DISUSE-INDUCED ELBOW FLEXOR STRENGTH LOSS OCCURS INDEPENDENT OF MUSCLE ATROPHY AND IS ACCOMPANIED BY IMBALANCES IN CORTICOSPINAL OUTPUT FOLLOWING 14-DAYS OF UNILATERAL UPPER-ARM IMMOBILIZATION IN YOUNG WOMEN

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LIST OF ABBREVIATIONS

%MSO – percentage of maximum stimulator output

1RM – one-repetition maximum

APB – abductor pollicis brevis

ATP – adenosine triphosphate

AUC – area under the curve

BDNF – brain-derived neurotropic factor

BOLD – blood oxygenation level dependent

Ca^{2+} – calcium

CAF – C-terminus agrin fragment

CE – cross-education training

CSA – cross-sectional area

CSE – corticospinal excitability

CT – computed tomography

DEXA – dual X-ray absorptiometry

EMG – electromyography

FDI – first dorsal interosseous

fMRI – functional magnetic resonance imaging

GRAPPA – GeneRalized Autocalibrating Partial Parallel Acquisition

IGF-1 – insulin-like growth factor 1

M1 – primary motor cortex

MEP – motor evoked potential

MI – motor imagery training

MPS – muscle protein synthesis

MRI – magnetic resonance imaging

NCAM – neural cell adhesion molecule

NMES – neuromuscular electrical stimulation

NMJ – neuromuscular junction

PCr – phosphocreatine

RDA – recommended dietary allowance

RMT – resting motor threshold

S1 – primary somatosensory cortex

SR – sarcoplasmic reticulum

SR curve – stimulus-response curve

TMS – transcranial magnetic stimulation

VA – voluntary muscle activation capacity

vPMC – ventral premotor cortex

ABSTRACT

Purpose: Evaluate the effects of upper arm immobilization on muscle strength, muscle size, and neuromuscular function in young women using current standards of measurement.

Methods: Using a within-subject, unilateral design, 12 healthy women aged 18-35 years underwent 14 days of nondominant upper arm immobilization using a brace and sling. Changes in elbow flexor and extensor muscle strength (isometric and isokinetic) and size (cross-sectional area, CSA; and volume) were measured pre- and post-immobilization using isokinetic dynamometry and magnetic resonance imaging, respectively. Measures of neuromuscular function included voluntary activation capacity of the biceps brachii quantified via twitch interpolation and corticospinal excitability of the biceps brachii using transcranial magnetic stimulation (TMS). Corticospinal excitability was inferred from resting motor threshold (RMT), as well as slope, inflection point, and area under the TMS stimulus-response (SR) curve. An additional assessment of corticospinal excitability took place 24 hours following immobilization to evaluate the short-term effect of immobilization on neuromuscular function.

Results: Immobilization induced a significant decline in isometric elbow flexion (-21.3±19.2%, $P = 0.040$) and extension (-19.9±15.7%, $P = 0.021$) strength in the immobilized arm only, with no effect on isokinetic strength ($P > 0.05$). There was no significant effect of immobilization on elbow flexor CSA or volume, whereas there was a significant decrease in elbow extensor CSA (-2.9±2.9%, $P = 0.018$) and volume (-2.5±2.5%, $P = 0.043$) in the immobilized arm. Immobilization did not significantly alter voluntary activation capacity, RMT, or inflection point of the SR curve. Corticospinal excitability was significantly lower at 2 weeks post-immobilization compared to baseline as evidenced by a decrease in slope of the SR curve ($P = 0.006$), an effect that was driven predominantly by a decrease in the non-immobilized arm. There was a trend for an increase in

excitability in the immobilized arm, based on differences in the relative change in area under the SR curve (-2.7±54.5 and +58.8±90.6% in non-immobilized and immobilized, respectively, P = 0.083).

Conclusion: Immobilization-induced strength loss in the upper limb can occur rapidly and independent of significant muscle atrophy in young women. This change may be accompanied by an imbalance in corticospinal excitability between limbs.

RÉSUMÉ

Objectif : Évaluer les effets de l'immobilisation du bras supérieur sur la force musculaire, la taille des muscles et la fonction neuromusculaire chez les jeunes femmes en utilisant les normes de mesure actuelles.

Méthodes : Dans le cadre d'une étude intra-sujet et unilatérale, 12 femmes droitières en bonne santé âgées de 18 à 35 ans ont subi une immobilisation du bras gauche pendant 14 jours à l'aide d'une attelle et d'une écharpe. Les modifications de la force (isométrique et isocinétique) et de la taille (surface de section transversale, SST; et volume) des muscles fléchisseurs et extenseurs du coude ont été mesurées avant et après l'immobilisation, respectivement par dynamométrie isocinétique et imagerie par résonance magnétique. Les mesures de la fonction neuromusculaire comprenaient la capacité d'activation volontaire du biceps brachial quantifiée par interpolation du twitch et l'excitabilité corticospinale du biceps brachial par stimulation magnétique transcrânienne (SMT). L'excitabilité corticospinale a été déduite du seuil moteur au repos (SMR), ainsi que de la pente, du point d'inflexion et de l'aire sous la courbe stimulus-réponse (SR) de la SMT. Une évaluation supplémentaire de l'excitabilité corticospinale a eu lieu 24 heures après l'immobilisation pour évaluer l'effet à court terme de l'immobilisation sur la fonction neuromusculaire.

Résultats : L'immobilisation a entraîné un déclin significatif de la force de flexion (-21,3±19,2 %, $P = 0,040$) et d'extension (-19,9±15,7 %, $P = 0,021$) isométrique du coude dans le bras immobilisé uniquement, sans effet sur la force isocinétique ($P > 0,05$). Il n'y a pas eu d'effet significatif de l'immobilisation sur la SST ou le volume des fléchisseurs du coude, alors qu'il y a eu une diminution significative de la SST des extenseurs du coude (-2,9±2,9%, $P = 0,018$) et du volume (-2,5±2,5%, $P = 0,043$) dans le bras immobilisé. L'immobilisation n'a pas modifié de manière significative la capacité d'activation volontaire, le SMR ou le point d'inflexion de la courbe SR.

L'excitabilité corticospinale était significativement plus faible 2 semaines après l'immobilisation par rapport à la ligne de base, comme en témoigne la diminution de la pente de la courbe SR (P = 0,006), un effet qui était principalement dû à une diminution dans le bras non immobilisé. Il y avait une tendance à l'augmentation de l'excitabilité dans le bras immobilisé, d'après les différences dans le changement relatif de l'aire sous la courbe SR (-2,7±54,5 et +58,8±90,6 % dans les bras non-immobilisés et immobilisés, respectivement, P = 0,083).

Conclusion : La perte de force induite par l'immobilisation dans le membre supérieur peut se produire rapidement et indépendamment d'une atrophie musculaire significative chez les jeunes femmes. Ce changement peut s'accompagner d'un déséquilibre de l'excitabilité corticospinale entre les membres.

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CONTRIBUTION OF AUTHORS

Freddie Seo (first author): contributed to the conception and design of the research, led and carried out all data collection, analyzed the muscle strength, neuromuscular function, and dietary data, performed all statistical analyses, interpreted the study findings, prepared the figures, drafted the thesis, read and approved the final thesis, and holds primary responsibility for the content of the published work along with the principal investigator (Dr. Tyler A. Churchward-Venne)

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CHAPTER 1: INTRODUCTION

1. INTRODUCTION

Skeletal muscle is the largest tissue in the human body, comprising 30-40% of body mass.[1] Due to the plastic nature of skeletal muscle, its mass and function are susceptible to environmental stimuli such as diet and exercise.[2] While it is well known that skeletal muscle is used for locomotion, it additionally plays a role in glucose and lipid metabolism.[3,4] Furthermore, skeletal muscle acts as the most abundant amino acid repository for the sustenance of organismal function during critical life conditions.[5] Given its multidimensional role in the genesis and prevention of disease, the maintenance of skeletal muscle is crucial for the preservation of overall health, particularly during aging.

Like skeletal muscle, the brain is a highly plastic organ and is well known to undergo remarkable changes in response to different stimuli such as exercise and learning, as well as certain disease states such as addiction, depression, and stroke.[6,7] It is well known that the frontal lobe of the brain houses the structures responsible for the origin of voluntary skeletal muscle contraction. In conjunction with the parietal lobe, prefrontal cortex, basal ganglia, cerebellum, and other frontal cortices, the primary motor cortex (M1) sends the majority of electrical signals to skeletal muscle to evoke voluntary contraction.[8] The brain shares a physiological connection with skeletal muscle and controls its function; hence, its own function is inevitably linked to skeletal muscle health.

Whether it be due to injury, surgery, or illness, people of all sorts (e.g., older adults, clinical populations, athletes) may undergo prolonged periods of reduced physical activity such as experienced during limb immobilization or bed rest. Due to disuse (i.e., a reduction in voluntary muscle contraction frequency relative to normal living conditions), the size and strength of muscles controlling the affected limb can decrease significantly, possibly resulting in physical impairment and/or lower quality of life during the recovery phase.[9] Among the most prevalent

situations/models of muscle disuse, limb immobilization is considered among the most restrictive due to the fact that it severely reduces movement about the joint(s) of interest, thus resulting in dramatic reductions in muscle activity.[10] Additionally, the recovery phase following immobilization, defined as the convalescence of muscle strength, has been shown to be longer than the immobilization period itself.[11–13] This is particularly important for older adults, as the loss of functional independence with aging is thought to be due to the accumulation of intermittent periods of reduced physical activity, at frequencies impermissible of sufficient muscle mass and strength recovery in between.[14] To minimize functional loss and facilitate rehabilitation, a concrete understanding of the physiological changes that take place during muscle disuse is of interest.

Prior immobilization studies have shown that the rate and degree of decline in muscle strength exceeds that of muscle size, indicating that determinants of muscle strength unrelated to muscle size further contribute to functional changes during immobilization.[15] In fact, the decrease in muscle strength during immobilization has predominantly been attributed to changes in neuromuscular function as opposed to muscle size, particularly during the early immobilization period (first 1-2 weeks).[12,15–17] Yet overall, research on the mechanisms and prevention of immobilization-induced muscle weakness have primarily focused on the muscle itself.[18] The brain (the governor of voluntary muscle contraction) and the nervous system in general has been relatively understudied in this regard. In addition, despite representing approximately a quarter of the total participants in neuromuscular research pertaining to limb immobilization, women may experience disproportionately greater losses in strength following immobilization relative to men.[15] The purpose of this study was to examine changes in muscle strength, muscle size, and neuromuscular function in young women following 2 weeks of single-arm immobilization.

CHAPTER 2: LITERATURE REVIEW

2. LITERATURE REVIEW

2.1 Introduction

The function of the brain is inextricably linked to that of the muscle and has been reviewed extensively in the context of exercise training.[19–21] Lesser known is the relationship between the brain and the muscle during prolonged bouts of reduced physical activity, such as during limb immobilization or other models of muscle disuse. This literature review will begin with an overview of the proposed physiological changes that occur in response to muscle disuse in the neuromuscular system. Criterion methods to measure muscle atrophy and neuromuscular function in humans *in vivo* (magnetic resonance imaging (MRI) and transcranial magnetic stimulation (TMS), respectively) will then be described. A discussion of sex differences in disuse-related muscle atrophy and strength loss will follow. Thereafter, the review will conclude with an examination of various therapeutic approaches to muscle disuse situations, and how study findings in this area further highlight the importance of considering the central nervous system in addition to the muscle for the prevention of functional decline with muscle disuse.

2.2 Neuromuscular changes during muscle disuse

In humans demonstrating exceptional or enhanced use of a particular body structure (e.g., Braille readers, string musicians), researchers have observed architectural differences in the brain when compared against the general population.[22,23] It is therefore of interest to determine whether the restricted use of a limb due to immobilization would have a similarly profound effect on brain plasticity, and whether these effects would be antagonist to those observed with motor skill training. Additionally, the function of a mature skeletal muscle fiber is highly regulated by its respective motor neuron.[24] As such, the neuromuscular junction (NMJ, synapse responsible for the translation of action potentials sent by the nervous system into mechanical muscle contraction),

may also be a site of interest in the context of muscle disuse. Nonetheless, there are few studies that have investigated the effects of muscle disuse on the brain, NMJ, and the neuromuscular system as a whole. Among the possible molecular changes in the brain with disuse, animal studies have suggested that insulin-like growth factor 1 (IGF-1) signaling may contribute to a reorganization of the sensorimotor cortex.[25] In humans, insight from functional magnetic resonance imaging (fMRI) studies have revealed changes in cortical activity and connectivity in response to acute and long-term immobilization.[26–28] Beyond the brain itself, evidence from muscle biopsies have suggested that neurophysiological changes local to the muscle may further impair its ability to produce force, such as denervation and reduced excitation-contraction coupling efficiency.[29,30] And finally, while the mechanisms remain unclear, immobilization-induced weakness may be associated with a reduced capacity of the central nervous system to voluntarily activate the immobilized musculature, especially in the upper limb.[10]

2.2.1 Reduced cerebral IGF-1 signaling

Studies on the metabolic effects of muscle hypoactivity on the brain are scarce and have exclusively been conducted in animals. Given the well-documented effects of exercise on neurogenesis,[31] much of the research on muscle disuse has tested whether the opposite effect is observed with physical inactivity through the measurement of neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and IGF-1, with only the latter presently being supported as a determinant of cortical plasticity during muscle disuse.[25,32] IGF-1 is particularly important for the genesis of neurons during growth; but it also plays an additional role in neuron differentiation and cortical plasticity modulation during adulthood.[33]

Using a hindlimb unloading model of disuse, Mysoet et al. (2014)[25] observed a reduction in IGF-1 levels in the sensorimotor cortex, as well as an associated reduction in anabolic signaling

within this area and (to a lesser extent) the striatum. To discern the functional implications of these findings, another study was conducted in which experimental rats received chronic IGF-1 infusion during unloading.[32] IGF-1 may be a determinant in modulating cortical representations of fine motor structures in animals, as IGF-1 infusion prevented shrinkage of the hindpaw somatotopic map, which is relevant for tactile perception and possibly the coordination of locomotion following significant disuse.[32] Similarly, in patients who underwent unilateral ankle immobilization in response to injury, motor cortical maps of the tibialis anterior muscle of the immobilized limb were found to be smaller relative to those of the non-immobilized limb, but these differences were negated during voluntary muscle contraction.[34] Despite evidence suggesting that IGF-1 mediates changes in cortical representations, it is not entirely clear whether these metabolic perturbations have any relevance to muscle weakness or dysfunction in response to disuse. In humans, it was observed that with immobilization due to injury of the dominant arm, thickness of the motor cortex decreased and increased in the contralateral and ipsilateral hemispheres, respectively.[35] Interestingly, increases in cortical thickness of the right motor cortex were associated with improvements in performance on a series of fine motor tasks with the nondominant hand, suggesting an adaptive reorganization of the motor cortex in response to increased use of the nondominant limb.[35] Therefore, while structural changes in the brain may occur with prolonged muscle disuse, researchers should interpret their findings with caution, since it is not always evident as to whether these changes are maladaptive, adaptive, or neutral in relation to functional capacity. Further research should attempt to identify possible links between brain plasticity and physical function through continued identification and integration of reliable motor and sensory performance-based tests.

2.2.2 Changes in brain activity and connectivity

Though there are few studies, limb immobilization has been demonstrated to induce changes in brain activity and connectivity in humans using fMRI, including within cortical regions that do not play a direct role in skeletal muscle contraction.[26,27] However, when considering the potential effect of muscle disuse on the motor system, the conditions under which fMRI measurements are acquired appear to influence results, as resting state fMRI studies employing a discrete, pre- and post-immobilization acquisition model have failed to observe any change in M1 activity following immobilization.[27,28,36] On the other hand, task-based fMRI analyses have revealed that immobilization decreases M1 activity during motor imagery[26] and execution of hand movements.[27] In fact, changes in M1 activity during motor imagery were observed following only 24 hours of hand immobilization.[26] Activity of the ventral premotor cortex (vPMC), which is involved in the post-movement discrimination between planned and executed movements, has also been shown to change with immobilization.[27] Specifically, vPMC increased in activation in the hemisphere corresponding to an immobilized hand during attempted movement immediately following cast immobilization, but was unchanged compared to the unaffected hemisphere when attempting the same task after the cast was worn for a week.[27] This suggests a learning effect, in which vPMC activity did not change during attempted movement as it was no longer anticipated that the movement could be executed by the immobilized limb.[27] Finally, cast immobilization of the hand and arm was demonstrated to cause a decrease in activity in the finger region of the primary somatosensory cortex (S1), which was restored to baseline within two to three weeks following the immobilization.[37] Altogether, while immobilization is suggested to modulate brain activity, changes in M1 do not appear to be evident during resting conditions. In contrast, the impact of muscle disuse on the motor system may be reflected during imagined and performed movements,

however evidence supporting this notion remains preliminary.

Given the limitations of analyzing fMRI data at discrete pre- and post-intervention intervals, Newbold et al. (2020)[28] conducted a pilot study on 3 healthy subjects in which functional connectivity was evaluated daily before, during, and after a hand and arm cast immobilization period of 2 weeks. Upon analyses of each separate participant, it was found that functional disconnection occurred between disused regions of the somatomotor cortex and cerebellum within 48 hours, while internal connectivity increased within these regions (**Figure 1**).[28] Changes were specific to internal circuits corresponding to the upper extremity, and were restored to baseline shortly after remobilization.[28] Interestingly, functional disconnection in response to the disuse reached a magnitude similar to that of stroke patients,[38] a remarkable finding given that the study was conducted on young, healthy subjects. Additionally, closer examination of fMRI signals revealed a greater frequency of spontaneous, high amplitude pulses in the disused somatomotor cortex of all participants, which propagated to the contralateral supplementary motor area and ipsilateral cerebellum relative to the immobilized arm.[28] These spikes in activity, termed 'disuse pulses', were proposed by the authors to reflect an attempt by the brain to maintain or change its organization in response to the disuse,[28] as they resembled the spontaneous activity pulses observed during brain development in utero.[39] Nevertheless, though muscle disuse may induce significant changes in resting state functional connectivity, the actual relevance of these changes remains in question. Measures of baseline and relative change in resting and task-based functional connectivity in motor skill training and acquisition studies have observed a correlation between fMRI measurements and improvements in motor performance in a variety of different training paradigms.[22,23,40,41] As a result, it is possible that functional connectivity could also predict the rate at which motor performance declines, however this claim is yet to be substantiated. To validate

the usefulness of including fMRI measurements in studies of muscle disuse, future research should aim to confirm whether significant changes in resting brain activity and connectivity occur, including a variety of disuse models such as those affecting the lower limbs (i.e., leg immobilization, bed rest), and determine whether these changes are relevant to the concomitant loss of muscle strength and function.

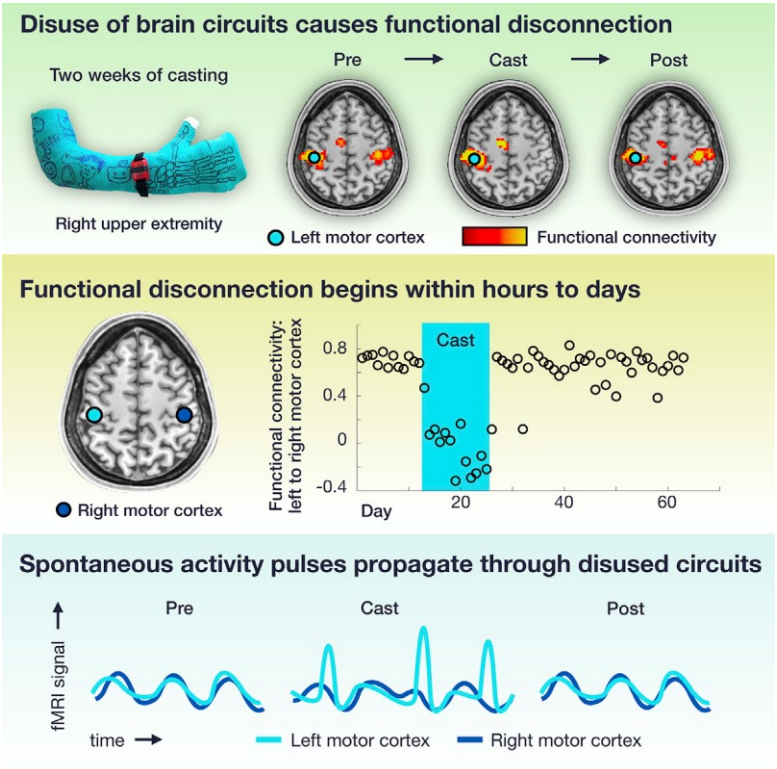

Figure 1. Summary of changes in functional connectivity in response to forearm cast immobilization. Adapted from Newbold et al. (2020).[28]

2.2.3 Signs of muscle denervation and reduced excitation-contraction coupling

At the level of the muscle, changes in motor nerve innervation and excitation-contraction coupling efficiency have been inferred from a small number of human studies.[29,30] Neural cell adhesion molecule (NCAM), which is typically responsible for the innervation of muscle fibers during maturation, is known to accumulate at the surface of adult muscle cells in response to denervation.[42] Since NCAM expression is minimal following myotube maturation, its presence in

adult muscle suggests an attempt to reinnervate axon-deficient muscle fibers and is therefore used as a surrogate measure of muscle denervation.[42] Bed rest has been demonstrated to increase the proportion of NCAM-positive muscle fibers in humans, however the magnitude of effect is rather small and is not consistently observed among individual subjects.[29,30]

Similarly, serum C-terminus agrin fragment (CAF), a biomarker associated with neuromuscular impairment due to injury, disease, and aging,[43,44] has been shown to be elevated following 10 days of bed rest in young men.[29] However, this finding was not reproduced in a recent study in which serum CAF was analyzed in young men and women at 4 discrete time points throughout 60 days of bed rest.[45] Elevated CAF is indicative of NMJ instability, as its excessive release in the bloodstream precedes NMJ degeneration in animals.[46]

Analysis of single muscle fibers by Monti et al. (2021)[29] suggests that muscle calcium dynamics are altered with disuse, as a decrease in ionic calcium (Ca^{2+}) content in the sarcoplasmic reticulum (SR) has been observed following 10 days of bed rest. Ca^{2+} release from the SR was additionally shown to be impaired, further suggesting that the SR may be a critical organelle in the control over neuromuscular plasticity following muscle disuse.[29] However, tests of calcium dynamics in single muscle fibers by Monti et al. were carried out by exposing samples to varying concentrations of caffeine. Not only does this fail to mimic the in vivo mechanism of excitation-contraction coupling in human skeletal muscle, but maximal Ca^{2+} release is achieved using concentrations of caffeine that would otherwise be toxic within a living subject.[47] Nonetheless, these novel findings highlight the possibility that disuse impacts not only skeletal muscle mass, but also muscle function on an individual fiber level.

Prolonged muscle disuse may induce changes in motor nerve innervation, NMJ stability, and SR function, however investigation in this area has begun only recently. Seeing as the majority of

studies in this context have focused on bed rest, it is possible that limb immobilization, which imposes greater restrictions on muscle activity at the target joint,[10] could have a more pronounced effect on neuromuscular dynamics; but further research is needed.

2.2.4 Reduced capacity to voluntarily activate skeletal muscle

The effects of disuse are not exclusive to muscular atrophy; the ability of the nervous system to voluntarily activate skeletal muscle has also been suggested to decline. Voluntary muscle activation capacity (VA) may be measured *in vivo* through the use of transcranial magnetic or peripheral electrical stimulation to elicit the contraction of a target muscle.[48] With peripheral stimulation, the force or torque produced by stimulation of a target muscle at a supramaximal intensity at rest is compared against that produced during a maximal voluntary contraction. Since the stimulation is delivered at an intensity intended to wholly recruit the target musculature, the force or torque ratio between the twitch superimposed during maximal contraction and the resting twitch is representative of VA. Several studies have demonstrated that muscle disuse decreases VA in humans, particularly through an increase in the superimposed twitch force (**Figure 2**).[13,49–53] In fact, using a multiple regression analysis, Clark et al. (2006)[18] found that neural factors explained a greater proportion of the variability in strength decline with 4 weeks of unilateral lower limb suspension relative to muscle atrophy (48% vs. 39%). And among the 8 neural factors included in the analysis, VA was the principal predictor variable for strength loss due to limb unweighting.[18] Similarly, a systematic review of limb immobilization studies revealed that not only are neural factors such as VA, resting twitch force, and electromyography (EMG) signal amplitude moderately to strongly correlated with muscle strength change, but that there is no significant relationship between muscle atrophy and strength among studies in which both variables are considered (**Figure 3**).[15] VA and resting twitch force, however, are associated with

muscle strength only in studies of the upper limb, which may be reflective of the greater density of corticospinal projections in the upper versus lower limbs.[54] Nevertheless, studies on the impact of immobilization on VA within the upper limb specifically are limited. Several unilateral lower limb suspension studies have demonstrated that muscle disuse has no significant effect on VA;[55–57] but this may simply be due to the lesser degree of restriction imposed by limb suspension in comparison to limb immobilization. As a result, there is a demand for more research on the effects of upper limb immobilization on VA to ascertain whether the relationship between muscle strength loss and neural drive is stronger in the upper relative to the lower limb. In essence, while muscle atrophy during disuse certainly contributes to the associated decline in strength, researchers may have underestimated the role of the central nervous system in this regard, particularly the ability to maximally recruit the disused musculature.

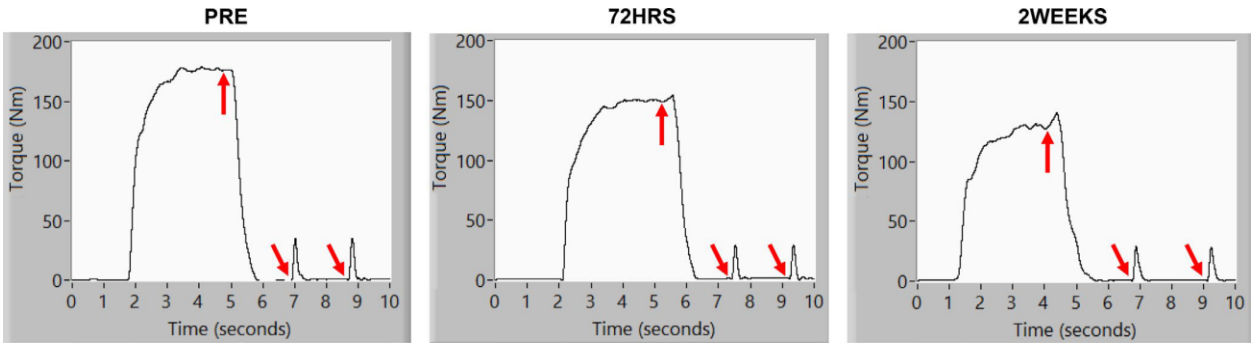

Figure 2. Single participant data displaying the time course of changes in voluntary activation of the knee extensors during lower limb immobilization. Adapted from MacLennan et al. (2020).[49]

There is additional evidence that the degree to which neural and muscle factors contribute to strength loss may depend on the muscle disuse paradigm employed. A systematic review of bed rest studies revealed that muscle atrophy accounts for up to 79% of the variance in muscle strength loss using a mathematical model including age, bed rest duration, sex, and bed rest modality as covariates.[58] However, the purpose of this study was to evaluate only the relationship between

muscle atrophy and strength, without considering noteworthy variables pertaining to the nervous system such as VA. In fact, in a 20-day bed rest study by Kawakami et al. (2001),[52] the association between reductions in VA and knee extensor strength appeared stronger than that between strength and quadricep physiological CSA measured using MRI. The researchers acknowledge that this result must be interpreted with caution, as due to a limited number of subjects, the regression analysis was conducted by pooling subjects from two different groups, one receiving an exercise countermeasure and a control group.

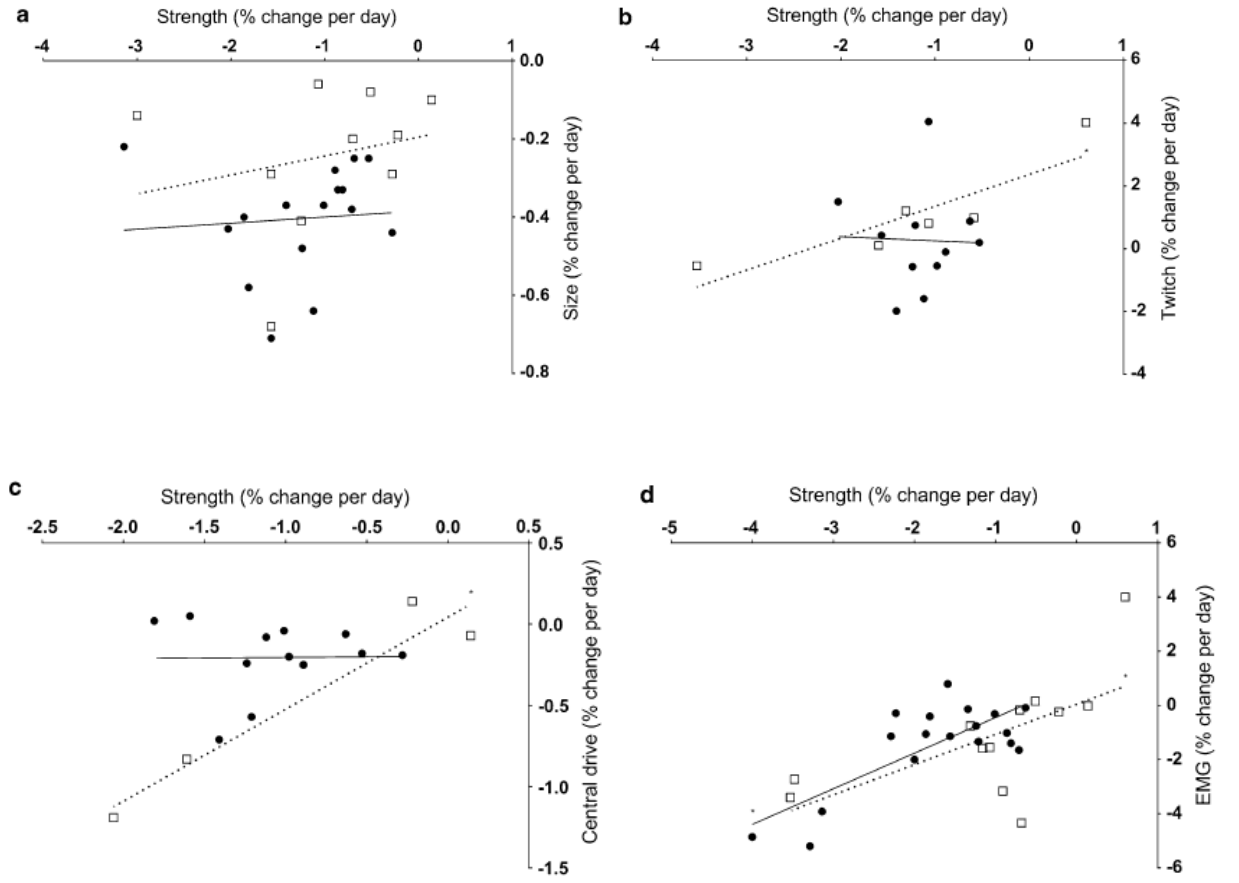

Figure 3. Association between change in muscle strength and (a) muscle size, (b) resting twitch force, (c) voluntary activation ("central drive"), and (d) electromyography (EMG) signal amplitude during upper (open squares) and lower (shaded dots) limb immobilization. Adapted from Campbell et al. (2019).[15]

Despite a lack of conclusive evidence, it remains possible that muscle atrophy may be more

relevant to muscle strength loss during bed rest versus limb immobilization, as the latter is more restrictive (i.e., induces a more marked suppression of muscle activity, as well as greater lower limb muscle atrophy and strength loss during the disuse period (**Figure 4**)).[10,59] Future reviews should aim to determine whether differences in neural activation of disused muscles using different paradigms (i.e., bed rest, immobilization, limb suspension, microgravity) translates to variance in the association between muscle atrophy and muscle strength loss. Regardless, given that the rate of muscle atrophy remains disproportionate to the rate of muscle strength decline among all major models of muscle disuse, it is evident that the loss of muscle strength in response to disuse is multifactorial.

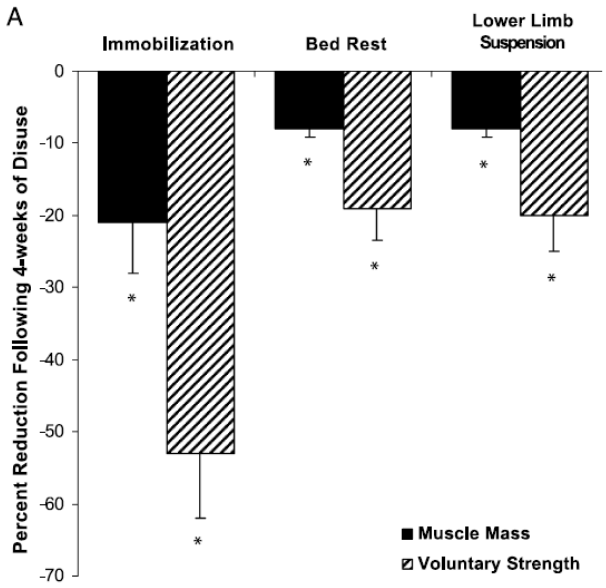

Figure 4. Decline in relative muscle mass and strength in response to various muscle disuse paradigms. Adapted from Clark (2009).[10]

2.3 Methods to assess skeletal muscle and brain plasticity *in vivo*

2.3.1 Magnetic resonance imaging to measure muscle size

MRI is a form of tomographic imaging in which the magnetic properties of protons within bodily tissues are exploited to create a series of sectional images. MRI is not only a common diagnostic tool for neuropathology, tumors, and musculoskeletal injury, but it is also particularly useful for the study of muscle hypertrophy or atrophy since it allows for great contrast between different types of soft tissue (i.e., muscle and adipose tissue); furthermore, it is one of the few assessment tools that can be used to discriminate between different muscles in a given body segment (**Figure 5**).[60] MRI creates images of internal physiological structures using a magnetic field that aligns protons (especially those of hydrogen nuclei) within tissues. Once aligned, a strong radiofrequency pulse is administered to cause brief misalignment of the protons. Once the pulse is stopped, the protons eventually move back to their equilibrium position, releasing electromagnetic energy as per the 2nd law of thermodynamics. The energy released is measured by a computer to construct a detailed image of the structures of interest, as tissues of varying proton densities will exhibit different intensities. Generally, the brightness or intensity of a particular tissue in an MRI image is determined primarily by the rate at which its protons return to equilibrium, and also the amount of energy released upon realignment with the magnetic field; it may additionally be determined by the parameters selected for the scan.[61] With computer software, images obtained from an MRI scan can be used to assess muscle size using a manual, automated, or blended approach. Manual methods are pseudo-quantitative and traditionally involve using assistive software to trace around the borders of the muscle.[62,63] Using the appropriate scale, the number of pixels within the traced image can be converted into a value corresponding to the CSA of the muscle.[63] On the other hand, automated methods are able classify tissues by comparing their precise intensity values against a

tissue-specific reference, which is useful for excluding any tissue-invading 'impurities' (e.g., intramuscular fat) that are not obvious to the naked eye.[62]

One of the principal advantages of using MRI as an assessment tool is its ability to provide volumetric measurements of a given tissue. For example, upon determining the two-dimensional CSA of a muscle of interest within each image, it is possible to arrive at an approximation of muscle volume using CSA, the image/slice thickness, and the distance between each slice during the scan.[64] Naturally, this method is only viable for as long as an adequate number of slices are used for analysis. MRI methods for muscle size assessment also demonstrate high intra-rater reliability.[65,66] Lastly, in comparison to computed tomography (CT), another reliable imaging modality used for muscle size measurement, there is no radiation exposure associated with MRI. Given that MRI is a safe procedure that can acquire high-resolution images for specific and reliable muscle size measurement, including volumetrics, it is largely considered the reference standard for quantifying changes in muscle size.[60]

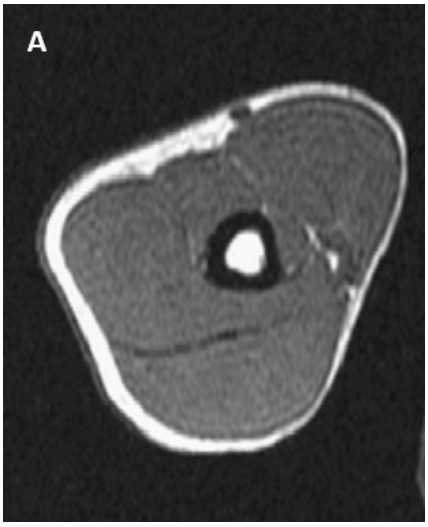

Figure 5. Axial MRI image of the human (right) arm. Adapted from Holzbaur *et al*. (2007).[67] A, Anterior.

Nonetheless, there are several limitations to MRI. First, scans are costly, and for that reason MRI is not often used in studies of muscle hypertrophy or atrophy.[60] MRI images are also subject to artifactual error, as voluntary or involuntary movements, even those associated with breathing, are known to disrupt image resolution.[68] Finally, there are a number of contraindications to MRI scans, namely claustrophobia (as the cylindrical corridor of the scanner is narrow), pregnancy, and the presence of certain metal implants or non-removable medical devices.

Despite its limitations, MRI remains the preferred method for measuring segmental changes in muscle size. Alternative tomographic imaging modalities include the aforementioned CT, as well as ultrasound imaging. CT is quite similar to MRI in the sense that it produces relatively high-quality images of the muscle with excellent measurement reliability.[69] However, the key difference is that scan times must be kept shorter since CT involves exposure to potentially harmful radiation, resulting in lower spatial resolution that often does not allow for the segmentation of different muscles.[68,70] Relative to MRI and CT, ultrasound imaging produces lower quality images; and when used in isolation, can only really allow researchers to measure muscle thickness and not CSA.[71] However, one of the main advantages of ultrasound is its accessibility: it is generally less costly, the ultrasound machine can be individually owned and operated by a research group and is relatively portable. When combined with motion capture technology, 3-D ultrasound may be used to quantify muscle volume and is not subject to motion artifact error during image acquisition.[71] Nevertheless, as with other imaging modalities, the machine operator must be careful not the compress the muscle of interest, which can be particularly difficult with ultrasound since the imaging procedure involves manual operation of an ultrasound probe.[71]

2.3.2 Transcranial magnetic stimulation to measure cortical excitability

TMS is a form of non-invasive brain stimulation in which a metal wired coil generates a

magnetic field in a pulsative manner. The magnetic pulses permeate the scalp and produce an electric current within a target area of the brain via electromagnetic induction. One of the major advantages of using TMS as a measurement tool is its precision. Currents induced by TMS are quite focal, meaning that by manipulating the placement of the coil, it is possible to study specific regions of the brain.[72] Typically, laboratories studying a particular brain region will place a marker on a tight-fitted cap to ensure adequate pulse-to-pulse measurement reproducibility.[73] However, coil placement can be made even more accurate using Neuronavigation software, which uses motion capture technology and the individual's anatomical or functional brain MRI scan to provide the user with specific information regarding coil distance, orientation, and tilt relative to a targeted brain region. Neuronavigation software, while not necessary, further reduces human measurement error and may be particularly advantageous for the study of smaller or more 'quiet' brain areas.[74]

When stimulating an area that lies within the M1, pulses of sufficiently high intensity can further transmit electrical energy to contralateral skeletal muscles, which occasionally results in a brief muscle twitch. When the electrical signal recorded at the level of the muscle (via EMG) following a TMS pulse surpasses a particular threshold (usually >0.05-0.10mv), the response is considered a motor-evoked potential (MEP, **Figure 6**).[75] The percentage of the magnetic stimulator's maximum output at which MEPs are evoked reliably (e.g., ≥5 out of 10 trials) is referred to as the resting motor threshold (RMT).[75] RMT is measured frequently in TMS studies so that pulses can be administered at a standardized intensity relative to each subject's individual threshold in a given testing session. Single-pulse TMS is predominantly used to measure corticospinal excitability (CSE), which is the ability of a specific cortical region to transmit electrical signals to a target muscle via the corticospinal tract. CSE is usually quantified as mean MEP amplitude across several pulses at a given TMS intensity or set of intensities, with a higher amplitude indicating greater

excitability. The first dorsal interosseous (FDI) and abductor pollicis brevis (APB) muscles in the hand are among the most popular targets for recording MEPs, as distal upper limb muscles involved in fine motor control are strongly implicated in corticospinal tract integrity and function, owing to their high degree of pyramidal tract neuron innervation.[76]

Measuring CSE with TMS is valuable within the field of neuroscience because it provides general mechanistic information on the neurophysiology behind a variety of pathologies and environmental adaptations in a safe, non-invasive manner. Certain modes of TMS in which pulses are administered repetitively in rapid succession can even be used to modulate CSE for up to an hour following stimulation, with the direction of effect (increase or decrease) depending on the frequency or pattern of stimulation.[77] Interestingly, CSE has been shown to differ significantly from age-matched, healthy controls in patients experiencing neuropsychiatric disorders or recovering from stroke; and in some cases, modulating CSE using therapeutic brain stimulation to reduce hyper- or hypoexcitability is followed by a subsequent reduction in symptom severity.[78,79] In healthy adults, CSE has also been shown to increase transiently during motor imagery of task performance as well as action observation, thus highlighting it's involvement in motor control.[80] While it is clear that CSE is sensitive to change in response to a wide array of stimuli, the fundamental mechanisms underpinning these changes are not well understood, nor are they always clinically or practically relevant such as in the study of neural adaptations to strength training.[81]

In the context of muscle disuse, CSE has not been demonstrated to change in a predictable manner, as studies have observed a decrease,[82–84] increase,[51,85–88] or no change[73] in CSE with limb immobilization or bed rest. It is possible that heterogeneity among limb immobilization studies may be a product of differences in study design, particularly the duration of immobilization, as CSE appears to decrease when evaluated within the first few hours (3-24 hours post-

immobilization),[26,83] but remains stable or increases after several days or weeks of disuse.[51,73,86,88] Therefore, it is possible there is a difference between the short- and long-term effects of immobilization on the corticospinal tract; however, a study has yet to test this hypothesis directly.

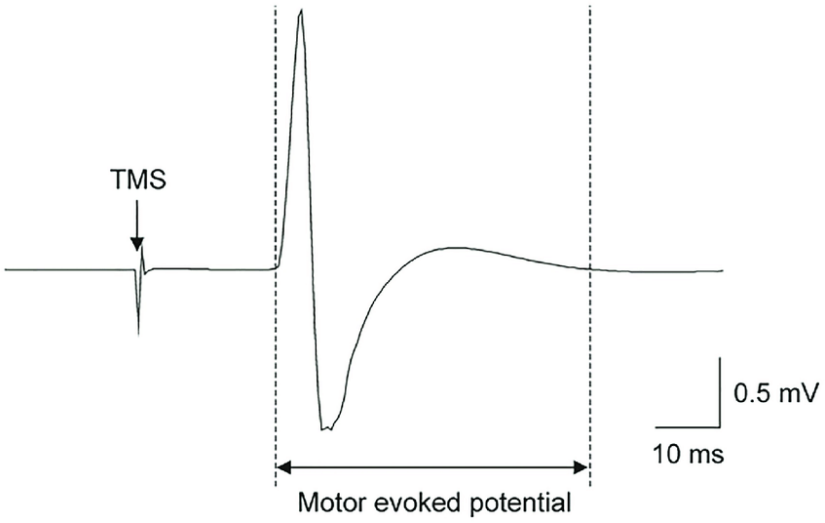

Figure 6. Depiction of a motor evoked potential (MEP) induced by transcranial magnetic stimulation (TMS). MEP amplitude (mV) is calculated as the difference between the peak and the trough in the EMG signal following each TMS pulse. Adapted from Dilena *et al*. (2019).[89]

2.4 Sex differences in immobilization outcomes

Though there are few studies, direct comparisons between men and women have suggested that women lose more relative muscle strength than men with immobilization, despite there being no difference in the relative loss of muscle size.[90–92] In contrast, one study of the atrophic response to unilateral arm suspension has suggested that elbow flexor muscle volume actually decreases at a greater rate in men compared to women,[93] however this study allowed the arm to be mobile during sleeping and bathing. Without the use of activity and sleep logs or accelerometers to confirm the extent of muscle disuse, differences between the sexes could have been confounded by varying activity patterns during the experimental period. In addition, unilateral suspension, as opposed to immobilization, is not known to significantly decrease muscle activity, perhaps due to its less

restrictive nature;[94] ergo, the overall extent of muscle disuse in the above study does not appear to be sufficient. Sex-based differences in muscle strength decline may be related to differential changes in neuromuscular function, as EMG signal amplitude recorded from disused musculature has been demonstrated to decrease nearly four times more in women compared to men following the same immobilization protocol.[92] However, the above study did not measure changes in muscle size, meaning that without confirmation that muscle atrophy was similar between the sexes, it cannot be concluded that the sex-based differences in strength loss were due to differential changes in EMG signal amplitude alone. Furthermore, there is yet to be a plausible biological mechanism to explain how changes in the neural activation of skeletal muscle differ between the sexes in response to disuse; without such evidence, it remains unclear whether differences in strength loss can be explained purely by sexual dimorphism. There exists one study, in which the nondominant forearm was immobilized for 3 weeks, that observed no difference in strength loss between men and women.[95] However, closer examination of the individual subject data reveals that muscle strength decreased by approximately 15% in all female subjects whereas only one of five male subjects experienced a similarly substantial decline in strength, thereby suggesting that the study may have not been adequately powered to detect a statistically significant difference in strength loss between the sexes.[95] On the other hand, despite finding no difference between the sexes in strength loss during immobilization, the same study revealed that women were not able to recover their strength as quickly as men upon being tested a week after remobilization.[95] But unfortunately, other than instructing participants not to engage in any deliberate exercise training during the recovery period, there were no clear control measures over the participants' lifestyle following the immobilization. It is therefore possible that sex differences in recovery could have been confounded by physical activity level after cast removal.

The overall finding within the current literature suggesting that there are sex differences in the response to muscle disuse is particularly important, as per the systematic review by Campbell et al. (2019),[15] women represent only ~24% of the participants among limb immobilization studies measuring changes in muscle strength and neuromuscular function. Given that a large majority of studies in this area have been conducted exclusively on men, we cannot conclude with as much certainty the extent to which muscle strength, muscle size, and neuromuscular function change in response to limb immobilization specifically in women. There is a clear demand for more research on the response to limb immobilization in women, especially given the fact that women generally exhibit lower levels of muscle size and strength in comparison to men.[96]

In summary, the current literature suggests that women are more susceptible to immobilization-induced strength loss compared to men, and that this phenomenon cannot be explained by differences in relative muscle atrophy. In light of this, neural factors such as VA or excitation-contraction coupling may be responsible, but studies have yet to test this hypothesis.

2.5 Prior approaches to preventing disuse-related atrophy and weakness

2.5.1 Dietary approaches

In an attempt to offset the immobilization-induced decline in muscle size, a variety of nutritional strategies acting on regulators of muscle protein turnover have been investigated. Regarding the preservation of muscle strength, these types of interventions have largely been unsuccessful, perhaps because they exclusively target muscle atrophy.[97–101] Hypothetically, protein supplementation during immobilization may act to maintain muscle mass through its known effect on facilitating muscle protein synthesis (MPS, the metabolic process in which intrinsically produced and dietary-derived amino acids are used to create new muscle proteins). However, immobilization has been shown to reduce the regular rate of feeding-induced MPS;[102,103] in fact,

it has been shown to be reduced by half within as little as five days of muscle disuse.[104] As a result, elevated protein intakes up to double the amount (1.6 g/kg body weight per day) of the recommended dietary allowance (RDA) for protein was shown to have no effect on rates of MPS nor muscle mass and strength loss following 3 days of leg immobilization.[105] Other interventions targeting oxidative stress and inflammation (e.g. omega-3 fatty acids), which are related to decreased rates of MPS, have only occasionally resulted in the retention of muscle size, while exerting no influence on measures of muscle strength.[97,106,107] Insofar as dietary interventions have not succeeded in preserving muscle function, daily creatine supplementation has once been shown in the upper limb to preserve muscle size, strength, and endurance during immobilization.[108] Creatine is an over the counter supplement known to facilitate exercise-induced gains in muscle mass and performance,[109] possibly due its role as a substrate (phosphocreatine, PCr) in the reversible regeneration of the adenosine triphosphate (ATP) required for muscle contraction. However, the abovementioned study stands in opposition to several others, which revealed no effect of creatine supplementation on preserving muscle strength, endurance, or performance on tests of physical function.[101,110–113] When examining specifically the lower limb, immobilization does indeed reduce muscle phosphocreatine content, which can be prevented through supplementation.[110,114] However, it has additionally been revealed that first, not all individuals respond to creatine supplementation when administered using a standard dosing procedure,[101] and that second, creatine loading may appear to offset muscle atrophy due to the retention of water via osmotic pressure.[115,116] In other words, regardless of whether an individual responds to creatine supplementation, a supposed preservation of muscle mass may not represent the retainment of myofibrillar proteins. And while some have proposed that cellular swelling due to creatine loading mechanically induces MPS in skeletal muscle to increase hypertrophy/decrease atrophy,[117] this

hypothesis is not necessarily supported by the current literature.[118–120] It is also possible that differences in experimental design explain the discrepancy in results. The studies revealing no effect of creatine supplementation randomized participants into either a supplement or placebo group whereas the study in favour of creatine supplementation employed a cross-over design in which all participants received both conditions, with a 7-day washout period in between. And to its detriment, the crossover study conducted by Johnston et al. (2009)[108] had all participants undergo the placebo prior to the creatine condition, meaning that the order of intervention may have confounded results. All things considered, nutritional strategies targeting regulators of muscle mass have generally been unsuccessful at preserving muscle function during immobilization. Since current dietary compounds of interest are not known to act upon the neuromuscular system as a whole, it is likely that nutritional interventions alone would not be the optimal approach for countering the consequences of muscle disuse.

2.5.2 Neuromuscular electrical stimulation

Given that local changes in gene expression during disuse are attributed primarily to a lack of mechanical load on the muscle,[121] researchers have also tested the potential of using neuromuscular electrical muscle stimulation (NMES) to preserve muscle mass and function. As a measure to prevent or attenuate muscle atrophy, NMES or NMES combined with protein supplementation appears to be superior to diet-related approaches alone.[99,122] Regarding the effectiveness of NMES as a means to preserve muscle function, it may depend on the health status of the patient as well as the type of functional assessment used. In healthy subjects, NMES is shown to be effective at preventing nearly all lower limb muscle atrophy, without any impact on preserving muscle function.[99,122] However, this is a rather contradictory finding, as although muscle atrophy may not be the sole factor in muscle strength loss during disuse, it is difficult to

explain how such a profound impact on preserving muscle mass would not result in a somewhat noticeable impact on strength loss. Perhaps the person's ability to voluntarily activate the retained muscle mass declined, since NMES prompts muscle contraction without direct involvement of the central nervous system; but this can only be speculated. Alternatively, it is more likely that the unforeseen outcomes above are explained by shortcomings in the methods of measurement employed. Isokinetic dynamometry and MRI or CT are the criterion measures for muscle strength and size, respectively.[60,123,124] Unfortunately, neither of the above NMES studies by Reidy et al. (2017)[99] and Dirks et al. (2014)[122] used the standard of measurement for both muscle mass and strength simultaneously.[60,123,124] Among these studies, one used one-repetition maximum (1RM) on a leg extension machine to quantify muscle strength whereas the other used dual X-ray absorptiometry (DEXA) to measure muscle mass. First, while 1RM measurements of lower limb strength have indeed been shown to correlate well with isokinetic dynamometry,[125] the two methods may not agree when assessing a change in strength.[126] Furthermore, 1RM has been shown to overestimate symmetry in muscle strength between lower limbs,[127] which is an issue for within-subject comparisons between an immobilized and non-immobilized limb as is the case for the NMES study by Dirks et al. (2014)[122]. Symmetry between limbs is likely overestimated with 1RM due to the fact that the load cannot be precisely controlled on a typical exercise machine to the same degree as a dynamometer. Secondly, DEXA does not directly quantify muscle mass. Instead, DEXA measures fat-free mass, a large proportion of which is assumed to be composed of muscle.[128] Using mathematical models, DEXA can indeed be used to predict muscle mass in a single limb with a relatively high degree of accuracy,[128,129] however such a model was not employed in the NMES study by Reidy et al. (2017).[99]

The potential of using NMES to combat both muscle atrophy and strength loss during disuse

becomes apparent when examining the literature on clinical populations. For instance, NMES has been shown to exert a partially protective effect on muscle strength in patients in rehabilitation for anterior cruciate ligament injury.[130] In the clinical context, noteworthy advantages of NMES are that it is relatively inexpensive and easy to use; the patient does not even need to be conscious for the intervention to be administered. As such, NMES has successfully been used for the attenuation of muscle atrophy and weakness in critically ill patients in intensive care[131] and even comatose patients.[132] Additionally, early NMES intervention for intensive care patients has been shown to reduce the time dependent on mechanical ventilation as well as time spent in the hospital.[131] This is quite remarkable given the severe impact that critical illness can have on a patient's nutrition and overall lifestyle, and in the case of comatose patients, a complete loss of voluntary muscle activity.[132] And in light of the suggestion that prolonged muscle disuse exacerbated by inflammatory conditions leads to more pronounced muscle atrophy,[133] it is possible that patients in intensive care benefit more from therapies that target primarily the regulation of muscle size when considering the preservation of physical function. Nonetheless, it is important not to overlook the possible benefits that NMES may have on the nervous system. NMES is a longstanding therapy for restoring physical function in patients suffering from stroke-induced hemiplegia and spinal cord injury and has known effects on activating the somatomotor system.[134,135] While the site of stimulation may not directly include the brain, an fMRI study by Francis et al. (2009)[136] demonstrated that involuntary ankle dorsiflexion triggered by electrical stimulation actually activates the secondary somatosensory system more than volitional contraction. Therefore, given that daily NMES intervention is capable of interrupting prolonged mechanical unloading during muscle disuse, while simultaneously activating cortical regions relevant to motor rehabilitation, it currently stands among the most effective interventions for

slowing muscle atrophy and functional decline, particularly for patients in intensive care.

2.5.3 Motor imagery

Interventions acting primarily on neural mechanisms have further advanced the importance of the central nervous system in preventing disuse-related strength loss. Motor imagery training (MI, routine imagination of executing a movement in the absence of voluntary muscle contraction) for example, has been shown to reduce the decline in muscle strength and VA by approximately 50% when performed 5 days/week during a 4-week period of forearm immobilization.[50] MI has further been suggested to offset acute changes in the sensorimotor system within the first few hours of hand immobilization, such as changes in corticospinal excitability, resting state functional connectivity, and sleep waves,[137,138] however the functional significance of these adaptations are not fully understood. Due to a lack of studies, further investigation is necessary to determine whether MI is valuable enough to incorporate into treatment plans for patients undergoing muscle disuse. Furthermore, the efficacy of MI has yet to be demonstrated using other models of disuse, as well as within the lower limbs.

2.5.4 Cross-education training

For reasons not entirely clear, unilateral limb strength can be augmented or preserved by repetitive, resisted muscular contraction of the contralateral limb, a phenomenon termed the 'cross-education' effect.[139,140] Cross-education training (CE) has further been suggested to prevent disuse muscle atrophy,[36,139,141,142] however this has primarily been demonstrated using ultrasound to measure changes in muscle thickness, rather than directly measuring muscle mass such as with MRI or CT. CE is known to elicit a small degree of muscle activity in the untrained limb,[143,144] which may explain its ability to maintain muscle size. However, muscle activity in the contralateral limb can vary significantly between individuals (2-29% of activity during maximal voluntary

contraction) and may be dependent on the muscle trained.[143,144] And currently, there exists no evidence confirming that the degree of involuntary muscle activity in the opposite limb during CE is associated with muscle size maintenance. It is also not entirely clear whether resting limb activity during CE is cortical in origin, as involuntary EMG signal amplitude of the homologous muscle group in the resting limb is correlated with increases in bilateral motor cortex activity during wrist flexion, but not wrist extension, arm flexion, or arm extension.[145]

On the other hand, the purported mechanisms through which CE can influence strength of the untrained contralateral limb are more thoroughly described. First, CE has been shown to increase VA of the wrist extensors,[146] implying that the mechanism through which CE prevents disuse-related muscle strength loss is predominantly neural. There are several possible ways through which CE may facilitate the maintenance of VA and strength, but remain untested during immobilization: 1) a reduction in interhemispheric inhibition; 2) concurrent activation of regions that are functionally related to M1 (e.g., premotor cortex, supplementary motor area, anterior cingulate cortex); and, in the event that the person training is able to observe their own movements, 3) training of the mirror neuron system through action observation to acquire or retain motor skills (for detailed review, see Frazer et al. (2018)[147]).

It is important to note that although therapeutic strategies targeting the nervous system have been effective for treating disuse-related strength loss, those discussed in this review have only been studied using a limb immobilization model. Current neural-based approaches to treating muscle disuse also do not seem widely applicable in a clinical context – MI requires the patient to be conscious while CE is restricted to situations in which a mobile, contralateral limb is available to train, and further depends on the person being able to perform intense exercise. Overall, current approaches to treating patients undergoing obligatory muscle disuse have highlighted the

importance of considering the central nervous system, but these approaches do not appear to be practical for patients in bed rest or suffering from severe illness or injury. Nevertheless, the importance of treating the neuromuscular system as a whole should not be overlooked when developing new therapies for disuse-related muscle atrophy and weakness.

CHAPTER 3: MANUSCRIPT

3.1 INTRODUCTION

Obligatory periods of skeletal muscle disuse (e.g., limb immobilization, bed rest) in response to injury, surgery, or hospitalization are known to result in significant strength loss and muscle atrophy. Research has largely demonstrated that the rate of disuse-related strength decline exceeds that of muscle atrophy, suggesting that factors other than muscle size may contribute to changes in muscle function. Given that the brain is ultimately responsible for the onset of voluntary skeletal muscle contraction, the nervous system may be a potential site of interest for understanding disuse-induced strength loss. It is well accepted that neural adaptations precede observable muscle hypertrophy during the first several weeks of resistance exercise training in untrained individuals.[148] As such, it is possible that the nervous system is the first site of adaptation during upper limb disuse within untrained individuals. In fact, prior studies have revealed that limb immobilization can elicit significant changes in corticospinal excitability within as little as 3 hours;[83] and with longer periods of immobilization, changes in brain anatomy and activity.[26,27,35]

In a recent systematic review by Campbell et al. (2019),[15] it was found that among limb immobilization studies measuring changes in muscle strength, size, and neuromuscular function, there was no significant association between changes in muscle size and strength. On the other hand, in the upper limb specifically, several measures indicative of neuromuscular function such as voluntary activation capacity (VA) and resting twitch force have been shown to be significantly and more strongly associated with muscle strength loss relative to muscle atrophy.[15] Indeed, unlike many of the muscles in the lower limb, upper limb muscles exhibit a greater density of corticospinal projections and are not responsible for weight bearing.[54] It is therefore possible that muscle atrophy plays a less significant role in disuse-related strength loss in the upper compared to the lower limb, particularly during the early disuse period. However upper limb immobilization

studies often lack the use of criterion measures, namely isokinetic dynamometry and either magnetic resonance imaging (MRI) or computed tomography (CT) for muscle strength and size assessment, respectively.[15] To our knowledge, no single study has employed current standards of measurement for the assessment of both muscle strength and size in response to upper limb immobilization.

It has further been suggested that the nervous system plays a proportionally larger role in disuse related strength loss in women relative to men.[91] Women have been demonstrated to lose more relative muscle strength with lower limb immobilization despite no differences in relative muscle atrophy compared to men.[90–92] This observation may be attributed to differences in neuromuscular plasticity, as it was additionally found that women lost greater than four times the amount of surface electromyographical (EMG) activity recorded during maximal voluntary knee extension compared to men following lower limb immobilization.[92] In contrast, a study of sex differences in response to upper limb suspension revealed similar declines in relative strength between men and women, however only the men experienced a significant reduction in elbow flexor volume, suggesting that mechanisms apart from muscle atrophy were responsible for the strength loss in women.[93] Despite this, women represent only ~24% of the participants studied among limb immobilization studies measuring both changes in muscle size and strength.[15] As such, the extent to which females lose muscle size and strength in response to upper limb immobilization is less known.

Altogether, current research suggests that early strength loss in response to muscle disuse may be attributed primarily to changes in neuromuscular function, particularly in women. The purpose of this study was to evaluate changes in muscle strength, muscle size, VA, and corticospinal excitability in response to 2 weeks of upper arm immobilization in young women using current

standards of measurement. We hypothesized that immobilization would result in a significant decrease in muscle strength, muscle size, and VA after 2 weeks. We additionally hypothesized that immobilization would induce a transient decrease in corticospinal excitability at 24 hours post-immobilization.

3.2 METHODS

3.2.1 Participants and sample size determination

We recruited a convenience sample of 12 right-hand dominant women aged 18-35 years, with a BMI between 18.5 and 30 kg/m^2 (inclusive). All participants reported having a regular menstrual cycle and engaged in deliberate exercise or sports from 0-6 days per week. Participants were excluded from the study if they met any of the following criteria: use of tobacco; pregnancy; history of brain trauma, neurological disease, movement disorder, or mental illness; peripheral nerve damage; use of certain medications or supplements known to affect protein metabolism (e.g., corticosteroids, non-steroidal anti-inflammatory drugs, prescription strength acne medications, creatine, fish oil); or the possession of any metal implants, non-removeable medical devices, or any other relevant contraindications. Accounting for the unilateral within-subject study design, it was determined using G*Power software (version 3.1.9.7) that 12 volunteers would be sufficient to detect a medium effect size (Cohen's f = 0.25) with an alpha of 0.05 and a power level of at least 0.9 for isometric muscle strength and muscle size.

3.2.2 Research ethics approval

This study was approved by the Faculty of Medicine Institutional Review Board at McGill University (date of last approval April 23, 2021, approval no. A01-M01-21A) and was carried out in accordance with the Helsinki Declaration of 1975 as revised in October 2013. All participants were informed by a study investigator regarding the purpose of the study, the procedures involved, and the possible risks associated with participation in the study before providing informed written consent.

3.2.3 Overview of study design

The study was prospectively registered at https://clinicaltrials.gov/:NCT05115643 on November 10, 2021. A schematic representation of the experimental design is shown in **Figure 7**. The study followed a within-subject, unilateral study design comparing the immobilized versus non-immobilized arm of each participant. Upon receiving informed consent, participants provided baseline measures indicative of their general health status and underwent a familiarization session with the isokinetic dynamometer used for strength testing. During the experimental period, each participant's left arm was immobilized with the elbow joint fixed at 90° flexion for 14 consecutive days. The following outcome measures were obtained prior to and following the immobilization period: bilateral elbow flexor and extensor muscle size; anatomical and resting state functional MRI scans of the brain; bilateral elbow flexor and extensor muscle strength; bilateral VA of the biceps brachii; bilateral corticospinal excitability of the biceps brachii; and ovarian hormone concentrations (oestradiol and progesterone). To evaluate the short-term effects of the intervention on the nervous system, bilateral corticospinal excitability of the biceps brachii was additionally measured 24 hours following arm immobilization.

In consideration of potential confounding by changes in dietary protein, participants recorded their food intake using standardized forms during the first two days before and last two days of the immobilization period. Participants were asked not to engage in any form of moderate to vigorous physical activity from the 48-hour period prior to the pre-immobilization testing visit to the end of the study. They were additionally asked not to consume any alcohol throughout the immobilization period, and not to consume any caffeine 24 hours prior to all study visits.

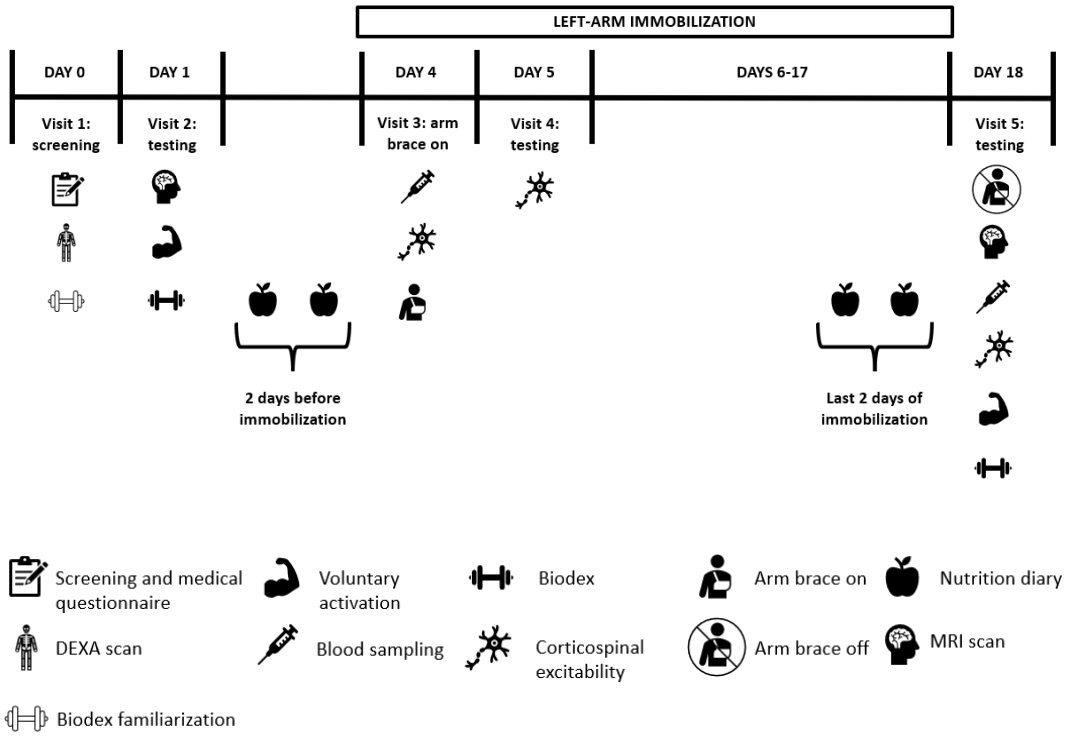

Figure 7. Experimental design

3.2.4 Preliminary testing

Prior to the intervention, information regarding each participant's overall health status was collected. This included a basic medical questionnaire, as well as measurement of anthropometrics (height and weight), body composition (by dual-energy X-ray absorptiometry; GE Healthcare; Madison, WI, USA), and resting heart rate and blood pressure (Omron 10 series, Model BP786CANN). Familiarization with the dynamometer for strength assessment included one maximal isometric and isokinetic (maximum velocity of 120°/s) contraction of the elbow flexors and extensors of each arm.

3.2.5 Blood collection and ovarian hormone analysis

Blood samples were analyzed for serum concentrations of progesterone (nmol/L) and oestradiol (pmol/L) to validate their normality while also confirming whether their levels fluctuated significantly between pre- and post-immobilization measurement points (Clinical Biochemistry

Laboratory of the McGill University Health Centre). This is due to the possibility that normal fluctuations in ovarian hormones throughout the female menstrual cycle alters day-to-day corticospinal excitability.[149] 8 mL of blood was collected in a serum vacutainer, centrifuged at 1000 RCF and stored at -80°C in 1.5 mL Eppendorf tubes until ready for analysis.

3.2.6 Magnetic resonance imaging

Two T2-weighted (echo time = 9.6 ms; repetition time = 3300 ms; matrix 256x256x44 slices; 0.8x0.8x5 mm), one T1-weighted (echo time = 2.95 ms; inversion time = 900; repetition time = 2300 ms; matrix 256x256x192; GRAPPA = 2; 1.0 mm isotropic), and one resting state blood oxygenation level dependent (BOLD) functional (echo time = 33 ms; repetition time = 800 ms; matrix 104x104x60 slices; SMS = 6; 2.5 mm isotropic) MRI scans were obtained before and following immobilization for the following measures: right and left elbow flexor and extensor muscle size; M1 cortical thickness; and resting state functional connectivity (McConnell Brain Imaging Centre, Montreal Neurological Institute, Montreal, CA). Size of the right and left elbow flexor and extensor muscle groups were defined by their cross-sectional area (CSA) and volume using the open-source computer software MRtrix3. Beginning at the proximal edge of the humerus, the borders of the muscle were manually traced by a sole investigator (Y.H.) until the elbow joint was visible. For CSA, the largest value was recorded at both pre- and post-immobilization. As there was no gap in between slices, volume was calculated by summating the individual CSAs and multiplying the result by the individual slice thickness. The number of slices evaluated was kept consistent between timepoints of measurement within the arms of each participant. To assess the degree of intra-rater measurement error, muscle CSA and volume measurements were repeated for three subjects, with a minimum of four days between measurements. The coefficient of variation (CV) was calculated by dividing the standard deviation of the two measurements by the mean.

3.2.7 Muscle strength

Muscle strength was measured as maximal voluntary isometric and isokinetic (maximum velocity of 120°/s) contraction in both arms using an isokinetic dynamometer (Biodex 4 ProTM, Biodex medical instruments, Shirley, USA). The order in which the arms were measured was pre-determined randomly in a 1:1 ratio using randomizer.org and kept consistent among all measurements and testing days for each individual participant. Before measurement, participants were strapped to the seat of the Biodex with their elbow joint fixed at 90° and aligned with the machine's axis of rotation. Participants performed three, 5-second maximal isometric contractions to measure the strength of the elbow extensor, then flexor muscles. For isokinetic measurements, participants were instructed to contract as hard and as fast as possible through their range of motion at the elbow joint. Verbal encouragement was provided during all strength tests. All contractions were separated by 90 seconds of rest to minimize the effect of fatigue. The highest peak torque achieved among the three trials for each contraction type was recorded as muscle strength.

3.2.8 Voluntary activation

VA of the biceps brachii was measured in both arms using the twitch interpolation technique[48] via peripheral muscle stimulation (Digitimer, Welwyn Garden City, Herfordshire, UK). Torque data was obtained using the same isokinetic dynamometer used to measure muscle strength and processed and recorded using an analog-to-digital converter (Micro 1401, CED, Cambridge, UK) connected to a laptop running Spike2 software (version 10, CED, Cambridge, UK). Two, 5x10 cm oval neurostimulation electrodes (Axelgaard Manufacturing Co., Lystrup, DK) were used to transfer electrically evoked doublets to the biceps brachii muscle (100 μs pulse width; 10 ms interstimulus interval). The anode was placed on the distal tendon of the biceps, over the antecubital fossa, whereas the cathode was moved around the muscle belly of the biceps until the

site of stimulation yielding the highest evoked elbow flexion torque was located. Upon identification of the optimal site of stimulation, the intensity of the stimulus was gradually increased until a plateau in torque was reached; stimuli at 120% of this intensity were administered during the measurement procedure to ensure maximal recruitment of the target muscle. For the measurement of activation capacity, the subject was asked to perform three maximal elbow flexion contractions on each arm, during and after which an electrically evoked doublet was administered to obtain the superimposed and resting twitch, respectively. The superimposed doublet was triggered manually once the subject reached peak torque, which was identified by a study investigator using the monitor of the dynamometer system's computer. The resting doublet was triggered several seconds later once torque returned to baseline level. Following each trial, the participant was questioned to confirm that they had been contracting at maximal effort immediately prior to feeling the first stimulus. Trials in which the subject and/or the torque data indicate that the electrical stimulus was not administered at or near the subject's maximal torque were discarded. A minimum of three trials were performed on each arm, and the highest value was recorded for analysis. VA was calculated as a percentage within Spike2 by comparing the additional elbow flexion torque produced by suprathreshold stimulation of the biceps brachii during maximal contraction, to the torque evoked at rest using the following equation:

$$Voluntary\ activation\ (\%) = \left(1 - \frac{superimposed\ twitch}{resting\ twitch}\right) X\ 100$$

VA was determined to be 100% for trials in which the stimulus was administered at peak torque but failed to produce a noticeable superimposed twitch.

3.2.9 Transcranial magnetic stimulation (TMS) procedure

TMS was administered using a Super Rapid^2 TMS system (Magstim Company, UK) connected to a dome-shaped coil. With the participant seated comfortably, the TMS coil was positioned tangentially to the participant's right or left M1 at a 45° angle from the mid-sagittal line to stimulate the motor region controlling the upper arm muscles. Electromyographic (EMG) activity of the biceps brachii was recorded using disposable surface electrodes (Biopac Systems, Inc., California, USA) positioned in a belly-tendon montage. EMG data was processed by a Biopac MP150 acquisition system (Biopac Systems, Inc., California, USA), sampled at 10 kHz on a 16-bit analog-to-digital board, and amplified and bandpass filtered at 10-5000 Hz. The biceps brachii 'hotspot', defined as the stimulation site yielding the greatest EMG response in the biceps brachii, was located using Neuronavigation software (Brainsight, Rogue Research Inc, Montreal, CA) outfitted with a 3-dimensional reconstruction of the participant's brain obtained from their T1-weighted anatomical MRI scan.

Corticospinal excitability of the biceps brachii was inferred using both resting motor threshold (RMT) and stimulus-response (SR) curves. RMT of the biceps brachii was defined as the minimum TMS intensity required to elicit a motor evoked potential (MEP) of 0.05mV in ≥10 out of 20 trials.[150] Data for SR curves was acquired through the administration of 10 pulses at various intensities separated by increments of 10% of maximum stimulator output (%MSO, range = 30-90), in a randomized order. Individual MEPs were excluded if they occurred in the presence of noticeable muscle activity or if they were classified as an outlier using the Tukey method.[151] Individual SR curves were constructed by fitting mean peak-to-peak MEP amplitudes at each TMS intensity against a sigmoidal curve using the Solver function of Microsoft Excel with the following equation:

$$MEP\ amplitude\ (mv) = \frac{MEP_{max}}{1 + e^{(\frac{S_{50} - S}{k})}}$$

Where S is the %MSO, S_{50} is the %MSO at which 50% of the maximum MEP amplitude is observed (i.e., the inflection point of the curve, the point at which higher threshold neurons are recruited), and k is the slope of the tangent passing through the inflection point. A coefficient of determination (R^2) >0.7 was considered an acceptable fit. The parameters of interest were inflection point; slope; and area under the curve (AUC), which was calculated using the trapezoidal method.

Two of the twelve subjects were not able to tolerate some of the higher TMS intensities. Appropriate measures, such as replacing these intensities with stimuli at lower %MSOs were undertaken so that a relationship between stimulus and response could still be evaluated. Certain individuals were also found to have low RMTs (<30%MSO). For these subjects, an additional 10 stimuli at 20%MSO were administered for both arms to extract the portion of the SR curve at which MEP amplitudes of zero are observed. The array of intensities was kept consistent between sessions for all instances in which the TMS procedure had to be adapted.

3.2.10 Arm immobilization

Following pre-immobilization testing, participants underwent immobilization of their left arm using a telescoping arm brace (Donjoy, Lewisville, USA) fixed at 90° elbow flexion and a sling. To ensure participant compliance to the immobilization, zip ties were placed around the brace to prevent its removal. Unique code words were written in ink by a study investigator on each zip tie so that any zip ties removed could not be replaced without the investigator's knowledge. The brace was worn at all times, including during sleep and bathing. Participants were permitted to remove the sling momentarily when bathing, as this allowed them to put on a waterproof brace cover

provided to them to prevent the brace from getting wet, as well as when changing clothes and sleeping.

3.2.11 Statistical analysis

Baseline measures of body composition (i.e., BMI, %body fat) and resting heart rate and blood pressure are used for descriptive purposes. Changes in physiological and behavioural measures following immobilization were analyzed using a two-way, repeated measures ANOVA, with time (pre- versus post-immobilization) and arm (immobilized versus non-immobilized) as within-subject factors. If significant effects were to be found, the nature of these effects were further elucidated using Bonferroni-adjusted pairwise comparisons. Dependent variables of interest were bilateral elbow flexor and extensor muscle strength (isometric and isokinetic); and bilateral elbow flexor and extensor muscle size (CSA and volume). For RMT and SR curve parameters, an additional level was added to time as a within-subject factor to include measurements at 24 hours post-immobilization.

Since stimuli at higher intensities (70-90%MSO) in some of the subjects with lower RMTs were saturated by the stimulus artifact, we were not able to identify MEPs at these TMS intensities and therefore standardized the procedure for calculating AUC to include only the values of the independent variable at which curves were fitted based on real data at all timepoints of measurement in both arms. Because the procedure for calculating AUC was no longer standardized between individual subjects, we opted to express the values as a percent change from baseline and analyze the results using a paired-samples t-test. As many individuals began with VA levels near or at 100%, VA was also expressed as a %change from baseline and analyzed using a paired-samples t-test. Serum ovarian hormone concentrations and dietary protein (absolute (g) and relative (g/kg/d) daily protein intake) before and after immobilization were compared using a

paired-sample t test. Tests of normality (Shapiro-Wilk) and sphericity (Mauchly) were conducted to test assumptions of the statistical models. For all analyses, a p-value <0.05 was considered statistically significant. All statistical calculations were performed using IBM SPSS statistical software (version 26).

3.3 RESULTS

3.3.1 Participant characteristics, dietary intake, and serum ovarian hormones

Baseline participant characteristics are presented in **Table 1**. There was no significant change in daily absolute (66±18g to 57±27g) and relative (1.2±0.3g/kg/d to 1.0±0.5g/kg/d) protein intake between the period before and during immobilization. Serum oestradiol (218.8±239.9 to 346.8±406.3pmol/L) and progesterone (5.0±3.9 to 5.9±5.0nmol/L) levels were not significantly different between pre- and post-immobilization testing visits.

Table 1. Participant characteristics

Age (yrs)	Height (cm)	Weight (kg)	BMI (kg/m^2)	Body fat (%)	Heart rate (bpm)	Systolic BP (mmHg)	Diastolic BP (mmHg)
20.6±2.1	164.3±6.3	58.4±10.4	21.5±3.2	32.5±8.2	88±16	109±11	76±8

3.3.2 Muscle strength

Changes in all strength variables in response to the immobilization are displayed in **Figure 8**. Certain strength parameters failed to meet the assumption of normality required for ANOVA (Shapiro-Wilk test, $P < 0.05$). An individual with exceptionally high baseline strength compared to the rest of the sample was identified as an outlier using the Tukey method. Upon removal of this outlier, all strength variables satisfied the conditions of normality. Statistical tests were performed both with and without the outlier to confirm whether the interpretation of the results remained consistent. The results reported below are with the outlier excluded from the analysis, whereas the figure includes all subjects.

There were no baseline differences between arms for all measures of strength ($P > 0.05$). There was a significant time × arm interaction effect for both isometric elbow flexion ($P = 0.040$) and extension ($P = 0.021$). Post-hoc comparisons indicated that strength decreased significantly

following the intervention in the immobilized arm (flexion: 30.2±6.4 to 22.9±3.5Nm, $P = 0.004$; extension: 32.7±7.4 to 25.3±4.9Nm, $P = 0.005$) but did not change in the non-immobilized arm (flexion: 32.7±9.2 to 31.9±8.4Nm, $P = 0.422$; extension: 30.7±10.1 to 29.8±7.9Nm, $P = 0.553$). The mean relative change in isometric strength in the immobilized arm was -21.3±19.2% and -19.9±15.7% for elbow flexion and extension, respectively. There were no significant interaction effects for isokinetic elbow flexion and extension. Statistical interpretations of the strength results remained consistent when the outlier was included in the analysis.

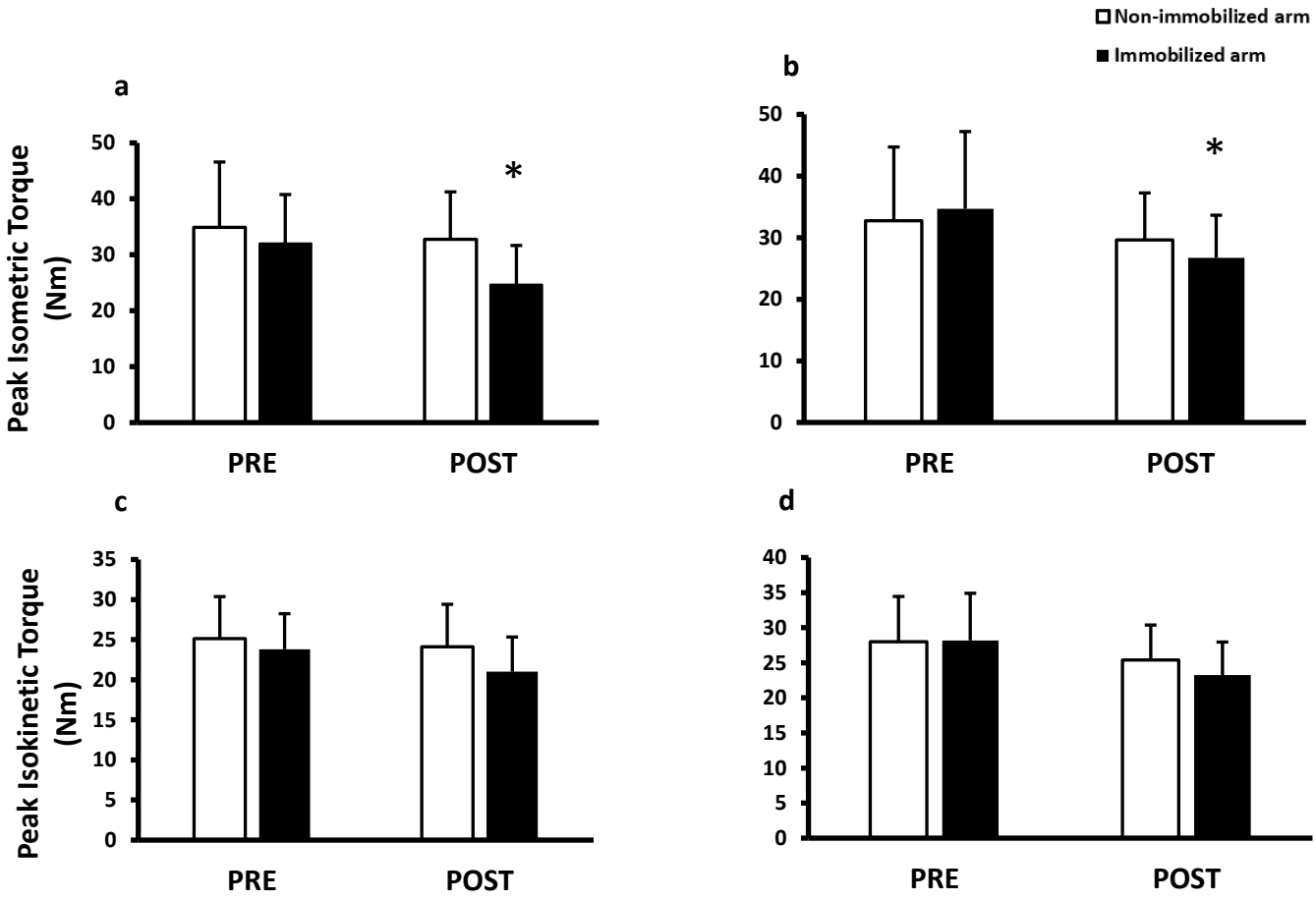

Figure 8. Peak torque during isometric elbow flexion (a), isometric elbow extension (b), isokinetic elbow flexion (c), and isokinetic elbow extension (d) before (PRE) and after 2 weeks (POST) of nondominant upper arm immobilization. Values are mean ± SD. *Significantly different from baseline within respective arm ($P < 0.01$).

3.3.3 Muscle cross-sectional area and volume

Changes in relative elbow flexor and extensor muscle volume are displayed in **Figure 9**. Mean CVs for repeated muscle size assessments were 0.70 (flexor volume), 0.47 (extensor volume), 0.76 (flexor CSA), and 0.67 (extensor CSA). Elbow flexor cross-sectional area data was not normally distributed in the right arm at pre-immobilization ($P = 0.044$) as well as in the left arm at pre- ($P = 0.030$) and post-immobilization ($P = 0.029$). Frequently used data transformations (i.e., log, square root, cubic root) failed to shift the data toward a normal distribution. Since significance levels were not far from the cut-off of 0.05, we visually inspected the Q-Q residual plots to determine whether these data met an acceptable level of normality (**Figure S1, Supplementary material**). It was concluded that the data was adequately proximal to a normal distribution, thus the raw elbow flexor CSA data was considered to be acceptable for analysis using repeated measures ANOVA.

There were no baseline differences between arms for all measures of muscle size. There were no significant effects of the immobilization on elbow flexor CSA (immobilized: 10.9±2.3 to 10.9±2.3cm^2, $P = 0.539$; non-immobilized: 10.6±2.1 to 10.6±2.2cm^2, $P = 0.772$). There was a significant time × arm interaction effect for elbow flexor volume ($P = 0.035$), however post-hoc tests did not reveal any significant differences between means regardless of whether the data was stratified by arm or timepoint of measurement (immobilized: 120.5±21.8 to 119.2±22.5cm^3, $P = 0.114$; non-immobilized: 119.4±23.1 to 119.5±24.1cm^3, $P = 0.917$). There were significant interaction effects for both elbow extensor CSA and volume, such that they decreased significantly in the immobilized arm (CSA: 15.3±3.8 to 14.8±3.5cm^2, $P = 0.018$; volume: 166.8±41.2 to 162.1±38.3cm^3, $P = 0.043$) but did not change in the non-immobilized arm (CSA: 15.3±3.5 to 15.2±3.3cm^2; volume: 167.0±38.3 to 166.0±34.9cm^3). The mean relative change in elbow extensor

CSA and volume in the immobilized arm from pre- to post-immobilization was -2.9±2.9% and -2.5±2.5%, respectively.

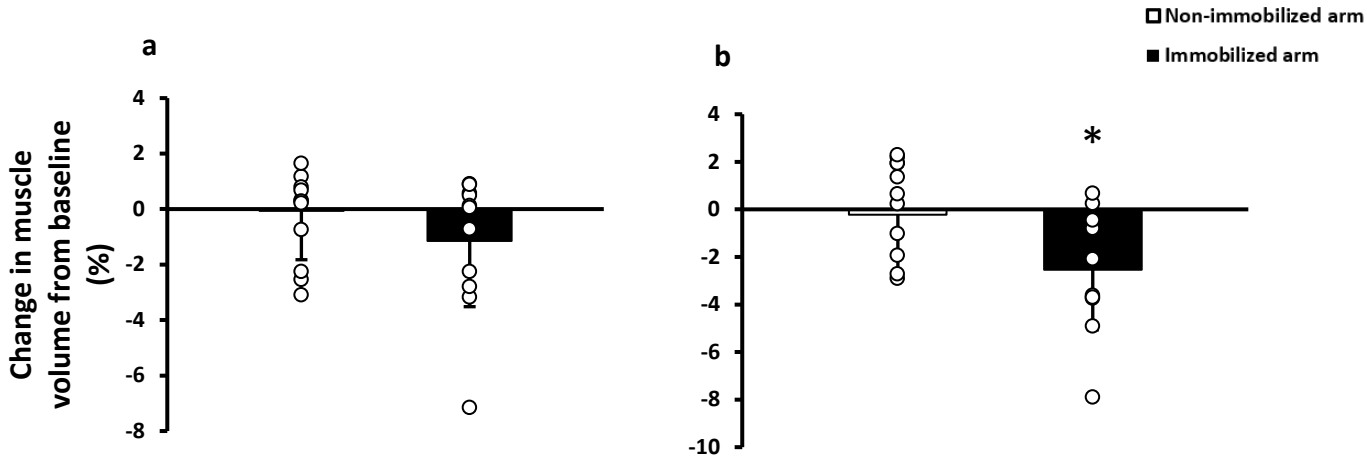

Figure 9. Relative change in elbow flexor (a) and extensor (b) muscle volume following 2 weeks of nondominant upper arm immobilization. Values are mean ± SD. Open circles represent individual subject values. *Significant change from baseline within respective arm ($P < 0.05$).

3.3.4 Voluntary activation capacity

VA data was available in eleven of the twelve subjects. There was no significant difference in the relative change in VA between arms following the intervention (**Figure 10**; -4.3±14.1% in immobilized versus 0.418±5.9% in non-immobilized arm, $P = 0.354$).

3.3.5 Corticospinal excitability

3.3.5a Resting motor threshold

Changes in RMT throughout the study are displayed in **Figure 11**. RMT data did not satisfy the assumption of normality in the non-immobilized arm at pre-immobilization ($P = 0.044$). Upon inspection of the Q-Q plots, it was determined that this data was adequately normally distributed for analysis (**Figure S1, Supplementary material**). Mean baseline RMT was approximately 31±7% and 32±7%MSO in the non-immobilized and immobilized arms, respectively, with no

differences between arms. There were no significant effects detected for RMT throughout the immobilization period.

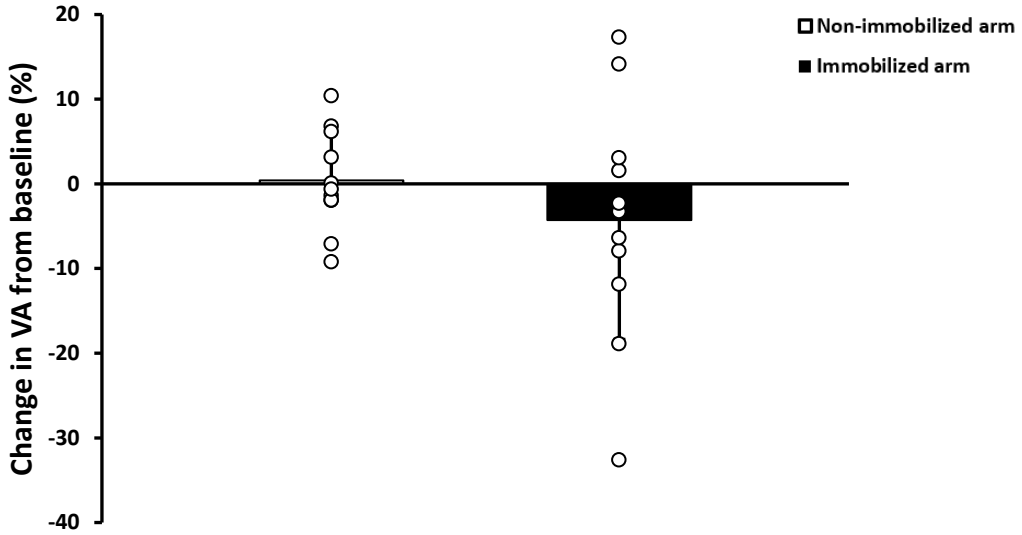

Figure 10. Relative change in voluntary activation capacity (VA) of the biceps brachii in response to 2 weeks of nondominant upper arm immobilization. Values are mean ± SD. Open circles represent individual subject values.

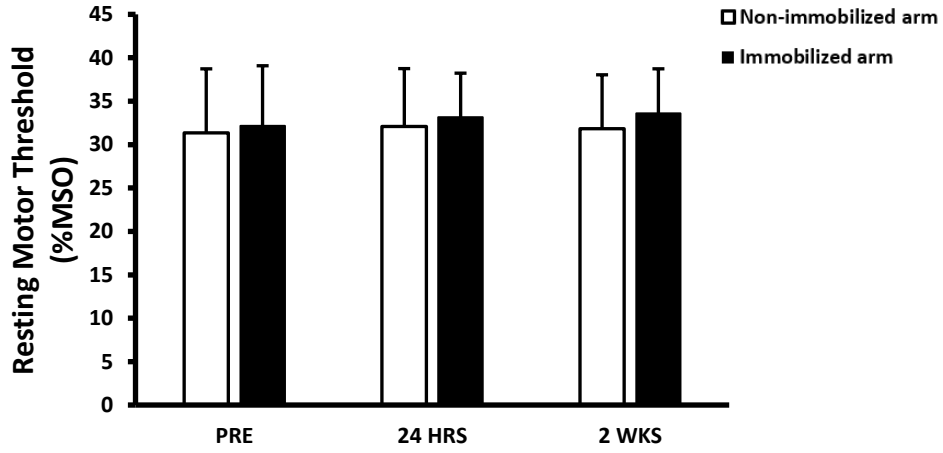

Figure 11. Resting motor threshold expressed as a percentage of maximum transcranial magnetic stimulator output (%MSO) before (PRE), 24 hours after (24 HRS), and 2 weeks after (2 WKS) nondominant upper arm immobilization. Values are mean ± SD.

3.3.5b Inflection point

Changes in mean peak-to-peak MEP amplitudes at each stimulus intensity are displayed in **Figure 12**. The mean R^2 for individual TMS SR curves was 0.92±0.07. Mean baseline inflection point of the TMS SR curve was estimated to be at 62±9%MSO and 61±11%MSO in the non-immobilized and immobilized arm, respectively, with no baseline differences between arms. There were no significant effects detected for inflection point throughout the immobilization period.

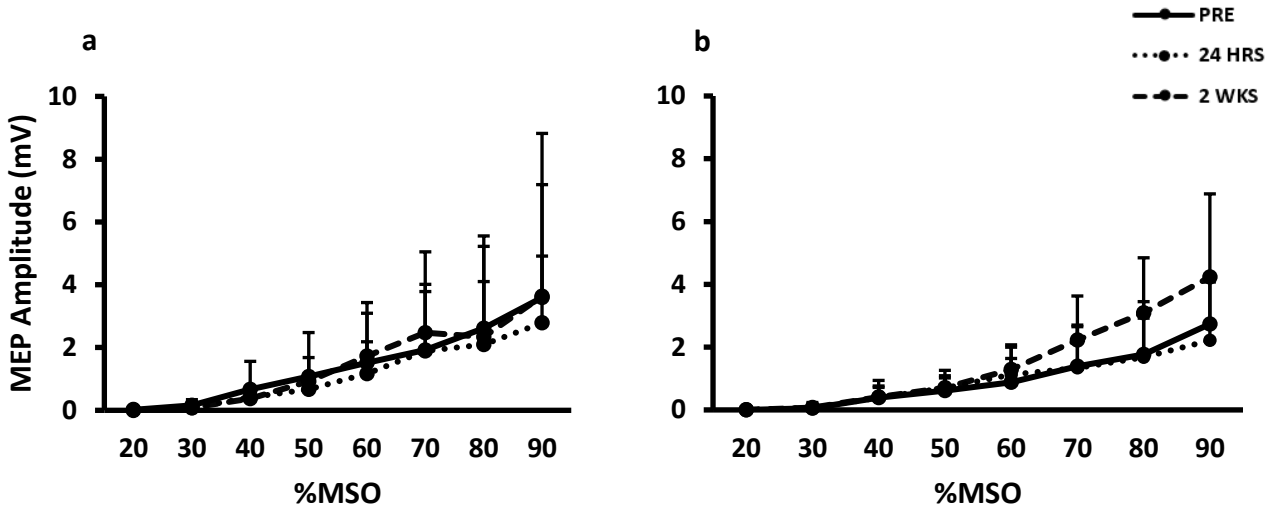

Figure 12. Peak-to-peak motor-evoked potential (MEP) amplitudes from 20-90% of maximum stimulator output (%MSO) using transcranial magnetic stimulation before (PRE), 24 hours after (24 HRS), and 2 weeks after (2 WKS) nondominant upper arm immobilization. Values are mean ± SD. a, non-immobilized arm; b, immobilized arm.

3.3.5c Slope

Changes in SR curve slope throughout the study are displayed in **Figure 13**. Mean baseline slope of the TMS SR curve was 12.0±4.1 and 9.3±3.9 in the non-immobilized and immobilized arms, respectively, with no significant difference between arms. There was a main effect of time such that slope at 2 weeks post-immobilization was significantly lower than at baseline ($P = 0.006$). It

was noted however that the mean difference in slope between arms at baseline was numerically identical to that between pre- and 2 weeks post-immobilization in the non-immobilized arm. Since the change in slope in the immobilized arm was ~3.5 times less than the mean baseline difference in slope between arms, pairwise comparisons of each timepoint stratified by arm were conducted to identify whether the significant main effect of time was driven primarily by the change in non-immobilized arm. Results revealed that there was a significant decrease in mean slope in the non-immobilized arm (12.0±4.1 to 8.2±3.8. P = 0.015), but not the immobilized arm (9.3±3.9 to 8.2±1.9, P = 0.650) after 2 weeks.

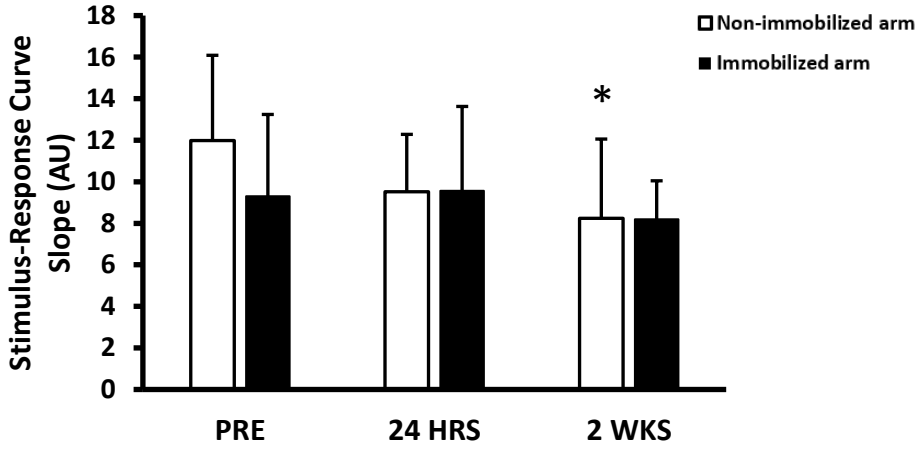

Figure 13. Slope of TMS stimulus-response curves before (PRE), 24 hours after (24 HRS), and 2 weeks after (2 WKS) nondominant upper arm immobilization. Values are mean ± SD. *Significantly different from PRE within respective arm (P < 0.05).

3.3.5d Area under the curve

The relative change in AUC at 24 hours and 2 weeks post-immobilization are displayed in **Figure 14**. The mean relative change in AUC from baseline at 24 hours post-immobilization was -3.2±42.7% and 30.4±70.7% in the non-immobilized and immobilized arm, respectively, with no

significant differences between arms (P = 0.173). Two outliers that skewed the data away from a normal distribution were identified at the 2 weeks post-immobilization timepoint, one showing a 242.8% increase in AUC in the non-immobilized arm, and another with a 645.3% increase in the immobilized arm. Upon removal of these two outliers, the data met the conditions of normality. The mean relative change in AUC from baseline at 2 weeks post-immobilization was -2.7±54.5% and 58.8±90.6% in the non-immobilized and immobilized arm, respectively (n=10). Paired-samples t-test revealed a trend for a greater relative change in the immobilized versus non-immobilized arm (P = 0.083). To account for the removal of data points, we also performed the nonparametric Wilcoxon signed-ranks test on the full sample; using this test, there was no significant difference between the mean relative change in AUC between arms (P = 0.139). The mean relative change from baseline in AUC at 2 weeks post-immobilization was 21.9±86.5% and 119.4±189.1% in the non-immobilized and immobilized arm, respectively (n=12).

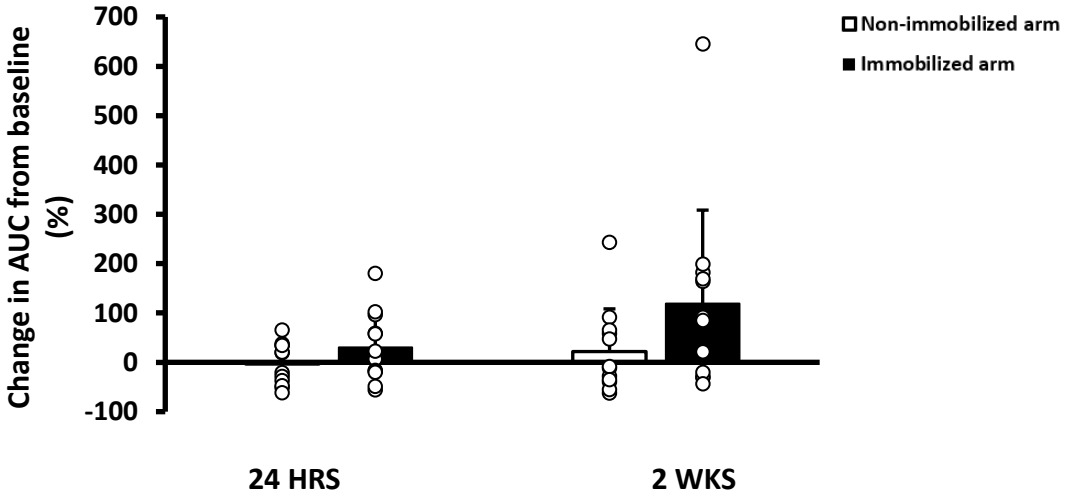

Figure 14. Relative change in area under the TMS stimulus-response curve (AUC) following 24 hours (24 HRS) and 2 weeks (2 WKS) of nondominant upper arm immobilization. Values are mean ± SD. Open circles represent individual subject values.

3.4 DISCUSSION

This study demonstrated that elbow flexor strength loss in response to upper limb immobilization can occur independent of elbow flexor muscle atrophy in young women, and that the nervous system may represent an early site of physiological adaptation to muscle disuse, as changes in corticospinal output were suggested to have occurred following the 2-week immobilization period. To our knowledge, this was also the first study of upper limb immobilization in which current standards of measurement for assessing changes in both muscle strength and muscle size were employed.

The 21.3% (flexion) and 19.9% (extension) decreases in isometric strength, which translate to strength losses of -1.5%/day and -1.4%/day, respectively, are marginally higher compared to other studies of upper arm immobilization using a brace/cast model (elbow flexion: -0.9 to -1.3%/day; extension: -0.6 to -1.3%/day).[152–154] This may be due to the shorter duration of the immobilization in our study, as immobilization-induced adaptations are thought to occur most rapidly during the early disuse period.[15]

Another notable finding in the present study was that immobilization had no significant impact on isokinetic muscle strength. This may have simply been due to the observation that muscle atrophy induced by the intervention was negligible. It may also have been due to the velocity of contraction, as not only is it well known that concentric force decreases with increasing contraction velocity, but isokinetic elbow flexion contraction at 120°/s has specifically been demonstrated to achieve a submaximal level of integrated EMG activity, work, and power relative to maximum.[155] These results are in agreement with another study by MacLennan et al. (2021),[49] in which isokinetic knee extension strength decreased at a contraction velocity of 180°/s, but not 360°/s in young women in response to 2 weeks of knee immobilization. It is therefore possible that the

isokinetic strength protocol used in this study was not challenging enough to detect strength loss in response to the immobilization, thus highlighting that quantifying functional loss in response to muscle disuse depends on the type of evaluation used.

In contrast to our hypothesis, immobilization did not significantly alter elbow flexor muscle size. This aligns with a previous study by Miles et al. (2005),[93] in which 3 weeks of upper arm suspension induced a statistically significant decline in elbow flexor volume in untrained men (-10.6%), but not women (-1.4%). Even in light of this study, we nonetheless hypothesized that immobilization would induce a noticeable degree of elbow flexor atrophy, as the above study did not actually immobilize the elbow joint with a brace or cast and allowed participants to remove the arm sling during sleep. It had also been observed previously that both elbow flexor CSA and volume decreased in response to 4 weeks of upper arm cast immobilization by around 11%, and so we anticipated that relative elbow flexor atrophy to have been at least half this value. However, prior to the current study, changes in muscle size with upper arm immobilization had not previously been evaluated using MRI in a study as short as 2 weeks, let alone in a single-sex sample of women. It is therefore possible that women are indeed less susceptible to muscle atrophy with upper arm muscle disuse, however further evidence on sex-based differences is needed in this regard.

It was additionally found that immobilization induced a statistically significant decrease in elbow extensor CSA and volume, a result that we did not anticipate based on the previous observation that elbow extensor muscle size did not change with 4 weeks of upper arm immobilization.[154] Nevertheless, the relative change from baseline in mean elbow extensor CSA (-2.9%) and volume (-2.5%) in the current study was arguably negligible. In fact, following immobilization in the aforementioned 4-week study, relative changes in mean elbow extensor CSA

and volume were -3.9±1.9% and -1.6±2.8%, respectively.[154] Instead, despite the immobilization period being twice the duration in the previous study, it is possible that we detected a statistically significant effect of immobilization on elbow extensor muscle size in our study due to being sufficiently powered to detect such a difference, owing to both the inclusion of the non-immobilized arm as an internal control and four additional subjects for the analysis of muscle size change.

We wish to highlight that while the effect of the intervention on elbow extensor muscle size was deemed to be statistically significant, we cannot conclude that the change observed in our study was clinically significant. Using the known density of mammalian skeletal muscle mass (1.0597g/cm), it is estimated that our sample lost on average 4.9 grams of muscle tissue based on the absolute change in elbow extensor volume. With a previously determined linear regression equation derived from the relationship between elbow extensor torque and muscle volume, we are able to estimate that the mass lost from the elbow extensor muscle group in our study would correspond to a ~0.6Nm reduction in isometric torque,[156] which falls within the absolute measurement error of the isokinetic dynamometer used in the present study.[124] Furthermore, it is possible that the decrease in elbow extensor muscle size in response to the intervention did not exclusively represent a loss of contractile tissue, but also the movement of water molecules due to osmotic pressure in response to changes in intramuscular substrate concentrations. Though it is not a consistent finding, lower limb immobilization has previously been demonstrated to reduce muscle glycogen content.[157] Given that changes in glycogen content can have a profound impact on water distribution, as 3-4 grams of water is estimated to be bound to each gram of glycogen in human skeletal muscle,[158] it is unlikely that the muscle mass lost measured using MRI is fully attributable to a loss of contractile tissue.

While this study further reinforces the idea that muscle atrophy plays a lesser role in early immobilization-induced strength loss, it is important to consider the possibility that other muscle-specific factors could have contributed to the decline in muscle function. Prior studies have revealed that muscle architecture and tendon properties are altered with disuse, in which factors such as single-fiber thin filament density and tendon stiffness decrease substantially;[55,159,160] these changes would presumably result in a reduction in strength of individual sarcomeres as well as a decrease in angular limb displacement with the same degree of muscle force output (i.e., a reduction in joint torque), respectively. In fact, in contrast to muscle size changes with disuse, the rate of decrease in tendon stiffness has been demonstrated to accelerate with increasing duration of disuse, to the point at which it exceeded the rate of muscle atrophy.[55] Similarly, other studies have revealed that single-fiber muscle contractility declines in response to bed rest, which may be due to a reduction in sarcoplasmic reticulum function and Ca^{2+} content.[29,161] In other words, muscle atrophy in response to disuse is likely accompanied by unrelated structural and functional changes in and around the muscle that reduce its ability to generate force. Future research should therefore consider factors beyond mere muscle size to ascertain the extent to which changes in skeletal muscle structure and function are responsible for disuse-related strength loss.

We did not observe a significant change in VA of the biceps brachii in either arm following immobilization, a finding that was contrary to our hypothesis. We had originally hypothesized that VA would decrease significantly and exclusively in the immobilized limb as a systematic review of limb immobilization studies revealed a very strong (Pearson's correlation coefficient, $r = 0.96$), significant correlation between changes in VA and muscle strength in the upper limb.[15] However, in comparison to other measures of neuromuscular function, studies on the effect of immobilization on VA are limited. To our knowledge, only 4 studies have assessed changes in VA

with immobilization in the upper limb, 3 of which immobilized the wrist joint rather than the elbow as in the present study.[50,51,141,162] Therefore, we may have not observed a significant effect of immobilization on VA of the biceps brachii as corticospinal tract output from the primary motor cortex (M1) is proportionally less toward proximal relative to distal muscles.[54] Furthermore, our failure to observe a change in VA in response to immobilization may have been due to methodological differences. Studies demonstrating a decrease in wrist flexor VA with immobilization assessed VA using cortical as opposed to peripheral stimulation.[50,51,162] Though peripheral methods of measuring VA are nonetheless appropriate for detecting a deficit in muscle activation at the level of the central nervous system,[48] our method may have not been specific enough to observe a decline in VA due to the potential maintenance of functional integrity within systems downstream relative to the cortex. Indeed, VA did not change in response to unilateral arm suspension using peripheral stimulation;[141] however, it is important to note that this study did not detect a significant change in elbow flexor strength following the intervention. Further research should aim to elucidate whether the neural mechanisms underpinning disuse-related strength loss are predominantly central or peripheral, assuming that these systems are affected disproportionally by muscle disuse.

The observation that immobilization did not alter RMT largely agrees with the literature.[86,88,162,163] Prior studies on the short-term effects of upper limb immobilization have observed a decrease in corticospinal excitability.[26,83,164] In our investigation however, we did not observe a change in corticospinal excitability at 24 hours post-immobilization. This may be due to heterogenous methodology between studies, as those prior demonstrating a decrease in excitability immobilized and/or tested muscles controlling the dominant wrist and hand rather than the nondominant elbow. Furthermore, excitability was quantified either as MEP amplitude or RMT in

prior studies, without consideration of specific TMS SR curve properties nor the use of Neuronavigation software for precise pulse administration as in the present study. The significant decrease and trend ($P = 0.083$) for an increase in corticospinal excitability in the non-immobilized and immobilized arms, respectively was a novel finding in the present study. To our knowledge, a decrease in excitability in the non-immobilized arm has not been observed before in this context, however this may be due to a lack of studies employing a unilateral, within-subject design in which both the immobilized and non-immobilized sides are evaluated. On the other hand, the concept that muscle disuse due to immobilization elicits opposing neuroplastic changes between immobilized and non-immobilized sides is not unfamiliar. For instance, patients who underwent immobilization of their dominant upper arm in response to injury displayed a decrease in motor cortical thickness of the hemisphere contralateral to the affected limb, which was accompanied by an increase in motor cortical thickness of the opposite hemisphere.[35] Similarly, 72 hours of dominant hand immobilization has been shown to result in an increase in cortical activation during performance of a finger-tapping task with the non-immobilized hand, but a decrease in activation when the same task was performed with the immobilized hand.[165] In fact, it has previously been reported that 10 hours of dominant arm suspension reduced corticospinal excitability of the M1 contralateral to the immobilized side while increasing excitability of the corresponding brain region in the opposite hemisphere for as long as the non-restricted arm was used adequately during the intervention,[164] a finding that was opposite to ours. Overall, prior research on the effects of limb immobilization on corticospinal excitability have yielded heterogenous outcomes. The observation of a trend for an increase in excitability in the immobilized arm in the present study is in congruence with others in which a significant increase in excitability was reported[51,85,86,88] but stands against several other studies in which excitability decreased[83,163,164] or did not change.[73,166]

It could be possible that the time course of corticospinal plasticity is fundamentally different between different muscle groups, as none of the abovementioned studies that contradict ours recorded MEPs from the biceps brachii. Furthermore, many studies of upper limb immobilization on neuromuscular function, particularly those in which the period of the immobilization was short, immobilized the dominant limb. It has previously been suggested that immobilizing the dominant hand has a greater impact on neuroplasticity compared to the nondominant hand,[167] and so the less burdensome nature of our immobilization model may explain our failure to detect early changes in excitability. Nevertheless, our study demonstrated that immobilization of the nondominant arm was sufficient to elicit changes in excitability after 2 weeks. The current study also suggests that the divergent changes in excitability between arms may have occurred through different mechanisms, as adaptations in the non-immobilized and immobilized arms were implied by changes in slope and AUC, respectively. Specifically, the slope of the TMS SR curve is suggested to be a surrogate measure of the gain or sensitivity of descending pyramidal tract neurons,[168] whereas AUC is a reflection of overall corticospinal output strength and is positively correlated with maximum MEP amplitude.[169] Several plausible explanations have been proposed to explain hyperexcitability of the M1 in response to immobilization. First, the deprivation of sensory feedback has been purported as a potential driver for increased corticospinal output as a compensatory mechanism,[86] however this appears to be more relevant in the context of fine motor sensory feedback, such as with hand immobilization.[37] Changes in muscle innervation may also drive corticospinal adaptations. Bed rest has been demonstrated to increase the proportion of muscle fibers bound to neural cell adhesion molecule (NCAM), which accumulates around mature muscle fibers that are axon deficient.[29] A reduction in innervation of disused muscles may partially explain an increased output from higher control centers such as the M1, however this can only be

speculated. Another hypothesis is that the mere restoration of autonomy over the immobilized limb induces a transient increase in excitability from M1 to the disused musculature as the subject begins to acclimate themselves to their environment and 'relearn' to incorporate the use of both limbs into their daily living.[88] Motor skill acquisition has previously been shown to be accompanied by increases in excitability within as little as 30 minutes.[170] It has further been revealed that 10 days of lower limb immobilization induced a delayed increase in corticospinal excitability 24 hours following cast removal, which returned to baseline levels the following the day.[88] Therefore, it is possible that the sudden barrage of sensory input upon removal of the brace prior to measurement was responsible for a momentary imbalance in corticospinal output designed to facilitate the recovery of sensorimotor control of the immobilized limb. This effect may have been more pronounced in our study due to the fact that brace removal was necessary prior to the MRI measurements of muscle size that took place before TMS measures; however, arm use was restricted while participants underwent MRI. Future research should aim to confirm whether shifts in interhemispheric balance occur in response unilateral limb disuse, with a particular interest in the time course of the possible neurophysiological adaptations during and following the intervention.

In summary, these findings suggest that upper limb immobilization can incur rapid, substantial losses of isometric muscle strength without significant muscle atrophy, while also provoking imbalances in corticospinal output in women. This highlights the importance of considering sites of adaptation apart from the muscle itself such as the nervous system in the attempt to understand the physiological mechanisms underlying disuse-related muscle weakness.

4 OVERALL CONCLUSION AND SUMMARY

Advancing our understanding of the physiological changes that occur during muscle disuse is an important topic in human health research, particularly for individuals who are vulnerable to such periods such as athletes, older adults, and several clinical populations. Muscle strength is arguably the most meaningful clinical outcome in the context of muscle disuse, as it is a non-invasive, easy to measure analog of overall change in functional capacity and is unequivocally linked to the muscle's ability to carry out activities of daily living. Given the longstanding relationship between muscle size and strength, it is interesting to observe that muscle strength loss is disproportionately greater than muscle atrophy and that there is apparently no significant relationship between the change in muscle strength and size during limb immobilization.[15] As it became quite well known that individuals naïve to resistance exercise training can experience rapid increases in strength and performance that occur independent of muscle hypertrophy, researchers lately have highlighted the importance of the nervous system in the determination of muscle function and adaptation.[148] On the other hand, while the role of the nervous system has certainly been investigated in studies of muscle disuse, it is far easier for humans to lose than to gain skeletal muscle mass, and so muscle atrophy is nonetheless an important consideration in this area. It is also thought that limb immobilization specifically induces rapid reductions in muscle size in comparison to other disuse models, as it restricts movement around the joint to substantially reduce the frequency of voluntary muscle contractions.[10]

As a result, our objective was to evaluate changes in muscle strength, muscle size, and various measures of neuromuscular function following a 2-week period of upper limb immobilization in young women. To our surprise, we found not only substantial declines in muscle strength that occurred independent of significant muscle atrophy in response to the intervention, but also the

suggestion of an imbalance in corticospinal excitability between limbs, and that this imbalance was realized through different neural mechanisms. Nevertheless, we encourage readers to interpret these findings with caution, as although we observed a statistically significant effect of the intervention on SR curve slope in the non-immobilized arm, there was no interaction effect despite the change only being significant in the non-immobilized arm. Furthermore, we observed only a trend for a significant effect on AUC in the immobilized arm despite the relative change from baseline being over twenty-five times greater in magnitude in the immobilized arm; and this finding was not reproduced when a nonparametric test was performed on the full dataset to accommodate for non-normality in this data.

4.1 Limitations and future directions

This study benefited from the use of criterion measures to assess changes in muscle strength and size, as well as Neuronavigation software for the precise administration of TMS. Nonetheless, this study is not without limitations. First, neurophysiological measures were acquired from the biceps brachii only. This was in consideration for the time-sensitive nature of the possible changes that could occur in response to the immobilization. It has previously been noted that changes in MEP amplitude in response to short-term cast immobilization began to normalize within 1 hour following cast removal and returned to baseline levels after 3 hours.[83] Should we have acquired neurophysiological measures from the triceps in addition to the biceps brachii, it would have been possible that participants spend a total of either more or less than 3 hours without the brace upon completion of the post-immobilization TMS assessment. Secondly, although the study was adequately powered to detect meaningful changes in isometric muscle strength, it was conducted on a relatively small and specific sample. Therefore, we were not in a position to determine the relative associations between measures of muscle size and neuromuscular function and the

immobilization-induced decline in strength. Finally, although we highlight that changes in the corticospinal tract may represent an early physiological adaptation to upper limb immobilization that precedes muscle atrophy, we are unable to pinpoint where specifically within the nervous system these changes occurred. As we did not measure maximal M-wave amplitude in our subjects via peripheral stimulation, changes in excitability may be representative of alterations in peripheral excitability. We nonetheless revealed that changes in corticospinal output in response to unilateral limb immobilization may be mediated through different mechanisms. Future investigations should aim to advance our knowledge of the precise neural mechanisms that either explain or accompany disuse-related muscle weakness. We recommend the use of electrophysiological techniques that can estimate motor unit recruitment and firing rate such as fire wire electromyography as well as the inclusion of additional TMS measures such as intracortical facilitation and inhibition.

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6. SUPPLEMENTARY MATERIAL

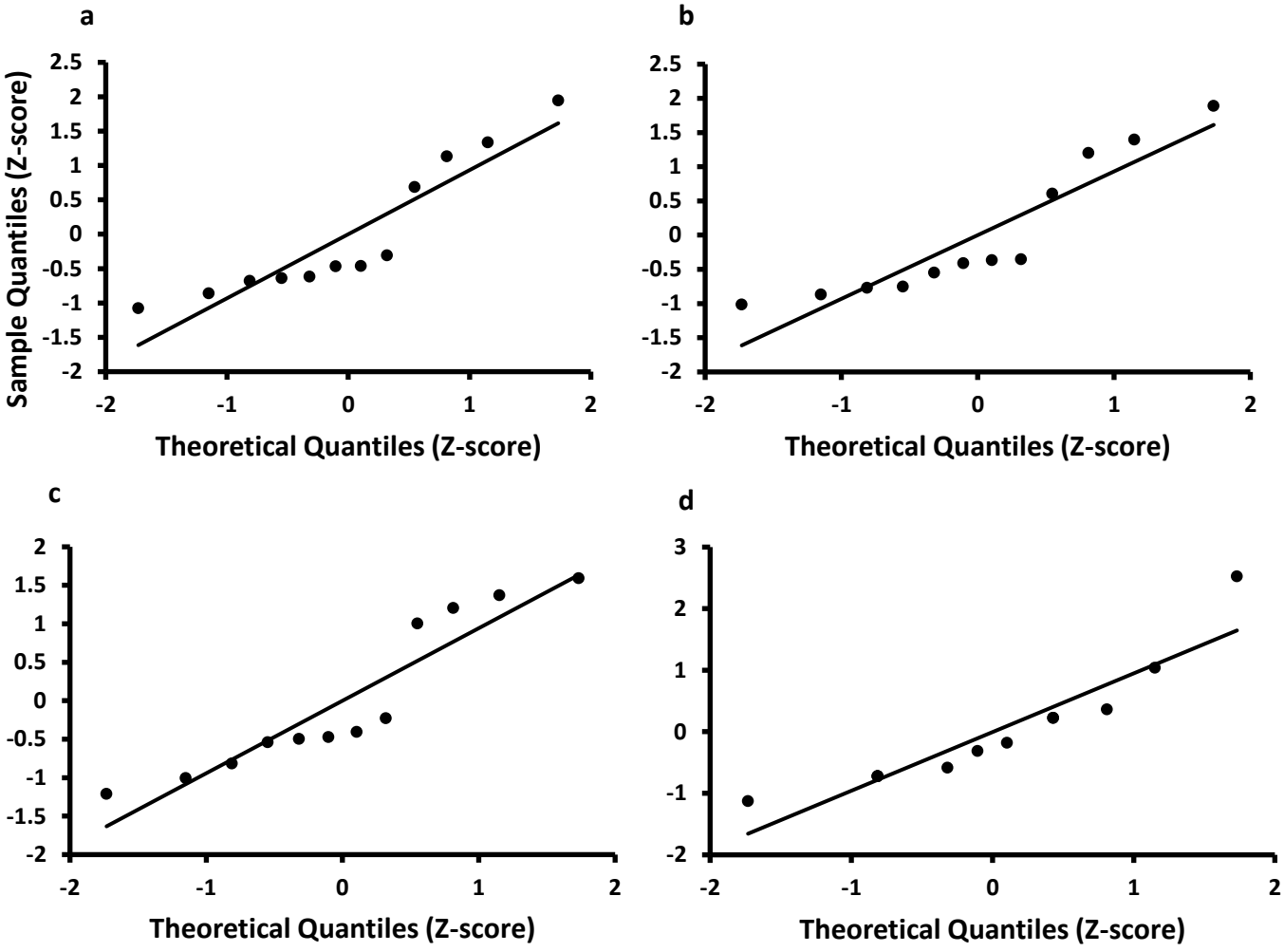

Figure S1. Q-Q plots indicating the degree of univariate normality for elbow flexor cross-sectional area in the immobilized arm before (a) and after (b) immobilization; elbow flexor cross-sectional area in the non-immobilized arm after immobilization (c); and resting motor threshold in the non-immobilized arm before immobilization (d).