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Neuromuscular activation strategies of voluntary and electrically elicited muscle fatigue

Underlying mechanisms and clinical implications

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Résumé

Introduction et objectif

La prise en charge thérapeutique de la fatigue musculaire par le biais d'exercices physiques vise à améliorer la qualité de vie et implique habituellement des exercices unilatéraux ou l'électrostimulation neuromusculaire pour compenser une fonction musculaire altérée aussi bien chez les personnes saines ou dites vulnérables (*e.g.* atteintes d'une pathologie ou d'un traumatisme). La fatigue musculaire est une réduction de la capacité de production maximale de force, induite par l'exercice, que la tâche puisse être maintenue ou non (Bigland-Ritchie et al. 1983; Gandevia 2001; Enoka and Duchateau 2008). L'objectif général de cette thèse était d'étudier les stratégies d'activation neuromusculaire lors de la fatigue musculaire, de l'endurance musculaire et sur la performance musculaire au cours et/ou après des contractions volontaires et évoquées électriquement chez des personnes saines ou vulnérables tels que des enfants atteints d'infirmité motrice cérébrale (IMC) et des patients atteints de dystrophie musculaire facio-scapulo-humérale (DMFSH).

Méthodes

Quatre études ont été conduites pour répondre à l'objectif général: 1) L'article 1 a examiné l'effet controlatéral de la fatigue musculaire après des exercices fatiguant isométriques maximaux unilatéraux des extenseurs du genou; 2) L'article 2 a étudié si un exercice induit par électrostimulation neuromusculaire délivré au niveau du tronc nerveux des muscles fléchisseurs plantaires de la cheville conduirait à des contributions périphériques et centrales de la fatigue musculaire différentes si l'intensité du courant était maintenue constante ou progressivement modulée; 3) L'article 3 a étudié si les muscles fléchisseurs du coude chez les enfants atteints d'IMC avaient une endurance musculaire aussi limitée que la force, et si les enfants atteints d'IMC pouvaient compenser la fatigue musculaire par le recrutement de nouvelles unités motrices comme le font les enfants sains; 4) L'article 4 a évalué l'effet d'un programme de rééducation par électrostimulation neuromusculaire (ESNM) sur la force et l'endurance du muscle tibialis anterior, et sur les fonctions motrices de patients atteints de DMFSH.

Principaux résultats et conclusions

Les principaux résultats indiquent que: 1) Un exercice fatigant unilatéral réduit la production maximale de moment de force du membre controlatéral non soumis à un exercice, mais cette perte de production de moment de force est différente selon le membre, et est retardée dans le membre controlatéral. Cet effet dit « croisé » serait une stratégie de compensation pour réduire la différence de performance musculaire entre le membre affaibli par l'exercice fatigant et le membre controlatéral et ce, probablement pour maintenir la coordination entre les membres. 2) Un protocole d'ESNM délivré au niveau du tronc nerveux, que l'intensité du courant soit maintenue constante ou progressivement augmentée, a induit une fatigue musculaire identique impliquant des adaptations périphériques et centrales semblables. Ainsi, pour une demande énergétique comparable, maintenir l'intensité du courant comme l'augmenter produirait une quantité de fatigue musculaire équivalente. 3) Même si la capacité de production de force des muscles fléchisseurs du coude et le temps de maintien de l'exercice étaient similaires dans les deux groupes, les enfants atteints d'IMC présentaient une plus faible augmentation du signal électromyographique (EMG) pendant la tâche d'endurance. Ceci suggère que les enfants atteints d'IMC n'ont pas été capable de recruter des unités motrices supplémentaires pour pallier à la fatigue musculaire pendant la contraction isométrique prolongée de flexion du coude à faible niveau de force. 4) Il n'y a pas eu d'amélioration de la force et de l'endurance musculaire du muscle tibialis anterior, ni des fonctions motrices après l'entraînement par ESNM. Il est possible que le programme d'ESNM n'ait pas été suffisamment intense, et/ou que les paramètres de stimulation n'aient pas été adéquats dans la mesure où aucun gain n'a également été observé chez les participants sains. Cependant, le groupe de patients atteints de DMFSH a montré une perte de force moindre pendant la contraction maximale volontaire prolongée de deux minutes, indiquant qu'ils ont éprouvé moins de fatigue musculaire que le groupe contrôle.

Sur le plan clinique, identifier les méthodes permettant de limiter la fatigue musculaire et développer des exercices physiques de rééducation est fondamental et une recherche plus approfondie doit être conduite sur les stratégies d'activation musculaire, en particulier chez les patients atteints de maladies neuromusculaires. Les effets bénéfiques

potentiels du phénomène « croisé » de la fatigue, voire de l'entraînement, et de la sollicitation musculaire par ESNM devraient être examinés tout en considérant la spécificité des troubles neuromusculaires et les altérations de l'activation musculaire qui y sont associées.

Sammendrag

Bakgrunn og hensikt

Den kliniske behandlingen av muskeltretthet ved hjelp av treningsterapi tar sikte på å forbedre livskvaliteten og involverer normalt unilaterale øvelser eller nevromuskulær elektrostimulering for å kompensere for svekket muskelfunksjon hos friske og pasient grupper. Muskeltretthet er en reduksjon i maksimal kraftproduksjon, indusert av aktivitet, uavhengig av om en gitt oppgave kan opprettholdes eller ikke (Bigland-Ritchie et al. 1983a; Gandevia 2001a; Enoka and Duchateau 2008). Hensikten med denne avhandlingen var å studere effekten av nevromuskulære aktiveringsstrategier under muskeltretthet, utholdenhet og muskelprestasjon etter frivillige og elektrisk induserte muskelsammentrekninger hos friske og personer med cerebral parese (CP) og facioscapulohumeral muskeldystrofi (FSHD).

Metode

Fire studier er gjennomført i henhold til hensikten med avhandlingen: 1) artikkel I undersøkte overføringseffekten av muskeltretthet til den kontralaterale siden etter en unilateral maksimal isometrisk utmattende kneekstensjonsøvelse; 2) artikkel II undersøkte om en nevromuskulær elektrostimuleringsøvelse påført over nerven til ankel plantarfleksorene ville føre til forskjellige sentrale og perifere bidrag i forhold til muskeltretthet når man sammenlignet en konstant stimuleringsstrøm med en progressiv modulering av strømstyrken; 3) artikkel III evaluerte om albuefleksorene hos barn med cerebral parese har en like begrenset utholdenhet som styrke og hvorvidt de kan kompensere for muskeltretthet med rekrutering av fler motoriske enheter som ved friske; 4) artikkel IV undersøkte effekten av et rehabiliteringsprogram, som benyttet seg av nevromuskulær elektrostimulering (NMES) av tibialis anterior, på muskelstyrke, utholdenhet og motorisk funksjon hos pasienter med FSHD.

Hovedfunn og konklusjoner

Hovedfunnene i avhandlingen er: 1) En unilateral utmattende øvelse svekket det maksimale dreiemomentet hos det kontralaterale, ikke arbeidende, lemmet. Det skilte også i tidsavhengig tap av dreiemoment mellom lemmene, hvor tap av dreiemoment var

forsinket i det ikke arbeidende lemmet. Denne overføringseffekten kan være en kompensasjonsstrategi for å redusere forskjellen i muskelprestasjon mellom utmattete og den ikke-utmattet side, trolig for å opprettholde koordinasjon; 2) En NMES protokoll påført over nerven med konstant eller økende strømstyrke, induserte lignende perifere og sentrale tilpasninger til muskeltretthet. Derfor, for et likt energiforbruk, vil å opprettholde strømstyrken, som ved å øke den, føre til en lignende muskeltretthet; 3) Selv om både styrke og tid til man ikke lenger var i stand til å gjennomføre oppgaven var lik i begge gruppene, viste barna med CP en lavere økning i EMG amplitude. Dette indikerer at barn med CP ikke er i stand til å rekruttere ytterligere motoriske enheter for å kompensere for muskeltretthet under en varig, lavbelastnings isometrisk albuefleksjonskontraksjon; 4) Rehabiliteringsprogrammet førte ikke til forbedring i verken styrke, utholdenhet eller motorisk funksjon i tibialis anterior. Kanskje var ikke NMES protokollen tilstrekkelig belastende og/eller parameterne i forhold til stimuleringen var utilstrekkelige ettersom heller ikke hos friske noen forbedring ble sett. Derimot viste pasientene med FSHD lavere krafttap under en 2 minutter maksimal frivillig kontraksjon, noen som antyder at de opplevde en lavere grad av muskeltretthet sammenlignet med den friske gruppen.

Å begrense muskeltretthet og å utvikle rehabiliteringsstrategier trengs videre forskning med tanke på muskelaktiveringsmønstre ved muskeltretthet og spesielt hos de med nevromuskulære tilstander. De potensielt gunstige overførings og NMES effektene bør undersøkes videre med tanke på det store spekteret av nevromuskulære tilstander og tilhørende muskel aktiveringssvekkelserne.

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Preface

This thesis is a collaborative work within a cotutelle agreement between the University of Nice-Sophia Antipolis, France (UNS) and the Norwegian University of Science and Technology, Norway (NTNU), co-supervised by Pr Serge Colson (UNS) and Pr Karin Roeleveld (NTNU).

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List of papers

Paper I

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Paper II

Doix A-CM, Matkowski B, Martin A, Roeleveld K, Colson SS. Effect of neuromuscular electrical stimulation intensity over the tibial nerve trunk on triceps surae muscle fatigue. Article manuscript.

Paper III

Doix A-CM, Gulliksen A, Brændvik SM, Roeleveld K (2013). Fatigue and muscle activation during submaximal elbow flexion in children with cerebral palsy. J Electromyogr Kinesiol 23:721–726. doi: 10.1016/j.jelekin.2012.12.005. Epub 2013 Jan 31.

Paper IV

Doix A-CM, Roeleveld K, Colson SS. Neuromuscular electrical stimulation training of the tibialis anterior in facioscapulohumeral muscular dystrophy patients. Article manuscript.

Abstract

Background and objective

The clinical care of muscle fatigue with exercise therapies aim at quality of life improvement and usually involve unilateral exercises or neuromuscular electrical stimulation to compensate impaired muscle function in both healthy and health-compromised people. Muscle fatigue is a decline in maximal force production, induced with exercising, whether or not the task can be maintained (Bigland-Ritchie et al. 1983a; Gandevia 2001a; Enoka and Duchateau 2008). The overall objective of this thesis was to study the effect of neuromuscular activation strategies during muscle fatigue, endurance and muscle performance after voluntary and electrically evoked contractions in healthy and health-compromised populations such as children with cerebral palsy (CP) and patients with facioscapulohumeral muscular dystrophy (FSHD).

Methods

Four studies aimed at fulfilling the overall objective: 1) paper I investigated the cross-over effect of muscle fatigue following unilateral knee extensors maximal isometric fatiguing exercise; 2) paper II studied whether a neuromuscular electrical stimulation exercise, delivered over the nerve trunk of the ankle plantar flexors would lead to differential peripheral and central contributions of muscle fatigue when comparing a constant intensity with a progressive modulation of the current intensity; 3) paper III evaluated whether the elbow flexor muscles of children with cerebral palsy have a muscle endurance as hampered as strength, and whether they can compensate muscle fatigue with recruitment of additional motor units as typically developing peers; 4) paper IV examined the effect of a rehabilitation program using neuromuscular electrical stimulation (NMES) on tibialis anterior muscle strength, endurance and motor function in patients with FSHD.

Main findings and conclusions

The main results indicate that: 1) A unilateral fatiguing exercise impaired the maximal torque production of the contralateral non-exercising limb, but the time course of torque production loss differed between limbs, being delayed in the non-exercising limb. This

cross-over effect could be a compensatory strategy to reduce the gap of muscle performance between the weaker, exercised, limb and the non-exercised one likely to warrant interlimb coordination; 2) A NMES protocol delivered over the nerve trunk, maintaining constant or increasing the current intensity induced similar peripheral and central adaptations of muscle fatigue. Therefore, for similar energy expenditure, maintaining the current intensity constant, as when increasing it, would yield the same amount of muscle fatigue. 3) Even though strength and time to task failure were similar in both groups, the children with CP exhibited a lower increase in EMG amplitude compared to typically developing peers. This suggests that the children with CP were not able to recruit additional motor units to compensate for muscle fatigue during a sustained low-force level isometric elbow flexion contraction; 4) No tibialis anterior muscle strength, endurance and motor function improvements were observed after the training. Maybe, the NMES protocol was not strenuous enough and/or parameters of stimulation were not adequate as no improvements were observed in healthy participants either. However, the group of patients with FSHD showed lower force losses during the 2-minute sustained MVC, suggesting that they were experiencing a lower amount of muscle fatigue compared to the healthy participants group.

Limiting muscle fatigue and developing rehabilitation strategies is needed and further research should be conducted regarding the muscle activation patterns with muscle fatigue, especially in neuromuscular disorders. The potential beneficial cross-over and NMES effects should be further investigated with regards to the variety of neuromuscular disorders and the associated muscle activation impairments.

1 Introduction

Muscle fatigue is an exercise induced reduction in maximal force production, whether or not the task can be sustained. Muscle fatigue is caused due to a combination of processes from the performing muscles and the nervous system (Bigland-Ritchie et al. 1983a; Gandevia 2001a; Enoka and Duchateau 2008).

The combination of surface electromyography (EMG) with percutaneous nerve stimulation is a method commonly used to evaluate changes of the neuromuscular system by investigating their electrical activity. The action potential originating from the central nervous system (CNS) travels towards the muscle where it propagates over the membrane of the muscle fibres, causing activation. The EMG technique measures the physiological electrical activity with the use of electrodes positioned over the surface of the active muscles. Percutaneous nerve stimulation bypasses the voluntary drive and activates muscles directly by depolarising the motor nerve. This allows the use of the interpolation twitch technique (ITT) (Merton 1954) which consists of superimposing electrical stimulation onto a maximal voluntary contraction to assess the voluntary activation level. This voluntary activation level refers to the quantity of voluntary descending drive to the motorneuron pool and muscles. Both techniques can be combined to investigate the neuromuscular system in humans (Bigland-Ritchie and Woods 1984; Gandevia 2001a). When used together, percutaneous nerve stimulation and EMG can unveil central and peripheral components of muscle fatigue; this is used widely in the field of neuromuscular physiology. In this thesis, these techniques were applied on both healthy and health-compromised population for the purpose of investigating muscle fatigue.

Health status is a key factor in determining the ability to perform prolonged exercise or daily activities, and fatigue is a widely reported symptom in patients with neuromuscular disorders (Chaudhuri and Behan 2004; Féasson et al. 2006; Schillings et al. 2007a; Garssen et al. 2007; Kalkman et al. 2008; Angelini and Tasca 2012). The variety of pathophysiological mechanisms and associated symptoms of neuromuscular disorders challenge the understanding and the clinical care of muscle fatigue. Focusing on muscle fatigue in a rehabilitation setting is crucial since it is among the top ranked

symptoms of neuromuscular disorders and patients experience difficulties to perform high intensity or long duration exercises (Féasson et al. 2006; Zwarts et al. 2008). Moreover, besides the actual survival goal of the therapeutic care in neuromuscular disorders, the improvement of the quality of life has become an important rehabilitation goal, often involving improvement of daily activity through exercising. The numerous therapeutic strategies to overcome deficits due to fatigue usually focus on unilateral exercise. When one limb is injured, or more seriously impaired than the contralateral limb, a common approach is to focus on the rehabilitation of the impaired limb. The goal is to enhance strength and endurance of the weaker limb, but it has been shown that this type of rehabilitation also leads to the improvement of the non-trained, contralateral limb. This limb interplay is the cross-education effect (Enoka 1988; Carroll et al. 2006; Fimland et al. 2009) and a similar cross-transfer of muscle fatigue occurs as well (Rosenbaum 1977; Zijdewind et al. 1998). Amongst the variety of therapeutic strategies, neuromuscular electrical stimulation (NMES) has gained in popularity and has become broadly used for rehabilitation purposes (Burridge and Ladouceur 2001; Bax et al. 2005a; Maffiuletti 2010). The goal of NMES is to generate a muscle contraction to replace or complete the voluntary muscle activation via direct depolarisation of the motor axons. This action also indirectly activates the spinal motorneurons via Ia afferents in mixed nerves (with stimulation over the nerve trunk) or intramuscular axonal branches (with stimulation over the muscle) (Hultman et al. 1983; Collins 2007; Bergquist et al. 2011b). However, a limitation of the use of NMES is the greater muscle fatigue rate compared to voluntary contractions because of the repeated and fixed recruitment of the same muscle fibres (Vanderthommen et al. 2003; Maffiuletti 2010).

The present introduction is three-fold where the first part will focus on the underlying mechanisms of muscle fatigue and the cross-over effect during muscle fatigue. The second part will address muscle fatigue in neuromuscular disorders and finally, the third part will discuss the use of neuromuscular electrical stimulation and its effects on the muscle activation and fatigue.

2 Muscle fatigue

The motor pathway carries signals from the brain to muscles and involves processes occurring at the central nervous system (CNS) and at the peripheral level until the muscle, where force is produced. A volitional muscle contraction arises from a neural descending motor command initiated at the motor cortex level that activates the skeletal muscles. The motor command originates from central processes, either of motivational or of sensory origins, and a volley of action potentials (AP) is generated. The action potential travels through the sensorimotor cortex and the cerebellum and thereafter travels along the spinal cord towards the alpha-motorneurons (α-MNs). Eventually, the AP hits the targeted muscle fibres via the neuromuscular junction at the synapse level (Enoka 2008). Then, the muscle membrane depolarises via the voltage-gated sodium (Na^+) potassium (K^+) ion channels (Na^+-K^+ pump) which triggers the release of the calcium ions (Ca^{2+}), from the sarcoplasmic reticulum that bind to the troponin. The adenosine triphosphate (ATP) is hydrolysed on the myosin head which rotates and binds to the actin, and splits into adenosine diphosphate (ADP) and inorganic phosphate (Pi). This causes the muscle to contract.

Muscle fatigue may arise at any level of these motor pathway processes. It refers to an evolving and reversible state modifying the functional performance of the motor system. Muscle fatigue is widely defined in the literature as a decrement of maximal force production, induced by sustained or repeated muscle contractions, whether or not the task can be maintained (Bigland-Ritchie et al. 1983a; Gandevia 2001a; Enoka and Duchateau 2008). Since a muscle contraction depends on a combination of the muscle fibres and of the CNS activities, the limiting sites yielding to muscle fatigue can be distinguished at two levels. Muscle fatigue can arise at the peripheral level (*i.e.* within the muscle fibre itself) (Allen et al. 2008a) here the muscle is simply incapable of sustaining the initial force production. Muscle fatigue can also develop at the central level, above the neuromuscular junction (Gandevia 2001a), here the central nervous system is unable to provide enough input to the muscle which is still able to contract and produce force.

2.1 Peripheral fatigue

Peripheral fatigue comprises ionic and metabolic changes at the muscle fibre level that could result in impaired action potential propagation and excitation-contraction coupling, which leads to a decrease of the mechanical muscle response (Allen et al. 2008a; Enoka and Duchateau 2008).

Muscle fatigue induces ionic changes such as the electrical activity of the membrane will be impaired and the Na^+ / K^+ balance will no longer be respected, K^+ ions accumulated outside of the membrane block Na^+ channels and the conduction velocity of the AP over the membrane decreases (from 2 up to 6 $m.s^{-1}$ in rested condition to <0.3 $m.s^{-1}$ when fatigued) (Nakajima and Gilai 1980; Lännergren and Westerblad 1987; Allen et al. 2008a). The sarcolemma and the T-tubules may no longer be capable of conducting the AP and the membrane excitability fails with fatigue. The Ca^{2+} ions accumulate in the T-tubules as they become unable to release and reuptake the Ca^{2+} ions from the sarcoplasmic reticulum (Bianchi and Narayan 1982). First, the Ca^{2+} release decreases, attenuating binding of the Ca^{2+} - Troponin and thus a failure in the actin-myosin cross-bridges (Westerblad et al. 1991). Besides the alteration in the excitation-contraction coupling, ionic changes impair the ability of the muscle fibre to contract. The metabolites and a reduced pH (lactate accumulation and acidosis) will also reduce the binding of Ca^{2+} on Troponin (Westerblad et al. 1991). Also, the hydrolysis of creatine phosphate (PCr) to creatine and Pi impairs the contractile function in fatigue (Westerblad et al. 2002a; Allen et al. 2008a).

The M-wave (the compound muscle action potential) is the motor response evoked by maximal percutaneous nerve stimulation that propagates orthodromically in the motor axons, generally observed 5-8 ms after the onset of electrical stimulation, reflecting the maximal muscle activation (Tucker et al. 2005). As the AP propagation and the excitability of the membrane are impaired, the amplitude of the M-wave is found to decrease. When a reduced M-wave is observed, the neuromuscular propagation is impaired (Fuglevand et al. 1993; Tucker et al. 2005; Allen et al. 2008a), suggesting the occurrence of fatigue of peripheral origin. As the membrane excitability decreases, the amplitude of the M-wave is reduced and also changes in the M-wave shape (*e.g.* widening) can also result (Brody et al. 1991).

The muscles afferents provide a link between the periphery and the CNS, as they inform the α-MNs and supraspinal centres in a feedforward and feedback control loop (Enoka and Stuart 1992; Gandevia 2001a; Dimitriou and Edin 2010). The integration of afferent feedback information is processed by the CNS via sensory receptors that can evoke rapid responses known as reflexes (Pierrot-Deseilligny and Burke 2005).

The main afferents are categorised in four groups: I, II, III and IV (Prochazka 2010). The muscle spindles, Ia and II afferents, are located in parallel to the muscle fibres and are innervated by the gamma motorneurons (γ-MNs) concurrently activated with the α-MNs. They are sensitive and discharge according to the change and rate of change in muscle length (Edin and Vallbo 1990). The Golgi tendon organs (Ib) are located in the tendons and are involved in the force exerted by the muscles. Also, the small diameter muscle afferents (groups III and IV) provide information regarding the strain and the metabolic milieu modifications in the muscle (Ellaway et al. 1982; Mense and Stahnke 1983; Kaufman and Rybicki 1987; Clarke et al. 1996; Hayes et al. 2005). Finally, cutaneous mechanoreceptors are also involved in the regulation of the muscle contraction as they specifically provide the CNS with the mechanical and kinematic stimuli (Edin and Abbs 1991). The afferent axons enter the spinal cord through the dorsal roots and make connections with α-MNs via one or several interneurons (Pierrot-Deseilligny and Burke 2005; Prochazka 2010) and are the link between the periphery and the CNS.

2.2 Central fatigue

Modifications due to prolonged exercise also occur at the nervous system level. Central fatigue entails adaptations occurring above the neuromuscular junction. The neural drive or the transmission of the drive to the muscle fibres through the α-MNs at the spinal level can be modified (Gandevia 2001a; Enoka and Duchateau 2008). At the motor cortex level, it has been shown that several brain areas increase their activity with fatigue. Indeed, the sensorimotor cortex, prefrontal cortex, cingulate gyrus, supplementary motor area, and cerebellum showed a steady increment during a fatiguing task (Liu et al. 2003). The common descending drive to the muscles increases

to compensate for fatigue, and additional MUs are recruited (Gandevia 2001a; Enoka and Duchateau 2008). Also, fatigue may impair the voluntary descending neural drive - *i.e.* the level of firing frequency of the stimulus which reduces the MUs activation, hence causing a decline of α-MN discharge rate (Bigland-Ritchie et al. 1986; Taylor et al. 2006). Muscle fatigue has been shown to raise the sensitivity of Ia and II afferents (Nelson and Hutton 1985; Christakos and Windhorst 1986; Zytnicki et al. 1990), to reduce (Bongiovanni and Hagbarth 1990; Macefield et al. 1991) responsiveness of the α-MNs and to decrease the MUs discharge rate. Concomitantly, groups III and IV afferents have been shown to act as a negative feedback loop onto the α-MNs (Bigland-Ritchie et al. 1986; Woods et al. 1987) and are also likely to increase the presynaptic inhibition (Duchateau and Hainaut 1993; Kostyukov et al. 2005). Thus, muscle afferents (muscle spindles, Golgi tendon organs, groups III and IV muscles afferents) inhibition mechanisms also explain the decrement of firing rate (Kernell and Monster 1982a; Kernell and Monster 1982b; Bigland-Ritchie et al. 1986).

At the spinal level, because of the accumulation of metabolites due to slowing down of Na^+-K^+ pumps along the membrane, the activation threshold of motor axons increases (Vilin and Ruben 2001; Kiernan et al. 2004; Miles et al. 2005) thus decreasing the number of active motor units with a similar input. It has been observed that the constant descending drive increases the threshold of low-threshold MUs and decreases the threshold of high-threshold MUs (De Luca and Erim 1994; Carpentier et al. 2001), even though a reduction of threshold can be measured directly after a α-MN is recruited (Gorassini et al. 2002). Eventually, the increased release of serotonin from the brain stem reduces α-MN excitability (Taylor et al. 1999; Gandevia 2001a; Andersen et al. 2003; Butler et al. 2003; Martin et al. 2006) and thus affects MU recruitment (Bigland-Ritchie et al. 1983a; Bigland-Ritchie et al. 1986), or impairs MUs discharge rates. Not only the decrease in MUs discharge rate is explained by a reduction in the descending drive, but also by an increase in inhibitory input and also by a modification in α-MN responsiveness due to their intrinsic properties (Andersen et al. 2003; Butler et al. 2003; Martin et al. 2006).

The use of voluntary contraction and non-invasive percutaneous nerve stimulation combined with surface electromyography allows the exploration of fatigue in humans

(Bigland-Ritchie and Woods 1984; Gandevia 2001a). This classical approach permits the investigation of the modification of the descending drive to the α-MNs, which reflects the individual capacity of MU activation. If extra force can be evoked by motor nerve stimulation with the interpolation twitch technique (ITT) (Merton 1954) it can be concluded that some MUs were not recruited or were not firing fast enough to produce fused contractions at the moment of stimulation (Herbert and Gandevia 1999). With fatigue, when using the ITT, an increase of evoked force indicates that the voluntary activation level progressively decreases, as the descending neural drive becomes suboptimal, but the absence of evoked force does not discard it (Gandevia, 2001). Percutaneous nerve stimulation is a useful technique to distinguish central fatigue from peripheral fatigue (Merton 1954; Gandevia et al. 1995), while simultaneously analysing: 1) the maximal voluntary contractions, 2) the maximal voluntary activation level (Merton 1954), and 3) the mechanical and electrophysiological responses (the M-wave, the Hoffman reflex and the V-wave seen in the EMG) elicited by the electrical nerve stimulation (see Figure 1).

Furthermore, the study of the Hoffman reflex (H-reflex) due to a percutaneous nerve stimulation, permits the assessment of the changes in spinal reflex pathways (Schieppati 1987; Zehr 2002). While the M-wave is the direct evoked motor response, the H-reflex is elicited because the electrical volley propagates antidromically, bypassing the gamma motorneurons and the muscle spindle discharge and involves both afferent sensory and efferent axons (Schieppati 1987; Zehr 2002). With the electrical stimulation, Ia afferent fibres are depolarised and cause neurotransmitter release at the Ia afferent / α-MN synapse within the spinal cord generating a motor response (Schieppati 1987; Zehr 2002). Because of the longer distance involving the spinal circuitry, the H-reflex latency is greater than the M-wave and is observable in the EMG signal 30-45 ms after the onset of electrical stimulation (Schieppati 1987; Zehr 2002; Tucker et al. 2005). Moreover, since the Ia afferent fibres are, on average, larger than the efferent α-MNs fibres, the activation threshold of the H-reflex is lower than the M-wave's (Schieppati 1987; Zehr 2002; Tucker et al. 2005). With muscle fatigue, the H-reflex is found to be depressed, such as it accounts for the decreased responsiveness of the α-MNs (Schieppati 1987; Gandevia 2001a). When the stimulation at the M-wave intensity is

superimposed over the MVC, an electrophysiological variant of the H-reflex is observed, this is the first volitional wave, the V-wave (Upton et al. 1971). The activation of motorneurons via the descending neural drive collide with the antidromic electrical volley and a part of the evoked reflex response will reach the muscle (Aagaard et al. 2002). The V-wave is used to study the central, supraspinal adaptations to muscle fatigue, as it represents the magnitude of the neural drive to the α-MNs (Upton et al. 1971; Aagaard et al. 2002).

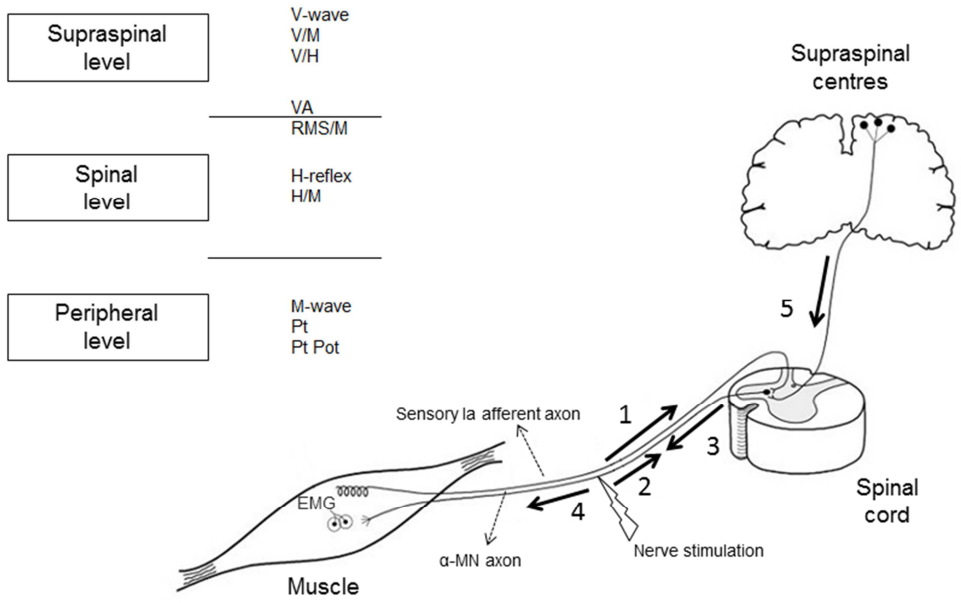


Figure 1. Schematic view of the different evoked potentials to investigate sites of muscle fatigue with the use of electrical nerve stimulation, adapted from (Aagaard et al. 2002; Millet et al. 2011). When the nerve is stimulated by a low-intensity electrical current, the electrical volley travels towards the CNS along the sensory Ia afferent axons (1) because of their lower activation threshold compared to the alpha-motorneuron axon (α-MN). At the spinal cord it activates the postsynaptic transmission and the electrical volley is conducted to the muscles via the α-MN axons (3) this is known as the H-reflex. When the nerve is stimulated at a higher intensity electrical current, the electrical volley travels directly along the α-MN axons towards the muscle (4), this is the M-wave. At the same time, the electrical volley propagates antidromically towards the spinal cord (2) and collides with the electrical volley of the evoked reflex response (1 and 3), partially cancelling the reflex response. With the stimulation increase the antidromic collision will increase and while the M-wave increases, the H-reflex decreases. When the nerve stimulation is applied during maximal voluntary muscle contraction, the voluntary descending drive will collide with the antidromic electrical volley (2), and a part of the evoked reflex response will reach the muscle, this is the V-wave. V/M: V-wave normalised to the M-wave; V/H: V-wave normalised to the H-reflex; VA: Voluntary activation level assessed with the interpolation twitch technique: RMS/M: EMG root-mean-square measured during maximal voluntary contraction normalised to the M-wave; H/M: H-reflex normalised to the M-wave; Pt: Peak twitch, mechanical response associated with the M-wave; Pt Pot: Potentiated Peak twitch, potentiated mechanical response associated with the M-wave following a maximal voluntary contraction.

Muscle fatigue is a multifaceted phenomenon, comprising peripheral, central and also cognitive factors (Gandevia 2001a). Thus, some cognitive factors such as motivation or

perception of the task can have an impact on central fatigue. All stimuli from the cortical area are mediated by neurotransmitters that will disturb the cerebral homeostasis and contribute to central fatigue. Furthermore, some parameters including the intensity, the time of the exercise, the type of load, the fibre composition of the measured muscle, the gender, the age, the training level, the health status or the type of contraction, will each induce specific neuromuscular modifications. In this thesis, submaximal and maximal voluntary force contractions have been used to induce muscle fatigue. Investigations have been directed at determining which force levels can be attributed to different neuromuscular strategies.

2.3 Level of contraction on muscle fatigue

2.3.1 Fatiguing isometric contractions at submaximal force level

During repetitive or sustained submaximal contractions, muscle activity as measured with EMG increases in amplitude (Basmajian and De Luca 1985). Furthermore, EMG signals show decreases in spectral frequencies (De Luca 1997; Karlsson et al. 2009). In addition, an increase of activity bursts are also seen in the EMG signal (Hunter and Enoka 2003; Enoka and Duchateau 2008). These changes in the EMG signal can be explained by an increase in the neural drive (Enoka and Duchateau 2008), recruitment of additional higher threshold MUs (Enoka et al. 1989), a decrease in firing rate, an increase in firing synchronisation and/or a decrease in the muscle fibre action potential propagation velocity (Karlsson et al. 2009). The amplitude increase observed in the EMG signal during submaximal sustained contractions suggests that muscle fatigue is compensated by the recruitment of additional MUs (De Luca 1997; Farina et al. 2004). However, a simulation study (Yao et al. 2000), showed that MU synchronisation can be responsible for the EMG amplitude increase and therefore responsible for force fluctuations in submaximal contractions. But it seems that the increase in EMG amplitude is likely to be dependent on the number of active MUs (Suzuki et al. 2002). During a constant submaximal force level contraction, the pool of active MUs undergoes an important compensatory mechanism of rotation (Westgaard and de Luca 1999; Bawa et al. 2006). The discharge rate of MUs varies and a cyclic alternative

substitution of a MU activation occurs, providing a metabolic rest for the contractile apparatus to offset muscle fatigue (Bawa et al. 2006). This cyclic unsynchronised activity is necessary to ensure a continuous contraction as it is acknowledged that MUs synchronisation and increase in force fluctuations are correlated (De Luca et al. 1982). However, fatigue impairs the ability of neural control to increase synchronisation of MU discharge (Sears and Stagg 1976). The synchronisation of MU activation contributes to the change of frequency content of the EMG signal towards lower frequencies observed due to fatigue (Krogh-Lund and Jørgensen 1992), as well as the decline in conduction velocity (CV) of the AP over the muscle fibre membrane (Merletti et al. 1990; De Luca 1997; Farina et al. 2004). This decline of conduction velocity can be attributed to the alteration in the Na^+/ K^+ transmembrane flow balance reducing the velocity of the AP propagation (Arendt-Nielsen and Zwarts 1989).

2.3.2 Fatiguing isometric contractions at maximal force level

The use of maximal voluntary contractions to investigate muscle fatigue is insightful because it tests the entire motor pathway (Taylor and Gandevia 2008). During maximal contractions, fatigue develops within seconds, the maximal voluntary force decreases almost immediately, and the superimposed twitch evoked by motor nerve stimulation increases (Bigland-Ritchie et al. 1983b; Gandevia et al. 1996). Maximally sustained contractions involve both central and peripheral level adjustments. At the peripheral level, after a 4-minute sustained isometric MVC of the ankle dorsiflexion muscles (Kent-Braun 1999), the pH decreases as the H^+ concentration increases and PCr is synthesised; this accounts for 80% of the developed fatigue. As the force production declines within seconds, force fluctuations increase and the MU firing rate reduces within 10 to 20 seconds (Bigland-Ritchie et al. 1983a; Bigland-Ritchie et al. 1986). It is probable that the slowing down of the MU firing rate is due to a reduction in the neural drive and/or to a diminished excitability of α-MNs. Additionally, the amplitude of the EMG signal during sustained maximal voluntary contraction is found to decrease (Bigland-Ritchie et al. 1983b). Indeed, supraspinal fatigue induces a suboptimal corticaloutput (Gandevia et al. 1996) and group III and IV muscles afferents are likely to negatively impact the descending neural drive (Taylor et al. 2006; Amann et al.

2008). Also, it seems that intracortical inhibition increased during a 2-minute MVC of the elbow flexor muscles (McNeil et al. 2009).

2.4 The cross-over effect of muscle fatigue

Rehabilitation exercises are mostly performed unilaterally and it has been shown that an unilateral exercise may modify the performance of the contralateral homologous muscle (Zijdewind et al. 1998; Zijdewind and Kernell 2001; Todd et al. 2003; Rattey et al. 2006; Martin and Rattey 2007; Post et al. 2008) or limb (McLean and Samorezov 2009; Berger et al. 2010a; Paillard et al. 2010). During a unilateral task, the muscle activity can irradiate in the non-exercising muscles (Zijdewind and Kernell 2001). Also, it has been shown that unilateral muscle fatigue can spread to the non-targeted contralateral homologous muscles and this phenomenon is known as the contralateral effect of muscle fatigue (Zijdewind et al. 1998; Zijdewind and Kernell 2001; Todd et al. 2003; Rattey et al. 2006; Martin and Rattey 2007; Post et al. 2008).

The MVC of the rested contralateral muscle group has been shown to decline after a unilateral fatiguing exercise of the ipsilateral homologous muscle both after maximal voluntary contraction fatiguing protocol (Martin and Rattey 2007; Post et al. 2008). On the other hand, some studies failed to show a MVC reduction of the homologous contralateral muscles after an unilateral fatiguing exercise in the upper limbs (Zijdewind et al. 1998; Zijdewind and Kernell 2001; Todd et al. 2003) and in the lower limbs (Rattey et al. 2006; Berger et al. 2010a; Paillard et al. 2010). Therefore, a decline of MVC of the contralateral limb after a unilateral fatiguing exercise does not always result. Yet, the cross-over effect of muscle fatigue has been observed (Zijdewind et al. 1998; Todd et al. 2003; Rattey et al. 2006; Martin and Rattey 2007; Post et al. 2008). Some authors have reported a concomitant decline of the voluntary activation with the decline in MVC (Martin and Rattey 2007; Post et al. 2008). The heterogeneity of fatiguing protocols used may partly account for these discrepancies.

In line with reports showing cross-over effects, functional impacts have been shown as a lower-limb unilateral fatiguing exercise alters bipedal postural control (Vuillerme et al. 2009; Vuillerme and Boisgontier 2010; Berger et al. 2010a; Paillard et al. 2010). This contralateral effect of muscle fatigue has been shown not only to be present in the

homologous contralateral muscle, but can spread towards other muscles groups (Kennedy et al. 2012). It is likely that central mechanisms at the spinal and supraspinal levels occur: at the spinal level, the amplitude of the contralateral H-reflex has been shown to decrease (McIlroy et al. 1992; Hortobágyi et al. 2003; Carson et al. 2004); at suprapsinal level, possible inter-hemispheric regulation through transcallosal pathways (Meyer et al. 1995).

Nonetheless, these aforementioned mechanisms, although of a broad variety, may differ according to the integrity of the neuromuscular system and muscle fatigue is a frequently reported symptom in patients with a neuromuscular disorder (Chaudhuri and Behan 2004; Féasson et al. 2006; Schillings et al. 2007a; Garssen et al. 2007; Kalkman et al. 2008).

3 Neuromuscular disorders

3.1 Neuromuscular disorders and fatigue

The expression neuromuscular disorders itself is broad as it comprises neurogenic (*e.g*. cerebral palsy) and myogenic disorders (*e.g.* facioscapulohumeral muscular dystrophy). These diseases are acquired or inherited and encompass a variety of impairments arising either from the brain, the anterior horn of the spinal cord, the neuromuscular junction or even from the muscle fibre itself (Féasson et al. 2006). Because of motor impairments associated with these pathologies, they are commonly referred to as neuromuscular disorders. It has been reported that fatigue is a common symptom in neuromuscular disorders (Chaudhuri and Behan, 2004; Féasson et al., 2006; Garssen et al., 2007; Schillings et al., 2007; Kalkman et al., 2008).

For instance, fatigue has been reported in several neuromuscular disorders such as in patients with cerebral palsy (Jahnsen et al., 2003), post-polio syndrome (Sunnerhagen and Grimby, 2001), facioscapulohumeral muscular dystrophy (Kalkman et al., 2008), myasthenia gravis (Paul et al., 2000), or Guillain-Barré syndrome (Merkies et al., 1999). However, it must be noted that fatigue in these cases is often subjective and refers to an

overall feeling of weakness or a lack of energy, rather than the muscle/physiological fatigue. This experienced fatigue relates to two factors: a psychological one and a physiological one (Zwarts et al., 2008). Additionally, associated symptoms such as spasticity, muscle weakness, pain, cramps or myotonia (Zwarts et al., 2008) alter motor control and muscle force production. In this thesis, the muscle performance in children with cerebral palsy and in adults with facioscapulohumeral muscular dystrophy is investigated.

3.2 Neuromuscular impairments in Cerebral Palsy

With a European prevalence of 1.5-3 per 1000 live births (Cans, 2000), cerebral palsy (CP) is the most frequent neurological disorder in children. Cerebral palsy is a clinically descriptive term that comprises a variety of motor and postural impairments due to brain injuries occurring *in utero* or up to two to three years after birth (Bax et al., 2005b). The physical symptoms associated with CP are spasticity, selective decreased motor control, muscle weakness and fatigue (Jahnsen et al., 2003; Rose and McGill, 2005; Morris, 2007; Rosenbaum et al., 2007).The variety of symptoms and their range of magnitude are classified according to scales, which include the Gross Motor Classification System – GMCS (Palisano et al., 1997) and the Manual Ability Classification System – MACS (Eliasson et al., 2006). They respectively classify ordinary motor function and manual capacities, on a scale from I to V, where I is the best level, and V the greatest impairment.

Alteration of the neuromuscular function can be summarised as follows. First, the muscles in CP show an altered distribution and predominance to type I, slow-twitch fatigue-resistant muscle fibres (Rose et al., 1994; Ito et al., 1996). Additionally, the structural properties of the muscle fibres are altered. It has been shown that 95% of healthy muscle bundles comprise of muscle fibres, while in spastic muscle bundles in CP, this value is only 40% (Lieber et al., 2003). The size of CP spastic muscle fibres is one-third the size of healthy muscle fibres with a greater amount of intramuscular fat and connective tissue (Lieber et al., 2003). Hence, since the muscle cells' structural properties are altered, their contractile capacities are diminished; this contributes to the

muscle weakness observed in CP compared to healthy peers. Second, besides this purely peripheral component, muscle weakness has also a strong central component that lies in the deficit of voluntary activation. It has been shown that children with CP are not capable of fully activating their muscles during a MVC (Rose and McGill, 2005). Because of the cerebral lesion, the neural descending drive is altered (Mockford and Caulton, 2010) and thus decrease the amount of MUs being activated. Some authors have suggested that there might be impairments between the motor cortex and the motorneurons (Brouwer and Ashby, 1990) possibly explaining the low level of voluntary activation in children with CP (Elder et al., 2003; Rose and McGill, 2005).

Therefore, these modifications of the neuromuscular activation hamper the resistance to muscle fatigue as it has been previously suggested (Leunkeu et al., 2010); this can be due to coactivation of the agonist and antagonist muscles that is found to be greater in CP than in healthy populations (Unnithan et al., 1996; Burtner et al., 1998; Stackhouse et al., 2005; Braendvik and Roeleveld, 2012). This coactivation would lead to higher rates of energy expenditure (Unnithan et al., 1996; Feltham et al., 2010) therefore muscle fatigue.

However, some studies show that in CP, muscles exhibit a lower decrease in MVC, and in median frequency of the EMG signal; therefore displaying less muscle fatigability than healthy participants (Stackhouse et al., 2005; Moreau et al., 2009). At the peripheral level, the neuromuscular junction is found to be altered as a greater number of acetylcholine receptors in children with CP has been reported (Theroux et al., 2005). Muscle fibre alteration, increased sarcomere length, decreased muscle volume, greater metabolic demand and energy expenditure (Lundberg, 1978; Unnithan et al., 1996; Smith et al., 2011) are also suggested to be among peripheral mechanisms of muscle fatigue (Brunton and Rice, 2012). At the central level, a diminished inhibitory control in the motor cortex has been observed (Heinen et al., 1999), as well as damaged corticospinal tract (Hodapp et al., 2007) due to altered corticospinal projections to the α-MNs (Cheney, 1997).

3.3 Neuromuscular impairments in fascioscapulohumeral muscular dystrophy

A recent report shows that the facioscapulohumeral muscular dystrophy (FSHD) is the most prevalent muscular dystrophy in Europe (Orphanet Report Series, 2013) with 7 cases per 100 000. The facioscapulohumeral muscular dystrophy is associated with a deletion of an integral number of tandemly arrayed 3.3 kb repeat units, termed D4Z4, located on chromosome 4q35 (Wijmenga et al., 1992). Healthy people show 11 to 100 repeats, while this tandem repeats are found to be of 1 to 10 in FSHD. The lower the number of repetitions, the more severe the disease (van der Maarel and Frants, 2005). This myogenic disorder is characterised by a selective and asymmetric weakness of facial muscles, progressively descending to the scapular, then hip-girdle or anterior lower leg muscles(Tawil and Van Der Maarel, 2006).

In an animal model, the muscle weakness has been found to be due to impaired intrinsic contractile strength and could be linked to the severity of the disease while no sarcolemmal impairments could be observed (D'Antona et al., 2007). It is suggested that a decrease in myosin concentration specifically affects more fast fatiguing fibres. Furthermore, a shift in muscle fibre composition changes were observed from predominantly fast to predominantly slow muscle fibre types (D'Antona et al., 2007). Recently, the overexpression of the DUX4 gene has been found to be involved in sarcomeric protein degradation pathways (Wallace et al., 2011) leading to muscle weakness in patients with FSHD (Lassche et al., 2013).

A recent 10-year prospective study (Stübgen and Stipp, 2010) demonstrated that the disease showed a stable progression in terms of increased muscle weakness, decrease of motor function and daily life activities, despite variability in the tested group. In the extensor digitorum brevis, a decreased number of MUs were measured while those remaining were found to be abnormally large (Sica and McComas, 1971).

Experienced fatigue is a major burden in daily life activities as reported by 61% of patients with FSHD (Kalkman et al., 2005). However, only a few studies focused on muscle fatigue in patients with FSHD (Schillings et al., 2007; Kalkman et al., 2008). Besides higher rates of experienced fatigue, the maximal force production is found to be

of lower magnitude in participants with FSHD (Schillings et al., 2007). Also, during a two-minute isometric elbow flexion sustained MVC, the torque declined significantly although the rate of decline was slower than in control participants and less peripheral fatigue occurred in patients with FSHD (Schillings et al., 2007). The lower amount of peripheral fatigue in patients with FSHD is proposed to be linked to reduced blood flow occlusion, keeping a better intracellular environment in this patient group than in healthy populations (Schillings et al., 2007).

The central activation level, assessed with the twitch interpolation technique, was found to be unchanged compared to healthy participants after a two-minute sustained MVC even though staring and end values were lower than the control group (Schillings et al., 2007). Yet the voluntary activation level was also found to be of greater magnitude, this possibly linked to pain (Kalkman et al., 2008). Sensory afferent pathways are found to be altered (Fierro et al., 1997) and pain is widely reported in patients with FSHD (Bushby et al., 1998). A study made on 102 participants with FSHD showed that 82% reported pain, mostly in the lower back and legs (Jensen et al., 2008). Since pain can reduce the voluntary activation level (Rutherford et al., 1986; Gandevia et al., 1996; Graven-Nielsen et al., 2002), it is likely to be a limiting factor in muscle fatigue investigation in patients with FSHD since voluntary activation decline might be a strategy to limit pain.

Recent genetic studies have highlighted the complexity of FSHD and two genetic impairments have been shown to lead to FSHD development (van Overveld et al., 2003; de Greef et al., 2007, 2009; Zeng et al., 2009). The first, and the most common, the FSHD 1, is characterized by a reduced number of tandem repeats 3.3 kb, D4Z4, on chromosome 4q35 (Wijmenga et al., 1992), inducing chromatin structure changes and loss of DNA methylation (van Overveld et al., 2003). In the second, FSHD 2 concerning about 5% of the patients, similar chromatin changes are observed without a D4Z4 contraction (van Overveld et al., 2003; de Greef et al., 2007, 2009; Zeng et al., 2009). Even though advances have been made, the underlying pathophysiological mechanisms of the disease remain misunderstood. Hence, no therapeutic treatments are available (Tawil, 2008) and rehabilitation strategies have been investigated to prevent the progressive muscle atrophy and the loss of motor function. Aerobic exercises have

been proposed to improve the neuromuscular function in patients with FSHD, but authors (van der Kooi et al., 2004, 2005; Voet et al., 2010) failed to show the improvements of such training on muscle strength, even though no deleterious effects were induced. However, a recent study (Colson et al., 2010) using neuromuscular electrical stimulation (NMES) demonstrated strength and muscle function gains after a 5-month training period. Such a positive result highlights the potential of using NMES for rehabilitation purposes in FSHD patients.

4 Neuromuscular electrical stimulation

Neuromuscular electrical stimulation (NMES) is a widely used tool in rehabilitation settings to enhance muscle strength in healthy participants (Currier et al., 1979; Selkowitz, 1985; Colson et al., 2000; Bax et al., 2005a; Gondin et al., 2005; Russ et al., 2012a), or to compensate for a diminished or lost muscle function in patients (Bélanger et al., 2000; Burridge and Ladouceur, 2001; Harridge et al., 2002; Bax et al., 2005a; Colson et al., 2010) and even to treat pain in knee osteoathritis (Imoto et al., 2013), or chronic low back pain (Coghlan et al., 2011).

Neuromuscular electrical stimulation is generally used with electrodes applied on the muscles bulks to generate a muscle contraction (Vanderthommen and Duchateau, 2007). The recruitment of muscle fibres occurs through the excitation of α-MN axons and indirectly with the activation of spinal motorneurons via the excitation of Ia afferents evoked in mixed nerves (stimulation over the nerve trunk, see Figure 1) or intramuscular axonal branches (stimulation over the muscle bulk) (Hultman et al., 1983; Collins, 2007; Bergquist et al., 2011b). Moreover, force production depends on the stimulation parameters (current amplitude, pulse duration and frequency) (Gregory et al., 2007; Gorgey et al., 2009; Gondin et al., 2010). Therefore the muscle activation differs from voluntary contractions, NMES would induce higher rates of muscle fatigue (Adams et al., 1993; Maffiuletti, 2010).

4.1 Muscle activation pattern and fatigue in electrically evoked contractions

4.1.1 Stimulation parameters

Electrical stimulation amplitude, pulse duration and frequency influence the muscle activation and muscle fatigue. Increasing amplitude (current in mA or voltage in V) may activate muscle fibres deeper below the surface, increasing spatial recruitment (Theurel et al., 2007), yielding production of greater forces. Therefore, the greater the current amplitude, the more the recruitment of the muscle will occur via peripheral pathways (Bergquist et al., 2011b). The augmentation of the current amplitude increases the activated muscle area and the evoked torque and maintains the metabolic demand per activated MU but does not necessarily increase muscle fatigue when the stimulation is applied on the muscle (Adams et al., 1993; Gorgey et al., 2009).

The pulse duration may differentially activate nerve axons: the shorter durations (50-400 µs) depolarise motor axons, while longer durations (> 500 µs) depolarise more sensory axons (Bergquist et al., 2011b) as they have a longer strength-duration time constant and lower rheobase (minimal current intensity required to evoke a motor response). Therefore, it is suggested that larger pulse durations involve greater central components, both in muscle and nerve stimulation (Collins, 2007; Lagerquist and Collins, 2010; Maffiuletti, 2010; Bergquist et al., 2011b), and its effect on muscle fatigue remains unclear (Maffiuletti, 2010).

Finally, the frequency of stimulation (*i.e.* the number of pulses per second, in Hz) is usually divided in low frequencies (< 50 Hz) and high frequencies (> 50 Hz). It has been suggested that to reach a tetanic state, the frequency needed will vary according to muscle characteristics (Edwards et al., 1977). To maximise the muscle force production, the frequency may be increased (Maffiuletti, 2010) in the high-frequency range. Whether the increase of frequency induces more fatigue is debated. Low (Jones, 1996; Matsunaga et al., 1999) and high frequencies (Kebaetse and Binder-Macleod, 2004; Kebaetse et al., 2005; Kesar et al., 2008) are reported to induce the greatest fatigue rates, although the latter has been challenged (Gondin et al., 2010).

Since muscle fatigue is influenced by NMES, the difference in muscle activation patterns are likely to explain these differences, also because NMES can either be applied with surface electrodes over the muscle bulks or directly stimulating over the nerve trunk.

4.1.2 Muscle activation pattern: Muscle and Nerve stimulation

The muscle recruitment pattern in NMES has been shown to be random, spatially fixed, spatially incomplete and temporally synchronous (Adams et al., 1993; Vanderthommen et al., 2003; Gregory and Bickel, 2005). While during a sustained voluntary submaximal contraction a rotation in MUs activity occurs to provide a metabolic rest for the contractile apparatus (Westgaard and de Luca, 1999; Bawa et al., 2006), NMES does not permit such a rotational recruitment and, therefore, do not follow the orderly recruitment of the size principle.

Using of electrodes over the muscle bulk, the recruitment of muscle fibres occur beneath and within the vicinity of the electrodes as the axonal branches are being activated through direct pathways (Gregory and Bickel, 2005; Maffiuletti, 2010). The muscle contractions elicited via electrodes over the muscle bulks are not only the results of depolarisation of axons through direct, peripheral pathways but also of central pathways (Collins, 2007), both are responsible for muscle fatigue (Boerio et al., 2005). When the stimulation is delivered at a current intensity that evoked an H-reflex (*i.e.* lower than a stimulation intensity that evoked an M-wave), the recruitment of spinal motorneurons by afferent excitation seems also to occur as the activation of larger-diameter afferent axons are primarily activated (Collins, 2007). A recent study demonstrated that a low-frequency (20 Hz) stimulation over the muscle belly of the triceps surae would preferentially generate torque via peripheral pathways (Bergquist et al., 2011a), as M-waves were of greater amplitude than H-reflexes. Similar observations have been made on the stimulation of the quadriceps bulks (Bergquist et al., 2012). Because of the fixed position of electrodes over the muscle bulks, the recruitment of fibres diminishes as their distance from the electrodes increases (Vanderthommen et al., 2000). Increasing the current intensity, suggested to be a factor in strength gains

(Colson et al., 2009), might overcome the distance and activate deeper fibres (Theurel et al., 2007). However, NMES with electrodes over the muscle bulks is likely to induce a random recruitment of MUs (Gregory and Bickel, 2005) activating both fast fatigable and slow fatigue-resistant MUs. Consequently, the metabolic cost and the energy expenditure are increased (Vanderthommen et al., 2003) since the same muscle fibres are activated. It is suggested that the glycogen depletion occurs at a higher rate in fast fatigable fibres than in slow twitch fibres (Sinacore et al., 1990) and even though this is also the case in high intensity voluntary contractions it is likely that the metabolic cost is higher in NMES (Gregory and Bickel, 2005; Gorgey et al., 2009; Maffiuletti, 2010).

When the stimulation is delivered over the nerve trunk, the central and peripheral contributions to the muscle contraction can differ. Central contributions have been shown to be of significant importance when a low-frequency stimulation at ≈20% MVC over the tibial nerve trunk was applied (Klakowicz et al., 2006; Bergquist et al., 2011a), and also over the femoral nerve trunk, when the quadriceps were stimulated (Bergquist et al., 2012). Indeed, H-reflexes were found to be of larger amplitude, and it can been suggested that central pathways via activation of spinal motorneurons contributed mainly to the torque production (Klakowicz et al., 2006; Bergquist et al., 2011a, 2012). When short duration of electrical stimulation of increase in frequency was applied (100 Hz), these central contributions were found to be of greater magnitude (Klakowicz et al., 2006; Bergquist et al., 2011a). However, the stimulation site may be a factor limiting factor, since responses have been found to be more variable when the femoral nerve was stimulated compared to the tibial nerve, likely due to the more superficial location of the latter.

It is possible that axons are activated differently depending on whether muscle or nerve stimulation has been applied. While sensory and motor axons are bundled in the nerve, they are spread within in the muscle increasing the variability of the evoked muscle contraction. Contributions through central and peripheral pathways differ when NMES is applied on the nerve trunk compared to the muscle bulks (Baldwin et al., 2006; Bergquist et al., 2011a). Nerve stimulation is more likely to activate a greater amount of Ia afferent axons in a more synchronous manner, increasing the spinal contributions to the torque production (Bergquist et al., 2011a). Consequently, since the central

pathways evoke a muscle contraction in nerve trunk stimulation activate more slow type fatigue-resistant fibres, a physiological recruitment via the size principle should activate the muscle (Collins, 2007; Maffiuletti, 2010; Bergquist et al., 2011b).

5 Aims of the thesis

The overall objective of this thesis is to investigate the effect of neuromuscular activation strategies during muscle fatigue as well as, endurance and muscle performance after voluntary and electrically evoked contractions in healthy and health-compromised populations. Furthermore, given the challenges that clinicians and patients must face, this thesis aims to extend the current knowledge base for rehabilitation of muscle fatigue in rehabilitation settings. Rehabilitation involves a number of possibilities including, for example, unilateral exercises or neuromuscular electrical stimulation. This implies that neuromuscular adaptations, which can vary according to the health status of the populations.

To fulfil the overall objective, the specific aims are:

I: Investigate the cross-over effect of muscle fatigue following a unilateral knee extensors maximal isometric fatiguing exercise (paper I).

II: Study whether a neuromuscular electrical stimulation exercise, delivered over the nerve trunk of the ankle plantar flexors would lead to differential peripheral and central contributions of muscle fatigue when comparing constant current intensity with a progressive modulation of current intensity (paper II).

III: Evaluate whether the elbow flexors muscles of children with cerebral palsy have an endurance as hampered as strength at a low force level, and whether they can compensate muscle fatigue with recruitment of additional motor units (paper III).

IV: Examine the effect of a rehabilitation program using neuromuscular electrical stimulation on muscle strength, endurance and motor function in patients with facioscapulohumeral muscular dystrophy (paper IV).

6 Methods

6.1 Studies samples and participants

Four studies were conducted on either healthy / health-compromised or a combination of populations. Each study involved a minimum of 8, maximum of 17 subjects, with ages ranging between 10 and 70 years old. The experiments were performed in accordance with the Declaration of Helsinki (1964), and a written informed consent was obtained from participants or from the parents when participants where below 18 year-old.

An overview of participant characteristics is shown in Table 1.

1. The sample of paper I comprised 15 healthy participants (HP), young male adults; they were recruited from the Faculty of Sport Sciences of the University of Nice - Sophia Antipolis (Nice).

2. The sample of paper II consisted of 8 healthy young male adults; Participants of the study were recruited from the Faculty of Sport Sciences of the University of Burgundy (France).

3. The sample of paper III consisted of 12 children or adolescents (8 boys, 4 girls) with cerebral palsy (CP) and 17 typically developing (TD – 11 boys, 6 girls) peers matched in gender and age; Children with CP were recruited from the outpatient records of the neuro-orthopaedic team at St. Olav's Hospital of Trondheim in the health region of Mid-Norway (Norway).

4. The sample of paper IV consisted of 10 adults with facioscapulohumeral muscular dystrophy (FSHD - 4 males and 6 females) and 6 healthy participants (HP) adults matched in gender and age (3 males and 3 females); Participants with FSHD were recruited from the outpatient record of the physical medicine and rehabilitation department at the University Hospital Archet 1 of Nice (France).

Table 1. Participants characteristics summary for papers I to IV.

	Sample Paper I	**Sample Paper II**	**Sample Paper III**		**Sample Paper IV**	
Health Status	HP	HP	CP	HP	FSH	HP
n	15	8	12	17	10	6
Age (mean ± SD)	21.7 ± 3.1	27 ± 4.2	12.3 ± 2.9	12.4 ± 2.8	61.5 ± 10	57.8 ± 4.8
Gender (male/female)	15/0	8/0	8/4	11/6	4/6	3/3

6.2 Neuromuscular assessments

6.2.1 Strength measures

Isokinetic dynamometers were used to standardise position and measure torque production during voluntary and electrically evoked contractions in papers I-III (Biodex System 3 Pro, Biodex Medical Systems, Shirley, NY, US). In paper IV, a custom made system consisting of a fixed footplate was used, where the foot was firmly strapped to avoid any movement and ensure the quality of the isometric force measurement. A load cell was attached to the footplate to measure ankle plantar and dorsiflexion force production (in N).

Strength is commonly measured as the voluntary capacity to perform the highest torque during a maximal voluntary contraction (MVC). In papers I, II and IV, the MVC was computed as the average over a 500 ms period around the highest torque value during the maximal plateau. The highest average value of the two trials was used as the maximal torque production (in N.m). In paper III, the MVC was determined as the highest peak torque value of the three trials.

In this thesis, the MVC of the following muscles groups were considered: in paper I, randomly selected side knee extensors and flexors; in paper II, right hand side ankle

plantar and dorsi flexors; in paper III, elbow flexors and extensors of the affected arm of children with CP and non-dominant arm of TD; in paper IV, both sides ankle dorsi and plantar flexors.

6.2.2 Muscle function evaluation for children with CP and adults with FSHD

6.2.2.1 Children with Cerebral palsy

Cerebral Palsy (CP) is a clinical descriptive term that comprises a variety of motor or postural impairments caused by injuries in the central nervous system during early development (from *in utero* until 2 to 3 years after birth) (Bax et al., 2005). Most common physical symptoms are spasticity, muscle weakness, decreased control and fatigue (Jahnsen et al., 2003; Rose and McGill, 2005; Rosenbaum et al., 2007). The Manual Ability Classification System (MACS) (Eliasson et al., 2006) is used to classify the bi-manual ability of children with CP to manipulate objects in daily-life activities. The MACS is made up of 5 levels where level I represents the best manual ability and level V represents the absence of hand function. In paper III, among the 15 children with CP, 9 were ranked at level I of MACS, 5 at level II and 1 at level III.

6.2.2.2 Adults with Facioscapulohumeral Dystrophy

The facioscapulohumeral muscular dystrophy (FSHD) is associated with a deletion of an integral number of tandemly arrayed 3.3 kb repeat units, termed D4Z4, located on chromosome 4q35 (Wijmenga et al., 1992). This myogenic disorder is characterised by a selective and asymmetric weakness of facial, scapular muscles, progressively descending to hip-girdle or anterior lower leg muscles (Tawil and Van Der Maarel, 2006). The Manual Muscle Testing (MMT) is used to assess the isolated muscle strength (Lacôte et al., 2008), ranking from 0 where muscle is no longer capable of force production to 5 that represents the absence of muscle impairment. In paper IV, left and right tibialis anterior muscles were tested, prior to and after NMES strength training. Table 2 gathers individual scores of MMT from the tibialis anterior for all adults with FSHD before the training as well as the 3.3Kb tandem repeats.

Table 2. Participants with facioscapulohumeral muscular dystrophy scores of the Manual Muscle Testing from the tibialis anterior and number of tandem 3.3 kb repeats.

FSHD participant ID	TA Right PRE	TA Left PRE	3.3Kb tandem repeats
S1	2	5	7
S2	2	2	8
S4	2	2-	6
S5	2	2	6
S6	3	4	8
S8	2-	4-	4
S11	5	5-	5
S12	4+	4	5
S13	2-	1+	9
S14	4-	4-	9

6.2.3 Surface Electromyography

In all papers, muscle activity was recorded with the use of bipolar surface electromyography (sEMG). In paper I, the concurrent muscle activity of the vastus medialis (VM), vastus lateralis (VL), rectus femoris (RF) and semitendinosous (ST) muscles were recorded. In paper II, the concurrent muscle activity of the soleus (SOL), gastrocnemius lateralis (GL), gastrocnemius medialis (GM) and tibialis anterior (TA) were recorded. In paper III, the muscle activity of the biceps brachii (BB), the lateral head of the triceps brachii (LTB) and the triceps longus (MTB) were recorded

simultaneously. In paper IV, the concurrent muscle activity of the tibialis anterior (TA) and the soleus (SOL) were recorded.

Bipolar sEMG electrode positions (10 mm diameter, 20 mm inter-electrode distance) followed the SENIAM recommendations (Hermens et al., 2000), except for the soleus electrodes in paper II that were positioned 2 cm below the gastrocnemii's insertion, over the calcaneal tendon. The skinfold thickness was measured in the group of children with CP and the group of TD in paper III. Skin was cleaned prior to placement of electrodes, and low resistance between electrodes (<5 kΩ) was obtained for papers I, II and IV. Surface EMG was recorded at a sampling frequency of 2000 Hz in studies I and IV, 3000 Hz in paper II, and 4000 Hz in paper II.

Root mean square (RMS) values of sEMG recordings were averaged over a 500 ms non overlapping window in all papers. The RMS values were normalised to the maximal peak-to-peak amplitude of the corresponding M-wave at rest (RMS/M_{max}) in paper I, and to the superimposed M-wave obtained during the MVC (RMS/M_{Sup}) in paper II. In paper III, the RMS values and the median frequency of the power density curve (MDF (Hz)) were calculated over the entire duration of the task in 1-second periods for the BB, LTB and MTB muscles.

6.2.4 Fatiguing exercises

In paper I, the fatiguing protocol consisted of two exercises (hereafter referred to as Fatigue 1 and Fatigue 2) of all-out 100 second MVC of isometric knee extension of the exercising limb (EL). The side of the EL was randomly selected across the participants. Participants were asked to relax the non-exercising limb (NEL) during fatiguing exercises.

In paper II, the fatiguing exercise consisted of three different protocols of intermittent submaximal isometric plantar flexion contractions, including two NMES protocols and a voluntary one of the ankle plantar flexors of the right leg at 20% of MVC. A visual feedback was provided on the monitor of the dynamometer. The torque-time integral was matched between the three fatiguing exercises.

In paper III, the endurance task consisted of a sustained submaximal isometric elbow flexion contraction at elbow angle of 90° until the task could no longer be maintained. A 3D accelerometer and a 2D inclinometer (Noraxon USA, Inc.) were placed respectively, on the proximal phalanx of the first finger and on the forearm. The inclinometer was used to provide visual feedback displayed on a computer screen (37.7 cm x 30.3 cm) placed at eye level. When the inclinometer feedback deviated by at least 5°, the task was considered as failed.

In paper IV, the fatiguing task consisted of an isometric ankle dorsiflexion single exercise of sustained 2-minute MVC. Participants were seated on the Biodex chair. The foot was placed in a custom-made ankle torque measurement system (see Figure 2).

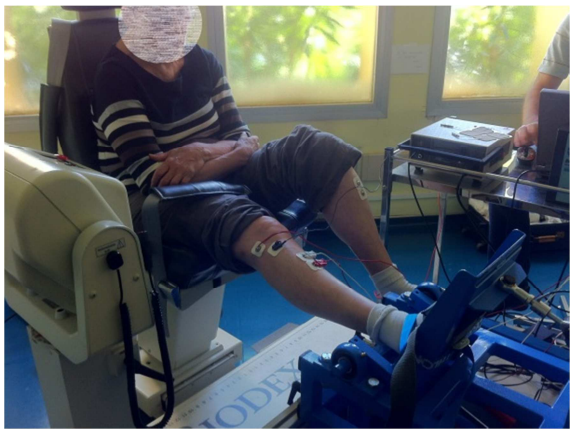


Figure 2. Experimental position of participants in study IV

6.2.5 Nerve Stimulation and evoked potentials

In papers I, II and IV, nerve stimulation was induced using a high-voltage constant current stimulator (model DS7AH and DS7A, Digitimer, Hertfordshire, United-Kingdom) to evoke the compound muscle action potential (M-wave), the Hoffman reflex (H-reflex) and the first volitional wave (V-wave). In paper I, the femoral nerve was stimulated with a tungsten cathode ball electrode (0.5 cm diameter) pressed onto the femoral triangle. The same researcher applied the stimulation during the entire experiment.. The anode was a 45 cm² rectangular electrode (Stimex, Wetzlar, Germany) placed between the great trochanter and the iliac spine. Stimulation parameters were

400 V, and a rectangular pulse of 2 ms pulse width. To elicit a supramaximal stimulation, the maximal stimulation intensity was increased by 10%. This intensity was used to induce doublet stimuli, separated by 10 ms at 100 Hz that were delivered over the MVC plateau (superimposed doublet), and 4 seconds after the MVC (potentiated doublet) (Behm et al., 1996).

In paper II, tibial nerve stimulation was induced at the popliteal fossa with a single rectangular pulse (400 V, 1 ms pulse width).The stimulation site was first located by a hand-held cathode ball electrode (0.5 cm diameter) and when the optimal stimulation site was determined, an electrode was firmly fixed with the use of rigid straps and taping to ensure constant stimulation location. The self-adhesive cathode (8 mm diameter, Ag-$AgCl^-$) was placed at the popliteal fossa while the anode (5 x 10 cm, Cefar-Compex, DJO France SAS, Mouguerre, France) was placed over the patella tendon.

In paper IV, common peroneal nerve stimulation was induced with a single rectangular pulse (400 V, 500 µs pulse width), at a position that produced dorsiflexion of the foot without eversion, with a self-adhesive cathode (8 mm diameter, Ag-$AgCl^-$) placed at the level of the fibula neck while the anode (8 mm diameter, Ag-$AgCl^-$) was placed over the head of the fibula, near the TA insertion.

6.2.5.1 Voluntary activation level

To disclose the level of voluntary activation, the twitch interpolation technique (Merton, 1954) has been used. The principle is to superimpose an electrical stimulus over the motor nerve during a MVC. If the muscle group is maximally activated, the stimulation does not generate extra torque. If extra force can be evoked, this suggests a submaximal activity of the muscle group, MUs recruitment is incomplete and/or, MUs discharge rate is suboptimal (Belanger and McComas, 1981).

In paper I, the maximal voluntary activation level of knee extensors was calculated using the following formula (Allen et al., 1995):

$$\text{Voluntary Activation (\%)} = \left(1 - \left(\frac{Superimposeddoublet}{Potentiateddoublet}\right)\right) \times 100$$

In paper II, the maximal voluntary activation level of knee extensors was calculated using the following formula (Allen et al., 1995):

$$\text{Voluntary Activation (\%)} = \left(1 - \left(\frac{Superimposed\ twitch}{Potentiatedtwitch}\right)\right) \times 100$$

In papers I and II, a correction was consistently applied to the original equation when the superimposed doublet was elicited slightly before or after the real MVC (Strojnik and Komi, 1998). In these cases, the maximal voluntary activation level was calculated as follows:

$$\left[1 - \left(\frac{\dfrac{Superimposed\ doublet\ \times\ Voluntary\ torque\ level\ just\ before\ the\ superimposed\ doublet}{Maximal\ Voluntary\ torque\ jk}}{Potentiated\ doublet}\right)\right] \times 100$$

6.3 Neuromuscular electrical stimulation strength training

The neuromuscular electrical stimulation (NMES) strength training carried out for paper IV was performed with an electrostimulator (Rehab 400, Cefar-Compex, DJO France SAS, Mouguerre, France).

During the bilateral NMES strength training sessions of the tibialis anterior muscle, participants were seated (hips, knees and ankles angle joints at 90°), and feet were fixed to avoid movement during the elicitation of dorsiflexion evoked by the stimulation. The self-adhesive electrodes (2 mm thick) made of elastomer (5 cm x 5 cm) were positioned as follows: the positive electrode was placed on the superior part of the muscle, the negative electrode was placed on the medial part of the muscle, over the muscle bulk.

The NMES strength training lasted for 8 weeks, 3 sessions per week, and each session was comprised of a 2-minute warm-up, followed by a 20-minute work out session, finishing with 3 minutes of relaxation. The NMES program consisted of 6 seconds of contraction, followed by 7 seconds of rest (35 Hz, 200 µs pulse-width). This program was chosen according to literature which showed that, over a five-month period, this NMES training improved muscle function and was well tolerated in FSH muscular dystrophy patients (Colson et al., 2010).

Participants either exercised at home or were trained by a physiotherapist. In the case where participants trained at home, a weekly appointment was established with a physiotherapist or researcher to answer questions and/or to control the quality of the training. Participants were asked to increase stimulation intensity progressively throughout each session up to individual tolerance threshold since strength gains would be dependent on the stimulation intensity (Colson et al., 2009).

7 Summary of results

7.1 Paper I: Time course of the cross-over effect of fatigue on the contralateral muscle after unilateral exercise

The aim of the study was to investigate the time course of the cross over effect of a unilateral fatiguing exercise onto the non-exercised contralateral limb (NEL). Fifteen healthy young males performed two bouts of a unilateral 100-second maximal isometric knee extension with the exercising limb (EL) and neuromuscular function for both limbs was assessed prior to (PRE), between (MID) and after (POST) the two fatiguing efforts. In the EL, the maximal torque production reduced between PRE and MID tests, between MID and POST tests ad between PRE and POST tests. In the NEL the maximal torque declined between PRE and POST tests (Figure 3). While the decrease in muscle performance of the EL was attributed to peripheral factors, the overall decline in voluntary activation level of knee extensors after the second fatiguing exercise was likely to explain the reduction of maximal torque production in the NEL. Differential adjustments of the central and peripheral levels might concur to the decline of maximal torque production in the EL and the NEL. These results indicate that, even though 2 bouts of 100-second MVC induced a quick reduction of MVC in the EL, the time course of torque production loss was postponed in the NEL and thus differed between both limbs.

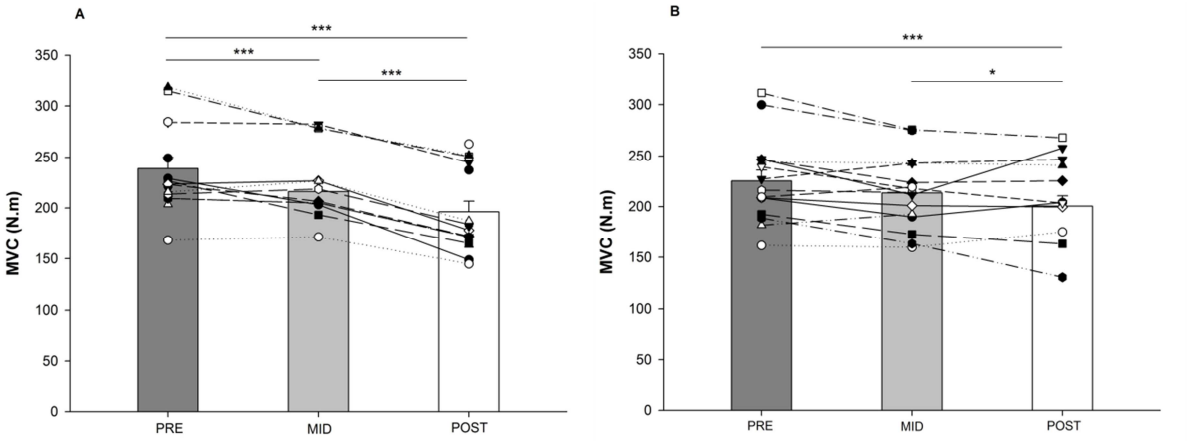


Figure 3. Maximal voluntary isometric torque. Maximal voluntary isometric torque tests of the knee extensor muscles measured at PRE, MID and POST tests for the exercising limb (A) and the non-exercising limb (B). Columns represent group mean values, while triangles, squares black and white symbols show individual values. Error bars are the standard error of the group mean. Significant differences $p<0.05$ (*) and $p<0.001$ (***).

7.2 Paper II: Effect of neuromuscular electrical stimulation intensity over the tibial nerve trunk on triceps surae muscle fatigue

The aim of the study was to investigate whether the intensity modulation of a neuromuscular electrical stimulation (NMES) protocol delivered over the nerve trunk of the plantar flexors would lead to differential peripheral and central contributions of muscle fatigue. Eight healthy young males performed an intermittent (duty cycle 50%) voluntary contraction protocol (VOL) at 20% of the maximal voluntary contraction (MVC) and two intermittent protocols induced by NMES where a constant stimulation intensity that induced 20% MVC at the start of the contraction was maintained (CST); and where the stimulation intensity level was progressively adjusted to maintain 20% of MVC during the whole protocol (PROG). The torque-time integral was matched between conditions to ensure that similar energy expenditure was met in all three protocols. A similar decline in MVC occurred after the three fatiguing protocols. Moreover, although the voluntary activation of the plantar flexors did not change, similar central (Figure 4) and peripheral adaptations were observed after the three fatiguing protocols. It seems that the soleus, gastrocnemii lateralis and medialis muscles of the triceps surae, underwent different adaptations. The results indicate that, for a similar TTI, a NMES protocol, whether or not the current intensity was increased, yield to similar muscle fatigue adaptations as voluntary contractions likely through similar pathways.

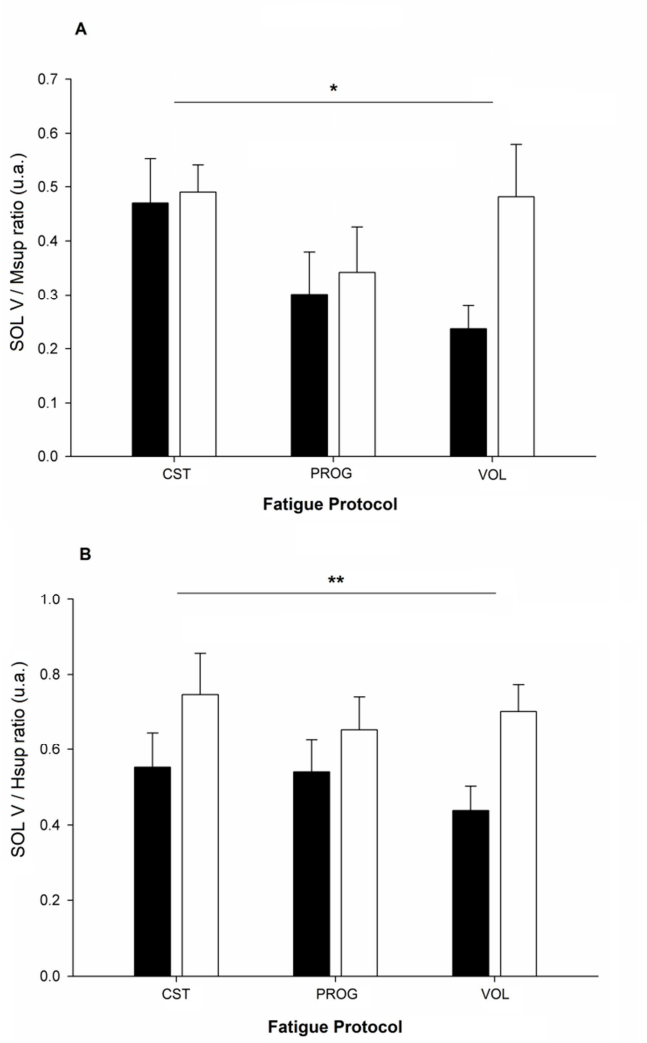


Figure 4. Maximal amplitude of the soleus (SOL) V-wave-to-Msup ratio (V/Msup; Top-panel, A) and of the V-to-Hsup (V/Msup ratio, bottom panel B) of the soleus muscle measured at PRE (black) and POST (white) tests for the constant intensity fatiguing protocol (CST), the progressive intensity fatiguing protocol (PROG) and the voluntary fatiguing protocol (VOL). Columns represent group mean values and error bars are the standard error of the group mean. Significant differences of main time effect, $p<0.05$ (*) and $p<0.01$ (**).

7.3 Paper III: Fatigue and muscle activation during submaximal elbow flexion in children with cerebral palsy

The aim of the study was to investigate whether children with cerebral palsy (CP) would compensate for muscle fatigue by recruitment of additional motor units (MUs), like typically developing peers, during a sustained low-force level isometric elbow flexion contraction. Twelve children with CP and 17 typically developing peers performed one submaximal isometric elbow flexion contraction until the task could no longer be sustained. Even though strength and the endurance time were similar in both groups, children with CP showed a lower increase in biceps brachii EMG amplitude (Figure 5) compared to the control group. Also, a lower change in median frequency EMG and a lower amount of acceleration fluctuations from the start of the contractions to exhaustion were present in the group of children with CP. This suggests that children with CP would stop their task at a lower level of muscle fatigue. These results indicate that children with CP were not able to recruit additional motor units to compensate for muscle fatigue throughout the task.

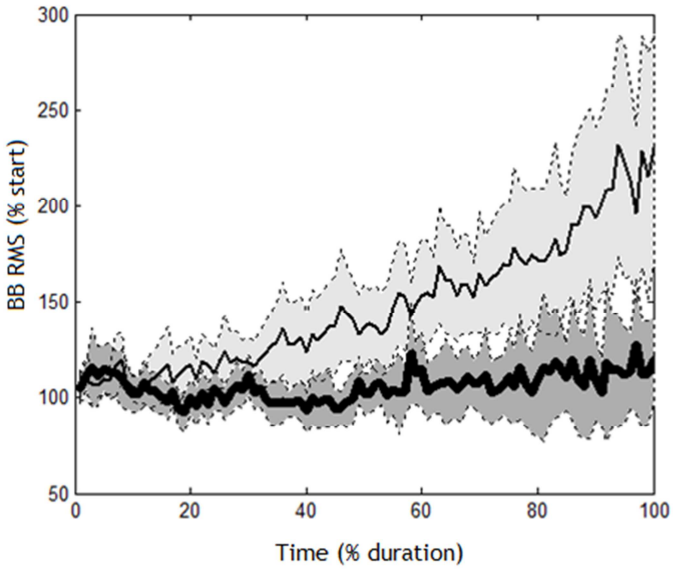


Figure 5. Average (solid line) and 95% confidence interval (area comprised between dotted lines) of the RMS amplitude of the EMG from the biceps brachii (BB) throughout the sustained elbow flexion task, of the patients with cerebral palsy (average: thick black line; 95% confidence interval: dark grey area) and the typically developing peers (average: thin black line; 95% confidence interval: light grey area). RMS amplitude is expressed as the percentage from the initial value (% start) and time as the percentage of total duration (% duration).

7.4 Paper IV: Neuromuscular electrical stimulation training of the tibialis anterior in facioscapulohumeral muscular dystrophy patients

The aim of the study was to investigate whether a bilateral 8-week neuromuscular electrical stimulation (NMES) training of the tibialis anterior (TA) muscles in adults with facioscapulohumeral muscular dystrophy (FSHD) would improve muscle endurance and the maximal voluntary force production of ankle dorsiflexion. Ten patients with FSHD and 6 age and gender matched healthy participants (HP) achieved the NMES training. Maximal voluntary contractions (MVCs) of ankle dorsi and plantar flexions were measured, a muscle endurance test consisting of a 2-minute sustained isometric ankle dorsiflexion MVC and functional tests were performed prior to and after the NMES training to disclose training effects. During the 2-minute sustained MVC, the percentage of force loss was lower for the FSHD patients before and after the training (Figure 6). Neither the functional tests, nor ankle dorsiflexion strength and endurance were improved after the NMES training in any of the tested groups. The results indicate that the FSHD patients experienced a lower amount of muscle fatigue compared to the HP group. Moreover, the weaker ankle dorsiflexion MVC and lower associated TA EMG amplitude, suggest that the intrinsic contractile properties of the TA were altered in patients with FSHD. The lack of apparent training effect may be explained by the NMES training which may not have been strenuous enough to trigger any improvement in patients with FSHD, while stimulation parameters targeted to the patient group were not adequate to induce strength gains in the HP group.

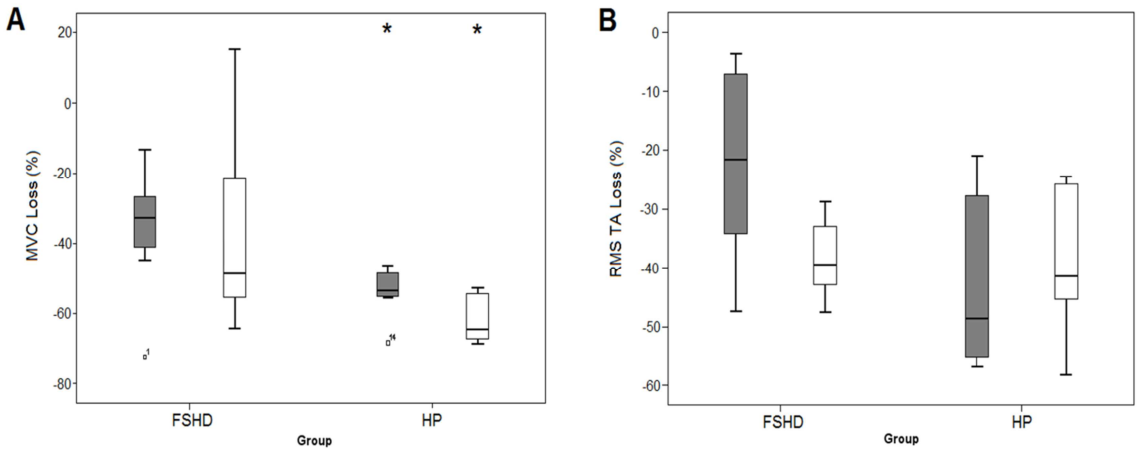


Figure 6. Box-plot of the percentage of force production loss (A, left panel) and of the RMS of the tibialis anterior (TA) (B, right panel) during the 2-minute sustained ankle dorsiflexion endurance exercise, before (grey) and after (white) the 8-week training for patients with facioscapulohumeral muscular dystrophy (FSHD) and healthy participants (HP). Boxes represent group median and interquartile range values and whiskers are the highest and lowest values. Significant group differences: $p<0.05$ (*)

8 Discussion

The overall aim of this thesis is to investigate the effect of neuromuscular activation strategies on muscle fatigue, endurance and muscle performance after voluntary and electrically evoked contractions in healthy and health-compromised populations. The underlying mechanisms of a maximal sustained voluntary isometric contraction and its cross-over effect is addressed first. Subsequently, the effect of the use of fatiguing neuromuscular electrical stimulation on the neuromuscular activation is discussed. This is followed by a discussion of the muscle fatigue and muscle performance in children with cerebral palsy and adults with facioscapulohumeral muscular dystrophy. Thereafter, methodological considerations are addressed, and eventually a conclusion regarding the contribution of the findings reported in this thesis and an outlook is provided.

8.1 The muscle activation strategies of a fatiguing maximal effort and its cross-over effect of muscle fatigue

In muscle contractions, the level of force produced influences the rate at which muscle fatigue develops and the origins (*i.e*. along the motor pathway) of muscle fatigue vary according to the task performed. Muscle fatigue can arise either at central, supraspinal, spinal level and/or within the muscle fibre itself (Gandevia, 2001; Enoka and Duchateau, 2008). During sustained maximal efforts, the capacity of generating the target force reduces considerably over time. For instance, participants experienced a decline of 64% and 65% during the first and the second 100 second MVC exercise in paper I while similar magnitude declines of 54% and 62% during the 2 minute MVC were observed for the control group before and after the training period in paper IV. During maximal efforts, the amplitude of the EMG signal decreases with increasing fatigue (Bigland-Ritchie et al., 1983). The participants in paper I showed a decline in the amplitude of the knee extensors EMG-to-M_{max} ratios throughout the first and the second 100 second MVC fatiguing exercise. A similar pattern was observed in both groups described in paper IV, where the EMG amplitude of the tibialis anterior muscle decreased between the start and the end of the 2 minute MVC fatiguing exercise. Two

central mechanisms can explain the decline in EMG amplitude with fatigue. The supraspinal level when the descending neural drive modifies and becomes suboptimal (Gandevia et al., 1996). This is supported by the decline of ≈ 9% of the voluntary activation level observed after the two 100 second MVC exercises in paper I. In paper I, the voluntary activation level declined after the second 100 second MVC, while it remained unchanged after the first. Furthermore, at the spinal level, the α-MNs' responsiveness can decrease because of their intrinsic properties (Andersen et al. 2003; Butler et al. 2003; Martin et al. 2006). Therefore, both spinal and supraspinal mechanisms can induce a decrease of the MU firing rate (Bigland-Ritchie et al., 1983).

At the peripheral level, the M-waves showed no significant change with fatigue in paper I, suggesting that the neuromuscular transmission was preserved. In the paper I, peak twitches evoked by single and double stimulation decreased significantly in the exercising limb after the two fatiguing exercises. A study (Kent-Braun, 1999) showed that after a 4 minute sustained isometric MVC of ankle dorsiflexion. The H^+ concentration increased causing 80% of the fatigue developed. In paper IV, force production reductions of 54% and 62% during the 2 minute MVC were seen for the control group before and after the training period. Thus, in paper I, and in paper IV, it is likely that modifications occurred within the muscle fibres, including pH and/or Pi increases and/or a reduction in Ca^{2+} ions sensitivity, hindering Ca^{2+} binding to troponin C on the myofilaments, leading to a lower number of active cross-bridges reducing force production (Westerblad et al., 2002; Allen et al., 2008; Place et al., 2009).

Through inhibition mechanisms, the muscles afferents (muscle spindles, Golgi tendon organs, groups III and IV afferents) possibly cause a decreased firing rate (Kernell and Monster 1982a; Kernell and Monster 1982b; Bigland-Ritchie et al. 1986), providing feedback to the α-MNs and the supraspinal centres. Groups III and IV afferents are leading candidates as they have been shown to limit the descending voluntary drive (Amann et al., 2008), and intracortical inhibition increases during a 2 minute isometric MVC of elbow flexion (McNeil et al., 2009). As the groups III and IV afferents provide information regarding the strain and the metabolic milieu modifications in the muscle (Ellaway et al. 1982; Mense and Stahnke 1983; Kaufman and Rybicki 1987; Clarke et al. 1996; Hayes et al. 2005), the accumulation of metabolites during high force level

contractions increases the activation threshold of motor axons; thus decreasing the number of active motor units (Vilin and Ruben, 2001; Kiernan et al., 2004; Miles et al., 2005).

It has been shown that muscle fatigue can spread to non-targeted contralateral homologous muscles, and a cross-transfer occurs as the muscle activity of the exercising limb irradiates to the non-exercising muscles where an electrical activity can be measured (Muellbacher et al., 2000; Zijdewind and Kernell, 2001; Shinohara et al., 2003). This contralateral effect of muscle fatigue has been shown not only to be present in the homologous contralateral muscle, but can spread towards other muscles groups (Kennedy et al. 2012). In paper I, results showed not only a cross-over effect of fatigue on the non-exercising limb after two unilateral 100 second isometric MVCs of the knee extensors, but results also showed that the time-course of fatigue differed between the exercising limb and the non-exercising limb, being postponed in the latter. The decreased force resulting from the MVC was significant in the exercising limb after the first exercise, whereas the maximal torque production in the contralateral non-exercising limb reduced after the second 100 second fatiguing exercise. The decline of the voluntary activation level that likely occurred after the second fatiguing exercise was correlated with the MVC loss. The contralateral decrease in MVC relies on the cross-over effect of fatigue (Zijdewind and Kernell, 2001; Todd et al., 2003; Rattey et al., 2006; Martin and Rattey, 2007; Post et al., 2008; Paillard et al., 2010; Elmer et al., 2013) and central mechanisms at both supraspinal (Muellbacher et al., 2000; Liu et al., 2003; Carson et al., 2004; Liang et al., 2008) and spinal levels (Rothwell et al., 1991; Stedman et al., 1998; Hortobágyi et al., 2003; Todd et al., 2003; Carson et al., 2004; Zijdewind et al., 2006) have been demonstrated to have a role in this limbs interplay.

During a unilateral contraction, the activity of the ispilateral hemisphere of the brain increases (Muellbacher et al., 2000; Liu et al., 2003; Carson et al., 2004; Liang et al., 2008). Interaction of the hemispheres, both inhibitory (Ferbert et al., 1992) and facilitatory (Meyer et al., 1995) occur through transcallosal pathways. At this time, one active motor cortex contributes to the increased activity of the other, even when the second is not involved in the task. It has been shown (Liu et al., 2003; Post et al., 2007) that similar regions of the brain (sensorimotor cortex, cerebellum, premotor cortex,

supplementary motor area) are activated during unilateral and bilateral contractions (Stedman et al., 1998). More precisely, the supplementary motor area increases its activity during fatigue, while decreases of voluntary activation, force output and EMG are observed during maximal effort (Post et al., 2009). Also, an increase in excitability of the ipsilateral motor cortex would increase the probability of the activation of the non-targeted muscle (Zijdewind and Kernell, 2001).

At spinal level, the interhemispheric neural activity mentioned above can partly explain changes observed in spinal activity (Stedman et al., 1998; Muellbacher et al., 2000; Hortobágyi et al., 2003; Carson et al., 2004). Furthermore, it has been suggested that a modification of contralateral motorneuron properties can take place when high forces are generated (Stedman et al., 1998). Motor evoked potentials (MEP), induced by transcranial magnetic stimulation (TMS) have been found to be facilitated in unilateral exercising muscles (Rothwell et al., 1991) as well as in the contralateral non-exercising muscles (Hortobágyi et al., 2003; Todd et al., 2003; Carson et al., 2004; Zijdewind et al., 2006) showing that there is an actual albeit involuntary activity. This is confirmed by depression of the H-reflex in non-exercising muscles (McIlroy et al., 1992; Brooke et al., 1997; Hortobágyi et al., 2003; Carson et al., 2004) indicating a decline in motorneuron excitability. Possibly, the interneurons within the spinal cord inhibit contralateral motorneuron activity, depressing the amplitude of the H-reflex (Gerloff et al., 1998; Grillner et al., 1998; Muellbacher et al., 2000).

Even though these cross-over mechanisms are not yet fully understood, it can be suggested that the decrease in peak torque production of the non-exercising contralateral limb is a compensatory strategy of central nervous system decreasing the voluntary activation to reduce differences between the weaker, exercised side and the non-exercised side, likely to warrant interlimb coordination. This idea is supported by recent literature showing that unilateral fatigue of lower limb muscles disturbs bipedal postural control (Vuillerme et al., 2009; Berger et al., 2010; Paillard et al., 2010; Vuillerme and Boisgontier, 2010). Contralateral neural projections in the spinal cord of unilateral limb exercise can contribute to the central pattern generator in order to regulate bilateral rhythmic activities (McCrea, 2001; Kiehn and Butt, 2003). Therefore, the cross-over effect of fatigue occurs to balance bilateral activity for two-limb coordinated and

automatic tasks (*e.g*. balance or locomotion) and more generally to maintain lower limb homeostasis.

This result can have implications in the therapeutic context where the repetition of unilateral contractions is widely believed to enhance muscle strength. The cross-education effect (strength gain of the non-trained contralateral limb after unilateral strength training) (Enoka, 1988; Carroll et al., 2006; Fimland et al., 2009) may be attributed to neural adaptations. Thus, admitting that muscle fatigue would be a necessary stimulus for strength gains (Rooney et al., 1994), it can be suggested that neural pathways that facilitate cross-transfer in the contralateral limb after an unilateral fatiguing exercising could be associated to those involved in the cross-education effect.

8.2 Neuromuscular electrical stimulation and muscle fatigue

Neuromuscular electrical stimulation (NMES) is a widely used tool in rehabilitation settings as it has been shown to enhance muscle strength in healthy (Currier et al., 1979; Selkowitz, 1985; Colson et al., 2000; Bax et al., 2005; Gondin et al., 2005), elderly (Caggiano et al., 1994) and spinal cord injured (Dudley et al., 1999) participants. It also limits muscle atrophy due to immobilisation (Gould et al. 1982). It is possible to apply NMES using electrodes on the skin surface above the muscle bulk or close to a nerve trunk. In both cases, the muscle contraction occurs because of the excitation of the α-MN and indirectly due to the activation of spinal motorneurons via the excitation of Ia afferents axons evoked in nerve or intramuscular axonal branches (Hultman et al. 1983; Collins 2007; Bergquist et al. 2011b). In this thesis, both muscle and nerve stimulations have been used.

A major hindrance with the use of NMES in rehabilitation to overcome functional deficits is the increased rate of muscle fatigue compared to voluntary contractions (Vanderthommen et al. 2003; Maffiuletti 2010). Therefore, the overall goal of paper II was to evaluate peripheral and central contributions of muscle fatigue of an NMES protocol delivered over the nerve trunk of the plantar flexors, when increasing or maintaining the current intensity. The testing of this paradigm is important given that little is known about intensity modulation of NMES training protocols on muscle

fatigue. Furthermore, the inconsistency of the protocols used in clinical settings makes practical recommendations challenging. In isometric conditions, it is possible to estimate the work load by calculating the torque-time integral. When the constant intensity, the progressively increasing intensity and voluntary contraction protocols were torque-time matched, suggesting a similar energy expenditure (Bergström and Hultman 1988; Russ et al. 2002), the observed reduction in MVC was similar (≈ 12%), and both peripheral and central contributions to the muscle fatigue did not seem to differ between protocols. Considering the peripheral level, both NMES and voluntary protocols were likely to have induced similar adaptations. Since the evoked peak twitch at rest and potentiated peak twitch declined after all protocols and in the absence of the soleus and gastrocnemius lateralis M_{max} alterations, this may suggest an impairment of cross-bridges cycles (most probably involving Ca^{2+} handling). However, a decline of the gastrocnemius medialis maximal M-wave was observed, suggesting that the AP propagation was hampered in this muscle only.

It appears that similar central adaptations occurred in the voluntary and the two NMES protocols. As already reported in literature after voluntary contractions (Lévénez et al. 2005), the increase of the soleus V/H_{sup} and H/M_{sup} ratios after the CST, PROG and VOL protocols, suggests an enhancement of the descending neural drive to the α-MN pool. Even though this was not observed for the gastrocnemii muscles, the stimulation site and the current intensity were primarily optimised for soleus muscle activation. The typology difference between the soleus and the gastrocnemii muscles may explain the differences observed. While the soleus is mostly composed of slow-twitch MUs, the gastrocnemii contain approximately an equal amount of slow-twitch and fast twitch MUs (Tucker et al. 2005). Therefore since the targeted force level was rather low (20 % of MVC), the soleus muscle may have been more largely involved than the gastrocnemii, especially given that the nerve stimulation intensity was optimised for the soleus. In addition to supraspinal adaptations , spinal contributions also occurred, as the H_{max}-to-M_{max} ratio increased in both the soleus and the gastrocnemius medialis regardless of the protocol used. Since the electrical signal is conducted towards the spinal cord via sensory Ia afferents, this can cause the H_{max}-to-M_{max} ratio to increase. The H_{max}-to-M_{max} ratio potentiation is likely to be caused due to a facilitation of

neurotransmitter release in the spinal cord via the Ia afferents (Lloyd 1949; Hirst et al. 1981; Van Boxtel 1986; Kitago et al. 2004) or a reduced synaptic inhibition (Schieppati 1987). As a consequence, it can be suggested that the excitability of both the Ia fibres and the motorneurons was increased (Hugon 1973; Schieppati 1987). Because of the greater spinal facilitation, the nerve trunk stimulation, regardless of the intensity modulation, would have evoked a physiological-like recruitment of motorneurons. This is also supported by literature (Baldwin et al. 2006; Klakowicz et al. 2006; Collins 2007; Dean et al. 2007; Bergquist et al. 2011b). The stimulation parameters (1 ms pulse duration, 50 Hz) used in paper II, may have activated a greater amount of Ia afferents in more synchronous manner, increasing the central contributions to the torque production (Bergquist et al. 2011b). The activation of the α-MNs via Ia fibres and the reflex arc would produce muscle contractions in accordance with the physiological recruitment order, since the Ia fibres would first recruit the smallest α-MNs, fatigue resistant MUs, according to the size principle (Henneman et al. 1965; Bawa et al. 1984). Nerve stimulation with wide pulse duration, as used in paper II (1 ms pulse duration, 50 Hz), may activate a greater amount of sensory fibres in a more synchronous manner; this will likely increase the central contributions to torque production (Bergquist et al. 2011b). Consequently, this physiological-like recruitment order would possibly activate additional, non-fatigued MUs, while other MUs have their threshold activation level raised (Vilin and Ruben, 2001; Kiernan et al., 2004; Miles et al., 2005) and stop discharging, mimicking the rotation of physiological MU activity. The activation of the α-MNs via sensory axons and reflex arc enhances spinal cord neuronal circuitry (Perez et al., 2003) and can modify cortical activity (Hamdy et al., 1998). In this way, maintaining stimulation intensity and targeting a constant torque level, may generate muscle contractions through a combination of peripheral and central pathways, similarly to voluntary contractions, this is likely to be beneficial to maintaining and restoring muscle function.

A cross-transfer effect may occur after a unilateral electrically stimulated contraction (Hortobágyi et al., 1999). After 6 weeks of NMES training on knee extensor muscles, strength of the contralateral limb increased more than after voluntary contractions (Hortobágyi et al., 1999). This has been recently confirmed in an animal study (Song et

al., 2012) where an electrical stimulation trial was conducted over a 6-week period on a unilateral muscle. Results of this research showed that ipsi- and contralateral changes occurred to the muscle tissue. The time-course differed between the limbs; ispilateral effects occurred after week 1, while contralateral effects took place beginning from week 3. Tissue changes, both degenerative and regenerative in both sides, with increased developmental myosin heavy chain (MyHC) were observed (Song et al., 2012) and were suggested to be mediated via central pathways. Changes in MyHC with NMES are observed in literature (Delitto et al., 1989; Minetto et al., 2013). Specifically MyHC changes are inconsistent across literature: shift from either MyHC type 1 or MyHC type 2X towards the intermediate type 2A (Pérez et al., 2002; Maffiuletti et al., 2006; Minetto et al., 2013) are observed as well as shifts from fast-twitch fibres to slow-twitch fibres (Delitto et al., 1989; Gondin et al., 2011). A recent study has highlighted the variability in the modification of muscle composition in response to NMES (Minetto et al. 2013). Since central and muscle adaptation mechanisms contribute to muscle fatigue and strength gains when using NMES, and considering that a unilateral exercise alters the MVC of the non-exercising limb, as shown in paper I is possibly cause by spinal and/or supraspinal interplay, it can be speculated that the cross-education of muscle strength after NMES would follow similar pathways. These results imply that muscle fatigue is a necessary stimulus for the cross-education effect.

8.3 Muscle fatigue and muscle performance in neuromuscular disorders

The papers III and IV investigated the muscle performance in two neuromuscular disorders. First, muscle activation during muscle fatigue was investigated in children with cerebral palsy. Second, the effects of a bilateral 8-week NMES training of the tibialis anterior on muscle strength and muscle endurance in adults with facioscapulohumeral muscular dystrophy were studied.

Muscle fatigue and muscle performance are likely to differ in different neuromuscular disorders. In children with CP (paper III), the muscle endurance was tested during a submaximal (≈ 25% MVC) sustained isometric contraction of the elbow flexors. While it was expected that children with CP would have had a better endurance time than

typically developing peers (TD), the task duration for children with CP was similar to the control group (3 to 4 minutes).Throughout the task, the group of TD experienced an increased amplitude of the EMG signal, resulting from the increase in the descending neural drive (Enoka and Duchateau 2008) to recruit additional MUs (De Luca 1997; Farina et al. 2004; Enoka et al. 2011) required to maintain the force level. However, unlike what was observed in the TD group, the muscle activation appeared to be impaired in children with CP since no increase in the amplitude of the biceps brachii EMG was recorded. The children with cerebral palsy seemed unable to recruit additional motor units to compensate for the task's demand. The recruitment of motor units was most likely hampered, despite a normal recruitment at low-force level in a non-fatigued state (Rose and McGill 2005). The lack of increase of the amplitude of the biceps brachii EMG and the absence of changes of the EMG power density spectrum indicated that the MUs recruitment to compensate the muscle fatigue in children with CP is hindered. Moreover, and in agreement with literature (Damiano et al. 2001; Rose and McGill 2005; Stackhouse et al. 2005; Mockford and Caulton 2010; Moreau et al. 2012), the lower absolute EMG amplitude recorded during MVCs would indicate a reduction in voluntary activation and therefore underestimating the actual maximal muscle capacity. Thus, this makes the submaximal load applied a relatively low percentage of MVC and children with CP were expected to hold the load for a longer duration than typically developing peers. Children with CP seem to have muscle endurance as impaired as strength; this is also suggested in a recent study (Eken et al. 2013).

Lower EMG amplitude of the tibialis anterior muscles during MVCs was also observed in patients with facioscapulohumeral muscular dystrophy associated with a weaker ankle dorsiflexion MVC compared to healthy adults (paper IV). The fat infiltration in the muscles (Olsen et al. 2006; Kan et al. 2010; Friedman et al. 2012) and the alteration of the sarcomeric contractile properties (D'Antona et al., 2007; Lassche et al., 2013) affect the ability of the muscle to contract, possibly reducing the amplitude of the EMG signal. In addition, when performing a 2 minute sustained ankle dorsiflexion MVC, the group of patients with FSHD exhibited a much lower percentage of force production loss compared to the control group, regardless of the training. As suggested in literature

(Schillings et al., 2007), this observation would indicate that the group of patients with FSHD experienced a lower amount of muscle fatigue, which may be attributed to a number of reasons. First, the amplitude of the tibialis anterior EMG reduced similarly during the 2-minute MVC, suggesting that the descending neural drive modified with fatigue (Gandevia, 2001) for both groups in the same fashion during the endurance task. Second, the muscle composition of patients with FSHD show a shift towards more slow type fatigue resistant muscle fibres than healthy muscle composition (D'Antona et al., 2007). Third, results have shown that weaker participants are less fatigable than stronger participants (Hunter and Enoka, 2001). Consequently, with a similar tibialis anterior EMG amplitude decline but with a lower force loss, it can be suggested that at the task truncation, the group of patients with FSHD was experiencing a lower amount of muscle fatigue compared to the healthy participants.

The specificity of the FSHD is that it affects the peripheral component of the motor pathway. Over a total of 10 patients with FSHD, an M-wave could be elicited in approximately half. Since the M-wave reflects the maximal activation of motor units (Tucker et al., 2005), when no M-wave can be recorded, then the muscle is no longer responsive to the electrical current. This phenomenon can be explained by the alteration of the contractile properties of the muscle (D'Antona et al., 2007; Lassche et al., 2013). This is also supported by the amplitude of the associated evoked mechanical response that was of much lower amplitude in the patient group than in the control group. In some participants, even though eliciting an M-wave was possible, the mechanical response was either of very low amplitude or it would induce an ankle inversion/eversion or even a plantar flexion. It is possible that, although the common peroneal nerve is superficial enough, the stimulation would not induce a mechanical response (Millet et al., 2011). While no difference was found in the amplitude of the M-wave between groups, our results showed clear amplitude differences in the evoked mechanical twitch between groups, suggesting that the contractile properties of the tibialis anterior of the group of patients with FSHD were altered by the disease.

Moreover, the aim of paper IV was to investigate whether an 8-week bilateral NMES training of the tibialis anterior muscle in adults with FSHD would improve muscle strength, muscle endurance and motor function. Surprisingly, the NMES training failed

to trigger any improvements in either group. Plausible reasons for the failure of the NMES training can be attributed to the protocol. Neuromuscular electrical stimulation has been shown to induce strength gains in young and old healthy adults (Caggiano et al., 1994; Gondin et al., 2005), in patients with muscular dystrophies (Scott et al., 1986, 1990; Zupan, 1992; Zupan et al., 1993) and also patients with FSHD (Milner-Brown and Miller, 1988; Colson et al., 2010). However, no improvement of muscle strength, muscle endurance of the TA muscle or motor function in the group of FSHD patients was observed. The failure of improvements could be attributed to the frequency of the sessions and/or the duration of the NMES training. Twenty minutes per session, 3 times per week, for 8 weeks was a less strenuous protocol than that of Milner-Brown and Miller (1988) where they performed sessions of 2 hours, 5 days per week and over a period of 14 months. Similarly, Colson et al. (2010) performed sessions of 20 minutes for 5 days per week, over a 5-month training period. However, the latter study (Colson et al., 2010) showed a positive effect of a similar NMES training (6 s ON / 7s OFF; 35 Hz, 200 µs pulse-duration) in patients with FSHD. Therefore, it is valid to expect that the NMES protocol described in this thesis would be effective on the tibialis anterior of patients with FSHD. Moreover, benefits of NMES training programs on muscle strength are widely reported in literature for similar training periods (Thériault et al., 1996; Colson et al., 2000; Pérez et al., 2003; Gondin et al., 2005; Colson, 2009) in healthy participants. However, the absence of the training effect in the control group suggests that the effects of of the stimulation parameters may be questioned. The stimulation parameters used were 35 Hz with a 200 µs pulse duration, while literature suggests that high frequency (> 50 Hz) and a wider pulse duration (> 400 µs) can maximise the effect involving more central, rather than peripheral, mechanisms (Collins, 2007; Dean et al., 2007; Maffiuletti et al., 2011). The stimulation parameters used in the research of this thesis were selected since they showed an increase muscle strength in patients with muscular dystrophies (Scott et al., 1990; Colson et al., 2010). Possibly, a NMES protocol with wider pulse duration than 200 µs pulse duration and higher frequency, above 50 Hz, should be considered for training of the tibialis anterior in healthy participants. One major reason for the absence of strength increase in the groups of patients with FSHD may be related to the frequency of the NMES sessions and/or to the duration of the training protocol. In paper II, with 50 Hz and a 1 ms pulse

duration, whether or not the current intensity was increased, the H_{max}-to-M_{max} ratio was potentiated. This suggests that central contributions are of similar amplitude when the stimulation is delivered over the nerve trunk with wide pulse duration. Moreover, the intrinsic state of the tibialis anterior (altered contractile properties, and fat replacement) at the beginning of the study can be suggested as a possible reason for the training to be ineffective. Since no improvements were observable in the HP group, this cannot be stated with certitude. Even though the 8-week bilateral training did not improve ankle dorsiflexion or plantar flexion strength, nor did it improve motor function, these parameters did not show signs of deterioration over the training period. It is difficult to estimate over such a short duration but it is possible that training helped to maintain the existing muscle strength.

8.4 Methodological considerations and limitations

In paper I, the knee extensors muscles' electrical activity of the contralateral non-exercising limb have not been recorded during the 100-second MVC bouts. Therefore, even though participants were asked to relax the contralateral limb during the fatiguing exercises, clearly it cannot be excluded that contralateral muscles were activated. However, since RMS/M_{max} and evoked mechanical responses (both single and doublet) did not modify, it may be fair to suggest that the contralateral muscle activity did not contribute to decrease in MVC of the exercising limb.

The small samples sizes in paper II (n=8) and in the paper IV (FHSD group = 10; HP group = 6) may also have weakened the results. Therefore, the lack of significant protocol effect on the evoked potentials results (V-waves, H-reflexes) of paper II cannot be taken with certainty. In paper IV, the absence of an additional group of patients with FSHD, and even a healthy control group not performing the training may be considered as a limitation.

The use of the twitch interpolation technique also involve a limitation, namely that; stimulation at rest does not always induce a mechanical response (Millet et al., 2011), as presented in paper IV.

Finally, the use of maximal voluntary contractions to assess the level of muscle fatigue can be questioned because of cognitive factors linked to pain in health-compromised populations as well as possible motivational factors. Therefore, the sustained maximal efforts described in papers I and IV may have influenced the decrease of force production. However, in paper I the torque production decline respectively of 64.2% and 65.1% at the end of each effort of 100-second MVC indicates that participants had no pacing strategies. In paper IV, the maximal sustained fatiguing effort may have influenced patients with FSHD performance because of pain. Submaximal efforts in health-compromised populations should be considered in future studies.

Finally, in paper III, the sustained load of isometric elbow flexion ranged from 8% to 36% of MVC across participants of both groups and may have influenced the holding time results. However, the mean relative sustained load and the time to task failure were both similar and found to be correlated in both groups.

9 Conclusion and outlook

The main objective of this thesis was to investigate the effect of neuromuscular activation strategies on muscle fatigue, endurance and muscle performance after voluntary and electrically evoked contractions in healthy and health-compromised populations. Because patients with neuromuscular disorders may experience problems when performing exercises, exercise therapies aim to increase the quality of life of both healthy and pathological populations. The main findings of this thesis contribute to the understanding of muscle fatigue in the rehabilitation setting.

As unilateral exercise therapy is common in the rehabilitation context, this thesis examined to what degree muscle fatigue affects the muscle activation of the non-exercising limb when a unilateral fatiguing exercise was performed. Two unilateral exercises of 100 second MVC induced a cross-over effect of muscle fatigue on the non-exercising limb, time-shifted with respect to the exercising limb. The cause can possibly be attributed to crossed reflex pathways at spinal and cortical levels. This cross-over

effect may be used to reduce the gap of muscle performance between the weaker, exercised and the non-exercised limbs, likely to warrant interlimb coordination.

Moreover, neuromuscular electrical stimulation is widely used in rehabilitation settings to enhance muscle strength or to compensate for a diminished or lost function but is characterised by higher rates of muscle fatigue compared to voluntary contractions. Delivering NMES over the nerve trunk, when matching a similar TTI, increasing the current intensity of stimulation, or maintaining it constant, seems to lead to similar peripheral and central adaptations even though the muscles contributions seemed to differ. The nerve trunk stimulation with wide pulse duration would have generated muscle contractions via spinal pathways. It is possible that contractions evoked by spinal circuitry would induce preferential recruitment of fatigue-resistant motor units. Consequently, when the mechanical output is matched, the decrease in amplitude of the MVC due to NMES is similar regardless of the intensity change. It can be suggested that maintaining the stimulation intensity, targeting a constant torque level, may generate muscle contractions via a combination of peripheral and central pathways, similarly to voluntary contractions. Neuromuscular electrical stimulation applied on the nerve trunk is likely to generate physiological-like muscle activation, whether or the current intensity is increased for a similar amount of TTI reached, although this observation has to be further confirmed when the NMES is applied over the muscle bulk.

Finally, neuromuscular activation strategies in response to fatigue differs depending on the population in question, namely healthy participants with respect to both CP children and adults with FSHD. During a sustained submaximal contraction, children with CP were unable to increase their neural descending drive to recruit additional motor units to compensate for muscle fatigue. The children with CP seemed to be as affected in muscle endurance as in strength. When considering the FSHD population, weaker ankle dorsiflexion maximal voluntary contraction and lower associated TA EMG amplitude suggests that the intrinsic contractile properties of the TA were altered in this population. Neuromuscular electrical stimulation training of the TA did not improve motor function nor ankle dorsiflexor strength or endurance in patients with FSHD. It is possible that this result is due to the choice of stimulation parameters or the

strenuousness of the NMES protocol and/or to the initial altered intrinsic contractile properties of the muscles.

The results of this thesis reveal the need to further investigate the change in muscle activation patterns with muscle fatigue. The potentially beneficial cross-over effect is encouraging when considering unilateral training if one limb is more affected than the other. Finally, populations suffering from neuromuscular disorders have a need for limiting muscle fatigue and development of clear rehabilitation strategies.

10 References

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PAPER I

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Time Course of the Cross-Over Effect of Fatigue on the Contralateral Muscle after Unilateral Exercise

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Abstract

We investigated the cross-over effect of muscle fatigue and its time course on the non-exercising contralateral limb (NEL) after unilateral fatiguing contractions of the ipsilateral exercising limb (EL). For this purpose, 15 males performed two bouts of 100-second maximal isometric knee extensions with the exercising limb, and neuromuscular function of both the EL and NEL was assessed before (PRE), after a first fatiguing exercise (MID) and after a second fatiguing exercise (POST). Maximal voluntary isometric torque production declined in the EL after the first bout of exercise (−9.6%; $p<0.001$) while in the NEL, the decrease occurred after the second bout of exercise (−10.6%; $p<0.001$). At MID, torque decline of the EL was strictly associated to an alteration of the mechanical twitch properties evoked by neurostimulation of the femoral nerve (*i.e.*, peak twitch torque, maximal rate of twitch development). According to these markers, we suggest that peripheral fatigue occurred. At POST, after the second bout of exercise, the voluntary activation level of the knee extensor muscles was altered from PRE (−9.1%; $p<0.001$), indicating an overall central failure in both the EL and NEL. These findings indicate that two bouts of unilateral fatiguing exercise were needed to induce a cross-over effect of muscle fatigue on the non-exercising contralateral limb. Differential adjustments of the motor pathway (peripheral fatigue *vs*. central fatigue) might contribute to the respective torque decline in the EL and the NEL. Given that our unilateral fatiguing exercise induced immediate maximal torque reduction in the EL and postponed the loss of torque production in the NEL, it is also concluded that the time course of muscle fatigue differed between limbs.

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Introduction

Muscle fatigue is a broad research field and its mechanisms are widely investigated, but data are scant regarding the cross-over effect of fatigue. This cross-over effect relates to the fact that one type of activity can negatively impact other types of action. For example, Mosso [1] initially observed a decreased endurance in a manual task after a day of intense intellectual activity. Recently, Kennedy et al. [2] showed that the maximal voluntary contraction of the plantar flexor muscles was decreased after bilateral sustained handgrip contractions. However, the cross-over effect of fatigue has been mainly investigated after a unilateral exercise onto the performance of the contralateral homologous muscle [3–8] or limb [9–12].

Generally, muscle fatigue is defined as a progressive and transient reduction of maximal force production induced by sustained or repeated muscle contractions, no matter whether the task can be sustained or not [13], [14]. This definition implies that the maximal force production of the non-exercising contralateral muscle must decrease to conclude that cross-over effect of muscle fatigue occurred. So far, only two studies have shown that the maximal voluntary contraction (MVC) of the rested contralateral muscle declined after a unilateral fatiguing exercise of the ipsilateral homologous muscle [7], [8]. Martin and Rattey [7] reported an average reduction of 16% in women and 25% in men of the contralateral MVC of the knee extensor muscles after a 100-second sustained maximal isometric knee extension. In the first dorsal interosseous muscle of the contralateral hand, Post et al. [8] observed an average MVC decline of 10% in a group of men and women after two distinct fatiguing protocols consisting of either a 120-second sustained isometric MVC or a submaximal intermittent exercise at 30% of MVC maintained until exhaustion. Other authors have highlighted that the motor performance of the rested contralateral limb was negatively affected by using biomechanical [9] or postural control approaches [10], [11]. Conversely, it is noteworthy that many studies have failed to observe significant MVC reduction of the homologous contralateral muscles after a unilateral fatiguing exercise either for muscles of the upper limbs [3–5] or the lower limbs [6],[10], [11]. The heterogeneity of the experimental procedures can easily account for these discrepancies and not all the results can be equally compared. As a consequence, the cross-over effect of muscle fatigue remains to be debated.

The manifestations of muscle fatigue can occur through either peripheral or central pathways. Specifically, peripheral fatigue refers to a failure of the muscle in generating force at the level of or distal to the neuromuscular junction [15]. Central fatigue, instead, relates to a progressive reduction in voluntary activation that encompasses supraspinal and spinal circuitry. In the literature,

most of aforementioned studies agreed that the cross-over effect of fatigue pertains to central fatigue mechanisms. Nonetheless, few have effectively investigated the cross-over effect of central fatigue [3], [5–8]. Indeed, two studies have found that the reduced MVC of the non-exercising contralateral muscle was associated to a significant decline in voluntary activation [7], [8]. As mentioned earlier, Post et al. [8] also examined the effect of sustained maximal and repetitive submaximal exercise, and their results indicated that the maximal effort induced a more pronounced decrease of voluntary activation in the non-exercising contralateral muscle. In line with this observation, Kennedy et al. [2] concluded that a maximal fatigue protocol of handgrip contractions affected voluntary activation and MVC of the ankle plantar flexor muscles more severely than a submaximal protocol. Overall these observations emphasised that the cross-over effect of fatigue may relate to the intensity of the contraction performed. Conversely, high-intensity single-leg cycling did not compromise maximum power capacity of the rested contralateral limb nor the maximum isometric handgrip force [12]. For all these reasons, the mechanisms underlying the cross-over effect of fatigue have yet to be clarified.

An interesting approach to delineate the effect of a unilateral fatiguing exercise on the maximal force production of the non-exercising contralateral muscle could be implemented by analysing the time course of the cross-over effect of fatigue. At present, there is evidence in the literature supporting this approach. Indeed, it has been suggested that multiple muscle contractions progressively impairs voluntary activation and thus increases the cross-over effect of fatigue [3], [5]. Therefore, the aim of the present study was to investigate the time course of muscle fatigue in both knee extensor muscles after unilateral maximal isometric fatiguing exercise consisting of two bouts of 100-second MVC. It was hypothesised that the first bout of exercise would induce muscle fatigue in the exercising limb, and we expected that the cross-over effect of fatigue of the non-exercising contralateral limb would occur after the second bout of exercise.

Methods

Participants

Fifteen healthy males (age: 21.7±3.1 years; height: 181±5 cm; body mass: 76.8±7.9 kg; mean ±SD) accepted to take part in the study. Participants were physical education students and were recreationally active (≈ 5 to 10 hours a week). Participants did not report any pathology, neurological complications, muscular, tendon or joint injury within six months prior to the study.

Ethics Statement

Participants were informed about potential risks and gave informed written consent prior to enrolment in the investigation. The experiment was approved by the Ethics Committee on Human Experiments in Life and Health Sciences of the University of Nice - Sophia Antipolis and in accordance with the Helsinki Declaration (1964).

Equipment Set-up

Torque measurements. A stationary Biodex® dynamometer (System 3 Pro; Biodex Medical Systems, Shirley, NY, USA) in isometric mode was used to record isometric MVCs and the evoked contractions of knee extension. The axis of the dynamometer was aligned with the anatomical axis of the knee flexion-extension, and the lever arm was attached on the shank, about 2 cm above the lateral malleolus of the ankle. Participants were positioned on the seat with a hip angle of 120° and a knee angle of 60° (0° being considered as the full knee extension). Upper-body movements of participants were constrained by two cross-over shoulder belts and a belt across the abdomen. Participants were allowed to grip cross-over shoulder belts during the testing procedure, but they were not allowed to hold the seat. To allow semitendinosus (ST) surface electromyography recordings, a board (thickness ≈5 cm) was placed underneath the subject with a hole where the electrodes were placed to avoid any compression between the surface electrodes and the wire on the seat.

Surface electromyography (sEMG) recordings. Bi-polar sEMG electrodes (silver chloride, recording diameter of 1cm, 2 cm inter-electrode distance, Contrôle Graphique Médical, Brie-Comte-Robert, France) were positioned over the VL (Vastus Lateralis), the VM (Vastus Medialis), the RF (Rectus Femoris) and the Semitendinosus (ST) on both limbs, according to the SENIAM recommendations [16]. The reference electrode was placed on the medial bony party of the right wrist. Low-resistance impedance between electrodes (<5 kΩ) was obtained abrading the skin and cleaning it with alcohol. A Biopac MP 100 system (Biopac systems, Inc., Holliston, MA, USA) was used to record sEMG data at a sampling rate of 2000 Hz. EMG signals were amplified with a bandwidth frequency ranging from 1 Hz to 500 Hz (common mode rejection ratio = 11 dB; impedance input = 1000 MΩ; gain = 1000).

Femoral nerve stimulation. Femoral nerve stimulation was induced using an electrical stimulator (Digitimer DS7AH, Digitimer, Herthforshire, United-Kingdom) to evoke the compound muscle action potential and associated mechanical response. The femoral nerve was stimulated with a tungsten cathode ball electrode (0.5 cm diameter) pressed onto the femoral triangle by the same experimenter during the whole session. The anode was a 45 cm^2 rectangular electrode (Stimex, Wetzlar, Germany) placed between the great trochanter and the iliac spine. The stimulus was a 400 V and 2 ms rectangular pulse. The optimal intensity of stimulation was set by increasing intensity by 10 mA steps. The maximal intensity was reached once the compound muscle action potential (M_{max}) and the torque output of the associated mechanical response were found to be maximal and stable. Then, to elicit a supramaximal stimulation, the maximal stimulation intensity was increased by 10%. This intensity was used to induce doublet stimuli, separated by 10 ms at 100 Hz. Individual supramaximal intensities were between 70 and 140 mA.

Experimental Procedure

Participants were required to attend two sessions at the laboratory. The first session served to familiarise the participants with the equipment and testing procedures. At the second session, participants were equipped with sEMG electrodes on both lower limbs. The participants performed a 5-minute standardised warm-up at 2 watts.kg^{-1} on a cycling ergometer (Monark, 818E, Vansbro, Sweden) with a pedaling frequency of 70 rpm [17]. After warming-up, volunteers were transferred and secured to the isokinetic dynamometer. The neurostimulation intensity was rapidly adjusted. While fatiguing exercises were performed unilaterally, hereby the exercising limb (EL), neuromuscular tests were done on both the EL and the non-exercising limb (NEL) to evaluate fatigue on both limbs.

Pre-, mid- and post-fatigue neuromuscular tests. Figure 1 illustrates the experimental design and procedures used for data collection. A sequence of neuromuscular tests was performed before (PRE), after a first fatiguing exercise (MID) and after a second fatiguing exercise (POST). These tests were conducted as follows: 1) three single stimuli were delivered at rest,

separated by 5 seconds; 2) three doublet stimuli spaced by 5 seconds delivered at rest; 3) two 4-second isometric MVCs of knee extension (separated by a 2-minute rest) with doublet stimuli delivered respectively over the isometric plateau (superimposed doublet), and 4 seconds after the MVC (potentiated doublet) [18]. Strong verbal encouragement was provided by the experimenters throughout MVCs. During PRE, two MVCs were achieved, while only one MVC was carried out during MID and POST tests assessments. Although the sequence of neuromuscular tests was standardised, the order of EL or NEL assessments was randomly selected for the PRE, MID and POST tests.

Fatiguing exercise. The fatiguing exercise consisted of two bouts (thereafter called Fatigue 1 and Fatigue 2) of 100-second MVC knee extension of the EL. The side of the EL (*i.e.*, right or left) was randomly selected across the participants. No visual feedback was provided to participants and they were asked to perform an all-out effort, and received strong verbal encouragement. Participants were also asked to relax the NEL during fatiguing tasks. To avoid teleoanticipation [19], [20], the participants were not aware that two bouts of MVC were to be performed.

Data Analysis

Torque and sEMG recordings. The MVC was considered as the mean value over a 500-ms period when the torque output reached a maximal plateau. In PRE tests, the best MVC trial was analysed. All sEMG were analysed over the same window width. Root mean square (RMS) of the VL, VM and RF muscles were calculated (AcqKnowledge® 3.8.2, BiopacSystems, Inc., Holliston, MA, USA) and normalised to their respective M_{max} peak-to-peak amplitude (*i.e.*, RMS/M_{max} ratio) obtained at PRE, MID and POST. This normalisation procedure reduces the variability in the EMG signals due to changes at the skin level and therefore allows the interpretation of the RMS/M_{max} modification as a central nervous adaptation. RMS of the ST muscle was computed and the coactivation level was expressed by the ratio between the ST RMS value and the sum of the RMS/M_{max} values of VM, VL and RF muscles [21]. During the fatiguing exercises, torque production, torque-time integral, as well as sEMG values of the different muscles were analysed in ten consecutive periods that represented 10% of the total duration (100%) of each fatiguing bout. The RMS values of the VM, VL and RF were normalised to the M_{max} of the respective muscles obtained before each fatiguing exercise (PRE and MID). The coactivation level was expressed by the ratio between the ST RMS value and the sum of the RMS/M_{max} values of VM, VL and RF muscles computed during the fatiguing exercise.

Evoked responses. M_{max} signals elicited by the three single stimuli delivered at rest were averaged and peak-to-peak amplitude (A) and the peak-to-peak duration (D) of the VM, VL and RF muscles were analysed. Mechanical twitch responses were obtained (average of the three) from single-peak twitch (Pts). The mechanical response induced by doublet stimuli after the MVC was considered as the potentiated twitch (PtdPot). The maximal voluntary activation level of knee extensors was calculated using the following formula [22]:

$$\text{VoluntaryActivation}(\%) = \left(1 - \left(\frac{\textit{Superimposed doublet}}{\textit{Potentiated doublet}}\right)\right) * 100$$

A correction was consistently applied to the original equation when the superimposed doublet was elicited slightly before or after the real MVC [23]. In these cases, the maximal voluntary activation level was calculated as follows:

$$\left[1 - \left(\frac{\textit{Superimposed doublet} * \textit{Voluntary torque level just before the superimposed doublet}}{\frac{\textit{Maximal voluntary torque}}{\textit{Potentiated doublet}}}\right)\right] * 100$$

Statistical analysis. Statistical processing was performed in Statistica (Statsoft, version 8.0 Tulsa, OK, USA). The Kolmogorov-Smirnov test was used to test whether outcome measures were normally distributed. The statistical significance was set at $p<0.05$. Since data were normally distributed, a repeated measures two-way ANOVA (limb × time) was performed to assess fatigue-induced changes between pre-, mid- and post-fatigue neuromuscular tests. Separate two-factor ANOVA (fatiguing exercise × periods) with repeated measures on fatiguing exercise and periods were used to compare torque, RMS/M_{max} values and coactivation during the fatiguing exercises. Post-hoc analyses (Bonferroni) were used to test for differences among pairs of means when appropriate. A Pearson correlation coefficient was used to assess the relation between the MVC torque production and the voluntary activation level changes. Effect size was computed from partial eta^2 values ($\eta^2 p$). Unless specified, all data are expressed as means±SE (standard error of the mean) in the entire manuscript and in the tables and figures.

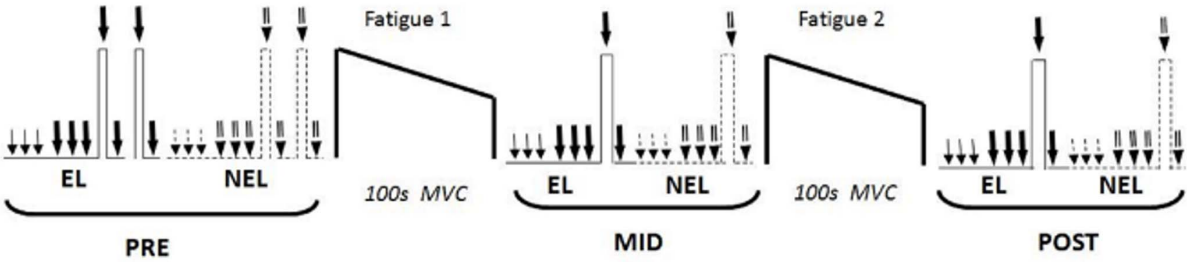


Figure 1. Graphical overview of the experimental protocol. Neuromuscular tests comprised single stimuli (single arrows), doublet stimuli (double arrows), MVC with superimposed doublet stimulus and followed by a doublet stimulus delivered at rest. Two MVCs of the exercising limb (EL; uninterrupted line) and the non-exercising limb (NEL; dashed lines) were performed at PRE, while only one was respectively realised at MID and POST. Testing order for the EL and NEL was randomly selected. The fatiguing exercise of the EL consisted of two bouts of 100-second MVC.
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Results

Regardless of the variable, no differences between the EL and the NEL have been observed during the PRE test, before the first fatiguing exercise.

MVCs, Voluntary Activation and sEMG

A significant limb × time interaction was observed for MVCs values ($F = 3.26$; $p<0.05$; $\eta^2p = 0.10$). In the EL (Figure 2A), a significant reduction in maximal torque was observed between PRE and MID tests ($-9.63\pm1.67\%$; $p<0.001$), MID and POST tests ($-9.24\pm1\%$; $p<0.01$), and between PRE and POST tests ($-17.91\pm1.93\%$; $p<0.001$) whereas, the maximal torque decline of the NEL was only significant between PRE and POST test ($-10.58\pm3.45\%$; $p<0.001$; Figure 2B).

No limb × time interaction was noted for voluntary activation of the knee extensor muscles, but a significant time effect was observed ($F = 9.98$; $p<0.001$; $\eta^2p = 0.26$; Figure 3). Regardless of the tested limb, voluntary activation was significantly depressed from MID and POST tests ($-6.07\pm2.22\%$; $p<0.05$), as well as from PRE to POST test ($-9.14\pm2.74\%$; $p<0.001$).

A significant correlation ($r = 0.451$; $p<0.05$) was found between the relative decline in MVC torque and the relative loss in voluntary activation of both limbs pooled data. This relationship indicated that the greater the MVC loss was, the greater the voluntary activation reduction.

No significant change was found in the RMS/M_{max} ratios of VL, RF, and VM muscles, nor in the coactivation level of the ST muscle recorded during MVCs ($p>0.05$; Table 1).

Mechanical Twitch and M_{max} Responses

A significant limb × time interaction was found ($F = 3.37$; $p<0.05$; $\eta^2p = 0.11$) for the mechanical twitch response evoked by single stimulus (Pts). A significant decrease was observed in the EL between PRE and POST tests ($-24.39\pm7.21\%$; $p<0.001$), while no modification was found in the NEL (Table 2).

A significant time effect was noted for potentiated doublet-peak twitch (PtdPot; $F = 5.54$; $p<0.01$; $\eta^2p = 0.17$). Independently of the limb, PtdPot values decreased from PRE to POST test ($-7.20\pm2.95\%$; $p<0.01$).

No significant interaction was observed for M_{max} responses of VM, VL and RF muscles ($p>0.05$).

Fatiguing Exercises

No significant fatiguing exercise × periods interaction was found for the torque produced during the fatiguing exercises but significant fatiguing exercise ($F = 17.27$; $p<0.001$; $\eta^2p = 0.55$) and periods ($F = 87.22$; $p<0.001$; $\eta^2p = 0.86$) effects were noted. On average and over the total duration, the torque production was significantly greater during Fatigue 1 compared to Fatigue 2 (136.8 ± 4.7 *vs.* 123.7 ± 4.6N.m; $p<0.001$). Also, across the ten periods, the torque production for both fatiguing exercises significantly decreased comparing the first period with the fourth and so on until the last period ($p<0.01$; Figure 4 A).

Additionally, RMS/M_{max} values were found to be significantly different across the ten periods for the RF ($F = 31.35$; $p<0.001$; $\eta^2p = 0.71$) between the first from the fifth and until the last period (as well as for the VM ($F = 16.3$; $p<0.001$; $\eta^2p = 0.54$) and for the VL ($F = 18.69$; $p<0.001$; $\eta^2p = 0.57$) between the first from the sixth and until the last period. However, no significant difference was noted for the coactivation during the fatiguing exercises (Figures 4 B and C).

Discussion

Main findings of this study were: 1) a unilateral fatiguing exercise reduced the MVC of both the ipsilateral (EL) and the contralateral (NEL) limbs; 2) the MVC torque production decrement of both limbs appeared to be related to an overall central failure; 3) the EL also exhibited fatigue due to peripheral impairments; 4) fatigue time course differed between the EL and the NEL after a unilateral fatiguing exercise.

Maximal Voluntary Contractions Decline in the EL and NEL

In line with previous reports in the first dorsal interosseus [8] and knee extensor muscles [7], the present work showed a decrement in maximal torque production in both the ipsilateral and contralateral limbs after a unilateral knee extensors fatiguing exercise. After the first fatiguing exercise, the MVC was significantly depressed in the EL whereas it remained unchanged

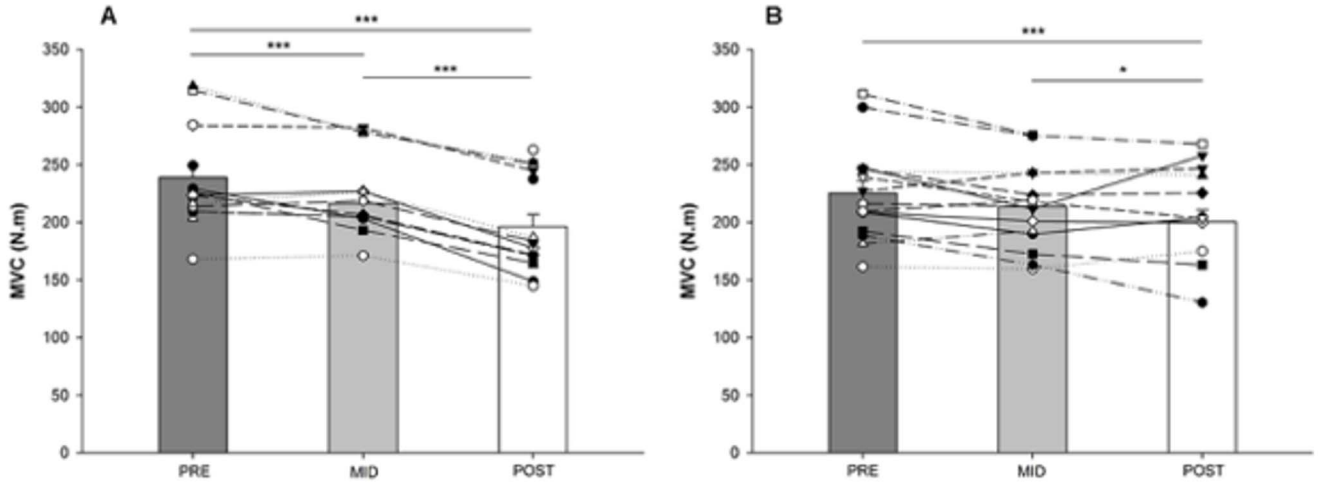


Figure 2. Maximal voluntary isometric torque. Maximal voluntary isometric torque tests of the knee extensor muscles measured at PRE, MID and POST tests for the exercising limb (A) and the non-exercising limb (B). Columns represent group mean values, while triangles, squares black and white symbols show individual values. Error bars are the standard error of the group mean. Significant differences $p<0.05$ (*) and $p<0.001$ (***).
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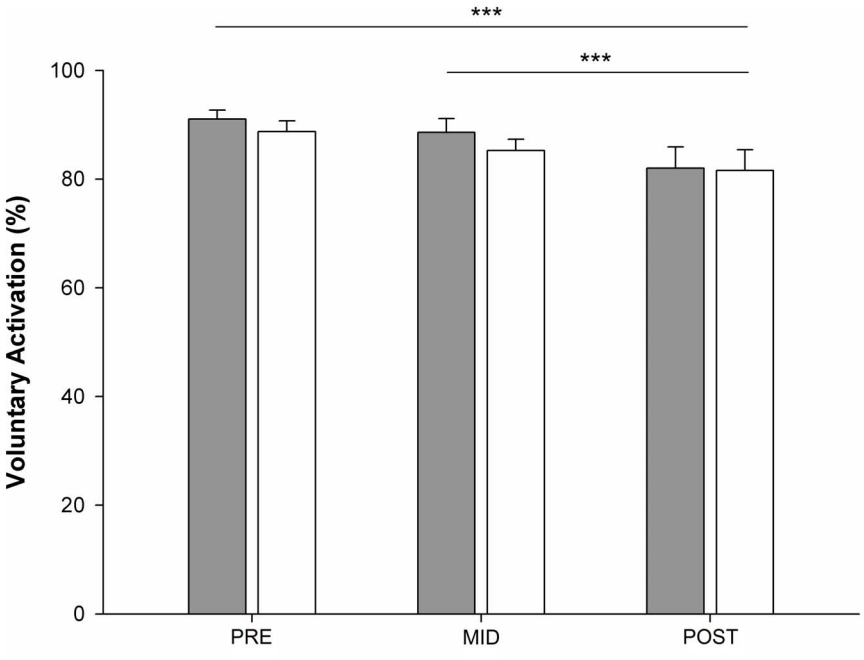


Figure 3. Voluntary activation level of the knee extensor muscles. Voluntary activation level measured at PRE, MID and POST tests for the exercising limb (in grey) and the non-exercising limb (in white). Columns represent group mean values and error bars are the standard error of the group mean. Significant differences $p<0.001$ (***).
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in the NEL [6]. After the second fatiguing exercise, the MVC declined in both the EL and NEL demonstrating a cross-over effect of fatigue [3], [5–8], [11], [12]. Previous studies initially reported that a single bout of 100-second maximal effort could lead to force decline up to 25% during knee extension in the exercising limb[6], [7]. Here, we found significant maximal torque reductions in the EL of 9.6% after the first, and 17.9% after the second bout of 100-second MVC. Differences in the experimental procedures could partly explain these discrepancies since these authors have used MVCs with short rest periods (*i.e.*, 30 seconds) prior to the fatiguing exercise. Thus, it might be suggested that repetitive muscle contractions could participate to greater force reductions.

In the NEL, we observed a significant torque decline of 10.6% after the second fatiguing exercise, while a 4.9% non-significant decline occurred after the first bout. Similar non-significant force decline of ≈4.1% was also observed by Rattey et al [6]. In opposition, Martin and Rattey [7] found a significant force decline (≈13%) just after one bout of 100-second MVC exercise in the non-exercising lower limb in men while this reduction (*i.e.*, 8%) was not significant in women. In their study, participants performed MVCs of the non-exercising lower limb immediately after the fatiguing exercise of the exercising lower limb, whereas in the present study, the NEL and the EL were randomly assessed one after each other after the fatiguing exercise. Hence, it might be suggested that maximal torque of the NEL could have partially recovered when this limb was tested after the EL. This assumption is reinforced by the results of Rattey et al. [6], where no significant force reductions were found in the non-exercising lower limb when lower limbs were tested in the same way as the present study. Even though experimental procedures, as well as physical activity level of the participants and/or gender could partly account for the difference in results between our study and the previous ones [6], [7], we originally observed a difference in the time course of maximal torque decline between both limbs *(i.e.*, a progressive muscle fatigue occurring in the EL after the first fatiguing exercise and then in the NEL after the second fatiguing exercise).

Table 1. sEMG RMS/M_{max} values of the knee extensors and coactivation level for both limbs.

		PRE				MID				POST			
		RMS/ MVL	RMS/ M RF	RMS/ M VM	CO-A	RMS/ M VL	RMS/ M R	RMS/ M VM	CO-A	RMS/ M VL	RMS/ M RF	RMS/ M VM	CO-
EL	Mean (SE)	0.065 (0.008)	0.111 (0.015)	0.068 (0.009)	0.307 (0.081)	0.066 (0.007)	0.106 (0.012)	0.077 (0.013)	0.217 (0.026)	0.062 (0.007)	0.095 (0.010)	0.067 (0.010)	0.213 (0.024)
NEL	Mean (SE)	0.077 (0.013)	0.093 (0.011)	0.067 (0.010)	0.178 (0.019)	0.077 (0.012)	0.088 (0.008)	0.064 (0.009)	0.181 (0.016)	0.078 (0.012)	0.081 (0.008)	0.066 (0.012)	0.186 (0.022)

Mean and standard error of sEMG RMS/M_{max} values of the vastus lateralis (VL), vastus medialis (VM), rectus femoris (RF) muscles and coactivation level (CO-A)of the semitendinosus (ST) muscle obtained during PRE, MID and POST tests for both the exercising (EL) and non-exercising (NEL) limbs. VL, VM and RF values were normalised to the respective muscle M_{max} responses of the test.
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Table 2. Mechanical responses evoked by single stimulation and potentiated doublet for both limbs.

		Pts (N.m)			PtdPot (N.m)		
		PRE	MID	POST	PRE	MID	POST
EL	Mean (SE)	37.97 (2.68)	32.61 (3.17)	28.66*** (3.56)	102.73 (5.22)	97.09 (5.36)	89.19## (5.16)
NEL	Mean (SE)	35.92 (2.95)	34.33 (3.74)	34.45 (3.75)	94.40 (5.34)	94.24 (5.55)	91.11## (5.83)

Mean and standard error of mechanical twitch response evoked by single stimulation (Pts), potentiated twitch evoked with a double stimulation (PtdPot) measured at PRE, MID and POST tests for the exercising limb (EL) and the non-exercising limb (NEL). Significant differences of the EL values between PRE and POST: $p<0.001$ (***). Significant differences of pooled data for the exercising limb (EL) and the non-exercising limb (NEL) between PRE and POST: $p<0.01$(##).
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Peripheral Adaptations in the EL and NEL

Although, M-wave values of both the vastus medialis [6] and the first dorsal interosseus [8] muscles in the exercising limb have been previously reported to decrease and the values in the contralateral non-exercising limb have been reported to remain unchanged, we found here that M_{max} responses of VM, VL and RF muscles were not altered by the fatiguing exercises in both the EL and NEL [7], suggesting preservation of the neuromuscular transmission. Yet, the mechanical twitch response (Pts) evoked at rest diminished in the EL (≈24%) after the two bouts of 100-second maximal effort exercise, while it remained constant in the NEL. This result is consistent with previous reports [6], [7], although in these studies declines of over 50% were found in the exercising limb after only a single bout of 100-second MVC exercise. The use of twitch responses evoked at rest to evaluate peripheral modifications has been questioned [24] and it has been shown that potentiated responses were more sensitive for the quantification of peripheral fatigue [25], [26]. In our study, the potentiated Ptd (PtdPot) was significantly reduced in both the EL and NEL after the two bouts of 100-second MVC exercise. Even though no significant difference could be observed between limbs, a 12% decline (effect size = 0.58) of the PtdPot was noted in the EL, whereas only 2% (effect size = 0.11) was found in the NEL. Thus, our results suggest that peripheral fatigue mainly affected the EL, while this seems unlikely to occur in the NEL [6], [7]. In the absence of M_{max} impairments, this may suggest that impaired cross-bridges cycles (most probably involving Ca^{2+} handling) would have played a major role in the peripheral fatigue that occurred in the EL.

Voluntary Activation of the EL and NEL

The reduction of voluntary activation observed in the present work is in accordance with previous literature [5–8]. The decline likely occurred after the second fatiguing exercise for both the EL and the NEL and was found to be correlated with the MVC loss [7]. WhileRattey et al. [6] reported, after only one bout of 100-second MVC exercise, a decline of voluntary activation of 17% in the exercising limb and 9% in the non-exercising lower limb our results showed an overall decrement of ≈10% after two bouts of 100-second maximal effort exercise. Martin and Rattey [7] reported a decline of 19 to 30% in the exercising limb and from 8 to 14% in the non-exercising limb, after one bout of 100-second MVC in women and men, respectively. In our study, the lack of significant difference in voluntary activation between the EL and the NEL is likely related to multifaceted methodological reasons. First, as argued above, these authors have used more strenuous protocols (*i.e.*, multiple MVCs with rest periods) prior to the fatiguing exercise which led to greater force declines and may also have induced greater voluntary activation decline. Second, differences can arise from the interpolation twitch technique procedures. Rattey et al. [6] used unpotentiated twitch and even though Martin and Rattey [7] quantified voluntary activation with potentiated twitch,they split their experimental design in two different days, thus maximising the potential effects of the exercise on the post fatigue measurements. In opposition with literature [6] and even though the overall activation level significantly decreased, the RMS/M_{max} ratio of the knee extensors muscles did not exhibit any change, neither in the EL nor in the NEL. This could be partly explained by the fact that assessment of voluntary activation by means of twitch interpolation technique is more reproducible than RMS/M_{max} ratios [26], [27–29]. Moreover, one limitation of this study relates to the time interval between the end of the sustained contraction and both the voluntary activation and MVC assessments. Indeed, and in agreement with previous reports on knee extensors [7] and elbow flexor muscles [30], [31], [32], we observed a loss of torque production respectively of 64.2% and 65.1% at the end of each bout of 100-second MVC exercise. However, the MVC torque decrements in the EL were −9.63% at MID and −9.24% at POST. Although these observations demonstrate that the participants had no pacing strategy when performing the first and second fatiguing exercises [20], a partial recovery could have occurred, thus explaining the non significant change of the RMS/M_{max} ratios. Finally, not only the electrical activity of agonist muscles was not altered, but the coactivation level of the ST muscle remained unchanged. Although this observation is unique in the literature regarding the cross-over effect of fatigue, we only recorded the sEMG of the ST muscle amongst the hamstrings muscles Interestingly, the present results confirmed that, since no peripheral fatigue occurred in the NEL, the decline of MVC in the NEL relied on a cross-over effect of fatigue likely occurring at a central level whereas the MVC impairment in the EL might likely relate to both central and peripheral fatigue factors.

Possible Mechanisms Explaining Central Failure and Limbs Interactions

Central failure encompasses adjustments at both spinal and supraspinal levels that induce reduced excitation of the motorneurone pool or decrease motorneurones responsiveness [33]. Although the twitch interpolation technique does not permit differentiation of the supraspinal from spinal mechanisms responsible for central fatigue [28], spinal inhibition arising from muscles afferents (muscle spindles and group III and IV afferents) in the EL could disturb the motorneurones excitation of the contralateral NEL. Indeed, it has been shown that unilateral task can depress H-reflex of the contralateral homologous muscle in both the upper [34], [35] and lower limbs [36]. Then, the reduced MVC of the NEL observed in our study could result from altered activity at spinal level of the ipsilateral EL through commissural interneurons.

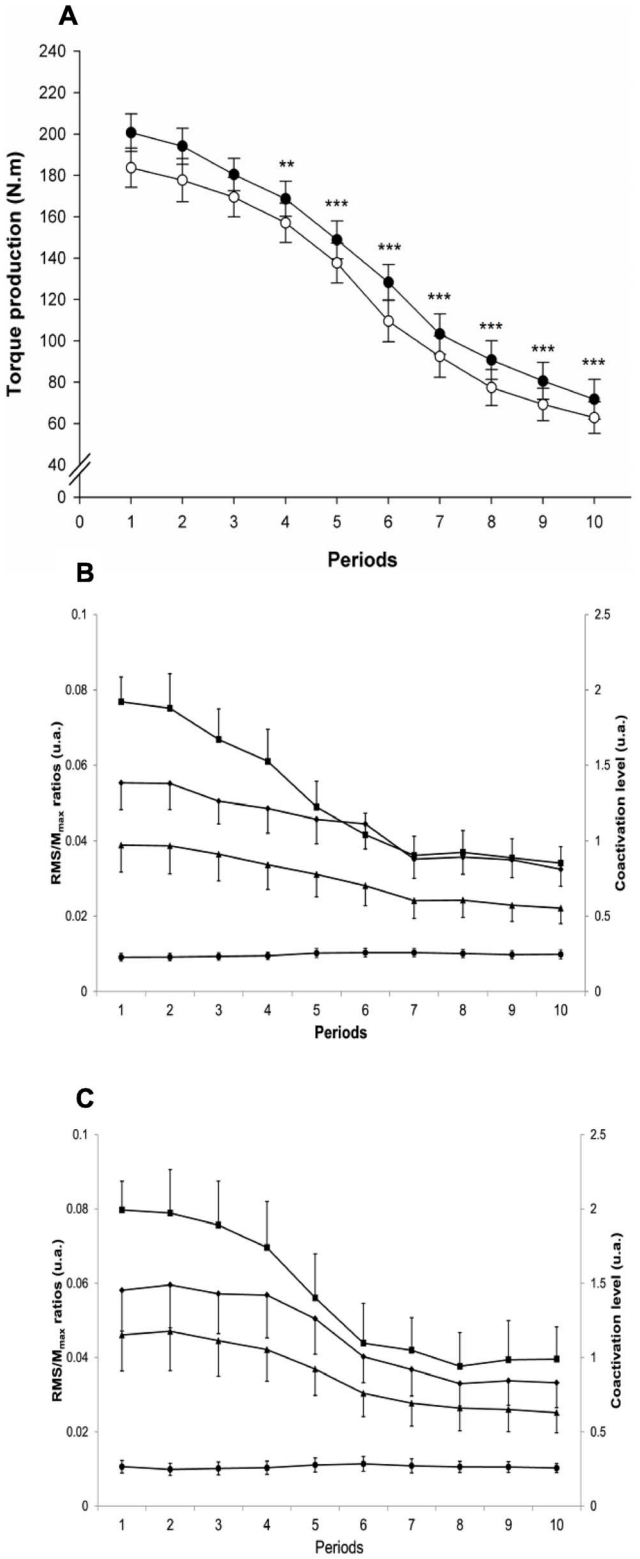


Figure 4. Torque production capacity and sEMG activity during the fatiguing exercises. Torque production capacity measured during the 10 periods of the first (black circles) and the second (white circles) fatiguing exercise for the exercising limb (A). Pooled data of the considered period significantly lower from the pooled data of the first period: $p<0.01$ (**) and $p<0.001$ (***). sEMG RMS/Mmax ratios of the vastus lateralis (diamonds), the vastus medialis (triangles), the rectus femoris (squares) muscles and coactivation level (circles) of the semitendinosus (ST) muscle during the 10 periods of the first (B) and second (C) fatiguing exercise. Values are mean and standard error of the mean.

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Supraspinal fatigue has been related to an inadequate cortical output [30] and group III and IV muscles afferents are likely to limit the circuits that generate voluntary drive [37], [38]. In addition, there is recent evidence that intracortical inhibition increased during a 2-minute MVC of the elbow flexor muscles [31]. In this context, the MVC decline of the NEL observed in the present study could also be related to inter-hemispheric neural regulation through transcallosal pathways [39]. Nonetheless, there is evidence that supraspinal fatigue was minimal in the elbow flexor muscles [5] and spinal mechanisms were proposed as the major contributor of central fatigue during a 2-minute MVC of the elbow flexor muscles [5]. Based on these observations and recent conclusions [40], we assume that changes at a spinal cord are likely to contribute to the overall voluntary activation decline. Although speculative, this regulation from spinal crossed reflex pathways during fatiguing exercise [11] has been observed after unilateral strength training [41] and requires further investigation. Our results are nevertheless of importance for functional activities suggesting that the cross-over effect of fatigue would occur to balance bilateral activity for two-limb coordinated and automatic tasks (*e.g.*, balance, locomotion…) and more generally to maintain lower limbs homeostasis. Recent studies have highlighted that unilateral fatigue of lower limb musculature disturbs bipedal postural control [10], [11], [42], [43]. In addition, unilateral fatigue induced by single-leg squats has been shown to provoke a cross-over effect of central fatigue to the contralateral limb during single-leg landings in elite female athletes which is substantially sufficient to support dangerous postural adjustments [9]. In this instance, we suggest that the central nervous system develops a compensating strategy to reduce MVC of the NEL through lower voluntary activation to cope with the weaker MVC of the EL, and therefore to warrant bilateral coordination of the lower limbs. The current data may have also direct implications in both the therapeutic and the physical training contexts where repetitive unilateral exercise is often performed for strengthening purpose. For example, neural adaptations are likely to be an explanative mechanism of the cross-education effect observed when a unilateral strength training leads to a strength gain to the non-trained contralateral limb [44–46]. Here, we emphasised that cross-over effects of fatigue induced by a unilateral exercise was of central origin. Considering that muscle fatigue contributes at least in part to the strength training stimulus [47], it might be speculated that unilateral exercise would activate central pathways that facilitate cross-transfer in the contralateral limb. As a consequence, contrary to some previous beliefs [3–5], [8] and in agreement with others [6], [7], [9] we concluded that the magnitude of the cross-over effect of central fatigue is important in the lower limbs.

Conclusion

This study has highlighted that a unilateral fatiguing exercise consisting of two bouts of 100-second MVC knee extension leads to cross-over fatigue to the contralateral limb and that the time course of muscle fatigue differed between both limbs. It seems that peripheral fatigue of the EL arose through adaptations involving intramuscular processes located beyond the sarcolemma whereas spinal crossed reflex pathways were proposed as the main factor of voluntary activation failure of the EL and the NEL. However, identification of central mechanism that is responsible for the

interlimbs adjustment seem worthy of consideration and exploration.

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Author Contributions

Conceived and designed the experiments: ACMD SSC. Performed the experiments: ACMD FL SSC. Analyzed the data: ACMD FL SSC. Contributed reagents/materials/analysis tools: ACMD FL SSC. Wrote the paper: ACMD SSC.

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PAPER II

Effect of neuromuscular electrical stimulation intensity over the tibial nerve trunk on triceps surae muscle fatigue

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KEYWORDS: M-wave; H-reflex; V-wave; Torque-time integral; Rehabilitation

ABSTRACT

Purpose. This study was designed to investigate whether the intensity modulation of a neuromuscular electrical stimulation (NMES) protocol delivered over the nerve trunk of the plantar flexors would lead to differential peripheral and central contributions of muscle fatigue. ***Methods.*** Three fatiguing isometric protocols of the plantar flexors matched for the same amount of isometric torque-time integral (TTI) were randomly performed including a volitional protocol (VOL) at 20% of the maximal voluntary contraction (MVC) and two NMES protocols (one at constant intensity, CST; the other at intensity level progressively adjusted to maintain 20% of MVC, PROG). ***Results.*** No time x protocol interaction was found for any of the variables. The MVC decreased similarly (≈ 12%; $p<0.001$) after all protocols, so did the potentiated twitch responses ($p=0.001$). Although voluntary activation of the plantar flexors did not change, maximal H-reflex to M-wave ratio of the soleus (SOL) and the gastrocnemius medialis (GM) muscles showed an overall increase (SOL: $p=0.037$; GM: $p=0.041$), while it remained stable for the gastrocnemius lateralis (GL) muscle ($p=0.221$). A main time effect was observed only for the SOL maximal V-wave to the superimposed M-wave ratio ($p=0.024$) and to the superimposed H-reflex ($p=0.008$). While similar central and peripheral adaptations were observed after the three fatiguing protocols, the individual contribution of the 3 different triceps surae muscles was differential. ***Conclusion.*** Whether or not the current intensity was increased, the adaptations after a NMES protocol yield to similar muscle fatigue adaptations as voluntary contractions likely through similar pathways matching a similar TTI.

INTRODUCTION

Neuromuscular electrical stimulation (NMES) is broadly used in rehabilitation to enhance muscle strength or to compensate for a diminished or lost function (Bélanger et al. 2000; Burridge and Ladouceur 2001; Bax et al. 2005; Gondin et al. 2005; Colson et al. 2010). A hindrance of the use of NMES in rehabilitation to overcome functional deficits is the greater muscle fatigue rates induced compared to voluntary contractions (Vanderthommen et al. 2003; Maffiuletti 2010). The principle of NMES is to initiate a muscle contraction through the excitation of the muscle fibres recruited directly by motor axons depolarisation, but also indirectly by activation of spinal motorneurons via Ia afferent volleys evoked in mixed nerve (stimulation over the nerve trunk) or intramuscular axonal branches (stimulation over the muscle) (Hultman et al. 1983; Collins 2007; Bergquist et al. 2011b). Whatever the stimulation site (muscle bulks or nerve trunk), the recruitment pattern of motor units (MUs) in NMES has been shown to be random, spatially fixed, spatially incomplete, temporally synchronous (Adams et al. 1993; Vanderthommen et al. 2003; Gregory and Bickel 2005) and differs from muscle activation in voluntary contractions and inducing higher rates of muscle fatigue (Adams et al. 1993; Maffiuletti 2010). During constant submaximal voluntary contractions, the MU discharge rate varies and MU rotation (alternative substitution of one MU activation to another in a cyclic fashion) (Westgaard and de Luca 1999; Bawa et al. 2006) takes place, delaying muscle fatigue since it provides a metabolic rest for the contractile apparatus (Bawa et al. 2006). However, NMES does not permit a recruitment rotation since usually the stimulation settings remain constant, fatigue occurs, and the force production progressively declines (Adams et al. 1993; Collins 2007; Maffiuletti 2010). Although these explanations for a higher fatigue rate with NMES seem logic, there is an alternative explanation. In these studies, the mechanical levels were different between voluntary and evoked contractions (Adams et al. 1993; Vanderthommen et al. 2003), plausibly being the main cause for the

difference in fatigue. It might therefore be that when the mechanical output is the same, fatigue rates are similar.

The force production during a NMES protocol depends on the stimulation parameters applied such as frequency, pulse width, and voltage and current intensity (Gregory et al. 2007; Gorgey et al. 2009; Gondin et al. 2010). Literature reports contradictory results regarding frequency effects on fatigue: lower (Jones 1996; Matsunaga et al. 1999) and higher (Kebaetse and Binder-Macleod 2004; Kebaetse et al. 2005; Kesar et al. 2008) frequencies are reported to induce the greatest fatigue rates, although the later has been challenged (Gondin et al. 2010). While frequency, voltage and pulse width can be standardised, the current intensity is usually set at a fixed force production level. It seems that with the increase of current intensity, more MUs are activated (Bergquist et al. 2011b), compensating for fatigued MUs and permitting force production maintenance (Theurel et al. 2007; Maffiuletti 2010; Bergquist et al. 2011b). Moreover, since sensory axons because of their larger fibres have a lower activation threshold than alpha-motorneurons (α-MN), increasing the current intensity depolarises more sensory axons and sends a larger sensory volley towards the CNS, especially at low contraction levels with a reduced antidromic volley (Bergquist et al. 2011b). Therefore, increasing current intensity is expected to give a larger central contribution to muscle fatigue. However, the effect of current intensity modulation on recruitment pathways and fatigue remains to be investigated.

In the lower limb, when comparing muscle stimulation with nerve stimulation, it has been shown that muscle fibre recruitment resulted from relatively different contributions of peripheral and central pathways (Baldwin et al. 2006; Bergquist et al. 2011a; Bergquist et al. 2012). The stimulation of the nerve trunk, not only activates the muscle directly through

motor axons depolarisation (muscle compound - M-wave), but also indirectly contracts the muscle, activating sensory axons Ia fibres (Hoffman reflex- H reflex) and facilitates the neurotransmitters release at the spinal level, thus increasing α-MNs activation (Hirst et al. 1981). Nerve stimulation increased the amplitude of the H-reflex and diminished the amplitude of the M-wave, thus suggesting a greater spinal contribution to force production (Bergquist et al. 2011a; Bergquist et al. 2012). Therefore, it has been suggested that stimulation of the nerve trunk would follow the size principle recruitment of MUs as it involves central pathways that would recruit fatigue-resistant MUs and would reduce muscle fatigue compared to the stimulation over the muscle belly (Collins 2007; Lagerquist and Collins 2010; Bergquist et al. 2011a; Bergquist et al. 2012).

The aim of the study was to investigate muscle fatigue of the triceps surae after NMES protocols where the current intensity was maintained constant or increased and compare that with fatigue after voluntary contractions. For a matched mechanical output (torque-time integral (TTI)), we expected a similar muscle fatigue assessed by the plantar flexion force production between protocols. Furthermore, it was hypothesised that increasing current intensity would yield to greater central contributions to muscle fatigue than maintaining the current intensity constant.

METHODS

Participants

Nine physically active males volunteered to take part in the study. However, one of the participants could not perform NMES sessions due to the discomfort experienced. Thus, the results of 8 participants were analysed in this study (mean ± standard error of the mean (SE): age 26.6 ± 2.02 year; height: 180 ± 2.38 cm; body mass: 74.4 ± 2.07 kg). None of the

participants had a history of neurological or neuromuscular disorder. Participants were informed about potential risks and gave informed written consent prior to enrolment in the experiment. The study was performed according to the Declaration of Helsinki and was approved by the local ethics committee.

Experimental procedure

Figure 1illustrates the overall experimental design used for data collection. The right leg was tested and a sequence of neuromuscular tests (Figure 1B) were performed prior to and after the fatiguing protocols to assess their effects on the muscle performance. The procedure consisted of three different plantar flexors fatiguing protocols (Figure 1A), including two NMES protocols and a voluntary one. The torque-time integral was matched between the three fatiguing protocols in order to ensure comparison of experimental protocols. Protocols were done in a stratified randomised order during three different days spaced at least by 48 hours, and done at the same period of the day.

Torque measurements

Measurements of ankle plantar and dorsi flexion isometric torques were performed with the use of a dynamometer (Biodex, System 3, Shirley Corporation, New-York, United-States). Participants were seated with a hip joint angle of 90°, the knee joint angle at 120° (full extension as 180°) and the ankle joint angle at 90°. The ankle extension-flexion axis was aligned with the centre of rotation of the dynamometer shaft. The foot was placed in a shoe screwed at the heel on the footplate of the dynamometer, and firmly strapped to prevent any movements of the forefoot and heel during efforts. Participants' pelvis and trunk were secured

with a waist belt and two cross-over shoulder belts, to avoid any motion from the upper-body. Participants were instructed to hold the shoulder belts during the testing procedure.

Electromyography

Bipolar surface electromyographic (EMG) electrodes (10 mm diameter, Ag-$AgCl^-$, 20 mm inter-electrode distance – Contrôle Graphique Médical, Brie-Comte-Robert, France) were placed over the soleus (SOL), the gastrocnemius lateralis (GL), the gastrocnemius medialis (GM) and the tibialis anterior (TA) muscles. The soleus EMG electrodes were positioned 2 cm below the gastrocnemii's insertion, over the calcaneal tendon, GL, GM and TA EMG electrodes were placed on the bulk of the muscles according to the SENIAM recommendations (Hermens et al. 2000). A common reference electrode was positioned over the contralateral patella. Low resistance between the two electrodes (<5 kΩ) was obtained abrading the skin, and remaining dirt was removed using alcohol. Electromyographic signals were amplified with a bandwidth frequency from 15 Hz to 2 kHz (common mode rejection ratio=90dB; impedance input=100MΩ; gain=1000). Synchronized data from EMG and torque measures were digitised on line at a sampling rate of 4000 Hz and stored for analysis with commercially available software (Tida 5.0, Heka Elektronik, Lambrecht/Pfalz, Germany).

Nerve stimulation

Tibial nerve stimulation was induced using a high-voltage (400V) constant current stimulator model DS7A, Digitimer, Hertfordshire, United-Kingdom) to evoke the compound muscle action potential (M-wave), the Hoffman reflex (H-reflex) of the SOL, GL and GM muscles and the stimulated fatiguing protocols. The stimulation site, giving the greatest amplitude of the evoked potentials of the SOL muscle, was first located by a hand-held ball electrode (0.5 cm diameter) with a single rectangular pulse (1 ms width). Once the optimal stimulation site

was determined, a self-adhesive electrode (8 mm diameter, Ag-AgCl$^-$) was firmly fixed with the use of rigid straps and taping to ensure constant stimulation location. The anode (5 x 10 cm, Compex Médical SA, Ecublens, Switzerland) was placed over the patella tendon of the ipsilateral knee.

Neuromuscular tests

First, only prior to the fatiguing protocols, a passive isometric recruitment curve was performed to establish the current intensity to elicit maximal amplitudes of the H-reflexand the M-wave of the soleus muscle. Intensity was increased with a 2 mA increment from the H-reflex threshold until the maximal M-wave of the SOL was obtained. Three stimuli separated by 5 seconds were delivered at each current intensity. Afterwards, ten minutes of rest were given to participants before starting the entire protocol.

To disclose the effects of each fatiguing protocol, several tests were achieved, before and after each experimental session (PRE, POST) in a stratified randomised order. The tests were conducted as follows (Figure 1B):

1) Electrically evoked twitches separated by 3 seconds, at SOL M_{max} and H_{max} intensities, while participants were asked to relax. At POST, intensity eliciting the maximal amplitude of the H-reflex was re-determined.
2) Four plantar flexors MVC of 4 to 5 seconds were performed with superimposed single stimulations delivered before, over the MVC and 3 seconds after the contraction. A rest period of minimum one minute was respected between each trial. Two trials were realised at H_{max} intensity (MVC H_{max}) and the other 2 at M_{max} intensity (MVC M_{max}), in a stratified randomised order, with a stimulation delivered at rest before the MVC (PtH_{max}; PtM_{max}) and (potentiated twitch – hereafter Pt Pot M_{max}; $PtPotH_{max}$).Strong

verbal encouragement was provided by the experimenters throughout MVCs. While participants were asked to perform the MVCs, stimuli at H_{max} and M_{max} intensities were applied over the contraction to record the superimposed H-reflex (H_{sup}) at H_{max} intensity, the superimposed M wave (M_{sup}) and the first volitional wave (V wave - V) at M_{max} intensity of the SOL, GL and GM muscles. At POST tests, only one trial at H_{max} and one at M_{max} intensity were performed.

3) Two dorsiflexion MVC were done at PRE whereas, one trial was performed at POST.
4) At last, and before the fatiguing protocols, the stimulation intensity to evoke a plantar flexion of 20% of MVC was determined. Thereafter 3 electrically induced twitches separated by 3 seconds were evoked at this intensity ($Pt_{Stim20\%}$). Three electrically induced twitches separated by 3 seconds were also evoked at the intensity reached at the end of the PROG protocol only.

The current intensity to evoke the maximal amplitude of the SOL H-reflex changed after each fatiguing protocols (mean ± standard error of the mean): CST: from 22.25 ± 2.09 mA to 21.67 ± 2.60; PROG: from 23.50 ± 4.14 mA to 23.50 ± 4.45 mA; VOL: from 23.11 ± 2.91 mA to 22.43 ± 3.13 mA.

Fatiguing protocols

A second a high-voltage (400V) constant current stimulator model DS7A, Digitimer, Hertfordshire, United-Kingdom) was used for the NMES protocols through the same stimulation site used to induce evoked potentials. Repeated tetanic isometric plantar flexion contractions were elicited over the trunk of the tibial nerve during 6 seconds and followed by 6 seconds of rest (duty cycle of 50%). Current characteristics (pulse duration: 1ms, frequency:

50 Hz) stimulation and the current intensity required (range: 10.2-73.6 mA) to produce 20% of the MVC caused only limited discomfort.

The NMES Progressive (PROG) protocol was designed to evoke 20% MVC throughout the entire session, and thus necessitated to manually adjust the stimulation intensity to keep on matching the 20% MVC controlled through a visual feedback of the torque signal displayed on the dynamometer screen. The current intensity at start (mean ± SE) was 20.95 ± 3.39 mA and reached 30.03 ± 7.69 mA at the end of the protocol.

For the NMES Constant (CST) protocol, the stimulation intensity was set to reach 20% of MVC for the first 6-second electrically elicited contraction, thereafter, the intensity was kept constant (20.60 ± 1.77 mA) until the end of the session. The third protocol (VOL) consisted of voluntary isometric plantar flexion contractions maintained at 20% of MVC with visual feedback.

Torque-time integral (TTI)

The torque time integral produced during each fatiguing protocol was controlled with a custom made system made of a voltmeter (Cen-Tech P30756, Digital multimeter) that measured the tension of the current developed. The digital voltmeter, synchronised to the dynamometer, measured the instantaneous torque produced throughout the fatiguing protocols.

As protocols were performed in a stratified randomised order, the first one consisted in 30 to 40 contractions. The following protocols were stopped when the TTI value reached the one from the first protocol. The number of contractions was on average (mean ± standard error of the mean) for the CST of 33.4 ±1.7, for the PROG of 28±1.4 and for the VOL of 22.6 ±2.1 contractions.

Data analysis

All outcome measures, EMG and torque data were analysed with Matlab R2010b (The MathWorks, Inc., Natick, MA, United-States).

Mechanical recordings

The MVC was computed as the average of the 500ms around the highest torque value from the best of the two trials of MVC M_{max}. The magnitude of the superimposed twitch and the potentiated twitch following the best MVC were analysed.

Mechanical parameters of the stimuli corresponding to M_{max}, H_{max} and 20% of MVC intensities were analysed, and the average of the three evoked peak twitches (PtM_{max}, PtH_{max}, $Pt_{Stim20\%}$) were calculated. In addition, the Pt Pot M_{max}-to-PtM_{max} ratio ($PtPotM_{max}/PtM_{max}$) was also computed.

The maximal voluntary activation level of plantar flexors was calculated using the following formula (Allen et al. 1995): (1 - (superimposed twitch / potentiated twitch)) x 100.

A correction was applied to the original equation when the superimposed twitch was elicited slightly before or after the real MVC accordingly to Strojnik and Komi (Strojnik and Komi 1998).

Evoked potentials

At rest. The evoked responses elicited at rest (H_{max}, M_{max}, Intensity Stimulation 20% MVC) were averaged to determine the following parameters: the maximal amplitude of the M wave (M_{max}); the maximal amplitude of the peak-to-peak of the H reflex (H_{max})and the M wave (M_{Hmax}) associated with the H_{max} peak-to-peak amplitudes of the H-reflex ($H_{Stim20\%}$) and the M wave ($M_{Stim20\%}$) at 20% of MVC of the SOL, GL and GM muscles.

Superimposed Tests.

The amplitude of the SOL, GL, and GM M_{sup}, the SOL, GL, and GM H_{sup} and V wave (electrophysiological variant of the H reflex recorded during the contraction (Upton et al. 1971)) of the analysed MVCs were recorded. The following ratios were then computed: H_{max}/M_{max}, H_{sup}/M_{Sup}, V/M_{Sup} and V/H_{sup} for the SOL, GL, and GM muscles.

Surface EMG recordings analysis

The root mean square (RMS) of EMG values of the SOL, GL, GM and TA associated with MVCs (RMS) were analysed over a 500-ms window width, the same as MVC computation. The EMG RMS of the SOL, GL and GM were then normalised to the peak-to-peak amplitude of their respective superimposed M-wave obtained during the MVC (RMS/M_{Sup}) to assess the level of EMG background.

Torque-time integral

An index to measure the quantity of isometric work is the calculation of the torque-time integral (TTI) since it is acknowledged to reflect the metabolic cost of the muscle contractions (Bergström and Hultman 1988; Binder-Macleod and Russ 1999; Russ et al. 2002b). Therefore, the torque-time integral (TTI) was computed as the area under the torque-time curve during muscle contraction (Bergström and Hultman 1988; Russ et al. 2002b).

Statistical analysis

Statistical processing was performed in PASW Statistics version 20 (SPSS Inc., Chicago, IL, USA). The Shapiro-Wilk test was used to test whether outcome measures were normally

distributed, and depending on the results the appropriate statistical test was performed. A one-way Kruskal-Wallis test was used to compare the amount of energy developed and the TTI between the three protocols. When data were normally distributed, a two-way ANOVA (protocol × time) with repeated measures on time was performed, while the Friedman test was applied when one of the variables did not follow a normal distribution. In all statistical analysis the significance level was set at $p<0.05$. Effect size was computed from partial eta² values (η^2p) and from the Kendall's coefficient of concordance (Kendall's W). Unless specified, all data are expressed as means ± SE (standard error of the mean) in the entire manuscript and in the tables and figures.

RESULTS

For any of the variables, no significant difference between protocols was observed in PRE, and neither interaction protocol × time.

Torque-time integral

The torque-time integral was not found to be significantly different between all three protocols ($H(2)=0.090$; $p=0.956$).

Maximal voluntary contraction, voluntary activation level and EMG activity

A main time effect on the maximal voluntary torque production decline was found to be significant after the three fatiguing protocols ($F=46.18$; $p<0.001$; $\eta^2p=0.69$).

As figure 2 illustrates, after the CST protocol, the MVC loss was of 11.94 ± 3.86 %, after the PROG the loss was of 13.21 ± 2.26 % and after the VOL the reduction was of 10.90 ± 2.42 %. Overall, no significant change was found in the level of voluntary activation ($Chi^2=0.17$;

p=0.683) after any of the fatiguing protocols (Table 2), also supported by the unchanged RMS/M_{sup} ratio of all muscles observed after the three protocols (Table 1).

Mechanical twitches

Results of the mechanical twitch variables obtained at PRE and POST for the 3 different fatiguing protocols are presented in Table 2. No significant time effect was found in the amplitude of the evoked peak twitch at M_{max} intensity, PtM_{max} (Chi^2=1.50; *p*=0.221) after the protocols (Table 2). Despite that, an overall time effect was found on the $PtPotM_{max}$ that diminished significantly (F=18.75; *p*<0.001; η^2p=0.47; Table 2). The magnitude of the potentiated peak twitch decreased after PROG of 11.08 ± 2.94 %, VOL of 9.67 ± 2.46 % and after CST of 5.97 ± 5.05 %

Finally, an overall time effect was also found on the ($PtPotM_{max}/PtM_{max}$) ratio (F=23.72; *p*<0.001; η^2p=0.53; Table 2) which decreased after CST of 12.84 ± 3.37 %, after PROG of 12.95 ± 8.02 % and after VOL of 15.12 ± 3.38%.

Evoked potentials

A time effect was found on the maximal amplitude of the SOL H-reflex (F=5.86, *p*=0.025; η^2p=0.218) whereas, the SOL M_{max} remained unchanged after all protocols (F=0.23, *p*=0.604; η^2p=0.013) as shown in the Figure 4. Therefore, an overall main time effect was observed for the ratio between the H_{max} and M_{max} of the SOL (F=4.98; *p*=0.037; η^2p=0.19; Figure 3). After the CST protocol, the SOL H_{max}/M_{max} ratio increased of (median ±interquartile range) 36% (7.90; 97.04), of 10.4% (8.64; 25.61) after the PROG protocol, and of 8.45% (-9.08; 70.39) after the VOL protocol.

Neither, the H_{sup}/M_{sup} ratio, the M_{Hmax}-to-M_{max} ratio nor the ratio between the amplitude of the H-reflex elicited at the intensity evoking 20% of MVC and the M_{max}, the $H_{Stim20\%}$ -to-M_{max} ratio for the SOL, GL and GM muscles were found to modify (respectively: SOL: p=0.390; p=0.280; ; p=0.542; GL: p=0.289; p=0.102; p=0.414; GM: p=0.394; p=0.683; p=0.221),.

The amplitudes of the GL and GM H_{max} were found to be potentiated after all protocols (GL: Chi^2=4.167; p=0.041; Kendall's W: 0.17; GM: Chi^2=6.000; Kendall's W: 0.25;p=0.014 - Table 3). A time effect on the M_{max} amplitude of the GM was found to decrease (Chi^2=8.167; p =0.004; Kendall's W: 0.34) but no change was observed for the GL M_{max} (p=0.414 – Table 3). The GL H_{max}-to-M_{max} ratio was found to remain stable (p=0.221), while the one of the GM increased (Chi^2=4.167; p=0.041; Kendall's W: 0.17 – Table 3).

Finally, an overall potentiation of the ratio between the amplitude of the SOL V wave and the superimposed M-wave (V/M_{sup}) after all three protocols was found (F=7.39; p=0.024; η^2p=0.45; Figure 5).The SOL V/M_{sup} ratio increased of 104 ± 96.9 % after the CST protocol, of 121 ± 111.9% after the PROG protocol; and of 117 ± 34.2 % after the VOL protocol.

Also, a time effect on the SOL V/H_{sup} ratio was found (F=11.60; p=0.008; η^2p=0.56; Figure 5). It increased after the CST of 70 ± 28.8 %, after the PROG protocol of 95 ± 50.2 % and of 82 ± 26 % after the VOL protocol. However, the V/M_{sup} and the V/H_{sup} ratios did not modify for the GL and GM muscles after any of the fatiguing protocols (respectively: GL: p=0.768; p=0.865; GM: p=0.940; p=0.816; Table 4).

DISCUSSION

In agreement with our first hypothesis, the results show a similar decline in maximal torque production across all conditions with a similar TTI. However, we failed to show a difference in relative central and peripheral contributions to triceps surae muscle fatigue between the two NMES protocols. Below, it will be discussed why: 1) the decline in MVC torque production appeared to be related to similar origins across the protocols; 2) increasing or maintaining the current intensity might have induced both peripheral and central contributions to muscle fatigue differing between triceps surae muscles; 3) at equivalent TTI, nerve stimulation at constant current intensity likely induced similar muscle fatigue as voluntary contractions.

Torque-time integral and maximal voluntary contraction

It is acknowledged that the TTI reflects the energy expenditure of an isometric contraction, and thus correlates to the ATP consumption (Bergström and Hultman 1988; Russ et al. 2002b). Moreover, a recent study showed that the metabolic demand was independent of the modulation of the stimulation parameters (frequency and pulse duration) for a standardised TTI (Gondin et al. 2010). In the present study, the TTI produced across protocols led to an average MVC loss of 12%. Even though we did not measure the metabolic cost, based on previous findings (Bergström and Hultman 1988; Russ et al. 2002a; Gondin et al. 2010), we suggest that the metabolic demand was of the same magnitude between CST, PROG and VOL protocols. Although, performed over the nerve trunk, our results are in line with previous studies showing that 9% and 10% of MVC loss were observed after 30 (Boerio et al. 2005) and 34 contractions (Papaiordanidou et al. 2010) respectively, when electrical stimulation was delivered over the triceps surae muscle bulks.

Peripheral mechanisms

Despite a decrease of the gastrocnemius medialis M_{max} amplitude, the present results show no modification of the soleus and gastrocnemius lateralis M_{max} suggesting both the impairment and the preservation of the neuromuscular transmission (Fuglevand et al. 1993; Allen et al. 2008). Our result concerning the soleus and gastrocnemius lateralis muscles is consistent with previous studies (Klakowicz et al. 2006; Lagerquist and Collins 2010), where fatiguing protocols were induced by a 7-second stimulation train on the tibial nerve at high frequencies with pulse duration of 1 ms as used in the present study. However, overall, the M-wave of the gastrocnemius medialis muscle decreased, suggesting that this muscle may have been adapting in a different fashion to the exercise. Although the M_{max} and its associated mechanical response (PtM_{max}) remained steady after all fatiguing protocols, the potentiated twitch at M_{max} intensity ($PtPotM_{max}$) was found to be reduced after CST, PROG and VOL (respectively 6%, 11% and 10%) without any protocol effect. Moreover, the ($PtPotM_{max}/PtM_{max}$) ratio was significantly reduced after CST, PROG and VOL (13%, 13% and 15% respectively). Importantly, these results seem to indicate a strong trend towards a similar decline of the potentiated mechanical evoked responses after the three fatiguing protocols.

In the present study, likely underlying mechanisms of peripheral impairments may lie in an alteration in Ca^{2+} release from the sarcoplasmic reticulum and/or impaired Ca^{2+} uptake/reuptake and accumulation within and by the T-tubules (Allen et al. 2008). Additionally, potentiated twitches are more sensitive to reflect peripheral changes related to muscle fatigue (Kufel et al. 2002), suggesting a reduction in Ca^{2+} ions sensitivity, hindering Ca^{2+} ions to bind on troponin C on the myofilaments leading to a lower number of active cross-bridges (Westerblad et al. 2002; Allen et al. 2008; Place et al. 2009). Taken together, our results indicate that peripheral adaptations occurred after all three protocols and none of

the protocols induced a different effect on peripheral adaptations, but central mechanisms are also to take into account.

Central mechanisms

Even though many studies have been performed on the modulation of stimulation parameters on muscle fatigue when NMES was applied over the muscles bulks (Binder-Macleod and Scott 2001; Boerio et al. 2005; Gregory et al. 2007; Kesar et al. 2008; Papaiordanidou et al. 2010; Gondin et al. 2010), a few focused on the effects of nerve stimulation on the muscle fatigue (Baldwin et al. 2006; Klakowicz et al. 2006; Lagerquist and Collins 2010; Bergquist et al. 2011a). Surprisingly, no modifications of the voluntary activation level, nor of the RMS/M_{sup} of the triceps surae occurred after any of the three protocols. This might likely be attributable to our method. In fact, even though the use of single twitch, compare to doublets would not influence the evaluation of the voluntary activation (Behm et al. 1996; Shield and Zhou 2004), including in the plantar flexor muscles (Scaglioni and Martin 2009), it is debated whether the use of the interpolated twitch technique is the best technique to determine voluntary activation (Herbert and Gandevia 1999; Taylor 2009). Additionally, the twitch interpolation technique does not differentiate the supraspinal from spinal mechanisms of central fatigue (Millet et al. 2011). Thus, it cannot be excluded that, even though the RMS/M_{sup} ratio did not modify, partial compensations of the voluntary activation occurred although it is unknown in which muscles and to what extent.

Nonetheless, the results of the present study show that neural modifications occur at both supraspinal and spinal levels. The steadiness of the soleus and gastrocnemii H_{sup}-to-Ms_{up} ratio suggests that pre- and post-synaptic inhibitions were not modified after the fatiguing protocols (Aagaard et al. 2002). Even though the voluntary activation level and the soleus and gastrocnemii RMS/M_{sup} ratios did not modify after any of the protocols, the soleus V/M_{sup} and

V/H_{sup} ratios significantly increased while they remained steady in for the gastrocnemii regardless of the protocol.

In spite of the absence of modification of the voluntary activation level, greater soleus V/M_{sup} and V/H_{sup} ratios found after the fatiguing exercises, without a protocol dependence. Since the V wave reflects the magnitude of the descending neural drive to the motorneurons pool (Upton et al. 1971; Aagaard et al. 2002), our results suggest that the number of α-MNs activated as well as the neural drive to the motorneurons were similarly enhanced after the CST, PROG and VOL protocols as previously reported after voluntary contractions (Lévénez et al. 2005). However, this may not hold for the gastrocnemii as the V/M_{sup} and V/H_{sup} ratios of these muscles did not modify. These results suggest that the fatiguing protocols, CST, PROG, and VOL led to a modification of the neural descending drive during the MVC towards the different muscles of the triceps surae with the muscle fatigue. Additionally, spinal adaptations might also have occurred.

In voluntary contractions, it has been shown that a facilitation of presynaptic inhibition on Ia afferent fibres occurs (Hultborn and Pierrot-Deseilligny 1979) but if the contraction remains at a constant level of force production, the presynaptic inhibition is restored to its starting threshold (Meunier and Pierrot-Deseilligny 1989). Therefore, it is likely that inhibitions did not affect the soleus and gastrocnemii H_{max}/M_{max} ratios, also supported by the unchanged soleus and gastrocnemii H_{sup}-to-Ms_{up} ratios.

The potentiation of the H-reflex after tetanic nerve stimulation has already been reported in literature after high frequency stimulation (above 50 Hz) (Lloyd 1949; Hagbarth 1962; Kitago et al. 2004; Klakowicz et al. 2006; Collins 2007; Lagerquist and Collins 2010; Bergquist et al. 2011b). Yet, to our knowledge, only one study (Kitago et al. 2004) investigated long duration

tetanic nerve stimulation protocol effects on the facilitation of the H-reflex. After 30 trains of 10-second contractions (duty cycle 50%), these authors found that their protocol potentiated the amplitude of the H-reflex, although it was not normalised to the maximal amplitude of the M-wave. Because caution should be taken when studying H-reflexes (Schieppati 1987; Zehr 2002), literature suggests to evoke H-reflexes with a sufficient corresponding M-wave and in the ascending part of the recruitment curve to ensure stimulus consistency and similar excitation/inhibition mechanisms (Schieppati 1987; Pierrot-Deseilligny and Mazevet 2000). Moreover, it is shown that the normalisation of the electrophysiogical responses should be normalised to the respective M-wave recorded under a similar condition to ensure same stimulus conditions between protocols (Aagaard et al. 2002; Gondin et al. 2006). Therefore, to account for the steadiness of the stimulation and recording conditions between protocols, the soleus and gastrocnemii M_{Hmax}-to-M_{max} ratios, as well as the $H_{Stim20\%}$-to-M_{max} ratios were computed, and no significant differences were found between PRE and POST tests suggesting that stimulations' conditions were stable.

In agreement with Kitago et al. (Kitago et al. 2004), the soleus and the gastrocnemii H_{max} was increased, and so did the soleus and the gastrocnemius medialis H_{max}/M_{max} ratio regardless of the protocol. However, the absence of a protocol effect suggests that spinal adaptations were of similar magnitude after all protocols. The increase in the amplitude of the H-reflex indicates that the enhanced stimulation of the sensory axons to the spinal cord due to the increase in stimulation likely induced an increase in neurotransmitters release from the Ia afferents (Lloyd 1949; Hirst et al. 1981; Van Boxtel 1986; Kitago et al. 2004), or a reduced presynaptic inhibition (Schieppati 1987). Considering that nerve stimulation does not only activate motor axons, but also sensory axons (Collins 2007), the overall potentiation observed in the soleus and the gastrocnemii H_{max} and H_{max}/M_{max} ratio after the three fatiguing protocols, are likely to be attributable to spinal mechanisms where the facilitation of the H-reflex would

be induced by a higher excitability of Ia fibres and α-MNs (Hugon 1973; Schieppati 1987). Moreover, the potentiation of soleus V/M_{sup} and V/H_{sup} ratios and of soleus and gastrocnemius medialis H_{max}/M_{max} ratios can be explained by an increased Ca^{2+} sensitivity due to regulatory light chain phosphorylation (Sale 2002). The potentiation is shown to coexist with muscle fatigue (Rassier and Macintosh 2000) as observed in the present study.

Altogether, the results showed that the soleus and the gastrocnemii seemed to be differently involved in the muscle fatigue, regardless of the protocol. As it appeared a neuromuscular transmission alteration (decrease of the M_{max}) in the gastrocnemius medialis, this did not seem to be the case for the lateral head of the gastrocnemius and the soleus muscles. Moreover, the spinal excitability increased for the gastrocnemius medialis and the soleus muscles, while this was probably of lower magnitude for the gastrocnemius lateralis as, despite an increase of the H-reflex, the H_{max}-to-M_{max} ratio remained steady. Therefore, it seems that the gastrocnemii muscles adaptations were mediated through peripheral and reflex pathways, while the muscle fatigue of the soleus muscle was compensated by an increase in spinal excitability and in the neural descending drive (increase of V/M_{sup} and V/H_{sup} ratios). The different adaptations across muscles can be attributable to the fibres typology difference between the soleus and the gastrocnemii muscles. While the first is mainly composed of slow-twitch MUs, the gastrocnemii contain approximately an equal amount of slow-twitch and fast twitch MUs (Tucker et al. 2005). Therefore as the force production targeted was rather low (20 % of MVC), the soleus muscle might have been more largely involved than the gastrocnemii, especially since the nerve stimulation intensity was optimised for the soleus.

Interest for NMES

The present findings suggest that similarly to voluntary contractions, a progressive or constant current intensity NMES protocol induced similar peripheral and central modifications when the same amount of TTI was matched. It can be suggested that when delivered over the nerve trunk, increasing or maintaining the current intensity induced adaptations of triceps surae muscle fatigue similar to voluntary contractions. Therefore, the muscle fatigue may have arisen from similar origins across protocols. Indeed, nerve trunk stimulation, regardless of the intensity modulation, may have evoked a physiological-like recruitment of motorneurons, also supported by literature (Baldwin et al. 2006; Klakowicz et al. 2006; Collins 2007; Dean et al. 2007; Bergquist et al. 2011b). This may partly due to our stimulation parameters (1 ms pulse duration, 50 Hz) as it has been shown that nerve stimulation may activate a greater amount of Ia afferents axons in more synchronous manner, increasing the central contributions to the torque production (Bergquist et al. 2011b). Since the Ia fibres would first recruit smallest α-MNs, accordingly to the size principle (Henneman et al. 1965; Bawa et al. 1984), the activation of the α-MNs via sensory axons and reflex arc would produce muscle contractions following the physiological recruitment order. Therefore, the Ia fibres would first recruit smallest and slow type fatigue-resistant fibres, a physiological recruitment should activate the muscle (Dean et al. 2007; Maffiuletti 2010; Bergquist et al. 2011b).

During voluntary contractions, a rotation amongst the MUs pool occurs, ensuring a continuous contraction (Bawa and Murnaghan 2009) to compensate with cumulative effects of fatigue (Bawa et al. 2006). Then, it is possible that regardless of the intensity modulation, the nerve stimulation followed a physiological-like recruitment order (through Ia fibres, as seen in the potentiation of the H_{max}-to-M_{max} ratio), recruited additional and fresh MUs, while other MUs had their threshold activation level increased (Vilin and Ruben 2001; Kiernan et al. 2004; Miles et al. 2005) and ceased firing, mimicking the rotation of MUs. The activation of the α-MNs via sensory axons and reflex arc enhances spinal cord neuronal circuitry (Perez

et al. 2003) and can modify cortical activity (Hamdy et al. 1998). Then maintaining the stimulation intensity, targeting a constant torque level, may generate muscle contractions through a combination of peripheral and central pathways, similarly to voluntary contractions likely beneficial to restore muscle function. Here, when a similar TTI is reached, and at 20% of MVC, our results showed that central and peripheral adaptations to the muscle fatigue were of similar magnitude as voluntary contractions whether or not the intensity is increased. The current intensity of stimulation is usually set subjectively at the highest threshold of tolerance in clinical settings. Thus, performing NMES sessions at a constant intensity current rather than sessions with increased current intensity would yield to the same amount and likely similar origins of muscle fatigue, reducing pain sensation along the NMES session. However, because of the fixed position of electrodes, the recruitment of fibres diminishes as their distance from the electrodes increases (Vanderthommen et al. 2000) and increasing the current intensity might overcome the distance and activate deeper fibres (Theurel et al. 2007).

CONCLUSION

When matching a similar TTI using voluntary contractions, constant intensity NMES or increasing intensity NMES protocols the fatigue developed was found to be of similar magnitude and origins in the plantar flexor muscles. Therefore, since peripheral and central modifications are likely to explain the decrease in MVC after the three fatiguing protocols, it seems that the neural drive from the supraspinal centres increased to perform the maximal effort (Gandevia 2001; Enoka and Duchateau 2008), also mediated through spinal modifications. The two NMES protocols induced similar peripheral and central adaptations of the triceps surae muscle fatigue as voluntary contraction, even though the muscles contributions seemed to differ. Nerve trunk stimulation is likely to generate physiological-like

muscle activation, whether or the current intensity is increased for a similar amount of TTI reached, although this observation has to be further confirmed when the NMES is applied over the muscle bulk.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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TABLES

Table 1.RMS/M_{sup} ratio of the soleus and the gastrocnemiilateralis and medialis

	SOL RMS/M_{sup}		GL RMS/M_{sup}		GM RMS/M_{sup}	
	PRE	***POST***	***PRE***	***POST***	***PRE***	***POST***
CST	1.93 (1.56 ; 2.75)	2.29 (1.75 ; 3.48)	3.36 (1.34 ; 7.49)	2.64 (2.35 ; 6.56)	2.48 (1.41 ; 3.59)	2.50 (1.62; 3.52)
p	0.157		1.000		1.000	
PROG	1.78 (1.59 ; 2.87)	1.78 (1.27 ; 2.49)	2.07 (1.55; 6.04)	2.81 (1.52 ; 4.15)	2.65 (1.37 ; 4.28)	2.18 (1.30 ; 3.51)
p	0.157		0.480		0.157	
VOL	2.57 (1.86 ; 2.80)	2.02 (1.56 ; 2.48)	4.03 (1.69 ; 16.53)	3.51 (2.07 ; 7.96)	2.69 (2.50 ; 3.96)	3.52 (1.77 ; 4.40)
p	1.000		0.480		0.480	

Table 2.Voluntary Activation Level, the rest evoked mechanical twitch response, and the twitch potentiated responses

	Voluntary Activation level (%)		PtM_{max} (N.m)		Pt Pot M_{max} (N.m)		Pt Pot M_{max} / PtM_{max}	
	PRE	***POST***	***PRE***	***POST***	***PRE***	***POST***	***PRE***	***POST***
CST	96.61 (85.85 ; 99.75)	90.41 (85.18 ; 96.84)	14.04 (10.95 ; 15.64)	15.45 (12.07 ; 16.50)	22.29 (1.55)	20.93 (1.70)	1.66 (0.08)	1.45 (0.1)
p								
PROG	95.12 (86.52 ; 98.51)	92.06 (86.26 ; 95.72)	13.08 (12.11 ; 16.00)	13.91 (12.51 ; 15.90)	21.33 (1.30)	19.03 (1.47)	1.57 (0.08)	1.33 (0.06)
p								
VOL	99.90 (92.02 ; 100)	95.92 (93.68 ; 99.97)	15.06 (13.01 ; 20.05)	15.98 (14.82 ; 23.60)	26.50 (2.34)	23.66 (1.71)	1.62 (0.08)	1.36 (0.04)
p	0.683		0.221		**<0.001**		**<0.001**	

Table 3. Maximal M-waves and H-reflex amplitudes and H_{max}-to-M_{max} ratio of the gastrocnemii lateralis and medialis

	GL M_{max} (mV)		GM M_{max} (mV)		GL H_{max} (mV)		GM H_{max} (mV)		GL H_{max} / M_{max} (u.a.)		GM H_{max} / M_{max} (u.a.)	
	PRE	**POST**	**PRE**	**POST**	**PRE**	**POST**	**PRE**	**POST**	**PRE**	**POST**	**PRE**	**POST**
CST	3.54 (2.59 ; 9.46)	4.05 (2.60 ; 8.62)	6.91 (4.01 ; 8.49)	3.50 (2.77 ; 6.52)	1.22 (0.64 ; 2.32)	1.41 (0.80 ; 2.54)	1.83 (0.81 ; 3.57)	2.20 (1.78 ; 2.89)	0.25 (0.20 ; 0.45)	0.41 (0.28 ; 0.49)	0.40 (0.12 ; 0.62)	0.44 (0.35 ; 0.80)
PROG	2.14 (0.90 ; 3.76)	2.55 (0.83 ; 3.99)	5.82 (3.14 ; 9.80)	3.69 (1.84 ; 5.38)	1.56 (0.93 ; 2.00)	1.80 (1.30 ; 2.23)	1.70 (1.26 ; 4.52)	2.39 (1.42 ; 4.63)	0.87 (0.42 ; 1.32)	0.71 (0.47 ; 1.62)	0.36 (0.17 ; 0.81)	0.76 (0.29 ; 1.13)
VOL	3.81 (0.83 ; 5.14)	3.51 (1.86 ; 5.56	4.97 (2.49 ; 8.14)	4.30 (2.58 ; 7.49)	1.23 (0.91 ; 2.50)	1.31 (0.72 ; 2.83)	1.32 (0.86 ; 4.33)	1.58 (0.94 ; 5.09)	0.55 (0.26 ; 1.89)	0.38 (0.25 ; 0.78)	0.40 (0.18 ; 0.89)	0.39 (0.26 ; 0.60)
p	0.414		**0.004**		**0.041**		**0.014**		0.221		**0.041**	

Table 4. V-to-M_{sup} and V-to-M_{sup} ratios of the gastrocnemii lateralis and medialis

	GL V / M_{sup} (u.a.)		GM V / M_{sup} (u.a.)		GL V / H_{sup} (u.a.)		GM V / H_{sup} (u.a.)	
	PRE	**POST**	**PRE**	**POST**	**PRE**	**POST**	**PRE**	**POST**
CST	0.47 (0.08)	0.39 (0.04)	0.56 (0.10)	0.44 (0.09)	0.70 (0.10)	0.90 (0.05)	0.49 (0.05)	0.80 (0.19)
PROG	0.28 (0.07)	0.29 (0.07)	0.49 (0.09)	0.35 (0.07)	0.65 (0.13)	0.68 (0.11)	0.81 (0.04)	0.70 (0.11)
VOL	0.13 (0.31)	0.06 (0.08)	0.44 (0.10)	0.57 (0.11)	0.63 (0.11)	0.54 (0.06)	0.67 (0.14)	0.75 (0.09)
p	0.768		0.940		0.865		0.816	

LEGENDS TO TABLES AND FIGURES

Table 1. Median and interquartile range of the RMS/M_{sup} ratio of the soleus (SOL) and the gastrocnemii lateralis (GL) and medialis (GM), prior to (PRE) and after (POST) constant (CST), progressive (PROG) and voluntary (VOL) protocols. *p*: p-value of main time effect. Significant p-values are in bold font.

Table 2. Median and interquartile range of the voluntary activation level, the rest evoked mechanical twitch response in N.m, and mean and standard error of the mean of the evoked mechanical twitch potentiated response at M_{max} intensity (Pt Pot Mmax) in N.m, of the Pt Pot M_{max}-to-PtM_{max} ratio (Pt Pot M_{max} / PtM_{max}), prior to (PRE) and after (POST) constant (CST), progressive (PROG) and voluntary (VOL) protocols. *p*: p-value of main time effect. Significant p-values are in bold font.

Table 3. Median and interquartile range of the maximal amplitudes of the M-waves (M_{max}) and H-reflex (H_{max}) and H_{max}-to-M_{max} ratio (H_{max} / M_{max}) for the gastrocnemii lateralis (GL) and medialis (GM), prior to (PRE) and after (POST) constant (CST), progressive (PROG) and voluntary (VOL) protocols. *p*: p-value of main time effect. Significant p-values are in bold font.

Table 4. Mean and standard error of the mean of the V-to-M_{sup} (V/ M_{sup}) and V-to-H_{sup} (V/ H_{sup}) ratios for the gastrocnemii lateralis (GL) and medialis (GM), prior to (PRE) and after (POST) constant (CST), progressive (PROG) and voluntary (VOL) protocols. *p*: p-value of main time effect.

Figure 1. Graphical overview of the experimental procedure and the neuromuscular tests**. A.** The fatiguing protocols consisted of 6 seconds of contraction followed by6 seconds of rest. Before (PRE test) and after (POST tests) each of the fatiguing protocols, a set of neuromuscular tests was performed. At the start of the experimental procedure, a passive recruitment curve was achieved to set stimulation intensities that elicited maximal amplitudes of the maximal muscle compound and of the Hoffman reflex. **B.** The set of neuromuscular tests performed before and after the protocols was conducted such as 3 electrically twitches separated by 3 seconds, at M_{max} and H_{max} intensities were evoked while participants were asked to relax. Thereafter, 4 plantar flexors MVC (MVC PF) were performed with superimposed single stimulations, at M_{max} and H_{max} delivered before, over the MVC and 3 seconds after the contraction. Finally, 2 dorsiflexion MVC (MVC DF) torques were measured at PRE whereas, one trial was performed at POST.

Figure 2. Percentage of maximal voluntary isometric torque loss of the plantar flexor muscles after the constant intensity fatiguing protocol (CST), the progressive intensity fatiguing protocol (PROG) and the voluntary fatiguing protocol (VOL). Columns represent group mean values and error bars are the standard error of the group mean.

Figure 3. A. H_{max}-to-M_{max} ratio (H_{max}/M_{max} ratio) of the soleus (SOL) muscle measured before (PRE, in black fill-in) and after (POST, in white fill-in) the constant intensity fatiguing protocol (CST),the progressive intensity fatiguing protocol (PROG) and the voluntary fatiguing protocol (VOL). Columns represent group mean values, error bars are the standard error of the group mean. Significant differences of main time effect, $p<0.05$ (*).

Figure 4. Maximal amplitude of the M-waves (M_{max}) of the soleus (SOL) muscle measured before (PRE, in black fill-in) and after (POST, in white fill-in) the constant intensity fatiguing

protocol (CST), the progressive intensity fatiguing protocol (PROG) and the voluntary fatiguing protocol (VOL). Columns represent group mean values, error bars are the standard error of the group mean.

Figure 5. Maximal amplitude of the soleus (SOL) V-wave-to-M_{sup} ratio (V/M_{sup}; Top-panel, A) and of the V-to-H_{sup} (V/M_{sup} ratio, bottom panel B) of the soleus muscle measured at PRE (in black fill-in) and POST (in white fill-in) tests for the constant intensity fatiguing protocol (CST), the progressive intensity fatiguing protocol (PROG) and the voluntary fatiguing protocol (VOL). Columns represent group mean values and error bars are the standard error of the group mean. Significant differences of main time effect, $p<0.05$ (*) and $p<0.01$ (**).

Figure 1

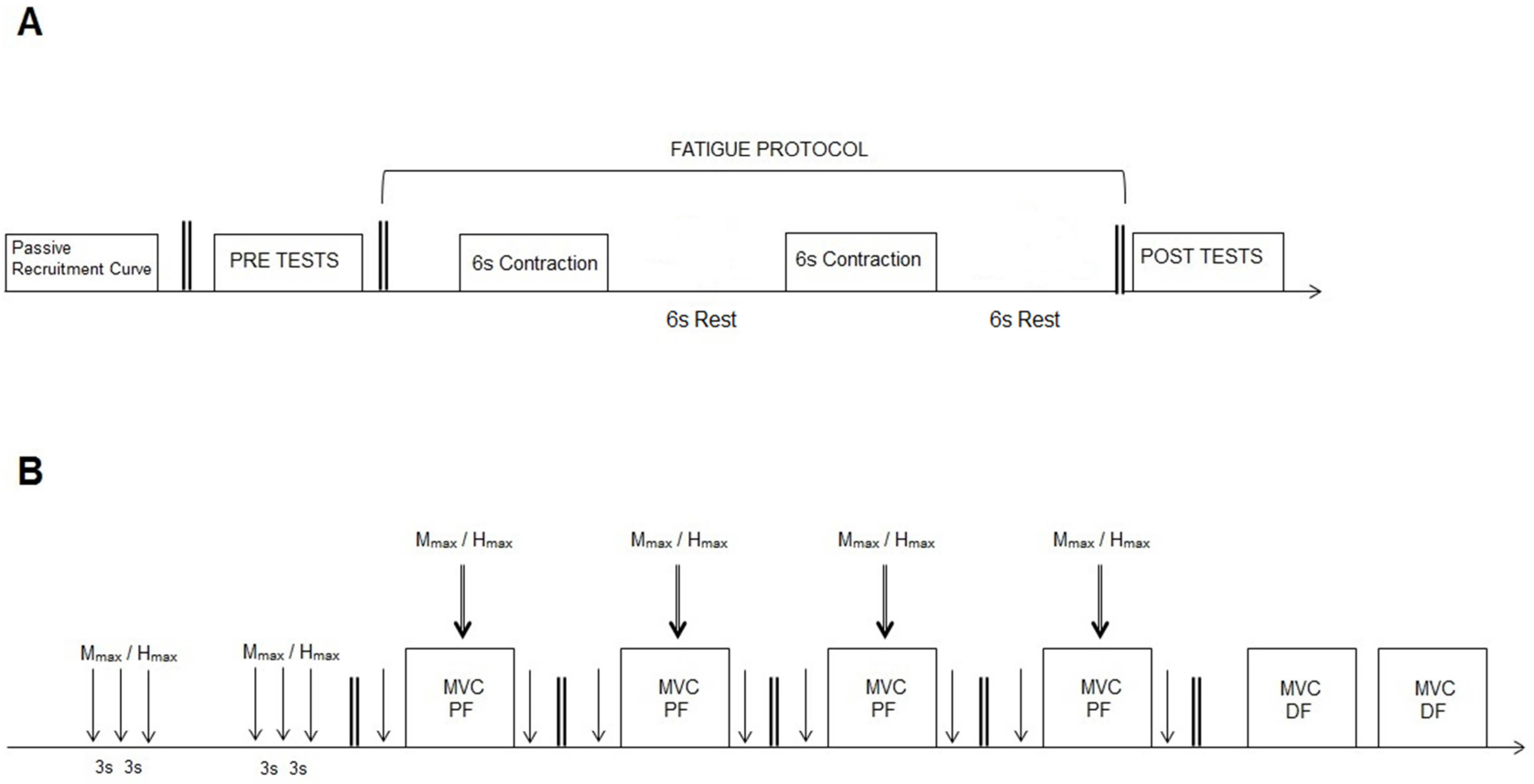

A
FATIGUE PROTOCOL
Passive Recruitment Curve
PRE TESTS
6s Contraction
6s Contraction
POST TESTS
6s Rest
6s Rest
B
Mmax / Hmax
Mmax / Hmax
Mmax / Hmax
Mmax / Hmax
Mmax / Hmax
Mmax / Hmax
MVC PF
MVC PF
MVC PF
MVC PF
MVC DF
MVC DF
3s 3s
3s 3s

Figure 2

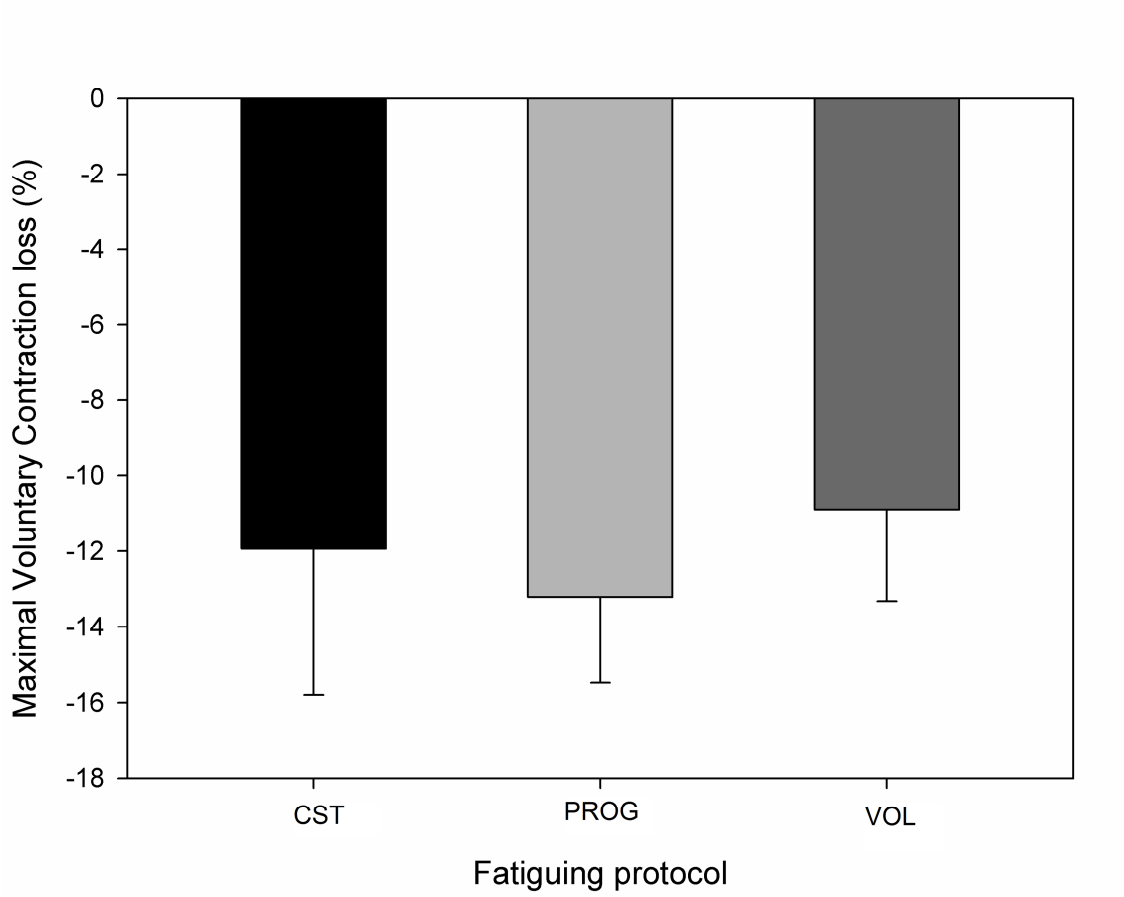

0
-2
-4
-6
-8
-10
-12
-14
-16
-18
Maximal Voluntary Contraction loss (%)
CST
PROG
VOL
Fatiguing protocol

Figure 3.

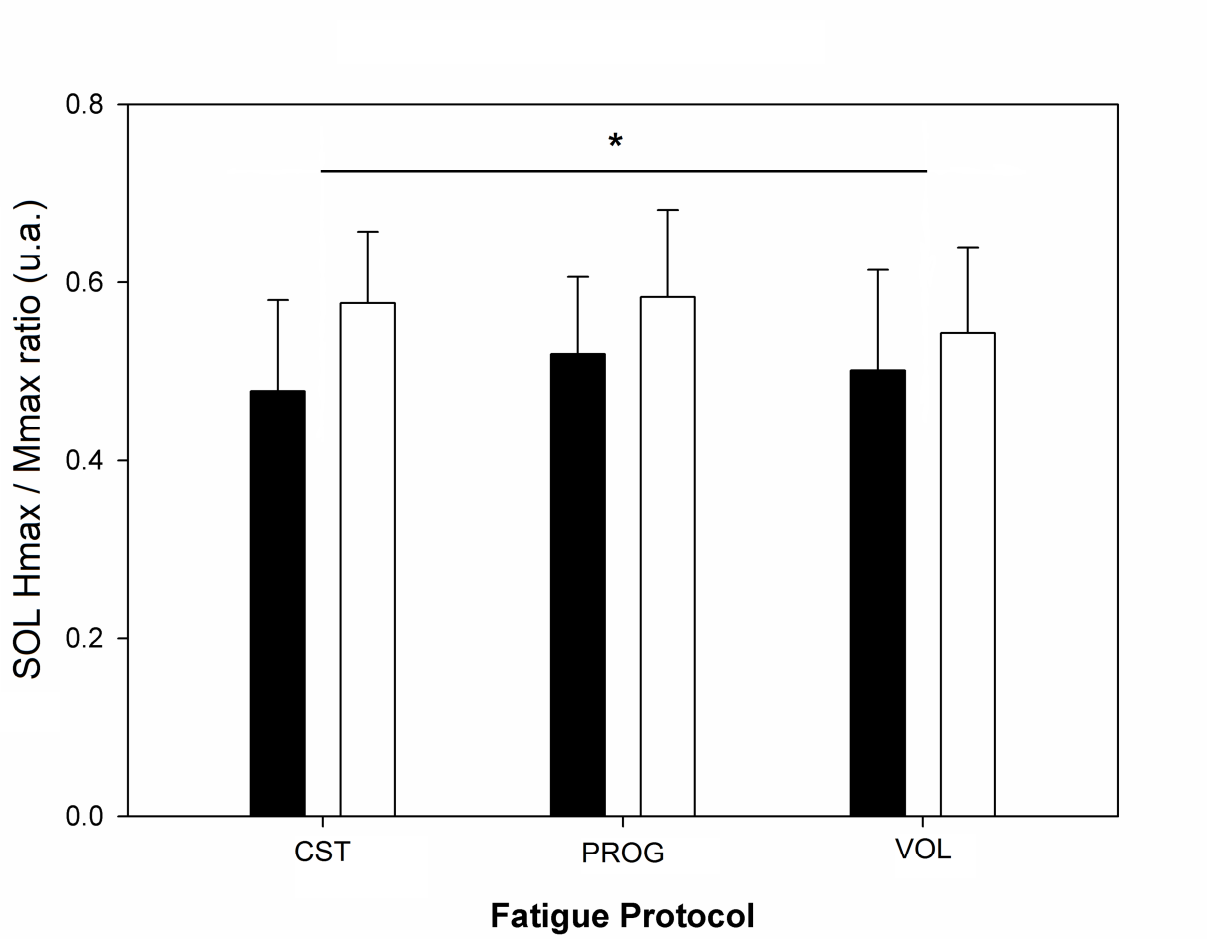

0.8
0.6
0.4
0.2
0.0
SOL Hmax / Mmax ratio (u.a.)
*
CST
PROG
VOL
Fatigue Protocol

Figure 4.

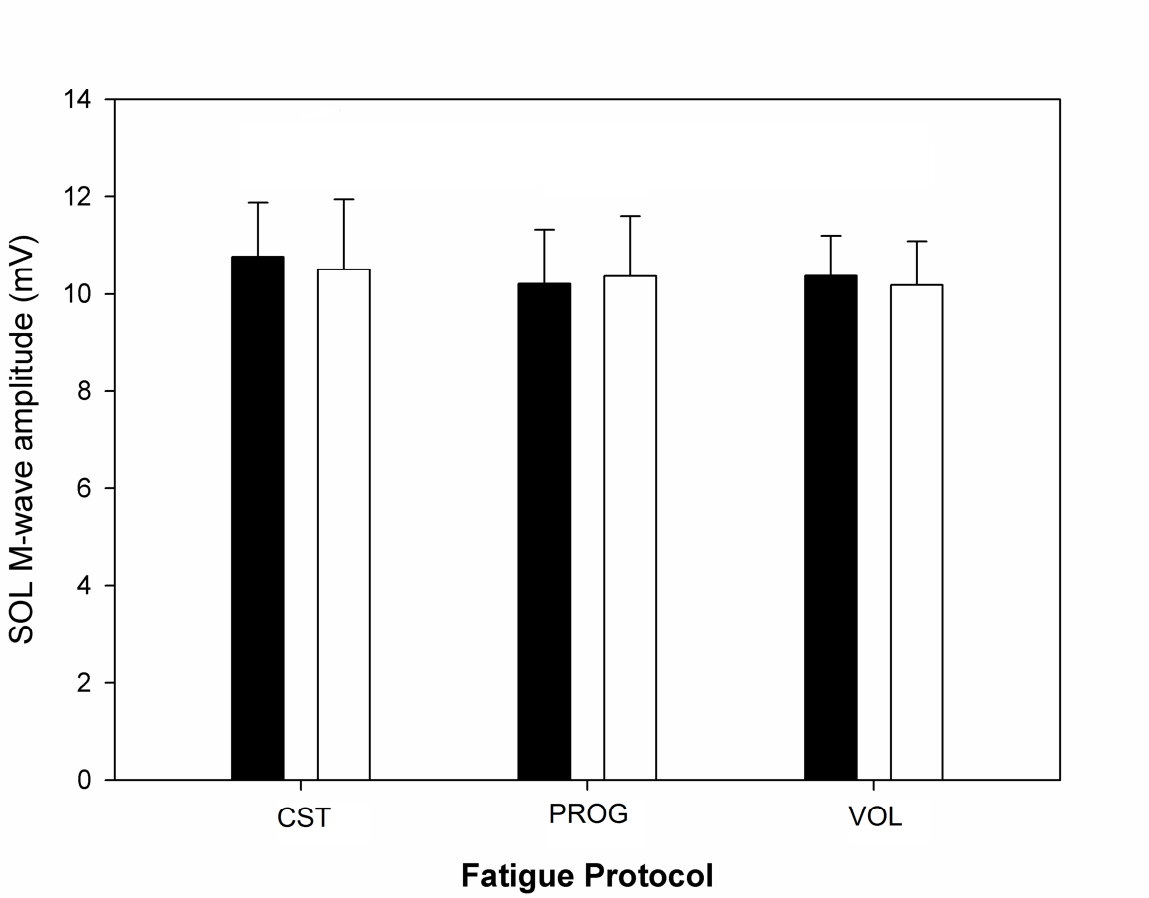
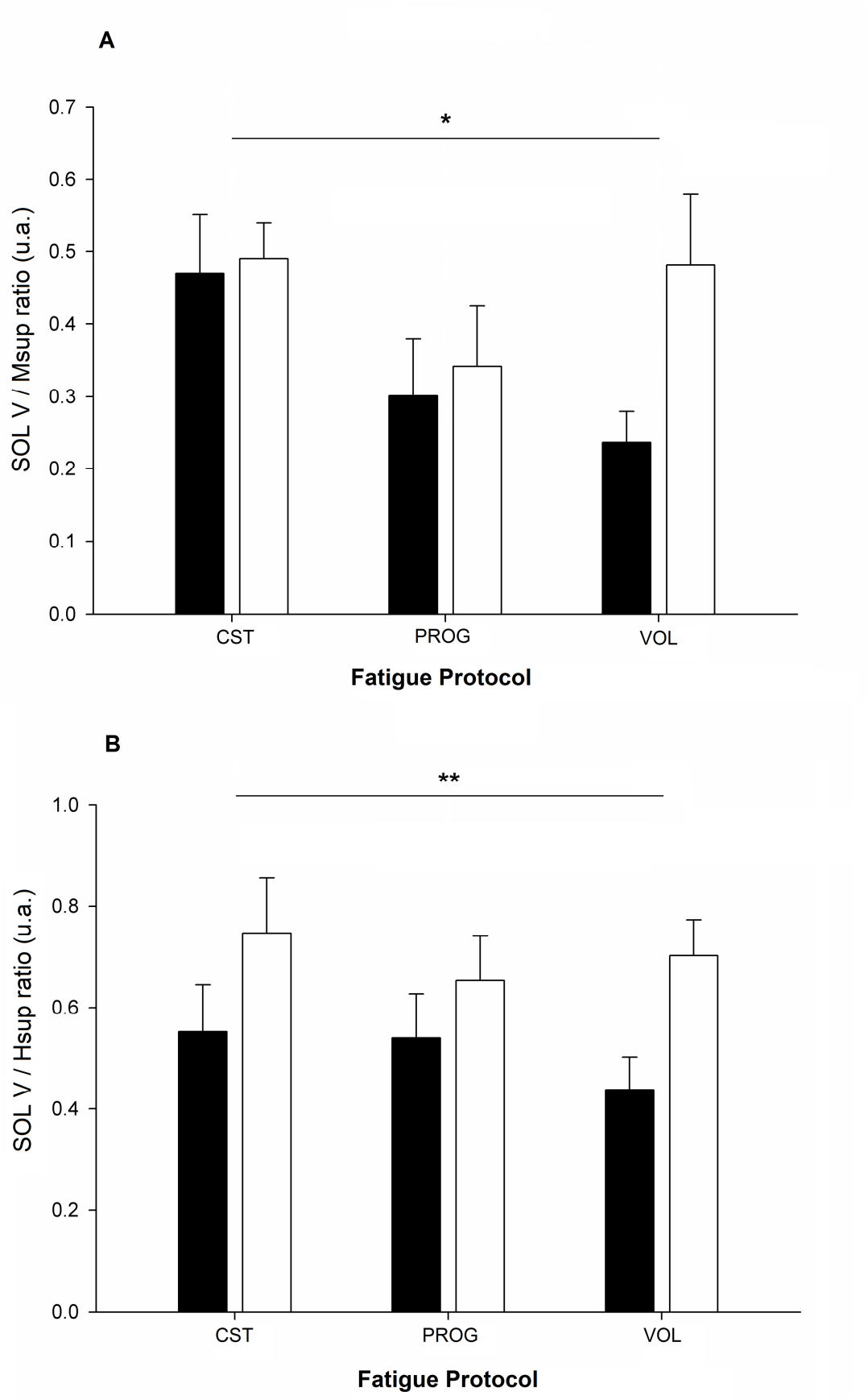

14
12
10
8
6
4
2
0
SOL M-wave amplitude (mV)
CST
PROG
VOL
Fatigue Protocol

Figure 5.


A
*
SOL V / Msup ratio (u.a.)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
CST
PROG
VOL
Fatigue Protocol
B
**
SOL V / Hsup ratio (u.a.)
0.0
0.2
0.4
0.6
0.8
1.0
CST
PROG
VOL
Fatigue Protocol

PAPER III

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Fatigue and muscle activation during submaximal elbow flexion in children with cerebral palsy

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ABSTRACT

The purpose of this study was to investigate whether children with cerebral palsy (CP), like typically developing peers, would compensate for muscle fatigue by recruiting additional motor units during a sustained low force contraction until task failure.

Twelve children with CP and 17 typically developing peers performed one submaximal isometric elbow flexion contraction until the task could no longer be sustained at on average 25% (range 10–35%) of their maximal voluntary torque. Meanwhile surface electromyography (EMG) was measured from the biceps brachii and triceps brachii, and acceleration variations of the forearm were detected by an accelerometer. Slopes of the change in EMG amplitude and median frequency and accelerometer variation during time normalised to their initial values were calculated.

Strength and time to task failure were similar in both groups. Children with CP exhibited a lower increase in EMG amplitude of the biceps brachii and triceps brachii during the course of the sustained elbow flexion task, while there were no significant group differences in median frequency decrease or acceleration variation increase. This indicates that children with CP do not compensate muscle fatigue with recruitment of additional motor units during sustained low force contractions.

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1. Introduction

Cerebral palsy (CP) is the most common neurological disorder in children, with a European prevalence of 1.5–3 per 1000 live births (Cans, 2000). CP is a term that encompasses motor or postural impairments caused by injuries in the central nervous system during early development; from *in utero*, birth or within the child's first 2–3 years (Bax et al., 2005). The most common physical symptoms are spasticity, muscle weakness, decreased control, and fatigue (Rosenbaum et al., 2007; Rose and McGill, 2005; Jahnsen et al., 2003). Yet, fatigue in CP is often described as a general feeling of weakness (Jahnsen et al., 2003), and, as described later in this introduction, only few reports relate about neuromuscular activation strategies during a fatiguing task in CP.

Muscle fatigue is generally defined as a decrease in maximal force capacity induced by sustained or repeated muscular contractions whether or not the task can be maintained (Gandevia, 2001; Enoka and Duchateau, 2008). Muscle fatigue comprises ionic and metabolic changes in the muscle fibre causing impaired action potential propagation and excitation–contraction coupling, and changes in the neural – central – drive conducted via the cortico-spinal pathway to alpha motorneurons which innervate muscle fibres (Allen et al., 2008; Gandevia, 2001). A decreased propagation velocity over muscle fibre membranes is represented by a decrease in the median/mean frequency of the power density spectrum (MDF) of the electromyogram (EMG) (De Luca, 1997; Farina et al., 2004). When a fatiguing contraction at a constant submaximal force level is sustained, the amplitude of the measured EMG signal increases. Even though the synchronisation of activated motor units (MUs) firing pattern partly accounts for the increment of the EMG signal (Yao et al., 2000) with fatigue, it is widely acknowledged that this muscle fatigue is usually compensated by recruitment of additional MUs that contribute to the amplitude of the measured signal (Enoka and Duchateau, 2008; De Luca, 1997; Farina et al., 2004).

One of the main motor impairments in CP widely discussed in literature is muscle weakness. Indeed, reduced strength in children with CP compared to typically developing peers is reported both in the lower (Engsberg et al., 2000; Leunkeu et al., 2010) and the upper (Akataki et al., 1996; Vaz et al., 2006; Smiths-Engelsman et al., 2005) limb. The cerebral lesion in CP alters the neural drive (Mockford and Caulton, 2010), decreasing the number of MUs that can be activated and their maximal firing rate during voluntary contractions (Rose and McGill, 2005). Moreover, in the gastrocnemius lateralis, the medial hamstring and the iliacus the muscle

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fibre distribution has been shown to be altered, with a predominance of fibre type I (Rose et al., 1994). Thus, patients with cerebral palsy would not be able to drive their MUs to higher firing rates, and the force capacity production is lowered (Rose and McGill, 2005). Although MU recruitment is reduced during maximal voluntary contractions (MVCs), at contraction levels up to 25% of MVC the MU recruitment in CP is found to be normal (Rose and McGill, 2005). Therefore, it might be expected that neuromuscular adaptations to fatigue during low level force contractions would be similar to healthy typically developing peers, but would differ at higher force levels.

There are a few studies investigating fatigability in children with CP. Children with CP have been shown to have less decrease in peak torque and thus less fatigability following electrically elicited isometric and voluntary elicited isokinetic maximal knee extensions (Stackhouse et al., 2005; Moreau et al., 2009). Moreover, children with CP had similar endurance time sustaining a load of 50% of MVC, with a larger decrease in EMG MDF and a smaller increase in EMG amplitude (Leunkeu et al., 2010). Yet, literature mentioned above focused on lower-limb muscles, and investigated fatigue during moderate to high contraction levels, from above 40% of MVC, while normal recruitment was found at low contraction levels.

The aim of the present study was to evaluate whether the upper limb muscles of children with CP at a low force level have an endurance as hampered as strength and whether they show an increase in EMG amplitude during the task like typically developing peers indicating muscle fatigue compensation by MU recruitment. Therefore, muscle activation of elbow flexors and extensors were studied during elbow flexion at on average 25% (range 10–35%) of the maximal voluntary contraction until task failure in children with CP and typically developing peers. We hypothesised to observe a similar increase in EMG amplitude during the sustained low force task in children with CP as in typically developing peers due to muscle fatigue compensation through normal additional MU recruitment at such low force levels. Moreover, we hypothesised that this normal MU recruitment would result in a longer endurance time in children with CP sustaining the same relative load, since children with CP have muscles with relatively more slow type, fatigue resistant muscle fibres (Ito et al., 1996; Stackhouse et al., 2005), are weaker (and weaker people are less fatigable than stronger ones (Hunter and Enoka, 2001)) and cannot activate all MUs during an MVC (Rose and McGill, 2005). The latter results in an underestimation of their muscle capacity regard to their strength observed during MVC, which makes the submaximal load applied as a percentage of MVC relatively low.

2. Methods

2.1. Participants

Fifteen children diagnosed with unilateral CP according to their medical records took part in the study. The participants were recruited from the outpatient records of the neuro-orthopaedic team at St. Olav's Hospital in the health region of Mid-Norway. All participants were classified at level III or better (1 child at level III; 5 children at level II; 9 children at level I – see Table 1) on the Manual Ability Classification System (Eliasson et al., 2006) and were able to take instructions. Exclusion criteria comprised orthopaedic or any other important surgery in the upper limb in the past 2 years, and Botulinum Toxin A injections in the upper limb in the past 6 months. Due to practical reasons, three children with CP could not participate in the study. Therefore, the results of 12 children with CP (8 boys and 4 girls) with an age range of 8–17 years were analysed in this study. Seventeen healthy typically developing peers (TD), gender and age matched (11 boys and 6 girls; age range of 8–16 years), were recruited as controls. See Table 1 for more participant characteristics information. The study was performed according to the Declaration of Helsinki and was approved by the Regional Committee for Medical Research Ethics. A written informed consent was obtained from the parents before participation.

2.2. Equipment set-up

A stationary Biodex dynamometer (System 3 Pro; Biodex Medical Systems, Shirley, NY, USA); and a Noraxon TeleMyo™ 2400T Direct Transmission system with wireless bipolar surface EMG sensors, a 3D accelerometer and a 2D inclinometer (Noraxon USA, Inc.) were used in this study. Signals from the Biodex (torque) and Noraxon sensors were synchronised and simultaneously collected by the Noraxon system. Data were recorded with a sampling rate of 1500 Hz. At sensor location, EMG data were sampled with 3000 Hz, low pass filtered with a cut of frequency of 1500 Hz and resampled to 1500 Hz prior to telemetric transmission. Also, prior to data sampling, the EMG signals were high pass filtered with a cut off frequency of 10 Hz and Biodex data was low pass filtered with a cut off frequency of 500 Hz.

2.3. Experimental procedure

The affected arm of the CP participants and the non-dominant arm of the TD participants (the arm normally not used for writing and throwing) were tested. First, the following general information and anthropometric measures were taken: gender, age, height, weight and skinfold thickness over the biceps brachii (BB) and triceps brachii (TB) with the use of a calliper.

Thereafter, bipolar EMG electrodes (1 cm diameter, 2 cm interelectrode distance) were placed over the BB, the triceps longus (MTB) and the triceps lateralis (LTB) of the arm under investigation, according to the SENIAM recommendations (Enschede, The Netherlands – Hermens et al., 2000).

During the remaining tests, a wrist orthosis and a flexion glove were used on both CP and TD participants to fix the wrist and to secure a good grip around the handle, since participants with CP might have problems with their grip strength and grip ability.

2.3.1. Maximal voluntary contractions (MVCs)

After sensor placement, maximal voluntary contractions were performed using the Biodex isokinetic dynamometer. The participants were seated and strapped in the individually adjusted Biodex chair with the elbow aligned with the rotation axis of the Biodex, an elbow angle of 60° (from full extension), the shoulder slightly flexed and abducted and the forearm in neutral position. Following a single submaximal test trial, three isometric MVCs in elbow flexion and elbow extensions were performed with a 1-min rest between each contraction in the same direction and a 10-s rest in the opposite one. Participants were asked to perform their maximal effort – as much as they could, as fast as they could within a 5 s period. Torque and EMG were recorded during these contractions.

2.3.2. Endurance task

The endurance task was performed about 5 min after the MVCs. Participants kept a similar position in the Biodex chair, but changed the elbow flexion angle to 90° and shoulder abduction to 0°. The participants were unfastened from the dynamometer, an accelerometer was placed on the proximal phalanx of the first finger and an inclinometer on the forearm. The inclinometer was used to give visual feedback about the arm position displayed on a computer screen of 19 in. (37.7 cm × 30.3 cm), placed on eye level

Table 1
Participants characteristics. Mean (and standard deviation) of participant characteristics and performance of the maximal and the endurance test for patients with cerebral palsy (CP) and typically developing peers (TD).

	Gender		Age (year)	MACS (levels)			Calliper (mm)		BMI (%)	MVC (N m)		Load		Time (s)
	Girls (#)	Boys (#)		I	II	III	BB	TB		EF	EE	(kg)	(%MVC)	
CP	4	8	12.3 (2.9)	9	5	1	7.1 (2.4)	11.1 (3.9)	18.6 (2.2)	21.8 (4.7)	26.7 (4.4)	1.9 (1.1)	23.7 (8.1)	210 (149)
TD	6	11	12.4 (2.8)				6.6 (2.6)	10.8 (3.9)	18.6 (2.4)	29.2 (3.8)	32.3 (3.7)	2.7 (1.3)	22.8 (3.1)	255 (167)
p			0.97				0.62	0.83	0.98	0.23	0.34	0.11	0.72	0.46

#: Number of participants. MACS: Manual Ability Classification System. Calliper represents skin fold thickness over the biceps brachii (BB) and the triceps brachii (TB). BMI: Body Mass Index. MVC: Maximal voluntary contraction torque of elbow flexion (EF) and elbow extension (EE). Load: load sustained during the endurance task, expressed in kg and as a percentage of the MVC (%MVC). Time: time to task failure. *p*: *p*-Value of between groups differences tested with a *t*-test.

around 1 m from the participants. The time range of the inclinometer feedback was 5 s, and the amplitude range was ±20°. During the endurance task, the participants were instructed to keep the forearm positioned horizontally while sustaining in elbow flexion a submaximal constant load until task failure. This external load was fixed on the wrist of the participant and calculated as a percentage of the highest peak torque (N m) during elbow flexion MVC divided by moment arm (m) and gravity (9.81 m s^{-2}). The participants were continuously reminded to keep their proper position. Task failure was set when the inclinometer feedback deviated of at least 5°, despite encouragement to keep their position.

2.4. Data analysis

All outcome measures, including torque, time to task failure, EMG data, and accelerometer data, were analysed with Matlab R2009a (The MathWorks, Inc., Natick, MA, USA). Prior to further analyses all EMG signals were band pass filtered with a recursive Butterworth filter with cut of frequencies of 10 and 500 Hz.

2.4.1. MVC analysis

First, the torque signal was low pass filtered with a cut off frequency of 6 Hz and corrected for arm weight. Then the highest peak torque (N m) of the three trials was obtained for elbow extension and flexion. Root mean square (RMS (μV)) of the BB EMG amplitudes around peak flexion torque and of MTB and LTB EMG amplitudes around peak extension torque were calculated with a 500 ms window width.

2.4.2. Endurance analysis

The time to task failure was defined as the time in seconds from start with stable inclinometer feedback, to stop, when the inclinometer feedback deviated of at least 5°. The RMS of the EMG amplitudes (μV) and median frequency of the power density curve of the EMG signal (MDF (Hz)) were calculated over the whole duration of the task in 1-s periods for all three muscles. The power density curve was calculated using Welch's averaged, modified periodogram method (Welch, 1967) including hamming windowing. The standard deviation of the horizontal acceleration (ACC SD) was also obtained in 1-s periods over the whole duration of the task RMS, MDF and ACC SD data were normalised to the percentage of their starting values (the average of the two first seconds). Thereafter, linear regression was used to determine the slopes of the change of RMS, MDF and ACC SD in time (%/s).

In addition, for illustrative purposes, the RMS (% start) development of the EMG of the BB muscle during the endurance task was resampled with 100 values using a spline fit, generating a time normalised signal (1–100%).

2.5. Statistics

Statistical processing was performed in PASW Statistics version 19 (SPSS Inc., Chicago, IL, USA). The Shapiro-Wilk test was used to test whether outcome measures were normally distributed. When variables were normally distributed, one-way ANOVA tests were performed to compare CP and TD groups, otherwise, the non-parametric Mann–Whitney *U* test was performed to compare groups. Group differences in time to exhaustion were evaluated by an ANOVA with time to exhaustion as dependent and load (%MVC) as covariate. For both groups, significance of the MDF, RMS and ACC SD slopes were evaluated by one-Sample Wilcoxon Signed Rank Tests.

The association between changes in the EMG signal (RMS slope and MDF slope) and the time to task failure in both groups (TD and CP) was investigated by taking Spearman's correlation coefficients. In all statistical analysis the significance level was set at $p < 0.05$.

3. Results

Table 1 shows the participant characteristics, and the mean and standard deviation (SD) of the performance of the strength test and the endurance test for CP and TD groups. Although on average the

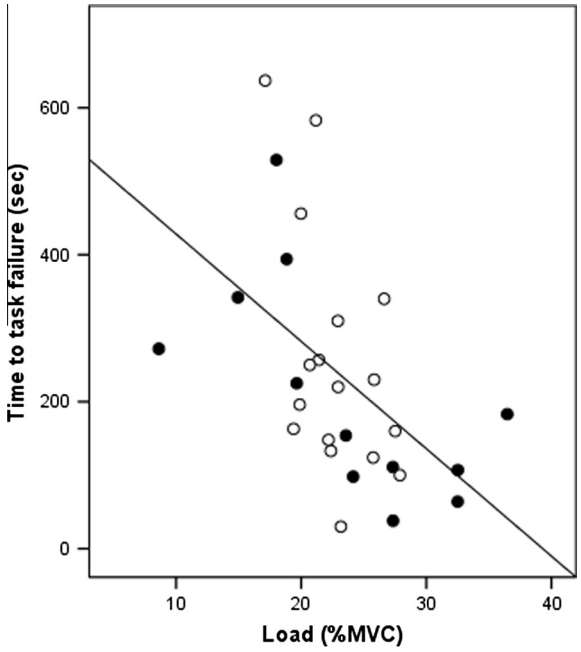


Fig. 1. Time to task failure expressed as a function of the relative sustained load (in percentage of maximal voluntary contraction – % MVC). Cerebral palsy group: Black dots; typically developing peers: White dots. The regression line is inserted ($r^2 = 0.266$).

Table 2
Maximal and initial EMG. Median (and interquartile range) of the root mean square (RMS) amplitudes of the EMG from the biceps brachii (BB) and medial and lateral heads of the triceps brachii (MTB and LTB) muscles during maximal voluntary contraction (MVC) in μV, and at the start of the elbow flexion endurance task in μV and as percentage of MVC for patients with cerebral palsy (CP) and typically developing peers (TD).

	EMG RMS MVC (μV)			EMG RMS start (μV)			EMG RMS start %MVC		
	BB	MTB	LTB	BB	MTB	LTB	BB	MTB	LTB
CP	183 (103; 513)	169 (12; 255)	236 (150; 432)	54 (35; 124)	7 (7; 11)	14 (9; 19)	22.0 (15.4; 28.1)	4.7 (2.5; 6.2)	3.6 (1.8; 7.9)
TD	453 (372; 856)	477 (194; 558)	362 (251; 597)	106 (87; 188)	12 (7; 14)	13 (11; 19)	16.9 (13.6; 17.8)	2.5 (1.1; 3.6)	3.1 (2.3; 4.1)
p	**0.027**	**0.004**	0.180	**0.021**	0.140	0.811	**0.031**	**0.024**	0.471

p: *p*-Value of differences between groups tested with Mann–Whitney *U* test. Significant *p*-values are in bold font.

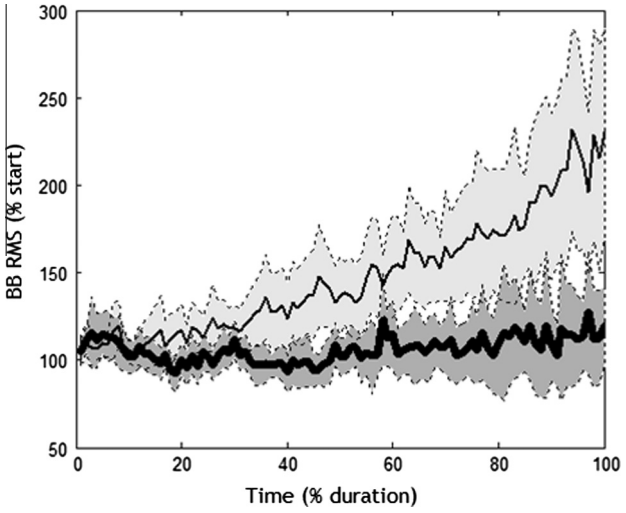


Fig. 2. Average (plain line) and 95% confidence interval (area comprised between dotted lines) of the RMS amplitude of the EMG from the biceps brachii (BB) throughout the sustained elbow flexion task, of the patients with cerebral palsy (average: thick black line; 95% confidence interval: dark grey area) and the typically developing peers (average: thin black line; 95% confidence interval: light grey area). RMS amplitude is expressed as the percentage from the initial value (% start) and time as the percentage of total duration (%duration).

TD group was able to develop higher elbow flexion and extension torques, these differences did not reach any statistical significance.

The time to task failure (duration) of CP and TD participants was comparable and without any statistical difference (Table 1). Moreover, time to task failure was similarly affected by the load in both groups (Fig. 1). More specific, there was no group effect ($p = 0.546$) on time to task failure when using an ANOVA with load (% of MVC) as covariate, while load (% of MVC) was a significant ($p = 0.005$) factor, without a significant group-load interaction.

Table 2 presents non-parametric statistical results (median, interquartile range and *p*-value of the Mann–Whitey *U* test) for EMG RMS amplitudes of the BB during elbow flexion MVC and of the LTB and MTB during elbow extension MVC and of the three investigated muscles at the start of the sustained elbow flexion.

During the MVCs, EMG RMS amplitudes of the muscles acting as agonists were lower in the CP group. These differences were significant for the BB ($p = 0.027$) and the MTB ($p = 0.004$). The absolute EMG RMS amplitude of the BB at the start of the sustaining elbow flexion task was significantly lower in the CP group, but when taken relative to its maximal voluntary value (during MVC), the EMG RMS was significantly higher in the BB ($p = 0.031$) and in the MTB ($p = 0.024$) of the CP group compared to the TD group. In addition, the MDF of the BB and LTB EMG were similar in both groups (TD and CP) at the start of the holding task. However, the MDF of the MTB EMG was significantly lower in TD than in CP at the start ($p = 0.034$). The standard deviation of acceleration at the start of the sustained contraction (ACC SD) was similar in both groups ($p = 0.314$).

Fig. 2 illustrates the average increase in RMS of the BB EMG signal from the start to the end of the sustained task for both groups. Non-overlapping 95% confidence intervals indicate significant larger RMS increase in the TD group from 30% of the contraction duration to task failure.

Moreover, the RMS slopes of all three muscles of the TD group were significantly positive and larger than in the CP group (Table 3). This group difference was significant for the BB and the LTB (Table 3). On average, TD and CP groups experienced a decrease (negative slope) in MDF of the EMG of all three muscles. The negative slope was significant for all three muscles in TD and for MTB in CP (Table 3). No significant group differences were found. Similarly, the standard deviation of forearm acceleration increased on average (ACC SD slope was positive) for both groups. This was significant for TD only, without a significant group difference.

No significant correlations between time to task failure and RMS- or MDF slopes were found.

4. Discussion

The purpose of this study was to investigate whether endurance of a sustained isometric task at low force-level is as hampered as strength in children with CP, and whether children with CP can compensate muscle fatigue during such a low-force level contraction by recruiting additional MUs, measured by an increase in EMG amplitude, as seen in healthy children. The main results of this study are as follows: (1) during the isometric elbow flexion sustaining a load between 8% and 36% of MVC, the time to task failure was found to be of comparable duration in CP and TD groups; (2) in control children, an increment of EMG amplitude was present throughout the task, but not in the CP group; (3) the EMG median frequency decreased similarly and acceleration fluctuations increased similarly in both groups. Below, the implications of these results on children with CP will be discussed.

In agreement with literature (De Luca, 1997; Enoka et al., 2011), healthy participants in our study showed an increase in the amplitude of the EMG signal and a shift towards lower frequencies in the EMG power density spectrum while sustaining an external load until the task cannot be sustained. However, the children with CP did not show this typical change in EMG signal with fatigue. Since the increase in EMG amplitude reflects additional recruitment of MUs (Enoka and Duchateau, 2008; Enoka et al., 2011) this indicates that the recruitment of additional MUs in the CP group was most likely hampered. Thus, in contrast to our hypothesis and like during maximal contractions (Stackhouse et al., 2005; Moreau et al., 2009) and fatiguing contractions at moderate to high force levels (Leunkeu et al., 2010), motor unit recruitment in children with CP is hampered during fatiguing contractions at low force levels, despite normal recruitment at this force level in non-fatigued muscles (Rose and McGill, 2005).

The time to task failure of the submaximal sustaining task was similar in both study groups, while there are three reasons why we hypothesised the children with CP to have a relatively less reduc-

Table 3
Changes during the sustained test. Median (and interquartile range) of the root means square amplitude (RMS) and median frequency (MDF) slopes of the EMG from the biceps brachii (BB) and medial and lateral triceps brachii (MTB and LTB) muscles and the standard deviation of acceleration of the forearm (ACC SD) for patients with cerebral palsy (CP) and typically developing peers (TD). The slopes are expressed as the percentage change from the initial value per second (% start/s).

	EMG RMS slope (% start/s)			EMG MDF slope (% start/s)			ACC SD slope (% start/s) forearm
	BB	MTB	LTB	BB	MTB	LTB	
CP	0.01 (−0.14; 0.10)	0.05 (−0.01; 0.22)	0.04 (−0.03; 0.27)	−0.03 (−0.06; −0.01)	−0.05* (−0.09; −0.03)	−0.04 (−0.11; −0.01)	0.17* (−0.02; 1.24)
TD	0.34* (0.08; 0.94)	0.30* (0.08; 0.43)	0.26* (0.05; 1.03)	−0.04* (−0.07; −0.01)	−0.06* (−0.10; −0.02)	−0.05* (−0.13; −0.03)	0.89* (0.46; 1.48)
p	**0.001**	0.092	**0.045**	0.682	0.779	0.414	0.37

p: *p*-Value of differences between groups tested with Mann–Whitney *U* test. Significant *p*-values are in bold.
* Slope significant different from zero (one-Sample Wilcoxon Signed Rank Test).

tion in time to task failure and therefore a longer endurance time sustaining the same relative load. First, children with CP have muscles with relatively more slow type, fatigue resistant muscle fibres (Ito et al., 1996; Stackhouse et al., 2005). Second, since participants with CP cannot activate all MUs during an MVC (Rose and McGill, 2005), the strength observed during MVC underestimates people's muscle capacity, which makes the submaximal load applied as a percentage of MVC relatively low. Finally, weaker subjects are less fatigable than stronger ones (Hunter and Enoka, 2001) since the intramuscular pressure is lower, as stronger subjects have a stronger occlusion of blood flow, the role of muscle afferents is attenuated (Barry and Enoka, 2007; Hunter and Enoka, 2001).

The most plausible reason for the not enhanced time to task failure is that CP had to terminate the task due to hampered motor unit recruitment to compensate muscle fatigue. Other factors could be our not so weak CP group and increased co-activation during the submaximal task. Even though the studied group of children with CP was not significantly weaker than the TD group, the other results indicate a reduction in voluntary activation (lower absolute EMG levels during MVC and lower increase in RMS during the sustained contraction) even in the strongest children with CP. Therefore, it is not likely that the strength of our study sample is the cause of the lack of difference in endurance time. In line with this, our results indicate that children with CP seem to be as affected in muscle endurance as in strength.

Increased antagonist co-activation would require a higher level of agonist activation to produce the same joint torque, implying a higher muscle load and reduced endurance time. Although at the start of the contraction the normalised (relative to maximal) EMG amplitudes of the agonist (BB) and antagonist (TB) muscles of the patients with CP were higher than the ones of TD (Table 2), the rate of fatigue indicated by negative MDF slope and positive ACC SD slope were not increased in the CP group (Table 3). An alternative explanation of the increased normalised EMG amplitudes could be an altered EMG-force relationship, possibly caused by the reduced possibility of patients with CP to voluntary drive their motor units to maximal capacity (Herda et al., 2011).

Albeit in the present study no differences were found in MVCs, other reports found more reduced strength and reduced EMG amplitudes during voluntary contractions in children with CP (Akataki et al., 1996; Mockford and Caulton, 2010; Rose and McGill, 2005; Damiano et al., 2001; Stackhouse et al., 2005; Moreau et al., 2012). This might be caused by our inclusion criteria where we included well-functioning children. The advantage of our relatively strong CP group is that group differences in fatigue and EMG amplitude changes are not caused by group differences in strength.

It is to be noted that participants did not hold exactly the same relative weight during the holding task, which most likely increased the variation in the time to task failure and could have hidden time to the task failure differences between the groups. However, means of the relative loads were approximately the same in both groups and the relation between load and time to task failure was similar in both groups. The effort of the participants is another factor that could affect the variation in time to task failure. However, all participants received strong verbal encouragement during the task in order to sustain the position of the forearm and the test leader got the impression that all children tried to keep the position as long as they could. Moreover, we expected a relatively longer time to task failure in children with CP at lower load levels. This could not be observed in our data, since the two CP participants holding the lowest relative weight had relatively short time to task failure (under the regression line, Fig. 1).

Finally, the use of 1-cm diameter recording EMG electrodes for children who have smaller muscles than adults might potentially have had induced a crosstalk component on the signal of the muscles under investigation (Staudenmann et al., 2010). This can be particularly problematic for the recording of the two heads of the triceps brachii where the placement of electrodes with respect of the edge of the muscle's heads and their relative proximity may account for a possible amount of crosstalk. Even though, spatial filtering of EMG data have been applied to reduce the likelihood of crosstalk (Farina et al., 2002, 2004), the closeness of electrodes on the TB might make difficult the interpretation of the TB activation.

5. Conclusion

In summary, our results show that during a sustained elbow flexion contraction until task failure between 10% and 35% MVC, like during contractions at higher force levels, children with CP are not able to increase their neural drive to recruit higher threshold MUs or arouse active MUs towards greater discharge rates to compensate muscle fatigue like typically developing peers. This resulted in similar and not increased time to task failure in the CP group holding a load at the same low force level relative to voluntary capacity.

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PAPER IV

Neuromuscular electrical stimulation training of the tibialis anterior in facioscapulohumeral muscular dystrophy patients

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KEYWORDS: Muscle fatigue; Muscle endurance; Electromyography; Neuromuscular disorder ; M-wave.

ABSTRACT

The purpose of this study was to investigate whether 8-week neuromuscular electrical stimulation (NMES) training of the tibialis anterior (TA) muscles in adults with facioscapulohumeral muscular dystrophy (FSHD) would improve motor function, muscle strength and endurance.

Ten patients with FSHD and 6 age and gender matched healthy participants achieved 8-weeks NMES training of the TA, on both legs for 20 minutes per session, 3 sessions per week. Ankle dorsiflexion (DF) and plantar flexion (PF) maximal voluntary isometric contractions (MVC), a 2-minute sustained maximal isometric ankle dorsiflexion with surface electromyography recordings (EMG) of the TA and the soleus (SOL) muscles were measured and functional tests were performed prior to and after the NMES training to disclose training effects.

No training effect was found in any investigated variable for either group ($p>0.1$). Patients with FSHD showed lower MVC ($p<0.05$) and a lower maximal TA EMG amplitude during the DF MVC ($p<0.01$). During the 2-minute sustained MVC, the percentage of force loss was lower for the FSHD patients ($p<0.05$) (Pre-training: HP: 54.2 %; FSHD: 35.9 %; Post-training: HP: 62 %; FSHD: 37.6 %) , but the percentage of TA EMG loss amplitude after the 2-minute MVC were found to be similar in both groups before (HP: 43 %; FSHD: 25.3 %) and after the training (HP: 39.4 %; FSHD: 38.4 %).

The results confirm that patients with FSHD are weaker than healthy participants, and our results also suggest that the intrinsic contractile properties of the TA are altered in patients with FSHD. Moreover, the lack of training effect suggests that the NMES protocol was not strenuous enough and/or parameters of stimulation were not adequate to improve ankle strength, muscle endurance and motor function for the group of patients with FSHD neither for the healthy participants. Alternatively, the absence of training effects may be explained by

the NMES protocol which was designed for the patients with FSHD, likely not appropriate to induce strength gains in healthy participants. Finally, the group of patients with FSHD showed lower force losses during the 2-minute sustained MVC, suggesting that they were experiencing a lower amount of muscle fatigue compared to the HP group.

INTRODUCTION

With a European prevalence of 7/100,000, the facioscapulohumeral muscular dystrophy (FSHD) is the most common inherited muscular dystrophy syndrome (Orphanet Report Series, 2013). Recently, it has been shown that two genetic impairments lead to FSHD development (van Overveld et al., 2003; de Greef et al., 2007, 2009; Zeng et al., 2009). The FSHD 1, the most common form, is characterised by a reduced number of tandem repeats 3.3 kb, D4Z4, on chromosome 4q35 (Wijmenga et al., 1992), inducing chromatin structure changes and loss of DNA methylation (van Overveld et al., 2003). In the FSHD 2, concerning about 5% of the patients, similar chromatin changes are observed without a D4Z4 contraction (van Overveld et al., 2003; de Greef et al., 2007, 2009; Zeng et al., 2009). The FSHD 2 has been found to be associated mutation of the SMCHD1 gene (Lemmers et al., 2012). Whereas, in FSHD 1, the overexpression of the DUX4 gene has been found to be involved into sarcomeric degradations pathways, explaining the muscle weakness (Wallace et al., 2011; Lassche et al., 2013). A decrease in myosin concentration affects predominantly fast fatigable fibres, and a shift from fast to slow muscle fibres types was observed (D'Antona et al., 2007).

Independently of the type of FSHD (*i.e.* 1 or 2),the disease is associated with an asymmetric weakness of facial, scapular girdle muscles, progressively descending to hip-girdle and anterior foreleg muscle (de Greef et al., 2010; Tawil and Van Der Maarel, 2006). However, the variability of disabilities varies considerably amongst patients and a recent study showed that the 6 year risk to become wheelchair-bound for patients was of 24%, with a greater risk with a greater D4Z4 contractions (≤18kb) (Statland and Tawil, 2013). Moreover, the experienced fatigue, reported by 61% of patients with FSHD (Kalkman et al., 2005), is a major burden in daily life activities. Since no therapeutic treatments are available (Tawil, 2008), it is of interest to propose alternative procedures to restraint the progressive loss of strength, endurance and muscle function.

Aerobic exercises have been proposed to improve the neuromuscular function in patients with FSHD, but many studies failed to show the improvements of such training on strength, even though no deleterious effects were induced (van der Kooi et al., 2004, 2005; Voet et al., 2010, 2013). Neuromuscular electrical stimulation is another type of exercise broadly used in rehabilitation settings as it has been shown to enhance muscle strength in health compromised participants such as chronic obstructive pulmonary disease (Neder et al., 2002), chronic heart failure (Quittan et al., 1999), spinal cord injury (Dudley et al., 1999) patients and it also limits atrophy caused by immobilisation (Gould et al., 1982). Moreover, when NMES training was performed on muscular dystrophy, such as Duchenne and Becker dystrophies, tolerance and efficacy were shown to maintain or even improve muscle strength (Scott et al., 1986, 1990; Zupan, 1992; Zupan et al., 1993). Comparable results in the tibialis anterior and the quadriceps muscles were reported in a group of mixed patients with neuromuscular disorders including patients with FSHD (Milner-Brown and Miller, 1988). Additionally, a recent study using NMES demonstrated strength and muscle function gains in patients with FSHD after a 5-month training program on the deltoid, the trapezius transversalis, the vastii lateralis and medialis muscles (Colson et al., 2010). Such a positive result highlights the interest of the use of NMES for patients with FSHD.

A recent study on patients with FSHD showed that the tibialis anterior and the deltoid muscles, mainly disabled by the disease, exhibited greater electrophysiological changes (*e.g*. lower amplitude of the electromyographic signal) than the vastus lateralis and the biceps brachii, and were correlated with motor function alterations(Dorobek et al., 2013).The tibialis anterior can be more affected than other muscles in the lower extremity(Olsen et al., 2006; Tawil and Van Der Maarel, 2006; Dorobek et al., 2013) and the odds of using of ankle brace increase throughout time, regardless of D4Z4 contractions (Statland and Tawil, 2013). Since the tibialis anterior has a strong functional role in gait and balance, the aim of the study was to

investigate whether a bilateral 8-week NMES training on the tibialis anterior in adults with FSHD would improve muscle strength and muscle endurance. First, beneficial effects of short term NMES training programs (< 8 weeks) on muscle strength and/or endurance have been reported on healthy (Thériault et al., 1996; Colson et al., 2000; Pérez et al., 2003; Gondin et al., 2005; Colson, 2009) and health compromised participants (Quittan et al., 1999; Neder et al., 2002). Second, NMES training was showed to be safe and effective in patients with FSHD (Colson et al., 2010). In light of these observations, we hypothesised to observe muscle endurance and strength gains in the tibialis anterior as well as improved motor function in patients with FSHD as healthy participants would.

METHODS

Participants

Ten adults with FSHD 1 (mean ± standard error of the mean (SE):6 females and 4 males; age 61.5 ± 3.16 year; height: 166.4 ± 4.11 cm; mass: 71.1 ± 5.42 kg) and 6 healthy participants (HP), gender and age matched (3 females and 3 males; age 57.83 ± 3.16 year; height: 176.33 ± 3.94 cm; mass: 80 ± 5.70 kg) volunteered to take part in the study. A written informed consent was obtained from all participants. The study was conducted according to the Declaration of Helsinki and was approved by the local Institutional Human Ethics Committee (CPP10.067).

Adults with FSHD were recruited from the outpatient record of the physical medicine and rehabilitation department at the University Hospital Archet 1 of Nice (France) and were included into the study according to the following criteria: number of 4q35 D4Z4 ≤ 35 kb or < 11 repeats; muscle weakness of ankle dorsiflexion from 2 to 4 assessed by manual muscle testing (MMT) (Lacôte et al., 2008) ranking from 0 where not muscle is no longer capable of

force production to 5 that represents the absence of muscle impairment. Exclusion criteria comprised previous NMES training of the lower-limb; history of cancer, joints pathologies, nor collagenopathies, parturient, or breast-feeding woman or simultaneous participation to another research study.

Neuromuscular electrical stimulation training

Neuromuscular electrical stimulation (NMES) training sessions of the tibialis anterior were performed with a Compex (Rehab 400, Cefar-Compex, DJO France SAS, Mouguerre, France) portable battery-powered stimulator. Participants either exercised at home or were trained by one of the experimenters or a physiotherapist. In the case participants trained at home, a weekly appointment was set-up with a physiotherapist or one of the experimenters to answer questions and/or to control the quality of the training. During these training sessions participants were seated (hips, knees and ankles angles at 90°), with their feet fixed. The participants were simultaneously stimulated bilaterally with self-adhesive electrodes (2 mm thick) made of elastomer (5 cm x 5 cm) that were positioned as follows: the positive electrode was placed on the superior part of the muscle, whereas the negative electrode was placed on the medial part of the muscle, over the muscle bulk.

The NMES strength training lasted for 8 weeks, with 3 sessions a week. Each session was composed of a 2-minute warm-up, followed by the 20-minute working out session, and finishing with 3 minutes of relaxation. The NMES program consisted in 6 seconds of contraction, followed by 7 seconds of rest (6s ON / 7s OFF, 35 Hz, 200µs pulse-width).Participants were encouraged to increase stimulation intensity progressively every 5 minutes throughout each session up to individual tolerance threshold since strength gains would be dependent on the stimulation intensity (Colson et al., 2009).

Pre and post training measurements

Equipment and sensor placement

Maximal voluntary contractions were carried out in a custom made device consisting of a fixed footplate, where the foot was firmly strapped to avoid any movement and ensure the quality of the isometric force measurement. A strain-gauged transducer(model OMF06M, linear range 0-15 kN, precision ± 0.5 %, sensitivity 10 mV/kN; OMICRO'N, Gambais, France) was placed on the footplate to measure ankle plantar and dorsiflexions force production.

Bipolar sEMG electrodes (10 mm diameter, 20 mm inter-electrode distance) recorded the muscle activity of the tibialis anterior (TA) and the soleus (SOL), according to the SENIAM recommendations (Hermens et al., 2000).The reference electrode was placed on the bony part of the contralateral patella. Skin was cleaned and abraded prior to the placement of electrodes, and low resistance impedance between electrodes (<5 kΩ) was obtained. A Biopac MP 150 system (Biopac systems, Inc., Holliston, MA, USA) was used to record sEMG data at a sampling rate of 2000 Hz. Electromyographic signals were amplified with a bandwidth frequency ranging from 1 Hz to 500 Hz (common mode rejection ratio = 11 dB; impedance input = 1000 MV; gain = 1000).

Common peroneal nerve stimulation was induced with a constant current electrostimulator (Digitimer DS7A, Digitimer, Herthforshire, United-Kingdom) to evoke the compound muscle action potential and associated mechanical response. The common peroneal nerve was stimulated with a self-adhesive cathode (8 mm diameter, Ag-$AgCl^-$) placed at the level of the fibula neck while the anode (8 mm diameter, Ag-$AgCl^-$) was placed over the head of the fibula. The stimulus was 400 V with a pulse width of 500 µs. The optimal intensity of stimulation was set by increasing intensity by 10 mA steps. The maximal intensity was reached once the compound muscle action potential (M_{max}) and the force output of the

associated mechanical response were found neither to increase nor to decrease with increasing stimulation intensity.

Experimental procedure

To disclose the effect of the 8-week NMES training on the tibialis anterior, the muscle performance was assessed prior to and after the training of both limbs, one at a time. The different tests were performed in a random order with at least 10 minutes of rest period between each limb.

During all contractions, participants were seated on a chair, with their leg extended with a slight flexion accordingly to the comfort of the participant, and the foot was place in the footplate with an ankle angle of 90°. The participants were not constrained and were allowed to seat as comfortably as they could to avoid any pain due to the position, however, during MVCs, they were not allowed to hold the seat and were asked to remain as steady as they could.

To assess the maximal amplitude of the M-wave, three single stimuli were delivered at rest, separated by 3 seconds on both limbs prior to the fatiguing exercise. Thereafter, two MVCs of plantar- and dorsi-flexion were performed to determine the maximal strength production.

The fatiguing task, performed on both sides, consisted of an isometric dorsiflexion performed as one single 2-minute MVC (Schillings et al., 2007). No visual feedback was provided to the participants and they were asked to perform an all-out effort. Participants received strong verbal encouragement.

Functional tests: Three functional tests were performed: 1) Muscle function and strength of the TA were assessed manually by a physiotherapist, by manual muscle testing - MMT

(Lacôte et al., 2008). Depending on the amplitude of the ankle dorsiflexion, without extension of the hallux, scores ranked from 0 where the muscle is no longer capable of force production to 5 that represents the absence of muscle impairment; 2) The Motor Function Measurement (MFM) assessed the functional capacity of daily life activities: standing still, weight transfers, sitting, proximal and distal motor ability of muscles, walking, raising up, raising up arms, stepping up stairs, brush hairs *etc*. After evaluation, the total score is a percentage, healthy participants reaching 100% (Bérard et al., 2005); 3) A six-minute walking test (6MWT) (ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories, 2002) was used to assess the greatest distance participants could walk in 6 minutes on a 20-m shuttle.

Data analysis

Force and sEMG analysis

The MVC was considered as the mean value over a 500-ms period around the peak force. The best of the two trials was analysed. All EMG data were analysed over the same window width. Root mean square (RMS) of the EMG of the TA and the SOL (TA RMS and SOL RMS) were calculated with commercially available software (AcqKnowledge4.1, Biopac Systems, Inc., Holliston, MA, USA). All outcome measures, EMG and force data were analysed with Matlab R2010b (The MathWorks, Inc., Natick, MA, United-States).

M-wave and mechanical twitch

The amplitudes of the three M-waves elicited by single stimuli at rest were averaged and the peak-to-peak amplitude of the TA muscle was analysed. Mechanical twitch responses were obtained (average of the three) from a single-peak twitch (Pts)

Fatiguing exercise

The percentage of MVC loss was calculated as the difference of a 1-second plateau at the start and a 1-second plateau at the end of the 2-minute MVCs. The loss amplitude of the TA EMG was calculated over the same windows.

Statistical analysis

Statistical processing was performed in PASW Statistics version 20 (SPSS Inc., Chicago, IL, USA). The Shapiro-Wilk test was used to test whether outcome measures were normally distributed, and depending on the results the appropriate statistical test was performed. When data were normally distributed, a paired t-test was used to disclose training-induced changes within in group (FSHD and HP). Then, a one-way ANOVA was performed to compare FSHD and HP groups. When data did not follow a normal distribution, equivalent non-parametric tests, the Wilcoxon signed ranks test and the Mann-Whitney *U* test were performed. Data from both limbs were pooled. In all statistical analysis the significance level was set at $p<0.05$. Normal distributed data are expressed as means ± SE (standard error of the mean), in the entire manuscript and in the tables and figures, while non-normally distributed data are expressed as median ± IQR (inter quartile range) in tables and box-plots are used in figures.

RESULTS

Maximal voluntary contractions and EMG activity

Peak force during ankle dorsiflexion MVC (Figure 1) was significantly lower in FSHD patients than in HP prior to and after the training (Pre: U=59.000; Z=3.141;p<0.001; Post: U=53.000; Z=3.064;p=0.001).No significant training effect was found in dorsiflexion strength in either group (FSHD: p=0.767; HP: p=0.173).

The TA RMS during dorsiflexion MVC (Figure 1) was lower in the FSHD group compared to the HP group before (F=9.477; p=0.008) and after (F=18.723; p=0.001) the training. No training effect was found in either group (FSHD: p=0.240; HP: p=0.493).Moreover, during the ankle dorsiflexion MVC, the SOL RMS significantly decreased with the training in the HP group (T=3.547; p=0.016) while it did not modify in the group with FSHD (p=0.437). The SOL RMS during dorsiflexion was lower in the FSHD group, but this did not reach a statistical significance prior to the training (F=4.544; p=0.051) while it did after the training (F=6.463; p=0.023).

Peak force during plantar flexion MVC was higher in the HP group than the group with FSHD (Table 1), before (U=53.000; Z=2.495; p=0.011) and after the training (U=49.000: Z=2.062;p=0.042). No training effect was found in the group of patients with FSHD (p=0.386) nor in the HP group (p=0.463).No group differences were found in the SOLRMS and TA RMS prior to and after training, nor any training effect for both groups in these variables.

Mechanical twitches and M-wave

The M-wave could not be elicited in all participants (Figure 2, Table 2). As shown in Table 2, no group differences were found in the amplitude of the M-wave prior to (p=0.106) and after

training (p=0.171). No significant training effect was found in the amplitude of the M-wave for both groups (FSHD: p=0.905; HP: p=0.290).

The amplitude of the evoked peak twitch (Pt) was greater in the HP group than in the group of patients with FHSD (Table 2) before (F=7.213; p=0.023) and after (F=7.802; p=0.017) the 8-week training, while there was no training effect for either group (FSHD: p=0.723; HP: p=0.236).

Fatigue protocol

- *Force production*

The group of patients with FSHD showed a significant reduction from start to end in the force production throughout the 2-minute sustained MVC before (Z=2.521; p=0.012) and after the training (Z=-2.240; p=0.025), and so did the HP group before (Z=-2.201; p=0.028) and after the training (Z=-2.201; p=0.028).

As illustrated in the figure 3A, FSHD patients had a significant less loss in the percentage of force production during the 2-minute MVC than HP before (F=5.673; p=0.035) and after training (U=6.000; Z=-2.324; p=0.020). There was a greater decline after the training for the HP group (Z=-2.201; p=0.028) but not for the group of patients with FSHD (p=0.889).

- *EMG activity*

The group of patients with FSHD showed a significant reduction in the TA RMS from start to end throughout the 2-minute sustained MVC before (Z=-2.666; p=0.008) and after the

training (T=3.222; p=0.015) and so did the HP group before (Z=-2.201; p=0.028) and after the training (T=5.750; p=0.002).

As illustrated in figure 3B, there was no significant difference in the percentage of loss of TA RMS during the endurance task, between groups before (p=0.074) and after the training (p=0.840). The percentage of TA RMS loss during the 2-minute MVC was greater after the training in the group with FSHD (T=2.435; p=0.045), but did not show any change for the HP group after the training (p=0.652).

Functional tests

There were no observable differences in the MMT score between groups before (p=0.428) and after the training (p=0.263) even though all HP participants ranked 5 on the MMT score for both sides. Also, the training did not modify MMT score for both groups (FSHD: p=0.172; HP: p=0.655) (Table 3). No significant training effect was observable on the 6MWT on both groups (FSHD: p=0.424; HP: p=0.279). Moreover, the distance performed was much shorter in the group with FSHD before (p=0.007) and after (p=0.022) the training (Table 4). Nevertheless, even though the 8-week training did not modify the MFM score in the group with FSHD (p=0.285), it was clear that the group with FSHD was significantly impaired compared to the healthy group before (U=60.000; Z=3.341; p<0.001) and after (U=54.000; Z=3.286;p<0.001) the 8-week training (Table 4).

DISCUSSION

The purpose of this study was to investigate whether an 8-week bilateral NMES training on the tibialis anterior in adults with FSHD would improve muscle strength, endurance and

motor function. The main results are as follows: 1) The 8-week training period did neither improve ankle dorsiflexion MVCs muscle strength, muscle endurance nor motor function in patients with FSHD; 2) The patients with FSHD were weaker than healthy participants and showed impaired activation of the tibialis anterior during ankle dorsiflexion MVC;3) The patients with FSHD experienced lower force losses and showed impaired activation of the tibialis anterior during the 2-minute MVC tests compared to healthy participants, before and after the training.

Strength training with the use of NMES is widely shown to be effective on young and old healthy adults (Caggiano et al., 1994; Gondin et al., 2005), on patients with muscular dystrophies (Scott et al., 1986, 1990; Zupan, 1992; Zupan et al., 1993) and also on patients with FSHD (Milner-Brown and Miller, 1988; Colson et al., 2010). Surprisingly, we failed to show such a positive effect on muscle strength, muscle endurance of the TA muscle and motor function in our group of FSHD patients. Although the stimulation parameters (*i.e.* 6s ON / 7s OFF; 35 Hz, 200µs pulse-width) of our NMES protocol as well as session duration were similar with the previous study recently published by Colson et al. (2010), the lack of significant improvements could be attributable to the frequency of the sessions and/or the duration of our training. Here, 3 sessions per week, for 8 weeks were performed, whereas Colson et al. (2010) trained their participants 5 days per week over a 5-month training period. Similarly, Milner-Brown and Miller (1988) obtained significant improvement in strength after a 2-hour session performed 5 days per week during 14 months. Another unexpected result is the absence of improvements in the HP group. Considering that benefits of NMES training programs on muscle strength are widely reported in literature for similar training periods (Thériault et al., 1996; Colson et al., 2000; Pérez et al., 2003; Gondin et al., 2005; Colson, 2009), it was valid to expect this NMES protocol to be effective on the tibialis anterior muscle

in our healthy participants. Since no improvements were seen the characteristics of stimulation parameters may be questioned. Here a 35-Hz frequency and a200-µs pulse duration were used, while literature suggests that high frequency (> 50 Hz) and wider pulse duration (> 400 µs) can maximise the training effect on muscle strength (Collins, 2007; Dean et al., 2007; Maffiuletti et al., 2011). These NMES parameters were chosen since they were beneficial in rehabilitation settings for patients with muscular dystrophies (Scott et al., 1990; Colson et al., 2010). Besides, the tibialis anterior muscle is mainly composed slow-type fibres in healthy participants (Johnson et al., 1973) and this fibre type proportion is even greater in FSHD patients since a shift from fast to slow muscle fibres types was reported (D'Antona et al., 2007). Consequently, although it may be suggested that a NMES protocol with pulse duration wider than 200 µs and frequency above 50 Hz, should be considered for training of the tibialis anterior muscle of healthy participants, it seems that the main reason for the absence of strength increase in our FSHD patients has to be related to the frequency of the NMES sessions and/or to the duration of the training protocol. Moreover, in the FSHD group, the intrinsic contractile properties of the tibialis anterior at the beginning of the study can be suggested as a possible reason candidate for the training to be ineffective. Since no training effect was seen in the HP group, this cannot be stated with certitude. Even though the 8-week bilateral training did not improve ankle dorsiflexion strength and endurance, neither improve the motor function, the training did not deteriorate these parameters either. Perhaps the training helped to maintain the already existing the maximal muscle strength. At last, another explanation could be related to the reliability of our measurements. However, interclass correlation coefficient for the ankle dorsiflexion MVC was 0.98 and 0.92 for the associated tibialis anterior RMS amplitude. Therefore, although the reliability of strength measurements is often questionable in health compromised populations, our measurements seemed to be sufficiently fair.

As expected, and consistently with literature (Schillings et al., 2007) the group of patients with FSHD showed much weaker ankle dorsiflexion MVCs, regardless of training. Confirming literature (Dorobek et al., 2013), this is related to the lower amount TA EMG amplitude observed in the group of patients with FSHD compared to the healthy participants. The patients with FSHD have fat infiltration in tibialis anterior muscles (Olsen et al., 2006; Kan et al., 2010; Friedman et al., 2012) and strong alteration of the sarcomeric contractile properties (D'Antona et al., 2007; Lassche et al., 2013) reducing the overall capacity of the muscle to contract and explaining the muscle weakness. Since the amplitude of the EMG signal reflects the activation of motor units, the lower amplitude in the group of patients with FSHD then demonstrates that the muscle activation is altered (*i.e.* a lower number of motor units is available and the force output is mainly generated by type I motor units) during ankle dorsiflexion MVCs, and the force production is hampered. Moreover, and regardless of the training, the peak force production of maximal plantar flexion was weaker in the group of patients with FSHD than in the HP group. The soleus EMG amplitude however was not found to differ between groups prior to and after the training. The gastrocnemii are strong contributors to the ankle plantar flexion, and the gastrocnemius medialis is also severely affected by the disease(Olsen et al., 2006). Thus, it may be suggested that other plantar flexor muscles than the soleus were altered, explaining the weakness of the ankle plantar flexion peak force in the group of patients with FSHD and the comparable soleus EMG amplitude.

During the 2-minute fatiguing effort, the MVC loss was much lower in the FSHD patients than in the control group (Figure 3). This may indicate that patients with FSHD experienced a lower amount of muscle fatigue (Schillings et al., 2007) before and after the training, likely

for several reasons. First, as the amplitude of the tibialis anterior EMG reduced similarly, this suggests that the neural drive would have become suboptimal with fatigue for both groups in the same fashion during the fatiguing exercise (Gandevia, 2001). Second, patients with FSHD show a shift of muscle fibres towards more slow type fatigue resistant muscle fibres (D'Antona et al., 2007). Third, weaker participants are shown to be less fatigable than stronger ones (Hunter and Enoka, 2001), as the intramuscular pressure is lower the negative feedback from groups III and IV is therefore diminished (Hunter and Enoka, 2001; Barry and Enoka, 2007). Even though, the group of patients with FSHD showed a greater tibialis anterior EMG amplitude loss after the training in comparison to the loss before the training, the tibialis anterior EMG amplitude decrease was similar in both groups. And, since the patients group showed lower force losses, it can be suggested that at the task truncation, the group of patients with FSHD were experiencing a lower amount of muscle fatigue compared to the HP group.

The specificity of the FSHD is that it affects the peripheral component of the motor pathway, and it is particularly striking when looking at the nerve stimulation evoked responses. Over a total of 10 patients with FSHD, we managed to elicit an M-wave in only about a half of them. Several plausible explanations can be suggested. First, as the M-wave reflects the maximal activation of motor units(Tucker et al., 2005), in cases where no M-waves were measurable (see Figure 2), it can be suggested that the muscle was not responsive anymore to the electrical current then supporting the alteration of the contractile properties of the muscle. This is also supported by the amplitude of the associated evoked mechanical response (Pt) that was of much larger amplitude in the HP group. In some participants, even though an M-wave was recordable, the mechanical response was either of very low amplitude or even would induce an ankle inversion/eversion or even a plantar flexion. Second, it is possible that,

even though the common peroneal nerve is superficial enough, the stimulation would not induce a mechanical response (Millet et al., 2011). This could be attributed to anatomical factors, as the stimulation would have involved the activation of other lower-leg muscles. Yet, although no difference was found in the amplitude of the M-wave between groups, our results showed clear amplitude differences in the evoked mechanical twitch between groups suggesting that the contractile properties of the tibialis anterior of the group of patients with FSHD was altered by the disease.

Additionally, the functional tests performed prior to and after the NMES training here, did not show any modification. When considering the MMT on both sides, results did not reveal, first any improvement with the training, but mostly no differences between groups. This result is surprising considering the ankle dorsiflexion weakness experienced by group of patients with FSHD compared to the control group. Likely, during MMT tests, patients with FSHD may have developed compensation strategies to overcome the deficit of the tibialis anterior, and other muscles, amongst them the extensor halluces longus, may have participated to the relatively high scores. Thus, although it is widely used in clinical practice, the validity of a manual test to assess the ankle dorsiflexion strength in FSHD can be questioned since this form of testing has been criticised regarding its reliability(Cuthbert and Goodheart, 2007), reproducibility and sensitivity (Bohannon, 2005). Then, it can be suggested to test the tibialis anterior strength with hallux flexion, or to investigate the link between the tibialis anterior M-wave and the tibialis anterior MMT score.

The MFM score was lower in the group of patients with FSHD compared to the healthy participants and this did not improve, nor worsened with training. The MFM score aims at evaluating the functional abilities of the patients, as well as the severity and progression of the

disease (Bérard et al., 2005), and our results showed that the tibialis anterior NMES training did not affect the overall MFM score because of it little role in the functional abilities of the patients.

Regarding the 6MWT, as expected patients with FSHD walked shorter distances, but no effect due to the training was observed. Walking involves a variety of muscles, and bigger muscles than the tibialis anterior like the knee and hip extensors and flexors, back and abdominal muscles. It has been shown that walking speed is more likely related to the balance between muscle groups (knee extensors and flexors, ankle plantar and dorsiflexors) than muscle strength (Willén et al., 2004). Therefore, it can be suggested that the tibialis anterior has a little role in the overall performance for a 6MWT, explaining the lack of training effect.

Conclusion

In summary, present results show that an 8-week bilateral NMES training (20 minutes per session, 3 sessions per week) of the tibialis anterior muscle did not improve muscle strength, endurance and motor function in patients with FSHD. Whether this non-responsiveness is due to the altered intrinsic contractile properties of the muscle and/or to the too short and not strenuous enough NMES protocol still remains to be shown. As suggested by Colson et al. (Colson et al., 2010), it is likely that the efficacy of the NMES training would depend on rapidity of starting a NMES training as soon as the FSHD diagnosis is made to maximise the effect of a training.

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TABLES

Table 1. Median and interquartile range (IQR) of the peak force during maximal voluntary contraction in ankle plantar flexion and the mean and standard error of the mean (SE) of the associated RMS amplitude of the soleus (SOL RMS), before (PRE) and after (POST) the 8-week training period for patients with facioscapulohumeral muscular dystrophy (FSHD) and healthy participants (HP).p: *p*-value of differences between groups. Significant p-values are in bold italic font.

		Force (N)			**SOL RMS (mV)**	
		PRE	**POST**		**PRE**	**POST**
FSHD	Median (IQR)	141 (88 ; 184)	165 (76 ; 181)	Mean (SE)	0.1099 (0.0194)	0.1263 (0.0190)
HP		307 (178 ; 666)	439 (189 ; 631)		0.1677 (0.0276)	0.1190 (0.0278)
p		***0.011***	***0.042***		0.180	0.467

Table 2. Number of valid and missing values and mean and standard error of the mean (SE) of the amplitude of the maximal M-wave (M-wave) and the associated mechanical responses (Pt), before (PRE) and after (POST) the 8-week training period for patients with facioscapulohumeral muscular dystrophy (FSHD) and healthy participants (HP). p: *p*-value of differences between groups. Significant p-values are in bold italic font.

			M-wave (m.V)		**Pt (N)**	
			PRE	**POST**	**PRE**	**POST**
FSHD	N	Valid	6	6	6	7
		Missing	4	4	4	3
	Mean (SE)		2.19 (0.29)	1.81 (0.34)	2.94 (1.63)	3.30 (1.86)
HP	N	Valid	6	6	4	5
		Missing	0	0	2	1
	Mean (SE)		3.57 (0.72)	2.93 (0.68)	8.57 (1.31)	9.97 (1.39)
p			0.106	0.171	***0.023***	***0.017***

Table 3. Participants with facioscapulohumeral muscular dystrophy (FSHD) scores of the Manual Muscle Testing from the tibialis anterior (TA) before (PRE) and after (POST) the 8-week training.

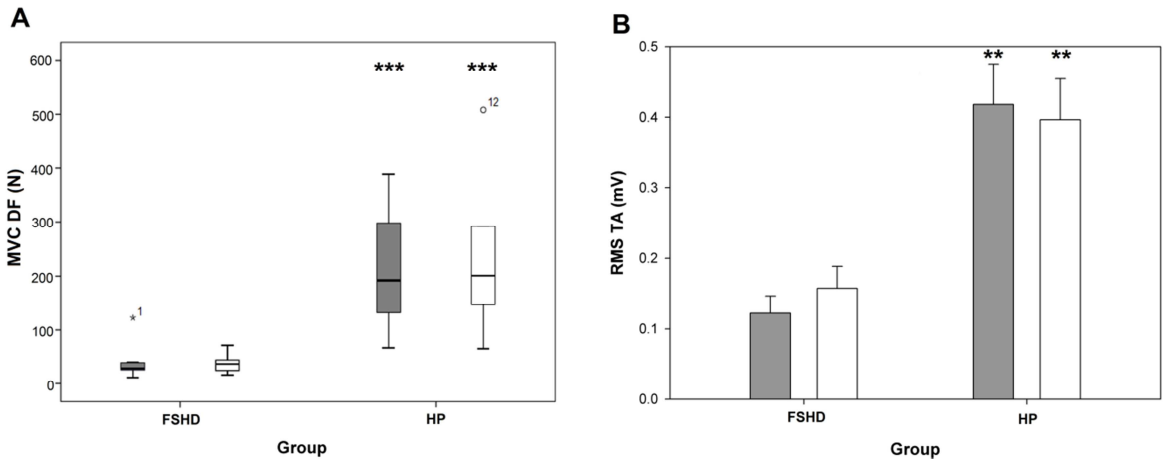
FSHD participant ID	TA Right PRE	TA Left PRE	TA Right POST	TA Left POST
S1	2	5	2	5
S2	2	2	4+	4+
S4	2	2-	4-	3+
S5	2	2	2	2
S6	3	4	5	2+
S8	2-	4-	1	5
S11	5	5-	5	5-
S12	4+	4	4+	4
S13	2-	1+	0	0
S14	4-	4-	5-	5-

Table 4. Mean and standard error of the mean (SE) of the 6-minute walking test (6MWT) distance in meters, and median and interquartile range (IQR) of the Motor Function Measurement score (MFM), before (PRE) and after (POST) the 8-week training period for patients with facioscapulohumeral muscular dystrophy (FSHD) and healthy participants (HP). p: *p*-value of differences between groups. Significant p-values are in bold italic font.

		6MWT (m)			**MFM (%)**	
		PRE	**POST**		**PRE**	**POST**
FSHD	Mean (SE)	303.63 (45.78)	286.00 (49.79)	Median (IQR)	72.12 (52.56 ; 86.13)	69.23 (51.28 ; 77.00)
HP	Mean (SE)	500.00 (32.14)	491.25 (42.85)	Median (IQR)	100 (100 ; 100)	100 (100 ; 100)
p		***0.007***	***0.002***		***<0.001***	***<0.001***

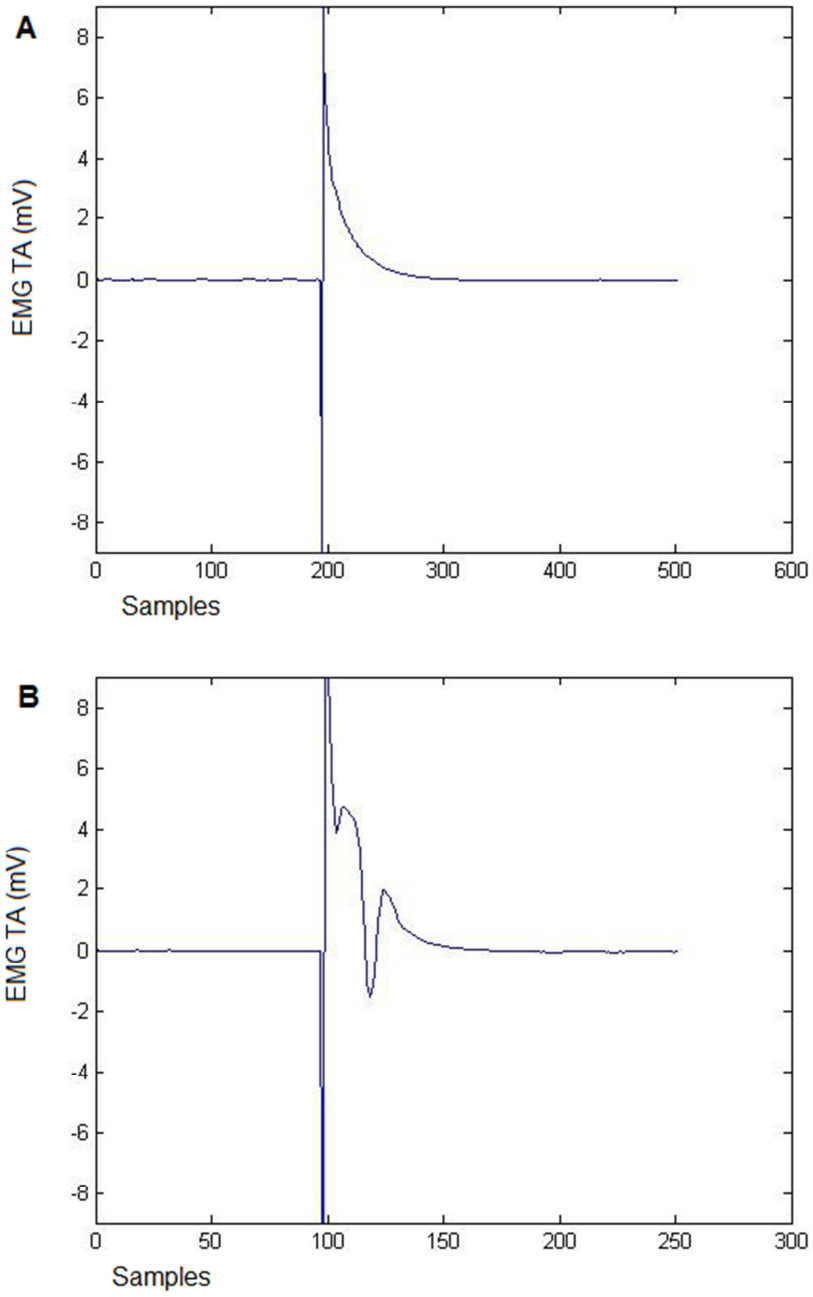
FIGURES

Figure 1



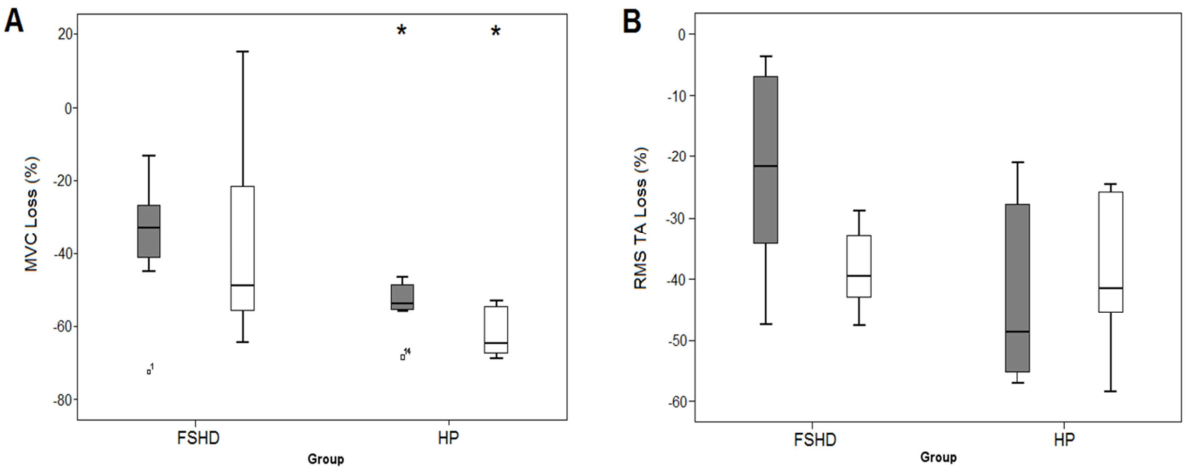
A, left side panel: peak force during isometric ankle dorsiflexion maximal voluntary contraction before (in grey fill-in) and after (in white fill-in) the 8-week training period for patients with facioscapulohumeral muscular dystrophy (FSHD) and healthy participants (HP). Boxes represent group median and interquartile range values and whiskers are the highest and lowest values; B, right side panel: the RMS amplitude of the tibialis anterior (TA), before (in grey fill-in) and after (in white fill-in) the 8-week training period for patients with facioscapulohumeral muscular dystrophy (FSHD) and healthy participants (HP).Columns represent group mean values and error bars the standard error of the group mean. Significant group differences $p<0.01$ (**) and $p<0.001$ (***).

Figure 2



Typical recording of M-wave (in mV) of the tibialis anterior (TA) of one patient with facioscapulohumeral muscular dystrophy (A, top panel) and one healthy participant (B, bottom panel). While on the healthy participant an M-wave could be seen on the EMG signal, the EMG signal patient with facioscapulohumeral muscular dystrophy did not measure any M-wave, only the stimulation artefact could be seen.

Figure 3.



Box-plot of the percentage of force production loss(A, left panel) and of the RMS of the tibialis anterior (TA) (B, right panel) during the 2-minute sustained ankle dorsiflexion endurance exercise, before (in grey fill-in) and after (in with fill-in) the 8-week training for patients with facioscapulohumeral muscular dystrophy (FSHD) and healthy participants (HP). Boxes represent group median and interquartile range values and whiskers are the highest and lowest values. Significant group differences: $p<0.05$ (*)