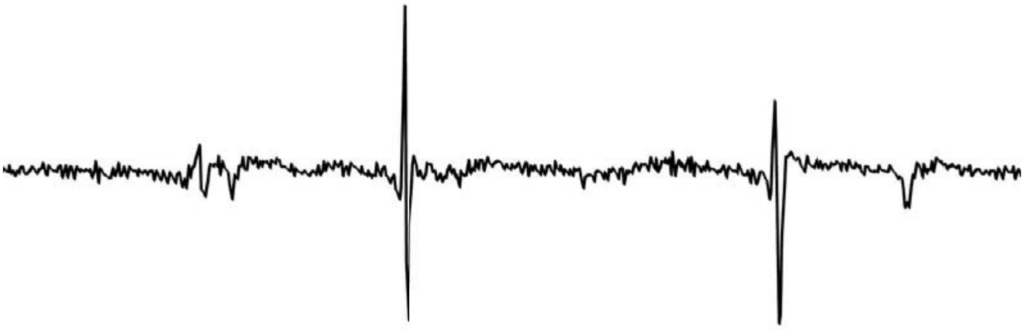
STUDIES IN SPORT, PHYSICAL EDUCATION AND HEALTH

189

Jouni Kallio

Motor Unit Activation and Spinal Excitability in Young and Elderly Males During Isometric and Dynamic Muscle Actions



JYVÄSKYLÄN YLIOPISTO

STUDIES IN SPORT, PHYSICAL EDUCATION AND HEALTH 189

Jouni Kallio

Motor Unit Activation and Spinal Excitability in Young and Elderly Males During Isometric and Dynamic Muscle Actions

Esitetään Jyväskylän yliopiston liikuntatieteellisen tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston Vanhassa juhlasalissa S 212
helmikuun 9. päivänä 2013 kello 12.

Academic dissertation to be publicly discussed, by permission of
the Faculty of Sport and Health Sciences of the University of Jyväskylä,
in building Seminarium, Auditorium, on February 9, 2013 at 12 o'clock noon.

UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2013

Motor Unit Activation and Spinal Excitability in Young and Elderly Males During Isometric and Dynamic Muscle Actions

STUDIES IN SPORT, PHYSICAL EDUCATION AND HEALTH 189

Jouni Kallio

Motor Unit Activation and Spinal Excitability in Young and Elderly Males During Isometric and Dynamic Muscle Actions

UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2013

Editors
Taija Juutinen
Department of Biology of Physical Activity, University of Jyväskylä
Pekka Olsbo, Harri Hirvi
Publishing Unit, University Library of Jyväskylä

Cover picture by Jouni Kallio

URN:ISBN:978-951-39-5060-6
ISBN 978-951-39-5060-6 (PDF)

ISBN 978-951-39-5059-0 (nid.)
ISSN 0356-1070

Jyväskylä University Printing House, Jyväskylä 2013

Having all the answers just means you've been asking boring questions
J. Comeau

ABSTRACT

Kallio, Jouni
Motor unit activation and spinal excitability in young and elderly males during isometric and dynamic muscle actions
Jyväskylä: University of Jyväskylä, 2013, 65 p.
(Studies in Sport, Physical Education and Health
ISSN 0356-1070; 189)
ISBN 978-951-39-5059-0 (nid.)
ISBN 978-951-39-5060-6 (PDF)

The aim of the present study was to examine the effects of muscle contraction type and age on motor unit (MU) control in the soleus muscle. The main purposes were to: 1) gain information about MU control in muscles and conditions more relevant to balance and locomotion by being able to record single MU firings in dynamic contractions of the soleus, 2) to examine the effects of aging and contraction type on H-reflex excitability and MU activation patterns in dynamic and isometric contractions, 3) to compare motor unit discharge rate (MUDR) and variations in both force and discharge rate in isometric contractions between the age-groups and 4) to investigate the age-related changes to MUDR of the soleus muscle in dynamic contractions and at high force levels. The current results showed a clear age-difference in the soleus MUDR in isometric contractions. The longer single muscle twitch duration in elderly subjects (OLD) suggests that the neuromuscular system may tune the discharge rate to match the changes in MU physiology. Differences in MUDR between both the age-groups and contraction levels were larger at forces close to MVC. It appears that coinciding changes in MU recruitment make the comparison of MUDR more difficult. This is supported by our analysis of MU pairs, which showed a steeper slope in MUDR with increasing force compared to a pooled analysis of all units. This may explain partially why no age-differences were found in dynamic contractions at the measured low force levels. Also the ability to hold a given isometric steady force-level was lower in OLD, but MUDR variation was not significantly different. Based on previous findings, it can be assumed that MUDR is only one of several factors that contribute to the lower steadiness in elderly. In line with previous literature, the H-reflex excitability was lower in OLD, which may be an indication of increased presynaptic inhibition. In dynamic measurements, concentric (CON) contractions always required a higher MUDR compared to eccentric or isometric muscle actions. The higher MUDR in CON can only partially be explained by the lower maximal force-capacity, since the MUDR was highest in CON even when muscle activation was matched between conditions. The present study displays age-related changes in the neuromuscular system at many functionally important levels. All these changes may affect balance control and thus the ability to avoid falling and injuries.

Keywords: aging, electromyography, motor unit, dynamic contraction, neuromuscular control

Author's address	Jouni Kallio Department of Biology of Physical Activity PL 35, 40014 University of Jyväskylä jouni.kallio@jyu.fi
Supervisors	Prof. Vesa Linnamo Department of Biology of Physical Activity University of Jyväskylä
	Prof. Emeritus Paavo Komi Department of Biology of Physical Activity University of Jyväskylä
Reviewers	Prof. Jacques Duchateau Laboratory of Applied Biology Université libre de Bruxelles
	Prof. Andrew Cresswell The Centre of Clinical Research Excellence in Spinal Pain, Injury and Health The University of Queensland
Opponent	Prof. Catherine Disselhorst-Klug Institute of Applied Medical Engineering RWTH Aachen University

ACKNOWLEDGEMENTS

This study was carried out in the Neuromuscular Research Center, Department of Biology of Physical Activity, University of Jyväskylä. The journey to reach this goal has been long, and my appreciation to all the helpful people that have aided me through the years is great.

I would like to thank my supervisor Prof. Vesa Linnamo for your patient guidance over the years. Your door has always been open for me and your help has never been limited or delayed, even when you have been in the middle of other work or on a vacation. I'm really thankful for all the things I have learned from you over the years. You are a fair man with a big heart, and I am glad I've gotten to know you both on and off "the court".

I am honored to have had Prof. Emeritus Paavo Komi as my other supervisor. His example and legacy has given all of us PhD-students in biomechanics a head start in research and his unprecedented experience and knowledge has helped me enormously. I'm especially thankful to him for introducing the international field of science to us students. His worldwide network has opened doors to many valuable contacts and friendships.

I want to thank Prof. Janne Avela for his help, both in my PhD project and in my two assignments as an organizer of scientific congresses. Getting a chance to work in an organizing team with Professors Avela and Komi, Dr. Jyrki Komulainen and Director Eino Havas gave me a unique opportunity to broaden my knowledge and view of the international scientific community.

I was very fortunate to get the opportunity to cooperate with our skilled colleagues in Denmark. I am grateful to Professor Karen Søgaard for tutoring me on motor unit analysis and later, for her valuable input on the manuscripts. I would also like to thank Henrik Baare Olsen for being my tech-support with the Daisy-program and the entire team at the National Institute of Occupational Health in Copenhagen for welcoming me so generously during my stay.

I would also like to acknowledge the great input of my other co-authors. The studies could not have been completed without their help. In particular, I would like to thank Prof. Toshio Moritani for his help in our first steps in the field of motor unit analysis and Dr. Harri Selänne for covering the medical aspects of working with the elderly. Last but not least, I want to thank Mr. Mika Kanervo, with whom I started my first measurements. Working with him made the beginning of this project much easier, and much more fun.

I wish to express my gratitude to Professors Andrew Cresswell and Jacques Duchateau for the valuable comments and criticism on the manuscript of this thesis, and to Prof Taija Finni and Dr. Neil Cronin for the proofreading. I also want to thank my current boss Dr. Tuija Tammelin for her important support in the final exhausting meters of the thesis.

My decade in the department has given me the pleasure of working with many great professionals. I would especially like to thank Prof. Taija Finni for her input in my learning-process. Her dedication to research and teaching is an inspiration and her helpfulness is a joy. Great thanks also to Dr. Mikko Virma-

virta and Mr. Teemu Pullinen and Heikki Kyröläinen for the co-work in educating the next generation of PhD students.

Working with fellow PhD students has been the most enjoyable part of this project. We have shared many unforgettable experiences, both in the lab and in our trips to international congresses. Learning from, and with you has been a real pleasure. I want to thank Dr. Masaki Ishikawa for all the help and the great hospitality you have shown to all of us who have visited your lab in Osaka. Thanks to my first office roommate Sami Kuitunen for your guidance in the beginning of my project. Work is great if you can do it with friends: I'm lucky to have crossed paths with three gentlemen, with whom my discussions in science and beyond have been truly inspirational. Simon, Neil and Jussi, I'm honored to have you as friends.

Many PhD-students share my passion for research. However I am yet to meet a person who would enjoy applying for funding. I have been fortunate to work with a full-time job in a lab that is internationally envied for its equipments. I want to thank the head of the department, Prof. Keijo Häkkinen and all the other professors for their hard work on providing us students with an excellent environment to create and learn. I am also grateful for the financial support I have received from the University of Jyväskylä and the TULES graduate school.

The researchers could not be productive without the support of an excellent cast of people, both in the lab and in the offices. I want to give a big hug to Katja and Minna for all the times you have rescued me from the wheels of bureaucracy! I owe a lot to the lab team of Mrs. Sirpa Roivas, Mrs Pirkko Puttonen, Mr. Markku Ruuskanen, Mr. Seppo Seppälä, Mr Jouni Tukiainen and Mr. Terho Kaipio-Sarja: from handcrafted wire-electrodes to weight pulleys made of old moped parts, your skill and creativity has been essential to my research. Further, I want to acknowledge the input of Dr. Matt Holmes in executing the measurements and of all the volunteers for dedicating their time for our project. Big thanks also to Mr. Marko Havu for the technical and theoretical support on just about any difficult topic I've faced. You are a true renaissance man!

Finally, I want to thank my family for giving me a solid base from which to reach for my goals. I can never repay the love and care I have received from my parents. My interest in science comes from a culture of curiosity and discussions that my family enjoyed at home, and my dad is still the one whose help I can always rely on when challenges arise. I am truly grateful for having a loving sister, Kaisa, with whom to share the best and the worst in life.

When I started my PhD, I had just begun dating a fellow biomechanist – who since then has become my wife and a mother to our three precious children. You, Siru, are the love of my life, and having you by my side brings serenity to the fascinating chaos that life – and our lively offspring offers.

Jyväskylä, January 2013
Jouni Kallio

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles, which are referred to in the text by their Roman numerals.

I Kallio J., Avela J., Moritani T., Kanervo M., Selänne H., Komi P. & Linnamo V. 2010. Effects of ageing on motor unit activation patterns and reflex sensitivity in dynamic movements. *Journal of Electromyography & Kinesiology* 20 (4), 590-598.

II Kallio, J., Søgaard, K., Avela, J., Komi, P., Selänne, H. & Linnamo, V. 2012. Age-related decreases in motor unit discharge rate and force control during isometric plantar flexion. *Journal of Electromyography and Kinesiology* 22 (6), 983-989.

III Kallio, J., Søgaard, K., Avela, J., Komi, P. V., Selänne, H. & Linnamo, V. 2013. Motor unit firing behaviour of soleus muscle in isometric and dynamic contractions. *PLos ONE* (accepted for publication).

III Kallio, J., Søgaard, K., Avela, J., Komi, P. V., Selänne, H. & Linnamo, V. 2013. Effects of ageing on Soleus motor unit discharge rate in dynamic movements. *Journal of Applied Physiology* (submitted for publication).

CONTENTS

ABSTRACT
ACKNOWLEDGEMENTS
LIST OF ORIGINAL PUBLICATIONS
CONTENTS

1 INTRODUCTION 11

2 REVIEW OF THE LITERATURE 13
  2.1 Muscle contraction 13
  2.2 Motor unit 15
    2.2.1 Motor unit structure 15
    2.2.2 Motor unit activation 16
  2.3 Effects of aging 17
  2.4 Intramuscular EMG- recordings and signal decomposition 19

3 PURPOSE OF THE STUDY 21

4 RESEARCH METHODS 23
  4.1 Subjects 23
  4.2 Experimental design 24
    4.2.1 Experiment 1 (I, II) 25
    4.2.2 Experiment 2 (III, IV) 26
  4.3 Recording procedures and analyses 27
    4.3.1 Force and angle 27
    4.3.2 EMG 28
    4.3.3 Electrical stimulation 32
    4.3.4 Statistical methods 32

5 RESULTS 34
  5.1 Effects of aging 34
    5.1.1 Range of motion 34
    5.1.2 Force and activation levels 34
    5.1.3 Motor unit firing 36
    5.1.4 Steadiness 38
    5.1.5 H-reflex and twitch properties 39
  5.2 Effect of muscle contraction type and intensity 39
    5.2.1 Force and activation levels 39
    5.2.2 Motor unit firing 40
    5.2.3 H-reflex 41

6 DISCUSSION 42
  6.1 Effects of age 43

6.1.1 Motor unit activation 43
6.1.2 Variability in force and discharge rate 45
6.1.3 H-reflex and twitch properties 46
6.2 Effects of contraction type on motor unit activation 46
6.2.1 Isometric contractions 46
6.2.2 Dynamic contractions 47
6.3 Limitations and practical implications of the study 49

7 PRIMARY FINDINGS AND CONCLUSIONS 52

YHTEENVETO 54

REFERENCES 56

1 INTRODUCTION

Muscle force is controlled via activation of motor units (MU) that consist of an alpha-motorneuron and the muscle fibers it connects to. With increasing activation, force increases with newly recruited units and the more rapid firing of the already active units. It is commonly agreed that at least in isometric contractions, the units are recruited and derecruited in an orderly manner, beginning from the smaller, more durable, and ending with the largest, most powerful, but also the most fatiguable units. This so-called size principle, as introduced by Henneman (Denny-Brown 1929, Henneman , Somjen & Carpenter 1965, Henneman, Somjen & Carpenter 1965), has also been consistently shown to take place in dynamic contractions (for review, see Chalmers 2008) with a few exceptions (Nardone, Romano & Schieppati 1989, Howell et al. 1995). In contrast, the motor unit discharge-rates have been found to be higher in concentric compared to isometric or eccentric contractions at equal submaximal force levels (Howell et al. 1995, e.g. Sogaard et al. 1996, Pasquet, Carpentier & Duchateau 2006).

Aging is characterized by changes in the neuromuscular system that not only diminish the capability for independent living, but also increase the risk of accidents and serious injuries, as the ability to recover from sudden balance disturbances is reduced. A decline in muscle mass (Cunningham et al. 1987, Doherty, Vandervoort & Brown 1993) especially in the population of the fast Type II fibers (Brown, Strong & Snow 1988) and the accompanying decrease in muscle activation lead to a decrease in force production, which is most prominent in fast contractions (Häkkinen et al. 1998). Motor control also seems to get worse, at least in some muscles (Laidlaw, Bilodeau & Enoka 2000, Tracy et al. 2005) and at low activation levels (Galganski, Fuglevand & Enoka 1993, Tracy & Enoka 2002).

Due to a decreased number of fast MUs and changes in plasticity it is likely that also the MU activity (recruitment and rate coding) would differ between young and older subjects. Based on previous results it appears that the age-related changes in motor unit discharge rate (MUDR) are muscle-dependent and most prominent in distal muscles, in the oldest subjects, and at higher relative force levels. It seems that the decreased MUDR would be an adaptation to the increased twitch duration to optimize force generation. As the twitch duration increases with age, tetanus theoretically could be achieved with lower discharge rates. (For review, see Roos, Rice & Vandervoort 1997).

A large body of knowledge exists about motor unit control in different muscles, but despite their importance for locomotion and balance, only a few studies have investigated how the units function in plantarflexors (Mochizuki, Ivanova & Garland 2005, Bellemare et al. 1983) and how aging affects them (Mochizuki, Ivanova & Garland 2005, Dalton et al. 2009, Dalton et al. 2008). As most daily activities involve dynamic contractions, and as it has been shown that age-related differences in balance are more pronounced in dynamic conditions than in static stance (Piirainen et al. 2010), it is of importance to extend the research on the soleus muscle motor unit control also to dynamic tasks. Therefore the purpose of this thesis was to extend the current knowledge on motor unit control to the soleus muscle in dynamic contractions, and to investigate the effects of aging on this important plantarflexor muscle.

2 REVIEW OF THE LITERATURE

2.1 Muscle contraction

The amount of force a given muscle can produce is dependent on the muscle length and the velocity of the contraction. According to the sliding filament hypothesis, developed by A.F. Huxley and colleagues in the 1950s, thick and thin filaments move past each other in muscle contractions. The most common theory suggests that force is produced via cross-bridges forming between the thick and thin filaments (Rayment et al. 1993). The force produced by each fibre is a function of the number of cross-bridges formed between the filaments. The muscle length determines the amount of possible actin-binding sites and therefore the maximal number of cross-bridges. At intermediate levels the overlap between filaments is optimal, and force production at its peak (Gordon, Huxley & Julian 1966). However, the force-length relationship is also affected by the passive elements in the muscle. These elements (connective tissue and cytoskeleton) that resist the lengthening of the muscle produce force regardless of muscle activation. The total force is therefore the sum of these active and passive elements. (Enoka 2008, Kandel, Schwartz & Jessell 2000).

For the topic of this thesis, the more important factor affecting force production is the velocity of the contraction. If the external force is equal to the force produced by the muscle, the length of the muscle stays constant and the contraction is called isometric. In many natural contractions the muscle force is either larger than the external resistance, enabling it to shorten (concentric), or smaller, causing it to lengthen (eccentric). The faster the muscle is shortening, the less force it is produced due to the missed potential cross-bridge binding sites during the movement. When the shortening velocity of a muscle fiber exceeds the maximal cycling rate of the cross-bridges the force becomes zero (Figure 1). In contrast, the force produced by single muscle fibers is higher during eccentric than in isometric contractions, possibly due to the stretching of incompletely activated sarcomeres, a higher force production of each cross-bridge cycle and a faster reattachment phase (Proske & Morgan 2001, Lombardi & Piazzesi 1990). (Enoka 2008, 230, Kandel, Schwartz & Jessell 2000, 683).

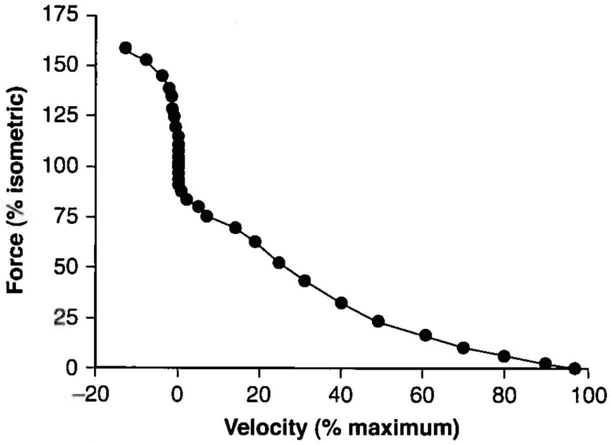


FIGURE 1 Force-velocity relationship. From (Edman 1988) in (Enoka 2008, 230).

The abovementioned differences in cross-bridge function also affect force production at the whole muscle level. The amount of neural activation required to produce a given muscular force in submaximal contractions is dependent on muscle action type. In eccentric (ECC) contractions equal force is produced with lower, and in concentric (CON) with higher levels of neural activity compared to isometric (ISO) conditions (Bigland & Lippold 1954). Conversely, with matched neural activation, the force is highest in ECC and lowest in CON (Katz 1939). In maximal contractions the force output has been shown to be largest either in ECC (Linnamo, Strojnik & Komi 2002, Komi 1973) or in ISO (Komi et al. 2000, Seger & Thorstensson 2000, Seger & Thorstensson 2000, Singh & Karpovich 1966). Although muscle fibers (Edman, Elzinga & Noble 1978) and whole muscle groups (Linnamo, Strojnik & Komi 2002, Komi et al. 2000, Linnamo, Strojnik & Komi 2006) produce more force when stretched following isometric preactivation, neural inhibition during maximal ECC contractions may cancel this advantage (Seger & Thorstensson 2000, Westing, Seger & Thorstensson 1990).

Compared to isometric conditions, eccentric contractions are also associated with a decrease in maximal voluntary activation (Amiridis et al. 1996) and reflex excitability (Romano & Schieppati 1987, Nakazawa, Yamamoto & Yano 1997). The differences in the activation strategy may be related to the lower motorneuron pool excitability in ECC, as indicated by a decrease in the electrically induced Hoffmann (H-) reflex. It seems that both presynaptic inhibition and homosynaptic postactivation depression increase in eccentric contractions (Romano & Schieppati 1987, Hultborn et al. 1996, Pinniger et al. 2001).

2.2 Motor unit

2.2.1 Motor unit structure

Each motor neuron activates several muscle fibers, forming the smallest controllable section of the muscle, the motor unit. The motor units can be classified to different types based on structure or function (Figure 1). When divided to groups based on the morphological features, the smallest units have less muscle fibers and produce less force, but are more fatigue resistant than the larger units (Enoka 2008). The most commonly used classification is based on the histochemical analysis of the muscle fiber myosin ATPase enzyme (Barany 1967). The type I fibers are usually called slow-twitch, and the type II fibers fast-twitch muscle fibers. All fibers within a unit are the same type (Edstrom & Kugelberg 1968). These units vary in size and type within the muscle, and the proportions of the different units are defined by genetics and training, both within and between individuals. For example, 80% of the motor units in soleus are type I, whereas in the lateral gastrocnemius more than 50% of the units are type II (Trappe et al. 2001, Johnson et al. 1973).

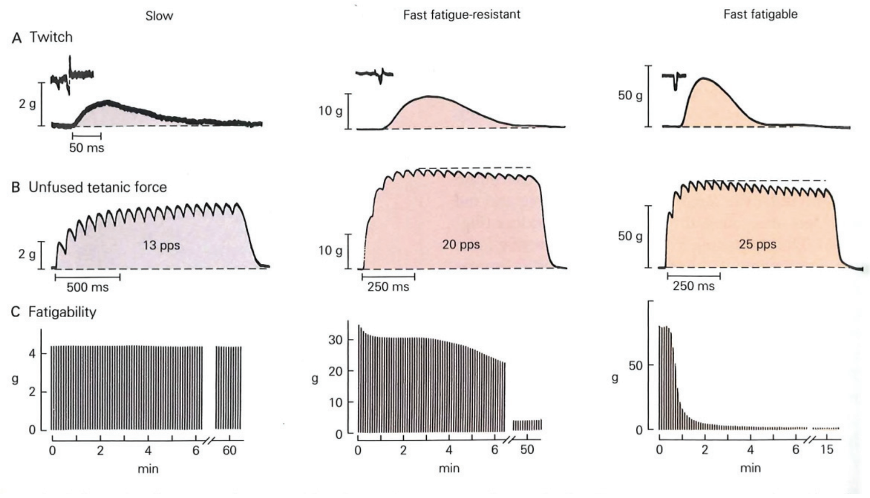


FIGURE 2 Slow, fast fatigue resistant, and fast fatigable motor units. The slow units have the lowest and slowest force-production in both muscle twitch (A) and in unfused tetanic contraction (B), but are the least fatigable (C). In contrast, the fast fatigable units have the highest and quickest force production in both twitches and tetanic contractions, but are the least resistant to fatigue. The fast units also require a higher motor unit discharge rate in order to achieve a tetanic contraction (B). From (Burke et al. 1973) in (Kandel, Schwartz & Jessell 2000, 684).

2.2.2 Motor unit activation

In a muscle contraction force is controlled by the amount of active motor units and by the rate at which the active units are discharging (Adrian & Bronk 1929). When force is increased and decreased in a ramp contraction, motor units are normally recruited in an orderly fashion and derecruited in reverse order (Figure 3). This so called Henneman's size principle states that the smallest and weakest units are always recruited first and the largest, fastest units last (Denny-Brown 1929, Henneman , Somjen & Carpenter 1965, Henneman, Somjen & Carpenter 1965). When looking at force production, modeling studies have estimated the contributions of recruitment and rate coding to be 25% and 75%, respectively (Barry et al. 2007, Fuglevand, Winter & Patla 1993).

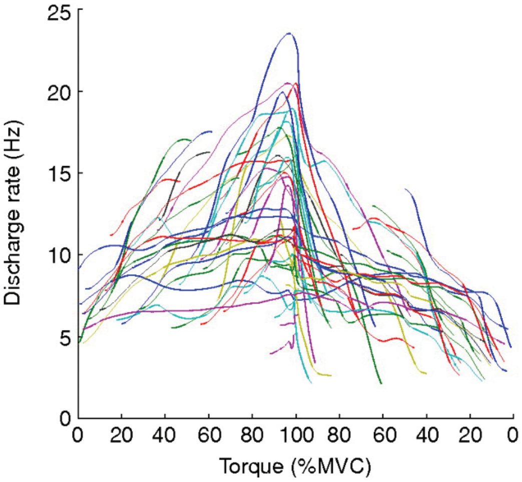


FIGURE 3 Recruitment, derecruitment and rate coding of motor units in a soleus ramp contraction with torque increase up to 100% MVC (Oya, Riek & Cresswell 2009).

The activation level at which all units have been recruited varies between muscles. The upper threshold for recruitment can be as low as 50%, like in adductor pollicis (Kukulka & Clamann 1981) and can extend to almost MVC, like in soleus (Oya, Riek & Cresswell 2009). It seems that the MUDR properties vary between muscles according to their physiological characteristics, and that in muscles like soleus with a high proportion of slow-twitch fibers, the capability of force increase with rate coding is limited (Bellemare et al. 1983, Oya, Riek & Cresswell 2009, Gydikov & Kosarov 1974).

The discharge rate of motor units is not constant even in isotonic contractions, but rather varies from discharge to discharge. This variation in the interspike intervals of successive discharges is largest at lowest rates and decreases as the rate increases (Person & Kudina 1972). The variation in discharge rate affects the steadiness of the force production especially at low force levels (Galganski, Fuglevand & Enoka 1993). Another form of variation, called double discharge, occurs sometimes at the beginning of activation, as a unit discharges twice with a very short (<10ms) interspike interval (Garland & Griffin 1999).

Due to the technical challenges of recording during dynamic contractions, most studies investigating single motor unit activation have concentrated on isometric contractions. However, the effect of contraction type on the neural activation strategy of the muscle can also be seen at the motor unit level. The recruitment thresholds have been reported to be lower in dynamic compared to ISO actions (Ivanova, Garland & Miller 1997, Tax et al. 1989, Linnamo 2003). A few studies have even found signs of deviation from the orderly recruitment of motor units in eccentric contractions (Nardone, Romano & Schieppati 1989, Howell et al. 1995), although there is a large body of evidence in both upper and lower limb muscles supporting the size principle also in ECC (Sogaard et al. 1996, Pasquet, Carpentier & Duchateau 2006, Tax et al. 1989, Bawa & Jones 1999, Stotz & Bawa 2001, Christensen et al. 1995, Christova & Kossev 2000, Garland et al. 1996, Kossev & Christova 1998a). (For review, see Chalmers 2008).

Also the motor unit discharge rates (MUDR) have been found to differ between contraction types. The earlier studies comparing muscle contraction types in dynamic movements have mostly been performed in upper extremity muscles and have shown the largest motor unit discharge rates (MUDR) in concentric compared to eccentric and isometric contractions. These studies examined the elbow flexors (Sogaard et al. 1996, Tax et al. 1989, Kossev & Christova 1998a, Moritani, Muramatsu & Muro 1987) and extensors (Del Valle & Thomas 2005), wrist flexors (Sogaard et al. 1998), the first dorsal interosseus (Howell et al. 1995, Laidlaw, Bilodeau & Enoka 2000), and these results were confirmed in studies on the knee extensors (Altenburg et al. 2009) and tibialis anterior (Pasquet, Carpentier & Duchateau 2006).

2.3 Effects of aging

Aging is associated with a decrease in muscle force and power, beginning after the age of 50 (Cunningham et al. 1987, Doherty, Vandervoort & Brown 1993). This decrease is in large part due to a loss of muscle mass (sarcopenia), which is caused by a progressive decrease in the number of muscle fibers (Brown, Strong & Snow 1988, Frontera et al. 1991). The decrease in fiber number is more pronounced in type II units (Frontera et al. 1991, Lexell 1993), which leads to major decreases, not only in maximal force, but also in fast force production (Häkkinen K. et al. 1998). The predominant loss of type II fibres is counteracted with MU remodeling that leads to an increase in MU size (Larsson 1995, Andersen, Terzis & Kryger 1999). The functional significance of aging is not limited to the decrease in the maximal level of force or power production. The optimal use of the neuromuscular system is also dependent on sufficient control of muscle activation. One way to assess the differences in motor control is to measure how accurately the subjects are able to hold a given submaximal target force, and how much the motor unit discharge rate varies during the contraction. Both of these variations are dependent on several factors, including activation level, the muscle tested, and age. Some of the previous studies with elderly subjects have shown a decrease in force control, while others have not. It seems that age-related differences are more prominent in small (Laidlaw, Bilodeau & Enoka 2000, Tracy et al. 2005) rather than large muscles (Graves, Kornatz & Enoka 2000, Christou & Carlton 2002), at low activation levels (Galganski, Fuglevand & Enoka 1993, Tracy & Enoka 2002), and with visual feedback (Sosnoff & Newell 2006b) rather than without (Barry et al. 2007). Tracy (Tracy 2007) has shown an age-related decrease in plantarflexor steadiness, which has subsequently been found to be related to diminished postural control (Kouzaki & Shinohara 2010).

The functional and physiological changes in the muscle are coupled with modifications in muscle activation, such as possible decreases in maximal voluntary activation (Klass, Baudry & Duchateau 2007) and changes in the degree of agonist/antagonist coactivation (Häkkinen K. et al. 1998). The literature on the age-related changes at the spinal level, like the amount of presynaptic inhibition (PI), is somewhat conflicting (Burke et al. 1996, Delwaide 1973, Koceja & Mynark 2000, Morita et al. 1995, Butchart et al. 1993). For example, an increase in PI was found in the study by Koceja & Mynark 2000, while Butchart and coworkers (Butchart et al. 1993) reported equal or even lower values in the elderly. However, evidence on the reduced ability to modify afferent input to the motorneuron pool via PI in elderly is more uniform (Chalmers & Knutzen 2000, Koceja, Markus & Trimble 1995). Both contraction type and aging have been found to cause changes in H-reflex excitability. H-reflex is partly affected by PI that may also be an important factor in the control of MU activation. It is not clear, however, how aging affects MU firing properties and H-reflex excitability in dynamic contractions.

Previous studies on aging have found eccentric strength to decrease less than isometric or concentric (Porter, Vandervoort & Kramer 1997, Poulin et al. 1992, Vandervoort & McComas 1986). This smaller decrease in eccentric strength has been attributed to greater passive resistance from the elastic tissue structures and to a slowing of cross-bridge cycling (Narici & Maganaris 2007, Vandervoort 2002). Due to decreased number of fast MUs and changes in plasticity it is likely that the MU activity (recruitment and rate coding) would differ between young and older subjects. Not much information is available about MU behavior in the elderly in dynamic conditions, and previous results concerning even isometric conditions are not very consistent. An age related difference in MUDR has been shown in maximal contractions in several muscles, like the first dorsal interosseous (FDI) (Kamen et al. 1995), tibialis anterior (Klass, Baudry & Duchateau 2008, Connelly et al. 1999), and adductor digiti minimi (Nelson, Soderberg & Urbscheit 1984), while no such differences were found when comparisons were made at recruitment threshold in biceps brachii, triceps brachii, TA or FDI in the studies by Howard et al. (Howard, McGill & Dorfman 1988) and Galganski et al. (Galganski, Fuglevand & Enoka 1993). Further, in the study by Dalton et al. (Dalton et al. 2009) an age difference in soleus

MUDR was reported at 25 and 50% MVC, but not at higher contraction levels. It appears that the age-related changes are muscle-dependent and most prominent in distal muscles and at higher relative force levels. It seems that the decreased MU discharge rate would be an adaptation to the increased twitch duration to optimize force generation. As the twitch duration increases with age, tetanus theoretically could be achieved with lower discharge rates. (Roos, Rice & Vandervoort 1997).

2.4 Intramuscular EMG- recordings and signal decomposition

Electromyography (EMG) is the most widely used technique in the study of muscle activation and the neural input to the muscle. Since the first measurements of electrical activity in the muscle in 1838 by Dr. Carlo Matteucci, several different kinds of electrodes have been constructed. The most commonly used type is the surface electrode, which, due to its relatively large detection area, provides a representation of the global activity of the muscle. Surface electrodes are easy to use, cheap and non-invasive. However, since the measured output is the sum of a large number of signals, and is measured some distance from the action, it can be influenced by many factors unrelated to the behavior of the motor units, like the inter-electrode (Fuglevand et al. 1992) and electrode to muscle –distances (Farina & Rainoldi 1999), skin contact (Basmajian & De Luca 1985) movement (Martin & MacIsaac 2006) and cancellation of overlapping actionpotentials (Day & Hulliger 2001).

When the goal is to study the activation patterns of the motor units, instead of measuring the sum of action potentials, a more accurate approach is to investigate the firings of single motor unit action potentials. Although recent advancements in matrix electrode design and analysis have enabled the decomposition of surface EMG to single motor unit action potential trains, this method is limited to superficial muscles and motor units, and low level isometric contractions (Farina et al. 2010). The more traditional method for more selective action potential data collection is the use of intramuscular electrodes. Several different designs exist, all with their advantages and drawbacks.

The needle electrode, first described by Adrian and Bronk (1929), is the most commonly used intramuscular electrode, due to its small pick-up area and ease of repositioning. Due to its rigid structure, needle electrodes have mainly been used in isometric contractions (e.g. (Dalton et al. 2008, Bigland & Lippold 1954, Kamen et al. 1995, De Luca et al. 1982, Desmedt & Godaux 1977, Christie & Kamen 2010). Only a few studies have utilized the needle in dynamic contractions, mainly at low activation levels (Sogaard et al. 1996, Pasquet, Carpentier & Duchateau 2006, Stotz & Bawa 2001, Del Valle & Thomas 2005, Sogaard et al. 1998).

The other often used intramuscular electrode is the fine-wire electrode, introduced by (Basmajian & Stecko 1962). The electrodes consist of two wires that are inserted to the muscle with a hypodermic needle which, upon removal leaves only the wires intact. While removing the needle limits the repositioning of the electrode, the flexibility of the wires means that the method is quite painless and electrode movement is minimal, even while the muscle moves. For this reason the wire electrode has been used extensively in both isometric (e.g. (Jonsson & Bagge 1968, Komi & Buskirk 1970, Duchateau & Hainaut 1990, Vieira et al. 2012) and dynamic (Tax et al. 1989, Vieira et al. 2012, Harwood, Davidson & Rice 2011, Semmler 2002, Linnamo et al. 2003b) contractions.

Before useful information can be gained from the intramuscular EMG signal, the data needs to be processed (Figure 4). The two different methods relevant to this thesis will be introduced here. The more crude analysis method is the so called intramuscular spike-amplitude-frequency (ISAF) histogram (Moritani et al. 1986), where individual spikes in the EMG signal are isolated and counted based on their amplitude in 100 µV increments. Mean spike amplitude and frequency can be counted from the temporal spike-data. Since this method does not separate the firings of different MU's with similar amplitudes, the data on frequency represents the mean firing rate of a selected population of MU's.

A more time-consuming, but also more accurate method for the interpretation of intramuscular EMG involves the identification and classification of individual motor unit action potentials (MUAP) from the recorded motor unit action potential trains (MUAPTs). Since the recorded signal generally consists of several overlapping trains, this method is commonly combined with the decomposition of the signal. The MUAP recognition is based on template matching of the action potential waveform. From the decomposed data the inter-spike intervals can be registered and instantaneous motor unit discharge rate calculated for each MU (Sogaard et al. 1996, Basmajian & De Luca 1985, Farina et al. 2001, Olsen, Christensen & Sogaard 2001).

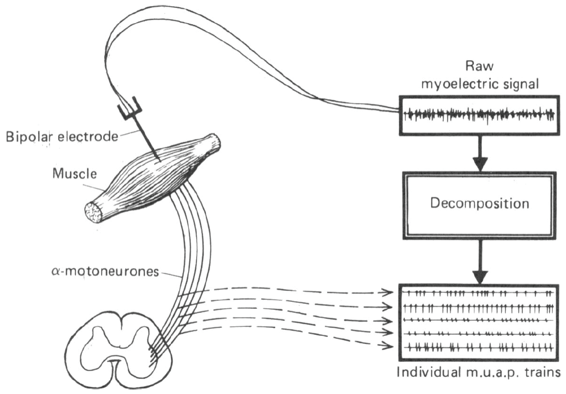


FIGURE 4 Illustration of the investigation of single motor unit action potentials. The muscle contraction is the sum of individual motor unit action potential trains (MUAPTs). An electromyographic recording made with a sufficiently selective electrode can be decomposed to identify the individual MUAPTs (De Luca et al. 1982).

3 PURPOSE OF THE STUDY

The increase in the proportion of elderly people in industrial countries brings great socio-economic challenges. It is well known that aging is related to changes at the muscular level, leading to a decline in motor performance. This loss in function increases the risk of falling and injury, with serious economic and personal consequences (Hamacher et al. 2011, Heinrich et al. 2010). Studies have shown the risk of falling to be associated with lower extremity strength, especially at the ankle joint level (Wolfson et al. 1995). It is of great importance to determine the mechanisms behind the decrease in muscle force and control. It is known that with age there is a loss of muscle cells which is coupled with a diminished neural drive to the muscle. While previous studies have implied these age-related changes to be muscle and intensity-specific, most results concerning motor unit control are from small and/or upper limb muscles and in isometric contractions. However, since most of the daily activities that determine the capability to continue independent living (e.g. raising out of a chair, stair climbing, walking) are dynamic in nature, it is important to gain more understanding of the mechanisms of aging in both concentric and eccentric muscle actions. Therefore the aims of this study were as follows:

In order to better understand the mechanisms of human movement it is important to investigate motor unit activation in plantar flexors as well. Of the ankle joint muscles, the plantar flexors have been shown to be most affected by aging (Simoneau, Martin & Van Hoecke 2005). Of the plantar flexors, the soleus muscle plays a role also in balance control (Basmajian & De Luca 1964, Di Giulio et al. 2009). However, to the best of our knowledge, soleus MUDR has only been studied in isometric contractions (Bellemare et al. 1983, Oya, Riek & Cresswell 2009, Dalton et al. 2010). Therefore the purpose of the present study was to identify possible differences in soleus motor unit activation strategies between isometric and dynamic contractions.

1) In order to get information about motor unit control in muscles and conditions more relevant to balance and locomotion, the first aim was to be able to record single motor unit firings in dynamic contractions of the soleus muscle and to find suitable methods to analyze them (Original articles I, II).

2) In submaximal contractions the amount of neural activation required to produce a given muscular force is dependent on muscle action type (Katz 1939). Contraction type also affects H reflex excitability, partly via presynaptic inhibition. Since presynaptic inhibition is affected by aging and may be an important factor in the control of MU activation, the second purpose of the thesis was to examine the effects of aging and contraction type on H-reflex excitability and motor unit activation patterns in dynamic and isometric contractions (Original article I).

3) The previous findings concerning the age-related changes in MUDR are somewhat contradictory, and only a few studies have investigated the effects of aging on force and motor unit control in the lower leg extensors. Therefore, the third aim of the thesis was to compare MUDR and variations in both force and discharge rate in isometric contractions between the age-groups (Original article II).

4) According to previous studies, the age-related difference in MUDR may be greater at high force levels, while no studies have investigated this change in dynamic contractions. In order to get a step closer to measuring motor unit control in natural locomotion, the final aim of the thesis was to investigate the age-related changes to MUDR of the soleus muscle in dynamic contractions and at high force levels in isometric contractions (Original articles II, IV).

4 RESEARCH METHODS

4.1 Subjects

In total 37 physically active males volunteered for the experiments – some of them for both experiments (Table 1). In both experiments subjects were recruited from two distinctively different age-groups (YOUNG: 20-30y, OLD: 65-80y). Physical activity of the subjects was determined with a questionnaire, to which the subjects wrote the type and frequency of their regular exercise routine. All men in both groups performed moderate to strenuous physical exercise three times a week or more. Before the measurements all of the subjects in the OLD group underwent a medical examination. Only subjects without any history of neuromuscular or vascular disease were approved for the study. Height, weight and ankle joint range of motion (ROM) were measured from all the subjects. Full advice about possible risks and discomfort was given to the subjects and all gave their written informed consent to the procedures prior to the commencement of the study. The investigation was conducted according to the declaration of Helsinki and approved by the ethics committee of the University of Jyväskylä.

TABLE 1 Mean physical characteristics of subjects in both experiments.

		N	Age (yr.)	Height (m)	Weight (kg)
Experiment I	Young	10	27.3(3)	1.77(0.07)	73.2(5)
	Old	13	70.4(5)	1.75(0.05)	81.8(10)
Experiment II	Young	11	26.1(2.2)	1.81(0.05)	81.7(9.4)
	Old	8	69.1(5.1)	1.69(0.04)	75.3(6.2)

4.2 Experimental design

The thesis consists of four original manuscripts that are based on data collected from two separate experiments (Figure 5). In both experiments the subjects performed isometric and dynamic plantarflexions while seated in an ankle dynamometer (Figure 6). The main difference between the two experiments was the method of normalizing submaximal contractions. In the first experiment (EXP I) the contraction intensity was measured from the soleus surface EMG and related to the maximal value obtained during the isometric maximal voluntary contraction (MVC). In the second experiment (EXP II) the trials were normalized according to the ankle angle torque during isometric MVC. In EXP I the contraction intensities were only 10, 20 and 40% MVC, while in EXP II the isometric trials were measured up to 100%MVC. All measurements were conducted in relation to the individual range of motion of the subject. The individual zero angle (IZA), was determined as being 15 deg. plantar from the maximal voluntary dorsiflexion without a load (Allinger & Engsberg 1993). In EXP I the knee was straight (180 deg), while in EXP II it was flexed to 110 deg in order to decrease gastrocnemius activity.

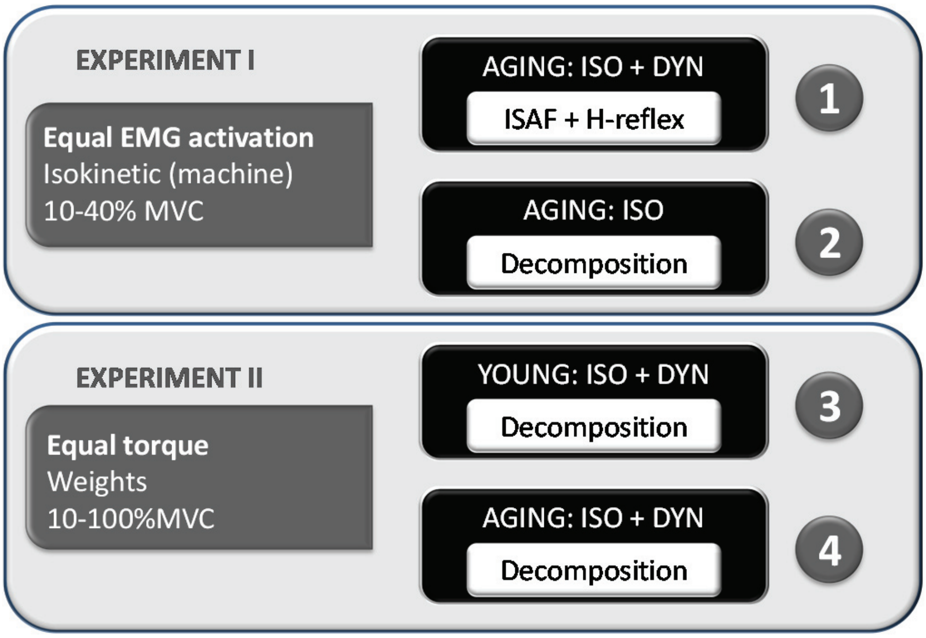


FIGURE 5 The experimental design of the thesis. The differences in the measurements of the two experiments are described in the dark grey rectangles. Both experiments (light gray rectangle) resulted in two publications (gray circle). These manuscripts differed either on the focus (black rectangle) or on the method of analysis (white rectangle). Both isometric (ISO) and dynamic (DYN) contractions were used in the studies. ISAF = intramuscular spike-amplitude-frequency analysis.

FIGURE 6 The setup in EXP I.

4.2.1 Experiment 1 (I, II)

In order to investigate the age related changes in H-reflex excitability, twitch properties and motor unit control in isometric and dynamic contractions, 10 younger (YOUNG) and 13 older (OLD) males participated in two measurement sessions separated by one to three weeks. One session was for the motor unit recordings, and the other for H-reflex measurements. After warm-up, range of motion and maximal isometric voluntary contraction (MVC) measurements, the subjects performed submaximal plantarflexions in isometric (ISO), eccentric (ECC) and concentric (CON) conditions (Figure 7). The activation levels used in the submaximal trials were 10, 20 and 40% of the surface soleus (SOL) EMG root mean square (RMS) value of the isometric MVC. In ISO motor unit recruitment thresholds were investigated with ramp contractions, where the subjects were asked to increase the plantar flexion force at a slow rate (~2% of MVC/s). In H-reflex measurements, also passive trials were measured. The orders of activation levels and dynamic trials were randomized. Subjects were instructed to hold the plantar flexion force at the given target level for 10 s. Activation levels were analyzed online in order to ensure successful trials at each condition. In the dynamic trials the ankle was rotated by a strong motor connected to the foot-pedal. The rotation of the ankle joint was ± 10° of IZA at 30 deg/s. The subject was instructed to increase the triceps surae activation before the start of the movement and hold the activation steady throughout the following concentric or eccentric trial. Activation was measured online in four 100ms windows, ±200ms around the individual zero angle (IZA). The trial was considered successful if the EMG activation of SOL (RMS) was a) within 5% of target activation level ±100ms around IZA, and b) within 10% of target activation level ±200-100ms around *IZA*. The minimum time interval between two trials was 2 minutes in active and 1 min in passive conditions.

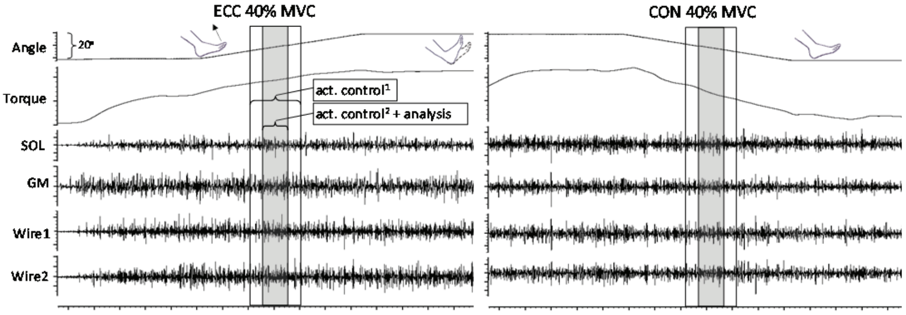


FIGURE 7 An example of surface (SOL, GM) and wire-electrode recordings of eccentric (ECC) and concentric (CON) trials. Analysis was performed ±100ms and activation level controlled ±200ms and ±100ms around the measurement angle.

4.2.2 Experiment 2 (III, IV)

The aim of the second experiment was to investigate the effects of contraction type and aging on motor unit activation patterns. For this, 11 YOUNG and 8 OLD males performed isometric and dynamic plantar flexions in relation to the individual range of motion of the subject. After warm-up, range of motion and maximal isometric voluntary contraction (MVC) measurements, the subjects performed trials in isometric (ISO), eccentric (ECC) and concentric (CON) conditions. In ISO the used force levels were 10, 20, 40, 60, 80 and 100% of the isometric MVC, while in dynamic contractions only the three lowest force levels were used. In ISO, the subjects were instructed to increase the force to the target level and hold it as steady as possible for several seconds, ranging from 10s at 10 & 20% MVC to a couple of seconds at 100% MVC. Contrary to the first experiment, the dynamic contractions in EXP II were performed by controlling the movement velocity against a constant inertial load. The subjects lifted (CON) or lowered (ECC) a weight stack that was attached to the foot pedal via a cable pulley system at a voluntarily controlled velocity (Figure 8). The weight was adjusted so that the ankle torque during movement was identical to the corresponding ISO condition. The range of motion was set to enable a measurement window clearly separated from the acceleration or deceleration phase of the movement (Figure 9).

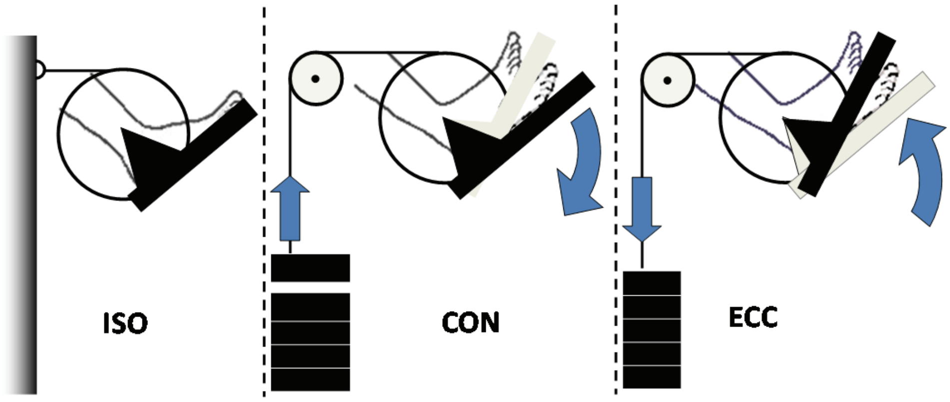


FIGURE 8 Weight system used in the isometric (ISO), concentric (CON) and eccentric (ECC) trials. Pedal was fixed in ISO, while an adjustable amount of weight was lifted in CON and lowered in ECC. The range of motion was 20 deg, and the velocity 10 deg/s.

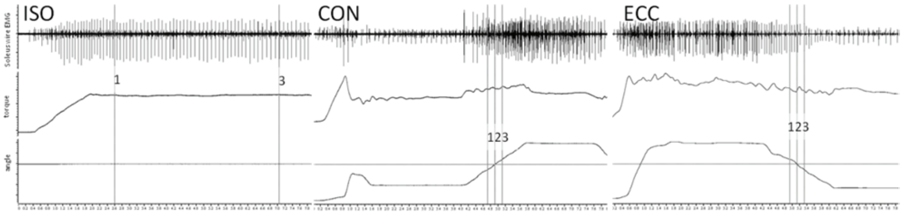


FIGURE 9 Examples of ISO, CON and ECC trials from one subject at 10% MVC. Cursors 1 and 3 mark the beginning (Cursor 2 – 200ms) and end (Cursor 2 + 200ms) of analysis, respectively. Cursor 2 indicates the point in time when the angle in the dynamic trials crosses the angle used in the ISO trial.

4.3 Recording procedures and analyses

4.3.1 Force and angle

4.3.1.1 Measurements

Trials were performed while subjects sat with the foot attached to an ankle dynamometer pedal mounted with torque and angle sensors. To maximize stability during testing, subjects were strapped to the chair, the chest with a 4-point seatbelt and the thigh and ankle with straps. In EXP I the dynamometer was attached to a motor, used previously in similar experiments with passive and active muscle (Avela et al. 2004, Dousset et al. 2007, Kallio, Linnamo & Komi 2004). In EXP II the foot pedal was attached to a weight stack via a pulley. The measured torque and angle signals were sent to a computer via a CED 1401+

(Cambridge Electronic Design) and sampled at a frequency of 50 kHz. Signals were collected and analyzed using Signal software (Cambridge Electronic Design, UK). Before analysis, torque and angle signals were down sampled to 2 kHz, and then filtered with a low-pass filter (cutoff frequencies 75 and 150 Hz, respectively).

4.3.1.2 Analyses

In MVC both sEMG and torque data were analyzed ±100 ms around the peak torque. In the isometric ramp and isotonic contractions, the force analysis window was determined by motor unit analysis, ranging from the first 10 firings in the ramp contractions to several seconds in the low level isotonic trials. In dynamic contractions analysis was done from a 200 ms window around the ISO angle (IZA). sEMG, angular velocity and torque were analyzed online, in order to ensure a sufficient number of successful trials in each condition.

Steadiness was calculated as the variability in force, determined by coefficient of variation (CV), and calculated as standard deviation (SD) of force curve/mean force multiplied by 100% (Enoka 2008, 345).

4.3.2 EMG

4.3.2.1 Measurements

Global EMG activity of the soleus (SOL) and gastrocnemius medialis (GM) muscles was recorded using bipolar silver chloride miniature surface electrodes (Beckman 650437, USA) with a 20 mm inter-electrode distance. Electrodes were prepared and placed in accordance with the recommendations by SENIAM (Hermens et al. 1999). The surface EMG (sEMG) signals were amplified and sampled at a frequency of 50 kHz.

For the intramuscular EMG recordings, the methods to prepare the electrode differed from the original design of Basmajian and Stecko (Basmajian & Stecko 1962). In the present study four separate bipolar fine-wire electrodes were inserted into the soleus muscle. This provided four potential signals that could be used for the decomposition. In EXP I trials the electrodes were inserted to soleus around the surface electrode, since the trials were matched according to sEMG activation. In EXP II the wires were inserted from the lateral side of the leg in order to avoid the gastrocnemius tendon. A ground electrode was placed on the medial malleolus.

The wire-electrodes consisted of two 50 µm polyurethane insulated wires that were glued together. Insulation was mechanically removed from the outer sides of the wires over a 0.5 mm distance from the tip, resulting in an inter-electrode distance of approximately 100µm. A hook was formed at the end of the wires to ensure fixation during the measurements (Figure 10). Electrodes were autoclaved before the experiment in order to minimize the risk of infection. The wire electrode pairs were inserted into the muscle with a 22 gauge needle that was subsequently withdrawn. Wire-electrodes were inserted after the MVC trials to avoid possible electrode migration due to high force levels. To optimize signal quality, the signal was pre-amplified with a non-commercial amplifier (amp 200, bandwidth 8-4500) attached to the calf of the subject. The amplified signal was sent to a CED 1902 programmable signal conditioner (Cambridge Electronics Design Ltd, Cambridge, UK) and digitized with CED 1401+ (Cambridge Electronic Design) with a sampling frequency of 20 kHz. Since the decomposition software allows visualization of three channels, the wire channel was duplicated twice and the two new channels were filtered with different fourth order butterworth band-pass filters (300-8000 and 400-10000). Observing three differently filtered channels gave the analyzer more information for the decomposition.

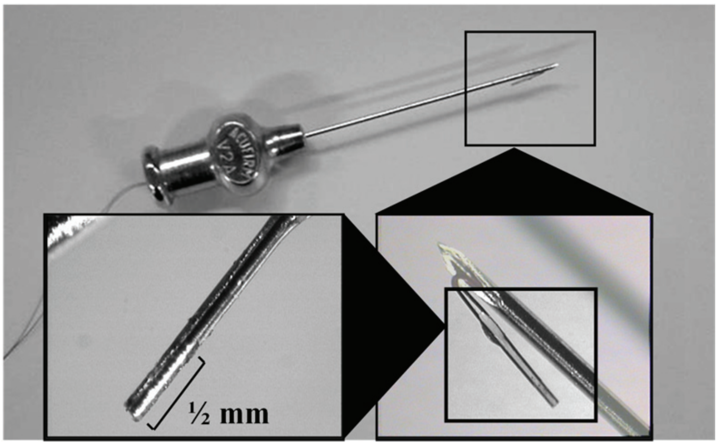


FIGURE 10 The wire electrode. The electrode consists of two insulated wires that are glued together. The insulation is mechanically removed from the outer sides of the wires over a 0.5 mm distance from the tip.

4.3.2.2 Analyses

The surface EMG signals were down sampled to 2 kHz, band-pass filtered at 20 to 1 kHz and analyzed using Signal software (Cambridge Electronic Design, UK). Surface EMG activities of SOL and GM were calculated as root mean square (RMS) of the signal. For the maximal voluntary contractions (MVC), both sEMG and torque data were analyzed ±100 ms around the peak torque. In the isometric ramp and isotonic contractions the sEMG analysis window was determined by motor unit analysis, ranging from the first 10 firings in the ramp contractions to several seconds in the low level isotonic trials. In both experiments, the final analysis of the dynamic contractions was always done from a 200 ms window around the ISO angle (IZA). In addition, in EXP I the activation was analyzed online in four 100ms windows, ±200ms around IZA. The trial was considered successful if the EMG activation of SOL (RMS) was a) within 5% of target activation level ±100ms around IZA, and b) within 10% of target activation level ±200-100ms around IZA.

Two different methods were used to analyze the intramuscular EMG data. For the first manuscript the analysis was done with intramuscular MU spike-amplitude-frequency (ISAF) histograms. This method has been described in more detail elsewhere (Moritani et al. 1986). In short, individual spikes in the EMG signal were isolated and counted based on their amplitude in 100 µV increments (Figure 11). Mean spike amplitude and frequency were used for statistical comparison between groups and conditions. Figure 12 shows an example of mean spike frequency and mean spike amplitude when an analyzing window of 100ms is overlapped by each ms during an isometric ramp condition from zero to 40% MVC. With this method, it is not possible to separate the firings of different MU's with similar amplitudes. Therefore, the data on frequency represents the mean firing rate of a selected population of MU's.

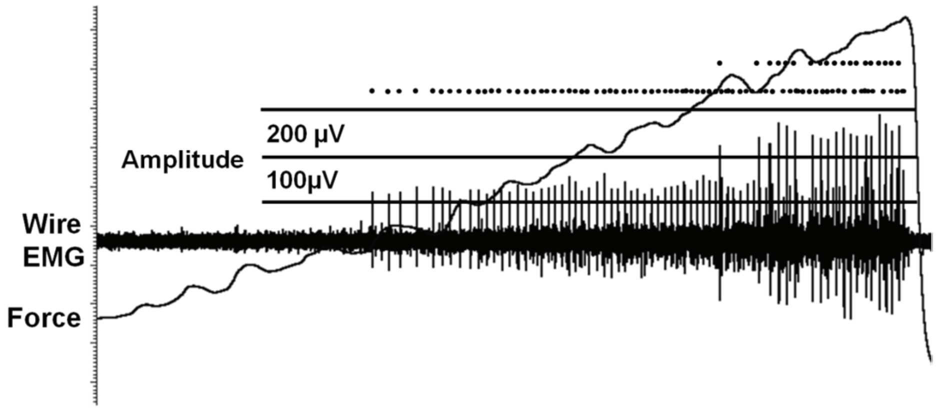


FIGURE 11 Illustration of intramuscular spike-amplitude-frequency (ISAF) analysis in a ramp contraction. Firings in two amplitude windows are shown as dots.

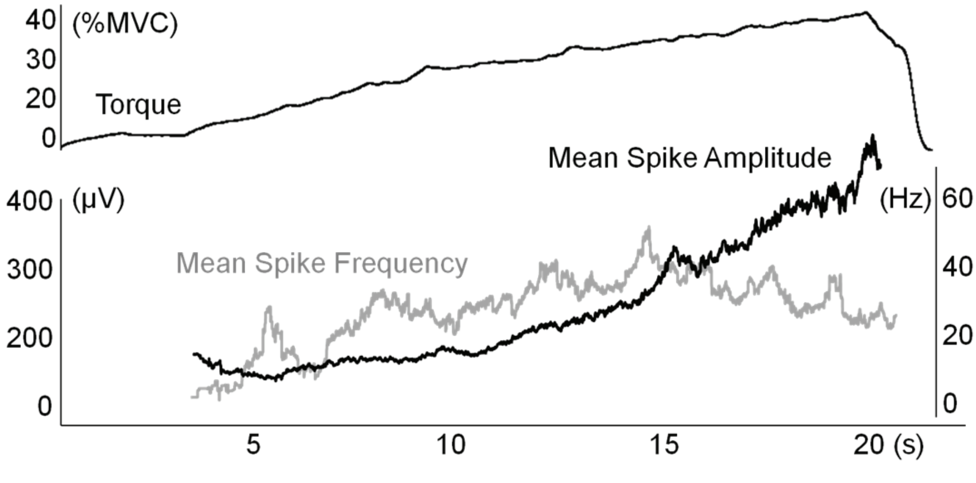


FIGURE 12 Example of intramuscular spike-amplitude-frequency (ISAF) analysis in an isometric ramp contraction showing mean spike frequency and amplitude.

For manuscripts 2, 3 and 4 the intramuscular EMG signal was decomposed to individual motor unit action potentials (MUAP). The motor unit identification and data analysis were performed by utilizing the three channel decomposition technique computer algorithm, “Daisy”, described in more detail in (Sogaard et al. 1996, Farina et al. 2001, Olsen, Christensen & Sogaard 2001). Motor unit action potential (MUAP) recognition is based on template matching of the waveform of the three channels (unfiltered and two band-pass filtered), created from the same intramuscular EMG recording (Figure 13). The MU classification was performed semi-automatically with a high degree of operator interaction. For each train of MU discharges the inter-spike intervals were registered and instantaneous motor unit discharge rate (MUDR) for each MU was calculated as the mean of the inverse of each inter-spike interval. In addition, for manuscript 3, units that could clearly be recognized in at least two of the three contraction types were analyzed in order to investigate the firing rate behavior in more detail. Analyses were checked by comparing MUDRs to eliminate the inclusion of the same motor unit by more than one bipolar wire set.

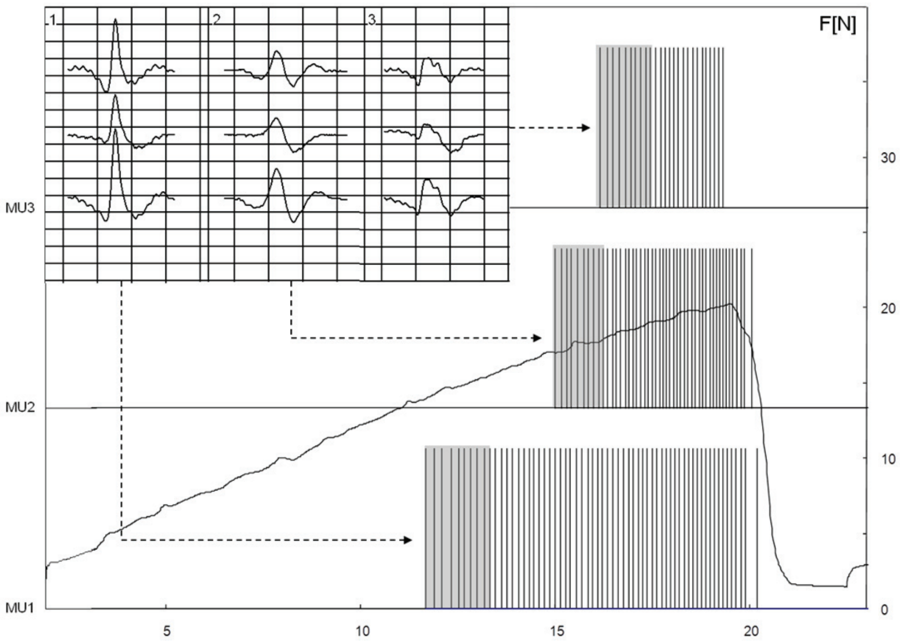


FIGURE 13 Decomposed motor unit discharge data from a submaximal ramp contraction in Daisy software. In this case, three units (MU1-3) were identified, and the corresponding motor unit action potential trains (arrows) are illustrated with vertical bars. The top left figure shows the shapes of the action potentials from raw (top row) and band pass filtered (300-8000 and 400-10000) signals. Threshold discharge rate was determined as an average from the 10 first discharges, as illustrated by the shaded area. The same time window was used to calculate the surface EMG for recruitment threshold.

Motor unit discharge rate (FR) at recruitment threshold was calculated in ramp contractions by averaging the first 10 inter firings intervals after the unit began firing constantly. For threshold discharge rate analysis, motor units were divided into two groups: recruitment before 10% (RECR-10%) and between 10 and 20 % (RECR-20%) according to the surface EMG activation level. For each recorded MU action potential train in the isometric isotonic contractions, the amount of activity above each unit's recruitment threshold was determined. Averaging these relative activity levels for both groups enabled the detection of a possible bias in MU selection. The variability in DR was determined by coefficient of variation (CV), calculated as standard deviation (SD) of instantaneous DR divided by the mean FR, multiplied by 100%. Similarly, variability in force was calculated as SD of force curve/mean force*100% (Enoka 2008, 345).

4.3.3 Electrical stimulation

Electrical stimulation was used in EXP I. Hoffmann reflexes were obtained utilizing a standard methodology. The skin was prepared, and the stimulation electrodes (pregelified Ag-AgCl electrodes, Niko, Japan) were placed on the leg. The stimulating cathode electrode (1.5 x 1.5 cm) was placed over the tibial nerve in the popliteal fossa, and the anode electrode (5 x 8 cm) was placed above the patella. The stimulation site resulting in the greatest M-wave amplitude was first located with a handheld cathode electrode (0.7-cm diameter). Once the stimulation site was determined, stimulation electrode was firmly fixed to this site with rigid straps and taping. Stimulus pulses (rectangular, 1ms duration) were delivered from an evoked-potential- measuring system (Digitimer stimulator (model DS7, Hertfordshire, UK). The stimulation of the tibial nerve elicited an M-wave (direct stimulation of a-motoneuron axons) and an H-wave (reflex response to stimulation of Ia muscle afferents). Maximal M-wave (M_{max}) was measured by delivering a stimulus at supramaximal intensity (1.5 times the maximum M-wave stimulus intensity) three times, and the mean value of the recorded M-wave amplitudes was considered as (M_{max}). Passive twitch force properties were measured as peak force (Pf) during maximal M-wave (Mmax). The contraction time was defined as the time from onset to peak force and the half-relaxation time was defined as the time from peak force to 50% reduction of peak force. Stimulation intensity in the H-reflex measurements was controlled with M-wave amplitude. The target M-wave amplitude was 25% of M_{max}. In each condition, the average of 4 successful trials was used, which has been found to be enough to reach reliability in both young and old subjects [intraclass correlations 0.9883 and 0.9667 for young and old, respectively, in (Mynark 2005)].

4.3.4 Statistical methods

All statistical analyses were performed by the SPSS™ program (SPSS Inc, USA). The normality of distribution was tested with the Shapiro-Wilk test. In the pooled units an analysis of variance (ANOVA) was used to compare the effects of contraction types and intensities, while repeated measures ANOVA was used for the units recognized in more than one condition. When a significant difference was detected, Bonferroni’s method was used to locate the difference. The critical level of significance was $P < 0.05$. Descriptive statistics include mean and standard deviation.

5 RESULTS

This chapter gives an overview of the results of the two experiments. For more details the original papers (I-IV) should be consulted.

5.1 Effects of aging

5.1.1 Range of motion

The range of motion was measured in both experiments and reported in papers comparing YOUNG and OLD (I, II, IV), mainly to see the effect of aging on the individual zero angle (IZA) used in the measurements. The maximal active dorsiflexion and the IZA calculated from it were slightly greater in OLD, but neither of the differences were statistically significant.

5.1.2 Force and activation levels

In both experiments the average maximal isometric voluntary contraction (MVC) in YOUNG was 12-41% greater ($p < .01 - .06$) than in OLD (Table 2), while the lack of significant reductions between pre- and post-measurements indicated that the measurement protocol did not fatigue the subjects.

Different submaximal trials were normalized to either the soleus EMG or torque values of MVC. Because activation- and torque-levels could be monitored online and only the trials fulfilling the inclusion criteria were accepted for the final analysis, no consistent or statistically significant differences in the control variables were found between groups or contraction types. In EXP I trials were normalized relative to the maximal RMS-value of the soleus sEMG. Although the relative activation was equal, the relative torque was found to be higher in all submaximal conditions in YOUNG compared to OLD ($p<.05$). In EXP II the MVC torque was used to normalize the trials. Also in this experiment the control-variables were equal, but the relative sEMG activities of SOL and

GM were consistently higher (p< .001 - n.s) in OLD compared to YOUNG in all conditions and torque levels (Figure 14). The age-related differences were largest in dynamic contractions, while statistically more significant differences were found in ISO, possibly because of the higher N in that condition.

TABLE 2 Maximal isometric voluntary contraction (MVC) of subjects in both experiments. P-values are between groups within experiments.

		N	MVC (Nm)	p
Experiment I	Young	10	219.2(35)	.06
	Old	13	192.2(42)	
Experiment II	Young	11	211.1(36)	.01
	Old	8	149.2(36)	

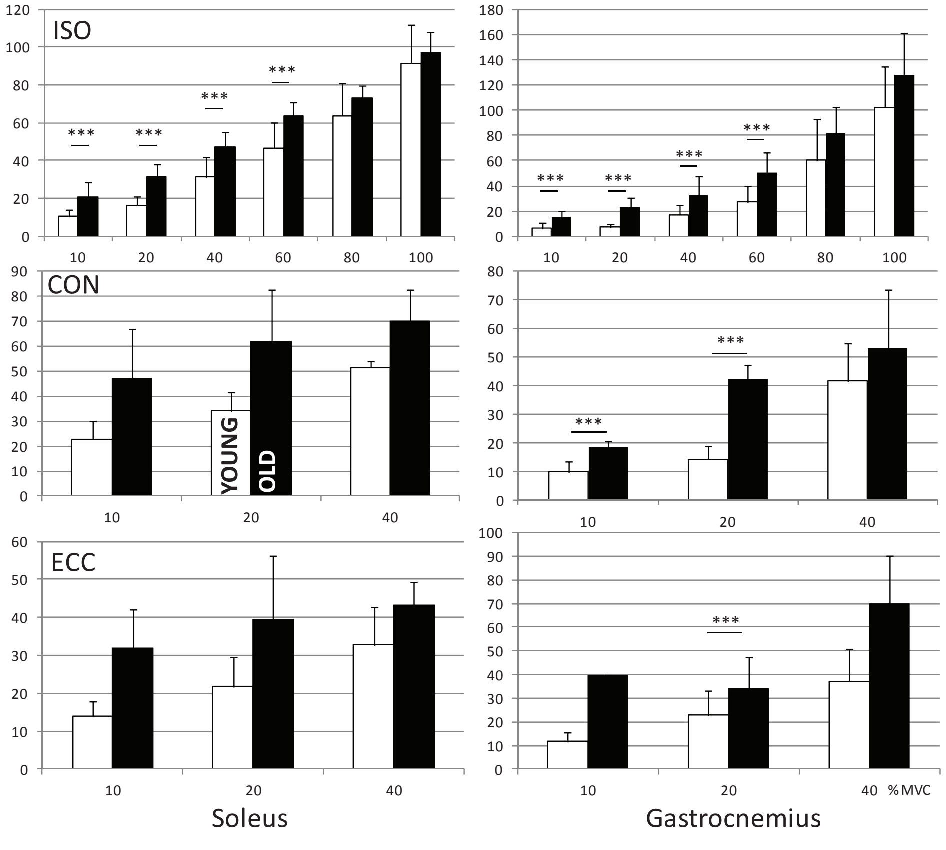


FIGURE 14 Relative activities (%of surface EMG in MVC) of soleus and gastrocnemius in YOUNG (white) and OLD (black) in isometric (ISO), concentric (CON) and eccentric (ECC) contractions. Significant differences between age-groups: * = p<.05, ** = p<.01, *** = p<.001. Differences between relative torque-levels are significant in ISO (p< .001 - .05), but not in soleus or gastrocnemius.

5.1.3 Motor unit firing

5.1.3.1 Intramuscular motor unit spike-amplitude-frequency (ISAF)

For the first manuscript the signal from the wire-electrodes was analyzed utilizing the intramuscular MU spike-amplitude-frequency (ISAF) histogram method. The mean spike frequency was higher in YOUNG in all trials ($p<0.05$ in ECC and CON), while no group-differences were found in the mean spike amplitude (Figure 15).

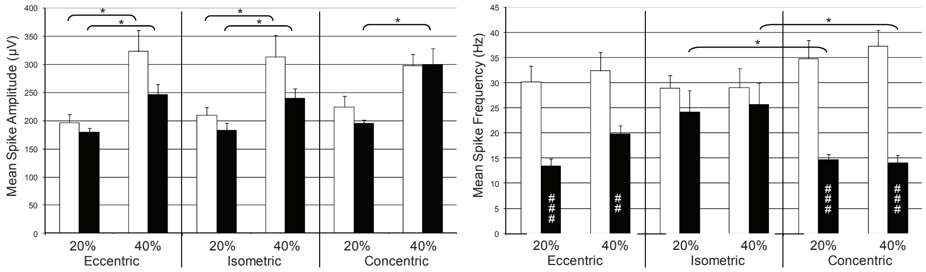


FIGURE 15 The mean spike amplitude (left) and the mean spike frequency (right) of YOUNG (white) and OLD (black) at 20 and 40% activation. *: significant difference between activation levels, #: significant difference between age-groups.

5.1.3.2 Motor unit discharge rate

For manuscripts II, III and IV, the wire-EMG signal was decomposed to individual motor unit action potentials in order to calculate the motor unit discharge rate. In EXP I the analysis was based on 4242 motor unit discharges from 136 different motor units and in EXP II on 8372 discharges from 210 different units. The number of subjects with decomposable units in each condition and the number of units can be seen in figures 16 – 18.

The instantaneous motor unit discharge rate (MUDR) in isometric contractions included:

- recruitment threshold and isotonic contractions at 10 & 20% $\mathrm{SOL}_{\mathrm{RMS}}$ in MVC (EXP I; Figure 16)
- Isotonic contractions at 10, 20, 40, 60, 80 and 100% of MVC torque (EXP II; Figure 17),

The dynamic contractions were performed at:

- 10, 20 and 40% of MVC torque (EXP II; Figure 15)

In isometric contractions MUDR was found to be consistently lower in OLD in both EXP I (14-18%, p< .001-.05, except ISO-20%; see Figure 16) and in EXP II (4-20%, p< .01-.06; see Figure 17). In isometric contractions the age-difference increased to higher force levels. However, no group-difference was seen in the dynamic contractions at the three measured force levels (10, 20 and 40% MVC; Figure 18).

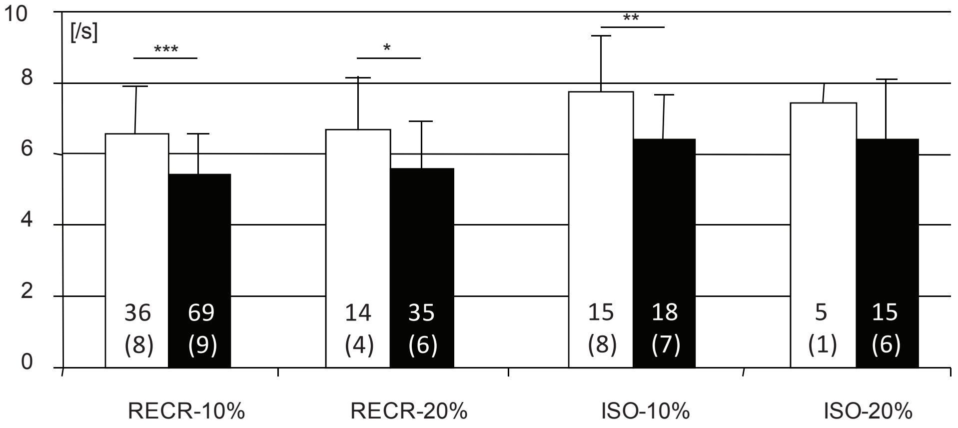


FIGURE 16 Motor unit discharge rate (mean pulses per second+SE) in YOUNG (black) and OLD (grey) in isometric contractions. Data are analyzed from recruitment thresholds (RECR) and during isotonic contractions (ISO). Number of units and subjects (in parenthesis) used in the analysis are in the columns. Activation level was calculated as 10 and 20% of soleus EMG at MVC. Significant differences between age-groups: * = p<.05, ** = p<.01, *** = p<.001.

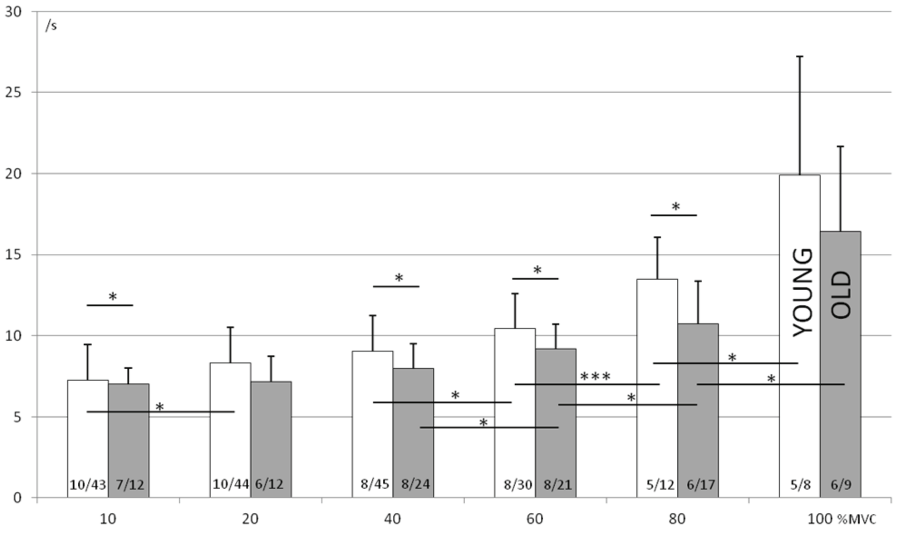


FIGURE 17 Motor unit discharge rate (pps; mean±SD) in YOUNG and OLD at different force levels in isometric contractions. Number of subjects/motor units used in the analysis is displayed in the columns. Significant differences between age-groups and levels: * = p<.05, ** = p<.01, *** = p<.001.

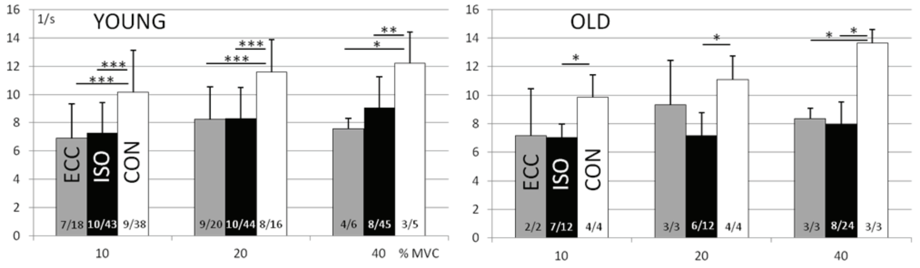


FIGURE 18 Motor unit discharge rate (1/s; mean±SD) in YOUNG and OLD in eccentric (ECC), isometric (ISO) and concentric (CON) contractions at different force levels. Number of subjects/motor units used in the analysis is displayed in the columns. Significant differences between contraction types: * = p<.05, ** = p<.01, *** = p<.001.

5.1.4 Steadiness

The average force variations (CV%) in the isotonic contractions were significantly smaller in YOUNG compared to OLD (p< .001 - .05; Figure19). However, the group-difference in the average coefficient of variation of instantaneous discharge rate did not reach statistical significance.

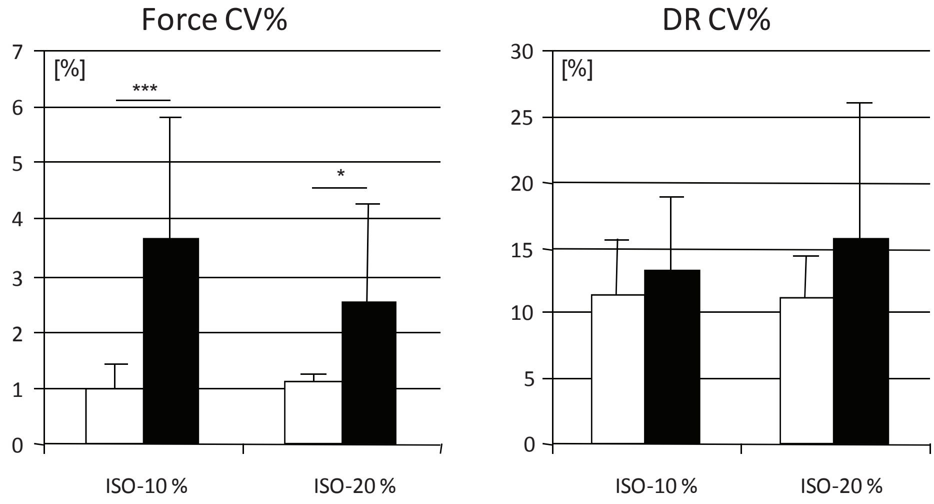


FIGURE 19 Fluctuations in force and discharge rate (DR) during isometric contractions in YOUNG (white) and OLD (black) at two submaximal activation levels (CV%+SE). Significant differences between age-groups: * = p<.05, *** = p<.001.

5.1.5 H-reflex and twitch properties

The average H/M-ratio was consistently higher in YOUNG regardless of activation level or contraction type (p< .001 - n.s; Figure 20) while no significant difference was found in mean maximal M-wave twitch force between groups (Table 3). The mean twitch contraction time (CT) and the ratio of peak twitch force and contraction time (PT/CT) were significantly longer in OLD (p< .001) while no significant difference was observed in the mean half relaxation time (HRT).

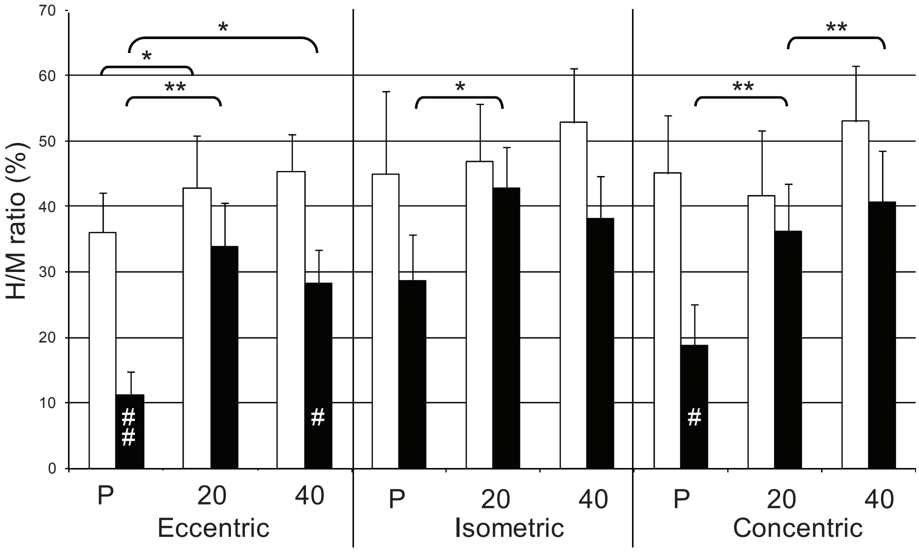


FIGURE 20 Mean H/M ratio (%+SE) of YOUNG (white) and OLD (black) in passive (P), 20 and 40% activation. *: significant difference between activation levels, #: significant difference between YOUNG and OLD.

TABLE 3 Mechanical twitch properties in maximal M-wave stimulation in YOUNG and OLD. Data include peak torque (PT), contraction time (CT), mean half relaxation time (HRT) and the ratio of peak twitch force and contraction time (PT/CT). Significant differences between groups: *** = p<.001.

	PT (Nm)	CT (ms)	HRT (ms)	PT/CT (Nm/ms)
YOUNG	24.1±11.0	117±22	151±124	0.20±0.07
OLD	21.5±8.8	150±28***	184±129	0.14±0.05***

5.2 Effect of muscle contraction type and intensity

5.2.1 Force and activation levels

In EXP I different submaximal trials were normalized relative to the maximal RMS-value of the soleus sEMG. Although the relative activation was equal, the average torque was highest in ECC and lowest in CON in both groups ($p < .05$). In EXP II the MVC torque was used to normalize the trials. The relative sEMG activity of soleus increased with every step increase in force in both groups and in all contraction types, but the difference between levels and groups was only statistically significant in ISO ($p < .001$ - $.05$). In gastrocnemius the difference between groups and levels was always significant in ISO ($p < .001$ - $.05$), while the age-difference was significant also in some dynamic conditions (see Figure 11 for details). In general, producing the same force required more activation in CON than in ISO or ECC.

5.2.2 Motor unit firing

5.2.2.1 ISAF

The mean spike amplitude increased with activation level ($p<.05$, except YOUNG in CON; see Figure 15 for details), whereas no significant differences were found between contraction types. In OLD the mean spike frequency was significantly smaller in CON compared to ISO ($p<.05$).

5.2.2.2 MUDR

In ISO contractions of EXP II, where force levels were explored up to 100% MVC, the MUDR followed the relative force levels (Figure 17). The MUDR increased significantly from 10 to 20 (YOUNG only), 40 to 60, 60 to 80 and 80 to 100% MVC ($p<.01$-$.05$). In both age-groups the increase followed a similar second order polynomial equation, showing that the MUDR increase was greater at higher intensities.

At the three lowest force levels all three contraction types were measured and showed that the MUDR was significantly higher ($p<.05$) in CON compared to ISO or ECC, regardless of whether the comparison was based on all analyzed units (Figure 18) or only on units that could clearly be recognized in at least two of the three contraction types (Figure 21). No significant differences in MUDR were found between ISO and ECC in either of the comparison methods. Of the 14 MUs recognized in both ISO and ECC, 9 fired with a higher discharge rate in ISO and 5 in ECC.

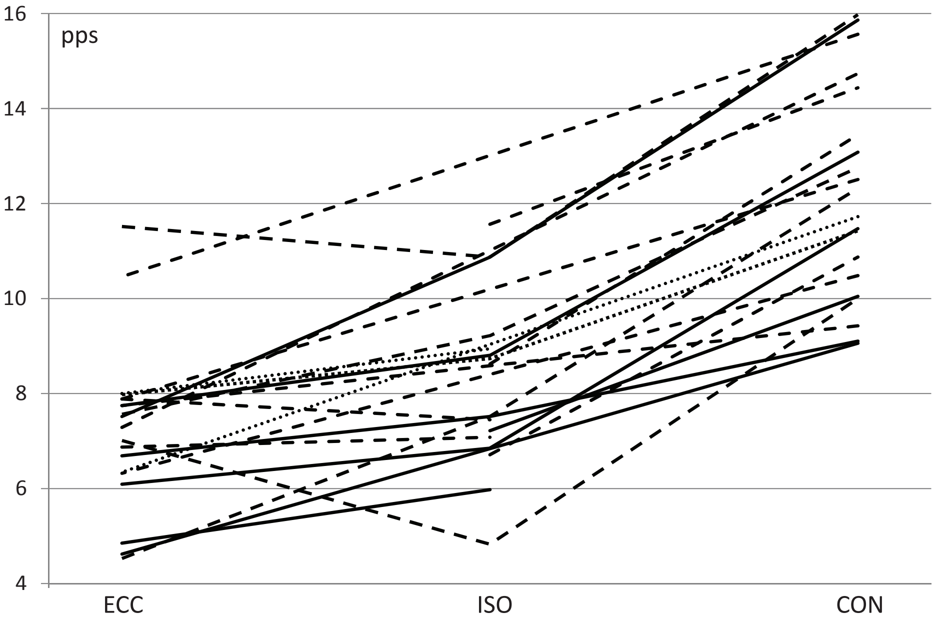


FIGURE 21 Discharge rates of motor units in YOUNG that could clearly be recognized in at least two of the three contraction types at 10 (solid line), 20 (dashed line) and 40% of MVC (dotted line).

5.2.3 H-reflex

In both groups, and at all activation levels, the average H/M ratio was lowest in ECC (n.s.) and higher in active compared to passive contractions (p< .01 - n.s; see Figure 20 for details).

6 DISCUSSION

The main findings of the present study are as follows:

1) In isometric contractions the soleus motor unit discharge rate (MUDR) was found to be lower in OLD at all measured levels, both when normalization was based on maximal global muscle activation (sEMG) and on isometric MVC torque (Original articles II, IV). Also the mean spike frequency, an indirect measure of MUDR, was lower in OLD (I).

2) In dynamic contractions at low force levels, no age-difference was found in MUDR (II, IV), although the mean spike frequency was lower in OLD (I). Also steadiness, the ability to hold a given isometric steady force-level, was lower in OLD. However, the corresponding difference in MUDR variation was smaller and not significantly different (II).

3) The H-reflex excitability was higher and the single muscle twitch duration shorter in YOUNG. Also the average H/M ratio was slightly lower in ECC compared to other contraction types (n.s.) (I).

4) In dynamic measurements CON contractions always required a higher MUDR compared to ECC or ISO, regardless of age or the method of normalization (III, IV).

5) In isometric contractions the MUDR increased with contraction level up to 100% MVC, the largest increases being at the high end of the force-range. Paired MU analysis showed a larger DR increase with increasing force compared to pooled analysis of all active units (III, IV). Also, ISAF analysis showed the mean spike amplitude to increase with activation level (I).

6.1 Effects of age

6.1.1 Motor unit activation

6.1.1.1 Isometric contractions

The main focus of the present study was to investigate the effects of aging on soleus motor unit activation. This phenomenon was researched with two different methods of normalization (% of maximal surface EMG activity or maximal voluntary contraction), in several different modes of contraction and by utilizing two different methods of intramuscular EMG analysis. In isometric contractions the motor unit discharge rate (MUDR) was lower in OLD at all measured activation levels (10-100%), in isotonic and ramp contractions, and with both methods of normalization. Also the ISAF analysis showed a group-difference in motor unit activation as the mean spike frequency was slightly lower in OLD (n.s.), suggesting a lower average MU firing rate. Since in Experiment I the trials were normalized by muscle activation (% of sEMG RMS in MVC), the measured lower MUDR in OLD would suggest that they either produce these submaximal contractions by recruiting more units or by having units with larger amplitudes. Animal studies have shown that the alpha-motorneurons of slow units can sometimes "adopt" fast muscle fibers that no longer receive neural input due to the loss of the fast efferent neuron (Larsson 1995). If this so-called remodeling of motor units takes place in soleus, it would explain this result, as it leads to an increase in MU size.

The current findings are in line with previous findings in other distal muscles, e.g. tibialis anterior (Klass, Baudry & Duchateau 2008, Connelly et al. 1999), first dorsal interosseous (Kamen et al. 1995), adductor digiti minimi (Nelson, Soderberg & Urbscheit 1984) and also the study on soleus by (Dalton et al. 2008). In another study, Dalton et al. (Dalton et al. 2009) found a similar age difference in soleus MUDR at 25 and 50% MVC, but not at higher contraction levels. In that study the force-related increase in MUDR was greater at high intensities in the elderly, very much like both age-groups in the current study. However, the young subjects in Dalton's study were increasing the MUDR at a reduced pace with increasing contraction intensity, which resulted in a 17% lower maximal MUDR compared to the current study (16.5 vs. 19.9 /s). The present results are in line with several studies that have shown the age-difference in MUDR to be larger at high force levels (Barry et al. 2007, Kamen et al. 1995, Kamen & Knight 2004, Patten & Kamen 2000). The subjects in both age-groups in the present study were physically very active, as were the elderly subjects in the study by Dalton and co-workers. In the latter, the younger subjects were "recruited from the university population". Although they were reported to be "recreationally active", it is possible that the young subjects were less accustomed to the kinds of maximal contractions used in the measurements than the older men.

6.1.1.2 Dynamic contractions

In the dynamic contractions the decomposed signals showed no clear age-differences in MUDR (IV), even though the mean spike frequency was lower in OLD (I). There are a few possible reasons for this. Firstly, in the second experiment (IV) the relative surface EMG levels were higher in OLD, which could lead to an increase in MUDR and thus reduce the group-difference. In the first experiment (I) the groups were matched on relative sEMG levels, and thus eliminating this bias. Higher muscle activation with similar MUDR would indicate a higher reliance on recruitment rather than rate coding in the elderly. Secondly, previous studies have found an age-related increase in coactivation during dynamic contractions like walking (Schmitz et al. 2009), which could explain the observed difference in EMG/force relationship between the two groups. Conversely, some studies have found no such age-difference in isometric (Simoneau, Martin & Van Hoecke 2005) or dynamic (Ochala et al. 2004) contractions. Thirdly, as the ISO trials show, the differences in MUDR increased with increasing torque level (Figure 17), a phenomenon that has been shown previously by (Barry et al. 2007, Patten & Kamen 2000) (Kamen et al. 1995, Kamen & Knight 2004) but not in (Connelly et al. 1999) or (Dalton et al. 2009). If the difference is more pronounced at high forces, less difference could be expected at the low dynamic levels.

Especially with elderly subjects, derecruitment at the start of the movement in ECC contractions was very common, which decreased the number of recorded units significantly (Figure 19). In the present study low force levels were selected because they are generally easier to decompose. Due to the abovementioned reasons, future research should try to concentrate on measuring also higher force levels. As shown in Figure 22, with modern technology this can already be done.

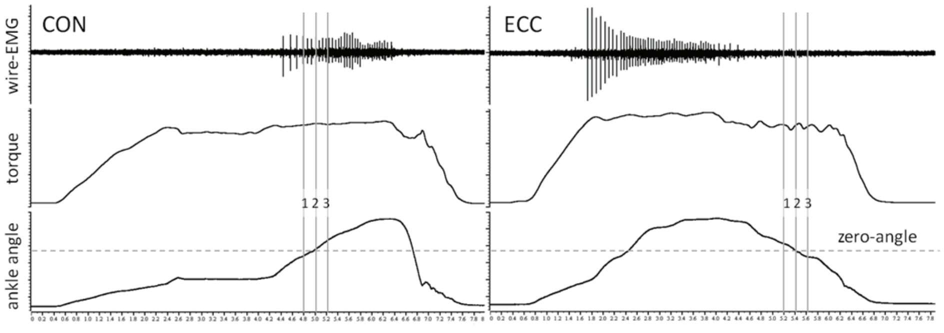


FIGURE 22 Examples of CON and ECC trials from one OLD subject at 60%MVC. The figures illustrate that identifying MUAP's in dynamic contractions is possible even at high force levels (CON, left). However, in many cases collecting MUAP's in ECC is quite challenging due to derecruitment (ECC, right).

6.1.2 Variability in force and discharge rate

In the present study the ability to hold a steady submaximal force was found to be significantly reduced in the elderly. Our findings are in line with previous reports in other muscles, such as the FDI (Laidlaw, Bilodeau & Enoka 2000) and the knee extensors (Tracy & Enoka 2002). Tracy (Tracy 2007) showed similar age-related differences in force fluctuations with ankle plantarflexors at the lowest force levels (2.5 and 5% MVC), while other force levels (10-80%) in that study did not show such an effect.

We also found the variability of motor unit discharge rate to be somewhat larger (n.s.) in elderly subjects. The activation levels used in the present study were quite low and, based on a previous FDI muscle study by Galganski et al. (Galganski, Fuglevand & Enoka 1993), it is possible that the group difference would have been smaller at higher levels of activation. It is interesting to note that the age-related differences in force fluctuations appear to be larger compared to the underlying changes in discharge rate variability. This finding is, however, in line with a previous study by Barry et al. (Barry et al. 2007), where both experimental data from the FDI muscle and simulation data using a modified Fuglevand model were compared. They found a slight increase in force variability even when there was no change in discharge rate variability. It is thus likely that the increased discharge rate variability reduces steadiness in conjunction with the other changes in motor unit physiology; for example reduced discharge rate, more fibres per MU, and different distribution of twitch forces. In addition, it is possible that the observed age-related differences in steadiness could be related to impairments in the visual feedback of the elderly (Sosnoff & Newell 2006b, Sosnoff & Newell 2006a). Recent studies have shown that removing visual feedback leads to similar force and discharge rate variabilities in young and elderly (Barry et al. 2007, Welsh, Dinenno & Tracy 2007). We did not observe any differences in DR between 10 and 20% activation levels within groups. This may be due to the fact that the same units were not measured at different force levels. This also prevents us from knowing what happened to DR coefficient of variation (CV) as activation level increased. Moritz et al (Moritz et al. 2005) and Barry et al (Barry et al. 2007) have shown that discharge rate variability decreases exponentially as the activation level increases after initial recruitment. This is a potential source of error in submaximal measurements. In order to make sure that the two pools of motor units we used in our isotonic analysis were comparable, we averaged for each group the amount each measured unit was above or below the measured recruitment threshold. We found the difference in mean to be very small (0.6 and 1.0 percentage points in 10 and 20%, respectively) and not statistically significant. Furthermore, the minor difference could not explain the observed larger CV% in OLD, as the analyzed units in OLD were on average more above the recruitment threshold compared to the units in YOUNG.

6.1.3 H-reflex and twitch properties

Similarly to previous literature (Morita et al. 1995, Sabbahi & Sedgwick 1982, Scaglioni et al. 2002) the H-reflex to M-wave ratio was lower in OLD in all conditions. There are numerous potential factors at the gamma motorneuron, spindle, Ia afferent and spinal level that may affect this age-related decrease [for review, see (Mynark & Koceja 2001)]. However, it has been suggested that the main cause would be an increase in presynaptic inhibition (Morita et al. 1995, Sabbahi & Sedgwick 1982, Scaglioni et al. 2002). These changes in the function of spinal reflexes are a likely factor in the deteriorating balance with aging (Koceja, Markus & Trimble 1995).

Also in agreement with the literature (Romano & Schieppati 1987, Pinniger et al. 2001, Duclay & Martin 2005) there was a clear trend of lowest average H/M ratio in ECC (n.s.) in both groups and at all activation levels. The same phenomenon that has also been found in stretch-reflexes (Nakazawa, Yamamoto & Yano 1997, Kallio, Linnamo & Komi 2004) is most likely due to presynaptic inhibition of Ia afferents (Romano & Schieppati 1987, Pinniger et al. 2001, Duclay & Martin 2005) or to homosynaptic postactivation depression (Hultborn et al. 1996).

The decreased MUDR has been suggested to be an adaptation to the increased twitch duration to optimize force generation (Roos, Rice & Vandervoort 1997). As the twitch duration increases with age, tetanus can be achieved with lower discharge rates. The present results confirm the previous results, that show the twitch contraction time in YOUNG to be significantly shorter compared to OLD (Dalton et al. 2009, Dalton et al. 2010).

6.2 Effects of contraction type on motor unit activation

6.2.1 Isometric contractions

MUDR was investigated both in ramp contractions at recruitment threshold and in isotonic contractions up to MVC. In YOUNG the average MUDR ranged from 6.6pps at the lowest recruitment thresholds to 19.9pps at MVC, while in OLD the corresponding rates were 5.4pps and 16.4pps. Compared to a previous study by Dalton et al. (Dalton et al. 2010) the maximal MUDRs in YOUNG were slightly higher (16.4pps) while in OLD the results were almost identical (16.4pps). In an older paper by Bellemare et al. (Bellemare et al. 1983) the reported maximal MUDR (10.7pps) in adult men was almost 40% lower. Since this value is much lower than the one reported from elderly subjects in more recent studies, it can’t be explained by the higher average subject age. Training background and activity status (not reported) might be another explaining factor, as well as the inclusion of both sexes as subjects. Women have generally a higher proportion of slow-twitch units that are known to have a lower MUDR (Celichowski & Drzymala 2006). The current data adds support to the suggestion that soleus force control works with a much more limited range of motor unit discharge rates compared to most other studied muscles, like adductor pollicis (30pps), biceps brachii (31pps), tibialis anterior (33pps) and abductor digiti minimi (52pps) (Bellemare et al. 1983, Van Cutsem et al. 1997, Patten, Kamen & Rowland 2001).

In the current study the increase in MUDR followed a second order polynomial equation with a steeper increase at higher torque levels. It is likely that increases in soleus force at high force levels rely more on an increase in MUDR than MU recruitment, even though some units have been observed to have thresholds above 90% MVC (Oya, Riek & Cresswell 2009). In the decomposition studies (II, III, IV) recruitment was not quantified, as each force and activation level was investigated separately. However, in the ISAF analysis assumptions could be made based on the two measured values, the mean spike amplitude and frequency. Increased amplitude would imply recruitment of larger motor units, whereas changes in mean spike frequency parallel changes in the firing rate of active motor units (Moritani et al. 1986). As expected, both values increased with activation level. As the activation-related increase was more pronounced in amplitude, it would indicate a stronger contribution from motor unit recruitment compared to rate coding at the analyzed moderate (20, 40%) activation levels.

6.2.2 Dynamic contractions

Our main interests in the study of motor unit control in dynamic conditions were the effects of contraction mode on MUDR and on the recruitment order. In the present study both age-groups showed a larger MUDR in CON compared to ISO or ECC at low submaximal force levels (III, IV). Even when the different contraction modes were matched by EMG activation levels (I), the intramuscular mean spike frequency (an indirect measure of MUDR) was slightly higher (n.s.) in CON than in ISO or ECC.

Two possible reasons for the lower MUDR in ECC have been suggested previously (Duchateau & Enoka 2008). Mechanically, less torque is required to lower (ECC) the same load compared to lifting it (CON). We controlled the weights so that the absolute plantarflexion torque in all contraction types was the same. The relative load in ECC contraction was possibly lower due to the higher maximal torque capacity in ECC. It is likely that the cross-bridges generate more torque when resisting pull (ECC) than they do when producing movement (CON) (Joyce, Rack & Westbury 1969). We measured MVC only in ISO, so the dynamic torques relative to their corresponding maxima are unknown. In an earlier study on plantarflexors the difference between MVCs in CON and ECC was 17% (Linnamo et al. 2003a). In the present study the difference in MUDR between CON and ECC at the three force levels was between 29-38%. It thus seems that the difference between contraction types is larger in MUDR than absolute torque production. This is in accordance with the study of (Altenburg et al. 2009) which showed a higher MUDR in CON even when force-velocity-related differences in force capacity were taken into account.

Global muscle activity of soleus ($sEMG_{SOL}$) behaved similarly to MUDR in different measurement trials. Both MUDR and $sEMG_{SOL}$ were largest in CON, however only $sEMG_{SOL}$ increased with torque levels in dynamic contractions. The torque related increase in neural drive to the muscle is probably more visible in $sEMG_{SOL}$ due to motor unit recruitment. At the lowest activation levels, new, slowly firing units were recruited as force level was increased. This would have a diminishing effect on the average MUDR, while the recruited units would still increase the amplitude in $sEMG_{SOL}$ (Farina, Merletti & Enoka 2004).

The behaviour of gastrocnemius activity differed from soleus in the dynamic contractions, as the gastrocnemius activity in ECC was as at the same level as in CON. This phenomenon has previously been reported by (Nardone & Schieppati 1988) and could reflect the more dominant role of gastrocnemius in normal dynamic contractions. In two classic papers, Nardone et al. reported the shift to a faster muscle (gastrocnemius) and the EMG findings as evidence for reversal of the recruitment order of motor units (Nardone, Romano & Schieppati 1989, Nardone & Schieppati 1988). In the present study we did not analyze recruitment order of the decomposed units systematically. However, in ECC trials we observed several clear cases of derecruitment of units that had been active in ISO and/or CON, but none vice versa. Further, the ISAF analysis did not show differences in the mean spike amplitude between contraction types in either age-group. This would also indirectly support Henneman's size principle on the orderly recruitment of MU's (Henneman , Somjen & Carpenter 1965, Henneman, Somjen & Carpenter 1965), as the selective recruitment of larger units in ECC should have increased the mean spike amplitude either through new, larger spikes, or through the derecruitment of spikes with smaller amplitudes. As the comparison between trials was not based on following individual MUs, but pools of several MUs, distinct conclusions about the recruitment order of slow and fast units are difficult to make. However, the ability for ISAF analysis to show recruitment of MUs is supported by an increase in the mean spike amplitude along with an increase in isometric force in the present study as well as in earlier studies with biceps brachii (Linnamo et al. 2003b, Moritani & Muro 1987).

The preservation of size principle has been found in ECC contractions in several studies (Sogaard et al. 1996, Pasquet, Carpentier & Duchateau 2006, Bawa & Jones 1999, Garland et al. 1996, Kossev & Christova 1998a, Kossev & Christova 1998b). The present results of similar mean spike amplitudes in all contraction types would also not imply a previously reported lower threshold of MU recruitment in dynamic contractions (Ivanova, Garland & Miller 1997, Tax et al. 1989). This difference could be due to muscle specific MU control strategies both within a muscle and between synergists. The aforementioned data is from biceps brachii, which, compared to soleus, has a much larger proportion of fast motor units. The present data does not allow comparison of recruitment thresholds between dynamic and isometric contractions in gastrocnemius muscle in which the fiber type distribution is more close to biceps brachii.

6.3 Limitations and practical implications of the study

The main challenges of the experiments were connected to the difficulty of recording and analyzing single motor unit action potentials, especially in large muscles and in dynamic contractions.

Motor units are often studied in muscles that are small and/or do not have many synergists (for example FDI, TA). We chose to study motor unit behaviour in soleus due to its important role in balance and locomotion. However, soleus is a large muscle with large synergists, like the gastrocnemii, which makes interpreting the results more difficult.

Due to the challenge of signal decomposition the sample size/trial in the current study was modest. Also, the recorded units may not represent the average of all SOL units, as all the wires in both experiments were located in the vicinity of the surface electrode, approximately 1/3 medial from the lateral end of the muscle. We do not know how the different fiber-types are distributed longitudinally along the muscle. However, an MRI study by (Kinugasa, Kawakami & Fukunaga 2005) has shown the soleus activation to be distributed evenly in plantarflexion exercises. It has been reported by Knight and Kamen (Knight & Kamen 2005) that larger MUs are located more superficially. In order to avoid such motor unit type bias wire electrodes were located at various depths. As the amount of adipose tissue increases with aging, it could be possible that the wire electrodes that were inserted to equal depths in both groups would not penetrate as deep into the muscle of elderly subjects, leading to a biased motor unit sample. However, since our elderly subjects were physically very active and lean we find such bias unlikely.

A further limitation in dynamic single motor unit measurements is the confinement to only low contraction levels, in this case to 40% MVC and below. This restriction reduces the amount of units analyzable in ECC contractions, as in many cases none of the active units that were visible in CON and/or ISO were active in ECC. However, as the present measurements showed (Figure 22), analyzing dynamic contractions at higher force levels is possible and should be an aim in future studies.

In measurements with intramuscular electrodes, there is always a risk of electrode movement, especially during dynamic contractions. In order to minimize a possible bias from electrode migration, we randomized the order of trials in both experiments. Also, since in many cases we were clearly able to follow the same decomposed units throughout several conditions, it would appear that electrode movement was not a significant source of error.

In experiment I the different trials were normalized to the RMS amplitude of the soleus sEMG at MVC. Since sEMG is the sum of all the motor unit action potential trains within its reach, the overlapping positive and negative phases of the potentials can cancel each other, resulting in a phenomenon called amplitude cancellation (Day & Hulliger 2001). When the signal is normalized to maximal level, as in the present study, the error is minimized, but could lead to a 13%

overestimation of motor unit activity (Keenan et al. 2005). However, since Keenan et al. (2005) have reported the overestimation to be smallest at MVC and largest when recruitment is complete, this error should be minimal in soleus, where new units are recruited up to 95% MVC (Oya, Riek & Cresswell 2009). The effect of cancellation on the current results is further minimized by the fact that this overestimation should affect both age-groups and all contraction types similarly.

On average motor units were recorded at the same ankle angle in all conditions, although the start and end angles in dynamic conditions differed from ISO. Thus, in ECC for example, the possible effect of shorter fascicle length in MUDR at the start of recording would be cancelled by the adversary effect of a longer fascicle in the end position. Several studies have shown fascicle length to be quite independent of total muscle-tendon length in dynamic movements, like jumping (Finni et al. 2003), walking and running (Lichtwark & Wilson 2006). We did not measure fascicle length directly, but as average joint angle and force were equal in ISO, CON and ECC, average fascicle length can also be assumed to be the same. This suggestion is supported by the study by Pasquet and colleagues (Pasquet, Carpentier & Duchateau 2006), showing that the fascicle length change in tibialis anterior varied linearly with ankle joint angle.

A very slow joint rotation velocity (10 deg/s) was selected for the dynamic contractions of the two experiments in order to enable the analysis of single motor unit action potentials. This velocity is significantly lower compared to most natural movements. For example, in walking, the angular velocity in dorsiflexion is ~30 deg/s and in plantarflexion ~220 deg/s (estimated from Lichtwark, Bougoulias & Wilson 2007). We also measured trials with a velocity that was twice as fast as the reported trials (20 deg/s). However, as the range of motion in ankle angle is limited, and some movement is required for the acceleration and deceleration phases, too few firings could be recorded from each movement to calculate a rate for motor unit discharges. This is a challenge that has to be overcome in order to be able to see how the activation functions in normal locomotion.

Despite being physically quite active, the OLD showed a significantly lower isometric MVC. One possible explanation for the observed lower discharge rate in the elderly would be an inability to fully activate their muscles during MVC. This would lead to erroneously low submaximal target levels that would require less activity from the motor units. However, a number of previous studies have shown that the voluntary activation of plantar flexors during MVC is unchanged in old subjects (Dalton et al. 2009, Vandervoort & McComas 1986, Simoneau, Martin & Van Hoecke 2005).

To what degree is the age-related deterioration in muscle performance, like decreasing maximal force and slowing of force production an inevitable part of life, and how much can be explained by changes in everyday activity? In the present study all the subjects in both age-groups were quantitatively equally active, as they performed moderate to strenuous physical exercise at least three times a week. However, the open-ended questionnaire showed clear group-differences in the types of activities they engaged in. The young subjects were more active in sports requiring strength and speed (e.g. running, soccer, ice-hockey, weight-training, tennis) than the elderly (e.g. walking with and without poles, swimming, gardening). More studies are thus needed to investigate whether there are training modes that would be more suited for preventing or possibly even reversing the observed age-differences. Since the subjects in both groups were physically very active, they do not represent the average of that age-level. However, for this reason we think that the observed group-differences are more the result of the unavoidable process of aging instead of the reduced physical activity that often accompanies aging.

The main goal of the present study was to investigate age-related changes in the activation and control of the soleus muscle. The present study clearly displays age-related deterioration of the neuromuscular system at many functionally important levels. The elderly had a lower MVC, slower force production (twitch CT) and reduced steadiness and MU discharge rate. All these changes may affect balance control and thus the ability to avoid falling and injuries.

7 PRIMARY FINDINGS AND CONCLUSIONS

1) The current results showed a clear age-difference in the soleus MUDR in isometric contractions. This lower MUDR in OLD adds evidence to the previous theory, whereby the most prominent age-related changes can be seen in distal muscles. The longer single muscle twitch duration in OLD suggests that the neuromuscular system may tune the discharge rate to match the changes in motor unit physiology.

2) Differences in MUDR between both age-groups and contraction levels were larger at forces close to MVC. It appears that coinciding changes in MU recruitment make the comparison of MUDR more difficult. This is supported by our analysis of MU pairs, which showed a steeper slope in MUDR with increasing force compared to a pooled analysis of all units. This may explain partially why no age-differences were found in dynamic contractions at the measured low force levels.

3) Also steadiness, the ability to hold a given isometric steady force-level, was lower in OLD although the corresponding difference in MUDR variation was not significantly different. Based on previous findings, it can be assumed that MUDR is only one of several factors that contribute to the decreased steadiness in elderly people.

4) In line with previous literature, H-reflex excitability was lower in OLD, which may be an indication of increased presynaptic inhibition (PI). In both age-groups the average H/M ratio was also always slightly lower in ECC compared to other contraction types, which is in line with previous findings in stretch-reflexes, and could be due to either PI or homosynaptic postactivation depression.

5) In dynamic measurements concentric contractions always required a higher MUDR compared to eccentric or isometric muscle actions, regardless of age or the method of normalization. The higher MUDR in CON can only partially be explained by the lower maximal force-capacity, since the MUDR was highest in CON even when muscle activation was matched between conditions.

YHTEENVETO

Motoristen yksiköiden aktivointi ja selkäydintason aktivaatioherkkyys nuorilla ja vanhoilla miehillä isometrisessä ja dynaamisessa lihastyössä

Lihaksen pienin hallittavissa oleva osa on yhden alfamotoneuronin ja sen hermottamien lihassolujen muodostama motorinen yksikkö. Voimaa vaativassa työssä aktivoimme lihaksen, jossa otetaan käyttöön uusia motorisia yksiköitä ja lisätään jo aktiivisten yksiköiden syttymistiheyttä. Voimatason kasvaessa yksiköt rekrytoidaan todennäköisesti aina samassa järjestyksessä. Tämän nk. Hennemanin kokoperiaatteen mukaan ensin aktivoidaan ne yksiköt, jotka ovat kaikkein kestävimpiä, mutta kooltaan pienimpiä sekä voimantuotossa hitaimpia ja heikoimpia. Suurimmat, voimakkaimmat ja nopeimmat, mutta helpoimmin väsyvät yksiköt rekrytoidaan puolestaan viimeisenä ja ne ovat aktiivisia ainoastaan korkeilla voimatasoilla ja mahdollisesti nopeissa suorituksissa. Motoristen yksiköiden toimintaa voidaan tutkia rekisteröimällä elektrodeilla niiden aktivoinnissa syntyviä lihassolukalvon jännitevaihteluita. Ihon pinnalle kiinnitettävillä elektrodeilla mitataan normaalisti lukuisten motoristen yksiköiden aktiivisuutta kerralla, kun taas lihaksen sisään sijoitettavien elektrodien avulla saavutetaan jopa yksittäisten yksiköiden erottelutarkkuus. Lihastyön tuottamaan voimaan vaikuttavat lihaksen aktiivisuuden lisäksi myös sen pituus ja työtapa. Lihasta pitää aktivoida enemmän punnusta nostettaessa eli lihaksen lyhentyessä (konsentrinen), kuin punnusta paikallaan pidettäessä (isometrinen) tai lihaksen pidentyessä tätä samaa painoa laskettaessa (eksentrinen).

Ikääntymisen johdosta hermolihasjärjestelmän toiminta heikkenee monella tavalla, mikä lisää vanhemman väestön onnettomuusriskiä mm. alentuneen voimantuottonopeuden ja siihen liittyvän heikentyneen tasapainokontrollin johdosta. Lihasvoiman ja voimantuottonopeuden pieneneminen ovat seurausta lihassolujen koon ja määrän vähenemisestä, erityisesti suurimpien yksiköiden kohdalla. Monissa aikaisemmista tutkimuksista on havaittu muutoksia myös yksiköiden aktivoinnissa. On todennäköistä, että hitaiden yksiköiden määrän suhteellinen kasvu on syynä havaittuun yksiköiden aktivaatiotiheyden laskuun.

Tämän tutkimuksen lähtökohtana oli selvittää lihastyötavan ja iän vaikutusta motoristen yksiköiden kontrolliin pohjelihaksistoon kuuluvassa soleus-lihaksessa. Päätavoitteina oli 1) saada tietoa motoristen yksiköiden kontrollista tasapainon ja liikkumisen kannalta tärkeällä lihaksella ja näiden kannalta oleellisilla lihastyötavoilla mittaamalla soleus-lihaksen motoristen yksiköiden syttymistä dynaamisessa lihastyössä ja suurilla voimatasoilla, 2) tutkia ikääntymisen ja lihastyötavan vaikutuksia selkäydintason aktivaatioherkkyyteen (H-refleksi) ja motoristen yksiköiden aktivaatioon dynaamisissa ja isometrisissä lihassupistuksissa ja 3) vertailla motoristen yksiköiden syttymistiheyden ja voiman kontrollia ikäryhmien välillä.

Tutkimukseen osallistui nuoria (20-30v) ja vanhoja (65-80v), fyysisesti aktiivisia miehiä, jotka suorittivat nilkan plantaarifleksioita eli ojennuksia dynamometrissä istuen. Motoristen yksiköiden aktiivisuutta rekisteröitiin lihaksen sisään laitettavilla lankaelektrodeilla.

Tulokset osoittavat selkeän eron motoristen yksiköiden syttymistiheydessä nuorten ja ikääntyneiden välillä. Sähköstimulaation tulokset tukevat olettamusta, jonka mukaan lihaksen kontrollia muokataan ikääntymisen myötä siten, että lihassolujen supistusnopeuden hidastuessa myös niiden syttymistiheyttä lasketaan. Syttymistiheydessä tapahtuvien muutosten havaitsemista vaikeuttaa aktiivisten yksiköiden määrän vaihtelu, mikä saattaa selittää sen, että ikäryhmien välinen ero näkyi ainoastaan isometrisessä lihastyössä. Useat hermolihasjärjestelmässä tapahtuvat muutokset ovat todennäköisesti syynä siihen, että myös kyky ylläpitää tasaista voimaa oli heikompi ikääntyneiden ryhmässä. Konsentrinen lihastyö edellytti aina isometristä ja eksentristä korkeampaa motoristen yksiköiden syttymistiheyttä, silloinkin kun lihaksen pinnalta rekisteröity aktiivisuus oli yhtä suuri eri lihastyötavoilla.

Tässä tutkimuksessa todennettiin ikääntymisen vaikutuksia monissa, tasapainon ja liikunnan kannalta tärkeissä, hermolihasjärjestelmän toiminnoissa. Nämä muutokset saattavat lisätä ikääntyneiden kaatumis- ja loukkaantumisriskiä.

REFERENCES

Adrian, E. D. & Bronk, D. W. 1929. The discharge of impulses in motor nerve fibres: Part II. The frequency of discharge in reflex and voluntary contractions. The Journal of Physiology 67 (2), i3-151.

Allinger, T. L. & Engsberg, J. R. 1993. A method to determine the range of motion of the ankle joint complex, in vivo. Journal of Biomechanics 26 (1), 69-76.

Altenburg, T. M., de Ruiter, C. J., Verdijk, P. W., van Mechelen, W. & de Haan, A. 2009. Vastus lateralis surface and single motor unit electromyography during shortening, lengthening and isometric contractions corrected for mode-dependent differences in force-generating capacity. Acta Physiologica 196 (3), 315-328.

Amiridis, I. G., Martin, A., Morlon, B., Martin, L., Cometti, G., Pousson, M. & van Hoecke, J. 1996. Co-activation and tension-regulating phenomena during isokinetic knee extension in sedentary and highly skilled humans. European Journal of Applied Physiology & Occupational Physiology 73 (1-2), 149-156.

Andersen, J. L., Terzis, G. & Kryger, A. 1999. Increase in the degree of coexpression of myosin heavy chain isoforms in skeletal muscle fibers of the very old. Muscle & Nerve 22 (4), 449-454.

Avela, J., Finni, T., Liikavainio, T., Niemela, E. & Komi, P. V. 2004. Neural and mechanical responses of the triceps surae muscle group after 1 h of repeated fast passive stretches. Journal of Applied Physiology 96 (6), 2325-2332.

Barany, M. 1967. ATPase activity of myosin correlated with speed of muscle shortening. The Journal of General Physiology 50 (6), Suppl:197-218.

Barry, B. K., Pascoe, M. A., Jesunathadas, M. & Enoka, R. M. 2007. Rate coding is compressed but variability is unaltered for motor units in a hand muscle of old adults. Journal of Neurophysiology 97 (5), 3206-3218.

Basmajian, J. V. & De Luca, C. J. 1985. Muscles alive. Their Functions Revealed by Electromyography. (5th edition) Baltimore, MD, USA: Williams & Wilkins.

Basmajian, J. V. & De Luca, C. J. 1964. Muscles Alive. Their Functions Revealed by Electromyography. Baltimore: Williams & Wilkins.

Basmajian, J. V. & Stecko, G. 1962. A new bipolar electrode for electromyography. Journal of Applied Physiology 17(5), 849.

Bawa, P. & Jones, K. E. 1999. Do lengthening contractions represent a case of reversal in recruitment order? Progress in Brain Research 123, 215-220.

Bellemare, F., Woods, J. J., Johansson, R. & Bigland-Ritchie, B. 1983. Motor-unit discharge rates in maximal voluntary contractions of three human muscles. Journal of Neurophysiology 50 (6), 1380-1392.

Bigland, B. & Lippold, O. C. 1954. Motor unit activity in the voluntary contraction of human muscle. Journal of Physiology 125 (2), 322-335.

Brown, W. F., Strong, M. J. & Snow, R. 1988. Methods for estimating numbers of motor units in biceps-brachialis muscles and losses of motor units with aging. Muscle & Nerve 11 (5), 423-432.

Burke, J. R., Schutten, M. C., Koceja, D. M. & Kamen, G. 1996. Age-dependent effects of muscle vibration and the Jendrassik maneuver on the patellar tendon reflex response. Archives of Physical Medicine and Rehabilitation 77 (6), 600-604.

Burke, R. E., Levine, D. N., Tsairis, P. & Zajac, F. E.,3rd 1973. Physiological types and histochemical profiles in motor units of the cat gastrocnemius. The Journal of physiology 234 (3), 723-748.

Butchart, P., Farquhar, R., Part, N. & Roberts, R. 1993. The effect of age and voluntary contraction on presynaptic inhibition of soleus muscle Ia afferent terminals in man. Experimental Physiology 78 (2), 235-242.

Celichowski, J. & Drzymala, H. 2006. Differences between properties of male and female motor units in the rat medial gastrocnemius muscle. Journal of Physiology & Pharmacology 57 (1), 83-93.

Chalmers, G. R. 2008. Can fast-twitch muscle fibres be selectively recruited during lengthening contractions? Review and applications to sport movements. Sports Biomechanics 7 (1), 137-157.

Chalmers, G. R. & Knutzen, K. M. 2000. Soleus Hoffmann-reflex modulation during walking in healthy elderly and young adults. Journals of Gerontology Series A-Biological Sciences & Medical Sciences 55 (12), B570-9.

Christensen, H., Sogaard, K., Jensen, B. R., Finsen, L. & Sjogaard, G. 1995. Intramuscular and surface EMG power spectrum from dynamic and static contractions. Journal of Electromyography and Kinesiology 5 (1), 27-36.

Christie, A. & Kamen, G. 2010. Short-term training adaptations in maximal motor unit firing rates and afterhyperpolarization duration. Muscle & Nerve 41 (5), 651-660.

Christou, E. A. & Carlton, L. G. 2002. Age and contraction type influence motor output variability in rapid discrete tasks. Journal of Applied Physiology 93 (2), 489-498.

Christova, P. & Kossev, A. 2000. Human motor unit activity during concentric and eccentric movements. Electromyography & Clinical Neurophysiology 40 (6), 331-338.

Connelly, D. M., Rice, C. L., Roos, M. R. & Vandervoort, A. A. 1999. Motor unit firing rates and contractile properties in tibialis anterior of young and old men. Journal of Applied Physiology 87 (2), 843-852.

Cunningham, D. A., Morrison, D., Rice, C. L. & Cooke, C. 1987. Ageing and isokinetic plantar flexion. European Journal of Applied Physiology & Occupational Physiology 56 (1), 24-29.

Dalton, B. H., Harwood, B., Davidson, A. W. & Rice, C. L. 2010. Recovery of motoneuron output is delayed in old men following high-intensity fatigue. Journal of Neurophysiology 103 (2), 977-985.

Dalton, B. H., Harwood, B., Davidson, A. W. & Rice, C. L. 2009. Triceps surae contractile properties and firing rates in the soleus of young and old men. Journal of Applied Physiology (Bethesda, Md.: 1985) 107 (6), 1781-1788.

Dalton, B. H., McNeil, C. J., Doherty, T. J. & Rice, C. L. 2008. Age-related reductions in the estimated numbers of motor units are minimal in the human soleus. Muscle & Nerve 38 (3), 1108-1115.

Day, S. J. & Hulliger, M. 2001. Experimental simulation of cat electromyogram: evidence for algebraic summation of motor-unit action-potential trains. Journal of Neurophysiology 86 (5), 2144-2158.

De Luca, C. J., LeFever, R. S., McCue, M. P. & Xenakis, A. P. 1982. Behaviour of human motor units in different muscles during linearly varying contractions. Journal of Physiology 329, 113-128.

Del Valle, A. & Thomas, C. K. 2005. Firing rates of motor units during strong dynamic contractions. Muscle & Nerve 32 (3), 316-325.

Delwaide, P. J. 1973. Human monosynaptic reflexes and presynaptic inhibition. An interpretation of spastic hyperreflexia. In J. E. Desmedt (Ed.) New Developments in Electromyography and Clinical Neurophysiology.Basel, Switzerland: Karger, 508-522.

Denny-Brown, D. 1929. On the Nature of Postural Reflexes. Proceedings of the Royal Society of London - B 104, 252-301.

Desmedt, J. E. & Godaux, E. 1977. Ballistic contractions in man: characteristic recruitment pattern of single motor units of the tibialis anterior muscle. Journal of Physiology 264 (3), 673-693.

Di Giulio, I., Maganaris, C. N., Baltzopoulos, V. & Loram, I. D. 2009. The proprioceptive and agonist roles of gastrocnemius, soleus and tibialis anterior muscles in maintaining human upright posture. The Journal of Physiology 587 (Pt 10), 2399-2416.

Doherty, T. J., Vandervoort, A. A. & Brown, W. F. 1993. Effects of ageing on the motor unit: a brief review. Canadian Journal of Applied Physiology 18 (4), 331-358.

Dousset, E., Avela, J., Ishikawa, M., Kallio, J., Kuitunen, S., Kyrolainen, H., Linnamo, V. & Komi, P. V. 2007. Bimodal recovery pattern in human skeletal muscle induced by exhaustive stretch-shortening cycle exercise. Medicine & Science in Sports & Exercise 39 (3), 453-460.

Duchateau, J. & Enoka, R. M. 2008. Neural control of shortening and lengthening contractions: influence of task constraints. Journal of Physiology 586 (Pt 24), 5853-5864.

Duchateau, J. & Hainaut, K. 1990. Effects of immobilization on contractile properties, recruitment and firing rates of human motor units. Journal of Physiology 422, 55-65.

Duclay, J. & Martin, A. 2005. Evoked H-Reflex and V-Wave Responses During Maximal Isometric, Concentric, and Eccentric Muscle Contraction. Journal of Neurophysiology 94 (5), 3555-3562.

Edman, K. A. 1988. Double-hyperbolic force-velocity relation in frog muscle fibres. The Journal of Physiology 404, 301-321.

Edman, K. A., Elzinga, G. & Noble, M. I. 1978. Enhancement of mechanical performance by stretch during tetanic contractions of vertebrate skeletal muscle fibres. The Journal of Physiology 281, 139-155.

Edstrom, L. & Kugelberg, E. 1968. Histochemical composition, distribution of fibres and fatiguability of single motor units. Anterior tibial muscle of the rat. Journal of Neurology, Neurosurgery, and Psychiatry 31 (5), 424-433.

Enoka, R. 2008. Neuromechanics of Human Movement. (4th edition) Champaign IL. USA: Human Kinetics.

Farina, D., Colombo, R., Merletti, R. & Olsen, H. B. 2001. Evaluation of intramuscular EMG signal decomposition algorithms. Journal of Electromyography and Kinesiology 11 (3), 175-187.

Farina, D., Holobar, A., Merletti, R. & Enoka, R. M. 2010. Decoding the neural drive to muscles from the surface electromyogram. Clinical neurophysiology 121 (10), 1616-1623.

Farina, D., Merletti, R. & Enoka, R. M. 2004. The extraction of neural strategies from the surface EMG. Journal of Applied Physiology 96 (4), 1486-1495.

Farina, D. & Rainoldi, A. 1999. Compensation of the effect of sub-cutaneous tissue layers on surface EMG: a simulation study. Medical engineering & physics 21 (6-7), 487-497.

Finni, T., Ikegawa, S., Lepola, V. & Komi, P. V. 2003. Comparison of force-velocity relationships of vastus lateralis muscle in isokinetic and in stretch-shortening cycle exercises. Acta Physiologica Scandinavica 177 (4), 483-491.

Frontera, W. R., Hughes, V. A., Lutz, K. J. & Evans, W. J. 1991. A cross-sectional study of muscle strength and mass in 45- to 78-yr-old men and women. Journal of Applied Physiology (Bethesda, Md.: 1985) 71 (2), 644-650.

Fuglevand, A. J., Winter, D. A. & Patla, A. E. 1993. Models of recruitment and rate coding organization in motor-unit pools. Journal of Neurophysiology 70 (6), 2470-2488.

Fuglevand, A. J., Winter, D. A., Patla, A. E. & Stashuk, D. 1992. Detection of motor unit action potentials with surface electrodes: influence of electrode size and spacing. Biological Cybernetics 67 (2), 143-153.

Galganski, M. E., Fuglevand, A. J. & Enoka, R. M. 1993. Reduced control of motor output in a human hand muscle of elderly subjects during submaximal contractions. Journal of Neurophysiology 69 (6), 2108-2115.

Garland, S. J., Cooke, J. D., Miller, K. J., Ohtsuki, T. & Ivanova, T. 1996. Motor unit activity during human single joint movements. Journal of Neurophysiology 76 (3), 1982-1990.

Garland, S. J. & Griffin, L. 1999. Motor unit double discharges: statistical anomaly or functional entity? Canadian Journal of Applied Physiology 24 (2), 113-130.

Gordon, A. M., Huxley, A. F. & Julian, F. J. 1966. The variation in isometric tension with sarcomere length in vertebrate muscle fibres. The Journal of Physiology 184 (1), 170-192.

Graves, A. E., Kornatz, K. W. & Enoka, R. M. 2000. Older adults use a unique strategy to lift inertial loads with the elbow flexor muscles. Journal of Neurophysiology 83 (4), 2030-2039.

Gydikov, A. & Kosarov, D. 1974. Some features of different motor units in human biceps brachii. Pflugers Archiv : European Journal of Physiology 347 (1), 75-88.

Häkkinen K., Newton RU., Gordon SE., McCormick M., Volek JS., Nindl BC., Gotshalk LA., Campbell WW. & Evans WJ. 1998. Changes in muscle morphology, electromyographic activity, and force production characteristics during progressive strength training in young and older men. Journals of Gerontology Series A-Biological Sciences & Medical Sciences 53 (6), B415-23.

Hamacher, D., Singh, N. B., Van Dieen, J. H., Heller, M. O. & Taylor, W. R. 2011. Kinematic measures for assessing gait stability in elderly individuals: a systematic review. Journal of the Royal Society, Interface 8 (65), 1682-1698.

Harwood, B., Davidson, A. W. & Rice, C. L. 2011. Motor unit discharge rates of the anconeus muscle during high-velocity elbow extensions. Experimental Brain Research 208 (1), 103-113.

Heinrich, S., Rapp, K., Rissmann, U., Becker, C. & Konig, H. H. 2010. Cost of falls in old age: a systematic review. Osteoporosis International 21 (6), 891-902.

Henneman , E., Somjen, G. & Carpenter, D. O. 1965. Functional Significance of Cell Size in Spinal Motoneurons. Journal of Neurophysiology 28, 560-580.

Henneman, E., Somjen, G. & Carpenter, D. O. 1965. Excitability and inhibitability of motoneurons of different sizes. Journal of Neurophysiology 28 (3), 599-620.

Hermens, H. J., Freriks, B., Merletti, R., Stegeman, D., Blok, J., Rau, G., Disselhorst-Klug, C. & Hägg, G. 1999. SENIAM 8: European recommendations for surface electromyography. Roessingh Research and Development b.v. ISBN: 90-75452-15-2.

Howard, J. E., McGill, K. C. & Dorfman, L. J. 1988. Age effects on properties of motor unit action potentials: ADEMG analysis. Annals of Neurology 24 (2), 207-213.

Howell, J. N., Fuglevand, A. J., Walsh, M. L. & Bigland-Ritchie, B. 1995. Motor unit activity during isometric and concentric-eccentric contractions of the human first dorsal interosseus muscle. Journal of Neurophysiology 74 (2), 901-904.

Hultborn, H., Illert, M., Nielsen, J., Paul, A., Ballegaard, M. & Wiese, H. 1996. On the mechanism of the post-activation depression of the H-reflex in human subjects. Experimental brain research. 108 (3), 450-462.

Ivanova, T., Garland, S. J. & Miller, K. J. 1997. Motor unit recruitment and discharge behavior in movements and isometric contractions. Muscle & Nerve 20 (7), 867-874.

Johnson, M. A., Polgar, J., Weightman, D. & Appleton, D. 1973. Data on the distribution of fibre types in thirty-six human muscles. An autopsy study. Journal of the Neurological Sciences 18 (1), 111-129.

Jonsson, B. & Bagge, U. E. 1968. Displacement, deformation and fracture of wire electrodes for electromyography. Electromyography 8 (4), 329-347.

Joyce, G. C., Rack, P. M. & Westbury, D. R. 1969. The mechanical properties of cat soleus muscle during controlled lengthening and shortening movements. Journal of Physiology 204 (2), 461-474.

Kallio, J., Linnamo, V. & Komi, P. V. 2004. The effects of muscle history on short latency stretch reflex response of soleus muscle. Journal of Electromyography & Kinesiology 14 (3), 411-421.

Kamen, G. & Knight, C. A. 2004. Training-related adaptations in motor unit discharge rate in young and older adults. Journals of Gerontology Series A-Biological Sciences & Medical Sciences 59 (12), 1334-1338.

Kamen, G., Sison, S. V., Du, C. C. & Patten, C. 1995. Motor unit discharge behavior in older adults during maximal-effort contractions. Journal of Applied Physiology 79 (6), 1908-1913.

Kandel, E. R., Schwartz, J. H. & Jessell, T. M. 2000. Principles of Neural Science. (4th edition) New York, US: McGraw-Hill.

Katz, B. 1939. The relation between force and speed in muscular contraction. The Journal of Physiology 96 (1), 45-64.

Keenan, K. G., Farina, D., Maluf, K. S., Merletti, R. & Enoka, R. M. 2005. Influence of amplitude cancellation on the simulated surface electromyogram. Journal of Applied Physiology 98 (1), 120-131.

Kinugasa, R., Kawakami, Y. & Fukunaga, T. 2005. Muscle activation and its distribution within human triceps surae muscles. Journal of Applied Physiology (Bethesda, Md.: 1985) 99 (3), 1149-1156.

Klass, M., Baudry, S. & Duchateau, J. 2008. Age-related decline in rate of torque development is accompanied by lower maximal motor unit discharge frequency during fast contractions. Journal of Applied Physiology 104 (3), 739-746.

Klass, M., Baudry, S. & Duchateau, J. 2007. Voluntary activation during maximal contraction with advancing age: a brief review. European Journal of Applied Physiology 100 (5), 543-551.

Knight, C. A. & Kamen, G. 2005. Superficial motor units are larger than deeper motor units in human vastus lateralis muscle. Muscle & Nerve 31 (4), 475-480.

Koceja, D. M., Markus, C. A. & Trimble, M. H. 1995. Postural modulation of the soleus H reflex in young and old subjects. Electroencephalography & Clinical Neurophysiology 97 (6), 387-393.

Koceja, D. M. & Mynark, R. G. 2000. Comparison of heteronymous monosynaptic Ia facilitation in young and elderly subjects in supine and standing positions. The International Journal of Neuroscience 103 (1-4), 1-17.

Komi, P. V. 1973. Relationship between muscle tension, EMG and velocity of contraction under concentric and eccentric work. In J. E. Desmedt (Ed.) New Developments in Electromyography and Clinical Neurophysiology.Basel: Krager, 596-606.

Komi, P. V. & Buskirk, E. R. 1970. Reproducibility of electromyographic measurements with inserted wire electrodes and surface electrodes. Electromyography 10 (4), 357-367.

Komi, P. V., Linnamo, V., Silventoinen, P. & Sillanpaa, M. 2000. Force and EMG power spectrum during eccentric and concentric actions. Medicine & Science in Sports & Exercise 32 (10), 1757-1762.

Kossev, A. & Christova, P. 1998a. Discharge pattern of human motor units during dynamic concentric and eccentric contractions. Electroencephalography and Clinical Neurophysiology 109 (3), 245-255.

Kossev, A. R. & Christova, P. 1998b. Motor unit recruitment and discharge behavior in movements and isometric contractions. Muscle & Nerve 21 (3), 413-415.

Kouzaki, M. & Shinohara, M. 2010. Steadiness in plantar flexor muscles and its relation to postural sway in young and elderly adults. Muscle & Nerve 42 (1), 78-87.

Kukulka, C. G. & Clamann, H. P. 1981. Comparison of the recruitment and discharge properties of motor units in human brachial biceps and adductor pollicis during isometric contractions. Brain Research 219 (1), 45-55.

Laidlaw, D. H., Bilodeau, M. & Enoka, R. M. 2000. Steadiness is reduced and motor unit discharge is more variable in old adults. Muscle & Nerve 23 (4), 600-612.

Larsson, L. 1995. Motor units: remodeling in aged animals. Journals of Gerontology Series A-Biological Sciences & Medical Sciences 50 (Spec No), 91-95.

Lexell, J. 1993. Ageing and human muscle: observations from Sweden. Canadian Journal of Applied Physiology 18 (1), 2-18.

Lichtwark, G. A., Bougoulias, K. & Wilson, A. M. 2007. Muscle fascicle and series elastic element length changes along the length of the human gastrocnemius during walking and running. Journal of Biomechanics 40 (1), 157-164.

Lichtwark, G. A. & Wilson, A. M. 2006. Interactions between the human gastrocnemius muscle and the Achilles tendon during incline, level and decline locomotion. The Journal of Experimental Biology 209 (Pt 21), 4379-4388.

Linnamo 2003. Motor unit activation patterns during isometric, concentric and eccentric actions at different force levels. Journal of Electromyography and Kinesiology 13 (1), 93.

Linnamo, V., Avela, J., Kallio, J., Dousset, E., Kuitunen, S., Ishikawa, M., Kyröläinen, H. & Komi, P. V. 2003a. Maximal torque and activation level during eccentric, concentric and isometric actions before and after damaging exercise. In Gantchev N. (Ed.) From motor Control to Functional Recovery III.Varna, Bulgaria:361-368.

Linnamo, V., Moritani, T., Nicol, C. & Komi, P. V. 2003b. Motor unit activation patterns during isometric, concentric and eccentric actions at different force levels. Journal of Electromyography & Kinesiology 13 (1), 93-101.

Linnamo, V., Strojnik, V. & Komi, P. V. 2006. Maximal force during eccentric and isometric actions at different elbow angles. European Journal of Applied Physiology 96 (6), 672-678.

Linnamo, V., Strojnik, V. & Komi, P. V. 2002. EMG power spectrum and features of the superimposed M-wave during voluntary eccentric and concentric actions at different activation levels. European Journal of Applied Physiology 86 (6), 534-540.

Lombardi, V. & Piazzesi, G. 1990. The contractile response during steady lengthening of stimulated frog muscle fibres. The Journal of Physiology 431, 141-171.

Martin, S. & MacIsaac, D. 2006. Innervation zone shift with changes in joint angle in the brachial biceps. Journal of Electromyography and Kinesiology 16 (2), 144-148.

Mochizuki, G., Ivanova, T. D. & Garland, S. J. 2005. Synchronization of motor units in human soleus muscle during standing postural tasks. Journal of Neurophysiology 94 (1), 62-69.

Morita, H., Shindo, M., Yanagawa, S., Yoshida, T., Momoi, H. & Yanagisawa, N. 1995. Progressive decrease in heteronymous monosynaptic Ia facilitation with human ageing. Experimental Brain Research 104 (1), 167-170.

Moritani, T., Muramatsu, S. & Muro, M. 1987. Activity of motor units during concentric and eccentric contractions. American Journal of Physical Medicine 66 (6), 338-350.

Moritani, T. & Muro, M. 1987. Motor unit activity and surface electromyogram power spectrum during increasing force of contraction. European Journal of Applied Physiology & Occupational Physiology 56 (3), 260-265.

Moritani, T., Muro, M., Kijima, A. & Berry, M. J. 1986. Intramuscular spike analysis during ramp force output and muscle fatigue. Electromyography and Clinical Neurophysiology 26 (2), 147-160.

Moritz, C. T., Barry, B. K., Pascoe, M. A. & Enoka, R. M. 2005. Discharge rate variability influences the variation in force fluctuations across the working range of a hand muscle. Journal of Neurophysiology 93 (5), 2449-2459.

Mynark, R. G. 2005. Reliability of the soleus H-reflex from supine to standing in young and elderly. Clinical Neurophysiology 116 (6), 1400-1404.

Mynark, R. G. & Koceja, D. M. 2001. Effects of Age on the Spinal Stretch Reflex. Journal of Biomechanics 17 (3), 188-202.

Nakazawa, K., Yamamoto, S. I. & Yano, H. 1997. Short- and long-latency reflex responses during different motor tasks in elbow flexor muscles. Experimental Brain Research 116 (1), 20-28.

Nardone, A., Romano, C. & Schieppati, M. 1989. Selective recruitment of high-threshold human motor units during voluntary isotonic lengthening of active muscles. The Journal of Physiology 409, 451-471.

Nardone, A. & Schieppati, M. 1988. Shift of activity from slow to fast muscle during voluntary lengthening contractions of the triceps surae muscles in humans. The Journal of Physiology 395, 363-381.

Narici, M. V. & Maganaris, C. N. 2007. Plasticity of the muscle-tendon complex with disuse and aging. Exercise and sport sciences reviews 35 (3), 126-134.

Nelson, R. M., Soderberg, G. L. & Urbscheit, N. L. 1984. Alteration of motor-unit discharge characteristics in aged humans. Physical Therapy 64 (1), 29-34.

Ochala, J., Lambertz, D., Pousson, M., Goubel, F. & Hoecke, J. V. 2004. Changes in mechanical properties of human plantar flexor muscles in ageing. Experimental Gerontology 39 (3), 349-358.

Olsen, H. B., Christensen, H. & Sogaard, K. 2001. An analysis of motor unit firing pattern during sustained low force contraction in fatigued muscle. Acta Physiologica et Pharmacologica Bulgarica 26 (1-2), 73-78.

Oya, T., Riek, S. & Cresswell, A. G. 2009. Recruitment and rate coding organisation for soleus motor units across entire range of voluntary isometric plantar flexions. Journal of Physiology 587 (Pt 19), 4737-4748.

Pasquet, B., Carpentier, A. & Duchateau, J. 2006. Specific modulation of motor unit discharge for a similar change in fascicle length during shortening and lengthening contractions in humans. Journal of Physiology 577 (Pt 2), 753-765.

Patten, C. & Kamen, G. 2000. Adaptations in motor unit discharge activity with force control training in young and older human adults. European Journal of Applied Physiology 83 (2-3), 128-143.

Patten, C., Kamen, G. & Rowland, D. M. 2001. Adaptations in maximal motor unit discharge rate to strength training in young and older adults. Muscle & Nerve 24 (4), 542-550.

Person, R. S. & Kudina, L. P. 1972. Discharge frequency and discharge pattern of human motor units during voluntary contraction of muscle. Electroencephalography and Clinical Neurophysiology 32 (5), 471-483.

Piirainen, J. M., Avela, J., Sippola, N. & Linnamo, V. 2010. Age dependency of neuromuscular function and dynamic balance control. European Journal of Sport Science 10 (1), 69.

Pinniger, G. J., Nordlund, M. M., Steele, J. R. & Cresswell, A. G. 2001. H-reflex modulation during passive lengthening and shortening of the human triceps surae. The Journal of Physiology Online 534 (3), 913-923.

Porter, M. M., Vandervoort, A. A. & Kramer, J. F. 1997. Eccentric peak torque of the plantar and dorsiflexors is maintained in older women. Journals of Gerontology Series A-Biological Sciences & Medical Sciences 52 (2), B125-31.

Poulin, M. J., Vandervoort, A. A., Paterson, D. H., Kramer, J. F. & Cunningham, D. A. 1992. Eccentric and concentric torques of knee and elbow extension in young and older men. Canadian journal of sport sciences 17 (1), 3-7.

Proske, U. & Morgan, D. L. 2001. Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. The Journal of Physiology 537 (Pt 2), 333-345.

Rayment, I., Holden, H. M., Whittaker, M., Yohn, C. B., Lorenz, M., Holmes, K. C. & Milligan, R. A. 1993. Structure of the actin-myosin complex and its implications for muscle contraction. Science (New York, N.Y.) 261 (5117), 58-65.

Romano, C. & Schieppati, M. 1987. Reflex excitability of human soleus motoneurones during voluntary shortening or lengthening contractions. The Journal of Physiology 390, 271-284.

Roos, M. R., Rice, C. L. & Vandervoort, A. A. 1997. Age-related changes in motor unit function. Muscle & Nerve 20 (6), 679-690.

Sabbahi, M. A. & Sedgwick, E. M. 1982. Age-related changes in monosynaptic reflex excitability. Journal of Gerontology 37 (1), 24-32.

Scaglioni, G., Ferri, A., Minetti, A. E., Martin, A., Van Hoecke, J., Capodaglio, P., Sartorio, A. & Narici, M. V. 2002. Plantar flexor activation capacity and H reflex in older adults: adaptations to strength training. Journal of Applied Physiology 92 (6), 2292-2302.

Schmitz, A., Silder, A., Heiderscheit, B., Mahoney, J. & Thelen, D. G. 2009. Differences in lower-extremity muscular activation during walking between healthy older and young adults. Journal of Electromyography and Kinesiology 19 (6), 1085-1091.

Seger, J. Y. & Thorstensson, A. 2000. Electrically evoked eccentric and concentric torque-velocity relationships in human knee extensor muscles. Acta Physiologica Scandinavica 169 (1), 63-69.

Semmler 2002. Motor unit synchronisation is enhanced during slow lengthening contractions of a hand muscle. The Journal of Physiology 545 (2), 681.

Simoneau, E., Martin, A. & Van Hoecke, J. 2005. Muscular performances at the ankle joint in young and elderly men. The journals of gerontology.Series A, Biological sciences and medical sciences 60 (4), 439-447.

Singh, M. & Karpovich, P. V. 1966. Isotonic and isometric forces of forearm flexors and extensors. Journal of Applied Physiology 21 (4), 1435-1437.

Sogaard, K., Christensen, H., Fallentin, N., Mizuno, M., Quistorff, B. & Sjogaard, G. 1998. Motor unit activation patterns during concentric wrist flexion in humans with different muscle fibre composition. European Journal of Applied Physiology & Occupational Physiology 78 (5), 411-416.

Sogaard, K., Christensen, H., Jensen, B. R., Finsen, L. & Sjogaard, G. 1996. Motor control and kinetics during low level concentric and eccentric contractions in man. Electroencephalography & Clinical Neurophysiology 101 (5), 453-460.

Sosnoff, J. J. & Newell, K. M. 2006a. Aging, visual intermittency, and variability in isometric force output. Journals of Gerontology Series B-Psychological Sciences & Social Sciences 61 (2), P117-24.

Sosnoff, J. J. & Newell, K. M. 2006b. Information processing limitations with aging in the visual scaling of isometric force. Experimental Brain Research 170 (3), 423-432.

Stotz, P. J. & Bawa, P. 2001. Motor unit recruitment during lengthening contractions of human wrist flexors. Muscle & Nerve 24 (11), 1535-1541.

Tax, A. A., Denier van der Gon, J. J., Gielen, C. C. & van den Tempel, C. M. 1989. Differences in the activation of m. biceps brachii in the control of slow isotonic movements and isometric contractions. Experimental Brain Research 76 (1), 55-63.

Tracy, B. L. 2007. Force control is impaired in the ankle plantarflexors of elderly adults. European Journal of Applied Physiology 101 (5), 629-636.

Tracy, B. L., Maluf, K. S., Stephenson, J. L., Hunter, S. K. & Enoka, R. M. 2005. Variability of motor unit discharge and force fluctuations across a range of muscle forces in older adults. Muscle & Nerve 32 (4), 533-540.

Tracy, B. L. & Enoka, R. M. 2002. Older adults are less steady during submaximal isometric contractions with the knee extensor muscles. Journal of Applied Physiology 92 (3), 1004-1012.

Trappe, S. W., Trappe, T. A., Lee, G. A. & Costill, D. L. 2001. Calf muscle strength in humans. International Journal of Sports Medicine 22 (3), 186-191.

Van Cutsem, M., Feiereisen, P., Duchateau, J. & Hainaut, K. 1997. Mechanical properties and behaviour of motor units in the tibialis anterior during voluntary contractions. Canadian Journal of Applied Physiology 22 (6), 585-597.

Vandervoort, A. A. 2002. Aging of the human neuromuscular system. Muscle & Nerve 25 (1), 17-25.

Vandervoort, A. A. & McComas, A. J. 1986. Contractile changes in opposing muscles of the human ankle joint with aging. Journal of Applied Physiology 61 (1), 361-367.

Vieira, T. M., Loram, I. D., Muceli, S., Merletti, R. & Farina, D. 2012. Recruitment of motor units in the medial gastrocnemius muscle during human quiet standing: is recruitment intermittent? What triggers recruitment? Journal of Neurophysiology 107 (2), 666-676.

Welsh, S. J., Dinenno, D. V. & Tracy, B. L. 2007. Variability of quadriceps femoris motor neuron discharge and muscle force in human aging. Experimental Brain Research 179 (2), 219-233.

Westing, S. H., Seger, J. Y. & Thorstensson, A. 1990. Effects of electrical stimulation on eccentric and concentric torque-velocity relationships during knee extension in man. Acta Physiologica Scandinavica 140 (1), 17-22.

Wolfson, L., Judge, J., Whipple, R. & King, M. 1995. Strength is a major factor in balance, gait, and the occurrence of falls. The journals of gerontology.Series A, Biological sciences and medical sciences 50 Spec No, 64-67.

ORIGINAL PAPERS

I

Effects of ageing on motor unit activation patterns and reflex sensitivity in dynamic movements

by

Jouni Kallio, Janne Avela, Toshio Moritani, Mika Kanervo, Harri Selänne, Paavo V. Komi, Vesa Linnamo, 2010

Journal of Electromyography and Kinesiology, vol 20(4):590-8

Effects of ageing on motor unit activation patterns and reflex sensitivity in dynamic movements

J. Kallio, J. Avela, T. Moritani, M. Kanervo, H. Selänne, P. Komi, V. Linnamo

Jouni Kallio (M.Sc.): Assistant of Biomechanics, Neuromuscular Research Center, Department of Biology of Physical Activity, University of Jyväskylä.
Janne Avela (Ph.D.): Professor of Biomechanics, Neuromuscular Research Center, Department of Biology of Physical Activity, University of Jyväskylä
Toshio Moritani (MD, Ph.D.): Professor of Applied Physiology, Graduate School of Human and Environmental Studies, Kyoto University
Mika Kanervo (M.Sc.) CEO, White House Finland Ltd
Harri Selänne (MD/Ph.D.) Chief Physician, LIKES – Foundation for Sport and Health Sciences
Paavo V. Komi (Ph.D.): Professor Emeritus, Neuromuscular Research Center, Department of Biology of Physical Activity, University of Jyväskylä
Vesa Linnamo (Ph.D.): Professor of Sports Technology, Vuokatti Snowpolis, Neuromuscular Research Center, Department of Biology of Physical Activity, University of Jyväskylä

Correspondence to:
Jouni Kallio
Neuromuscular Research Center
Department of Biology of Physical Activity
University of Jyväskylä
P.O.Box 35, FIN-40351, Jyväskylä
Finland
Tel. +358 (0) 14 260 2084
Fax. +358 (0) 14 260 2071
E-mail. jouni.kallio@jyu.fi

ABSTRACT

Both contraction type and ageing may cause changes in H reflex excitability. H reflex is partly affected by presynaptic inhibition that may also be an important factor in the control of MU activation. The purpose of the study was to examine age related changes in H-reflex excitability and motor unit activation patterns in dynamic and in isometric contractions. Ten younger (YOUNG) and 13 elderly (OLD) males performed isometric (ISO), concentric (CON) and eccentric (ECC) plantarflexions with submaximal activation levels (20, 40% of maximal soleus surface EMG). Intramuscular EMG data was analyzed utilizing an intramuscular spike amplitude frequency histogram method. Average H/M ratio was always lowest in ECC (n.s.). Mean spike amplitude increased with activation level ($P<.05$), whereas no significant differences were found between contraction types. Both H-reflex excitability, which may be due to an increase in presynaptic inhibition, and mean spike frequency were higher in YOUNG compared to OLD. In OLD the mean spike frequency was significantly smaller in CON compared to ISO. Lack of difference in mean spike amplitude and frequency across contraction types in YOUNG would imply a similar activation strategy, whereas the lower frequency in dynamic contractions in OLD could be related to synergist muscle behavior.

Keywords: ageing, intramuscular electromyography, isometric, concentric, eccentric

INTRODUCTION

In submaximal contractions the amount of neural activation required to produce a given muscular force is dependent on muscle action type. In eccentric (ECC) contractions equal force is produced with lower, and in concentric (CON) with higher levels of neural activity compared to isometric (ISO) conditions. Conversely, with matched neural activation, the force is highest in ECC and lowest in CON [Katz, 1939].

Contraction type has also an effect on the neural activation strategy of muscle. Motor unit (MU) firing rates have been found to differ between contraction types [Moritani et al, 1987; Sogaard et al, 1996] and recruitment thresholds to be lower in dynamic compared to ISO actions [Ivanova et al, 1997; Linnamo et al, 2003; Tax et al, 1989]. Differences in activation strategy in different conditions may be related to motoneuron pool excitability. One way to study changes in this excitability has been the utilization of an electrically induced Hoffmann (H) reflex. However, the H-reflex response is susceptible to inputs from many sources, like presynaptic inhibition (PI). H-reflex response has been found to decrease in ECC contractions, which is probably due to an increase in the amount of PI to Ia afferents and, to some extent, to homosynaptic postactivation depression [Hultborn et al, 1996; Pinniger et al, 2001; Romano and Schieppati, 1987]. Slow motor units (MU) have been found to receive greater amounts of PI [Pierrot-Deseilligny and Burke, 2005], which has been hypothesized to cause the observed deviation from the orderly recruitment [Henneman et al, 1965a; Henneman et al, 1965b] to a preferential recruitment of fast units in some ECC contractions [Nardone et al, 1989].

Ageing is associated with a decrease in muscle force and power, beginning after the age of 50 [Cunningham et al, 1987; Doherty et al, 1993]. This decrease is in large part due to a loss of muscle mass (sarcopenia), which is caused by a progressive decrease in the number of muscle fibers [Brown et al, 1988; Frontera et al, 1991]. The decrease in fiber number is more pronounced in type II units [Frontera et al, 1991; Lexell, 1993] and is probably counteracted with MU remodeling [Larsson, 1995] that leads to an increase in MU size.

Previous studies on ageing have found eccentric strength to decrease less than isometric or concentric [Porter et al, 1997; Poulin et al, 1992; Vandervoort and McComas, 1986]. This smaller decrease in eccentric strength has been attributed to greater passive resistance from the elastic tissue structures [Narici and Maganaris, 2007; Vandervoort, 2002]. Due to decreased amount of fast MUs and changes in plasticity it is likely that the MU activity (recruitment and rate coding) would differ between young and older subjects. Especially when comparing submaximal ECC actions to ISO or CON, because of increased stiffness, one would expect greater differences in MU activity in the elderly. Not much information is available about MU behavior in the elderly in dynamic conditions, and previous results concerning even the isometric conditions are not very consistent. For example Kamen et al. [1995] and Klass et al. [2008] showed the elderly to have a lower firing rate in maximal first dorsal interosseous (FDI), and fast tibialis anterior (TA) contractions, respectively. In contrast, no such differences were found when comparisons were made at recruitment threshold in biceps brachii, triceps brachii, TA or FDI in the studies by Howard et al. [1988] and Galganski et al. [1993]. It appears that the largest changes exist in distal muscles, in oldest subjects and in higher normalized force levels [for review, see Roos et al, 1997].

In addition to the progressive degenerative process in the muscle physiology, ageing may be associated with some changes in the spinal level. Literature on the age-related changes to the amount of PI is somewhat conflicting [Burke et al, 1996; Delwaide, 1973; Koceja and Mynark, 2000; Morita et al, 1995], while the evidence on the reduced ability to modify afferent input to the motorneuron pool via PI in elderly is more uniform [Chalmers and Knutzen, 2000; Koceja et al, 1995].

The increase in the proportion of elderly in the industrial countries brings great socio-economical challenges. Age-related deterioration of the neuromuscular system increases the risk of accident that lead to injuries. Since most of the daily activities that determine the capability to continue independent living (e.g. raising out of a chair, stair climbing, walking) are dynamic in nature, it is important to gain more understanding on the mechanisms of ageing in both concentric and eccentric muscle actions. Both contraction type and ageing have been found to cause changes in H-reflex excitability. H-reflex is partly affected by PI that may also be an important factor in the control of MU activation. It is not clear, however, how ageing affects MU firing properties and H-reflex excitability in dynamic contractions.

Therefore the purpose of the present study was to examine the age related changes in H-reflex amplitude and MU activity in dynamic and in isometric contractions. To match the conditions between age groups, same relative levels of activation were chosen. Further, soleus muscle was chosen for its important role in balance locomotion.

METHODS

Subjects

Twenty-three physically active males volunteered as subjects in the study. There were 10 males in the younger (YOUNG) group (27.3±3yr. 1.77±0.07m 73.2±5kg) and 13 males in the older (OLD) group (70.4±5yr. 1.75±0.05m 81.8±10kg). Before the measurements all the subjects in the OLD group underwent a medical examination. Only subjects without any history of neuromuscular or vascular disease were approved for the study.

Full advice about possible risks and discomfort was given to the subjects and they all gave their written informed consent to the procedures prior to the commencement of the study. The investigation was conducted according to the declaration of Helsinki and approved by the ethics committee of the University of Jyväskylä.

Protocol and measurements

Each subject participated in two measurement sessions separated by one to three weeks. One session was for the motor unit recordings, and the other for H-reflex measurements. Prior to data collection all subjects performed a warm-up consisting of 10 minutes light cycling on a bicycle ergometer and 5-10 submaximal isometric plantar flexions.

During the measurements the subjects performed plantar flexions while seated in an ankle dynamometer. The test battery used in the both sessions was as follows:

1) Maximal isometric voluntary contraction (MVC)
2) Submaximal trials
    a. Isometric (ISO) trials
    b. Dynamic trials: Eccentric (ECC) and concentric (CON)
3) Maximal isometric voluntary contraction (MVC)

The activation levels used in the submaximal trials were 20 and 40% of the surface Soleus (SOL) EMG root mean square (RMS) value of the isometric MVC. In H-reflex measurements, also passive trials were measured. The orders of activation levels and dynamic trials were randomized.

Preparations

All measurements were conducted in relation to the individual range of motion of the subject. In order to determine the individual zero angle (IZA), the voluntary range of motion was determined according to a protocol by [Allinger and Engsberg, 1993]. Subject was asked to perform slow, maximal, dorsi and plantar flexions in the ankle dynamometer without a load. Range of motion was calculated as the difference between the two maximum angles. IZA was determined as being 15 deg. plantar from the maximal voluntary dorsiflexion. The knee was fixed straight (180 deg). All isometric contractions were performed at each subject's IZA.

Maximal voluntary contraction

The subject was instructed to perform a maximal plantar flexion for approximately 5 s. Minimum of two successful trials were performed and consecutive trials were performed if the maximal torque value between trials differed by more than 10%. MVC measurement was repeated in the end of the protocol to examine the amount of possible fatigue caused by the session. Each subject practiced maximal contractions during warm-up before the actual measurements.

Submaximal trials.

Subjects were instructed to hold the plantar flexion force at the given target level for 10 s. Activation levels were analyzed online in order to ensure successful trials at each condition.

In the dynamic trials the ankle was rotated by a strong motor connected to the foot-pedal. The rotation of the ankle joint was ± 10° of IZA at 30 deg · s-1 (Figure 1). The subject was instructed to increase the triceps surae activation before the start of the movement and hold the activation steady throughout the following concentric or eccentric trial. Activation was measured online in four 100ms windows, ±200ms around the individual zero angle (IZA). The trial was considered successful if the EMG activation of SOL (RMS) was a) within 5% of target activation level ±100ms around IZA, and b) within 10% of target activation level ±200-100ms around *IZA*. The minimum time interval between two trials was 2 minutes in active and 1 min in passive conditions.

Apparatus

Trials were performed while the subject was seated in a motor driven ankle dynamometer. To maximize stability during testing, subjects were strapped to the chair from the thigh and the ankle. The rotational axis of the device was aligned with the axis of the tibio-tarsal joint. The dynamometer was controlled by a digital feedback system, used previously in similar experiments with passive and active muscle [Avela et al, 2004; Dousset et al, 2007; Kallio et al, 2004]. Maximum torque capacity of the dynamometer was 150 Nm. The angular movement of the ankle joint with respect to the plane of the ergometer is monitored by a linear potentiometer and the moment by piezoelectric crystal transducer (Kistler, Switzerland). The torque and angle signals were sent to a computer via a CED 1401+ (Cambridge Electronic Design) and sampled at a frequency of 20 kHz. Signals were collected and analyzed using Signal software (Cambridge Electronic Design). Before analysis, torque and angle signals were filtered with a low-pass filter (cutoff frequencies 75 and 150 Hz, respectively).

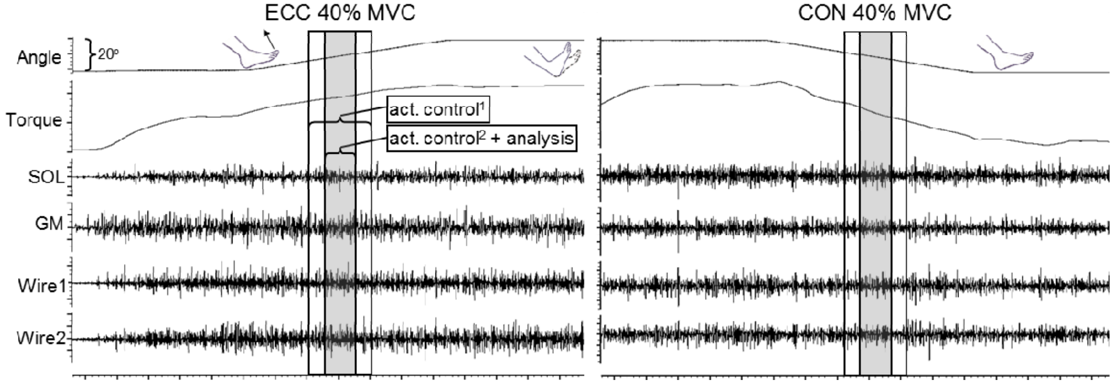


Figure 1. An example of surface (SOL, GM) and wire-electrode recordings of eccentric (ECC) and concentric (CON) trials. Analysis was performed ±100ms and activation level controlled ±200ms and ±100ms around the measurement angle.

Measurements

Global EMG activities of Soleus (SOL) and Gastrocnemius medialis (GM) muscles were recorded utilizing bipolar silver chloride miniature surface electrodes (Beckman 650437, USA) with 20 mm inter-electrode distance. Electrodes were prepared and placed according to the recommendation by SENIAM [1999]. The EMG signals were amplified and sampled at a frequency of 20 kHz. The signals were subsequently downsampled at 2 kHz, band-pass filtered at 20 to 1 kHz and analyzed using the Signal software (Cambridge Electronic Design).

For intramuscular EMG recordings, four novel bipolar fine-wire electrodes were inserted into SOL. The wire-electrodes consisted of two 50 µm polyurethane insulated wires that were glued together (cutting one wire 2mm shorter than the other one). Insulation was mechanically removed from the wires over 0.5 mm distance from the tip. A hook was formed to the end of the wires to ensure the fixation during the measurements. The wire electrode pairs were inserted into the muscle with a 27 gauge needle that was subsequently withdrawn. Electrodes were autoclaved before the experiment, in order to minimize the risk of infection. Wire-electrodes were inserted after the MVC-trials to avoid possible electrode migration due to high force levels. Electrodes were placed in different depths around the surface EMG electrode and a ground electrode was placed on the medial malleolus. To ensure the best possible signal quality, the signal was pre-amplified with a non-commercial amplifier (amp 200, bandwidth 8-4500), attached to the subjects calf, and subsequently sent to the CED 1902 programmable signal conditioner (Cambridge Electronics Design Ltd, Cambridge, CB4 4FE, UK). This signal was digitized with CED 1401+ (Cambridge Electronic Design) with a sampling frequency of 20 kHz. Figure 1 shows an example of the EMG recordings from one subject in concentric and eccentric 40% condition.

Hoffmann reflexes were obtained utilizing a standard methodology. The skin was prepared, and the stimulation electrodes (pregelified Ag-AgCl electrodes, Niko, Japan) were placed on the leg. The stimulating cathode electrode (1.5 x 1.5 cm) was placed over the tibial nerve in the popliteal fossa, and the anode electrode (5 x 8 cm) was placed above the patella. The stimulation site resulting in the greatest M-wave amplitude was first located with a handheld cathode electrode (0.7-cm diameter). Once the stimulation site was determined, stimulation electrode was firmly fixed to this site with rigid straps and taping. Stimulus pulses (rectangular, 1ms duration) were delivered from an evoked-potential- measuring system (Digitimer stimulator (model DS7, Hertfordshire, UK). The stimulation of the tibial nerve elicited an M-wave (direct stimulation of a-motoneuron axons) and an H-wave (reflex response to stimulation of Ia muscle afferents). Maximal M-wave (M_{max}) was measured by delivering a stimulus at supramaximal intensity (1.5 times the maximum M-wave stimulus intensity) three times, and the mean value of the recorded M-wave amplitudes was considered as (M_{max}). Stimulation intensity in the H-reflex measurements was controlled with M-wave amplitude. The target M-wave amplitude was 25% of M_{max}. In each condition, the average of 4 successful trials was used, which has been found to be enough to reach reliability in both young and old subjects [intraclass correlations 0.9883 and 0.9667 for young and old, respectively, in Mynark, 2005]

Analysis

For the maximal voluntary contractions (MVC), both EMG and torque data was analyzed ±100 ms around the peak torque. Maximal EMG activities of SOL and GM were calculated as root mean square (RMS) of the signal.

The recorded H-reflex and M-wave signals were analyzed trial by trial, and averaged for each subject. H-reflex peak-to-peak amplitudes were expressed in relation to the corresponding M-wave amplitudes (H/M ratio). The aim of this method is to minimize the effects of changes in the peripheral excitability.

Intramuscular EMG data was analyzed by utilizing intramuscular MU spike-amplitude-frequency (ISAF) histograms. This method has been described in more detail elsewhere [Moritani et al, 1986]. In short, individual spikes in the EMG signal were isolated and counted based on their amplitude in 100 μV increments. Mean spike amplitude and frequency were used for statistical comparison between groups and conditions. Figure 2 shows an example of mean spike frequency and mean spike amplitude when analyzing window of 100ms is overlapped by each ms during isometric ramp condition from zero to 40% MVC. With this method, it is not possible to separate the firings of different MU's with similar amplitudes. Therefore, our data on frequency represent the mean firing rate of a selected population of MU's.

Statistical analysis

A two-way analysis of variance for repeated measurements (rANOVA) was used to test for significant differences between contraction types and activation levels. T-test was used to find differences between groups. When a significant difference was detected, Bonferroni's method was used to locate the difference. The critical level of significance in the present study was $P < 0.05$. Descriptive statistics include mean and standard deviation.

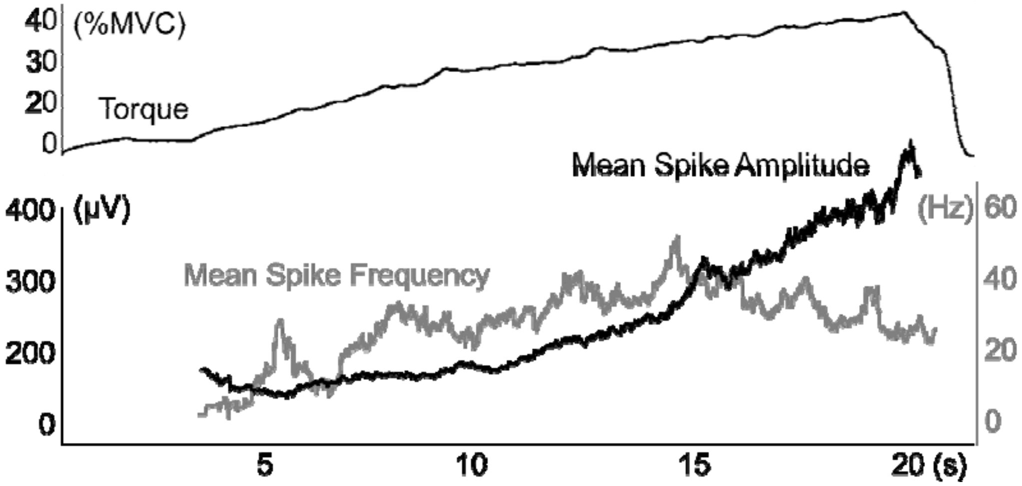


Figure 2. Example of intramuscular EMG analysis in isometric in ramp contraction showing mean spike frequency and amplitude.

RESULTS

10 YOUNG and 13 OLD subjects were measured in the current study. However, data was successfully collected and analyzed from 8 YOUNG and 7 OLD in the H-reflex-measurements and 7 YOUNG and 10 OLD in the EMG-measurements. As each subject had four wire-electrodes during the measurements, and each successful wire recording was considered as one MU population, a total of 51 OLD and 19 YOUNG populations were analyzed. The results for MVC, background activity, torque and coactivation are reported as average of all subjects (10 YOUNG and 13 OLD), as the results did not differ significantly from the corresponding sub-group averages.

ROM The maximal active range of motion was 4.1% (n.s.) larger in YOUNG (65.1+8.1 deg) compared to OLD (62.5+6.2) deg. Also the maximal active plantar flexion in YOUNG was 8.1% larger (151.3+13.1) than in OLD (139.1+5.4 deg). However, the maximal active dorsiflexion in OLD (69.2+2.3 deg) was 7.6% (p=0.01) larger than in YOUNG (74.9+6.5 deg). Therefore the zero angle used in the measurements was on average 5.1 deg more dorsal in group OLD (5.6%; n.s.).

Table 1: Results of H-reflex measurements (H/M ratio), ISAF amplitude and frequency

		H/M ratio (%)			Mean spike amplitude			Mean spike frequency		
		YOUNG	OLD	Diff %	YOUNG	OLD	Diff %	YOUNG	OLD	Diff %
E	P	36.1+17	11.2+9	-69.0**						
C	20	42.8+24	33.9+18	-20.8	196.3+57	179.0+47	-8.8	30.2+12	13.5+9	-55.4***
C	40	45.4+17	28.2+12	-37.9*	322.5+141	246.0+100	-23.7*	32.4+14	19.7+10	-39.0**
I	P	45.0+36	28.6+17	-36.4						
S	20	47.0+25	42.8+17	-8.8	209.8+72	182.9+51	-12.8	29.0+13	24.2+17	-16.4
O	40	52.9+25	38.2+17	-27.7	313.0+116	240.1+76	-23.3	29.0+12	25.7+19	-11.4
C	P	45.1+25	18.7+17	-58.4*						
O	20	41.7+30	36.2+18	-13.1	224.2+84	195.1+47	-13.0	34.8+16	14.7+8	-57.7***
N	40	53.1+25	40.7+8	-23.3	296.9+87	300.2+143	1.1	37.2+13	14.1+8	-62.2***

Values are presented as mean + SD. *: sig. difference between groups YOUNG and OLD

Contraction type

In the submaximal contractions (20, 40% SOL_{RMS} at MVC) the average torque was highest in ECC and lowest in CON in both groups. All differences between contraction types are significant ($p<0.05$) excluding ECC vs. ISO at 40% MVC.

The relative soleus (SOL) activation level (% of SOL_{RMS} in MVC) did not differ between contraction types or groups. The corresponding relative activation of gastrocnemius medialis (GM) was generally higher than SOL activity in both groups and in all conditions (15-42%, n.s.- $p<0.01$). No differences were found between groups or contraction types.

In both groups, and in all activation levels, the average H/M ratio was lowest in ECC (n.s.) and higher in active compared to passive contractions (Figure 3). Mean spike amplitude increased with activation level (Figure 4), whereas no significant differences were found between contraction types. In OLD the mean spike frequency was significantly smaller ($p<0.05$) in CON compared to ISO (Figure 5).

Age

The maximal isometric voluntary contraction torque (MVC) was 12.3% (p=0.06) higher in YOUNG (219.2+34.9 Nm) than in OLD (192.2+41.8 Nm). No significant difference was found between pre- and post-measurement MVC-values in either group.

The average torque was found to be larger in YOUNG all active trials. The differences between groups in 20%-trials were 41.7% in ISO, 30.7% in ECC and 45.4% in CON. In the 40%-trials the corresponding differences were 33.8, 27.8 and 47.5%. The average relative torque (related to the values measured in MVC) was found to be higher in all conditions in YOUNG compared to OLD. The differences between groups in 20%-trials were 22.8% in ISO, 15.7% in ECC and 33.9% in CON. In the 40%-trials the corresponding differences were 15.6, 11.8 and 33.8% ($p<0.05$). In both groups torque was consistently larger in ECC compared to CON, while the difference between the torques in these two contraction types was significantly larger in OLD ($p<0.05$) in both activation levels.

H-reflex amplitude was 9-69% higher in YOUNG compared to OLD depending on the activation level and contraction type. Also the mean spike frequency was higher in YOUNG in all trials, ($p<0.05$ in ECC and CON) (Figure 5).

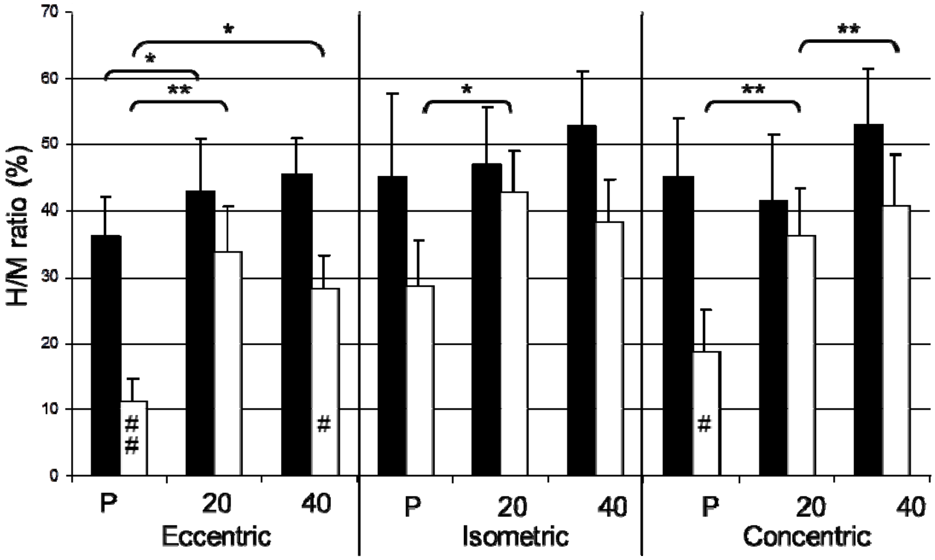


Figure 3: Mean H/M ratio (%+SE) of YOUNG (black) and OLD (white) in passive (P), 20 and 40% activation. *: sig. difference between activation levels, #: sig. difference between YOUNG and OLD.

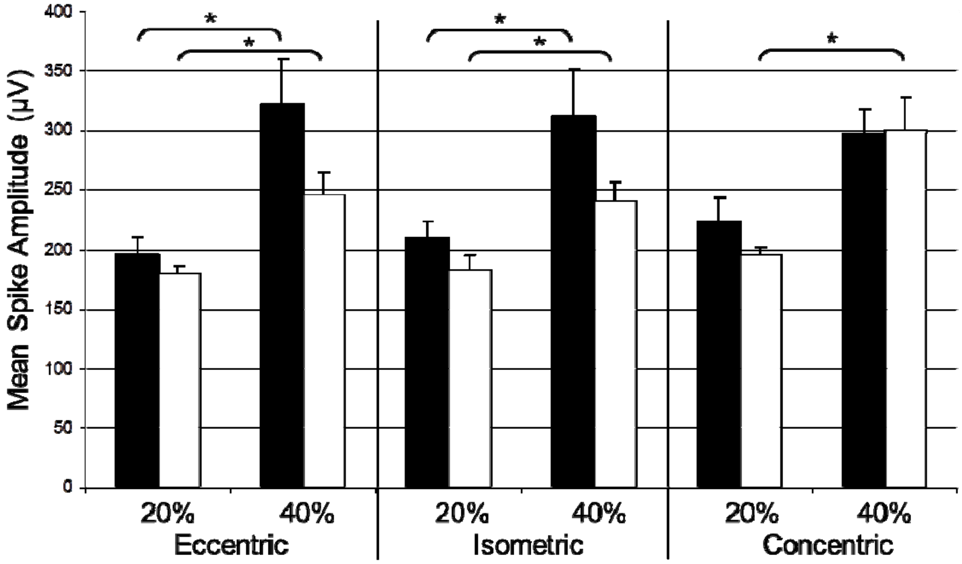


Figure 4: The mean spike amplitude (+SE) of YOUNG (black) and OLD (white) at 20 and 40% activation. *: sig. difference between activation levels.

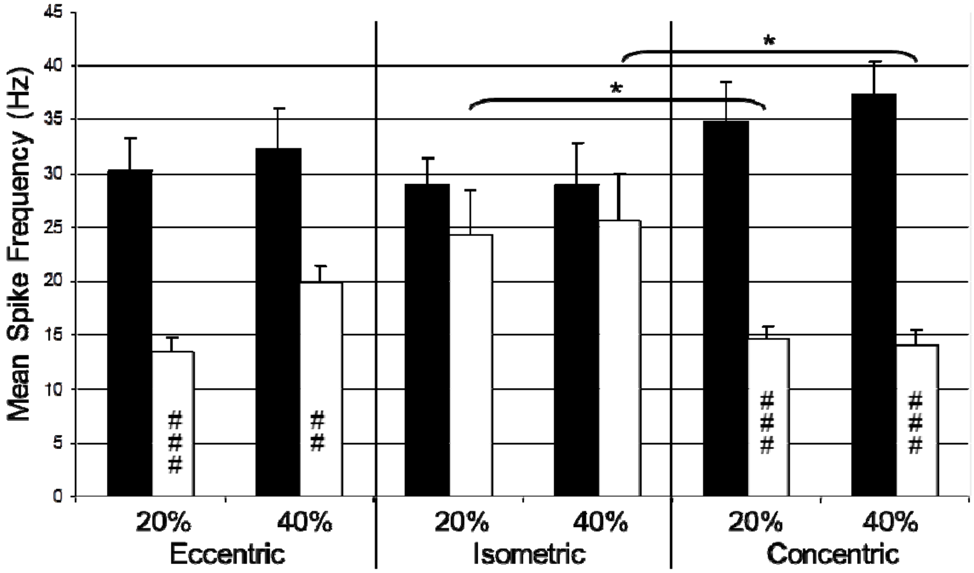


Figure 5: The mean spike frequency (+SE) of YOUNG (black) and OLD (white) at 20 and 40% activation. *: sig. difference between activation levels, #: sig. difference between YOUNG and OLD.

DISCUSSION

In order to examine the age-related mechanisms in dynamic muscle actions motor unit activity and H-reflex measurements were combined. The current results imply that young men use a similar motor unit activation strategy regardless of muscle contraction type, whereas older men activate the motor units with a lower firing rate accompanied with a lower H/M ratio and may vary activation between isometric and dynamic contractions.

Contraction type

To our knowledge, this is the first study to combine motor unit firing property and H-reflex excitability measurements in dynamic contractions. As could be expected, based on previous studies, the submaximal torque was largest in ECC and smallest in CON [for review, see Duchateau and Enoka, 2008; Katz, 1939]. Also in agreement with literature [Duclay and Martin, 2005; Pinniger et al, 2001; Romano and Schieppati, 1987] there was a clear trend of lowest average H/M ratio in ECC (n.s.) in both groups and in all activation levels. The same phenomenon that has also been found in stretch-reflexes [Kallio et al, 2004; Nakazawa et al, 1997] is most likely due to presynaptic inhibition of Ia afferents [Romano and Schieppati, 1987] or to homosynaptic postactivation depression [Hultborn et al, 1996].

As expected, there was an increase in both mean spike amplitude and frequency with activation level. The lack of statistical significance in concentric contractions of YOUNG is likely due to insufficient number of subjects. Increased amplitude would imply recruitment of larger motor units, whereas changes in mean spike frequency parallel changes in the firing rate of active motor units. [Moritani et al, 1986]. As the activation-related increase was more pronounced in amplitude, it would indicate a stronger contribution from motor unit recruitment compared to rate coding at these moderate (20, 40%) activation levels. Amplitude did not vary between contraction types in either group. This would indirectly support Hennemans size principle on the orderly recruitment of MU's [Henneman et al, 1965a; Henneman et al, 1965b], as the selective recruitment of larger units in ECC should have increased the mean spike amplitude either through new, larger, spikes, or through the derecruitment of spikes with smaller amplitudes. The preservation of size principle has been found in ECC contractions in several studies [Bawa and Jones, 1999; Garland et al, 1996; Kossev and Christova, 1998; Pasquet et al, 2006; Sogaard et al, 1996], while some evidence of a reversal of recruitment exists [Howell et al, 1995; Nardone et al, 1989]. The present results of similar mean spike amplitudes in all contraction types would not imply a previously reported lower threshold of MU recruitment in dynamic contractions [Ivanova et al, 1997; Linnamo et al, 2003; Tax et al, 1989]. This difference could be due to muscle specific MU control strategies both within a muscle and between synergists. The aforementioned data is from biceps brachii, which, compared to soleus, has a larger proportion of fast motor units. The present data does not allow comparison of recruitment thresholds between dynamic an isometric in gastrocnemius muscle in which the fiber type distribution is more close to biceps brachii.

As the individual MUs were not analyzed in the present study, but rather pools of several MUs, the distinct conclusions about the recruitment order of slow and fast units are difficult to make. However, the ability for ISAF analysis to show recruitment of MUs is supported by an increase in the mean spike amplitude along with an increase in isometric force in the present study as well as in earlier studies with biceps brachii [Linnamo, 2003; Moritani and Muro, 1987]. In measurements with intramuscular electrodes, there is always a risk of electrode movement, especially during dynamic contractions. We have also decomposed and analyzed the MU firings in isometric contractions with decomposition software (unpublished). Since we were able to follow the same decomposed units throughout the measurements, it would appear that the electrode movement was not significant.

Ageing

The average isometric MVC was 12.3% higher in YOUNG (n.s.). Lanza et al. [2004] found a similar 14% difference (n.s.) in plantar flexors, while also larger differences have been found in other studies with the same muscle group [40% in study by Simoneau et al, 2005]. We also found the difference between torques in ECC and CON to be larger in the OLD group. As the relative activation level was equal in all contraction types, this would indicate a better preservation of ECC strength with ageing, which supports previous findings in elbow flexors [Pousson et al, 2001] and extensors [Poulin et al, 1992].

Similarly to previous literature [Morita et al, 1995; Sabbahi and Sedgwick, 1982; Scaglioni et al, 2002] H-reflex amplitude was lower in OLD in all conditions. . There are numerous potential factors in the gamma motorneuron, spindle, Ia afferent and spinal level that may affect this age related decrease [for review, see Mynark and Koceja, 2001]. However, it has been suggested that the main cause would be an increase in presynaptic inhibition [Morita et al, 1995]. These changes in the function of spinal reflexes are a likely factor in the deteriorating balance with ageing [Koceja et al, 1995].

The mean spike frequency was higher in YOUNG in all trials, ($p<0.05$ in ECC and CON). This would suggest a higher average MU firing rate, which supports earlier findings in distal muscles in isometric contractions [Connelly et al, 1999; Kamen et al, 1995; Klass et al, 2008; Roos et al, 1999]. The decreased MU firing rate may be an adaptation to the increased twitch duration to optimize force generation [Roos et al, 1997]. As the twitch duration increases with age, tetanus can be achieved with lower firing rates. The difference in mean spike frequency was not statistically significant in ISO, which may be due to the low activation levels used in the study. It has been previously shown that this difference is more evident in higher relative levels of activation [Barry et al, 2007; Kamen et al, 1995; Leong et al, 1999]. It has been shown by Knight and Kamen [2005] that larger MUs are located more superficially. In order to avoid such motor unit type bias wire electrodes were located in various depths. As the amount of adipose tissue increases with ageing, it could be possible that the wire electrodes that were inserted to equal depths in both groups would not penetrate as deep into the muscle of elderly subjects. However, this should result in more recordings of larger MU's in OLD, which, according to the onion skin principle [Person and Kudina, 1972], should decrease the average FR in submaximal contractions. Furthermore, our elderly subjects were physically very active and lean, so we find such bias unlikely.

In YOUNG subjects contraction type did not affect mean spike amplitude or frequency with matched levels of surface EMG activation. This would imply a similar activation strategy across conditions. However, in OLD the mean spike frequency was lower in dynamic contractions, especially in concentric contractions. What is the mechanism behind this observation is difficult to answer based on the present data, but it could related to synergist muscle behaviour. As mentioned above, in relation to recruitment threshold, we do not know the activation strategy of the gastrocnemii muscles. Although the surface EMG of gastrocnemius medialis did not differ between contraction types, we do not know recruitment and firing rate patterns in the gastrocnemii. As the data from soleus demonstrated, equal surface EMG values can be obtained with different MU strategies.

CONCLUSIONS

The elderly subjects showed lower H/M ratio and mean spike frequency compared to the young subjects. The reason for the lower H-reflex amplitude in elderly can't be answered based on these results, but one factor may be an increase in presynaptic inhibition. The observed lack of difference in mean spike amplitude and frequency across contraction types in YOUNG would imply a similar activation strategy, whereas the lower frequency in concentric contractions in OLD could be due to differences in the co-contraction between synergist muscles.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the valuable contribution of Dr. Masaki Ishikawa in the coding of signal analysis, and Ms S. Roivas, Mr. M. Ruuskanen, Ms P. Puttonen and Mr S. Seppänen for technical assistance.

REFERENCES

SENIAM 8: European recommendations for surface electromyography. Hermens HJ, Freriks B, Merletti R, et al, editors. ; 1999

Allinger TL, Engsberg JR. A method to determine the range of motion of the ankle joint complex, in vivo. J Biomech. 1993;26(1):69-76

Avela J, Finni T, Liikavainio T, Niemela E, Komi PV. Neural and mechanical responses of the triceps surae muscle group after 1 h of repeated fast passive stretches. J Appl Physiol. 2004;96(6):2325-32

Barry BK, Pascoe MA, Jesunathadas M, Enoka RM. Rate coding is compressed but variability is unaltered for motor units in a hand muscle of old adults. J Neurophysiol. 2007;97(5):3206-18

Bawa P, Jones KE. Do lengthening contractions represent a case of reversal in recruitment order? Prog Brain Res. 1999;123:215-20

Brown WF, Strong MJ, Snow R. Methods for estimating numbers of motor units in biceps-brachialis muscles and losses of motor units with aging. Muscle Nerve. 1988;11(5):423-32

Burke JR, Schutten MC, Koceja DM, Kamen G. Age-dependent effects of muscle vibration and the jendrassik maneuver on the patellar tendon reflex response. Arch Phys Med Rehabil. 1996;77(6):600-4

Chalmers GR, Knutzen KM. Soleus hoffmann-reflex modulation during walking in healthy elderly and young adults. Journals of Gerontology Series A-Biological Sciences & Medical Sciences. 2000;55(12):B570-9

Connelly DM, Rice CL, Roos MR, Vandervoort AA. Motor unit firing rates and contractile properties in tibialis anterior of young and old men. J Appl Physiol. 1999;87(2):843-52

Cunningham DA, Morrison D, Rice CL, Cooke C. Ageing and isokinetic plantar flexion. European Journal of Applied Physiology & Occupational Physiology. 1987;56(1):24-9

Delwaide PJ. Human monosynaptic reflexes and presynaptic inhibition. an interpretation of spastic hyperreflexia. In: Desmedt JE, editor. New Developments in Electromyography and Clinical Neurophysiology. Basel, Switzerland: Karger, 1973:508-22.

Doherty TJ, Vandervoort AA, Brown WF. Effects of ageing on the motor unit: A brief review. Can J Appl Physiol. 1993;18(4):331-58

Dousset E, Avela J, Ishikawa M, Kallio J, Kuitunen S, Kyrolainen H, Linnamo V, Komi PV. Bimodal recovery pattern in human skeletal muscle induced by exhaustive stretch-shortening cycle exercise. Med Sci Sports Exerc. 2007;39(3):453-60

Duchateau J, Enoka RM. Neural control of shortening and lengthening contractions: Influence of task constraints. J Physiol (Lond). 2008;586(Pt 24):5853-64

Duclay J, Martin A. Evoked H-reflex and V-wave responses during maximal isometric, concentric, and eccentric muscle contraction. J Neurophysiol ; J Neurophysiol. 2005;94(5):3555-62

Frontera WR, Hughes VA, Lutz KJ, Evans WJ. A cross-sectional study of muscle strength and mass in 45- to 78-yr-old men and women. J Appl Physiol. 1991;71(2):644-50
Galganski ME, Fuglevand AJ, Enoka RM. Reduced control of motor output in a human hand muscle of elderly subjects during submaximal contractions. J Neurophysiol. 1993;69(6):2108-15
Garland SJ, Cooke JD, Miller KJ, Ohtsuki T, Ivanova T. Motor unit activity during human single joint movements. J Neurophysiol. 1996;76(3):1982-90
Henneman E, Somjen G, Carpenter DO. Functional significance of cell size in spinal motoneurons. J Neurophysiol. 1965a;28:560-80
Henneman E, Somjen G, Carpenter DO. Excitability and inhibitability of motoneurons of different sizes. J Neurophysiol. 1965b;28(3):599-620
Howard JE, McGill KC, Dorfman LJ. Properties of motor unit action potentials recorded with concentric and monopolar needle electrodes: ADEMG analysis. Muscle Nerve. 1988;11(10):1051-5
Howell JN, Fuglevand AJ, Walsh ML, Bigland-Ritchie B. Motor unit activity during isometric and concentric-eccentric contractions of the human first dorsal interosseus muscle. J Neurophysiol. 1995;74(2):901-4
Hultborn H, Illert M, Nielsen J, Paul A, Ballegaard M, Wiese H. On the mechanism of the post-activation depression of the H-reflex in human subjects. Exp Brain Res. 1996;108(3):450-62
Ivanova T, Garland SJ, Miller KJ. Motor unit recruitment and discharge behavior in movements and isometric contractions. Muscle Nerve. 1997;20(7):867-74
Kallio J, Linnamo V, Komi PV. The effects of muscle history on short latency stretch reflex response of soleus muscle. J Electromyogr Kinesiol. 2004;14(3):411-21
Kamen G, Sison SV, Du CC, Patten C. Motor unit discharge behavior in older adults during maximal-effort contractions. J Appl Physiol. 1995;79(6):1908-13
Katz B. The relation between force and speed in muscular contraction. J Physiol (Lond); The Journal of Physiology. 1939;96(1):45-64
Klass M, Baudry S, Duchateau J. Age-related decline in rate of torque development is accompanied by lower maximal motor unit discharge frequency during fast contractions. J Appl Physiol. 2008;104(3):739-46
Knight CA, Kamen G. Superficial motor units are larger than deeper motor units in human vastus lateralis muscle. Muscle Nerve. 2005;31(4):475-80
Koceja DM, Mynark RG. Comparison of heteronymous monosynaptic ia facilitation in young and elderly subjects in supine and standing positions. Int J Neurosci. 2000;103(1-4):1-17
Koceja DM, Markus CA, Trimble MH. Postural modulation of the soleus H reflex in young and old subjects. Electroencephalography & Clinical Neurophysiology. 1995;97(6):387-93
Kossev A, Christova P. Discharge pattern of human motor units during dynamic concentric and eccentric contractions. Electroencephalogr Clin Neurophysiol. 1998;109(3):245-55
Lanza IR, Russ DW, Kent-Braun JA. Age-related enhancement of fatigue resistance is evident in men during both isometric and dynamic tasks. J Appl Physiol ; J Appl Physiol. 2004;97(3):967-75
Larsson L. Motor units: Remodeling in aged animals. J Gerontol A Biol Sci Med Sci. 1995;50(Spec No):91-5
Leong B, Kamen G, Patten C, Burke JR. Maximal motor unit discharge rates in the quadriceps muscles of older weight lifters. Med Sci Sports Exerc. 1999;31(11):1638-44
Lexell J. Ageing and human muscle: Observations from sweden. Can J Appl Physiol. 1993;18(1):2-18
Linnamo. Motor unit activation patterns during isometric, concentric and eccentric actions at different force levels. Journal of Electromyography and Kinesiology. 2003;13(1):93
Linnamo V, Moritani T, Nicol C, Komi PV. Motor unit activation patterns during isometric, concentric and eccentric actions at different force levels. J Electromyogr Kinesiol. 2003;13(1):93-101

Morita H, Shindo M, Yanagawa S, Yoshida T, Momoi H, Yanagisawa N. Progressive decrease in heteronymous monosynaptic ia facilitation with human ageing. Exp Brain Res. 1995;104(1):167-70

Moritani T, Muro M. Motor unit activity and surface electromyogram power spectrum during increasing force of contraction. Eur J Appl Physiol. 1987;56(3):260-5

Moritani T, Muramatsu S, Muro M. Activity of motor units during concentric and eccentric contractions. Am J Phys Med. 1987;66(6):338-50

Moritani T, Muro M, Kijima A, Berry MJ. Intramuscular spike analysis during ramp force output and muscle fatigue. Electromyogr Clin Neurophysiol. 1986;26(2):147-60

Mynark RG. Reliability of the soleus H-reflex from supine to standing in young and elderly. Clinical Neurophysiology. 2005;116(6):1400-4

Mynark RG, Koceja DM. Effects of age on the spinal stretch reflex. J Appl Biomech. 2001;17(3):188-202

Nakazawa K, Yamamoto SI, Yano H. Short- and long-latency reflex responses during different motor tasks in elbow flexor muscles. Exp Brain Res. 1997;116(1):20-8

Nardone A, Romano C, Schieppati M. Selective recruitment of high-threshold human motor units during voluntary isotonic lengthening of active muscles. J Physiol. 1989;409:451-71

Narici MV, Maganaris CN. Plasticity of the muscle-tendon complex with disuse and aging. Exerc Sport Sci Rev. 2007;35(3):126-34

Pasquet B, Carpentier A, Duchateau J. Specific modulation of motor unit discharge for a similar change in fascicle length during shortening and lengthening contractions in humans. J Physiol (Lond). 2006;577(Pt 2):753-65

Person RS, Kudina LP. Discharge frequency and discharge pattern of human motor units during voluntary contraction of muscle. Electroencephalogr Clin Neurophysiol. 1972;32(5):471-83

Pinniger GJ, Nordlund MM, Steele JR, Cresswell AG. H-reflex modulation during passive lengthening and shortening of the human triceps surae. J Physiol. 2001;534(3):913-23

Porter MM, Vandervoort AA, Kramer JF. Eccentric peak torque of the plantar and dorsiflexors is maintained in older women. Journals of Gerontology Series A-Biological Sciences & Medical Sciences. 1997;52(2):B125-31

Poulin MJ, Vandervoort AA, Paterson DH, Kramer JF, Cunningham DA. Eccentric and concentric torques of knee and elbow extension in young and older men. Can J Sport Sci. 1992;17(1):3-7

Pousson M, Lepers R, Van Hoecke J. Changes in isokinetic torque and muscular activity of elbow flexors muscles with age. Exp Gerontol. 2001;36(10):1687-98

Romano C, Schieppati M. Reflex excitability of human soleus motoneurones during voluntary shortening or lengthening contractions. J Physiol. 1987;390:271-84

Roos MR, Rice CL, Vandervoort AA. Age-related changes in motor unit function. Muscle Nerve. 1997;20(6):679-90

Roos MR, Rice CL, Connelly DM, Vandervoort AA. Quadriceps muscle strength, contractile properties, and motor unit firing rates in young and old men. Muscle Nerve. 1999;22(8):1094-103

Sabbahi MA, Sedgwick EM. Age-related changes in monosynaptic reflex excitability. J Gerontol. 1982;37(1):24-32

Scaglioni G,Ferri A, Minetti AE, Martin A, Van Hoecke J, Capodaglio P, Sartorio A, Narici MV. Plantar flexor activation capacity and H reflex in older adults: adaptations to strength training. J Appl Physiol. 2002; 92: 2292-2302

Simoneau E, Martin A, Van Hoecke J. Muscular performances at the ankle joint in young and elderly men. J Gerontol A Biol Sci Med Sci. 2005;60(4):439-47

Sogaard K, Christensen H, Jensen BR, Finsen L, Sjogaard G. Motor control and kinetics during low level concentric and eccentric contractions in man. Electroencephalogr Clin Neurophysiol. 1996;101(5):453-60

Tax AA, Denier van der Gon JJ, Gielen CC, van den Tempel CM. Differences in the activation of m. biceps brachii in the control of slow isotonic movements and isometric contractions. Exp Brain Res. 1989;76(1):55-63

Vandervoort AA. Aging of the human neuromuscular system. Muscle Nerve. 2002;25(1):17-25

Vandervoort AA, McComas AJ. Contractile changes in opposing muscles of the human ankle joint with aging. J Appl Physiol. 1986;61(1):361-7

II

Age-related decreases in motor unit discharge rate and force control during isometric plantar flexion

by

Jouni Kallio, Karen Søgaard, Janne Avela, Paavo V. Komi, Harri Selänne, Vesa Linnamo, 22(6); 983-89, 2012

Journal of Electromyography and Kinesiology, vol 22

Age-related decreases in motor unit discharge rate and force control during isometric plantar flexion

J. Kallio [a], K. Søgaard [b], J. Avela [c], P. Komi [d], H. Selänne [e], V. Linnamo [c]

[a] Neuromuscular Research Center, Department of Biology of Physical Activity, University of Jyväskylä, Finland
[b] Professor, Institute of Sports Science and Clinical Biomechanics, University of Southern Denmark
[c] Professor, Neuromuscular Research Center, Department of Biology of Physical Activity, University of Jyväskylä, Finland
[d] Professor Emeritus, Neuromuscular Research Center, Department of Biology of Physical Activity, University of Jyväskylä, Finland
[e] Chief Physician, LIKES, Foundation for Sport and Health Sciences, Jyväskylä, Finland

Keywords:
Ageing, Intramuscular, Electromyography, Isometric, Soleus, Motor unit

Correspondence to:

Jouni Kallio
Neuromuscular Research Center
Department of Biology of Physical Activity
University of Jyväskyla
P.O.Box 35, FIN-40351, Jyvaskyla
Finland
Tel. +358 (0) 40 805 3741
E-mail. jouni.kallio@jyu.fi

ABSTRACT

Ageing is related to multiple changes in muscle physiology and function. Previous findings concerning the effects of ageing on motor unit discharge rate (DR) and fluctuations in DR and force are somewhat contradictory. Eight YOUNG and nine OLD physically active males performed isometric ramp (RECR) and isotonic (ISO) plantar flexions at 10 and 20% of surface-EMG at MVC. Motor unit (MU) action potentials were recorded with intramuscular fine-wire electrodes and decomposed with custom build software "Daisy". DR was lower in OLD in RECR-10% (17.9%, $p<.001$), RECR-20% (15.8%, $p<.05$), ISO-10% (17.7%, $p<.01$) and ISO-20% (14%, n.s.). In YOUNG force fluctuations were smaller at ISO-10% (72.1%, $p<.001$) and ISO-20% (55.2%, $p<.05$) which were accompanied with a slight increase in DR variation [n.s.]. The observed lower DR in OLD is in line with earlier findings in small distal muscles. Also the larger force fluctuation in OLD was in line with previous studies with smaller hand muscles. These findings suggest that the age-related changes in MU control do exist also in large leg extensors that play an important role in human locomotion and balance control.

INTRODUCTION

Ageing is related to muscle atrophy that is accompanied with decreases in muscle force. The largest changes in fibre size are found in fast muscle fibres [Frontera et al, 1997; Lexell, 1993], which leads to major decreases, not only in maximal force, but also in fast force production [Häkkinen K. et al, 1998]. The predominant loss of type II fibres is counteracted with MU remodeling that leads to an increase in MU size [Andersen et al, 1999; Larsson, 1995].

The functional and physiological changes in the muscle may be coupled with modifications in muscle activation, such as possible decreases in voluntary activation [Klass et al, 2007] and changes in the degree of agonist/antagonist coactivation [Häkkinen K. et al, 1998]. However, previous results concerning motor unit activation are inconsistent. Several studies have found the elderly to have a lower discharge rate during maximal contractions in muscles, for example the first dorsal interosseous (FDI) and tibialis anterior (TA) [Kamen et al, 1995; Klass et al, 2008]. In contrast, no such differences were found when comparisons were made at recruitment threshold in biceps brachii, triceps brachii, TA or FDI in the studies by Howard et al. (1988) and Galganski et al. (1993). It appears that the largest changes exist in the oldest subjects, in distal muscles, and at higher relative force levels. It seems that the decreased MU discharge rate would be an adaptation to the increased twitch duration to optimize force generation. As the twitch duration increases with age, tetanus theoretically could be achieved with lower discharge rates. (For review, see Roos et al, 1997).

The functional significance of ageing is not limited to the decrease in the maximal level of force or power production. The optimal use of the neuromuscular system is also dependent on sufficient control of muscle activation. One way to assess the differences in motor control is to measure how accurately the subjects are able to hold a given submaximal target force, and how much the motor unit discharge rate varies during the contraction. Both of these variations are dependent on several factors, including activation level, the muscle tested, and age. Some of the previous studies with elderly subjects have shown a decrease in force control, while others have not. It seems, that age-related differences are more prominent in small [Laidlaw et al, 2000; Tracy et al, 2005] rather than large muscles [Christou and Carlton, 2002; Graves et al, 2000], in low activation levels [Galganski et al, 1993; Tracy and Enoka, 2002], and with visual feedback [Sosnoff and Newell, 2006a] rather than without [Barry et al, 2007]. Tracy (2007) has shown an age-related decrease in plantarflexor steadiness, which has subsequently been found to be related to diminished postural control [Kouzaki and Shinohara, 2010]. Even though plantar flexors are important for locomotion and balance control, to the best of our knowledge, no data on motor unit discharge properties in the plantar flexors of the elderly exists.

Therefore, the purpose of this study was to assess the underlying mechanisms behind age-related changes in plantarflexor force and force control. The aim was to examine possible differences in motor unit discharge rate, variability of discharge rate and force, and twitch properties between young and elderly adults.

METHODS

Subjects

Seventeen physically active males volunteered as subjects for the study. There were 8 males in the younger (YOUNG) group (27.1±3.2yr. 1.77±0.08m 71.7±4.7kg) and 9 in the older (OLD) group (70.2±4.1yr. 1.76±0.05m 84.9±10.7kg). Physical activity of the subjects was determined with an open-ended questionnaire, where the subjects wrote the type and frequency of their regular exercise routine. All men in both groups performed moderate to strenuous physical exercise three times a week or more. Before the measurements all of the subjects in the OLD group underwent a medical examination. Only subjects without any history of neuromuscular or vascular disease were approved for the study. Height, weight and ankle joint range of motion (ROM) were measured from all the subjects.

Full advice about possible risks and discomfort was given to the subjects and all gave their written informed consent to the procedures prior to the commencement of the study. The investigation was conducted according to the declaration of Helsinki and approved by the ethics committee of the University of Jyväskylä.

Protocol

The testing protocol employed in the present study consisted of isometric and dynamic plantar flexions. However, only the results from the isometric contractions are reported here. We have previously published the results from isometric and dynamic measurements that have been analyzed with a more automated analysis method (intramuscular spike amplitude frequency histogram) [Kallio J. et al, 2010]. Each subject participated in two measurement sessions separated by one to three weeks. One session was for the motor unit recordings and another for H-reflex (reported in Kallio J. et al, 2010) and twitch properties.

The subjects performed plantar flexions while seated in an ankle dynamometer (University of Jyväskylä, Finland). Prior to data collection all subjects performed a warm-up consisting of 10 minutes light cycling on a bicycle ergometer and 5-10 submaximal isometric plantar flexions. All measurements were conducted in relation to the individual range of motion of the subject. The protocol for determining the individual zero angle (IZA), has been described in detail in [Kallio J. et al, 2010].

The test battery used in the both sessions was as follows:

I) Maximal voluntary contractions (MVC)

The subject was instructed to perform a maximal isometric plantar flexion for approximately 5 s. A minimum of two successful trials were performed and further trials were performed if the maximal force value between trials differed by more than 10%. The MVC measurement was repeated at the end of the protocol to examine the amount of possible fatigue caused by the session. Each subject practiced maximal contractions during the warm-up before the actual measurements.

II) Maximal electrically evoked contractions

The setup for the twitch measurements has been explained in more detail in [Kallio J. et al, 2010]. Passive twitch force properties were measured as peak force (Pf) during maximal M-wave (Mmax). The contraction time was defined as the time from onset to peak force and the half-relaxation time was defined as the time from peak force to 50% reduction of peak force.

III) Submaximal trials

a) Isometric ramp contractions

Ramp contractions were used to detect motor units, and to specify their recruitment thresholds (as % of SOL surface EMG in MVC). The subjects were asked to increase the plantar flexion force at a slow rate (~2% of MVC/s). Before data collection, subjects practiced for several trials in order to learn how to produce a smooth, rising force-curve. Multiple ramps were then recorded (0-20, 10-30, 20-40% of MVC force) for the threshold analysis of all the detected units.

b) Isometric isotonic contractions

The activation levels used in the isotonic contractions were 10% (ISO-10%) and 20% (ISO-20%) of the Soleus (SOL) surface EMG root mean square (RMS) value of the isometric MVC. The subjects were given a target force-level that would correspond to the desired activation level. The subjects were instructed to increase the force to this level and hold it as steadily as possible for approximately 10s. Each subject practiced the task several times before the recorded measurements. Soleus surface EMG (sEMG) was analyzed online, and if the activation level was not within the accepted range of ±2.5%, the target force level was adjusted accordingly and the trial was repeated.

Force and surface EMG

Trials were performed while the subject was seated in a motor driven ankle dynamometer, used previously in similar experiments with passive and active muscle [Avela et al, 2004; Dousset et al, 2007; Kallio et al, 2004]. To maximize stability during testing, subjects were strapped to the chair from the thigh and the ankle. Maximum torque capacity of the dynamometer was 150 Nm. The torque signal was sent to a computer via a CED 1401+ (Cambridge Electronic Design) and sampled at a frequency of 20 kHz. Signals were collected and analyzed using Signal software (Cambridge Electronic Design, UK). Before analysis, force and angle signals were filtered with a low-pass filter (cutoff frequencies 75 and 150 Hz, respectively).

Global EMG activities of the Soleus (SOL) and Gastrocnemius medialis (GM) muscles were recorded utilizing bipolar silver chloride miniature surface electrodes (Beckman 650437, USA) with 20 mm inter-electrode distance. Electrodes were prepared and placed according to the recommendation by SENIAM [Hermens et al, 1999]. The surface EMG signals were amplified and sampled at a frequency of 20 kHz. The signals were subsequently downsampled to 2 kHz, band-pass filtered at 20 to 1 kHz and analyzed using the Signal software (Cambridge Electronic Design, UK). For the maximal voluntary contractions (MVC), both sEMG and torque data were analyzed ±100 ms around the peak torque. Maximal sEMG activities of SOL and GM were calculated as root mean square (RMS) of the signal and used to normalize the sEMG RMS values during submaximal contractions.

Intramuscular EMG

For intramuscular EMG recordings, four bipolar fine-wire electrodes were inserted into SOL. The wire-electrodes consisted of two 50 µm polyurethane insulated wires that were glued together (cutting one wire 2mm shorter than the other one). Insulation was mechanically removed from the wires over 0.5 mm distance from the tip, resulting in an inter-electrode distance of approximately 100µm. A hook was formed to the end of the wires to ensure fixation during the measurements. The wire electrode pairs were inserted into the muscle with a 27 gauge needle that was subsequently withdrawn. Electrodes were autoclaved before the experiment in order to minimize the risk of infection. Wire-electrodes were inserted after the MVC-trials to avoid possible electrode migration due to high force levels. Electrodes were placed in different depths around the sEMG electrode and a ground electrode was placed on the medial malleolus. To ensure the best possible signal quality, the signal was pre-amplified with a non-commercial amplifier (amp 200, bandwidth 8-4500), attached to the subjects calf. This signal was sent to the CED 1902 programmable signal conditioner (Cambridge Electronics Design Ltd, Cambridge, CB4 4FE, UK) and digitized with CED 1401+ (Cambridge Electronic Design) with a sampling frequency of 20 kHz. The channels were copied twice, and the two new channels were filtered with different fourth order butterworth band-pass filters (300-8000 and 400-10000) to give more information for decomposition.

Signal decomposition, motor unit identification, and data analysis were performed with our application of the three channel decomposition technique computer algorithm, "Daisy", described in more detail in [Farina et al, 2001; Olsen et al, 2001; Sogaard et al, 1996]. Motor unit action potential (MUAP) recognition is based on template matching of the waveform of the three channels (unfiltered and two band-pass filtered), created from the same intramuscular EMG recording. The MU classification was performed semi-automatically with a high degree of operator interaction. For each train of MU discharges the inter-spike intervals were registered and instantaneous discharge rate (FR) was calculated as the inverse of each inter-spike interval. The recorded units from OLD and YOUNG subjects were pooled within the group for calculating the age-related differences. The analysis was based on 4242 motor unit discharges (1669 in YOUNG, 2573 in OLD) from 40 different motor units in YOUNG and 76 in OLD. The number of discharges analyzed in RAMP trials was always 10, whereas in the isotonic contractions the average number of discharges in each trial was 48.2±29.4.

Motor unit discharge rate (FR) at recruitment threshold was calculated in ramp contractions by averaging the first 10 inter firings intervals after the unit began firing constantly. For threshold discharge rate analysis, motor units were divided into two groups: recruitment before 10% (RECR-10%) and between

10 and 20 % (RECR-20%) according to the surface EMG activation level. For each recorded MU action potential train in the isotonic contractions, the amount of activity above each unit's recruitment threshold was determined. Averaging these relative activity levels for both groups enabled the detection of a possible bias in MU selection. The variability in DR was determined by coefficient of variation (CV), calculated as standard deviation (SD) of instantaneous DR divided by the mean FR, multiplied by 100%. Similarly, variability in force was calculated as SD of force curve/mean force*100% [Enoka, 2008].

Statistical analysis

An independent t-test was used to find differences between groups. The critical level of significance in the present study was $P < 0.05$. Descriptive statistics include mean and standard deviation.

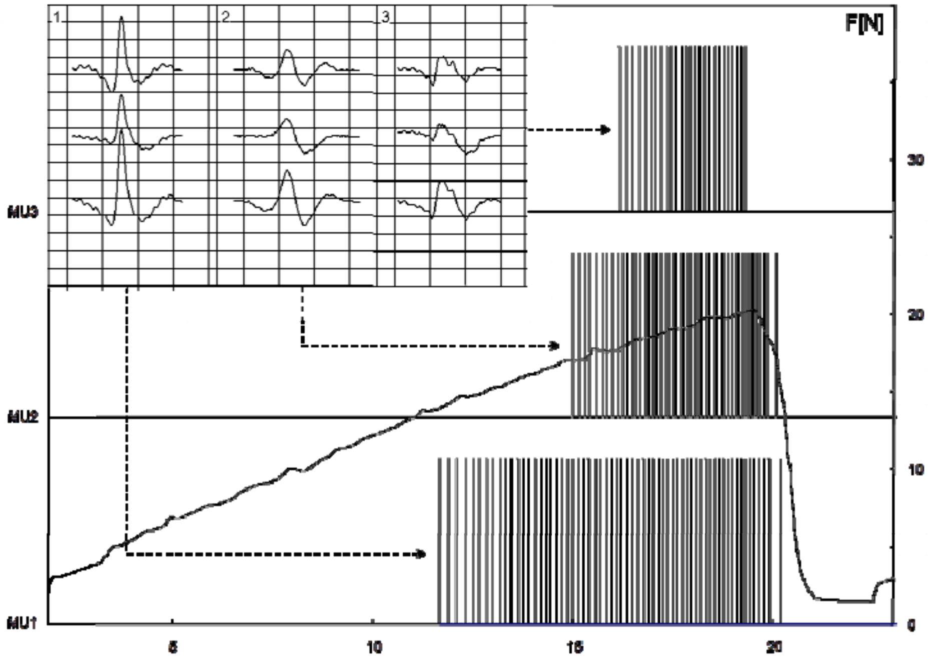


Figure 1. Motor unit decomposition with Daisy software during a submaximal ramp contraction. In this case, three units were identified, and the corresponding motor unit action potential trains (arrows) are illustrated with vertical bars. The top left figure shows the shapes of the action potentials from raw (top row) and band pass filtered (300-8000 and 400-10000) signals. Threshold discharge rate was determined as an average from the 10 first discharges, as illustrated by the shaded area. Also the surface EMG for recruitment threshold was calculated from the duration of the first 10 discharges.

RESULTS

Range of motion

The maximal active range of motion was 4.1% (n.s.) larger in YOUNG (65.1 ± 8.1^{o}) compared to OLD (62.5 ± 6.2 ^{o}). Also, the maximal active plantar flexion in YOUNG was 8.1% larger (151.3 ± 13.1^{o}) than in OLD (139.1 ± 5.4^{o}). However, the maximal active dorsiflexion in OLD (69.2 ± 2.3^{o}) was 7.6% (p = 0.01) larger than in YOUNG (74.9 ± 6.5^{o}). Therefore, the individual zero angle (determined as being 15 deg. plantar from the maximal voluntary dorsiflexion) used in the measurements was on average 5.1^{o} more dorsal in group OLD (5.6%; n.s.).

MVC and submaximal activation level

The maximal voluntary plantarflexion force was 21.4% lower ($p<0.05$) in OLD as compared to YOUNG (Table 1). There was a non-significant reduction in MVC between pre and post measurements in both groups. Based on force level the ramp contractions were divided into force level below 10% and between 10 and 20 % and the corresponding EMG level calculated normalized to maxEMG in Soleus. No consistent or statistically significant differences in submaximal activation levels between YOUNG and OLD were found in any of the conditions (Table 1).

Stimulation

There was no significant difference in mean maximal M-wave twitch force between groups (Table 1). The mean twitch contraction time (CT) was significantly longer in OLD, whereas no significant difference was observed in the mean half relaxation time (HRT). The ratio of peak twitch force and contraction time (PT/CT) was significantly lower in OLD.

Motor unit discharge rate

The analysis was based on 4242 motor unit discharges from 136 different motor units. The number of discharges analyzed in RAMP trials was always 10, whereas in the isotonic contractions the average number of discharges in each trial was 48.2±29.4. Number of MU's in the different conditions are given in figure 2.

Table 1: Descriptive information about maximal voluntary contraction (MVC), stimulation and surface-EMG of both groups. MVC torque includes change between before and after-measurements in %. Maximal M-wave data include contraction time (CT), mean half relaxation time (HRT) and the ratio of peak twitch force and contraction time (PT/CT). Activation is measured as % of soleus surface EMG in MVC. Significant differences between groups: * = p<.05, *** = p<.001.

	MVC		Maximal M-wave				Activation (%SOL MVC)			
	Before (Nm)	change(%)	PT (Nm)	CT (ms)	HRT (ms)	PT/CT (Nm/ms)	RAMP-10%	RAMP-20%	ISO-10%	ISO-20%
YOUNG	103.6±18.3	-2.0	24.1±11.0	117±22	151±124	0.20±0.07	7.1±3.9	18.8±2.7	8.9±3.1	22.3±0.0
OLD	81.4±20.4*	-4.4	21.5±8.8	150±28***	184±129	0.14±0.05***	8.9±2.9	24.6±7.7	10.9±2.4	20.0±2.0

The instantaneous motor unit discharge rate measured at recruitment threshold in the ramp contractions was significantly lower in OLD at both measured activation levels (Fig. 2). In the units recruited at 10% of maximal activation (RECR-10%) the mean discharge rate in OLD (5.4±1.2/s) was 17.9% lower (p<.001) compared to YOUNG (6.6±1.4/s). The difference in the discharge rates for the units recruited at 20% activation level (RECR-20%) between OLD (6.7±1.5/s) and YOUNG (5.6±1.3/s) was 15.8% (p<.05).

In the isotonic contractions at 10% of maximal activation the mean discharge rate (FR) in OLD (6.4±1.3/s) was 17.7% lower (p<.01) compared to YOUNG (7.8±1.6/s). The difference in DR (14.0%) at ISO-20% (YOUNG 7.4±0.6/s; OLD 6.4±1.7/s) did not reach statistical significance (p=0.06), probably due to a low number of MU identified. The mean activation levels for the analyzed motor units at 10% trials were 1.1±5.0 and 1.7±3.1 percentage points above the recruitment threshold in YOUNG and OLD, respectively [n.s.]. For the 20% trials, the corresponding numbers were 2.98±2.6 for YOUNG and 3.99±7.2 for OLD [n.s.].

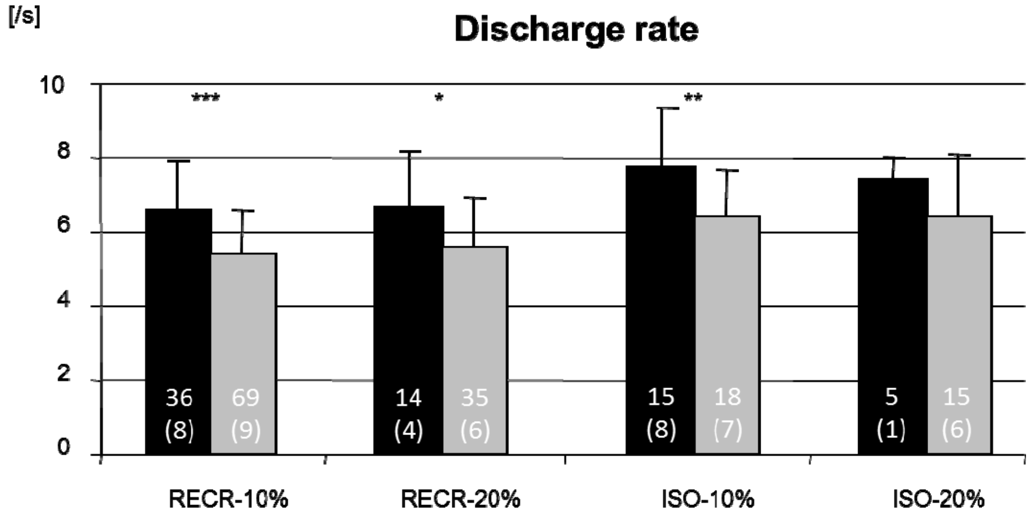


Figure 2. Motor unit discharge rate (mean+SE) in YOUNG (black) and OLD (grey) at recruitment thresholds (RECR) and during isotonic contractions (ISO). Number of units and subjects (in parenthesis) are in the columns. Activation level was calculated as 10 and 20% of soleus EMG at MVC (see text for details). *: significant difference between age-groups.

Force variability

The average force variations (CV), in the isotonic contractions at ISO-10% in YOUNG (1.0±0.4%) were 72.1% smaller (p<.001) than in OLD (3.7±2.2%). At ISO-20% the difference between YOUNG (1.1±0.1%) and OLD (2.5±1.7%) was 55.2% (p<.05).

Motor unit discharge rate variability

The average coefficient of variation in instantaneous discharge rate (DR CV%) at ISO-10% in YOUNG (11.5±4.1%) was 13.7% smaller than in OLD (13.3±5.5%). At ISO-20% there was a 28.5% difference in DR CV% (YOUNG 11.3±3.0%; OLD 15.7±10.1%) in both cases the differences were not statistically significant (p=0.3 and p=0.1), probably due to a limited number of MUs identified. (Fig.3)

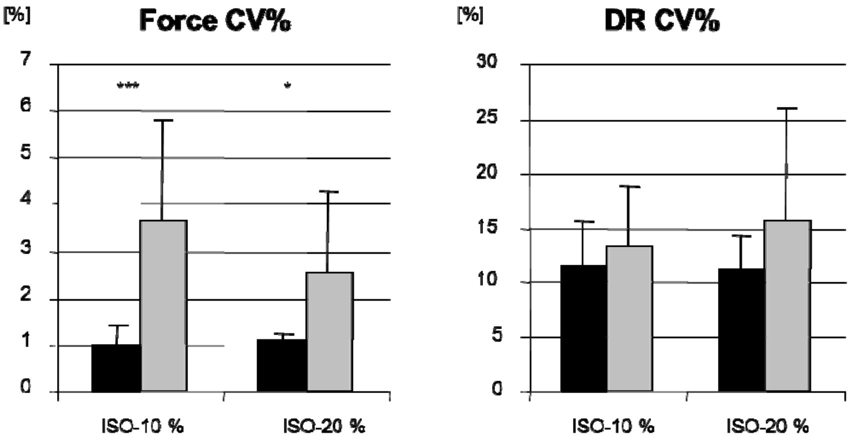


Figure 3. Fluctuations in force and discharge rate (FR) during isotonic contractions at two submaximal activation levels (CV%+SE). *: significant difference between age-groups.

DISCUSSION

In the present study the elderly showed a lower motor unit discharge rate across all measured submaximal conditions. In addition, their ability to hold a steady isometric force was lower, although the variations in motor unit discharge rates were not significantly different.

Discharge rate

The mean soleus motor unit discharge rate was significantly lower in OLD in all measured conditions, which is in line with previous findings in other smaller distal muscles, e.g. Tibialis Anterior [Connelly et al, 1999; Klass et al, 2008], first dorsal interosseous [Kamen et al, 1995] and adductor digiti minimi [Nelson et al, 1984]. These results also confirm the results of our previous, cruder analysis of the data collected both during isometric and dynamic contractions [Kallio J. et al, 2010]. The decreased MU discharge rate has been suggested to be an adaptation to the increased twitch duration to optimize force generation [Roos et al, 1997] and reduce fatigue [Kent-Braun, 2009]. As the twitch duration increases with age, tetanus can be achieved with lower discharge rates. The present analysis shows the contraction time in YOUNG to indeed be significantly shorter compared to OLD even though the peak force was not significantly decreased.

Motor units are often studied in muscles that are small and/or do not have many synergists (for example FDI, TA). We chose to study motor unit behaviour in soleus due to its important role in balance and locomotion. However, soleus is a large muscle with many synergists, which makes interpreting the data more difficult. We chose to normalize the submaximal trials, not relative to plantarflexion force in MVC, but instead as percentage of soleus surface EMG activity during MVC. This enabled us to focus on the activation patterns of soleus at the same level of common drive to the muscle. As the present data demonstrates, equivalent surface EMG RMS-values can be obtained with different MU activation strategies.

Despite being quite physically active, the OLD showed a significantly lower (21.4%) isometric MVC. Previous findings on age-group differences on plantar flexion MVC have ranged from none [Piirainen et al, 2010] to 40% [Simoneau et al, 2005]. One possible explanation for the observed lower discharge rate in the elderly would be an inability to fully activate their muscles during MVC. This would lead to erroneously low submaximal target levels that would require less activity from the motor units. However, a number of previous studies have shown that the voluntary activation of plantar flexors during MVC is unchanged in old subjects [Simoneau et al, 2005; Vandervoort and McComas, 1986].

Variability in force and discharge rate

We found the ability to hold a steady submaximal force to be significantly reduced in the elderly. Our findings are in line with previous reports in other muscles, such as the FDI [Laidlaw et al, 2000] and the knee extensors [Tracy and Enoka, 2002]. Tracy [Tracy, 2007] showed similar age-related differences in force fluctuations with ankle plantarflexors at the lowest force levels (2.5 and 5% MVC), while other force levels (10-80%) in that study did not show such an effect.

We also found the variability of motor unit discharge rate to be somewhat larger (n.s.) in elderly subjects. The activation levels used in the present study were quite low and, based on a previous FDI muscle study by Galganski et al. (1993), it is possible that the group difference would have been smaller at higher levels of activation. It is interesting to note, that the age-related differences in force fluctuations appear to be larger compared to the underlying changes in discharge rate variability. This finding is, however, in line with a previous study by Barry et al. (2007), where both experimental data from the FDI muscle and simulation data using a modified Fuglevand model were compared. They found a slight increase in force variability even when there was no change in discharge rate variability. It is thus likely that the increased discharge rate variability reduces steadiness in conjunction with the other changes in motor unit physiology; for example reduced discharge rate, more fibres per MU, and different distribution of twitch forces. In addition, it is possible that the observed age-related differences in steadiness could be related to impairments in the visual feedback of the elderly [Sosnoff and Newell, 2006a; Sosnoff and Newell, 2006b]. Recent studies have shown that removing visual feedback leads to similar force and discharge rate variabilities in young and elderly [Barry et al, 2007; Welsh et al, 2007].

We did not observe any differences in DR between 10 and 20% activation levels within groups. This may be due to the fact that the same units were not measured at different force levels. This also prevents us from knowing what happened to DR coefficient of variation (CV) as activation level increased. Moritz et al (2005) and Barry et al (2007) have shown that discharge rate variability decreases exponentially as the activation level increases after initial recruitment. This is a potential source of error in submaximal measurements. In order to make sure that the two pools of motor units we used in our isotonic analysis were comparable, we averaged for each group the amount each measured unit was above, or below the measured recruitment threshold. We found the difference in mean to be very small (0.6 and 1.0 percentage points in 10 and 20%, respectively) and not statistically significant. Furthermore, the minor difference could not explain the observed larger CV% in OLD, as the analyzed units in OLD were on average more above the recruitment threshold compared to the units in YOUNG.

The present study clearly displays the age-related deterioration of the neuromuscular system in many, functionally important levels. The elderly had a lower MVC, slower force production (twitch CT) and reduced steadiness and MU discharge rate. All these changes may affect balance control and thus the ability to avoid falling and injuries.

Limitations of the study

Due to the challenge of signal decomposition the sample size in the current study was modest (5-69 units/age-group/condition). The recorded units may not represent the average of all SOL units, as all the wires were located around the surface electrode, approximately 1/3 medial from the lateral end of the muscle. The wires were inserted to various depths (15-25 mm) to avoid the possible bias of recording predominantly large (superficial) or slow (deep) units [Knight and Kamen, 2005]. The elderly subjects in the study were physically very active, and in that regard do not represent the average of that age-level. However, for this reason we think that the observed differences are the result of the unavoidable process of aging instead of the reduced physical activity which is often accompanied with aging.

Conclusions

These findings suggest that age-related differences in motor unit control do exist also in large leg extensors that play an important role in human locomotion and balance control. The observed lower motor unit discharge rate and force control have potential implications on the balance and risk of falling in the elderly.

References

Andersen JL, Terzis G, Kryger A. Increase in the degree of coexpression of myosin heavy chain isoforms in skeletal muscle fibers of the very old. Muscle Nerve. 1999;22(4):449-54

Avela J, Finni T, Liikavainio T, Niemela E, Komi PV. Neural and mechanical responses of the triceps surae muscle group after 1 h of repeated fast passive stretches. J Appl Physiol. 2004;96(6):2325-32

Barry BK, Pascoe MA, Jesunathadas M, Enoka RM. Rate coding is compressed but variability is unaltered for motor units in a hand muscle of old adults. J Neurophysiol. 2007;97(5):3206-18

Christou EA, Carlton LG. Age and contraction type influence motor output variability in rapid discrete tasks. J Appl Physiol. 2002;93(2):489-98

Connelly DM, Rice CL, Roos MR, Vandervoort AA. Motor unit firing rates and contractile properties in tibialis anterior of young and old men. J Appl Physiol. 1999;87(2):843-52

Dousset E, Avela J, Ishikawa M, Kallio J, Kuitunen S, Kyrolainen H, Linnamo V, Komi PV. Bimodal recovery pattern in human skeletal muscle induced by exhaustive stretch-shortening cycle exercise. Med Sci Sports Exerc. 2007;39(3):453-60

Enoka RM. Neuromechanics of human movement 4th ed. Champaign, IL: Human Kinetics; 2008

Farina D, Colombo R, Merletti R, Olsen HB. Evaluation of intra-muscular EMG signal decomposition algorithms. J Electromyogr Kinesiol. 2001;11(3):175-87

Frontera WR, Grimby L, Larsson L. Firing rate of the lower motoneuron and contractile properties of its muscle fibers after upper motoneuron lesion in man. Muscle Nerve. 1997;20(8):938-47

Galganski ME, Fuglevand AJ, Enoka RM. Reduced control of motor output in a human hand muscle of elderly subjects during submaximal contractions. J Neurophysiol. 1993;69(6):2108-15

Graves AE, Kornatz KW, Enoka RM. Older adults use a unique strategy to lift inertial loads with the elbow flexor muscles. J Neurophysiol. 2000;83(4):2030-9

Häkkinen K., Newton RU., Gordon SE., McCormick M., Volek JS., Nindl BC., Gotshalk LA., Campbell WW., Evans WJ. Changes in muscle morphology, electromyographic activity, and force production characteristics during progressive strength training in young and older men. J Gerontol A Biol Sci Med Sci. 1998;53(6):B415-23

Hermens HJ, Freriks B, Merletti R, Stegeman D, Blok J, Rau G, et al. SENIAM 8: European recommendations for surface electromyography. ; 1999

Howard JE, McGill KC, Dorfman LJ. Age effects on properties of motor unit action potentials: ADEMG analysis. Ann Neurol. 1988;24(2):207-13

Kallio J., Avela J., Moritani T., Kanervo M., Selänne H., Komi P., Linnamo V. Effects of ageing on motor unit activation patterns and reflex sensitivity in dynamic movements. J Electromyogr Kinesiol. 2010;20(4):590-8

Kallio J, Linnamo V, Komi PV. The effects of muscle history on short latency stretch reflex response of soleus muscle. J Electromyogr Kinesiol. 2004;14(3):411-21

Kamen G, Sison SV, Du CC, Patten C. Motor unit discharge behavior in older adults during maximal-effort contractions. J Appl Physiol. 1995;79(6):1908-13

Kent-Braun JA. Skeletal muscle fatigue in old age: Whose advantage?[see comment]. Exercise & Sport Sciences Reviews. 2009;37(1):3-9

Klass M, Baudry S, Duchateau J. Voluntary activation during maximal contraction with advancing age: A brief review. Eur J Appl Physiol. 2007;100(5):543-51

Klass M, Baudry S, Duchateau J. Age-related decline in rate of torque development is accompanied by lower maximal motor unit discharge frequency during fast contractions. J Appl Physiol. 2008;104(3):739-46

Knight CA, Kamen G. Superficial motor units are larger than deeper motor units in human vastus lateralis muscle. Muscle Nerve. 2005;31(4):475-80

Kouzaki M, Shinohara M. Steadiness in plantar flexor muscles and its relation to postural sway in young and elderly adults. Muscle Nerve. 2010;42(1):78-87

Laidlaw DH, Bilodeau M, Enoka RM. Steadiness is reduced and motor unit discharge is more variable in old adults. Muscle Nerve. 2000;23(4):600-12

Larsson L. Motor units: Remodeling in aged animals. J Gerontol A Biol Sci Med Sci. 1995;50(Spec No):91-5

Lexell J. Ageing and human muscle: Observations from sweden. Can J Appl Physiol. 1993;18(1):2-18

Moritz CT, Barry BK, Pascoe MA, Enoka RM. Discharge rate variability influences the variation in force fluctuations across the working range of a hand muscle. J Neurophysiol. 2005;93(5):2449-59

Nelson RM, Soderberg GL, Urbscheit NL. Alteration of motor-unit discharge characteristics in aged humans. Phys Ther. 1984;64(1):29-34

Olsen HB, Christensen H, Sogaard K. An analysis of motor unit firing pattern during sustained low force contraction in fatigued muscle. Acta Physiol Pharmacol Bulg. 2001;26(1-2):73-8

Piirainen JM, Avela J, Sippola N, Linnamo V. Age dependency of neuromuscular function and dynamic balance control. European Journal of Sport Science. 2010;10(1):69

Roos MR, Rice CL, Vandervoort AA. Age-related changes in motor unit function. Muscle Nerve. 1997;20(6):679-90

Simoneau E, Martin A, Van Hoecke J. Muscular performances at the ankle joint in young and elderly men. J Gerontol A Biol Sci Med Sci. 2005;60(4):439-47

Sogaard K, Christensen H, Jensen BR, Finsen L, Sjogaard G. Motor control and kinetics during low level concentric and eccentric contractions in man. Electroencephalogr Clin Neurophysiol. 1996;101(5):453-60

Sosnoff JJ, Newell KM. Information processing limitations with aging in the visual scaling of isometric force. Exp Brain Res. 2006a;170(3):423-32

Sosnoff JJ, Newell KM. Aging, visual intermittency, and variability in isometric force output. J Gerontol B Psychol Sci Soc Sci. 2006b;61(2):P117-24

Tracy BL. Force control is impaired in the ankle plantarflexors of elderly adults. Eur J Appl Physiol. 2007;101(5):629-36

Tracy BL, Maluf KS, Stephenson JL, Hunter SK, Enoka RM. Variability of motor unit discharge and force fluctuations across a range of muscle forces in older adults. Muscle Nerve. 2005;32(4):533-40

Tracy BL, Enoka RM. Older adults are less steady during submaximal isometric contractions with the knee extensor muscles. J Appl Physiol ; J Appl Physiol. 2002;92(3):1004-12

Vandervoort AA, McComas AJ. Contractile changes in opposing muscles of the human ankle joint with aging. J Appl Physiol. 1986;61(1):361-7

Welsh SJ, Dinenno DV, Tracy BL. Variability of quadriceps femoris motor neuron discharge and muscle force in human aging. Experimental Brain Research. 2007;179(2):219-33

III

Motor unit firing behaviour of soleus muscle in isometric and dynamic contractions

by

Jouni Kallio, Karen Søgaard, Janne Avela, Paavo V. Komi, Harri Selänne, Vesa Linnamo, 2013

PLOS ONE, accepted for publication

Motor unit firing behaviour of soleus muscle in isometric and dynamic contractions

Jouni Kallio [a], Karen Søgaard [b], Janne Avela [c], Paavo V. Komi [d], Harri Selänne [e], Vesa Linnamo [c]

[a] Neuromuscular Research Center, Department of Biology of Physical Activity, University of Jyväskylä, Jyväskylä, Finland
[b] Institute of Sports Science and Clinical Biomechanics, University of Southern Denmark, Odense, Denmark
[c] Neuromuscular Research Center, Department of Biology of Physical Activity, University of Jyväskylä, Jyväskylä, Finland
[d] Neuromuscular Research Center, Department of Biology of Physical Activity, University of Jyväskylä, Jyväskylä, Finland
[e] LIKES Foundation for Sport and Health Sciences, Jyväskylä, Finland

Keywords:
Electromyography, Dynamic, Intramuscular, Soleus, Motor unit

Correspondence to:
Jouni Kallio
Neuromuscular Research Center
Department of Biology of Physical Activity
University of Jyväskyla
P.O.Box 35, FIN-40351, Jyvaskyla
Finland
Tel. +358 (0) 40 805 3741
E-mail. jouni.kallio@jyu.fi

1

Motor unit firing behaviour of soleus muscle in isometric and dynamic contractions

Introduction: Understanding the detailed control of human locomotion and balance can be improved, when individual motor units can be isolated and their firing rates followed in natural movement of large, fuctionally important muscles. For this reason the present study investigated the motor unit discharge rate (MUDR) in isometric and dynamic contractions of the soleus muscle. Methods: Eleven males performed isometric (10-100% MVC) and dynamic (10-40% MVC) plantar flexions. Intramuscular EMG was measured from Soleus with bipolar wire-electrodes and decomposed with custom built “Daisy” software. Results:The Soleus MUDR was significantly higher in concentric compared to isometric or eccentric contractions at all submaximal force levels ($P < 0.05$). In isometric contractions MUDR increased up to 100% MVC. Conclusion: Motor unit discharge properties of a large plantarflexor can be measured in dynamic and maximal contractions. For a given torque output, MUDR is dependent upon contraction type, as set by the major mechanical differences between concentric and eccentric actions.

INTRODUCTION

It is well known that to produce a certain force requires less motor unit activation in eccentric as compared to concentric action [1]. This has been clearly demonstrated for both upper arm and lower leg muscles (e.g. [2-11]. Compared to isometric conditions, eccentric contractions are associated with lower reflex excitability [12,13]. Contraction type has also been shown to relate to different motor unit activation patterns, like synchronization [14], while changes in motor unit recruitment order seem to be small or non-existent (for review see: Chalmers 2008 [15]).

Due to the challenges of recording during dynamic contractions, most studies investigating single motor unit activation have concentrated on isometric contractions. The few earlier studies comparing muscle contraction types in dynamic movements have mostly been performed in upper extremity muscles and have shown the largest motor unit discharge rates (MUDR) in concentric compared to eccentric and isometric contractions. These studies examined the elbow flexors [7,9,10,16] and extensors [17], wrist flexors [18], the first dorsal interosseus [8,19], and these results were confirmed in the studies on the knee extensors [20] and Tibialis Anterior [6].

In order to better understand the mechanisms of human movement it is important to investigate motor unit activation also in the large plantar flexors. The soleus muscle plays an important role in locomotion and balance control [21,22] and seems to be modulated bilaterally, to some degree, by common drive in quiet standing [23]. As it has been shown that for example age-related differences in balance are more pronounced in dynamic conditions than in static stance [24], it is of importance to extend the research on the soleus muscle motor unit control to dynamic tasks. As soleus MUDR has only been studied in isometric contractions (e.g. [25-29], the main purpose of the present study was to identify possible differences in Soleus motor unit discharge rates between isometric, concentric and eccentric contractions. This is possible by utilizing highly selective wire-electrodes that enable the recording of single motor units from a large muscle with a high number of motor units and a large range of movement in dynamic contractions. In addition, the aim was to investigate the possibility of recording MUDR's up to maximal contractions with wire electrodes that enable performance of dynamic contractions.

MATERIALS AND METHODS

Subjects

11 physically active males (26.1±2.2yr. 1.81±0.05m 81.7±9.4kg) volunteered as subjects for the study. Subjects' physical activity and health were assessed with a questionnaire. Only subjects without any history of neuromuscular or vascular disease were approved for the study.

Full advice about possible risks and discomfort was given to the subjects and they all gave their written informed consent to the procedures prior to the commencement of the study. The investigation was conducted according to the declaration of Helsinki and approved by the ethics committee of the University of Jyväskylä.

Protocol

The subjects performed isometric and dynamic plantar flexions while seated in a custom made ankle dynamometer (University of Jyväskylä, Finland). Prior to data collection all subjects performed a warm-up consisting of 10 minutes light cycling on a bicycle ergometer, 5-10 submaximal and for practice 5 maximal isometric plantar flexions. All measurements were conducted in relation to the individual range of motion of the subject. In order to determine the individual zero angle (IZA), the voluntary range of motion was determined according to the protocol of [30]. Subjects were asked to perform slow maximal dorsi and plantar flexions in the ankle dynamometer without external/additional load. The range of motion was calculated as the difference between the two maximum angles. IZA was determined by subtracting 15 degrees from the maximal voluntary dorsiflexion angle. On average the IZA was 87.6±1.8 degrees. All isometric contractions were performed at each subject's IZA and with a fixed straight knee (180 deg).

The test battery was as follows:

I) Maximal voluntary contractions (MVC)

The subject was instructed to perform a maximal isometric plantar flexion for approximately 5 s. A minimum of two trials were performed and further trials were performed if the maximal force value between trials differed by more than 10%. The MVC measurement was repeated at the end of the protocol to examine the extent of possible fatigue caused by the session. A three minute rest was given between successive MVC trials.

II) Submaximal trials

a) Isometric contractions (ISO)

The force levels used in the contractions were 10, 20, 40, 60, 80 and 100% of the isometric MVC. The target force level was displayed on the monitor for the subjects to match. The subjects were instructed to increase the force to this level and hold it as steady as possible for several seconds, ranging from 10s at 10 & 20% MVC to a couple of seconds at 100% MVC. Analysis was done from a time-window when the force was stationary and on target. Each subject practiced the task several times before the insertion of wires.

b) Dynamic contractions

Dynamic contraction measurement tasks can be performed in different setups either by controlling the force variation as the limb is moved with a constant velocity by an isokinetic machine or controlling the movement velocity against a constant inertial load. The present study used the latter setup, as the subjects lifted (concentric: CON) or lowered (eccentric: ECC) a weight stack that was attached to the foot pedal via a cable pulley system at a voluntarily controlled velocity of 10 deg/s. The weight was adjusted so that the ankle torque during movement was identical to the corresponding ISO condition. The submaximal force levels in dynamic measurements corresponded to 10, 20 and 40% of the isometric MVC. In some subjects also higher dynamic contractions were measured, but due to the low number of successful trials with identifiable units in the highest levels, only the lowest three levels are reported here. The subjects controlled the movement velocity by guidance from visual feedback of the ankle angle on a monitor that had cursors set for both the correct start and end times and the start and end angles of the movement. The range of motion was set to enable a measurement window clearly separated from the acceleration or deceleration phase of the movement. Analysis was done from a 400 ms window around the ISO angle (Figure 1). Both angular velocity and torque were analyzed online and a trial was only included in the study if both of the values were within 2.5 percentage points of the target.

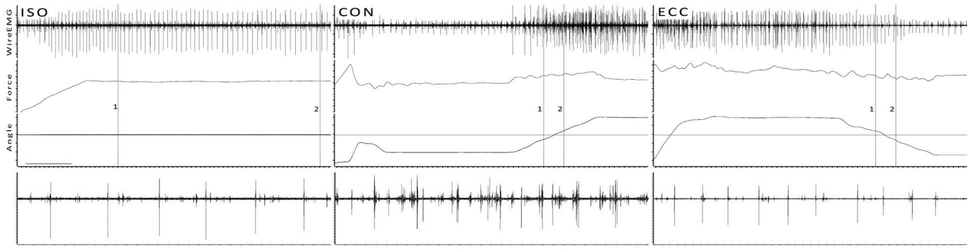


FIGURE 1 Examples of ISO, CON and ECC trials from one subject at 10% MVC. TOP: Cursors 1 and 2 mark the beginning and end of analysis, respectively. In dynamic contractions Cursor 1 is 200ms prior and Cursor 2 200ms after the angle crosses the angle of the ISO trial, as indicated by the horizontal cursor. BOTTOM: 400 ms close-ups of the wire EMG signal of each trial.

Force and surface EMG

Trials were performed while subjects sat with the foot attached to an ankle dynamometer pedal mounted with torque (HPM, Germany) and angle (Vishay 357-0-0-103, USA) sensors. To maximize stability during testing, subjects were strapped to the chair, the chest with a 4-point seatbelt and the thigh and ankle with straps. The foot pedal was attached to a weight stack via a pulley. The measured torque signal was sent to a computer via an A-D board CED 1401+ (Cambridge Electronic Design) and sampled at a frequency of 50 kHz. Signals were collected and analyzed using Signal software (Cambridge Electronic Design, UK). Before analysis, force and angle signals were downsampled to 2 kHz, and then filtered with a low-pass filter (cutoff frequencies 75 and 150 Hz, respectively).

Global EMG activity of the soleus (SOL) and gastrocnemius medialis (GM) muscles was recorded using bipolar silver chloride miniature surface electrodes

(Beckman 650437, USA) with 20 mm inter-electrode distance. Electrodes were prepared and placed in accordance with the recommendations by SENIAM [31]. The surface EMG (sEMG) signals were amplified and sampled at a frequency of 50 kHz. The signals were subsequently downsampled to 2 kHz, band-pass filtered at 20 to 1 kHz and analyzed using Signal software (Cambridge Electronic Design, UK). Surface EMG activities of SOL and GM were calculated as root mean square (RMS) of the signal. For the maximal voluntary contractions (MVC), both sEMG and torque data were analyzed ±100 ms around the peak torque.

Intramuscular EMG

For the intramuscular EMG recordings, four separate bipolar fine-wire electrodes were inserted into the soleus muscle. This provided four potential signals that could be used for the decomposition. Electrodes were inserted to soleus from the lateral side of the sEMG electrode at different depths (approximately 10-25 mm), and a ground electrode was placed on the medial malleolus. The wire-electrodes consisted of two 50 µm polyurethane insulated wires that were glued together. Insulation was mechanically removed from the outer sides of the wires over 0.5 mm distance from the tip, resulting in an inter-electrode distance of approximately 100µm. A hook was formed at the end of the wires to ensure fixation during the measurements. Electrodes were autoclaved before the experiment in order to minimize the risk of infection. The wire electrode pairs were inserted into the muscle with a 22 gauge needle that was subsequently withdrawn. Wire-electrodes were inserted after the MVC trials to avoid possible electrode migration and/or loss of signal due to MVC force levels. Further, all measurements were started with the lowest force-levels to secure data from submaximal levels before risking the possible breaking at the largest force levels. In some cases a wire stopped recording during the measurements for an unknown reason. To optimize signal quality, the signal was pre-amplified with a non-commercial amplifier (amp 200, bandwidth 8-4500 Hz) attached to the calf of the subject. The amplified signal was sent to a CED 1902 programmable signal conditioner (Cambridge Electronics Design Ltd, Cambridge, UK) and digitized with CED 1401+ (Cambridge Electronic Design) with a sampling frequency of 20 kHz. Since the decomposition software allows visualization of three channels, the wire channel was duplicated twice and the two new channels were filtered with different fourth order butterworth band-pass filters (300-8000 Hz and 400-10000 Hz). Observing three differently filtered channels gave the analyzer more information for decomposition.

Signal decomposition, motor unit identification and data analysis were performed by utilizing the three channel decomposition technique computer algorithm, "Daisy", described in more detail in [9,32,33]. Motor unit action potential (MUAP) recognition is based on template matching of the waveform of the three channels (unfiltered and two band-pass filtered), created from the same intramuscular EMG recording. The MU classification was performed semi-automatically with a high degree of operator interaction. The identification is based on the definition of templates from the three channels. The templates were updated as a running average of the last 10 detected MUAPs, allowing for the unavoidable gradual amplitude and shape changes of the MUAPs due to slight changes in the position of the wire relative to the detected unit. All firings were checked or sorted manually via visual inspection, and only the units with either a stable or a gradual and systematical change in shape and amplitude were included for further analysis. For each train of MU discharges the inter-spike intervals were registered and instantaneous motor unit discharge rate (MUDR) for each MU was calculated as the mean of the inverse of each inter-spike interval. The decomposed units from all subjects were pooled for calculating the differences between contraction types and levels. In addition, units that could clearly be recognized in at least two of the three contraction types were analyzed in order to investigate the firing rate behavior in more detail. Analyses were checked by comparing MUDRs to eliminate the inclusion of the same motor unit by more than one bipolar wire set.

Statistical analysis

The normality of distribution was tested with the Shapiro-Wilk test. In the pooled units an analysis of variance (ANOVA) was used to compare the effects of contraction types and intensities, while repeated measures ANOVA was used for the units recognized in more than one condition. When a significant difference was detected, Bonferroni's method was used to locate the difference. The critical level of significance was $P < 0.05$. Descriptive statistics include mean and standard deviation.

RESULTS

Submaximal force levels

Submaximal force levels were compared between contraction types and no consistent or statistically significant differences were found at any of the force levels (Table 1). Furthermore, MVC before and after was not significantly different indicating that fatigue did not develop during the experiment.

Motor unit discharge rate

The analysis was based on 5773 motor unit firings from 187 different motor units. The number of subjects with decomposable units in each condition ranged between 3 and 10, and the number of units between 5 and 45 (Table 1).

In ISO contractions, where force levels were explored up to 100% MVC, the MUDR increased significantly with force. The increase followed the relative force levels (Figure 2, Table 1). The MUDR increased significantly from 10 to 20, 40 to 60, 60 to 80 and 80 to 100% MVC. The increase followed a second order polynomial equation $y = 0.64x^2 - 2.23x + 9.43$ ($R^2 = 0.9766$).

Type	Level	N(subj)	N(MU)	MUDR (1/s)	Force%	SOL%	GM%
ISO	**10**	10	43	7.3 ± 2.2†	8.8 ± 0.9	10.5 ± 3.9†‡	6.2 ± 4.2†‡
	20	10	44	8.3 ± 2.2*†	17.7 ± 0.7	16.5 ± 4.8*†‡	7.4 ± 2.6†‡
	40	8	45	9.1 ± 2.2†	34.9 ± 1.5	31.3 ± 10.3*	17.3 ± 7.6*†‡
	60	8	30	10.4 ± 2.2*	55.7 ± 6.5	46.3 ± 14.0*	27.3 ± 13.2*
	80	5	12	13.5 ± 2.6*	74.7 ± 7.0	63.7 ± 17.4*	60.0 ± 33.4*
	100	5	8	19.9 ± 7.3*	99.4 ± 6.1	91.6 ± 20.2*	102.4 ± 32.2*
CON	**10**	9	38	10.1 ± 3.0	9.4 ± 1.3	22.2 ± 8.0	9.6 ± 3.9
	20	8	16	11.6 ± 2.3	19.2 ± 0.7	34.0 ± 7.6*	14.4 ± 4.6*
	40	3	5	12.2 ± 2.2	37.7 ± 0.5	51.4 ± 2.9*	41.6 ± 15.4*
ECC	**10**	7	18	6.9 ± 2.4†	9.6 ± 1.3	13.9 ± 3.9†	11.7 ± 3.8
	20	9	20	8.2 ± 2.3†	20.8 ± 1.9	21.7 ± 7.8*†	22.8 ± 10.2*
	40	4	6	7.6 ± 0.7†	38.5 ± 1.8	32.7 ± 10.0*†	37.1 ± 13.8

TABLE 1 Number of subjects and motor units (MU), motor unit discharge rate (MUDR) and relative force and sEMG levels of soleus (SOL) and gastrocnemius medialis (GM) in different conditions. Force and sEMG levels are calculated relative to isometric MVC. *: significantly larger than in lower force level. † : significantly smaller than in CON. ‡ : significantly smaller than in ECC (P<0.05).

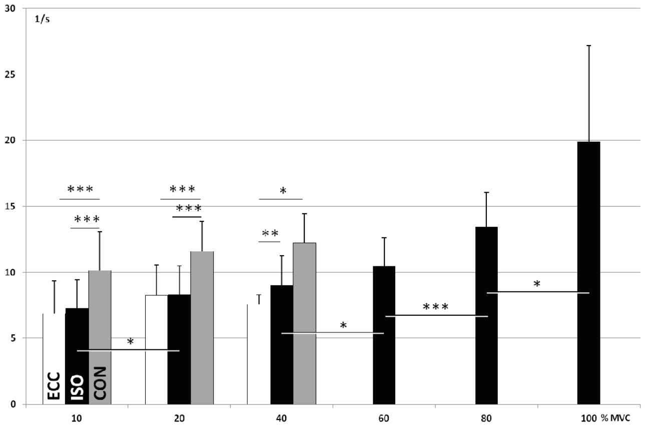


FIGURE 2 Motor unit discharge rate (1/s; X±SD) at different relative force levels (%MVC) in eccentric (ECC), isometric (ISO) and concentric (CON) contractions. Significant differences between conditions and force-levels: * = p<.05, ** = p<.01, *** = p<.001.

With increasing force new units were recruited, with generally lower initial firing rates compared to units that were already active at lower force levels. Therefore the average difference in MUDR between force-levels was always larger within MU pairs when compared to the average of all analyzed units (table 2).

Level1	Level2	N	MUDR 1	MUDR 2	MUDR diff%
		Pairs			
10	20	21	7.6 ± 2.1	9.3 ± 1.9	23.2*
20	40	15	7.9 ± 1.9	10.0 ± 2.4	25.5*
40	60	9	8.9 ± 2.1	11.4 ± 1.9	28.5*
60	80	5	8.7 ± 0.5	11.8 ± 0.7	34.3*
		Pooled			
10	20	43/44	7.3 ± 2.2	8.3 ± 2.2	13.7*
20	40	44/45	8.3 ± 2.2	9.1 ± 2.2	9.6*
40	60	45/30	9.1 ± 2.2	10.4 ± 2.2	14.3*
60	80	30/12	10.4 ± 2.2	13.5 ± 2.6	29.8*

TABLE 2 The difference in motor unit discharge rate (MUDR) between force levels in motor unit pairs (top) and all pooled units (bottom) in isometric contractions. *: significant difference ($P<0.05$).

At the three lowest force levels all three contraction types were measured and showed that the MUDR was significantly effected by contraction type. MUDR was significantly higher ($p<.05$) in CON compared to ISO or ECC, regardless of whether the comparison was based on all analyzed units (Figure 2, Table 1) or only on units that could clearly be recognized in at least two of the three contraction types (Figure 3). No significant differences in MUDR were found between ISO and ECC in either of the comparison methods. Of the 14 MUs recognized in both ISO and ECC, 9 fired with a higher discharge rate in ISO and 5 in ECC.

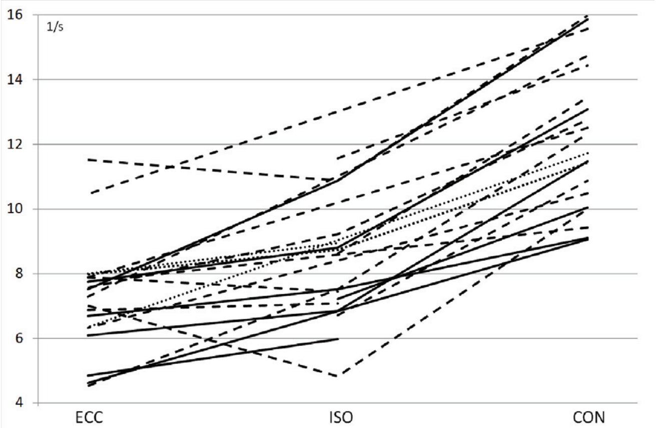


FIGURE 3 Discharge rates of motor units that could clearly be recognized in at least two of the three contraction types at 10 (solid line), 20 (dashed line) and 40% of MVC (dotted line).

The global sEMG activity of Soleus, as measured by the surface electrode, increased with increasing relative force in all contraction types (Figure 4A). In general, concentric contractions required more activation than ISO or ECC and at the two lowest force levels sEMG was significantly higher in ECC than in ISO. In gastrocnemius the relative sEMG also increased with increasing force (significant in all conditions except ISO 10-20% and ECC 20-40%). However, in this muscle the smallest sEMG was measured in ISO (Figure 4B).

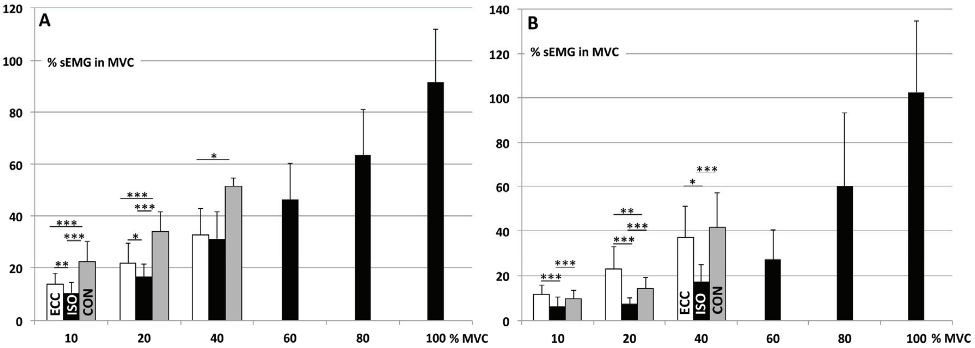


FIGURE 4 Relative muscle activity in different trials. sEMG levels of soleus (A) and gastrocnemius medialis (B) at different force levels in eccentric (ECC), isometric (ISO) and concentric (CON) contractions. sEMG is expressed relative to maximal isometric contraction (MVC). Significant differences between conditions and force-levels: * = p<.05, ** = p<.01, *** = p<.001.

DISCUSSION

The main result from the present study is that the soleus motor unit discharge rate was found to 1) increase with activation level up to 100% MVC and 2) be highest in concentric contractions in all measured submaximal trials when absolute torque levels were matched between contraction types.

Isometric contractions

We found the average soleus MUDR to increase with increasing torque level from 7.3±2.2/s at 10% MVC to 19.9±7.3/s at MVC. The average maximal MUDRs were slightly higher than those reported in soleus by Dalton et al. 2010[25] (16.4±5.3/s) and almost twice the values of Bellemare et al. 1983[27] (10.7±2.9/s). The differences between our results and those of Bellemare's study could be partly due to a higher average subject age (30-55 vs. 26±2 yr), as MUDR decreases with aging in Soleus [34] and this age-related difference is largest for maximal contractions (for review see Roos et al. 1997[35]). The current data adds support to the suggestion that soleus force control works with a much more limited range of motor unit discharge rates compared to most other studied muscles, like adductor pollicis (30/s), biceps brachii (31/s), tibialis anterior (33/s) and abductor digiti minimi (52/s) [27,36,37].

The torque-related increase in MUDR followed a second order polynomial equation with a steeper MUDR increase at higher torque levels. In our earlier intramuscular EMG study [4] the increase in MU activity at moderate activation levels from 20 to 40% EMG at MVC was more pronounced in intramuscular mean spike amplitude than in mean spike discharge frequency, indicating a stronger contribution from motor unit recruitment compared to rate coding. However, at high force levels it is likely that increases in Soleus force rely more on an increase in MUDR than MU recruitment, even though some units have been observed to have thresholds above 90% MVC [26].

Dynamic contractions

On average motor units were recorded at the same ankle angle in all conditions, although the start and end angles in dynamic conditions differed from ISO. Thus, in ECC for example, the possible effect of shorter fascicle length in MUDR at the start of recording would be cancelled by the adversary effect of a longer fascicle in the end position. Several studies have shown fascicle length to be quite independent of total muscle-tendon length in dynamic movements, like jumping [38], walking, running [39] and isokinetic maximal contractions[40]. We did not measure fascicle length directly, but as average joint angle and force were equal in ISO, CON and ECC, average fascicle length can also be assumed to be the same. This suggestion is supported by the study by Pasquet and colleagues [6], showing that the fascicle length change in tibialis anterior varied linearly with ankle joint angle.

A quite modest joint rotation velocity (10 deg/s) was chosen in order to have a sufficiently wide time window for the analysis. Even at this slow pace, the analysis was on average based on only 6.6 firings per trial. However, in order to reduce the error, the MUDR calculation included only the inter-firing-intervals of the firings that were detected within the measurement window (not the time before the first and after the last firing). The rotation velocity was quite slow compared to most natural movements. For example, in walking, the angular velocity in dorsiflexion is ~30 deg/s and in plantarflexion ~220 deg/s (estimated from Lichtwark 2007[41]). Therefore this study is a step in the direction of investigating how the motor unit activation functions in normal locomotion.

The present study showed a larger MUDR in CON compared to ISO or ECC at low submaximal force levels. Our previous experiments [4] also showed the intramuscular mean spike frequency (an indirect measure of MUDR) to be slightly higher (n.s.) in CON than in ISO or ECC when different contraction modes were matched by surface EMG. In the current measurements the difference in the required neural input to produce equal torque may have amplified the difference in MUDR.

Two possible reasons for the lower MUDR in ECC have been suggested previously [42]. Firstly, mechanically, less torque is required to lower (ECC) the same absolute load compared to lifting it (CON). However, in the present study we controlled the weights so that the weight in CON was lower than in ECC, making the absolute plantarflexion torque in all contraction types the same.

Secondly, the relative load in ECC contraction may be lower due to the higher maximal torque capacity in ECC. It is likely that the cross-bridges generate more torque when resisting pull (ECC) than they do when producing movement (CON) [43]. We measured MVC only in ISO, so the dynamic torques relative to their corresponding maxima are unknown. In an earlier study on plantarflexors the difference between MVCs in CON and ECC was 17% [44]. In the present study the difference in MUDR between CON and ECC at the three force levels was between 29-38%. It thus seems that the difference between contraction types is larger in MUDR than absolute torque production. This is in accordance with the study of [20] which showed a higher MUDR in CON even when force-velocity-related differences in force capacity were taken into account.

Surface muscle activity of soleus ($sEMG_{SOL}$) behaved similarly to MUDR in different measurement trials. Both MUDR and $sEMG_{SOL}$ were largest in CON, however only $sEMG_{SOL}$ increased with torque levels in dynamic contractions. The torque related increase in neural drive to the muscle is probably more visible in $sEMG_{SOL}$ due to motor unit recruitment. At the lowest activation levels, new, slowly firing units were recruited as force level was increased. This would have a diminishing effect on the average MUDR, while the recruited units would still increase the amplitude in $sEMG_{SOL}$ [45].

The behaviour of gastrocnemius activity differed from soleus in the dynamic contractions, as the gastrocnemius activity in ECC was as at the same level as in CON. This phenomenon has previously been reported by Nardone and colleagues [46] and could reflect the more dominant role of gastrocnemius in normal dynamic contractions. In two classic papers, Nardone et al. reported the shift to a faster muscle (gastrocnemius) and the EMG findings as evidence for reversal of the recruitment order of motor units [46,47]. With equal torques, an increase in gastrocnemius activity could remove some of the load from soleus. In the present study 9 of the 14 units that could be followed in both ECC and ISO showed a lower DR in ECC while in 5 units the DR was unaltered or higher in ECC. It therefore seems that the motor unit rate coding in ECC varies more than in CON, where the MUDR was always higher than in the other recognised conditions. It is possible that the increased activation of gastrocnemius affected the soleus activation more in recruitment than in rate coding. Recruitment threshold has also been observed to be lower in nonisometric contractions [7,48]. In the present study the focus was on the firing rate of a random sample of motor units and thus we did not analyze recruitment order systematically. However, in ECC trials we observed several clear cases of derecruitment of units that had been active in ISO and/or CON, but none vice versa. This is in line with a number of other studies on dynamic contractions (for review see Chalmers [15]).

Conclusion

Soleus motor unit discharge rate was found to be highest in concentric contractions in all measured submaximal trials when absolute torque levels were matched between contraction types. Being able to measure functionally important extensor muscles at maximal levels and in dynamic contractions offers possibilities for future research in various contexts, such as natural locomotion, dynamic balance control and ageing.

Acknowledgements:
Henrik Baare Olsen, Sirpa Roivas, Markku Ruuskanen and Seppo Seppänen for technical assistance, Dr. Neil Cronin for proofreading.

References

1. Bigland B, Lippold OC. (1954) Motor unit activity in the voluntary contraction of human muscle. Journal of Physiology 125: 322-335.
2. Komi PV, Buskirk ER. (1972) Effect of eccentric and concentric muscle conditioning on tension and electrical activity of human muscle. Ergonomics 15: 417-434.
3. Eloranta V, Komi PV. (1980) Function of the quadriceps femoris muscle under maximal concentric and eccentric contractions. Electromyography and Clinical Neurophysiology 20: 159-154.
4. Kallio J, Avela J, Moritani T, Kanervo M, Selänne H (2010) Effects of ageing on motor unit activation patterns and reflex sensitivity in dynamic movements. Journal of Electromyography & Kinesiology 20: 590-598.
5. Katz B. (1939) The relation between force and speed in muscular contraction. The Journal of Physiology 96: 45-64.
6. Pasquet B, Carpentier A, Duchateau J. (2006) Specific modulation of motor unit discharge for a similar change in fascicle length during shortening and lengthening contractions in humans. Journal of Physiology 577: 753-765.
7. Tax AA, Denier van der Gon JJ, Gielen CC, van den Tempel CM. (1989) Differences in the activation of m. biceps brachii in the control of slow isotonic movements and isometric contractions. Experimental Brain Research 76: 55-63.
8. Howell JN, Fuglevand AJ, Walsh ML, Bigland-Ritchie B. (1995) Motor unit activity during isometric and concentric-eccentric contractions of the human first dorsal interosseus muscle. Journal of Neurophysiology 74: 901-904.
9. Sogaard K, Christensen H, Jensen BR, Finsen L, Sjogaard G. (1996) Motor control and kinetics during low level concentric and eccentric contractions in man. Electroencephalography & Clinical Neurophysiology 101: 453-460.
10. Kossev A, Christova P. (1998) Discharge pattern of human motor units during dynamic concentric and eccentric contractions. Electroencephalography and Clinical Neurophysiology 109: 245-255.
11. Bawa P, Jones KE. (1999) Do lengthening contractions represent a case of reversal in recruitment order? Progress in Brain Research 123: 215-220.
12. Romano C, Schieppati M. (1987) Reflex excitability of human soleus motoneurones during voluntary shortening or lengthening contractions. The Journal of Physiology 390: 271-284.
13. Nakazawa K, Yamamoto SI, Yano H. (1997) Short- and long-latency reflex responses during different motor tasks in elbow flexor muscles. Experimental Brain Research. Experimentelle Hirnforschung. Experimentation Cerebrale 116: 20-28.
14. Semmler JG, Kornatz KW, Dinenno DV, Zhou S, Enoka RM. (2002) Motor unit synchronisation is enhanced during slow lengthening contractions of a hand muscle. Journal of Physiology 545: 681-695.

15. Chalmers GR. (2008) Can fast-twitch muscle fibres be selectively recruited during lengthening contractions? review and applications to sport movements. Sports Biomechanics 7: 137-157.
16. Moritani T, Muramatsu S, Muro M. (1987) Activity of motor units during concentric and eccentric contractions. American Journal of Physical Medicine 66: 338-350.
17. Del Valle A, Thomas CK. (2005) Firing rates of motor units during strong dynamic contractions. Muscle & Nerve 32: 316-325.
18. Sogaard K, Christensen H, Fallentin N, Mizuno M, Quistorff B, et al. (1998) Motor unit activation patterns during concentric wrist flexion in humans with different muscle fibre composition. European Journal of Applied Physiology & Occupational Physiology 78: 411-416.
19. Laidlaw DH, Bilodeau M, Enoka RM. (2000) Steadiness is reduced and motor unit discharge is more variable in old adults. Muscle & Nerve 23: 600-612.
20. Altenburg TM, de Ruiter CJ, Verdijk PW, van Mechelen W, de Haan A. (2009) Vastus lateralis surface and single motor unit electromyography during shortening, lengthening and isometric contractions corrected for mode-dependent differences in force-generating capacity. Acta Physiologica 196: 315-328.
21. Basmajian JV, De Luca CJ. (1964) Muscles alive. their functions revealed by electromyography. Baltimore: Williams & Wilkins.
22. Di Giulio I, Maganaris CN, Baltzopoulos V, Loram ID. (2009) The proprioceptive and agonist roles of gastrocnemius, soleus and tibialis anterior muscles in maintaining human upright posture. The Journal of Physiology 587: 2399-2416.
23. Mochizuki G, Semmler JG, Ivanova TD, Garland SJ. (2006) Low-frequency common modulation of soleus motor unit discharge is enhanced during postural control in humans. Experimental Brain Research.Experimentelle Hirnforschung.Experimentation Cerebrale 175: 584-595.
24. Piirainen JM, Avela J, Sippola N, Linnamo V. (2010) Age dependency of neuromuscular function and dynamic balance control. European Journal of Sport Science 10: 69.
25. Dalton BH, Harwood B, Davidson AW, Rice CL. (2010) Recovery of motoneuron output is delayed in old men following high-intensity fatigue. Journal of Neurophysiology 103: 977-985.
26. Oya T, Riek S, Cresswell AG. (2009) Recruitment and rate coding organisation for soleus motor units across entire range of voluntary isometric plantar flexions. Journal of Physiology 587: 4737-4748.
27. Bellemare F, Woods JJ, Johansson R, Bigland-Ritchie B. (1983) Motor-unit discharge rates in maximal voluntary contractions of three human muscles. Journal of Neurophysiology 50: 1380-1392.
28. Mochizuki G, Ivanova TD, Garland SJ. (2005) Synchronization of motor units in human soleus muscle during standing postural tasks. Journal of Neurophysiology 94: 62-69.

29. Monsour M, Ivanova TD, Wilson TD, Garland SJ. (2012) Influence of vestibular afferent input on common modulation of human soleus motor units during standing. Motor Control .
30. Allinger TL, Engsberg JR. (1993) A method to determine the range of motion of the ankle joint complex, in vivo. Journal of Biomechanics 26: 69-76.
31. Hermens HJ, Freriks B, Merletti R, Stegeman D, Blok J, et al. (1999) SENIAM 8: European recommendations for surface electromyography. .
32. Olsen HB, Christensen H, Sogaard K. (2001) An analysis of motor unit firing pattern during sustained low force contraction in fatigued muscle. Acta Physiologica Et Pharmacologica Bulgarica 26: 73-78.
33. Farina D, Colombo R, Merletti R, Olsen HB. (2001) Evaluation of intra-muscular EMG signal decomposition algorithms. Journal of Electromyography and Kinesiology 11: 175-187.
34. Kallio J, Søgaard K, Avela J, Komi P, Selänne H, et al. (2012) Age-related decreases in motor unit discharge rate and force control during isometric plantar flexion. Journal of Electromyography and Kinesiology .
35. Roos MR, Rice CL, Vandervoort AA. (1997) Age-related changes in motor unit function. Muscle & Nerve 20: 679-690.
36. Van Cutsem M, Feiereisen P, Duchateau J, Hainaut K. (1997) Mechanical properties and behaviour of motor units in the tibialis anterior during voluntary contractions. Canadian Journal of Applied Physiology 22: 585-597.
37. Patten C, Kamen G, Rowland DM. (2001) Adaptations in maximal motor unit discharge rate to strength training in young and older adults. Muscle & Nerve 24: 542-550.
38. Finni T, Ikegawa S, Lepola V, Komi PV. (2003) Comparison of force-velocity relationships of vastus lateralis muscle in isokinetic and in stretch-shortening cycle exercises. Acta Physiologica Scandinavica 177: 483-491.
39. Lichtwark GA, Wilson AM. (2006) Interactions between the human gastrocnemius muscle and the achilles tendon during incline, level and decline locomotion. The Journal of Experimental Biology 209: 4379-4388.
40. Reeves ND, Narici MV. (2003) Behavior of human muscle fascicles during shortening and lengthening contractions in vivo. Journal of Applied Physiology (Bethesda, Md.: 1985) 95: 1090-1096.
41. Lichtwark GA, Bougoulias K, Wilson AM. (2007) Muscle fascicle and series elastic element length changes along the length of the human gastrocnemius during walking and running. Journal of Biomechanics 40: 157-164.
42. Duchateau J, Enoka RM. (2008) Neural control of shortening and lengthening contractions: Influence of task constraints. Journal of Physiology 586: 5853-5864.
43. Joyce GC, Rack PM, Westbury DR. (1969) The mechanical properties of cat soleus muscle during controlled lengthening and shortening movements. Journal of Physiology 204: 461-474.

44. Linnamo V, Avela J, Kallio J, Dousset E, Kuitunen S, et al. (2003) Maximal torque and activation level during eccentric, concentric and isometric actions before and after damaging exercise. In: Gantchev N, editor. From motor Control to Functional Recovery III. Varna, Bulgaria. pp. 361-368.
45. Farina D, Merletti R, Enoka RM. (2004) The extraction of neural strategies from the surface EMG. Journal of Applied Physiology 96: 1486-1495.
46. Nardone A, Schieppati M. (1988) Shift of activity from slow to fast muscle during voluntary lengthening contractions of the triceps surae muscles in humans. The Journal of Physiology 395: 363-381.
47. Nardone A, Romano C, Schieppati M. (1989) Selective recruitment of high-threshold human motor units during voluntary isotonic lengthening of active muscles. The Journal of Physiology 409: 451-471.
48. Theeuwen M, Gielen CC, Miller LE. (1994) The relative activation of muscles during isometric contractions and low-velocity movements against a load. Experimental Brain Research 101: 493-505.

IV

Effects of ageing on Soleus motor unit discharge rate in dynamic movements

by

Jouni Kallio, Karen Søgaard, Janne Avela, Paavo V. Komi, Harri Selänne, Vesa Linnamo, 2012

Journal of Applied Physiology, submitted for publication

Differences between young and elderly in soleus motor unit discharge rate in dynamic movements.

J. Kallio [a], K. Søgaard [c], J. Avela [a], P. Komi [d], H. Selänne [e], V. Linnamo [f]

[a] Neuromuscular Research Center, Department of Biology of Physical Activity, University of Jyväskylä, Finland
[b] Professor, Neuromuscular Research Center, Department of Biology of Physical Activity, University of Jyväskylä, Finland
[c] Professor at the Institute of Sports Science and Clinical Biomechanics, University of Southern Denmark
[d] Professor Emeritus, Neuromuscular Research Center, Department of Biology of Physical Activity, University of Jyväskylä, Finland
[e] Chief Physician, LIKES, Foundation for Sport and Health Sciences, Jyväskylä, Finland
[f] Professor of Sports Technology, Vuokatti Snowpolis, Neuromuscular Research Center, Department of Biology of Physical Activity, University of Jyväskylä, Finland

keywords:
Aging, Electromyography, Isometric, Dynamic, Intramuscular, Soleus, Motor unit

Correspondence to:
Jouni Kallio
Neuromuscular Research Center
Department of Biology of Physical Activity
University of Jyväskylä
P.O.Box 35, FIN-40351, Jyvaskyla
Finland
Tel. +358 (0) 50 324 5993
E-mail. jouni.kallio@likes.fi

ABSTRACT

Aging is related to changes at the muscular level, leading to a decline in motor performance increasing the risk of falling and injury. It seems that the age-related changes in motor unit activation are muscle- and intensity dependent. The purpose of this study was to examine possible differences in soleus MUDR between young and elderly adults. 11 young (YOUNG) and 8 elderly (OLD) males participated in the study. The subjects performed isometric and dynamic plantar flexions while seated in an ankle dynamometer. The force levels studied were 10, 20, 40, 60, 80 and 100% of the isometric (ISO) MVC in ISO and 10, 20 and 40% in concentric (CON) and eccentric (ECC) contractions. Soleus intramuscular EMG was recorded with bipolar fine-wire electrodes and decomposed to individual trains of motor unit discharges. In ISO motor unit discharge rate (MUDR) was higher in YOUNG in all measured force levels, while no age-difference was seen in the dynamic contractions. MUDR was highest in CON compared to ISO or ECC in both age-groups. The relative sEMG activities of SOL and GM were higher in OLD compared to YOUNG in all conditions. The decreased MUDR in OLD may be an adaptation to the increased twitch duration to optimize force generation. The lack of an age-difference in dynamic contractions could be due to differences in recruitment-strategies, coactivation or a lack of recording from high force levels.

INTRODUCTION

It is well known that ageing is related to changes at the muscular level, leading to a decline in motor performance. This loss in function increases the risk of falling and injury, with serious economical and personal consequences (Hamacher et al. 2011, Heinrich et al. 2010). Studies have shown the risk of falling to be associated with lower extremity strength, especially at the ankle joint level (Wolfson et al. 1995).

The age-related functional and physiological changes in the muscle are associated with modifications in muscle activation, such as a possible decrease in voluntary activation (Klass, Baudry & Duchateau 2007) and changes in the degree of agonist/antagonist coactivation (Häkkinen K. et al. 1998). However, it seems that changes in motor unit activation are muscle- and intensity dependent. Several studies have found the elderly to have a lower discharge rate during high intensity contractions in muscles, like the first dorsal interosseous, tibialis anterior (Kamen et al. 1995, Klass, Baudry & Duchateau 2008) and vastus lateralis (Kamen & Knight 2004). In contrast, soleus motor unit discharge rate (MUDR) was found to be lower in the elderly only at low force-levels, and no age related differences were found in MUDR at recruitment threshold in biceps brachii, triceps brachii, TA or FDI in the studies by Howard et al. (Howard, McGill & Dorfman 1988) and Galganski et al. (Galganski, Fuglevand & Enoka 1993).

So far the age-related differences in motor unit discharge behavior have almost entirely been investigated in isometric contractions, even though a large number of studies have shown the neuromuscular system of younger individuals to function quite differently in the kind of dynamic contractions that most daily activities involve. These studies have found MUDR to be higher in concentric compared to eccentric and isometric contractions in both upper (Sogaard et al. 1996, Kossev & Christova 1998, Tax et al. 1989, Del Valle & Thomas 2005, Sogaard et al. 1998, Howell et al. 1995) and lower (Pasquet, Carpentier & Duchateau 2006, Altenburg et al. 2009, Kallio et al. 2013) limb muscles. Further, some studies have found the eccentric contractions to be associated with a lower MUDR at a given force level (Tax et al. 1989), a larger degree of motor unit synchronization (Semmler 2002), changed MU activation patterns (Linnamo 2003, Linnamo et al. 2003a, Linnamo et al. 2003b) and possibly even changes in recruitment order (Howell et al. 1995, Nardone, Romano & Schieppati 1989). For dynamic compared to static contractions more irregular discharge patterns involving intermittent activity (Sogaard et al. 1998, Sogaard 1995) and double discharges (Sogaard et al. 2001) have also been reported.

Considering how central dynamic contractions are in human locomotion, it is interesting to see whether the observed age-related changes in isometric motor unit activation also apply to dynamic contractions. This paper extends the scope of our previous study from the effects of contraction type on MUDR in young males (Kallio et al. 2013) to the effects of aging. Thus, the purpose of this study was to examine possible differences in soleus motor unit discharge rate in static and dynamic contractions between young and elderly adults. Our hypothesis was that the elderly will show a decreased firing rate also in dynamic contractions, especially at higher force levels.

METHODS

Subjects

Nineteen physically active males of the ages 20-30 and 65-80 years volunteered as subjects for the study. There were 11 males in the younger (YOUNG) group (age: 26.1±2.2yr. height:1.81±0.05m body mass: 81.7±9.4kg) and 8 in the older (OLD) group (age: 69.1±5.1yr. height:1.69±0.04m body mass: 75.3±6.2kg). Physical activity of the subjects was determined with a questionnaire, where the subjects wrote the type and frequency of their regular exercise routine. All men in both groups performed moderate to strenuous physical exercise three times a week or more. Before the measurements all of the subjects in the OLD group underwent a medical examination. Only subjects without any history of neuromuscular or vascular disease were approved for the study. Height, weight and ankle joint range of motion (ROM) were measured from all the subjects.

Full advice about possible risks and discomfort was given to the subjects and all gave their written informed consent to the procedures prior to the commencement of the study. The investigation was conducted according to the declaration of Helsinki and approved by the ethics committee of the University of Jyväskylä. Most of the results from the YOUNG have been described in Kallio et al. (2013, accepted for publication) and serves mainly as a reference in the present study focused on motor unit pattern in dynamic contractions among an elderly group of subjects.

Protocol

The subjects performed isometric and dynamic plantar flexions while seated in a custom made ankle dynamometer (University of Jyväskylä, Finland). Prior to data collection all subjects performed a warm-up consisting of 10 minutes light cycling on a bicycle ergometer and for familiarization to the test procedure also 5-10 submaximal and 5-10 maximal isometric plantar flexions. In order to determine the individual zero angle (IZA), the voluntary range of motion was determined according to the protocol of (Allinger & Engsberg 1993). Subjects were asked to perform slow maximal dorsi and plantar flexions in the ankle dynamometer without external/additional load. The range of motion was calculated as the difference between the two extreme angles. IZA was determined by subtracting 15 degrees from the maximal voluntary dorsiflexion angle. On average the IZA was 87.6±1.8 degrees. All isometric contractions were performed at each subject's IZA and with a fixed straight knee (180 deg).

The test battery was as follows:

I) Maximal voluntary contractions (MVC)

The subject was instructed to perform a maximal isometric plantar flexion for approximately 5 s. A minimum of two trials were performed and further trials were performed if the maximal force value between trials differed by more than 10%. The MVC measurement was repeated at the end of the protocol to examine the extent of possible fatigue caused by the session. A three minute rest was given between successive MVC trials.

II) Submaximal trials

a) Isometric contractions of the plantar flexor muscles.

The force levels used in the contractions were 10, 20, 40, 60, 80 and 100% of the isometric MVC in plantar flexion. The target force level was displayed on the monitor for the subjects to match. The subjects were instructed to increase the force to this level and hold it as steady as possible for several seconds, ranging from 10s at 10 & 20% MVC to a couple of seconds at 100% MVC. For each contraction data was analyzed off-line for a time-window with stationary target force defined by visual inspection. Each subject practiced the task several times before the insertion of wires.

b) Dynamic contractions

There are two common setups for measuring isokinetic dynamic contractions. In a previous experiment we have used an isokinetic machine and given the subjects a pre-set force trace to follow on a monitor (Kallio et al. 2012). In the present study the task was performed as a free movement against a constant inertial load, and the subjects tried to follow a pre-set velocity trace. The subjects lifted (CON) or lowered (ECC) a weight stack that was attached to the foot pedal via a cable pulley system at a voluntarily controlled velocity of 10 deg/s. The weight was adjusted so that the ankle torque during movement was identical to the corresponding ISO condition. The submaximal force levels in dynamic measurements corresponded to 10, 20 and 40% of the isometric MVC. The subjects controlled the movement velocity by guidance from visual feedback of the ankle angle on a monitor that had cursors set for both the correct start and end times and the start and end angles of the movement. The range of motion was set to enable a measurement window clearly separated from the acceleration or deceleration phase of the movement. Analysis was done from a 400 ms window around the ISO angle (Figure 1). Both angular velocity and torque were analyzed online and a trial was only included in the study if both values were within 2.5 percentage points of the target.

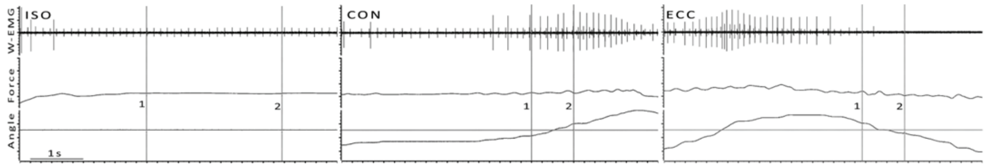


FIGURE 1 Examples of ISO, CON and ECC trials from one OLD subject at 10% MVC. TOP: Cursors 1 and 2 mark the beginning and end of analysis, respectively. In dynamic contractions Cursor 1 is 200ms prior and Cursor 2 200ms after the angle crosses the angle of the ISO trial, as indicated by the horizontal cursor. This figure is a typical example of recruitment of an additional motor unit in CON and a derecruitment of active units in ECC.

Force and surface EMG

Trials were performed while subjects sat with the foot attached to an ankle dynamometer pedal mounted with torque and angle sensors. To maximize stability during testing, subjects were strapped to the chair, the chest with a 4-point seatbelt and the thigh and ankle with straps. The foot pedal was attached to a weight stack via a pulley. The measured torque signal was sent to a computer via a CED 1401+ (Cambridge Electronic Design) and sampled at a frequency of 50 kHz. Signals were collected and analyzed using Signal software (Cambridge Electronic Design, UK). Before analysis, force and angle signals were downsampled to 2kHz, and then filtered with a low-pass filter (cutoff frequencies 75 and 150 Hz, respectively).

Global EMG activity of the soleus (SOL) and gastrocnemius medialis (GM) muscles was recorded using bipolar silver chloride miniature surface electrodes (Beckman 650437, USA) with 20 mm inter-electrode distance. Electrodes were prepared and placed in accordance with the recommendations by SENIAM (Hermens et al. 1999). The surface EMG (sEMG) signals were amplified and sampled at a frequency of 50 kHz. The signals were subsequently downsampled to 2 kHz, band-pass filtered at 20 to 1 kHz and analyzed using Signal software (Cambridge Electronic Design, UK). Surface EMG activities of SOL and GM were calculated as root mean square (RMS) of the signal. For the maximal voluntary contractions (MVC), both surface EMG (sEMG) and torque data were analyzed ±100 ms around the peak torque.

Intramuscular EMG

For the intramuscular EMG recordings, four separate bipolar fine-wire electrodes were inserted into the soleus muscle. This provided four potential signals that could be used for the decomposition. Electrodes were inserted to soleus from the lateral side at different depths around the sEMG electrode, and a ground electrode was placed on the medial malleolus. The wire-electrodes consisted of two 50 µm polyurethane insulated wires that were glued together. Insulation was mechanically removed from the outer sides of the wires over 0.5 mm distance from the tip, resulting in an inter-electrode distance of approximately 100µm. A hook was formed at the end of the wires to ensure fixation during the measurements. Electrodes were autoclaved before the experiment in order to minimize the risk of infection. The wire electrode pairs were inserted into the muscle with a 22 gauge needle that was subsequently withdrawn. Wire-electrodes were inserted after the MVC trials to avoid possible electrode migration and/or loss of signal due to MVC force levels. Further, all measurements were started with the lowest force-levels to secure data from submaximal levels before risking the possible breaking at the larger force levels. However, in spite of all the care taken, in some cases during the measurements, recording for unknown reasons stopped from one of the 4 pairs of wires. To optimize signal quality, the signal was pre-amplified with a non-commercial amplifier (amp 200, bandwidth 8-4500 Hz) attached to the calf of the subject. The amplified signal was sent to a CED 1902 programmable signal conditioner (Cambridge Electronics Design Ltd, Cambridge, UK) and digitized with CED 1401+ (Cambridge Electronic Design) with a sampling frequency of 20 kHz. Since the decomposition software allows visualization of three channels, the wire channel was duplicated twice and the two new channels were filtered with different fourth order butterworth band-pass filters (300-8000 and 400-10000Hz). Observing three differently filtered channels provided the analyzer more information for decomposition.

Signal decomposition, motor unit identification and data analysis were performed by utilizing the three channel decomposition technique computer algorithm, "Daisy", described in more detail in (Sogaard et al. 1996, Olsen, Christensen & Sogaard 2001, Farina et al. 2001). Motor unit action potential (MUAP) recognition is based on template matching of the waveform of the three channels (unfiltered and two band-pass filtered), created from the same intramuscular EMG recording. The MU classification was performed semi-automatically with a high degree of operator interaction. For each train of MU discharges the inter-spike intervals were registered and instantaneous motor unit discharge rate (MUDR) was calculated as the mean of the inverse of each inter-spike interval. The decomposed units from all subjects within an age group were pooled for calculating the differences between contraction types, force-levels and age-groups. Analyses were checked to avoid recording of the same motor unit by more than one bipolar wire set.

Statistical analysis

The normality of distribution was tested with the Shapiro-Wilk test. In the pooled units an analysis of variance (ANOVA) was used to compare the effects of contraction types and intensities. When a significant difference was detected, Bonferroni's method was used to locate the difference. An independent t-test was used to find differences between OLD and YOUNG. The critical level of significance was $P < 0.05$. Descriptive statistics include mean and standard deviation.

RESULTS

MVC, force levels and ankle angle

The maximal voluntary isometric plantarflexion force was 29.3% lower ($p<0.01$) in OLD (149.2±35.7 Nm) than in YOUNG (211.1±35.6 Nm). The average maximal active dorsiflexion range did not differ between groups (72.0 ± 1.8 deg in YOUNG; 71.1 ± 1.0 deg in OLD). Therefore, also the individual zero angles (IZA, determined as being 15 deg. plantar from the maximal voluntary dorsiflexion) used in the measurements differed on average only 0.9 deg (1.2%; n.s.).

Number of identified motor units

The analysis was based on 8372 motor unit discharges (YOUNG: 5772, OLD: 2600) from 210 different motor units (135 YOUNG, 75 OLD). The number of subjects with decomposable units in each condition and the number of units can be seen in Table 1.

		N		N(MU)		Difference between groups			
Condition	**level(%)**	**Y**	**O**	**Y**	**O**	**DR**	**F%MVC**	**SOL%**	**GM%**
ISO	**10**	10	7	43	12	3,5*	0,2	-10,3***	-9,5***
	20	10	6	44	12	15,6	-0,9	-14,7***	-15,8***
	40	8	8	45	24	13,5*	-3,9	-16,2***	-15,5***
	60	8	8	30	21	13,8*	-2,5	-17,2***	-23,2***
	80	5	6	12	17	25,4*	-4,3	-9,6	-21,8
	100	5	6	8	9	21,0	-4,6	-5,2	-25,9
CON	**10**	9	4	38	4	2,8	1,4	-24,3	-8,5***
	20	8	4	16	4	4,5	1,6	-27,8	-27,8***
	40	3	3	5	3	-10,3	0,5	-18,6	-11,4
ECC	**10**	7	2	18	2	-4,0	1,4	-17,8	-28,0***
	20	9	3	20	3	-11,6	2,8	-17,8	-11,1
	40	4	3	6	3	-9,4	0,0	-10,6	-32,9

* $P<0.05$, *** $P<0.001$ between groups

TABLE 1 Number of subjects (N) and motor units (N-MU) in young (Y) and old (O) group and differences between groups in discharge rate (DR), relative force levels (F%MVC) and relative EMG levels in soleus (SOL%) and gastrocnemius medialis (GM%). Positive difference indicates a larger value in group YOUNG. In DR difference is calculated as % between groups, in F%MVC, SOL% and GA% difference is calculated by subtracting the relative values (YOUNG-OLD).

Effect of age

In isometric contractions (ISO) the discharge rate was higher in YOUNG in all measured force levels Table 1, Figure 2). The average age-related difference ranged between 3.5 and 25.4%, and increased with force levels. In contrast, no age-related difference in MUDR was seen in the dynamic contractions at the three measured force levels (10, 20 and 40% MVC; Figure 3).

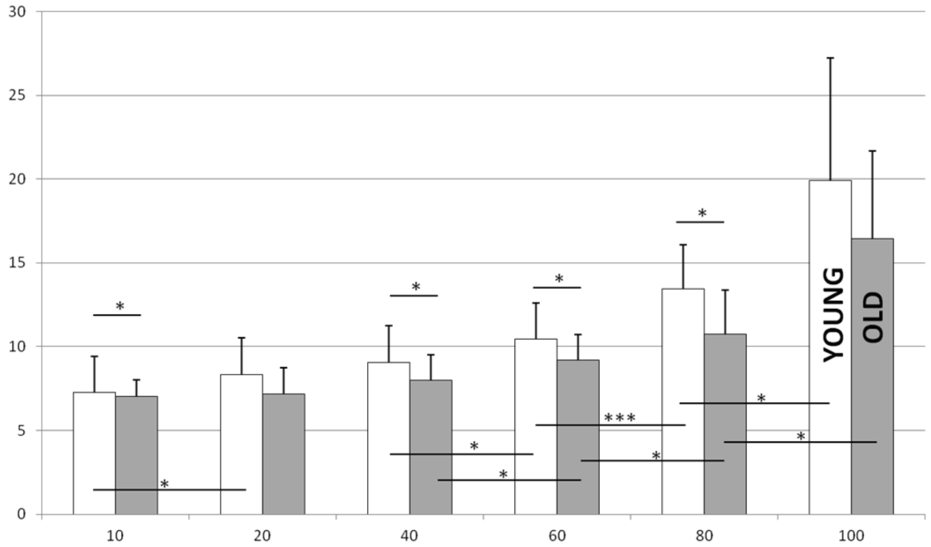


FIGURE 2 Motor unit discharge rate (1/s; X±SD) in YOUNG and OLD at different force levels in isometric contractions. * P<0.05, *** P<0.001 between groups.

The relative global sEMG activities of SOL and GM, as measured by the surface electrode, were higher in OLD compared to YOUNG in both dynamic and isometric conditions and in all force levels (p = n.s. – 0.001) despite the fact that the relative force levels did not differ between the age-groups (Table 1). The age-related differences were largest in dynamic contractions, while statistically more robust results were found in ISO, due to the higher number of MUs in that condition.

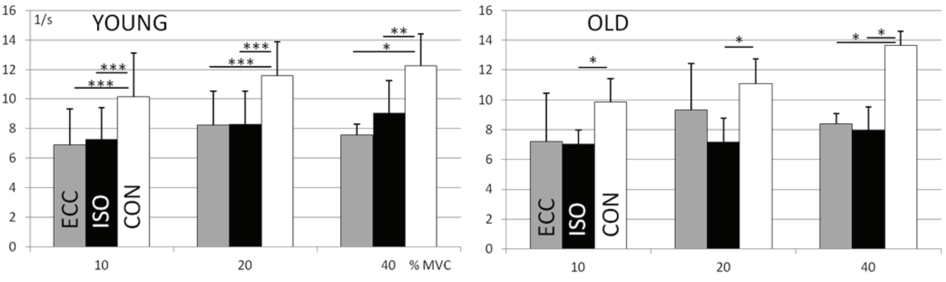


FIGURE 3 Motor unit discharge rate (1/s; X±SD) in YOUNG and OLD in eccentric (ECC), isometric (ISO) and concentric (CON) contractions at different force levels. There were no significant differences between YOUNG and OLD in dynamic contractions (see Figure 2 for age-comparison in ISO). Significant differences between contraction types: * = P<0.05, ** = P<0.01, *** = P<0.001.

Effect of contraction type and level

At the three lowest force levels all three contraction types were measured and showed that the MUDR was higher in concentric (CON) compared to isometric

(ISO) or eccentric (ECC) in both age-groups (Figure 3). No significant differences in MUDR were found between ISO and ECC.

In ISO contractions, where force levels were explored up to 100% MVC, the MUDR increased significantly along with the increase in relative force level (Figure 2). In both age-groups the increase followed a similar second order polynomial equation (YOUNG: $y = 0.6x^2 - 2.2x + 9.4$, $R^2 = 0.98$; OLD: $y = 0.6x^2 - 2.2x + 9.0$, $R^2 = 0.96$).

The relative sEMG activity of Soleus (as compared to isometric MVC) increased with every step increase in force in both groups and in all contraction types, however the difference between levels and groups was only statistically significant in ISO. In general, producing the same force required more activation in CON than in ISO or ECC for both OLD and YOUNG (Table 2).

Age	Type	Level	SOL%	GA%
YOUNG	ECC	10	13,9 ± 3,9#	11,7 ± 3,8#
	ECC	20	21,7 ± 7,8*#	22,8 ± 10,1*#
	ECC	40	32,7 ± 9,9*#	37,1 ± 13,8#
	ISO	10	10,5 ± 3,9#	6,2 ± 4,1#
	ISO	20	16,5 ± 4,8*#	7,4 ± 2,6#
	ISO	40	31,3 ± 10,3*	17,3 ± 7,5*#
	ISO	60	46,3 ± 14,0*	27,3 ± 13,1*
	ISO	80	63,7 ± 17,3*	60,0 ± 33,3*
	ISO	100	91,6 ± 20,2*	102,4 ± 32,1*
	CON	10	22,8 ± 7,0	9,9 ± 3,6
	CON	20	34,0 ± 7,5*	14,4 ± 4,5*
	CON	40	51,4 ± 2,5*	41,6 ± 13,3*
OLD	ECC	10	31,8 ± 10,1	39,7 ± 0,2#
	ECC	20	39,5 ± 16,7	34,0 ± 13,5
	ECC	40	43,3 ± 6,0#	70,1 ± 20,1
	ISO	10	20,9 ± 7,5	15,8 ± 4,1#
	ISO	20	31,2 ± 6,7*	23,3 ± 7,2*#
	ISO	40	47,5 ± 7,7*	32,8 ± 14,7*
	ISO	60	63,6 ± 6,9*	50,5 ± 16,0*
	ISO	80	73,3 ± 6,2*	81,8 ± 20,3*
	ISO	100	96,9 ± 10,9*	128,4 ± 32,2*
	CON	10	47,2 ± 19,6	18,4 ± 2,1
	CON	20	61,8 ± 20,7	42,2 ± 5,1
	CON	40	70,0 ± 12,6	53,1 ± 20,5

TABLE 2 Relative surface EMG levels of soleus (SOL) and gastrocnemius medialis (GM) in different levels and conditions, calculated relative to isometric MVC. *: significantly smaller than in lower force level. #: significantly smaller than in CON (P<0.05).

DISCUSSION

The main finding in the present study is that in contrast to the lower motor unit discharge rate in elderly across all measured isometric contraction levels there was no age-related decrease in dynamic submaximal conditions. The elderly displayed a similar motor unit control to YOUNG, whereby concentric contractions required a higher motor unit discharge rate than eccentric or isometric contractions.

Despite the OLD group being quite physically active, the isometric MVC was almost thirty percent lower compared to YOUNG. In previous reports on plantar flexors the MVC in elderly has been shown to be lower (e.g. Kallio et al. 2012, in press; (Simoneau, Martin & Van Hoecke 2005, Dalton et al. 2009) or in par with young (Piirainen et al. 2010). One possible cause for the lower MVC in OLD could be the inability to fully activate their muscles during MVC, which would also affect the discharge rate, as it would lead to erroneously low submaximal target levels that would require less activity from the motor units. However, a number of previous studies have shown that the voluntary activation of plantar flexors during MVC is unchanged in old subjects (Simoneau, Martin & Van Hoecke 2005, Dalton et al. 2009, Vandervoort & McComas 1986).

In line with earlier findings for isometric contraction the mean soleus MUDR was significantly lower in OLD compared to YOUNG in all measured levels (Kallio et al. 2012, Dalton et al. 2008). This is also in line with findings in other muscles, e.g. tibialis anterior (Klass, Baudry & Duchateau 2008, Connelly et al. 1999), first dorsal interosseous (Kamen et al. 1995) and adductor digiti minimi (Nelson, Soderberg & Urbscheit 1984). Dalton et al. (Dalton et al. 2009) found an age difference in soleus MUDR at 25 and 50% MVC, but none at higher contraction levels. Like the current study Dalton also reported a force-related increase in MUDR that was greater at high intensities in the elderly, but the young subjects in Dalton's study were increasing the MUDR at a reduced pace with increasing contraction intensity, which resulted in a 17% lower maximal MUDR compared to OLD. This is in contrast to the increased difference from 16.5 at the low contraction level to 19.9 /s at the highest level found in the present study This is however, in line with several other studies that have shown the age-difference in MUDR to be larger at high force levels (Kamen et al. 1995, Kamen & Knight 2004)(Barry et al. 2007, Patten & Kamen 2000).

The observed age-difference in MUDR also confirms the results of our previous measurements in isometric (Kallio et al. 2013, accepted for publication, Kallio et al. 2012), and dynamic (Kallio J. et al. 2010) contractions when low, submaximal contractions were not matched by relative force, but instead by relative surface EMG. The decreased MUDR has been suggested to be an adaptation to the increased twitch duration to optimize force generation (Roos, Rice & Vandervoort 1997). Previous studies have confirmed that the soleus twitch duration actually increases with age, suggesting that tetanus can be achieved with lower discharge rates (Kallio et al. 2012, Dalton et al. 2009, Dalton et al. 2010).

It can be questioned if the age-related deterioration in muscle performance, like decreasing maximal force and slowing of force production, is just an inevitable part of ageing or if it can be partly explained by an age related change in everyday activity. In the present study all the subjects in both age-groups were quantitatively equally active, as they performed moderate to strenuous physical exercise at least three times a week. However, the open-ended questionnaire showed clear group-differences in the types of activities they engaged in. The young subjects were more active in sports requiring strength and speed (e.g. running, soccer, ice-hockey, weight-training, tennis), than the elderly who mainly were walking with and without poles, swimming and gardening).

No clear differences in MUDR were found between age-groups in the dynamic contractions. There are a few couple of possible reasons for this. Firstly, the relative surface EMG levels, especially in the dynamic contractions, were higher in OLD than in YOUNG, which could lead to an increase in MUDR and thus reduce the group-difference. Higher muscle activation with similar MUDR would indicate a higher reliance on recruitment rather than rate coding in the elderly. Previous studies have also found an age-related increase in coactivation during dynamic contractions like walking (Schmitz et al. 2009), which could explain the observed difference in EMG/force relationship between the two groups. Secondly, as the ISO trials show, the age related differences in MUDR increased with increasing torque level (Figure 2). This is a phenomenon that has been reported previously by some studies (Barry et al. 2007, Patten & Kamen 2000, Kamen et al. 1995, Kamen & Knight 2004) while others have not fully supported this (Connelly et al. 1999, Dalton et al. 2009). If the age related difference in general is more pronounced at high forces, less difference could also be expected at the low dynamic levels. Especially with elderly subjects, the derecruitment at the start of the movement in ECC contractions was very common, which decreased the number of recorded units significantly. In the present study low force levels were selected because they are generally easier to decompose. The abovementioned reasons set a clear need for the technical development that would enable recording units at high force levels in dynamic contractions.

The present study showed the largest MUDR in CON in both groups. Since we controlled the weights so that the absolute plantarflexion torque in all contraction types was the same, the most likely reason for the difference is found in the cross-bridge physiology. It has been shown that the force production of the cross-bridge is decreased when producing movement in concentric contractions (Joyce, Rack & Westbury 1969). For this reason, to generate an equal amount of force, more activity either in terms of more recruited motor units or higher discharge frequency is required in CON compared to ISO or ECC contractions, which was also apparent as an increased surface EMG.

The joint rotation velocity (10 deg/s) in the current study was quite slow compared to most natural movements. For example, in walking, the angular velocity in dorsiflexion is ~30 deg/s and in plantarflexion ~220 deg/s (estimated from Lichtwark 2007). The next challenge for motor unit studies would be to see how the activation functions in normal locomotion.

Conclusions

These findings suggest that age-related differences in motor unit control do exist also in large leg extensors that play an important role in human locomotion and balance control. The lack of age-difference in dynamic contractions could be due to the low force levels measured in the study, differences in recruitment-strategies or an age-related increase in coactivation.

References

Allinger, T. L. & Engsberg, J. R. 1993. A method to determine the range of motion of the ankle joint complex, in vivo. Journal of Biomechanics 26 (1), 69-76.

Altenburg, T. M., de Ruiter, C. J., Verdijk, P. W., van Mechelen, W. & de Haan, A. 2009. Vastus lateralis surface and single motor unit electromyography during shortening, lengthening and isometric contractions corrected for mode-dependent differences in force-generating capacity. Acta Physiologica 196 (3), 315-328.

Barry, B. K., Pascoe, M. A., Jesunathadas, M. & Enoka, R. M. 2007. Rate coding is compressed but variability is unaltered for motor units in a hand muscle of old adults. Journal of neurophysiology 97 (5), 3206-3218.

Connelly, D. M., Rice, C. L., Roos, M. R. & Vandervoort, A. A. 1999. Motor unit firing rates and contractile properties in tibialis anterior of young and old men. Journal of applied physiology 87 (2), 843-852.

Dalton, B. H., Harwood, B., Davidson, A. W. & Rice, C. L. 2010. Recovery of motoneuron output is delayed in old men following high-intensity fatigue. Journal of neurophysiology 103 (2), 977-985.

Dalton, B. H., Harwood, B., Davidson, A. W. & Rice, C. L. 2009. Triceps surae contractile properties and firing rates in the soleus of young and old men. Journal of applied physiology (Bethesda, Md.: 1985) 107 (6), 1781-1788.

Dalton, B. H., McNeil, C. J., Doherty, T. J. & Rice, C. L. 2008. Age-related reductions in the estimated numbers of motor units are minimal in the human soleus. Muscle & nerve 38 (3), 1108-1115.

Del Valle, A. & Thomas, C. K. 2005. Firing rates of motor units during strong dynamic contractions. Muscle & nerve 32 (3), 316-325.

Farina, D., Colombo, R., Merletti, R. & Olsen, H. B. 2001. Evaluation of intra-muscular EMG signal decomposition algorithms. Journal of electromyography and kinesiology 11 (3), 175-187.

Galganski, M. E., Fuglevand, A. J. & Enoka, R. M. 1993. Reduced control of motor output in a human hand muscle of elderly subjects during submaximal contractions. Journal of neurophysiology 69 (6), 2108-2115.

Häkkinen K., Newton RU., Gordon SE., McCormick M., Volek JS., Nindl BC., Gotshalk LA., Campbell WW. & Evans WJ. 1998. Changes in muscle morphology, electromyographic activity, and force production characteristics during progressive strength training in young and older men. Journals of Gerontology Series A-Biological Sciences & Medical Sciences 53 (6), B415-23.

Hamacher, D., Singh, N. B., Van Dieen, J. H., Heller, M. O. & Taylor, W. R. 2011. Kinematic measures for assessing gait stability in elderly individuals: a systematic review. Journal of the Royal Society, Interface 8 (65), 1682-1698.

Heinrich, S., Rapp, K., Rissmann, U., Becker, C. & Konig, H. H. 2010. Cost of falls in old age: a systematic review. Osteoporosis International 21 (6), 891-902.

Hermens, H. J., Freriks, B., Merletti, R., Stegeman, D., Blok, J., Rau, G., Disselhorst-Klug, C. & Hägg, G. 1999. SENIAM 8: European recommendations for surface electromyography.

Howard, J. E., McGill, K. C. & Dorfman, L. J. 1988. Age effects on properties of motor unit action potentials: ADEMG analysis. Annals of Neurology 24 (2), 207-213.

Howell, J. N., Fuglevand, A. J., Walsh, M. L. & Bigland-Ritchie, B. 1995. Motor unit activity during isometric and concentric-eccentric contractions of the human first dorsal interosseus muscle. Journal of Neurophysiology 74 (2), 901-904.

Joyce, G. C., Rack, P. M. & Westbury, D. R. 1969. The mechanical properties of cat soleus muscle during controlled lengthening and shortening movements. Journal of Physiology 204 (2), 461-474.

Kallio J., Avela J., Moritani T., Kanervo M., Selänne H., Komi P. & Linnamo V. 2010. Effects of ageing on motor unit activation patterns and reflex sensitivity in dynamic movements. Journal of Electromyography & Kinesiology 20 (4), 590-598.

Kallio, J., Søgaard, K., Avela, J., Komi, P., Selänne, H. & Linnamo, V. 2012. Age-related decreases in motor unit discharge rate and force control during isometric plantar flexion. Journal of Electromyography and Kinesiology (0).

Kallio, J., Søgaard, K., Avela, J., Komi, P. V., Selänne, H. & Linnamo, V. 2013. Motor unit firing behaviour of soleus muscle in isometric and dynamic contractions. PLos ONE, accepted for publication .

Kamen, G. & Knight, C. A. 2004. Training-related adaptations in motor unit discharge rate in young and older adults. Journals of Gerontology Series A-Biological Sciences & Medical Sciences 59 (12), 1334-1338.

Kamen, G., Sison, S. V., Du, C. C. & Patten, C. 1995. Motor unit discharge behavior in older adults during maximal-effort contractions. Journal of applied physiology 79 (6), 1908-1913.

Klass, M., Baudry, S. & Duchateau, J. 2008. Age-related decline in rate of torque development is accompanied by lower maximal motor unit discharge frequency during fast contractions. Journal of Applied Physiology 104 (3), 739-746.

Klass, M., Baudry, S. & Duchateau, J. 2007. Voluntary activation during maximal contraction with advancing age: a brief review. European Journal of applied physiology 100 (5), 543-551.

Kossev, A. & Christova, P. 1998. Discharge pattern of human motor units during dynamic concentric and eccentric contractions. Electroencephalography and clinical neurophysiology 109 (3), 245-255.

Lichtwark, G. A., Bougoulias, K. & Wilson, A. M. 2007. Muscle fascicle and series elastic element length changes along the length of the human gastrocnemius during walking and running. Journal of Biomechanics 40 (1), 157-164.

Linnamo 2003. Motor unit activation patterns during isometric, concentric and eccentric actions at different force levels. Journal of Electromyography and Kinesiology 13 (1), 93.

Linnamo, V., Avela, J., Kallio, J., Dousset, E., Kuitunen, S., Ishikawa, M., Kyröläinen, H. & Komi, P. V. 2003a. Maximal torque and activation level during eccentric, concentric and isometric actions before and after damaging exercise. In Gantchev N. (Ed.) From motor Control to Functional Recovery III.Varna, Bulgaria:361-368.

Linnamo, V., Moritani, T., Nicol, C. & Komi, P. V. 2003b. Motor unit activation patterns during isometric, concentric and eccentric actions at different force levels. Journal of Electromyography & Kinesiology 13 (1), 93-101.

Nardone, A., Romano, C. & Schieppati, M. 1989. Selective recruitment of high-threshold human motor units during voluntary isotonic lengthening of active muscles. The Journal of physiology 409, 451-471.

Nelson, R. M., Soderberg, G. L. & Urbscheit, N. L. 1984. Alteration of motor-unit discharge characteristics in aged humans. Physical Therapy 64 (1), 29-34.

Olsen, H. B., Christensen, H. & Sogaard, K. 2001. An analysis of motor unit firing pattern during sustained low force contraction in fatigued muscle. Acta Physiologica et Pharmacologica Bulgarica 26 (1-2), 73-78.

Pasquet, B., Carpentier, A. & Duchateau, J. 2006. Specific modulation of motor unit discharge for a similar change in fascicle length during shortening and lengthening contractions in humans. Journal of Physiology 577 (Pt 2), 753-765.

Patten, C. & Kamen, G. 2000. Adaptations in motor unit discharge activity with force control training in young and older human adults. European journal of applied physiology 83 (2-3), 128-143.

Piirainen, J. M., Avela, J., Sippola, N. & Linnamo, V. 2010. Age dependency of neuromuscular function and dynamic balance control. European Journal of Sport Science 10 (1), 69.

Roos, M. R., Rice, C. L. & Vandervoort, A. A. 1997. Age-related changes in motor unit function. Muscle & nerve 20 (6), 679-690.

Schmitz, A., Silder, A., Heiderscheit, B., Mahoney, J. & Thelen, D. G. 2009. Differences in lower-extremity muscular activation during walking between healthy older and young adults. Journal of electromyography and kinesiology : official journal of the International Society of Electrophysiological Kinesiology 19 (6), 1085-1091.

Semmler 2002. Motor unit synchronisation is enhanced during slow lengthening contractions of a hand muscle. The Journal of Physiology 545 (2), 681.

Simoneau, E., Martin, A. & Van Hoecke, J. 2005. Muscular performances at the ankle joint in young and elderly men. The journals of gerontology.Series A, Biological sciences and medical sciences 60 (4), 439-447.

Sogaard, K. 1995. Motor unit recruitment pattern during low-level static and dynamic contractions. Muscle & nerve 18 (3), 292-300.

Sogaard, K., Christensen, H., Fallentin, N., Mizuno, M., Quistorff, B. & Sjogaard, G. 1998. Motor unit activation patterns during concentric wrist flexion in humans with different muscle fibre composition. European Journal of Applied Physiology & Occupational Physiology 78 (5), 411-416.

Sogaard, K., Christensen, H., Jensen, B. R., Finsen, L. & Sjogaard, G. 1996. Motor control and kinetics during low level concentric and eccentric contractions in man. Electroencephalography & Clinical Neurophysiology 101 (5), 453-460.

Sogaard, K., Sjogaard, G., Finsen, L., Olsen, H. B. & Christensen, H. 2001. Motor unit activity during stereotyped finger tasks and computer mouse work. Journal of Electromyography & Kinesiology 11 (3), 197-206.

Tax, A. A., Denier van der Gon, J. J., Gielen, C. C. & van den Tempel, C. M. 1989. Differences in the activation of m. biceps brachii in the control of slow isotonic movements and isometric contractions. Experimental Brain Research 76 (1), 55-63.

Vandervoort, A. A. & McComas, A. J. 1986. Contractile changes in opposing muscles of the human ankle joint with aging. Journal of applied physiology 61 (1), 361-367.

Wolfson, L., Judge, J., Whipple, R. & King, M. 1995. Strength is a major factor in balance, gait, and the occurrence of falls. The journals of gerontology.Series A, Biological sciences and medical sciences 50 Spec No, 64-67.