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## Age-Related Reductions of Motor Unit Discharge Rates in the Human Hamstrings

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Graduate Program in Kinesiology  
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## Abstract

Neuromuscular age-related differences of human limb muscles have been widely described with the notable exception of the hamstring muscles. The purpose was to assess contractile function and spinal motor neuron output expressed as motor unit discharge rates in the hamstrings of 11 young ( $26 \pm 4$  y) and 10 old ( $80 \pm 5$  y) men. Maximal voluntary isometric contractions (MVC), stimulated contractile properties and motor unit discharge rates from sub-maximal to MVC were recorded from the lateral (biceps femoris) and medial (semimembranosus-semitendinosus) posterior thigh. In the old men, knee extension and flexion at MVC were lower ( $P < 0.05$ ) and voluntary activation as assessed by the twitch interpolation technique was reduced ( $P < 0.05$ ) compared with the young. Electrically evoked twitches were lower in amplitude and increased in duration of old hamstrings ( $P < 0.05$ ) compared with the young. At sub-maximal to maximal contraction intensities the old had lower motor unit discharge rates as compared to the young ( $P < 0.001$ ). At MVC, mean motor unit discharge rates in the biceps femoris and semimembranosus-semitendinosus of old hamstrings were  $15.6 \pm 6.4$  and  $15.3 \pm 5.9$  Hz, as compared to  $26.1 \pm 10.1$  and  $27.9 \pm 7.8$  Hz in the young, respectively ( $P < 0.001$ ). To date, the hamstrings show the greatest age-related reductions in motor unit discharge rates of any major limb muscle. These findings, in relation to motor unit discharge rates from other flexors and extensors support that in ageing, greater reductions are associated with limb flexor muscles.

## Keywords

Ageing; maximal isometric voluntary contraction (MVC); dynamometry; electromyography (EMG); human; hamstrings; motor unit (MU); muscle; neuromuscular system; voluntary activation

## Co-authorship statement

Portions of Chapter 2 and 3 co-authored by Charles L. Rice and Kevin J. Gilmore have been published. Charles L. Rice and Kevin J. Gilmore also participated in collection and interpretation of all experimental data. Experimental data were collected, analyzed and interpreted by Eric A. Kirk.

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Eric Andrew Kirk

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## List of abbreviations

ANOVA - analysis of variance

BF - biceps femoris (long head)

CD - contraction duration

EC - excitation-contraction

EMG - electromyography

HRT - one-half relaxation time

ITT - interpolated twitch technique

MU - motor unit

MVC - maximal voluntary isometric contraction

Pt - peak twitch amplitude

PTP - potentiated peak twitch amplitude

RMS - root-mean-square

RTD - rate of torque development

SS - semimembranosus-semitendinosus

TPT - twitch time to peak tension

VA - voluntary activation

## Chapter 1

### 1 General introduction

Ageing is an inevitable human condition within a finite biological window. Throughout the existence of known life on earth, expected to have begun up to 4.28 billion years ago (Dodd *et al.*, 2017), the ageing condition within an organism has been countered by the transfer of genomic information to a newer, younger form. As humans, we must acknowledge the importance of ageing as it enabled evolution to get us to our present state of being able to rationalize and appreciate our sentience. Indeed, “*nothing in biology makes sense except in the light of evolution*” (Theodosius Dobzhansky, 1973). Perhaps we can further extrapolate that *nothing in human physiology makes sense except in the light of ageing*. In almost all forms of animal life, the neuromuscular system is a central attribute. It enables voluntary movement giving us the ability to interact with our environment and has likely been examined well before the beginning of the Holocene era. If you are lucky enough to age beyond maturity, there will be an ever-narrowing capacity for voluntary movement that is accelerated towards functional thresholds of limitation. We are far from complete in understanding mammalian neuromuscular physiology, further complicated by the diversity of ageing. In characterizing and understanding these systems it will help us better develop preventative methods and enhance action to extend the ageing process in the hope to one day bypass or reverse it altogether.

#### 1.1 Neuromuscular transmission and motor control – a brief review

Voluntary movement is possible due to the neuromuscular system (Figure 1). Movement is initiated in the pre-motor cortices of the brain occurring on the order of 100 ms before muscle activation (Chen *et al.*, 1998). Electro-chemical signals from the central nervous system are directed through the corticospinal tract acting on alpha motor neurons in the spinal cord (Liddell & Sherrington, 1925). These spinal motor neurons are connected to a network of multiple afferent and efferent synapses, and support neurons within the

corticospinal tract. Simply, one spinal motor neuron innervates multiple fibres at the muscle level thus being defined as the motor unit. Within the mammalian central nervous system, the motor unit provides the principle motor output by converting intrinsic, sensory and descending neural inputs into force, and ultimately generating movement (Heckman & Enoka, 2012). Within the motor unit, action potentials are generated through regulatory processes at the neuron due to depolarization from the resting potential (-70 mV) to a threshold of action potential initiation (-50 mV). The origin of action potential discharges occurs at the axon hillock propagating in an ‘all-or-none’ response throughout the motor unit with invariant amplitude. This temporal control (rate of impulses per second) is a principle mechanism regulating the intensity of electro-chemical signals within each individual motor unit to the muscle, ultimately affecting muscle force.

For each muscle, motor units are variable in innervation ratio and number (McNeil *et al.*, 2005; Boe *et al.*, 2006; Stevens *et al.*, 2013). At the muscle level, electro-chemical signals are transduced from the neuromuscular junction enabling excitation-contraction (E-C) coupling and cross-bridge cycling. Simply, the neuromuscular junction is activated at the terminal axon releasing vesicles that contain acetylcholine which diffuse throughout the synaptic cleft. Acetylcholine then interacts with receptors on the motor end plate of muscle fibres opening ligand-gated ion channels resulting in depolarization of the muscle membrane. The motor end plate potential propagates throughout the muscle fibre membrane to then activate T-tubules. This activation at the T-tubule then begins E-C coupling which releases calcium ion from the sarcoplasmic reticulum, which then binds to troponin on the actin filament exposing cross-bridge binding sites on actin enabling interactions with myosin. The cross-bridge cycling of the active actin-myosin complex results in sarcomere shortening producing tension (Huxley, 1957). This force generation acts to shorten muscle which through the musculotendinous junction transfers the force onto bone levers at joint fulcrums producing angular joint rotation.

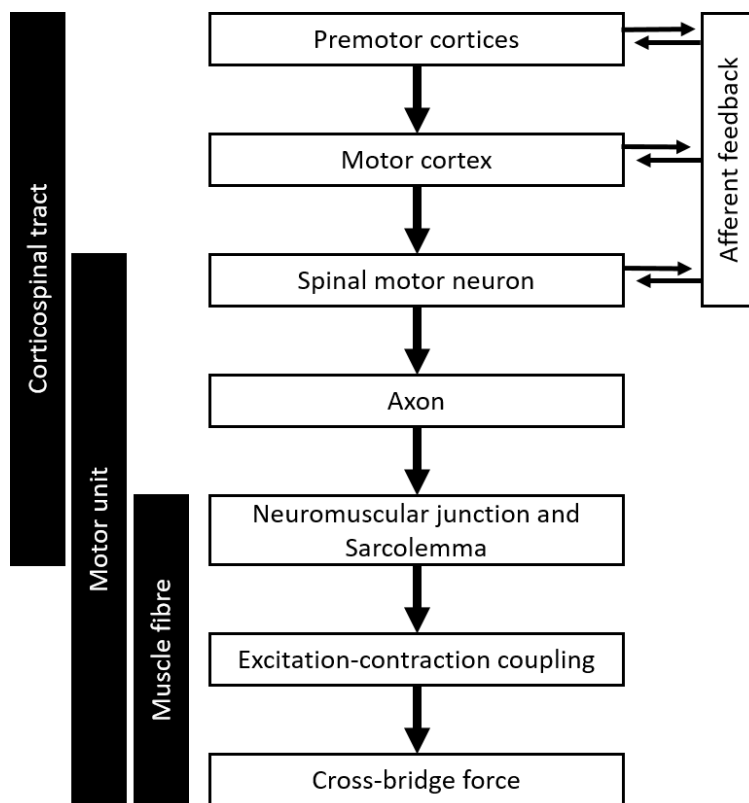


Figure 1. Basic diagram of the motor pathway

In building upon neuromuscular transmission of each individual motor unit, volitional action is achieved through the regulation of multiple motor units within each muscle's motor unit pool in coordination with other muscles. For motor unit pools supplying individual muscles, two principle mechanisms are employed to generate force gradations: 1) temporal regulation and 2) spatial regulation (Adrian & Bronk, 1928). Temporal regulation, also known as rate coding involves modulating the rate at which action potentials are transmitted throughout the motor unit. Spatial regulation, also known as motor unit recruitment involves increasing or decreasing the number of active motor units within a muscle. Recruitment order, according to the size principle is determined by the size of the motor unit's soma (Henneman *et al.*, 1964) having an activation pattern of smallest to largest. In theory, to achieve maximal voluntary force within a muscle, complete recruitment of all motor units with each being activated at its highest absolute discharge rate would occur. However, this scenario is rarely the case, due to multiple

known and unknown factors that modulate and protect the neuromuscular system during action (Enoka & Fuglevand, 2001).

## 1.2 Age-related changes of human motor units

In humans, there are many neuromuscular factors implicated in ageing (Johnston *et al.*, 2008; Ryall *et al.*, 2008; Alway & Siu, 2008; Hepple & Rice, 2016), including: motor neuron loss, altered neural drive, motor unit remodeling and intrinsic changes within the muscle (Power *et al.*, 2013; Cruz-Jentoft *et al.* 2014; Hepple & Rice, 2016). In ageing, motor unit remodeling is regarded as an inevitable compensatory process (Hepple & Rice, 2016). Over decades, from early adulthood into advanced age, the motor unit population declines and subsequently orphaned muscle fibres will degrade or be collaterally reinnervated by surviving motor units (Campbell *et al.*, 1973; McNeil *et al.*, 2005). At the spinal level, it is postulated that there is selective loss of larger motor neurons that innervate type II fibres, and due to collateral reinnervation, remaining viable motor units become larger and slower (Doherty *et al.*, 1993b; Hepple & Rice, 2016). With this remodeling, discharge rates of viable aged motor units are commonly thought to be lower than those for young adults, especially at moderate to high contraction intensities; however, these reductions are not observed in all muscles.

## 1.3 Force, contractile properties and voluntary activation in ageing

With advanced ageing, there is associated loss of skeletal muscle mass and strength, defined as *sarcopenia* (Cruz-Jentoft *et al.*, 2014). This ‘poverty of the flesh’ is further described as a degenerative loss of muscle mass at a rate of ~1 % per year starting in the 6<sup>th</sup> decade of life being accelerate in very old age. Accompanying this loss of skeletal muscle mass are co-morbidities and a finite functional threshold for independence and locomotion (Baumgartner *et al.*, 1998; Janssen *et al.*, 2002). Currently, 1 in 3 community dwelling adults are predicted to be sarcopenic (Cruz-Jentoft *et al.*, 2014). Furthermore, in non-community dwelling active older adults (who were assessed in their 9<sup>th</sup> decade of life) there is a significant loss of force production and slower contractile quality

(Vandervoort & McComas, 1986; Connelly *et al.*, 1999; Roos *et al.*, 1999; Kamen & Knight, 2004; Dalton *et al.*, 2009; Kirk *et al.*, 2016). However, unlike many muscles especially of the lower limb, the elbow extensors and flexors do not show significant contractile slowing, despite diminished force production when old were compared with young (Dalton *et al.*, 2010).

With sarcopenia, strength losses are not merely due to available contractile tissue, but are more likely due to a compromised neuromuscular system. The ability of older adults to fully activate their muscle is an interplay within the entire neuromuscular system (Klass *et al.*, 2007; Clark & Taylor, 2011). Voluntary activation is technique to measure to the level of voluntary drive to a muscle during an effort (Gandevia, 2001). Voluntary activation is commonly assessed by the interpolated twitch technique (ITT) established by Merton 1954 (Merton, 1954) and has been applied to paradigms of ageing. In findings from limb muscles and intrinsic hand muscles, age-related differences are variable and muscle specific (Klass *et al.*, 2007). And with sufficient practice of maximal efforts, old adults are able to activate their muscles to high levels that are similar to young (Jakobi & Rice, 2002). The ability to achieve complete voluntary activation becomes more variable with advanced age, dependent on muscle specific and training factors.

## 1.4 Motor unit numbers in ageing

With advanced age there is observed spinal motor neuron decline (Tomlinson & Irving, 1977), with reductions by as much as 70 % in very old age (Campbell *et al.*, 1973). To assess the motor neuron population in vivo, various electromyographical techniques are used resulting in a motor unit number estimate (Gooch *et al.*, 2014). In muscles of the brachium, motor units are lost with ageing in both men and women (Doherty *et al.*, 1993a). Since then, motor unit loss with ageing has revealed that loss may be muscle specific and may be attenuated with behavior or genetic factors. One example of such a muscle is the soleus, where minimal reductions in motor unit number estimates are observed in the old (Dalton *et al.*, 2008). However, in muscles having significant age-related reductions, the initial slow decline in motor neuron loss does not have immediate functional significance, suggesting that there is a secondary critical threshold associated

with accumulating motor unit loss amplifying muscle strength deficits (McNeil *et al.*, 2005). Apart from the soleus, muscles that are more distal may be more susceptible to motor unit loss by the ageing process. In using a surface EMG model, Galea (1996) assessed the biceps brachii, thenar and extensor digitorum brevis in four age groups (20-39, 40-59, 60-79 and 80-98) finding a 13% reduction in the proximal biceps as compared to a 47 and 55% reduction in the thenar and extensor digitorum brevis of the oldest group (Galea 1996). In two reports assessing the biceps brachii (Power *et al.*, 2012) and tibialis anterior (Power *et al.*, 2016b), there was an observed phenotype rescue as compared to the age-matched controls. Those who were master's athletes had a preservation of motor units postulated to have a neuroprotective advantage, this observed difference points to the unknown roles that behavior and genetics may have on motor unit loss and neuroprotection in ageing.

## 1.5 Motor unit discharge rates in ageing

In assessing temporal regulation in humans, motor neuron discharge rates are quantified by recording from muscle fibres (Adrian & Bronk, 1928). Commonly, motor unit discharge rates are assessed by intramuscular and surface array methodologies; with each method having mutually exclusive advantages, such as sampling from multiple motor units at high contraction intensities and non-invasiveness, respectively. The technique used in this thesis is described as the 'gold standard' in recording motor unit discharge rates at maximal intensities. Simply, it is a microelectrode inserted into the muscle belly and is manipulated during voluntary isometric contractions to record from as many discrete motor units during voluntary efforts (Bellemare *et al.*, 1983; Stashuk & Qu, 1996; Rich *et al.*, 1998).

It was originally proposed that motor unit discharge rates in old humans (80-85 y) were lower to maintain the speed-match with slowed muscle contractile properties (Connelly *et al.*, 1999). However, as more limb muscles were explored it became apparent that this relationship was too simplistic, and other factors likely were involved to explain the varying degrees of age-related differences in discharge rates among different limb muscles (Dalton *et al.*, 2010). At moderate to maximal contraction intensities, age-related

reductions of discharge rates occur in the first dorsal interossei (Kamen *et al.*, 1995; Erim *et al.*, 1999), abductor digiti minimi (Patten *et al.*, 2001), tibialis anterior (Connelly *et al.*, 1999), vastus lateralis (Kamen & Knight, 2004), biceps brachii and triceps brachii (Dalton *et al.*, 2010). In opposition to these findings, discharge rates in the vastus medialis (Roos *et al.*, 1999b), soleus (Dalton *et al.*, 2009) and gastrocnemii (Kirk *et al.*, 2016) have no observed or minimal age-related reductions at high contraction intensities. For limb muscles that do show a reduction, such as the tibialis anterior, discharge rates collected in old participants who were healthy and moderately active seniors over 80 years of age, showed age-related reductions of 26 % at maximal contraction intensities (Connelly *et al.*, 1999). Similarly, in the vastus lateralis, initial discharge rates between young and old were significantly different from non-resistance trained individuals, but during 6 weeks of isometric training, discharge rates in the old were increased to that of the young (Kamen & Knight, 2004). In the soleus muscle at 75 and 100 % maximal voluntary isometric contraction (MVC) old men showed nominal age-related reductions in discharge rates (Dalton *et al.*, 2009) despite 34 % lower plantar flexion strength. Furthermore, in the vastus medialis and gastrocnemii, there were no significant age-related differences in discharge rates, despite 50 % reductions in MVC between young and old men (Roos *et al.*, 1999; Kirk *et al.*, 2016). In contrast to lower limb muscles, upper limb muscles have all reported age-related reductions in motor unit discharge rates, but when comparing flexors and extensors within the same individual, age-related reductions were greater for the biceps brachii as compared to the triceps brachii (Dalton *et al.*, 2010). Further compared across many muscles, it appears that discharge rates in extensor limb muscles either have the capacity to increase to match those of the young (Kamen & Knight, 2004) or are more resilient to age-related decreases (Roos *et al.*, 1999b; Dalton *et al.*, 2009, 2010; Kirk *et al.*, 2016). Whereas flexors have greater reductions within the same individual (Dalton *et al.*, 2010) and present significant age-related reductions (Connelly *et al.*, 1999).

## 1.6 Human hamstrings

Major human limb muscles have been studied in assessing age-related motor unit properties, including: intrinsic hand muscles, elbow extensors, elbow flexors, knee



extensors, plantar flexors and dorsiflexors, with the notable exception of the hamstrings. Recently, muscle contractile properties and motor unit discharge rates of the hamstrings were characterized in young adults at two different muscle lengths (Kirk & Rice, 2016), but no study has explored these properties in aged humans. The hamstrings are a large proximal muscle group and developmentally considered as flexor muscles (as compared with quadriceps, a well-studied extensor group) comprising the biceps femoris on the lateral aspect and the semimembranosus and semitendinosus muscles on the medial aspect of the posterior thigh (Moses *et al.*, 2005; Sadler & Langman, 2012). The biceps femoris consists of a long and short head, the long head originates on the ischial tuberosity whereas the short head originates on the lateral lip of the linea aspera and lateral supracondylar line of the femur. Both the long and short heads insert on the head of the fibula and lateral condyle of the tibia. The semimembranosus originates on the upper and lateral ischial tuberosity inserting on the posterior part of the medial condyle of the tibia. The semitendinosus originates on the upper and medial ischial tuberosity inserting on the superior part of the medial surface of the tibia (Moses *et al.*, 2005). The tibial division of the sciatic nerve (L5 to S2) innervates the semimembranosus, semitendinosus (SS) and long head of the biceps femoris (BF), whereas the short head of the biceps femoris is innervated by the common fibular division of the sciatic nerve (L5 to S2). The perforating branch of the deep artery of the thigh and superior muscular branches of the popliteal artery are the common blood supply to all four muscles (Moses *et al.*, 2005). In cadaveric preparations, fibre type distributions in the hamstrings are reported to be like those for the quadriceps (which range from 40-55% type-I). The composition of the semimembranosus and semitendinosus are reported to be ~50 and ~45% type-I, and the long head of the biceps femoris is reported to be 45-67% type-I (Johnson *et al.*, 1973; Garrett *et al.*, 1984).

## 1.7 Purpose and hypothesis

The aim of the present investigation was to assess age-related neuromuscular properties in the hamstrings by comparing a group of young ( $26 \pm 4$  y) and old ( $80 \pm 5$  y) men. For this investigation, maximal voluntary strength of the knee flexors and extensors was assessed, and for the hamstrings: stimulated contractile properties, voluntary activation

and intramuscular EMG were recorded. For voluntary activation, the interpolated twitch technique was used. For motor unit discharge rates, microelectrodes were inserted into the biceps femoris (BF) and semimembranosus-semitendinosus (SS) of the hamstrings during submaximal to maximal voluntary isometric contractions. In age-related motor unit remodeling in limb muscles, motor unit discharge rates in flexors appear to have greater age-related reductions, likely facilitated by anatomical location and functional requirements of the constitutive motor unit pools. Like the tibialis anterior and biceps brachii the hamstrings are embryological flexors (Sadler & Langman, 2012) and based on this classification, it was expected that motor unit discharge rates of the hamstrings would have significant age-related reductions. Given the evidence that motor unit discharge rates are not associated with fibre type (Bellemare *et al.*, 1983), contractile strength (Roos *et al.*, 1999b) and contractile speed (Dalton *et al.*, 2010) it was hypothesized that motor unit discharge rates of the hamstrings (a flexor) would have significant age-related reductions.

## Chapter 2

# 2 Age-related changes in force, contractile properties, voluntary activation and motor unit discharge rates in the human hamstrings

## 2.1 Introduction

In bipeds, the hamstrings represent the largest limb flexor muscle group and are functionally required for posture and locomotion. With ageing, voluntary strength is reduced due to numerous factors that are muscle specific. Some of these factors may include: motor neuron loss, altered neural drive, motor unit remodeling and intrinsic changes within the muscle (Power *et al.*, 2013; Cruz-Jentoft *et al.*, 2014; Hepple & Rice, 2016). Apart from the hamstrings, age-related motor unit properties have been investigated in the triceps brachii, biceps brachii, vastus lateralis, vastus medialis, tibialis anterior, soleus, gastrocnemii and intrinsic hand muscles. In most of these muscles motor unit discharge rates (rate coding) have been quantified in relation to voluntary isometric contractions. From these cross-generation studies using intramuscular electromyography (EMG) methods, motor unit discharge rates in flexor limb muscles have age-related decline (Connelly *et al.*, 1999) that is greater within the same individual as compared to an extensor (Dalton *et al.*, 2010). In contrast, motor unit discharge rates in extensor limb muscles have shown no age-related decline (Roos *et al.*, 1999; Kirk & Rice, 2016), minimal age-related decline (Dalton *et al.*, 2009) and the capacity to be increased by resistance training (Kamen & Knight, 2004). Investigating force, contractile properties, voluntary activation and motor unit discharge rates in the hamstrings allows for comparisons to be made among all major limb muscle groups tested to date. Therefore, the aims were to quantify force, contractile properties and voluntary activation specific to the hamstrings, and discharge rates of functioning motor units in the long head of the biceps femoris, semimembranosus and semitendinosus in a group of young ( $26 \pm 4$  y) and old ( $80 \pm 5$  y) men.

## 2.2 Methods

### *Participants*

Twenty-one male participants volunteered for this investigation (Table 1). All participants were recreationally active. One young male participant was excluded from surface and intramuscular electromyography testing due to unavailability. Exclusion criteria included: known neuromuscular, orthopedic and metabolic diseases; alcoholism and recreational drug use. This study conformed to the local University's research ethics board for human experimentation and the latest revision of the Declaration of Helsinki. Participants were instructed to refrain from intense exercise and caffeine consumption within 48 h prior to testing and provided both oral and written informed consent. The study required each participant to visit the neuromuscular laboratory for several 2-3 h testing sessions each separated by 2–7 days.

### *Experimental set-up*

For the first session, participants were seated in a dynamometer (Cybex HUMAC NORM; CSMi Medical Solutions, MA, USA) with the non-dominant leg fixed to an adaptor arm located on the anterior tibia just proximal to the malleoli. The hip was extended to 100 degrees, the ankle was positioned at 90 degrees and the lateral femoral condyle was aligned to the dynamometers axis of rotation. Collection occurred at two knee joint angles which affected the length of the hamstrings. In the first position the knee joint was flexed to 90 degrees (shortened length) and in the second position the knee was extended to 160 degrees (lengthened). In both positions participants were firmly positioned in the dynamometer chair by seatbelts at the shoulder and hip, and a Velcro strap secured the thigh, just proximal to the knee. Torque was recorded from the dynamometer and sampled at 500 Hz. Torque production was displayed on a computer screen for visual feedback.

### *Muscle stimulation*

For electrical stimulation, large stimulation electrodes (5 x 15 cm, aluminum foil) covered in electro-stimulation gel, were wrapped in paper towel and saturated in saline water (Edwards *et al.*, 1977). The electrodes were securely taped transversely over the proximal aspect of the posterior thigh inferior to the gluteal fold and slightly medial to avoid activation of the vastus lateralis. The second electrode was placed distally over the posterior thigh muscles superior to the popliteal fossa. Stimulation (stimulator model DS7AH, Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK) was applied through the electrodes at 400 V with a pulse duration of 50  $\mu$ s for the tetanic stimulation protocol and 200  $\mu$ s for the modified twitch stimulation protocol. Stimulation current intensities for all participants, ranged between 250-450 mA. For all stimulation events the current intensity was incrementally increased to a level that activated as much of the hamstring muscles as possible (as measured by torque output) with minimal activation of muscles in the anterior or medial thigh compartments assessed by maximal torque output, visual inspection and manual palpation.

*Voluntary activation, evoked twitch and post-activation evoked twitch*

To assess maximal voluntary torque production, MVCs were performed without stimulation for knee extension and knee flexion at the two knee joint angles (90 and 160 degrees). Electrically stimulated twitch properties of the hamstrings were recorded in the extended knee joint position (160 degrees). This was found to be the optimal position to record stimulated contractile responses in this muscle group (Kirk & Rice, 2016). The twitch interpolation technique (Hales & Gandevia, 1988; Todd *et al.*, 2004) was used to test the ability of the participants to maximally activate the hamstring muscles during MVC. For this test, electrical impulses (doublets; two pulses at 100 Hz) were applied to the muscle at rest ~1 s before contraction started, during the peak plateau of the 5-7 s MVC and ~1 s following the MVC when at rest (Figure 2). Stimulated twitches were maximized for each participant; the current intensity was incrementally increased for the optimal twitch torque response. Visual feedback and strong verbal encouragement were given during the MVCs. Two to three separate MVC trials were performed with 5 min rest between contractions to control for fatigue, with the highest MVC value being recorded as the maximum.

### *Force-frequency stimulation*

The force-frequency relation is the sigmoid relationship between the isometric force (torque) response of the muscle to varying frequencies of excitation. In following the method Edwards et al., 1977 used for the quadriceps, the hamstring muscle was stimulated at 1, 5, 8, 10, 12, 15, 20, 30, 40, 50, 80 and 100 Hz for a duration of 1 s with the muscle at rest (Edwards *et al.*, 1977). A 30 s rest interval was given between each level of stimulation. The stimulus intensity was determined by the maximum tolerable intensity at the 100 Hz stimulation, this remained the intensity for all subsequent stimulation frequencies with this force-frequency protocol. The torque output at the 100 Hz stimulation for each participant ranged from 21-49 % MVC in the old and 17-35 % MVC in the young. The order of stimulation frequencies was randomized.

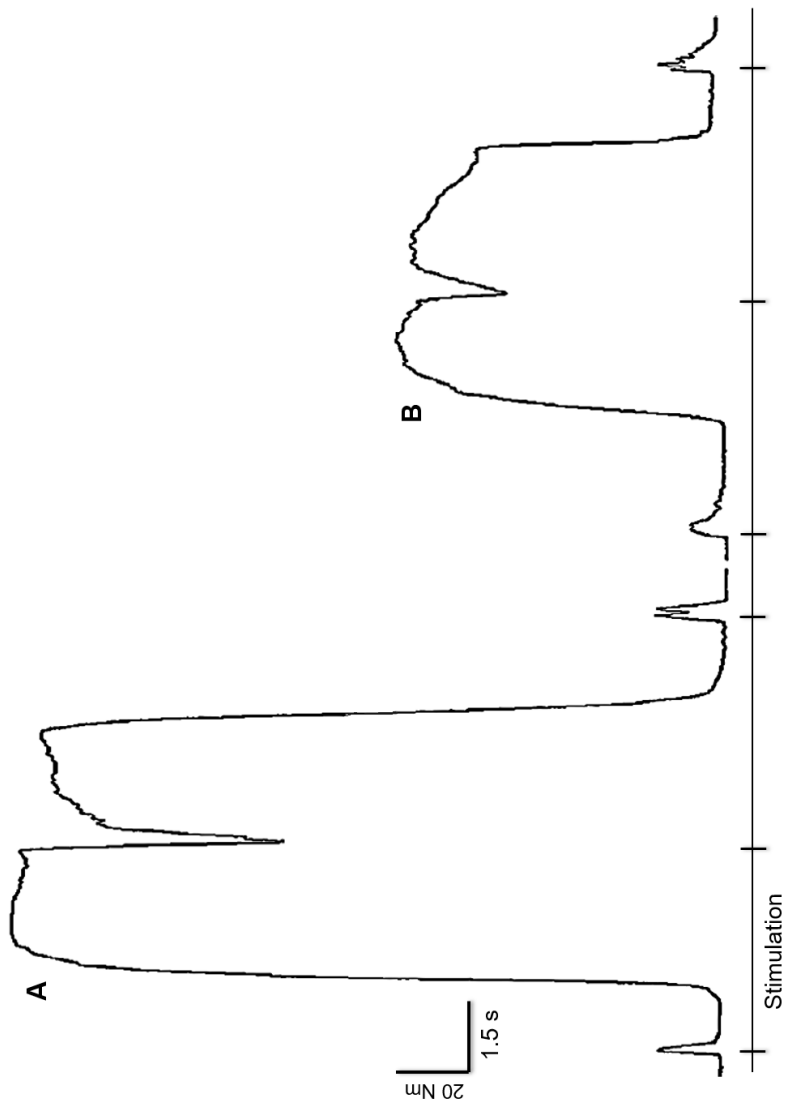


Figure 2.

Raw data tracing from the hamstrings in the 160-degree knee flexion position of an evoked stimulated twitch, seated maximal voluntary contraction, superimposed evoked stimulated twitch and post-activated potentiated evoked stimulated twitch. Panel A is the strongest young participant (25 y) and panel B is the strongest old participant (92 y).

### *Experimental set-up*

On a separate day, participants were positioned prone on the same dynamometer with the same leg fixed to the adaptor arm as described above. The hip was extended to 170 degrees with the ankle fixed at 90 degrees. The lateral femoral condyle was aligned to the axis of rotation and the knee was extended to 160 degrees. All participants were firmly fastened by large Velcro straps located on the lower back and posterior distal thigh to prevent extraneous movements. Intramuscular electromyography (EMG) was only possible in this position (muscle lengthened) due to accessibility of the posterior thigh for needle insertions and manipulations.

### *Surface electromyography*

To assess overall neuromuscular activation, surface EMG (sEMG) in a bipolar configuration for the medial and lateral hamstrings muscle groups was used. An inter-electrode distance of 2 cm centre-to-centre was used with a common ground located on the patella. Electrode pairs were placed over the lateral mid-thigh on the biceps femoris and medial mid-thigh over the semimembranosus and semitendinosus muscles. sEMG signals were recorded using self-adhering cloth Ag-AgCl electrodes (H59P monitoring electrodes, Kendall, Mansfield, MA, USA). The sEMG electrode placement sites were swabbed vigorously with 70 % ethanol prior to placement. All sEMG signals were amplified (x1000), wide-band filtered between 10 Hz and 1 kHz (Neurolog; NL844, Digitimer, Welwyn Garden City, UK) and sampled at 2 kHz (Power 1401, Cambridge Electronic Design Limited, Cambridge, UK).

### *Intramuscular electromyography*

For sampling motor unit discharge rates during voluntary contractions, intramuscular tungsten microelectrodes (Bellemare *et al.*, 1983) were inserted into the hamstring muscles. This technique can discriminate individual motor unit discharge rates from submaximal to maximal voluntary isometric contraction intensities to build a representative sample of discharge rates from the motor unit population supplying the muscle. Differential motor unit discharge rates are recorded within the same contraction



event and among multiple attempts at the different intensities. In recording motor units using this technique the probability of recording from two or more muscle fibres from the same motor unit when modelled to have muscle fibres distributed along the length of the whole muscle follows a Poisson distribution with a mean of 0.88, 0.44 and 0.22 for 25, 50 and 100 % MVC, respectively (Rich *et al.*, 1998). For this model, the probability of motor unit duplications is inversely related to contraction intensity (Rich *et al.*, 1998), and the impact of perhaps recording the same MU more than once on average motor unit discharge rates over the range of contraction intensities is minimal. The electrodes were insulated tungsten wire needles (123  $\mu\text{m}$  in diameter and 45 mm in length; Freddy Haer Company, Bowdoin, ME, USA). One electrode was inserted into the lateral musculature (long head of biceps femoris) and one into the medial (semimembranosus and semitendinosus) musculature halfway the distance from the head of the fibula to the greater trochanter near to the surface electrodes. Prior to insertion, each site was cleansed with 70 % ethanol on the skin surface over the muscle bellies. The two sterilized intramuscular electrodes were connected to separate channels and each was manipulated independently by an operator. The intramuscular EMG signals were amplified ( $\times 100$ ), wide-band filtered between 10 Hz and 10 kHz (Neurolog; NL844, Digitimer, Welwyn Garden City, UK) and sampled at 20 kHz per channel (Power 1401, Cambridge Electronic Design Limited, Cambridge, UK). Reference electrodes for the intramuscular electrodes were positioned over the patella of the lower limb being tested. Audio and visual feedback was provided to each operator independently. Voluntary contractions were held for 5-10 s at each of the three (25, 50 and 100 % of MVC) contraction levels with rest periods between contractions of 3-5 min to mitigate fatigue. The order was pseudorandomized with motor unit trains being sampled during the steady-state plateau portion of the different contraction intensities (Figure 3). Visual feedback of torque and strong verbal encouragement were provided to each participant. To sample from as many discrete motor units as possible, each intramuscular electrode was manually manipulated and advanced slowly through the muscle at a rate of  $\sim 3\text{-}5$  mm per contraction (Figure 3). Intramuscular electrodes were repositioned and reinserted into different portions of the muscle in a similar region of the thigh to achieve a representative sample from each muscle, thus building an overall motor unit profile range at each contraction intensity.

Several (3-6) contractions were made at each contraction intensity until the participant's MVC level was ~5 % lower than their initial MVC. To acquire many motor unit trains without the influence of fatigue, some participants returned to the lab to repeat the protocol on additional days.

#### *Data acquisition and analyses*

Analyses were performed offline with Spike2 (Cambridge Electronic Design; Cambridge, UK) as described previously (Connelly *et al.*, 1999; Roos *et al.*, 1999). For contractile properties of the evoked twitch the following measures were made: peak twitch amplitude (Pt), twitch time to peak tension (TPT), rate of torque development (RTD), one-half relaxation time (HRT), contraction duration (CD) and potentiated peak twitch amplitude (PTP) (Vandervoort & McComas, 1986; Roos *et al.*, 1999). Voluntary activation was calculated from the interpolated twitch technique as previously described (Todd *et al.*, 2004). The force-frequency relation was normalized to the 100 Hz stimulation torque. To assess sEMG, 1 s epochs were measured during the steady state portion of voluntary contractions. The root-mean-squared (RMS) amplitude during MVC torque of the BF and SS was used to normalize sEMG of each individual muscle for the 25 and 50 % contraction intensities.

For motor unit train analysis, a template shape algorithm was used, requiring visual inspection by an experienced operator to confirm the algorithm allocation to each specific motor unit train. For inclusion, motor unit discharges were analyzed with a shape overlay of action potentials to ensure sequential discharges had analogous shape profiles confirming they were from the same motor unit (Stashuk & Qu, 1996). A minimum of 5 contiguous action potentials (4 inter-spike intervals) and a coefficient of variation <30 % for the inter-spike interval durations of each train were required for inclusion (Fuglevand *et al.*, 1993). Doublets (>100 Hz) were excluded from analysis. An example of four discrete motor unit action potential trains extracted during MVC are shown (Figure 3). For statistical comparisons, motor unit trains were grouped into 3 bins based on torque level: a 100 % bin contained torque levels between 100–87.5 % of MVC; a 50 % bin contained 62.5–37.5 % of MVC and lastly a 25 % bin contained 37.5–12.5 % of MVC.

### *Statistics*

A statistical application (R, version 3.2.3) was used for analyses. Anthropometric, voluntary and stimulated contractile properties were compared between age groups using unpaired t-tests. To assess differences in sEMG, a 1x3 analysis of variance (ANOVA) was used (sEMG | age x muscle x contraction intensity). To assess differences in motor unit discharge rates a 1x3 ANOVA was used (motor unit discharge rates | age x muscle x contraction intensity). When statistical significance was found ( $P < 0.05$ ) a TukeyHSD post-hoc was used to determine differences within the interactions. Multiple linear regression analyses were used to determine relationships between voluntary contraction intensity and motor neuron discharge rates for each age and muscle group. Effect sizes (Cohen's d) were calculated to assess the influences of age and muscle on discharge rates. Numerical outcomes are reported as means  $\pm$  standard deviation.

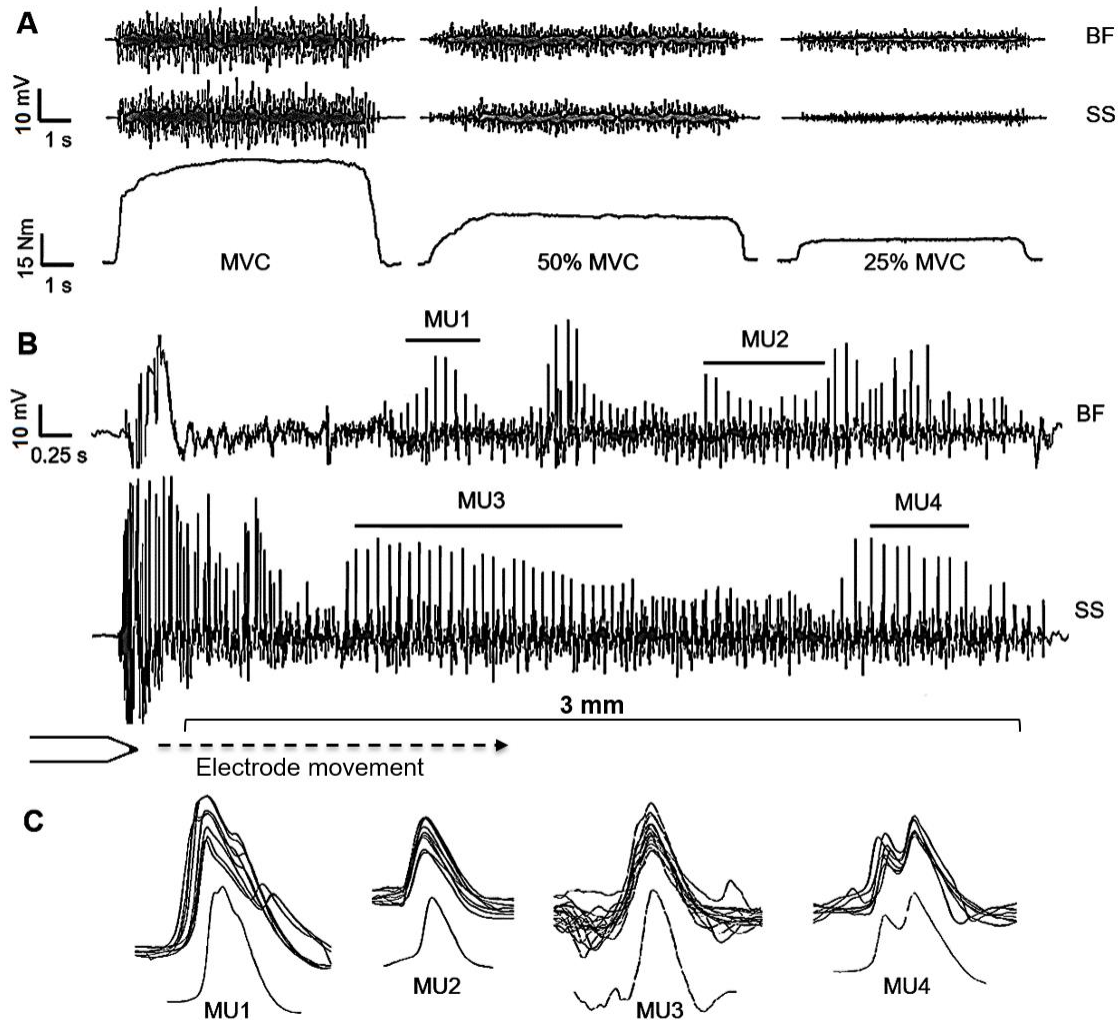


Figure 3.

Raw data from an old participant (78 y) who had the fastest observed discharge rates. (A) Surface electromyogram of the biceps femoris (BF) and semitendinosus-semimembranosus (SS) muscles and torque tracings at 100, 50 and 25 % of maximal voluntary contraction. (B) An expanded view of unprocessed intramuscular electromyogram collected during an independent maximal voluntary contraction event using tungsten electrodes individually inserted into the BF and SS. (C) Examples of action potential shape and overlay of all action potentials from four identified motor unit (MU) trains: MU1 is an overlay of 7 action potentials with a coefficient of variation 12.8, discharging at 21.6 Hz. MU2 is an overlay of 10 action potentials with a coefficient of

variation 9.4, discharging at 17.8 Hz. MU3 is an overlay of 25 action potentials with a coefficient of variation 9.1, discharging at 19.3 Hz. MU4 is an overlay of 8 action potentials with a coefficient of variation 12.6, discharging at 14.9 Hz.

## 2.3 Results

### *Anthropometric, strength and voluntary activation*

Age, height, body mass and maximal voluntary strength are presented in Table 1. The old exhibited >50 % weaker knee flexion and extension strength than the young ( $P < 0.05$ ), despite no statistically significant differences in body mass. In the prone position, both groups were equally capable of targeting the various contraction intensities (25, 50 and 100 %) relative to MVC in the young ( $23.0 \pm 3.7$ ,  $45.9 \pm 5.0$  and  $93.3 \pm 4.1$  %) and old ( $25.5 \pm 5.9$ ,  $46.9 \pm 7.26$  and  $94.0 \pm 3.7$  %). During knee flexion MVCs the old had a lower voluntary activation ( $P < 0.05$ ) as compared to the young (Table 2).

Table 1. Anthropometric and maximal voluntary isometric contractions

Anthropometric			Young (n = 11)	Old (n = 10)
Age (y)			$26 \pm 4$	$80 \pm 5$ *
Height (m)			$1.82 \pm 0.04$	$1.72 \pm 0.03$ *
Body mass (kg)			$82.9 \pm 9.3$	$76.4 \pm 9.2$
Maximal voluntary contraction (Nm)				
Seated	Knee joint 90°	Flexion	$101.3 \pm 24.2$	$47.4 \pm 8.60$ *
		Extension	$320.1 \pm 85.4$	$118.7 \pm 16.9$ *
	Knee joint 160°	Flexion	$172.3 \pm 37.3$	$84.7 \pm 15.8$ *
		Extension	$106.3 \pm 19.1$	$49.2 \pm 17.0$ *
Prone		Flexion	$86.3 \pm 16.5$	$43.0 \pm 9.6$ *

Values are presented as mean  $\pm$  standard deviation. \* denotes ( $P < 0.05$ ).

*Stimulated contractile properties*

In the seated position, resting evoked peak twitches in the old were significantly lower ( $P < 0.05$ ) as compared to the young (Table 2). For the evoked twitch, the time to peak tensions, one-half relaxation and contraction duration were 165-196 % longer ( $P < 0.05$ ) in the old as compared to the young (Table 2). In addition, the post-activation potentiated resting twitch was 46 % lower ( $P < 0.05$ ) in the old (Table 2). When the hamstrings were stimulated at incremental frequencies (1 to 100 Hz), the old reached fused tetanus at lower frequencies of stimulation than the young (Figure 4). The average stimulus frequency required to reach half-maximum tetanic force was 15 Hz for the old and 18 Hz for the young. Both the young and old achieved maximum fused tetanus between 40 and 50 Hz.

Table 2. Stimulated contractile properties of the hamstrings

Neuromuscular property	Young (n = 11)	Old (n = 10)
Voluntary activation (%)	98.7 ± 1.2	92.9 ± 3.2 *
Evoked peak twitch (Nm)	24.7 ± 8.0	11.7 ± 4.6 *
Time to peak tension (ms)	95.8 ± 41.6	159 ± 53.3 *
Rate of torque development (Nm•s <sup>-1</sup> )	350.9 ± 317.1	84.0 ± 48.7 *
One-half relaxation time (ms)	85.6 ± 50.8	167.7 ± 63.1 *
Contraction duration (ms)	181.5 ± 64.6	327.1 ± 75.8 *
Potentiated peak twitch (Nm)	28.1 ± 6.6	15.1 ± 5.2 *

Values are presented as mean ± standard deviation. \* denotes ( $P < 0.05$ ).

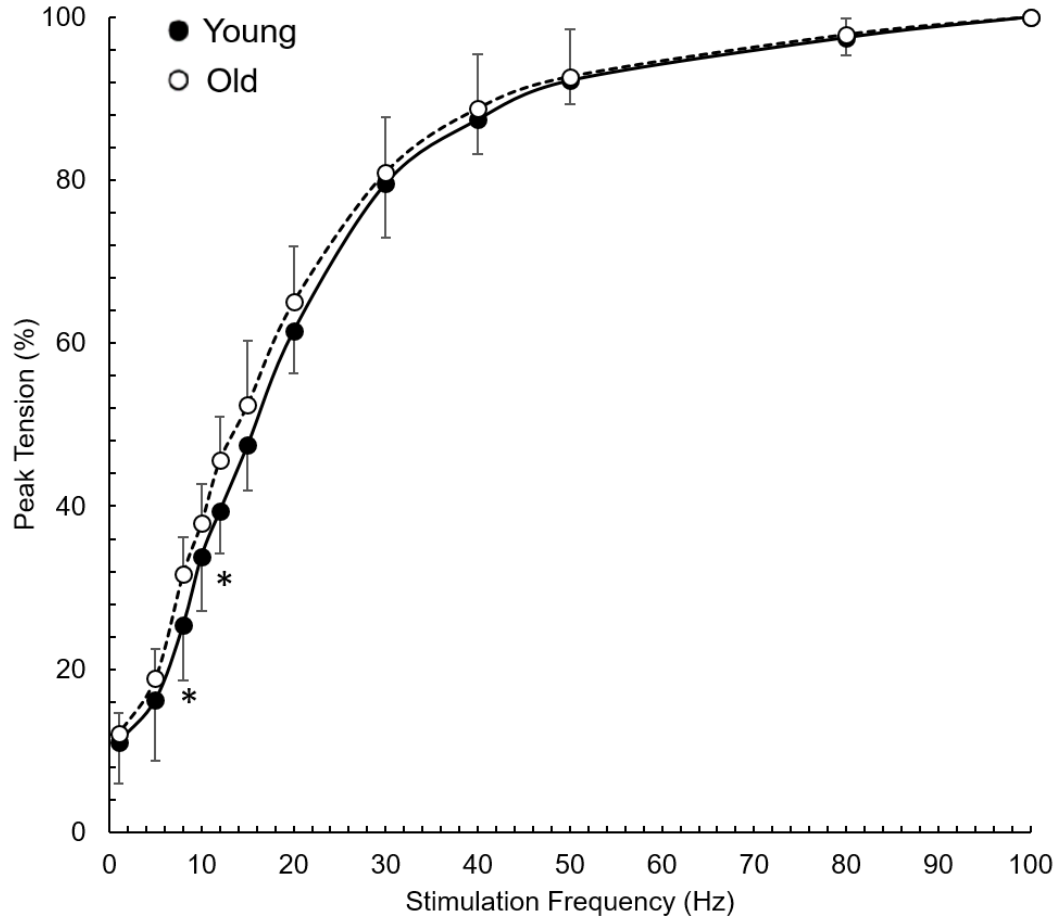


Figure 4.

Stimulated force-frequency relationship of the hamstrings in the seated position. Peak tetanic tension was normalized to the maximal peak tension of each participant at 100 Hz stimulation. Mean maximal peak tension achieved in the young and old relative to percent maximal voluntary contraction was  $30.0 \pm 5.2$  and  $35.1 \pm 8.6$  %, respectively. Error bars are standard deviation. \* denotes ( $P < 0.05$ );  $n = 21$ .



### *Surface electromyography*

In the prone position, surface and intramuscular EMG were sampled simultaneously (Table 3). For sEMG 25 and 50 % MVC were normalized to the 100% MVC for the BF and SS separately. There was a between-group interaction for age ( $P < 0.05$ ) and contraction intensity ( $P < 0.01$ ). There was a significant difference between 25 and 50 % MVC within the BF and SS for each age group ( $P < 0.01$ ).

Table 3. Surface EMG properties of the hamstrings

Muscle	MVC (%)	RMS (%)	
		Young (n = 10)	Old (n = 10)
SS	25	21.7 ± 5.7	29.4 ± 7.9
	50	40.8 ± 10.3	50.0 ± 11.9
BF	25	24.9 ± 10.8	24.1 ± 9.6
	50	45.6 ± 13.3	51.2 ± 12.9

Surface electromyogram (EMG) of the hamstrings. Root mean square (RMS) values are normalized to the RMS amplitude of the maximal voluntary contraction for knee flexion. There was a between-group interaction for the young and old ( $P < 0.05$ ). For each muscle at 25 and 50 % maximal voluntary contraction there was an interaction within age groups ( $P < 0.01$ ); n = 20. Values are presented as mean ± standard deviation.

### *Motor unit discharge rates*

A total of 2613 motor unit spike trains were separately identified with 1196 from the young and 1378 from the old. In the young, 567 motor unit trains were identified from the BF and 629 identified from the SS. In the old, 681 motor unit trains were identified in the BF and 736 identified from the SS (Figure 5). When sorted into three bins of 25, 50 and 100 % relative to MVC, the number of trains in the young was 225, 213 and 93 in the BF, and 307, 211 and 76 in the SS, respectively. In the old, the number of trains was 293, 173 and 131 in the BF, and 327, 173 and 125 in the SS. For all motor unit trains in the young, the range of inter-spike intervals defining a train was 4-88 spikes with a mean of  $9.4 \pm 7.4$ . The coefficient of variation for inter-spike intervals ranged from 0.8-29.8 with the mean being  $14.2 \pm 5.8$ , and discharge rate frequencies were between 4.5-76.2 Hz. The contribution by each young participant to the sum of collected motor unit trains was  $10.2 \pm 3.1$  %. For all motor unit trains in the old, the range of inter-spike intervals defining a train was 4-88 with a mean  $9.3 \pm 6.7$ . The coefficient of variation for inter-spike intervals ranged from 0.8-29.6 with the mean  $11.9 \pm 5.2$ , and discharge rate frequencies were between 4.1-45.2 Hz. The contribution by each old participant to the sum of collected motor unit trains was  $10.0 \pm 3.2$  %.

The normalized distribution of all collected motor unit trains had a positive skew of discharging rate frequencies in the motor unit population from the old compared with the young. In the BF, the percentage of individual motor unit discharge rates <15 Hz was 20.4 and 25.7 % in the young and old, respectively, whereas in the SS, the percentage of individual motor unit discharge rates <15 Hz was 11.9 and 28.5 % in the young and old, respectively (Figure 7). To compare the relationship between increasing motor unit discharging rates and increasing contraction intensity influenced by age and muscle group, the data set was modelled by linear regression (Figure 5). There was no interaction for muscle group ( $P = 0.15$ ) and significant interactions of age ( $P < 0.001$ ) and contraction intensity ( $P < 0.001$ ) on discharge rates. For each study participant, all collected motor unit discharge rates are modelled against contraction intensity in the BF and SS by linear regression (Figure 6).

Motor unit discharge rates were binned into three target contraction intensities normalized to the MVC to generate a mean discharge rate value for comparisons (Figure 8). In the BF, mean discharge rates in the old at 50 and 100 % MVC were significantly lower than the young ( $P < 0.001$ ). Mean discharge rates in the old were  $12.8 \pm 4.0$  and  $15.6 \pm 6.4$  Hz as compared to  $16.1 \pm 8.6$  and  $26.1 \pm 10.1$  Hz in the young at contraction intensities of 50 and 100 % MVC, respectively. In the SS, mean discharge rates in the old at 25, 50 and 100 % MVC were significantly lower than the young ( $P < 0.001$ ). Mean discharge rates in the old were  $10.5 \pm 3.2$ ,  $11.2 \pm 3.3$  and  $15.3 \pm 5.9$  Hz as compared to  $15.6 \pm 6.3$ ,  $20.3 \pm 7.8$  and  $27.9 \pm 7.8$  Hz in the young at contraction intensities of 25, 50 and 100 % MVC, respectively. Furthermore, mean discharge rates within muscles of the young were significantly greater in the SS as compared to the BF at contraction intensities of 25 and 50 % MVC ( $P < 0.001$ ). However, there was no muscle by age interaction in mean discharge rates within the old group. To explore whether age-related differences in discharge rates were larger for the BF or SS, effect sizes were calculated at each contraction intensities (Figure 8). The effect size was greater in the SS at all contraction intensities (Cohen's  $d$  1.01, 1.52 and 1.82 at 25, 50 and 100% MVC, respectively) as compared to the BF (Cohen's  $d$  0.14, 0.49 and 1.24 at 25, 50 and 100% MVC, respectively).

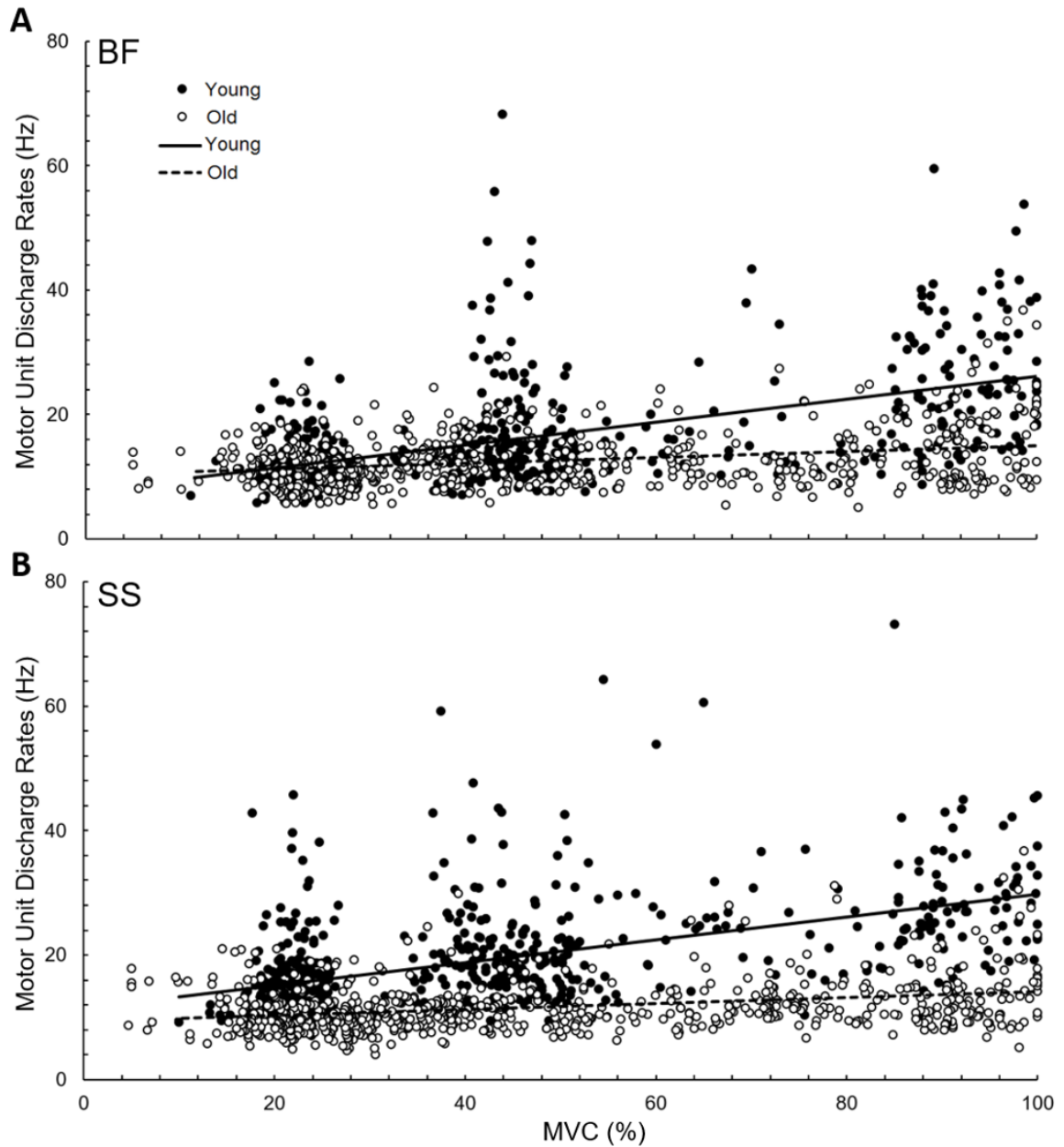


Figure 5.

Scatterplots of normalized voluntary contraction levels of 2613 individual motor unit discharging rates with 1196 (young) and 1378 (old). A) Scatterplot of 1248 motor unit trains in the biceps femoris (BF) between young and old. Linear regression equations for young and old are:  $\text{discharge rate}_{(\text{Hz})} = 0.18 \times \text{torque}_{(\text{MVC } \%)} + 7.76$ ,  $R^2 = 0.29$  [solid line];  $\text{discharge rate}_{(\text{Hz})} = 0.05 \times \text{torque}_{(\text{MVC } \%)} + 10.28$ ,  $R^2 = 0.09$  [dashed line], respectively. B) Scatterplot of 1365 motor unit trains in the semimembranosus-semi-tendinosus (SS)

between young and old. Linear regression equations for young and old are: discharge rate<sub>(Hz)</sub> = 0.18 x torque<sub>(MVC %)</sub> + 11.48, R<sup>2</sup> = 0.28 [solid line]; discharge rate<sub>(Hz)</sub> = 0.05 x torque<sub>(MVC %)</sub> + 9.34, R<sup>2</sup> = 0.09 [dashed line], respectively; n = 20.

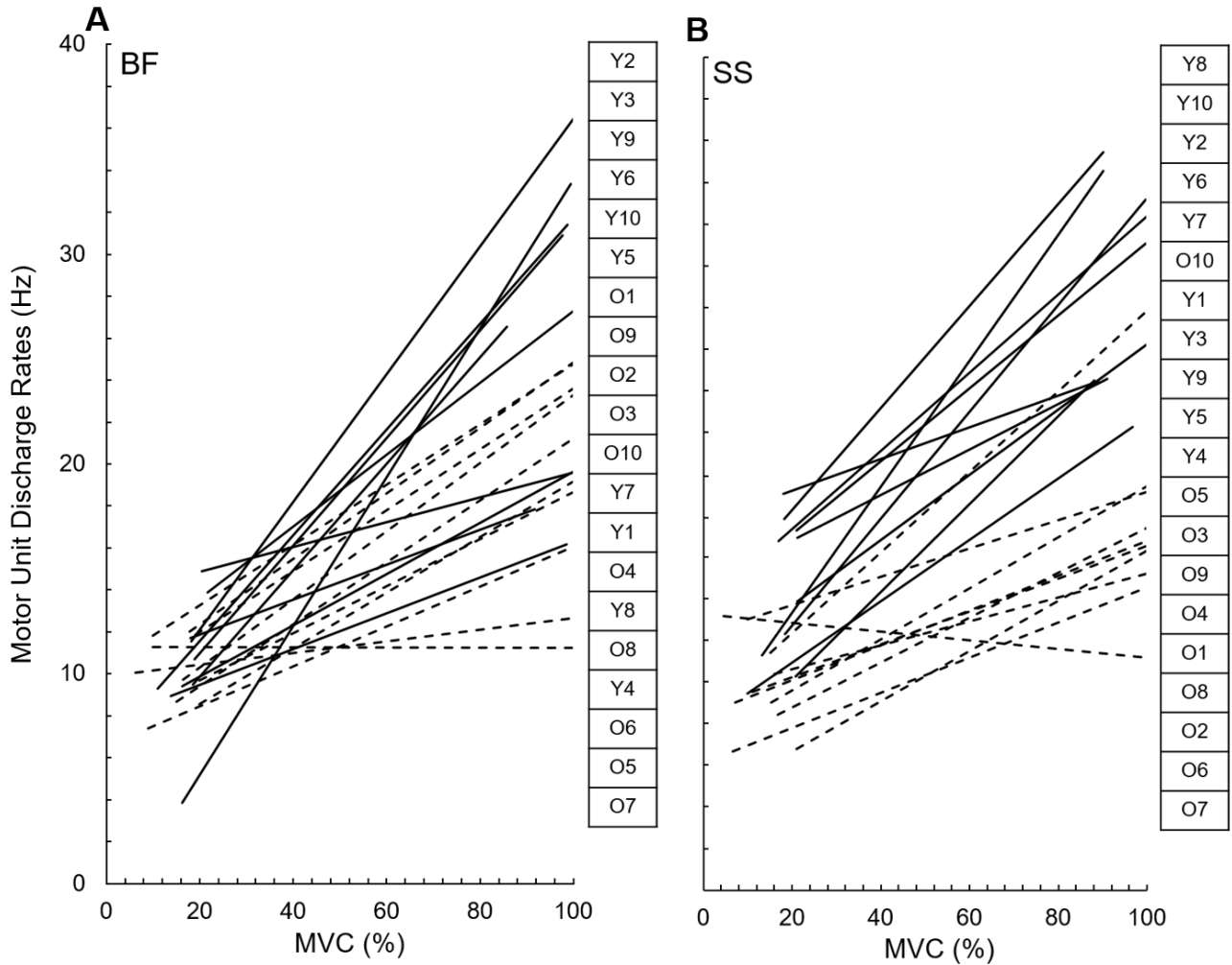


Figure 6.

Linear regression lines modelled for each study participant in the A) biceps femoris and B) semimembranosus-semi-tendinosus. Each regression line represents multiple different motor units collected at different target contraction intensities. Solid lines represent young and dashed lines represent old. For alpha-numeric notation of the within figure table, Y denotes young, and O denotes old, in the same order as the regression lines from top to bottom; n = 20.

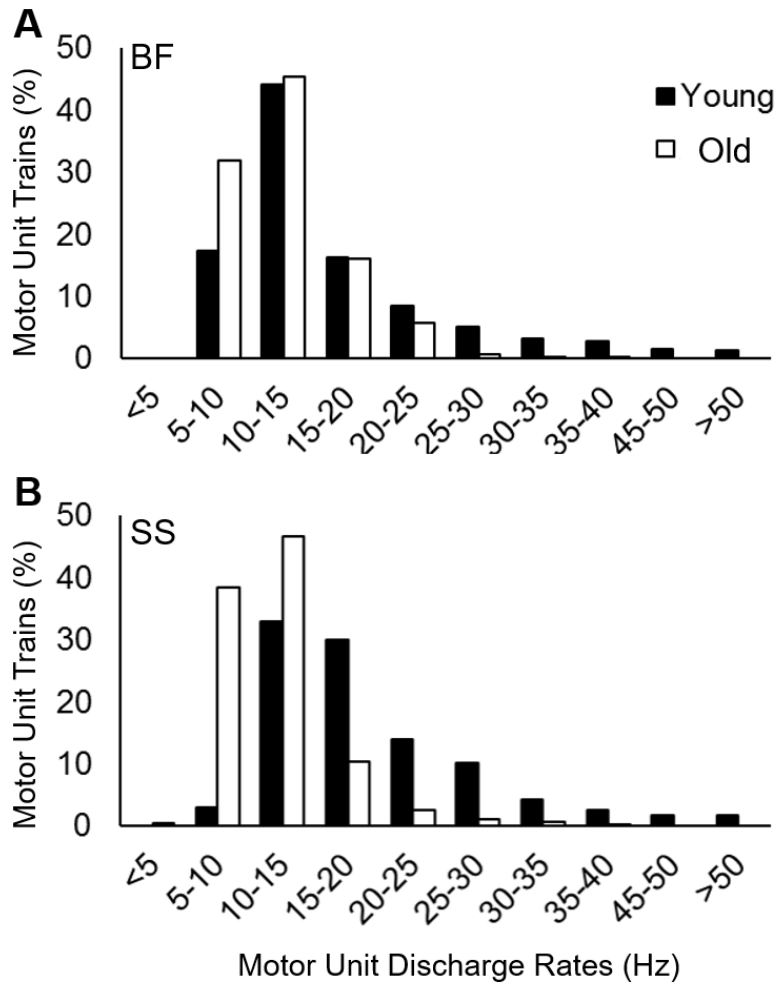


Figure 7.

Histograms of the distribution of motor unit discharge rates from all voluntary contraction levels in young and old in the (A) biceps femoris and (B) semimembranosus-semitendinosus muscle groups. Percentage of total motor unit trains are sorted into 5 Hz bins;  $n = 20$ .

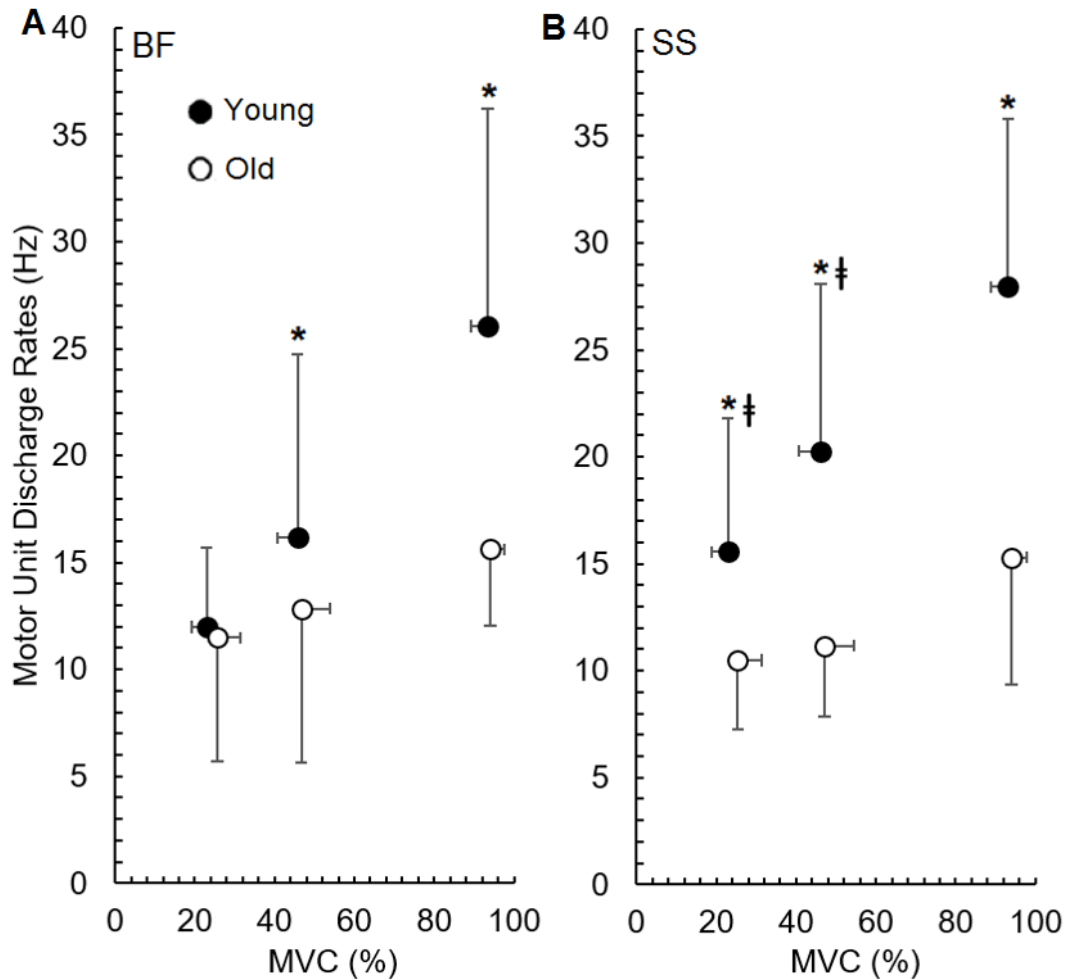


Figure 8.

Mean motor unit discharge rates from the young and old in the biceps femoris (BF) and semimembranosus-semi-tendinosus (SS) at each voluntary contraction level in panels A and B, respectively. Mean motor unit discharge rates represent a total of 2344 MU trains with 531 (young) and 597 (old) in the BF, and 594 (young) and 622 (old) in the SS.

Discharge rates increased with voluntary contraction in both muscle groups ( $P < 0.001$ );  $n = 20$ . Values are mean with error bars representing standard deviation. \* denotes an interaction between young and old within the same muscle ( $P < 0.001$ ), ‡ denotes an interaction between muscle groups of the same age group ( $P < 0.001$ ).



## Chapter 3

### 3 Discussion and summary

#### 3.1 Discussion

This thesis reports a comprehensive evaluation of age-related neuromuscular properties in human hamstrings. Importantly, the depiction of age-related changes of voluntary activation, stimulated contractile properties and motor unit discharge rates in major human limb muscles has been advanced (Connelly *et al.*, 1999; Roos *et al.*, 1999; Kamen & Knight, 2004; Dalton *et al.*, 2009, 2010; Kirk *et al.*, 2016). Compared to other muscles reported to date, the hamstrings show the greatest age-related reductions in motor unit discharge rates at MVC (Figure 8). These findings support that not all neuronal populations are equally affected during the ageing process. When compared with observations from other limb muscles, results from this investigation support that discharge rates of motor units controlling flexor muscle groups (as determined by embryological origin) show greater differences due to the ageing process than do extensor muscle groups.

At the muscle, when electrically stimulated, evoked twitches in the hamstrings of the old were lower in force and slower to generate tension, with the evoked peak twitch force being 47 % in the old as compared to young and with 165-196 % slower stimulated tension (Table 2, Figure 4). There are numerous muscle specific factors related to ageing, including: motor unit remodeling, muscle fibre membrane function, sarcoplasmic reticulum function, calcium ion kinetics and structural elements influencing contractile twitch properties that are likely implicated in explaining the observed differences (Narici *et al.*, 2008; Hepple & Rice, 2016).

Voluntary strength is lower in muscles of aged humans due to numerous factors (Clark & Taylor, 2011; Mitchell *et al.*, 2012), and in the present study old men were 50% as strong (Table 1) with lower voluntary activation ( $92.9 \pm 1.2$  %) as compared with the young ( $98.7 \pm 1.2$  %). Age-related reductions in voluntary activation are variable (Klass *et al.*,

2007) and have been extensively reported in the elbow flexors, elbow extensors, knee extensors, plantar flexors and dorsiflexors. There are broad technical and muscle specific limitations of the interpolated twitch technique in assessing supraspinal versus spinal mechanisms (Gandevia, 2001), and it is hard to delineate where the present limitation occurred in the hamstrings of the older men. However, from prior investigations, it is known that voluntary activation in the old is less consistent across trials (Jakobi & Rice, 2002) and the cause of variable performance may be supraspinal (Hunter *et al.*, 2008). In almost all muscles there is a correlation of reduced force capacity with ageing, and in some muscles like the tibialis anterior that have significant reductions in force, the majority of investigations report no difference in voluntary activation between young and old (Klass *et al.*, 2007). Voluntary activation in hamstrings of the old men was significantly lower when compared with young, however, it remains unknown where within the neuromuscular system and to what extent the motor unit population is affected.

In the lower limb, motor unit discharge rates of flexor muscles (hamstrings and tibialis anterior) have significant age-related reductions. Specifically, at MVC, discharge rates in the hamstrings and tibialis anterior were 40-45 % and 26 % lower in the old compared with young, respectively (Connelly *et al.*, 1999). However, in the vastus medialis, soleus and gastrocnemii, which had comparable strength reductions (up to 50% in the old) there were no significant age-related differences in mean motor unit discharge rates at maximal contraction intensities (Roos *et al.*, 1999; Dalton *et al.*, 2009; Kirk *et al.*, 2016). For extensor muscles in the lower limb, motor unit discharge rates can also be increased with exercise. In the vastus lateralis of untrained individuals, initial motor unit discharge rates in the old were significantly lower than those for the young, but over the course of a 6 month isometric training intervention, discharge rates in the old were increased to the starting level of the young (Kamen & Knight, 2004). At the end of the training intervention, discharge rates at MVC were increased by 49 and 15 % in the old and young, respectively. A greater increase in the old, was thought to implicate an acute mechanism of increased facilitation to the motor pool observed to occur in an extensor muscle. Like the lower limb, muscles of the upper limb also have age-related reductions in strength. In an investigation comparing strength, contractile properties, voluntary activation and motor unit discharge rates of the triceps brachii and biceps brachii

muscles, MVC was shown to be up to 40 % less with 33 % lower discharge rates at MVC in the old. However, unlike the lower limb, significant reductions in discharge rates were observed in an extensor muscle at MVC, despite this, the biceps brachii had greater age-related differences than the triceps brachii within the same individuals (Dalton *et al.*, 2010). Therefore, although with large differences in strength among various muscles there is no clear relationship with age-related changes in motor unit discharge rates. Indeed, within the hamstring group, motor unit discharge rates have a greater age-related difference in the SS as compared to the BF (Figure 6 and Figure 8), highlighting that despite similar sEMG results (Table 3), there seems to be differential age-related influence on neural drive between the medial and lateral muscles of the hamstrings. However, motor unit discharge rate reductions within muscles from aged people do not seem to follow a preference for medial or lateral compartments, because in the medial and lateral gastrocnemii both were able to achieve comparable firing rates and with no age-related reductions (Kirk *et al.*, 2016).

In humans, age-related reductions in contractile force are related to decreases in composition of both type I and II fibres (Yu *et al.*, 2007). From *in vitro* animal models, the contractile speed of muscle fibres match motor unit discharge rates (Kernell, 1979), however, this matching was not shown to associate to muscles of different fibre types in young humans (Bellemare *et al.*, 1983). In ageing, in the tibialis anterior, there was a reported match of a 26 % reduction in motor unit discharge rates at MVC corresponding to contractile slowing, thus it was proposed that the matching concept of slowing of contractile speed might be related to lower discharge rates with adult ageing (Connelly *et al.*, 1999). However, this relationship of muscle contractile changes in ageing did not associate with motor unit discharge rates in limb muscles (Dalton *et al.*, 2010) indicating other mechanisms were involved. In the thigh, both the anterior and posterior compartments show significant reductions in stimulated contractile speed when old are compared with young men (Roos *et al.*, 1999). Although fibre type distributions are not substantially different between the quadriceps and hamstrings at approximately 50 % Type I (Johnson *et al.*, 1973; Garrett *et al.*, 1984), the hamstrings have significantly lower discharge rates in old men compared with no observed changes in medial quadriceps (Roos *et al.*, 1999). Unlike muscles of the thigh, leg muscles have large proportions of

type I fibres in the tibialis anterior (~70 %), soleus (~80 %) and gastrocnemii (~50 %) (Johnson *et al.*, 1973), however only in the tibialis anterior (dorsiflexor) were age-related reductions of motor unit discharge rates found (Connelly *et al.*, 1999; Dalton *et al.*, 2009; Kirk *et al.*, 2016). The addition of hamstrings motor unit discharge rates supports that an age-related speed matching concept is likely an oversimplification and that other factors are involved (Hepple & Rice, 2016).

In electrophysiological techniques used to estimate motor unit numbers, there are significant age-related reductions in the motor unit populations by as much as 70 % in very old age (Campbell *et al.*, 1973; McNeil *et al.*, 2005). These findings are in agreement with the age-related decline of the spinal motor neuron population (Tomlinson & Irving, 1977). In ageing human limb muscles, motor unit number estimates have been used to quantify viable motor units in the tibialis anterior (McNeil *et al.*, 2005; Power *et al.*, 2010), soleus (Dalton *et al.*, 2008), vastus lateralis (Piasecki *et al.*, 2016) and biceps brachii (Doherty *et al.*, 1993a; Power *et al.*, 2012) revealing that with ageing, there is muscle specific spinal motor neuron loss. Therefore, it is highly likely that the BF and SS also undergo age-related motor neuron loss, although this has not been quantified.

Regardless of age-related motor neuron degradation, motor unit discharge rates are affected by numerous intrinsic motor neuron properties coordinating with excitatory and inhibitory factors (Heckman & Enoka, 2012). By comparing these differences in motor unit discharge rates among muscles it becomes more apparent, here with the addition of the hamstrings, that extensor muscles (quadriceps, triceps surae and triceps brachii) have comparatively fewer age-related deficits in discharge rates at MVC (Roos *et al.*, 1999; Dalton *et al.*, 2009, 2010; Kirk *et al.*, 2016) and have the capacity to be increased (Kamen & Knight, 2004). In the vastus medialis mean motor unit discharge rates at all contraction intensities had non-significant differences between young and old, with MVC mean motor unit discharge rates at  $26.4 \pm 7.6$  Hz in the young and  $25.5 \pm 7.2$  in the old (Roos *et al.*, 1999). Whereas in the vastus lateralis after a 6 week training intervention, mean motor unit discharge rates of the old matched initial mean discharge rates of the young group at ~ 27 Hz (Kamen & Knight, 2004). In addition, the old group had greater increase in motor unit discharge rates throughout the training intervention (Kamen &

Knight, 2004). In the triceps surae, mean discharge rates at 75 and 100 % MVC in the soleus, and at 25, 50, 75 and 100 % MVC in the medial and lateral gastrocnemii had non-significant age-related differences. At MVC, mean discharge rates in the soleus were  $16.5 \pm 6$  Hz in the young and old (Dalton *et al.*, 2009), and in the medial gastrocnemius were  $21.8 \pm 8.0$  and  $20.4 \pm 9.8$  Hz, and in the lateral gastrocnemius were  $23.5 \pm 8.8$  and  $21.4 \pm 9.5$  Hz, in the young and old, respectively (Kirk *et al.*, 2016). In contrast to extensors, flexor muscles (biceps brachii, tibialis anterior, BF and SS) exhibit significant (Connelly *et al.*, 1999; Dalton *et al.*, 2010) and comparatively greater (Dalton *et al.*, 2010) age-related reductions in motor unit discharge rates. At MVC, mean motor unit discharge rates in the tibialis anterior were  $42 \pm 7$  and  $31 \pm 8.5$  Hz in the young and old, respectively. And in the upper limb comparing the biceps brachii and triceps brachii within the same individual, mean motor unit discharge rates were 38 and 42 Hz in the young, respectively, and 28 Hz for each muscle in the old. In combining motor unit discharge rates from all force levels for each muscle and age group, the effect size was greater in the biceps brachii (Cohen's  $d$  8.25) as compared to the triceps brachii (Cohen's  $d$  4.79) (Dalton *et al.*, 2010). From previous outcomes of investigated limb muscles and now with addition of the hamstrings, limb flexors present greater age-related reductions in motor unit discharge rates as compared to extensors.

Developmentally, the hypaxial limb bud contains ventral primary rami which innervate the ventral (flexor) and dorsal (extensor) compartments (Sadler & Langman, 2012). Descending and ascending innervation characteristics are different between flexors and extensors, although there is limited data. In humans, in descending pathways to the motor neuron, flexors are shown to have a greater monosynaptic component to the spinal motor neuron (Brouwer & Ashby, 1990, 1991; Capaday *et al.*, 1999; Petersen *et al.*, 2002) and extensors have greater polysynaptic drive (Cowan *et al.*, 1986). In using cortical magnetic stimulation, the duration of latency (such as the measure of short latency) is attributed to projections of the fast corticospinal pathway inferring monosynaptic projections to motor neurons, whereas the strength of facilitation is considered to reflect the density of corticospinal projections to the motor neuron pool (Brouwer & Ashby, 1990, 1991). With experimental arrangements using cortical magnetic stimulation, the corticospinal tract is more closely linked with segmented motor circuits controlling the

tibialis anterior (flexor) as compared to the soleus (extensor) during motor bursts within the step cycle (Capaday *et al.*, 1999). Furthermore, the tibialis anterior has stronger short latency as compared to the triceps surae (extensor) (Brouwer & Ashby, 1991). Similarly, the biceps brachii (flexor) contains a large excitatory input with a dominant efferent monosynaptic component to the spinal motor neuron (Petersen *et al.*, 2002). Evidence from cat and primate models also found strong facilitation to flexors and inhibition to extensors of the hip and thigh muscles (Uemura & Preston, 1965). In humans, if flexors and extensors of the thigh follow the same innervation pattern as other flexors and extensors, these innate differences may provide important neurophysiological insight to help explain differences among muscles for ageing motor units.

In addition to outcomes based on neuromuscular innervation characteristics, age-related differences could be affected at the cortical and spinal levels by chronic activity (Papegaaij *et al.*, 2014), which likely has an important role in attenuating neuromuscular ageing decline. For example, masters athletes (>65 y) have better preservation of motor unit numbers and stability in flexor muscles of the tibialis anterior and biceps brachii when compared to age-matched controls. Such life-long chronic activity could provide neuroprotection, contributing to more robust motor units (Power *et al.*, 2010, 2016a).

## 3.2 Conclusions

In the hamstrings, we report that contractile properties and motor unit discharge rates are lower in old compared with young, with the hamstrings presenting the greatest age-related reduction in motor unit discharge rates of any muscle tested to date. With the addition of the hamstrings, these findings help advance a depiction of neuromuscular ageing in all major limb muscle groups, indicating that not all motor unit populations are equally affected by the ageing process.

### 3.3 Limitations

Although the hamstrings model provides insight to explore age-related changes in neural drive to the largest limb flexor of the body during high intensity voluntary contractions, there are limitations associated with this investigation. These limitations are classified into the anatomical, technical and electrophysiological.

Anatomically, the hamstrings are a two-joint muscle that acts to extend the thigh and flex the knee. Using the tungsten microelectrode technique, we were only able to test isometric voluntary activation of the hamstrings during knee flexion in a thigh-extended position. In dynamic movements at high intensity contractions the muscle movement would displace the needle and cause pain to the participant. Secondly, we are only able to measure one volitional aspect of the hamstrings which is a static contraction, we might hypothesize that if done dynamically, motor unit discharge rates would increase by 2-3 times the current rate (Harwood *et al.*, 2011). And lastly, insertion locations of the microelectrodes were limited to one main region of these large muscles. To assess inter-muscle differences within the hamstrings, the medial and lateral aspects of the posterior thigh were chosen. This however, due to the anatomical proximity between the semimembranosus and semitendinosus made it uncertain to assess which muscle the microelectrode was in given our current methodology. Instead, we chose to denote that the semimembranosus and semitendinosus are instead similar and therefore compared them against the long head of the biceps femoris. The biceps femoris itself has a long and short head, with the long head of the muscle crossing two joints and the short head acting to only flex the knee. To compare the biceps femoris against the semimembranosus and semitendinosus muscles, we chose to compare the long head, so that all muscles being assessed through the microelectrode technique act over two joints and are innervated by the same common nerve supply.

Technical limitations of how the tungsten microelectrode technique was used in this investigation relate to the population sampling concept which precludes: following the same motor unit during recruitment, discharge rate modulations and de-recruitment. These limitations are logical given the objective of the technique to record from as many

discrete motor units as possible during voluntary contractions, up to maximum intensity. In analysis, limitations exist in the possibility of erroneous classification by the algorithm or investigator, although a conservative and well-established technique was employed (Stashuk & Qu, 1996; Rich *et al.*, 1998).

Electrophysiological limitations are inherent in that with any electrode insertion you rely on recording motor units that are active near the electrode. Furthermore, with ageing, it allows us to make inferences on set factors within the neuromuscular system, which is a highly complex and continually diverging system.

### 3.4 Future directions

This investigation has helped further the depiction of age-related changes in neural drive to major human limb muscles. In contrast to the well-studied extensor muscles of the thigh, the hamstrings have important potential to be investigated in aspects of: fatigue, trainability, motor unit recruitment thresholds, synchronization, sex differences, dynamic voluntary contractions and spatial recruitment.

To further the hypothesis that motor unit discharge rates in flexor limb muscles have greater reductions in ageing, other muscles should be characterized in both longitudinal and cross-generational models. Muscles of the antebrachium, hip, torso and neck are virtually untouched electro-physiologically for these types of questions. Furthermore, assessing multiple muscles within individuals would provide even more insight.



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## Appendix

### Appendix A. Ethical Approval

	<b>Western Research</b>	Research Ethics
<b>Western University Health Science Research Ethics Board HSREB Annual Continuing Ethics Approval Notice</b>		
<b>Date:</b> February 24, 2017 <b>Principal Investigator:</b> Dr. Charles Rice <b>Department &amp; Institution:</b> Schulich School of Medicine and Dentistry\Anatomy & Cell Biology, Western University		
<b>Review Type:</b> Full Board <b>HSREB File Number:</b> 107505 <b>Study Title:</b> Motor neuron and muscle fiber resilience in humans <b>Sponsor:</b> Natural Sciences and Engineering Research Council		
<b>HSREB Renewal Due Date &amp; HSREB Expiry Date:</b> Renewal Due -2018/02/28 Expiry Date -2018/03/07		
<p>The Western University Health Science Research Ethics Board (HSREB) has reviewed the Continuing Ethics Review (CER) Form and is re-issuing approval for the above noted study.</p> <p>The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice (ICH E6 R1), the Ontario Freedom of Information and Protection of Privacy Act (FIPPA, 1990), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.</p> <p>Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.</p> <p>The HSREB is registered with the U.S. Department of Health &amp; Human Services under the IRB registration number IRB 00000940.</p>		
_____ _____, on behalf of Dr. Joseph Gilbert, HSREB Chair		
EO: Erika Basile ___ Nicole Kaniki ___ Grace Kelly ___ Katelyn Harris ___ Nicola Morphet ___ Karen Gopaul ___		



## Curriculum vitae

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### Education

The University of Western Ontario

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BSc - honors specialization in genetics

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Teaching Assistant - The University of Western Ontario

1. 2015 and 2016, muscle function and metabolism 4430F
2. 2017, exercise biochemistry 3360B

### Peer reviewed publications

1. **Kirk, E.A.**, Rice, C.L. (2016) Contractile functions and motor unit firing rates of the human hamstring. *Journal of Neurophysiology*. 1:243-250. PMID: 27784806
2. **Kirk, E.A.**, Moore, C.W., Chater-Diehl, E.J., Singh, S.M., Rice, C.L. (2016) Human COL5A1 polymorphisms and quadriceps muscle-tendon mechanical stiffness in vivo. *Experimental Physiology*. 12:1581-1592. PMID: 27717059
3. **Kirk, E.A.**, Copithorne, D.B., Dalton, B.H., Rice, C.L. (2016) Motor unit firing rates of the gastrocnemii during maximal and sub-maximal isometric contractions in young and old men. *Neuroscience*. 330:376-385. PMID: 27298006

### Peer reviewed contributions

1. **Kirk, E.A.**, Rice, C.L. (2016) Characterization of neuromuscular properties of the human hamstring muscle group at two knee-joint angles. (POSTER PRESENTATION) October 13-2016, *Appl. Physiol. Nutr. & Metab.* 41:9 (Suppl. 4): S366. Canadian Society for Exercise Physiology annual meeting, Victoria, BC, Canada.