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Maximal motor unit discharge rates of the medial and lateral gastrocnemeii of young males

Mitchell T. Graham, *The University of Western Ontario*

Supervisor: Dr. Charles L. Rice, *The University of Western Ontario*
A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Kinesiology

MAXIMAL MOTOR UNIT DISCHARGE RATES OF THE MEDIAL AND LATERAL GASTROCNEMEII OF YOUNG ADULT MALES

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by

Mitchell T. Graham

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ABSTRACT

The triceps surae is composed of the mono-articular soleus and the bi-articular gastrocnemeii. Mean maximal motor unit discharge rates (MUDRs) reported for the soleus (~16Hz) are lower than other limb muscles tested (Dalton et al., 2009). Because of differences in fibre-type and functional anatomy it is important to determine maximal MUDRs in the two heads of gastrocnemeii, as compared with the soleus, to fully understand the interplay of these three muscles for plantar flexion. The purpose of the study was to record maximal MUDRs of the medial (MG) and lateral gastrocnemeii (LG) in 9 recreationally active, young men (age 24.2 ± 1.6y; 81.3 ± 8.1kg; 180.3 ± 5.3cm). During 3 separate visits to the lab, participants performed a series of 6-8, 7s maximal voluntary isometric contractions (MVC) of the plantar flexors with a knee joint angle of 90 degrees, with 5 minutes of rest between contractions. Maximum voluntary activation of the plantar flexors using the interpolated twitch technique was calculated. Custom tungsten microelectrodes were inserted individually into the belly of the lateral and medial gastrocnemeii and gently manipulated during the contractions to sample from as many distinct motor units throughout the muscle as possible. Action potential trains were analyzed offline to calculate discharge rates for each identified motor unit. All subjects were capable of high % of voluntary activation (>96%) and achieved a mean maximal plantar flexor torque of 194.6 ± 57.1Nm. A total of 198 and 117 motor unit action potential trains were identified in the MG and LG, respectively. The mean maximal motor unit discharge rates were 22.7 ± 8.6Hz and 22.4 ± 8.1 Hz (Range: 5.5 - 64Hz) in the MG and LG, respectively, and were not significantly different from one another, $p > 0.05$. The coefficient of variation of discharge frequencies in the identified trains were 14.6 ± 1.4% and 14.3 ± 2.2% in the MG and LG respectively, and were not significantly different, $p > 0.05$. Maximal MUDRs in both heads of the gastrocnemeii are greater than in the soleus, but are not different from one another. Despite their similar roles in plantar flexion, the amount and degree of habitual activation (phasic vs. tonic) or their functional role (flexor vs. extensor) may account for motor unit discharge rate differences between the gastrocnemeii and soleus during plantar flexion actions.

Keywords: motor unit, muscle, isometric, torque, maximal

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LIST OF ABBREVIATIONS

ANOVA	-	Analysis of variance
CD	-	Contraction duration
CoV	-	Coefficient of Variation
EMG	-	Electromyography
FDI	-	First dorsal interosseus
HRT	-	Half-relaxation time
ITT	-	Interpolated twitch technique
LG	-	Lateral gastrocnemeii
L_F	-	Fascicle length
MG	-	Medial gastrocnemeii
MVC	-	Maximum voluntary contraction
MUNE	-	Motor unit number estimate
PA	-	Pennation angle
Pt	-	Peak twitch tension
RMS	-	Root mean square
RMSamp	-	Root mean square amplitude
RT	-	Recruitment threshold
SD	-	Standard deviation
ST	-	Slow-twitch muscle (Type I)
sEMG	-	Surface EMG
SOL	-	Soleus
TA	-	Tibialis anterior
TPT	-	Time-to-peak tension
VA	-	Voluntary activation

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MAXIMAL MOTOR UNIT DISCHARGE RATES OF THE MEDIAL AND LATERAL GASTROCNEMEII OF YOUNG ADULT MALES

CHAPTER 1: INTRODUCTION

1.1 TRICEPS SURAE

1.1.1 General

In the human body many muscle groups work synergistically to create distinct and purposeful movements of the body. Whereas there are several examples of synergistic muscle groups, the triceps surae is an important group and one that has in many ways been well-studied especially in relation to bipedal locomotion; a unique human characteristic. The triceps surae is comprised of 3 distinct muscles, crossing 2 joints, all acting through one common tendon, responsible for approximately 80% of total plantar flexion torque (15, 59). The soleus and the medial and lateral gastrocnemeii cross the ankle joint and insert on the posterior calcaneus via the calcaneal tendon. These muscles are innervated by the tibial nerve, a major branch of the sciatic nerve, from spinal root levels S1 and S2. The soleus is a mono-articular, multi-pennate muscle, and originates at the posterior aspect of the fibular head, the superior fourth of the posterior fibula and the medial tibial border. It is a habitually active muscle, and is imperative to both standing posture and gait (38). According to both cadaveric (37) and muscle biopsy studies (71), the soleus is almost entirely comprised of histochemically (~88%) slow-twitch muscle fibres. The two heads of the gastrocnemeii however, are bi-articular, unipennate, originating at the medial and lateral condyles of the femur, thus crossing both the knee and the ankle joints. Unlike its primarily slow-twitch triceps surae counterpart, the gastrocnemeii are composed of about 45-50% histochemically slow-twitch (Type I) muscle fibres, with the medial head being slightly slower in fibre-type composition (36, 37). Figure 1 illustrates the components of the triceps surae.

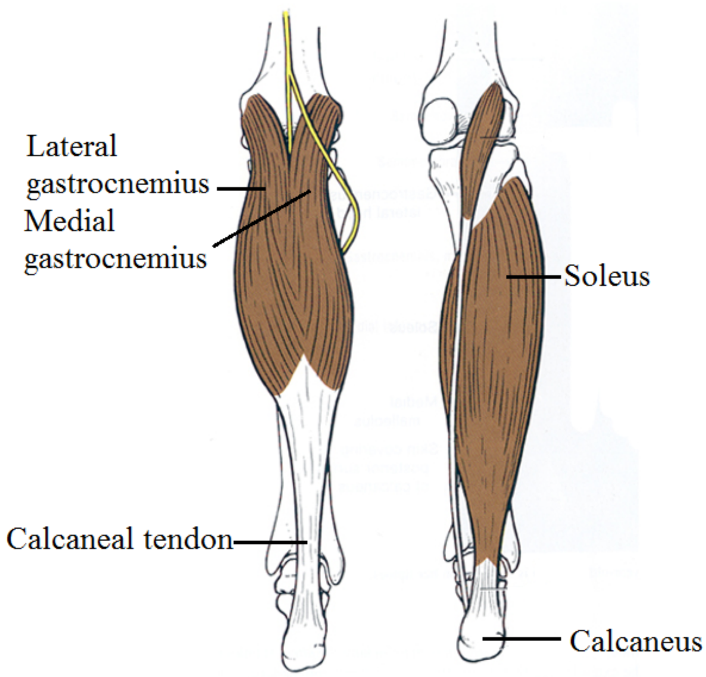

Figure 1. Triceps surae muscle arrangement.

1.1.2 Muscle Architecture and Length

Muscle architecture has been defined as "the arrangement of muscle fibers within a muscle relative to the axis of force generation" (50). Changes in muscle architecture will ultimately affect the force production of a muscle. Two critical measures that are often identified when explaining muscle architecture are fascicle length (L_F) and pennation angle (PA). Fascicle length is used as a proxy to represent a change in muscle fibre length, as a bundle of muscle fibres (known as a fascicle) usually extends from the proximal to distal tendon (43). In addition, pennation angle is the orientation of the muscle fibres (thus the fascicles) with respect to the force-generating axis. These two key architectural parameters can be mathematically combined to calculate the physiological cross sectional area (PCSA), the area of muscle mass perpendicular to the line of pull. PCSA is directly proportional to the force generating capabilities and is a better indicator than anatomical cross-sectional area which fails to consider a muscle's pennation angle (49).

Because the gastrocnemeii are bi-articular, their architecture, that is muscle length, fibre length and pennation angle, differ with changes in both knee and ankle joint angles. Using ultrasound technology, these measures can be found, at both rest and MVC and at differing joint angles. At rest, from a neutral ankle angle of 0°, the MG has a L_F of 52 ± 7mm with an extended knee, and this changes to 35 ± 5mm when the knee is flexed to 90°; a 32% decrease. During an MVC with an extended knee, the MG L_F shortens to 31 ± 5mm, but only decreases 16% to 26 ± 3mm if the knee is flexed to 90°. Thus, a change from a longer (extended knee) to a shorter (flexed knee) muscle length results in a 25% and 40% decrease in MG L_F at rest compared to MVC, respectively (43). However, Arampatzis et al. (2006) found that while there were differences in fascicle length at rest in both the extended and flexed knee positions, these differences became non-significant when tested at MVC (1). These findings however were in distance runners as opposed to non-athletes as in the Kawakami study (43), which may influence the results as runners may have training-related adaptations to their triceps surae architecture. Pennation angle for the MG at rest with a flexed knee was 39 ± 9° and was 62 ± 8°, at MVC; a 60% increase. The PA increased about 67% from 24 ± 2° to 40 ± 4° with an extended knee from rest to MVC (43).

The LG L_F at rest is 18% shorter with a flexed knee at 46 ± 4mm, compared to an extended knee position at 56 ± 8mm. During MVC, LG L_F is 31 ± 5mm and 38 ± 6mm in the flexed and extended knee angles respectively; a 21% difference. This results in a 32% decrease in L_F between rest and MVC at both knee angles. The PAs of the LG increase more from rest to MVC in both the flexed and extended knee positions compared to the MG, however they differ less with a change in knee angle within the relative torque produced. The PA of the LG more than doubles between rest and MVC (14 ± 1°; 29 ± 6°) with a flexed knee, and increases 85% from 13 ± 1° to 24 ± 4° with an extended knee (43).

These data follow the usual finding that fascicle length decreases while pennation angle increases from rest compared to MVC (1, 43, 53). The soleus also exhibits a decrease in L_F from rest to MVC (~35mm to ~31mm) albeit a smaller change than that of the gastrocnemeii. The soleus PA increases from 25 to 40 between rest and MVC; a 60% change (53). Studies have also been conducted examining the changes in gastrocnemeii and soleus architecture with changes in ankle angle (52, 63). These studies find similar changes in both L_F and PA, as both muscles lengthen with dorsiflexion and shorten with plantar flexion. It is important to appreciate that architectural features likely play a role in the ability to successfully record motor unit discharge rates during different levels of voluntary contraction. Changes in muscle and fascicle length with respect to motor unit behaviour will be further discussed later in this chapter.

1.1.3 Muscle Volume

With respect to absolute muscle mass there is roughly a 4:2:1 relationship from the soleus, to the medial, to the lateral gastrocnemius (28, 31, 33). Using magnetic resonance imaging, Fukunaga et al. (1992) identified the soleus to be approximately 489cm^3, with a physiological cross-sectional area (PCSA) of 230cm^2, while the MG and LG were 245cm^3 and 140cm^3, with PCSAs of 68cm^2 and 28cm^2, respectively (31). Using an algorithm to correct for artifacts within the MRI scans, Elliot et al. (1997) found the total muscle volumes to be smaller in the soleus at 432cm^3, yet larger in the MG and LG at 281cm^3 and 154cm^3, respectively (28), than the prior study. This group however only had 7 subjects, 3 of which were female, compared to 11 males and 1 female in the 1992 study by Fukunaga and colleagues. Although muscle is primarily a contractile tissue made of contractile protein, there are many non-contractile elements, such as intramuscular fat deposits and fibrous support structures such as collagen and elastin which outline its basic architecture (67). Hasson et al. (2011) identified the actual contractile tissue volume to be approximately 95% of total muscle mass in young men in both the gastrocnemii and the soleus (33). Overall, the PCSA of the soleus is larger than the combined values of the LG and MG by almost 2.5 times. Therefore, functionally the soleus generates about 70% of plantar flexor MVC regardless of knee joint position, yet the LG and MG are likely compromised due to their shorter lengths with a flexed knee position (31).

1.1.4 Strength and Twitch Contractile Properties

While muscles have a variety of differentiating characteristics, one of the more common measures examines how they respond to a single electrical pulse, or a twitch, delivered either directly to the muscle's motor point or to the nerve innervating the muscle. These measures include peak twitch tension (Pt), time-to-peak tension (TPT), and half-relaxation time (HRT). Contraction duration (CD) is also often reported, which is the summation of TPT and HRT. These traits are one of a number of features used to discriminate muscle as either fast-twitch or slow-twitch. TPT is indicative of the rapidity to which a muscle releases calcium and breaks down ATP to perform a contraction. Conversely, HRT indicates how quickly a muscle can reuptake the released calcium back into the sarcoplasmic reticulum, thus CD is a good measure of overall intramuscular calcium kinetics (10). It is notable that the amount and quality of non-contractile tissue in a musculotendinous unit also can affect contractile responses (64).

Since the soleus and gastrocnemeii are both innervated by the tibial nerve, a stimulus applied to the nerve elicits a twitch of the entire triceps surae, making it challenging to differentiate the twitch contractile properties of the individual muscles. However in 1983, Vandervoort and McComas stimulated each of the 3 muscles directly over their motor points allowing for discrimination of each of their respective contractile properties (72). This was conducted with a flexed knee and 10° of dorsiflexion, corresponding to the optimal region of the length-tension curve according to Sale et al. (68). They found the LG to be slightly faster than the MG with respect to both TPT, HRT and thus CD by ~ 11%. The soleus however was significantly slower in these measures with a CD of over 300ms. Because the soleus makes up more than half of the triceps surae by volume, the twitch contractile properties of the entire group are dominated by the soleus with a TPT and HRT much more similar to the soleus itself then to gastrocnemeii values. (72). Thus the overall contraction duration of the triceps surae is reported from many studies to be between 250ms and 300ms, (10, 15, 17, 20, 72). Peak twitch tension of the triceps surae is a length-dependent measure, but also dependent on training status and absolute muscle mass. Using a commercially available Biodex dynamometer, both Cresswell et al. (1995) and Dalton et al. (2014) found a 27% difference between Pt with extended and flexed knee positions, with twitch tensions of 29Nm and 21Nm (15, 16). Using a custom made isometric dynamometer, Vandervoort and McComas (1986) reported Pt as ~16Nm with a slightly dorsiflexed ankle and a flexed knee (73). The twitch contractile properties of a muscle or muscle group are important because previously, there has been a demonstrated link between these properties and the motor unit discharge rates in a muscle such as in the TA (14).

With dynamometric testing, muscle strength, reported as a force about an axis (torque), can be measured either isometrically, isotonically or isokinetically. While isotonic and isokinetic testing are dynamic measures, the most often tested and reported measure of torque are reported with the isometric testing parameter at a constant joint angle. The triceps surae isometric torque seems to be rather variable across populations even when testing at the same ankle and knee angles. With a fully extended knee and the ankle at 90°, Dalton et al. (2014) reported triceps surae MVC torque as 192 ± 37.1Nm (16), whereas Cresswell et al. (1995) reports it as 134 ± 23Nm with a slightly plantar flexed ankle angle of 5° (15). Subjects were the same age (26y) and all male, although they were larger in Dalton et al. (2014) with an average weight of 80kg. The isometric triceps surae torque has also been reported as low as 100Nm by Miyamota & Oda (2003) but these subjects despite being young men, only weighed an average of 67kg (56). Clearly, overall static strength is strongly related to muscle size as reflected by differences in bodyweight and likely activity patterns. The degree to which plantar flexion torque decreases in a flexed knee position also varies among studies with decreases of 17%, 24% and 35% in these 3 mentioned studies (15, 16, 56). Table 1 summarizes the triceps surae anatomical and functional features.

	Muscle volume (cm^3)	**MVC Torque**	**Pt (Nm)**	**TPT (ms)**	**HRT (ms)**	**CD (ms)**
MG	240 - 290	□ 20%		113.7	111.0	224.7
LG	130 - 160	□ 10%	12 - 30	100.0	100.5	200.5
SOL	425 - 500	□ 70%		156.5	151.5	308.0
Triceps surae		**100 - 200Nm**		□ **140**	□ **122**	**250-300**

Table 1. Summary of triceps surae and its respective heads' twitch contractile properties, torque and muscle volume. Pt = peak twitch torque; TPT = time-to-peak tension; HRT = half-relaxation-time; CD = contraction duration. Data from Cresswell et al. (1995) (15), Dalton et al. (2014) (16), Elliot et al. (1997) (28), Fukunaga et al. (1992) (28), Hasson et al. (2011) (33), Miyamota and Oda (2003) (56), Vandervoort and McComas (1983, 1986) (72, 73).

In addition to assessing the stimulated twitch properties of a muscle (as outlined above) muscles also can be electrically stimulated at varying frequencies to create a force-frequency relationship (Figure 2). Slower muscles will have a steep initial response to increasing stimulation intensity and then reach a plateau at full tetanus. Conversely, faster muscles will have a slower rate of increase and thus a less steep force-frequency tracing prior to plateauing at a particular stimulation intensity (11, 12, 54).

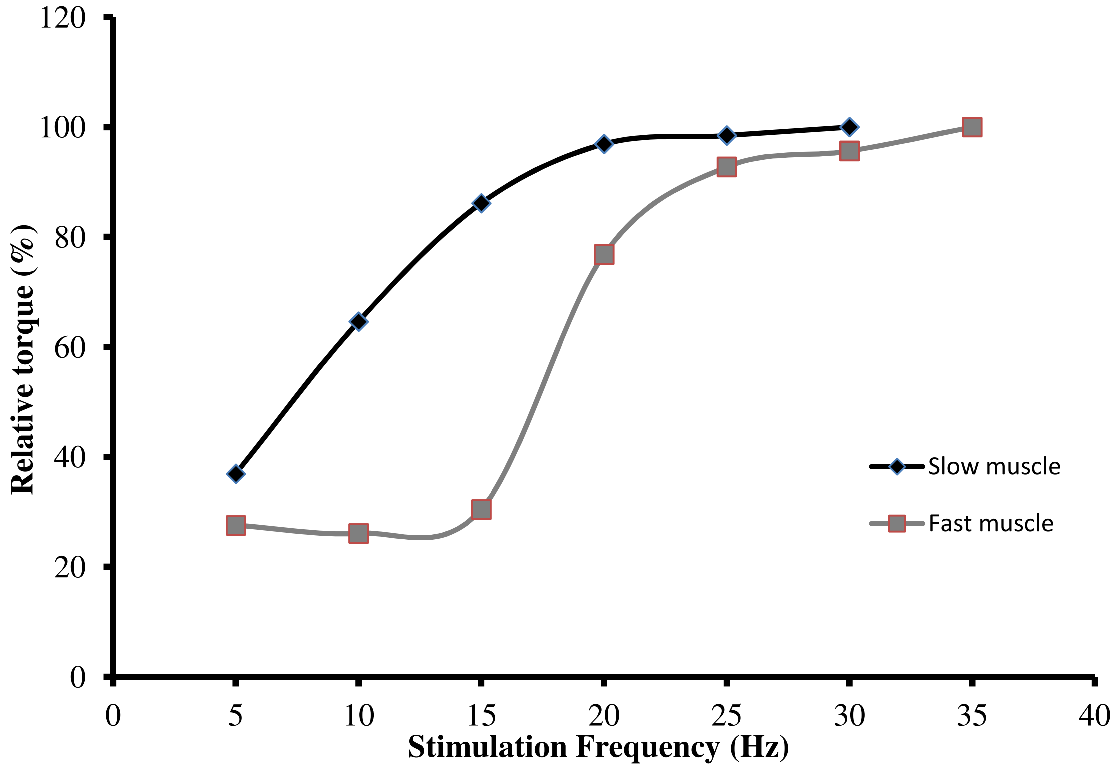

Figure 2. Comparing a fast and slow muscle stimulated force-frequency recruitment curve. Note the delayed increase in force in the fast muscle, thus resulting in a rightward shift. Adapted from Head and Arber (2013) (34).

In 1982, Sale et al. examined the force-frequency relationship of the triceps surae at different ankle angles. It took about 60Hz of stimulation with an ankle of angle of 10° plantar flexion to induce a full tetanus (68). Because of the anatomical connection between the gastrocnemeii and soleus it is not possible to assess force-frequency relationships separately for these 3 muscles. By comparison, the quadriceps, which based on fibre-type composition is very similar to the gastrocnemeii, require between 40 and 50Hz to achieve full tetanus (66). Even though the soleus dominates the muscle group by volume, it seems the faster nature of the gastrocnemeii may increase the stimulus frequency required to achieve full tetanus.

Functionally, the soleus is critical in standing balance with low levels of tonic activity (57) and is phasically active during the gait cycle, with minimal variability in activation. The MG however is intermittently active and the LG is almost entirely inactive during quiet standing in young healthy adults (35, 74), and although the gastrocnemeii are phasically active during gait, there is large variability in their patterns of activation (75).

1.2 MOTOR UNIT

1.2.1 History and Definition

In 1925, Liddell and Sherrington first coined the term "motor unit" (MU) as the "common final pathway" and identified it as the smallest functional unit of the neuromuscular system. It includes an alpha motor neuron, originating from the anterior horn of the spinal cord, its axon and dendrites, as well as all the muscle fibres which it innervates (48). Because of this important link, and in healthy subjects of the faithful transmission of action potentials from nerve to muscle, inserting wires or needles into contracting muscles provides a direct recording of the output of the spinal alpha-motoneuron. Many electrophysiological techniques have been utilized to study human motor unit properties; however there is an inherent limitation with these techniques when assessing motor unit output above approximately 60% MVC. At this point, it becomes increasingly difficult to discriminate individual motor unit trains because of large numbers of activated fibres.

Muscle force is graded or controlled by a combination of the amount of active fibres (recruitment) and their respective discharge rates (rate coding). Architectural and muscle composition features (ie. fibre-type, elasticity, and others) can substantially modify the expression of force realized across a joint. Some muscles seem to be more recruitment – oriented, perhaps to be more functionally relevant for gross movements, such as the elbow flexors and shoulder muscles with recruitment occurring up to approximately 85% MVC (25, 46). Conversely, other muscles are more rate-coding oriented for finer controlled movements, such as the intrinsic hand muscles (46, 58) . However, it seems all limb muscles use some combination of these two basic features when grading muscle force. From the study of many human limb muscles during voluntary contractions of varying forces it seems that most motor units of a muscle are able to be fully activated during brief MVCs (<10s). Thus at MVC recruitment is complete and rates of excitation are maximized and optimal to achieve peak force output, possibly in relation to inherent contractile muscle speed. Perhaps because of architectural differences, motor unit composition, or habitual activity patterns when presumably, all MUs are recruited there are large differences in mean maximal steady-state discharge rates across the various muscles studied to date (see Figure 8).

1.2.2 Maximal discharge rates

Although there have been studies identifying motor unit discharge rates during varying intensity submaximal contractions, the data for maximal motor unit discharge rates remains scarce. This is because of the difficulties of extracting individual MU trains during an MVC when so many units are active, thus creating a very large intramuscular interference signal. While submaximal discharge rates are most commonly collected using intramuscular bi-polar fine wire, it is nearly impossible to collect individual motor unit trains during MVC with this technique as the fine wire is unable to discriminate individual trains with all of the units active at once. During maximal contractions, either a multichannel quadrifillar needle electrode or tungsten needle microelectrodes have been used to better focus selectivity on individual active units (6, 23). The tungsten needles use a population sampling approach to create a profile of active units because it is known that the mean maximal discharge rates are not the same for all units (6, 22, 24, 30), whereas the quadrifillar type electrode may sample just a few units per subject per contraction. The small surface recording tip of the tungsten electrode is slowly advanced through the muscle during the contractions in an attempt to focus and record from a number of individual units during repeated maximal non-fatiguing contractions (6).

To date the only muscles to have identified mean maximal motor unit discharge rates (MUDR) are the first dorsal interosseus (FDI) (42), the abductor digiti minimi (69), the adductor pollicis (5), the extensor hallucis longus, (51), the tibialis anterior (TA) (14), the vastus medialis (66) and lateralis (41), the biceps brachii (18), the triceps brachii (18) and the soleus (5, 17, 40). Of these muscles, the FDI exhibits the greatest rates at 51 ± 20Hz. The biceps brachii and triceps brachii have similar maximal motor unit discharge rates at 42 ± 15 and 38 ± 10Hz, respectively. The adductor pollicis discharges maximally at 29.9 ± 8.6Hz. The knee extensors vastus medialis and vastus lateralis are similar at 26 ± 5Hz and 24 ± 7Hz, respectively. The TA has the highest rates in a lower limb muscle at approximately 42 ± 8Hz. The small muscles, extensor hallucis longus and abductor digiti minimi have low maximal rates of ~15Hz and ~11Hz, respectively. However the EHL was measured over 30s which may have allowed the development of fatigue factors to lower the mean rates (6) and the ADM was studied in an older age group (22 to 54 years) also known to result in lower mean rates (14, 42). Apart from these two muscles, the soleus is markedly lower than the other muscles tested, which has been tested in three studies to date. Bellemare et al. (1983) reported rates of 11 ± 3Hz, but this was only done in 3 subjects with large age variability (5). In 2009, Dalton et al. found the mean max MUDR to be slightly higher (16.5Hz) than the study by Bellemare et al (1983) but still very low compared to other muscles and in both young and old adults (17). Most recently, Kallio et al. (2013) found the mean MUDR in the soleus to be 19.9 ± 7.3Hz, however these data were collected using a different recording technique than the prior two studies and there were only 8 motor unit trains analyzed from 5 subjects at MVC (40). Thus, because of the known range of maximal discharge rates among units from the same muscle (for example 7 to >25Hz in soleus; Dalton et al. 2009), under-sampling may have skewed the results in the Kallio report (40).

1.2.3 Triceps Surae and Length-Dependent Motor Unit Properties

To date, there exists minimal data on the behaviour of the MG and LG motor unit pools during various submaximal contractions, despite a larger body of knowledge regarding the soleus motor unit behaviour. The soleus, consistent with its distinct slow max MUDR, is reported to have rates ranging from 6Hz to 15Hz, during submaximal contraction intensities between 25 and 75% MVC (17). In 2010, a Dalton et al. abstract reported that both heads of the gastrocnemeii, with a knee angle of 90°, exhibited discharge rates between 6 and 24Hz at torques ranging from 25 – 75% MVC (21). These were about 20% greater than that of the soleus at the same torque outputs. Because the quadriceps are similar in fibre-type to the gastrocnemeii, reporting their submaximal motor unit behaviour may be relevant. Two different studies reported the mean MUDR at 50% MVC to be ~15Hz, with one reporting a discharge rate of 9.3Hz at 10% MVC, and the other reporting rates of ~20Hz at 75% MVC (41, 65). Slightly lower rates were identified in the vastus medialis (~10, 14, 17Hz at 25, 50, 75%MVC, respectively) (66).

To my knowledge, there are no intramuscular data identifying gastrocnemeii motor unit behaviour exceeding 75% MVC with either a flexed or extended knee. One study, however, identified significant decreases (~50%) in the surface EMG of the gastrocnemeii during MVC with a flexed knee when compared with an extended knee joint (15). Motor unit behaviour has been studied previously with changes in muscle length both submaximally and maximally. With regard to motor unit recruitment, Ballantyne et al. (1993) examined MG recruitment with varying knee and ankle angles and found that MG recruitment was dependent on the linear combination of both plantar flexion and knee flexion torques exceeding a certain combined force (3). Kennedy and Cresswell (2001) found a significantly lowered recruitment threshold in the MG with an extended knee compared to a flexed knee position (3Nm vs. 32Nm, respectively). They however, only exerted torque with a ramp contraction until a single train could be detected in the MG, and initial discharge rates were not reported. They suggested that there exists presynaptic inhibition of the MG motor unit pool in a shortened muscle state to prevent unnecessary MG activation while being mechanically disadvantaged (44). Conversely, in the TA, Pasquet et al. (2005) found a reduced recruitment threshold for motor units in a shortened position, with 10° of dorsiflexion. However, they too terminated the ramp contraction once motor unit activity was detected at less than 10% MVC, similarly attributing the change to afferent proprioception altering the motor unit pool excitability (63). In 1998, Christova and Kossev found no difference in recruitment thresholds after normalizing for differences in torque in the biceps brachii at 3 different elbow angles, yet found an increased discharge rate at a short muscle length in approximately half of the units detected at about 8% MVC (13). The only study to have examined motor unit behaviour in the LG was by Heroux et al. (2014), during ramp and hold standing balance trials in which they compared MU recruitment thresholds to anterio-posterior and medio-lateral centre of pressure displacements. While they found MG motor units to be recruited at twice the threshold of the SOL, no motor unit activity was detected in the LG until a 20 – 35x greater threshold. They attributed this absence of activity to the LG having a distinct range of joint angles in which it operates, which is unique to that of the MG and SOL (35).

Two studies have reported maximal MUDRs during MVC at different muscle lengths. In 1992, Bigland-Ritchie et al. found no differences in the TA MUDRs between a control and a short muscle length once normalized for relative torque, despite differences in twitch contractile properties. They also found no differences in MUDRs at both 50% and 75% MVC (7). In the triceps brachii, Del Valle and Thomas (2004) also found no differences in discharge rates at any torque level at different muscle lengths, after normalizing for differences in torque. They did however find differences in rates when comparing to absolute torque at each of the 5 elbow angles; that is to produce a certain torque at one joint angle required a different discharge rate to produce the same absolute torque at a different angle (26).

1.3 PURPOSE & HYPOTHESIS

Although the submaximal motor unit behaviour of the medial gastrocnemeii, and all levels of the soleus are fairly well reported, the maximal MUDRs are not known for either head of the gastrocnemeii during short, maximal voluntary isometric contractions. As contributors to plantar flexion, it is important to gain insight into the upper limit of gastrocnemeii rates required to sustain brief maximal isometric plantar flexor contractions, and to compare these to the rates already described for the soleus under similar contractile conditions. This permits further insight into the functional interplay among the triceps surae muscles by providing baseline data for comparison in adapted states (aging, disease, training) and in relation to submaximal excitation levels during tasks of daily living such as walking. There is also minimal information regarding the behaviour of the LG motor units in any condition. Thus the purpose of this thesis was to determine mean maximal MUDR in both the medial and lateral gastrocnemeii. Despite the MG and LG behaving differently during submaximal postural tasks, based on their differences in both fibre-type and mode of activation from the soleus, it was hypothesized that the MG and LG will exhibit similar mean MUDR at maximal contraction intensity, but both will be greater than that of the soleus.

CHAPTER 2: METHODS

2.1 PARTICIPANTS

Nine recreationally active males (age 24.2 ± 1.6; weight 81.3 ± 8.1kg; 180.3 ± 5.3cm) volunteered as subjects in this study. All were free of any known neuromuscular and cardiovascular ailments, and none were athletes or systematically trained. Each subject provided both oral consent and signed an informed consent form (Appendix A) approved by the local University's ethics board in accordance with the Declaration of Helsinki.

2.2 EXPERIMENTAL SETUP

Subjects were seated on a chair with their right leg placed in a custom plantar flexor isometric dynamometer, placing the knee, hip and ankle at 90° (Figure 3). Their foot rested on a footplate to which a force transducer was affixed, while two Velcro straps were fastened over the dorsum of the foot to prevent any foot movements. A C-clamp was pressed firmly against the distal aspect of the right thigh to prevent any anterior movement of the shank and hip while plantar flexing, ensuring all force was transmitted directly through the force transducer.

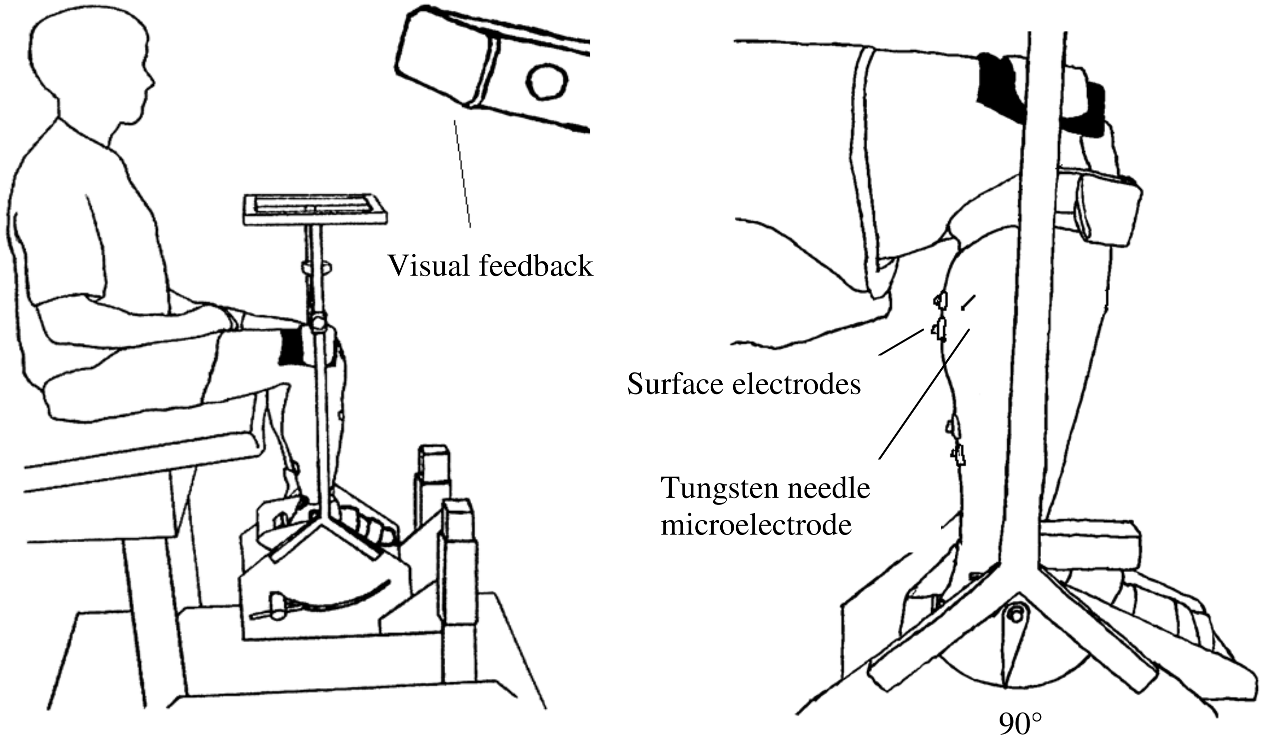

Figure 3: Experimental Setup

2.3 RECORDING

2.3.1 Surface EMG

A bi-polar electrode set-up was used to record surface EMG (sEMG) of the soleus, and medial and lateral gastrocnemeii. Repositionable Ag-AgCl electrodes were used (H59P Monitoring Electrodes, Kendall; Mansfield, MA, USA). The active electrodes of each pair were placed distal to the reference electrodes over the muscle bellies of the gastrocnemeii. For the soleus, the pair was placed just below the inferior border of the gastrocnemeii. For all 3 pairs, the inter-electrode distance was 2cm. A subject ground electrode was placed over the patella. All sEMG signals were pre-amplified (Model NL844, Digitimer; Welwyn Garden City, UK) by

100x, then amplified 2x before being sampled through a 16-bit analog-to-digital board (micro1401 *mkII*, CED; Cambridge, UK) at 2000Hz each.

2.3.2 Intramuscular EMG

Custom-made tungsten needle microelectrodes (see Appendix B for needle specifications) (FHC; Bowdoin, ME, USA) were used to collect single motor unit action potential trains during maximal voluntary contractions. Before being used the needles were first sterilized by an autoclave (NAPCO Model 900-D, Precision Scientific; Chicago, IL, USA) for 45 minutes at 130°C. Sterilization was maintained by keeping the needles in a beaker of 95% ethyl alcohol until they were inserted using sterilized instruments. The insertion site was swabbed with 70% ethyl alcohol before needle insertion. Subjects made a light contraction as the needle was inserted into either the medial or lateral gastrocnemeii muscle belly, at about the midpoint of the muscle, close to the surface electrodes, simultaneously. In order to sample from as many different motor units as possible the needle was slowly advanced into the muscle during each of the MVCs. To improve sampling of many different units of each muscle, between MVCs the needles were withdrawn and reinserted within 1-2 cm and at different angles and depths.

The reference surface electrodes for the needles were placed over the medial and lateral malleoli. The raw signal was pre-amplified by 1000 times, then again by either 2 or 5x at the main amplifier depending on the signal-to-noise ratio required to maximize the size of the action potentials of a train within the voltage limitations. Action potentials for each channel were sampled at a minimum rate of 10 000Hz. The filter bandwidth ranged from 10 to 10 000Hz, with a 60Hz notch.

2.4 PROTOCOL

To normalize surface EMG, and to assess twitch contractile properties, an M-wave and maximal twitch response were electrically evoked for the plantar flexors. This was done by stimulating the tibial nerve in the distal popliteal fossa between the heads of the gastrocnemeii with a Digitimer stimulator (Model DS7AH; Digitimer, Welwyn Garden City, UK) using a 100µs pulse-width, 400V stimulus. The current was increased until there was no further increase in soleus M-wave amplitude, and then the current was further increased by 15% to a supramaximal level. The soleus recording electrodes were used to assess the M-wave for the plantar flexors. The twitches corresponding to the maximal M-waves were used to assess contractile properties and for twitch interpolation.

A plantar flexor MVC was then conducted with a supramaximal twitch delivered at rest before the MVC (pre twitch), during the brief (~7s) maximum MVC (superimposed twitch), and immediately (1-2s) following the MVC (post twitch) in order to assess percent voluntary activation (VA). No more than 2 practice MVCs were required for subjects to produce a consistently well-activated MVC (>95%) to be used as their baseline value.

Subsequently, each of the two operators inserted one tungsten needle each into the belly of either the MG or LG using the methods outlined above. Subjects were then asked to perform a small contraction of about 25% MVC in order to determine if motor units were detectable, and repositioned if necessary.

A total of 6 or 7 brief MVCs (~7s each) were performed while the tungsten needles recorded individual motor unit trains from the MG and LG, simultaneously. Each operator received separate auditory and visual feedback from either the MG or LG needle to improve the high quality sampling from as many individual units as possible during the contractions. Five minutes of rest was provided between each contraction in order to ensure the subject could produce maximal torque each time. If a subject was unable to perform a maximal contraction to within 95% of the baseline MVC, the testing session was concluded. Subjects returned to the lab for 3 separate sessions with at least two days between sessions and no more than 5 days between sessions. The total time per each session was about 1.5h. These methods have been shown to be reproducible from day to day (70).

2.5 ANALYSIS

All data were sampled using Spike2 software (CED; Cambridge, UK). Following data collection, twitch contractile properties, and motor unit train analyses were conducted offline using a custom script for Spike2. For contractile properties, peak voluntary torque was measured during the baseline MVCs and was the highest of the 2 baseline contractions. For stimulated twitch properties, measures of peak twitch torque (Pt), time to peak torque (TPT) and one-half relaxation times (HRT) were made from the non-potentiated twitch with the highest torque. Contraction duration (CD) was the sum of TPT and HRT. Voluntary activation (VA) was assessed using the interpolated twitch technique (ITT) with the formula:

$$VA\% = (1-(superimposed\ twitch/post\text{-}twitch))*100$$

Motor unit train analysis required a window discriminator, shape recognition and operator-directed overlaying of successive action potentials within a train. While the software facilitated this process, visual inspection was required to confirm spike allocation by an experienced operator. A minimum of 5 potentials (4 inter-spike intervals) were required to qualify a set of contiguous MU potentials, a ‘train’. Doublet discharges (>100Hz) were not included. If the torque produced during the ~7s MVC dropped to less than 95% of maximal torque, those action potential trains at those lower torques were not analyzed. In addition, a coefficient of variation (CoV) of no more than 30% among inter-spike intervals of the train was required for inclusion (30). Figure 4 provides a visual representation of this process.

For surface EMG, a 1-second epoch was taken about maximal torque during MVC to calculate the root-mean-squared (RMS) of the EMG signal. The RMS amplitude (RMSamp) was normalized to the corresponding muscle's M-waves.

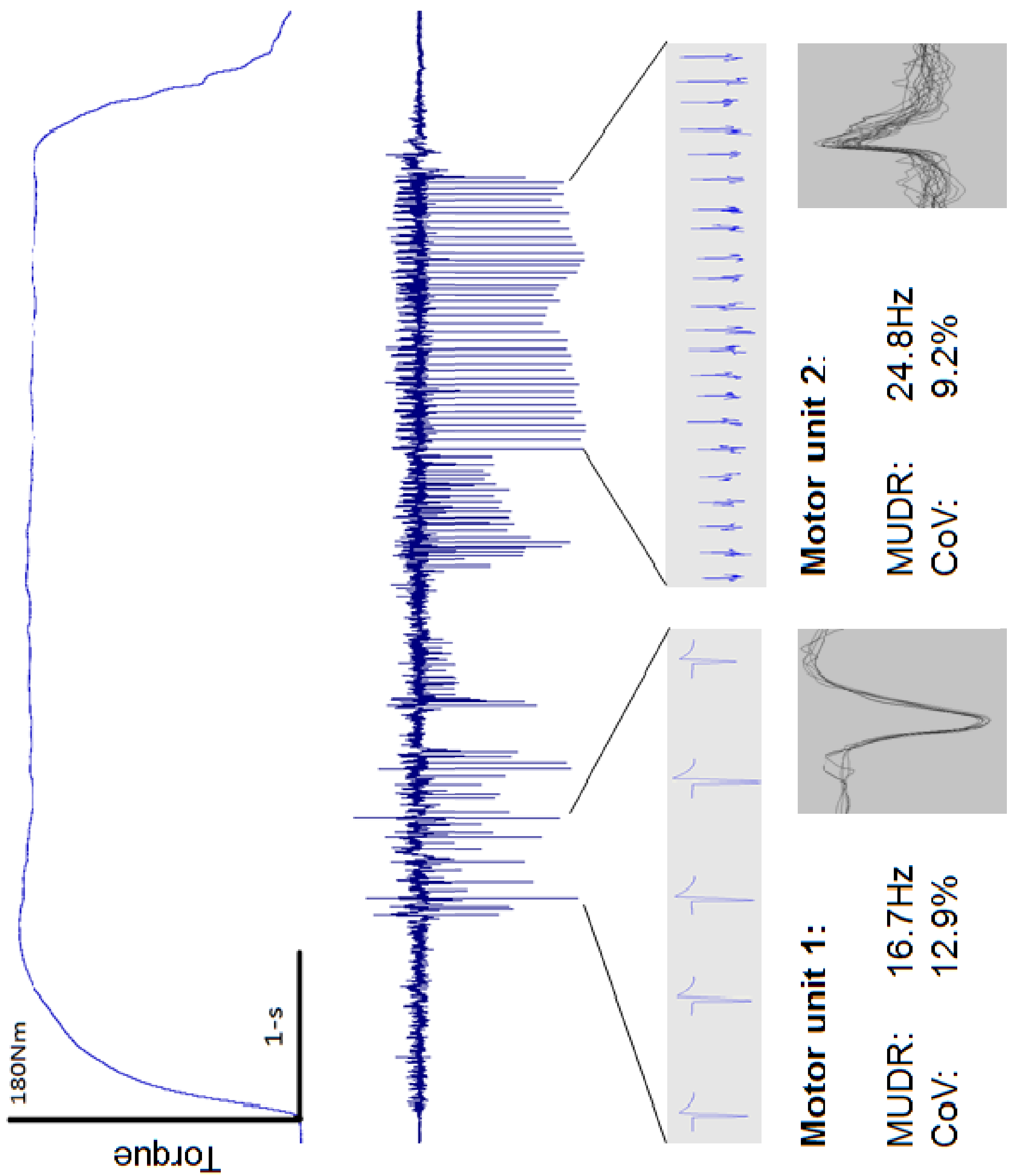

Figure 4. An example of the torque, raw tungsten data and the extracted motor unit trains and their overlays. Two motor unit trains can be identified from the tungsten tracing, each with their own unique discharge rates, waveform and coefficient of variation (CoV).

2.6 STATISTICS

All statistical analyses were carried out using SPSS Version 20 (SPSS; Chicago, IL, USA). A Shapiro-Wilk's test was used to assess data normality, while a Levene's test was conducted to test for homogeneity of variance. A one-way ANOVA was used to compare the surface EMG between the MG, LG, and SOL. Because group sizes were different by more than 1.5 times, a non-parametric test, the Mann-Whitney U, was used to assess differences between the maximal motor unit discharge rates of the medial and lateral gastrocnemeii. Results were considered significant with a $p < 0.05$.

CHAPTER 3: RESULTS

3.1 SURFACE ELECTROMYOGRAPHY

Levene's test was non-significant for sEMG indicating that the data displayed homogeneity of variance, $p > 0.05$. The mean RMSamp during plantar flexion MVCs (as a percent of M-wave) of the sEMG for the MG and LG were 13 ± 4% and 11 ± 4%, respectively. For the SOL the mean RMSamp (as a percent of M-wave) was 10 ± 3%. Overall, there was no significant difference in RMSamp between muscles, $p > 0.05$.

3.2 MVC TORQUE & TWITCH CONTRACTILE PROPERTIES

Table 2 identifies the mean twitch contractile properties (Pt, TPT, HRT, and CD) and voluntary measures (MVC torque, and voluntary activation). The average rate of twitch torque development (RTD) was calculated by normalizing the Pt to TPT.

	Pt (Nm)	**TPT (ms)**	**HRT (ms)**	**CD (ms)**	**RTD (Nm/ms)**	**MVC Torque (Nm)**	**VA (%)**
Mean	20.8	133.2	85	218.2	0.16	194.6	96
SD	5.9	8.4	10.2	15.5	0.04	57.1	3

Table 2. Mean and standard deviation of triceps surae twitch contractile properties, MVC torque and voluntary activation. Pt = peak-twitch tension; TPT = time-to-peak tension; HRT = half-relaxation time; CD = contraction duration; RTD = rate of torque development; VA = voluntary activation.

3.3 MOTOR UNIT DATA

3.3.1 Motor Unit Trains

There were a total of 315 motor unit trains identified in the two heads of the gastrocnemeii. The medial gastrocnemeii had 198 motor unit trains, while the lateral gastrocnemeii had 117 motor unit trains. Table 3 indicates the subject-by-subject contribution to the total sampled motor unit train pool and their respective mean MUDRs.

Subject	MVC torque (Nm)	# of MG MU trains	MG mean maximal MUDR (Hz)	# of LG MU trains	LG mean maximal MUDR (Hz)
1	161.0	24	18.1	21	19.7
2	156.8	35	27.2	9	24.9
3	247.8	26	24.3	23	26.4
4	174.8	33	19.6	26	18.4
5	128.0	9	23.1	10	23.6
6	294.6	19	24.4	10	24.4
7	177.8	16	24.0	9	22.4
8	182.8	19	18.9	0	n/a
9	175.9	17	24.0	9	23.9

Table 3. Subject-by-subject contribution to total sampled motor unit pool and their respective mean MUDR for both the MG and LG.

3.3.2 Maximal Motor Unit Discharge Rates

The Shapiro-Wilk's test showed neither the MG nor LG MUDRs were normally distributed, $p < 0.05$, but Levene's test confirmed they were homoschedastic, $p > 0.05$. Therefore, while the data are skewed about the mean (ie. non-normal), the two distributions are equivalent. The mean maximal discharge rate for the medial gastrocnemius was 22.7 ± 8.6Hz, with a range of 7.6Hz to 64.4Hz. The mean maximal discharge rate for the lateral gastrocnemius was 22.4Hz ± 8.1Hz, with a range of 5.5Hz to 59.8Hz. There was no statistical difference in maximal motor unit discharge rates, $z=0.287$, $p > 0.05$ (Figure 5). From Figure 5 it can be appreciated that the range of discharge rates also did not differ between the two muscles.

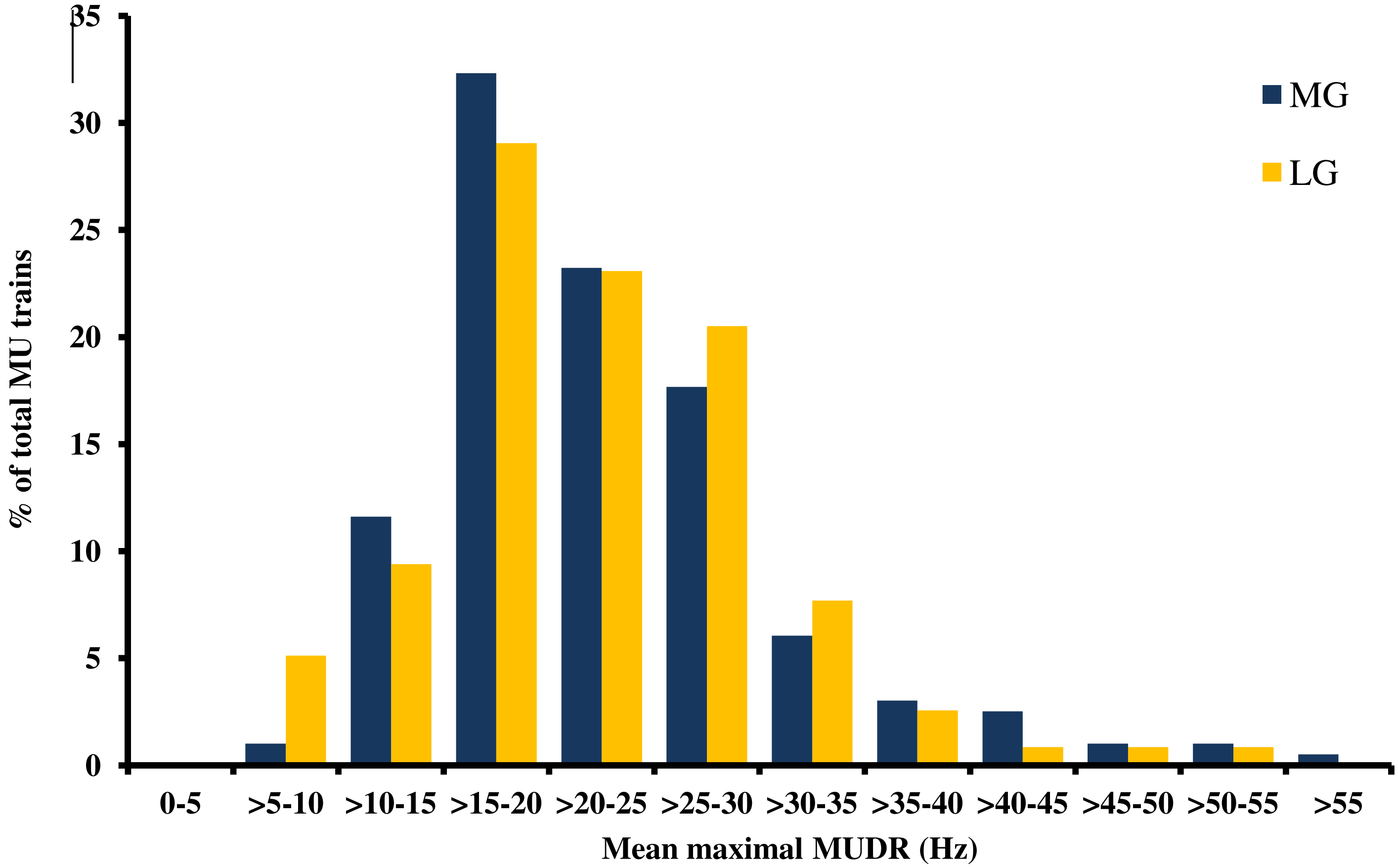

Figure 5. Relative distribution of medial (MG) and lateral gastrocnemeii (LG).

As mentioned in the 'Methods' section, in order for the motor unit train to be considered valid, its discharge rate coefficient of variation must be below 30%. The motor units sampled from the MG and LG had a mean CoV of 14.6 ± 1.4% and 14.3 ± 2.2%, respectively. There were no significant differences in mean CoV between the MG and LG, either between or within subjects, p > 0.05 (Table 4).

SUBJECT	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**MEAN ± SD**	
MG	15.4	14.2	14.6	14.3	16.5	17.0	13.0	12.8	13.6	*14.6*	±1.4
LG	15.0	14.0	13.5	10.0	13.8	18.6	14.1	NA	15.1	*14.3*	±2.2
MEAN	*15.2*	*14.1*	*14.1*	*12.1*	*15.1*	*17.8*	*13.5*	*12.8*	*14.4*	*14.4*	±1.8

Table 4. Subject-by-subject coefficient of variations (CoV) for the medial (MG) and lateral gastrocnemeii (LG). There is no statistical difference between muscles or subjects, p > 0.05.

3.4 CORRELATIONS

To assess whether there were any relationships between twitch contractile speeds and maximal discharge rates, or peak torques and maximal rates correlations were done between the subject-by-subject rates in the MG and LG, with the overall triceps surae twitch contraction durations (Figure 6) and with the MVC torques (Figure 7). Neither of these correlations were significant, and thus those with longer CDs did not have lower maximal rates nor did those with larger MVC torques have higher rates.

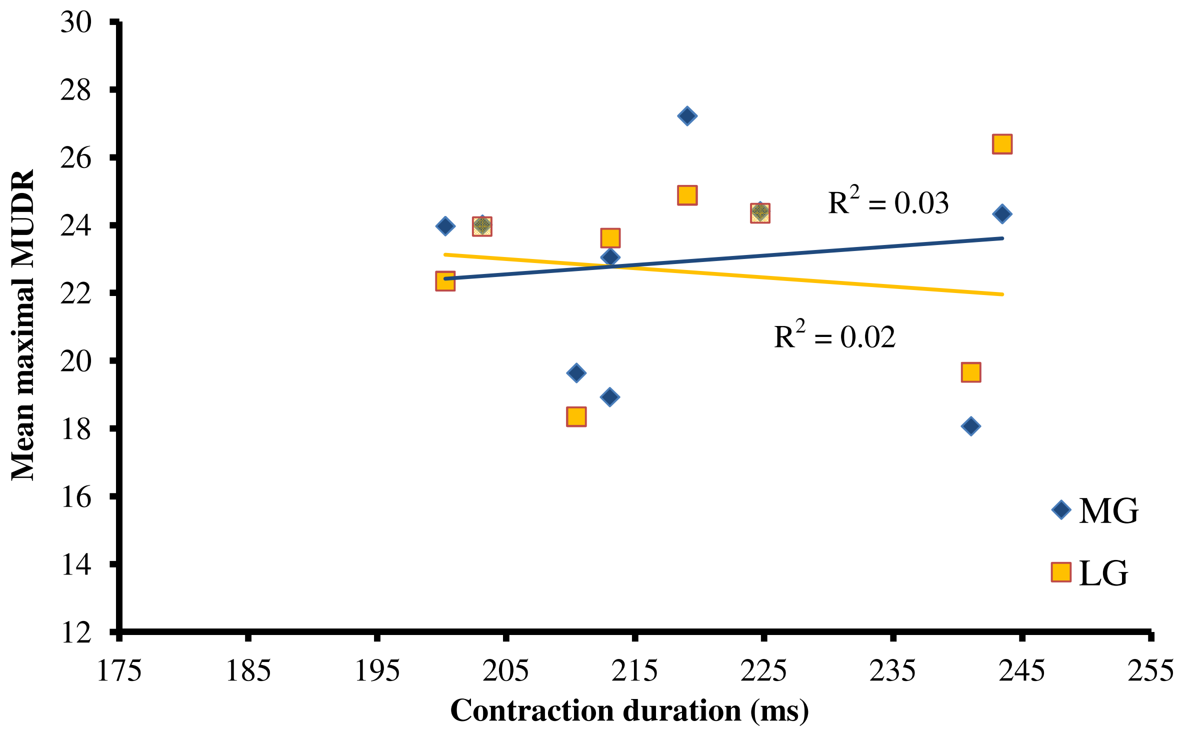

Figure 6. Correlations of triceps surae twitch contraction duration (TPT+HRT) vs. mean maximal motor unit discharge rates for all subjects. MG $R^2 = 0.03$; LG is $R^2 = 0.02$.

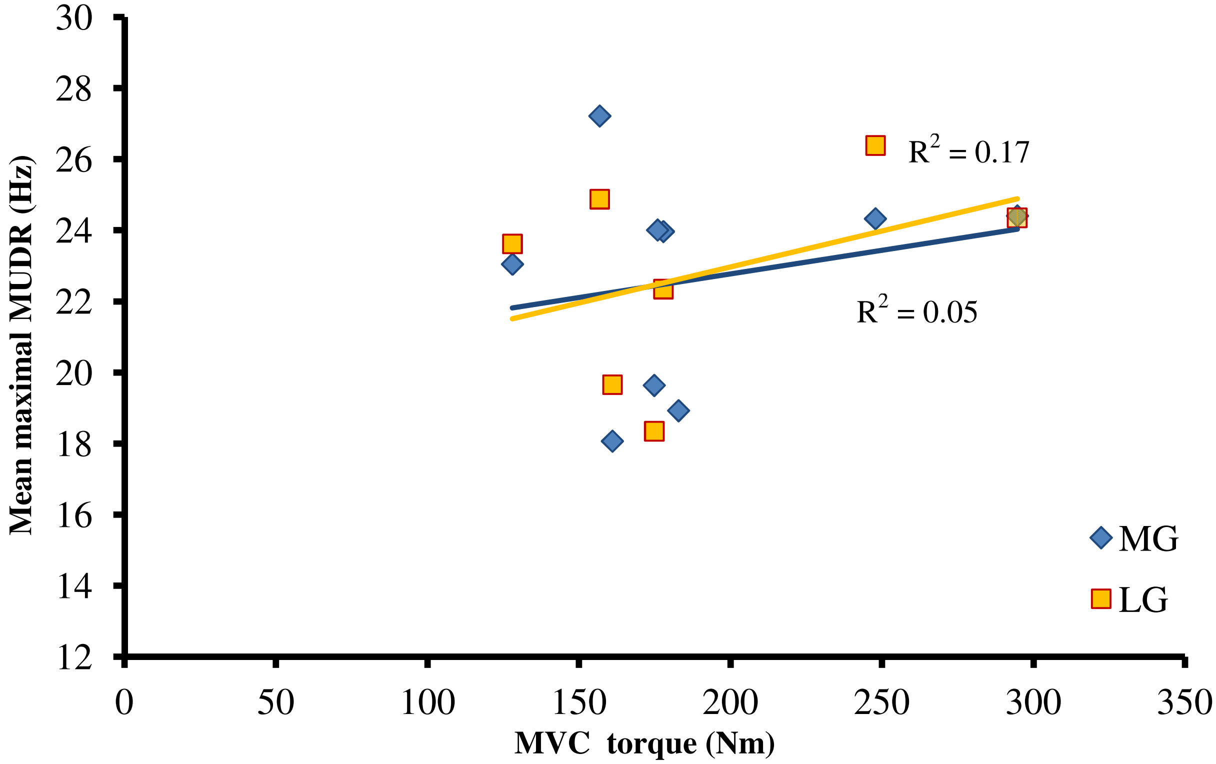

Figure 7. Correlations of the triceps surae MVC plantar flexor torque vs. mean maximal motor unit discharge rates of the two gastrocnemeii. MG $R^2 = 0.17$; LG $R^2 = 0.05$.

CHAPTER 4: DISCUSSION

4.1 MAJOR FINDINGS

This thesis is the first report of mean maximal motor unit discharge rates of the medial and lateral gastrocnemeii (22.7 ± 8.6Hz and 22.4Hz ± 8.1Hz, respectively), during brief sustained maximal voluntary efforts. Furthermore, despite some functional and anatomical differences between these two portions of the triceps surae, the mean maximal rates are not different, but they are substantially faster than the soleus motor units rates (~16Hz) reported elsewhere using the same set-up and methods (17). The results are consistent with the hypothesis that rates will not differ between heads, yet will be greater than the soleus.

4.2 VOLUNTARY TORQUE & TWITCH CONTRACTILE PROPERTIES

The overall relatively large mass of the plantar flexors and the mechanical advantage of the main contributor, the triceps surae provided by the Achilles tendon arrangement, results in relatively strong plantar flexion torques during voluntary efforts. Therefore, despite the less than optimal (shortened) position of the gastrocnemeii in the current setup, the young adult males in the present study generated a mean torque of 194.6 Nm with a range of 128 to 294 Nm. A baseline study that used this same setup and similar version of this isometric dynamometer reported lower mean torques (~170 Nm) in males of the same age range, albeit, ~10Kg lighter and ~4cm shorter than those in the present study (73). Various other studies have reported values in different devices and knee angles to range from 100 (56) to 200Nm (16). Flexing the knee by about 90 degrees results usually in a decrease in isometric MVC torque of 25-30% compared with the straight knee position (15, 16, 56, 68).

In the present study, voluntary activation assessed by the interpolated twitch technique, resulted in average activation of 96%. It has been reported previously that the triceps surae can be challenging to fully activate (ie. 100%), but the value found here agrees well with several other studies (4, 16, 20). Voluntary activation using the interpolated twitch technique has been tested systematically in the plantar flexors with a changing knee angle and was demonstrated that there is no difference in muscle activation (16, 20). Ankle angle also has no effect on triceps surae activation across a multitude of angles, except when the ankle is well plantar flexed to 20° or more (8). Other muscles such as the abductor digiti minimi, the biceps brachii and the tibialis anterior have also shown no difference in muscle activation at shortened muscle lengths (32).

For stimulated properties, peak twitch torques reported here were slightly higher than the original baseline study of Vandervoort and McComas (1986) (73), but similar to the MVC findings, likely reflect the overall larger size (both height and mass) of the subjects in this study, which presumably was reflected in calf mass. Compared with the flexed knee angle, triceps surae peak twitch torques are usually found to be 25 – 30% larger with an extended knee (15, 16, 20). With an average Pt of ~21Nm in the present study, it falls within the previously reported ranges. For contractile quality (speed), the overall contraction duration (CD), derived as the sum of TPT and HRT, was somewhat faster here (218ms) than the original baseline study which had a CD of about 260ms (72). In most studies however, the range of contractile speeds are rather large and likely reflects modest variations in intrinsic structure and function and are affected by activity status. Overall, from many studies using different devices, the plantar flexors in comparison to other limb muscle groups have very slow contractile speeds (17, 72, 73). This is due to the large influence of the rather slow soleus muscle, contributing ~70% (or more with a flexed knee) to torque and speed to the triceps surae contractile properties (31). Indeed, although it is not easy to test the contractile quality of each of the 3 parts of the triceps surae separately, one study found that the CD of the soleus was over 300ms, while the MG and LG were closer to

200ms (72). Also, while antagonistic muscle action was not measured in the present study, it is known that due the relatively smaller size and torque generating abilities of the TA, with respect to that of the plantar flexors, co-activation of the TA is minimal during isometric plantar flexion MVCs and does not significantly affect agonist torque output (8).

4.3 FIRING RATES AND CONTRACTILE FUNCTION

The main new findings in the present study were that the mean maximal MUDR of the gastrocnemeii are about 23Hz, and there was no difference between the LG and MG muscles. These findings have not been previously documented, but as part of the triceps surae muscle group are on average faster than the slow soleus (~16.5Hz) reported by Dalton et al. (17). For other muscles of the lower limb, the vastus medialis and lateralis are the most similar to the gastrocnemeii at 26Hz (66) and 24Hz (41), respectively. The tibialis anterior has mean maximal rates of ~42Hz (14). Because the soleus seems to be a unique muscle in fibre composition (88% ST) (37) and in function it was expected that the MG and LG would have higher average maximal rates than the soleus.

According to observations first reported in human muscles in 1983 (5) but well-studied in reduced preparations (2, 45), is that discharge rates are fundamentally matched to the contractile speed of the muscle fibres innervated by the motoneuron. This seems physiologically sensible in order to optimize force output and has sometimes been found in adapted states like adult aging in which age-related muscle slowing was matched by lower maximal discharge rates in the tibialis anterior (14). The gastrocnemeii have contraction durations of ~210ms which are ~36% faster than those reported for the soleus (72) and the maximal rates of the gastrocnemeii at ~23Hz are ~32% faster than the soleus. In addition because of the very low maximal rates in soleus it seems much of its force regulation relies more on recruitment than rate coding (61). This may also relate to its role as an active postural muscle (57) whereas the gastrocnemeii are activated sporadically (74) and could be considered as more phasic muscles. Thus, a larger rate coding range found in the gastrocnemeii suggests that this aspect plays a greater role in force gradation than in the soleus. Because contractile properties in humans can be influenced by factors not directly related to MU function per se (such as tendon and connective tissue compliance, muscle architecture, muscle synergies, sensory feedback, contractile history and contractile task) a matching between contractile speed and discharge rates may not appear tightly coupled when comparing between muscles or conditions. Therefore, for example the contraction duration of the quadriceps is slightly faster than the gastrocnemeii but the maximal discharge rates are nearly identical.

In this study, no relationship was observed between contraction duration of the whole triceps surae and the mean maximal MUDRs with a narrow range of variations from this sample of subjects (Figure 6). This relationship also was shown to be poor when comparing the triceps surae CD and the soleus maximal MUDR (17). This may be attributed to the poorly discriminated twitch contraction duration ability in the gastrocnemeii as part of the triceps surae. If direct motor point stimulation had been applied to the heads of the gastrocnemeii, there may have been a stronger correlation. With generally slow maximal rates in the soleus at ~16Hz and the MG and LG at ~23Hz, it is possible that the “average” triceps surae maximal discharge rates of ~20Hz among the 3 parts may show a matching to the overall slow triceps surae contraction duration.

There was also a poor correlation between max torque generated and discharge rates, as the subjects with greater plantar flexion MVC did not have faster rates (Figure 7). Although previous reports have shown a matching when considering submaximal torques in the soleus (17) and TA (14) currently no studies have found a good correlation during MVC in young adults. Strong correlations between maximum MVC torque and maximal firing rates was reported in old weightlifters, which considers both age-related motor unit remodelling and potential training effects thus making it a unique population (41).

It has been assumed that fibre-type composition is related to maximal discharge rates following the idea that contractile speed is determined by fibre-type, and that muscles with a high proportion of slow twitch or Type I fibres will have slow contractile speeds. From a survey of several studies that have reported maximal discharge rates in various upper and lower limb muscles and from separate studies examining fibre-type composition, it is clear that fibre-type composition is not related to maximal rates (Figure 8). The results from my study importantly solidify this lack of a simple relationship by helping to complete the story for major lower limb muscles and in this interesting triceps surae model. Although the gastrocnemeii are near 50% ST as compared with the soleus at ~85% ST, the discharge rates of gastrocnemeii are not twice as fast for example. Note also from the figure that the both TA and the FDI have a large proportion of ST fibres yet have high rates, and that upper limb muscles seem to have higher rates regardless of fibre-type. One factor, although not well studied, is that flexor muscles may receive preferential or facilitated corticospinal activation during voluntary contractions as compared with extensors (62). This may explain the very high rates in the TA (an ankle flexor) when compared with the gastrocnemeii (ankle extensors) and vasti (knee extensors). Knee flexor (hamstrings) maximal MUDRs are currently unknown. The biceps brachii (elbow flexor) also exhibits slightly higher maximal MUDRs than the triceps brachii (elbow extensor), however it is a possibility that the muscles of the upper limb, typically which work in a more phasic nature, exhibit greater maximal MUDRs overall than the more tonically active muscles of the lower limb. Thus, this summary figure highlights issues noted above that in a whole muscle system many factors may affect contractile quality and discharge rates differently among muscles.

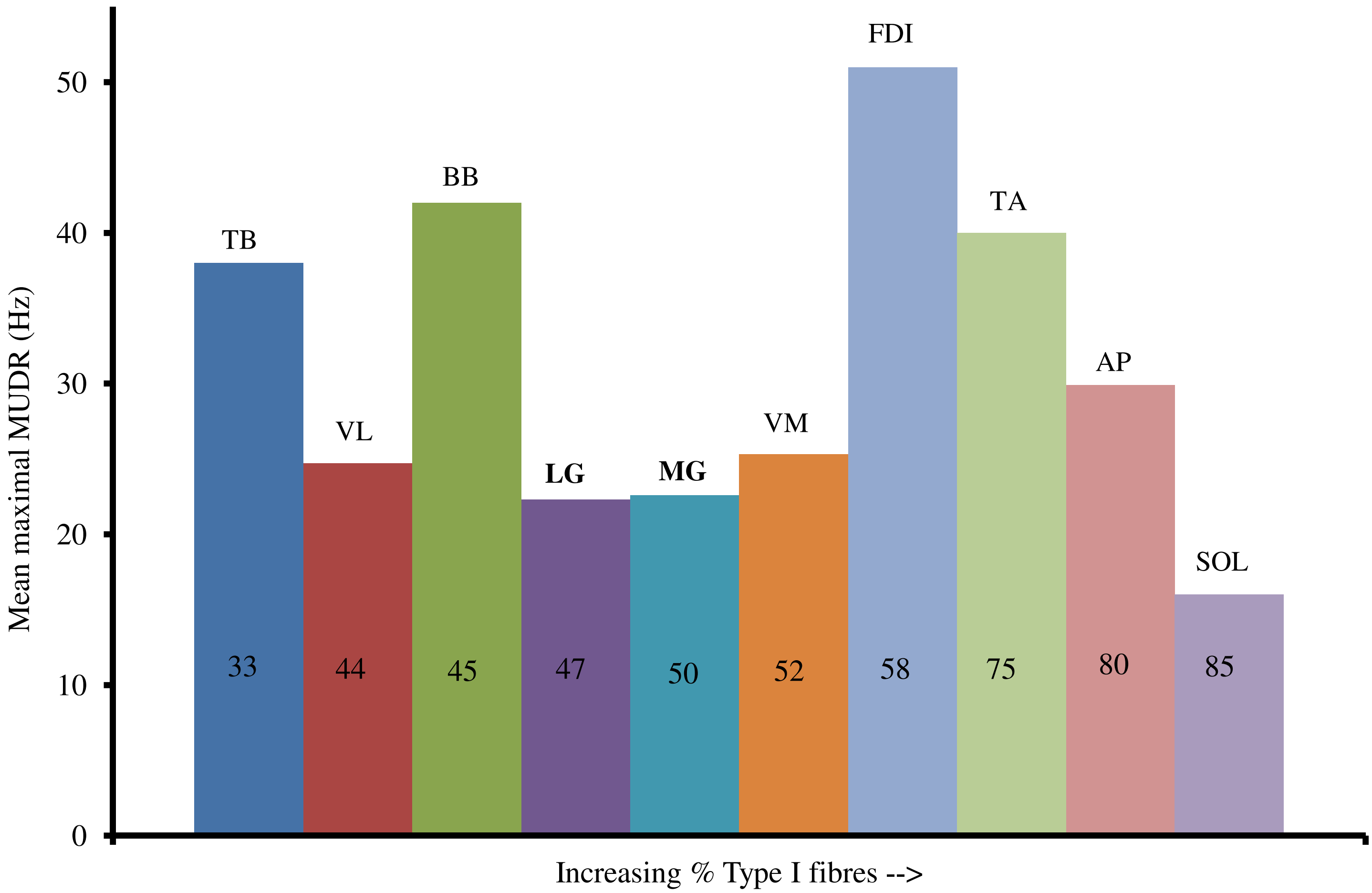

Figure 8. Maximal motor unit discharge rates for muscles with known fibre-types from the literature. MG and LG data from present study are bolded. Numbers within bars indicate % Type I fibres. It appears there is no relationship between fibre-type and mean maximal MUDRs. TB=triceps brachii; VL=vastus lateralis; BB=biceps brachii; LG=lateral gastrocnemius; MG=medial gastrocnemius; VM=vastus medialis; FDI=first dorsal interosseus; TA=tibialis anterior; AP=adductor pollicis; SOL=soleus. Fibre-type data from Johnson et al. (1973); MUDR data from Enoka et al. (2001), Dalton et al. (2009).

4.4 OTHER CONSIDERATIONS

According to Sale and colleagues it requires a stimulus of about 60Hz applied directly over the muscles, to elicit a full tetanus in the entire triceps surae while seated (68). This represents an almost 3-fold difference between the mean maximal discharge rates of the gastrocnemeii and a 3.5-fold difference of that of the soleus. However, using this method, the induced tetanus elicits a much lower amount of torque generated than the voluntary triceps surae MVC. This dissociation has also been reported in the vastus medialis (66) and adductor pollicis (60) where tetanus frequencies were 4 and 2 times greater, respectively, than the maximal MUDRs recorded.

Because there is a range in maximal discharge rates (5 - 60Hz) in the gastrocnemeii, it is apparent that there is large variability in the neural frequency output required to induce maximal torque between the different units within the entire motor unit pool when, during an MVC presumably with nearly complete activation, all units are recruited and firing at optimal maximal rates to achieve maximal torque. However when a muscle (group) is stimulated using a constant-frequency input, as is the case when determining force-frequency curves, very few units are operating at their optimal stimulation frequency. Furthermore, a voluntary MVC employs all the synergistic muscles, which in the case of the triceps surae, includes the accessory plantar flexors such as the flexor hallucis longus, flexor digitorum longus, and tibialis posterior (71).

In the present study, subjects produced a 6-8 second MVC in order to sample as many motor unit trains at maximal torque as possible without inducing fatigue. The limitation of the recording method here is that rates are sampled when the force is sustained and plateaued which requires several hundred milliseconds after the initiation of the contraction. Although previous studies on maximal MUDRs tend to use this MVC duration, there are data reported from brief, maximal ballistic-type contractions when units are first recruited that indicate that rates could briefly be higher than those recorded during the plateaued force. Seminal work with ballistic contractions by Desmedt and Godaux (1979) have identified extremely high initial discharge rates, up to 120Hz in an assortment of muscles, including the FDI and masseter (27). These rapid discharges are termed doublets, and they may serve to rapidly increase muscle force and responsiveness when first recruited. In the present study, only units identified during a steady-state MVC were analyzed and therefore brief higher discharge rates may have been missed. This may help explain some of the discrepancies found between induced frequencies required to achieve tetanus and the rates recorded during an MVC.

The total sampled motor unit pool in the present study, exhibited a positive skew (tail in positive direction) and a non-normal distribution in both the MG and LG, as the means were approximately one third of the fastest sampled discharge rates (~60Hz). In both heads, less than 15% of the sampled motor unit trains discharged at rates above 30Hz, and almost 75% discharged at rates between 15-30Hz. From the literature, some muscles (biceps brachii, adductor pollicis, soleus (5)) exhibit a normal distribution, while others (VM (66); TA (14)) are also positively skewed, thus it remains unclear whether maximal MUDRs are evenly distributed with respect to the mean, or whether there is some sampling bias due to technical recording limitations. With this uncertainty regarding the typical distribution of discharge rates during MVC, it is possible that the mean maximal MUDR in the present study could be greater than 23Hz, but it seems unlikely that they would substantially shift to more closely match the tetanic rates of stimulation reported for full tetanus. Because the average number of MUs in the gastrocnemeii muscles remains unknown, it is unclear what percentage is being sampled using this MU recording technique. Sampling from 10% of a total motor unit pool is an adequate representation (55) and thus with an average yield of 22 and 13 units per person in the MG and

LG respectively, the total number of MUs would need to be between ~130 and 220 for these muscles in order to have a representative sample. From motor unit number estimate (MUNE) studies in other limb muscles and based roughly on the size and function of the gastrocnemeii muscles this number of MUs seems reasonable (9, 19, 55) indicating that this study likely provides a representative sample.

Extrapolating findings from Cresswell and colleagues (1995) regarding surface EMG of the triceps surae during MVC at varying degrees of knee flexion, the MG and LG exhibited a 40% and 65% lower overall EMG signal with a flexed compared with an extended knee position (15). While this suggests there is decreased neural activity in the shortened gastrocnemeii, there was no difference in voluntary activation as measured by the twitch interpolation technique. There may also be inherent limitations in comparing a surface EMG signal at different joint angles. In the present study, combined with the voluntary activation results and the torque outputs that agree with the literature, it appears that the plantar flexors and particularly the triceps surae are well activated during isometric MVCs in this position. However, perhaps in the extended knee position maximal MUDRs would be higher which might contribute to the greater surface EMG signal found with an extended knee, despite previous reports that maximal MUDRs are not affected by changes in muscle length (7, 26). These studies were done on the lateral head of the triceps brachii and the tibialis anterior, which are both mono-articular muscles, and the gastrocnemeii as a bi-articular muscle may act uniquely.

With the anatomical and functional interdependence among the three heads of the triceps surae, it is difficult to ascertain the relative activation of the gastrocnemeii in any knee position. Indeed, there is evidence that the LG is not recruited during standing or with small postural perturbations, and has a recruitment threshold as high as 35x that of the soleus (35) suggesting that the lateral gastrocnemeii may add critical extra torque only during strong plantar flexion contractions. In this study, the sample of MUs from the LG, although smaller in number than the MG (Table 3), are larger when compared on a volumetric basis of muscle tissue (28, 31, 33) and therefore this muscle was well-activated with the same average maximal discharge rates as the MG. There was also a trend for subjects who had higher rates in one head of gastrocnemius to have high rates in the other head of the gastrocnemius, suggesting at maximal torque outputs, the MG and LG may behave similarly. Whether rates at various submaximal levels of contraction are different between these two muscles is not known.

4.5 SUMMARY

Maximal motor unit discharge rate for the medial and lateral gastrocnemeii were not different from each other at ~23Hz. Making up ~35% of the triceps surae, they were substantially greater than those reported for the soleus previously. Also, the rates seem to be appropriate when compared with other lower limb extensor muscles. It appears in conjunction with other limb muscles sampled to date, that maximal rates are not governed by muscle fibre-type per se suggesting that their functional role may be a more important factor in determining discharge rates of motor units during MVC. Because of the challenges of assessing contractile properties of each gastrocnemius muscle independent of the remainder of the triceps surae, it is not entirely clear whether these rates are strongly related to contractile speed. Unlike the soleus, the gastrocnemeii are often considered more phasic rather than tonic muscles in nature and require stronger plantar flexions to recruit their motor unit pool. In addition, as two-joint muscles, the gastrocnemeii also function to flex the knee joint and are active in helping control knee flexion during gait (75). My results have added to this understanding of motor unit function of the gastrocnemeii in this complicated muscle model, and have provided important baseline data from which future studies in health, exercise, fatigue, training, disease, or with knee joint position can compare.

CHAPTER 5: LIMITATIONS & FUTURE DIRECTIONS

5.1 LIMITATIONS

As with many studies across a wide range of research, it is critical to have accurately surveyed a representative sample of the entire population; in this case, motor units from each muscle. As mentioned in the 'Discussion' section, a 10% sample is considered adequate when assessing motor unit behaviour, however because the population size, that is, the number of motor units in either the medial and lateral gastrocnemeii, is currently unknown, it remains difficult to determine whether this sample was sufficient. By having the subjects return for further visits, more motor units could have been sampled thus increasing the total pool, therefore making it more representative of the entire population. However, in relation to other studies in other limb muscles it seems likely that the sample is adequate (29).

Another challenge with this model is the anatomical link between the gastrocnemeii and soleus. Therefore it is difficult to explore in humans, a more direct relationship between muscle properties and motoneuron properties in these two gastrocnemeii muscles because one cannot selectively activate in isolation and record from either one individually. For example, the twitch properties reported here are a compilation of the three heads of the triceps surae, and a plantar flexion MVC involves all of the triceps surae as well as smaller plantar flexor muscles (flexor hallucis longus, tibialis posterior, flexor digitorum longus, and fibularis longus and brevis). Stimulation applied directly to the motor point of each head of the triceps surae separately would be useful to make comparisons between motoneuron output and muscle contractile properties.

The main results of this thesis are also limited to young, healthy, recreationally active men, and may not be generalizable to other populations, such as women, older adults, or those with neuromuscular disorders.

5.2 FUTURE DIRECTIONS

Although it has been previously reported that there is no difference in maximal MUDR with changes in muscle length, these reports were from the lateral head of the triceps brachii (26) and the TA (7), both mono-articular muscles. At submaximal intensities at the same relative torques however, there are mixed reports as to whether discharge rates differ with changes in muscle length (7, 13, 26, 63). It is evident however, that the bi-articular gastrocnemeii have a complex behaviour, with changes in knee and ankle angle affecting both neural and mechanical activity. Because of its unique interaction with the soleus by acting through a common tendon, it would be advantageous to understand the maximal motor unit behaviour at varying knee and ankle positions. It would also be beneficial to identify the submaximal discharge rates and to identify the recruitment and derecruitment thresholds, with both a flexed and extended knee, throughout a range of contraction intensities.

These data of maximal rates reported here provide a baseline, to now make comparisons with special populations or adapted states such as aging, fatigue, training and disease. For example, a future study could examine the maximal discharge rates of the gastrocnemeii in older adults in order to understand potential differences in the how the triceps surae motor unit pool is affected by adult aging. Typically, maximal MUDRs decrease with age (14, 18, 39, 41, 42) however the soleus seems to be an exception (17), therefore understanding the potential differential aging process of the triceps surae would be advantageous. A future study could also examine the maximal discharge rates in the gastrocnemeii in women, both young and old in order to elucidate potential sex differences.

With changes in joint angle, there are subsequent changes in muscle architecture importantly including both fascicle length and pennation angle. It has been reported at submaximal contraction intensities in the triceps surae, that fascicle length is a better predictor of soleus and medial gastrocnemius motor unit behaviour than is joint angle per se (47). By using ultrasound, the soleus and MG muscle architecture could be measured during stronger contractions, with the sampling of maximal MUDRs, at different muscle lengths. This would test whether the previously reported relationship between fascicle length and discharge rates holds true at greater contraction intensities including at MVC.

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APPENDIX A: Ethics approval documentation

Research Western

Use of Human Participants - Ethics Approval Notice

Principal Investigator: Dr. Charles Rice
Review Number: 18097
Review Level: Full Board
Approved Local Adult Participants: 100
Approved Local Minor Participants: 0
Protocol Title: Neuromuscular control of human movement
Department & Institution: Anatomy & Cell Biology,University of Western Ontario
Sponsor: Natural Sciences and Engineering Research Council

Ethics Approval Date: July 22, 2011 **Expiry Date:** August 31, 2015

Documents Reviewed & Approved & Documents Received for Information:

Document Name	Comments	Version Date
UWO Protocol		
Letter of Information & Consent		

This is to notify you that the University of Western Ontario Health Sciences Research Ethics Board (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this HSREB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request form.

Member of the HSREB that are named as investigators in research studies, or declare a conflict of interest, do not participate in discussions related to, nor vote on, such studies when they are presented to the HSREB.

The Chair of the HSREB is Dr. Joseph Gilbert. The UWO HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Signature

Ethics Officer to Contact for Further Information

X Janice Sutherland	____ **Grace Kelly**	___ **Shantel Walcott**

This is an official document. Please retain the original in your files.

***The* University *of* Western Ontario**
Office of Research Ethics
Support Services Building Room 5150 • London, Ontario • CANADA - N6A 3K7

TO SPECIFY FHC METAL MICROELECTRODES:

CATALOG# UE 1234567890 n/n/n...

Metal micro-electrode — Base Electrode — Finishing Options — Modifier(s)

Modifier Note:
Any X's or numbers in the catalog # indicate that modifiers are required.

1 Material:

W: Tungsten
S: Stainless Steel
P: Platinum/Iridium 80% / 20%
X: Special / Specify

2 Length Range:

Total Electrode Length will be range maximum (+/- 5mm) plus termination addition unless length is specified in step 9.
S: = up to 60mm
M: = 61mm to 70mm
L: = 71mm to 145mm
V: = 146mm to 295mm

3 Shank Diameter:

C: .003" / 75µm
D: .004" / 100µm
E: .005" / 125µm
F: .008" / 200µm
G: .010" / 250µm
H: .020" / 500µm
X: Special / Specify

4 Final Taper Angle:

The last 120 microns

A: Extra Blunt
B: Rounded Extra Blunt — 30°-25°
C: Standard Blunt (1)
D: Rounded Blunt — 25°-20°
E: Standard Medium (2)
F: Rounded Medium — 20°-15°
G: Standard Fine (3)
H: Rounded Fine — 15°-10°
J: Standard Ultra-fine (4)
K: Rounded Ultra-fine — 10°-5°

Note: Fine and Ultra-fine tapers (G, H, J, K) not available with Glassed Tungsten

X: Special / Specify

Standard Tip: <1 micron dia. at tip
Rounded Tip: ~3 micron dia. at tip
120µm

5 Microelectrode Profile:

Specialty Profiles

S: Standard Profile
V: Short Convex
C: Short Concave (~0.35mm)
T: Short Thinned (70 Microns, ~4 mm)
L: Long Thinned (60% Shank dia., ~15 mm)
Note: Profile T and L not available with Glass Insulation
X: Special / Specify

6 Insulation Options:

E: Epoxylite Insulation
M: 'Max dips' Epoxylite
G: Glass, immersed tip (Pt/Ir or W, only)
Standard Insulation Length: 15mm from tip.
*See Shank Modification Note **
S: Glass, exposed tip (Pt/Ir, only)
Standard Insulation Length: 15mm from tip
*See Shank Modification Note **
N: No Insulation
X: Special / Specify

G: Glass Insulation, No Ridge Where Glass Starts
E: Epoxylite Insulation Standard ~10% O.D increase
S: Glass Insulation Definite Ridge Where Glass Starts
M: Epoxylite Insulation Maximum Dips ~20% O.D increase

7 Impedance:

Measured at 1000Hz

A: 50k Ω 100k Ω	1: 1M Ω+/-.2M Ω
B: 300k Ω 500k Ω	2: 2M Ω+/-.4M Ω
C: 800k Ω 1.2M Ω	3: 3M Ω+/-.6M Ω
D: 2M Ω 4M Ω	4: 4M Ω+/-.8M Ω
E: 5M Ω 7M Ω	5: 5M Ω+/-1M Ω
F: 8M Ω 10M Ω	6: 6M Ω+/-1M Ω
G: 13M Ω 15M Ω	7: 7M Ω+/-1M Ω
H: 18M Ω 20M Ω	8: 8M Ω+/-1M Ω
J: 22M Ω 25M Ω	9: 9M Ω+/-1M Ω
K: 2M Ω 5M Ω	0: 10M Ω+/-1M Ω
L: 9M Ω 12M Ω	X: Special / Specify
N: No Zap (High)	

8 Tip Conditioning:

N: None, Standard
P: Platinum Black Discontinued, replaced by D.ZAP
T: Platinum Black Discontinued, replaced by D.ZAP
X: Special / Specify

Note: D.ZAP is standard for impedances 1.2M Ω and lower (A, B, C, 1)

9 Shank Modifications:

Please provide required specifications for any shank modifications

Note: For glass insulated electrodes a shank modification of #4 Shank Enlargement with Polymide Tubing is the default. Modifications #3, 5, and 6 also available. Standard extension (glass insulation) is 15mm. Other lengths may be specified between 5 and 20mm. Polymide length will be total length minus the extension and termination.*

N: None, standard
1: Cut electrode to a specified length +/- 2mm, (specify electrode length in mm)
NOTE: If pin termination is specified in 10, total electrode length will be specified length + pin addition. (see UEWMGBSE5N1M,65mm example below)

Rounded Extra Blunt Taper, Standard Profile — Male 220-PO2 Termination
Epoxylite insulated; Z = 5 Meg-ohms
Tungsten microelectrode, 0.010" dia, 65 mm
Total Length 73 mm

2: Heatshrink over Termination. Specify either dimension shown below in diagram
Extension Specify Length — Heat Shrink 15mm or Specify — Typical termination: WPI #220-PO2

3: Shank Enlargement with Polyimide and Stainless Steel Tubing. Specify dimensions shown below in diagram. Tubing sizes are indicated on the chart unless otherwise specified
4: Shank Enlargement with Polyimide Tubing Only. Specify dimensions shown below in diagram w/ exception of SS Tube length. Tubing sizes are indicated on the chart unless otherwise specified

Extension 10mm, or specify — Polyimide Tube Specify Length — Exposure 10mm, or specify
Any Micro-electrode — Stainless Steel Tubing Insulated from Electrode Specify Length

Shank Diameter vs. Final Diameter with and without Stainless Steel Tubing
.003" / .013" / .005"
.004" / .018" / .008"
.005" / .020" / .009"
.008" / .028" / .013"
.010" / .032" / .016"

5: Shank Extension up to 150mm, specify dimensions shown in digram below, Tubing sizes are indicated on the chart unless otherwise specified
6: Shank Extension from 151mm to 300mm, specify dimensions in digram below, Tubing sizes are indicated on the chart unless otherwise specified

Extension 10mm, or specify — Polyimide Tube Specify length — Exposure 10mm, or specify
Epoxy Seal — 3mm — Stainless Steel Tube — Polyimide Tubing

Shank Diameter vs. Tubing used and Polyimide diameter
.003" / 31 ga / .013"
.004" / 30 ga / .016"
.005" / 29 ga / .016"
.008" / 26 ga / .024"
.010" / 23 ga / .030"

X: Special / Specify

10 Termination Options:

Special terminations available upon request, see Microneurography Needle Data sheet for additional options, specify X.

D: No pin, Insulation not stripped
E: No Pin, Insulation stripped 5mm
G: No Pin, Insulation stripped 10mm (X mm)
H: No Pin, Insulation stripped 15mm
J: No Pin, Insulation stripped 20mm
M: Male 220-P02 (Standard) — 2.4 mm — 8mm
F: Female 220-S02

PIN ADDITION: WPI pins add 8mm to the length of the electrode

C: "Cut off" Chronic termination, see diagram: — 2mm — Exposure: cut off after implantation — Epoxy cap — 38ga insulated copper lead, stripped and tinned

K: Friction Fit Extension — 2mm — Extension: slide tube off and cut after implantation — SS Tube, 50 mm — Bent Electrode to prevent SS tube from slipping off — Epoxy cap — 38ga insulated copper lead, stripped and tinned

L: Floating termination, see diagram: — Epoxy Cap — 38ga insulated copper lead, stripped and tinned x 50um

X: Special (not listed)

(K3-73B) V\L022\CAD cat pgs\UE 20130314

FHC
Neural microTargeting™ Worldwide

CURRICULUM VITAE

Education:

The University of Western Ontario: 2012 – 2014
London, ON, Canada
MSc. Kinesiology: Neuromuscular Physiology
Thesis: Max MUDRs of Gastrocnemeii in Humans
Supervisor: Dr. Charles L. Rice

Wilfrid Laurier University: 2008-2012
Waterloo, ON, Canada
BSc. Honours in Kinesiology

Research Contributions:

Summary:
Articles Published: **1**
Abstracts Published and Presented: **4** (presenting author: 4)

Articles Published in Refereed Journals

1. McKinnon N.B., **Graham, M.T**. & Tiidus, P.M. (2012). Effect of creatine supplementation on muscle damage and repair following eccentrically-induced damage to the elbow flexor muscles. *J Sports Sci Med. 11(4):653-9.*

Abstracts Presented:

1. **Graham, M.T**, Smith, C.B., Dalton, B.H., Rice, C.L. Maximal motor unit firing rates in the human gastrocnemeii. (2014). *Med Sci Sp and Ex. Suppl. 46, No. 5 S147*
    - Poster presented at ACSM 2014; Orlando, FL, May 27th – 31st
2. **Graham, M.T.,** Allen, M.D., Paturel, J.R., Choi, I.H., Rice, C.L. Neuromuscular properties and fatigability of knee extensors in human coronary artery disease. *APNM 2013, 38(10): 1003-1091.*
    - Poster presented at CSEP 2013; Toronto, ON, Oct 16th - 19th.
3. **Graham, M.T.,** Allen M.D., Choi, I.H., Paturel, J.R. Neuromuscular strength and fatigability of knee extensors in human coronary artery disease. Exercise Neuroscience Group.
    - Oral communication at ENG 2013; Oshawa, ON, June 13th – 14th

4. **Graham, M.T.,** McKinnon N.B., Tiidus, P.M. Effect of creatine supplementation of muscle damage and repair following eccentrically-induced damage to the elbow flexor muscles. *APNM 2012: 37(S1):S1-S38*
    - Poster presented at CSEP 2012; Regina, SK, Oct 10^{th} – 13^{th}.