CORTICOSPINAL ADAPTATIONS TO STRENGTH TRAINING

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Johdanto. Lihasvoimaharjoittelun aikaan saamat kortikospinaaliset adaptaatiot ovat harjoittelu spesifejä ja ovat yhteydessä maksimaalisen voimantuoton kasvuun. Kortikospinaalisten adaptaatioiden on havaittu tapahtuvan niin supraspinaalisella, että spinaalisella tasolla. Supraspinaalisia adaptaatioita on tutkittu paljon, mutta tutkimustulokset ovat ristiriitaisia. Spinaalisen tason motoneuroni altaaseen keskittyviä tutkimuksia on puolestaan niukasti. Lisäksi vain muutama tutkimus on keskittynyt tutkimaan harjoittelun jälkeisiä muutoksia harjoittelemattomuusjakson aikana. Tämän takia tutkimuksen ensisijaisena tavoitteena oli tutkia lihasvoimaharjoittelun aikaan saamia sekä harjoittelemattomuusjakson jälkeisiä kortikospinaalisia adaptaatioita. Tarkennettuna tavoitteena oli selvittää kortikospinaalisen radan supraspinaalisen ja spinaalisen tason muutoksia, sekä arvioida kummalla tasolla tapahtuu merkittävämpiä muutoksia voimaharjoittelun ja harjoittelemattomuusjakson seurauksena.

Menetelmät. Tutkimuksen populaatio koostui yhdestätoista vapaaehtoisesta tutkittavasta. Kaikki tutkittavat toteuttivat seitsemän viikon lihasvoimaharjoittelujakson ja viiden viikon harjoittelemattomuusjakson. Kortikospinaalisten adaptaatioiden tutkimiseen käytetyt menetelmät sisälsivät rectus femoris lihaksen transkraniaalisen magneettistimulaation (TMS), lannerangan sähköstimulaation (LS) ja maksimaalisen M-aallon (M-max). Lisäksi voiman kehitystä mitattiin oikean polven isometrisellä maksimaalisella tahdonalaisella supistuksella (MVC) ja polvien ojennuksien yhden toiston maksimitestillä (1RM). TMS:ta ja lannerangan sähköistä stimulaatiota käytettiin herätys potentiaalien aikaan saamiseen. Herätys potentiaalit analysoitiin laskemalla huipusta huippuun amplitudit, normalisoimalla herätys potentiaalit M-max amplitudiin ja keskiarvoistamalla kymmenen yksittäisen herätys potentiaalin amplitudit. Mittauspisteet olivat ennen harjoittelua, heti harjoittelujakson jälkeen ja harjoittelemattomuusjakson jälkeen.

Tulokset. Tutkimuksessa havaittiin seitsemän viikon voimaharjoittelujakson jälkeen tilastollisesti merkittävä voiman kasvu (14%, 19kg ± 5kg), polvien ojennuksen yhden toiston maksimitestissä ($F = 51{,}113$, $p < 0.05$). Lisäksi harjoittelun jälkeen havaittiin merkittävä kasvu (60%) spinaalisella tasolla herätys potentiaalien amplitudeissa ($F = 11{,}073$, $p < 0.05$). Yhden toiston maksimitesti osoitti myös merkittävää laskua (4%, 5kg ± 1kg) kohti lähtötasoa harjoittelemattomuusjakson jälkeen ($p<0.05$). Missään muissa parametrissa ei havaittu tilastollisesti merkitseviä eroja.

Johtopäätös. Johtopäätöksenä seitsemän viikon voimaharjoittelujakso lisäsi polvien ojennuksen yhden toiston maksimikuormaa, jonka selittävänä tekijänä voi olla spinaalisen tason adaptaatiota. Tarkennettuna, spinaalisen tason adaptaatiot voivat johtua spinaalisten refleksien ja motoneuronialtaan herkkyyden kasvusta. Lisäksi havaittiin, että viiden viikon harjoittelemattomuusjakso on riittävä vähentämään polvien ojennuksen yhden toiston maksimikuormaa kohti lähtötasoa. Tämä on ensimmäinen tutkimus, joka on löytänyt lihasvoimaharjoittelun aiheuttavan merkittävän kasvun lannerangan herätyspotentiaaleissa.

Asiasanat: voimaharjoittelu, kortikospinaalinen adaptaatio, transkraniaalinen magneettistimulaatio, lannerangen sähköstimulaatio, yhden toiston maksimitesti

ABSTRACT

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Introduction. The corticospinal adaptations to resistance training are based on use-dependent plasticity, which are rapid and are associated with increase in maximal force production and strength development. In addition, these changes happen without detectable changes in hypertrophy. The corticospinal adaptations to strength training have been found to happen in the supraspinal and spinal level. However, there are still conflicting results on the corticospinal adaptations. In addition, there are only limited number of studies that have investigated the corticospinal adaptations at the level of spinal motor neuron pool and the adaptations to detraining. As such the primary aim of the study was to investigate the corticospinal adaptations to strength training and detraining and to evaluate, whether the supraspinal or spinal level adaptations are more prominent.

Methods. The study consisted of eleven volunteer participants, who completed a seven-week strength training and five-week detraining period. The methods used to study corticospinal adaptations included transcranial magnetic stimulation (TMS), electrical stimulation of the lumbar spine (LS), and maximal M-wave (M-max) of the rectus femoris muscle. Additionally, strength development was measured by isometric maximal voluntary contraction (MVC) of the right knee and one-repetition maximum (1RM) of the bilateral knee extension. The TMS and electrical stimulation of the LS were used to induce evoked potentials. The evoked potentials peak-to-peak amplitudes were analyzed, normalized to the amplitude of the M-max, and averaged across ten single stimuli. Measurement time points were before training, immediately at post-training, and after detraining.

Results. The study found a significant increment in strength as in one-repetition maximum of bilateral knee extension of 14% (19kg ± 5kg) ($F = 51.113$, $p<0.05$) and a significant increment in the evoked potentials at the spinal level of 60% ($F = 11.073$, $p<0.05$) after 7-week strength training. The one-repetition maximum also showed a significant decrease toward back to baseline after detraining period of -4%, 5kg ± 1kg ($p<0.05$). No significant differences were found in any of the other parameters.

Conclusion. In conclusion, seven-week strength training induced an increase in the 1RM of the bilateral knee extension, which could be due to the increment in the evoked potentials at the spinal level. More specifically, the spinal level adaptations could be due to an increase in spinal reflexes and or motor neuron pool excitability. The study also found that five-week detraining period is sufficient to decrease the one-repetition maximum towards back to baseline. This study is to my knowledge the first study that has found a corticospinal adaptation at the spinal level in the form of increased LEPs, after strength training.

Key words: strength training, corticospinal adaptation, transcranial magnetic stimulation, electrical stimulation of the lumbar spine, one-repetition maximum, neurophysiology

ABBREVIATIONS

aMT	active motor threshold
EMG	Electromyography
LEP	lumbar evoked potential
LS	lumbar spine
MEP	motor evoked potential
M-max	maximal compound wave
MVC	maximal voluntary contraction
TMS	transcranial magnetic stimulation
SP	silent period
1RM	one-repetition maximum

CONTENTS

1 INTRODUCTION

Resistance training is the most effective way to develop strength and increase muscle hypertrophy (Krzysztofik et al. 2019). Siddique et al. (2020) states that strength training induces strength gains, but the strength gains are at least partly task specific. According to Hart & Buck (2019) these adaptations are associated with improved quality of life, life expectancy (Kraschnewski et al. 2016) and better sport performance (Otero-Esquina et al. 2017). Usually, resistance training with high loads (>80-85% 1RM), with a low number of repetitions (1-5), and long rest intervals (3-5min) causes higher mechanical loading and greater neural adaptations accompanied by a greater increase in strength and maximal force production (ACSM 2009; Jenkins et al. 2017).

According to Tallent et al. (2021) neuromuscular adaptations to resistance training are based on use-dependent plasticity. Also, Nuzzo et al. (2017), Mason et al. (2019) Siddique et al. (2020) & Tallent et al. (2021) states that neuromuscular adaptations to strength training are rapid and are associated with increase in maximal force production and strength development. According to Naciri et al. (1989) and Nuzzo et al. (2017) these changes happen without detectable changes in hypertrophy. These adaptations are prominent in the corticospinal tract at the supraspinal and spinal level. These adaptations lead for example to decrease in antagonist activation, changes in motoneuron excitabilities, and changes in motor unit recruitment (Mason et al. 2019; Leung et al. 2017; Kidgell et al. 2010). Even though there are some conflicting results about the adaptations (Mason et al. 2019; Leung et al. 2010; Ruotsalainen et al. 2013; Kidgell et al. 2010).

There are still conflicting results on the corticospinal adaptations. In addition, there are only limited number of studies that have investigated the corticospinal adaptations at the level of spinal motor neuron pool and the adaptations to detraining. As such the primary aim of the study was to investigate the corticospinal adaptations to strength training and detraining and to evaluate, whether the supraspinal or spinal level adaptations are more prominent. Additionally, the secondary aim of this study was to find out if these supraspinal and spinal level adaptations would correlate with changes in strength (1RM or isometric MVC).

2 NEUROMUSCULAR ADAPTATIONS TO STRENGTH TRAINING AND DETRAINING

The improvement in maximal strength has been found to be based mainly on neuromuscular adaptations in the early stages of strength training and only in the later stages on hypertrophic adaptations (Folland & Williams 2007; Naciri et al. 1989). Nuzzo et al. (2017), Siddique et al (2020) and Tallent et al. (2021) also state that neuromuscular adaptations to strength training are rapid and are associated with an increase in maximal force production and strength development. Strength training seems to affect the central nervous system from the very first session and the changes continue with each session (Dayan & Cohen 2011) as use-dependent manner (Tallent et al. 2021). On the other hand, significant hypertrophy has been reported to be observed usually after two months of training in untrained individuals strength training (Folland & Williams 2007).

The majority of the neuromuscular adaptations induced by a single and repeated bouts of strength training appear to occur in the motor cortex and corticospinal pathway (Carroll et al. 2009; Duchateau et al. 2006; Mason et al. 2020). The corticospinal adaptations can happen at the level of motor cortex, at the corticospinal tract and at the spinal level (Aagaard et al. 2002; Ansdell et al. 2020; Bestmann & Krakauer 2015; Del Balso & Cafarelli 2007; Kidgell et al. 2010; Kidgell et al. 2011; Mason et al. 2020; Tallent et al. 2017). The corticospinal adaptations induced by strength training are both activation and inhibition related. As strength training has been shown to induce changes in the coordination of muscle activation, by antagonistically reducing muscle coactivation and synergistically improving muscle activation during performance. (Häkkinen 2000; Mason et al. 2020) As well as improve the recruitment and activation of motor units (Christie & Kamen 2014; Farina & Holobar 2016). Improvements in recruitment and activation occur through an increase in the frequency of motor unit firing and synchronization, and a decrease in the threshold of motor unit arousal (Christie & Kamen 2014; Farina & Holobar 2016).

2.1 Corticospinal adaptations to strength training

Strength training has been shown to improve corticospinal tract excitability at the level of motor cortex and spinal cord (Beck et al. 2007; Christie & Kamen 2014; Griffin & Cafarelli 2007; Mason et al. 2020; Kidgell et al. 2010; Kidgell et al. 2011; Tallent et al. 2017). The

improvement in corticospinal excitability are, but not limited to enhanced synaptic function, synchronization, recruitment, and enhanced short- and long-term potentiation (Christie & Kamen 2014; Griffin & Cafarelli 2007; Kidgell at al. 2010).

One way to measure corticospinal pathway excitability is through use of transcranial magnetic stimulation which can be used to induce motor evoked potentials (MEPs) in the target muscle. These induced MEPs can be detected by surface EMG located in the target muscle (Bestmann & Krakauer 2015; Kidgell et al. 2010) and an increase in MEP amplitude can be inferred as an improvement in neural drive of the corticospinal tract to the target muscle (Bestmann & Krakauer 2015; Griffin & Cafarelli 2007; Kidgell et al. 2010). A number of studies have shown that strength training seems to increase corticospinal excitability and as a result increase the amplitude of MEPs in the target muscle (Griffin & Cafarelli 2007; Kidgell et al. 2010; Kidgell et al. 2011; Tallent et al. 2017). These improvements in the neural drive to the target muscle can be seen after two to four weeks of strength training and are accompanied by increase in maximal voluntary contraction (Griffin & Cafarelli 2007; Kidgell et al. 2010; Kidgell et al. 2011; Mason et al. 2020; Tallent et al. 2017). However, these improvements seem to be found most consistently only, when measured during voluntary contraction (Siddique et al. 2020).

However, not all studies have found these improvements, like Latella et al. (2012) and Carrol et al. (2002) which reported that maximal strength increased with strength training, but no significant changes in corticospinal excitability were observed. Similarly, in a study by Kidgell and Pearce (2010) found no differences in the amplitudes of MEPs after four weeks of strength training.

In addition, it has been shown that strength training also induces changes at the spinal level (Aagaard et al. 2002; Del Balso & Cafarelli 2007). These changes have been measured in previous studies by Hoffman's reflex (H-reflex) and volitional wave (V-wave). The amplitude of the H-reflex and V-wave has been shown to increase after strength training. However, both of these adaptations more or less reflect the monosynaptic adaptations at the spinal cord level. (Aagaard et al. 2002; Del Balso & Cafarelli 2007) Additionally, Ansdell et al. (2020) have shown that the strength training causes acute effects in the spinal cord, which include increment in the amplitude of lumbar evoked potentials (LEPs) after strength training. The LEPs reflects the overall spinal motor neuron pool excitability to the target muscle (Ansdell et al. 2020).

Studies have shown that strength training also decreases the corticospinal inhibition (Latella et al. 2012; Leung et al. 2017; Mason et al. 2020; Pearce 2010; Weier et al. 2012). Strength training seems to induce changes in inhibition at both the spinal and cortical levels, which could be partly related to GABA-mediated inhibition processes (Kidgell & Pearce 2010). A decrease in inhibition has been shown to be related to the movement regulation and motor performance through the centralization of neural control from M1 and the increased of excitatory drive from corticospinal neurons to the muscles (Kidgell & Pearce 2010; Latella et al. 2012; Perez & Cohen 2008).

Reductions in corticospinal inhibition (reduction in silent period duration) have been found to be associated with increases in maximal voluntary contractions (MVC) in a number of studies (Christie & Kamen 2014; Kidgell & Pearce 2010; Latella et al. 2012). Additionally, the Kidgell and Pearce (2010) found only reduction silent period duration, but not in the amplitude of MEPs. Thus, according to the research of Kidgell and Pearce (2010) it seems that reduction in silent period duration could happen without changes in corticospinal excitability. Similar to corticospinal excitability, changes in corticospinal inhibition may be seen even after only two weeks of strength training (Mason et al. 2020). However, not all studies have found reduction in the silent period duration after strength training (Kidgell et al. 2011).

2.2 Corticospinal adaptations to detraining

Detraining refers to a period when a person reduces or stops their regular exercise or strength training routine. The detraining period without sufficient training stimulus can cause a reduction in the maximal force (Bosquet et al. 2013; Häkkinen et al. 2000). According to (Mujika & Padilla 2012) the maximal voluntary contraction decreases even after 2-weeks without sufficient training of the muscle. This reduction in the maximal voluntary contraction seems to be accompanied by decrease in the EMG (Andersen et al. 2005; Häkkinen et al. 2000). In addition, Tallent et al. (2017) found a decrease in corticospinal excitability measured as amplitude of MEPs, after 2-weeks of the detraining period. Additionally, Yamanaka et al. (1999) found a decrease in the H-reflex after 20 days of bed rest, but not in the amplitude of MEPs. As Yamaka et al. (1999) study used a complete bed rest, these findings should be carefully implemented when discussing the effects of detraining. Ultimately as there are only a few studies published, which have investigated the effects of detraining on neuromuscular

adaptations, these studies should be conducted more to make firm conclusions about the effects of detraining.

3 METHODS TO STUDY NEUROMUSCULAR ADAPTATIONS TO STRENGTH TRAINING

Neuromuscular adaptations to strength training have been mostly studied at the corticospinal tract in the supraspinal and spinal level (Aagaard et al. 2002; Bestmann & Krakauer 2015; Del Balso & Cafarelli 2007; Kidgell et al. 2010; Kidgell et al. 2011; Mason et al. 2020; Tallent et al. 2017). Supraspinal adaptations have been mostly studied by the transcranial magnetic stimulation (TMS) (Kidgell et al. 2010; Kidgell et al. 2011; Mason et al. 2020; Tallent et al. 2017). While the spinal level adaptations have been mostly studied by the Hoffman's reflex (H-reflex) and volitional wave (V-wave). However, both of the spinal level methods to study adaptations have been more or less reflecting the monosynaptic adaptations at the spinal cord level. (Aagaard et al. 2002; Del Balso & Cafarelli 2007) Additionally, in recent years new research method has been developed to study the spinal level motor neuron pool excitability, which consist of stimulating the spinal motor neuron pool (Ansdell et al. 2020).

3.1 Transcranial magnetic stimulation

Transcranial magnetic stimulation (TMS) is a non-invasive brain stimulation procedure that is based on electromagnetic induction and can be used to stimulate the corticospinal tract, the motor output from the brain. TMS can be used for research purposes to measure activity and function of specific brain circuits and connections within the central nervous system. (McKinley et al. 2012) However, transcranial magnetic stimulation faces the problem of delivering stimulation across the high resistance barriers, like scalp, skull, meninges and cerebrospinal fluid (Rossini et al. 2015).

Transcranial magnetic stimulation generates suprathreshold current in the brain by exploiting electromagnetic induction which is based on Faraday's law of electromagnetic induction (Rossini et al. 2015). According to Hallett (2007) magnetic field is produced by lines of current fluxes passing perpendicularly to the plane of the TMS coil and the electrical field is induced perpendicularly to the magnetic field, figure 1. In a homogeneous medium, the spatial changes of electrical field cause current to flow in loops parallel to the plane of TMS coil. This means that the loops with the strongest current are near the circumference of the coil itself and becomes weak near the center of the coil. (Hallett 2007) Although Yang et al. (2006) has shown that the exact current distributions can only be predicted by modelling because the human brain is not

homogeneous, and the induced currents and their paths are distorted by regional differences in tissue conductivity.

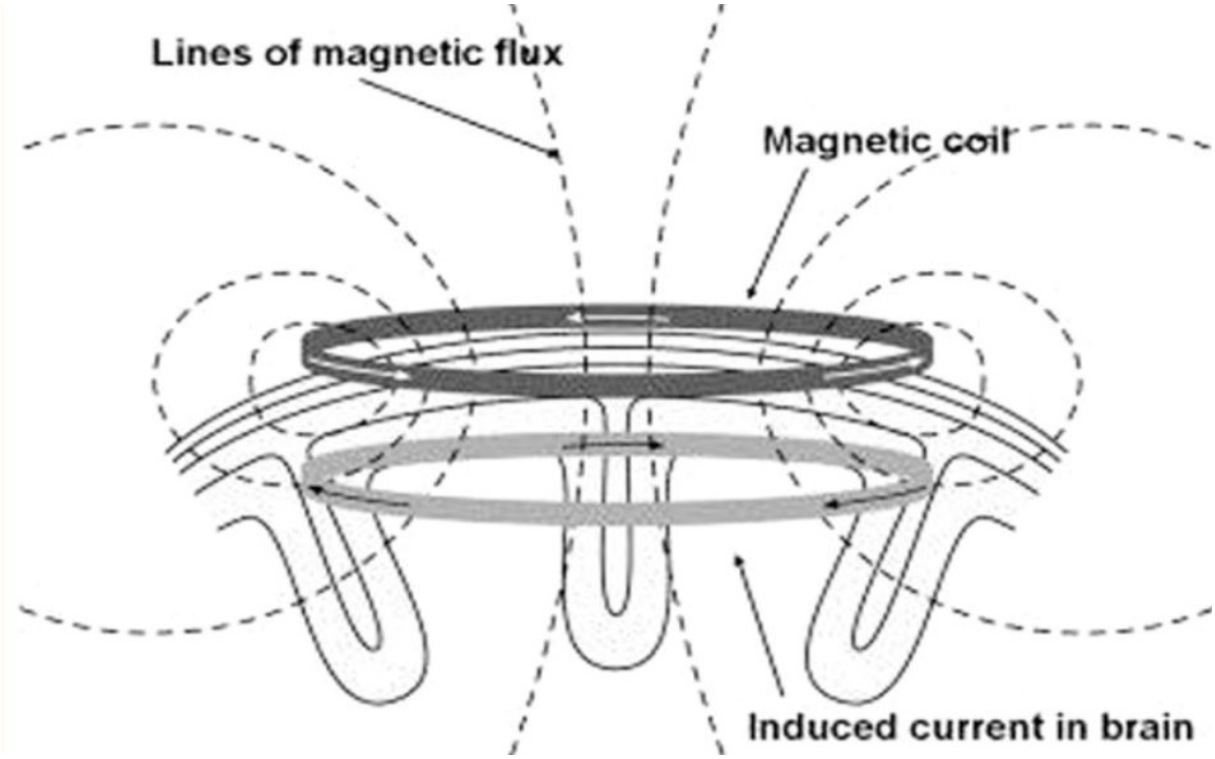

FIGURE 1. Direction of current flows in magnetic coil and the induced current in the brain (Hallett 2007)

TMS operates through a magnetic coil positioned at the head of the subject. Coil designs differ from each other in shape and size and the magnetic field they are producing, depending on the purpose of use. (Hallett 2007; McKinley et al. 2012) Figure-of-eight-shaped coil produce focal stimulation, and the maximal current is produced at the intersection of the two coils (Hallett 2007; Rossi et al. 2009). To produce even more power at the intersection of the coils, the cone-shaped coil has been created, which is figure-of-eight-shaped coil with the two components at an angle. The cone-shaped coil fits perfectly for the stimulation of human leg muscles as it produces more powerful stimulation at the intersection of the coils, as shown in figure 2. (Hallett 2007)

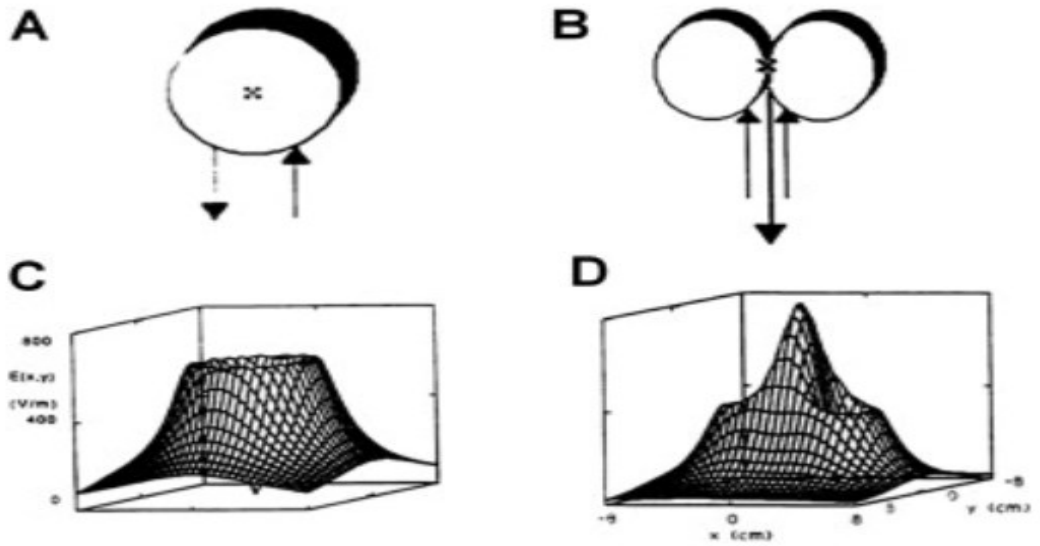

FIGURE 2. Pattern of electrical fields is determined by magnetic coil shapes (Hallett 2007)

Orientation of the TMS coils should be based on targeted cortex area and sulcus, so that the coil location is perpendicular to the sulcus. As this can affect the recruitment order of pyramidal neurons, figure 3 shows the usual orientation of the coil when targeting the leg muscles. (Groppa et al. 2012; Rossini et al. 2015; Richter et al. 2018)

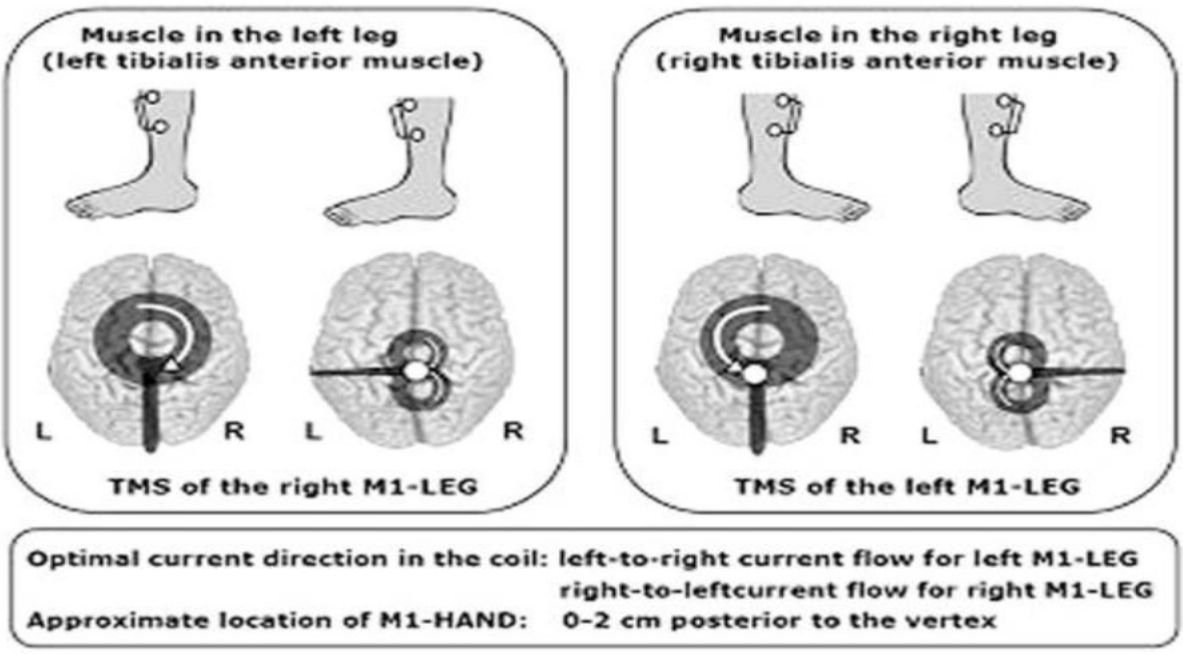

FIGURE 3. Optimal orientation of the coil and current direction for stimulating TA muscle (Groppa et al. 2012)

Groppa et al. (2012) has shown that the optimal orientation of the coil and current direction produces the greatest motor evoked potential (MEP). However, these differences are less evident at higher stimulation intensities. However, because of individual variability in neuroanatomy and tissue anisotropy same coil orientation and current direction produces different responses across subjects. (Rossini et al. 2015) Additionally, as shown by the work of Kesar et al. (2018) it is important to understand that the TMS stimulates not only the cortex area intended, but also the cortex areas close to it.

Identification of specific muscle representation area in primary motor cortex is usually performed by using specific procedure, called hotspotting. Defining hotspot is crucial, because it allows researcher to find optimal location for TMS stimulations that evokes maximal MEP responses. (Sivaramakrishnan et al. 2016) According to Rossini et al. (2015) muscle representation hotspot can be determined by evoking TMS stimulations, while simultaneously gradually moving the coil over the assumed muscle representation area, until the largest MEP response will be found. The location, angle and tilt of the coil should be kept consistent as possible across measurements and sessions. (Rossini et al. 2015)

The number of stimuli should be determined carefully, because of the random jitter of MEP responses across TMS stimulations. Because the random jitter of MEP responses could especially affect the reliability of the TMS results. (Cavaleri et al. 2017; Rossini et al. 2015) According to Rossini et al. (2015) 5-6 stimuli per protocol should be usually enough for TMS for reliable results. However, Cavaleri et al. (2017) suggest at least 10 stimulations per protocol for more reliable TMS responses if stimulations are given in multiple sessions. He also, suggest that even more stimulations could be needed when stimulating lower limb musculature. Chang et al. (2016) even suggests higher number of stimulations per protocol than Cavaleri et al. (2017), as they suggest minimum of 20 pulses for reliable measurements.

3.1.1 Motor threshold

According to Groppa et al. (2012) motor threshold reflects the integrated excitability of corticomotor projections to the target muscle, including the excitability of spinal level motor neurons. Motor threshold can be defined as lowest TMS intensity to produce an MEP response in the target muscle with over 50μV peak-to-peak MEP amplitude (Rossini et al. 2015). Motor threshold for different muscles representations in motor cortex differ from one to another as

shown by the review of Rossini et al. (1994). Usually more superficial muscle representation areas over motor cortex require lower TMS intensity to produce over 50μV peak-to-peak MEP amplitude (Rossini et al. 2015).

Motor threshold can be divided and measured during resting and active conditions. Resting motor threshold (RMT) determined while target muscle is at rest, which means no background muscle activity. On the other hand, active motor threshold (aMT) is determined during muscle contraction. In that case the intensity of muscle contraction should be determined. Usually, active motor threshold measurements are performed during slight muscle contraction, around 5% - 20% of MVC. (Rossini et al. 2015)

Motor threshold can be measured by using relative frequency method, adaptive method, two-threshold method and supervised parametric estimation (Gropppa et al. 2012). Relative frequency method is mostly used in laboratory setting and measurements, so this paper only explains and refers to that one. According to Groppa et al. (2012) and Rossini et al. (2015) active motor threshold measurements should be started with subthreshold TMS intensity, around 35% of maximal stimulator output (MSO). Determining of RMT should be done by gradually increasing MSO in steps of 5% until TMS evokes >200μV peak-to-peak MEP amplitudes (positive). After that MSO is gradually decreased in steps of 1% until there are less than 3/5 trials with >200μV MEP responses. The TMS intensity that produced >200μV peak-to-peak MEP amplitudes 3 out of 5 trial, with minimal MSO is then defined as active motor threshold. (Groppa et al. 2012; Rossini et al. 2015) Usually, 3/5 or 5/10 positive trials are accepted for determining of aMT, even though according to Rossini et al. (2015) the accuracy of AMT determination increases with increasing the number of stimuli per intensity level. He also states that 10 out of 20 trials would be a more reliable way to determine RMT and aMT. (Rossini et al. 2015)

3.1.2 Motor evoked potentials

Motor evoked potentials (MEPs) are defined as net output of corticospinal tract from corticomotor and spinal level neurons to the target muscle (Groppa et al. 2012; Rossini et al. 2015). Stimulating cortex with transcranial magnetic stimulation, the electrical fields produced by magnetic fields of TMS recruits mostly I-waves, but also D-waves if the intensity of the TMS stimulation is high enough (Rossini et al. 2015). The recruitment of I- and D-waves could

also be muscle specific as shown by Nielsen et al. (1995) as they produced only D-waves in leg area muscles. These descending volleys of I- and D-waves are recorded as motor evoked potentials (MEPs) from the target muscle by electromyographic, figure 4. The D-waves are defined as direct activation of cortex pyramidal tract axons. On the other hand, I-waves are defined as indirect activation of corticospinal neurons which means that I-waves are produced at least partly from synaptic activity of cortical interneurons to the pyramidal neurons. Even though TMS usually (at least in lower intensities) recruits only I-waves, it's worth to mention that recruitment of I- and D-waves could also be affected by orientation of the coil. (Rossini et al. 2015) According to Ziemann (2020) we mostly only want to recruit I-waves with TMS, because it has cortical component and less variability in synchronization.

According to Groppa et al. (2012) and Rossini et al. (2015) the size of a single MEP amplitude can be expressed as peak-to-peak amplitude, area under the curve or amplitude from pre-MEP baseline. Peak-to-Peak amplitude of MEP is measured by calculating the difference from the maximal negative to maximal positive value (Groppa et al. 2012). Because at least several MEPs are usually recorded per force level or per TMS intensity level, the MEP amplitudes are averaged across for each force level or TMS intensity level (Rossini et al. 2015). MEPs are usually normalized to the maximal M-wave (Groppa et al. 2012).

MEP amplitude is affected by TMS stimulus intensity as the MEP amplitude increases in sigmoid curve fashion with increasing stimulus intensity. This means that after motor threshold TMS intensity it has a linear relationship between MEP amplitude and TMS intensity until the curve flattens, because MEP amplitude plateaus. When MEP amplitude has plateaued the increase in stimulus intensity doesn't increase the size of MEP amplitude anymore. This sigmoid curve relationship between MEP amplitude and TMS intensity differs between muscles and whether muscle is at rest or under contraction. Because sigmoid curve fashion relationship between MEP amplitude and TMS intensity, it is also possible to measure stimulus-response characteristics by using different TMS intensities during muscle activity or rest. (Gropppa et al. 2012; Rossini et. al 2015)

TMS measurements has always variability in MEP amplitude from trial-to-trial, no matter how standardised TMS protocols are planned and performed (Groppa et al. 2012; Rossini et al. 2015). According to Groppa et al. (2012) and Rossini et al. (2015) MEP variability is caused by intrinsic fluctuations of the excitability of cortical and spinal neurons, technical noise,

environmental noise and psychology state of the subject. Also, Keil et al. (2014) has shown by using EEG that variability of MEP amplitude is also affected by cortical brain states. To reduce this variability of trial-to-trial MEP amplitude the TMS measurements should be standardised, which means standardising coil placement and current direction, background activity of muscle, arousal level of the subject and environmental noise et cetera. (Groppa et al. 2012; Rossini et. al 2015)

Muscle activity during stimulation is another factor that could affect the produced MEPs and its latencies. According to Hallett (2005) and Rossini et al. (2015) this can be seen when comparing TMS intensity high enough to produce MEPs in relaxed muscle and during low level muscle activity. For example, if specific motor cortex area during low level voluntary contraction is stimulated with TMS intensity of AMT, the same intensity wouldn't probably produce MEP under relaxed condition. Also, the MEPs are larger and latency of MEPs during TMS stimulation could be shorted when stimulated during voluntary contraction. (Hallet 2005; Rossini et al. 2015) According to Rossini et al. (2015) this could be because during low level voluntary contraction more neurons are near their threshold level compared to relaxed state. On the other hand, it could be also because TMS stimulation during relaxed condition reflects activation of low-threshold and slowly propagating pyramidal tract neurons. While stimulation during voluntary contraction reflects activation of higher-threshold and faster propagations neurons. (Rossini et al. 2015)

Because of the variability of MEP amplitude caused by many factors the from trial to trial, large number MEPs are needed to obtain reliable estimation of probabilistic distribution the MEP amplitudes (Rossini et al 2015).

3.1.3 Silent period

Silent period can be only measured during muscle activity and is defined as period of silence in EMG activity, which occurs immediately after the MEP until the return of EMG activity back to baseline, figure 4. Typically, silent period is measured from single-pulse stimulus TMS response. Silent period is complex phenomenon, and it is thought has different supraspinal and spinal sources contribute to it. At spinal level it is thought that several inhibitory mechanisms are involved in silent period, including inhibition due to Renshaw cells, refractoriness of spinal neurons after excitation and postsynaptic inhibition due to activation of Ia inhibitory

interneurons. (Gropppa et al. 2012; Rossini et al. 2015) On the cortical level it is thought that different intra-cortical inhibitory mechanisms are involved in the silent period. According to Fuhr et al. (1991) first part of the silent period (initial 50ms) is caused by spinal inhibitory mechanisms. On the other hand, the later part of the silent period is thought be caused by intra-cortical inhibitory mechanisms (Rossini et al. 2015). Like MEPs amplitude, also the cortical silent period has variability, which can be divided according to Orth and Rothwell (2004) inter-hemispheric, interindividual and inter-session variability. Inter-hemispheric variability is typically small, but interindividual differences and inter-session variability can be large, around 20% - 35%. It has also been shown by Kimiskidis et al. (2005) that there's relationship between silent period duration and TMS intensity. This relationship has positive trend, which means that the silent period duration increases gradually with an increase in TMS intensity (Kimiskidis et al. 2005).

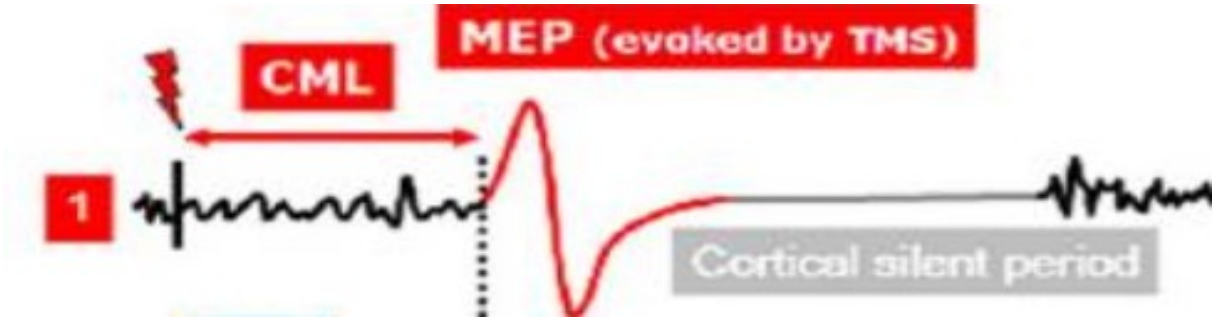

FIGURE 4. Schematic picture of motor evoked potential and cortical silent period (Groppa et al. 2012)

Arccoding to Groppa et al. (2012) one method for analysing cortical silent period, consist of calculating the mean silent period duration from trial-to-trial measurements and then the silent period durations are averaged across trials. The silent period is usually defined as the time elapsing from the onset of the MEP until return of EMG activity back to baseline. (Groppa et al. 2012)

3.2 Electrical stimulation of the lumbar spine

The electrical stimulation of the lumbar spine involves placing two self-adhesive electrodes on the spine. The electrical stimulation produces similar kind of responses in the target muscle EMG as TMS stimulation. These responses consist of lumbar evoked potentials (LEPs) and silent period, when stimulating during voluntary activation of the muscle. The amplitude of

LEPs can be analysed the same way as MEPs, which consist of calculating the peak-to-peak amplitude of LEPs and normalizing them to the obtained amplitude of M-max. Additionally, the stimulation intensity of should be determined beforehand, which could be based on the amplitude of M-max (Ansdell et al. 2020)

According to the modelling studies by Kuck et al. (2017) the electrodes should be placed on to the first lumbar (L1) spinous process and the second one on to the eight thoracic spinous process (T8), when stimulating the quadriceps femoris muscle. As this kind of placement of the electrodes should create the greatest quadriceps femoris muscle motor neuron pool activation, located in the spinal cord activation between L1 and L5. (Kuck et al. 2017)

In addition, when stimulating the quadriceps femoris muscle three validation processes should be conducted to ensure the proper placement of the electrodes and to minimize the risk of stimulating the dorsal or ventral roots (Martin et al. 2008; Taylor & Gandevia 2004). First, the latency of the lumber evoked potential (LEP) should not change with the increase in stimulation intensity (Taylor & Gandevia 2004). Secondly, paired pulse with 50 ms inter-pulse interval should be used assess that stimulation does not excite the dorsal roots (Courtine et al. 2007; Minassian et al. 2007). Thirdly, the amplitude of LEP should increase with the increment in contraction strength (Martin et al. 2008).

3.3 Peripheral nerve stimulation femoral nerve

Peripheral nerve stimulation is usually included in the methods when studying neuromuscular adaptations to strength training. According to Mason et al. (2020) and Siddique et al. (2020) peripheral nerve stimulation consists of stimulation of nerve and recording this M-wave response from target muscle by surface electromyography (sEMG). Rodriguez-Falces and Place (2017) states that M-wave represents the summated activity of motor units, which has been depolarized by the stimulation. Peripheral nerve stimulation is used to define the excitability of peripheral target nerve and muscle. M-wave is affected by many physiological and anatomical factors, which are mainly the same factors that also affect the EMG signal. One of the main ones is the placement of EMG electrodes, which affects the M-wave shape. Phase cancellation also occurs in M-wave. (Rodriguez-Falces and Place 2017)

The femoral nerve is originating from the L2, L3 and L4 anterior rami at the lumbar plexus and traverses through the psoas major muscle. Then the femoral nerve passes inguinal ligament in the femoral triangle and travels next to the femoral artery before branching into anterior division, which travels to the rectus femoris muscle. (Singh et al. 2020) Two adhesive electrodes can be used for peripheral nerve stimulation of the femoral nerve. These adhesive electrodes can be placed next to the femoral nerve at the femoral triangle, in line with the inguinal ligament. (Nuzzo et al. 2021)

The peripheral nerve stimulation of femoral nerve protocol consists of stimulating the nerve with gradually increasing stimulus intensity, until the recorded sEMG response plateaus. This is usually also confirmed with supramaximal stimulus. According to Rossini et al. (2015) peripheral nerve stimulation should be done at rest, because stimulation during voluntary activity does not provide any advantages. Additionally, the contraction of the muscle would represent noise in the signal (Rossini et al. 2015).

According to Rodriguez-Falces and Place (2017) M-wave can be used as a normalization factor of EMG activity by expressing the percentage of response to some other stimulation or task. As such the amplitude of M-wave is usually used to normalize MEPs and LEPs (Ansdell et al. 2020; Groppa et al. 2012). M-wave can be analysed by calculating the amplitude. Amplitude (peak-to-peak) is more reliable way to analyse it, as it is not affected so much by time (Rodriguez-Falces & Place 2017).

3.4 Electromyography of the rectus femoris muscle

According to Gohel and Mehendale (2020) the electromyography is used to record myoelectrical signals, which are generated by neuromuscular activity of muscle. There are basically two types to electrodes, which can be used to measure EMG signals. These two types are needle and surface electrode. Two types of surface electrodes exist, which are gelled and dry electrodes. There's also different width of surface electrodes, ranging from 0.5 to 2.5 cm wide. (Gohel & Mehendale 2020)

Surface electrodes detects the changes in chemical equilibrium between muscle surface and skin through electrolytic conduction. Raez et al. (2006) states, that this means that surface electromyography (sEMG) signal is composed of all muscle fiber action potentials, which are

occurring in the muscle underneath the electrodes. These action potentials are generated by the activity of motor units and order of activation, firing rate and amplitudes. This means that action potentials are developed in random order and intervals, which means that any instance sEMG signal can be positive or negative. (Raez et al. 2006) The motor evoked potentials are usually recorded from the target muscle by surface electrodes. Placement of electrodes and skins preparations should be done following validated technique, as the SENIAM. The recorded EMG should be filtered, amplified and digitized with appropriate configurations as with peripheral nerve stimulations. (Rossini et al. 2015) For the rectus femoris muscle the sEMG electrodes should be placed on the halfway between anterior spina iliaca superior and superior part of the patella with 20mm inter-electrode distance, while knee is slightly flexed (SENIAM).

According to Raez et al. (2006) EMG signal is mainly affected by two main issues, which are signal-to-noise ratio and distortion of signal. Distortion of the signal means that frequency components from the recorded EMG signal should not be altered. Noise is caused by many factors, which can be categorized into inherent noise, ambient noise, motion artifact, inherent instability of signal, cross talk, electrode contact, transducer noise and baseline shifts. The signal-to-noise ratio can be increased by proper placement of electrodes and setup, removal of unnecessary electrical equipment nearby and using proper filters on EMG signal EMG signal is also affected by anatomical and physiological properties of muscles and tissues underneath electrodes. (Amrutha & Arul 2017; Raez et al. 2006) Raez et al. (2006) states, that EMG signal should be analysed with an appropriate method, depending on the specific interest of the study. According to Farfán et al. (2010) EMG signal time domain analysis can be performed by using root mean square parameter. The root mean square EMG is commonly used when the recruitment of muscle fibers during contraction is analysed (Farfán et al. 2010).

4 STUDY AIM AND OBJECTIVIES

Most of the previous studies have shown that neuromuscular adaptations to strength training include adaptations at the corticospinal tract at the supraspinal and spinal level, which are associated with the increment in strength (Aagaard et al. 2002; Ansdell et al. 2020; Bestmann & Krakauer 2015; Del Balso & Cafarelli 2007; Kidgell et al. 2010; Kidgell et al. 2011; Mason et al. 2020; Tallent et al. 2017). However, there are still some conflicting results about these adaptations (Carrol et al. 2002; Kidgell & Pearce 2010; Kidgell et al. 2011; Latella et al. 2012). In addition, spinal level adaptations have been mostly studied in the context of monosynaptic motor neuron adaptations due to methodologic restrictions (Aagaard et al. 2002; Del Balso & Cafarelli 2007). As such the study will strengthen knowledge about the corticospinal adaptations to strength training at the supraspinal level. Additionally, the study brings up fairly new methodology for studying spinal level adaptations in investigating the lumbar level motor neuron pool adaptations to strength training.

On the other hand, there are only limited number of studies about the neuromuscular adaptations to detraining. The studies have shown that detraining induce decrease in strength gains (Bosquet et al. 2013; Häkkinen et al. 2000), corticospinal excitability (Tallent et al. 2017). Additionally, it could decrease the spinal excitability (Yamanaka et al. 1999). As such the study will strengthen the knowledge about these adaptations by also investigating the neuromuscular adaptations to detraining.

As such the primary aim of the study was to determine if the strength training induce changes in strength immediately after training and after the detraining period. In addition, the aim was to explore the supraspinal and spinal level adaptations to strength training and detraining, and to compare which of these adaptations were more prominent. The secondary aim of the study was to find out if these supraspinal and spinal level adaptations would be related with the change in strength (one-repetition maximum of the bilateral knee extension or isometric MVC of the right knee). The specific research question, hypothesis, and objective for the primary and secondary aims are listed below.

Research question 1) Does strength training and detraining induce changes in the strength parameters?

Hypothesis 1) Strength training and detraining induce changes in strength parameter(s). More specifically, strength training increases strength while detraining causes decrease in strength.

The objective 1) Determine the changes of a one-repetition maximum (1RM) of the bilateral knee extension and isometric MVC of the right knee from pre- to post- and from post- to detraining.

The first hypothesis is based on the previous research studies, which have shown multiple times that strength training induces increment in the strength parameters (Aagaard et al. 2002; Ansdell et al. 2020; Bestmann & Krakauer 2015; Del Balso & Cafarelli 2007; Kidgell et al. 2010; Kidgell et al. 2011; Mason et al. 2020; Tallent et al. 2017). While detraining period has been shown to cause a reduction in strength (Bosquet et al. 2013; Häkkinen et al. 2000).

Research question 2) Does strength training and detraining induce corticospinal adaptations at the supraspinal and spinal level?

Hypothesis 2) Strength training and detraining induce corticospinal adaptations at the supraspinal and spinal level.

The objective 2) Identify the changes in the averaged MEP and LEP amplitudes and silent period durations from pre- to post- and from post- to detraining.

The second hypothesis is based on the fact, that most of the previous studies have shown that strength training induces corticospinal adaptations at the supraspinal and spinal level (Aagaard et al. 2002; Ansdell et al. 2020; Bestmann & Krakauer 2015; Del Balso & Cafarelli 2007; Kidgell et al. 2010; Kidgell et al. 2011; Mason et al. 2020; Tallent et al. 2017). In addition, the limited number of studies about detraining also point out that detraining period causes corticospinal adaptations, towards back to baseline (Tallent et al. 2017; Yamanaka et al. 1999).

Research question 3) In which of the corticospinal tract level is the adaptations to strength training and detraining more prominent?

Hypothesis 3) Strength training and detraining induced corticospinal adaptations are more prominent in the spinal level.

The objective 3) Compare the changes in the averaged MEP and LEP amplitudes and silent period durations from pre- to post- and from post- to detraining.

The third hypothesis is based on the previous theory that postulates, that the corticospinal supraspinal centre could modulate the spinal-level neurons (Kidgell et al. 2017; Siddique et al. 2020). Thus, it could be that spinal level neurons and motor neuron pool could be more enhanced by the strength training, and these adaptations could be more transient compared to the supraspinal centre.

Research question 4) Are the corticospinal adaptations related to the change in strength?

Hypothesis 4) The corticospinal adaptations are related to the change in strength parameters.

The objective 4) Define the correlation between the change in 1RM of the bilateral knee extension or isometric MVC of the right knee and corticospinal adaptations between pre- to post-and post- to detraining.

The fourth hypothesis is based on the previous studies that have shown that strength training induces an increment in strength (Aagaard et al. 2002; Bestmann & Krakauer 2015; Del Balso & Cafarelli 2007; Kidgell et al. 2010; Kidgell et al. 2011; Mason et al. 2020; Tallent et al. 2017), and this increment in strength happens without changes in hypertrophy (Folland & Williams 2007). In addition, it has been shown that an increment in strength happens parallel with corticospinal adaptations (Aagaard et al. 2002; Bestmann & Krakauer 2015; Del Balso & Cafarelli 2007; Kidgell et al. 2010; Kidgell et al. 2011; Mason et al. 2020; Tallent et al. 2017).

5 METHODOLOGIES

The study was conducted in the laboratory of the University of Jyväskylä, Finland and had the statement of University of Jyväskylä Human Sciences Ethics Committee (1597/13.00.04.00/2020). All methodologies used were used only by researcher who were specialised in the used methodology.

5.1 Study population and inclusion/exclusion criteria

Screening resulted in seventeen volunteer subjects who were healthy young adults (28±5 years). By the end of the experiment three subjects had dropout from the experiment due to personal reasons. In addition, three subject's data was excluded from the final analysis, due to possible measurement error. Therefore, final data include 11 subjects. All subjects included in the final data analysis did not miss more than one training session throughout the intervention. Background characteristics of subjects (N=11) are presented in table 1.

TABLE 1. Background characteristics of subjects, reported in mean and standard deviation.

Gender	Age	Height (cm)	Weight (kg)	BMI
Male (N=5)	27 ± 4	181 ± 9	100 ± 21	30 ± 5
Female (N=6)	28 ± 5	170 ± 10	70 ± 18	24 ± 4
Total (N=11)	28 ± 5	174 ± 11	83 ± 24	27 ± 5

Predefined inclusion criteria were 1) healthy male and females, in age of 18 to 35; 2) no strength training experience in six months prior to the experiment. Exclusion criteria were 1) acute musculoskeletal injury; 2) musculoskeletal injury or neurological disease (such as epilepsy, seizures, depression) that affects the nervous system or training response or training safety; 3) medications that affect the nervous system; 4) contraindication for TMS, such as any implantable metal/electronic device e.g. cochlear implants, cardiac pacemaker.

The subjects were also screened for amount of systematic endurance training performed, prior six months, and for the use of nutritional supplements that could potentially affect the exercise

responses. Reported endurance training was recreational endurance training of approximately 1–3 hours per week. No subject reported use of additional nutritional supplements.

5.2 Study flow

Recruitment of the participants were conducted through the University mailing list and social media platforms. Subjects received a written information about the study, which had the information about the study design, methods, and objectives. If subject wanted more information about the study, after information document, it was verbally given to the subject. Possible risks and benefits of the study and methodologies were explained to subjects and a signed informed consent was obtained. Participants were informed about their legal rights, such as allowed to withdraw from the experiment at will, without a justification.

The project began with 1-week familiarization period, followed by a 2-week control period. Strength training period was 7-week long, and detraining period was 5-week. Neural measurements were divided into two separate sessions, which were defined as TMS-session and lumbar-session. Strength measurements were conducted in one session.

5.3 Study design

The study design was defined as within-subjects design. The study consisted of a 1-week period familiarization, 2-week control period, 7-week strength training period and 5-week detraining period. Familiarization session consisted of both neural and strength measurements. The study design is presented figure 5.

During the whole study period the subjects were advised to retain their habitual level of daily physical activity but to retreat from other forms of exercise. For the detraining period, subjects were carefully instructed on not to start any new kind of sport or perform any high-intensity workouts or to continue strength training. Additionally, if subject were performing endurance training prior to the study, that subject was told not to increase the volume of that training. Normal daily activity and same volume of endurance training as prior to the study was allowed.

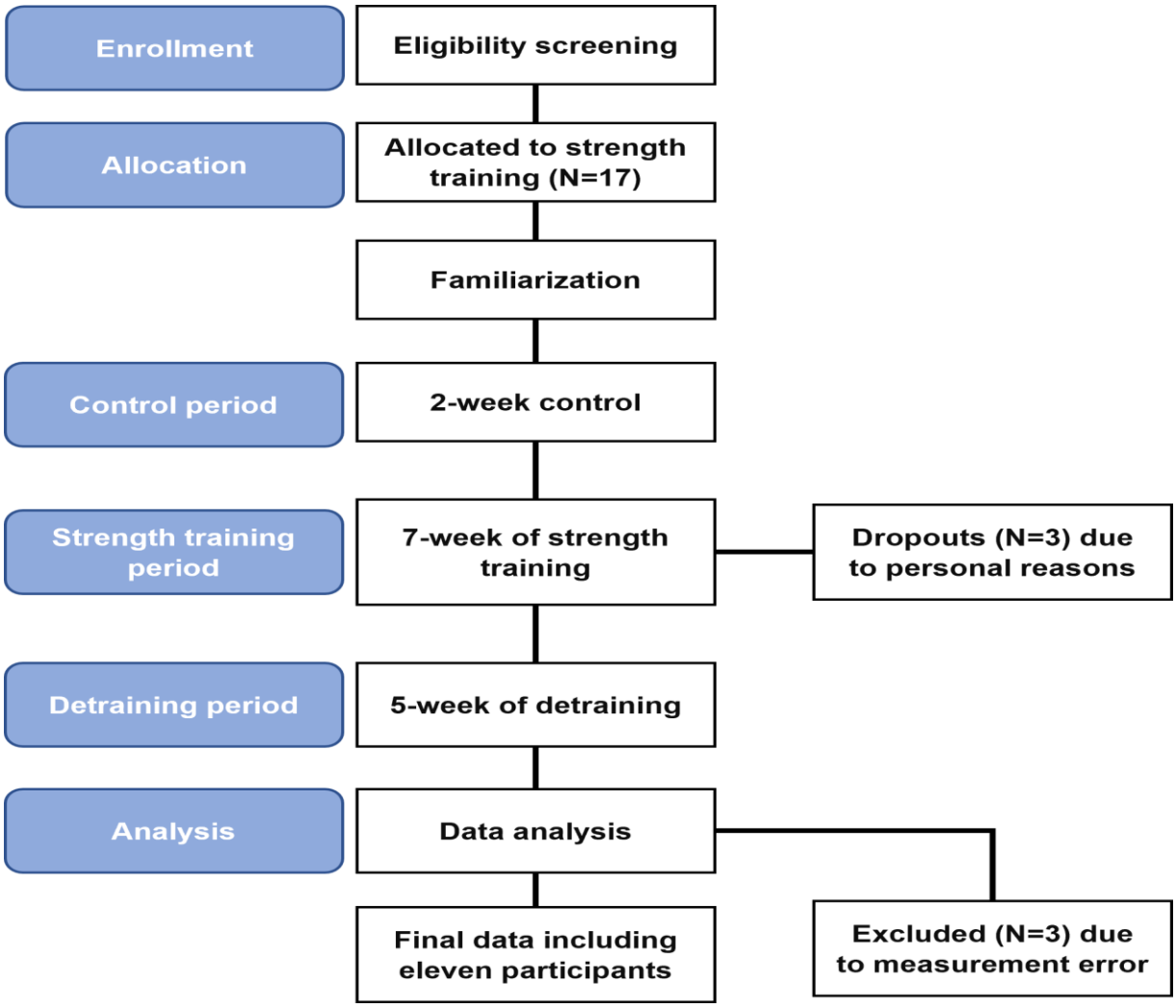

FIGURE 5. The study design.

As the study design was defined as the within-subject design each participant served as his own control. The control values for each variable were defined in the control -2 measurement and compared to control 0 values. The study endpoint was defined as last subject's last measurement.

5.4 Strength training

During the 7-week strength training intervention, subjects performed conventional strength training two times a week, separated by 48 hours, resulting in a total of 13 training sessions. All sessions were monitored by research staff to ensure the safety of the training, sufficient technique, proper use of the machines, and tempo of each exercise. The training was performed at a consistent time of day and week. The subjects were not allowed to perform additional training during the intervention.

The training session consisted of 5-minute warmup, followed up strength training. The 5-minute warmup consisted of cycling with self-selected tempo and dynamic mobility exercises. The strength training exercises consisted of single and multi-joint exercises such as leg press, knee-extension, bench press, bicep curl, and chest-supported seated row. The strength training included 3 sets with 8–10 repetitions for leg press, chest-supported seated row and bench press, and 5 sets with 8-10 repetitions for knee-extension and bicep curl. Rest time between inter-sets were two-minutes. The repetitions concentric phase was performed in explosive fashion followed by two second eccentric phase, with no isometric holding. In the beginning of the strength training period, the loading was designed based on pre-intervention 1RM (knee-extension and biceps curl) or 3–5RM (leg press, bench press, and chest-supported seated row) strength tests. Additionally, appropriate loading was redetermined for each week in the last training session of the week using a failure set assessment, which was based on the number of repetitions until failure for each exercise.

5.5 Descript of study measures

The study measurement time points for one-repetition maximum of bilateral knee extension (1RM) and neural measurements were at week -2, week 0, week 8 and week 14. The study measurement time points are presented in figure 6. At each measurement time point there was two neural measurement sessions (lumbar stimulation session and transcranial magnetic stimulation session) and one one-repetition maximum test session. This equals to a total of 8 neural measurement sessions during the study. The neural measurement session included electrical stimulations of the peripheral femoral nerve, maximal isometric voluntary contraction (MVC) test, electrical stimulations of the lumbar spine or transcranial magnetic stimulations of the motor cortex. Each measurement time point lasted for one week, and each measurement session was separated by 48-hour to minimize possible neural fatigue caused by the measurements.

Measurement time points	Week -2 (control)	Week 0 (control)	Strength training (week 1 – 7)	Week 8 (post measurement)	Week 14 (detraining measurement)
	↓	↓		↓	↓
Lumbar stimulation session: M-max Maximal isometric voluntary contraction of the right knee Electrical stimulation of the lumbar spine	X	X		X	X
Transcranial magnetic stimulation session: M-max Maximal isometric voluntary contraction of the right knee Transcranial magnetic stimulation of the rectus femoris	X	X		X	X
Strength session: One-repetition maximum of the bilateral knee extension	X	X		X	X

FIGURE 6. The study measurement time points.

The subjects first visited the laboratory for a familiarization session. During the familiarization session subjects were familiarized to the neural (peripheral nerve stimulation of femoral nerve, electrical stimulation of lumbar spine, and transcranial magnetic stimulation) and strength measurement (maximal isometric voluntary contraction and 1RM test). This was done in order to minimize the risk of measurement bias, which could be caused by new unfamiliarized somatic sensation and unfamiliarized on how to produce maximal voluntary contraction.

The purpose of the control period was to assess the baseline variation in the measurements for each subject, and to define the measurement time of the day and day of the week. For each subject, the defined measurement time of the day and of the week was kept consistent and only approximately 4-hour fluctuation from the measurement time was allowed. This was conducted to minimize the measurement error due to fluctuations in measurement time, which could affect neural responsiveness. Additionally, the control measurement sessions acted as a test-retest reliability measurement. While, the week 0 measurement time point acts as a pre-measurement time point, for which post- and detraining results were compared.

All subjects were given instructions about what are not allowed before or during any of the measurements. In the instructions, the subjects were not allowed to: 1) drink coffee or caffeine-containing drinks 12 hours before measurements; 2) perform any high-intensity workouts 48 hours before measurements; 3) eat chocolate 12 hours before the measurements; 4) smoke cigarette or use nicotine-containing products 12 hours before the measurements; 5) eat gum during the measurements. In addition, the subjects were instructed to keep good hydration status during the whole study and drink 500 ml of water one to two hours before the measurements. As any of these could have an influence on the neuromuscular measurements.

5.6 Neuromuscular system measurements

The neuromuscular measurement sessions were identical in the order of measurements, except in the other measurement session electrical stimulation of lumbar spine was conducted, while in the other one the TMS was performed. The order of the measurements are presented in figure 7. Each measurement session lasted about 90 minutes.

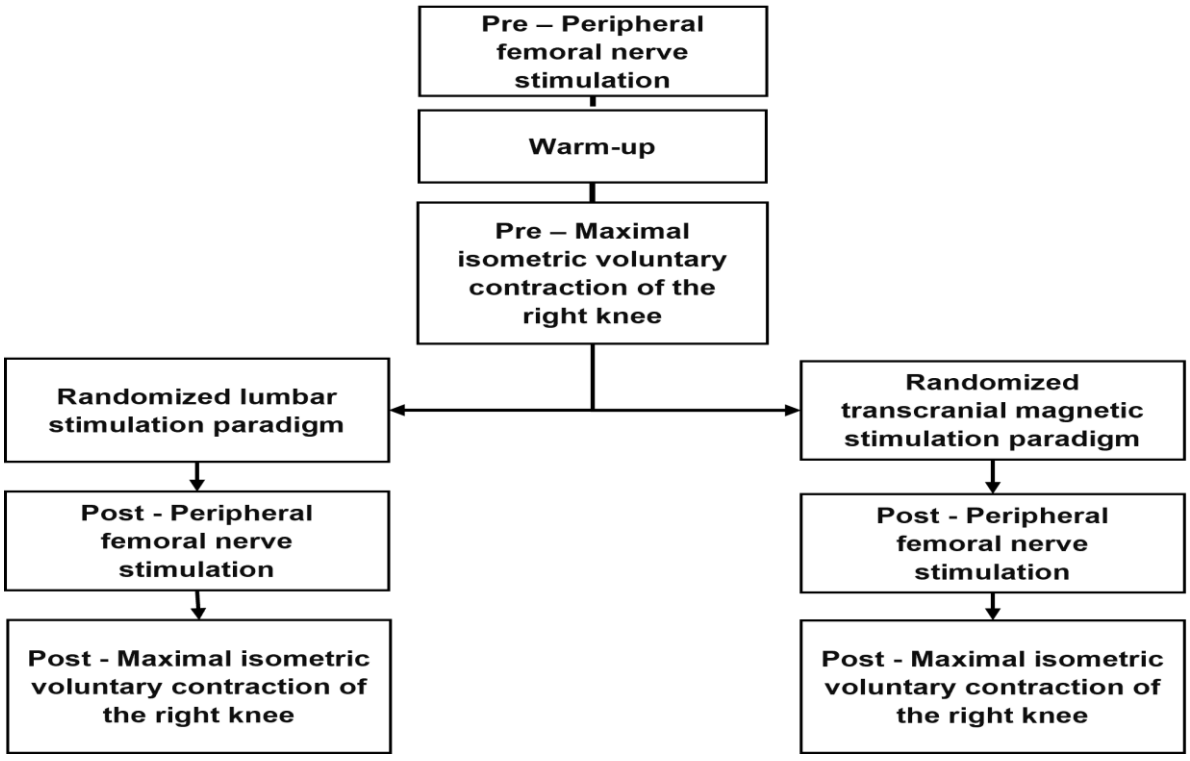

FIGURE 7. Measurement order in the neural measurement sessions.

The subject sat on a rigid custom-built force chair (University of Jyväskylä, Finland) during the neural and MVC measurements, figure 8. Custom-built force char restrained joint movement

and distension to avoid uncontrolled changes in the joint angle to standardize the joint angles during force production. The joint angles were also standardized across all subjects by measuring the distance of the back of the seat, and the height of the ankle strap (2.0 cm) above from the right leg lateral malleoli. In addition, the hip, knee, and ankle angles were fixed to a 90° angle, which was measured by a goniometer. Finally, the subjects were fixed on the chair with a belt around the waist, around the shoulders, and a strap on the mid-thigh. All measured distances and joint angles were documented during the first measurement session, and the documented distances and joint angles were used during the subsequent sessions.

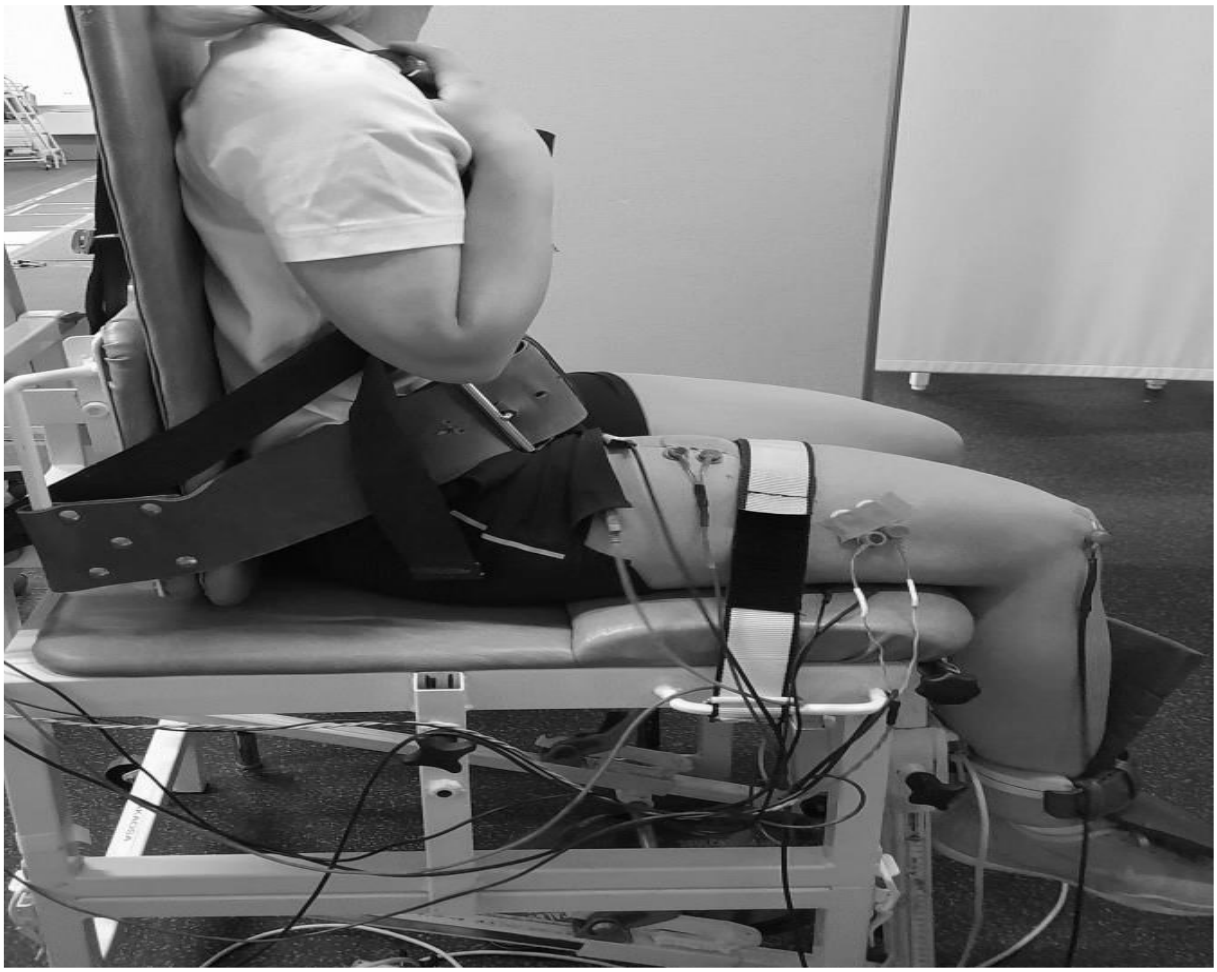

FIGURE 8. Position of the subject on the custom-built force chair.

5.6.1 Transcranial magnetic stimulation of the rectus femoris

TMS (Magstim 2002 stimulator 9-cm, Magstim, Whitland, UK) was used to detect changes in motor evoked potentials (MEPs) and silent period (SP) in the corticospinal tract. These MEPs

and SPs were detected by surface EMG, located in the rectus femoris muscle on the right leg. First, the optimal coil location (hotspot) for stimulating the rectus femoris muscle was determined by placing the double cone coil over the assumed location of the rectus femoris muscle representation area over the motor cortex. After that, the coil was moved around the representation area over the motor cortex, while simultaneously stimulating, until the largest MEP amplitude was found. Multiple stimulations were delivered at different sites, to confirm the place of the hotspot. After confirmation of the hotspot, the hotspot was marked by drawing the placement of the coil on the subject head with a permanent marker. The location of the coil was kept on the marked hotspot over the duration of a single session and the hotspot was redetermined at the beginning of each new session.

Active motor threshold (aMT) was defined as the lowest intensity to produce 3 out of 5 positive (≥200μV peak-to-peak) MEPs. Active motor threshold was determined under voluntary activation of predetermined 10% of MVC using the relative frequency method. At the first session determination of aMT began at subthreshold TMS intensity of 35% from maximal stimulator output (MSO). After that the MSO was gradually increased or decreased by 1% - 5% at a time until a positive MEP response (≥200μV peak-to-peak) was evoked. At each MSO intensity, at least 3 stimulations were given. When 3 out of 5 stimulations were defined as positive, the MSO was decreased by 1% until the lowest MSO intensity that produced 3 positive (≥200μV peak-to-peak) MEPs was determined.

The randomized TMS stimulation paradigms consisted of three different stimulation intensities (120%, 140% and 160% of aMT) and one isometric force level (20% of MVC). One paradigm consisted of one stimulation intensity and isometric force level. The MVC was defined before the start of TMS measurement. During each paradigm, the stimulations were delivered 10 times resulting in a total of 30 stimulation in each session. Before each session the TMS stimulation order of paradigm was randomized. The inter-stimulus interval was 10 seconds, and the interval between different paradigms was 1 minute. The TMS stimulation paradigms are presented in table 2. During all paradigms, the stimulations were delivered manually on the computer and only after the subject achieved the 20% force level.

TABLE 2. TMS paradigms, which consisted of one intensity, force level and number of stimulations.

Stimulation intensity	Force level	Number of stimulations
120% of aMT	20% of MVC	10
140% of aMT	20% of MVC	10
160% of aMT	20% of MVC	10

5.6.2 Electrical stimulation of the lumbar spine

The electrical stimulation of the lumbar spine was used to detect changes in lumbar evoked potentials (LEPs) and silent period (SP) in the corticospinal tract on the level of the lumbar spine. These LEPs and SPs were detected by surface EMG, located in the rectus femoris muscle on the right leg. Electrical stimulation of the lumbar spine was conducted by placing two electrodes on the lumbar spine. A rectangular electrode (Polar Trode® 50x100 mm Rectangular) was placed on top of the spinous process of lumbar vertebrae (L1) and a circular electrode (Polar Trode® 32 mm diameter) was placed on top of the eighth thoracic vertebrae (T8). The inter-electrode space variance was 3.5 to 5.0 cm depending on the height of the subject lumbar spine. The location of L1 and T8 was identified by first palpating the L3, which was assumed to be in the midpoint of the top of the ilium bones. After that palpation and counting of the vertebrae towards L1 and T8 were conducted.

The placement of lumbar stimulation electrodes was validated in the familiarization and at both control sessions by predefined validation protocol and during subsequent sessions, the same spot was used for electrical stimulation of the lumbar spine. More in-depth details about the placement of lumbar stimulation pads are described in the next chapter (5.6.3).

Electrical stimulation of the lumber spine was conducted by manually triggering each stimulation from a computer. Electrical stimulation was delivered via a constant current stimulator (DS7AH, Digitimer Ltd, Welwyn Garden City, UK) on the lumbar spine. The threshold for electrical stimulation of LS was defined as 25% of M-max. The stimulation intensity for the 25% of the M-max threshold was determined by gradually increasing the stimulus intensity until the stimulus intensity produced lumbar evoked potentials (LEPs) of 25% of M-max. In addition, the defined intensity was accepted if the average LEPs amplitude of 3 out of 5 stimulations produced 25% of the M-max response.
The randomized electrical stimulation of LS paradigms consisted of two different stimulation intensities (25% and 50% of M-max) and one isometric force level (20% of MVC). Electrical

stimulation of LS with the intensity of 50% of M-max is not part of this master thesis and will not be discussed further. Electrical stimulation of the LS with 25% of the M-max intensity paradigm consisted of one stimulation intensity and one isometric force level. The MVC was defined before starting of lumbar stimulation measurement. During the paradigm, the stimulations were delivered 10 times, during 20% of MVC contraction. Resulting in a total of 10 stimulation in each session. Before each session the order of the electrical stimulation of the LS paradigm was randomized. The inter-stimulus interval was 10 seconds, and the interval between different paradigms was 1 minute. The electrical stimulation of the LS paradigm is presented in table 3.

TABLE 3. The electrical stimulation of the lumbar spine, stimulation paradigm.

Stimulation intensity	**Force level**	**Number of stimulations**
25% of M-max	20% of MVC	10

5.6.3 Validation of the placement of electrodes for the lumbar stimulation

The validation of the electrode placements for the electrical stimulation of the LS was conducted with three different validation processes. The first two validation processes were done in resting condition, which was controlled by monitoring the EMG. If muscle activity was detected prior to 100ms of the stimulation, the stimulation response was not accepted, and the stimulation was repeated. In addition, it was instructed to keep neutral position of the back and spine during all the validation processes, and not to contract their back muscles. The validation of the electrode placement was performed during familiarization and at both control sessions. The main reason for the validation process was to confirm that the electrical stimulation of the LS did not stimulate the motor neuron pool at the ventral or dorsal roots. As this could cause inaccurate results. If during any of the three validation processes it was defined that the stimulation targeted the ventral or dorsal roots, the placement of the electrodes was changed and the whole validation process was started again from the first validation process.

In the first validation process, two thresholds were set to 25 and 50% of the M-max amplitude. The stimulation intensity for the 25% and 50% of the M-max thresholds was determined by gradually increasing the stimulus intensity until the stimulus intensity produced lumbar evoked potentials (LEPs) of 25% or 50% of M-max. The defined intensity was accepted if the average LEPs amplitude of 3 out of 5 stimulations produced 25% or 50% of the M-max response. In

addition, during the determination of threshold intensities the onset latency from stimulus artifact to start of the LEP was screened. If the decrease in the onset latency of more than 1ms was detected during increasement of stimulation intensity, it was defined by caused by targeting the ventral roots.

In the second validation process a paired stimulation of 50ms apart between stimulations was applied using the stimulation intensity of 50% of M-max. The paired stimulation peak-to-peak amplitude of LEPs were compared to each other. If the second LEP peak-to-peak amplitude was 10% smaller than the first LEP, the paired stimulation response was defined to target the dorsal roots. If the peak-to-peak amplitude of both LEPs were the same or the amplitude of the second LEP was less than 10% smaller, the paired stimulation response was defined not to stimulate the dorsal roots.

In the last validation process the electrical stimulation of the LS was conducted by evoking LEPs at the intensity of 25% of M-max during rest and during four different voluntary contraction levels. The voluntary contraction levels were predefined to be 10%, 20%, 50% and 60% of MVC. The order of the voluntary contraction levels was randomized. If the peak-to-peak amplitude of LEP increased in relationship with the increase in the contraction level, the placement of the electrodes was accepted. However, if the peak-to-peak amplitude decreased during higher contraction levels, it was defined to be caused by the stimulation of the ventral or dorsal roots. As such, the position of the electrodes was replaced.

After the validation was conducted in the familiarization and in both control sessions, the placement of electrodes for the subsequent sessions was defined to be the last accepted placement in the last session. After the placement of the electrodes were validated the position of the electrodes were documented and marked. Marking was performed by drawing the position of the electrodes using a permanent marker. Documentation included the measuring of the inter-electrode distance and the distance from C7 to the electrode placed on the T8. Additionally, a picture of the placement of the electrodes was taken on the agreement of the subject.

5.6.4 Peripheral nerve stimulation of femoral nerve

The peripheral nerve stimulation of femoral nerve was used in each session to determine the peak-to-peak amplitude of M-max, which was recorded by the surface EMG. The amplitude of M-max was determined at the start and at end of each session. The amplitude of M-max at the start of the session was used for the normalization of collected MEPs and LEPs. The peripheral nerve stimulation of femoral nerve was conducted in resting condition. Muscle activity was monitored and controlled by the root mean square (RMS) of the surface EMG placed on the rectus femoris.

The peripheral nerve stimulation was conducted by using single electrical stimuli of the femoral nerve vie constant current stimulator (DS7AH, Digitimer Ltd, Welwyn Garden City, UK). The M-max was defined as the maximal peak-to-peak amplitude of M-wave acquired from the surface EMG placed on the rectus femoris, during M-max determination process. The M-max determination process included gradually increase in the stimulus intensity until the peak-to-peak amplitude of M-wave did not increase from the further increase in stimulation intensity. In addition, the maximal peak-to-peak amplitude of M-wave was verified by increasing the stimulation intensity by 150%, from the intensity where the plateau of M-wave occurred. If the peak-to-peak amplitude of M-wave did not increase by the verification process, the previous maximal peak-to-peak amplitude of M-wave was accepted as M-max. However, if the amplitude of M-wave increased in the verification process, then the stimulation process continued by gradually increasing the stimulation until the amplitude of M-wave plateaued once again. The absolute intensity in which the M-max was acquired was documented in milli amperes (mA), as well as the peak-to-peak amplitude of M-max in milli volts (mV).

The placement of electrodes for peripheral nerve stimulation of femoral nerve was defined by palpating the pulse of common femoral artery on the right leg. Two circular surface electrodes (Polar Trode® 32mm diameter) were placed on each side of the artery, in the direction of inguinal ligament.

5.6.5 Electromyography of the rectus femoris muscle

The surface electromyography (sEMG) recordings were collected from the rectus femoris (RF), vastus lateralis (VL) and biceps femoris (BF) muscles. However, the primary muscle of interest in the study was RF muscle. The sEMG recordings from multiple muscles allowed the monitoring of agonist-antagonist muscle activity in different stimulation paradigms and during

force production. In addition, the multiple sEMG was used to find a more optimal site of location in different stimulation paradigms.

Electrodes used for acquiring sEMG recordings were bipolar surface electrodes (Ambu® BlueSensor N 22x44 mm) with 10-mm area. The interelectrode distance was defined to be 20-mm. The analogue to digital converter used in acquiring sEMG signals was (CED Power 1401-3, CED, Cambridge, UK). The software used during the acquiring of the signals was Signal 4.10 software (Cambridge Electronic Design Ltd., Milton, Cambridge, UK). The acquired sEMG recordings sample rate was 3kHz with x1000 gain. The common-mode rejection ratio was defined as >120dB and the signals were filtered by bandpass filter (16-1000Hz). Additionally, the sEMG recordings were synchronized in time with the force signal recordings.

In the preparation and placement of sEMG electrodes on the RF, VL and BF muscles, the guideline of Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscle (SENIAM) was followed. The preparation of the defined sEMG electrodes spot included shaving excessive hair on the skin, cleaning of the skin with etanol and rubbing the skin with sandpaper. The sEMG electrodes electrical conductance of below 2 kΩ was accepted, which was determined by the volt-ampere-ohmmeter. As this ensures good electrode-skin contact, which minimizes the risk of artefacts, imbalance between electrodes and ensures the lowest noise in the EMG. If above 2 kΩ conductance was recorded, the rubbing of the skin with sandpaper was repeated until below 2 kΩ conductance was recorded.

The sEMG electrodes for the RF muscle were placed halfway between distance on the line from anterior spina iliac superior to the superior edge of the patella. While the sEMG electrodes for the VL muscle were placed to two thirds of the distance between the trochanter major to the lateral side of the patella. In addition, the electrodes for the BF muscle were placed halfway in line between ischial tuberosity and the lateral epicondyle of the tibia. The sEMG electrodes were placed in parallel with the orientation of the muscle fibers. In addition, the ground electrode was placed on the patella.

In the familiarization session the sEMG electrode locations on each muscle were documented and marked by using a permanent marker. The documentation included distance between the anatomical landmarks to the electrode, discussed in the previous chapter. The subject was advised to renew the markings with a permanent marker. In addition, the exact location of the

electrodes was measured on each subsequent session and compared to the documented locations.

5.6.6 The isometric maximal voluntary contraction of right knee

The isometric maximal voluntary contraction (MVC) of the right knee extension was collected in each session before and after the TMS or LS stimulation paradigms. The protocol for defining of MVC of the right knee included two-minute warm up prior MVC. The isometric MVC was defined as the highest peak knee torque (N/m) achieved during 3 attempts. The rest interval between MVC attempts was defined to be 1 minute. If the peak torque of the third MVC attempt was >5% higher, than in the previous attempt, a fourth attempt was conducted. The isometric MVC of the knee was instructed to perform in an explosive fashion, contract as much as possible, and hold the contraction for 2 – 3 seconds. In addition, verbal encouragement and visual feedback about the force produced were given to the subject on each attempt.

The MVC was recorded by the force sensor, which was located within the ankle strap. The analog-to-digital converter used in acquiring force signals from the sensor was (CED Power 1401-3, CED, Cambridge, UK). The software used for the recording of the signals was Signal 4.10 software (Cambridge Electronic Design Ltd., Milton, Cambridge, UK), with a sample rate of 1kHz. The built-in force sensor, located within the ankle strap was located 2cm upwards from the lateral malleoli of the right leg. In addition, the distance from the midpoint of the force sensor to the lateral epicondyle of the femur was measured. This distance was used to convert the force from newtons (N) to knee torque (N/m).

Prior to isometric MVC attempts the subjects performed a two-minute warm-up. The two-minute warm-up process consisted of at least 2 attempts to produce the assumed 50% and 80% of MVC with 10 – 20 second rest intervals between attempts. In addition, the subjects were instructed to hold the contraction for 3 – 4 seconds.

5.7 One-repetition maximum of bilateral knee extension

Dynamic knee extensor muscle strength was assessed by determining the one-repetition maximum (1RM) of bilateral knee extension on a leg extension machine (David 210, David Health Solutions Ltd, Helsinki, Finland). The subjects sit on the leg extension machine with

their shin against the shin pad and belt secured around their hips. The subjects were required to lift the load to a fully extended position (180° knee angle) from a beginning knee angle of approx. 60°. The knee angle was subjectively estimated, and as such no goniometer was used. 1RM of bilateral knee extension was defined in the three to five attempts, with the accuracy of 2.5kg. More attempts were completed, if needed. Rest periods between attempts were 3 minutes.

Before the 1RM assessment, a warm-up was performed to ensure the neuromuscular system was ready for the 1RM attempts. Warm-up consisted of progressively increasing weights. The first warm-up set consisted of 10 repetitions with 40-60% of 1RM weight, and the second set consisted of 5 repetitions with 60-80% of 1RM weight. The 1RM weight was estimated, based on the 1RM defined in the previous measurement session. The rest time between sets was 1 minute.

5.8 Data analysis

The motor evoked potentials (MEPs) and lumbar evoked potentials (LEPs) were analysed from the rectus femoris muscle. From the MEPs and LEPs the peak-to-peak amplitudes were automatically analysed in Matlab R2021a (The MathWorks, Inc., US) by a custom-made script. The script calculated the peak-to-peak amplitude of each individual MEP or LEP. The peak-to-peak amplitude was defined as the difference between the maximal positive and negative peak value. The individual MEPs and LEPs were also manually screened, if deemed so. The normalization of MEPs and LEPs were conducted on the averaged amplitude of M-max for each session. The average M-max was calculated by averaging the M-max values obtained from both (TMS and LS) sessions on each measurement time point together. Finally, the normalized peak-to-peak amplitude of MEPs and LEPs was averaged across 10 stimuli per each intensity (120%, 140%, and 160% of aMT and 25% of M-max). The isometric MVC level on all stimulation intensities was 20% of MVC.

The silent period was calculated from the TMS stimulations and from the electrical stimulation of the lumbar spine of the rectus femoris muscle. The silent period was analyzed from the raw data and calculated for each stimulation from the time of the stimulation to the return of sEMG back to baseline. The silent period was calculated for all TMS and the electrical stimulation of

LS intensities (120%, 140%, and 160% of aMT and 25% of M-max). Finally, the silent periods were averaged across 10 stimuli per intensity.

The maximal compound wave of rectus femoris (M-max) was determined at the start of each session. The M-max was defined as the highest peak-to-peak amplitude obtained during peripheral nerve stimulation of the femoral nerve. The peak-to-peak amplitude was defined as the difference between the maximal positive and negative peak values. The amplitudes of M-max acquired from both sessions on each measurement time point were averaged. The averaged amplitude of M-max was used for the normalization of MEPs and LEPs.

The isometric maximal voluntary contraction (MVC) was determined at the start of each session. The highest knee torque obtained from the three attempts was defined as the MVC of the knee. The MVC was analyzed by calculating the peak torque attained from the best attempt. Baseline correction to torque level zero, was conducted when needed.

5.9 Statistical analysis

All statistical analyses were conducted using IBM SPSS Statics 28® (IBM Corporation, US). The imputation was conducted for missing values by using the linear regression method. Missing values were due to not attending in the session on the measurement time points.

The normal distribution of each variable was tested by the Shapiro-Wilk test. If the p-value was smaller than $p<0.05$, the distribution of that parameter was considered to be non-normally distributed. If non-normal distribution of the parameter was detected, then the variable was log transformed and the Shapiro-Wilk test was repeated.

The repeated measures of Analysis of Variance (ANOVA) was used to analyze parameters that were normally distributed to detect whether there were significant differences in group mean between different time points. Additionally, a post hoc test with Bonferroni was used for pairwise comparison between different time points. If the Mauchly's Sphericity test p-value was lower than $p<0.05$ then the sphericity had been violated and Greenhouse-Geisser was used to identify significant differences across time. Otherwise, the Sphericity-assumed was used to identify significant differences across time. For the non-normal distributed data, the Friedman's

2-way ANOVA by ranks analysis was conducted to identify significant differences between time points.

The analysis of the correlation between neural adaptations and changes in strength were conducted by the Pearson correlation coefficient for normally distributed variables and the Spearman correlation for the non-normally distributed variables. The delta% changes were used in the analysis of the correlation. If at least one of the variables were non-normally distributed, then the Spearman correlation was used for the analysis of correlation. The statistical level of significance in all analysis tests was set to $p<0.05$.

6 THE STUDY RESULTS

The study was conducted in a within-subject design, only one group-level mean, standard deviation (SD) and or standard error (SE) was calculated for each variable. As such descriptive statics include group-level mean and standard error and or standard deviation for each parameter. The study measurement time points for strength measurements and neuromuscular measurements were at week -2, week 0, week 8 and week 14. Strength training was conducted from the week 1 to week 7.

6.1 Changes in knee extension strength

The strength during the study were assessed by the isometric maximal voluntary contraction (MVC) of the right knee and one-repetition maximum of the bilateral knee extension. There was no significant difference in the mean isometric MVC of right knee between different measurement time points ($p>0.05$). However, there was almost a significant difference in the MVC between control 0 and post-training ($p=0.071$) of 4% increment. The MVC in newton-meter (N/m) as group level mean in different time points are presented in figure 9.

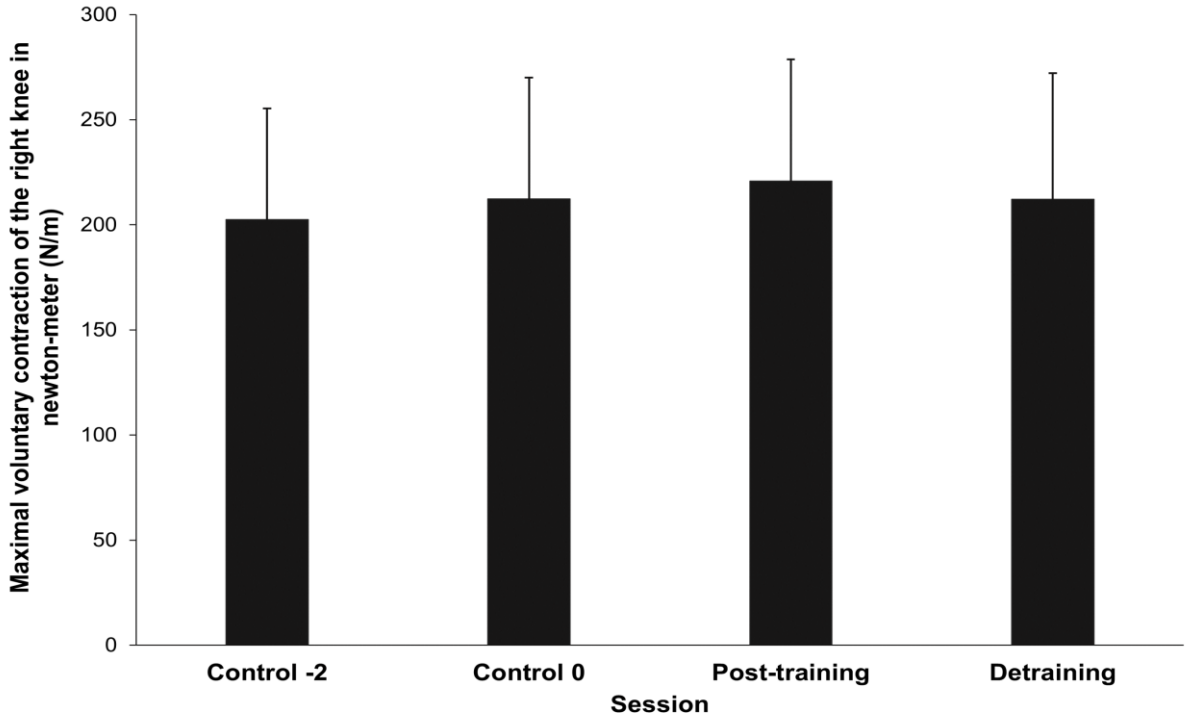

FIGURE 9. The isometric MVC of right knee (N/m) in different measurement time points. The values are presented as the mean value with SD.

There was significant increment of in the mean one-repetition maximum of the bilateral knee extension from control -2 to control 0 of 3% (4kg ± 1kg), from control 0 to post-training of 14% (19kg ± 5kg) and decrement of -4% (5kg ± 1kg) from post-training to detraining ($F = 51.113$, $p<0.05$). The one-repetition maximum of the bilateral knee extension as group level mean in different time points are presented in figure 10.

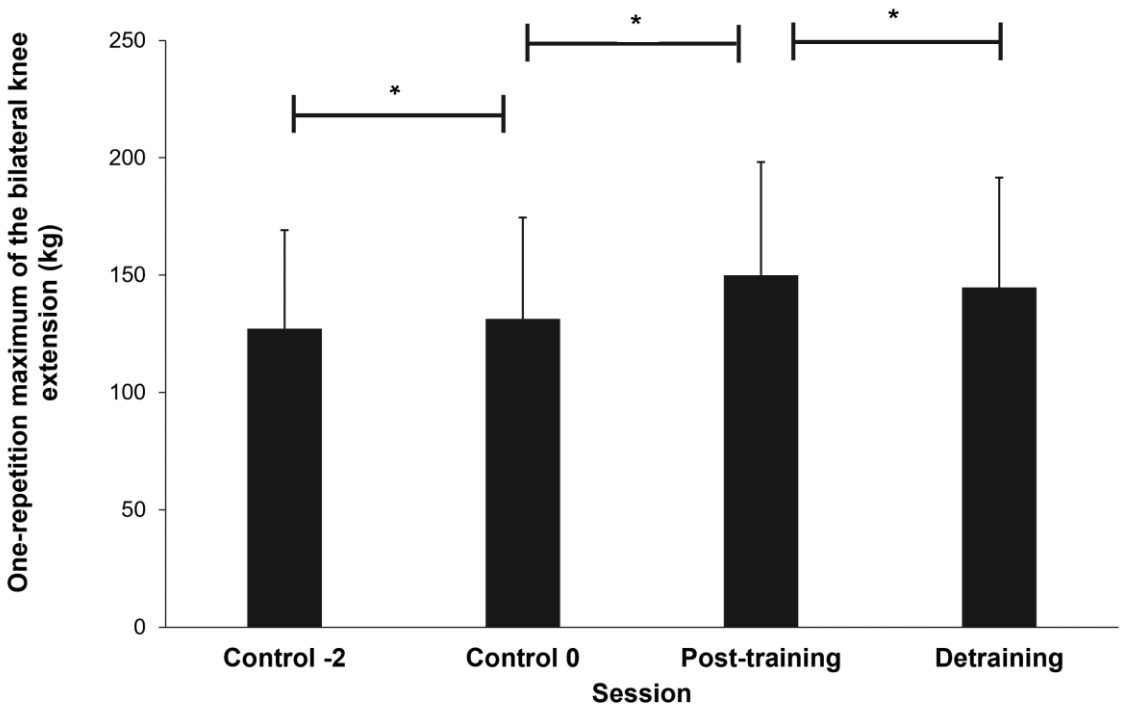

FIGURE 10. The one-repetition maximum of the bilateral knee extension in different measurement time points. The values are presented as the mean value with SD. The symbol * presents significant difference between measurement time points ($p<0.05$).

6.2 Corticospinal adaptations of the rectus femoris

The neuromuscular adaptation results include changes in motor evoked potentials (MEPs), lumbar evoked potentials (LEPs), silent period duration (SP) and active motor threshold (aMT). The peak-to-peak amplitude of the MEPs, LEPs and the duration of SPs are all averaged across 10 stimuli. In the following subchapter each of these variables are discussed further.

The averaged amplitude of M-max attained from LS and TMS sessions in different time points presented in table 4. As the peak-to-peak amplitude of averaged MEPs and LEPs are normalized to the averaged M-max of each time point. There were no statistical significance differences in the amplitudes of M-max across the study ($p>0.05$).

TABLE 4. The peak-to-peak amplitude of M-max in different measurement time points. The values are presented as the mean value with SD.

	Control -2	Control 0	Post-training	Detraining
Peak-to-peak amplitude of the M-max	2.6 ± 1.3	2.5 ± 0.7	2.4 ± 0.7	2.5 ± 0.6

6.2.1 Active motor threshold of the rectus femoris

There was a significant difference across time in the mean active motor threshold MSO% ($F = 3.286$, $p<0.05$). However, post hoc test with Bonferroni pairwise comparisons of different measurement time points, did not reveal significant differences between time points ($p>0.05$). The aMT as group level mean in different time points are presented in figure 11.

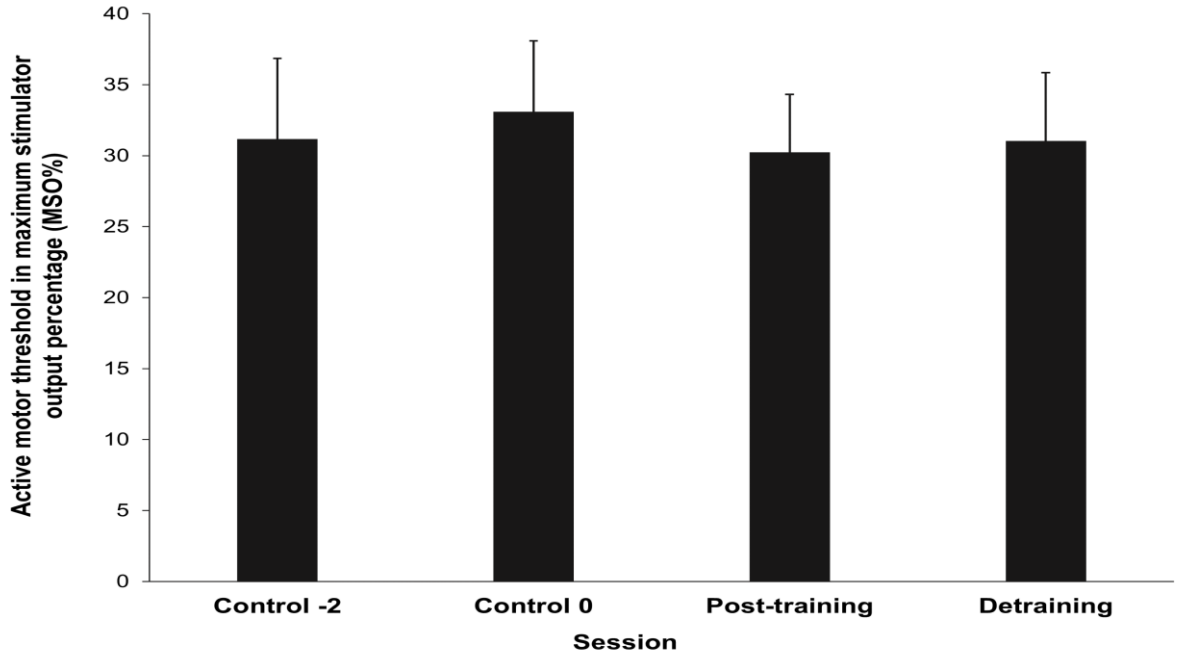

FIGURE 11. The active motor threshold value (MSO%) in different measurement time points. The values are presented as the mean value with SD.

6.2.2 Motor evoked potentials of the rectus femoris

There were no significant differences in the mean normalized peak-to-peak amplitudes of MEPs at any intensity between different measurement time points ($p>0.05$). The normalized peak-to-peak amplitude of MEPs as group level mean in different time points are presented in figure 12.

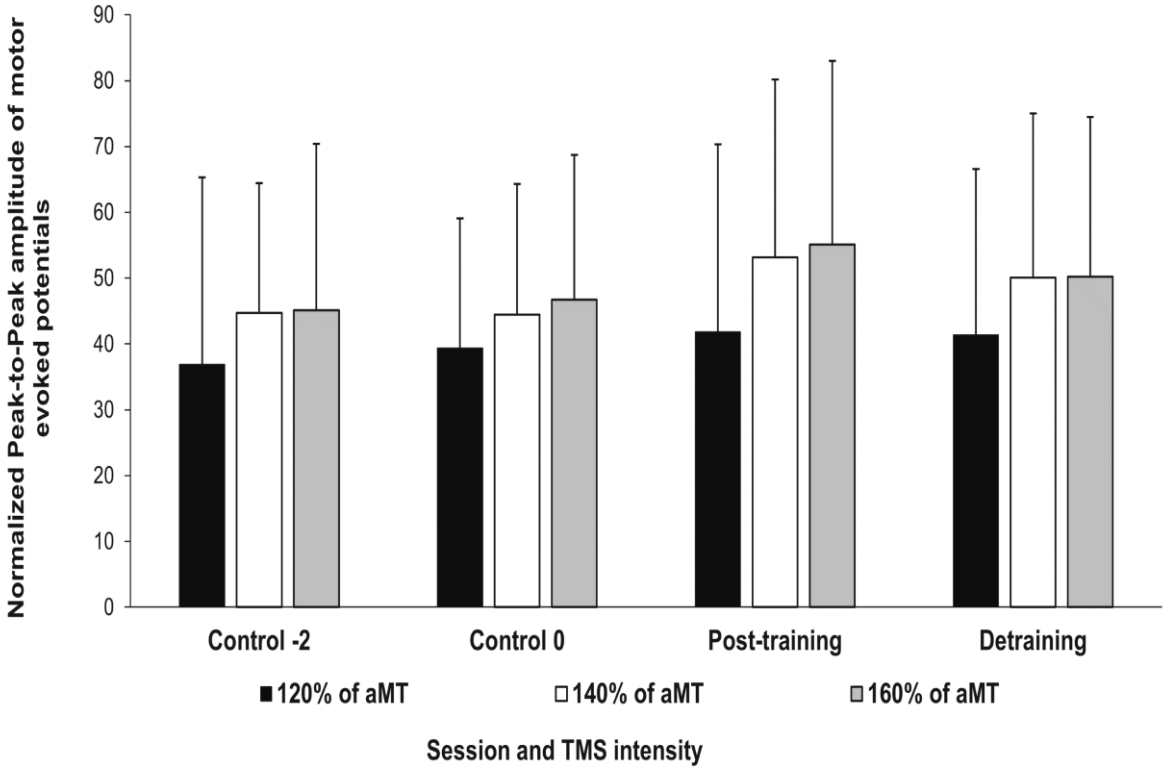

FIGURE 12. The normalized peak-to-peak amplitude of the MEPs in different measurement time points. The values are presented as the mean value with SD.

6.2.3 Lumbar evoked potentials of the rectus femoris

There was a significant increment of 60% in the mean normalized peak-to-peak amplitude of lumbar evoked potentials with intensity of 25% of M-max from control 0 to post-training ($F = 11.073$, $p<0.05$). The normalized mean peak-to-peak amplitude of lumbar evoked potentials at control 0 was 30% ± 9% of M-max, while at post-training it was 47% ± 22%. However, there were no significant differences between other measurement time points ($p>0.05$). The

normalized peak-to-peak amplitude of LEPs as group level mean in different time points are presented in figure 13.

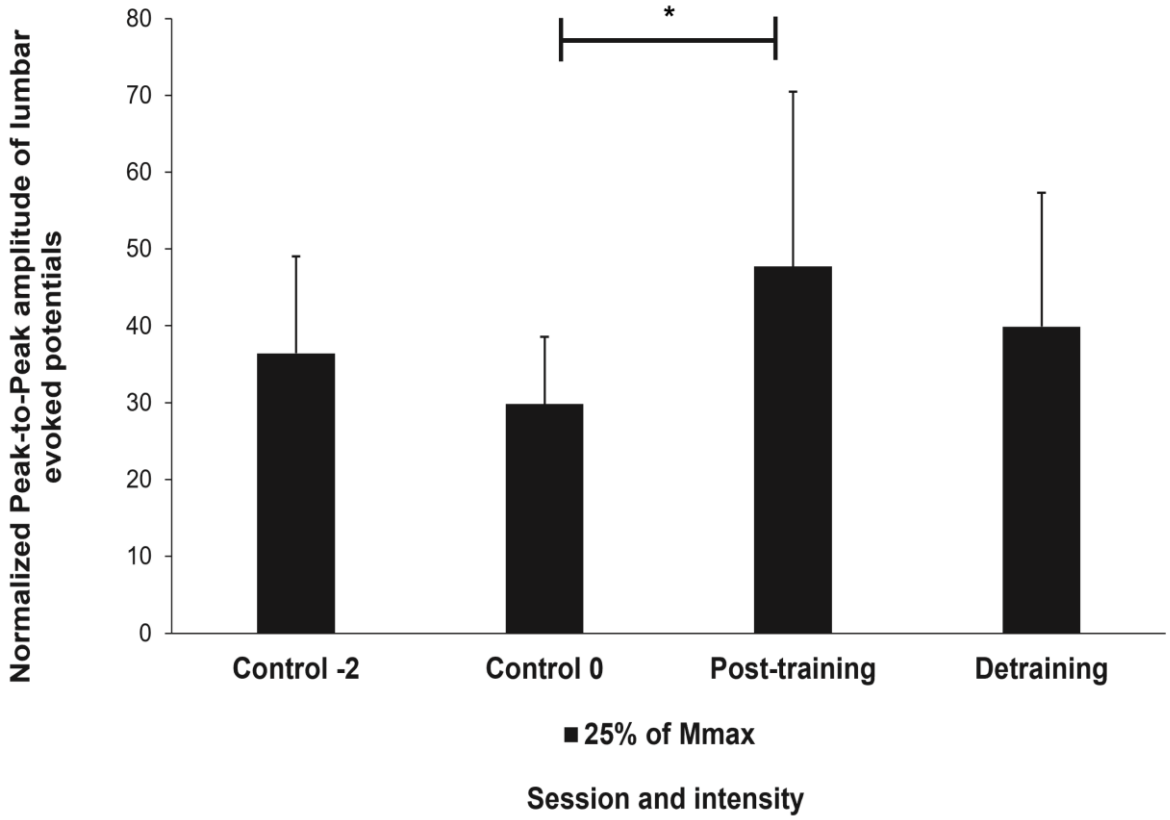

FIGURE 13. The normalized peak-to-peak amplitude of the LEPs in different measurement time points. The values are presented as the mean value with SD. The symbol * presents significant difference between measurement time points ($p<0.05$).

6.2.4 Silent period durations of the rectus femoris

There were no significant differences in the mean silent period durations induced by transcranial magnetic stimulation (TMS) between different measurement time points ($p>0.05$). The silent period duration as group level mean in different time points are presented in figure 14.

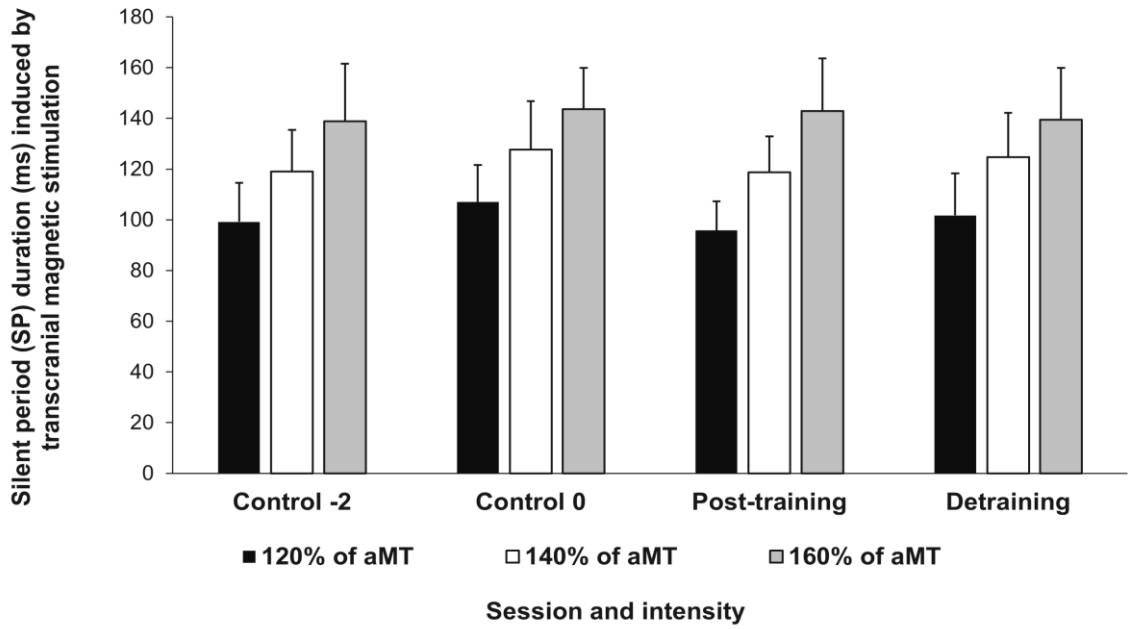

FIGURE 14. The silent period duration induced by TMS in different measurement time points. The values are presented as the mean value with SD.

In addition, there were no significant differences in the mean silent period duration induced by electrical stimulation of the lumbar spine (LS) with intensity of 25% of M-max between different measurement time points ($p>0.05$). The silent period duration as group level mean in different time points are presented in figure 15.

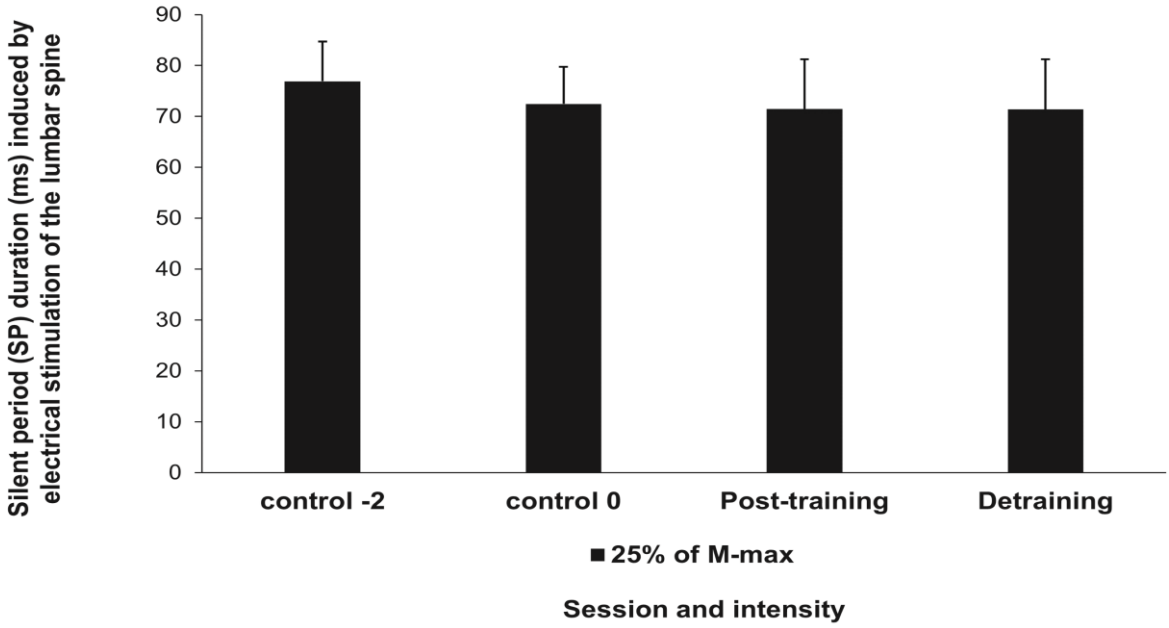

FIGURE 15. The silent period duration induced by electrical stimulation of LS in different measurement time points. The values are presented as the mean value with SD.

6.3 The correlation between corticospinal adaptations and changes in strength

There was no significant correlation found between MEPs or LEPs or silent period durations and isometric MVC of the right knee or one-repetition maximum of the bilateral knee extension ($p>0.05$). However, an almost significant correlation was found between the delta% change in the peak-to-peak amplitude of LEPs (25% of M-max intensity) and MVC, between control 0 to post-training ($p=0.077$). In addition, the effect size between these two variables was 0.66 estimated by the Hedges g.

7 DISCUSSION

As a summary of the study results, the study found a significant increment from control -2 to control 0 of 3%, and from pre- to post-training of 14% in the one-repetition maximum of bilateral knee extension ($p<0.05$). However, no significant difference was found in the isometric MVC of the right knee. In addition, the study found a significant increment in the peak-to-peak amplitude of LEPs at 25% of M-max intensity from pre- to post-training of 60% ($p<0.05$). There was also a significant change in the aMT over the course of the study, but the post hoc test with Bonferroni did not show this difference in the pairwise comparison. While the only significant difference after detraining was detected in the one-repetition maximum of bilateral knee extension as it decreased by -4% from post- to detraining ($p<0.05$).

The purpose of the study was to investigate and identify the corticospinal adaptations to strength training and detraining, and their associations to strength development. The duration of the strength training was 7-weeks and included a total of 13 training sessions, while the detraining period lasted 5-weeks.

The primary aim of the study was to investigate the corticospinal adaptations to strength training and to detraining at the supraspinal and spinal level. In addition, the aim was to compare which of these adaptations are more prominent. The secondary aim was to investigate if the corticospinal adaptations would be associated with the change in strength.

To answer the primary and secondary aims of the study transcranial magnetic stimulation (TMS) and electrical stimulation of the lumbar spine (LS) were used to evaluate the changes in the corticospinal tract. TMS was used for the assessment of the neural adaptations at the whole corticospinal tract level, while electrical stimulation of the LS was used to evaluate the adaptations at the level of the lumbar spine. In addition, two different strength methods were used to determine the changes in strength over the course of the study.

The null hypothesis could only be rejected from the first and partly for the second and third hypothesis. The alternative hypothesis can be accepted for the first hypothesis as the results show a significant increment in a one-repetition maximum of the bilateral knee extension at post-training ($p<0.05$) and decrement after detraining ($p<0.05$). In addition, the alternative hypothesis can be partly accepted for the second and third hypothesis as the study found a

significant increment in the amplitude of LEPs at post-training ($p<0.05$), but not after detraining period. Additionally, no significant differences were found in the MEPs or SP durations at post-training or detraining. For other hypotheses, the results did not meet the requirements to reject the null hypothesis ($p<0.05$).

7.1 Knee extension strength gains of strength training

The one-repetition of the bilateral knee extension showed a significant increment from control -2 to control 0 and from pre- to post-training, after 7-weeks of strength training. However, the isometric MVC of the right knee did not significantly change during the study, nor did it increase after strength training. However, the isometric MVC showed a similar pattern in change across time points as the one-repetition maximum.

The early strength increment, from control -2 to control 0 can be postulated to be induced by the skill acquisition phase. As all the subjects were untrained subjects, it could be that the bilateral knee extension movement was an unfamiliar movement for them. This can be considered to be in line with the research, about skill acquisition, which has shown that even after one session the motor skill of the movement can quite rapidly be enhanced, especially during simple movements (Dayan & Cohen 2012; Doyon & Benali 2005.)

The significant increase in the one-repetition maximum after strength training also is in line with previous research findings, which have shown that strength training a particular movement will result in an increment in maximum force production capabilities (Christie & Kamen 2014; Kidgell & Pearce 2010; Latella et al. 2012; Mason et al 2020 Tallent et al. 2017; Griffin & Cafarelli 2007). In addition, the reason for not significant differences found in the isometric MVC could be due to not high enough transfer effect between the strength training exercise and the isometric MVC. As the strength training of the knee was conducted as a dynamic bilateral exercise, not in an isometric exercise. Previous research also supports this idea, which has shown that the transfer effect of exercised movement could be relatively low (Siddique et al. 2020). This idea is also supported by the training specificity of the movement. As Stien et al. (2020) have shown that strength development in the lower limbs was significantly superior in the trained strength exercise compared to the untrained exercise.

7.2 Corticospinal excitability adaptations to strength training

In the active motor threshold, there was a detected significant change over the course of the study time, but the post hoc test with Bonferroni did not show this difference in the pairwise comparison between time points. This finding is in line with previously published research articles as they have also seen a reduction in the aMT after strength training (Jensen et al. 2005; Mason et al. 2020). This could be due to the reduction of threshold in the higher threshold motor neurons (Jensen et al. 2005; Rossini et al. 2015). However, as there was seen the highest aMT in the control 0 session, this could also be due to the hotspot detection paradigm or the position of the coil, which could have resulted in a measurement error. As it is hard to keep the same angle and tilt of the coil between measurement sessions, without a navigation system.

Additionally, as we defined aMT based on 3/5 positive trials with the lowest intensity, this could have also caused an error in the accuracy of the defined aMT. As the accuracy of aMT determination could increase with an increased number of stimulations per intensity level and 5/10 or 10/20 positive trials could be a more reliable way to measure aMT (Rossini et al. 2015).

The study found no significant changes in the MEPs induced by different intensities (120%, 140%, and 160% of aMT) at 20% of MVC during the control period or between pre- to post-training. However, there was a trend towards increment in all intensities used to induce MEPs, for example the peak-to-peak amplitude of the MEPs induced by 140% of aMT was grown by 22% from pre- to post-training. These findings are in line with the previous findings of Carrol et al. (2002), Kidgell and Pearce (2010), and Latella et al. (2012) who found no significant difference in the MEPs after 4 to 8 weeks of training. However, it is worth mentioning that multiple other studies have found an increment in the amplitude of MEPs after strength training, even after two to four weeks of training (Tallent et al. 2017; Griffin & Cafarelli 2007; Kidgell et al. 2010; Kidgell et al. 2011).

The reason for not finding significant changes in the MEPs could be due to the training specificity, as the strength training was performed as bilateral knee extension, but the neuromuscular adaptation measurements were conducted during isometric contraction of the right knee. There have been a lot of studies done about training specificity, which conclude that the adaptations to training are highly task or training specific (Häkkinen et al. 1996; Kidgell et al. 2010; Stien et al. 2020). This could have resulted into the fact, that the neuromuscular

adaptations of bilateral knee extension did not transfer into isometric knee extension of the right knee as the MVC did not increase significantly during the study.

It could also be that the TMS stimulation of the rectus femoris muscle representation area over the motor cortex resulted in the activation of all quadriceps muscles. As Davies (2022) has shown through navigated TMS, the hotspot for quadriceps femoris muscles overlap between each other in the primary motor cortex. In addition, it could be that the supraspinal adaptations induced by the bilateral knee extension happened in the two other quadriceps femoris muscles (VL or VM muscles), and not in the rectus femoris muscle. Thus, analysing MEPs only from the rectus femoris muscle could face the problem that it is not representative of the whole quadriceps femoris muscle group (Temesi et al. 2014).

The study found a significant increment from control 0 to post-training in LEPs induced by 25% of M-max intensity at 20% of MVC. This increment indicates that the strength training induced corticospinal adaptations at the spinal level. This finding is in line with the previous studies, as it has been shown that strength training induces changes at the spinal level (Aagaard et al. 2002; Balso & Cafarelli 2007). These changes have been measured in previous studies by Hoffman's reflex (H-reflex) and volitional wave (V-wave). The amplitude of the H-reflex and V-wave has been shown to increase after strength training. However, both of these adaptations more or less reflect the monosynaptic adaptations at the spinal cord level (Aagaard et al. 2002; Balso & Cafarelli 2007). Previously to my knowledge, only the study done by Ansdell et al. (2020) has found an increment in the LEPs acutely after strength training.

The reason why the study found a significant increment only at the spinal level could be due to the fact that transcutaneous lumbar stimulation stimulates both the sensory and motor neurons. In fact, it has been shown transcutaneous spinal stimulation could lead to the stimulation of sensory and motor neurons (Roy et al. 2012). As such the increment in the amplitude of LEPs could be the result of an increment in both the spinal reflexes and motor neuron pool excitability, which could have resulted in a positive summation. As previous studies have shown that strength training leads to an increment in the amplitude of MEPs, which reflects the excitability of the whole corticospinal tract (Tallent et al. 2017; Griffin & Cafarelli 2007; Kidgell et al. 2010; Kidgell et al. 2011). As well as an increment in the amplitude of H-reflex, which reflects the monosynaptic sensory-motor reflex arc (Aagaard et al. 2002; Balso & Cafarelli 2007).

Finally, the finding could suggest that strength training could lead to adaptations at the corticospinal and the reticular tract. The possible involvement of reticular adaptation to strength training has been studied only in monkeys (Glover & Baker 2020). However, it has been shown that reticular formation and reticular tract are at least partly controlling the spinal reflexes (Chai et al. 1990; Satoh et al. 2006).

7.3 Corticospinal inhibition adaptations to strength training

The study did not find any significant changes in the SP durations from control 0 to post-training measurement time points at any stimulation methods (TMS or lumbar stimulation) at 20% of MVC. However, the trend towards a decrease in the SP durations was noticed after strength training. The results indicate that the strength training did not induce significant adaptations in the corticospinal inhibition. This finding is controversial to the previously published research findings, which indicate that resistance training causes a decrease in the SP duration (Christie & Kamen 2014; Kidgell & Pearce 2010; Latella et al. 2012; Mason et al 2020). However, not all studies have found a reduction in the silent period time after strength training (Kidgell et al. 2011).

The reason for not finding significant changes in the SP durations could be due to the low sample size and the fact, that SP is known for having large (20% - 35%) inter-session variability (Orth & Rothwell 2004). However, the not significant change in the SP duration can be considered to be in line, with the fact that the study did not find any significant changes in the MEPs. As it has been postulated that part of the increased neural drive from the motor cortex to the target muscle after strength training could be due to a reduction in the synaptic efficacy of inhibitory networks within the primary motor cortex and corticospinal tract (Kidgell et al. 2017).

7.4 The relationship between corticospinal adaptations and strength gains

The study found no significant correlation between neuromuscular adaptations and the changes in strength parameters from pre- to post-training. However, the effect size between LEPs and isometric MVC was 0.66 estimated by the Hedges g, which could be carefully considered as a moderate effect. This finding could be carefully considered to be in line with previous research

studies that have reported that an increase in corticospinal excitability is accompanied by an increase in the strength or the force production (Tallent et al. 2017; Griffin & Cafarelli 2007; Kidgell et al. 2010; Kidgell et al. 2011; Mason et al 2020). In addition, this could be carefully considered to be in line with previous studies, which has demonstrated an increase in H-reflex or V-wave along with increase in strength (Aagaard et al. 2002; Vila-Châ et al. 2012). However, this study did not find any correlation between SP durations and changes in strength, which are controversial to the previous findings. As the previous research results have shown a reduction in the SP duration has been found to be associated with increase in the MVC (Christie & Kamen 2014; Kidgell & Pearce 2010; Latella et al. 2012).

The primary reason for not finding a significant correlation between corticospinal adaptations and changes in strength are probably due to the fact, that the study did not find a significant change in the MVC. As the MVC of the right knee and not the bilateral knee extension was used in the neuromuscular measurements. This again goes back to reflect that the training specificity, as previous studies have shown training a particular movement leads primarily to improvements in that specific movement (Häkkinen et al. 1996; Kidgell et al. 2010; Stien et al. 2020).

7.5 Neuromuscular adaptations to detraining

The study found only a significant reduction toward back to the baseline value in the one-repetition maximum of the bilateral knee extension after the detraining period. This finding is in line with the previous research, which has shown that after the third week of cessation of training a significant reduction in the maximal force was detected (Bosquet et al. 2013; Häkkinen et al. 2000). In addition, all other neural and strength parameters showed a trend back to baseline value after the detraining period, even though these changes were not significant.

These changes can be considered to be in line with the findings of Tallent et al. (2017), who found a decrease in corticospinal excitability after 2-weeks of the detraining period. Additionally, Yamanaka et al. (1999) found a decrease in the H-reflex after 20 days of bed rest, but not in the amplitude of MEPs. As Yamaka et al. (1999) study used a complete bed rest, these findings should be carefully implemented when discussing the effects of detraining. However, this could suggest that there could be spinal level adaptations as well during the detraining period.

Finally, as there are only a few studies published, which have investigated the effects of detraining on neuromuscular adaptations, these studies should be conducted more to make firm conclusions about the effects of detraining.

7.6 The study limitations

The study limitations consist of methodological issues, which could also have an effect on the study results. As the study used averaged M-max values obtained from LS and TMS measurement sessions, per measurement time point, it could have an influence over the neuromuscular results of the study. As MEPs and LEPs are both normalized to the amplitude of M-max. The averaging of the M-max from both sessions has most likely resulted in the fact that the M-max is not completely representing the peripheral excitability of the femoral nerve during the particular measurement session. This used methodology is controversial to the usually used normalization process and as such can be defined as a flaw in the methodology. As usual, the amplitude of M-max from the single session is used to normalize the other EMG responses from that particular session, such as MEPs (Millet et al. 2011; Tallent et al. 2019; Veira et al. 2015).

In addition, we should further discuss the training specificity as it can be considered the main reason (in addition to the small sample) size for not finding statistically significant changes in the MVC of the right knee, MEPs or SP durations. As many studies have shown that training particular kind of strength exercise movement leads to primarily improvement in the trained movement (Christie & Kamen 2014; Kidgell & Pearce 2010; Latella et al. 2012; Mason et al. 2020; Siddique et al. 2020; Stien et al. 2020; Tallent et al. 2017; Griffin & Cafarelli 2007). This fact can affect all of the neuromuscular adaptation results. As the main neuromuscular adaptations most likely happened in the bilateral knee extension movement and not in the isometric MVC of the right knee movement.

Additionally, only in a simple exercise movement like in knee extension. Gresswell & Ovendal (2002) have shown that bilateral knee extension results in 17% less muscle activity in the quadriceps femoris muscle, than the sum of unilateral knee extension. In addition, (Pincivero et al. 2006) has shown that the muscle activity during the concentric phase of knee extension is similar during all three quadriceps femoris muscles (VL, RF and BF muscles). However, during

isometric contraction the relative muscle activity of RF muscle is decreased, while the VL muscle activity is significantly increased. In addition, during 1RM of the bilateral knee extension the most active muscle seems to be VL muscle. (Pincivero et al. 2006) As such, the results of this study can be affected by the fact the study focused on neuromuscular adaptations of rectus femoris, while the main neuromuscular adaptations achieved by the bilateral knee extension could have happened in the vastus lateralis muscle.

7.7 The sources of errors

The sources of errors in the study design can be divided into different categories which are the study design, measurement protocol, and technical errors. The study design most likely causes a most serious error to the study results, as only eleven subjects were analyzed in the final data. As this can cause Type II error, caused by a small sample size which can result in the fact, that no significant result could not be found in many of the parameters as the variance of the variables is too high compared to the change in a variable. In addition, the training was conducted as a dynamic bilateral exercise and the neural measurements were performed during an isometric contraction. This could have resulted in that the transfer effect from the dynamic bilateral exercise to isometric contraction being too low, which could have caused the fact that no significant differences were found in the MVC or in most of the neuromuscular parameters.

The measurement protocol could have caused a systematic error to the data, as the one neural measurement lasted about 90 minutes, including multiple contractions. This could have caused muscle or mental fatigue to the subject, which could have influenced the measurement results. Even though, at least no neuromuscular fatigue was detected in the post-session M-max and isometric MVC.

The technical errors could have caused a random error in the data, especially in the MEPs results. As the finding of the hotspot with the TMS was conducted manually, which could have resulted in the false hotspot in the sessions. In addition, when manually identifying the hotspot the location of the hotspot between sessions or the true position of the TMS coil (angle, rotation, location) could not be identified. This could have affected the aMT values since the aMT is affected by the location of the hotspot and the location and position of the TMS coil.

8 CONCLUSION

In conclusion, the 7-week strength training resulted in a significant increase of 14% (19kg ± 5kg) in strength as in one-repetition maximum of bilateral knee extension ($F = 51.113$, $p<0.05$) and to a significant increment the spinal level as increment of 60% in the amplitudes of LEPs ($F = 11.073$, $p<0.05$). The one-repetition maximum also showed a significant decrease of -4% (5kg ± 1kg) towards back to baseline after detraining period ($p<0.05$). In addition, there was a significant difference across time in the mean active motor threshold of the rectus femoris muscle in MSO% ($F = 3.286$, $p<0.05$). However, post hoc test with Bonferroni pairwise comparisons of different measurement time points, did not reveal significant differences between time points ($p>0.05$). No significant differences were found in any of the other parameters.

These results show that 7-week strength training period increase the one-repetition maximum of the bilateral knee extension by 14%, which could be explained by the corticospinal adaptations at the spinal level. As the amplitude of LEPs increased by 60% after the strength training period. However, no correlation significant correlation was found between these two variables. The increase in the amplitude of LEPs could be due to increase in spinal reflexes and motor neuron pool excitability. As we only found a significant change in the LEPs, but not in the MEPs or SP durations. In addition, the study results show that the 5-week detraining period is sufficient to decrease the one-repetition maximum of bilateral knee extension strength towards back to baseline.

The study results can be considered as significant to the scientific community and public health. As this study is to my knowledge the first study that has found a corticospinal adaptation at the spinal level in the form of increased LEPs, after strength training. However, the implications of this data should be carefully considered due to the small sample size and the possible errors of the study, and the limitations of the study, which are discussed in the previous chapters. As such the results of this study cannot be generalized to the population level.

Further research will be needed about the corticospinal adaptations to strength training, particularly in the quadriceps femoris muscle group. In addition, further research will be needed on the electrical stimulation of the lumbar spine to make a firm conclusion about the site of adaptation and underlying adaptation mechanism at the spinal level.

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