

MOTOR FUNCTION:
MEASUREMENT, TRANSLATION, AND THE EFFECTS OF AGE

by

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ABSTRACT

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Motor Function: measurement techniques and influencing factors

Thesis directed by Professor Roger M. Enoka

Motor function encompasses the capacity of the nervous system to control the actions of muscles to exert forces and thereby produce movement. Motor function can be characterized by performance on such subdomains as locomotion, strength, dexterity, balance, endurance, steadiness, and performance fatigability. The measurement techniques used to assess these domains vary depending on the neuromuscular property being assessed, the species being investigated, and the age of the person or animal. The four projects in this dissertation examined the measurement of motor function and its modulation by age. The first study focused on the motor domain of performance fatigability in humans, and found that endurance time depends on the compliance of the load and also the rate of increase in muscle activation. The second study examined the influence of age on performance fatigability and found that, contrary to previously published works, fatigability of the dorsiflexor muscles is greater in older adults. The third study assessed the influence of metabolic biomarkers on motor function in older adults, and found that vitamin D hormone and fasting insulin are associated with walking endurance in relatively healthy older men and women. The fourth study developed a mouse model of multiple domains of motor function to further assess the influence of age on motor function. Taken together, these results reinforce the importance of assessing of multiple tasks and subdomains when characterizing motor function with advancing age in people and animals.

DEDICATION

My thesis is dedicated to my loving husband, Mike Pont, who has encouraged me in all things, loved me despite myself, but in particular for supporting me during all of my late nights, early mornings and desperate days; without him none of the following works would be possible. I also dedicate this to family: my boys, Jasper and Tobe, for their love, understanding, and comic relief; my parents, Dennis and Sharon Justice for not kicking me out of their house when I was young and wild, and for the time spent supporting me and playing with the boys while I put this all together; and finally to my dogs Fuller and Mabel who have warmed my feet and my heart during long hours at the computer. Lastly, I dedicate this to Roger Enoka, for being my mentor and my hero.

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CONTENTS

I. Review of Literature	1
Overview	2
Motor Function and Age-Related Dysfunction	3
The Integrated Motor System	20
Translational Models of Age-Related Declines in Function	32
Summary	35
II. Muscle activity differs with load compliance during fatiguing contraction with the knee extensor muscles	36
Abstract	37
Introduction	38
Methods	39
Results	46
Discussion	54
III. Fatigability of the dorsiflexors during a submaximal isometric contraction increases with age in old adults	61
Abstract	62
Introduction	63
Methods	63
Results	65
Discussion	67

IV. Association of motor function with 1,25(OH)₂D and indices of insulin-glucose dynamics in non-diabetic older adults	70
Abstract	71
Introduction	72
Methods	74
Results	80
Discussion	84
V. Battery of behavioral tests to quantify age-associated changes in motor function in mice	91
Abstract	92
Introduction	93
Methods	94
Results	101
Discussion	111
VII. Summary	119
VIII. References	123

Tables
Chapter IV.

Table 1. 81

Subject characteristics for primary health measures

Table 2. 83

Significant correlations and trends between motor function, ivGTT-derived measures, and vitamin D metabolites

Table 3. 84

Regression model to predict walking endurance

Chapter V.

Table 4. 102

Test battery outcomes for 3, 20, and 26-month C57Bl/6 male mice.

Table 5. 108

Regression model to determine the relative contribution of each test score in explaining the variance in the motor function summary score.

Table 6. 109

Regression model to determine the relative significance of each subdomain score in explaining the variance in the motor function summary score.

Table 7. 110

Comparison of age-related motor function declines in humans and mouse model.

Table 8. 116

Scores derived from individual tests in 3, 20, and 26-month male C57BL/6 mice.

Table 9. 117

Expanded: comparison of age-related motor function declines in humans and mouse model.

Figures

Chapter I

Figure 1. 28

Model of the mechanisms underlying the influence of 25(OH)D on neuromuscular function.

Chapter II

Figure 2. 40

Schematic drawing of experimental apparatus for knee extension force and position tasks.

Figure 3. 47

Representative data for a position task and a force task.

Figure 4. 48

Mean \pm SE for average EMG amplitude for the knee extensor and the knee flexor muscle during the two fatiguing contractions.

Figure 5. 50

Averaged frequency data of the interference EMG signal for the knee extensor muscles.

Figure 6. 51

Coefficient of variation (CV) for force % during the force and position tasks.

Figure 7. 53

Mean arterial pressure, heart rate, and ratings of perceived exertion during the force and position tasks.

Chapter III

Figure 8. 66

Observed versus predicted endurance time for an isometric contraction

Chapter IV

Figure 9. 77

Schematic drawings of the experimental setup.

Chapter V

Figure 10. 99

Experimental apparatus used to characterize subdomains of motor function in mice.

Figure 11. 105

Subdomain and summary scores of motor function for the three groups of mice.

Figure 12. 106

Power analysis.

CHAPTER 1

Review of Literature

1. Overview

Motor function is the ability to use and control muscles and movements, and encompasses coordinated actions of multiple physiological systems. Advancing age is often accompanied by declines in motor function, such as loss of strength, slowing gait speed, and balance impairments that can lead to a progression of functional decrements and may ultimately limit an older individual's ability to live independently in the community [Fried and Guralnik 1997]. Given the aging of the US population, disability and long-term care requirements will have an increasing impact on healthcare and budgetary needs in the US [Olshansky et al. 2009], and likely throughout the world. Therefore, understanding the causes and impacts of motor decline in aging populations is of the utmost importance.

The aim of this literature review is to examine: 1) the morphological and physiological factors underlying decrements in motor function with advanced age, 2) the consequences of functional declines on disability and mortality in older adults, and 3) animal models of aging to facilitate clinical translation of behavioral outcomes commonly studied in humans.

2. Motor Functions and Age-Related Dysfunction

Voluntary physical function is a complex phenomenon with movements catered to meet the demands of a task. Thus optimal motor function required for one task, such as walking, may not relate to function in a different task, such as the ability to manipulate objects with the fingers. Therefore, to measure and understand motor function, it is critical to define multiple functional domains. In 2012, the National Institutes of Health (NIH) defined motor function in terms of strength, locomotion, balance, dexterity, and endurance [Reuben et al. 2013]. These domains of motor function have been measured by standardized test batteries and incorporated into large research cohorts. Thus, as normed data exists for many of these domains, declines with age, and consequences of age-related dysfunction on disability, morbidity and mortality are well characterized. However, these actions involve orchestration of movements across multiple joints, and so simpler single joint tasks such as those used to determine performance fatigability and the ability to maintain a steady contraction are often employed.

A review of the motor function subdomains strength, locomotion, balance, and endurance as well as steadiness and fatigability follows. This chapter seeks to review subdomains of motor function, identify tasks used to assess each subdomain, and to highlight the importance each domain with respect to disability, morbidity and mortality in an aging population.

2.1 Strength

Strength is the ability to generate a maximal force against physical objects [Reuben et al. 2013]. In both clinical and experimental settings, strength is most commonly

measured as the maximum force that can be generated during an isometric contraction (maximal voluntary contraction, MVC), the maximal load that can be lifted once (one-repetition maximum, 1-RM), or the peak torque during either a shortening or lengthening contraction (peak isokinetic torque) [Enoka 2008]. Values achieved on these three tests are often uncorrelated as the performance on the MVC depends primarily on muscle size, whereas the 1-RM and peak isokinetic torque depends on both muscle size and the capacity of the nervous system to provide the requisite activation signals [Enoka 2008].

2.1.1 Strength and Power Declines with Age

Aging is associated with a progressive loss of strength of maximal voluntary contraction strength in young compared with older adults. After the age of 60 years these declines are on the order of 1 – 2.5% per year [Forrest et al. 2007; Vandervoort 2002]. For example, Vandervoort and McComas [1986] examined the effects of age on contractile properties of the ankle dorsiflexor and plantarflexor muscles in men and women, and found that that oldest group of men (aged 80-100 years) exhibited torques of 56% and 55% of the youngest group (20-32 years), and 63% and 48% for women. The decline in strength is not exclusive to the ankle muscles, though the magnitude of the reduction in strength varies between muscle groups. Hunter et al. [2000] demonstrated weaker maximal voluntary contractions for handgrip (35%), plantar flexors (37%), and knee extensors (50%) in older women (70-79 years) compared with young (20-29 years), reflecting declines of 6.2%, 7.4% and 9.3% declines per decade, respectively. The strength declines in older groups relative to young were similar of those observed by other groups

in men and women [Burke et al. 1953; Bohannon et al. 1997; Mathiowetz et al. 1985; Vandervoort et al. 1986; see Supplemental Table 8, Chapter V for additional details].

In addition to the declines in isometric maximal voluntary contractions across age, power is also an important determinant of motor function. Whereas strength refers to the ability to generate maximal force, power is the product of the force produced and the velocity of the muscle contraction [Reid and Fielding 2012], and often measured as the rate of force development (RFD). Similar to strength, RFD is markedly reduced with aging [Aagaard et al. 2010; Raj et al. 2010; Reid and Fielding 2012], whether normalized to body mass, maximal isometric or dynamic strength or not [Barry et al. 2005; Klass et al. 2008]. In a study of maximal strength and power characteristics in both isometric and dynamic actions in middle-aged and older men, Izquierdo et al. [1999] examined maximal isometric strength, and the shape of isometric force-time curves (RFD), and power-load curves during concentric and stretch shortening cycle during half-squat, squat jump, and bench-press tasks. Their primary finding was that RFD and power output decreased with increasing age even more than maximal isometric strength in lower and upper body actions [Izquierdo et al. 1999].

2.1.2 Age-Related Strength Loss and Disability, Morbidity and Mortality

The associations between strength and negative health outcomes are well documented in both cross-sectional and prospective analyses. Cross-sectional analysis from the Canadian NuAge cohort (n = 904, aged 67-84 years) found that individuals in the lowest and middle tertiles of handgrip and quadriceps strength relative to body mass were more likely to have impaired mobility [Choquette et al. 2010]. Handgrip and knee extensor

strength were also strongly correlated with motor disability as determined by the number of self-reported difficulties in grasping, lifting 10-lb, walking across a small room, walking ¼ mile, climbing 10-steps and performing heavy housework [Rantanen et al. 1999a]. The cross-sectional analysis by Rantanen et al. [1999a] from the Women's Health and Aging Study (n = 1002 disabled women aged >65 years) is of particular interest because physical activity was also measured. Modeling of the relations between physical activity, disability and strength indicated that muscle strength had a significant mediating role between physical inactivity and disability [Rantanen et al. 1999a]. Additionally, a smaller study (n = 84, aged 60-88 years) investigating cross-sectional associations between muscle strength, biomechanical functional assessment and health-related quality of life, found that average lower body isometric muscle strength was associated with quality of life including physical functioning, bodily pain, vitality, social functioning and emotional scores [Samuel et al. 2012]. Interestingly, knee flexion strength was one of the stronger predictors of health-related quality of life, including physical and social functioning, vitality, emotional, mental health and mental component scores [Samuel et al. 2012].

Many of the associations between strength and future health outcomes have been confirmed in prospective studies. For example, loss of knee extensor strength over ~6 years is highly predictive of severe mobility limitation in initially well-functioning older adults [Manini et al. 2007]. Further, in a hallmark investigation, Rantanen et al. [1999b] found handgrip strength was highly predictive of functional limitation and disability 25 years later. This conclusion was based the Honolulu Heart Program, in which 3,218 Japanese-American men were assessed for hand grip strength and functional limitations, including slow preferred walking speed (≤ 0.4 m/s), inability to rise unassisted from a

seated position, and self-reported upper extremity, mobility, and self-care disability outcomes at baseline and after a 25-year follow up [Rantanen et al. 1999b]. The finding that strength was predictive of functional limitation and disability represents a significant contribution as most of the information regarding age-related strength changes are largely based on cross-sectional data, and few longitudinal studies have been published with numbers substantial enough, and the intervening follow-up time long enough, to demonstrate such predictions [Rantanen 2003; Rantanen et al. 2003]. Furthermore, a recent 44-year prospective study in the same cohort has revealed that midlife grip strength is not just predictive of disability and functional limitation, but mortality up to age 100 years [Rantanen et al. 2012].

Although handgrip strength may not demonstrate the greatest magnitude of decline across age [Hunter et al. 2000], use of grip strength as a model to describe overall strength changes is supported by its significant correlation with other strength measures, including elbow flexion, knee extension, trunk extension, trunk flexion, and a sit-to-stand task [Bohannon et al. 2012; Rantanen et al. 1994]. The utility of grip strength in predicting future adverse health outcomes has been confirmed in a number of studies with shorter follow-up times. Grip strength has been shown to predict incident fragility fractures [Albrand et al. 2003], vertebral and non-vertebral fractures [Finigan et al. 2008], and low energy fractures [Kärkkäinen et al. 2008; Sirola et al. 2008], risk of cognitive decline or Alzheimer's disease [Alfaro-Acha et al. 2006; Buchman et al. 2007; Taekema et al. 2010; Wang et al. 2006], coronary heart disease and stroke [Silventoinen et al. 2009], long term nursing home stay [Rothman et al. 2008], and overall disability [Seidel et al. 2011; Taekema et al. 2010].

Despite the predictive utility of isometric handgrip strength on negative health outcomes, a new hypothesis has emerged proposing that lower extremity muscle power may be more critical to impairments, functional limitation and resultant disability than muscle strength [Reid and Fielding 2012]. Leg extensor power is associated with physical performance on walking speed, chair rise ability and repeated chair stands in institutionalized elders [Basseby et al. 1992], and in disabled elderly women peak muscle power was found to be more highly correlated with functional status than muscle strength [Fodlvari et al. 2000; Suzuki et al. 2001]. Also, contraction velocity on a leg press task, but not muscle strength, was a predictor of functional performance on low intensity tasks such as preferred walking speed in older women [Sayers et al. 2005], a finding that is in agreement with several other studies in older persons [Cuoco et al. 2004; Bean et al. 2008]. However, as muscle power or contraction velocity are more difficult to measure in large, longitudinal epidemiological studies, the predictive value of muscle power on outcomes such as disability, morbidity and mortality remains undetermined.

2.1.3 Strength Loss, Sarcopenia, Dynapenia

The term “sarcopenia”, has been adopted by the scientific community to describe the age-related loss of skeletal muscle mass. The name “sarcopenia” was first used by Rosenberg in 1988 to draw attention of clinicians, scientists, and policy makers to the phenomenon of the decline in lean body mass with advancing age, independently of substantial disease effects [Bautmans 2009; Rosenberg 1997]. However, some groups have also included screening of muscle weakness and low functional ability in the sarcopenia definition [Bautmans 2009; Janssen et al. 2002]. For example, isometric handgrip strength

has suggested for an operational definition of sarcopenia [Lauretani et al. 2003]. In more recent efforts to create criteria for diagnosing sarcopenia, teams of scientists and practitioners in Europe and the US have included aspects of physical function, primarily muscle strength, and muscle mass [Biomarkers Consortium: Sarcopenia Project; Cruz-Jentoft et al. 2010; Sayer et al. 2013]. The term “sarcopenia” which was initially defined exclusively as the age-related loss of muscle mass, has also become nearly synonymous with loss of strength [Carter et al. 2012; Manini and Clark 2012]. However, loss of strength and mass with aging are dissociated with the loss of strength occurring more rapidly than the concomitant loss of muscle mass [Clark and Manini 2008]. In fact, longitudinal data from the Health ABC study suggests that the change in quadriceps area only explains 6-8% of the variability in in the changes in knee extensor strength [Delmonico et al. 2009]. Based on this dissociation, the use of a single word to link changes in mass and strength has been met with resistance, as it implies that these phenomena are causally linked and that changes in skeletal muscle mass are “directly and fully responsible” for changes in strength [Manini and Clark 2012]. Some groups have adopted a new word, dynapenia, to describe the age-related loss of strength and power independently of the loss of muscle mass [Carter et al. 2012; Clark and Manini 2008], and working decision algorithms for defining or diagnosing dynapenia are being developed [Manini and Clark 2012].

2.2 Locomotion

Locomotion, or the ability to move from place to place, is a key to independent functioning [Bischoff et al. 2003; Hornyak et al. 2012], and the speed of walking is relevant to appropriate functioning within a community [Langlois et al. 1997; Mitchell 2006]. One

of the simplest, and clinically valuable, ways to quantify walking is to measure speed required to travel a given distance [Hornyak et al. 2012]. For example, the NIH Toolbox suggests measurement of locomotor function as gait speed while walking 4 m for use in clinical trials and large cohort studies [Reuben et al. 2013].

2.2.1 Gait speed and Changes with Age

Many tests of locomotion are relevant to daily function with aging, such as sit-to-stand or timed Up and Go tests [Janssen et al. 2000; Podsiadlo and Richardson 1991], step length, step frequency or other assessments of gait pattern [Bautmans et al. 2011; Ko et al. 2010]. However, this section will focus on changes in gait speed with age, as gait speed is a common measure both in geriatric clinical assessment and is broadly used in epidemiological research leading to robust findings of associations with negative health events in older age.

Whereas gait speed does slow with advancing age and with similar magnitudes in men and women, appreciable declines from young occur at later ages than strength loss. Bohannon and Andrews [2011] performed a meta-analysis to describe normal gait speed over courses from 3 to 30 m for healthy individuals within age and gender strata (10 year age bins from 20 – 99 years). Of the forty-one articles identified, data were consolidated to provide normative data from 23,111 subjects. Cumulatively, these cross-sectional findings suggest that gait speeds increase from young adults through middle age, and then begin to decline slowly at first, and rapidly at advanced ages. For example, preferred gait speed in men increases from the 20s through the 40s age bins (1.36-1.45 m/s; +7%) and does not decline until the 60s (1.34 m/s; -2% from 40s), with a -6% decline between 60s and 70s

and a -23% decline between 70s and 80-90 year groups, for a total of -29% from 20s to 80-90 years. In women, gait speeds increase from 20s to 40s (1.34 – 1.39 m/s; +4%) and begin declining in the 50-59 year bin (1.31 m/s; -6% from 40s) with additional -6% declines from 50s to 60s, -9% from 60s to 70s, and -15% from 70s to 80-90 year groups, for a total of -30% between 20s and 80-90 years [Bohannon and Andrews 2011]. These declines apply to habitual, comfortable gait speeds, as opposed to fast or maximum speed gait. In a study investigating changes in comfortable compared with maximum walk speeds over a 25 foot (7.62 m) track in men aged 20-79 years (n = 230), appreciable cross-sectional declines across ages were observed primarily in the maximal or fast speeds (maximal: -18%, habitual: -5% from 20s to 70s)[Bohannon 1997].

2.2.2 Locomotor Impairment, Disability, Morbidity and Mortality

Despite the later declines in habitual gait speed with age, habitual walking speed is a significant predictor of adverse outcomes [Abellan Van Kan et al. 2009], such as disability [Guralnik et al. 2000; Seidel et al. 2011], cognitive decline [Atkinson et al. 2007], falls [Dargent-Molina et al. 1996; Montero-Odasso et al. 2005], hospitalization [Studenski et al. 2003], nursing home admission [Bischoff et al. 2003], and mortality [Stanaway et al. 2011; Studenski et al. 2011]. Studenski et al. [2011] evaluated the relation between habitual gait speed and survival in a pooled analysis from 9 cohort studies using 34,485 community-dwelling older adults aged 65 or older. Gait speed was shown to predict survival and survival increased across the full range of gait speeds, with significant increments per 0.10 m/s. For example, at age 75 years 10-year survival in men ranged from 18% for those walking ≤ 0.4 m/s to 88% for those walking ≥ 1.4 m/s. Additionally, age, sex and gait speed

predicted survival as accurately as a more comprehensive model that included, age, sex, chronic conditions, smoking history, blood pressure, body mass index and hospitalization [Studenski et al. 2011]. These pooled data support several other studies from single cohorts demonstrating an association between gait speed and survival [Cesari et al. 2005; 2008; 2009; Ostir et al. 2007; Rolland et al. 2006; Rosano et al. 2008; Woo et al. 1999]. Given the associations with mortality, comorbidities [Cesari et al. 2006], and subclinical conditions such as atherosclerosis [Elbaz et al. 2005] and inflammatory status [Cesari et al. 2004], both habitual and fastest gait speed have been proposed as a “vital sign” for older persons [Cesari 2011; Lusardi 2012].

2.3 Balance & Falls

Balance refers to the ability to orient the body in space, maintain an upright posture under both static and dynamic conditions, and move and walk without falling [Reuben et al. 2013]. Maintaining balance during everyday tasks requires the ability to respond to internal and external disturbance, to realign body segments, and to protect oneself from falling [Shumway-Cook & Woollacott 2001]. Balance control is highly task specific and a number of clinical measures, typically involving corrective actions, have been proposed to assess standing balance and fall risk [Patla et al. 1992]. Assessments of corrective responses typically involve the ability to stay within one’s base of support with minimal movement, or responses to balance perturbation and protective strategies.

2.3.1 Balance Measures and Changes with Age.

Assessments requiring the person to maintain their balance include, bipedal, unipedal and tandem stance tasks [Newton 1989; Vellas et al. 1997; Rueben et al. 2013; Rossiter-Fornoff et al. 1995]. Performance on these quasi-static balance tests declines across age primarily for the unipedal eyes closed and tandem stance positions, whereas declines in bipedal, foam surface, and unipedal with eyes open are evident only at later ages (80-90 years) [Vereeck et al. 2008]. One experimental paradigm for assessing the response to balance perturbation and protective response experimentally is to assess stability during balance recovery from a task such as a static forward leaning position [Carty et al. 2011; Thelen et al. 1997]. Previous studies using this approach have demonstrated that older adults as compared with young exhibit a poorer capacity to recover [Wojcik et al. 1999; Madigan et al. 2005; Tang and Woollacott 1998], a shorter recovery step length [Luchies et al. 1994], a lower step velocity [Wojcik et al. 1999], greater reaction time [Wojcik et al. 1999], and are more likely to use multiple steps to recover balance [McIlroy and Maki 1996; Thelen et al. 1997], which may be predictive of future falls [Maki et al. 2006]. The protective response to perturbation can also be assessed clinically by maximal and rapid stepping measures, which also declines substantially from young in both unimpaired and balance-impaired older adults [Medell and Alexander 2000], and is a good predictor of mobility performance, frequent falls, self-reported function and balance confidence [Cho et al. 2004].

2.3.2. Balance and Falls, Morbidity and Mortality.

Falls are a serious threat to the health and wellbeing of older adults. Among community-dwelling people over the age of 65 years, 30% experienced a fall at least once a year [Berg et al. 1997; Syllias et al. 2009; Tinetti et al.1988], and the incidence of falls increases with age [Sattin 1992; Tinetti and Speechley 1989]. Falling is associated with increased risk of mortality, morbidity, and a loss of function and independence [Bruce et al. 2002; Cumming et al. 2000; Richmond et al. 2002; Sattin 1992]. Four of the most common risk factors for falls are muscle weakness, history of falls, gait deficit, and balance impairments [AGS Panel on Falls Prevention 2001]. Age-associated declines in motor performance that impact these risk factors may lead to a greater incidence of falls in older adults.

Many large studies investigating balance impairments with aging on falls or disability have been inconsistent. In studies incorporating quasi-static balance tests, impaired static balance has been related to falls in some cross sectional studies [Heitman et al. 1989; Lord et al. 1994; Studenski et al. 1991]. However, fall risk is more closely related with dynamic balance [Lord et al. 1994; Nevitt et al. 1989], impaired stepping [Cho et al. 2004; Clark et al. 1993], endurance [O'Loughlin et al. 1993], or mobility [Gabell et al. 1985; Nevitt et al. 1989; Svvenson et al. 1992] than static balance alone [Myers et al. 1996]. Perhaps more predictive of disability and fall risk, is the co-occurrence of balance impairments with other functional limitations [Studenski et al. 1991]. In a cross-sectional analysis of women aged 65 or older, Rantanen et al. [1999c] found that the burden of co-impairments, in this case strength and balance, seems to be greater than the sum of single impairments in predicting future fall-related disability.

2.4 Endurance

According to the NIH Toolbox motor domain, endurance refers the duration that a person can perform an activity at a particular intensity and focuses on overall physical fitness rather than individual muscle endurance (see 2.6 Performance Fatigability for individual muscle endurance). Measures of endurance, therefore, require cardiopulmonary, biomechanical and neuromuscular function [Ruebens et al. 2013].

2.4.1 Endurance Changes with Age.

A few common, functionally relevant measures of endurance for older adults are: time to complete a 400-m walk [Newman et al. 2006; Vestergaard et al. 2009], 1-km walk test [Malmberg et al. 2001], and distance traveled in a 2- [Reuben et al. 2013] and 6-minute walk test [Rikli and Jones 1999]. These tests are not often used to assess function in both young and older adults, and normative data do not currently exist to demonstrate endurance declines from young to older persons (NIH Toolbox anticipated release date Oct 2013). However, walk tests have been used extensively in older adults and average declines of 9.5% per decade from 60-90 years have been observed [Rikli and Jones 1999].

2.4.2 Endurance, Disability, Morbidity and Mortality

Endurance capacity in healthy middle aged and older adults predicts both cardiovascular and total mortality [Blair et al. 1989; Mora et al. 2003]. Endurance as measured by long distance walk performance is associated with mortality mainly in patient populations, such as those with chronic obstructive pulmonary disorder [Jenkins et al.

2007], stroke [Kosak et al. 2005], peripheral artery disease [Montgomery et al. 1998], fibromyalgia [Pankoff et al. 2000], and chronic heart failure [Peeters et al. 1996]. In an investigation of endurance walk performance in the Health, Aging, and Body Composition Study (Health ABC cohort), inability to complete the 400-m walk was associated with greater risk of cardiovascular disease, mobility disability and total mortality 10 years later in relatively healthy community-dwelling adults aged 70-79 years [Newman et al. 2006].

2.5 Fatigue and Performance Fatigability

Fatigue is multidimensional and can be defined in a number of ways: as a subjective feeling that interferes with usual activities, a sense of reduced energy, mismatch between effort expended and actual performance, or physical and mental exhaustion following exertion [Alexander et al. 2010; Enoka and Duchateau 2008; Kluger et al. 2013].

Perceptions of fatigue can be quantified by self-report scales, including momentary or chronic perceptions, physical or mental, and include impact of fatigue on function or severity of fatigue experienced [Kluger et al. 2013]. Additionally, a widely used psychophysical tool to assess fatigue is the rating of perceived effort [Borg 1982] during physical tasks or exercise [Scherr et al. 2013]. Fatigability refers to objective changes in performance [Alexander et al. 2010; Kluger et al. 2013], and will be referred to as performance fatigability in this review for clarity. Performance fatigability is the loss of force-producing capacity or rate of change in some performance metric relative to a reference [Bigland-Ritchie et al. 1978; Enoka 2012]. The distinction between fatigue and performance fatigability is important as perceptions of fatigue are often unrelated to measures of performance fatigability [Lou et al. 2003; Zwarts et al. 2008], and daily

perceptions of fatigue and fatigability may not just be distinct but possibly independent [Kluger et al. 2013; Zwarts et al. 2008].

Although fatigue is a common complaint among older adults, the clinical prevalence of fatigue varies widely across published results with no consistent observable change in self-reported fatigue across ages [Hickie et al. 1996; Lerdal et al. 2005; Stone et al. 2008]. In one large epidemiological investigation, older subjects reporting perceptions of fatigue experienced poorer functional ability, notably on the time to complete a 400-m walk test [Vestergaard et al. 2009]. The fatigue rating was based on two questions evaluating whether participants felt that “ everything was an effort ” and/or they “ could not get going ” on three or more days in the past week. Inconsistent findings are also observed in studies examining the perceived effort during tasks involving performance fatigability [Hutchinson and Tenebaum 2006]. Allman and Rice [2003] compared ratings of perceived exertion (RPE) of young (25 years) and old (84 years) during intermittent voluntary isometric contractions of the elbow flexors. Although all subjects reported a maximal perceived exertion at task failure, the older men reported a greater perceived exertion compared to the young men earlier during the task [Allman and Rice 2003]. However, in a sustained submaximal isometric elbow flexor contraction to task failure, RPE increased similarly for young and old men at the beginning and end of the contraction, but the duration of contraction was longer for the older men and, as such, the rate of increase in RPE was more gradual for the older adults [Hunter et al. 2005].

Changes in performance fatigability with age are similarly inconclusive; some studies report that older adults are less fatigable than young [Kent-Braun 2009; Christie et al. 2011], whereas others find older adults to be more fatigable [Baudry et al. 2007; McNeil

and Rice 2007]. These inconsistent findings of performance fatigability may be due to inherent task dependency. Classically, performance fatigability is quantified as the decline in maximal voluntary contraction force, or power, after performing a demanding activity [Enoka and Duchateau 2008; Gandevia 2001].

Early observations of performance fatigability by Mosso [1906] noted that the mechanisms underlying the declines in maximal force or power production depend on the characteristics of the task used to induce fatigue [Enoka and Stuart 1992]. For example, when strength matched young (18-31 years) and older (67-76 years) men sustained an isometric contraction at 20% of maximal voluntary contraction torque with the elbow flexors until task failure, the contraction could be held longer by the old (22.6 ± 7.4 min) compared with the young (13 ± 5.2 min) despite both groups exerting similar torques and experiencing similar declines in maximal force following the task (Hunter et al. 2005). This result is supported by other studies reporting increased resistance to performance fatigability in older individuals [Ditor and Hicks 2000; Kent-Braun et al. 2002; Lanza et al. 2004]. However, in a protocol incorporating a velocity-dependent power task performance fatigability increased progressively with age [McNeil and Rice 2007]. In this study, young, old, and older men (26, 64, and 84 years, respectively) performed 25 dorsiflexion contractions as fast as possible against a submaximal load, and muscle power decreased by 13% in young, 19% in old, and 24% in older men, indicating that the older men were not resistant to performance fatigability.

Consistent with these diverging examples, a systematic review and meta-analysis by Christie et al. [2011] of performance fatigability revealed that overall, the standardized effect of age on fatigability was positive, indicating that older adults were generally more

resistant to performance fatigability. However, when studies used dynamic contractions or muscle power as the index of performance fatigability, this effect was reversed such that older adults were less resistant to performance fatigability, thus demonstrating the task dependency in age-related changes in performance fatigability [Christie et al. 2011].

Despite these paradoxical findings, the study of performance fatigability is relevant as the ability to maintain force output is a critical aspect of neuromuscular function. Untangling the symptomology of perceptions of fatigue and its associations to fatigability in aging populations could lead to insights into the underlying physiology of idiopathic fatigue.

2.6 Steadiness

2.6.1 Aging and Steadiness.

Aging is accompanied by a remodeling of the neuromuscular system and a decline in the ability to control muscle force. One way to quantify the ability of older adults to control muscle force is to measure the fluctuations in force during a constant-force contraction, which is an index of steadiness. A consistent finding in our laboratory is that the relative force fluctuations during submaximal isometric contractions are often greater for older adults [Galganski et al. 1993; Tracy and Enoka 2002]. Moreover, our laboratory has recently shown that measures of strength and steadiness during an index finger task are good predictors of performance on functional hand tasks, including the Grooved Pegboard Test, which is the standard test of hand dexterity to be used in the NIH toolbox [Marmon et al. 2011]. It has been reported that steadiness during knee extension is reduced in older adults with a history of falls compared with non-falling older and younger adults [Carville et al. 2007]. Additionally, steadiness in the lower extremity is an independent predictor of

performance on some functional tasks [Seyennes et al. 2002], although this may depend on the population or task [Manini et al. 2005].

2.6.2 Aging, Steadiness, and Stressors.

Aging is accompanied by changes in neuroendocrine responses to psychosocial or physical stress, often due to an excessive activation of the hypothalamic-pituitary-adrenal (HPA) axis [Ferrari et al. 2001; Pedersen et al. 2001], which can impair motor performance. Previous work in our laboratory has found that heightened physiological arousal following exposure to a stressor reduces steadiness [Noteboom et al. 2001a; 2001b], and that this effect is even greater in older adults [Christou et al. 2004]. Therefore, aging is accompanied by an adverse response to stressors that can further diminish steadiness during submaximal contractions. This finding is consistent with clinical observations that motor performance deteriorates when older adults are confronted with an environmental stressor. The presence of a stressful psychological or physical event could, therefore, further impair steadiness in older adults who exhibit a higher incidence of falls.

3. The Integrated Motor System

Aspects critical for the motor functions described in the previous section are influenced by multiple integrated physiological systems. With advancing age, changes in these integrated systems will influence functional motor domains relevant to tasks of life. This section primarily explores the neuromuscular system, including motor unit anatomy and morphological adaptations with age. Additional changes that occur with age in

relevant physiological systems that influence motor functions will also be discussed, with focus on the vitamin D hormone system and insulin-glucose dynamics.

3.1 Neuromuscular System

3.1.1 Motor Unit Anatomy

Anatomical features of the motor unit that are critical for function and the control of force production include the number of motor units in the motor unit pool, the number of muscle fibers in each motor unit, and the organization of innervated fibers within the muscle.

The motor unit is the basic functional unit of the neuromuscular system, comprising a single motor neuron and the muscle fibers it innervates [Sherrington 1925]. The motor unit is the smallest quantity of muscle the nervous system can activate [Sherrington 1930], and is referred to as the “final common pathway” in the control of movement [Sherrington 1925]. Muscles are comprised of many motor units, ranging from 10 to 1,500 motor units depending on the muscle [Jenny and Inukai 1983], and the group of neurons innervating a single muscle is known as the motor nucleus, or motor unit pool [Burke et al. 1977].

The number of muscle fibers innervated by a motor neuron, or innervation number, varies within and between muscles and can range from an innervation number of 10 to up to several thousand muscle fibers [Buchthal and Schmalbruch 1980]. As the peak force generated by a single motor unit varies in relation to the innervation number such that the motor units with the largest innervation numbers generate the most force [Kanda and Hashizume 1989], the innervation number of a motor unit is a useful determinant of the force production of a single motor unit. Relatedly, another useful index of force production

is the average innervation number for a whole muscle, which is derived by dividing the number of muscle fibers in a muscle by the number of motor neurons innervating the muscle [Enoka 2008]. In general, muscles with fine force control, such as those in the hand responsible for control of movements related to dexterity, have small average innervation numbers, whereas muscles involving explosive and powerful force production, such as those in the lower extremity, have large innervation numbers [Feinstein et al. 1955].

3.1.2 Age-Related Motor Unit Remodeling

Ample evidence indicates that an age-related neuropathic process leads to the death of α -motor neurons both in animals [Hashizume et al. 1988] and humans [Gardner 1940; Tomlinson and Irving 1977]; consequently the number of functioning motor units in a muscle decreases [McNeil et al. 2005]. As a motor unit is comprised of an α -motor neuron and the muscle fibers it innervates, motor neuron apoptosis initiates a progressive motor unit remodeling process. For example, when a motor neuron dies, the muscle fibers it innervates are subsequently denervated; the abandoned fibers will degenerate if they are not re-innervated by a collateral sprout from a surviving motor unit [Kanda and Hashizume 1989]. The loss of both type I and type II muscle fibers as well as muscle fiber atrophy results in reduced muscle mass and strength. The loss of strength resulting from this remodeling process is often substantial in older adults. Electrophysiological studies using spike-triggered averaging in the elbow flexors of young and old subjects further indicate associations between estimated losses in motor units and reduced contractile strength in older men and women [Doherty et al. 1993]. However, although the estimated number of functioning motor units using motor unit number estimates (MUNE) in the tibialis anterior,

declined across young (27 years), old (66 years) and oldest studied adults (82 years), the maximal isometric muscle strength did not differ between young and old but was reduced in very old subjects. This demonstrated a dissociation between the estimated number of motor units and strength or functional impairment [McNeil et al. 2005; Aagaard et al. 2010], because strength declines across all age groups, not just in older adults over 75 to 80 years [Hunter et al. 2000].

Muscle fiber re-innervation by surviving motor neurons results in many morphological and physiological changes to motor unit properties [Doherty 2003], which could have implications for force production. For example, the size of the motor unit increases as the number of muscle fibers innervated by a given motor neuron increases with re-innervation [Roos et al. 1997]. An age-related increase in innervation number is evidenced by glycogen-depletion in rats [Ansved et al. 1991], and has been estimated in older adults by macro-EMG [Masakado et al. 1994] and spike-triggered averaging [Galganski et al. 1993]. Evidence from the spike-triggered averaging technique by Galganski et al. [1993] demonstrates that the average force contribution by a single motor unit to the whole muscle force during a voluntary contraction is greater in older (29.3 mN) compared with young adults (17.4 mN). The greater spike triggered average force is indicative of the larger motor unit size for older adults, but also shows that peripheral motor unit reorganization results in changes in force production in older adults [Galganski et al. 1993]. Furthermore, the change in innervation number could have consequences for tasks requiring fine control of force or movement, as each recruited motor unit contributes more to the overall force.

3.1.3 Additional Neuromuscular Changes with Age

In addition to changes resulting from motor unit remodeling, other age-related adaptations have been observed in muscle activation, the neuromuscular junction (NMJ), and excitation-contraction coupling.

Accompanying the motor unit remodeling, action potential discharge characteristics are augmented with advancing age [Barry et al. 2007; Erim et al. 1999]. Because the force exerted by a muscle during a voluntary contraction depends on the number of active motor units and the rate at which they discharge action potentials, the ability to modulate the discharge rate is critical for force production. Studies have demonstrated a decline in maximal discharge rates during maximal contractions in older adults [Kamen and Knight 2004; Rubinstein and Kamen 2005], which may contribute to the reduced rate of torque development during ballistic contractions [Klass et al. 2008]. Furthermore, when a single motor unit is tracked, older adults have a reduced range of discharge rate values [Barry et al. 2007], and thus a reduced capacity for discharge rate modulation [Pascoe et al. 2013]. The age-related reduction in rate modulation could be due to changes in either intrinsic properties of motor neurons, such as the afterhyperpolarization phase [Piotrkiewicz et al. 2007], the synaptic inputs to the motor neuron pool or both. Regardless, the augmented capacity for discharge rate modulation demonstrates a change in muscle activation with aging that could lead to changes in neuromuscular function.

Neural adaptations with advancing age occur at many locations from the spinal cord motor neurons to the neuromuscular junction (NMJ). In addition to changes in motor neuron soma size [Kanda & Hashizume 1989], deterioration of the myelin sheath [Ceballos et al. 1999], and axonal atrophy [Verdu et al. 2000], structural and functional alterations at

the NMJ have been noted [Jang et al. 2011]. Some studies have found that aging was associated with morphological changes that were confined primarily to nerve endings with little or no degeneration or loss of primary axons [Chai et al. 2011; Jang and Remmen 2011]. At the NMJ in mice, age-related declines in the number and variation in the size of synaptic vesicles, reduced mitochondrial content and altered mitochondrial shape, and large areas of junctional folds that lacked contact with the respective terminal axon were noted [Boaro et al. 1998]. Despite these morphological changes at the NMJ, a compensatory response may exist to recover increased vesicle recycling and maintain adequate neurotransmitter levels [Fahim and Robbins 1982; Robbins 1992]. Furthermore, Kelly and Robbins [1986] investigated the ability of intact neuromuscular junctions in old animals to maintain a one-to-one ratio of action potential transmission. They found that the safety factor of transmission was not compromised in old animals, as the output of quanta per impulse (the end-plate potential resulting from the release of transmitter) was greater in the old mice, thereby compensating for the reduced number of pre-synaptic vesicles [Kelly and Robbins 1986]. However, the compensatory effect of axonal sprouting and regrowth of functional NMJ's following denervation is drastically decreased in older aged mammals [Jang and van Remmen 2011]. The cumulative effect of the compromised NMJ is likely reduction in the successful reinnervation of denervated muscle fibers increasing the risk of skeletal muscle atrophy.

The excitatory signal transduction continues from the NMJ and leads to muscle fiber action potentials that are conducted to sarcolemmal infoldings called T-tubules. At the T-tubules, the electrical signals are transduced to chemical signals by electro-mechanical coupling of the dihydropyridine receptor (DHPR) and ryanodine sensitive calcium

channels (RyR1) to allow Ca²⁺ release into the intracellular space. This Ca²⁺ mobilization binds to the contractile proteins within muscle to allow for cross-linkage and force production [Delbono et al. 1997], and this entire transduction of electrical-chemical-mechanical transduction process is referred to as excitation-contraction coupling. Alterations in the transmission of membrane depolarization to Ca²⁺ release from the sarcoplasmic reticulum are referred to as excitation-contraction uncoupling, and may be responsible for decreased specific muscle fiber force [Payne and Delbono 2004]. Existing literature suggests that, with age, the DHPR are particularly susceptible to oxidative stress and decline in function and/or number, especially when considered in comparison to RyR1, which could result in excitation-contraction uncoupling [Renganathan et al. 1998]. Uncoupling of excitation-contraction is one of the major factors contributing to the age-dependent decline in the force generating capacity of individual muscle cells, although the results on functional control of force production are unknown [Delbono 2003].

3.2 Systemic Factors Influencing Motor Function

While loss of muscle mass and fiber atrophy due to remodeling processes are largely responsible for declines in function, other hormonal, metabolic, immunologic, and molecular factors may also contribute to impaired functional performance with aging. In this section, systems that exhibit concurrent changes in function that may impact the neuromuscular system and influence motor function will be reviewed. These include vitamin D regulation, insulin-glucose dynamics, and a brief overview of additional hormonal and systemic changes with age that could impair motor functions.

3.2.1 Vitamin D Hormone System

The vitamin D steroid hormone system has classically been associated with intestinal calcium absorption, normal growth and development, and skeletal integrity [Bischoff-Ferrari et al. 2004a; Cashman et al. 2007; Parfitt et al. 1982]. However, it is now evident that vitamin D has actions beyond bone health [Holick 2007; Bischoff-Ferrari 2008], and vitamin D deficiency appears to be associated with negative health consequences such as dental complaints [Krall et al. 2001; Dietrich et al. 2005], cardiovascular diseases [Schleithoff et al. 2006; Zitterman 2006; Pfiefer et al. 2001; Krause et al. 1998], type II diabetes [Knekt et al. 2008; Pittas et al. 2007], common cancers [Garland et al. 2007; Gorham et al. 2007], and autoimmune disorders [Merlino et al. 2004; Munger et al. 2006; Hypponen et al. 2001].

Vitamin D deficiency is a significant public-health concern, particularly among older adults [Allain and Dhesi 2003; Bischoff-Ferrari et al. 2004a; Gloth 1995; Hamilton 2010; Norman and Bouillon 2010]. Vitamin D deficiency negatively impacts long-term health [Bischoff-Ferrari 2010; Boucher et al. 1995; 1998; Ford et al. 2005; Holick 2007; Semba et al. 2000; 2009; Visser et al. 2006] by reducing quality of life and increasing the risks of disability and mortality [Baumgartner et al. 1998; Buchman et al. 2007; Rantanen et al. 1999; Rolland et al. 2008]. Vitamin D deficiency, for example, is associated with multi-system dysfunction and results in muscle weakness, functional deficits, and an increased risk of falls [Bischoff 1999; Bischoff-Ferrari et al. 2004b; Faulkner et al. 2006; Glerup et al. 2000; Janssen et al. 2002; Pfiefer et al. 2002; Wicherts et al. 2007], which is of great clinical significance as each year one in three people aged 65 years or older experiences at least

one fall, with 9% of falls leading to an emergency room visit [Blake et al. 1988; Graafmans 1996; Tinetti et al. 1988].

Vitamin D deficiency likely impairs motor performance in both skeletal muscle and motor neurons (Figure 1). These changes are mediated genomically [Boland 1986; Ceglia 2008], as evidenced by the presence and function of vitamin D receptors (VDRs) in both skeletal muscle [Bischoff et al. 2001; Boland 1985; Simpson et al. 1985] and motor neurons [Stumpf et al. 1988], and via rapid non-genomic effects [Fleet 2004]. The primary mode of action of vitamin D is to improve the Ca²⁺ handling system to provide neuroprotection in motor neurons [Alexianu et al. 1998; Brewer et al. 2001; Garcion 2002], and calcium kinetics, excitation-contraction coupling, and contractile properties in muscle fibers [Bauman et al. 1984; Glerup et al. 2000; Pleasure et al. 1979; Rodman and Baker 1978].

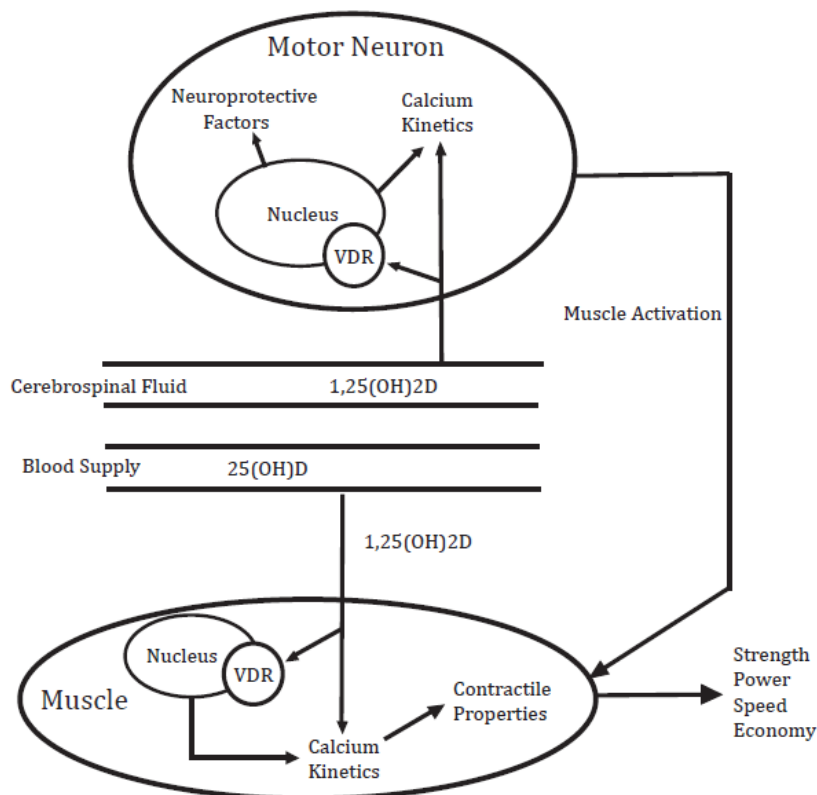


Figure 1. Model of the mechanisms underlying the influence of circulating vitamin D (25(OH)D) on neuromuscular function. The vitamin D metabolite 1,25(OH)₂D acts via genomic and non-genomic mechanisms in motor neurons and muscle fibers to augment calcium kinetics and modulation of neuroprotective factors. These adaptations modulate the contractile properties of muscle fibers and the functional capacity of motor neurons, thereby influencing such features of physical function as muscle strength and power, contraction speed, and the energetics of muscular work.

One of the purported consequences of vitamin D deficiency is a decline in neuromuscular function, particularly in older adults [Annweiler et al. 2009; Carlson & Kenny 2007; Janssen et al. 2002; Pfeifer et al. 2002]. The evidence, however, is somewhat equivocal. Cross-sectional studies of associations between 25(OH)D₃ and physical performance, for example, report either no statistically significant associations [Annweiler et al. 2009] or, at best, modest and mixed associations [Bischoff et al. 1999; 2000; Bischoff-Ferrari et al. 2004b; Gerdhem et al. 2005; Hicks et al. 2009; Houston et al. 2007; Rinaldi et al. 2007]. Similarly, longitudinal studies found that declines in performance were only modestly associated with 25(OH)D levels [Dam et al. 2009; Verreault et al. 2002; Visser et al. 2003; Wicherts et al. 2007], even when participants were given vitamin D supplements [Janssen et al. 2002; Verhaar et al. 2000]. However, many of the existing cross-sectional and longitudinal studies have focused mainly on the circulating form of vitamin D, 25(OH)D rather than the biologically active and hormonally regulated metabolite 1,25(OH)₂D. The few studies that have examined 1,25(OH)₂D have indicated associations between the hormonally regulated metabolite and muscle strength and function even in the absence of an association with 25(OH)D [Bischoff et al. 1999; Dukas et al. 2005; Marantes et al. 2011]. The one intervention to administer alphacalcidol, the form of vitamin D that is readily metabolized to 1,25(OH)₂D, demonstrated substantial improvements in motor functions such as knee extensor strength and 2-min walk time over the 6-mo intervention, despite only minimal increases in circulating 25(OH)D (7.3 ± 0.4 to 11.1 ± 0.08 ng/ml, both considered deficient levels) [Verhaar et al. 2000].

3.2.2 Insulin-Glucose Dynamics

Among the age-associated comorbidities that accompany decreases in motor function, diabetes and pre-diabetic insulin resistance have emerged as significant predictors of frailty and disability in older U.S. adults [Abbetacola and Paolisso 2008; Gregg et al. 2000]. For example, glucose abnormalities are associated with poor physical function in diabetic [Andersen and Jakobsen 1999; de Rekeneire et al. 2003; Giacomozzi et al. 2008] and insulin resistant adults [Abbetacola et al. 2005; Chen et al. 2008]. Prospective studies indicate that weak grip strength may predict the development of insulin resistance, especially among those at risk for diabetes [Chen et al. 2008; Lazarus 1997], although most of these associations were noted in large epidemiological studies and included people with overt diabetes. In an investigation of non-diabetic older adults, Barzilay et al. [2009] found that knee extensor strength relative to muscle mass was negatively associated with insulin resistance as assessed by the homeostasis model assessment of insulin resistance (HOMA-IR) in non-diabetic adults. Similarly, Kuo et al. [2009] found an association between habitual gait speed and HOMA-IR in non-diabetic men, but not in women.

The mechanisms underlying this association are uncertain. For example, an increase in fasting blood insulin, consistent with insulin resistance, could be explained by a decrease in skeletal muscle mass, a reduction in peripheral sensitivity to insulin, or a combination of both adaptations [Abbatecola et al., 2005; Lazarus et al., 1997]. The potential interactions include impairment in muscle glucose handling that reduces intracellular energy production and contributes to muscle weakness, and disturbances in insulin regulation that reduces protein synthesis, total body protein turnover, and body

protein mass, especially in the elderly [Fukagawa et al., 1988]. Thus, it is unclear if reduced insulin sensitivity is a contributor to or a result of age-related reductions in motor function.

3.2.3 Hormonal, Inflammatory and Metabolic Factors

Additional hormonal mediators. The relations between hormonal mediators of systemic, and possibly motor functions, such as testosterone, estrogen, IGF-1, and DHEA, and loss of strength and mass with age has been widely explored. Ample evidence demonstrates that serum levels of testosterone and the adrenal androgens decline with age [Tenover 1997; Tenover et al. 1987], and epidemiological evidence supports the relation between the drop in testosterone (and possibly estrogen through its conversion to testosterone) and declines in muscle mass, strength and functional status [Baumgartner et al. 1999; Perry et al. 2000; Roubenoff and Hughes 2000]. Furthermore, dehydroepiandrosterone (DHEA) and DHEA-sulfate levels decline markedly with age, which theoretically should contribute to changes in muscle mass and strength, as it can be converted to the potent androgen testosterone [Welle 2002]. In addition, levels of growth hormone and IGF-1 decline with age [Morley 1999], and IGF-1 have known anabolic effects, as well as neurotrophic effects to enhance re-innervation of muscle fibers [Butterfield et al. 1997; Caroni et al. 1994]. Therefore, the declining levels of these hormonal factors could lead to a reduction in anabolism and lean mass to an indirect catabolic effect on muscle [Doherty 2003].

Immunologic Factors. Aging is associated with chronic low-grade inflammation, characterized by increases in circulating pro-inflammatory mediators [“inflammaging” or “immunosenescence”; Franceschi 2007a; 2007b; Bruunsgaard et al. 2001; Krabbe et al.

2004]. Tumor necrosis factor- α (TNF- α), CRP and IL-6 predict all cause mortality in older persons [Bruunsgaard et al. 2003]. Also, in older adults low serum IL-6 is predicts disability [Ferrucci et al. 1999], and is associated with faster walk times [Blain et al. 2012], and greater grip strength [Peirera et al. 2009]. Paradoxically, experimental investigations have not been able to link IL-6 to loss of muscle mass or strength [Krabbe et al. 2004] possibly because IL-6 may just be a surrogate marker of pro-inflammatory status. The cytokine TNF- α , however, has been demonstrated experimentally to promote catabolism in mature muscle cells and induce apoptosis, that likely contribute to muscle wasting [Roubenoff 2003].

Metabolic Factors. Damaging agents are produced as a consequence of normal, metabolic processes, such as reactive oxygen species (ROS) from oxidative metabolism, and ROS accumulation [Harman 1956] could play a key role in triggering dysfunction the neuromuscular system, thereby contributing to a loss in motor function with age [Fulle et al. 2004]. Epidemiological investigations have determined that oxidative protein damage is associated with functional dependence in older adults [Gonzalo-Calvo et al. 2012], and oxidative damage as determined by biomarkers of lipid peroxidation is a predictor of mobility disability (difficulty walking 400 m or climbing 10 steps) as well as mortality over 1 year [Cesari et al. 2012].

4. Translational Models of Age-Related Declines in Function

Animal models of aging are extremely useful research tools. Relying exclusively on human subjects for aging research is complicated by long natural lifespan, environmental

influences, ethical issues, and other limiting factors [Vanhooren and Libert 2013]. In many model organisms, genetic background, diet, environment and health status can be strictly controlled. However, while controlling for intrinsic and extrinsic influences, the model species must also mimic the biological and behavioral changes that occur with age to realistically translate to human aging.

4.1.1 Healthspan versus Lifespan in Animal Models of Aging

Advances in the mechanistic understanding of aging have been made using experimental animal models, including research into interventions and genetic mutations that successfully enhance survival [Vanhooren and Libert 2013]. However, in some cases, lifespan may be enhanced at the expense of overall function; therefore, rather than extending lifespan at all costs, recent advances in aging research have begun trying to characterize and improve healthspan [Carter et al. 2012]. Healthspan is the portion of the lifespan during which an individual is able to maintain good health, where health is defined as the ability of a system to maintain or return to homeostasis in response to challenges [Tatar 2009]. Healthspan also assumes that function is sufficient to maintain independence, productivity and wellbeing [Kirkland 2013]. Thus there is a drive in the basic biology of aging and age-related interventions to develop biomarkers of healthspan in animal models for improved translations to clinical applications [Kirkland 2013; Kirkland and Peterson 2009; Tatar 2009]. As physical functioning is a key determinant of good health and independence in humans (see section 3. Motor Functions and Age-related Dysfunction), behavioral outcomes are being increasingly recognized as important healthspan biomarkers [Carter et al. 2012].

4.1.2 The Rodent as an Aging Animal Model

Rodent models are often used in aging research for many practical reasons: the husbandry is well-established, they are good breeders, develop quickly (~2-3 months) and exhibit relatively short lifespans for mammalian species (~2-4 years). Other experimental advantages include the genetic standardization of certain species as well as similarities to humans in many aspects of the aging phenotype [Ingram 2000]. The Fischer 344 x Brown Norway rat (F344BN) has been used extensively as a model organism given several conceptual similarities between age-related changes observed in this strain and those observed in humans [Carter et al. 2012]. For example, assessment of functional limitations near the 50% survival range (i.e., “mid-life”) is highly predictive of mortality in both humans [Rantanen et al. 1999] and F344BN rats [Carter et al. 2002], and changes in body composition across the lifespan mirror those observed in humans [Carter et al. 2012].

Despite the common use of rats, mice are increasingly used to investigate the impact of the aging process in different organ systems including bone [Brochmann et al. 2003], skeletal muscle [Hamrick et al. 2006], and kidney [Yabuki et al. 2006]. Mice are currently the most commonly used rodent model for laboratory experiments [Rosenthal and Brown 2007], and of these, the C57BL/6 mouse is the most widely used inbred strain and was the first to have its genome sequenced. Importantly, many age-associated changes that occur in humans also occur in C57Bl/6 mice, including age-related adaptations in basic metabolic status, body composition, hemodynamic measures, and activity levels [Parks et al. 2011]. Similar to the rat, C57Bl/6 mice also exhibit age-related impairments in motor functions

and physical activity [Ingram 1983; 2000] and the degree of functional impairment at 50% survival is predictive of lifespan [Fahlström et al. 2012; Ingram and Reynolds 1986].

5. Summary

In summary, the health and functional status of older adults is multifactorial and motor function must be examined with respect to different tasks or subdomains. It is well established that aging from maturity to senescence is associated with significant declines in neuromuscular function. These age-related declines ultimately lead to a progressive decline in functional status and adverse health outcomes including disability, reduced healthspan and mortality. Identifying underlying neuromuscular, hormonal, immunological, and metabolic factors associated with or leading to loss of function could lead to targets for interventions to improve healthspan, and could translate to clinical practice and community health. The projects outlined in this dissertation reinforce the importance of assessing of multiple tasks and subdomains when characterizing motor function with advancing age in people and animals. The first project investigated the task dependence of performance fatigability, while the second examined the influence of age on fatigability and motor functions. The influence of metabolic and hormonal factors on motor functions in older adults was explored in the third project. Finally, translation of motor function measurements to an animal model of aging was developed in the final project. Together, this literature review and the following four projects describe the measurement, translation and effects of age on motor function.

Chapter II

**Muscle activity differs with load compliance during fatiguing contraction with the
knee extensor muscles**

Abstract

The purpose of the study was to determine the influence of load compliance on the time to failure and rate of change in electromyographic (EMG) activity when the knee extensor muscles performed fatiguing contractions against submaximal loads. The low-compliance condition required the subject to exert a force against a rigid restraint (force control), whereas the high-compliance condition involved maintaining the knee joint angle while supporting an equivalent inertial load (position control). Both contractions were sustained for as long as possible. Each subject exerted a similar net torque about the knee joint during the force and position tasks; the target force corresponded to a force at the ankle equal to 20% of the maximum voluntary contraction force (MVC) force. Thirteen healthy adults (25 ± 7 year) participated in the study. MVC forces before the force and position tasks were similar (189 ± 40 N vs. 179 ± 43 N, $p = 0.4$), and the target force was 36 ± 8 N. The time to task failure was longer for the force task (224 ± 114 s) than for the position task (110 ± 36 s, $p < 0.05$), but MVC force declined to a similar level immediately after task failure for the two tasks ($-31 \pm 16\%$). The briefer time to failure for the position task was accompanied by greater rates of increase in agonist EMG amplitude and the pressor response. Coactivation ratios, in contrast, were similar for the two tasks and did not contribute to task differences in time to failure. These findings indicate that it was more difficult to sustain a submaximal contraction with the knee extensor muscles when the task required position control, despite comparable net muscle torques for the low- and high-compliance tasks.

Introduction

The mechanisms responsible for the decline in force during fatiguing contractions depend on the details of the task being performed, including the compliance of the load supported by the limb and the posture of the limb [Enoka and Duchateau 2008]. In a seminal study on the influence of load compliance, Hunter et al. [2002] found that the time to task failure for a submaximal contraction performed with the elbow flexors was twice as long when subjects exerted a constant force against a rigid restraint (force task) compared with maintaining the position of an equivalent inertial load (position task). Similar differences in time to failure for the force and position tasks have been reported for the elbow flexors [Klass et al. 2008; Rudroff et al. 2007a, 2007b], first dorsal interosseus [Maluf et al. 2005], wrist extensors [Baudry et al. 2011], and the dorsiflexor muscles [Hunter et al. 2008]. Despite each subject exerting a similar net muscle torque during the two tasks, Hunter et al. [2002] observed that the briefer time to failure for the position task was accompanied by greater rates of increase in indirect measures of central neural activity, and subsequent studies found that the difference in time to failure was not attributable to a difference in the amount of antagonist coactivation during the two tasks [Hunter et al. 2008; Rudroff et al. 2009].

Not all studies, however, have found a difference in the relative times to failure for the force and position tasks. Rudroff et al. [2007a, 2007b] found that the time to failure for the force task with the elbow flexors was longer than that for the position task when the forearm was either horizontal and neutral or vertical and supinated, but not when the forearm was vertical and neutral. Similarly, the interaction between load compliance and limb posture has produced mixed effects on time to task failure and muscle activation for

the knee extensor muscles [Justice et al. 2008; Rochette et al. 2003]. The current study is the first to compare the times to failure when the force and position tasks were performed with the functionally important knee extensor muscles. The purpose of the current study was to determine the influence of load compliance on the time to task failure and rate of change in EMG activity when the knee extensor muscles performed fatiguing contractions against submaximal loads. The hypothesis was that the time to failure for the position task would be briefer than that for the force task and this would be accompanied by greater rates of increase in EMG amplitude and other indices of neural activity.

Methods

Thirteen healthy adults (25 ± 7 years, 9 men) participated in the study. All subjects completed a general health screening and none of the subjects reported any neurological or cardiovascular disorders or history of knee injuries. All subjects provided informed, written consent before participating in the study. The Human Subjects committee at the University of Colorado approved the protocol.

Experimental arrangement

Each subject participated in three experimental sessions. The first was a familiarization session in which the subject visited the laboratory and was introduced to the equipment and procedures. The next two sessions involved performing the force and position tasks with the knee extensor muscles (Figure 2). The force task required the subject to maintain a force that was equal to 20% of the maximal voluntary contraction (MVC) force exerted at the ankle. The position task involved maintaining a target knee

joint angle while supporting an inertial load that was equivalent to the 20% MVC force. Both tasks were sustained for as long as possible. The net torque about the knee joint exerted by each subject was similar for the force and position tasks. Subjects were provided with visual feedback of a target force (1% MVC/cm) during the force task and target knee angle ($1^\circ / \text{cm}$) during the position task using a custom-made LabView program (version 8.2, National Instruments, Austin, TX). The visual-gain settings resulted in a similar amount of on-screen movement of the cursor caused by the typical fluctuations observed during the force and position tasks, respectively [Mottram et al. 2006].

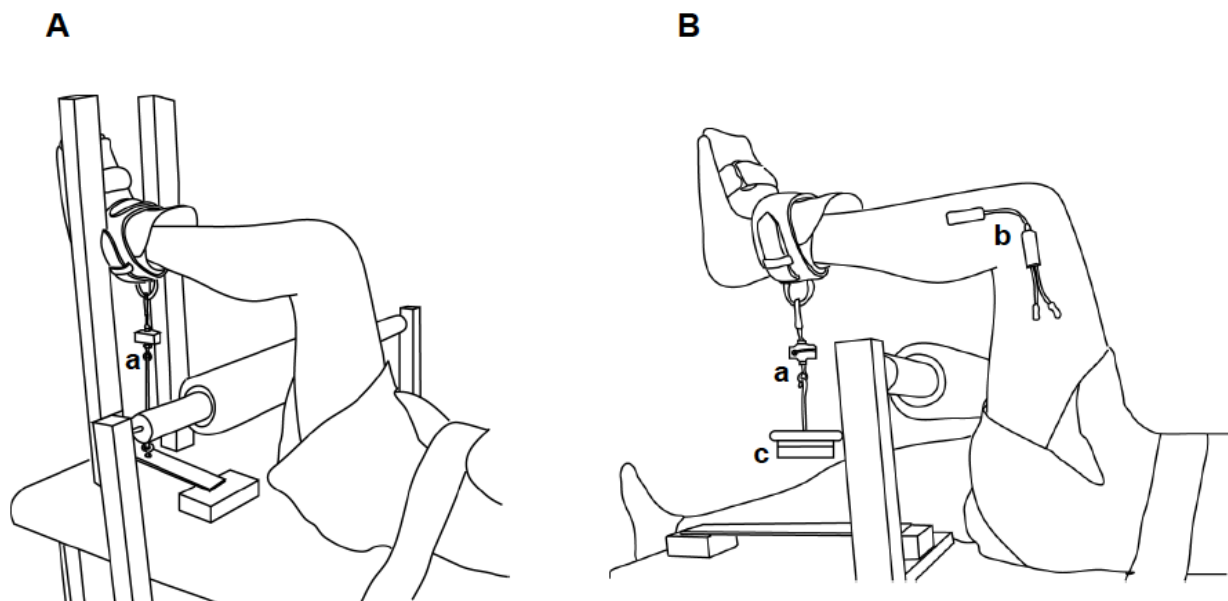


Figure 2. Schematic drawings of the subject placement and respective loads for the force (**a**) and position (**b**) tasks. The hip and knee joints of the left leg were at right angles (1.57 rad). The foot and lower leg were placed in an orthosis and attached to a force transducer (*a*) that was in series with the load in both tasks. The position task involved supporting an equivalent inertial load (*c*) suspended from the ankle. Knee angle was measured with a goniometer (*b*) during the position task.

Both tasks were performed in a supine posture to limit the influence of accessory muscles, especially those in the upper body. The two fatiguing contractions were performed at the same hip- and knee-joint angles (1.57 rad). The subject lay on a padded

treatment table with the right leg extended and the left hip and knee flexed to 1.57 rad. A padded bar was positioned behind the thigh approximately 15 cm below the knee and the subject was instructed to use the bar as a reference for the required hip-joint angle. A Velcro strap was placed around the waist to stabilize the subject.

The left foot and ankle were placed in a walking brace (SP Walker, Aircast, Vista, CA) and a leather strap was wrapped around the brace just above the ankle to connect the load to the leg. A strain gauge (300 lb range, MLP-300, S/N 234017, Transducer Techniques, Terneucula, CA) was placed in series between the ankle and the load (a in Figure 2). The cable connecting the ankle to the rigid restraint for the force task was adjustable to achieve the desired knee joint angle. Knee joint angle during the position task was measured with an electrogoniometer (SG110 and K800, Biometrics Ltd, Cwmfelinfach, Gwent, UK) secured to the lateral aspect of the knee joint (b in Figure 2b). The output of the goniometer was recorded, displayed on a monitor, and stored on a computer. The inertial load (20% MVC force) for the position task was suspended from the ankle at the same location that the restraint was applied during the force task.

EMG signals were recorded with bipolar surface electrodes (Ag-AgCL, 8-mm diameter; 20-mm distance between electrodes) that were placed over the vastus medialis oblique, vastus medialis longus, rectus femoris, vastus lateralis, and biceps femoris. The electrodes were attached according to landmarks between the innervation zone and the end of the tendon [Rainoldi et al. 2004, 2007], band-pass filtered (13-1,000 Hz; Coulbourn Instruments, Allentown PA) and recorded on a computer. The force, position, and EMG signals were digitized at 1,000 samples/s.

Heart rate and mean arterial pressure (MAP) were recorded at 200 samples/s during the sustained, submaximal contractions with an automated beat-by-beat blood pressure monitor (Finapres 2300, Ohmeda, Madison, WI). The blood pressure cuff was placed around the ring finger of the right hand, and the arms were crossed over the chest so that the hand was at heart level. Additionally, the rating of perceived exertion was measured with a modified Borg 10-point scale [Borg 1982]. The subjects were instructed to focus the assessment of effort on the knee extensor muscles performing the task. The scale was anchored so that one represented a resting state and 10 corresponded to the strongest contraction that the knee extensor muscles could perform.

Experimental protocol

The two experimental sessions included the following measurements: (1) MVC force and maximal EMG for the knee extensor muscles; (2) maximal EMG for biceps femoris performed in the same experimental apparatus; and (3) time to failure for the sustained submaximal contraction and subsequent MVC. The order of the two fatiguing contractions was counterbalanced across sessions. Subjects were not informed of the times to failure until the two sessions had been completed.

MVC Force

Each subject performed several MVC trials with the knee extensor muscles. The MVC task comprised a 3-s increase in force from zero to maximum with the maximal force held for 2-3 s, while subjects were verbally encouraged to achieve maximal force. There was a 60- to 90-s rest between trials. When the peak forces achieved in two of the three

trials differed by >5%, additional MVCs were performed until this criterion was met. The greatest force achieved by each subject was taken as the MVC force and used as the reference value to calculate the target force for the fatiguing contraction.

Fatiguing contractions

Subjects were required to sustain each contraction at 20% of MVC force until task failure. The criteria for terminating the force task were 5 s of not being able either to sustain the force within 5% of the target value or to maintain the target hip angle (1.57 rad) due to pushing against the padded support. Similarly, the position task was terminated when the subject was unable either to maintain the knee angle within 0.17 rad of the target value or to sustain the target hip angle (1.57 rad) without internally or externally rotating the hip for 5 s. The required postures were monitored by visual observation, and feedback was provided by the same investigator for all experiments. The knee extensor force and joint angle signals were monitored using LabView (version 8.2, National Instruments, Austin, TX), and a visual signal appeared on the monitor when the signals departed from target values for 5 s.

Data analysis

All data were analyzed off-line using the Spike2 data analysis system (Cambridge Electronic Design, Cambridge, UK) and MATLAB (version 7.2, R2006a, MathWorks, Natick, MA). Heart rate and MAP during the fatiguing contractions were quantified as 10-s averages at 20% increments of task duration. The blood pressure signal was analyzed in 10-s intervals for mean systolic blood pressure (SBP), mean diastolic blood pressure (DBP),

and the number of pulses per second to determine heart rate. MAP was calculated as $MAP = DBP + 1/3 (SBP-DBP)$. MAP, heart rate, and the scores for rating of perceived exertion were plotted as a function of absolute time.

The steadiness of the contraction was quantified as the coefficient of variation (CV) for the force measured with the same force transducer (a in Figure 2) during both tasks. Values were averaged over 10-s intervals. The rate of change in MAP, heart rate, and CV for force during each task was quantified by the slopes of a linear or exponential fit to the data for individual trials, whereas the change in rating of perceived exertion was quantified by the slope of an exponential fit to the data.

The maximal EMG for the knee extensor muscles was calculated as the average value over a 0.5-s interval about the peak force during the MVC with the knee extensor muscles, whereas the maximal EMG for the biceps femoris was calculated as the average value over a 0.5-s interval about the peak rectified EMG during maximal knee flexion. The maximal EMGs were recorded in the same experimental setup prior to the fatiguing contraction in each session. The EMG activity of the knee extensors and flexors during the fatiguing contractions was quantified by averaging the rectified EMG (aEMG) over the first and last 10 s of task time and over 10-s intervals centered about the 20, 40, 60, and 80% time points. The EMG values were normalized to the aEMG obtained during the MVC. The rate of change in aEMG during each task was quantified by the slope of a linear fit to the data for individual trials.

Coactivation ratios for the hamstrings and quadriceps femoris (biceps femoris/knee extensors) muscles were quantified by dividing the averaged, rectified and normalized

EMG values of the biceps femoris (antagonist) by that for the quadriceps muscle group (agonist) at start, 20, 40, 60, 80% and end time points.

In addition, a Fourier analysis was performed on the interference EMG signals [Neto and Christou 2010]. Autospectral analysis of the EMG signals was obtained using Welch's average periodogram method with a non-overlapping Hanning window (MATLAB). The length of the data segment was 10-s and the sampling frequency was 1kHz. The window size was 1,024, which gave a resolution of ~ 1 Hz. The spectral data for the EMG signal of each knee extensor muscle were divided into 0-10, 10-29, and 30-60 Hz frequency bands and analyzed over the first and last 10 s of task time and over 10-s intervals centered about the 20, 40, 60, and 80% time points. The power (%) in each band was expressed as a percentage of the total power from 0 to 300 Hz.

Statistical analysis

Prior to comparing each dependent variable the normality of the data was confirmed with the Kolmogorov-Smirnov test. The independent variables were the time to task failure, MAP, heart rate, rating of perceived exertion, coefficients of variation for force (CV), and aEMG activity. Independent *t*-tests were used to compare time to task failure and percent decline in MVC forces across the fatigue tasks. Two-factor ANOVAs (task \times time) with repeated measures on the two factors were used to compare the CV of force, coactivation ratios, MAP, heart rate and rating of perceived exertion. A three-factor ANOVA (task \times muscle \times time) with repeated measures on all factors was used to compare the aEMG of the knee extensors (vastus medialis oblique, vastus medialis longus, rectus femoris, and vastus lateralis) and biceps femoris during the submaximal contractions.

Another three-factor ANOVA (task × muscle × time) with repeated measures on all factors was used to compare the power spectra of the interference EMG in the three frequency bands (0-10, 10-29, and 30-60 Hz) for the knee extensors. Paired and unpaired *t*-tests with Bonferroni corrections were used as post hoc tests of differences among pairs of means when appropriate. Linear regression analyses were performed between rates of increase in low-frequency oscillations in the interference EMG of each knee extensor muscle and CV for force in both tasks. A significance level for all statistical tests was set at $p \leq 0.05$. Data are reported as means ± SD within the text and displayed as means ± SE in figures.

Results

MVC force did not differ before beginning the force and position tasks (189 ± 40 N and 179 ± 43 N, $p = 0.4$), which resulted in a similar target force (36 ± 8 N) for the two tasks. Time to failure was longer for the force task (224 ± 114 s) than for the position task (110 ± 36 s, $p = 0.0015$). In contrast, the decline in MVC force immediately after task failure was not different for the force and position tasks ($-31 \pm 17\%$ and $-30 \pm 15\%$, $p = 0.12$).

EMG amplitude

The EMG activity for all knee extensor muscles (Figure 3) increased during the fatiguing contraction (time main effect, $p < 0.001$) and did not differ between tasks (task × muscle, $p = 0.5$). The EMG did not differ in the first 10-s of the force and position tasks (34.5 ± 8.8 and 35.9% MVC, $p = 0.84$), but the rate of increase in EMG amplitude was faster during the position task ($0.13 \pm 0.15\%$ MVC/s) compared with the force task ($0.06 \pm 0.14\%$ MVC/s, $p = 0.01$).

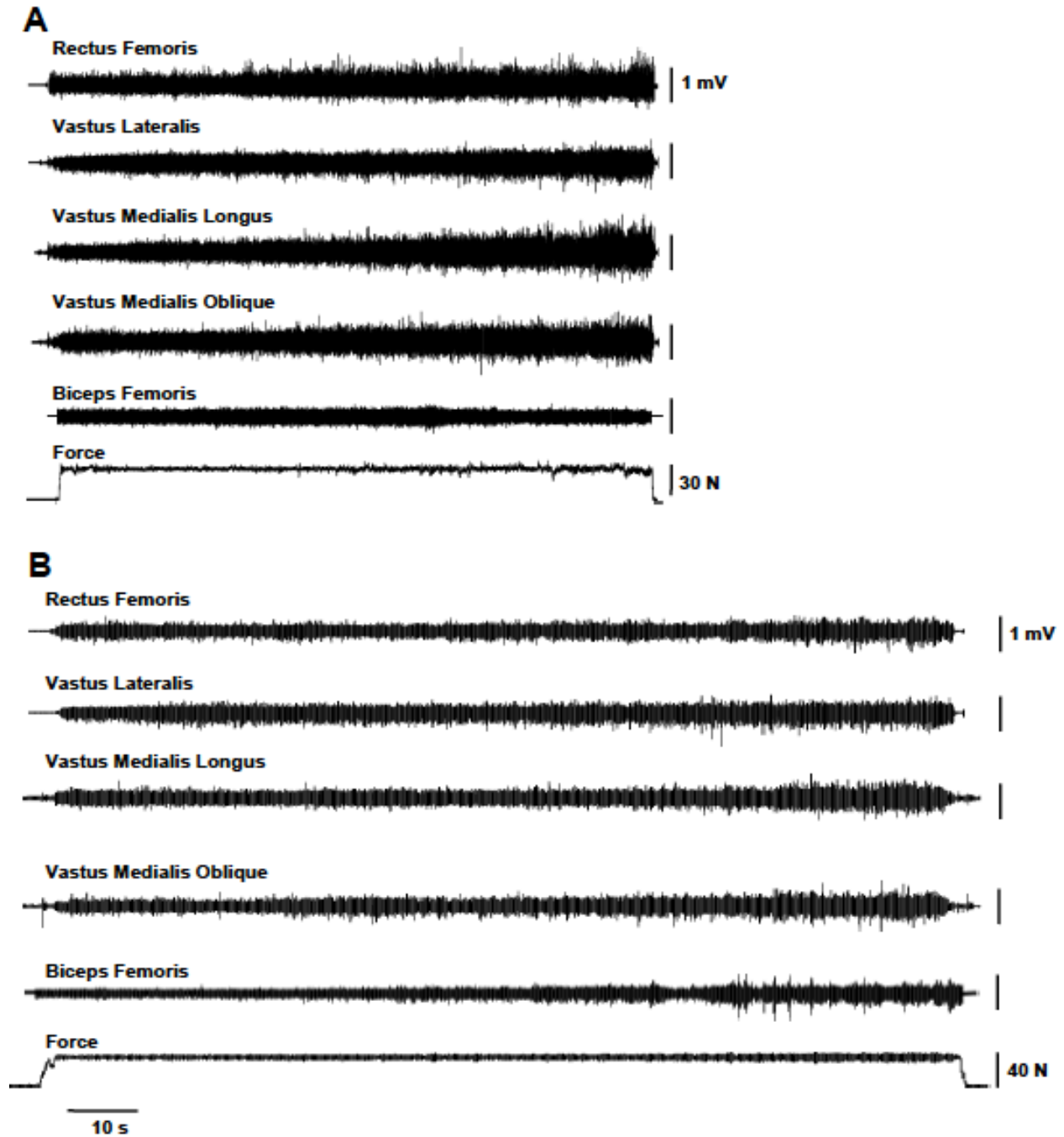


Figure 3. Representative data for a position task (a) and a force task (b) performed by one of the participants. The interference EMG of the knee extensor muscles (first four traces) increased progressively throughout the contraction, whereas the amplitude of the EMG signal for the knee flexor muscle (fifth trace) was more variable. The average force (bottom trace) remained constant until the contraction approached task failure.

The aEMG activity for biceps femoris (antagonist) increased with time (time main effect, $p = 0.01$) during both the force (11.6 ± 8.9 to $16.1 \pm 10.6\%$ MVC) and position tasks (11.9 ± 12 to $16.7 \pm 14.3\%$ MVC) (Figure 4). The amount of increase in aEMG for biceps femoris did not differ for the two tasks (task \times time, $p = 0.9$). The coactivation ratios [aEMG (%MVC) for biceps femoris / knee extensors] did not change during the sustained contractions and were comparable between tasks at each time point during the two contractions (force task, start: 0.34 ± 0.27 , end: 0.37 ± 0.35 ; position task, start: 0.33 ± 0.28 , end: 0.35 ± 0.3) (task \times time, $p = 0.5$).

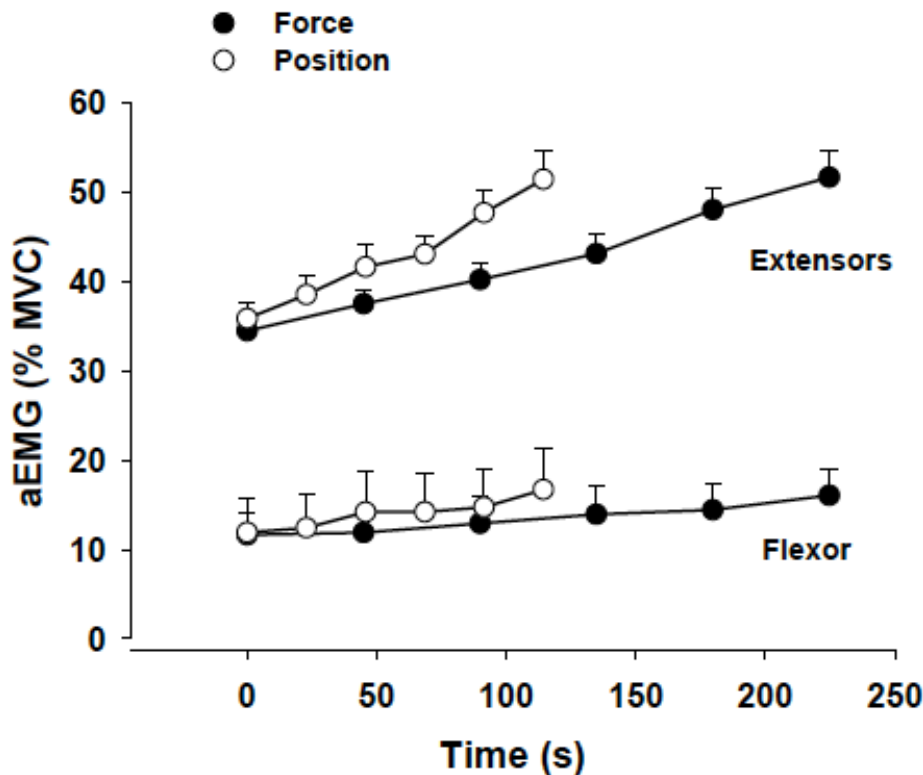


Figure 4. Mean \pm SE for average EMG amplitude (aEMG, normalized to the peak MVC value) for the knee extensor muscles (vastus medialis longus, vastus medialis obliquus, vastus lateralis, rectus femoris) and the knee flexor muscle (biceps femoris) during the two fatiguing contractions. *Filled symbols* indicate the force task and *open symbols* denote the position task. The aEMG was averaged over 10-s intervals for each task at 6 time points that correspond to the absolute times at start, 20, 40, 60, 80, and 100% of time to task failure.

Spectral analysis

The normalized power in the three frequency bands for the interference EMG of all knee extensor muscles (Figure 5) increased during the fatiguing contractions (time main effect, $p < 0.001$). A task \times frequency band \times time interaction ($p = 0.006$) indicated that power in the 0-10 Hz band was higher during the position task than the force task at each time point during the fatiguing contraction (post hoc tests, $p < 0.04$) (Fig 5a), whereas there was no statistical difference between tasks for the power in the 10-29 Hz and 30-60 Hz bands (post hoc tests $p > 0.3$) (Figure 5b, c).

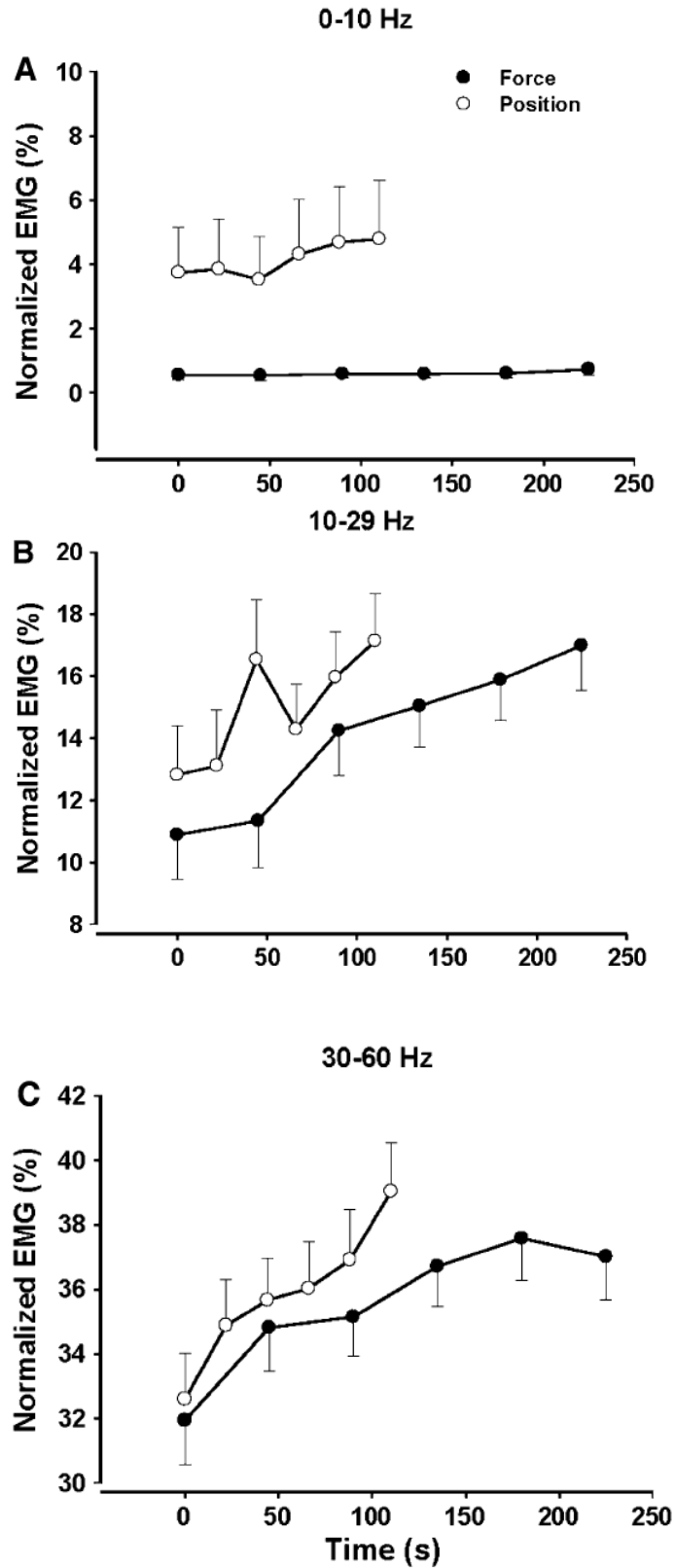


Figure 5. Averaged frequency data of the interference EMG signal for the knee extensor muscles in 0-10 Hz (a), 10-29 Hz (b), and 30-60 Hz (c) frequency bands. The data in each band were normalized to the total power in 0-300 Hz. Data were analyzed over the first and last 10 s of task time and over 10-s intervals centered about the 20, 40, 60, and 80% time points.

Fluctuations in force

The rate of increase in the coefficient of variation for force was not different during the force ($0.048 \pm 0.034\%/s$) and position tasks ($0.038 \pm 0.047\%/s$, $p = 0.58$). Due to the longer time to failure for the force task, however, the coefficient of variation for force was significantly greater at the end (80 and 100% of task duration) of the force task ($11.3 \pm 6.7\%$ and $13.7 \pm 7.6\%$) compared with the position task ($5.7 \pm 2.5\%$ and $8.5 \pm 5.6\%$) (post hoc tests $p < 0.05$) (Figure 6). No significant associations were found between the rates of increase in the low-frequency band of the interference EMG for any of the knee extensors and the rates of increase in CV during either the force task ($r^2 < 0.3$, $p > 0.1$) or position task ($r^2 < 0.25$, $p > 0.1$).

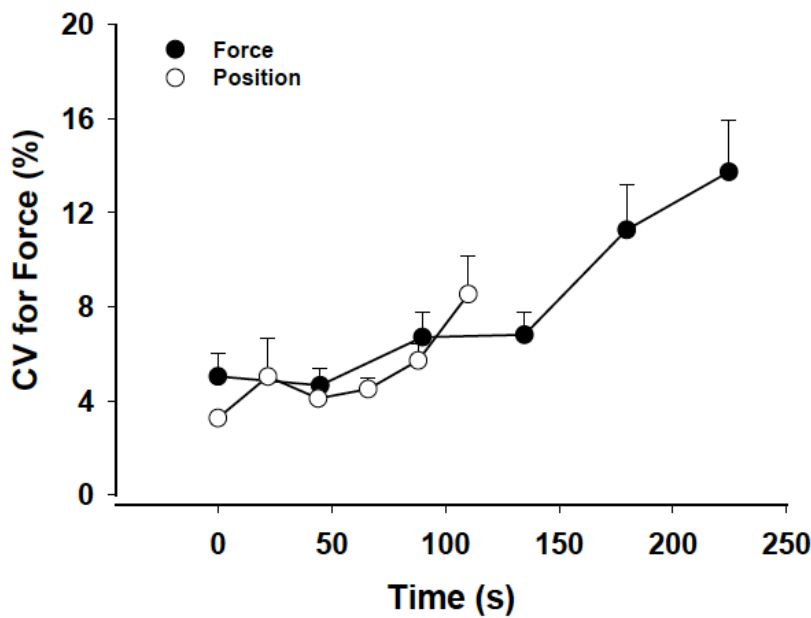


Figure 6. Coefficient of variation (CV) for force % during the force and position tasks. The CV for force (%) was averaged over 10-s intervals for each task at 6 time points that corresponded to the absolute times at start, 20, 40, 60, 80, and 100% of time to task failure.

MAP, heart rate, and rating of perceived exertion

MAP increased during both tasks ($p < 0.001$) but was not different either at the beginning of the force and position tasks (68 ± 8 mmHg and 77 ± 16 mmHg, respectively) or at task failure (94 ± 13 mmHg and 95 ± 19 mmHg, $p > 0.29$). The rate of increase, therefore, was greater during the position task (0.18 ± 0.07 mmHg/s) compared with the force task (0.12 ± 0.07 mmHg/s, $p = 0.03$) (Figure 7a).

Heart rate also increased during both tasks ($p < 0.001$) but was not different either at the beginning of the force and position tasks (84 ± 10 bpm and 87 ± 15 bpm, respectively) or at task failure (108 ± 12 bpm and 108 ± 13 bpm, $p > 0.63$). As with MAP, the rate of increase in heart rate was greater for the position task (0.19 ± 0.09 bpm/s) than the force task (0.08 ± 0.08 bpm/s, $p = 0.03$) (Figure 7b).

Rating of perceived exertion was not different at the beginning of the force and position tasks (4.2 ± 1 and 4.9 ± 1 , respectively, $p > 0.23$), and the rate of increase ($p < 0.001$) did not differ during the position (0.04 ± 0.07) and force task (0.03 ± 0.06 , $p = 0.21$) (Figure 7c).

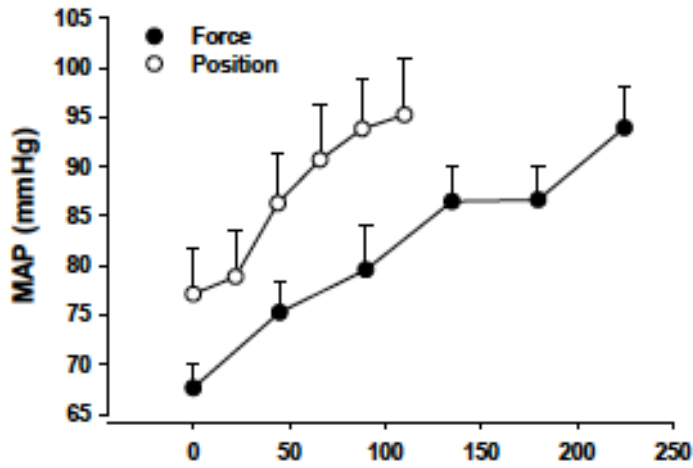
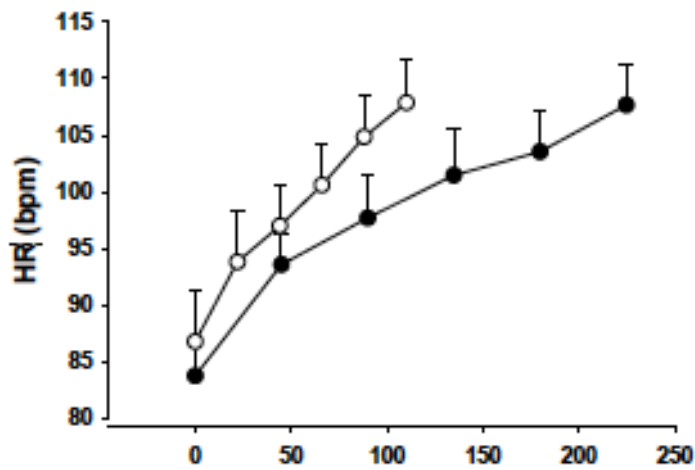
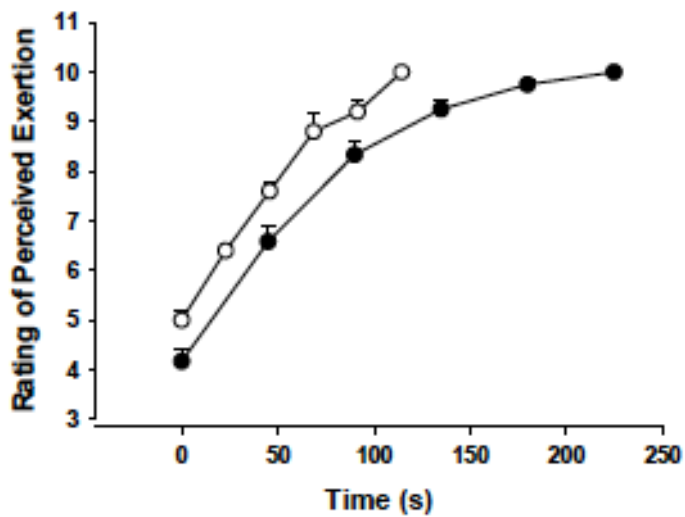
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Figure 7. Mean arterial pressure (MAP, heart rate, and ratings of perceived exertion during the force and position tasks. *Filled symbols* indicate force task and *open symbols* correspond to position task. Values are indicated as means \pm SE. Measurements were made at the beginning of each task and at 20% increments of task duration.

Discussion

The new findings in this study were as follows: (1) the knee extensor muscles exhibited a briefer time to failure for the position task than the force task; (2) the position task was characterized by a greater rate of increase in knee extensor EMG amplitude, mean arterial pressure, and heart rate; and (3) the levels of antagonist coactivation were not different for the force and position tasks. The briefer time to failure for the position task occurred despite each subject exerting a similar net muscle torque during the force and position tasks. There were no differences between the two fatiguing contractions in both the initial MVC force and its decline at task failure (~30%).

Control Strategy

The 49% difference in time to failure for the two tasks with the knee extensor muscles was similar to that observed for the elbow flexors when the upper arm was vertical (41-56%) [Hunter et al. 2002; Klass et al. 2008; Rudroff et al. 2007a, 2007b], the first dorsal interosseus muscle (40%) [Maluf et al. 2005], and the dorsiflexor muscles (53%) [Hunter et al. 2008], but is greater than that for the elbow flexor muscles when the upper arm was horizontal (22%) [Rudroff et al. 2005]. However, the elevated posture of the lower limb did result in time to failure for the two tasks that were much briefer (~167 s) than those observed previously (~750 s), presumably due to muscle perfusion being compromised (low mean arterial pressure) and the earlier recruitment of high-threshold motor units [MacDonald et al. 1998].

The mechanisms underlying the difference in time to failure for the two tasks when maintaining a submaximal contraction, therefore, appears to be independent of the muscle

group and not associated with either the anatomy of the involved muscles or their fiber-type composition. Rather, these findings suggest that the difference in load compliance was managed by activating unique control strategies that resulted in different times to failure.

The strongest evidence in support of different control strategies for the two tasks is the observations on the discharge characteristics of the same motor units during force and position control. Previous studies recorded the discharge of the same motor unit in biceps brachii when subjects performed the two tasks with the same relative load (~20% MVC force) either with the forearm horizontal [Mottram et al. 2005] or vertical and supinated [Rudroff et al. 2009]. Both studies showed that motor units exhibited the greatest decline in mean discharge rate and the most pronounced increase in discharge variability during the position task.

As the discharge of the same motor unit was monitored in the two tasks in both studies, the differential change in discharge characteristics likely involved synaptic mechanisms rather than intrinsic motor neuron properties. One potential source for the difference in synaptic input is the feedback provided by group III-IV afferents, which can increase the fluctuations in membrane trajectory during the after hyperpolarization phase [Windhorst et al. 1997] and thereby increase the variability in discharge times [Calvin and Stevens 1968; Matthews 1996]. The influence of group III-IV afferents on the discharge of action potentials by motor neurons is likely mediated by presynaptic inhibition of the group Ia afferents rather than by direct inhibitory effects onto the motor neuron pool [Butler et al. 2003; Duchateau et al. 2002]. Another potential source for the difference in discharge characteristics during the position task is a change in the balance of synaptic input onto motor neurons due to heightened activation of the stretch reflex. The amplitude

of the stretch reflex is enhanced when a limb acts against a compliant load compared with a rigid restraint [Akazawa et al. 1983; De Serres et al. 2002], which may be necessary to control limb position when supporting a compliant load. It has been suggested that an increase in the stretch reflex response during the position task involves greater net excitation from supraspinal sources that results in the earlier recruitment of the motor unit pool and an earlier task failure [Enoka and Duchateau 2008; Maluf et al. 2005; Mottram et al. 2005].

The likelihood that the control strategy differs for the two tasks is underscored by the greater rates of increase in average EMG amplitude, mean arterial pressure, and heart rate during the position task. The greater increase in EMG amplitude during the position task is attributable to the recruitment of additional motor units, which are presumably activated to compensate for the greater reduction in discharge rate of the previously recruited motor units [Mottram et al. 2005; Rudroff et al. 2009]. The increase in mean arterial pressure and heart rate is mediated by adjustments in central command and peripheral reflexes involving the group III and IV afferents [Alam and Smirk 1937; Fisher and White 1999; Gandevia and Hobbs 1990]. As peripheral reflexes dominate these adjustments after the start of a fatiguing contraction [Rowell and O'Leary 1990; Rowell 1993], the greater increase in the mean arterial pressure for the position task suggests a more rapid increase in the inhibitory input from group III and IV afferents onto the motor neuron pool [Bigland-Ritchie et al. 1986; Garland 1991; Macefield et al. 1993]. However, given the observation that the briefer time to failure for the position task can involve greater rates of increase in accessory muscle activity, mean arterial pressure and heart rate [Hunter et al. 2002; Rudroff et al. 2007a, 2007b], the position task in the supine posture

likely involved a greater active muscle mass [Seals 1989; 1993] that would have hastened task failure.

Despite the more rapid increase in mean arterial pressure and heart rate during the position task, ratings of perceived exertion increased at a similar rate during the two tasks. Increases in the rating of perceived exertion indicate an individual's sense of the relative intensity of the sustained physical activity and are probably derived from the descending voluntary command [Carson et al. 2002]. Although previous studies [Green et al. 2006; Noble et al. 1983] found associations between changes in heart rate and ratings of perceived exertion during physical activity others suggest that heart rate may be a more appropriate indicator of exercise intensity [Jacobs et al. 1997; Lewis et al. 2007]. Accordingly, the more rapid increase in heart rate indicates that descending drive increased at a greater rate during the position task compared with the force task.

Muscle activation and fluctuations in force

Although the aEMG increased more rapidly during the position task due to an augmented rate of motor unit recruitment, the coefficient of variation for force increased at the same rate during the two tasks and achieved greater values for the final two time points of the force task compared with the position task. This result contrasts with other findings that have shown the fluctuations in motor output to increase more rapidly during the position task [Hunter et al. 2002, 2008; Rudroff et al. 2005, 2007a, 2007b]. In the current study, however, a direct comparison of the force fluctuations in the two tasks was possible due to the placement of a force transducer in series with the load. The continued increase in force fluctuations during the force task was not attributable to either the distribution of

activity among synergistic muscles or the amount of antagonist coactivation as these two parameters did not differ between the two tasks. Furthermore, the explanation that the position task was terminated before all motor units were recruited could also be excluded, as the decrease in MVC force did not differ between tasks. Although alternating bursts of muscle activity between agonist and antagonist pairs can contribute to the increase in the coefficient of force [Rudroff et al. 2007a, 2007b; Riley et al. 2008], such activity was not observed in the agonist and antagonist EMG records.

As the force exerted by a muscle depends on the net motor unit activity, the analysis examined the association between the coefficient of variation for force and the spectral characteristics of the interference EMG as an index of net motor unit activity. The progressive increase in the coefficient of variation for force during the force task was not associated with a task difference in the increase of the normalized power in either the 10-29 Hz or the 30-60 Hz bands (Figure 5b, c). There was a task difference in the normalized power in the 0-10 Hz band, but this was due to power being greater during the position task (Figure 5a). Similarly, Negro et al. [2009] found only a weak association between the coefficient of variation for force during isometric contractions and the envelope of the surface EMG signal. However, they did observe a strong association between the coefficient of variation for force and the smoothed discharge rates, which suggests that the progressive increase in the coefficient of variation for force observed in the current study during the force task was likely attributable to an increase at common low-frequency modulation of motor unit discharge rate. This effect was not related to the control strategy used to perform the force task, but instead was a consequence of the longer duration that the task was sustained.

The task difference in the final values for the coefficient of variation for force was also not associated with a difference in antagonist coactivation. The rate of increase in EMG activity for biceps femoris did not differ during the force and position tasks, which is consistent with the findings from previous studies for upper [Rudroff et al. 2007a, 2007b, 2008; Maluf et al. 2005; Baudry et al. 2011] and lower [Hunter et al. 2008] limb muscles, and the absence of a difference in the coactivation ratio at the start and end of both fatiguing contractions indicates no difference in the increase in relative aEMG values for the biceps femoris and knee extensors. Furthermore, although coactivation of quadriceps and hamstrings is important for knee joint stabilization during many activities [Kellis 1998; Solomonow and D'Ambrosia 1991; Smith 1981], especially in the last part of the range of motion before full knee extension [Baratta et al. 1988], knee angle was fixed at a right angle for both tasks in the present study.

In summary, the time to task failure for the knee extensor muscles was briefer for the position task relative to the force task, despite each participant exerting a similar torque about the knee joint during the two tasks. The findings from this study are consistent with the results from studies on other limb muscles and demonstrate that different strategies are also used when the knee extensor muscles perform force and position control. Coactivation levels remained constant throughout both fatigue tasks and did not explain the difference in time to failure. The briefer time to failure for the position task involved greater rates of increase in neural adjustments that suggested an increased rate of descending drive and earlier recruitment of the motor unit pool during position control.

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Chapter III

**Fatigability of the dorsiflexors during a submaximal isometric contraction increases
with age in old adults**

Abstract

Old adults are often less fatigable than young adults, although there are few comparisons among old adults. The purpose of the study was to determine the association between age in older adults and fatigability of dorsiflexor muscles when supporting a submaximal inertial load. The strength, steadiness, and fatigability of leg muscles were assessed in 55 adults aged 65-90 years (75.2 ± 6.0 years). Fatigability was determined as endurance time for a submaximal (20% load) isometric contraction with the dorsiflexors. Endurance time was negatively correlated with age denoting an increase in fatigability with advancing age ($r = -0.47, p < 0.001$). A multiple regression model explained 61% of the variance in endurance with three predictor variables: age, steadiness during a submaximal (20% load) contraction with the dorsiflexors, and normalized knee flexor strength. These results demonstrate that fatigability while supporting an inertial load with the dorsiflexors increases across age among older adults.

Introduction

Advancing age is typically accompanied by reductions in strength, power, and motor performance [Baudry et al. 2007; Reid and Fielding 2012]. The adaptations in muscle fatigability, however, are more equivocal due to the influence of the fatigue protocol [Christie et al. 2011; Enoka and Duchateau 2008; Kent-Braun 2009; McNeil and Rice 2007] on the adjustments underlying activity-induced decreases in the force-generating capacity of muscle [Christou and Enoka 2011; Enoka 2012]. For example, old adults are usually less fatigable than young adults when the task involves isometric contractions against a rigid restraint [Christie et al. 2011; Enoka and Duchateau 2008; Kent-Braun 2009; Hunter et al. 2005], but not when performing dynamic contractions [Christie et al. 2011; McNeil and Rice 2007]. The purpose of the current study was to determine the association between age in a group of older adults and fatigability of the dorsiflexor muscles when supporting a submaximal inertial load.

Materials and Methods

Fifty-five older adults (65-90 years) were recruited by advertisement from Boulder, Colorado. Subjects were free of neurological disorders, chronic pain, diabetes and advanced chronic disease states. Similarly healthy young men and women (n = 9, 5 women) were also recruited for comparison with the old adults. All procedures were approved by the Institutional Review Board and conducted in accordance with the Declaration of Helsinki.

The protocol included tasks to measure the strength, steadiness, and fatigability of lower limb muscles. Strength was quantified with two measures: the maximal weight lifted

in 1 repetition (1RM) with the dorsiflexor muscles and the peak force achieved during 3-5 isometric maximal voluntary contractions (MVC) with the knee extensor, knee flexor, and dorsiflexor muscles. A strain gauge transducer (300 lb range, MLP-300, S/N 234017, Transducer Techniques, Terneucula, CA) was used to measure the force exerted by the limb during each MVC, which required subjects to increase muscle force gradually to maximum and sustain it for ~3 s.

Steadiness was assessed during 60-s isometric dorsiflexion contractions while supporting loads of 5 and 20% 1RM. Subjects were instructed to maintain a neutral foot position based on the measurement of ankle joint angle by an electrogoniometer (SG110 and K800, Biometrics Ltd, Cwmfelinfach, Gwent, UK). Subjects were provided with visual feedback ($1^{\circ}/\text{cm}$) of the actual and target joint angles using a customized Labview program (version 8.2, National Instruments, Austin, TX). Steadiness was quantified as the average of the absolute and relative fluctuations (standard deviation and coefficient of variation) in torque measured during 10-s epochs centered about 10, 20, 30, 40, and 50 s of the 60-s contractions.

Fatigability was quantified as endurance time of a submaximal isometric contraction with the dorsiflexors. Subjects were instructed to maintain the neutral position of the ankle joint while supporting a load equivalent to 20% of 1RM for as long as possible [Hunter et al. 2008]. The task was terminated when the ankle joint angle declined by 12° from neutral position despite strong verbal encouragement. The absolute and relative fluctuations in torque during the fatiguing contraction were quantified via a custom

MATLAB program at 10-s epochs at six time points: start, 20, 40, 60, 80, and 100% of endurance time.

Prior to primary analysis, outliers (>3 SD) were excluded and normality was assessed with the Kolmogorov-Smirnov test. The functional significance of the association between age and fatigability, which was quantified by Pearson correlation, was assessed with a multiple regression model that predicted endurance time with the strength and steadiness characteristics of the lower extremity muscles. The forward regression included variables obtained from the motor function battery as well as age, sex, height, body mass, and total physical activity as estimated by questionnaire (Modifiable Activity Questionnaire). The significant predictor variables from the forward regressions were entered into a stepwise multiple regression to verify the predictors and establish the model to explain the variance in endurance time. The α -level for all statistical analyses was set at 0.05, and all data are presented as the mean \pm SD. All statistical procedures were performed with SPSS Statistics (version 16.0.1; SPSS, Inc., Chicago, IL).

Results

The 55 older participants (75.2 ± 6.0 years, 24 women) were relatively healthy and presented with moderate values for BMI (26.8 ± 3.9 kg/m²), total fat mass as measured with dual x-ray absorptiometry ($34.7 \pm 8.8\%$), and estimated levels of habitual physical activity (38.7 ± 26.0 MET hr/wk). The fatigability task with the dorsiflexors was sustained for 558 ± 400 s. Endurance time was negatively correlated with age ($r = -0.51, p < 0.001$), indicating that fatigability increased with age. The significant predictors of endurance time were age, the coefficient of variation for torque during the steadiness task performed at

20% 1RM (1.55 ± 0.89), and knee-flexion MVC torque normalized to body mass ($1.04 \pm 0.28 \text{ N} \cdot \text{m} / \text{kg}$) (Figure 8, $p < 0.05$). The model explained 61% of the variance in endurance time for this sample of older men and women ($p < 0.001$).

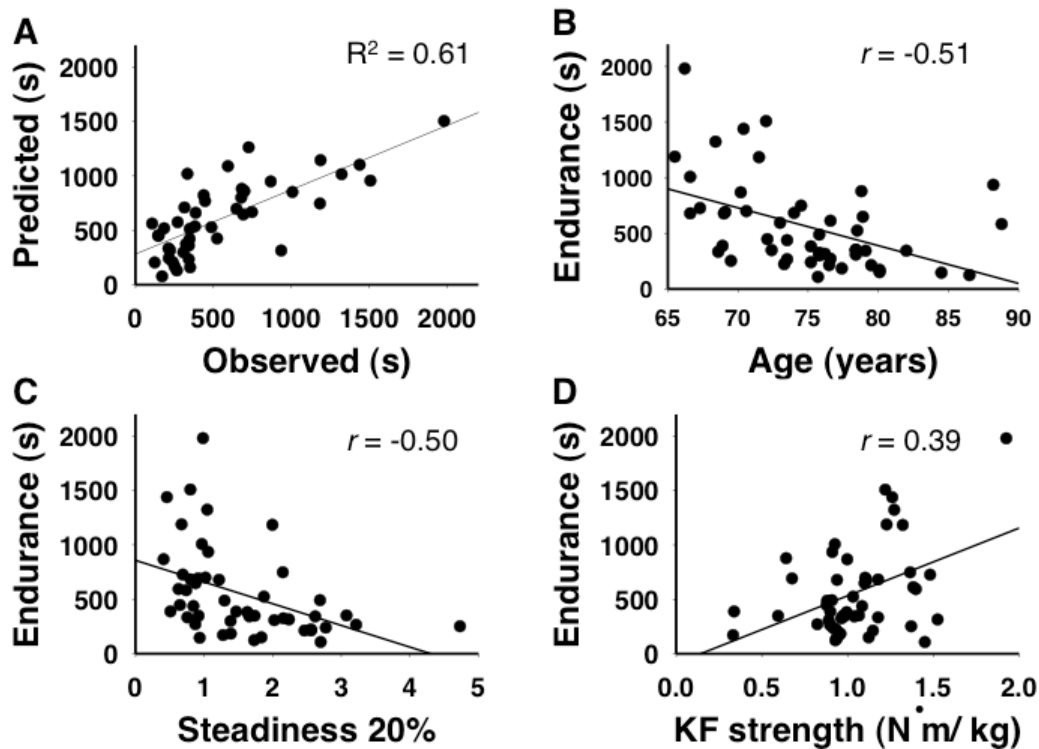


Figure 8. Endurance time for an isometric contraction in which the dorsiflexors supported a submaximal inertial load was significantly predicted by three dependent variables. A forward regression model indicated that 61% of the variance (R^2) in observed endurance time (A) was predicted by age (B), coefficient of variation of torque during a submaximal (20% load) steady contraction (C), and normalized MVC torque for the knee flexors (KF) (D). The part correlations for the significant predictor variables are indicated in panels B, C, and D

The 9 young men and women (aged 20-25) also completed the fatigability task with the dorsiflexors. Although the load supported by the young adults was also 20% of 1RM, the absolute value was greater than for the older adults ($5.8 \pm 1.5 \text{ kg}$ versus $4.1 \pm 1.2 \text{ kg}$, respectively, $p = 0.001$) because the young adults were stronger ($30.6 \pm 6.5 \text{ kg}$ vs. 20.7 ± 6.1

kg 1RM, respectively, $p < 0.001$). A trend was observed to suggest that the young adults were able to sustain the fatiguing contraction for a slightly longer duration than the older adults (13.5 ± 9.2 vs. 9.3 ± 6.1 min, $p = 0.09$), despite similar declines in MVC before and after the endurance task (young: $23.8 \pm 18.3\%$ vs. old: $24.6 \pm 22.3\%$, $p = 0.93$).

Discussion

The primary finding of this study was that endurance time for a submaximal isometric contraction with the dorsiflexors was inversely correlated with age in a group of older adults. The variance in endurance time was most strongly associated with age, but also significantly associated with steadiness during a submaximal contraction with the dorsiflexors and the strength of the knee flexors. The strength of the knee extensors and the dorsiflexors did not significantly explain the variance in endurance time.

The ability to maintain a steady contraction at 20% 1RM and knee flexor strength normalized to body mass were significant predictors of endurance time for the fatiguing contraction with the dorsiflexors. An association between a decline in steadiness during fatiguing contractions and endurance time has been noted in previous studies in the lower extremity [Hunter et al. 2008, Rudroff et al. 2010]. Additionally, fluctuations in force during fatiguing contractions, reported as CV for force, are predictive of time to failure for submaximal (20 and 30% MVC) isometric contractions in the elbow flexors [Rudroff et al. 2011]. However, this is the first study to demonstrate a relation between reduced steadiness during a brief isometric contraction and time to failure on a subsequent fatiguing contraction. A reduced ability to maintain a steady contraction demonstrates an impaired ability to control force or movement. Recent experiments provide evidence that

amplified motor output variability (reduced steadiness) can impair the ability to perform other functional movements [Christou 2011]. For example, reduced steadiness during isometric plantar flexion predicted the variability of center of pressure during a quiet standing task in older persons [Kouzaki and Shinohara 2010], and steadiness of the index finger abduction force can be explained by functional tasks requiring manual dexterity [Marmon et al. 2011]. Thus the unintentional variations in the force fluctuations at 20% 1RM, may reflect age-associated augmentation in motor output, such as common modulation [Negro et al. 2009], that result in deficits in a task requiring similar contraction mode and intensity: a 20% 1RM isometric task to failure.

In addition to age and steadiness, the variability in dorsiflexor endurance time was also explained by knee flexor strength. The subjects supported a mass with the ankle at a neutral position, while holding the knee angle constant at ~ 1.57 rad, which required activation of the accessory knee flexor and extensor muscles. An experimental observation was that the investigators made frequent verbal corrections for subjects to “pull their heel back” thus activating the knee flexors to maintain 1.57 rad at the knee. Therefore, the isometric dorsiflexion task to failure likely involved accessory activation of the knee flexors, although a limitation of the current study was that EMG activity of the biceps femoris, a primary knee flexor, was not recorded due to experimental constraints. However, previous reports have indicated that in isometric position holding tasks, briefer time to failure involved greater rates of increase in accessory muscle activity [Rudroff et al. 2005], and coactivity of the biceps femoris to vastus lateralis is greater in older adults than young adults [Hotobábyi et al. 2005; Hortobábyi and DeVita 2006]. As the knee flexor muscles were likely recruited to maintain the dorsiflexion task to failure, the strength of

the knee flexor muscle group contributed to the duration of the fatigue task. Knee flexor strength has been previously identified as a strong predictor of health-related quality of life, including physical and social functioning, vitality and mental health, in older persons [Samuel et al. 2012]. Thus, knee flexion strength may be related to overall functional status as well as fatigability.

The main finding of this study was that the primary predictor of time to sustain a submaximal contraction with the dorsiflexors is age, such that endurance time decreased with advancing age. Old adults often exhibit longer endurance times than young adults for submaximal isometric contractions, but not for dynamic contractions [Baudry et al. 2007; Christie et al. 2011; McNeil and Rice 2007]. The studies that contributed to this conclusion, however, typically compared the performance of young and old adults. In contrast, McNeil and Rice [2007] found that fatigability during rapid dynamic contractions with the dorsiflexors was greater for a group of oldest adults (80-90 yrs) relative to older adults (60-69 yrs). Furthermore, preliminary evidence indicates an emerging trend for greater endurance time in young adults compared with the older adults in the current study. In another investigation of dorsiflexion endurance time when supporting a submaximal inertial load (20% 1RM load) in young adults (21.1 ± 1.4 yrs), endurance times were similar to times reported for older adults in the current study (10.0 ± 6.2 vs 9.3 ± 6.7 min, young and old respectively) [Hunter et al. 2008]. Taken together, these findings suggest that the fatigability of the dorsiflexors when supporting a submaximal inertial load may remain relatively constant until the late 60s before fatigability begins to increase.

Chapter IV

Association of motor function with 1,25(OH)₂D and indices of insulin-glucose dynamics in non-diabetic older adults

Abstract

Advancing age is accompanied by metabolic changes, such as reduced insulin sensitivity and low vitamin D, which may exacerbate age-related declines in physical function. The goal of the study was to examine the associations between motor function, insulin-glucose dynamics, and vitamin D status in relatively healthy older adults. Sixty-nine community-dwelling men and women without diabetes (65-90 years) were recruited. Insulin-glucose dynamics were determined by an intravenous glucose tolerance test, and vitamin D metabolites were quantified as serum levels of 25(OH)D and 1,25(OH)₂D. Motor function was characterized by 500-m walk time (500mwt), chair-rise time, lower-body strength, dorsiflexor steadiness and endurance time, and muscle coactivation. 500mwt and leg muscle strength were associated with fasting levels of blood glucose and insulin along with 1,25(OH)₂D, whereas coactivation and fatigability of the dorsiflexor muscles were associated with fasting glucose ($p < 0.05$ for all). Significant correlations were also found for lower body strength and steadiness with indices of insulin secretion and sensitivity with ($p < 0.05$). 500mwt variability was explained by sex, circulating levels of 1,25(OH)₂D, and fasting blood insulin ($R^2 = 0.36$, $p < 0.001$). Poor motor function in non-diabetic older men and women was independently associated with multiple indices of insulin-glucose dynamics. Multiple regression models, however, indicated that superior functional performance in this relatively small sample of older adults was achieved by men, individuals with greater 1,25(OH)₂D levels, and those with lower fasting blood insulin. These findings indicate that differences in motor function (walk-speed, steadiness, muscle coactivation, and endurance) are associated with several metabolic factors that increase in prevalence in an aging population.

Introduction

Adults >65 years represent the fastest growing portion of the U.S. population and are projected to comprise 20% of the U.S. population by 2050 [Statistics FIF0A-R 2008]. As the number of older adults increases, maintaining physical function has emerged as a major health priority. Age-associated declines in motor function are tightly coupled with quality of life [Cooper et al. 2011], disability [Rantanen et al. 2003], and mortality [Buchman et al. 2007], and identifying how metabolic factors interact to influence functional health is of great significance in geriatric medicine [Kritchevsky 2012].

Among the age-associated comorbidities that accompany decreases in motor function, diabetes, pre-diabetic insulin resistance, and vitamin D deficiency have emerged as significant predictors of frailty and disability in older U.S. adults [Abbatecola and Paolisso 2008; Ferrucci et al. 2000; Gregg et al. 2000; Holick 2007]. For example, physical function is associated with glucose abnormalities in diabetic [Andersen and Jakobsen 1999; Giacomozzi et al. 2008] and insulin-resistant adults [Abbatecola et al. 2005], and may predict the development of insulin resistance [Lazarus et al. 1997; Chen et al. 2008], although these associations were noted in large epidemiological studies that included older adults with overt disease states of diabetes and pre-diabetes. Characterizing subclinical impairments is important because early intervention is associated with positive outcomes [Fried and Guralnik, 1997; de Rekeneire et al. 2003]; however, few investigations have explored the associations between motor function and insulin-glucose dynamics in non-disabled older adults in the absence of the overt disease state of diabetes.

Vitamin D deficiency, or circulating 25-hydroxyvitamin D (25(OH)D) levels <20 ng/ml, is also a public health concern, especially among older adults [Holick 2007], as it is associated with a diverse array of pathologies, including diabetes and insulin resistance

[Holick 2008; Pittas et al. 2007], and may also be related to physical function [Bischoff-Ferrari et al. 2004]. For example, vitamin D deficiency appears to be associated with muscle weakness, slower walking speeds, and less steady balance [Bischoff-Ferrari et al. 2004; Gerdhem et al. 2005; Glerup et al. 2000]. The evidence is often equivocal, however, with investigators reporting either an absence or mixed [Annweiler et al. 2009; Matheï et al. 2013] associations between physical function and circulating vitamin D (25(OH)D), and an under-reporting of associations with the biologically active vitamin D metabolite 1,25(OH)₂D [Bischoff et al. 1999; Dukas et al. 2005].

Although both vitamin D metabolites and insulin-glucose dynamics are independently associated with motor function, little is known about how they may interact to influence function in relatively healthy older men and women. Given that low vitamin D levels can occur concurrently with hyperglycemia and impaired glucose tolerance in older adults [Holick 2008; Pittas et al. 2007; Hirani 2011], the effects of both insulin-glucose dynamics and vitamin D metabolites on function may have clinical significance. The aim of the present study was to determine the associations between insulin-glucose dynamics, vitamin D metabolites, and performance on a battery of motor tasks in non-diabetic older adults. Motor function was characterized with tests of walking speed, the ability to rise from a chair, strength, steadiness, and endurance time. The functional significance of the associations was evaluated by assessing the relative importance of the metabolic factors in explaining the variance in performance on a test of walking endurance.

Methods

Sixty-nine older adults (65-90 years) were recruited for the study from Boulder, Colorado. All subjects were non-diabetic as determined by medical history and two fasting blood glucose measures of <126 mg/dL (MediSense, Precision PCX, Abbott, Quebec, Canada). Subjects reported no neurological disorders, chronic pain, advanced chronic disease states, or medical conditions that might limit safe participation, and all BMI values were <40 kg/m². Written informed consent was obtained prior to screening and testing. The Institutional Review Board approved all procedures.

Subjects participated in three sessions on different days within 1 month of consent: (1) to obtain a medical history, complete a physical examination, and receive a nutrition consultation; (2) to perform the frequently sampled intravenous glucose tolerance test (ivGTT) and vitamin D metabolite assessment; and (3) to complete a battery of physical tests to characterize walking speed, chair-rise ability, strength, steadiness, and endurance time.

Indices of Insulin-Glucose Dynamics

Whole-body glucose uptake and insulin sensitivity were determined by a frequently sampled ivGTT [Bergman et al. 1983] following a 24-hour research diet that comprised 50% carbohydrate, 30% fat, 15% protein, and overnight fast. Blood samples to determine fasting blood glucose and insulin were obtained every 5 min for 15 min, after which a glucose solution (0.3 g/kg) was administered as an intravenous bolus over one minute. A bolus of regular human insulin (0.03 U/kg; Novo Nordisk, Princeton, NJ) was given 20 min

later. Blood samples were collected to determine plasma concentrations of glucose and insulin at 21 time points over 3 hrs.

Fasting blood glucose and insulin were determined from baseline measurements. Subjects were considered pre-diabetic fasting blood insulin was 100-125 mg/dL. Insulin sensitivity was calculated using the Minimal Model Identification software as the fractional decrease in plasma glucose in relation to the change in plasma insulin, which indicates the degree of tissue sensitivity to the metabolic influence of insulin. The acute insulin response to glucose (AIRg) was calculated as the endogenous insulin response to the glucose bolus prior to insulin administration [Bergman et al. 1983]. As insulin secretion is augmented in response to reduced insulin sensitivity, a disposition index was calculated as the product of insulin sensitivity and AIRg to denote the relative appropriateness of the insulin secretion for the degree of insulin sensitivity.

Vitamin D

Serum concentrations of the vitamin D metabolites 25-hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D [1,25(OH)₂D] were determined with radioimmunoassay (DiaSorin Inc. Stillwater, MN) of a fasted blood sample.

Body Composition

Body composition was characterized by dual energy x-ray absorptiometry (DXA) (Lunar) estimates of total fat mass, fat-free mass, and bone density [Wang et al. 1999], and the measurement of height, weight, body mass index (BMI), and waist and hip circumferences.

Motor Function

The tests of motor function assessed walking speed, chair-rise ability, and the strength, steadiness, and fatigability of lower limb muscles (Figure 9). The force exerted by the limb during the strength, steadiness, and fatigability tasks was measured with a strain-gauge transducer (MLP-300, Transducer Techniques, Terneucula, CA). In addition, muscle activity (electromyography; EMG) was recorded with surface electrodes (Ag–AgCl, 8-mm diameter) above the tibialis anterior and medial gastrocnemius muscles. Coactivation was quantified as the ratio of EMG amplitude for medial gastrocnemius relative to the average amplitude for tibialis anterior and medial gastrocnemius during the dorsiflexion tasks (Falconer and Winter 1985).

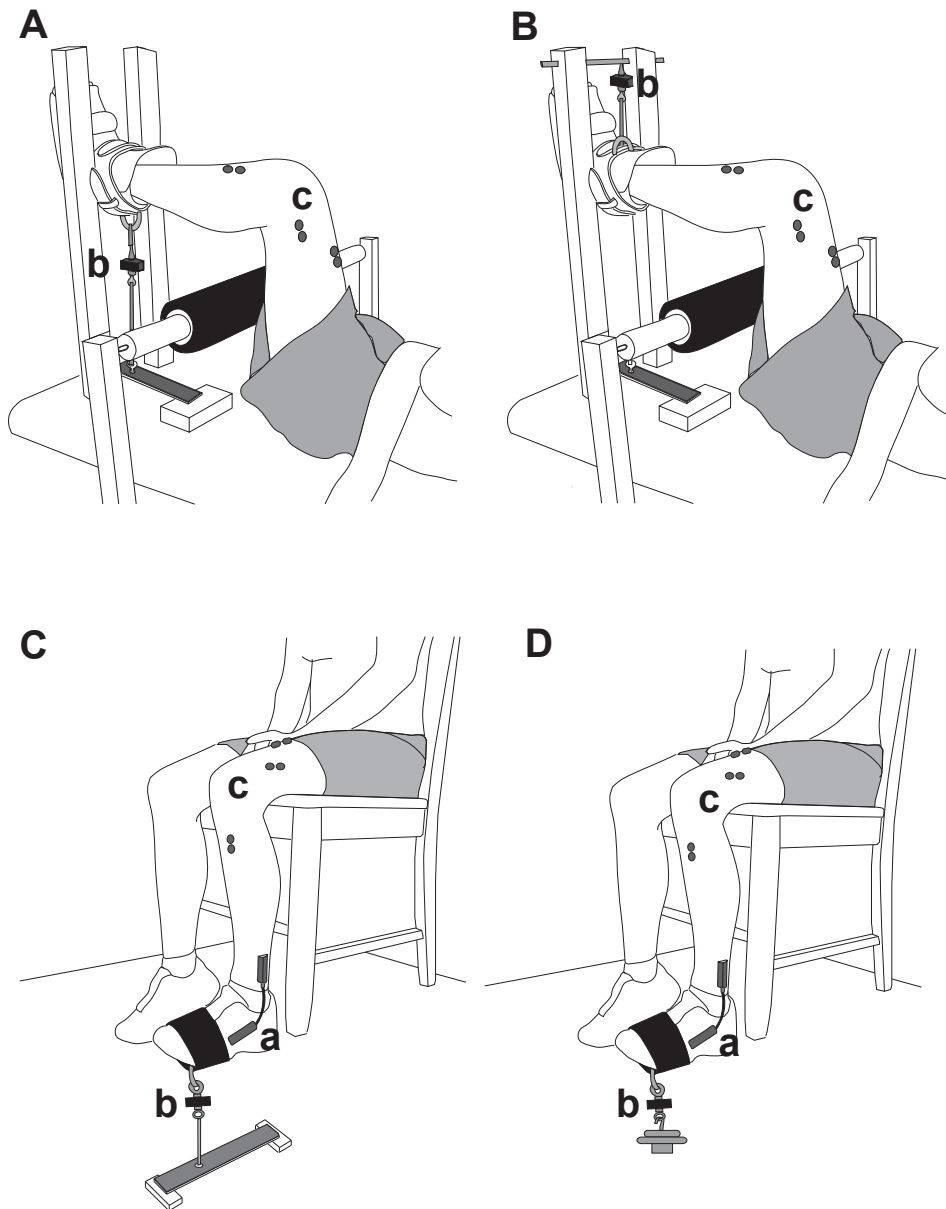


Figure 9. Schematic drawings of the experimental setup for knee extensor (A), knee flexor (B), and dorsiflexor (C) maximal voluntary contractions (MVCs), and dorsiflexor one-repetition maximum (1 RM), steadiness, and fatigability tests (D). The foot and lower leg were placed in an orthosis and fixed to a rigid restraint for the measurement of knee extensor (A) and flexor (B) strength. In the dorsiflexor tasks, maximal force was exerted against a rigid restraint (C), or weights equivalent to 5% or 20% 1-RM load were suspended from the foot at the same point of contact (D). The ankle joint was maintained at a neutral position (1.61 ± 0.08 rad), as determined by an electrogoniometer (a). A force transducer (b) was placed in series with the restraint or weight for each task.

Electromyography (EMG) was measured with surface electrodes (c) throughout each isometric contraction.

Walking Endurance

Walking endurance was assessed by a timed 500-m walk test (500mwt), in which subjects walked at a brisk pace for 3 laps around an indoor track (161 m per lap).

Chair-rise Ability

The ability to rise from a standard height chair was assessed by a timed chair-rise test, which involved standing up and sitting down five times from a chair as quickly as possible. The arms were folded across the chest.

Strength

Strength was quantified as the maximal weight that could be lifted once (1 repetition maximum; 1-RM) with the dorsiflexor muscles, and as the peak force achieved during 3-5 isometric maximal voluntary contractions (MVC) with the knee extensor, knee flexor, and dorsiflexor muscles. The maximal surface EMG for the primary agonist muscle was measured during MVCs and quantified as the maximal average rectified EMG about a sliding 0.5 s window via a custom MATLAB program (version 7.2, R2006a, MathWorks, Natick, MA).

Steadiness

Steadiness was assessed during 60-s isometric contractions with the dorsiflexors supporting 5 and 20% 1-RM loads. Subjects were instructed to maintain a neutral foot position during the contractions based on joint-angle measurement by an electrogoniometer (SG110 and K800, Biometrics Ltd, Cwmfelinfach, Gwent, UK). Steadiness was quantified as relative force fluctuations (coefficient of variation). The force fluctuations and coactivation were quantified as the average of 10-s epochs centered about 10, 20, 30, 40, and 50 s during the steady contractions.

Fatigability

Fatigability was quantified as the endurance time for an isometric contraction with the dorsiflexors [Hunter et al. 2008]. Subjects were instructed to maintain a constant ankle joint angle while supporting a 20% 1-RM load for as long as possible. The task was terminated when the ankle joint angle declined by 12° from neutral despite strong verbal encouragement. The average relative force fluctuations and coactivation ratio were quantified during the fatiguing contraction via a custom MATLAB program for 10-s epochs at six time points: start, 20%, 40%, 60%, 80%, and 100% of endurance time.

Statistical Analysis

Normality was assessed with the Kolmogorov-Smirnov test. Prior to primary analysis, one outlier ($\pm >3$ SD) was identified based on insulin sensitivity. Experimental notes revealed that this subject experienced an extreme drop in blood glucose at ~60 min post insulin injection (low of 34 mg/dL) leading to termination of the trial, and the subject's data was removed from further analysis. The associations between ivGTT

outcomes and motor function were determined by Pearson correlation coefficients (r). Linearity was verified by visual assessment. The influence of pre-diabetes and vitamin D status on motor function was analyzed by ANOVA.

Linear regression equations were used to describe the associations between metabolic factors, age, sex and total percent body fat on walking endurance. Race and comorbidities were not adjusted as the study participants were predominantly white (non-white $n=3$), and had few existing comorbidities. The variables included in the final multivariate analysis were identified with a backward regression model. A stepwise, multiple-regression model was then performed to evaluate the explanatory value, denoted by the coefficient of determination (R^2), of the identified factors on 500mwt. An absence of multicollinearity for the explanatory variables was verified. The α -level for all statistical analyses was set at 0.05, and all data are presented as the mean \pm SD. The statistical procedures were performed with SPSS Statistics (version 16.0.1; SPSS, Inc., Chicago, IL).

Results

Sixty-nine subjects aged 65-90 years were recruited for the study; 5 subjects were excluded due to high fasting blood glucose or medication use following enrollment, and 8 dropped out. Fifty-six subjects (34 women) completed all three experimental sessions and are included in the reported results. Selected characteristics of the participants are listed in Table 1. Significant correlations existed between markers of glucose-insulin dynamics, $1,25(OH)_2D$, and motor function (Table 2). The multiple regression model indicated that a significant amount of the variance in 500mwt could be explained by the sex of the

participant, the vitamin D metabolite 1,25(OH)₂D, and fasting levels of insulin (Table 3), but not insulin sensitivity or circulating 25(OH)D.

Table 1. Subject characteristics for primary health measures.

	All		Women		Men	
	n = 56		n = 34		n = 22	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	75.2	6.0	75.8	6.0	74.7	6.1
Fasting blood glucose (mg/dL)	94.9	8.1	92.1	8.3	97.7	7.0
Fasting blood insulin (μU/mL)	12.9	4.9	12.4	4.9	13.3	5.0
AIRg (μU/mL)	367	220	378	232	355	211
Si (min⁻¹/ mIU/mL)	2.39	1.4	2.51	1.3	2.28	1.4
25(OH)D (mg/mL)	35.2	10	37.1	7.5	33.3	12
1,25(OH)₂D (pg/mL)	41.3	13	43.3	13	39.2	12
BMI (kg/m²)	26.6	3.5	26.3	4.9	27.3	2.7

All subjects were non-diabetic as estimated by medical history and two fasting glucose measures <126 mg/dL: 41 subjects <100 mg/dL (90.8 ± 5.0 mg/dL, 29 women) and 15 subjects between 100-113 mg/dL (105.5 ± 4.0 mg/dL, 5 women). Women had lower fasting glucose levels than men ($p = 0.007$). Values for motor function were not statistically different between pre-diabetic (fasting glucose 100-125 mg/dL) and healthy (<100 mg/dL) participants.

Thirty-seven of the 56 subjects had sufficient levels of 25(OH)D (>30 ng/mL), 16 were insufficient (20-29 ng/mL), and two were deficient (<20 ng/mL). There were no statistically significant differences between the three 25(OH)D groups with respect to age, sex, or motor function.

The statistically significant and trend results from Pearson correlations between the metabolic factors and motor function are presented in Table 2. Notably, 25(OH)D was not significantly related to motor function, markers of insulin-glucose dynamics, or 1,25(OH)₂D ($r = 0.03$, $p = 0.85$). In contrast, 1,25(OH)₂D was associated with fasting blood glucose ($r = -0.44$, $p < 0.001$) and insulin ($r = -0.28$, $p = 0.04$).

Table 2. Significant correlations and trends between motor function, ivGTT-derived measures, and vitamin D metabolites.

	FBG	FBI	AIRg	Si	Disp	25(OH)D	1,25(OH)₂D
	Index						
500mwt	0.29	0.39	0.05	-0.26	-0.21	0.16	-0.44
Strength	-0.28*	-0.33*	-0.31†	0.30†	0.28†	0.15*	0.26
Steadiness at 5% 1-RM	-0.11	-0.18	0.30	0.03	0.03	-0.08	-0.16
Steadiness at 20% 1-RM	0.12	0.19	0.19	0.02	0.12	-0.19	-0.27
Coactivation ratio	0.34	-0.03	-0.18	-0.10	-0.09	0.09	-0.10
Fatigability	-0.27	-0.18	0.07	-0.04	0.15	0.03	0.02

Significant ($p < 0.05$) Pearson correlations (r) are shown in bold. FBG = fasting blood glucose, FBI = fasting blood insulin, AIRg = acute insulin response to glucose, Si = insulin sensitivity, and Disp Index = disposition index. Strength corresponds to lower body strength (*) or dorsiflexion one-repetition maximum (1-RM) (†) normalized to body mass. Coactivation ratio is an index of concurrent muscle activity during the steadiness contraction at 5% 1-RM. No significant correlations were observed for either 25(OH)D or chair-rise time and these variables have been omitted from the table.

To assess the relative significance of the associations between motor function and metabolic factors, a multiple regression model was developed to explain the variability in 500mwt with covariates age, sex and total percent body fat included (Table 3). Although the backward model identified sex, age, 1,25(OH)₂D, AIRg, and fasting blood insulin as variables most closely associated with 500mwt, verification with step-wise multiple regression analysis excluded age (partial $r = 0.27$, $p = 0.11$) and AIRg (partial $r = -0.32$, $p = 0.06$) as significant explanatory variables of 500mwt. Walking endurance, therefore, was

explained by the other three variables: the sex of the participant, 1,25(OH)₂D, and fasting insulin ($R^2 = 0.36$, $p < 0.001$) (Table 3).

Table 3. Regression model to predict walking endurance.

	Beta	Standard Error	p-value	Partial Correlations
1,25(OH) ₂ D (pg/mL)	-1.82	0.66	0.009	-0.40
Sex	44.4	15.8	0.007	0.41
Fasting blood insulin (mg/dL)	3.93	1.64	0.021	0.36

Coefficient of determination: $R^2 = 0.36$ ($p < 0.001$). Intercept: 317. Covariates age, sex and total % body fat were included with metabolic factors 1,25(OH)₂D, fasting blood insulin, and AIRg.

Discussion

The current study examined the association between motor function and metabolic factors related to insulin sensitivity and vitamin D status in relatively healthy, non-diabetic older men and women. We found significant independent associations between indices of insulin-glucose dynamics, vitamin D metabolite 1,25(OH)₂D, and motor function (500mwt, strength, steadiness, muscle coactivation, and endurance). Furthermore, walking endurance (500mwt) was better in men, subjects with greater circulating levels of 1,25(OH)₂D, and lesser fasting levels of blood insulin. The findings indicated that greater levels of fasting insulin and lower levels of the vitamin D metabolite [1,25(OH)₂D] were significantly associated with longer walk times in healthy older adults.

Insulin-Glucose Dynamics and Motor Function

The current study identified several significant associations between indices of insulin-glucose action and motor function. Fasting glucose and insulin were positively associated with many motor functions, suggesting that greater fasting glucose levels were associated with poorer performance. Although deficits in motor function have been shown to accompany diabetes [Andersen and Jakobsen 1999; Rekenire et al. 2003], the current study seems to be the first to identify an inverse relation between motor function and fasting glucose in non-diabetic adults. Similarly, greater fasting insulin was associated with longer walk time and lower strength, a finding consistent with a previous report of an association between hyperinsulinemia and lower grip strength [Lazarus et al. 1997].

In addition, greater fasting glucose was related to increased coactivation of agonist and antagonist muscles. Whereas coactivation can be a useful and necessary strategy in some contexts [Baratta et al. 1988], the excessive coactivation often observed in older adults [Baratta et al. 1988; Burnett et al. 2000] likely increases the energetic cost of performing muscular work [Hortobágyi et al. 2011]. The current study is the first to demonstrate an association between a neuromuscular activation strategy and fasting glucose, which underscores the need for further investigation.

Other ivGTT-derived measures, including AIRg, Si, and disposition index, were correlated with lower body strength. Greater insulin secretion in response to a glucose load (AIRg), which may coincide with insulin secretion at the tissue level [Mari et al. 2005], was related to lower strength and a decrease in steadiness when supporting a light load. Tissue sensitivity to insulin (insulin sensitivity index, Si) and the degree of insulin secretion relative to insulin sensitivity (disposition index) were related to lower body strength;

greater strength was associated with the insulin sensitivity and relative appropriateness of insulin secretion given insulin sensitivity.

Significant associations between insulin resistance and motor performance have been noted in epidemiological studies [Abbatecola et al. 2005; Barzilay et al. 2009; Kuo et al. 2009]. For example, Barzilay et al. [2009] found that knee extensor strength relative to muscle mass was negatively associated with insulin resistance, as assessed by the homeostasis model assessment of insulin resistance (HOMA-IR) in non-diabetic adults. Similarly, Kuo et al. [2009] found an association between habitual gait speed and HOMA-IR in non-diabetic men, but not in women. Although there was no such association between walking endurance and indices of insulin resistance in the current study, the discrepancy could be accounted for by the inclusion of women in our study and the large sample sizes in the epidemiological studies.

Vitamin D

Significant associations were identified between levels of the vitamin D metabolite 1,25(OH)₂D and motor function, but not circulating vitamin D as 25(OH)D. Although many investigations have examined the association between motor function and 25(OH)D, this circulating form of vitamin D is biologically inert. Despite demonstrated associations between 25(OH)D with strength, balance, and mobility in older adults [Bischoff-Ferrari et al. 2004; Gerdhem et al. 2005], particularly in those with severe deficiency [Wicherts et al. 2007], these relations are inconsistent [Annweiler et al. 2009; Dukas et al. 2005; Matheï et al. 2013]. Our results, and other cross-sectional studies [Bischoff et al. 1999; Dukas et al. 2005], demonstrate associations between 1,25(OH)₂D and motor function. For example,

Marantes et al. [2011] observed significant associations between low 1,25(OH)₂D levels and muscle mass and strength, but there was no consistent association with circulating 25(OH)D. These results indicate that influence of vitamin D on motor function should focus on the active metabolite 1,25(OH)₂D rather than 25(OH)D in healthy older adults. However, the biologically active metabolite 1,25(OH)₂D is dynamically regulated and circulating concentrations are thought to vary over 24-hours which may complicate the clinical interpretation of our results [Rejnmark et al. 2008].

Interactions

Due to the critical influence of physiologic factors on functional health [Kritchevsky 2012], the current study explored the relative importance of vitamin D metabolites and indices of insulin-glucose dynamics on motor function as characterized by walking endurance. When metabolic markers were considered in concert with age, sex, and total percent body fat, 36% of the variance in 500mwt could be explained by sex, 1,25(OH)₂D, and fasting blood insulin. 500mwt was longer in women, individuals with low 1,25(OH)₂D, and those with high fasting insulin. Notably, sex but not age explained some of the variability walk time, which likely was a consequence of the study including only older adults without physical limitations.

The stronger association between fasting insulin and motor function compared with insulin sensitivity was unexpected. Insulin resistance, or impaired stimulation of glycogen formation in skeletal muscle [Beck-Nielsen and Groop 1994], is presumed to be responsible for the development of hyperglycemia and diabetes, and can occur years in advance of an overt disease state even in the absence of impaired glucose tolerance [Eriksson et al. 1989].

Although we expected insulin sensitivity to be associated with motor function, the earliest pre-diabetic stage can also be phenotypically characterized by different degrees of metabolic abnormalities, including high fasting insulin [Beck-Nielsen and Groop 1994], which suggests that changes in fasting insulin may have coincided with altered insulin sensitivity that was not detected in our healthy older adults. Consequently, fasting levels of insulin in healthy older men and women who are normoglycemic may be a biomarker of functional status.

Despite the potential for fasting blood insulin to serve as a biomarker of motor function in non-diabetic older adults, the mechanisms underlying this association are uncertain. For example, the increase in fasting blood insulin could be explained by a decrease in skeletal muscle mass, a reduction in peripheral sensitivity to insulin, or a combination of both adaptations [Abbatecola et al. 2005; Lazarus et al. 1997]. The potential interactions include impairment in muscle glucose handling that reduces intracellular energy production and contributes to muscle weakness, and disturbances in insulin regulation that reduces protein synthesis, total body protein turnover, and body protein mass, especially in the elderly [Fukagawa et al. 1988]. Although the present study suggests that declines in motor function precede clinically identifiable disease states in older adults, it is unclear if reduced insulin sensitivity is a contributor to or a result of age-related reductions in motor function.

Limitations

Although the findings of the current study are intriguing, several limitations should be acknowledged. Given the cross-sectional design, the causation and direction of the

relations cannot be determined. Several potential covariates, including estimated physical activity, were excluded from analysis to preserve statistical power in the relatively small sample size. Similarly, other metabolic factors that change with age, such as renal function, were not measured and inclusion in the present study was based on two fasting blood glucose samples <126 mg/dL instead of determining diabetic state by HbA1c.

Conclusions

Indices of insulin-glucose dynamics and the vitamin D metabolite 1,25(OH)₂D were associated with motor function and muscle activation in non-diabetic older men and women. Fasting blood glucose and insulin were associated with multiple measures of motor function, including walking endurance, strength, steadiness, and fatigability. When age, sex, total percent body fat, indices of insulin-glucose handling, and vitamin D metabolites were considered in concert, 500mwt was significantly explained by sex, 1,25(OH)₂D, and fasting blood insulin. These findings indicate that differences in motor function are associated with several metabolic factors that increase in prevalence in an aging population. Among these associations, the active vitamin D metabolite 1,25(OH)₂D, and fasting blood insulin explain significant amounts of the variability in walking endurance of non-diabetic older men and women.

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Chapter V

Battery of behavioral tests to quantify age-associated changes in motor function in mice

Abstract

Motor function in humans can be characterized by locomotion, strength, balance, and endurance, and standardized test batteries and norms have been established for individuals aged 3-85 yrs (NIH Toolbox, Motor Function Domain). The aim of this project was to establish an initial, analogous test battery to assess motor functions in mice. Male C57BL/6 mice were studied at 3 (n = 87), 20 (n = 48) and 26 (n = 43) months of age. Tests assessed locomotion, strength, balance/coordination, and endurance capacity in mice. Motor function declines were observed with advancing age for the locomotion, strength, and endurance subdomains ($p < 0.001$). A motor function summary score was calculated and demonstrated declines of 7.4% between 3-to-20-month mice and 13.5% between 20-to-26-month mice. Based on comparison with previously published data in humans, the magnitude and relative time course of changes were similar in mice and humans in each subdomain except balance/coordination. Power calculations confirmed that the age-associated differences depicted by several of the individual tests and domain summary scores would be sufficient to assess the efficacy of interventions aimed at prevention or treatment of motor dysfunction with aging. In conclusion, the current study describes a mouse model that characterizes age-associated changes in clinically relevant domains of motor function. The model has utility for preclinical testing of strategies to preserve motor function with aging.

Introduction

The age-associated decline in motor function of humans is tightly coupled with quality of life [Cooper et al. 2011; Manini et al. 2007], disability [Guralnik et al. 1995; Rantanen et al. 1999], independent-living status [Bischoff et al. 2003], and mortality [Buchman et al. 2007; Rantanen et al. 2012; Stanaway et al. 2011]. Among middle-aged and older adults, the decline in motor function is a primary contributor to the increased risk of disability [Brach and VanSwearingen 2002; Fried and Guralnik 1997; Vestergaard et al. 2009], fall-risk [Cho et al. 2004; Lord et al. 1994; Sattin 1992; Tinetti et al. 1988], and hospitalization [Bohannon et al. 2002; Kerr et al. 2006]. Because the number of older adults is expected to double between now and 2050 [Statistics FIFoA-R 2008], these associations suggest that the incidence of physical impairment and associated costs will increase dramatically in the absence of effective interventions [Carter et al. 2012; Olshansky et al. 2009].

To evaluate the effectiveness of an intervention, however, there must first exist an adequate battery of tests to assess motor function. A recent NIH initiative has established such a foundation for humans (3-85 yrs) with sets of tests to assess subdomains of motor performance including balance, dexterity, endurance, locomotion and strength (NIH Toolbox motor domain, Ruebens et al. 2013). However, no analogous battery exists in rodent models of aging. This is a major current limitation for assessing promising mid- to late-life healthspan-enhancing preclinical interventions and translating those results to humans [Carter et al. 2012; Kirkland 2013; Kirkland and Peterson 2009].

The purpose of the present study was to develop and assess the validity of a battery of tests to characterize motor function in the mouse. Three cohorts of male C57BL/6 mice

were tested at ages that corresponded to young, middle-aged, and older adult humans. Analogous to the approach used with the NIH Toolbox, the tests quantified performance with measures of locomotion, strength, balance/coordination, and endurance. The model was validated descriptively by comparing the magnitude and time course of declines in performance across the three groups of mice with those observed in humans.

Materials and Methods

Animals

Male C57BL/6 mice were obtained from Charles River at 2 months of age (n = 87) and from the National Institute on Aging (n = 91; 48 at 19 months and 43 at 25 months). Upon arrival at the University of Colorado Boulder, mice were ear punched for identification and housed in groups (~3-4 per cage). Mice were acclimated to our existing colony in the animal care facility for 4 weeks under a 12-hr light/dark schedule (7am to 7pm light cycle) with food and water ad-libitum. Mice were tested approximately one month later at 3, 20, or 26 months of age. Each mouse was tested to assess locomotion, strength, balance and coordination, and endurance.

Motor Function Test Battery

All testing occurred in a subsection of the vivarium in which the animals were housed. Test sessions occurred in the afternoon hours of the light cycle (11 am – 5 pm), and each test occurred at the same time of day with little variation within or across cohorts. One investigator (JNJ) conducted each test in all sessions and was assisted by another

investigator who had extensive training with the procedures. The experimental apparatus (Figure 10) were cleaned with ethanol between the testing of each mouse.

In developing the test battery, commonly used assessments of motor performance were aligned with the subdomains that comprise the NIH Toolbox Motor Function Domain. Several of the tests selected for the battery have been described in detail previously and have demonstrated reliability and validity across multiple ages in mice or rats [Altun et al. 2007; Carter et al. 2002; Fahlström et al. 2012; Ingram 1983; Ingram 1988; Ingram & Reynolds 1986; Joseph et al. 1983; Sumien et al. 2006].

Locomotion

Locomotion was characterized with measures of exploratory behavior and gait speed as assessed by behavior in an open field arena, rearing cylinder, and walking track.

a. Open Field Distance. Explorative locomotion in mice was examined as the total distance traveled during 5 min in a novel arena, referred to as the open field (Figure 10A). The custom built open field apparatus comprised two side-by-side arenas (40 L x 40 W x 30 H cm, each) with matte white finish, diffuse lighting source, and top mounted video-recorder for off-line multi-arena video tracking (EthoVision XT, Noldus Information Technology, Leesburg, VA, USA). Open field tests were performed once for each mouse [Montiglio et al. 2010]. The distance covered during the open-field test correlates with distance traveled in voluntary wheel running in both male and female in-bred mouse strains [Careau et al. 2012]. Body mass was recorded at the end of testing.

b. Rearing Counts. Locomotor behavior was further assessed as exploratory rearing in a cylinder (Figure 10B). The mice were placed in a clear plexiglass cylinder (12.5 dia x

14 H cm) and an observer counted the number of rears during a single 3 min bout. A rear was defined as lifting forepaws, typically to reach the sidewalls of the cylinder, and extending upward from hindlimbs, and was completed when forepaws returned to the floor of the cylinder.

c. Scurry Speed. Scurry speed was determined as the average time to travel a straight track (Figure 10C). The walking track comprised a custom built elevated platform with a narrow channel (3.5 W x 80 L x 15 H cm) and a dark box with a removable melamine top panel. Mice were first allowed to acclimate to the dark box for ~1 min, then were placed on the elevated platform at the other end of the track. An air puff was used to stimulate the mice to traverse the straight track to reach the dark box. A timer was started once the mouse entered the track and stopped once the head crossed the threshold of the dark box. The time taken to traverse the track and the number of stimulations needed to complete the walk were recorded. Five trials were recorded: 2 initial practice trials and 3 experimental trials, with 30 s in the dark box between trials. Experimental trials were excluded when the mouse required more than 2 stimulations to reach the dark box; only 2 trials were excluded from young and old animal groups. Scurry speed was determined as the average speed to travel the 80 cm. The scurry speed device was not developed until after the 20-month mice were assessed.

Strength

Grip strength of the forelimbs was measured and normalized to body mass. A customized grip strength device was used that included a force transducer (0.5 kg, Imada PS Series, Northbrook, IL, USA) attached to a trapeze grip of ~1.5 mm diameter (Figure

10D). The method was similar to one reported previously [Cabe et al. 1978; Ingram 1983]. Briefly, the mouse was grasped by its tail, suspended just above the trapeze bar, and lowered until it successfully grasped the bar with both forepaws. A uniform horizontal tug was then applied until the mouse released its grip. Five trials were taken with 30 s between trials. Trials in which the mouse forcefully jerked the bar rather than simply releasing its grip were excluded. Body mass was recorded prior to strength testing.

Balance/Coordination

Balance was challenged under dynamic conditions with tests that required mice to respond to a perturbation: an accelerating rota-rod test and latency to hindlimb grasp when suspended from a tightrope.

a. Accelerating rota-rod test. The time to fall from a five-station accelerating rota-rod (Ugo Basile, Comerio, Italy) was recorded on 3 trials separated by an inter-trial interval of ~1 hour (Figure 10E). The rota-rod was accelerated during each trial from 4 to 40 rpm over a 5 min period and a cut-off time was set at 6 min [Kuleskaya et al. 2011]. On the day prior to testing, each mouse was introduced to the test by replacing it on the rota-rod until it could maintain its balance while the rod accelerated for 90 s.

b. Hindlimb grasp. The latency to hindlimb grasp was recorded as a measure of motor coordination during the tightrope suspension test (Figure 1F). The mouse was suspended above soft bedding by grasping a taut cotton string (2 mm dia, 50 cm L) with its forepaws. The test involved recording the time it took the mouse to grasp the string with its hindlimbs.

Endurance

Endurance was measured as how long the mouse could perform the tightrope test and run on the rota-rod.

a. Tightrope. Tightrope suspension time was determined with the same apparatus used to assess hindlimb grasp (Figure 10F) using a protocol described by Ingram [1983] and Miguel and Blasco [1978]. Briefly, the mouse was suspended by its forepaws from the string in the center until one of two events occurred: a) fall into soft bedding below; or b) an escape in which the mouse traversed along the string to the support panels. If a mouse fell, the time to the fall was recorded. If a mouse did not fall or escape, a score of 120 s was recorded. If a mouse escaped within 60 s, the recorded score was 120 s minus the time to escape.

b. Rota-rod run. The maximal time and distance run until falling off the rota-rod were recorded. The test was conducted 24-48 hours after the accelerating rota-rod test. The maximal time each mouse could remain on the three accelerating rota-rod trials was used to set the speed for the rota-rod run. Mice with similar maximal speeds were run at the same time on the 5-panel rota-rod. The test comprised four consecutive phases: refresh, warm-up, endurance 1, and endurance 2. In the refresh period, the rota-rod was accelerated to 25% of maximal baseline speed and maintained for 2 min. Next, the mice performed a warm-up run during which the rota-rod was accelerated to 50% of maximum for a 5 min. Mice that fell during either the refresh or warm-up phases were immediately replaced on the rota-rod to continue running. Subsequently, the rota-rod was accelerated to 75% of maximum speed for the endurance 1 phase and the time to falling off the rota-rod was recorded. When a mouse did not fall after 10 min, the rota-rod was further

accelerated to 100% of maximum speed for the endurance 2 phase for up to 30 min or until a fall. The speed of each phase was recorded and used to determine the total distance run in all phases.

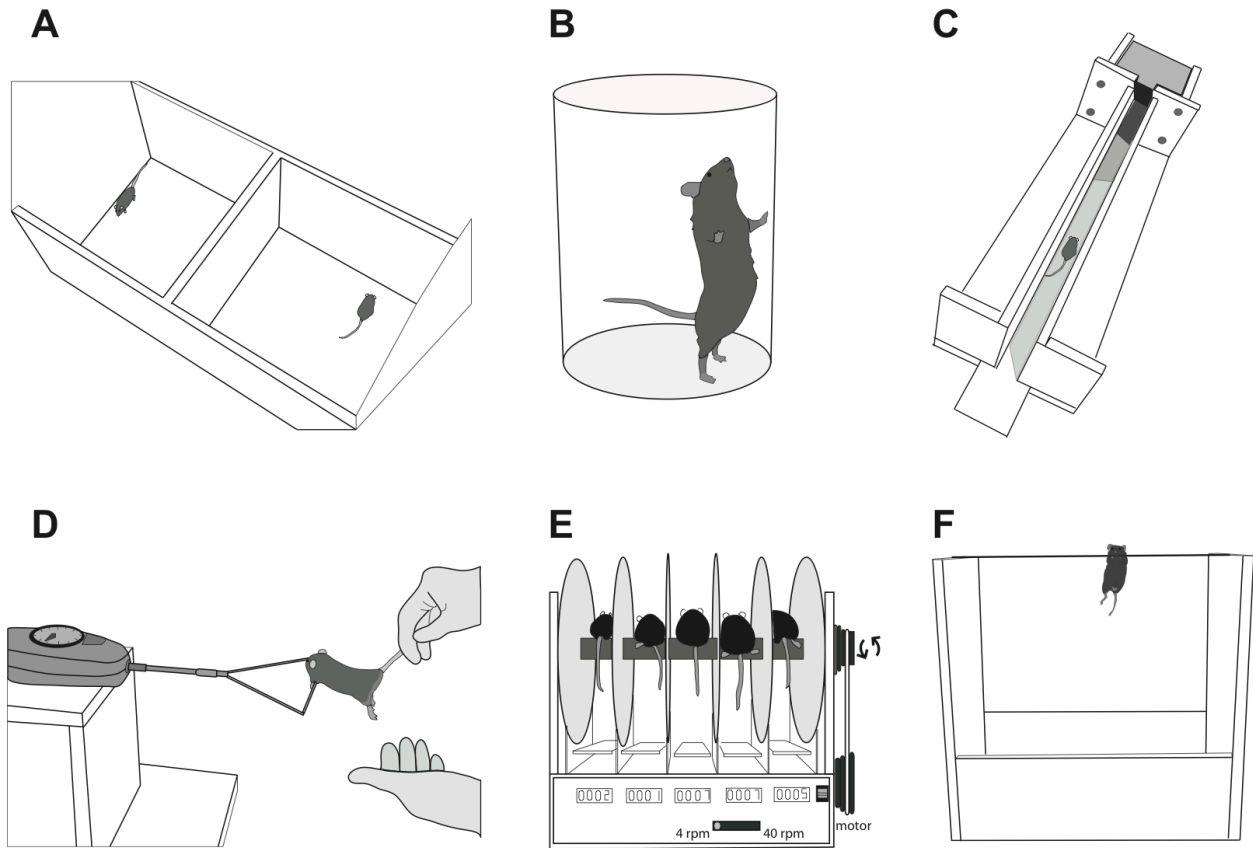


Figure 10. Experimental apparatus. Multiple devices were used to characterize subdomains of motor function in mice. Locomotion was quantified as the distance traveled during 5-min in an open field (A), rearing counts during 3-min in a 12.5 cm diameter cylinder (B), and average walking speed along a 40-cm track (C). Grip strength was measured with a custom-built device attached to a load cell (D). Balance was measured as the maximal time on a rota-rod that accelerated from 4 to 40 rpm over 5-min (E) and time to grasp a tightrope with hindlimbs (F). Endurance was quantified as tightrope hang time (F) and time and distance on a rota-rod rotating at a normalized speed (E).

Normalization of Motor Function Scores

Raw performance on each test was normalized to similar scale for direct comparison of test results within and between subdomains. Performance on each test was

rescaled to values ranging from 0 to 1, where 0 indicated the worst performance across all three age groups [Carter et al. 2002], with the formula: $\text{Score} = 1 - (\text{worst performance} / \text{current performance})$. Scores for each test within a subdomain were then averaged to create mean subdomain scores for locomotion, strength, balance and coordination, and endurance. An overall motor function summary score was then calculated as the average of the 4 subdomain scores.

Statistical Analysis

Prior to primary analysis, normality was assessed with the Shapiro-Wilkes test, and homogeneity of variance between age groups was examined with Levene's test for each measure. As the variance in performance within a group was greater in the oldest age group for all measures, except locomotion, comparisons of individual tests between age groups were performed using the Welch ANOVA (one-way, two degrees of freedom), and Games-Howell test for post-hoc group comparisons. Because 16 hypotheses of an age effect were tested, a Bonferroni-adjusted significance level of 0.003 was calculated to account for the increased possibility of type-I error. Linear regression equations were used to assess the relative significance of each test within the battery or subdomain to the motor function summary score. Stepwise multiple-regression models were used to explain the motor function summary scores based on individual test scores or average subdomain scores. The α -level for all statistical analyses was set at 0.05, and all data are presented as mean \pm SD within the text and tables and mean \pm SEM within figures. The statistical procedures were performed with SPSS Statistics (version 21; SPSS, Inc., Chicago, IL, USA).

Results

Test Outcomes Across Ages

Original data from each test in the motor function battery are presented in Table 4. Age-related declines were observed for each measure ($p < 0.001$ main effect for age, each test), but post hoc analyses indicated that the decreases across age groups varied among individual tests. The normalized motor function scores (0-1 scale, with 0 being the worst performer) for each test for the three groups of mice demonstrate similar age-related declines in performance to the raw scores shown in Table 4 are reported in Supplemental Data Table 8.

Table 4. Test battery outcomes for 3, 20, and 26-month C57Bl/6 male mice.

	3 month	20 month	26 month
	<i>n</i> = 87	<i>n</i> = 48	<i>n</i> = 42
Body mass (g)	26.0 ± 1.9	35.2 ± 2.4	31.2 ± 2.7
Locomotion			
Open field distance (cm)	1859 ± 265	1740 ± 431	1357 ± 398*†
Rearing counts	25.4 ± 6.7	10.6 ± 6.3*	8.6 ± 8.2*
Walking speed (cm/s)	11.0 ± 4.2	–	8.2 ± 3.8*
Strength			
Fore-paw grip strength (g)	130 ± 14	96.7 ± 15.1*	79.7 ± 13.3*†
Grip strength normalized to mass (g/g)	5.58 ± 0.55	2.80 ± 0.44*	2.55 ± 0.44*
Balance and Coordination			
Average accelerating rota-rod time (s)	240 ± 57	181 ± 76*	164 ± 62*
Time to hindlimb grasp (s)	5.0 ± 2.4	8.3 ± 7.2*	16.7 ± 13.1*
% capable of hindlimb grasp	100	77	36
Endurance			
Tightrope hang time (s)	84.0 ± 32.5	62.4 ± 34	23.6 ± 24.2*†
Tightrope score per mass (s/g)	3.5 ± 1.4	1.8 ± 1.0*	0.8 ± 0.8*†
Rota-rod run time (s)	864 ± 356	670 ± 478	461 ± 328*
Rota-rod run distance (m)	24.4 ± 9.9	15.7 ± 8.6*	14.2 ± 11.6*

Mean ± SD. * *p* < 0.0003 compared with young, † *p* < 0.0003 compared with 20-month old mice.

Locomotion

Open-field distance was less for 26-month mice compared with 3-month old mice ($p < 0.001$), but there was no significant difference between 3- and 20-month old mice ($p = 0.27$). Rearing counts were greater in 3-month mice compared with both older age groups ($p < 0.001$), but there was no additional decline with age from 20 to 26 months ($p = 0.53$). Scurry speed was only assessed in 3- and 26-month old mice and was faster for the 3-month mice ($p < 0.001$).

Strength

Forepaw grip strength decreased progressively across the three ages ($p < 0.001$). However, when grip strength was normalized to body mass (3 mo: 23.8 ± 2.4 g, 20 mo: 35.1 ± 2.4 g, 26 mo: 31.6 ± 2.6 g; $p < 0.001$), there was no longer a statistical difference in strength between 20- and 26-month old mice ($p = 0.01$) given the Bonferroni correction.

Balance Coordination

Average time that mice could remain on an accelerating rota-rod was less in both 20- and 26-month old mice compared with 3-month mice ($p = 0.001$ and $p < 0.001$, respectively), but was not different between 20- and 26-month old groups ($p = 0.57$). The same relation was observed for the time on the accelerating rota-rod for the last trial with the 3-month mice lasting longer (274 ± 71.7 s, $p < 0.001$) and no statistically significant difference between the two older groups (20-mo: 183 ± 77 s; 26-mo: 205 ± 84 s, $p = 0.42$). When coordination was assessed as the time to grasp a tightrope with the hind limbs when suspended by the forepaws, the time was less for the 3-month mice ($p < 0.001$) and not

statistically significant between 20- and 26-month groups ($p = 0.07$). Additionally, fewer mice in the older group were able to perform the hindlimb grasp. The same relations were observed when performance was normalized to a 0-1 scale for motor function scoring.

Endurance

Tightrope hang time declined across the three age groups when normalized to body mass ($p < 0.001$ all), but the absolute time was not statistically significant between 20- and 26-month mice ($p = 0.01$). Run time on the rota-rod was not significantly different between 3- and 20-month ($p = 0.12$) or 20- and 26-month ($p = 0.03$) mice, but run time run for the 26-month group was less than that for the 3-month group ($p < 0.001$). The 3-month mice ran further than both the 20-month ($p < 0.001$) and 26-month groups ($p < 0.001$), but the difference between the 20- and 26-month groups was not statistically significant ($p = 0.83$).

Subdomain and Summary Scores

The subdomain scores and motor function summary score are shown in Figure 11. Age-related declines were observed in each subdomain score and the motor function summary score (age main effect, $p < 0.001$ all). Post-hoc analysis indicated that the progressive decline from the 3- to 26-month groups were statistically significant for the motor function summary score, and for the strength and endurance subdomain scores ($p < 0.001$ all). An age-related decline in locomotion scores was observed between each age group, but a non-significant trend was observed between 3- to 20-months ($p = 0.01$). The coordination score was statistically greater for the 3-month group compared with the two older groups ($p < 0.001$), but the difference between the 20- and 26-month groups was not

statistically significant ($p = 0.58$).

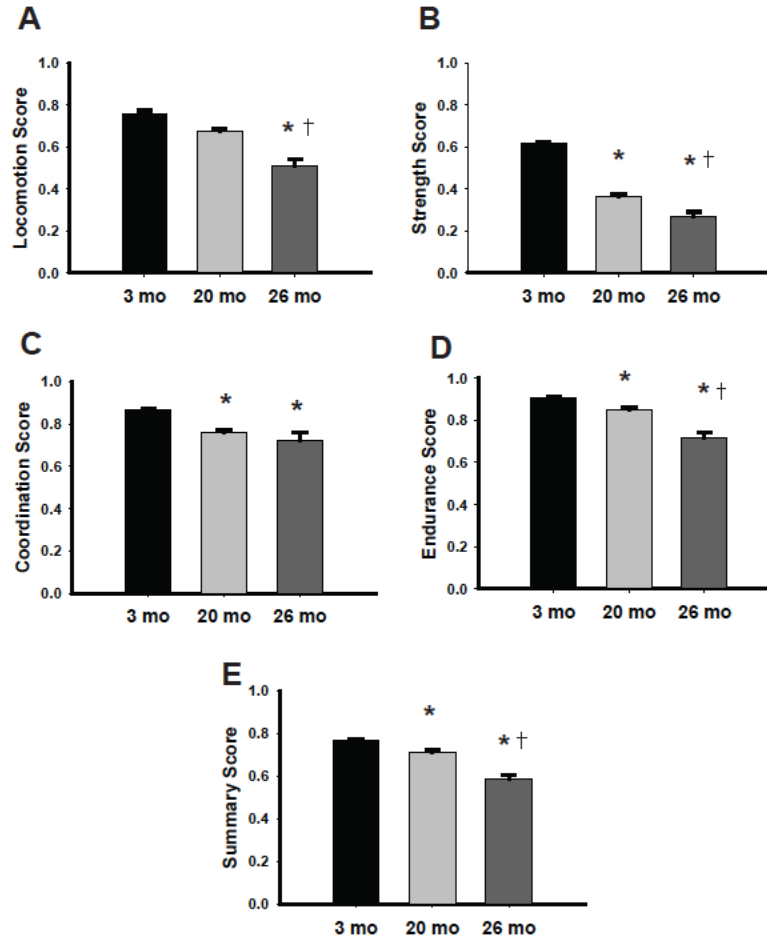


Figure 11. Subdomain and summary scores (mean \pm SEM) of motor function for the three groups of mice. Scores for each test in the motor function battery were converted to a 0-1 scale in which 0 was the worst performance. The scores for each test within a subdomain were averaged to create a subdomain score. Locomotion (A) was based on the average scores for distance traveled in an open field, rearing counts, and walking speed. Strength score (B) was based on the scores for grip strength and grip strength normalized to body mass. Coordination score (C) combined the scores for the time to hindlimb grasp and average time to remain on an accelerating rota-rod. Endurance score (D) corresponded to the scores for the tightrope time normalized to body mass and the time and distance for the rota-rod run. Summary score (E) represent the average score of the subdomain scores. * $p < 0.0003$ compared with 3-month old mice. † $p < 0.01$ between 20- and 26 month-old mice.

A power analysis was conducted to estimate the sample size needed to attenuate or reverse age-related deficits in the motor function summary score given a known effectiveness for an intervention (Figure 12).

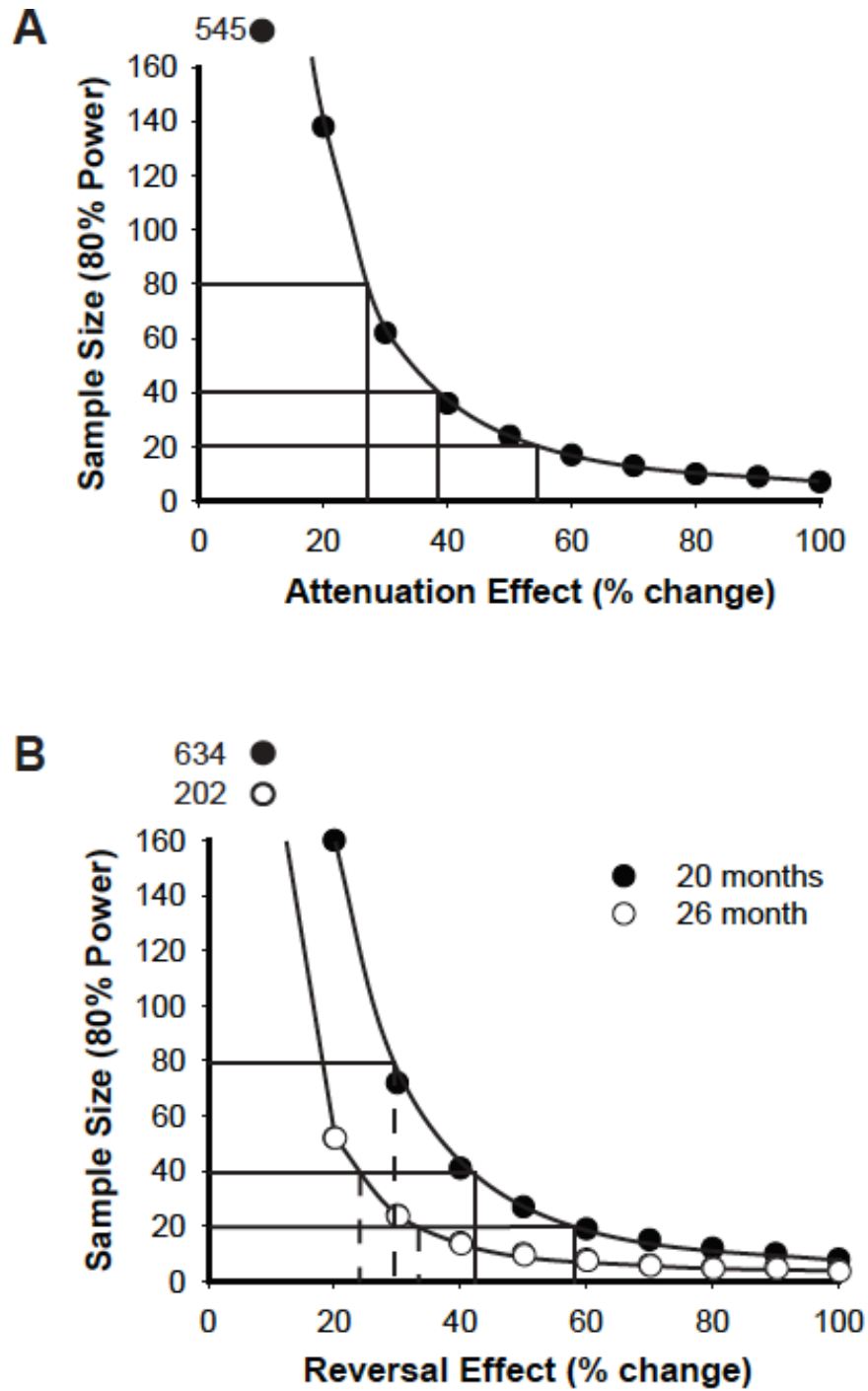


Figure 12. Power analyses to indicate the number of mice required to attenuate (A) or reverse (B) deficits in the summary performance score from the known effectiveness of an intervention. An attenuation effect of 100% indicates that an intervention has the capacity to improve the mean score in 26-month old mice to similar values for 20-month mice (A). An intervention that has a reversal effect of 100% can change the scores for 20-month (closed circles) and 26-month mice (open circles) to values similar to those for 3-month mice (B).

Relations Between Subdomains and Summary Scores

The relative contribution of each test and subdomain score to the motor function summary score was assessed with stepwise linear regression models (Tables 5 and 6). Absolute grip strength explained more of the variance in the summary score ($R^2 = 0.83$, $p < 0.001$) than any other single variable (Table 5). The inclusion of four additional test scores (endurance rota-rod distance, normalized tightrope time, walking speed, and accelerating rota-rod time) resulted in the model explaining 98% of the variance in the motor function summary score. In contrast, scores from all four subdomains were necessary to explain 98% of the variance in the motor function summary score (Table 6). The subdomain score with the greatest explanatory power was endurance ($R^2 = 0.65$, $p < 0.001$).

Table 5. Regression model to determine the relative contribution of each test score in explaining the variance in the motor function summary score.

	Beta	Standard Error	Part	R²
Summary Performance Score				
Grip strength	0.203	0.005	0.162	0.83
Rota-rod distance	0.087	0.005	0.069	0.90
Tightrope time per mass	0.190	0.004	0.178	0.94
Walking speed	0.060	0.004	0.067	0.97
Accelerating rota-rod	0.109	0.010	0.044	0.98
Rearing count	0.068	0.004	0.078	0.99
Rota-rod time	0.125	0.007	0.070	0.99
Time to hindlimb grasp	0.095	0.005	0.083	1.00
Open field distance	0.070	0.006	0.049	1.00

Variables (normalized scores) are listed in the order of their entry into the stepwise regression model. All of the listed variables explained statistically significant amounts of the variance in the motor function summary score ($p < 0.001$). Part correlations and the cumulative R² values as test scores are added to the regression equation indicate the relative contribution of each test in explaining the variance in the motor function summary score. The excluded variables were the scores for grip strength normalized to mass and absolute tightrope time.

Table 6. Regression model to determine the relative significance of each subdomain score in explaining the variance in the motor function summary score.

	Beta	Standard Error	Part	R²
Motor Function Summary Score				
Endurance	0.369	0.013	0.382	0.65
Strength	0.153	0.010	0.222	0.85
Locomotion	0.230	0.011	0.302	0.93
Coordination	0.199	0.012	0.227	0.98

Variables (average subdomain scores) are listed in order of their entry in the stepwise model and all explained statistically significant amounts of variance in the motor function summary score ($p < 0.001$). Part correlations and cumulative R² values as subdomains are added to the regression equation indicate the relative contribution of each subdomain in explaining the variance in the motor function summary score.

Comparison with Declines in Humans

The validity of the model was examined descriptively by comparing the current results with the magnitude and time course of declines for humans reported in peer-reviewed publications (Table 7). Criteria for inclusion in the comparison were: 1) standard tests consistent within the subdomain construct; 2) mean \pm SD for young adults aged 20-29 years; and 3) mean \pm SD for older adults with 10-year increments. Data from 21 published articles were included, with normative data from 30 standard tests (see Supplemental Data). The percent decline for each older group of humans from the young group (20-29 years) was calculated for each test and averaged to indicate the relative decrease in each subdomain score (Table 4). Similarly, the percent decline from young mice (3-month) was calculated for the 20- and 26-month groups and listed in Table 4 under the two age-increments of comparable biological age. Because the values are descriptive, no statistical comparisons were performed. The percent declines are similar for humans and mice in all

subdomains except balance/coordination. The difference in the latter was that declines in balance scores were observed earlier in the lifespan for mice than humans.

Table 7. Comparison of age-related % declines in motor function in humans and mice.

		Percentage Decline from Young (%)				
Measures			Age (yrs)			
		Sex	50-59	60-69	70-79	80-89
Locomotion						
Humans	Gait speed, up & go	M/F	2	8	14	42
Mice	Locomotion Score			8		31
Strength						
Humans	Grip, knee extension, plantar flexion	M/F	19	26	40	50
Mice	Strength Score			26		49
Balance & Coordination						
Humans	Static and dynamic	M/F	11	6	34	45
Mice	Coordination Score			35		39
Endurance and Cardiorespiratory Fitness						
Humans	Endurance run race times	M	17	23		48
Mice	Endurance Score			10		21
Mice	Endurance Run Distance			19		30

Percent declines from young (20-29 years) in humans were calculated from representative studies within each subdomain (see Supplemental Material). In the present study in mice, the percent declines from young (3-month) for each subdomain are presented for 20-month and 26-month old mice and listed between age categories that are at similar in terms of percent of mean lifespan.

Discussion

The main findings of this study were: (1) the development of a translational battery of tests to characterize age-related declines in motor function in mice; (2) establishing a normalization procedure to standardize tests scores for each of four subdomains and a motor function summary score; (3) identification of tests and subdomains that explained most of the variance in the motor function summary score; and (4) evidence validating that, in general, declines in subdomain scores for the mouse model advanced were similar to those observed in adult humans.

Normalized Test Battery

Although previous investigations have examined the influence of age on behavior in animals [Ingram1983], and have reported that age-related declines in motor function are predictive of lifespan in rats [Altun et al. 2007; Carter et al. 2002] and mice [Fählstron et al. 2012; Ingram and Reynolds 1986], the translation of these studies to human behavior are limited. There has been little attempt to describe changes in behavior in animals in terms relevant to humans other than physical versus cognitive. One approach to improve translation is not only to identify specific tests that may characterize function in subdomains that are clinically relevant, but also to normalize the raw performance outcomes to scores. By normalizing test outcomes, relative performance on individual tests can be compared, and combined to develop subdomain scores that can translate more readily to motor function in humans. Moreover, the collation of multiple measures into a composite index affords greater sensitivity in detecting the progressive decline in motor function with advancing age [Markowska and Breckler 1999; de Fiebre et al. 2006; Carter

et al. 2012]. The use of normalization procedures has been performed in rats as a way to combine performance outcomes to predict lifespan [Carter et al. 2002], however, these performance outcomes do not map to functional measures widely used in the human literature to predict disability, independent living status, and mortality [Guralnik et al. 1994, 1995, 2000; Rosano et al. 2008].

In the present study, multiple regression models were created to explain the variance in the motor function summary score in order to assess the relative significance of each test within and across the subdomains. The step-wise regression model demonstrated that although grip strength, rota-rod run distance, and tightrope test entered into the model first, grip strength and tightrope score normalized to body mass had the greatest part correlations ($r = 0.16$, and $r = 0.18$, respectively), and thus were more strongly and independently related to the motor function summary score (Table 5). However, when subdomain scores were used to predict performance, the endurance score explained most of the variance in the motor function summary scores ($r = 0.38$, Table 6). The likely reason for the greater predictive value of the endurance subdomain can be seen from the step-wise regression model from individual tests: whereas grip strength test alone was most predictive of summary motor function, two tests of endurance were also strongly predictive. When these two endurance tests were combined into a subdomain score, the endurance subdomain was more predictive overall. The regression analyses indicate the specific tests and subdomains that need to be included in studies that examine the effectiveness of interventions to reduce age-associated declines in motor function. Also, the set the tests included in the locomotion and coordination subdomains explained the least amount of variance in the motor function summary score.

Power and Validity

The power calculations depicted in Figure 12 demonstrate that the summary motor function score obtained from the battery should have sufficient power to detect the effects of an intervention with potential to attenuate or reverse age-related deficits in motor functions. If an intervention can attenuate 50% of the additional declines in motor function that occur from age 20- to 26-months, for example, then 24 animals would be needed to detect a significant effect. Similarly, an intervention that could reverse 50% of the decrease in motor function from 3 to 20 month mice, a sample of 27 mice per group would be needed to evaluate the intervention. The detection of smaller attenuation or reversal effects with this battery would require larger sample sizes.

In an attempt to establish the translational validity of the battery of tests used in the current study, we provided a descriptive comparison of age-associated changes in motor function for humans and mice (Table 7, and expanded in Supplemental Data Table 10). The differences in the four motor function subdomain scores across the three groups of mice approximated those reported for humans of similar relative biological age. The one difference was balance/coordination. Declines of 6-11% have been reported in adults aged 50-69 years in tests of static and dynamic balance, whereas coordination declined by 35% in 20-month mice based on accelerating rota-rod and time to hindlimb grasp. Thus, the tests used in the current study to assess this particular measure of motor function appear to translate less well to humans than the other tests. Although there appeared to be a difference in the magnitude of change in the endurance score for mice (10-21%) compared with humans (17-48%), the lack of a comparable literature on changes in endurance in

humans compromised the comparison for this subdomain. For example, the magnitude of decline was similar for rota-rod run score of mice (19-30%) and running race times for humans (17-48%).

Limitations and Future Directions

The battery includes 12 tests of motor function for C57BL/6 mice that mapped onto four subdomains of the NIH Toolbox Motor Domain for humans. Although the motor function summary score was sensitive to differences across the three groups of mice, the tests that contributed to the subdomain scores for locomotion and coordination explained the least amounts of the variance in the summary score. Interventions that target either locomotion or balance and coordination, therefore, may need to identify different tests to evaluate the effectiveness of the intervention, such as tasks requiring bridge walking [de Fiebre et al. 2006, Sumien et al. 2006], or beam balance [Altun et al. 2007; Fahlstrom et al. 2012].

Future work should be conducted to extend the model to other mouse strains and other preclinical model species such as rats, and to include comparisons of functional declines in both male and females. Also longitudinal studies would provide greater insight into rate of age-related declines in motor function across subdomains as well as underlying mechanisms leading to functional declines. The identification of functional biomarkers, such as those assayed in the current study, are essential to establishing the efficacy of interventions aimed at slowing declines in health-span [Carter et al. 2012; Rae et al. 2010], and future work should establish the sensitivity of the model to detect the capacity of late-life interventions to attenuate or reverse functional deficits.

Concluding Remarks

We have developed a test battery and composite scoring system to characterize declines in four subdomains of motor function across young and older C57BL/6 mice. Age-related declines were observed in locomotion, strength, balance and coordination, and endurance, as well as a motor function summary score. The cross-sectional declines in locomotion, strength, and endurance in mice reflect those observed in humans, thus demonstrating a degree of construct validity for the test battery. Although much work remains to validate and expand the preclinical model to other species and to determine its sensitivity to late-life intervention, the current model is the first to adequately describe functional scores that are relevant to humans, and thus represents a significant translational link in biomedical aging research.

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Supplemental Tables

Table 8. Normalized scores derived from individual tests in 3, 20, and 26-month male C57BL/6 mice.

	3 month <i>n</i> = 87	20 month <i>n</i> = 48	26 month <i>n</i> = 42
Locomotion			
Open field distance	0.60 ± 0.06	0.55 ± 0.13	0.40 ± 0.19*†
Rearing counts	0.96 ± 0.01	0.84 ± 0.17*	0.64 ± 0.40*
Walking speed	0.56 ± 0.11	–	0.37 ± 0.23*
Strength			
Fore-paw grip strength	0.57 ± 0.05	0.42 ± 0.11*	0.29 ± 0.13*†
Grip strength normalized to mass	0.66 ± 0.04	0.31 ± 0.18*	0.25 ± 0.13*
Balance and Coordination			
Average accelerating rota-rod time	0.85 ± 0.05	0.76 ± 0.11*	0.75 ± 0.16*
Time to hindlimb grasp	0.89 ± 0.05	0.75 ± 0.14*	0.62 ± 0.30*
% capable of hindlimb grasp	100	77	36
Endurance			
Tightrope hang score	0.95 ± 0.04	0.90 ± 0.08	0.69 ± 0.23*†
Tightrope score per mass	0.96 ± 0.03	0.91 ± 0.08*	0.72 ± 0.22*†
Rota-rod run time	0.98 ± 0.02	0.95 ± 0.05	0.90 ± 0.21*
Rota-rod run distance	0.79 ± 0.13	0.63 ± 0.21*	0.56 ± 0.26*

Mean ± SD. * ($p < 0.0003$) compared with 3-month group, † ($p < 0.0003$) compared with 20-month old mice. Scores were normalized on a 0-1 scale such that 0 represents the worst performer (score = $1 - (\text{worst} / \text{current})$).

Table 9. Comparison of age-related motor function declines in humans and mouse model.
Percentage Decline from Young (%)

Source	Measures	Sex	Age (yrs)			
			50-59	60-69	70-79	80-89
Locomotion						
Ble 2005	4 m walk	M	0	2	5	33
Bohannon 1997	7 m walk	M	0	2	5	
Lord 1996	7.2 m walk	F	7	18	22	30
Oberg 1993	5.5 m walk	M	+1 (gain)	+4 (gain)	4	
Bohannon 2011	Meta-analysis	M	+5 (gain)	1	7	29
Vereeck 2008	Timed Up & Go	M/F	11	27	52	77
Average			2	8	14	42
Present Study	Locomotion Score	M	8			31

Strength						
			Sex			
Burke et al. 1953	Handgrip	M	17	19	41	
Mathiowetz et al. 1985	Handgrip	M	11	25	38	
Kallman et al. 1990	Handgrip	M	9	16	29	
Hunter et al. 2000	Handgrip	F	16	22	35	38
Werle et al. 2009	Handgrip	M	2	15	27	50
Vandervoort et al. 1986	Plantar flexors	M		21	29	45
Vandervoort et al. 1986	Plantar flexors	F		20	22	55
Bemben et al. 1991	Plantar flexors	M	18		37	
Hunter et al. 2000	Plantar flexors	F	16	28	37	55
Bohannon et al. 1997	Knee extensors	M	19	34	36	

Bohannon et al. 1997	Knee extensors	F	30	42	56	
Hunter et al. 2000	Knee extensors	F	31	41	50	56
Average		M/F	19	26	40	50
Present Study	Strength Score	M		26		49
Balance and Coordination		Sex				
Gill 2001	One leg stance time	M/F	50		77	
Vereeck 2008	Foam stand	M/F	0	0	13	34
	Tandem Romberg	M/F	4	6	40	56
	1 leg stand eyes open	M/F	1	0	8	29
	1 leg stand eyes close	M/F	21	28	68	82
	Tandem gait	M/F	0	0	14	30
	Dynamic gait	M/F	0	0	3	8
Tang & Woollacott 1998	Sharpened Romberg	M/F				45
	1 leg stand eyes open	M/F				49
Average			11	6	34	45
Present Study	Coordination Score	M		35		39
Endurance and Cardiorespiratory Fitness		Train				
Pimentel et al. 2003	10km run time	ET	21		47	
Leyk et al. 2007	Marathon run time	ET	19	26	48	
Leyk et al. 2007	Half-marathon time	ET	11	25	48	
Average			17	26	48	
Present Study	Endurance Score	Sed		10		21
Present Study	Endurance Run Distance	Sed		19		30

Percent declines from young humans (20-29 years) were estimated from representative studies within each subdomain. In the present study in mice, the percent declines from young mice (3 month) for each subdomain are presented for 20-month and 26-month old mice between age categories that are at similar in terms of percent mean lifespan.

Chapter VI

Summary

The primary goal of this thesis was to examine the subdomain factors that influence motor function. The findings from the four studies show that functional outcomes (including fatigability, steadiness, strength, locomotion, balance, and endurance) depend on the demands of the task, the subtleties of measurement, and the age and species of the subject. An important feature of current biomedical research also explored in this dissertation is the translation of findings from the “bench to the bedside”, which involves extending basic or clinical research findings to clinical practice and public health arenas. However, the work in this dissertation illustrates the equal importance of reverse translation: looking to community health and epidemiological trends to focus research in traditional physiology to further understand mechanisms, influential factors, and targets for treatment that may then expand our ability to translate forward.

The first project clearly illustrates the intricacy of measurement technique on functional outcomes. In this project, two tasks were used to assess performance fatigability to the point of task failure: both the force task (push against rigid restraint) and position task (hold an inertial load) required the subject to sustain a submaximal isometric contraction with an equivalent net muscle torque. This project follows a long line of work in our laboratory that demonstrates a shorter time to failure for the position task [Hunter et al. 2002, 2008, Rudroff et al. 2005]. The position task could only be maintained with moderate loads for approximately half the time of the force task in the knee extensors (Dissertation Project 1, Thesis Chapter 2, Rudroff et al. 2010), and the two tasks are limited by impairments in different neuromuscular adjustments.

The second project demonstrates the task dependence of aging on motor functions. Whereas most functional tasks are reduced in older populations, the effect of age on

performance fatigability is inconsistent. Many studies reveal that performance fatigability is preserved or even improved in old compared with young adults [Hunter et al. 2005; Kent-Braun 2008; Snook et al. 2011]. However, the age-related changes in fatigability are also task dependent: in tasks requiring power, particularly of the dorsiflexors, old adults demonstrate greater performance fatigability compared with young [Baudry et al. 2007; Christie et al. 2011; McNeil and Rice 2007]. The second project provides additional support for the argument of task-dependency in the age-fatigability relation: in a group of older adults, endurance time for a submaximal isometric dorsiflexion contraction was best explained by age, such that fatigability performance was increased with advancing age. Furthermore, young adults demonstrated a trend such that young adults may be either less or similarly fatigable to the older adult cohort.

The third project investigated hormonal and metabolic factors that may influence motor function. Epidemiological evidence has revealed associations between diabetes / pre-diabetes and vitamin D status and motor function in older persons. However, many of these large-scale investigations were limited by the inclusion of persons with comorbid conditions that can influence the primary outcome. The third study in this dissertation examined the associations between insulin sensitivity and vitamin D metabolites with motor function in a cohort of relatively healthy, non-diabetic older persons. The primary findings of this study were that many associations were evident between markers of insulin-glucose dynamics, as measured by intra-venous glucose tolerance testing, and motor function. However, functional ability, as indexed by endurance walk time, was predicted by vitamin D hormone (1,25(OH)₂D), sex of the participant, and fasting blood

insulin levels. This project illustrates the strength of relations between motor function and systemic markers of health status, even in healthy older adults.

The final project outlined in this dissertation sought to translate the motor functional consequences of aging from humans to an animal model. The goal of the final project was to create a translational model of aging that incorporated multiple motor function subdomains. The NIH Toolbox subdomains of motor function were used as a framework to define function in animals. Common tests of physical function were assessed, and a final battery was established to demonstrate age-related declines in motor function. These tests were then normalized to a 0-1 scale and scored such that each test and subdomain could be compared to each other and to age-related declines observed in humans. This final project establishes a valid preclinical model of motor function that can be used to study mechanisms and interventions relevant to humans.

Taken together, these projects highlight the necessity to consider multiple subdomains in measuring motor function, especially in an aging-population and when considering translating functional outcomes.

Chapter VII

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