke=** Chronic	Exercise Stretching	**Mode:** Treadmill **Duration:** 6 months **Frequency:** 3/week **Intensity:** Vigorous **Time:** 40 min	fMRI	Brain activity	Compared to the stretching group, exercise showed significant increases in brain activity during paretic limb movement in the posterior lobe of the cerebellum and midbrain regions.
Xiao, 2017 (Pre-Post)	**N=** 8 **Sex (M/F)=** 6/2 **Age=** 58.38±9.91 **Stroke Type (I/H)=** 8/0 **Time poststroke=** Early subacute	Exercise	**Mode:** Virtual reality-enhanced treadmill **Duration:** 3 weeks **Frequency:** 5/week **Intensity:** Speed increased as normal step length was observed **Time:** 60 min	fMRI	Brain activity	Exercise exhibited pre-post increases in brain activity during paretic limb movement on ipsilesional primary sensorimotor cortex and bilateral supplementary motor areas.
Moriya, 2016 (Within-subjects study)	**N=**11 **Sex (M/F)=** 7/4 **Age=** 69.6±12 **Stroke Type (I/H)=** 5/6 **Time poststroke (m)=** NR	Exercise Rest	**Mode:** Bicycle ergometer **Duration:** Acute **Intensity:** Light **Time:** 15 min	fNIRS	Brain activity (Oxyhemoglobin, deoxyhemoglobin, total hemoglobin)	Compared to the control condition, exercise exhibited significant increases in oxyhemoglobin, particularly on the right prefrontal cortex while performing a working memory task.
Clark, 2021 (Randomized trial)	**N=** 38 **Sex (M/F)=** 23/15 **Age=** 59.55±9.15 **Stroke Type (I/H)=** NR **Time poststroke=** Chronic	Accurate adaptability walking Steady state walking	**Mode:** Treadmill and overground walking **Duration:** 12 weeks **Frequency:** 3/week **Intensity:** Vigorous **Time:** 30 min	fNIRS	Brain activity (Oxyhemoglobin)	Both exercise conditions showed significant reductions of oxyhemoglobin in the prefrontal cortex, with the accurate adaptability group exhibiting the largest effects.
Hsu, 2021 (Randomized trial)	**N=** 23 **Sex (M/F)=** 20/3 **Age=** 55.8±15.6	HIIT	**Mode:** Bicycle ergometer **Duration:** ~12 weeks **Frequency:** 2/3 week	fNIRS	Brain activity (Oxyhemoglobin,	Compared to the MICT, the HIIT group exhibited significant increases in deoxyhemoglobin and

	Stroke Type (I/H)= 15/8 **Time poststroke=** Chronic	MICT	**Intensity:** MICT= Moderate; HIIT= Vigorous **Time:** 36 min		deoxyhemoglobin, total hemoglobin)	total hemoglobin on the ipsilesional prefrontal cortex.
Boyne, 2022 (Time Series)	**N=** 10 **Sex (M/F)=** 6/4 **Age=** 59.8±6.8 **Stroke Type (I/H)=** NR **Time poststroke=** Chronic	HIIT Multiple baseline control	**Mode:** Treadmill and overground walking **Duration:** 4 weeks **Frequency:** 3/week **Intensity:** Vigorous **Time:** 45 min	MRI	Brain volume (Supratentorial brain volume)	Compared to an initial control phase, HIIT showed significant increases in supratentorial volume on both ipsilesional and contralesional hemispheres.
Moore, 2015 (Randomized trial)	**N=** 40 **Sex (M/F)=** 34/6 **Age=** 69±9.5 **Stroke Type (I/H)=** 37/3 **Time poststroke=** Chronic	Exercise Stretching	**Mode:** Multimodal training (functional mobility, stretching, strengthening, balance, agility and fitness) **Duration:** 19 weeks **Frequency:** 3/week **Intensity:** Very light to Vigorous **Time:** 45-60 min (15min CE)	MRI	Brain volume (Grey matter atrophy)	No significant between-group differences were observed. The stretching group exhibited significant bilateral brain atrophy, while no changes were shown in the exercise group.

Abbreviations: CE, cardiovascular exercise; F, female; fMRI, functional magnetic resonance imaging; fNIRS, functional near-infrared spectroscopy; H, hemorrhagic; HIIT, high-intensity interval training; I, ischemic; M, male; MICT, moderate-intensity continuous training; MRI, magnetic resonance imaging; N, number of subjects; NR, not reported.

Blood Biomarkers

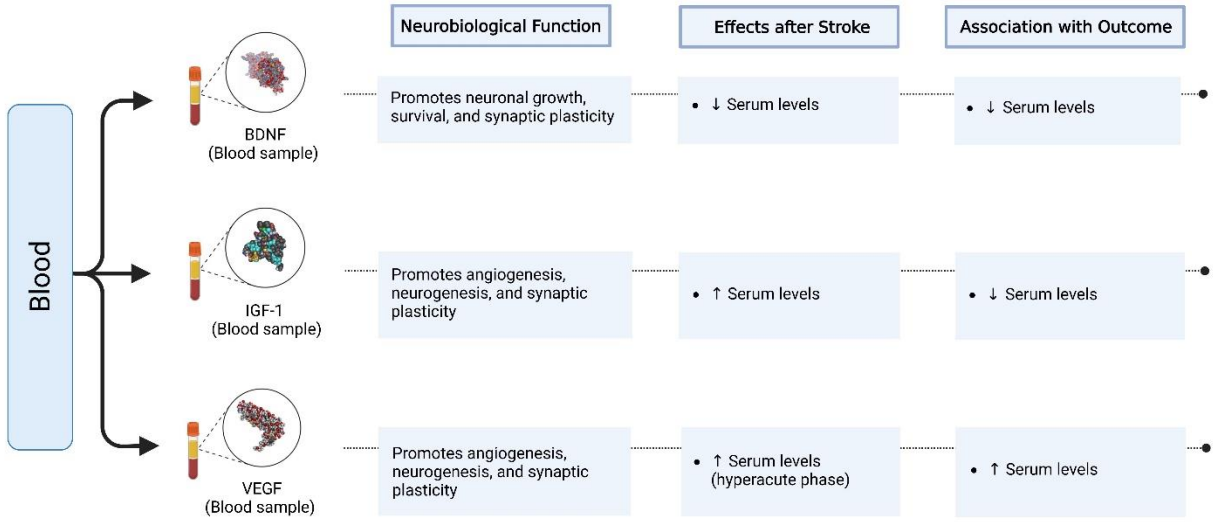

Figure 3. Blood biomarkers in stroke recovery

Box 3 | **Blood biomarkers in stroke recovery**

Brain-derived neurotrophic factor: Brain-derived neurotrophic factor (BDNF) is one of the most studied proteins in stroke recovery due to its central role in neuronal growth, survival, and synaptic plasticity. Reduced levels of peripheral BDNF, which have been reported in people after stroke, have been associated with poor functional outcomes. However, given the limited amount of BDNF that can cross the blood-brain barrier (BBB), to what extent peripheral levels reflect central neural processes is still debated.

Insulin-like growth factor 1: Insulin-like growth factor 1 (IGF-1) is primarily secreted by the liver but plays important neurotrophic actions when crossing the BBB. For example, IGF-1 has shown to promote angiogenesis, neurogenesis, and synaptic plasticity. Following a stroke, IGF-1 levels tend to increase and low levels have been associated with poor long-term functional outcomes.

Vascular Endothelial Growth Factor: In addition to its primary role in angiogenesis, vascular endothelial growth factor (VEGF) also contributes to neurogenesis and synaptic plasticity. In animal models, overexpression of VEGF levels during the hyperacute stages of stroke recovery has been associated with increased BBB disruption, while increased levels in later stages have been linked to neuroprotective effects. In humans, although its capacity to effectively cross the BBB remains unclear, elevated levels of VEGF have been associated with improved functional outcomes.

- **Brain-derived Neurotrophic Factor**

The inaccessibility to directly study molecular changes in the human brain makes blood biomarkers collected peripherally potential surrogates of central neurobiological processes and recovery poststroke (**Figure 3; Box 3**). Brain-derived neurotrophic factor (BDNF) is the most abundant neurotrophin in the brain, playing a central role in neuronal growth, survival, and synaptic plasticity [55]. Animal studies confirm that ensuring BDNF availability following brain

ischemia is critical for promoting neuroplasticity, recovery, and rehabilitation-induced motor improvements [56].

Six studies investigated acute BDNF changes after a single bout of exercise [11,57-61], with three showing significant changes (**Table 3**). One study revealed that 30 minutes of treadmill CE at vigorous intensity promoted significant increases in BDNF levels, while no changes were observed in the resting control group [59]. However, no significant associations between CE-induced BDNF changes and post-exercise improvements in the performance of a sensorimotor adaptation task were found.

Three studies examined the intensity-dependent effects of a single bout of CE on BDNF concentrations [11,60,61]. Twenty-five minutes of treadmill CE showed significantly larger BDNF increases following HIIT compared to MICT [11]. Furthermore, in the same study, significant increases in BDNF were observed following a maximal treadmill graded exercise test. In another study, walking at moderate intensity for 30 minutes resulted in greater BDNF elevation than walking at light intensity [60]. Finally, compared to five minutes of treadmill walking at light intensity, five minutes of moderate-intensity treadmill or vigorous-intensity ergometry did not elicit significantly larger increases in BNDF levels [61].

Only one of the two studies [46,62] examining the chronic effects of CE in BDNF reported significant changes following training (**Table 3**). Ploughman et al. examined the interaction between chronic and acute responses to CE by measuring basal BDNF levels post-training as well as before and immediately after a graded exercise test [62]. Compared to a group undergoing standard therapeutic activity, 10 weeks of vigorous-intensity treadmill training did not induce any acute or chronic significant change in BDNF. Intensity-dependent effects were investigated in another study comparing 12 weeks of cycling MICT and HIIT [46]. The HIIT group showed significantly greater increases in basal BDNF concentrations following training.

BDNF secretion in response to CE is highly variable and can be influenced by multiple factors. In non-disabled individuals, while peripheral levels of BDNF transiently increase following acute CE, especially when performed at higher exercise intensities, the long-term effects of chronic CE interventions are less consistent [63]. The results analyzed here indicate similar findings in stroke survivors, with circulating levels of BDNF transiently increasing following single vigorous exercise sessions and, less consistently, after a period of chronic CE. In any event,

whether the peripheral upregulation of BDNF triggered with CE translates to improvements in recovery has yet to be demonstrated.

- **Insulin Growth Factor 1**

Insulin growth factor 1 **(**IGF-1), a pleiotropic protein, involved in neuroplasticity and neurogenesis, promotes the upregulation of BDNF expression and VEGF production in the brain (**Figure 3; Box 3**). In animal models, elevated IGF-1 expression has been associated with improvements in recovery poststroke and neural repair through neovascularization and neurogenesis [64]. In people poststroke, similar findings support the association between increased IGF-1 levels and improved motor outcomes [65]. Research in neurotypical individuals has reported disparate results following CE, with most studies showing increases in circulating IGF-1 in response to acute CE [66] and chronic studies reporting reductions in basal levels at the end of the training intervention [67].

Two of the three studies [58,62,68] examining CE-induced effects on IGF-1 poststroke reported significant changes (**Table 3**). One study reported significant reductions in IGF-1 levels following a graded exercise test [58]. Another study investigated the intensity-dependent effects of circulating IGF-1 by comparing a single bout of HIIT to MICT [68]. Although no differences were observed between groups, 25 minutes of HIIT on either treadmill or recumbent stepper promoted significant increases in IGF-1 levels, while no changes were reported following treadmill MICT. Finally, Ploughman et al. examined the acute and chronic responses on IGF-1 concentrations [62], with no significant changes following 10 weeks of vigorous-intensity treadmill training paired with cognitive training.

- **Vascular Endothelial Growth Factor**

Besides playing a central role in angiogenesis, vascular endothelial growth factor (VEGF) is an essential protein regulating neurogenesis and synaptic plasticity via stimulation of neural stem cells, endothelial cells, and production of BDNF and IGF-1 (**Figure 3; Box 3**). In stroke, the overexpression of VEGF during early stages of recovery has been associated with suboptimal recovery. In contrast, in later stages poststroke, increased VEGF has been associated with neuroprotective effects facilitating recovery [69].

In neurotypical populations, CE upregulates VEGF following acute and chronic CE [66,67]. In stroke survivors, two studies were identified examining this neurotrophin in response to CE [68,70], with only one reporting significant changes (**Table 3**). Intensity-dependent effects on circulating VEGF were investigated by comparing a single 25-minute bout of treadmill HIIT and MICT [68]. Following the HIIT intervention, VEGF concentration levels increased significantly compared to MICT. Chronic effects were also investigated by comparing 12 weeks of home-based bicycle HIIT to standard care in patients at acute stages poststroke [70]. Compared to standard care, no significant changes in basal VEGF levels were reported following exercise.

Table 3. Main characteristics of the studies including blood biomarkers.

First author, Year (Design)	Demographics	Study Arms	Intervention	Technique	Biomarkers (outcomes)	Main Findings
Boyne, 2019 (Within-subjects study)	**N=** 16 **Sex (M/F)=** 9/7 **Age=** 57.4±9.7 **Stroke Type (I/H)=** 12/4 **Time poststroke=** Chronic	GXT-treadmill HIIT-treadmill HIIT- recumbent stepper MICT-treadmill	**Mode:** Treadmill; Recumbent stepper **Duration:** Acute **Intensity:** MICT treadmill=Moderate; HIIT treadmill=Vigorous; HIIT stepper= Vigorous. **Time:** GXT: 7.9±3.2 min, Exercise protocol: 25 min	Blood sample	Serum BDNF	Following a GXT, significant pre-post increases were observed in BDNF levels. The HIIT-treadmill condition showed significant increases in BDNF compared to the MICT-treadmill condition.
Charalambous, 2018 (Randomized trial)	**N=** 37 **Sex (M/F)=** 23/14 **Age=** 58±11.66 **Stroke Type (I/H)=** 25/12 **Time poststroke =** Chronic	Light intensity-treadmill Moderate intensity-treadmill Vigorous intensity-Upper&Lower limb cycle ergometer	**Mode:** Treadmill; Upper&Lower limb ergometer **Duration:** Acute **Intensity:** Treadmill=Light; Treadmill=Moderate; Upper&Lower limb Ergometer= Vigorous **Time:** 5 min	Blood sample	Serum BDNF	No significant changes were reported in BDNF levels following any of the exercise conditions.
De Morais, 2018 (Within-subjects study)	**N=** 10 **Sex (M/F)=** 5/5 **Age=** 58±12.8 **Stroke Type (I/H)=** 10/0 **Time poststroke=** Chronic	Light intensity walking Moderate intensity walking	**Mode:** Overground walking **Duration:** Acute **Intensity:** Light; Moderate **Time:** 30 min	Blood sample	Serum BDNF	No significant between-group differences were observed, but the moderate-intensity condition exhibited significant pre-post increases in BNDF levels, while no changes were reported after light intensity.
King, 2019 (Pre-Post)	**N=** 35 **Sex (M/F)=** 23/12 **Age=** 65.2±9.4 **Stroke Type (I/H)=** 26/9 **Time poststroke=** Chronic	GXT	**Mode:** Treadmill; Recumbent stepper **Duration:** Acute **Intensity:** Vigorous **Time:** 12.46±6.4 min	Blood sample	Serum BDNF, IGF-1	Following a GXT, significant pre-post decreases were observed in IGF-1 levels, while no changes were shown in BDNF levels after exercise.
Mackay, 2021 (Within-subjects study)	**N=** 20 **Sex (M/F)=** 15/5 **Age=** 60±14 **Stroke Type (I/H)=** NR **Time poststroke=** Chronic	Exercise Rest	**Mode:** Treadmill **Duration:** Acute **Intensity:** Vigorous **Time:** 30 min	Blood sample	Serum BDNF	Following exercise, significant pre-post increases in BDNF levels were reported, while no changes were shown in the control condition.

Silva, 2017 (Pre-Post)	**N=** 15 **Sex (M/F)=** 9/6 **Age=** 60.8±7.7 **Stroke Type (I/H)=** 15/0 **Time poststroke=** Chronic	Exercise	**Mode:** Overground Walking **Duration:** Acute **Intensity:** Light to Moderate **Time**: 40 min	Blood sample	Serum BDNF, proBDNF	Following a walking session, no significant pre-post changes were observed in proBDNF and BDNF levels.
Hsu, 2021 (Randomized trial)	**N=** 23 **Sex (M/F)=** 20/3 **Age=** 55.8±15.6 **Stroke Type (I/H)=** 15/8 **Time poststroke=** Chronic	MICT HIIT	**Mode:** Bicycle ergometer **Duration:** ~12 weeks **Frequency:** 2/3 week **Intensity:** MICT= Moderate; HIIT= Vigorous **Time**: 36 min	Blood sample	Serum BDNF	Compared to MICT, the HIIT group showed significant increases in basal levels of BDNF after training.
Ploughman, 2019 (Randomized trial)	**N=** 52 (BDNF: 46 chronic, 25 acute; IGF: 27 chronic, 23 acute) **Sex (M/F)=** 36/16 **Age=** 63.4±11.3 **Stroke Type (I/H)=** 40/12 **Time poststroke=** Chronic	Exercise+cognitive training Standard care+cognitive training	**Mode:** BWSTT **Duration:** Acute (GXT) + 10 weeks **Frequency:** 3/week **Intensity:** Vigorous **Time:** 20-30 min	Blood sample	Serum BDNF, IGF-1	Compared to the standard care group, no significant acute or chronic changes were observed in BDNF and IGF-1 levels following training.
Boyne, 2020 (Within-subjects study)	**N=** 16 **Sex (M/F)=** 9/7 **Age=** 57.4±9.7 **Stroke Type (I/H)=** 12/4 **Time poststroke=** Chronic	GXT-treadmill HIIT-treadmill HIIT-recumbent stepper MICT-treadmill	**Mode:** Treadmill; Recumbent stepper **Duration:** Acute **Intensity:** MICT treadmill= Moderate; HIIT treadmill= Vigorous; HIIT stepper= Vigorous **Time:** GXT: 7.9±3.2 min, Exercise sessions: 25 min	Blood sample	Serum VEGF, IGF	No significant changes were observed following a GXT. Compared to MICT-treadmill, HIIT-treadmill showed significant increases in VEGF concentration. Both HIIT conditions showed significant pre-post increases in IGF-1, with no significant changes after MICT.
Krawcyk, 2019 (Randomized trial)	**N=** 63 **Sex (M/F)=** 49/14 **Age=** 63.7±9.05 **Stroke Type (I/H)=** 63/0 **Time poststroke=** Acute	HIIT+standard care standard care	**Mode:** Home-based stationary bicycle **Duration:** 12 weeks **Frequency:** 5/week **Intensity:** Vigorous **Time:** 15 min	Blood sample	VEGF	Compared to standard care, exercise did not show significant changes in basal levels VEGF after training.

Abbreviations: HIIT, high-intensity interval training; I, ischemic; IGF-1, insulin-like growth factor 1; M, male; MICT, moderate-intensity continuous training; N, number of subjects; NR, not reported; VEGF, vascular endothelial growth factor

Gaps and Future Directions

This paper presented a critical analysis regarding the use of biomarkers to assess the neuroplastic response to CE in individuals poststroke. The summarized view of the main results for each biomarker is presented in **table 4**. From a methodological perspective, it should first be noted that only a few large studies examined the effects of CE on biomarkers after stroke, with the majority (78%) comprising studies with small sample sizes ($n \leq 30$). Furthermore, only 14 studies (41%) were RCTs with a comparative non-exercise control group, which is essential to determine any causal effects induced by the CE. To draw more definitive conclusions, future CE studies using biomarkers to assess neuroplasticity should be carefully designed and adequately powered RCTs including a control group not receiving the exercise intervention.

Table 4. Summarized View of the Main Results for Each Biomarker in Response to Cardiovascular Exercise.

Biomarker	Recovery Stage	Exercise Intensity	Acute Response	Chronic Response	Supporting Studies	Participants Number
Transcranial Magnetic Stimulation						
Resting motor threshold	C,LS	M,V	↔	↓IH, CH, ↔	12, 18, 19	44
Active motor threshold	C	M,V	↓IH	↔	11, 20	97
Resting MEP amplitude	C	M,V	↑IH,↔	?	10, 12, 15, 16, 17	92
Active MEP amplitude	C	L,M,V	↓IH,↔	↔	13, 14, 16, 20	140
MEP latency	C	M,V	↑IH	?	12	12
Cortical silent period	C	L,M,V	↔	?	11, 16, 20	64
Intracortical facilitation	C	M,V	↔	?	12, 16	34
Intracortical inhibition	C	L,M,V	↓IH,↔	?	10, 12, 14, 15, 16	85
Map size	C,LS	NA	?	↑ IH, CH	18, 19	32
Electroencephalography						
Brain activity	C	M	↑	↔	23, 25	21
Brain connectivity	C	NA	?	↑	24	18
Nerve and Vibration Stimulation						
Cutaneous reflex	C	L	?	↓↑	32, 33, 34	57
Stretch Reflex	C	L	?	↓↑	32, 33	38
Functional Magnetic Resonance Imaging						
Brain activity	C,ES	L,V	?	↑, ↔	38, 39, 40, 41	83
Functional Near-infrared Spectroscopy						
Brain activity	C	L,M,V	↑	↓	44, 45, 46	72
Magnetic Resonance Imaging						
Brain volume	C	V	NA	↑, ↔	53, 54	50
Blood Collection						
BDNF	C	L,M,V	↑, ↔	↑, ↔	11, 46, 57, 58, 59, 60, 61, 62	202
IGF-1	C	M,V	↓, ↑, ↔	↔	58, 62, 68	78
VEGF	A,C	M,V	↑	↔	68, 70	79

Abbreviations: Biomarker: BDNF, brain-derived neurotrophic factor; IGF-1, insulin-like growth factor 1; MEP, motor-evoked potential; VEGF, vascular endothelial growth factor. Recovery stage: A, acute; C, chronic; ES, early subacute; LS, late subacute. Exercise intensity: L, Light; M, moderate; V, vigorous. Response to exercise: CH, contralesional hemisphere; IH, ipsilesional hemisphere; NA, not applicable; ↑, significant increase; ↓, significant decrease; ↔, no significant change;?, not investigated.

The evidence reviewed herein suggests that there is little consensus regarding which biomarkers could best capture the neuroplastic changes taking place in response to CE and the implications for functional recovery after stroke. In most cases, the rationale for selecting the specific biomarker was not clearly articulated and presumably based on the availability of a specific technique rather than because the chosen biomarker was the most appropriate to investigate a specific aspect of neuroplasticity. Furthermore, associations between changes in biomarkers and recovery outcomes were either not investigated or not reported. Clearly, more investigation is needed to identify the most appropriate biomarkers to assess the neuroplastic effect of CE, standardize how they should be assessed and determine their implications in stroke recovery. In the next sections, we discuss the most important gaps in knowledge identified and suggest strategies to improve future studies.

- **Selection of Biomarkers**

Lack of consensus and methodological quality issues such as the absence of validation, lack of association with clinically important differences, and small sample sizes are the main issues using biomarkers in stroke [71]. This critical view aligns well with the evidence collected here in response to CE. When designing CE studies, it is important to select biomarkers, which are supported by the strongest scientific evidence and, ideally, are also associated with changes in recovery outcomes that are clinically relevant (see next section) [72]. The studies reviewed here that used techniques such as TMS, fMRI, or fNIRS, varied widely in their choice of primary measures, targeted brain regions, and timing of assessments. This heterogeneity contributed to the already large variability in the response to CE between individuals with stroke, making it difficult to compare results across studies and to determine the potential true effects of CE on neuroplasticity. Such variability undermines the understanding of which biomarkers and techniques can best capture mechanisms related to neuroplasticity and recovery [5]. Moreover, in addition to its effects on the nervous system, it is also important to consider the influence of CE on other mechanisms, including metabolic, vascular, or inflammatory processes. These mechanisms can also be independently implicated in the recovery process and may both interact with and influence neuroplasticity mechanisms. Finally, it is critical that advances in biomarkers progress in parallel with the evidence derived from preclinical animal models of stroke. The establishment of translational research, functioning in a bidirectional and iterative manner between animal and

human studies is essential to enable the comparison and validation of biomarkers. Overall, this information is critical not only to develop more precise and relevant biomarkers for stroke recovery but also to improve the measurement of treatment-induced neuroplasticity and create a better consensus for establishing consistent methodological procedures for future studies.

- **Assessment of Function**

Improving functional recovery through neural repair and plasticity is one of the primary goals of stroke rehabilitation [73,74]. While behavior is undeniably the ultimate determinant of treatment effectiveness, a mechanistic understanding is essential for identifying biological targets to help elucidate the potential reparative capacity of an intervention or lack thereof, as well as for tailoring its application to maximize its effect on recovery. However, to determine the functional validity of a specific biomarker, it is essential to examine whether it is associated with recovery outcomes [72]. While most studies (78%) included functional measures to study the effects of CE on recovery, only a few (37%) investigated the association between behaviour and biomarker changes. Although associations cannot confirm causality, without this analysis, it is impossible to determine whether neuroplasticity changes induced by CE can have a potential positive effect on functional recovery. To determine the neural reparative capacity of CE in people after stroke, future studies should investigate and report the associations between biomarker change and outcome improvement in response to this type of training [75]. Furthermore, in order to better capture mechanisms of true neuroplasticity and brain repair it is essential that future studies include the assessment of impairment in addition to activity limitation and participation outcomes [74].

- **Characterization of Patients**

The characteristics of the brain injury and baseline function directly impact the individual's capacity to recover after stroke [76]. Unlike animal models, where ischemic-induced lesions can be precisely induced, clinical studies include patients presenting with a broad range of lesion sizes, locations and resulting impairments. This variability increases heterogeneity and limits the ability to determine the reparative potential of rehabilitative interventions. Most of the studies reviewed (79%) classified stroke types as either ischaemic and hemorrhagic, with only a few (29%) providing more detailed information on aspects such as size and location of the stroke. Similarly,

while most studies (95%) provided some baseline measures of severity, impairment, or disability, these measures varied significantly, with only a few following consensus-based recommendations [75]. Stratifying patients while ensuring generalizability will require concerted efforts in designing multisite studies to increase sample sizes. Ultimately, this is the only way to investigate how the characteristics of the patient can affect the neuroplastic response to CE. Furthermore, we observed that most patients in the selected studies presented mild degrees of disability and mobility problems, suggesting recruitment bias towards less disabled individuals. Selection bias, which is a recurrent issue in stroke rehabilitation research, may lead to overlook the potential therapeutic effects of CE in a substantial portion of the stroke population.

- **Consideration of Recovery Stage**

Research in animals and humans has identified distinct critical timepoints after stroke, each involving unique neurobiological processes that interact with recovery [73]. While functional recovery can occur during chronic stages, a critical period for recovery exists within the first weeks to months post-stroke, when most functional improvements take place, and where greater gains can be achieved in response to rehabilitation [77,78]. It is unclear if this optimal window of opportunity also applies to the introduction of CE [79]. Furthermore, the large majority (95%) of studies identified focused on patients at chronic stages poststroke (>6 months), while only a few included individuals from earlier stages. This constitutes a significant gap in the literature that limits the understanding of how CE interacts with the unique time-dependent neurobiological processes that occur after stroke. Furthermore, is important to note that promoting neuroplasticity is not always beneficial. The results from many animal studies [80] and indirect evidence from humans studies [81] suggest that, especially during very acute phases of recovery, the brain is highly sensitive to further stressors, including physical exercise. These stressors may exacerbate infarct lesions and impact behavioral outcomes by altering different aspects of brain activity. Clearly, more clinical research is needed to determine the optimal timing where CE should be introduced after stroke and how it should be implemented to ensure that any potential neuroplastic effect leads to a positive outcome for these patients [74].

- **Quantification of Exercise Stimulus**

Individualizing exercise workloads and monitoring training stimulus is critical to minimize variability in response to CE and to better understand any potential dose-response relationship in neuroplasticity. Exercise stimulus in stroke patients can be quantified using the FITT (frequency, intensity, time, type) principle [82]. Furthermore, a symptom-limited graded exercise test can increase safety and enable the accurate individualization of workloads based on each patient's capacity [83]. Although all the reviewed studies reported and defined their exercise parameters, there was heterogeneity in the types of measures used, and only 34% of the studies employed an exercise test to individualize workloads. Employing well-established measures to quantify both internal and external exercise workloads would permit the accurate monitoring of training stimuli in patients with varying degrees of neurological impairment and fitness and examine potential associations with neuroplastic responses. Identifying significant associations will allow us to be more precise in the prescription of CE with the aim of promoting neuroplasticity. Finally, it is crucial to note that while studies performing a single exercise session can provide valuable mechanistic insights regarding the acute neurobiological responses to CE, whether these changes can be considered neuroplasticity, understood as persistent change in neural networks and synaptic connections is still open to debate. Implementing longitudinal interventions, coupled with measurements of neuroplasticity that capture more persistent effects, is indispensable to determine the potential effects of CE and their impact on recovery [7].

Summary

Given its multiple benefits, CE should be a core component of stroke rehabilitation. Preclinical evidence has linked CE-induced gains in functional recovery to neuroplastic changes in the nervous system. The use of biomarkers has opened a unique opportunity to investigate neuroplasticity processes during recovery and in response to treatment after stroke in humans. Although some biomarkers appear to be responsive to the effects of both acute and chronic CE interventions, the evidence supporting their use is still inconsistent. Small sample sizes, methodological variability, lack of information regarding patient's characteristics, and inadequate standardization of CE parameters, in addition to lack of reporting of associations with functional outcomes, are the main barriers precluding the quantification of the true reparative neuroplastic

effects of CE poststroke. To advance our mechanistic understanding of the impact of CE on neuroplasticity and recovery in people after stroke, it is imperative that we address all gaps and methodological issues identified in this paper. Only by addressing these challenges, we will be able to uncover the potential reparative capabilities of this intervention and inform clinical practice.

Declaration of Conflicting Interests

The Authors declare that there is no conflict of interest.

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Supplementary Material

Methods

Eligibility Criteria

The **PICOS** framework was used to operationalize the eligibility criteria of the included studies[1]. **Population:** human adults ≥ 18 years old with any type of stroke[2]. Studies with people with transient ischemic attack (TIA) were not included. **Intervention:** one (acute) or multiple (chronic) training sessions of CE with an intensity sufficient to increase the basal metabolism of the individual (e.g., heart rate increase) and with a well-defined protocol providing exercise parameters (frequency, intensity, time, type). Multimodal exercise interventions with an aerobic component were included. Cardiovascular exercise (CE) was defined as an activity that uses large muscle groups, can be maintained continuously and is rhythmic in nature[3]. Studies without well-defined parameters (e.g., self-selected speed) were excluded unless they provided evidence of significant workload increases in volume and/or intensity[4]. Studies combining CE with other non-exercise complementary interventions were excluded unless the control group was exposed to the same complementary intervention or one of the study arms performed only CE. **Comparison:** standard care, non-exercise control, waiting list, a control group receiving the same complementary intervention than the exercise group or a CE intervention employing different modes or intensities. Pre-post studies without comparison group were also included. **Outcomes:** biomarkers expressing neuroplasticity changes in the CNS[5,6]. Studies monitoring transient changes in biomarkers only during exercise or analyzing changes in cerebral blood flow[7] or inflammatory biomarkers were not included in the review. **Study design:** randomized controlled and non-randomized controlled trials, within-subject, one group pre-post, and time-series[8]. Observational studies, case series, qualitative studies, surveys, and protocols were excluded.

Search Strategy

The search for this review was conducted in accordance with PRISMA guidelines and registered in PROSPERO (CRD42022293109). Two authors independently performed the electronic search on the databases PubMed and Web of Science. Reference lists from previous reviews containing studies related to the topic of interest were also screened[9-11]. The electronic

search was neither language nor date-restricted, but it was limited to studies completed in human subjects. The search terms used for the primary search included keywords related to stroke, exercise, and neuroplasticity biomarkers following the PICOS framework and combined using Boolean operators **(Table 1).** A preliminary search occurred on July 20th, 2022, and the final search occurred on November 10th, 2022. An updated search was conducted on September 15th, 2023.

Study Selection and Data Extraction

Titles and abstracts of studies retrieved in the search were reviewed independently by two authors, who selected studies for full-text review according to the eligibility criteria. After reviewing the articles, both authors held a consensus meeting to compare their results and decide which articles should be included. Both authors independently extracted the following data from studies: first author, year and study design, demographics, study arms, parameters of exercise intervention, technique, biomarker, and main findings. When provided, the association between changes in the biomarker and functional recovery measure was also extracted.

Depending on if they used one or multiple bouts of CE, studies were classified as acute or chronic. Time poststroke was categorized as hyper-acute (0 to 24 hours), acute (1 to 7 days), early subacute (7 days to 3 months), late subacute (3 to 6 months), and chronic (>6 months)[12]. Exercise intensity was categorized as very light, light, moderate, vigorous, or maximal, according to ACSM guidelines[3]. When exercise intensities were not reported, training workloads (e.g., increases in speed) were retrieved.

Results

Search Results

The different steps of the search, with the number of studies reviewed at each stage and the reasons for exclusion are shown in **Figure 1**. The combined database search yielded a total of 8392 records. After removing duplicates, 4742 abstracts were reviewed. The abstract review yielded 167 articles to be reviewed at a full-text level. After the full-text review, a total of 133 studies were excluded. The main reasons for exclusion were exercise parameters not described (n=65), exercise interventions without a clear cardiovascular component (n=36), complementary interventions not received also by the control group (n=21), and the absence of neuroplasticity biomarkers (n=9).

One study was excluded because included patients with TIA[13] and another because brain activity measured with functional near-infrared spectroscopy (fNIRS) was monitored during exercise only[14]. Five studies that employed non-invasive brain stimulation (transcranial direct current stimulation -tDCS-, intermittent theta burst stimulation -iTBS-) as complementary intervention were included because they had both the exercise and control groups receiving the same intervention[15-17] or one of the groups performed exercise only[18,19]. After excluding the articles not meeting inclusion criteria, 34 studies were deemed eligible for review. Two of these studies investigated CE-induced neuroplastic changes via two distinct biomarker categories[20,21].

Characteristics of Studies

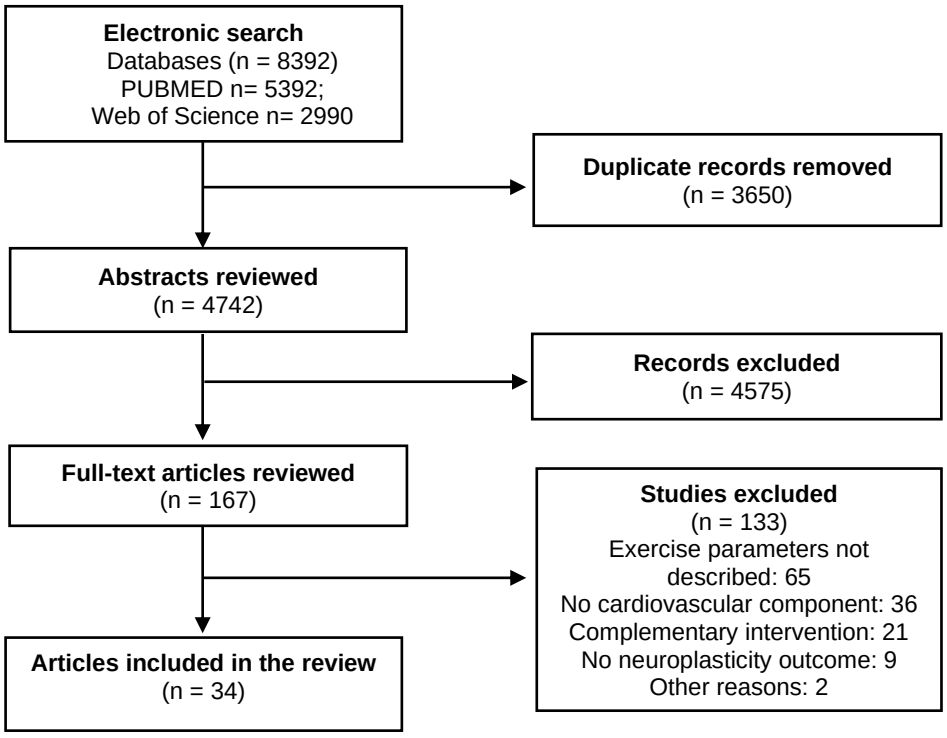
The most common study design was randomized control trial (n=13), followed by within subject (n=11), time series (n=5), and one group pre-post (n=5). In total, data from 807 patients (548 males and 259 females) with average ages ranging from 52.5 to 73 were included. Twenty studies included both patients with ischemic (n=328) and hemorrhagic stroke (n=136), six studies included patients with ischemic stroke only (n=146), and the type of stroke was not reported in nine studies (n=197). Including all studies, 716 patients were categorized as chronic, eight early subacute, nine late subacute, 63 acute, and the stage of recovery was not reported for 11 patients.

Fifteen studies used acute, 18 chronic, and one both acute and chronic CE interventions. The most common mode of exercise was treadmill walking, followed by bicycle ergometry, recumbent stepper, overground walking, upper and lower limb cycle ergometry, multimodal training, and upper limb cycle ergometry. The duration of training sessions ranged from five to 40 minutes in acute studies and from 15 to 60 minutes in chronic studies. Exercise intensity ranged from light to maximal and four studies reported changes in external workloads (i.e. speed, amount of body weight support) instead of participants' internal workload values (i.e. heart rate, rating of perceived exertion)[22-25].

Table 5. Terms used for primary search following PICOS framework.

Population	Intervention	Outcome
Stroke Cerebral vascular accident (Humans)	Exercise Physical training Motor activity Physiotherapy Physical conditioning	Biomarker Neuronal plasticity Neurophysiol* Imag* / Neuroimag* Neurotrophi* Growth factor Cortical excitability Functional connectivity Non-invasive brain stimulation Transcranial magnetic stimulation Magnetic resonance imaging Diagnostic techniques, neurological Functional magnetic resonance Electroencephalography Magnetoencephalography Positron-emission tomography Near-infrared spectroscopy

Figure 1. Flow chart with different phases of the search process



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Cortico-spinal Excitability: Measuring a Pathway to Recovery

Cortico-spinal excitability (CSE), measured through TMS, is a widely used brain biomarker in stroke recovery research due to its strong association with motor recovery [91]. Using single or paired-pulse protocols, TMS can activate a mixed population of inhibitory and excitatory cortical circuits that depolarize local and remote pyramidal tract neurons, providing measures of functional and structural integrity of the corticospinal tract, as well as intracortical facilitatory (glutamate) or inhibitory ($GABA_A$, $GABA_B$) neurotransmitter systems **(Figure 1)** [115,116]. These neurophysiological measures have been linked to motor impairment and used in predictive algorithms for upper-limb recovery [117].

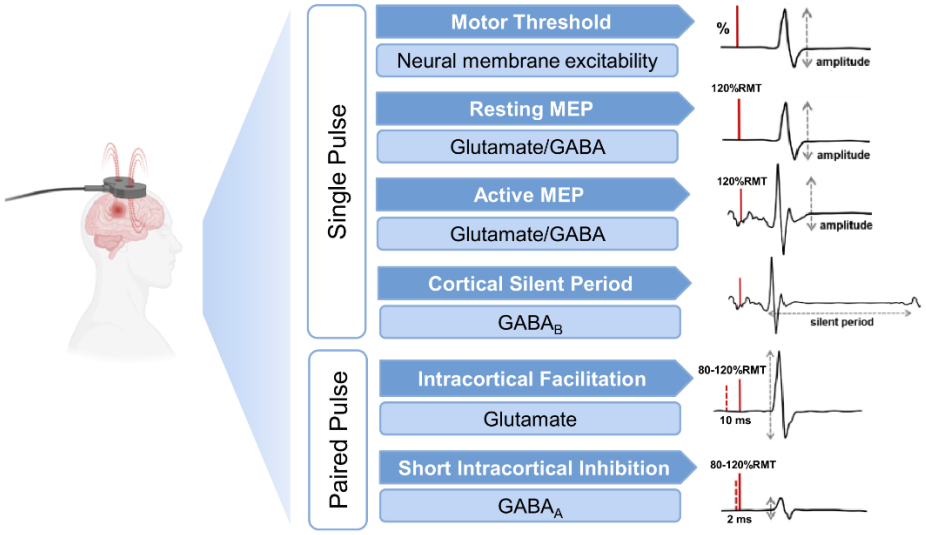

Figure 1. TMS-assessed corticospinal excitability measures. Single and paired pulse transcranial magnetic stimulation protocols used to assess different corticospinal excitability (CSE) measures and their putative underlying mechanisms. Resting Motor Threshold (RMT) is shown as a percentage of the stimulator output capacity, reflecting neural membrane excitability, with lower RMT indicating higher CSE. Motor evoked potential (MEP) amplitude measures excitability of cortical and spinal projections influenced by excitatory (glutamate) and inhibitory (GABA) circuits, with larger MEP amplitudes indicating higher CSE. Cortical Silent Period (CSP) reflects GABAB receptor-mediated inhibition, with longer CSP indicating greater inhibition. Intracortical Facilitation (ICF) measures facilitation mediated by NMDA receptors, and SICI (Short

Intracortical Inhibition) assesses inhibition mediated by GABAA receptors. Larger ICF values indicate greater facilitation, while smaller SICI values indicate greater inhibition. GABA, gamma-aminobutyric acid; ms, milliseconds; NMDA, N-methyl-D-aspartate.

Functional improvements resulting from motor rehabilitation have been associated with specific CSE changes, such as the restoration of interhemispheric balance [118], reduced motor thresholds [119], increased MEP amplitudes [120], shorter MEP latencies [121], longer CSPs [122], decreased intracortical inhibition [123], and increases in cortical map size on the ipsilesional hemisphere [124], suggesting treatment-induced neuroplastic changes leading to recovery. Additionally, single CE bouts have been shown to modulate CSE and promote plasticity-like states that may mediate improvements in motor behavior such as motor learning in both neurotypical and stroke populations [125,126].

Following our review in Chapter 1, we noticed that besides being one of the most extensively used biomarkers in human stroke research [91,127,128], CSE is also the most used biomarker for investigating brain changes in response to CE in individuals after stroke. Single-session studies have offered valuable insights into acute neurobiological responses to CE [126], while longitudinal interventions involving multiple sessions over time have reflected chronic neuroplastic adaptations [129,130]. However, the impact of CE on CSE during the subacute stages post-stroke remained unexplored. Notably, no studies have examined individuals in the early subacute stages of stroke recovery, a period when corticospinal circuits are highly malleable and receptive to treatment [41].

Our laboratory holds extensive expertise in TMS, with several studies investigating the neurophysiological mechanisms underlying motor learning and in response to CE interventions in both neurotypical and stroke populations [125,126]. We are also an important site of the Canadian platform for Non-invasive Brain Stimulation Trials (CanStim) aimed at improving motor recovery through large multi-site studies [131]. Thus, examining the effects of CE on CSE during the subacute stages of stroke recovery is not only a crucial research question but also one that can be feasibly answered in our laboratory. In the following Chapter, presented as a manuscript, we use TMS to measure acute and chronic CSE responses following CE in individuals during the subacute stages post-stroke.

Chapter 2: Acute and Chronic Effects of Cardiovascular Exercise on Corticospinal Excitability in People with Subacute Stroke

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Abstract

Cardiovascular exercise (CE) has shown promise as a motor intervention in stroke recovery, in part due to its potential to induce neuroplastic changes through excitatory neural signaling. While this is well-documented in animal models, the neurophysiological mechanisms of CE in post-stroke individuals remain largely unexplored, particularly during the early subacute phase (<3 months post-stroke) when the brain may be more responsive to treatment. In this study, 76 first-ever ischemic subacute patients were randomly assigned to either eight weeks of progressive CE using whole-body recumbent steppers in addition to standard care, or to standard care alone. Using single and paired-pulse transcranial magnetic stimulation (TMS) protocols, we assessed ipsi- and contralesional corticospinal excitability (CSE) at rest and following a single high-intensity interval training (HIIT) session at baseline, four weeks, and eight weeks, to evaluate both chronic and acute responses to CE. The influence of BDNF Val66Met polymorphism, a genetic variant that alters neuroplasticity processes including CSE, was also examined. At baseline, when combining both groups, a single HIIT session significantly increased CSE in the contralesional hemisphere, with no changes observed ipsilesionally. Over the study period, CE training did not significantly impact CSE measures either chronically or acutely when compared to standard care, despite notable improvements in cardiorespiratory fitness. Val66Met may have influenced the acute response to a single CE at baseline, with Val carriers exhibiting higher CSE increases compared to Met carriers. This study is the first to investigate the effects of CE on CSE in early subacute stroke patients. Our findings suggest that while CE can improve fitness levels significantly, it may have limited neuroplastic effects during the early stages of stroke recovery. We propose that these results could be influenced by inhibitory activity during subacute stages, intervention specificity, and patient variability.

Introduction

Stroke stands as the leading cause of disability among adults worldwide [1]. Despite significant medical advancements in acute care that have reduced mortality rates and disability levels, about half of survivors sustain chronic sequelae, impacting their functional capacity and quality of life [2,3]. Consequently, in the absence of more effective treatments, rehabilitation emerges as the primary strategy for minimizing functional disability in individuals post-stroke [4].

Despite initial phases of neural damage and inflammation, the brain following stroke exhibits a heightened yet time-limited state of neuroplasticity that contributes to recovery [5]. During this critical period, which spans about a month post-stroke in animal models, sensorimotor recovery is usually accompanied by profound structural and functional changes in the surviving neural systems. These changes include enhanced dendritic spine turnover, axonal sprouting, and cortical remapping, all dependent on alterations in cellular excitatory within neural circuits [6,7].

This transient state of neural malleability can be effectively harnessed through appropriate treatment interventions, with animal studies showing that early motor rehabilitation can lead to larger motor gains and neuroplastic changes compared to initiating rehabilitation at later stages [8,9]. In humans, evidence has suggested a similar critical period within the first week to three months post-stroke, known as the early subacute phase (Bernhardt et al., 2017), during which motor training interventions may lead to greater functional gains [10,11]. Therefore, implementing motor rehabilitative treatments during this period may hold recovery potential by interacting with stroke-induced neuroplastic processes.

Cardiovascular exercise (CE) has been recommended as a core component of stroke rehabilitation due to its capacity to simultaneously improve functional, cardiorespiratory, and metabolic recovery outcomes [12]. Animal studies have demonstrated that functional recovery in response to CE can be attributed to changes in the nervous system [13], including the upregulation of growth factors, angiogenesis, and neurogenesis, as well as enhanced synaptic plasticity [14,15], an event tightly regulated by neural excitability [16]. However, unlike in animal models, the effects of CE on neuroplasticity and, particularly, the excitability of neural circuits in people after stroke remain largely unknown [14,15].

Corticospinal excitability (CSE), assessed with transcranial magnetic stimulation (TMS), is one of the most extensively used biomarkers in stroke research, given its capacity to assess the structural and functional integrity of the corticospinal tract (CST) [17,18]. When applied to the primary motor cortex (M1), TMS can activate a mixed population of inhibitory and facilitatory circuits, which depolarize to local and remote pyramidal tract neurons, enabling the quantification of distinct CSE measures [19]. Some of these measures have proven valuable for predicting motor recovery outcomes (Stinear et al., 2017) and assessing neurophysiological responses to rehabilitation treatments [20].

Over the last decade, an increasing number of studies have investigated the impact of CE on CSE in post-stroke individuals. While findings varied among studies, both acute and chronic changes have been reported following a single CE session and training programs in specific aspects of CSE [21]. Surprisingly, however, all studies have primarily focused on chronic stages (> six months post-stroke), neglecting earlier periods when the brain may be more susceptible to changes in response to training. Understanding the neurophysiological mechanisms in response to CE, especially during the subacute period, is crucial for quantifying its potential reparative effects [6].

Additionally, genetic factors are believed to influence individual treatment responses after stroke by affecting mechanisms related to neuroplasticity and neural repair [22]. Specifically, the brain-derived neurotrophic factor (BDNF) Val66Met polymorphism, which affects activity-dependent BDNF expression, has been shown to alter CSE responses following neuromodulation treatments and potentially impact post-stroke recovery [23,24]. However, whether this polymorphism influences CSE responses to CE in stroke patients remains unexplored.

This randomized controlled trial (RCT) aims to investigate, for the first time, the acute and chronic effects of CE training on CSE in individuals during early subacute stages post-stroke, and to examine any potential associations with functional recovery outcomes, as well as the influence of the Val66Met polymorphism.

Methods and Materials

Experimental Design

In this registered RCT (NCT05076747), participants were randomly assigned in a 2:1 ratio to either an 8-week CE training in addition to standard care or standard care alone **(Figure 1)**. All assessments occurred at baseline (T0), four weeks (T1), and eight weeks (T2). Each assessment comprised three experimental sessions 48 hours apart, covering clinical motor outcomes, cardiorespiratory fitness, and CSE measures. Chronic and acute CSE measures were assessed at rest and following a 15-minute standardized high-intensity interval exercise (HIIT) session, respectively. Self-reported physical activity levels were measured at each time point using the physical activity scale for people with disabilities (PASIPD) [25]. Participants were instructed not to engage in moderate- or high-intensity physical activity 24 hours before assessments. Information regarding participant's characteristics and relevant clinical information was collected at T0. The local ethics review board approved the study (Centre de Recherche de Readaptation du Montréal, CRIR-1265-0817), and all participants provided written informed consent.

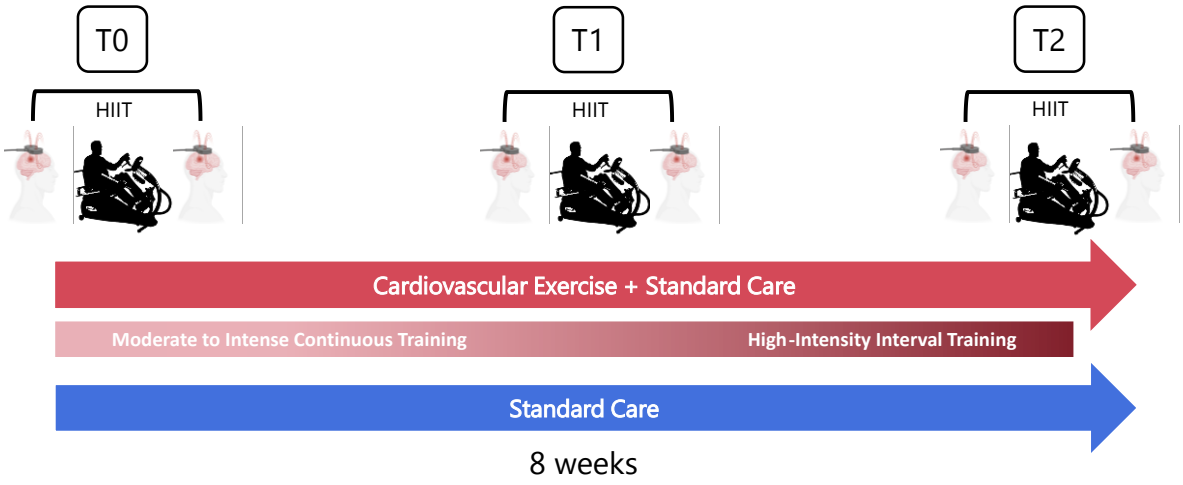

Figure 1. Study design with TMS evaluations at baseline (T0), four weeks (T1), and eight weeks (T2). To measure the chronic effects of CE training on CSE measures, TMS was applied at rest on both hemispheres at each time point. Acute CSE changes were determined by measuring the difference between pre- and post- a 15-minute HIIT session (post-HIIT–pre-HIIT), with loads individually adjusted based on a previous GXT. Acute responses were measured at T0 combining both groups and in response to both the CE+standard care and standard care groups at each time point. GXT: graded exercise test, HIIT: high-intensity interval training.

Patients

We included participants with first-ever ischemic stroke within the early subacute (7 days-3 months) stages of recovery [26]. Participants had to be between 40 to 80 years old, present no musculoskeletal or neurological conditions other than stroke, have sufficient ability/capacity to perform the exercise protocols, sufficient cognitive/communicative capacity to perform the protocol and understand instructions safely, and no TMS contraindications [19]. Individuals were excluded if they had a hemorrhagic stroke, cognitive impairment/dysphasia affecting informed consent, absolute contraindications to exercise, or were concurrently enrolled in another exercise program. Participants were categorized into cortical, subcortical, and cerebellar stroke groups using neuroimages acquired during acute stages (<5 days after stroke) confirmed by a clinical radiologist.

Assessments

Baseline Assessment

At baseline (T0), stroke severity and cognitive status were assessed with the National Institutes of Health Stroke Scale (NIHSS) [27] and the Montreal Cognitive Assessment (MoCA), respectively [28]. Additionally, the Charlson Comorbidity Index (CCI) age-adjusted was employed to assess pre-existing comorbidities, with higher scores indicating a greater comorbidity burden [29].

Cardiorespiratory Fitness

Measurement of peak oxygen uptake (VO_2peak in $mL.Kg^{-1}.min^{-1}$) during a graded exercise test (GXT) is the gold standard for determining cardiorespiratory fitness [30]. A symptom-limited GXT utilizing a whole-body recumbent stepper (NuStep T4r, Michigan, USA), validated for individuals with stroke, was performed [31]. During the GXT, with resistance levels rising in 2-minute blocks, heart rate (HR) was measured continuously while blood pressure and rate of perceived exertion (RPE 0-10) were taken every 2 minutes [32]. Electrocardiography was utilized to monitor cardiac function in individuals with cardiac comorbidities. The GXT was used to determine VO_2peak, along with its associated maximal HR values (HR_{max}) and peak power output (PPO). VO_2peak was the highest recorded value of oxygen consumption during the test. PPO expressed in Watts was used to adjust training loads based on the capacity of each individual [33]. The GXT was terminated if participants reached volitional fatigue, met any absolute termination

criteria per current guidelines, or failed to maintain a cadence of at least 80 steps per minute after two warnings [34].

Clinical Motor Outcomes

Trained assessors evaluated clinical outcomes, including upper-limb motor impairment and function. Upper-limb motor impairment changes were assessed with the Upper-Limb Fugl-Meyer Assessment (UL-FMA) (scores ranging from 0-64), with higher scores indicating lower impairment [35]. The UL-FMA assesses various arm and hand motor components, including reflex activity, movement patterns, coordination, and sensation. Changes in upper-limb function were assessed with the Box and Block Test (BBT) on both sides [36]. To this end, participants were instructed to move as many small wooden blocks as possible from one side of a partitioned box to the other within one minute. This test assesses manual dexterity, hand function, and arm strength.

Maximal Voluntary Contraction

We measured grip strength because this was needed for TMS protocols measuring CSE during an active muscle contraction (see CSE section for details). Handgrip strength was assessed with a maximal voluntary contraction (MVC) of the muscles of the affected and nonaffected hand using a custom script built on LabView (National Instruments, Austin, TX, USA). Patients were seated in front of a 27-inch computer screen, grasping a grip force response pad with their hand in a neutral, semi-prone position. A slider displaying the force applied was shown on the screen. Patients made a fist, "squeezing" the force pad as intensely as possible. They performed 3 MVCs of approximately 3 seconds each, separated by a 30-second pause. The highest MVC was recorded and saved.

Transcranial Magnetic Stimulation

Using neuronavigation (Brainsight, Rogue Research Inc., Montreal, QC, Canada), we first co-registered the patients' heads to a standard magnetic resonance image template to identify and mark the optimal coil position ("hot-spot") of M1 for eliciting motor-evoked potentials (MEPs). TMS was applied through a 70-mm coil with a Magstim BiStim stimulator (Magstim, Whitland, Wales, UK) oriented posteriorly at 45º to the midsagittal line [19] on the M1 representational area of the first dorsal interosseous muscle in both the ipsilesional and contralesional hemispheres. This muscle has a low resting motor threshold (MT) [37]. This facilitates MEPs to be elicited at relatively

low stimulation intensities, which is important in patients with stroke, who usually require higher intensities [19]. The "hot-spot" was determined through a mini-mapping procedure identifying the coil position that elicited the largest possible MEP amplitudes obtained at a fixed suprathreshold intensity. Electromyographic activity, recorded via two surface electrodes (Ambu Neuroline 70,010-K/12) placed over the first dorsal interosseous at ~1 cm of distance, was acquired through a CED Micro1401-4 data acquisition unit and controlled by Signal software (CED, Cambridge, UK) at 2000 Hz with a gain of 300 and filtered using a high- and low-pass cut off filter of 10 Hz and 500 Hz, respectively [38]. The level of background muscle activity was monitored continuously and trials with any excessive activity (>0.05 mV) 300 ms before stimulation were removed from analysis.

Corticospinal Excitability

The experiments took place in a room with dimmed lighting, where participants were seated in a semi-reclined chair. During the evaluation, they were instructed to relax, close their eyes, and rest their arms on a height-adjustable desk. The positions of the arms and the chair angle were measured with a goniometer at T0 and replicated at T1 and T2. To investigate the acute CSE responses to CE, TMS was performed before and 10 minutes after finalizing a 15-minute standardized HIIT protocol. The exercise protocol, which started and finished with 3 minutes of warm-up and cool-down at 15% PPO, consisted of six 30-second blocks of high-intensity at 90% PPO (3 minutes), interspersed with six 1-minute blocks at low intensity at 30% PPO (6 minutes). This protocol allows sustained high-intensity levels while minimizing excessive fatigue [39]. Participants were instructed to maintain a minimum stepping cadence of 80 steps/min [40], and intensity was continuously monitored through Watts, HR, and RPE. Except for resting MT, which was measured only at rest, the following CSE measures were obtained both before and immediately after HIIT through single or paired-pulse TMS protocols [41].

Single-pulse stimulation measures. *Resting motor threshold:* once we identified the "hot-spot," we determined the resting MT, defined as the minimum stimulation intensity required to elicit MEPs of >0.05 mV in at least 10 of 20 trials [19]. The resting MT is represented as a percentage of the stimulator output capacity and reflects neural membrane excitability of cortical axons, with higher resting MT representing lower CSE [17]. *MEP amplitude:* MEP amplitudes were assessed both at rest and during an active muscle contraction sustained at 20% of the MVC. To assess active

excitability, the LabView script used to measure MVC provided visual feedback while participants were asked to maintain the muscle contraction at the 20% MVC force level. MEP amplitude was quantified by measuring the average peak-to-peak of the MEP amplitude in 60 stimulations (30 resting and 30 active) elicited at an intensity of 120% resting MT [42]. The MEP amplitude quantifies excitability of cortical and spinal projections regulated by both excitatory (glutamate) and inhibitory (γ-aminobutyric acid, GABA) circuits [43] with larger MEP amplitudes reflecting higher CSE [44]. To minimize the potential effects of repetitive TMS on CSE, each stimulation was delivered 5 seconds apart. *Cortical silent period*: the CSP is a period of electrical silence in the surface EMG activity that occurs immediately after an MEP is elicited during an isotonic muscle contraction [19]. When elicited at relatively high intensities of stimulation (i.e., generating CSPs >100 ms), CSP provides information about the inhibitory activity modulated by $GABA_B$ receptors [19], with longer CSP reflecting greater inhibition [19]. The CSP was obtained from the 30 stimulations elicited at 120% resting MT to measure active MEP amplitudes. We used the EMG baseline signal amplitude measured 200 ms before stimulation to calculate the CSP, spanning from the end of the MEP until the recovery of the voluntary electromyographic activity (i.e., increase of two standard deviations above the mean baseline signal amplitude) [40]. The accuracy of the CSP detection was confirmed through visual inspection and the average of CSP duration was calculated.

Paired-pulse stimulation measures. *Intracortical facilitation and short intracortical inhibition:* Intracortical facilitation (ICF) provides information on facilitation mediated by N-methyl-d-aspartate (NMDA) and glutamatergic neural activity, while short intracortical inhibition (SICI) reflects inhibition mediated by phasic $GABA_A$-related signaling [43]. These two parameters were measured with a paired-pulse TMS protocol in which a conditioning pulse (80% resting MT) was followed by a suprathreshold pulse (120% resting MT) delivered after 10 (ICF) and 2 (SICI) milliseconds (ms), respectively [45]. The amplitude of the MEP elicited by the second pulse was normalized to the previously measured resting MEP amplitudes to estimate facilitation and inhibition [40]. Sixty paired-pulses (30 for ICF and 30 for SICI) were delivered with an interstimulus interval of 5 seconds [19].

Genotyping

DNA was extracted from red blood cells and saliva samples (DNA Genotek Inc., Canada), and genotyped using the Infinitum™ Global Diversity Array-8 v1.0 from Illumina. DNA

extraction and purification were processed by Genome Quebec (Quebec, Canada) using the QIAsymphony system (QIAGEN). Sixty-eight individuals were genotyped with sufficient DNA concentration for reliable genotyping (10ng/ul). Standard quality control was performed using PLINK v1.9 to exclude SNPs with high missingness in individuals (>5%). The genotype of subjects for the BDNF single nucleotide polymorphism rs6265 was classified as homozygous for the Val allele (Val/Val), heterozygous (Val/Met), and homozygous for the Met allele (Met/Met) using PLINK v1.9 **(Table 1)**. Individuals with Val/Met and Met/Met genotypes were combined to increase statistical power [46].

Intervention

Cardiovascular Exercise

Training sessions were performed at the Jewish Rehabilitation Hospital, Laval, Canada by trained therapists. The intervention group underwent a total of 24 CE training sessions over an 8-week period, with a frequency of 3 times a week and a 48-hour rest between sessions whenever possible to avoid overtraining. In the event of missed training sessions, makeup sessions were offered after the program. The CE intervention comprised four weeks of progressive moderate-to-vigorous intensity continuous training (MICT) followed by four weeks of progressive high-intensity interval training (HIIT), all conducted on a whole-body recumbent stepper ergometer. The initial four weeks of MICT served as preparation for higher intensities in the second half of the program. Each training session included 2.5 minutes of warm-up and cool-down at 35% of the PPO, along with the main training component at the targeted intensity. Blood pressure was measured at the beginning and end of each CE session. HR, and Watts were continuously monitored during training sessions via a pulse sensor (Polar H10, Kempele, Finland) and the stepper's digital console, respectively [47]. RPE (0-10) was assessed every 5 minutes throughout each training session during the MICT period, including at the end of the warm-up and the beginning of the cool-down, while during HIIT, RPE was collected in the final 5 seconds of each high-intensity bout with the modified Borg scale[32]. Training variables, including the average percentage of maximal HR (%HR_{max}) and PPO relative to the previous GXT, along with total step count, and average RPE, were calculated for each session to quantify the internal and external training workloads **(Table 2)** [48].

Moderate-to-vigorous Continuous Training (weeks 1-4): MICT has been typically employed as standard CE training in stroke rehabilitation programs [12]. Intensities were determined using the PPO associated with VO_2peak during the GXT at T0 and progressively increased by 5% weekly from 65% to 80% PPO, ensuring constant cardiovascular adaptations. Session durations also increased from 20 to 35 minutes. This intensity progression has been demonstrated as achievable and safe for individuals in subacute stages of recovery [49].

High-intensity Interval Training (weeks 5-8): This protocol, which has proven to be safe and effective for enhancing cardiorespiratory fitness in subacute and chronic stroke patients, enables even deconditioned individuals to reach higher exercise intensities [50]. HIIT intensities were determined using PPO corresponding to VO_2peak achieved during the GXT at T1. The HIIT protocol comprised 8 x 60-second high-intensity intervals (8 minutes) interspersed with 7 x 60-second low-intensity intervals (7 minutes), totaling 20 minutes per session. This 60:60 interval ratio is optimal for sustaining high intensities in stroke patients [39]. High-intensity intervals began at 85% PPO and increased by 5% weekly until reaching 100% PPO, while low-intensity intervals were set constantly at 35% PPO. To minimize sudden changes in BP while ensuring target intensity, the workload was progressively increased (15 seconds) before each high-intensity interval.

Standard Care Program

Standard care consisted of rehabilitation sessions conducted in the same center as the intervention and prescribed by the stroke clinical unit. In addition to routine health monitoring by physicians and nursing staff, standard care included physiotherapy, occupational therapy, and speech therapy sessions. The content, amount, and length of rehabilitation varied among patients and was tailored to individual needs by the stroke clinical unit. Each session consisted of 45-minute sessions of therapy. Therapists were blinded to the group allocation. To identify potential differences between groups in standard care, we recorded the type and number of therapy sessions received by each patient from the study's beginning to its conclusion **(Table 1)**.

Statistical Analysis

Data were inspected using normality plots and histograms. The Shapiro-Wilk test confirmed normality for each variable. Baseline differences in participant characteristics and

clinical variables between groups were assessed using t-tests or Wilcoxon tests for continuous variables. Chi-square tests (X^2) were used to compare groups in categorical variables.

Linear mixed models (LMM) were used to analyze differences in clinical motor outcomes (FMA, BBT), cardiorespiratory fitness, and CSE measures between groups across time points (T0-T2). Group differences for each CSE measure were analyzed separately for both ipsilesional and contralesional hemispheres. Chronic CSE changes were assessed at rest, while acute changes were determined as the change following 15-minute HIIT (*post-HIIT–pre-HIIT*). Each model included either acute or chronic CSE measures as a dependent variable, with time point (T0, T1, T2), group, and their interaction as fixed effects. Covariates included age, sex, handgrip MVC, and stroke severity. To examine the potential influence of the Val66Met polymorphism (Val/Val vs. Val/Met + Met/Met) in the model, it was nested within the Time x Group interaction. Exploratory sensitivity analyses compared individuals with cortical, subcortical, and cerebellar lesions, examining their impact on chronic and acute CSE responses in both groups. Detailed findings are provided in the supplementary files. Participants were treated as a random effect to account for baseline differences and an intention-to-treat approach was used for those who were assessed at least at T1. Auto-Regressive order 1 (AR1) was set as the repeated covariance structure.

Standard least squares multivariate linear regression, adjusted for the same covariates as the LMM, was used to investigate associations between CSE measures and changes in clinical motor outcomes and cardiorespiratory fitness for each group. Linear model assumptions were checked for residual normality. Extreme outliers were defined as values more than 3 times the interquartile range from the 0.1 and 0.9 quantiles of the data. Multicollinearity between predictor variables was assessed with the variance inflation factor (VIF) with a threshold of ≤ 5, indicating unacceptable multicollinearity [51]. Statistical analyses were performed with JMP (SAS Institute Inc, Cary, NC), version 17, and tested for significance at 0.05 alpha level ($p<0.05$).

Results

Table 1 displays participant characteristics and clinical information at baseline (T0). Of the 76 enrolled participants, 48 were randomized to the CE+standard care group and 28 to the standard care group. The trial flow, including dropouts, is shown in **Figure 2**. No training-related adverse events were reported.

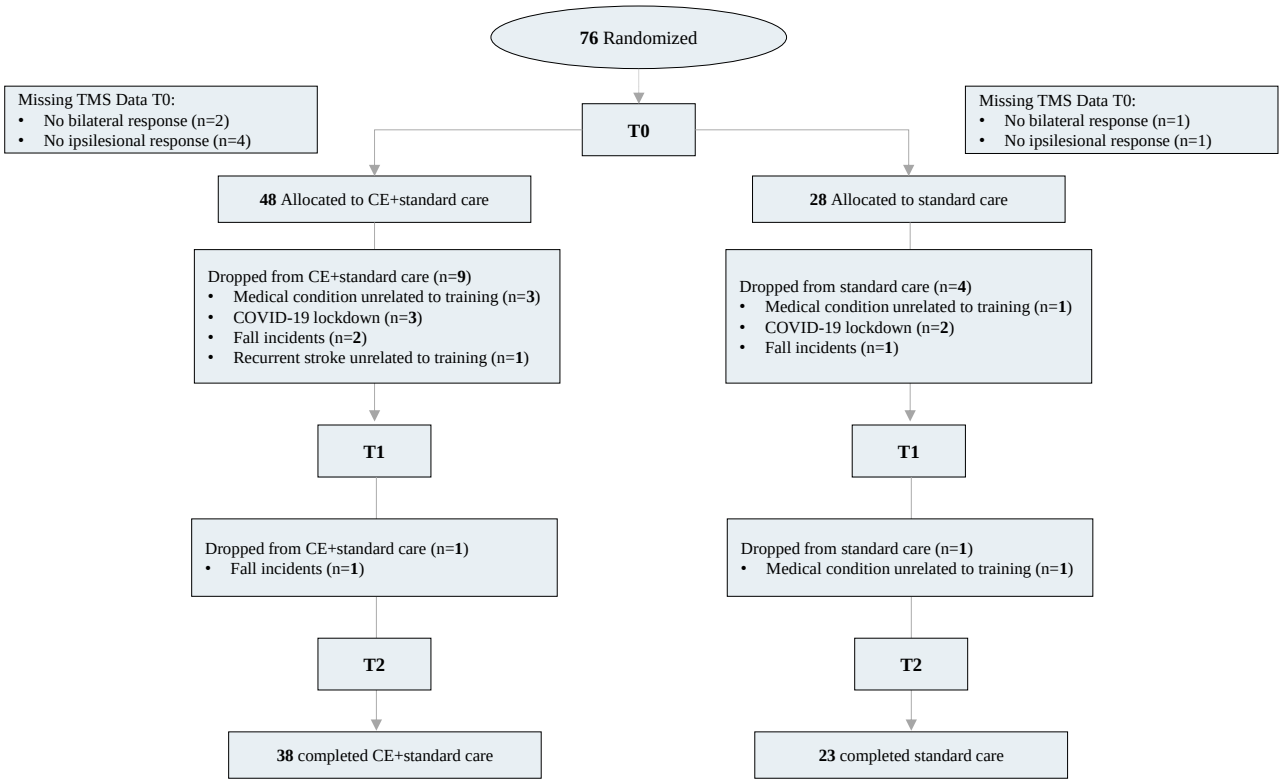

Figure 2. Flow chart of the Randomized Controlled Trial. CE: cardiovascular exercise, n: number of participants, TMS: transcranial magnetic stimulation, T0: baseline, T1: four weeks, T2: eight weeks.

On average, participants were 63.5±10.2 years old (mean ± SD) and initiated the study 65.1±22.8 days after stroke. Participants presented mild stroke severity (NIHSS=2.01±2.09) and an average MoCA score of 23.8±4.48. No significant differences were observed at T0 between groups in terms of age, sex, body mass index (BMI), time since stroke, lesion location, stroke severity, cognitive status, upper-limb impairment and function, pre-existing comorbidities (measured with age-adjusted CCI), walking aid dependence, smoking history, and the average number of prescribed medications (**Table 1**). The amount of standard care, including physiotherapy, occupational therapy, and speech therapy, as well as levels of physical activity outside the rehabilitation center (measured with the PASIPD), were similar between groups from T0 to T2. All participants assigned to the CE group who completed the study attended all 24 sessions.

Table 1. Baseline demographic and clinical outcomes.

	CE + Standard Care (n= 48)	Standard Care (n= 28)	p value
Age (years)	63±11.39	65.35±8.68	0.347
Sex (F/M)	11/37	10/18	0.290
BMI	27±3.46	26.52±4.01	0.624
Time since stroke (days)	68.12±22.07	58.75±24.03	0.088
Lesion location (%)			0.881
Cortical	36	36	
Subcortical	54	57	
Cerebellar	10	7	
NIHSS (0-42)	2.02±2.20	1.92±1.92	0.799
MoCA (0-30)	24.14±4.85	23.21±3.8	0.126
UL-FMA (0-66)	56.20±10.24	59.14±8.22	0.106
$BBT_{affected}$ (blocks/min)	46.8±13.25	48.10±12.65	0.678
$MVC_{affected}$	0.72±0.35	0.66±0.27	0.435
Cardiorespiratory fitness (VO_2peak, $mL.Kg^{-1}.min^{-1}$)	17.88±5.49	18.12±5.58	0.855
SNP rs6265			0.225
Val/Val	27	18	
Val/Met	13	7	
Met/Met	3	0	
CCI (Age-adjusted)	4.57±1.83	4.57±1.66	0.994
Walking aid dependence (%)	15	13	0.641
Smoking history (%)			0.242
Non-smoker	52	43	
Former smoker	40	56	
Current smoker	8	1	
Medications (n)	5.07±2.58	5±2.22	0.909
Classification (%)			
AC	60	53	
ACE	31	42	
AP	46	60	
BB	35	25	
PSY	33	28	
STA	79	100	
Therapy sessions (n)			
Physiotherapy	8.87±8.21	6.59±5.78	0.259
Occupational Therapy	11.5±8.26	7.45±5.98	0.051

Speech Therapy	5.02±8.81	2.15±5.48	0.212
Physical activity (METS hr/day)	8.37±4.99	9.26±6.44	0.259

AC, anticoagulant; ACE, angiotensin-converting Enzyme; AP, antiplatelet; BB, beta-blocker; BBT, box and block Test; BMI, body mass index; CCI, charlson comorbidity index; F, female; UL-FMA, upper-limb Fugl-Meyer assessment; M, male; MET, metabolic equivalent of task; Met, methionine; MoCA, montreal cognitive assessment; MVC, maximal voluntary contraction; NIHSS, national institutes of health stroke scale; PSY, psychoactive; SNP, single nucleotide polymorphism; STA, statin; Val, valine. Values are presented as mean ± standard deviation (SD) unless otherwise specified.

Table 2. Internal and external training workloads for the CE group.

	MICT	**HIIT**	**Total**
HR_{max} (%)	82.35±8.06	81.89±6.94	82.13±7.05
PPO (%)	63.69±8.74	67.49±13.74	65.59±10.96
Total Steps	29384±5932	20237±4366	49464±10183
RPE_{ave} (0-10)	4.58±1.31	5.15±1.61	4.86±1.35

CE group's average internal and external training loads during both MICT and HIIT periods, including the warm-up and cool-down phases of each session. Average percentages of HR_{max} and PPO achieved during both MICT and HIIT periods were calculated based on VO_2peak values at T0 and T1, respectively. Regarding RPE measurement, values were recorded every 5 minutes during the MICT period, including at the end of the warm-up and the beginning of the cool-down, while during HIIT, RPE was collected in the final 5 seconds of each high-intensity bout. Values are presented as mean and SD. HIIT, high-intensity interval training; HR, heart rate; MICT, moderate-to-vigorous continuous training; PPO, peak power output RPE, rate of perceived exertion. Values are presented as mean ± SD.

Cardiorespiratory Fitness and Clinical Motor Outcomes

No significant differences in cardiorespiratory fitness were observed between groups at T0. On average, all participants initially presented a VO_2peak of 18.43±5.63 $mL.Kg^{-1}.min^{-1}$. There was a significant effect of Time ($F(2,78)= 16.76$, $p <.0001$), and Time x Group interaction ($F(2,78)=13.46$, $p <.0001$). While no significant within-group VO_2peak changes were reported in the standard care group (+0.27 $mL.Kg^{-1}.min^{-1}$, 95% CI -2.19 to 1.64, p=0.998), the CE+standard

care group exhibited significant increases at T1 during MICT (+2.76 $mL.Kg^{-1}.min^{-1}$, 95% CI 1.58 to 3.93, p <.0001), and at T2 following HIIT (+1.64 $mL.Kg^{-1}.min^{-1}$, 95% CI 0.45 to 2.82, p <.0001), resulting in a total average increase of 4.43 $mL.Kg^{-1}.min^{-1}$ (95% CI 2.97 to 5.82, p <.0001), representing a +27.25% improvement in cardiorespiratory fitness.

There was a significant effect of Time on upper-limb motor impairment using the UL-FMA (F(2, 99)=15.61, p=0.0001), with no significant Time x Group interaction (F(2, 99)=1.04, p=0.355). Similarly, for upper-limb function measured using the BBT, there were significant effects of Time (F(2, 116)=15.73, p <.0001) but no significant Time x Group interaction (F(2, 116)=0.22, p=0.801).

Acute and Chronic Effects of Cardiovascular Exercise on Corticospinal Excitability

Except for three individuals from whom MEP responses were not elicited in either the ipsilesional or contralesional hemispheres, TMS data was collected from all participants **(Figure 2)**. Data from five participants who exhibited only contralesional responses were also included in the analysis. Chronic and acute changes for each CSE measure for both groups are detailed in **Supplementary Tables 3 and 4**, respectively.

No statistically significant differences were observed between groups for any CSE measures at T0. When comparing ipsilesional and contralesional hemispheres, no significant differences were found for any CSE measures, except for the CSP, which was significantly prolonged ipsilaterally at all time points compared to the contralesional hemisphere (F(1, 291.3)=53.63, p<.0001), suggesting increased ipsilesional inhibition. These differences remained significant after adjusting for handgrip MVC and group.

When combining both groups at T0 **(Figure 3)**, a 15-minute HIIT session resulted in significant acute increases in resting (F(1,70) = 7.77, p = 0.006) and active (F(1,70) = 7.43, p = 0.008) MEP amplitudes on the contralesional side, with no significant changes in CSE measures on the ipsilesional hemisphere.

No significant effects of Time or Time x Group interaction were found for chronic CSE responses in either ipsilesional or contralesional hemispheres **(Figure 4, Supplementary Table 1)**. Similarly, no significant acute effects of Time or Time x Group interaction were identified for

any CSE measures over time in either ipsilesional or contralesional hemispheres **(Figure 5, Supplementary Table 2)**.

Sensitivity analyses across different lesion groups (cortical, subcortical, cerebellar) found no significant impact of lesion location on either acute or chronic CSE responses to CE training (**Supplementary Tables 3-4**).

We also analyzed the influence of Val66Met polymorphism on CSE responses and changes in recovery outcomes. BDNF Val66Met polymorphism did not affect clinical recovery outcomes or CSE responses to CE training. However, combining both groups at T0, Val carriers exhibited significantly larger increases in resting MEP amplitudes in the ipsilesional hemisphere after a HIIT session ($F(1,60) = 5.83$, $p = 0.0188$) compared to Met carriers **(Supplementary Tables 5-7, Figure 13**).

Associations between Clinical Outcomes and Corticospinal Excitability

We investigated the potential associations between chronic and acute CSE responses and changes in cardiorespiratory fitness and clinical motor outcomes, finding no significant associations in either group (**Supplementary Tables 8-9**).

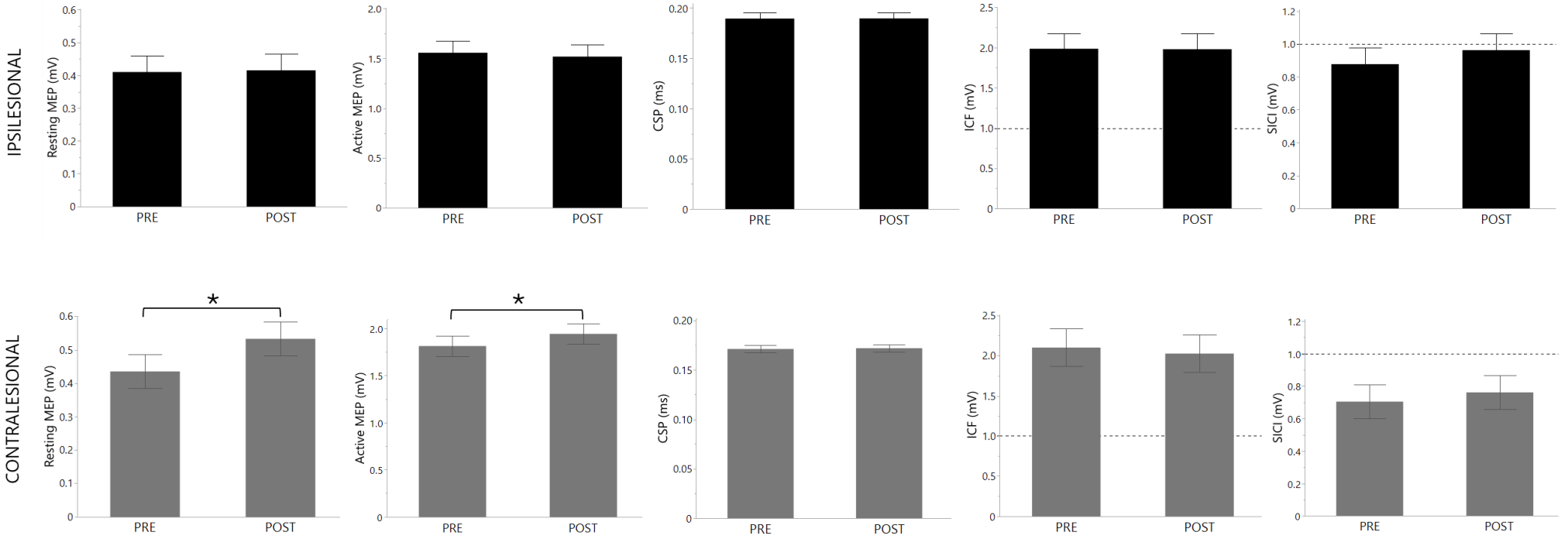

Figure 3. Acute CSE changes (PRE-POST) in response to a HIIT session at T0 (n=73). Following a 15-minute HIIT session, resting and active MEP amplitudes expressed in millivolts showed significant acute increases on the contralesional hemisphere, while no changes were observed ipsilesionally. * $p<0.05$. Dotted lines in intracortical facilitation and inhibition represent facilitation (>1) and inhibition (<1) thresholds, respectively. Data is presented as least squares means and error bars are standard errors of the means. CSP, cortical silent period; ICF, intracortical facilitation; MEP, motor evoked potential; ms, millisecond; mV, millivolt; SICI, short-intracortical inhibition.

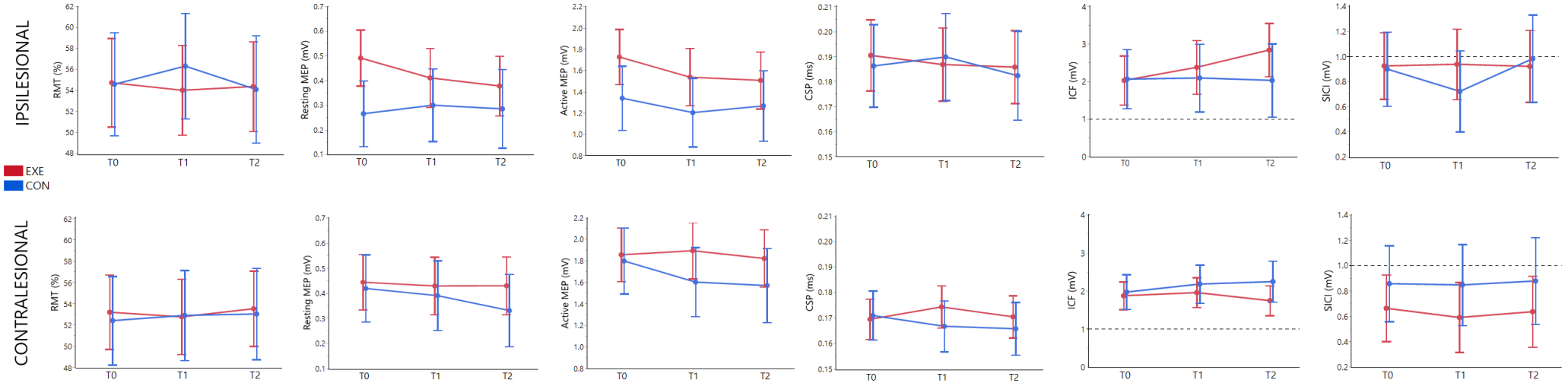

Figure 4. Chronic CSE changes in CE+standard care (EXE) and standard care (CON) groups through time points (T0,T1,T2). Dotted lines in intracortical facilitation and inhibition represent facilitation (>1) and inhibition (<1) thresholds, respectively. Data is presented as least squares means and error bars are standard error of the means. CSP, cortical silent period; ICF, intracortical facilitation; MEP, motor evoked potential; ms, millisecond; mV, millivolt; SICI, short-intracortical inhibition.

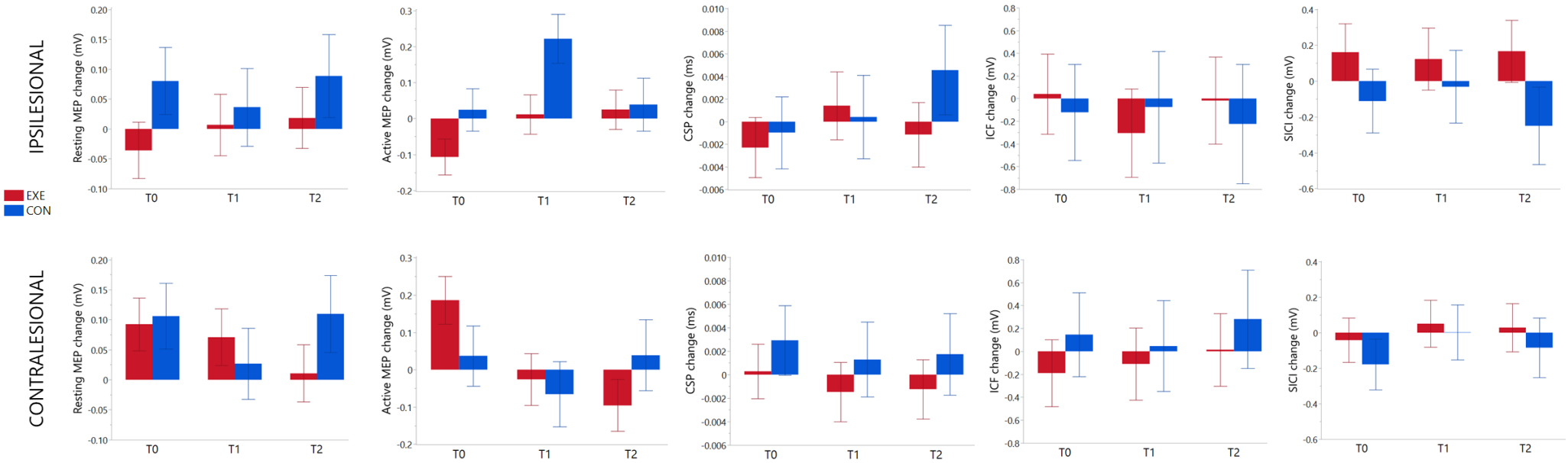

Figure 5. Acute CSE changes in CE+standard care (EXE) and standard care (CON) groups through time points following a single HIIT session (T0,T1,T2). Data is presented as least squares means and error bars are standard error of the mean. CSP, cortical silent period; ICF, intracortical facilitation; MEP, motor evoked potential; ms, millisecond; mV, millivolt; SICI, short-intracortical inhibition.

Discussion

We investigated the acute and chronic effects of cardiovascular exercise on motor cortex neurophysiology in individuals during the subacute stages of stroke recovery. Using single and paired-pulse TMS protocols, we assessed measures indicative of corticospinal and intracortical excitability in subacute patients undergoing eight weeks of exercise training alongside standard care, compared to a control group receiving standard care alone. Specifically, we examined: 1) the acute effects of a single HIIT session on CSE; 2) the chronic effects of CE training on CSE; and 3) the effects of CE training on the acute CSE response following a HIIT session. We also investigated whether these CSE responses were associated with changes in recovery motor outcomes or influenced by the BDNF Val66Met polymorphism following either CE training or standard care. In the next sections, we will discuss our findings in detail, focusing on the potential mechanisms underlying the observed effects and their implications for stroke recovery.

Acute CSE Responses to HIIT in Subacute Individuals after Stroke

Studies using TMS in neurotypical individuals have demonstrated that a single session of CE can modulate specific CSE parameters, with higher intensities evoking larger increases [52]. In neurotypical individuals, increased CSE following a brief, high-intensity CE session has been associated with motor skill learning [38], suggesting a potential mechanism for CE-induced motor gains [53]. In our study, we combined baseline data from both groups at baseline (T0) and found CSE increases in the contralesional hemisphere via resting and active MEP amplitudes following a 15-minute HIIT session, with no changes observed ipsilesionally **(Figure 3)**. These findings contrast with other stroke studies focusing on chronic stages, which have reported that the ipsilesional, but not contralesional hemisphere, can be modulated acutely through single high-intensity CE bouts [54]. Specifically, MEP latencies, MTs, and MEP amplitudes have been shown to respond to short, intense CE bouts, with higher intensities evoking more pronounced CSE increases ipsilesionally [55-57].

Discrepancies between chronic and subacute stages may be explained by the time-dependent neurobiological processes characterizing each post-stroke timepoint [58]. During the initial days to weeks post-stroke, neural connections are disrupted, resulting in diminished responses to afferent inputs in areas surrounding the lesion, causing an overall state of hypo-

excitability [59]. This phenomenon is observed in animals but also in humans, where reduced or even absent CSE is detected in the ipsilesional hemisphere during the acute and early subacute phases [60]. As recovery progresses, neuronal excitability begins to reemerge in affected neural networks yet remains reduced even in patients with significant recovery [61-66].

One factor contributing to this reduced CSE and impacting the responsiveness to a single HIIT session ipsilesionally is the increased inhibitory activity typically occurring during early stages of recovery. Early stages are characterized by an increase in GABAergic tone by approximately 50% in areas adjacent to the stroke, primarily via tonic extrasynaptic GABA signaling [67]. This inhibition serves as a neuroprotective mechanism against excitotoxic signaling during acute stages [68,69], but when prolonged excessively, this inhibitory effect can reduce the propensity of neurons to fire in response to excitatory stimuli, hindering essential neuroplastic processes for recovery [70,71]. Indeed, reducing tonic GABA signaling in rodents through genetic or pharmacological blockade during early stages has been shown to promote motor recovery by restoring excitability in neural circuits [71-74].

Our study found differences in M1 inhibition between brain hemispheres. Specifically, ipsilesional CSP, but not SICI, remained significantly elevated compared to the contralesional hemisphere at baseline (T0), 4 weeks (T1), and 8 weeks (T2), suggesting increased $GABA_B$ receptor-mediated activity throughout the subacute stages. Notably, these interhemispheric differences in CSP remained significant even after controlling for the intervention group and the MVC of the hand contralateral to the lesioned hemisphere, a covariate used to calculate CSP and often affected by stroke [19]. These findings coincide with previous evidence indicating increased ipsilesional $GABA_B$ but not $GABA_A$ synaptic activity in subacute post-stroke individuals [75]. This could be due to the fact that tonic extrasynaptic inhibition, rather than synaptic (phasic) inhibition, appears to be increased ipsilesionally after stroke [72], a molecular event undetectable through the 2ms interstimulus interval SICI protocol used in this study [76] but indirectly captured through CSP. Preclinical studies have demonstrated that activation of post-synaptic $GABA_B$ receptors, the mechanism underlying CSP, enhances tonic extracellular inhibitory currents, reducing in turn neural excitability [77]. Based on these findings, CSP might have captured the elevated extrasynaptic inhibition in the ipsilesional hemisphere, explaining why the affected corticomotor pathway may be less susceptible to the potential excitatory effects of CE. Future studies should aim to validate

this hypothesis using techniques that allow measuring extracellular neurotransmitter levels, such as magnetic resonance spectroscopy (MRS) or variations of SICI protocols [76,78].

The absence of acute changes in intracortical excitability measures following a HIIT session is consistent with prior studies involving stroke patients [40,55-57] but contrasts with evidence from neurotypical populations, where increased ICF and decreased SICI have been observed following single CE bouts [79-83]. Although no studies have directly compared the two population groups, these findings suggest a limited capacity of CE to modulate the facilitatory-inhibitory cortical balance in stroke patients, a required precursor for neuroplastic changes supporting recovery [84,85].

Effects of Cardiovascular Exercise Training on Chronic and Acute CSE Responses

While a single exercise session can provide valuable insights into the transient neurophysiological responses to CE, longitudinal interventions are necessary to determine lasting neuroplastic changes [13]. In this study, we measured CSE at rest and immediately after a single bout of HIIT to examine the chronic and acute responses to CE training, respectively. This latter approach is supported by both animal and human studies, indicating that repetitive CE sessions can amplify the acute response to a single CE session in neuroplasticity biomarkers such as BDNF [86-88]. Similar adaptive responses have also been suggested in humans, where physically active individuals exhibit enhanced acute responses following neuroplasticity-inducing interventions, such as brain stimulation [89] and CE [90], compared to sedentary populations.

No effects of CE were observed on the chronic CSE response following eight weeks of training **(Figure 4)**. Additionally, CE training did not significantly impact the acute response to HIIT at either T1 or T2 time points **(Figure 5)**. These findings contrast with pre-clinical evidence indicating increases in neuronal markers related to excitability, such as synaptogenesis and dendritic branching, following repetitive CE sessions [14]. In human stroke survivors, although only limited evidence exists, two studies involving participants in the late-subacute (3-6 months) and chronic stages (>6 months) reported increases in CSE after four weeks of gait training compared to standard care. Specifically, CE gait training resulted in reductions in resting MT on ipsilesional [91] and contralesional [92] hemispheres, as well as bilateral increases in cortical map size for lower-limb muscle cortical representations. Furthermore, one small study (n=11) suggested adaptive responses to CE training, showing enhanced CSE responses ipsilesionally following a single

vigorous-intensity exercise session in a treadmill-trained group of chronic stroke patients compared to an untrained group [93].

However, whether the changes in CSE are directly attributable to the training stimulus and adaptations from the CE intervention remains unclear in these studies, as they did not monitor training workloads or employ an exercise test to measure any improvements in cardiorespiratory fitness [94]. Our results indicate that the effects of CE training on CSE were unrelated to the effectiveness of our intervention in improving cardiorespiratory fitness levels. Compared to the control group receiving standard care alone, our progressive 8-week CE training program significantly improved cardiorespiratory fitness, with average VO_2peak increases of 4.43±3.24 $mL.Kg^{-1}.min^{-1}$, representing a 27.2% enhancement in fitness levels. These improvements surpassed previously reported values in subacute stroke populations undergoing high-intensity CE training interventions (+1.46 $mL.Kg^{-1}.min^{-1}$) [95], as well as the minimal clinical important difference (MCID) for VO_2peak (3.0 $mL.Kg^{-1}.min^{-1}$), which has been associated with reduced cardiovascular mortality [96], stroke hospitalization [97], and ischaemic stroke risk [98]. Additionally, no significant associations were identified between CSE responses and changes in cardiorespiratory fitness (VO_2peak) in either the CE or control groups, confirming that an insufficient training stimulus was unlikely the cause of the null effects on CSE outcomes. These findings are consistent with the only CE study to date examining chronic CSE responses in neurotypical individuals, which revealed no significant CSE changes following six weeks of intensive cycling training, despite notable improvements in cardiorespiratory fitness [99]. Together, these findings suggest that while CE training confers significant benefits in cardiorespiratory fitness, it may have limited effects on the excitability of central motor pathways and intracortical circuits, at least in the subacute stages of recovery.

Influence of Intervention Specificity on CSE Responses to Training

One possible reason for the absence of CSE effects following CE training could be the lack of specificity of our intervention. Unlike task-specific rehabilitative treatments such as gait or upper-limb motor training, which are based on motor learning principles, our intervention was primarily designed to induce physiological and neurophysiological adaptations through whole-body exercise using recumbent steppers, rather than improving a specific task [100,101].

Evidence from animal and human studies suggests that nonspecific repetitive movement activity alone, without any motor learning component, does not induce significant plastic changes in corticospinal motor circuits [102-105]. This may also apply to stroke motor rehabilitation, where nonspecific movement alone does not seem to induce the same degree of neuroplastic and functional recovery changes as when is paired with targeted, intensive, goal-oriented training [106-111]. This aligns with the observation that, along with no CSE changes, no significant effects on upper-limb motor outcomes were seen following CE, suggesting that treatment specificity and goal-oriented approaches are crucial both neurophysiologically and behaviourally [100,101].

Interestingly, using the same mode of CE intervention as in this study, we recently reported CSE increases in the ipsilesional hemisphere following 12 weeks of whole-body training [112]. This was observed in the largest RCT to date (n=56) measuring chronic CSE responses to CE training in people during chronic stages post-stroke. Although these findings may contradict the specificity hypothesis, they could indicate that nonspecific CE interventions alone may not be sufficient to counteract the unique neurobiological processes occurring during the early subacute stages, such as GABAergic inhibitory activity, thus hindering the capacity to induce neuroplastic and neural repair processes. Cardiovascular exercise has demonstrated to protect and maintain brain function via neurobiological mechanisms involved the nervous system [113]. Paired with goal-oriented rehabilitative treatments, CE could serve as a priming intervention to promote a neural environment conducive to neuroplasticity and functional recovery [108,111]. Future clinical studies investigating the potential synergetic effects of combining CE training with goal-oriented motor training in subacute phases of recovery are warranted [114].

Influence of BDNF Val66Met Polymorphism on CSE Responses to Cardiovascular Exercise Training

Brain-derived neurotrophic factor (BDNF) is the most abundant neurotrophin in the brain, playing a vital role in rehabilitation-induced recovery after stroke by promoting brain repair and activity-dependent forms of synaptic plasticity [115]. The expression of this protein mediates excitatory signaling and structural neural changes through AMPA and NMDA-type glutamate receptors [116]. Indeed, during early stages post-stroke, ipsilesional synaptic excitability changes underlying motor recovery have been shown to be mediated by BDNF release [117-119].

Consequently, genetic variants affecting BDNF protein transcription could influence neuroplastic responses to interventions such as CE, thereby impacting recovery [120].

In this study, we investigated the influence of the BDNF Val66Met single nucleotide polymorphism (rs6265)—which alters activity-dependent BDNF secretion and affects neuroplastic processes related to brain function [121,122]—on CSE responses following CE in subacute stroke patients. The Val66Met polymorphism of the BDNF gene encodes a substitution of a valine (Val) to methionine (Met) at codon 66, resulting in three genotypes (Val/Val, Val/Met, and Met/Met), with individuals carrying one or two copies of the Met allele potentially showing reduced neuroplastic responses to interventions like CE [123].

Consistent with Caucasian population frequencies [124], among the 68 individuals genotyped, 23 (33%) were Met carriers. Our findings indicate that Val66Met did not influence any CSE responses or clinical recovery outcomes following CE training. However, although no demographic and clinical differences were found between Val and Met carriers, we did observe an effect on the acute CSE response following a HIIT session when analyzing both groups combined at baseline (T0). Specifically, compared to Met carriers, Val carriers showed a trend ($p=0.051$) toward higher CSE acute increases ipsilesionally in resting MEP amplitudes **(Figure 6)**, which became statistically significant ($p=0.018$) when including a potential influential observation not considered an outlier by the quantile-based outlier detection method.

These results align with previous studies in neurotypical individuals showing reduced practice-dependent CSE increases in Met carriers [23] and diminished responses to brain stimulation protocols that modulate cortical excitability [125,126]. Val66Met has also been associated with impaired motor learning [127] and poorer stroke recovery outcomes, though findings are mixed [24,128]. Our findings suggest that Val66Met may influence the acute response to a single CE bout in CSE during subacute stages of stroke recovery.

However, these results should be interpreted with caution due to the large sample sizes required to detect true effects in genetic studies and the neuroanatomical and behavioral variability observed in clinical stroke studies, particularly in the subacute stages [129]. Human genetics can be a powerful approach for studying treatment response mechanisms and prognosis prediction. In stroke populations, controlling for these critical factors will be essential to better understand the genotype's role in functional recovery and responses to rehabilitation [130].

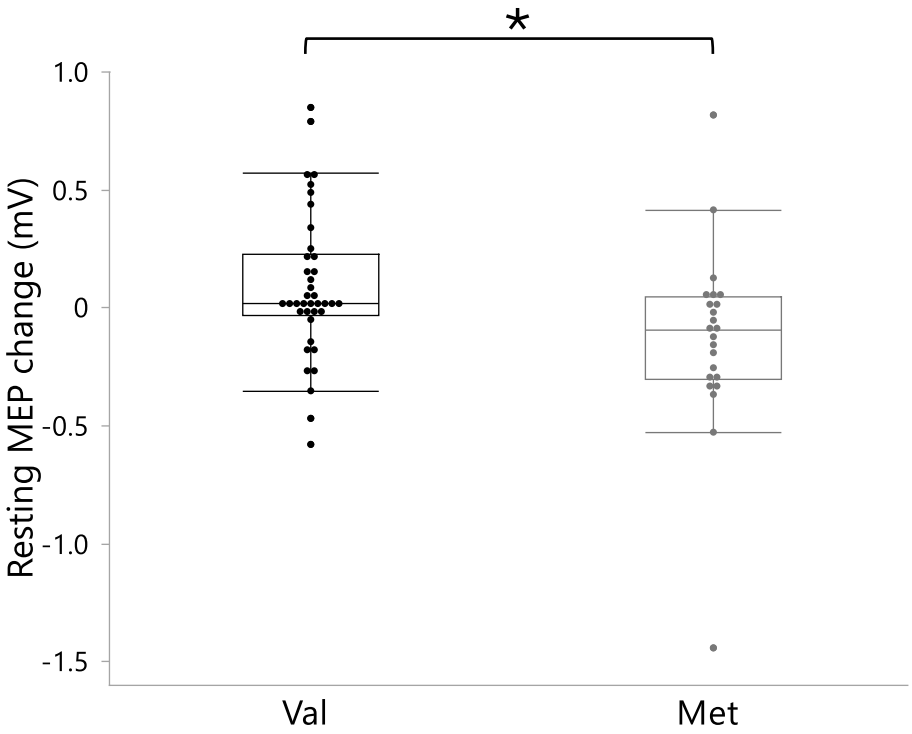

Figure 6. Effect of BDNF Val66Met polymorphism on acute CSE changes in response to a HIIT session at T0 (n=68). Val carriers exhibited higher increases in corticospinal excitability (CSE) via resting motor evoked potential (MEP) amplitude in the ipsilesional hemisphere after a 15-minute high-intensity interval training (HIIT) session compared to Met carriers (Val/Met + Met/Met). *$p < 0.05$. Data are presented as least squares means with error bars representing the standard errors of the means. MEP: motor evoked potential; mV: Millivolt.

Limitations

One limitation of this study was the absence of structural neuroimaging data, which could have provided a better characterization of lesion location and potentially influenced our findings, especially when using TMS to examine CSE. Transcranial magnetic stimulation offers a highly focused spatial resolution (1-2 cm) for assessing CSE from the CST [17,18]. Lesion location can impact CSE patterns, particularly when lesions affect the descending corticospinal pathways [131]. Although sensitivity analyses across different lesion groups (cortical, subcortical, cerebellar) found no significant impact on CSE, variability in CST damage among participants may still have obscured the potential effects of CE on CSE. Future studies using TMS should integrate precise

neuroimaging measures of CST damage to better understand how CSE can be modulated by rehabilitative interventions in stroke patients, where inter-subject variability is highly common [132].

Another limitation is the low levels of disability in our sample population, which affects the generalizability of our results to more severely affected individuals. Those with severe lesions may exhibit distinct neuroplasticity and CSE patterns post-stroke [133,134]. Additionally, obtaining complete CSE measures from individuals with significant CST disruption can be challenging or impossible [135]. One potential solution is the use of multimodal neuroimaging techniques to assess neural activity levels and neurotransmitter dynamics in individuals whose MEPs are not elicitable[76].

A stroke population with predominantly mild impairment may also experience ceiling effects in clinical motor outcomes such as the FMA [136]. These ceiling effects can mask treatment effects on recovery outcomes and their associations with brain biomarkers like CSE. Utilizing kinematic measures with finer granularity would allow us to better capture recovery gains and mechanisms of true neuroplasticity and neural repair following rehabilitative treatments [6]. Selection bias is a recurrent issue in rehabilitation studies, often due to recruitment challenges. Future research should, however, aim to include more severely affected individuals to enhance understanding of how treatments impact this population.

Conclusion

We investigated for the first time the acute and chronic effects of CE in stroke patients during the early phases of recovery. Although significant increases in CSE were observed in the contralesional hemisphere following a single HIIT session at baseline, CE training did not significantly modulate CSE measures either chronically or acutely, despite notable improvements in cardiorespiratory fitness. Factors such as increased inhibitory cortical activity, lack of intervention specificity, and individual patient variability may have influenced these results. Additionally, our findings revealed that while BDNF Val66Met polymorphism might have affected the acute CSE responses at baseline, this polymorphism does not seem to have a generalized modulatory effect. In summary, while our findings support the use of CE to improve cardiovascular outcomes in the subacute phase of stroke recovery, they do not support its use for promoting persistent neuroplastic changes at this stage.

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Supplementary Materials

Supplementary Table 1. Estimated within-group chronic changes and between-group differences in corticospinal excitability measures from T0 to T2.

	CE group		STA group		Between-Group Differences	
CSE Outcome	**Estimate (95% CI)**	**p value**	**Estimate (95% CI)**	**p value**	**Estimate (95% CI)**	**p value**
RMT						
Ipsilesional (%)	-0.44 (-3.22 to 2.22)	0.66	-0.24 (-3.73 to 3.24)	0.24	-0.20 (−4.62 to 4.22)	0.19
Contralesional (%)	0.30 (-1.17 to 1.77)	0.43	0.75 (-2.53 to 4.03)	0.83	-0.45 (−3.86 to 2.96)	0.70
MEP amplitude (resting)						
Ipsilesional (mV)	-0.10 (-0.28 to 0.07)	0.31	-0.009 (-0.11 to 0.09)	0.80	-0.091 (−0.29 to 0.11)	0.55
Contralesional (mV)	-0.003 (-0.10 to 0.09)	0.99	-0.09 (-0.23 to 0.04)	0.24	0.087 (−0.07 to 0.25)	0.45
MEP amplitude (active)						
Ipsilesional (mV)	-0.22 (-0.47 to 0.01)	0.06	-0.07 (-0.11 to 0.09)	0.33	−0.15 (−0.41 to 0.11)	0.66
Contralesional (mV)	-0.03 (-0.38 to 0.30)	0.94	-0.25 (-0.60 to 0.08)	0.10	0.22 (−0.26 to 0.70)	0.40
CSP						
Ipsilesional (ms)	-0.004 (-0.01 to 0.007)	0.60	-0.003 (-0.01 to 0.007)	0.27	−0.001 (−0.01 to 0.01)	0.54
Contralesional (ms)	0.001 (-0.005 to 0.008)	0.25	-0.005 (-0.01 to 0.005)	0.48	0.006 (−0.003 to 0.01)	0.18
ICF						
Ipsilesional (mV)	0.69 (-0.57 to 1.96)	0.36	0.18 (-0.79 to 1.15)	0.88	0.51 (−1.08 to 2.10)	0.74
Contralesional (mV)	-0.60 (-1.59 to 0.39)	0.34	0.44 (-1.02 to 1.90)	0.47	−1.04 (−2.80 to 0.72)	0.19
SICI						
Ipsilesional (mV)	0.005 (-0.36 to 0.37)	0.97	0.12 (-0.37 to 0.62)	0.24	−0.11 (0.73 to 0.5)	0.32
Contralesional (mV)	-0.39 (-1.12 to 0.34)	0.32	0.02 (-0.55 to 0.61)	0.96	−0.41 (−1.34 to 0.52)	0.54

CE, cardiovascular exercise; CI, confidence interval; CSE, corticospinal excitability; CSP, cortical silent period; ICF, intracortical facilitation; MEP, motor evoked potential; ms, millisecond; mV, millivolt; RMT, resting motor threshold; STA, standard care; SICI, short-intracortical inhibition. Data is presented as least squares means with 95% confidence intervals.

Supplementary Table 2. Estimated within-group acute changes and between-group differences in corticospinal excitability measures from T0 to T2.

	CE group		STA group		Between-Group Differences	
CSE Outcome	**Estimate (95% CI)**	**p value**	**Estimate (95% CI)**	**p value**	**Estimate (95% CI)**	**p value**
MEP amplitude (resting)						
Ipsilesional (mV)	0.03 (-0.13 to 0.21)	0.86	-0.006 (-0.17 to 0.16)	0.59	0.03 (−0.20 to 0.27)	0.69
Contralesional (mV)	-0.08 (-0.23 to 0.05)	0.34	-0.01 (-0.19 to 0.15)	0.38	−0.07 (−0.29 to 0.15)	0.34
MEP amplitude (active)						
Ipsilesional (mV)	0.16 (-0.02 to 0.35)	0.070	0.02 (-0.17 to 0.21)	0.96	0.14 (−0.12 to 0.40)	0.13
Contralesional (mV)	-0.26 (-0.53 to 0.002)	0.052	0.006 (-0.25 to 0.26)	0.60	−0.26 (−0.63 to 0.10)	0.23
CSP						
Ipsilesional (ms)	-0.003 (-0.02 to 0.02)	0.82	0.003 (-0.007 to 0.01)	0.37	−0.006 (−0.02 to 0.01)	0.75
Contralesional (ms)	-0.0026 (-0.01 to 0.006)	0.68	-0.0023 (-0.01 to 0.009)	0.67	-0.0003 (−0.01 to 0.01)	0.99
ICF						
Ipsilesional (mV)	0.02 (-1.21 to 1.26)	0.83	-0.18 (-1.76 to 1.38)	0.95	0.20 (−2.13 to 2.53)	0.87
Contralesional (mV)	0.34 (-0.59 to 1.28)	0.66	0.14 (-1.38 to 1.66)	0.88	0.20 (−1.58 to 1.98)	0.84
SICI						
Ipsilesional (mV)	0.04 (-0.48 to 0.57)	0.97	-0.14 (-0.70 to 0.41)	0.67	0.18 (−0.58 to 0.94)	0.69
Contralesional (mV)	0.30 (-0.32 to 0.94)	0.39	0.11 (-0.43 to 0.67)	0.66	0.19 (−0.64 to 1.02)	0.92

CE, cardiovascular exercise; CI, confidence interval; CSE, corticospinal excitability; CSP, cortical silent period; ICF, intracortical facilitation; MEP, motor evoked potential; ms, millisecond; mV, millivolt; STA, standard care; SICI, short-intracortical inhibition. Data is presented as least squares means with 95% confidence intervals.

Supplementary Table 3. Adjusted linear mixed models comparing chronic corticospinal excitability responses to cardiovascular exercise training across different lesion location groups—cortical, subcortical, and cerebellar—in both ipsilesional and contralesional hemispheres.

	DFNum	DFDen	F ratio	p value
RMT ipsilesional				
Group	1	63.5	1e-5	0.997
Time	2	104.2	0.50	0.605
Time*Group[Location]	6	104.4	0.57	0.747
Age	1	62.6	0.01	0.920
Sex	1	62.6	1.24	0.269
NIHSS	1	63.3	4.79	0.032*
MVC	1	62.2	5.42	0.023*
Resting MEP ipsilesional				
Group	1	63.5	2.48	0.120
Time	2	81.5	0.40	0.669
Time*Group[Location]	6	88.6	0.64	0.698
Age	1	59.9	0.41	0.520
Sex	1	59.0	0.32	0.573
NIHSS	1	60.9	1.002	0.321
MVC	1	57.8	10.31	0.002*
Active MEP ipsilesional				
Group	1	64.2	2.09	0.152
Time	2	102.5	2.89	0.060
Time*Group[Location]	6	102.4	0.75	0.611
Age	1	62.1	0.70	0.406
Sex	1	61.4	0.013	0.909
NIHSS	1	62.9	0.64	0.427
MVC	1	60.7	16.02	0.003*
CSP ipsilesional				
Group	1	64.3	0.13	0.715
Time	2	101.4	0.79	0.455
Time*Group[Location]	6	101.4	1.09	0.371
Age	1	62.7	0.55	0.459
Sex	1	62.3	7.38	0.009*
NIHSS	1	63.3	0.67	0.416
MVC	1	61.8	3.09	0.083
ICF ipsilesional				
Group	1	66.3	0.90	0.346
Time	2	86.9	0.40	0.671
Time*Group[Location]	6	94.8	1.67	0.137
Age	1	64.0	0.05	0.812
Sex	1	62.5	0.002	0.958
NIHSS	1	64.8	2.68	0.106
MVC	1	60.9	0.20	0.651
SICI ipsilesional				
Group	1	54.7	0.71	0.401

Time	2	69.2	0.71	0.492
Time*Group[Location]	6	76.1	0.67	0.674
Age	1	52.4	0.77	0.382
Sex	1	52.8	1.24	0.270
NIHSS	1	54.6	0.77	0.382
MVC	1	52.9	3.56	0.065
RMT contralesional				
Group	1	67.8	0.02	0.872
Time	2	111.3	0.24	0.781
Time*Group[Location]	6	111.6	0.50	0.805
Age	1	66.6	0.19	0.661
Sex	1	67.0	4.78	0.032*
NIHSS	1	67.5	0.68	0.413
MVC	1	66.5	0.003	0.957
Resting MEP contralesional				
Group	1	67.4	0.32	0.573
Time	2	112.3	1.04	0.356
Time*Group[Location]	6	112.7	0.90	0.491
Age	1	64.2	0.001	0.979
Sex	1	65.7	0.22	0.641
NIHSS	1	66.7	0.02	0.869
MVC	1	63.9	2.26	0.137
Active MEP contralesional				
Group	1	63.0	1.19	0.279
Time	2	80.5	0.73	0.481
Time*Group[Location]	6	92.2	0.97	0.446
Age	1	57.8	0.08	0.769
Sex	1	60.0	0.41	0.520
NIHSS	1	61.3	0.83	0.364
MVC	1	57.5	1.62	0.207
CSP contralesional				
Group	1	67.2	0.37	0.543
Time	2	112.1	0.54	0.579
Time*Group[Location]	6	112.5	0.73	0.626
Age	1	64.1	0.62	0.431
Sex	1	65.6	3.33	0.072
NIHSS	1	66.5	0.32	0.574
MVC	1	63.8	0.03	0.843
ICF contralesional				
Group	1	71.3	1.44	0.234
Time	2	96.0	0.33	0.719
Time*Group[Location]	6	107.0	0.44	0.849
Age	1	65.1	1.08	0.301
Sex	1	67.7	0.75	0.389
NIHSS	1	70.1	0.02	0.879
MVC	1	64.1	0.23	0.633
SICI contralesional				
Group	1	57.3	1.39	0.242
Time	2	70.1	0.06	0.940

Time*Group[Location]	6	82.7	0.22	0.966
Age	1	54.2	0.08	0.776
Sex	1	55.9	0.01	0.903
NIHSS	1	58.6	0.82	0.366
MVC	1	54.7	2.51	0.118

CSP, cortical silent period; ICF, intracortical facilitation; MEP, motor evoked potential; MVC, maximal voluntary contraction; NIHSS, national institutes of health stroke scale; RMT, resting motor threshold; SICI, short-intracortical inhibition. Location represents the three lesion location groups—cortical, subcortical, and cerebellar—nested in the model as a categorical variable. *p<0.05

Supplementary Table 4. Adjusted linear mixed models comparing acute corticospinal excitability responses to cardiovascular exercise training across different lesion location groups—cortical, subcortical, and cerebellar—in both ipsilesional and contralesional hemispheres.

	DFNum	DFDen	F ratio	p value
Resting MEP ipsilesional				
Group	1	63.4	1.76	0.189
Time	2	85.7	0.16	0.849
Time*Group[Location]	6	93.4	0.43	0.851
Age	1	62.3	2e-4	0.989
Sex	1	60.6	0.25	0.615
NIHSS	1	63.3	0.81	0.371
MVC	1	59.2	0.04	0.831
Active MEP ipsilesional				
Group	1	54.9	4.50	0.038*
Time	2	80.5	4.66	0.012*
Time*Group[Location]	6	85.5	0.83	0.543
Age	1	52.1	0.35	0.552
Sex	1	50.9	0.35	0.556
NIHSS	1	53.0	1.49	0.227
MVC	1	49.9	0.54	0.462
CSP ipsilesional				
Group	1	50.5	0.73	0.394
Time	2	106.6	0.62	0.539
Time*Group[Location]	6	106.6	1.13	0.350
Age	1	50.2	3.17	0.081
Sex	1	48.7	1.64	0.206
NIHSS	1	51.0	1.52	0.222
MVC	1	48.5	0.45	0.505
ICF ipsilesional				
Group	1	62.4	0.01	0.913
Time	2	111.1	0.06	0.934
Time*Group[Location]	6	110.1	1.64	0.142
Age	1	60.7	0.17	0.677

Sex	1	59.0	5e-4	0.983
NIHSS	1	61.6	0.006	0.937
MVC	1	57.5	0.47	0.495
SICI ipsilesional				
Group	1	54.5	3.11	0.083
Time	2	73.6	0.07	0.924
Time*Group[Location]	6	80.5	0.61	0.722
Age	1	52.8	0.75	0.388
Sex	1	53.1	2.84	0.098
NIHSS	1	55.3	2.55	0.115
MVC	1	53.0	1.27	0.264
Resting MEP contralesional				
Group	1	70.4	0.22	0.634
Time	2	122.8	0.65	0.520
Time*Group[Location]	6	123.7	0.60	0.724
Age	1	65.2	0.02	0.880
Sex	1	67.1	0.005	0.943
NIHSS	1	69.7	0.71	0.400
MVC	1	64.2	0.19	0.663
Active MEP contralesional				
Group	1	58.6	0.03	0.856
Time	2	96.8	2.49	0.088
Time*Group[Location]	6	104.5	1.82	0.102
Age	1	56.2	3.57	0.064
Sex	1	57.1	0.02	0.869
NIHSS	1	59.6	0.17	0.673
MVC	1	55.3	1.61	0.209
CSP contralesional				
Group	1	62.3	1.37	0.246
Time	2	122.1	0.19	0.825
Time*Group[Location]	6	123.3	0.67	0.671
Age	1	58.0	2.65	0.109
Sex	1	59.2	0.70	0.403
NIHSS	1	61.8	0.31	0.574
MVC	1	57.2	0.01	0.910
ICF contralesional				
Group	1	65.2	0.67	0.413
Time	2	93.9	0.16	0.850
Time*Group[Location]	6	105.5	0.13	0.991
Age	1	61.1	0.48	0.488
Sex	1	62.4	0.01	0.901
NIHSS	1	65.2	1e-4	0.991
MVC	1	59.7	0.03	0.844
SICI contralesional				
Group	1	53.7	0.17	0.677
Time	2	80.9	0.55	0.578
Time*Group[Location]	6	89.6	0.64	0.691
Age	1	51.3	0.89	0.349
Sex	1	52.5	0.19	0.659

NIHSS	1	55.6	1.09	0.299
MVC	1	51.8	2.91	0.094

CSP, cortical silent period; ICF, intracortical facilitation; MEP, motor evoked potential; MVC, maximal voluntary contraction; NIHSS, national institutes of health stroke scale; SICI, short-intracortical inhibition. Location represents the three lesion location groups—cortical, subcortical, and cerebellar—nested in the model as a categorical variable. * $p<0.05$

Supplementary Table 5. Adjusted linear mixed models examining the influence of Val66Met polymorphism on acute corticospinal excitability responses at T0 in both ipsilesional and contralesional hemispheres.

	DFNum	DFDen	F ratio	p value
Resting MEP ipsilesional				
Age	1	56.0	0.33	0.571
Sex	1	56.0	0.02	0.898
NIHSS	1	56.0	2.98	0.090
MVC	1	56.0	6.79	0.012*
Time	1	60.0	0.27	0.604
Val66Met	1	56.0	2.73	0.104
Time*Val66Met	1	60.0	5.83	0.019*
Active MEP ipsilesional				
Age	1	56.0	0.30	0.581
Sex	1	56.0	0.01	0.912
NIHSS	1	56.0	0.12	0.726
MVC	1	56.0	10.04	0.002*
Time	1	60.0	1.26	0.266
Val66Met	1	56.0	1.13	0.291
Time*Val66Met	1	60.0	0.18	0.671
CSP ipsilesional				
Age	1	56.0	0.30	0.585
Sex	1	56.0	1.89	0.174
NIHSS	1	56.0	1.26	0.266
MVC	1	56.0	0.12	0.725
Time	1	60.0	0.11	0.732
Val66Met	1	56.0	0.13	0.710
Time*Val66Met	1	60.0	0.79	0.376
ICF ipsilesional				
Age	1	56.0	0.242	0.625
Sex	1	56.0	0.255	0.615
NIHSS	1	56.0	1.612	0.210
MVC	1	56.0	0.322	0.573
Time	1	60.0	0.458	0.501
Val66Met	1	56.0	0.033	0.857
Time*Val66Met	1	60.0	4.819	0.322
SICI ipsilesional				

Age	1	50.0	0.26	0.613
Sex	1	50.0	0.04	0.828
NIHSS	1	50.0	1.004	0.321
MVC	1	50.0	2.78	0.101
Time	1	54.0	1.75	0.191
Val66Met	1	50.0	0.30	0.585
Time*Val66Met	1	54.0	1.19	0.280
Resting MEP contralesional				
Age	1	59.0	0.20	0.653
Sex	1	59.0	0.75	0.389
NIHSS	1	59.0	0.19	0.659
MVC	1	59.0	0.50	0.480
Time	1	63.0	5.97	0.017*
Val66Met	1	59.0	0.13	0.713
Time*Val66Met	1	63.0	0.33	0.563
Active MEP contralesional				
Age	1	59.0	0.002	0.967
Sex	1	59.0	0.36	0.548
NIHSS	1	59.0	0.19	0.664
MVC	1	59.0	1.81	0.183
Time	1	63.0	5.72	0.020*
Val66Met	1	59.0	0.02	0.868
Time*Val66Met	1	63.0	0.16	0.683
CSP contralesional				
Age	1	59.0	3.24	0.077
Sex	1	59.0	0.02	0.884
NIHSS	1	59.0	0.06	0.802
MVC	1	59.0	0.002	0.965
Time	1	63.0	0.84	0.361
Val66Met	1	59.0	0.03	0.848
Time*Val66Met	1	63.0	1.30	0.258
ICF contralesional				
Age	1	59.0	0.07	0.786
Sex	1	59.0	0.008	0.927
NIHSS	1	59.0	0.76	0.385
MVC	1	59.0	0.10	0.750
Time	1	63.0	0.09	0.758
Val66Met	1	59.0	1.96	0.166
Time*Val66Met	1	63.0	0.03	0.851
SICI contralesional				
Age	1	47.0	0.23	0.630
Sex	1	47.0	0.005	0.940
NIHSS	1	47.0	0.01	0.896
MVC	1	47.0	0.73	0.396
Time	1	51.0	1.35	0.250
Val66Met	1	47.0	2.10	0.154
Time*Val66Met	1	51.0	1.56	0.217

CSP, cortical silent period; ICF, intracortical facilitation; MEP, motor evoked potential; MVC, maximal voluntary contraction; NIHSS, national institutes of health stroke scale; SICI, short-intracortical inhibition. Time represents the two time points before and after the high-intensity interval training session. * $p<0.05$

Supplementary Table 6. Adjusted linear mixed models examining the influence of Val66Met polymorphism on chronic corticospinal excitability responses to cardiovascular exercise training in both ipsilesional and contralesional hemispheres.

	DFNum	DFDen	F ratio	p value
RMT ipsilesional	1	56.8	0.05	0.817
Age	1	56.7	1.64	0.205
Sex	1	57.5	5.62	0.021*
NIHSS	1	56.4	5.78	0.019*
MVC	1	56.9	1.65	0.203
Val66Met	2	103.8	0.99	0.372
Time	1	57.2	0.21	0.642
Group	4	104.1	1.61	0.176
Time*Group[Val66Met]	1	56.8	0.05	0.817
Resting MEP ipsilesional				
Age	1	55.3	1.03	0.313
Sex	1	53.9	0.55	0.460
NIHSS	1	55.9	0.92	0.341
MVC	1	52.9	9.79	0.003*
Val66Met	1	54.3	4.26	0.044*
Time	2	80.0	0.44	0.645
Group	1	56.7	1.96	0.166
Time*Group[Val66Met]	4	85.0	1.05	0.384
Active MEP ipsilesional				
Age	1	56.7	0.96	0.330
Sex	1	55.7	0.01	0.908
NIHSS	1	57.3	0.62	0.432
MVC	1	55.1	12.62	0.001*
Val66Met	1	56.0	0.52	0.474
Time	2	101.5	2.99	0.055
Group	1	57.6	1.43	0.237
Time*Group[Val66Met]	4	101.9	1.10	0.359
CSP ipsilesional				
Age	1	56.9	0.46	0.500
Sex	1	56.2	4.39	0.041*
NIHSS	1	57.3	0.76	0.387
MVC	1	55.8	2.19	0.144
Val66Met	1	56.4	0.80	0.373
Time	2	100.6	0.78	0.457
Group	1	57.6	0.25	0.614

Time*Group[Val66Met]	4	100.9	0.25	0.905
ICF ipsilesional				
Age	1	57.6	0.23	0.628
Sex	1	56.0	1e-5	0.997
NIHSS	1	57.8	2.79	0.100
MVC	1	54.5	0.24	0.620
Val66Met	1	56.6	1.61	0.209
Time	2	85.0	0.48	0.621
Group	1	58.5	1.55	0.217
Time*Group[Val66Met]	4	90.2	0.31	0.865
SICI ipsilesional				
Age	1	47.6	0.87	0.354
Sex	1	46.8	0.93	0.339
NIHSS	1	48.9	1.22	0.273
MVC	1	46.4	3.13	0.083
Val66Met	1	46.0	0.83	0.366
Time	2	67.4	1.22	0.301
Group	1	48.4	1.28	0.262
Time*Group[Val66Met]	4	72.0	1.01	0.406
RMT contralesional				
Age	1	56.8	0.05	0.817
Sex	1	56.7	1.64	0.205
NIHSS	1	57.5	5.62	0.021
MVC	1	56.4	5.78	0.019*
Val66Met	1	56.9	1.65	0.203
Time	2	103.8	0.99	0.372
Group	1	57.2	0.21	0.642
Time*Group[Val66Met]	4	104.1	1.61	0.176
Resting MEP contralesional				
Age	1	57.4	0.006	0.940
Sex	1	58.0	0.68	0.411
NIHSS	1	59.2	0.001	0.969
MVC	1	56.8	1.56	0.216
Val66Met	1	58.6	0.09	0.756
Time	2	108.2	0.95	0.390
Group	1	58.8	0.55	0.461
Time*Group[Val66Met]	4	108.8	0.46	0.762
Active MEP contralesional				
Age	1	53.1	0.02	0.878
Sex	1	53.8	0.20	0.654
NIHSS	1	55.4	0.31	0.575
MVC	1	52.2	1.17	0.283
Val66Met	1	54.8	0.43	0.515
Time	2	77.0	0.79	0.456
Group	1	55.1	0.68	0.413
Time*Group[Val66Met]	4	84.6	0.58	0.674
CSP contralesional				
Age	1	59.0	1.22	0.272
Sex	1	59.5	1.22	0.273

NIHSS	1	60.7	0.28	0.595
MVC	1	58.3	0.17	0.674
Val66Met	1	60.1	0.01	0.918
Time	2	109.6	0.38	0.682
Group	1	60.3	0.25	0.618
Time*Group[Val66Met]	4	110.1	0.69	0.596
ICF contralesional				
Age	1	59.9	0.57	0.452
Sex	1	60.6	0.50	0.482
NIHSS	1	62.9	0.003	0.956
MVC	1	58.3	0.42	0.519
Val66Met	1	63.6	1e-4	0.991
Time	2	88.8	0.22	0.798
Group	1	62.4	1.51	0.224
Time*Group[Val66Met]	4	96.7	0.20	0.935
SICI contralesional				
Age	1	50.4	0.09	0.764
Sex	1	50.7	0.10	0.751
NIHSS	1	53.2	0.74	0.391
MVC	1	49.8	2.75	0.103
Val66Met	1	51.5	0.004	0.950
Time	2	67.7	0.14	0.866
Group	1	51.6	1.59	0.213
Time*Group[Val66Met]	4	76.1	0.38	0.816

CSP, cortical silent period; ICF, intracortical facilitation; MEP, motor evoked potential; MVC, maximal voluntary contraction; NIHSS, national institutes of health stroke scale; RMT, resting motor threshold; SICI, short-intracortical inhibition. * $p<0.05$

Supplementary Table 7. Adjusted linear mixed models examining the influence of Val66Met polymorphism on acute corticospinal excitability responses to cardiovascular exercise training in both ipsilesional and contralesional hemispheres.

	DFNum	**DFDen**	**F ratio**	**p value**
Resting MEP ipsilesional				
Age	1	55.6	0.19	0.664
Sex	1	53.9	0.15	0.698
NIHSS	1	56.1	0.93	0.338
MVC	1	52.6	8e-6	0.998
Val66Met	1	54.4	3.33	0.073
Time	2	81.7	0.23	0.791
Group	1	55.8	1.70	0.197
Time*Group[Val66Met]	4	87.0	2.24	0.071
Active MEP ipsilesional				
Age	1	46.9	0.0003	0.986

Sex	1	45.5	0.19	0.663
NIHSS	1	47.4	2.95	0.092
MVC	1	44.6	0.56	0.458
Val66Met	1	46.5	0.10	0.752
Time	2	76.9	5.29	0.007*
Group	1	48.8	6.61	0.013*
Time*Group[Val66Met]	4	81.2	1.22	0.307
CSP ipsilesional				
Age	1	49.7	2.52	0.119
Sex	1	48.7	0.80	0.375
NIHSS	1	49.9	1.54	0.219
MVC	1	48.6	0.47	0.494
Val66Met	1	50.0	0.004	0.950
Time	2	105.0	0.74	0.479
Group	1	9.7	1.33	0.253
Time*Group[Val66Met]	4	105.3	2.56	0.420
ICF ipsilesional				
Age	1	54.0	0.115	0.736
Sex	1	52.4	0.007	0.933
NIHSS	1	54.2	0.049	0.825
MVC	1	50.9	0.675	0.415
Val66Met	1	53.0	5.888	0.019*
Time	2	106.4	0.036	0.965
Group	1	54.6	0.174	0.678
Time*Group[Val66Met]	4	106.5	0.802	0.526
SICI ipsilesional				
Age	1	48.1	0.77	0.383
Sex	1	47.1	2.27	0.138
NIHSS	1	49.5	2.04	0.159
MVC	1	46.2	0.74	0.394
Val66Met	1	46.4	2.57	0.115
Time	2	71.1	0.27	0.757
Group	1	48.3	1.86	0.178
Time*Group[Val66Met]	4	75.8	0.71	0.588
Resting MEP contralesional				
Age	1	58.8	0.218	0.643
Sex	1	59.2	0.013	0.910
NIHSS	1	61.8	1.735	0.193
MVC	1	57.3	0.193	0.662
Val66Met	1	61.1	0.092	0.763
Time	2	115.4	1.005	0.369
Group	1	61.0	0.55	0.461
Time*Group[Val66Met]	4	116.2	0.876	0.481
Active MEP contralesional				
Age	1	51.9	2.03	0.160
Sex	1	52.1	0.13	0.718
NIHSS	1	54.1	0.15	0.698
MVC	1	51.0	1.47	0.231
Val66Met	1	55.2	0.04	0.840

Time	2	90.4	2.43	0.093
Group	1	52.6	0.09	0.766
Time*Group[Val66Met]	4	96.4	1.09	0.364
CSP contralesional				
Age	1	54.0	0.34	0.558
Sex	1	54.1	0.36	0.549
NIHSS	1	56.2	1.47	0.229
MVC	1	52.9	0.06	0.804
Val66Met	1	57.4	0.20	0.652
Time	2	114.3	0.37	0.689
Group	1	55.9	2.21	0.142
Time*Group[Val66Met]	4	115.5	0.26	0.899
ICF contralesional				
Age	1	54.7	0.34	0.560
Sex	1	55.1	0.01	0.919
NIHSS	1	57.5	0.001	0.977
MVC	1	53.3	0.06	0.799
Val66Met	1	56.8	0.29	0.591
Time	2	86.3	0.13	0.873
Group	1	56.6	0.52	0.473
Time*Group[Val66Met]	4	94.0	0.54	0.705
SICI contralesional				
Age	1	47.7	1.10	0.299
Sex	1	47.6	0.17	0.676
NIHSS	1	50.0	0.78	0.381
MVC	1	47.4	3.93	0.053
Val66Met	1	49.2	1.31	0.258
Time	2	77.8	0.75	0.474
Group	1	48.2	0.21	0.648
Time*Group[Val66Met]	4	83.6	0.24	0.914

CSP, cortical silent period; ICF, intracortical facilitation; MEP, motor evoked potential; MVC, maximal voluntary contraction; NIHSS, national institutes of health stroke scale; SICI, short-intracortical inhibition. Time represents the two time points before and after the high-intensity interval training session. * $p<0.05$

Supplementary Table 8. Adjusted multivariate linear regression examining associations between chronic CSE responses and changes in clinical motor outcomes and cardiorespiratory fitness for both groups.

Predictor	CE+standard care			Standard care		
	Estimate (95% CI)	p value	R^2	Estimate (95% CI)	p value	R^2
UL-FMA T0-T2						
Ipsilesional						
Δ RMT	-0.06 (-0.32, 0.19)	0.596	0.27	0.03 (-0.30, 0.38)	0.817	0.57
Δ Resting MEP	0.18 (-3.18, 3.56)	0.910	0.28	3.94 (-4.03, 11.93)	0.299	0.61
Δ Active MEP	0.93 (-1.54, 3.41)	0.445	0.29	-0.02 (-4.04, 4.00)	0.990	0.57
Δ CSP	-3.29 (57.09, 50.50)	0.901	0.28	-53.58 (-135.04, 27.87)	0.175	0.64
Δ ICF	0.10 (-0.39, 0.60)	0.662	0.28	-0.37 (-1.31, 0.57)	0.403	0.59
Δ SICI	0.94 (-0.54, 2.44)	0.202	0.13	-0.65 (-2.32, 1.02)	0.408	0.59
Contralesional						
Δ RMT	-0.35 (-0.90, 0.19)	0.194	0.32	0.10 (-0.13, 0.34)	0.363	0.53
Δ Resting MEP	3.06 (-3.62, 9.76)	0.357	0.30	-3.58 (-11.50, 4.32)	0.345	0.60
Δ Active MEP	1.79 (-0.05, 3.64)	0.056	0.36	0.70 (-1.71, 3.11)	0.541	0.59
Δ CSP	-15.13 (-126.89, 96.63)	0.784	0.28	-8.36 (-76.33, 59.61)	0.794	0.57
Δ ICF	0.99 (-0.33, 2.27)	0.141	0.33	0.30 (-0.32, 0.94)	0.313	0.61
Δ SICI	0.96 (-1.06, 2.99)	0.336	0.17	0.59 (-1.05, 2.23)	0.450	0.59
BBT T0-T2						
Ipsilesional						
Δ RMT	0.09 (-0.33, 0.52)	0.659	0.03	0.30 (-0.34, 0.96)	0.320	0.50
Δ Resting MEP	4.22 (-1.22, 9.68)	0.123	0.10	-1.72 (-16.79, 13.33)	0.805	0.56
Δ Active MEP	0.53 (-3.68, 4.75)	0.796	0.01	-0.006 (-7.23, 7.22)	0.998	0.55
Δ CSP	-40.62 (-130.18, 48.93)	0.360	0.05	-4.16 (-163.93, 155.59)	0.955	0.55
Δ ICF	-0.47 (-1.29, 0.35)	0.249	0.06	0.89 (-0.75, 2.54)	0.256	0.61
Δ SICI	-2.54 (-6.78, 1.70)	0.226	0.10	1.77 (-1.10, 4.65)	0.202	0.62
Contralesional						
Δ RMT	-0.19 (-1.06, 0.68)	0.657	0.03	0.49 (0.05, 0.94)	0.310	0.65
Δ Resting MEP	-3.89 (-14.37, 6.58)	0.454	0.04	2.40 (-13.64, 18.46)	0.751	0.51
Δ Active MEP	-1.78 (-4.85, 1.27)	0.243	0.07	-1.89 (-6.56, 2.78)	0.397	0.53

Δ CSP	35.11 (-140.77, 210.99)	0.687	0.03	1.80 (-132.09, 135.70)	0.977	0.51
Δ ICF	-0.80 (-2.80, 1.19)	0.417	0.06	-0.90 (-2.08, 0.28)	0.124	0.59
Δ SICI	-2.60 (-6.53, 1.31)	0.181	0.12	-1.77 (-4.89, 1.35)	0.243	0.56
CRF T0-T2						
Ipsilesional						
Δ RMT	0.007 (-0.14, 0.17)	0.921	0.29	0.15 (-0.18, 0.48)	0.341	0.28
Δ Resting MEP	2.47 (0.46, 4.49)	0.173	0.39	-4.59 (-12.68, 1.50)	0.237	0.31
Δ Active MEP	0.77 (-0.86, 2.41)	0.341	0.28	2.04 (-1.87, 5.95)	0.275	0.30
Δ CSP	-11.60 (-47.08, 23.87)	0.508	0.27	29.83 (-59.44, 119.12)	0.477	0.25
Δ ICF	-0.34 (-0.64, 0.03)	0.284	0.38	-0.22 (-1.21, 0.77)	0.633	0.23
Δ SICI	0.03 (-1.70, 1.78)	0.963	0.30	0.30 (-1.46, 2.06)	0.715	0.22
Contralesional						
Δ RMT	-0.52 (-0.81, -0.23)	0.345	0.46	0.08 (-0.14, 0.32)	0.444	0.27
Δ Resting MEP	-0.16 (-4.26, 3.92)	0.933	0.23	2.62 (-5.40, 10.65)	0.492	0.27
Δ Active MEP	-1.19 (-1.37, 0.98)	0.740	0.23	-1.41 (-3.70, 0.87)	0.204	0.33
Δ CSP	-19.30 (-87.68, 49.07)	0.569	0.24	9.61 (-58.13, 77.37)	0.763	0.25
Δ ICF	-0.02 (-0.78, 0.73)	0.942	0.23	0.23 (-0.41, 0.87)	0.412	0.28
Δ SICI	-0.28 (-1.85, 1.28)	0.712	0.27	-0.35 (-2.01, 1.31)	0.654	0.25

BBT, Box and Blocks Test; CRF, cardiorespiratory fitness; CSP, cortical silent period; ICF, intracortical facilitation; MEP, motor evoked potential; RMT, resting motor threshold; SICI short-intracortical inhibition. UL-FMA, upper-limb Fugl-Meyer assessment.

Supplementary Table 8. Adjusted multivariate linear regression examining associations between acute CSE responses over time and changes in clinical motor outcomes and cardiorespiratory fitness for both groups.

Predictor	CE+standard care			Standard care		
	Estimate (95% CI)	p value	R^2	Estimate (95% CI)	p value	R^2
UL-FMA T0-T2						
Ipsilesional						
Δ Resting MEP	-1.02 (-4.99, 2.94)	0.602	0.29	-2.44 (-9.10, 4.22)	0.436	0.59
Δ Active MEP	0.49 (-3.17, 4.17)	0.784	0.28	-1.13 (-5.32, 3.06)	0.564	0.58
Δ CSP	12.18 (-36.98, 61.36)	0.616	0.28	-6.29 (-92.18, 79.60)	0.874	0.57
Δ ICF	0.30 (-0.31, 0.93)	0.324	0.31	0.15 (-0.48, 0.78)	0.609	0.58
Δ SICI	-0.32 (-1.45, 0.80)	0.559	0.08	0.10 (-1.28, 1.50)	0.866	0.57
Contralesional						
Δ Resting MEP	-1.11 (-6.33, 4.10)	0.666	0.29	0.91 (-4.31, 6.14)	0.710	0.58
Δ Active MEP	0.41 (-2.03, 2.85)	0.734	0.31	0.98 (-2.29, 4.26)	0.528	0.59
Δ CSP	-22.51 (-129.04, 84.02)	0.669	0.31	14.83 (-39.25, 68.93)	0.563	0.58
Δ ICF	-0.09 (-0.78, 0.59)	0.788	0.28	0.07 (-0.42, 0.57)	0.755	0.58
Δ SICI	-0.69 (-2.03, 0.64)	0.293	0.17	-0.43 (-1.95, 1.08)	0.293	0.17
BBT T0-T2						
Ipsilesional						
Δ Resting MEP	-4.91 (-11.46, 1.62)	0.135	0.09	-7.52 (-18.80, 3.74)	0.169	0.63
Δ Active MEP	-3.53 (-9.58, 2.52)	0.242	0.06	-2.31 (-9.82, 5.19)	0.511	0.57
Δ CSP	-11.71 (-44.06, 20.62)	0.464	0.27	-19.75 (-173.79, 134.27)	0.782	0.56
Δ ICF	0.50 (-0.55, 1.57)	0.337	0.05	0.008 (-1.14, 1.15)	0.987	0.55
Δ SICI	2.52 (-0.48, 5.54)	0.095	0.16	-0.30 (-2.80, 2.19)	0.793	0.56
Contralesional						
Δ Resting MEP	-7.07 (-14.79, 0.64)	0.071	0.12	-7.18 (-16.57, 2.20)	0.122	0.59
Δ Active MEP	2.76 (-1.12, 6.64)	0.157	0.08	2.29 (-4.10, 8.69)	0.452	0.53
Δ CSP	113.88 (-50.60, 278.38)	0.167	0.08	56.87 (-45.28, 159.03)	0.250	0.56
Δ ICF	1.11 (0.11, 2.10)	0.298	0.16	0.69 (-0.19, 1.58)	0.113	0.60
Δ SICI	1.76 (-1.03, 4.57)	0.204	0.08	2.45 (-0.10, 5.10)	0.067	0.62
CRF T0-T2						

Ipsilesional						
Δ Resting MEP	-2.52 (-5.00, -0.04)	0.469	0.36	5.52 (-0.50, 11.55)	0.068	0.42
Δ Active MEP	-1.66 (-4.03, 0.69)	0.159	0.31	-3.99 (-7.48, -0.50)	0.028*	0.50
Δ CSP	-11.71 (-44.06, 20.62)	0.464	0.27	-77.57 (-149.53, -5.60)	0.037*	0.48
Δ ICF	0.28 (-0.11, 0.69)	0.156	0.30	0.06 (-0.59, 0.72)	0.827	0.21
Δ SICI	0.24 (-1.03, 1.52)	0.696	0.31	-0.30 (-1.72, 1.11)	0.641	0.23
Contralesional						
Δ Resting MEP	-0.99 (-4.25, 2.26)	0.538	0.24	2.92 (-2.01, 7.87)	0.223	0.33
Δ Active MEP	-0.31 (-1.86, 1.23)	0.682	0.24	-0.08 (-3.41, 3.23)	0.956	0.24
Δ CSP	14.76 (-54.31, 83.85)	0.665	0.24	-21.87 (-74.97, 31.22)	0.389	0.29
Δ ICF	0.46 (0.07, 0.85)	0.218	0.35	-0.27 (-0.74, 0.20)	0.238	0.32
Δ SICI	0.02 (-1.16, 1.20)	0.968	0.28	0.71 (-0.76, 2.19)	0.314	0.30

BBT, Box and Blocks Test; CRF, cardiorespiratory fitness; CSP, cortical silent period; ICF, intracortical facilitation; MEP, motor evoked potential; SICI short-intracortical inhibition. UL-FMA, upper-limb Fugl-Meyer assessment.

From the Brain to the Periphery: Brain-derived Neurotrophic Factor

After measuring the effects of CE on central corticospinal networks through TMS, a critical yet unresolved question in neurorehabilitation and neuroscience is whether these central brain changes can be inferred from molecules measured in the periphery. The inability to directly study molecular changes in the human brain has led to the study of blood biomarkers collected peripherally as potential surrogates for central neural processes [61,132,133].

Among these biomarkers, BDNF is perhaps the most popular and studied due to its involvement in neuroplastic processes essential for brain function, such as dendritic and axonal sprouting, neural survival, and synaptic plasticity [134]. The mature form of BDNF, along with its receptor, tyrosine kinase receptor B (TrkB), have been shown to influence neural activity by eliciting long-term potentiation (LTP), a neurophysiological parameter underpinning learning and memory processes [135]. Studies on neurotypical animal models have shown that suppressing BDNF protein expression can hinder synaptic plasticity and impair learning capacity [136]. Conversely, increasing BDNF levels has been shown to enhance LTP induction in the hippocampus and improve memory [137]. In humans, reductions in peripheral BDNF concentration have been associated with aging and neurodegenerative processes [138], with clinical implications such as reductions in hippocampal volume and deficits in episodic memory [139]. In stroke survivors, reduced peripheral BDNF levels has been linked to poor functional outcomes [140].

Indeed, following stroke, BDNF also stands as the most studied protein due to its role in neuronal growth, survival, and plasticity. Upregulation of growth-promoting factors, including BDNF, during early periods post-stroke, has been observed both ipsilesionally and in distant brain areas, fostering neuroplastic environments and supporting recovery [141,142]. Additionally, ensuring BDNF availability during these early phases is crucial for inducing neuroplasticity and enhancing motor recovery in response to motor exercise rehabilitation [143].

Cardiovascular exercise has been shown to upregulate BDNF brain levels in animal models, an increase directly associated with neuroplasticity and behavioral improvements in learning and memory [144]. Inhibiting this BDNF expression can impair the brain's ability to undergo neural reorganization in response to exercise, consequently abolishing any behavioral gains [59]. In neurotypical individuals, a single bout of intense CE has been shown to transiently elevate

peripheral BDNF concentrations, with some studies reporting associations between these increases and improvements in motor learning [145], with the expectation that these increases may reflect central brain levels.

As reviewed in Chapter 1, circulating levels of BDNF in stroke patients show transient increases following single CE sessions and, to a lesser extent, after extended training interventions. However, these studies have been conducted exclusively in chronic patients, a period when neuroplasticity and recovery events tend to plateau. Similar to spontaneous changes in neural excitability, animal studies demonstrate that most growth factor upregulation takes place during early stages post-stroke, with interventions potentially enhancing this neurotrophic response [24]. Yet, no studies have examined the effects of CE on peripheral BDNF in individuals during the early subacute stages and whether these increases translate into recovery gains [146]. In the following Chapter, presented also as a manuscript, we measured acute and chronic circulating BDNF responses following CE in individuals during the subacute stages post-stroke and explored whether these responses were influenced by the Val66Met polymorphism, known to alter BDNF expression [98].

Chapter 3: The Effect of Cardiovascular Exercise on Brain-Derived Neurotrophic Factor in Subacute Stroke and its Interaction with the BDNF Val66Met Polymorphism

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Abstract

A stroke induces a profound yet time-limited growth-promoting response resulting in heightened neuroplasticity during subacute stages, a period where the brain may be more responsive to training experiences. Given its central role in regulating neuroplastic processes and brain repair in animal models, brain-derived neurotrophic factor (BDNF) has been investigated as a potential biomarker of stroke recovery in humans, with interventions aimed at increasing its levels holding therapeutical potential. Cardiovascular exercise (CE) is an effective treatment for improving functional recovery after stroke and promoting neuroplasticity, including upregulation of central BDNF concentration levels. However, studies examining the effects of CE on circulating BDNF levels in individuals at the subacute stroke stage of recovery have not yet been conducted. In this study, 76 first-ever ischemic subacute stroke (<3 months post-stroke) patients were randomly assigned to either eight weeks of CE+standard care or standard care alone. Blood samples were collected from participants before and immediately after a graded exercise test at baseline, four and eight weeks to measure the chronic and acute responses in serum BDNF levels. The influence of Val66Met, a BDNF polymorphism known to alter activity-dependent BDNF expression, was also examined. Despite significant increases in cardiorespiratory fitness, CE training did not result in any significant chronic and acute changes in BDNF concentration compared to the group receiving standard care alone. Similarly, BDNF response was not modulated by Val66Met polymorphism or associated with any changes in recovery outcomes. These findings indicate limited effects of CE in modulating circulating BDNF in people during subacute stages post-stroke. Factors potentially contributing to these outcomes, such as post-stroke inflammation and stress responses, and the capacity of circulating BDNF to reflect central nervous processes, are discussed.

Keywords: Cardiovascular exercise, BDNF, Brain Plasticity, Recovery, Biomarker.

Statements and Declarations

The authors have no relevant financial or non-financial interests to disclose.

Introduction

Following a stroke, a time-limited window of heightened neuroplasticity and increased responsiveness to training is initiated during the initial weeks, where most recovery gains occur [1]. During this critical period, extending for one month in animal models, a growth-promoting phase is activated, facilitating profound functional and structural alterations in the brain, leading to both spontaneous and treatment-induced recovery [2-4]. These changes include processes such as modifications in gene expression, neural excitability, dendritic spine turnover, axonal sprouting, and remapping of neural networks, all regulated by the expression of growth-promoting molecules [3,5]. In humans, this critical period is estimated to occur within the first week to three months post-stroke, known as the early subacute period [6]. During this stage, wherein nearly all recovery from impairment occurs [7], motor rehabilitation appears to induce greater gains compared to interventions initiated in later stages [8,9]. However, there is currently a dearth of evidence regarding the neurobiology of spontaneous and treatment-induced recovery in individuals during this critical period [10].

Neurotrophic factors, also referred to as neurotrophins, are secretory growth-promoting proteins widely expressed in the nervous system that play a central role in the differentiation, growth, and survival of neurons, as well as in activity-dependant forms of synaptic plasticity [11]. Brain-derived neurotrophic factor (BDNF) stands out as the most abundant neurotrophin in the brain and is by far the most extensively studied due to its crucial role in neuroplasticity and brain repair [12]. Binding to the high-affinity tyrosine kinase receptor B (TrkB), the mature isoform of BDNF initiates a downstream intracellular signaling pathway involving functional and structural neural changes by acting on presynaptic neurotransmitter release, and postsynaptic receptor responsiveness [13].

Post-ischemic lesion rodent studies have demonstrated the protective and restorative effects of BDNF activity, including mitigating cell death in acute phases [14] and facilitating synaptic plasticity, contributing to enhanced functional recovery during subacute stages [15]. Systemic administration of BDNF early after stroke has been shown to enhance motor recovery by reducing infarct size, promoting neurogenesis, and increasing synaptic plasticity [16-19], while local blockade of BDNF expression in the peri-lesional cortex impairs recovery by suppressing synaptic-related

plasticity mechanisms during the early stages [15]. Furthermore, a single nucleotide polymorphism in the BDNF gene, responsible for BDNF protein transcription, the Val66Met, has been associated with diminished post-stroke recovery, potentially by impairing BDNF secretion as well as neuroplastic reparative processes [20-23]. Taken together, these findings suggest that BDNF holds promise as a potential biomarker for stroke recovery.

Unlike most neurotrophins, BDNF is an activity-dependent neurotrophic factor, with its expression, secretion, and action susceptible to neural activity [24]. Consequently, interventions with the potential to enhance neural activity hold promise in facilitating neuroplastic changes and neural repair through BDNF signaling [25]. Cardiovascular exercise (CE) is a simple yet effective intervention to protect, maintain, and repair the nervous system by promoting neuroplasticity [26,27]. In animal models, CE interventions have been demonstrated to support functional recovery after stroke, in part by stimulating neurobiological processes such as synaptic plasticity, neurotransmitter signaling, neurogenesis, and upregulation of neurotrophic factors, including BDNF [28-30]. BDNF expression, in particular, has been shown to modulate the positive effects that CE has on neuroplasticity and brain repair after stroke, with rodent models showing no benefits on motor recovery when BDNF is blocked via antisense therapy [31].

In humans, the inaccessibility to study molecular changes directly in the brain has led to the study of blood biomarkers collected peripherally as potential surrogates for central neuroplastic processes and recovery post-stroke [32]. Reduced circulating BDNF has been observed in individuals following stroke and has been linked to poor functional outcomes [33,34]. Cardiovascular exercise in neurotypical populations has been shown to transiently increase circulating BDNF concentrations following a single bout ($BDNF_{acute}$) of exercise. Following long-term ($BDNF_{chronic}$) interventions, less consistent increases in circulating BDNF have been reported in some but not all studies [35]. Similar yet more inconsistent findings have been reported in individuals after stroke [36]. Importantly, repetitive sessions of CE have been found to amplify $BDNF_{acute}$ after a single bout of CE, suggesting enhanced responsiveness following regular CE training [37].

Previous studies investigating the effects of CE on BDNF in humans have exclusively focused on patients within chronic stages (> six months after stroke) [38], neglecting the period during which the brain might be more responsive to training [1]. To address this gap, we conducted a randomized controlled trial to evaluate the effects of CE on circulating BDNF levels in

individuals during the subacute stages of recovery. We assessed these effects under three different conditions: (1) basal BDNF levels after an 8-week progressive CE program, (2) a single session of CE, and (3) a session of exercise following a progressive CE program. We also examined the associations between BDNF response and changes in recovery outcomes, as well as the potential influence of the Val66Met polymorphism.

Methods and Materials

Experimental Design

In this randomized control trial (ClinicalTrials.gov Identifier: NCT05076747), participants were randomly assigned in a 2:1 ratio to either an 8-week CE training in addition to standard care or standard care alone (**Figure 1**). Evaluations occurred at baseline (T0), four weeks (T1), and eight weeks (T2). Each assessment comprised two experimental sessions 48 hours apart, comprising clinical motor outcomes and cardiorespiratory fitness with blood collection. Information regarding participant's characteristics and relevant clinical information were collected at T0. The site ethics board approved the study (Centre de Recherche de Readaptation du Montréal, CRIR-1265-0817), and all participants provided written informed consent.

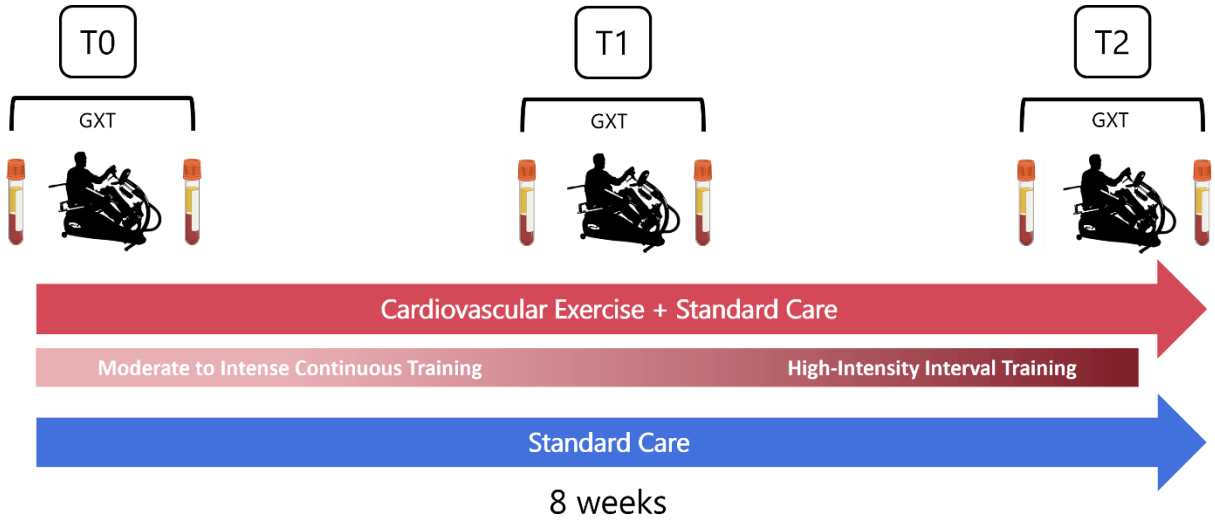

Figure 1. Study design with blood collection evaluations at baseline (T0), four weeks (T1), and eight weeks (T2). To measure the effects of CE training on circulating serum $BDNF_{chronic}$, blood samples were taken at rest before the GXT at each timepoint. $BDNF_{acute}$ was calculated as the difference between resting levels before the GXT and the average concentration levels at 3, 8, and 12 minutes after GXT termination. $BDNF_{acute}$ responses were measured at T0 combining both groups, and in response to CE+ standard care and standard care alone at each timepoint. GXT, graded exercise test.

Patients

We only included participants with first-ever ischemic stroke within the early subacute (7 days-3 months) stages of recovery [6]. Participants had to be between 40 and 80 years old, present no upper limb musculoskeletal or neurological conditions other than stroke, have sufficient ability/capacity to perform the exercise and assessment protocols safely, and have sufficient cognitive/communicative capacity to understand instructions. Individuals were excluded if they had a hemorrhagic stroke, cognitive impairment/dysphasia affecting informed consent, absolute contraindications to exercise, or were concurrently enrolled in another CE program.

Assessments

Baseline Assessment

At baseline (T0), stroke severity and cognitive status were assessed with the National Institutes of Health Stroke Scale (NIHSS) [39] and the Montreal Cognitive Assessment (MoCA) [40], respectively. The Charlson Comorbidity Index (CCI) age-adjusted was employed to assess the influence of pre-existing comorbidities [41]. Self-reported physical activity levels were measured at each time point using the physical activity scale for people with disabilities (PASIPD) (Washburn et al., 2002). Participants were instructed not to engage in moderate- or high-intensity physical activity 24 hours before the assessments.

Cardiorespiratory Fitness

Measurement of maximum oxygen uptake (VO_2peak in $mL.Kg^{-1}.min^{-1}$) is the gold standard for determining cardiorespiratory fitness [42]. To assess cardiorespiratory fitness, a symptom-limited GXT utilizing a whole-body recumbent stepper (NuStep T4r, Michigan, USA), validated for individuals with stroke, was performed [43]. During the GXT, heart rate (HR) was measured continuously while blood pressure and rate of perceived exertion (RPE) were taken every 2 minutes. The GXT was also used to determine VO_2peak, along with its associated maximal HR values (HR_{max}) and peak power output (PPO). PPO expressed in Watts was used to adjust training loads based on the capacity of each individual [44]. Indications for test termination followed current guidelines [45].

Clinical Motor Outcomes

Trained assessors evaluated clinical outcomes, including upper-limb motor impairment and function. Upper-limb motor impairment changes were assessed with the Upper-Limb Fugl-Meyer Assessment (UL-FMA), with higher scores indicating lower impairment [46]. Changes in upper-limb function were assessed with the Box and Block Test (BBT) [47]. To this end, participants were instructed to move as many small wooden blocks as possible from one side of a partitioned box to the other within one minute.

Blood Collection and Analysis

Blood collection was carried out by a registered nurse, with patients instructed to arrive in the laboratory 30 minutes prior to the procedure and to refrain from eating for at least two hours before the test. Upon participant arrival for each evaluation session, an antecubital intravenous line was placed in the non-paretic arm. Since the synthesis and release of BDNF increases transiently due to physical stimulus [48], chronic and acute BDNF changes were assessed at rest ($BDNF_{chronic}$) and following a GXT ($BDNF_{acute}$), respectively. Prior to each blood sample extraction, a waste sample was collected, and the line was flushed after each draw. A 5 mL blood sample was collected in a vacutainer serum separator tube 10 minutes before the GXT and at 3, 8, and 12 minutes post-GXT to capture BDNF peak levels after CE [49]. This procedure was repeated at T1 and T2 for both groups **(Figure 1)**.

While it was not always possible to collect blood samples at the same time of day across all participants due to schedule constraints, the collection times remained consistent throughout evaluation time points within each participant. Upon collection, blood samples were clotted for 1 hour, resting at room temperature, followed by 30 minutes at ~4ºC, and then centrifuged at 2200g for 15 minutes [50]. The resulting serum was then aliquoted into 250µL cryovials and stored in a -80ºC freezer (Thermo Fisher Scientific, Waltham, USA). Identified as the best-performing assay, the Biosensis Mature BDNF Rapid™ enzyme-linked immunosorbent assay (ELISA) Kit (Thebarton, Australia) was employed to determine BDNF concentrations [51]. All samples were measured in duplicate, with the average of both readings used for analysis to ensure reliability. The laboratory staff performing the analysis were blinded to the study aims, design, and exercise intervention associated with the samples.

Genotyping

Genomic DNA was extracted from red blood cells and saliva samples (DNA Genotek Inc., Canada), and genotyped using the Infinitum™ Global Diversity Array-8 v1.0 from Illumina. DNA extraction and purification were processed by Genome Quebec (Quebec, Canada) using the QIAsymphony system (QIAGEN). Sixty-eight individuals were genotyped with sufficient DNA concentration for reliable genotyping (10ng/ul). Standard quality control was performed using PLINK v1.9 to exclude SNPs with high missingness in individuals (>5%). The genotype of subjects for the BDNF single nucleotide polymorphism rs6265 were classified as homozygous for

the Val allele (Val/Val), heterozygous (Val/Met), and homozygous for the Met allele (Met/Met) using PLINK v1.9 **(Table 1)**. Individuals with Val/Met and Met/Met genotypes were combined to increase the statistical power [52].

Intervention

Cardiovascular Exercise

Cardiovascular exercise training was performed by trained therapists. The intervention group underwent a total of 24 CE training sessions over an 8-week period, with a frequency of 3 times a week and a 48-hour rest between sessions whenever possible. The CE intervention comprised four weeks of progressive moderate-to-vigorous intensity continuous training (MICT) followed by four weeks of progressive high-intensity interval training (HIIT), all conducted on a whole-body recumbent stepper ergometer **(Figure 1)**. The initial four weeks of MICT served as preparation for higher intensities in the second half of the program. Each training session included 2.5 minutes of warm-up and cool-down at 35% of the PPO, along with the main training component at the targeted intensity. Blood pressure was measured at the beginning and end of each CE session. To quantify the CE stimulus, HR, and Watts were continuously monitored during training via a pulse sensor (Polar H10, Kempele, Finland) and the stepper's digital console, respectively [53]. RPE (0-10) was assessed every 5 minutes throughout each training session during the MICT period, including at the end of the warm-up and the beginning of the cool-down, while during HIIT, RPE was collected in the final 5 seconds of each high-intensity bout with the modified Borg scale [54]. Training variables, including the average percentage of maximal HR (%HR_{max}), the average percentage of maximal watts (%W_{max}), total steps, and average RPE, were calculated for each session to quantify internal and external training workloads **(Table 2)** [55].

Moderate-to-vigorous Continuous Training (weeks 1-4): MICT has been typically employed as standard CE modality in stroke rehabilitation programs [56]. Intensities were determined using the PPO associated with VO_2peak during the GXT at T0 and progressively increased by 5% weekly from 65% to 80% PPO, ensuring constant cardiovascular adaptations. Session durations also increased from 20 to 35 minutes. This workload progression has been demonstrated as achievable and safe for individuals in subacute stages [57].

High-intensity Interval Training (weeks 5-8): HIIT is a proven safe and effective method for enhancing cardiorespiratory fitness in stroke patients that, enables even deconditioned individuals to reach higher exercise intensities [58]. HIIT intensities were determined using the PPO corresponding to the VO_2peak level achieved during the GXT at T1. The HIIT protocol comprised 8 x 60-second high-intensity intervals (8 minutes) interspersed with 7 x 60-second low-intensity intervals (7 minutes), totaling 20 minutes per session. This 60:60 interval ratio is optimal for sustaining high intensities [59]. While high-intensity intervals began at 85% PPO and increased by 5% weekly until reaching 100% PPO, low-intensity intervals were kept constant at 35% PPO. To minimize sudden changes in BP while ensuring target intensities, workload was progressively increased (15 seconds) before each high-intensity interval.

Standard Care Program

Standard care consisted of rehabilitation sessions conducted in the same center as the intervention and prescribed by the stroke clinical unit. In addition to routine health monitoring by physicians and nursing staff, standard care included physiotherapy, occupational therapy, and speech therapy sessions. The content, amount, and length of rehabilitation varied among patients and was tailored to individual needs determined by the stroke clinical unit. Each session consisted of 45-minute sessions of therapy. To examine potential differences between groups in standard care, we recorded the type and number of therapy sessions received by each patient from the study's beginning to its conclusion (**Table 1**).

Statistical Analysis

Data were plotted using normality plots and histograms for inspection. The Shapiro-Wilk test was used to confirm normality for each variable. Baseline differences in participant characteristics and clinical variables between groups were assessed using t-tests or Wilcoxon tests. Linear mixed models (LMM) were used to analyze differences in clinical outcomes (UL-FMA, BBT), cardiorespiratory fitness, and BDNF concentration levels between groups across time points (T0-T2). $BDNF_{chronic}$ were assessed by comparing the concentrations at rest throughout the study time points (T0-T2), while $BDNF_{acute}$ were determined as the difference between resting levels pre-GXT and the average concentration levels post-GXT (3, 8, and 12 minutes) also throughout the study time points. Each model included either $BDNF_{chronic}$ or $BDNF_{acute}$ as the dependent variable, with time point (T0, T1, T2), group, and their interaction as fixed effects. Covariates in

the model included age, sex, and stroke severity (NIHSS). Body mass index was also entered into the model as a covariate due to its significant effect on BDNF levels [60]. To examine the potential influence of the Val66Met polymorphism (Val/Val vs. Val/Met + Met/Met) in the model, it was nested within the Time*Group interaction. Participants were treated as a random effect to account for individual differences at baseline. Based on the Bayesian Information Criterion, log-likelihood ratio tests, and considering the temporal dependence of the data, AutoRegressive order 1 (AR1) was set as the most appropriate covariance structure. Tukey's HSD test was conducted to identify statistically significant pairwise differences. Assumptions for linear models, including normality in the distribution of random coefficients, were examined for all the variables in the model. Standard least squares multivariate linear regression analyses were used to investigate associations between $BDNF_{chronic}$ and $BDNF_{acute}$ with and changes in recovery outcomes. In the regression model, the same covariates—age, sex, stroke severity, and BMI—were included. Multicollinearity between predictor variables was assessed with the variance inflation factor (VIF) with a threshold of ≤ 5, indicating unacceptable multicollinearity [61]. All statistical analyses were performed with JMP (SAS Institute Inc, Cary, NC), version 17, and tested for significance at 0.05 alpha level ($p<0.05$).

Results

Table 1 presents the participant's characteristics and relevant clinical information for both groups at baseline (T0). Seventy-six participants were enrolled in the study, with 48 randomized to the CE+standard care group and 28 assigned to the standard care group. The trial flow, including dropouts, is detailed in **Table 2**. Data from all participants were included to measure BDNF responses at T0, and an intention-to-treat approach was used for those who were assessed at least at T1. No adverse events related to training were reported.

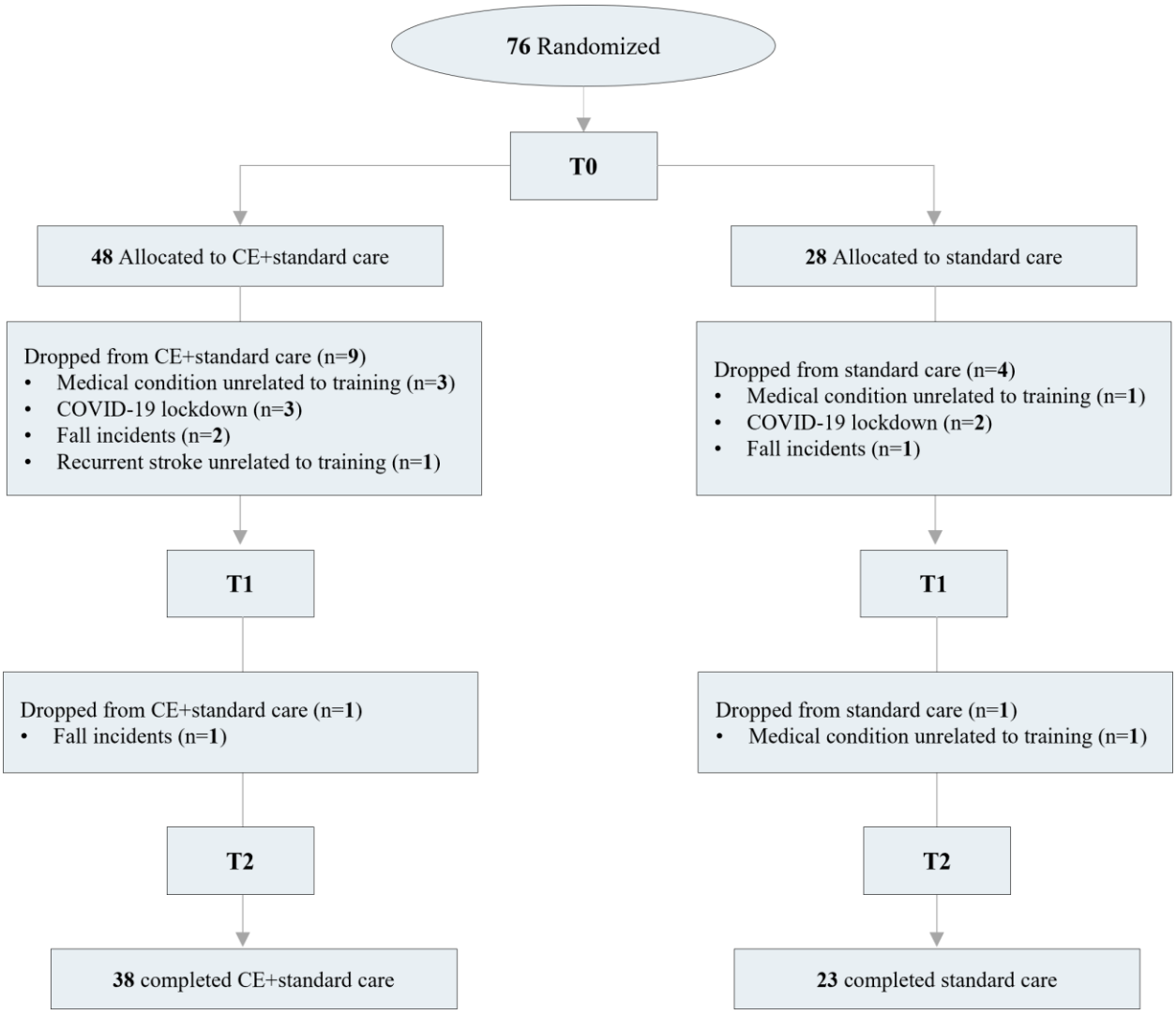

Figure 2. Flow chart of the Randomized Controlled Trial. CE: cardiovascular exercise; n: number of participants; T0: baseline; T1: four weeks; T2: eight weeks.

On average, participants were 63.5±10.2 years old (mean ± SD) and initiated the study 65.1±22.8 days after stroke. Participants presented mild stroke severity, with an average NIHSS score of 2.01±2.09, and an average MoCA score of 23.8±4.48. No significant differences were observed at T0 between groups in terms of age, sex, body mass index (BMI), time since stroke, lesion location, stroke severity, cognitive status, upper-limb impairment, upper-limb function, pre-existing comorbidities (measured with age-adjusted CCI), walking aid dependence, smoking history, and the average number of prescribed medications. The amount of standard care provided during the participation in the trial and levels of physical activity outside of the rehabilitation center

were similar between groups from T0 and T2. All participants assigned to the CE group who completed the study attended all 24 sessions.

Table 1. Baseline demographic and clinical outcomes.

	Exercise + Standard Care (n= 48)	**Standard Care (n= 28)**	**p value**
Age (years)	63 ± 11.39	65.35 ± 8.68	0.347
Sex (F/M)	11/37	10/18	0.290
BMI	27 ± 3.46	26.52 ± 4.01	0.624
Time since stroke (days)	68.12 ± 22.07	58.75 ± 24.03	0.088
Lesion location (%)			0.881
Cortical	16	21	
Cortico-subcortical	19	14	
Subcortical	54	57	
Cerebellar/Brainstem	10	7	
NIHSS (0-42)	2.02 ± 2.20	1.92 ± 1.92	0.799
MoCA (0-30)	24.14 ± 4.85	23.21 ± 3.8	0.126
UL-FMA (0-66)	56.20 ± 10.24	59.14 ± 8.22	0.106
$BBT_{affected}$ (blocks/min)	46.8 ± 13.25	48.10 ± 12.65	0.678
Cardiorespiratory fitness (VO_2peak, $mL.Kg^{-1}.min^{-1}$)	17.88 ± 5.49	18.12 ± 5.58	0.855
SNP rs6265			0.225
Val/Val	27	18	
Val/Met	13	7	
Met/Met	3	0	
CCI (Age-adjusted)	4.57 ± 1.83	4.57 ± 1.66	0.994
Walking aid dependence (%)	15	13	0.641
Smoking history (%)			0.242
Non-smoker	52	43	
Former smoker	40	56	
Current smoker	8	1	
Medications (n)	5.07 ± 2.58	5 ± 2.22	0.909
Classification (%)			
AC	60	53	
ACE	31	42	
AP	46	60	
BB	35	25	
PSY	33	28	
STA	79	100	
Therapy sessions (n)			

Physiotherapy	8.87 ± 8.21	6.59 ± 5.78	0.259
Occupational Therapy	11.5 ± 8.26	7.45 ± 5.98	0.051
Speech Therapy	5.02 ± 8.81	2.15 ± 5.48	0.212
Δ Physical activity (METS hr/day)	1.48 ± 5.39	-0.23 ± 5.28	0.245

AC, anticoagulant; ACE, Angiotensin-Converting Enzyme; AP, antiplatelet; BB, beta-blocker; BBT, Box and Block Test; BMI, body mass index; CCI, Charlson Comorbidity Index; F, female; M, male; MET, metabolic equivalent of task; MoCA, Montreal Cognitive Assessment; NIHSS, National Institutes of Health Stroke Scale; PSY, psychoactive; STA, statin. Values are presented as mean and standard deviation (SD).

Table 2. Internal and External Training Load for the CE Group.

	MICT	**HIIT**	**Total**
HR_{max} (%)	82.35 ± 8.06	81.89 ± 6.94	82.13 ± 7.05
$Watts_{max}$ (%)	63.69 ± 8.74	67.49 ± 13.74	65.59 ± 10.96
Total Steps	29384 ± 5932	20237 ± 4366	49464 ± 10183
RPE_{ave} (0-10)	4.58 ± 1.31	5.15 ± 1.61	4.86 ± 1.35

CE group's average internal and external training loads during both MICT and HIIT periods, including the warm-up and cool-down phases of each session. Average percentages of HRmax and PPO achieved during both MICT and HIIT periods were calculated based on VO2peak values at T0 and T1, respectively. Regarding RPE measurement, values were recorded every 5 minutes during the MICT period, including at the end of the warm-up and the beginning of the cool-down, while during HIIT, RPE was collected in the final 5 seconds of each high-intensity bout. Values are presented as mean and SD. HIIT, high-intensity interval training; HR, heart rate; MICT, moderate-to-vigorous continuous training; PPO, peak power output RPE, rate of perceived exertion. Values are presented as mean ± SD.**Cardiorespiratory Fitness**

No significant differences in cardiorespiratory fitness were observed between groups at T0. At baseline, all participants had an average VO_2peak of 18.43±5.63, a HR_{max} of 81±13% of the age-predicted maximum, and an average time to exhaustion of 10.49±2.50 minutes **(Table 3)**. There was a significant effect of Time (F(2,78) = 16.76, p = <.0001), and a significant Time x Group interaction (F(2,78) = 13.46, p = <.0001). The standard care group showed no significant change in VO_2peak (0.27 $mL.Kg^{-1}.min^{-1}$, 95% CI -2.19 to 1.64, p=0.998). In contrast, the

CE+standard care group exhibited significant increases at T1 during MICT (2.76 $mL.Kg^{-1}.min^{-1}$, 95% CI 1.58 to 3.93, p=<.0001), which continued following HIIT at T2 (1.64 $mL.Kg^{-1}.min^{-1}$, 95% CI 0.45 to 2.82, p=<.0001). This resulted in a total increase of 4.43 $mL.Kg^{-1}.min^{-1}$ (95% CI 2.97 to 5.82, p=<.0001), representing a 27.25% improvement in cardiorespiratory fitness, which is clinically significant [62,63].

Clinical Motor Outcomes

There was a significant effect of Time on upper-limb motor impairment using the UL-FMA ($F(2, 99) = 15.61$, $p = 0.0001$), with no significant Time x Group interaction ($F(2, 99) = 1.04$, $p = 0.355$). Similarly, for upper-limb function measured using the BBT, there were significant effects of Time ($F(2, 116) = 15.73$, $p < .0001$) but no significant Time x Group interaction ($F(2, 116) = 0.22$, $p = 0.801$).

Chronic and Acute BDNF Changes

Two participants did not go through blood sample collection, resulting in no BDNF data being available for analysis. $BDNF_{chronic}$ and $BDNF_{acute}$ for both CE and standard care groups are detailed in **Table 4**.

At T0, no statistically significant differences in basal BDNF concentration were observed between groups (p= 0.275). No significant effects of Time ($F(2,186) = 1.08$, $p = 0.340$) or Time x Group ($F(2,186) = 0.06$, $p = 0.937$) were identified for $BDNF_{chronic}$, indicating limited effects of CE training on basal BDNF concentrations **(Figure 4)**.

Combining both groups at T0 (n=74, **Figure 3)**, $BDNF_{acute}$ showed significant effects of Time ($F(3,282) = 2.67$, $p = 0.047$) **(Figure 3**). Although the model was significant, the increase from baseline to 3 minutes post-GXT was not statistically significant, with pairwise comparisons revealing significant decreases between 3 and 12 minutes post-GXT (-1345.19 pg/ml, 95% CI -2593 to 96.47, p=0.029).

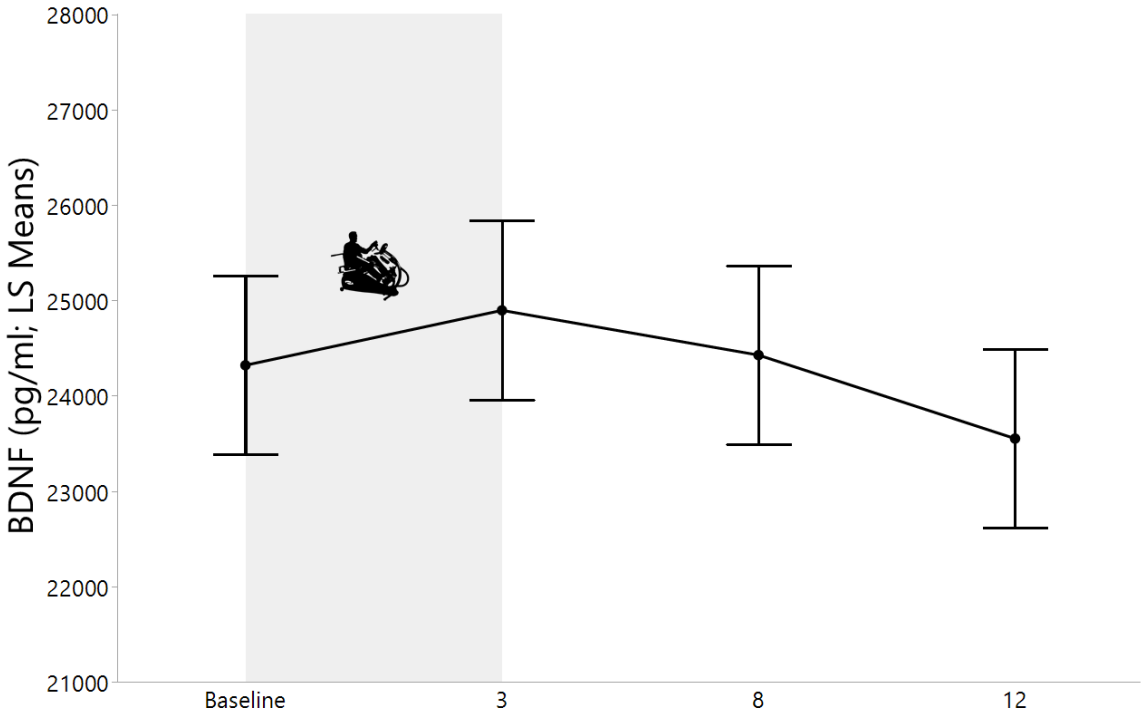

Figure 3. $BDNF_{acute}$ concentration at baseline (T0) following GXT (n=74). Blood samples were collected 10 minutes before the GXT and at 3, 8, and 12 minutes post-GXT to capture serum BDNF peak levels after CE. Data are presented as least squares means with and standard errors (SE). GXT: graded exercise test, Pg/ml: picograms per milliliter.

When investigating the effects of CE training on $BDNF_{acute}$ throughout the study (T0-T2), we found no significant effects of Time ($F(2,184) = 2.76$, $p= 0.065$) or Time x Group interaction ($F(2,184) = 1.01$, $p= 0.364$), indicating that CE training over time did not affect $BDNF_{acute}$ following GXT (**Figure 4**).

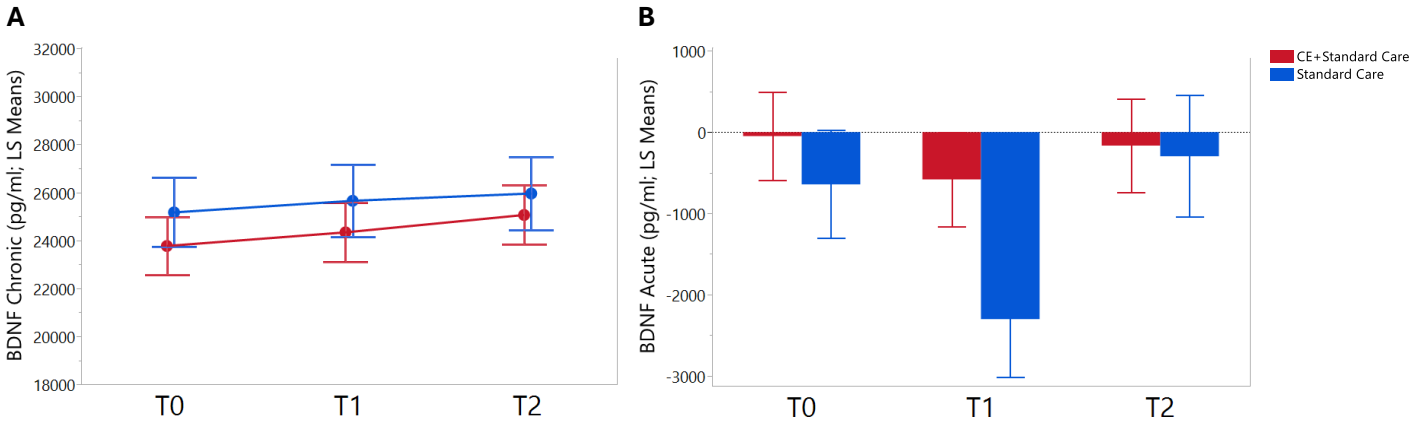

Figure 4. $BDNF_{chronic}$ (A) and $BDNF_{acute}$ (B) changes in serum BDNF concentration throughout the study at baseline (T0), four weeks (T1), and eight weeks (T2) following CE+standard care and standard care groups. $BDNF_{chronic}$ was assessed by comparing the basal concentrations at rest across the study time points, while $BDNF_{acute}$ was determined as the difference between resting levels pre-GXT and the average concentration levels post-GXT (3, 8, and 12 minutes). Data are presented as least squares means with and standard errors (SE). pg/mL: picograms per milliliter.

Table 3. GXT values at baseline (T0), four weeks (T1), and eight weeks (T2).

	T0	**T1**	**T2**
GXT(VO_2peak, mL.Kg^{-1}.min^{-1})			
CE+Standard care	17.19 (0.82)	19.99 (0.84)	21.65 (0.85)
Standard care	18 (1.01)	18.11 (1.03)	18.34 (1.05)
GXT (%HRmax)			
CE+Standard care	82.6 (1.87)	82.85 (2.05)	87.42 (2.0)
Standard care	78.89 (2.59)	80.73 (2.69)	81.66 (3.44)
GXT (minutes)			
CE+Standard care	11.23 (0.39)	13.02 (0.41)	13.97 (0.36)
Standard care	10.08 (0.55)	10.91 (0.47)	11.79 (0.64)

Measurements include maximum oxygen uptake (VO_2peak), HR_{max}, and average time to exhaustion in minutes. Data are presented as least squares means with and standard errors (SE). $mL.Kg^{-1}.min^{-1}$: milliliters per kilogram per minute.

Table 4. $BDNF_{chronic}$ and $BDNF_{acute}$ serum concentration at baseline (T0), four weeks (T1), and eight weeks (T2).

Serum concentration (pg/ml)	**T0**	**T1**	**T2**
$BDNF_{chronic}$			
CE+Standard care	24012 (1162)	24495 (1210)	25220 (1210)
Standard care	25192 (1431)	25660 (1478)	25972 (1506)
$BDNF_{acute}$			
Δ CE+Standard care	2210 (590)	1694 (630)	1755 (630)
Δ Standard care	1279 (722)	-320 (772)	2150 (806)

Data are presented as least squares means with and standard errors (SE). pg/mL: picograms per milliliter.

Associations and Predictors of BDNF Response

We examined potential associations between $BDNF_{chronic}$ and $BDNF_{acute}$ with changes in clinical motor outcomes (UL-FMA, BBT) and cardiorespiratory fitness. We also analyzed the influence of Val66Met polymorphism on BDNF responses and changes in clinical motor outcomes and cardiorespiratory fitness. No significant associations were observed between BDNF responses and any motor and fitness measures in either the CE+standard care or the standard care group **(Supplementary Table 1)**. Similarly, the Val66Met polymorphism did not influence $BDNF_{chronic}$ or $BDNF_{acute}$, nor did it affect clinical motor outcomes and cardiorespiratory fitness **(Supplementary Table 2)**.

Discussion

Rehabilitative treatments capable of inducing neuroplasticity, such as CE training, are believed to have significant therapeutic potential for stroke recovery, especially during the early post-injury stages when the brain is highly responsive to plasticity-inducing interventions [1]. This study is the first to examine the effects of CE on circulating BDNF levels in individuals with subacute stroke. Despite significant improvements in cardiorespiratory fitness, an 8-week progressive CE training intervention did not significantly affect $BDNF_{chronic}$ or $BDNF_{acute}$ responses in the peripheral circulation. Furthermore, BDNF responses were neither modulated by

Val66Met polymorphism nor associated with clinical outcomes of recovery following either CE training or standard care.

Cardiovascular exercise is recommended as a fundamental part stroke rehabilitation due its potential to reduce the risk of stroke recurrence while enhancing cardiorespiratory health, metabolic function, and neural recovery [64]. However, despite the well-documented cardiovascular and metabolic benefits for individuals with stroke, its effects on neuroplasticity and brain repair, especially in the early stages of recovery, remains largely unknown [38]. Studies on rodents have shown that several days of voluntary exercise increase BDNF expression and its receptor TrkB in the brain, a molecular response mediating activity-dependent neuroplasticity supporting learning and memory, as well as neural repair and recovery processes after stroke [31,65,66]

Our findings revealed no significant effects of CE training on $BDNF_{chronic}$ levels despite significant improvements in cardiorespiratory fitness (VO_2peak). Compared to a standard care group, CE demonstrated significant increases in VO_2peak (+27.25%). These values surpass those previously reported in individuals with subacute stroke undergoing high-intensity CE interventions (+1.46 mL/kg/min) [67] and previous BDNF studies involving chronic stroke populations (Ploughman et al., +1.7 mL/kg/min; Hsu et al., +3.4 mL/kg/min). Additionally, no significant associations were found between changes in cardiorespiratory fitness and $BDNF_{chronic}$, indicating that the lack of increase in basal BDNF concentration was unlikely due to an insufficient exercise stimulus.

Our results are consistent with the mixed evidence on the impact of CE training on circulating basal BDNF in humans. In non-disabled populations, studies have presented conflicting findings, with some investigations reporting increased concentrations following long-term CE interventions, while others showing no change or even reductions in BDNF levels (Dinoff et al., 2016; Knaepen, 2010). In the context of stroke, only two clinical trials have investigated the long-term effects of CE training on $BDNF_{chronic}$ levels in patients with chronic stroke, with divergent results. One study (n=23) reported significant increases in BDNF levels after 12 weeks of HIIT and significant decreases after MICT [68] whereas another study (n=52) reported no significant changes following ten weeks of vigorous-intensity treadmill training compared to a group undergoing standard care [69]. Our findings align with these inconsistencies but now in the subacute stages post-stroke.

In contrast to neurotrophins like nerve growth factor (NGF) that are secreted constitutively, BDNF remains within the cytoplasm under resting conditions and is only secreted in response to neural activity [24,70]. While current evidence does not consistently support that CE training can increase basal circulating BDNF concentrations, there is more robust evidence regarding the impact of a single CE session on increasing $BDNF_{acute}$ levels [35]. In mice, a single exercise session has been shown to upregulate intracellular signaling molecules in the brain, including BDNF [48], initiating a biochemical cascade responsible for exercise-induced synaptic changes [71]. Similarly, in neurotypical individuals, increased circulating BDNF levels have consistently been reported following a single CE session [49,72], with higher exercise intensities eliciting more pronounced increases compared to moderate intensities [73-75]. In individuals with stroke at chronic stages of recovery, similar yet mixed findings have been reported, with serum BDNF levels transiently increasing following a single CE bout at vigorous or maximal intensities such as HIIT or a GXT [36]. In this study, a GXT was performed to assess the $BDNF_{acute}$ to CE. By using a GXT at multiple time points, we were able to measure $BDNF_{acute}$ longitudinally while also evaluating changes in cardiorespiratory fitness (VO_2peak), which have been shown modulate BDNF responses to CE [76]. Previous studies have shown significant BDNF increases following GXT in neurotypical populations [77,78] and in people with chronic stroke [79]. Our findings revealed a significant yet moderate increase in BDNF levels when combining both groups at T0 **(Figure 2)**, with peak concentrations at 3 minutes post-exercise, gradually declining below pre-GXT levels at 12 minutes.

While CE training may not increase $BDNF_{chronic}$ significantly, both animal and human studies suggest that longitudinal CE interventions could prime the acute response following a single exercise session, indicating an adaptive response for $BDNF_{acute}$ induction [37,80,81]. However, our results did not reveal any priming effects of training on $BDNF_{acute}$. Unlike studies in neurotypical populations showing that several weeks of CE training can enhance $BDNF_{acute}$ response to a single bout of CE [82-84], our findings align with the only other stroke study that, revealed no effects of 10 weeks of vigorous-intensity treadmill training on enhancing $BDNF_{acute}$ immediately after a GXT [69].

One possible explanation for the limited effects of CE on both $BDNF_{chronic}$ and $BDNF_{acute}$ responses could be the stress and inflammatory status that characterizes the early stages post-stroke

and its interaction with CE. Stroke triggers a cascade of stress-related hormones (e.g. corticosterone, cortisol) and pro-inflammatory molecules (e.g. Interleukin-6, tumor necrosis factor-alpha, or C-reactive Protein) that can persist during acute and subacute stages [85-87] and have been shown to attenuate brain reparative processes, including BDNF mRNA levels and BDNF expression, potentially affecting functional recovery outcomes [88-92]. Additionally, vigorous-intensity CE can also stimulate pro-inflammatory cytokines and cause up to a 20-fold increase in corticosterone levels, potentially mitigating CE-induced BDNF expression [71,93,94]. Given the vigorous intensities reported both during the CE training program **(Table 2)** and the GXTs **(Table 3)**, it could be that our interventions mitigated CE-induced BDNF expression. Although we did not measure stress or inflammatory markers, this hypothesis will align with previous animal work where high-intensity motorized running implemented two weeks post-stroke resulted in an attenuated BDNF response alongside significantly elevated serum corticosterone levels [95]. This time-sensitive period post-injury, where CE's effects on BDNF might be more limited, has also been observed in rat models of traumatic brain injury, where early, but not late, initiation of CE resulted in dysregulated expression of BDNF and delayed recovery [96]. Taken together, our findings suggest that the inability of CE to promote both $BDNF_{chronic}$ and $BDNF_{acute}$ increases could be related to molecular alterations occurring early post-stroke and their interaction with intense CE. However, the fact that similar negative findings have been reported in both chronic stages post-stroke and neurotypical populations suggests that other factors may also contribute to these results.

In addition to the limited effects of CE on BDNF, we found no significant associations between BDNF responses and changes in clinical motor outcomes, including upper limb impairment and function, in either CE or standard care groups **(Supplementary Table 1)**. These results contrast with pre-clinical evidence demonstrating that BDNF is crucial in mediating the positive effects that CE has on functional stroke recovery [31]. Although the low disability levels of the individuals included in our study could have influenced these associations, our results align with previous evidence showing that peripherally measured BDNF has limited predictive value as a recovery biomarker post-stroke [97,98]. Furthermore, BDNF response, both $BDNF_{chronic}$ and $BDNF_{acute}$, and well as recovery outcomes were not influenced by BDNF Val66Met polymorphism, a genetic variant decreasing activity-dependent BDNF secretion [20]. Understanding the role of genetic variants in rehabilitation's effects on neuroplasticity biomarkers has been suggested as a method to better identify patients who are more likely to benefit from such

treatments [23]. It has been hypothesized that individuals with one or two copies of the met allele may show a decreased response to neuroplasticity-based interventions such as CE [99]. This study is the first to investigate the impact of the Val66Met polymorphism on serum BDNF levels in response to CE in individuals with stroke. Unlike studies in animals where Val66Met polymorphism consistently alters intracellular trafficking and activity-dependent BDNF expression, also in response to CE [100], our findings align with other human studies that have shown inconclusive results regarding the association between this genetic variant and BDNF concentration levels following motor interventions, as well as its impact on recovery outcomes post-stroke [52,101-106].

One possible explanation for these contradictory findings could be the different sources from which BDNF is typically measured between species. In animal models, BDNF can be measured directly in the brain, whereas in humans, it is measured peripherally, assuming its concentration reflects central neural processes. Previous studies suggest that BDNF can be transported unidirectionally from peripheral circulation to the brain by crossing the blood-brain barrier (BBB) [107] and that the brain might be the primary source of circulating BDNF both at rest and during CE [108]. This is supported by studies showing correlations between peripheral BDNF levels and central brain concentrations [109]. However, this notion has been challenged by evidence indicating that neurotrophins, including BDNF, do not cross the BBB in significant amounts unless they are conjugated with a molecular Trojan horse [110]. Intravenous administration of BDNF, when conjugated to a BBB molecular trojan horse, has been shown to reduce stroke volume and improve functional outcomes in rats with middle cerebral artery occlusion [111,112]. This disparity between BDNF sources has also been observed during early post-stroke stages in animal models, where BDNF concentrations increase in the brain [113], while no changes are reported in circulation [114,115]. This discrepancy underscores the need for caution in interpreting human studies and highlights the necessity for further studies to elucidate the role of circulating BDNF in central neural processes and its association with stroke recovery. Employing techniques such as positron emission tomography could provide precise measures of BDNF utilization in the brain by assessing the TrkB/BDNF system [116]. Ultimately, such analyses will be necessary to gain a clearer understanding of the neurotrophic effects of BDNF in the brain and its role in promoting neuroplastic changes underlying stroke recovery.

To the best of our knowledge, this study represents the largest trial investigating the effects of CE training on circulating BDNF levels in individuals recovering from a stroke. Given that animal evidence suggests a period of heightened neuroplasticity and responsiveness to training during early stages of recovery [1], we expected a significant effect of CE on enhancing BDNF levels in subacute stroke patients. However, our findings indicated that despite significant improvements in cardiorespiratory fitness, CE training had limited effects on both $BDNF_{chronic}$ and $BDNF_{acute}$ circulating levels. Similarly, BDNF responses were neither influenced by BDNF Val66Met polymorphism nor associated with changes in recovery outcomes. This aligns with previous studies that have not established a clear link between the upregulation of circulating BDNF following CE and stroke recovery improvements [38]. Factors such as inflammation and stress responses during early stages of recovery post-stroke may contribute to these results. Additionally, given the uncertainty about how well circulating BDNF represents central neural processes, these findings should be interpreted cautiously regarding CE's potential neurotrophic effects on the brain. It is crucial to emphasize that these findings should not affect current health policies regarding the implementation of CE in post-stroke individuals, considering its established benefits in improving functional capacity, daily living activities, quality of life, and reducing the risk of subsequent cardiovascular events [56].

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Supplementary Material

Supplementary Table 1. Adjusted multivariate linear regression examining associations between chronic and acute serum BDNF changes and changes in recovery outcomes, including upper-limb impairment, function, and cardiorespiratory fitness.

	CE+standard care			**Standard care**		
	Estimate (95% CI)	**p value**	**R^2**	**Estimate (95% CI)**	**p value**	**R^2**
UL-FMA T0-T2						
$BDNF_{chronic}$	-7.45 (-0.0004, 0.0002)	0.661	0.20	-6.06 (-0.0004, 0.0002)	0.717	0.38
$BDNF_{acute}$	0.0001 (-0.0002, 0.0006)	0.411	0.22	0.0001 (-0.0003, 0.0006)	0.464	0.38
BBT T0-T2						
$BDNF_{chronic}$	-0.0005 (-0.0001, -5.81)	0.209	0.16	-0.0004 (-0.0009, 9.79)	0.103	0.62
$BDNF_{acute}$	-0.0001 (-0.0004, 0.0008)	0.588	0.04	0.0003 (-0.0004, 0.001)	0.325	0.55
CRF T0-T2						
$BDNF_{chronic}$	-2.91 (-0.0002, 0.0001)	0.770	0.19	-0.0001 (-0.0001, 0.0003)	0.424	0.17
$BDNF_{acute}$	-0.0001 (-0.0001, 0.0003)	0.376	0.20	6.55 (-0.0003, 0.0004)	0.742	0.14

BBT, Box and Blocks Test; CRF, cardiovascular fitness; UL-FMA, upper-limb Fugl-Meyer assessment.

Supplementary Table 2. Adjusted linear mixed models examining the influence of Val66Met polymorphism on acute serum BDNF changes at T0, as well as chronic and acute changes following cardiovascular exercise training.

	DFNum	**DFDen**	**F ratio**	**p value**
$BDNF_{acute}$T0				
Age	1	61.5	0.02	0.876
Sex	1	61.5	0.23	0.630
NIHSS	1	61.5	0.19	0.664
BMI	1	61.6	3.02	0.087
Val66Met	1	61.6	0.009	0.923
Time[Val66Met]	6	254.0	1.48	0.185
$BDNF_{chronic}$				
Age	1	62.0	0.08	0.775
Sex	1	62.5	0.76	0.386
NIHSS	1	62.8	0.30	0.585
BMI	1	65.5	2.10	0.152
Val66Met	1	62.3	0.07	0.793
Group	1	62.3	0.44	0.507

Time	2	172.0	1.87	0.157
Time*Group[Val66Met]	4	172.0	0.11	0.978
$BDNF_{acute}$				
Age	1	60.1	0.18	0.673
Sex	1	62.4	3.15	0.081
NIHSS	1	62.4	0.004	0.952
BMI	1	71.9	0.11	0.737
Val66Met	1	61.2	0.61	0.436
Group	1	60.9	1.92	0.170
Time	2	171.0	2.67	0.072
Time*Group[Val66Met]	4	171.0	0.75	0.554

BMI, body mass index; NIHSS, national institutes of health stroke scale; * $p<0.05$

Chapter 4: General Discussion

In recent years, significant interest has emerged in identifying the optimal recovery period following stroke, understanding its underlying mechanisms, and determining the most effective treatments [147]. This expectation is primarily based on animal models, where motor rehabilitation within the first month post-injury yields greater neuroplastic changes and functional improvements, but also recently in humans, suggesting a similar critical period during the early subacute stages of recovery [29]. Identifying effective interventions during these early stages and determining their therapeutic capacity could improve rehabilitation approaches and mitigate long-term impairments in stroke patients.

In this project we focused on cardiovascular exercise (CE) as a rehabilitative intervention post-stroke due to its well-established effectiveness in promoting brain function, and because its effects closely parallel some of the mechanisms underlying stroke recovery. However, while animal studies have demonstrated the positive impact of CE on neuroplasticity and recovery, its effects on the human brain remain unclear, even more so when the brain has been damaged by stroke, thus limiting our understanding of its reparative capacity and clinical application.

To address these gaps, we used a two-step approach. First, we conducted a comprehensive review of the current literature on CE's effects on neuroplasticity biomarkers in stroke. This review allowed us to analyze how biomarkers are being used to measure neuroplasticity in response to CE in stroke patients, identify the main knowledge gaps, and propose directions for future research. One of the major gaps identified was the lack of studies focusing on the subacute stages of recovery. To address this, we conducted the first randomized controlled trial examining the effects of CE training on two widely studied biomarkers associated with stroke recovery, CSE and BDNF, in this specific patient group. Additionally, we investigated whether the individual genotype, specifically the Val66Met polymorphism, influences the neuroplastic response to CE, offering insights into factors that might contribute to variability in the neuroplastic response and recovery outcomes.

Our findings demonstrated that 8 weeks of progressive CE training is an effective intervention for improving cardiorespiratory fitness in people with subacute stroke. Specifically, 4 weeks of moderate-intensity continuous training (MICT) followed by 4 weeks of high-intensity

interval training (HIIT) resulted in a 27.25% improvement in cardiorespiratory fitness, with a total $VO2_{peak}$ increase of 4.43 $mL \cdot kg^{-1} \cdot min^{-1}$ in individuals with an average time-since stroke of 65 days. However, contrary to our expectations, CE training had minimal effects on both CSE and BDNF biomarkers, suggesting limited neuroplastic effects. Additionally, Val66Met polymorphism had minimal impact on how these biomarkers responded to CE or affect functional recovery outcomes.

Given the substantial evidence demonstrating a critical period for rehabilitation during early post-stroke stages in pre-clinical models, we anticipated that CE training would amplify the endogenous neuroplasticity processes underlying subacute stroke. We expected increases in CSE and BDNF as neuroplasticity biomarkers and improvements in motor recovery. These findings challenge the previous notion that early post-stroke interventions can induce significant neuroplastic changes leading to improved recovery. In the following sections, we will discuss the study's findings, offering neurobiological and methodological factors to explain the results, highlight the main limitations, and propose future directions to enhance our understanding of CE's effects on stroke recovery.

Neurobiological Hypotheses

- **Underlying Processes in Subacute Stages**

One often overlooked factor discussed in both Chapters 2 and 3, involving CSE and BDNF, is the dual nature of neuroplasticity following stroke. While the growth-promoting response associated with "reparative" neuroplasticity during early post-stroke stages has been well-documented and reported in the literature, there is also a gradual upregulation of growth-inhibitory responses as this critical period ends [24]. These inhibitory responses may limit potential aberrant neuroplastic changes but also can interfere with the neural repair processes, limiting outgrowth or repelling axon sprouting post-stroke. These two parallel yet competing processes interact during the early stages of recovery, regulating net neuroplasticity outcome and determining the final potential for recovery [148].

The main goal of any experimental rehabilitative approach is to find interventions that promote growth factors and inhibit limiting factors [149]. In both Chapters 2 and 3, we discuss

potential mechanisms that may explain the null effects of CE on CSE and BDNF biomarkers. Specifically, we discuss how increased intracortical inhibition ($GABA_B$) in the ipsilesional hemisphere, and inflammation and stress responses during early post-stroke stages, might have mitigated CE-induced excitability and neurotrophic changes, respectively.

At the same time, it has been well-documented in animals but also in humans, that CE can modulate GABAergic activity in the brain, as well as reduce inflammation and stress responses [150]. Despite these known effects, we did not observe a reduction in inhibitory excitability (i.e. CSP, SICI) with CE, and unfortunately, we did not measure inflammatory and stress blood markers. Although these findings may seem counterintuitive, they could suggest that the growth-inhibiting processes during the early stages post-stroke may be too profound for neuroplasticity-inducing interventions like CE to effectively promote significant changes in the brain on their own. Adjuvant treatments (e.g., intensive upper-limb motor training, brain stimulation, pharmacological) might be necessary to reduce growth-inhibiting responses and enable neuroplastic changes to occur following rehabilitative interventions such as CE during early recovery stages.

- **Inter-subject Variability in Subacute Stages**

Another potential explanation for our results could lie in the variability of neurobiological processes that characterize the early stages of recovery. Unlike the later chronic stages, where spontaneous recovery tends to plateau, individuals in the subacute stages often exhibit a more pronounced and variable pattern of behavioral change **(Figure 1)** [151]. This variability is well-established in motor recovery outcomes in humans, yet less is known about the underlying neurobiological mechanisms detected through biomarkers [152-155]. However, given the clear link between neurobiological processes and motor outcomes after stroke, it is plausible that similar variable patterns could be occurring neurobiologically, mirroring the behavioral changes.

Importantly, if not controlled, this variability can significantly impact the interpretation of rehabilitation studies investigating underlying mechanisms during these early stages. Although statistical models like linear mixed models (LMM) can accommodate the lack of homogeneity in regression intercepts (baseline) and slopes (longitudinal changes), failure to account for this variability in sample size calculations could result in a Type II error, falsely indicating null effects of treatment intervention when a significant effect exists. Without a doubt, more evidence is needed in humans to better understand how neurobiological events evolve during different stages post-stroke [17].

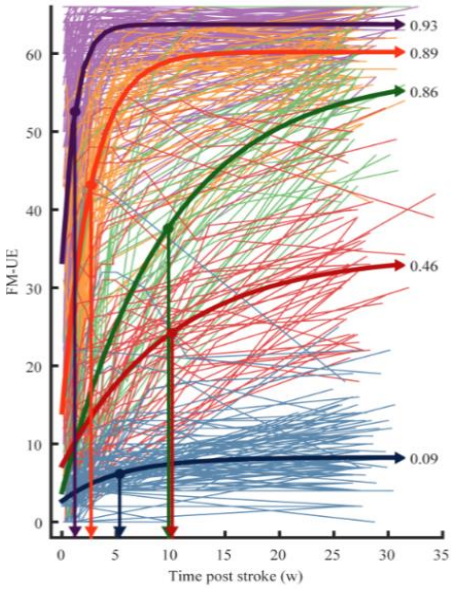


Figure 1. Inter-subject motor variability post-stroke. Longitudinal data of Fugl-Meyer Upper Extremity (FM-UE) scores from 412 ischemic stroke patients, as reported by van der Vliet et al. (2020).

Often, we mistakenly treat "stroke" as a homogenous condition, when it describes a very heterogenous group of disorders that evolve differently over time. To detect the true effects of treatment interventions, future studies should be adequately powered by adjusting for this variability, likely requiring larger sample sizes for subacute stages. Additionally, to confirm the existence of increased inter-subject neurobiological variability during early recovery stages, it would be essential to either (1) follow the same patients longitudinally during relevant time points post-stroke [156] or (2) select a homogeneous group of individuals with similar neuroanatomical and behavioral features — an approach commonly used in animal models but less frequently observed in humans [85].

Methodological Hypothesis

- **Characterization of the Patients**

Stroke-related characteristics, such as lesion location and baseline function, can significantly impact the response to rehabilitative interventions, influencing both the underlying

neurobiology and the individual's capacity to recover function [157]. Consequently, a heterogenous group of patients with lesions affecting different brain structures could increase variability in how certain biomarkers respond to treatments, thus limiting the capacity to detect any potential neuroplastic changes. One prime example of this is in TMS studies, a technique with excellent temporal resolution at the millisecond level but limited special resolution, targeting cortical areas as small as 1-2 cm in diameter [158]. In stroke research, TMS has been extensively used as it assesses functional integrity of the corticospinal tract (CST), a critical pathway whose structural integrity dictates both initial impairment and motor recovery [117].

In our study, we aimed to reduce inter-subject variability by applying stringent inclusion criteria (i.e. first-ever stroke, ischemic, 7 days to 3 months post-stroke, 40-80 years old), while at the same time, including patients with any lesion location in order to increase sample size. This approach enabled us to conduct the largest study examining neuroplastic responses to CE in people with subacute stroke, a population challenging to recruit for pragmatic and safety reasons [16], while also controlling for different lesion subgroups. However, despite sensitivity analyses between lesion groups (subcortical, cortical, cerebellar) suggested no influence of lesion location, lesion heterogeneity, and specifically CST lesion heterogeneity, could have influenced our findings, particularly for TMS outcomes. Without controlling for CST damage, and given the localized stimulation of TMS on this pathway, stroke location could have influenced CSE responses to CE, potentially masking any true effects.

In the review of the literature in Chapter 1, we observed that only 29% of the studies provided detailed information on key lesion-related aspects such as size and location [159]. To accurately determine the reparative potential of rehabilitative interventions like CE in stroke, where inter-subject variability is inherent, it is crucial that future studies control for neuroanatomical factors that can influence how specific biomarkers respond to interventions, such as CST integrity in TMS studies. This could be achieved through rigorous stratification of patients in large multisite studies or, as mentioned earlier, by selecting a homogeneous group of individuals with similar neuroanatomical and behavioral features. Without controlling for these factors, finding a true effect could be like trying to find a needle in a haystack, or even worse, result in a type I error [160]. Ultimately, this approach will be essential to investigate how CE, or any other interventions, affects neuroplasticity in individuals with stroke.

Lastly, another potential methodological explanation could reside in the mild levels of impairment (UL-FMA: 57±9) and severity (NIHSS: 2±2) of our patients. This is likely due to selection bias, a recurrent issue in rehabilitation research, where less affected individuals are more able and more likely to participate in interventions such as CE. Additionally, clinical stroke units might be hesitant to refer more severely impaired patients to research programs, fearing it might interfere with their regular therapy. This biased selection of participants may have led to ceiling effects, particularly in motor outcomes like FMA (0-66) known to have a ceiling effect [161], but also in the capacity to induce neuroplastic changes [162], potentially masking any true CE effects and biomarker-behaviour associations. Importantly, one of the main limitations of this bias is that our findings cannot be generalized to severely affected individuals, thus neglecting a substantial portion of the stroke population. Future studies should include more severely affected populations to investigate their response to treatments such as CE. Additionally, utilizing impairment outcome measures with sufficient granularity and no ceiling effect will be crucial to capturing true recovery gains and mechanisms of neuroplasticity following rehabilitative treatments [41].

- **Biomarkers: The Pathway to Neuronal Understanding?**

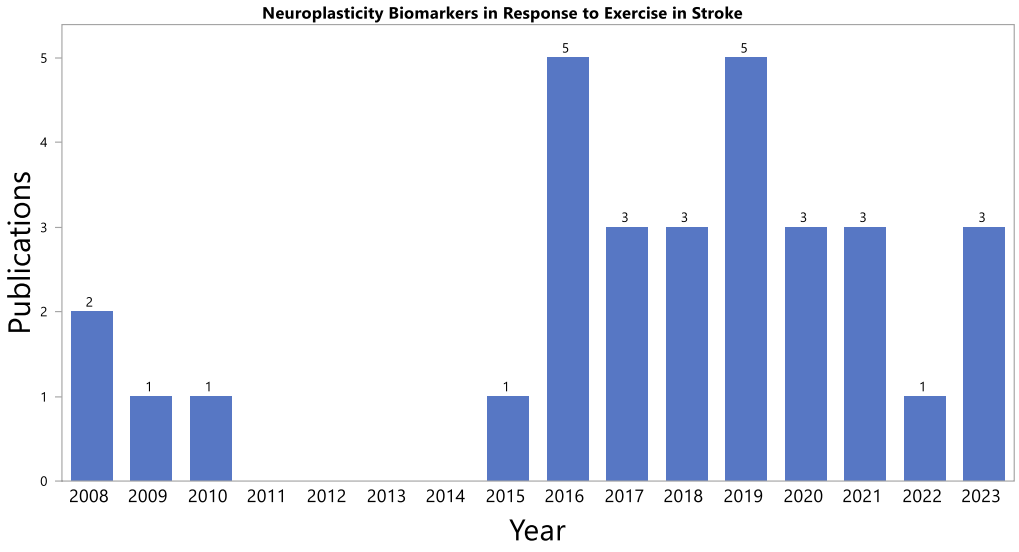
To better understand the brain's mechanisms following a stroke and how treatments can modulate these processes, it is crucial to examine them as closely as possible from the neurobiological source. In other words, "the closer we come to understanding the actual neural systems directly involved in recovery, the better positioned we will be to fully comprehend and effectively influence its process.". However, while this is feasible in animal models through invasive examination methods, it is far more challenging in humans due to the inaccessibility of the human nervous system. Non-invasive and indirect methods are advantageous in many ways, but they come with a trade-off: the further we are from the actual neurobiological processes in the brain, the lower the explanatory power (i.e. R^2) of our models, limiting our ability to identify neuroplastic changes and their behavioral associations.

The World Health Organization defines biomarkers as "any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease" [163]. The emergence of biomarkers has revolutionized the field of medicine, including neurorehabilitation, allowing us to infer neurobiological events in the central nervous system non-

invasively and safely. This information is critical as it can provide mechanistic insights into specific therapies and their potential to promote neuroplasticity and brain repair [91].

In stroke, however, several studies have demonstrated that the initial severity of hemiparesis, measured with disability or motor impairment scales, remains the best predictor of recovery. For instance, initial upper-limb FMA itself can explain up to 89% of the variability in long-term upper-limb recovery after stroke [164], while biomarkers such as CSE and peripheral BDNF have shown to be significantly less reliable and have lower predictive power [140,165]. These findings indicate that our understanding of stroke recovery through biomarkers is still incomplete and that more research needs to be done to "get closer to the neuron", enabling better identification of the biological processes affecting individual differences. Interestingly, recent algorithms have shown value in combining clinical predictors of severity (NIHSS) with biomarkers of CST integrity (MEP status) for predicting upper-limb function [166].

The primary issues with using biomarkers in stroke, including lack of consensus, absence of validation, lack of association with clinically significant differences, and small sample sizes [167], have similarly been observed in response to CE interventions in this population [159]. It has been only 15 years since biomarkers began to be implemented in stroke to examine the neuroplastic responses to CE, and the evidence is growing steadily **(Figure 2).** To enhance our understanding of neuroplastic changes underlying post-stroke recovery and treatments like CE, collaboration between the fields of technology and neuroscience is essential. This synergy will bridge the gap between what we measure through biomarkers and what is actually happening in the nervous system.



Ultimately, this will allow us to get “closer to the neuron," leading to more relevant biomarkers for stroke recovery and rehabilitation [85].

Cardiovascular Exercise in Subacute Stroke

Despite our findings in neuroplasticity biomarkers not aligning with our initial hypothesis, it is crucial to emphasize the impact of our CE intervention on cardiorespiratory fitness in our subacute population. Following stroke, deconditioning and sedentary lifestyles are highly prevalent, with individuals often exhibiting fitness and physical activity levels ~50% lower than non-disabled peers of the same age and sex, falling below the criterion for independent living (stroke-adjusted ≈ 19 mL.Kg^{-1}.min^{-1}) [70,168]. This initiates a vicious cycle of deconditioning that leads to decreased functional capacity, increased morbidity, mortality, and hospitalizations [69]. Additionally, this stroke-induced sedentary behavior can lead to psychological issues such as chronic fatigue, anxiety, and depression, which can in turn affect the quality of rehabilitation further limiting recovery potential [169]. Breaking this vicious cycle is therefore essential for secondary prevention, long-term recovery, and functional independence after stroke, particularly during early recovery stages, when most rehabilitation takes place.

Figure 2. Studies examining exercise effects on neuroplasticity biomarkers post-stroke.

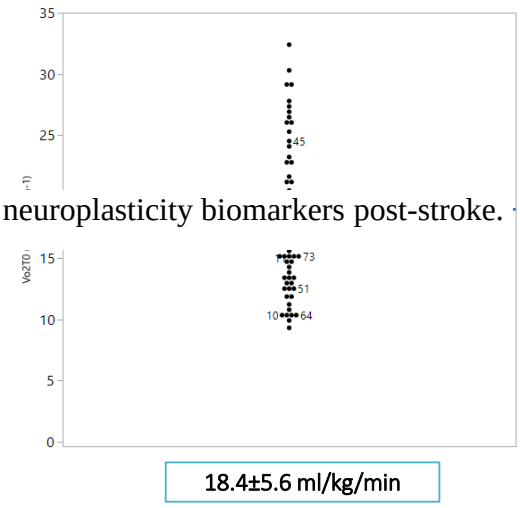


Figure 3. Baseline levels of cardiorespiratory fitness (VO_2peak) in our population.

Our study population exhibited an average cardiovascular fitness of 18.4±5.6 ml/kg/min, with 63% falling below the independent living threshold **(Figure 3)**. During the 8-week study, the control group receiving standard care alone showed some positive yet minimal increases in VO_2peak (0.2±2.4), indicating that some spontaneous recovery may also occur in cardiorespiratory

fitness during subacute stages. In contrast, the CE training group demonstrated VO_2peak increases of 4.4±3.2 ml/kg/min, representing a 27.25% improvement in fitness levels **(Figure 4).**

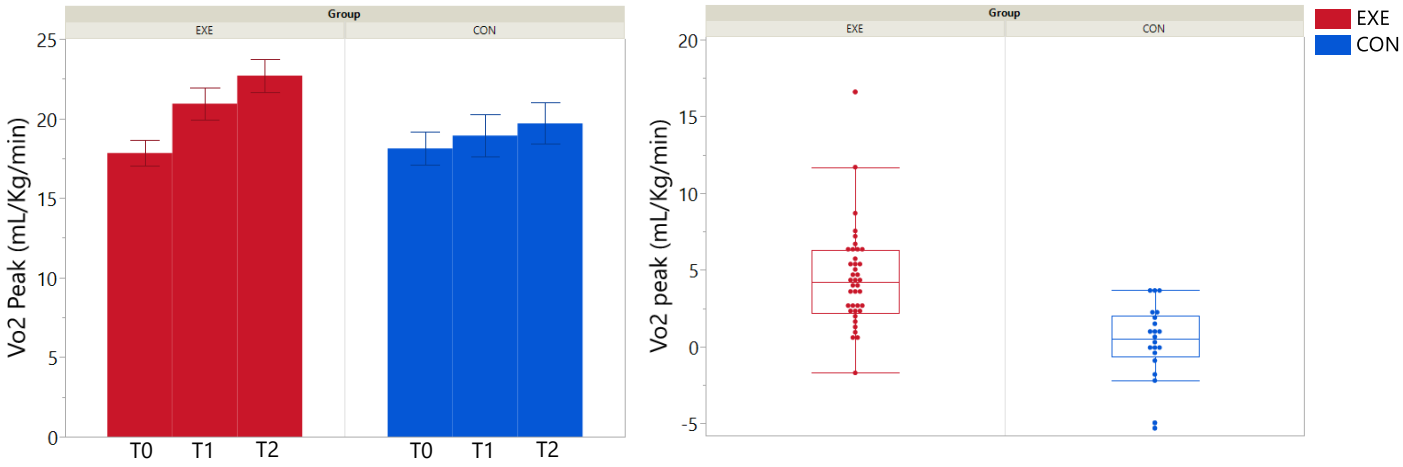


Figure 4. Changes in cardiorespiratory fitness (VO_2peak) in both the cardiovascular exercise (EXE) and standard care (CON) groups over the 8-week study period.

These findings represent the largest VO_2peak increase observed to date following a CE training intervention in subacute stroke individuals while at the same time, they confirm that an insufficient training stimulus was unlikely the cause of the null effects on neuroplasticity biomarkers [170-177]. Furthermore, these improvements surpass the minimal clinical important difference (MCID) for VO_2peak (3.0 ml/Kg/min), associated with reduced cardiovascular mortality, stroke hospitalization, and ischaemic stroke risk [178].

Although not the primary goal of this study, these results support the notion that a progressive CE training program, adhering to the exercise principles (specificity, progression, individualization, overload, recovery, and adaptation), is not only safe but also effective in improving fitness levels during the subacute stages post-stroke. Clinical guidelines now recommend incorporating CE into routine neurorehabilitation and long-term post-stroke

management [179]. However, the reality is that CE remains underutilized in stroke rehabilitation units, the period where most patients typically stay during early and late subacute stages of recovery [81]. Our findings suggest that an 8-week CE training program could be effectively implemented during patients' rehabilitation stays in hospitals and clinics, typically lasting 2-3 months before discharge [180].

Our study does not address an important question regarding the application of CE post-stroke, which is to determine the optimal time for implementation to achieve the greatest fitness benefits. A recent review suggested that early CE implementation during subacute stages might be more beneficial to improve clinical outcomes such as the 6-minute walking test and 10-meter walking time. However, initiating CE during these stages did not show larger gains in cardiorespiratory fitness, possibly due to the lower number of studies measuring VO_2peak, early termination of GXT for non-cardiovascular reasons, or simply because of the absence of time-dependent effects on VO_2peak post-stroke [83]. A recent, well-designed, multicenter, randomized study found no significant improvements in gait economy following 4 weeks of CE 28 days post-stroke [82]. Similarly, the AVERT study showed that higher dose mobilization within 24 hours post-stroke reduced the odds of favorable outcomes [20]. Altogether, more studies are warranted to determine the best time to introduce CE after stroke to achieve vascular, metabolic, and neural benefits (https://clinicaltrials.gov/study/NCT04742686).

Future Directions

- **Cardiovascular Exercise and Motor Recovery after Stroke**

The primary objective of any neurorehabilitation aimed at modulating neuroplasticity after stroke is to restore impairment and function to pre-stroke levels [41]. Despite the significant improvements in cardiorespiratory fitness, our findings show that our intervention had no effect on motor outcomes for upper-limb recovery, including Fugl-Meyer or the Box and Blocks Test **(Figure 5).** Furthermore, changes in those outcomes were not associated with changes in any of the neuroplasticity biomarkers, CSE and BDNF, following either CE or standard care.

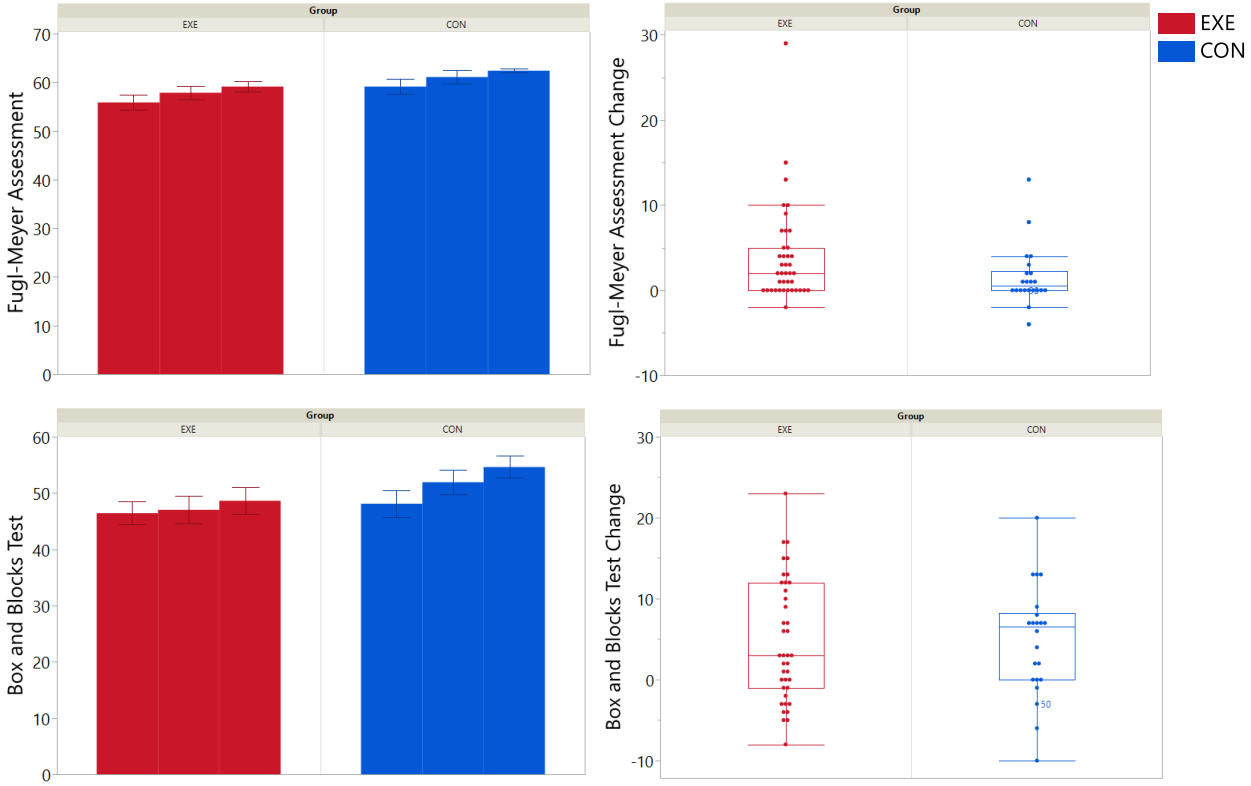


Figure 5. Changes in upper-limb recovery outcomes in the cardiovascular exercise (EXE) and standard care (CON) groups over the 8-week study period. Data are presented as raw values at each time point and as overall change (T2-T0). Both groups demonstrated spontaneous improvements in upper-limb recovery throughout the study period, with no significant effect attributable to the intervention.

A priori, these findings could be seen as unexpected, especially considering the rationale behind the effects of motor rehabilitation on motor outcomes and the relationship between these specific biomarkers with motor stroke recovery. Both CSE and BDNF have been associated with post-stroke motor outcomes and have been shown to be modulated by CE interventions [159]. The theory behind any therapeutic intervention, including CE, posits that if the intervention is able to modulate biomarkers related to a recovery outcome, such intervention could have a therapeutic effect [163]. In other words, if we see an effect on behavior, this should be the result of neuroplastic changes captured through biomarkers. Given that our intervention did not modulate any of these biomarkers or motor outcomes, it would be logical to suggest that the absence of significant behavioral changes would correspond with the absence of observable neuroplastic changes, and vice versa.

Previous evidence has demonstrated that, in addition to improving cardiorespiratory fitness, CE can enhance functional mobility outcomes such as walking capacity and balance [181]. However, it is important to note that these improvements in walking were primarily driven by specific walking interventions, underscoring the importance of intervention specificity in rehabilitation [182]. In simpler terms, it is unlikely that one would improve their basketball game solely by running and doing push-ups.

Previously in the introduction, I quoted Dr. Zatorre's sentence: "The brain is the source of behavior, but in turn, it is modified by the behaviors it produces". However, does this apply to all types of behavior? Overwhelming evidence from animal studies but also few human studies has demonstrated that unspecific repetitive activity alone, without any learning component, does not induce significant motor behavior gains or neuroplastic changes [183-187]. The same notion applies in stroke, where just unspecific movement alone does not seem to induce functional recovery and neuroplastic changes to the same extent as when paired with targeted goal-oriented training, suggesting that specificity and motor learning might be prerequisite factors [55,188-190]. Furthermore, neuroplastic changes are detected only in neural populations functionally related to the newly learned skill, indicating task-specific neuromodulation. [191,192]. The lack of specificity of our intervention in relation to upper-limb motor outcomes could be one of the reasons for the lack of functional changes in our study and perhaps also the null effects in neuroplasticity, particularly in

CSE [193]. Indeed, specificity and learning seem to matter in stroke rehabilitation, not only behaviourally but also neurophysiologically, as both concepts are tightly interdependent.

Surprisingly, however, recent large, well-designed clinical studies do not convincingly support superior recovery improvements when implementing goal-oriented motor interventions during subacute stages post-stroke [30,52,194-196]. This discrepancy between animal and human models raises the question of what is about pre-clinical rehabilitation studies that cause such superior recovery gains, a phenomenon rarely seen in humans. Although efforts are being currently made to see if this discrepancy between models could be explained by the upper-limb training dose (AVERT DOSE ACTRN:12619000557134), in my view, there is an important aspect that has been quite overlooked **(Figure 6).**

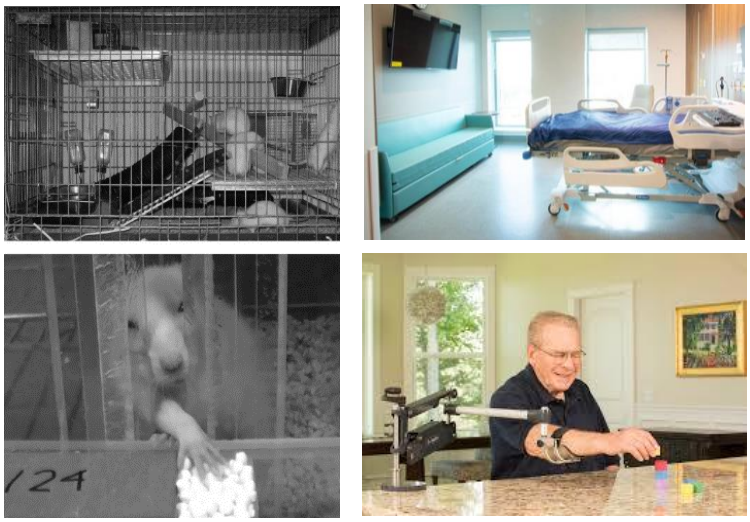


Figure 6. Post-stroke upper-limb interventions in animal and human models. The left section illustrates an animal study design where forelimb reaching training is paired with an enriched environment promoting exploration and physical activity. The right section depicts a typical clinical human trial design post-stroke, in which participants undergo upper-limb therapy programs of varying doses and intensities but remain otherwise inactive and isolated in rehabilitative settings. Images are sourced from Jeffers et al. (2018) and Winstein et al. (2016).

While animal studies have demonstrated remarkable motor gains nearing pre-stroke levels when combining specific goal-oriented training with "unspecific" physical activity interventions such as enriched environments or CE **(Figure 7)**, clinical human trials have primarily focused on

upper-limb motor training without any adjuvant treatments. Indeed, despite participating in standard care therapies and/or upper-limb training programs, which have been shown to inadequately stress the cardiovascular system [80], stroke patients are often inactive and isolated during the early stages of recovery [197]. Unfortunately, a visit to any inpatient stroke rehabilitation unit is sufficient to realize that animals experience a more enriched and physically active environment than human patients.

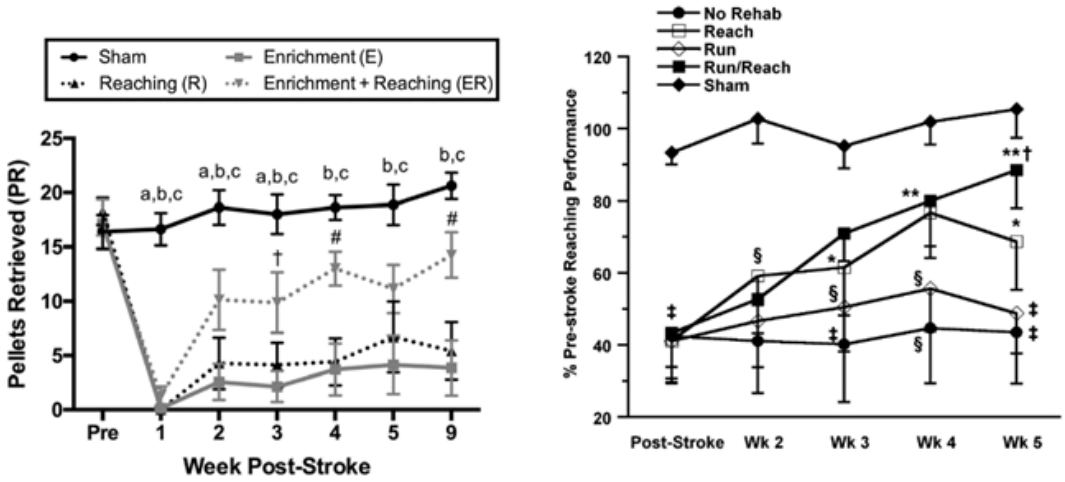


Figure 7. Priming effects of exercise post-stroke. Motor gains from combining specific goal-oriented training with "unspecific" physical activity interventions, such as enriched environments or CE, in animal models post-stroke. Images are sourced from Jeffers et al. (2018) and Ploughman et al. (2007).

It has been over twenty years since the seminal study by Biernaskie et al. [55] first demonstrated the synergistic effects of combining enriched environments with specific upper-limb motor training. Only this year, has the first clinical study investigating the interactive effects of CE and upper-limb training been conducted [198]. In this study, involving patients in the chronic stages of recovery, the authors found that CE implemented immediately prior to upper-limb training yielded similar improvements in upper-limb function (UL-FMA) as an upper-limb training program that was twice as long. This result suggests, for the first time, the potential for CE to serve as a priming intervention for upper-limb rehabilitation in humans after stroke. While more studies are underway [199,200], none have yet been conducted in the subacute phase, where most animal

research is based, and where the brain seems to be more responsive to training. Investigating this phase could significantly advance our understanding of post-stroke motor recovery.

Stroke is fundamentally a vascular disease. Attempting to improve stroke recovery without "targeting" the vascular system could be seen as trying to fix a car without touching the engine. Cardiovascular exercise is a perfect cost-effective intervention that stresses the vascular system and promotes a neural milieu in the brain that could be supportive of plasticity [201]. This neuroplasticity could then be harnessed with intense, goal-oriented motor interventions to direct neural changes in a direction that promotes functional recovery, much like a blacksmith heating metal before shaping a sword.

- **The Neglected Side in Stroke Recovery: The Subacute Period**

Given the ample evidence in animal models demonstrating a time-sensitive window of heightened neuroplasticity and recovery during the early stages post-injury and maybe also for logistical reasons, most pre-clinical studies have been focused on treatments (e.g., intensive upper-limb motor training, brain stimulation, pharmacological) during these early post-injury stages. This bulk of evidence, however, contrasts diametrically with human studies, where the majority of clinical research focuses on the chronic stages [45]. This discrepancy is well reflected in our critical review (Chapter 1), where only 2 out of 34 studies measuring the neuroplasticity responses to CE were focused on subacute stages [159].

This situation is surprising and alarming for several reasons. Firstly, the majority of stroke rehabilitation takes place during the acute and subacute stages in rehabilitation hospitals and clinics, with most patients being discharged before chronic stages. Secondly, emphasis only in chronic stages hinders translational research and the comparison of animal studies to human trials, compromising our understanding of the neurobiological mechanisms during the critical months when the brain may be more receptive to training. This discrepancy can in turn have serious clinical implications for how rehabilitation is implemented in patients, potentially limiting their recovery capacity [202]. Although recruiting patients during the subacute stages can be more challenging, conducting more studies during this period should be a priority in the field of stroke recovery and rehabilitation research [38].

Good partnerships and collaborations between researchers and clinicians in rehabilitation centers are essential to achieve this goal. The main mission of clinicians is to improve patient function as much as possible within the available time. However, this goal is often restricted by governmental policies that limit the number of sessions per patient (~15 sessions, 2/week) and session time (~45 minutes/session), often resulting in insufficient rehabilitation. With the right coordination with the clinical team, research could assist in providing additional relevant treatment to these patients, while simultaneously answering key questions that could help the work of clinicians and ultimately benefit patient recovery. I believe we accomplished this mission here at the Jewish Rehabilitation Hospital with the Memory Lab (https://memorylab.ca/training-program-stroke-survivors/), and this model could be replicated elsewhere.

- **From Variability to Opportunity: A Look into the Future of Stroke Recovery**

As previously mentioned, one of the main challenges perceived in stroke research preventing the identification of unique mechanisms underlying recovery and following treatments is the neuroanatomical variability observed between patients. If we were to randomly recruit 1000 stroke patients, we would likely observe 1000 different lesion characteristics, including variations in location, size, and resulting impairments. As stated in the latest consensus paper on stroke biomarkers: "stroke describes a very heterogeneous group of disorders that are unified by a vascular injury, but not by size, location, or impact of injury" [91]. Although this variability may initially appear to be challenge in rehabilitation and recovery, it paradoxically presents a unique opportunity to gain deeper insights into the functioning of the human brain in both health and disease.

Historically, much of our knowledge about brain functioning has come from anecdotal events such as work accidents or war injuries. Notable examples include Phineas Gage, a rail worker who survived an iron rod passing through his frontal lobe, resulting in significant changes to his decision-making and self-control, and the well-known patient H.M., who, after surgery removing the hippocampus to treat severe epilepsy, lost the ability to retain long-term episodic memories while his procedural memory remained largely intact. Although these are just examples, these cases provided clear causal links between brain function and behavior.

Annually, 15 million people worldwide suffer a stroke, each case affecting functional domains in a unique way (i.e. motor, cognition, language, mood, sensation) based on its lesion characteristics. At the same time, neuroimaging technology and healthcare digitalization have drastically advanced in recent years, allowing for routine precise brain scans and the storage of both clinical and neuroimaging information in large databases, a feat unimaginable a few years ago.

If properly controlled, this variability, rather than being a limitation, could offer valuable insights into the mechanisms underlying recovery and help identify more accurate therapeutical targets for designing individualized treatment approaches aimed at promoting recovery following stroke. We recently demonstrated that lesion location can dictate the patterns of CSE associated with motor skill acquisition [203], as well as impact long-term skill retention [204], information that could help guide recovery interventions such as non-invasive brain stimulation or upper-limb motor training.

Simultaneously, this information could, in turn, revolutionize our understanding of the brain and its function, a mystery yet to be fully solved despite remarkable progress over the last century. In other words, stroke could help us better understand the brain. A project of this magnitude would require collaboration among multiple health institutions, but the international community has already demonstrated that projects of this caliber are possible, as evidenced by initiatives like the Human Brain Project (https://www.humanbrainproject.eu/en/).

Chapter 5: Conclusions and Summary

Understanding how the brain responds to treatments is essential for improving recovery and rehabilitation after stroke. However, limited knowledge of the neurobiological mechanisms underlying these processes in humans restricts our ability to determine the brain's capacity to recover and the therapeutic potential of rehabilitative interventions. This thesis addresses this issue by reviewing the current use of biomarkers that measure neuroplastic changes in response to CE and empirically testing the main gaps in the literature. This was achieved through a randomized controlled trial examining the acute and chronic effects of CE on central and peripheral neuroplasticity biomarkers in individuals with subacute stroke.

Contrary to our expectations, and despite clinically significant improvements in cardiorespiratory fitness, 8 weeks of CE did not modulate BDNF or CSE biomarkers. Neurobiological and methodological factors such as underlying neurobiological processes, individual variability, biomarker reliability, and intervention specificity may account for these results. These findings suggest that CE does not promote neuroplasticity during the early stages post-stroke and prompt a revaluation of how CE is applied and assessed as a neuroplasticity-promoting intervention.

This study is the first to examine the effects of CE on CSE and BDNF as neuroplasticity biomarkers in individuals with subacute stroke. The findings offer valuable insights into how neuroplasticity-based interventions like CE interact with the underlying processes during these early stages of recovery. They also highlight the advantages and pitfalls of using CSE and BDNF as biomarkers for measuring putative neuroplastic changes.

As Dr. John Krakauer once said: “science is not here to please us”. While these findings may not align with our previous hypotheses, they will open new avenues for future research in stroke rehabilitation, offering a foundation for developing and testing further hypotheses.

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