



Fatigability and neuromuscular performance of old and very old healthy adults

Giorgio Varesco

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FATIGABILITY AND NEUROMUSCULAR PERFORMANCE OF
OLD AND VERY OLD HEALTHY ADULTS

FATIGUE ET PERFORMANCES NEUROMUSCULAIRES
D'ADULTES SAINS ÂGÉS ET TRÈS ÂGÉS

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LIST OF ABBREVIATIONS

EMG	Electromyography
CNS	Central nervous system
SR	Sarcoplasmic Reticulum
SERCA	Sarco-Endoplasmic Reticulum Calcium ATPase
MVIC	Maximal Voluntary Isometric Contraction
PNS	Peripheral Nerve Stimulation
CMAP	Compound Muscle Action Potential
MEP	Motor Evoked Potential
CMEP	Cervicomedullary Motor Evoked Potential
TMEP	Thoracic Motor Evoked Potential
PNMS	Peripheral Nerve Magnetic Stimulation
TMS	Transcranial Magnetic Stimulation
SP	Silent Period
GABA _B	Gamma-Aminobutyric Acid
PAP	Post-Activation Potentiation
ITT	Interpolated Twitch Technique
SIT	Superimposed Twitch
POT	Potentiated Resting Twitch
ERT	Estimated Resting Twitch
MU	Motor Unit

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EPIGRAPH

So, now you're a Professor?

Grandma

INTRODUCTION (FRANÇAIS)

D'ici 2050, l'espérance de vie mondiale à la naissance devrait passer d'environ 70 ans à 76 ans, la population âgée de plus de 65 ans devrait passer de ~9% de la population à ~17%, et la partie la plus âgée de la population (>80 ans) pourrait tripler, passant de 127 à 447 millions de personnes. Comme le vieillissement s'accompagne d'une augmentation du taux d'hospitalisation et des coûts des soins de santé, les conséquences d'une augmentation de la population âgée et très âgée sur le budget sanitaire seraient un lourd fardeau. Les altérations physiologiques liées au vieillissement ont montré un intérêt grandissant de la part de la communauté scientifique, afin de comprendre et d'atténuer ces détériorations. Les connaissances actuelles sur les processus du vieillissement mettent en évidence un déclin physiologique continu chez les individus en bonne santé, entraînant une limitation de la capacité à accomplir les tâches de la vie quotidienne (par exemple marcher, retrouver l'équilibre, porter des poids).

Ce déclin physiologique lié à l'âge, en l'absence de maladie particulière, commence approximativement à 30 ans. Chaque décennie, la consommation maximale d'oxygène diminue d'environ 10 % (Trappe et al. 1996; Baker et Tang 2010), la fréquence cardiaque maximale d'environ 7 bpm (Tanaka et al. 2001), et la masse musculaire d'environ 5% (Mitchell et al. 2012). Alors que des études sur des athlètes Master ont démontré qu'il était possible de maintenir une performance athlétique relativement bonne jusqu'à 50 ans ou plus (Baker et al. 2003 ; Knechtle et al. 2012), un déclin rapide est inévitablement observé à partir de la 7e décennie, tant en termes de performance que de fonction du système neuromusculaire (Faulkner et al. 2007). De plus, ce processus semble s'exacerber au cours de la 9e décennie. Par exemple, la perte de masse musculaire s'accélère, passant de ~0,5 % à ~1 % par an, tandis que la perte de force et de puissance est encore plus importante, de l'ordre de -3 % chaque année (Skelton, Greig, Davies, & Young, 1994; Mitchell et al. 2012). Ce déclin soudain de la qualité musculaire chez les octogénaires comprend des modifications de la taille, de la forme, de l'architecture, de la vascularisation, de la morphologie des fibres et de la synthèse des

protéines, accompagnées d'une réduction des concentrations de myonucléus et de cellules satellites, et d'une augmentation de la graisse intramusculaire et du stress oxydatif (Mitchell et al. 2012). De plus, cette détérioration ne se limite pas au muscle, car les facteurs centraux (la partie du système neuromusculaire qui s'étend du cortex moteur à la jonction neuromusculaire) sont également influencés par le processus de vieillissement (Clark et al. 2015 ; Sundberg et al. 2018b ; Rozand et al. 2020). Ainsi, l'étude des déficiences du système neuromusculaire qui surviennent avec le vieillissement est cruciale pour comprendre et prévenir les risques de fragilité, de perte d'autonomie et d'hospitalisations (Hunter 2016, 2018). La fonction du système neuromusculaire peut être divisée en quatre composantes principales : force, puissance, endurance et fatigabilité.

Alors que les altérations liées à l'âge de la capacité à générer et à maintenir la force ont été largement étudiées et semblent être dues à une perte progressive de la masse musculaire et du tissu neural, qui dépend de leur utilisation (Power et al. 2016b), la fatigabilité est plus complexe et n'a pas été étudiée en profondeur dans la population très âgée. Par exemple, les altérations de la fonction neuromusculaire liées à l'âge pourraient varier en fonction des différentes tâches/activités effectuées (Hunter 2018). Une revue récente (Krüger et al. 2018) a démontré que, lorsque la charge de travail était normalisée par la capacité maximale individuelle, les personnes âgées étaient moins fatigables après des exercices isométriques, c'est-à-dire qu'ils présentaient une perte de la force maximale isométrique moins importante. Lorsque l'exercice était dynamique, les personnes âgées démontraient une fatigabilité similaire à celle de leurs homologues plus jeunes. Bien que la normalisation de la charge de travail par les capacités maximales est une approche intéressante pour étudier les propriétés du système neuromusculaire, son application fonctionnelle reste limitée (Hureau et al. 2021). Régler la charge en pourcentage du poids de corps pourrait apporter des connaissances transférables aux activités de la vie quotidienne. En effet, les activités comme la marche ou monter des escaliers dépendent à la fois de charges relative (par exemple le poids du corps) et de charges absolues (par exemple soulever une charge) (Hureau et al. 2021).

Pour ajouter de la complexité, le niveau d'activité physique quotidien pourrait jouer un rôle clé dans le maintien de la bonne fonctionnalité du système neuromusculaire avec le vieillissement. Récemment, nous avons mis en lumière comment le comportement en matière d'activité physique peut être un facteur discriminant chez les personnes âgées (Varesco et al. 2021a) et, s'il n'est pas pris en compte, il pourrait conduire à des interprétations erronées des résultats. En évaluant des individus physiquement actifs, il serait possible d'évaluer les effets délétères du vieillissement uniquement plutôt que ceux du vieillissement combinés à l'inactivité physique (Harridge et Lazarus 2017).

Le but de cette thèse était d'investiguer la fonction neuromusculaire et la fatigabilité de personnes jeunes (<35 ans), âgées (>60 ans) et très âgées (>80 ans) lors de tâches isométriques et dynamiques avec différents types de charge. Seuls les individus actifs étaient intégrés aux études pour limiter les effets de l'inactivité physique.

INTRODUCTION (ENGLISH)

By 2050 the global life expectancy at birth is projected to increase from the actual ~70 years to ~76 years, the population over 65 years is expected to grow from ~9% of the population to ~17%, and the oldest part of the population (>80 years) could triplicate, from 127 to 447 million people. Because hospitalization rate and health care costs increase with age, the consequences of an increase in the old and very old populations on the sanitary budget would be a heavy burden. The age-related physiological alterations have shown increasing interest in the scientific community to understand and attenuate these deteriorations. Current understandings of the aging process evidence a continuous physiological decline in healthy individuals, leading to limitations in the ability to perform the tasks of the everyday life (e.g. walking, regain balance, carrying weights).

This age-related physiological decline, in absence of particular disease, starts approximatively at 30 years old. Each decade, maximal oxygen consumption declines by ~10% (Trappe et al. 1996; Baker and Tang 2010), maximal heart rate decreases by ~7 bpm (Tanaka et al. 2001), and muscle mass by ~5% (Mitchell et al. 2012). While studies on master athletes demonstrated that maintaining a relatively good athletic performance is possible until 50 years old or more (Baker et al. 2003; Knechtle et al. 2012), a rapid decline is inevitably observed starting about the 7th decade of human life, for both performance and neuromuscular function (Faulkner et al. 2007). Furthermore, this process seems exacerbated in the 9th decade. For instance, muscle mass loss accelerates from ~0.5% to ~1% per year, while force and power loss are even greater, approximatively -3% every year (Skelton, Greig, Davies, & Young, 1994; Mitchell et al., 2012). This sudden decline in muscle capacity in octogenarians includes changes in muscle size, shape, architecture, vascularization, fibers morphology and protein synthesis, accompanied by a reduction in the concentrations of myonuclei and satellite cells, and an increase in intramuscular fat and oxidative stress (Mitchell et al. 2012). Moreover, this deterioration is not limited to the muscle, as central factors (i.e. the part of the neuromuscular system that extends from the motor cortex to the neuromuscular junction) are also

influenced by the aging process (Clark et al. 2015; Sundberg et al. 2018b; Rozand et al. 2020). Thus, studying the impairments in the neuromuscular system that occur with aging is crucial to understand and prevent the risks of frailty, autonomy loss and hospitalizations (Newman et al. 2006; Cruz-Jentoft et al. 2010; Hunter 2016, 2018). The function of the neuromuscular system has been mainly investigated by measuring strength, power, endurance and fatigability [e.g. (Hunter et al. 2005; Justice et al. 2014)].

While the age-related alterations in the ability to generate and maintain force have been widely studied and seem to be due to a progressive loss in muscle mass and neural tissue (Power et al. 2016b), fatigability is more complex and has not been extensively studied in the very old population (> 80 years old). For instance, the age-related impairments in neuromuscular function could vary among tasks/activities (Hunter 2018). A recent review (Krüger et al. 2018) evidenced that, when the load was normalized to the individual maximal capacity, older adults were less fatigable after isometric exercises, i.e. lower decrease in maximal isometric force. When dynamic exercise was proposed, old individuals showed similar fatigability compared to young adults. Although workload normalization to maximal capacity is a useful approach to investigate the properties of the neuromuscular system, its functional application is limited (Hureau et al. 2021). Setting the workload as percentage of body weight could provide knowledge transferable to the activities of daily living. Indeed, actions like walking or climbing stairs depend on both relative (e.g. body weight) and absolute loads (e.g. carrying weights) (Hureau et al. 2021).

To further add complexity, the level of daily physical activity could play a key role in the maintenance of good functionality of the neuromuscular system with aging. Recently, we highlighted how physical activity behavior can be a discriminant factor among older adults (Varesco et al. 2021b) and could lead to misinterpretations of the results in absence of assessment. By evaluating physically active individuals it would be possible to assess the deleterious effects of inherent aging rather than aging in combination with physical inactivity (Harridge and Lazarus 2017).

The aim of the present thesis was to investigate neuromuscular function and performance fatigability in young (< 35 years), old (> 60 years) and very old (> 80 years) adults after isometric and dynamic fatiguing tasks with different workloads. Only active adults were considered to limit the effects of physical inactivity.

THEORETICAL FRAMEWORK

1. NEUROMUSCULAR FUNCTION AND FATIGABILITY

1.1 Fatigue and fatigability

Fatigue is a multifactorial and complex phenomenon that generally occurs following a stressor-induced change in the homeostasis of the organism. However, despite the large and evolving literature in the field of exercise physiology, a clear and complete definition of fatigue is still hard to find. In materials engineering, fatigue describes a structural failure that occurs following repeated cyclic loads, even when the experienced stress range is far below the static material strength (Britannica Online Encyclopedia 2021). This concept could be extended to exercise physiology, attributing 3 main characteristics to fatigue (Craig and Ratel 2009): i) an alteration in the homeostasis of one or more biological systems, ii) this alteration is reversible with recovery and iii) it can happen before (latent fatigue) an observable decrease in the performance (i.e. exhaustion). Since the pioneer works of the Italian physiologist Angelo Mosso (1846-1910), it has been acknowledged that exercise-induced fatigue is a complex phenomenon that encompass impairments from the central nervous system [CNS,(Enoka et al. 2011)], down to the skeletal muscle level. This results in both the sensation of tiredness and the decline in muscle force and performance (Enoka et al. 2011; Taylor et al. 2016a; Enoka and Duchateau 2016). To separate those two distinct phenomenon, in recent years some authors suggested that the term “fatigue” should be separated in “perceived fatigability” and “performance fatigability” (Enoka and Duchateau 2016). “Perceived fatigability” is usually adopted to indicate the sensation of discomfort and lack of energy caused by exercise or clinical conditions (Twomey et al. 2017) and generally measured through visual scales of perceived effort/fatigue that answer to general questions like “how do you feel?” (Micklewright et al. 2017; St Clair Gibson et al. 2018). “Performance fatigability” indicates a decrease in observable performance (e.g. force, power) following exercise (Enoka and Duchateau 2016) and is usually quantified using dynamometers.

Performance fatigability and its etiology are typically quantified by evaluating impairments in the neuromuscular system (Figure 1), which is composed by the motor cortex (supraspinal or cortical component), the spinal motoneuronal pool (spinal component), and the motor units down to the actin-myosin bridges (peripheral or muscular component; Figure 1). A current definition of performance fatigability could be “a response that is less than the expected or anticipated contractile response, for a given stimulation” (Macintosh and Rassier 2002). Usually, during exercise, afferent feedbacks stressed by exertion converge to the CNS, which could modulate the motor drive output to the muscles. This can be achieved by influencing the excitability/inhibition of three sites: the motor cortex, the motoneuron pool in the spinal cord pathway and/or the single motor units (Gandevia 2001; Ranieri and Di Lazzaro 2012; Heckman and Enoka 2012). Thus, performance fatigability could be caused by a lack in energetic substrates, oxygen supply, metabolite accumulation or structural alteration that happen within the muscle (impairments in contractile function), but also by alterations in the voluntary activation capacity of the skeletal muscle by the CNS (Figure 1).

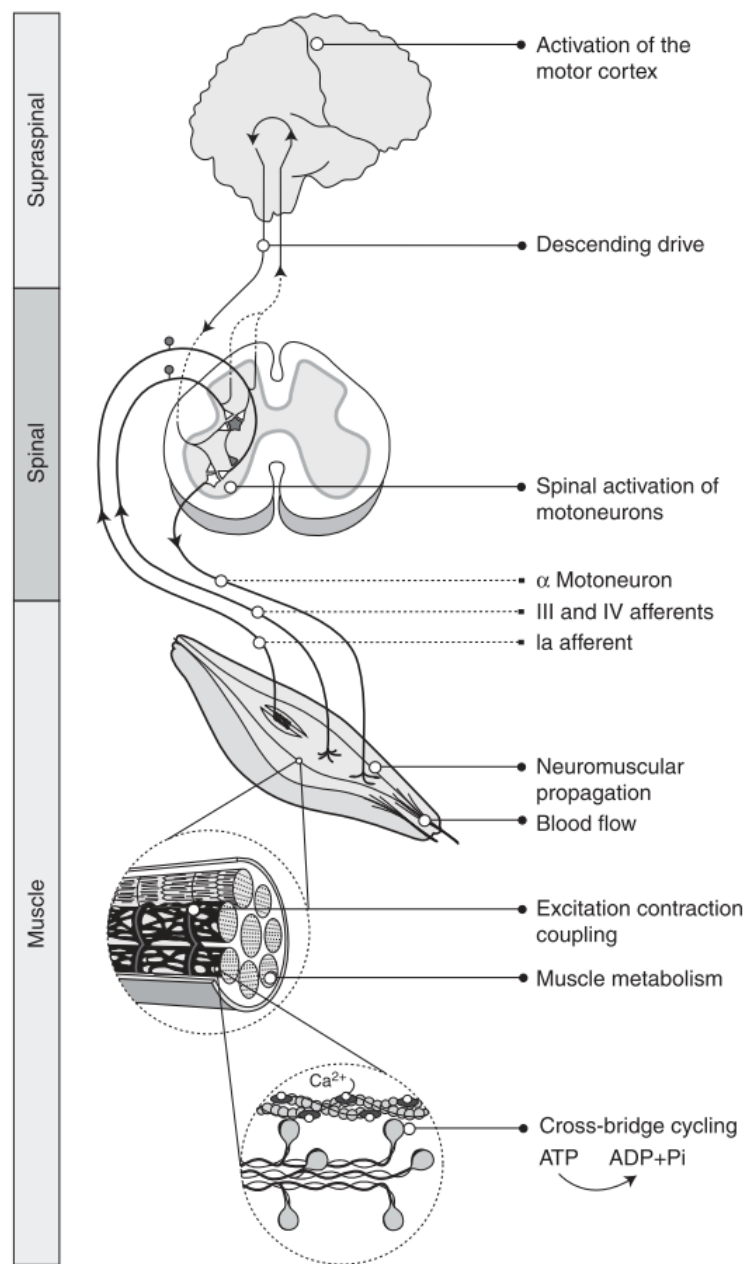


Figure 1. Schematic representation of the neuromuscular system and the sites / mechanisms that could be impaired during physical efforts. From: (Hunter 2018).

1.2 Central origin of performance fatigability

The central origin of performance fatigability is multifactorial and depends on the population considered, the environmental conditions and the kind of task performed. From a practical point of view, it indicates the inability of the CNS to recruit the α -motoneurons at a frequency sufficient to

provoke a tetanus during maximal contractions (Millet et al. 2011). Central alterations are usually defined as a decrease in voluntary activation of the muscle during exercise by processes not attributable to mental fatigue (Gandevia 2001).

1.2.1 Biological processes

Different biological processes have been hypothesized to cause fatigue-related alterations within the CNS. The first phenomenon has been observed during prolonged exercise and involves the synthesis and metabolism of some neurotransmitters of the CNS such as serotonin, dopamine and noradrenalin that influence the activity of the serotonergic system (Meeusen et al. 2006). Activity of the serotonergic system increases during fatiguing endurance exercise, causing sleepiness and general sensation of tiredness, and reduces the excitability (and thus the recruitment) of the motor unit pool (Newsholme et al. 1987; Newsholme and Blomstrand 1996). An increase in the serotonergic system activity has also been shown to impair motor coordination, proprioception and the ability to focus, and to increase the reaction time to an external stimulus (Gandevia 2001). Moreover, a decrease in the dopamine/serotonin ratio is well correlated with the decrease in maximal muscular voluntary activation (Meeusen et al. 2007).

Other biochemical processes have been proposed to contribute to the development of voluntary activation impairments, such as the influence of several neurotransmitters [GABA, ACTH, glutamate and adenosine; (Meeusen et al. 2006)] that could be released in the CNS during exercise. These neurotransmitters can i) influence the neuronal excitation/inhibition within the motor cortex (GABA, glutamate), ii) reduce the secretion of dopamine and noradrenaline (adenosine) or iii) influence the energetic balance of the organism (ACTH). Other possible contributors to the alteration of the nervous system include i) the production of interleukins during exercise that provide an hormonal-like feedback (Nybo and Secher 2004; Zajac et al. 2015), ii) an increased concentration of ammonium ions in the brain blood flow and iii) a reduction in the energetic precursors and oxygen to sustain the brain and muscle activity (Nybo and Secher 2004; Meeusen et al. 2006).

1.2.2 Neural feedback from the muscles

The capacity to generate and transmit the voluntary drive to the muscles could be directly influenced by a feedback neural circuitry, i.e. inhibitory neurons that are activated by high concentrations of metabolic byproducts generated during exercise. This function has been mainly attributed to the groups III and IV afferent inputs (Gandevia 2001; Zajac et al. 2015; Amann et al. 2015), which consist in myelinated and unmyelinated neural afferents to the CNS whose role is primarily to adjust the heart rate, respiratory frequency and other hemodynamics factors during exercise (Amann et al. 2015). In this view, fatigability at CNS level would be considered as secondary to peripheral alterations (Amann et al. 2011). Afferent inhibitory burst within the spinal circuitry has been recognized as the main responsible for the reduction in spinal transmission. Thus, the motor drive to the α -motoneurons and then to the muscle can be reduced importantly at spinal level. Finally, intrinsic changes within the motoneurons and interneurons due to repetitive activation could also be responsible for spinal fatigability (Kernell and Monster 1982; Kukulka et al. 1986). For instance, repetitive neuromodulator activation induces a dendritic persistent inward current that may increase the gain of motor excitability from 6 to 10 fold (Heckman et al. 2008). This phenomenon is mediated by the CNS, i.e. noradrenergic neurons (from the locus coeruleus) and serotonergic neurons (from the caudal raphe nucleus) send their axons down to the spinal motoneurons. Serotonin release may increase α -motoneuron excitability when acting on some specific receptors while high concentrations of serotonin leads to reduction in responsiveness of the targeted α -motoneuron (Cotel et al. 2013; Taylor et al. 2016b). As serotonin release in those areas might decrease with exercise, this would result in a progressive decrease in spinal loop excitability during long lasting fatiguing tasks (Kavanagh et al. 2019).

1.3 Peripheral origin of performance fatigability

The term “peripheral” denotes all the alterations that occur below the neuromuscular junction and that are associated with a loss in functionality of the contractile apparatus (e.g. diminution of

sarcolemmal excitability, impairments in the actin-myosin coupling, metabolites accumulation and acute structural damage). The mechanisms that could be impaired are dependent on the type, intensity and duration of the task. The cascade of mechanisms that goes from the electrical signal to the neuromuscular junction to the force generation are presented in Figure 2. Two main categories of phenomena are accounted for this loss in contractile function: ionic and metabolic impairments.

1.3.1 Ionic mechanism

Ionic phenomena linked to the synaptic and membrane transmission of the action potential have a large *safety factor*. This means that the concentration of ions displaced from the activity of the Na^+/K^+ pump and the displacement of Cl^- ions, largely overcome the quantity needed for an overshoot (action potential). Then, depletion is unlikely and this mechanism probably does not limit exercise performance (Allen et al. 2008b, a). However, exceptions occur in case of transient ions accumulations in small volumes (Sejersted and Sjøgaard 2000). This is the case for the action potential transmission across the T-tubules, because of their very small volume [less than 1% of the muscle; (Farrell et al. 2011)]. During the repeated activity of the Na^+/K^+ pump, there is an extracellular accumulation of K^+ ions and depletion of Na^+ ions. Those transitory events increase in magnitude with high discharge frequencies, inactivating the Na^+ channels and reducing the action potential transmission. Consequently, there is a lower activation of the voltage-dependent ryanodine receptors, and in turn a reduced release of Ca^{2+} from the sarcoplasmic reticulum (SR) (MacLaren and Morton 2012).

Nevertheless, these localized ionic phenomena *in vivo* have been considered secondary (Allen et al. 2008b). Indeed, other ionic mechanism have been hypothesized to compensate for the K^+ accumulation, such as a reduction in the conductance of the ions Cl^- caused by the drop in pH during exercise (Pedersen et al. 2005). Furthermore, other alterations in contractile function occur before the accumulation of ions in the T-tubules, and could play an important role in Ca^{2+} release (Allen et al. 2008a; MacLaren and Morton 2012). One of those involves the local depletion of ATP in the

interspace between T-tubules and SR (Stewart et al. 2007). In this interspace, the high concentration of glycolytic enzymes can be unable to produce ATP in case of lack in substrate supply (Ørtenblad et al. 2011). This ATP is necessary for the functioning of the ATP-dependent channels like the Na^+/K^+ pumps or the Sarco-Endoplasmic Reticulum Calcium ATPase (SERCA), which are responsible for the calcium reuptake (Ørtenblad et al. 2011). Reduction in calcium reuptake has been associated with the slowdown in the rate of muscle relaxation observed with fatigue (Vernillo et al. 2020).

Other ionic mechanisms of fatigability consists in ionic imbalance. Magnesium (Mg^{2+}) depletion favours an excessive quantity of Ca^{2+} to binds the troponin C, resulting in hypercontractility and muscular cramps/spasm (de Baaij et al. 2015). In case of ATP depletion, there is a rise in free Mg^{2+} (Westerblad and Allen 1992). Accumulation of Mg^{2+} could also have deleterious effects on SERCA and ryanodine receptors, and thus on Ca^{2+} release and reuptake (Blazev and Lamb 1999; Dias et al. 2006; de Baaij et al. 2015).

1.3.2 Metabolic mechanisms: the role of pH and free phosphates

Classically, a decrease in oxygen uptake from the blood flow has been linked to the utilisation of the lactic anaerobic system and accounted as responsible for the production of lactate (La^-) and H^+ ions. H^+ ions accumulation decreases cellular pH, inducing alterations in contractile function. In particular, the drop in pH seems associated with the slowing of the rate of force relaxation (Westerblad and Allen 2002). However, several studies showed that the drop in pH and the presence of lactate would not be determinant for other contractile function impairments (Stackhouse et al. 2001a; Westerblad and Allen 2002; Allen et al. 2008b; MacLaren and Morton 2012).

In the last few decades, research focusing on the metabolic causes of peripheral origin of neuromuscular fatigue found a key role played by the free phosphate (Pi) released following exercise, as it regulates several biochemical pathways (Baker et al. 2010). Accumulation of Pi has been shown to reduce or even directly inhibit the formation of actin-myosin bridges and bind the calcium released from the SR, blocking its affinity with the troponin C and SERCA (Dahlstedt et al. 2001; Westerblad

and Allen 2002; Allen et al. 2008b; Debold 2012). Concomitant with the increase in Pi production, high energetic turnover causes the depletion of glucose and glycogen. Impairments in glucose or energetic supply to the muscle leads to ATP depletion and the accumulation of ADP and AMP (Cooke et al. 1988; Allen et al. 2008b). ADP and AMP are important allosteric effectors involved in cell and glucose metabolisms, regulating the energetic production and consumption (Jensen et al. 2009).

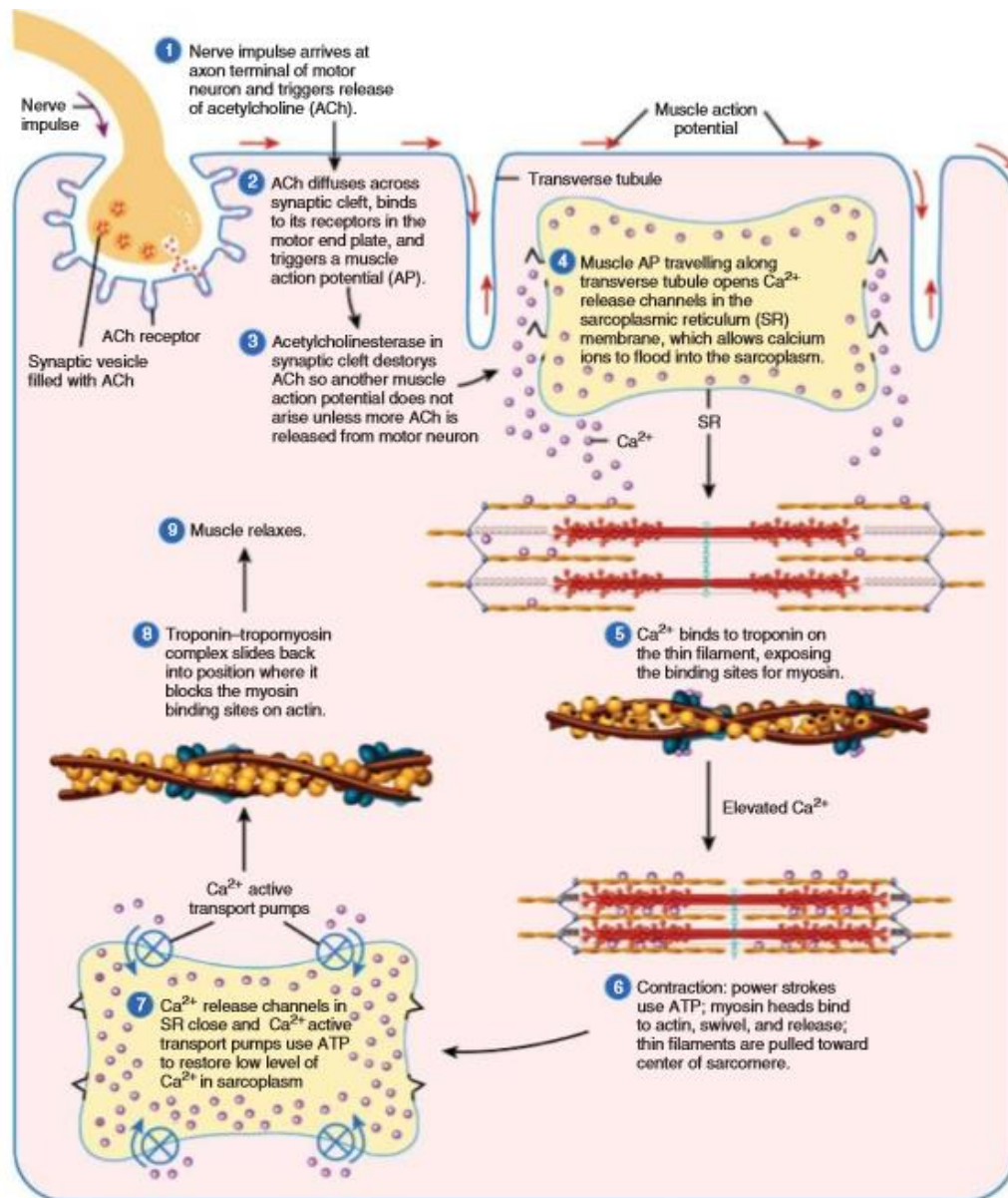


Figure 2. Representation of the main mechanisms that convert the nervous stimuli into contractile force. From: (Tortora and Derrickson 2014).

1.3.3 Concentration of free radicals

Prolonged energetic turnover during exercise causes the rise in concentration of by-products such as free radical species, i.e. the reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Reid 2008; Ferreira and Reid 2008), which could damage the cell structure. The production of free radicals could increase dramatically during intense and prolonged exercises as well as in ischemic conditions, dehydration, UV radiation exposure, heat, infections, lack of sleep or poor diets (Lewis et al. 2015). ROS/RNS accumulation causes muscle structural damage at the muscular proteins level, in particular to troponin C, myosin and actin, reducing in turn Ca^{2+} sensibility and contractile force (Scherer and Deamer 1986; Snook et al. 2008; Debold 2012). An association between those free radical species and performance fatigability has been directly observed by experimentally exposing muscle fibers to ROS-scavengers, which reduced the concentration of free radicals within the cell. As a result, force depression was attenuated (Moopanar and Allen 2005, 2006).

TAKE HOME MESSAGE: NEUROMUSCULAR FUNCTION AND FATIGABILITY

Fatigue can be separated in two main aspects: perceived fatigability and performance fatigability. Performance fatigability originates within the neuromuscular system. Impairments could occur within the muscle involved during exercise (peripheral alterations), impairing directly the force output produced by the muscle fibers, or at the neural level (central alterations), leading to suboptimal motor drive to the contractile structures. This chapter provided an overview over the main mechanisms attributed to contractile function and voluntary activation impairments.

À RETENIR: NEUROMUSCULAR FUNCTION AND FATIGABILITY

La fatigue peut être séparée en deux aspects principaux : la fatigabilité perçue et la objective. La fatigabilité objective trouve son origine dans le système neuromusculaire. Les altérations peuvent se produire au sein du muscle impliqué dans l'exercice (altérations périphériques), altérant directement la production de force des fibres musculaires, ou provenir du niveau neuronal (altérations centrales), conduisant à une commande motrice sous-optimale des structures contractiles. Ce chapitre a fourni un aperçu des principaux mécanismes attribués aux altérations de la fonction contractile et de l'activation volontaire.

2. NON-INVASIVE MEASUREMENT OF PERFORMANCE FATIGABILITY

2.1 Neuromuscular performance

The quantification of performance differs across studies depending on the definition of performance and the research question. Studies usually quantify performance as duration, time to exhaustion [e.g. (Justice et al. 2014)] and/or total work performed, i.e. force-time integral [e.g. (Zarzissi et al. 2020)]. Performance is influenced by the intensity/workload of exercise. To compare performance between different age populations, a typical approach in the laboratory setting is to normalize intensity to maximal capacity of each individual, e.g. percentage of the maximal voluntary contraction force or power (Krüger et al. 2018), and performing single-limb fatiguing tasks.

2.2 Performance fatigability

The standard approach for quantifying performance fatigability is measuring directly the difference in maximal force or power before and after a fatiguing task (Vøllestad 1997). In aging studies, the most diffused approach to quantify performance fatigability is to measure the loss in maximal isometric force (Krüger et al. 2018). The alterations in neuromuscular function varies if performance fatigability is quantified using single-limb isometric contractions (e.g. maximal isometric contractions) or single-limb (e.g. maximal concentric contraction) and multiarticular dynamic contractions (e.g. maximal sprints on bike ergometers) in both fresh and fatigued muscle (Krüger et al. 2018, 2019). Despite most of the activities of daily living are performed using dynamic multiarticular contractions, monoarticular isometric contractions allow the experimental investigation of performance fatigability etiology using artificial stimulations (electrical or magnetic) techniques. Indeed, it is technically challenging to perform neuromuscular evaluation during dynamic contractions, even if some attempt have been performed [e.g. (Sidhu et al. 2012; Weavil et al. 2016)].

2.3 Performance fatigability etiology

The most used technique to evaluate the etiology of performance fatigability and quantify the presence of peripheral (contractile function) vs. central (voluntary activation) alterations is the

interpolated twitch technique [ITT; (Shield and Zhou 2004)]. Briefly, the interpolated twitch technique consists in evoking a response to artificial stimulation during a maximal isometric contraction, and another at rest after the contraction. These responses are then compared before and after a fatiguing task (for further details see section 2.3.3). Different techniques are available to study the etiology of performance fatigability: magnetic stimulation that could be applied over the motor cortex or the peripheral nerve, or electrical stimulation that could be applied on the peripheral nerve or directly over the muscle belly. The frequency and intensity of stimulation can also be modulated. The parameters are selected depending on the population and the outcome studied. For instance, high-frequency doublets could better detect impairments in voluntary activation (Rozand et al. 2020) but the discomfort related to those procedures induce some research groups to use single stimulations when studying specific populations such as the old adults or patients (Millet et al. 2012; Bachasson et al. 2013).

2.3.1 Measurement of peripheral alterations

Contractile function can be measured analyzing the responses evoked at rest by artificial electrical or magnetic stimulation over the motor nerve or the muscle belly. Stimulation techniques allow to quantify peripheral neuromuscular alterations. However, it is not possible to assess directly the biological mechanisms that could be responsible for muscle fatigability with these techniques. However, biological techniques such as biopsies or blood sample require sampling biological tissues, which is invasive and usually limited to a small and superficial muscle compartment. The information are usually specific to the muscle and do not allow the investigation of the whole neuromuscular system including the central nervous system. Although stimulation being an indirect technique, it offers a vast possibility of application, from “on field” studies [e.g. (Besson et al. 2021)] to clinical populations [e.g. (Souron et al. 2021)]. Originally, high-frequency trains of peripheral nerve stimulations (PNS) evoked at rest were used to measure the maximal contraction capacity of the muscle measured on a dynamometer. With this procedure, it is possible to obtain a fused tetanus in

the studied muscle. However, the discomfort of the evoked stimuli induced the researchers to progressively substitute high-frequency tetanus with high-frequency octet, doublets, or single twitches (Millet et al. 2011). The choice is usually based on the number of stimulations delivered during the protocol and the tolerance of the single individual or the population considered, other than the safety of the procedures. For instance, high-frequency stimulation trains induce risks of knee cap dislocation (Bigland-Ritchie et al. 1978), limiting its use for aging studies. When evoking a response at rest (Figure 3), the intensity and duration of the stimulation are adjusted to recruit all the muscle mass available for contraction. Long impulses and high intensities of stimulation could help to maximally recruit large muscle masses such as the quadriceps [e.g.(Gregory et al. 2007; Zarkou et al. 2017)]. If EMG signal is recorded during single stimulations at rest, the maximal M-wave (Figure 3) obtained by increasing stimulation intensity corresponds to the simultaneous activation of all the motor units of the muscle (Hugon 1973). The parameters usually studied from the evoked response on the force channel (peak twitch, Figure 3) are its amplitude, contraction time and half-relaxation time. The comparison of the peak twitch amplitude prior and after a fatiguing task is adopted as global measure of the fatigue-induced impairments in contractile function. In several studies attempting to detect *in vivo* some age-related differences in calcium kinetic, the contraction time and half-relaxation time of the response evoked at rest by nerve stimulation has been used to quantify calcium release and reuptake, respectively (Krüger et al. 2018). However, the discordant results across studies, probably due to high between-subjects variability, and low sensibility of these parameters compared to the peak relaxation rate detected from the drop in force following a stimulation over the motor cortex [e.g.(Vernillo et al. 2020)] lower their usefulness in aging studies (Krüger et al. 2018).

To evaluate possible impairments in calcium release in fresh muscle, two different doublets, as surrogates of similar-frequencies evoked tetani (100 Hz and 10 Hz), are delivered for the assessment of the low-to-high frequency fatigue [LFF, (Verges et al. 2008)]. The decrement in force at low frequency (10 Hz) compared to high frequency (100 Hz), in absence of muscle damage, is mainly

attributed to a decrease in cytosolic free calcium concentration ($[Ca^{2+}]_i$) due to a lower release in calcium from the SR, indicating muscle damage. Due to the possible partial compensation of postactivation potentiation (i.e. transient increase in calcium sensitivity following an intense contraction) to LFF (Sale 2002), timing, number and order of stimulation, and position and length of the studied muscle (Kohn et al. 2018) must be kept constant before and after the task (Ruggiero et al. 2021).

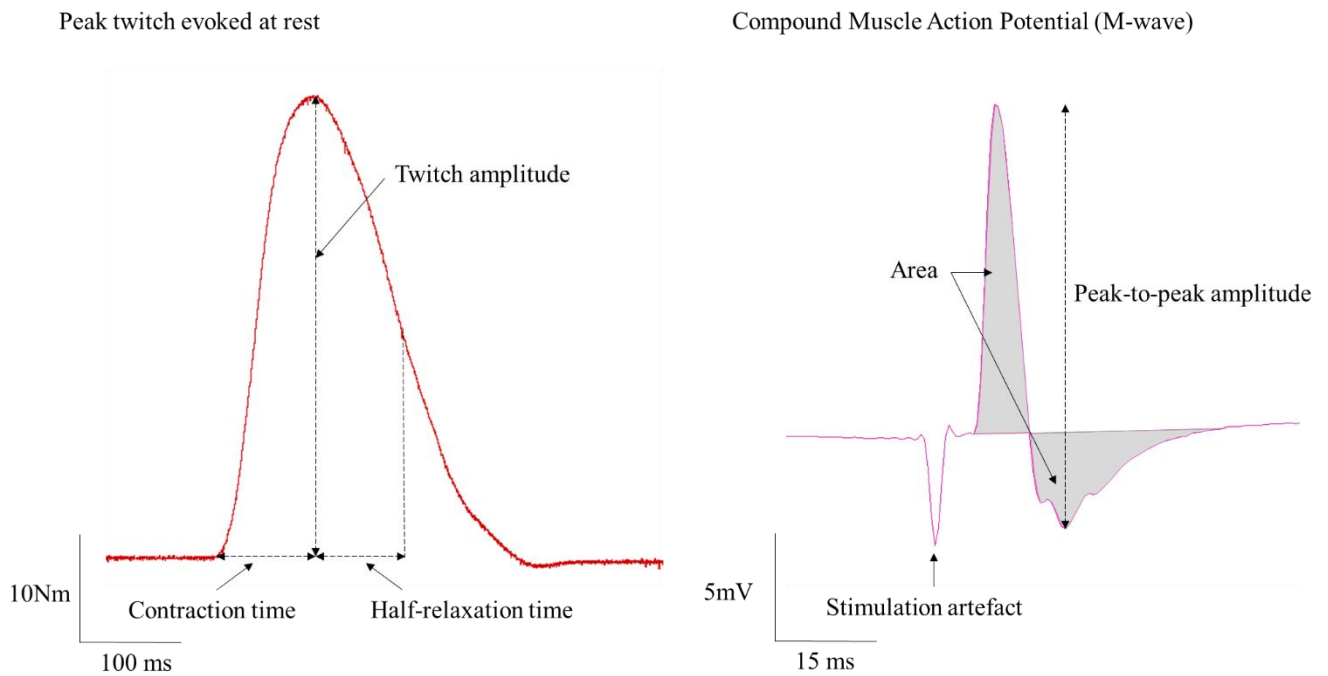


Figure 3. Responses obtained following electrical or magnetic stimulation on the force (left) and EMG channel (compound muscle action potential, right).

Pairing PNS and EMG signals, it is possible to measure the compound muscle action potential (CMAP, or M-wave, Figure 3), which represents the summated electrical activity of the motor units of the depolarized motor axons. The M-wave could provide useful information on the excitability of the sarcolemma, although it represents only the activity of the motor units within the recording range of the electrodes (Figure 3). Whether an increase or a decrease in the amplitude of the M-wave would indicate an impairment, however, is still under discussion (Rodriguez-Falces and Place 2018).

An alternative to electrical nerve stimulation is magnetic nerve stimulation (Verges et al. 2008). The advantage offered by peripheral nerve magnetic stimulation (PNMS) consists in reducing the discomfort induced by electrical stimulation over the peripheral nerve, which can be relevant when stimulating large muscle masses such as the knee extensors in old and very old adults. Despite some evidence suggest its validity [e.g. (Olney et al. 1990; Nilsson et al. 1992; Neyroud et al. 2015)], this technique has not been widely adopted in aging studies because of uncertainty of the stimulation site and because the fat tissue could interfere with the efficacy of the stimulation (Tomazin et al. 2011). Evoking high-frequency doublets evoked via PNMS could represent a possible solution because they are less painful than doublets elicited via electrical stimulations and the responses obtained are greater than that with single stimuli, possibly overcoming some of the limitations related to fat thickness (Tomazin et al. 2011). However, due to stimulation artifact contamination, the M-wave elicited via PNMS cannot be consistently recorded (Neyroud et al. 2015). Some efforts have been made to suppress artificially the artefact and synthetically reconstruct the contaminated signal (Liu et al. 2014), however, peripheral nerve electrical stimulation remains the gold standard for the assessment of M-waves.

2.3.2 Measurement of cortical and spinal excitability and inhibition

Stimulation techniques allow to test the excitability and behaviour of the motoneurons along the neuromuscular pathway. Variation in excitability is defined as the variation in the recruitment of more or less motoneurons to generate an action potential during or following an intervention (McNeil et al. 2013). This variation comes from the interaction of different mechanisms of inhibition and facilitation across the neuromuscular pathway (McNeil et al., 2013; Amann, Sidhu, Weavil, Mangum, & Venturelli, 2015). *In vivo*, the proprieties of the motoneuronal pool in humans are necessarily measured indirectly. Impairments in the transmission of the signal from the motor cortex to the motor units have been assessed for long time through the Hoffmann reflex (H-reflex): a submaximal percutaneous electrical stimulation is delivered to activate selectively the neural afferences firing to

the alpha-motoneuron. This allows the quantification of the spinal loop excitability (Zehr, 2002; Brinkworth, Tuncer, Tucker, Jaberzadeh, & Türker, 2007). More recently, other variables obtained by electrical or magnetic stimulation have been used to study the spinal and supraspinal changes in excitability/inhibition (Zehr, 2002 and McNeil et al., 2013) and have been applied in aging studies [e.g. (Hunter et al. 2008; Baudry et al. 2015)]. Stimulation of the corticospinal tract at the cervicomedullary level, over the cervical spine, evokes the so-called cervicomedullary motor evoked potential (CMEP). When elicited at the thoracic level, the EMG response is denominated thoracic motor evoked potential (TMEP). CMEP and TMEP, used respectively to investigate the upper and lower limbs motoneuron excitability, have the advantage to be mainly monosynaptic and unaffected by presynaptic inhibition (Taylor, 2006; Rösler, Scheidegger, & Magistris, 2009; McNeil et al., 2013). It is also possible to stimulate the primary motor cortex in order to elicit a motor evoked potential (MEP), via transcranial magnetic stimulation (TMS), to study the corticospinal excitability (Figure 4). To separate the excitability of the supraspinal tract (cortical excitability) from the corticospinal tract, the MEP could be normalized to the CMEP (upper limbs) or TMEP (lower limbs).

Another useful index when investigating the effect of an acute fatiguing task, the chronic adaptations to a training program or the effects of a treatment is the intracortical inhibition: following a MEP elicited during voluntary contractions, there is a flat period in the EMG signal: the so-called cortical silent period (SP, Figure 4). From the beginning of the SP, the first part is attributed to spinal process (up to 100 ms), while the second part represents the intracortical inhibition mediated by long-lasting GABA_B receptors (Sidhu et al., 2013; Gruet et al., 2013).

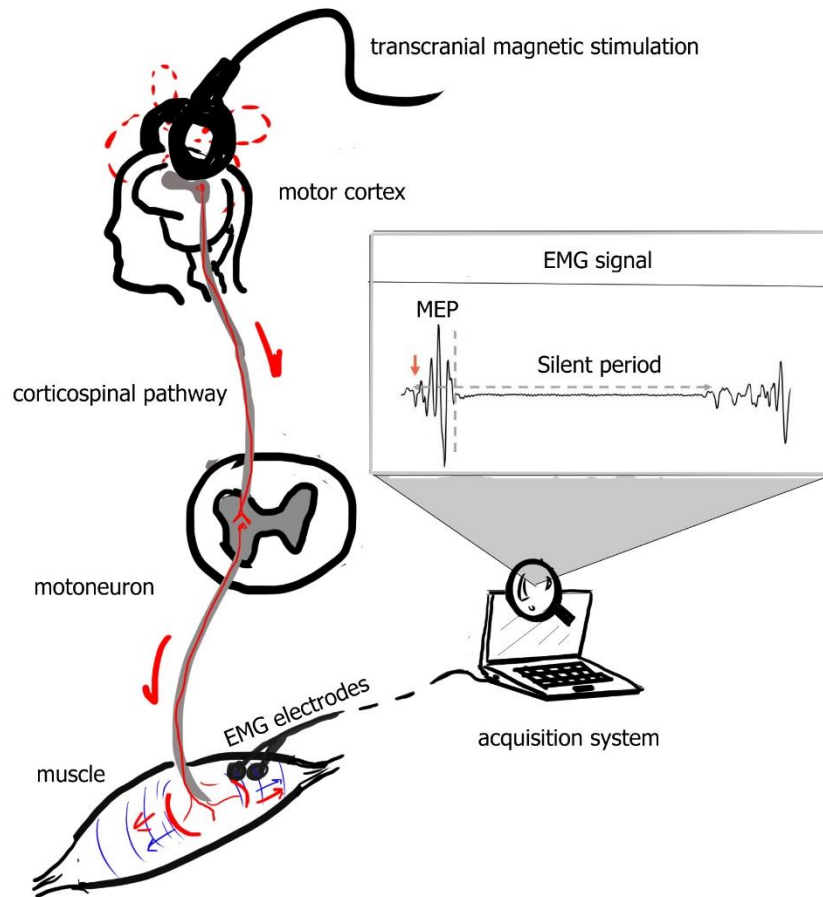


Figure 4. Schematic representation of the real time data acquisition of the silent period (SP) that follows the motor evoked potential (MEP).

2.3.3 Voluntary activation

The evaluation of voluntary activation was initially developed by Merton (Merton 1954) who used motor nerve electrical stimulation to elicit a tetanus and compared it to a maximal voluntary isometric contraction (MVIC) of the *abductor pollicis*. Merton reported a similar force expressed by the tetanus and the maximal voluntary contraction, concluding that this muscle could be fully activated. Despite the technological limitations, he demonstrated that a linear negative relationship exists between the evoked twitch amplitude and voluntary isometric force, i.e. the lower the target force level (expressed as % of MVIC), the higher the evoked force. He then used a technique based on electrical stimulations to study the voluntary activation, which he defined as the difference between the force derived from

the maximal level of voluntary drive produced by the CNS and the absolute maximal force that can be produced by the muscle.

Voluntary activation can be modulated or impaired potentially at every level of the neuromuscular pathway (Meeusen and Roelands 2018), starting from the motor cortex excitability, inhibition mediated by GABA_B receptors or maybe the effects of other neurotransmitter in the brain (Meeusen and Roelands 2018). It is possible to establish in a semi-quantitative-way the increasing influence of central origin of neuromuscular fatigue on performance by calculating the percentage of voluntary activation, using the ITT (Merton 1954): during a short MVIC (3-5 s), when the plateau is reached, a supramaximal stimulation is delivered to evoke a superimposed twitch (SIT). Another stimulation is delivered at rest with a minimal time delay from the MVIC to elicit a potentiated twitch (POT), to account for the postactivation potentiation (Sale 2002). The amplitude of the SIT is then normalized to the amplitude of the POT. This normalization is necessary to account for alterations in the drive transmission within the muscle cells (Shield and Zhou 2004).

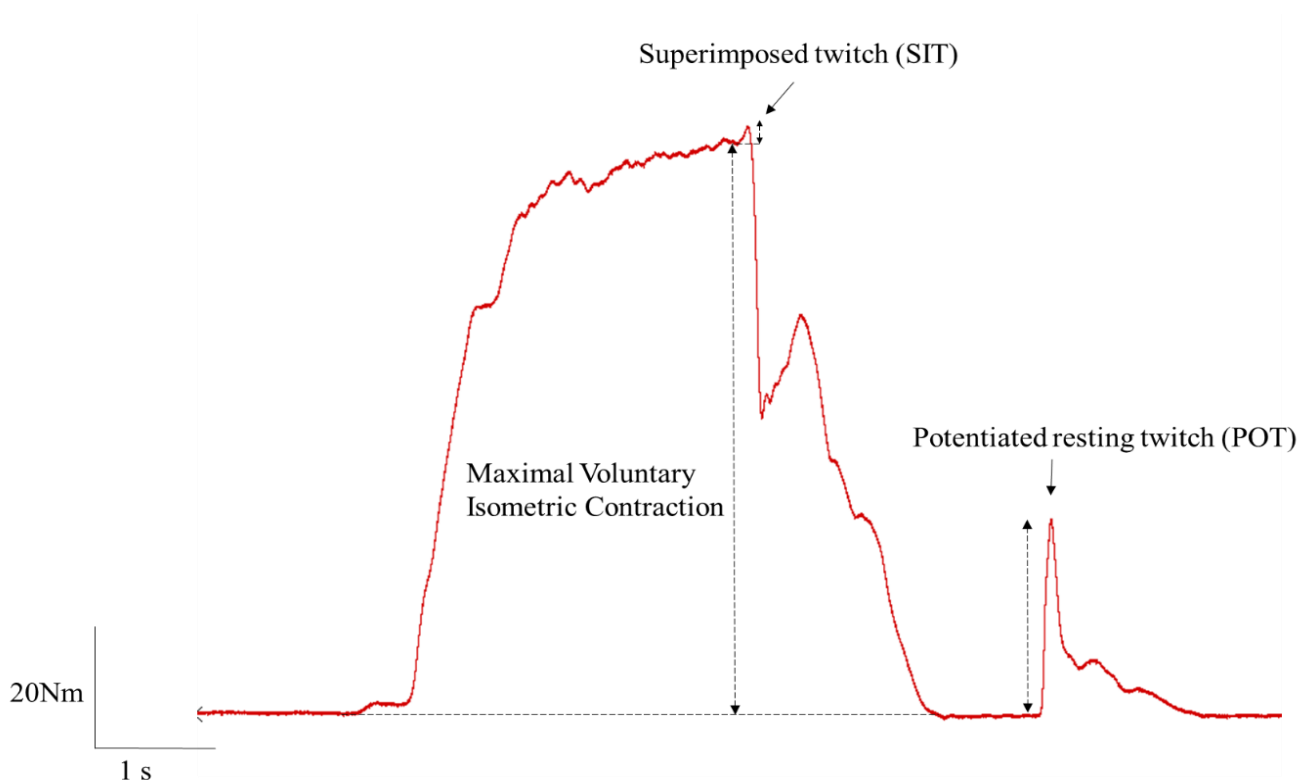


Figure 5. Typical assessment of Voluntary Activation via peripheral nerve stimulation

Voluntary activation is usually calculated using the following formula:

$$voluntary\ activation\ (\%) = \left[1 - \left(\frac{SIT}{POT} \right) \right] * 100$$

Equation 1

A sub-maximal voluntary activation would indicate that some motor units are not firing fast enough to produce a fused tetanus or are not recruited at all. Consequently, it can be inferred that some modulation in the motor drive prevents the muscle to be fully activated, and thus, that central alterations are present. Because the muscle is never fully activated (Herzog 2009), according to the literature, a level of activation voluntary activation $\geq 90\%$ in the fresh muscle can be considered as satisfactory (Lepers, Maffiuletti, Rochette, Brugniaux, & Millet, 2002; Millet, Martin, Lattier, & Ballay, 2003; Sidhu et al., 2009; Giandolini et al., 2015; Mira et al., 2017; Twomey et al., 2017) and could be obtained also in old or very old adults [e.g. (Sundberg et al. 2018b)]. Voluntary activation levels lower than 80% can be expected in pathological populations or in injured athletes during the rehabilitation phase (Shield and Zhou 2004). Low levels of activation in healthy subjects would indicate the presence of fatigue, lack of motivation or errors in measurement (e.g. position of the subject or the use of compliant instrumentation). The accuracy can increase, for example, with the introduction of a familiarization session in the protocol. Error in the calculation of voluntary activation could be induced if the stimulation is not delivered on the plateau of MVIC. In that case, a correction in the original equation can be applied (Strojnik and Komi 1998):

$$voluntary\ activation\ (\%) = 100 - D * (Tb/T_{MAX})/POT * 100$$

Equation 2

Where Tb is the torque developed just before the stimulation, T_{MAX} is the maximal torque obtained from the MVIC, POT is the amplitude of the potentiated twitch and D is the difference between Tb and the maximal torque obtained with the SIT.

Another potential limitation is the position of the muscle and the role of the series elastic slackness, which can be reduced by positioning the muscle at longer lengths or applying high-frequency doublets (100Hz) (Shield and Zhou 2004). The use of doublets is recommended because they are less influenced by postactivation potentiation than single stimulations and avoid the effect of antidromic collisions and spinal reflexes, activating more likely fibers which are in refractory period during the first stimuli (Duchateau 2009). This leads to a more accurate and less variable response in voluntary activation assessment (Rozand et al. 2020). However, in specific populations like older adults single twitches are usually preferred to doublets because of stimulation discomfort (Shield & Zhou 2004). In case of single twitches, it seems that longer stimuli durations (2 ms) are better correlated with doublet responses (Zarkou et al. 2017).

Doublets can be delivered to the peripheral nerve with magnetic stimulation to limit discomfort. Although the evoked responses are similar than that with electrical stimulations in young adults (Bachasson et al. 2013), this technique seems less effective with great skinfold thickness (Tomazin et al. 2011).

Magnetic stimulation can also be applied over the motor cortex to quantify voluntary activation through TMS (Todd et al. 2003). Because the supraspinal excitability is lower at rest and increases during contraction, it is inappropriate to normalise the TMS superimposed twitch to a twitch evoked at rest (Todd et al. 2003). The relationship between the TMS SIT and the voluntary force has been demonstrated to be linear from 50% to 100% MVIC. Thus, Todd et al. (2003) tried to estimate the resting twitch by extrapolation of the evoked responses during contractions at 50%, 75% and 100% MVC using this linear relationship (estimated resting twitch; ERT), showing that the linearity is maintained in the fatigued elbow flexors and concluding that this procedure can illustrate the impairments at corticospinal level (Todd et al. 2003, 2004). Several authors used TMS voluntary activation to provide insights to the etiology of performance fatigability in different tasks and conditions (e.g. Temesi, Rupp, et al., 2014; Temesi et al., 2015; Zghal et al., 2015; Jubeau et al., 2017). However, there are several methodological limitations. For example, the three evoked

responses used to interpolate the ERT should present a linear correlation with a pearson's r coefficient >0.90 (Hunter et al. 2006; Cadigan et al. 2017). However, this cannot usually be checked online, increasing the risk of missing data. Furthermore, the number of stimulation used to calculate the ERT and the long duration of the assessment procedures could influence the calculation of voluntary activation (Mira et al. 2017; Dekerle et al. 2019). More recently, the validity of the calculation of voluntary activation using TMS in the knee extensors has been questioned (Nuzzo et al. 2020). These authors argued that the optimization of the stimulation intensity is challenging as the motor evoked responses of the antagonists (hamstrings) is rarely measured (due to technical difficulties to effectively stimulate the sciatic nerve). They also argued that the ERT calculated in the knee extensors is systematically underestimated because of the consistent differences between ERT and POT evoked via electrical stimulation both at rest and in fatigued condition. Finally, they questioned the effectiveness of TMS to activate an appreciable number of motor units of the KE motoneuronal pool, as the SIT_{TMS}/SIT_{PNS} ratio rarely overcome the value of 0.80 and the MEP size overcome the 50% of the M-wave evoked via electrical stimulation.

For those methodological reasons, TMS could not be an appropriate technique to study age-related changes in voluntary activation of the knee extensors. To date, no study showed age-related changes in this parameters when using TMS, contrary to some evidences showing lower voluntary activation in older adults using PNS (Rozand et al. 2020).

TAKE HOME MESSAGE: NON-INVASIVE MEASUREMENT OF PERFORMANCE FATIGABILITY

The assessment and interpretation of fatigability and changes in the neuromuscular system need a clear and accepted methodology. Stimulation techniques coupled with force and EMG measures allow to quantify impairments in contractile function and/or voluntary activation in a non-invasive manner. The interpolated twitch technique is the most diffused procedure for evaluating the etiology of performance fatigability, and consists in delivering stimulations during and after an isometric maximal contraction. Stimulations can be delivered over the motor cortex (magnetic), over the motor nerve (magnetic and electrical) or over the muscle belly (electrical). Finally, duration and intensity of the stimulation can be adapted to the population and aim of the investigation.

À RETENIR: NON-INVASIVE MEASUREMENT OF PERFORMANCE FATIGABILITY

L'évaluation et l'interprétation de la fatigabilité et des changements dans le système neuromusculaire nécessitent une méthodologie claire et acceptée. Les techniques de stimulation couplées à des mesures de force et de signal électromyographique permettent de quantifier les altérations de la fonction contractile et/ou de l'activation volontaire de manière non-invasive. La technique de la secousse interpolée est la procédure la plus utilisée pour évaluer l'étiologie de la fatigabilité. Les stimulations peuvent être délivrées au niveau du cortex moteur (magnétique), du nerf moteur (magnétique et électrique) ou sur le ventre musculaire (électrique). Pour terminer, la durée et l'intensité de stimulation peuvent être adaptés à la population et au but de l'étude.

3. NEUROMUSCULAR CHANGES WITH AGING

3.1 Neuromuscular alterations with age

The neuromuscular system properties and functionality changes with advancing age. The progressive reduction in muscle mass (sarcopenia) with age induces a decrease in maximal force and power output (dynapenia) (Harridge and Lazarus 2017). From ~35 to ~60 years old, 10-20% of total muscle mass is lost (Mitchell et al. 2012). A further ~20% is lost in the next twenty years, when approaching the ninth decade of life (Vandervoort 2002). Sarcopenia and dynapenia are both associated with increased risk of falls, hospitalisation, loss of autonomy, frailty and mortality (Newman et al. 2006; Cruz-Jentoft et al. 2019).

Neuromuscular deterioration could be due to neurodegenerative disorders or cognitive impairments [e.g. (D'Angelo and Bresolin 2006)], however, in healthy older adults, the primary cause of these age-related alterations are due to a progressive loss of motor-units (MU) (Power et al. 2013; Hepple and Rice 2016; Hunter et al. 2016; McNeil and Rice 2018).

3.1.1 *Properties of the motor units*

The MU is usually described as the “final common path” of the nervous system (McNeil and Rice 2018), and consists in a α -motoneuron located in the spinal cord and all the individual muscle fibers innervated by this motoneuron (D'Angelo and Bresolin 2006; Power et al. 2013). All muscle fibers innervated by the same motoneuron are of the same fiber type, and are spread over a relatively large area within the muscle, called neuromuscular compartment (Peters 1989). This area can cover 15% to 75% of the muscle volume, depending on the muscle considered (D'Angelo and Bresolin 2006). Usually, type I muscle fibers compose smaller MU called *slow-twitch* MU, with lower threshold for full activation [tetanus at ~20 Hz (Enoka 2015)]. They are the most “fatigue-resistant” of the muscle fibers. On the other side of the spectrum, *fast-twitch* MU are large units composed by type IIx fibers, even if it is not excluded that they could be composed by type IIa fibers, depending on the method of categorization (Enoka 2015). *Fast-twitch* MU have an high full activation threshold [up to 80-100 Hz

for a tetanus (Enoka 2015)] and are mainly adopted during short and intense muscle action (e.g. jumping, sprinting). In between, medium-to-large size motor units, known as *fast fatigue-resistant* motor units, are intermediate MU and composed by type IIa fibers. Characteristics of the different types of muscle fibers are presented in Table 1.

Table 1. Muscle fibers type characteristics

	Type I fibre	Type IIa fibre	Type IIx fibre
<i>Diameter</i>	+	++	+++
<i>Motoneuron size</i>	+	+++	+++
<i>Nerve conduction speed</i>	+	+++	+++
<i>Contractile speed</i>	+	++	++
<i>Motor unit strength</i>	+	++	++
<i>Glycolitic capacity</i>	+	++	++
<i>Fatigue resistance</i>	+++	++	+
<i>Oxydative capacity</i>	+++	++	+
<i>Capillarity</i>	+++	++	+
<i>Myoglobin content</i>	+++	++	+

Adapted from: (Bruton 2002).

3.1.2 Age-related changes in the motor units

The loss in MU number with age, and thus muscle mass, is associated with a variety of factors that include DNA damage, alterations in blood flow, systemic inflammation, reduction in neurotrophic signalling, and decreased Schwann cell function leading to impairments in myelinated fibers (α -motoneuron) (Allen et al. 2021). The MU loss starts with the denervation of the α -motoneuron from the muscle fibers (Allen et al. 2021), probably due to mechanisms such as deterioration and death by apoptosis of the α -motoneuron (Faulkner et al. 2007), possible perturbations in the signaling network at the level of the neuromuscular junction or injury-related necrosis of the muscle fibers (Hepple and

Rice 2016). With the loss of the α -motoneuron, the muscle fibers can either be lost or reinnervated and integrated in a proximal MU via motor axon sprouting (Gordon et al. 2004). This axonal sprouting is a natural mechanism of the cycle of denervation-reinnervation of muscle fibers that occurs over the lifespan, for example to compensate for α -motoneuron losses following partial nerve injuries (Siu and Gordon 2003).

The process of denervation-reinnervation accelerates with age, resulting into fewer but larger MU, increasing the number of fibers innervated from an average of 3 to 5-fold to a maximum of 8-fold (Siu and Gordon 2003). This imply that only a finite number of muscle fibers could be incorporated in by a MU, with the exceeding fibers being lost. This “saturation” phenomenon have been traced back to the severe muscle atrophy of very old adults (i.e. > 80 years old), where muscle remodelling could be severely limited (McNeil and Rice 2018). Furthermore, larger MU have greater metabolic demands, which is related to increased oxidative stress and could result in an accelerated deterioration at advanced age (Gordon et al. 2004; McNeil and Rice 2018). Finally, despite the atrophy with age seems uniform between type I and II muscle fibers in humans (Power et al. 2013), motoneuronal death seem to occur preferentially in large motor neurons (i.e. *fast-twitch* MU), at least in animal models (Kanda and Hashizume 1989). This would result, for example, in a reinnervation of type IIa and type IIx fibers by *slow-twitch* MU.

These phenomenon associated with aging radically change some of the typical characteristics of MU observed in young men. First, fibers with different myosin-heavy-chain expression can be observed into the same MU, with the newly-acquired fibers adapting to the characteristics of the MU (Hepple and Rice 2016; McNeil and Rice 2018). Second, because axonal sprouting and reinnervation are limited to a small compartment of the muscle, MU fibers are clustered in a limited area [Figure 6; (Hepple and Rice 2016)].

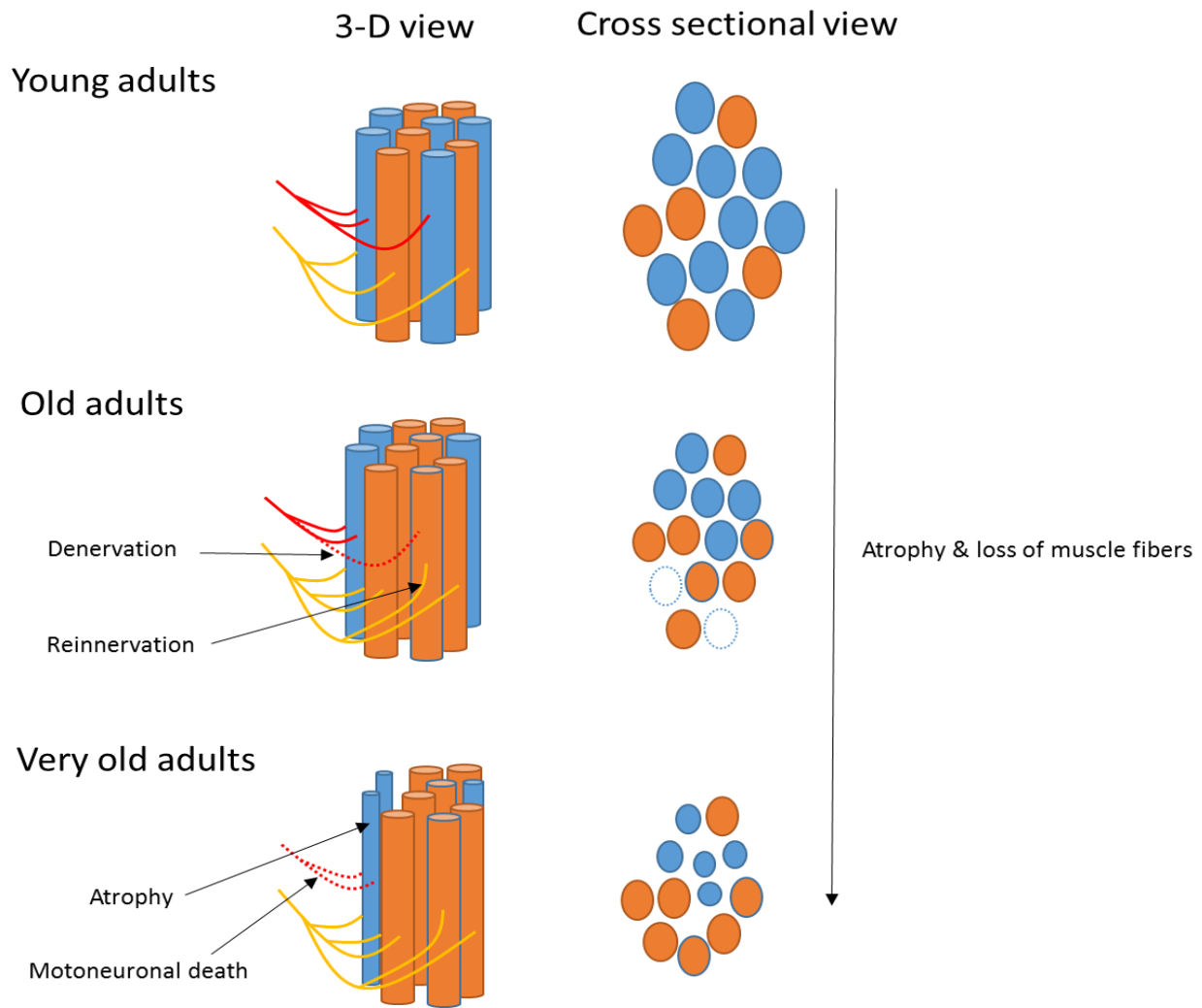


Figure 6. Illustration of the age-related transformation in the motor units. Adapted from (Hepple and Rice 2016).

With the age-related remodelling in MU, the neuromuscular system in old and very old adults would progressively perform with fewer and larger MUs, prevalently of *slow-twitch* type. This would result in higher reliance on the oxidative metabolism for the muscles of older adults. Fibers from the remaining MU in advanced age are typically smaller, with atrophy predominantly affecting large muscle mass such as the lower limbs [Figure 7, (Hunter et al. 2016)]. Deterioration in MU and loss in muscle mass are not uniform across individuals with age (Hunter et al. 2016). Indeed, heterogeneity in epigenetic factors, nutrition, hormonal status, inflammation status and disuse, increase between-subjects variability (Hunter et al. 2016; Hunter 2018).

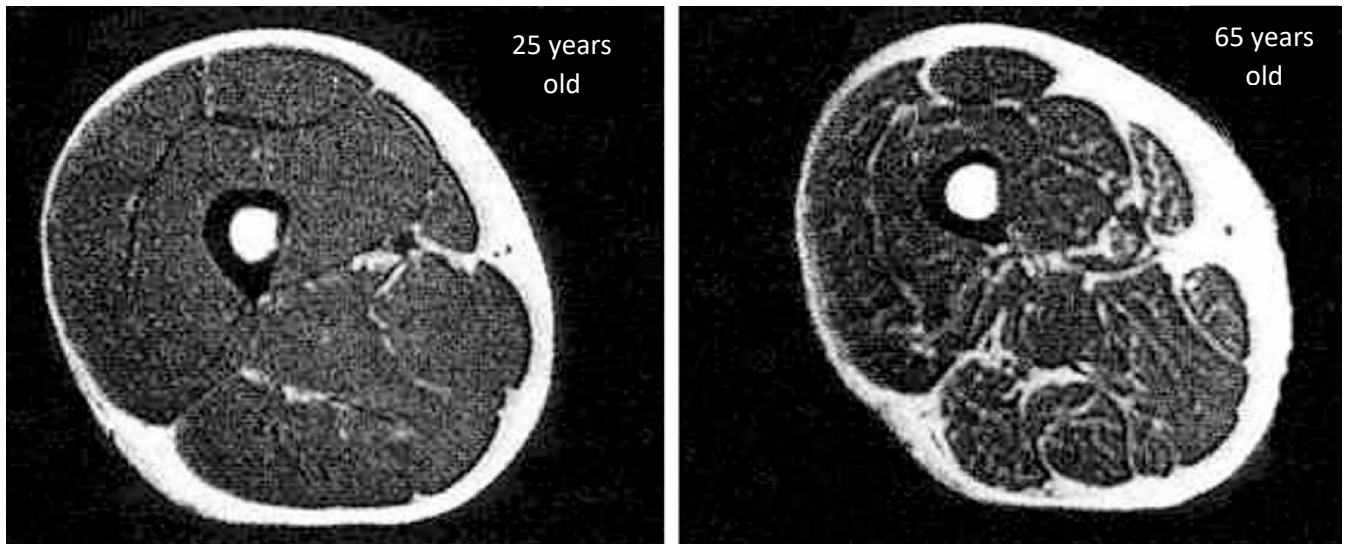


Figure 7. Cross-sectional view of the mid-tigh in healthy young (25 years old, left) and old (65 years old, right) men. From: (Sergi et al. 2016).

3.1.3 Changes in single muscle fibers function with age

The specific tension of the muscle fibers (force per unit) seems to be preserved with aging (Venturelli et al. 2018), although this is a debatable subject (Hunter et al. 2016). Conversely, contraction speed and relaxation rate seem to deteriorate, especially in very old adults (Baudry et al. 2005; Raj et al. 2010; Power et al. 2013; Hunter et al. 2016). This is probably due to slower cross-bridge formation and rates of Ca^{2+} uptake, and the reduced activity of the Ca^{2+} -ATPase activity, although physical activity could reduce part of these age-related deteriorations (Hunter et al. 1999). Finally, contractility of the muscle tissue could also be reduced by a progressive change in muscle architecture and fat infiltration within the contractile tissue (Figure 7), compromising muscle contractile properties of the whole muscle (Klein et al. 2001; Delmonico et al. 2009).

3.1.4 Age-related alterations in motor drive and voluntary activation

Other than structural integrity, the performance of the neuromuscular system also depends on an adequate activation of the motor units (Reid and Fielding 2012; Hunter et al. 2016; Hunter 2018). The ability to rapidly activate and contract the muscle, assessed with the rate of force development, is impaired with advancing age (Klass et al. 2008; Varesco et al. 2019a), suggesting alterations of the

voluntary drive to the muscle during rapid contractions. The impaired ability to generate or to maintain high discharge frequencies could lead to different or less efficient activation pattern with aging (Klass et al. 2008; Maffiuletti et al. 2016; Varesco et al. 2019a). Deteriorations of the motor nerve could also alter its excitability (Hunter et al. 2016) and the conduction velocity of the impulse (Campbell et al. 1973). These alterations could also contribute to a decrease in the capacity to activate the muscles in older adults. However, previous studies showed moderate [e.g. (Stevens et al. 2003; Škarabot et al. 2019)] or no impairments [e.g. (Roos et al. 1999)] in voluntary activation with age. In a recent meta-analysis pooling all the studies comparing voluntary activation level in young and old adults, Rozand et al. (2020) evidenced a moderate reduction in voluntary activation in older adults. Moreover, older adults are more variable across trials in their activation level, with studies showing that older adults needs more practice and multiple trials to achieve a similar level of activation compared to young adults (Jakobi and Rice 2002; Hunter et al. 2008). Changes in voluntary activation level depends on muscle groups and techniques adopted, with higher likelihood to observe age-related impairments with electrically evoked high-frequency doublets and trains of stimuli (Klass et al. 2007; Rozand et al. 2020). The decline in motor drive to the motor units pool might reflect a neural adaptation to slowing of the muscle contractile properties with aging (Kent-Braun 2009; Tevald et al. 2010). Because of the important deterioration of MU with advancing age, these results might change at very old age. Studies investigating voluntary activation in very old adults are limited in the literature, and do not allow a clear information on whether deficits in voluntary activation are greater in very old adults (>80 years old) than old adults (60–79 years old) (Rozand et al. 2020). Physical activity could play a role in the maintenance of voluntary activation in older adults. For instance, studies investigating the effects of resistance training in older individuals found improved voluntary activation and decreased inhibition (Christie and Kamen 2010), accompanied by greater MU firing rate (Christie and Kamen 2010; Unhjem et al. 2016) to a similar level than young adults (Hansen et al. 2017). Overall, impairments in voluntary activation seem modest compared to alterations in

muscle mass or maximal force but may be underestimated in a laboratory setting, where multiple attempt and familiarization are a common practice (Jakobi and Rice 2002; Rozand et al. 2020).

3.2 Muscle function: force and power

From the fourth to the ninth decade of life, humans can lost up to 40% of their maximal strength (Lindle et al. 1997). In very old age, force and power loss range approximatively from -1.5% to -3% every year (Skelton, Greig, Davies, & Young, 1994; Mitchell et al., 2012). Because of the large between-subjects variability, longitudinal studies suggest that these values may be underestimated (Frontera et al. 2000; Hughes et al. 2001). The lower limbs muscle, in particular, are greatly affected by the age-related loss in muscle force and power (Ditroilo et al. 2010). For example, Hughes et al., (2001) investigated the changes in maximal strength of the knee and elbow extensors in both men and women (46 to 78 years old) over ~10-years. These authors found a loss of ~14% of isokinetic strength per decade for knee extensors in both sexes. For the elbow extensors, men and women presented a decline of ~12% and ~2% per decade, respectively. Interestingly, these authors observed that the decrease in muscle strength was ~60% larger than the estimated loss from a cross-sectional study on the same population.

3.2.1 *Contraction mode affects the age-related differences*

The changes in maximal force with aging varies across muscles (Winegard et al. 1996; Hughes et al. 2001; Raj et al. 2010) and tasks performed (Raj et al. 2010; Hunter et al. 2016). The difference between young and older adults is greater in concentric vs. isometric and eccentric contractions (Klass et al. 2005; Raj et al. 2010). For concentric contractions, the age-related impairments in maximal torque increases with contraction velocity in the knee extensors, but not in the ankle dorsiflexors (Lanza et al. 2003; Klass et al. 2005). Because of the lower contractile speed due to muscle remodelling with advancing age, maximal contraction speed is slower in old than young adults, with further impairments in very old adults (Sundberg et al. 2018b). Older adults show ~15-40% lower maximal contraction velocities compared to young adults (Raj et al. 2010), with some

healthy older adults not being able to develop fast contraction velocities [e.g. $270^{\circ} \cdot s^{-1}$, (Callahan and Kent-Braun 2011)]. Because of the loss in maximal velocity and maximal force with age, there is a change in the force-velocity relationship that greatly affects maximal power production (Figure 8). Indeed, the decline in maximal power surpasses the decline in maximal isometric or concentric strength (Raj et al. 2010). When eccentric contractions are performed, maximal torque deficit has been shown to be less pronounced than during isometric and concentric contractions [e.g. (Hortobágyi et al. 1995; Lindle et al. 1997; Klass et al. 2005; Rozand et al. 2017)]. This difference seems independent of muscle mass, muscle fiber type or fiber size (Raj et al. 2010). Despite the underlying mechanisms remain to be fully understood (Raj et al. 2010), it has been suggested that the age-related increase in intramuscular connective tissue could increase passive resistance during muscle lengthening (Hortobágyi et al. 1995). Another possible explanation could be that the slower cross-bridge formation and thus slower rate of force development might provide some advantages during eccentric contractions (Porter et al. 1997). Finally, studies in vitro also found that passive properties were preserved in the muscle fibers of older adults compared to young adults (Ochala et al. 2006).

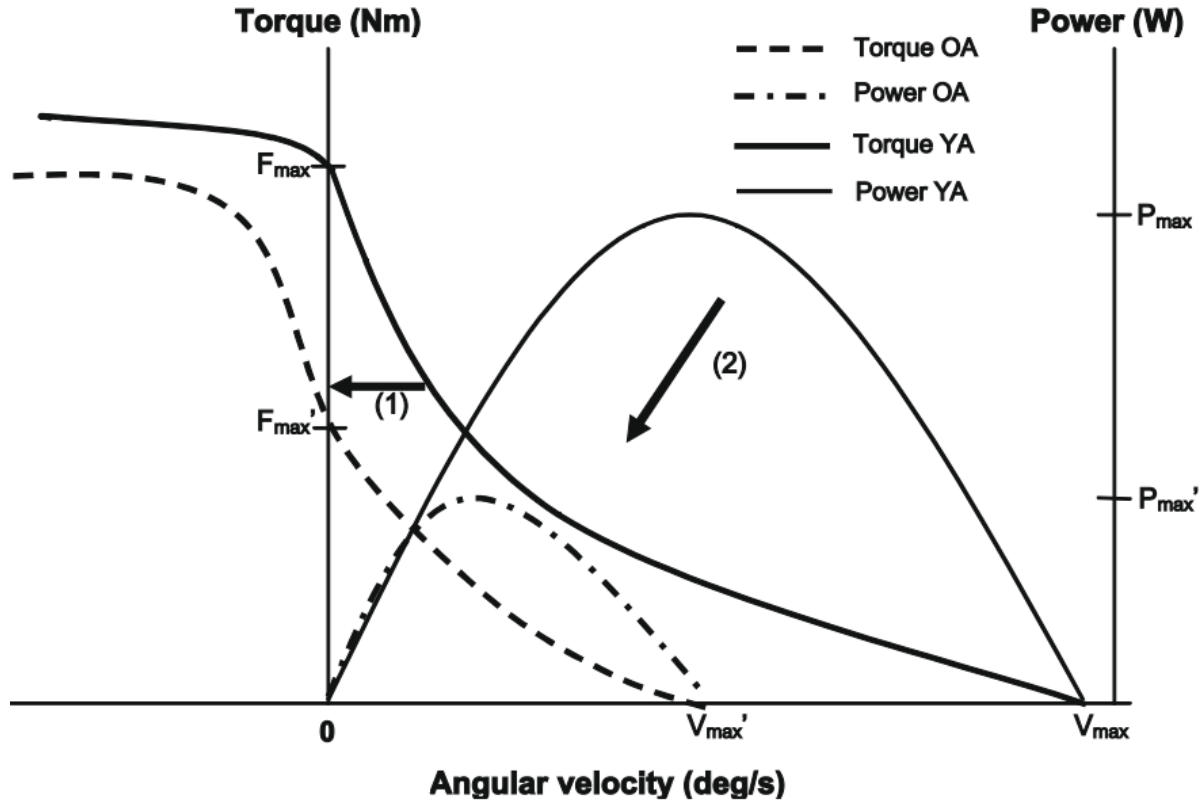


Figure 8. Changes in the torque-velocity relationship (1) and power-velocity relationship (2) with aging (dotted line). OA: old adults; YA: young adults; P_{max} : Maximal Power; F_{max} : Maximal force; V_{max} : Maximal velocity. From: (Raj et al. 2010).

3.2.2 Sex-related differences in muscle function with age

In general, men possess larger skeletal muscle mass than women and possess, at least for some muscle groups, a larger percentage of type II muscle fibers, that makes men rely more on anaerobic energetic metabolism (Hunter 2014). The loss in maximal strength with age seems not affected by sex in the knee extensors (Haynes et al. 2020). However, alterations in maximal concentric force at $60^{\circ} \cdot s^{-1}$ accelerate earlier in women (~40 years old) than men (~70 years old) (Haynes et al. 2020). Men shows a steep decline at very old age that reduces the sex-related differences in concentric force production (Haynes et al. 2020). However, eccentric torque might be better preserved in older women than men, possibly be due to the greater capacity of women to store and utilize elastic energy per unit of muscle or to the lower absolute force generated by women (Lindle et al. 1997; Klass et al. 2005). Specific age-related alterations in performance fatigability are discussed in chapter 4.

TAKE HOME MESSAGE: NEUROMUSCULAR CHANGES WITH AGING

Aging induces both structural and functional alterations in the neuromuscular system, including a remodeling and loss of motor units. However, impairments in the capacity to develop force and power are even greater, especially in very old adults. Despite alterations in the ability to rapidly develop force in older adults, which could be due to impairments in the neural drive to the muscle, maximal voluntary activation capacity seems only moderately impacted by age. These age-related impairments are dependent on the muscle group, contraction mode and velocity, . Finally, interindividual variability increases with age, making challenging to identify some of the age-related alterations in neuromuscular function.

À RETENIR: NEUROMUSCULAR SYSTEM CHANGES WITH AGING

Le vieillissement induit des altérations à la fois structurelles et fonctionnelles du système neuromusculaire, incluant le remodelage et la perte d'unités motrices. Cependant, les altérations de la capacité à développer la force et la puissance sont encore plus importantes, surtout chez les personnes très âgées. Malgré les altérations de la capacité à développer rapidement la force chez les personnes âgées, qui pourraient être dues à des altérations de la commande motrice du muscle, la capacité d'activation volontaire maximale ne semble que modérément affectée par le vieillissement. Ces altérations liées à l'âge varient en fonction du groupe musculaire, du mode et de la vitesse de contraction. Enfin, la variabilité interindividuelle augmente avec l'âge, ce qui rend difficile l'identification de certaines altérations de la fonction neuromusculaire avec l'âge.

4. AGE-RELATED ALTERATIONS IN PERFORMANCE FATIGABILITY

The age-related deterioration in muscle structure and activation capacity can have a major impact on activities of daily living that require repeated or prolonged exertion. The loss in muscle force and power with age would imply that simple activities like walking or gardening are performed at a higher percentage of maximal capacity in old compared to young adults, possibly influencing performance fatigability.

Performance fatigability is important to consider for quality of life in older adults. For example, the activities of daily living could progressively induce fatigue along the day, reducing the ability to perform motor tasks [e.g.(Murphy and Smith 2010)]. Similarly to the loss of strength and power, age-related differences in performance fatigability depend on the task characteristics, such as muscle groups involved, intensity, or mode of contraction (Hunter et al. 2016; Hunter 2018; Krüger et al. 2018).

4.1 Isometric tasks

Isometric contractions fatiguing tasks are commonly used to assess performance fatigability in various populations using sustained or intermittent contractions. Because maximal strength is lower in old compared to young adults, the contraction intensity is usually set relatively to maximal isometric force to normalize the workload. In this condition, healthy older adults are typically less fatigable than young adults for both maximal and submaximal isometric fatiguing tasks [e.g. (Callahan et al. 2009; Solianik et al. 2017)]. This could be explained by their higher percentage of *slow-twitch* motor units, or lower metabolite accumulation (e.g. inorganic phosphate, changes in pH) due to lower occlusion of the muscle mass (Moritani et al. 1992). The important role of muscle occlusion and metabolite accumulation is underlined by studies evaluating sex-related differences in the plantar flexors (Russ and Kent-Braun 2003), which found lower performance fatigability in free-flow condition for women than men, but similar performance fatigability when blood flow was occluded. It was argued that larger muscle mass in men compared to women produces higher

intramuscular pressure, decreasing oxygen delivery and increasing metabolite accumulation because of muscle ischemia induced by the isometric contractions (Russ and Kent-Braun 2003). However, when repeating similar experiments comparing young and older adults, the age-related difference in performance fatigability was maintained even during ischemia (Chung et al. 2007).

The results reported by Chung et al. (2007) suggest that oxygen supply might not be the main responsible for the lower fatigability per unit of muscle observed in older adults. Another possible explanation could be the slowing in muscle contractile properties in older adults that might result in lower MU firing rates and, consequently, less energy expenditure during a sustained isometric task (Connelly et al. 1999; Tevald et al. 2010). A model reporting the hypothetical causes of the increased metabolic economy in skeletal muscle in the older adult proposed by Kent-Braun (2009) is presented in Figure 9.

Importantly, lower performance fatigability for older adults is not consistently reported when using isometric protocols (Krüger et al. 2018). For example, when isometric intermittent protocols (5-s on/5-s off) were used, old adults and young adults showed similar performance fatigability on the knee extensors (Stackhouse et al. 2001b), while lower fatigability was observed for older adults in other muscle groups [e.g. (Kent-Braun et al. 2002; Lanza et al. 2004)].

Regarding very old adults, because of the great loss in MU and atrophy of muscle fibers, this relative fatigue resistance could be possibly lost or reduced. There is a limited body of evidence investigating this hypothesis, showing that very old adults were similarly (Allman and Rice 2001) or more (Justice et al. 2014) fatigable than young adults, depending on the muscle considered. Because the lower limbs are particularly affected by the age-related deterioration in muscle function (Janssen et al. 2000; Hughes et al. 2001; Ditroilo et al. 2010), the knee extensors performance fatigability in old and very old adults is of great interest. The function of the knee extensors is essential for everyday life activities such as walking (Hurley et al. 1998) and has been linked to the risk of fall and frailty (e.g. (De

Rekeneire et al. 2003; Pijnappels et al. 2008). Yet no evidence exists on the knee extensors performance fatigability of very old adults after isometric fatiguing tasks.

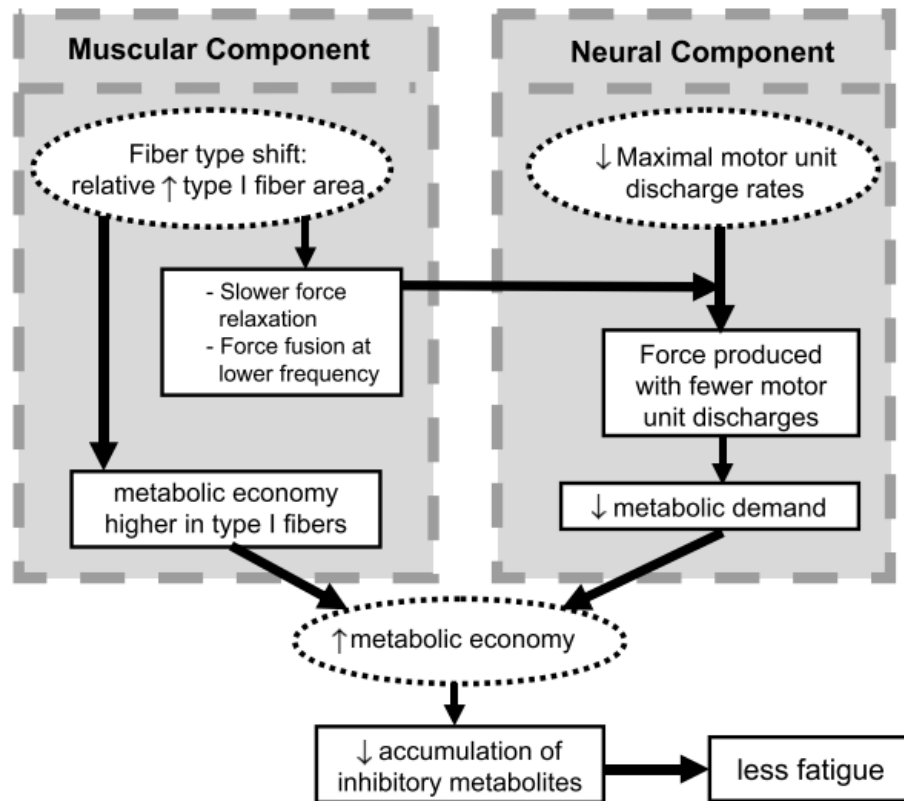


Figure 9. A model explaining the increased metabolic economy in the older adults muscle. From: (Kent-Braun 2009).

In both young and older adults, performance fatigability observed after isometric fatiguing tasks seem mainly explained by impairments in contractile function, with a relatively small decrease in voluntary activation (Krüger et al. 2018). After a 2-min sustained isometric contraction of the knee extensors, Solianik et al. (2017) observed greater impairments in contractile function and voluntary activation in young adults compared to old adults (Solianik et al. 2017), which could be explained by the large accumulation of metabolites and blood flow occlusion derived from the sustained high-intensity contraction. After an isometric intermittent task of the knee extensors for 4 minutes (5-s on/ 5-s off, maximal contractions), Callahan et al. (2009) observed greater impairments in contractile function in young than old adults, with no age-related difference in voluntary activation. The total work

performed was greater in young than old adults, and the origin of performance fatigability was more attributed to impairments in contractile function than voluntary activation (Callahan et al. 2009).

Taken together, those results suggest that, after an isometric fatiguing task of the knee extensors performed at an intensity relative to the maximal capacity, older adults show lower performance fatigability compared to young adults (Callahan et al. 2009; Solianik et al. 2017). The magnitude of this difference seems more mediated by alterations in contractile function than impairments in voluntary activation (Callahan et al. 2009; Solianik et al. 2017). While relative workloads could provide useful insights on the properties of the neuromuscular system, it could lead to some misinterpretation of the results, with limited transferability to activities of daily living (Hureau et al. 2021).

Studies exploring performance fatigability of the knee extensors in isometric condition using absolute workloads and including the investigation of very old adults have yet to be performed.

4.2 Concentric tasks

Concentric mode of contraction requires an active shortening of the muscle, which is closer to what is required from the activities of daily living (Figure 10). The majority of the studies investigating neuromuscular function of the knee extensors evaluated single limb tasks in both isotonic and isokinetic tasks, setting the workload as a percentage of maximal capacity. These studies showed that older adults are typically more fatigable than young at high contractions speed (e.g. $>180^{\circ} \cdot s^{-1}$) during both isokinetic maximal contractions at $270^{\circ} \cdot s^{-1}$ (Callahan and Kent-Braun 2011) and isotonic maximal velocity contractions at 20% of maximal isometric force (Dalton et al. 2012; Senefeld et al. 2017; Sundberg et al. 2018b, 2019). However, at slower contraction speed ($80-180^{\circ} \cdot s^{-1}$), similar performance fatigability was observed (Callahan and Kent-Braun 2011; Dalton et al. 2012). At $80^{\circ} \cdot s^{-1}$, performance fatigability was higher for the young adults (Weavil et al. 2018). When young and old adults performed a similar workload for a similar duration (iso-work condition), however, performance fatigability was lower in young compared to older adults (Weavil et al. 2018). The

modality of the concentric contraction (isokinetic vs. isotonic) could also influence performance fatigability. Dalton et al. (2015) compared the age-related differences following an isokinetic (fixed velocity) vs. isotonic (fixed load) fatiguing task performed until 40% of the maximal power was lost (Dalton et al. 2015). Older adults performed 20% fewer contractions at the isotonic task compared to the young, suggesting that the lower velocity production capacity might not explain the difference in performance and fatigability with age during concentric tasks. Indeed, no age-related differences were found for the isokinetic task (Dalton et al. 2015).

Regarding very old adults, after a fatiguing exercise at maximal speed during isotonic contractions (20% MVIC), very old adults showed greater impairments in force and power production than both old and young adults independent of sex (Sundberg et al. 2018b), suggesting accentuated impairments with advancing age. Whether or not the difference in performance fatigability between old and very old adults varies across different tasks remains unknown.

Studies evaluating the etiology of performance fatigability with concentric contractions suggest peripheral rather than central impairments with age (Dalton et al. 2015; Sundberg et al. 2018a, b; Weavil et al. 2018). Evidence of a reduced contraction efficiency have been shown in concentric mode with aging, especially in very old adults (Layec et al. 2018). This age-related impairment in contraction efficiency in concentric tasks does not seem to be associated with altered oxygen supply, as similar levels of tissue oxygenation (Layec et al. 2018) and blood flow (Weavil et al. 2018) were found in young and older adults. This would also suggest that differences in contraction economy between isometric and concentric contractions would not be due to oxygen supply. By studying the age-related differences in muscle bioenergetics of the knee extensors, Sundberg et al. (2019) did not observe any age-related differences in oxidative capacity during the concentric task, but greater metabolite accumulation (P_i , H^+ and dihydrogen phosphate) within the working muscle in older adults compared to young adults. These results suggest that the lower contractile economy would lead to higher metabolite accumulation, inhibiting directly the contractile function within the muscle.

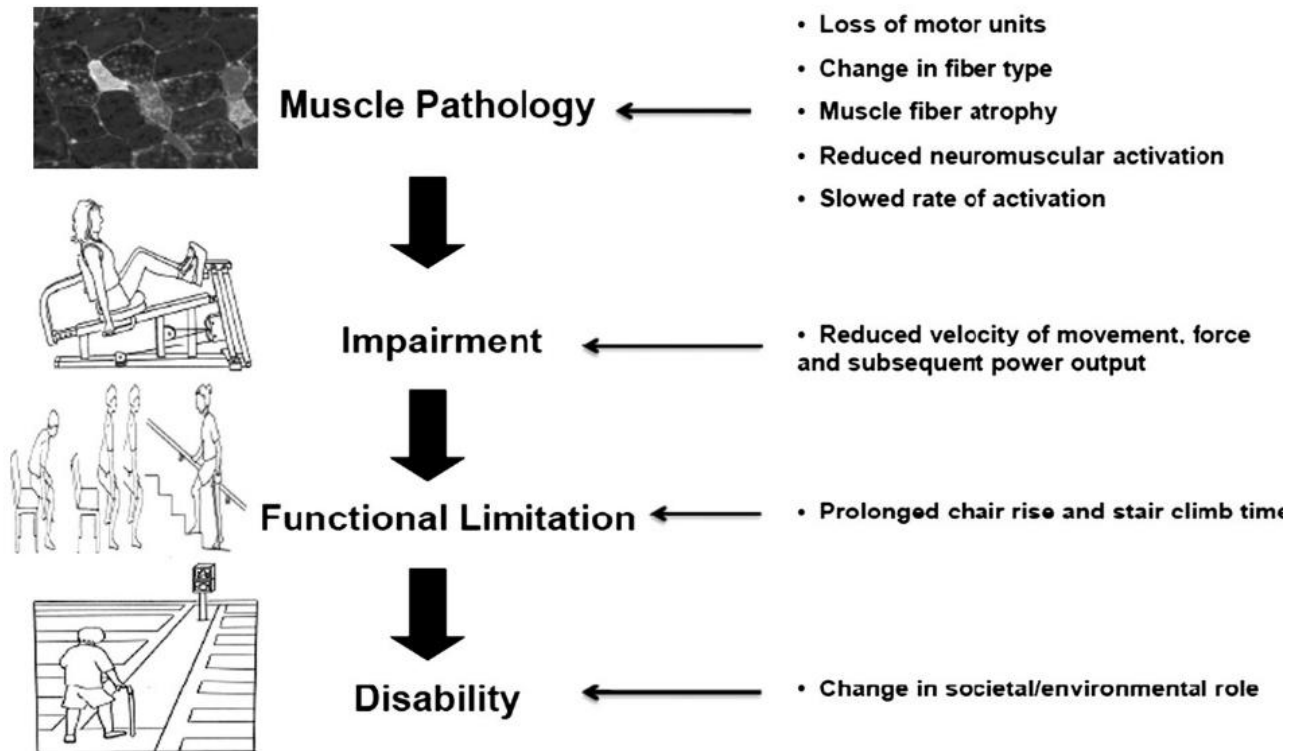


Figure 10. Impairments in power production could result in functional limitation and disability. From (Reid and Fielding 2012).

The fatigability observed in the knee extensors after concentric fatiguing tasks are associated with both walking endurance and balance in the elderly (Reid and Fielding 2012). However, further insights can be obtained by evaluating multiarticular or locomotor fatiguing tasks. When normalizing for the individual one-repetition maximum at the leg press, old and young adults showed similar performance fatigability, but lower total work performed for older adults (Hakkinen 1995; Bieuzen et al. 2010). During a cycling task at 80% of maximal power, Weavil et al. (2018) reported greater performance fatigability in young than older adults, which was accompanied by greater impairments in voluntary activation and contractile functions for young adults. However, when absolute intensity and duration were matched between age groups, young adults presented lower performance fatigability and lower impairments in contractile function and voluntary activation compared to old adults, indicating that absolute performance and fatigability are impaired with age during a cycling task (Weavil et al. 2018).

Krüger et al. (2020) investigated performance fatigability and its etiology following cycling fatiguing tasks of various intensities. They found similar levels of performance fatigability after a sprint (Wingate) and a long moderate-intensity task, while performance fatigability was greater for young than older adults when exercising at severe intensity (Krüger et al. 2020). Because young adults produced more total work than old adults for all fatiguing tasks, these authors suggested a relationship between performance at the cycling tasks, power development capacity, and performance fatigability (Krüger et al. 2020). Other than power development capacity, performance in older adults could be limited by the reduced maximal aerobic capacity and oxygen delivery caused by the decrease in cardiac output and maximal heart rate responses during exercise (Wilson and Tanaka 2000). This would lead to a higher utilization of anaerobic metabolisms and, because anaerobic capacities decrease with aging [e.g. (Reaburn and Dascombe 2009)], exercise cessation. Because the cardiovascular system is more stressed during a multiarticular (cycling) than a single-limb (isokinetic) task [e.g. (Weavil et al. 2018)], the reduction in cycling performance in old compared to young adults do not depend solely on impairments of the neuromuscular system (Poole et al. 2016; Krüger et al. 2020). Whether or not differences in performance and fatigability between a single-limb vs. multiarticular tasks exist in very old compared to older adults remains to be investigated.

TAKE HOME MESSAGE: AGE-RELATED ALTERATIONS IN FATIGABILITY

The age-related differences in performance fatigability vary across tasks. Older adults show lower performance fatigability after isometric fatiguing tasks. Those differences are attenuated during intermittent tasks that reduce the negative effects of blood flow occlusion. During isokinetic and isotonic contractions, performance and performance fatigability are impaired with age, with greater impairments at faster velocities. Finally, when performing multiarticular tasks like cycling, performance and performance fatigability impairments with age depend on the duration and intensity of exertion, with the cardiovascular system contributing greatly to these age-related differences during intense and prolonged efforts.

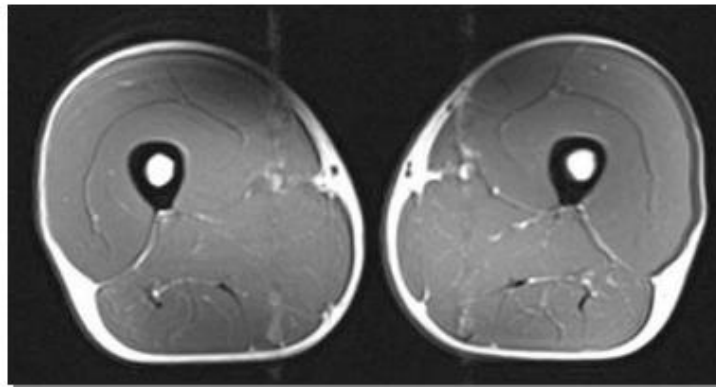
À RETENIR: AGE-RELATED ALTERATIONS IN FATIGABILITY

Les différences de fatigue avec l'âge varient en fonction des tâches effectuées. Les personnes âgées montrent une moindre fatigabilité après des tâches de fatigue isométriques. Ces différences sont atténuées lors de tâches intermittentes qui réduisent les effets négatifs de l'occlusion du flux sanguin. Lors de contractions isocinétiques et isotoniques, la performance et la fatigabilité diminuent avec l'âge, avec des altérations plus importantes à des vitesses de contraction plus rapides. Enfin, lors de tâches multiarticulaires comme le cyclisme, les détériorations de performance et de fatigabilité avec l'âge dépendent de la durée et de l'intensité de l'effort, le système cardiovasculaire contribuant largement à ces différences liées à l'âge lors d'efforts intenses et prolongés.

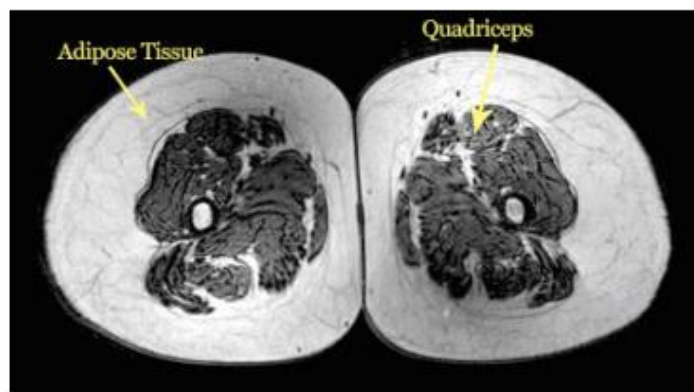
5. EFFECTS OF PHYSICAL ACTIVITY ON NEUROMUSCULAR FUNCTION

Physical activity level could influence the individual rate of muscle deconditioning with advancing age (Bouchard et al. 1994). Aging is associated with a reduction in quantity and intensity of physical activity level (Tudor-locke et al. 2011; Amireault et al. 2019; Varesco et al. 2021b). For instance, while younger adults perform a variety of exercises or sports, physical activity at very old adults mainly consists in walking (Amireault et al. 2019). Both cross-sectional and longitudinal studies assessing the effects of physical activity on aging highlight the gap in physiological function and physical performance between active and inactive older adults (Varesco et al. 2021b). For instance, muscle quality (strength per unit of muscle volume) has been shown to be preserved with age in active individuals from 50 to 80 years old, but decreases in inactive older adults (Barbat-Artigas et al. 2014). Physical activity could also play a role in attenuating the age-related decline in corticospinal excitability (Hassanlouei et al. 2017; Rozand et al. 2019), skeletal muscle lipid content (St-Jean-Pelletier et al. 2017) and skeletal muscle mitochondrial density (Distefano et al. 2018). A large body of studies investigated the physiological characteristics of master athletes as a model of inherent or healthy aging (Lazarus et al. 2019), without the negative effects of physical inactivity. The analyses of performance during endurance competitions showed that performance decreases by ~5 to 20% per decade starting from ~40 years old, arguing that this rate of decline represents the effect of age only (Hoffman and Wegelin 2009; Hoffmann and Hoffman 2010; Knechtle et al. 2012; Lazarus and Harridge 2017; Varesco et al. 2019b). Lifelong endurance exercisers showed greater maximal aerobic capacity, accompanied by greater left ventricular mass, muscle capillarization and aerobic enzyme activity compared to inactive older adults (Gries et al. 2018; Carrick-Ranson et al. 2020). The master athletes showed preserved muscle mass, reduced fat infiltration [e.g. Figure 11; (Wroblewski et al. 2011)], higher neuromuscular transmission stability (Power et al. 2016a) and greater power development per type I muscle fiber compared to inactive older adults (Gries et al. 2019).

40 year old tri-athlete



74 year old sedentary man



70 year old tri-athlete

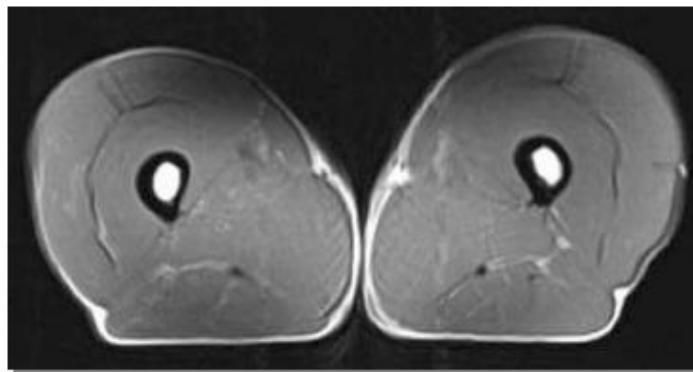


Figure 11. Effect of age and physical activity on muscle mass and fat infiltration of the mid-thigh. From: (Wroblewski et al. 2011).

The benefits of physical activity are also evident at the MU level, with active individuals presenting more stable neuromuscular transmission (Power et al. 2016a) and greater slow twitch muscle fibers power (Gries et al. 2019). MU number preservation in active older adults is still controversial, with

greater preservation observed in the tibialis anterior by Power et al. (2010), although not by others (Piasecki et al. 2016). There is also minimal preservation of the number of estimated MU in the vastus lateralis (Piasecki et al. 2019) and the biceps brachii (Power et al. 2012) muscles.

As for young adults, the type of physical activity triggers specific adaptations in older individuals. For instance, strength does not seem to be preserved in endurance-trained older adults (Chambers et al. 2020). Conversely, strength-trained old adults typically show preserved muscle strength and power (less of an age-related decline) when compared with inactive old adults (Mckendry et al. 2018). This relative preservation of strength and power is associated with greater limb muscle mass and neural drive to the contracting muscles than inactive older adults (Unhjem et al. 2016), counteracting sarcopenia and the reduced neural drive that could occur with advanced aging (Christie and Kamen 2010; Rozand et al. 2020). Taken together, these results indicate that the type of physical activity (endurance vs. resistance exercises) and quantity (active vs. non-active adults) need to be carefully evaluated and quantified when studying age-related changes in physiological function. Despite those differences between active and inactive older adults, physical activity is rarely used to discriminate participants such as sex or age, potentially masking the age-related differences between young and old adults in their physiological outcomes and function. (Varesco et al. 2021b). Future studies should quantify physical activity to separate or include only active or inactive older adults in order to differentiate the effects of inherent aging from those of physical inactivity.

TAKE HOME MESSAGE: EFFECTS OF PHYSICAL ACTIVITY ON NEUROMUSCULAR FUNCTION

Physical activity preserves or attenuates the age-related decline of neuromuscular and cardiovascular function. Because active and inactive older adults show different characteristics, physical activity should be checked when performing aging studies. Active and inactive participants should be separated to reduce the between-subjects variability and differentiate the effects of aging and physical inactivity.

À RETENIR: THE EFFECTS OF PHYSICAL ACTIVITY ON NEUROMUSCULAR FUNCTION

L'activité physique préserve ou atténue le déclin des fonctions neuromusculaire et cardiovasculaire avec l'âge. Étant donné que les personnes âgées actives et inactives présentent des caractéristiques différentes, le niveau d'activité physique doit être vérifié lors des études sur le vieillissement. Les participants actifs et inactifs doivent être séparés pour réduire la variabilité interindividuelle et différencier les effets du vieillissement de ceux de l'inactivité physique.

OBJECTIVES AND HYPOTHESES

Aging is associated with a progressive deterioration of the neuromuscular system that could result in impairments in performance fatigability. These age-related alterations seem exacerbated at very old age and could lead to impaired mobility, loss of autonomy and frailty. Moreover, it is important to study the function of large muscle masses used in locomotion such as the knee extensors, which are particularly affected by aging and are related to physical function. Furthermore, the effect of physical activity level could mediate some of these age-related alterations, with evidence showing differences in performance between active and inactive older adults. Including only active older adults would minimize the negative effects of physical inactivity.

The general aim of the thesis was to investigate performance fatigability of the knee extensors in young, old and very old active adults. Specifically, the present work aimed to:

- Characterize the neuromuscular, functional (using standard clinical tests) and biological characteristics (using muscle biopsies) of the knee extensors of healthy very old men and women and evaluate their association with habitual physical activity level (study 1)
- Evaluate performance fatigability in healthy young, old and very old men and women using a standardized isometric fatiguing protocol (study 2)
- Extend those results to more ecologically valid conditions by comparing performance fatigability after isometric, concentric and cycling tasks with a workload set relatively to individual body weight (study 3).

Study 1 aimed to evaluate whether habitual physical activity is associated with the knee extensors performance (i.e. isometric strength and fatigability) and biological parameters (muscle structural, microvascular and metabolic properties) in healthy very old men and women. Fatigability was evaluated with an intermittent isometric incremental test. Biological parameters were assessed using muscle biopsy in the *vastus lateralis* muscle. Finally, the study explored the sex-related differences in the analyzed parameters. We hypothesized that a greater physical activity level would be associated

with higher capillarization, enzymatic activity and maintenance of type IIA and IIX fibers in both very old men and women, and lower knee extensors fatigability. We did not expect however, an association between physical activity and knee extensors maximal force, according to previous evidence on older adults (Chambers et al. 2020)..

Study 2 aimed to investigate performance fatigability of the knee extensor muscles induced by the isometric quadriceps intermittent fatiguing test in healthy and active young, old and very old men and women. The workload was set relative to maximal force production capacity. It was hypothesized that young and very old adults would present similar fatigability after the isometric intermittent fatiguing test, while older adults would show a lower performance fatigability, according to previous evidence on other muscle groups (Allman and Rice 2001; Kent-Braun et al. 2002; Lanza et al. 2004). It was further hypothesized that old adults would show lower impairments in contractile function than young and very old adults, without age-related differences in voluntary activation impairments. No effect of sex on these age-related impairments in performance fatigability or etiology was expected.

Study 3 aimed to compare performance and fatigability in healthy and active young, old and very old men during isometric, isokinetic concentric, and cycling fatiguing tasks. For this study, workload was set as percentage of body weight. It was hypothesized that young adults would perform better than old and very old men independent of the task. It was also hypothesized that old, but not very old men, would present lower fatigability during the isometric fatiguing task only. Greater fatigability in old and very old adults compared to young adults was expected during the concentric and cycling fatiguing tasks. It was expected that performance fatigability would be mainly driven by impairments in contractile function for all the tasks independent of age, with no alteration in voluntary activation.

MATERIALS AND METHODS

1. EXPERIMENTAL APPARATUS

1.1 Force and Torque measurements

1.1.1 Isometric custom-made chair

For the neuromuscular evaluation of the knee extensors (study 1 and 2), participants seated upright on a custom-build chair, as presented in Figure 12. The position of the force transducer and the seat were adjusted so the knee angle was at 90° (180° = full extension). The hip angle was set at 120° to facilitate coil placement over the femoral triangle. Extraneous movements of the upper body were minimized using belts across the waist and the thorax. The force signal was acquired using a force transducer (Omega Engineering Inc., Stamford, CT, USA) firmly attached over the malleoli, and digitized at a sampling rate of 2 kHz by PowerLab System (16/30-ML880/P, AD Instruments, Bella Vista, Australia).

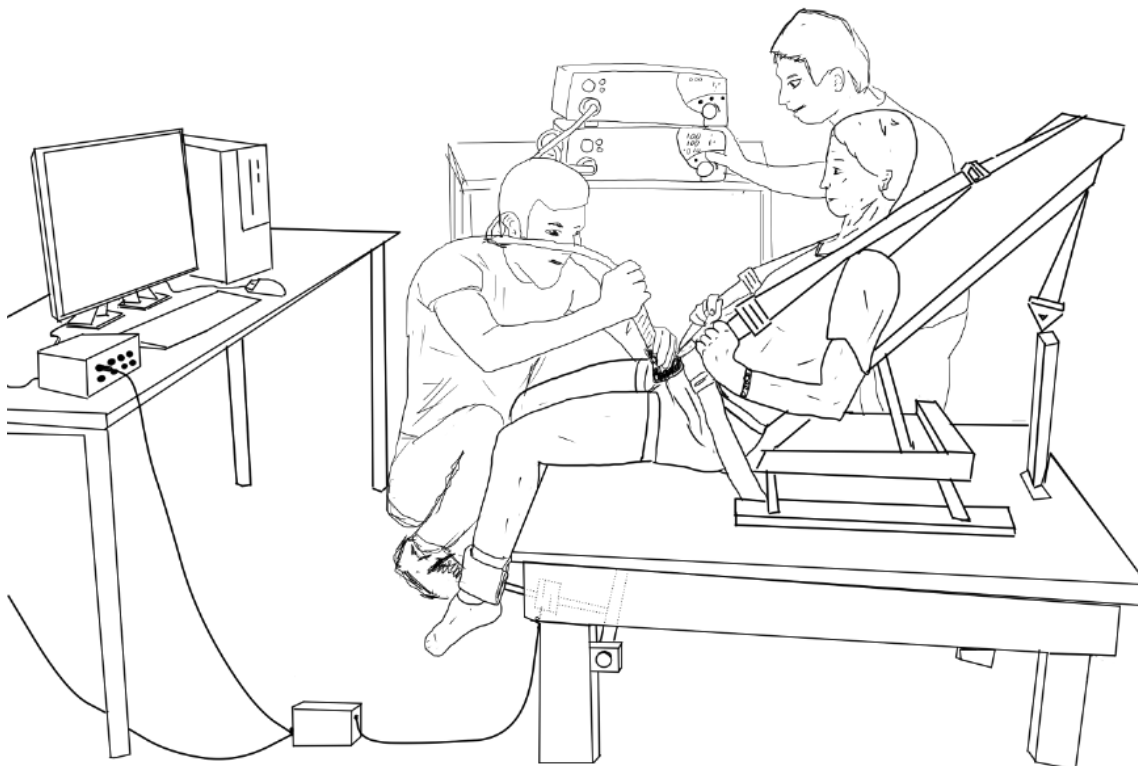


Figure 12. Illustration of the experimental setting for the neuromuscular evaluation in study 1 and 2 on the custom-made isometric chair.

1.1.2 Isokinetic dynamometer

For the torque measurements (study 3), participants were seated upright on a validated isokinetic dynamometer [Con-Trex MJ; CMV AG, Dubendorf, Switzerland; (Maffiuletti et al. 2007)]. Hip and knee angles were set at 95° and 90° , respectively (180° = full extension). Extraneous movements of the upper body were minimized using belts strapped across the waist and the thorax. A non-compliant pad was attached over the malleoli for torque measurements. Torque was sampled without analogical or digital filters at 2kHz (Powerlab 16/30-ML880/P). Experimental setting on the isokinetic dynamometer is presented on Figure 13.

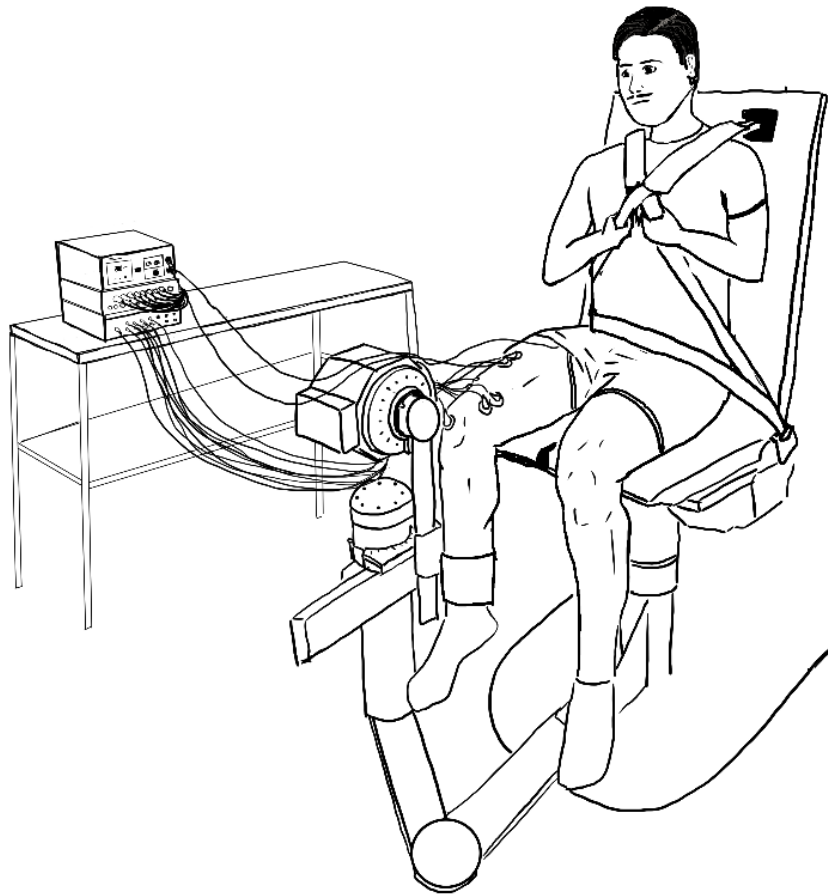


Figure 13. Illustration of the experimental setting for the neuromuscular evaluation on the isokinetic dynamometer.

1.1.3 Customized cycle-ergometer

The cycle ergometer (study 3) was built based on the validated one (Doyle-Baker et al., 2017). It consisted in a custom-made recumbent bike with electromagnetically-braked smart trainer (CycleOps H3, Saris, Madison, WI) working with Rouvy software (VirtualTraining s.r.o., Vimperk, Czech Republic), as presented in *Figure 14*. The smart trainer measured power, cadence, and speed. A mechanical block was built so the pedals could be locked instantaneously in horizontal position. Participants were positioned on the ergometer and the seat was adjusted so the hip and knee angles were at 95° and 90°, respectively (180° = full extension) when the pedals were blocked. Extraneous movements of the upper body were minimized using belts strapped across the waist and the thorax. A custom-made unlockable system for the ankle was used to facilitate the participants to maintain an ankle angle of 90° and reduce extraneous movements of the articulation. Participants were also instructed to maintain the ankle angle at 90° and push their foot forward. To start cycling, the ankle-lock system was first released, and then the pedals were unlocked (≤ 5 -s). Isometric force was measured using validated wireless force transducer installed on the pedals (PowerForce pedals, Radlabor GmbH, Freiburg, Germany). Force signal was transmitted at 500 Hz to a PowerLab System (16/30-ML880/P, AD Instruments, Bella Vista, Australia).

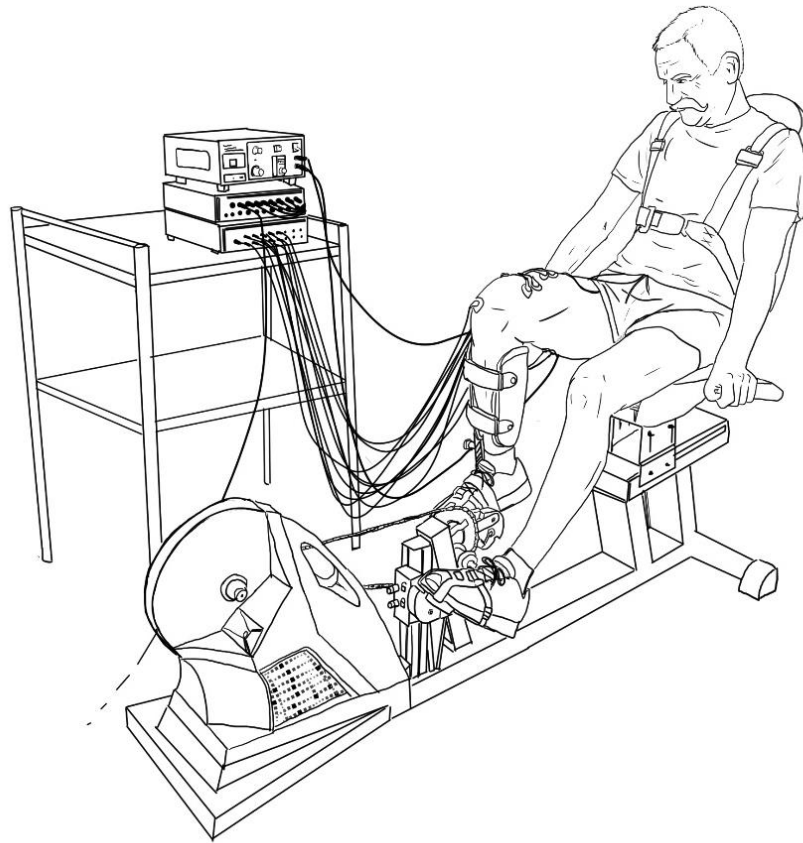


Figure 14. Illustration of the experimental setting for the neuromuscular evaluation on the cyclo-ergometer.

1.2 Electromyography

Electromyographic (EMG) activity was recorded from the *vastus lateralis*, *rectus femoris* and *vastus medialis* muscles of the right leg (study 3). Pairs of self-adhesive Ag/AgCl surface electrodes (recording diameter=10 mm, Kendall MediTrace 130 foam electrode, MA) were placed in bipolar configuration over the muscle belly following the SENIAM guidelines, except for the interelectrode (center to center) distance that was 30 mm due to electrode total diameter. A reference electrode was placed on the right patella. Additionally, two electrodes were placed over the right clavicle and the tenth left rib, respectively, to measure heart rate (study 3). The skin was shaved, abraded, and cleaned with alcohol swab before the electrode placement. EMG signals were amplified (gain = 500) using an octal bio-amplifier (ML138, AD Instruments) with a bandwidth frequency range of 10–500

Hz and digitized by a Power-Lab System (16/30-ML880/P, AD Instruments). An example of electrode placement is presented in Figure 15.

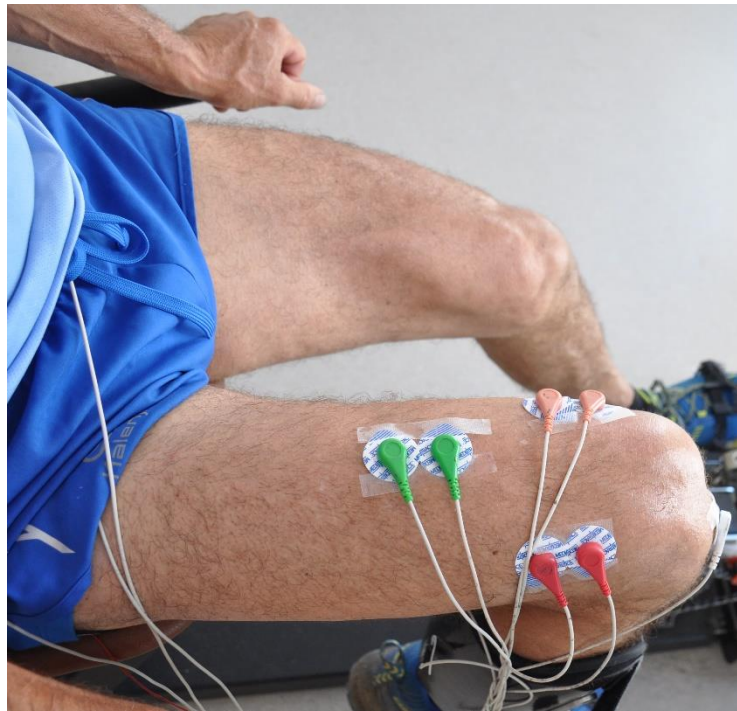


Figure 15. Electrode placement over the vastus lateralis (red), vastus medialis (pink) and rectus femoris muscles (green).

1.3 Stimulations

Magnetic and electrical stimulations were applied to the femoral nerve, which innervate the knee extensor muscles.

1.3.1 Femoral nerve magnetic stimulation

Femoral nerve magnetic stimulation (FNMS) was performed for study 1 and 2. FNMS was delivered through a 45-mm figure-of-eight coil powered by two linked Magstim 200 stimulators (peak magnetic field: 2.5 T, stimulation duration: 0.1 ms; Magstim, Whitland, UK). The coil was positioned above the femoral triangle to recruit the femoral nerve and all stimuli were given at 100% of the maximum stimulator output. Optimal stimulation site evoking the highest twitch force amplitude was determined with minor adjustments and marked on the skin. FNMS supramaximality was checked with decreasing stimulator power output (100%, 95%, and 90). Because the capacity of FNMS to

deliver supramaximal stimuli is altered when fat thickness below the coil increases (Tomazin et al. 2011), clear plateaus in maximal twitch could not be observed for all participants. Thus, only valid responses to FNMS were retained for analysis.

1.3.2 Peripheral Nerve Electrical Stimulation

Square-wave stimulus of 2-ms duration (400 V) were delivered over the femoral nerve through a constant current stimulator (DS7R, Digitimer, Welwyn Garden City, UK) (study 3). The cathode electrode (Kendall MediTrace) was placed on the inguinal triangle and the anode (50 × 90 mm Durastick Plus electrode; DJO Global, Vista, CA) was placed midway between the greater trochanter and the lower border of the iliac crest (Piponnier et al. 2019). The stimulation intensity was progressively increased by 10 mA until the torque and EMG (M-wave) responses reached a plateau. Stimulation intensity at the plateau was then increased by 30% to ensure supramaximality.

1.4 Physical activity

Participants wore an accelerometer on their wrist (wGT3X-BT, ActiGraph, Pensacola, USA) for at least 7 complete days (> 8 h of wear time per day), including one weekend (Migueles et al. 2017; Varesco et al. 2021b). Additionally, for study 1 daily energy expenditure was calculated using the Questionnaire d'Activité Physique de Saint-Etienne, a validated self-administered physical activity questionnaire that represented retrospectively the activity done over a period of one week (Bonney et al. 1996; Garet et al. 2004).

2. FATIGUING AND FUNCTIONAL TASKS

2.1 Fatiguing tasks

2.1.1 The isometric quadriceps intermittent fatigue test

The isometric quadriceps intermittent fatigue test (QIF test, study 1 and 2) consisted of incremental sets of 10 intermittent (5-s on / 5-s off) isometric contractions, starting at 10% MVIC for the first set with increments of 10% MVIC per set until exhaustion, as described by Bachasson et al. (Bachasson

et al. 2013) (Figure 17). Visual feedback of the target force level was provided (Figure 12), and participants listened to a soundtrack with the contraction-relaxation rhythm. The target force level was defined with a line of 4-N thickness (2 N under and 2 N above the target force). Exhaustion was defined as two consecutive contractions below the target force level for 2.5 s. Evaluations of maximal force and responses to stimulation were performed before the test, immediately (~2 s) after the end of each set and at task failure. The duration between the end of a set and the beginning of the following one was set at 25 s.

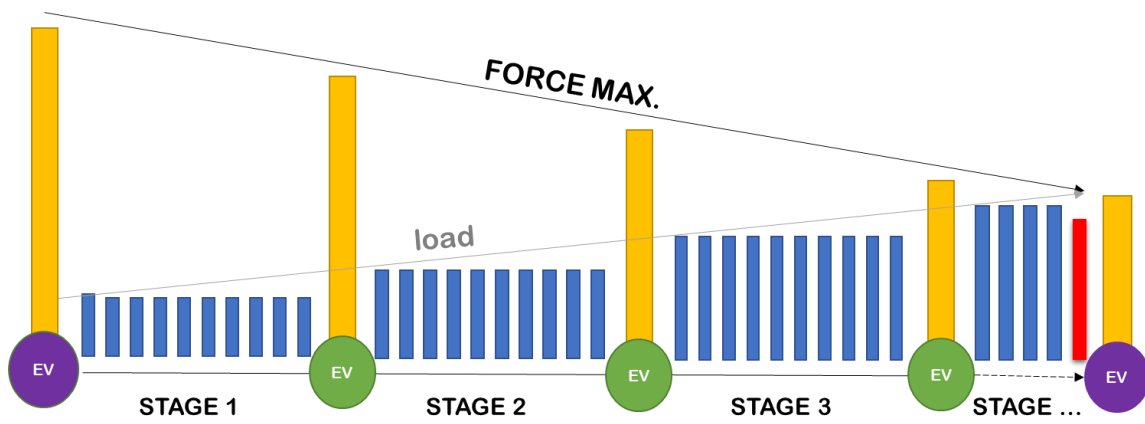


Figure 16. Schematic representation of the QIF test. EV: evaluation of maximal force and response to stimulation. MVIC: maximal voluntary isometric contraction force.

2.1.2 The isometric body-weight based QIF test

For study 3, a modified version of the classic isometric QIF test was used. The body weight based isometric QIF test consisted of stages of 75 contractions (0.8 s on/0.8 s off; stage duration = 120 s) starting at a load that corresponded to 10% of body weight. Because the isokinetic dynamometer measured torque (Nm), the body weight (kg) was first converted to newtons and successively multiplied by the lever arm of the leg expressed in meters, so the workload of the first stage (10%) was calculated as:

$$\text{workload first stage} = \frac{[\text{Body weight (Kg)} \times 9,80665] \times \text{lever arm (m)}}{10}$$

Equation 3.

Starting from the second stage, the increments were set at 5% BW. The target load was displayed on a large screen using a 2-Nm thickness green line (1Nm above – 1Nm below). Participants were instructed to follow the rhythm of contraction imposed by a metronome and to reach the line at each contraction, without surpassing the force level required. Stages were separated by 1 min. At the end of each stage and at exhaustion, an evaluation of maximal torque and responses to stimulation was performed (~10-s) and the rate of perceived exertion was asked on a 6-20 Borg scale (Borg 1982). Task failure was defined as 5 consecutive contractions below the target line. Before and after the test, a series of 3 maximal voluntary concentric contractions was performed.

2.1.3 The concentric body-weight based QIF test

The body-weight based concentric QIF test (study 3) is a modified version of the concentric QIF test (Varesco et al. 2021d). In the original version, 100 contractions were performed (~ 160 s). We set the duration of the stages at 120 s to increase the number of neuromuscular evaluations performed due to large expected difference in performance between young and older adults based on pilot studies.

The load and protocol were identical to the isometric body-weight based QIF test [stages of 75 contractions (0.8 s on/0.8 s off; stage duration = 2 min) starting at 10% of body weight, with increments of 5%]. The range of motion for the concentric contractions was from 90° to 150°, with 180° corresponding to full-extension of the knee, and the velocity set at 120°.s⁻¹. The return to the starting position was passive and performed at the same speed. Participants were instructed to start the contraction immediately after the machine returned the leg to the starting position.

The duration of the duty cycle (0.8 s on/0.8 s off) was measured during pilot studies and was due to the intrinsic delay of the device to attain 120°.s⁻¹. The duration was consistent throughout the exercise and the 75-contractions stages lasted systematically 120 s.

2.1.4 The body-weight based QIF test on the customized cyclo-ergometer

The body-weight based QIF test on the cyclo-ergometer (study 3) consisted of 120-s stages of continuous cycling (37.5 rpm) starting at a load that corresponded to 10% of body weight. Due to

different configuration of the experimental setting and consequent different lever arm, it was not possible to calculate the load similarly to the other two tests. The initial load was set at 0.6 W.kg^{-1} and successive increments at 0.3 W.kg^{-1} , based on pilot studies. The cadence was set at 37.5 rpm (75 pedal stroke for the 120-s duration of each stage) to match duty cycle for the three body-weight based QIF tests. Participants were provided visual feedback of the cadence and a metronome dictated the rhythm. Immediately after each 120-s stage, the pedals were blocked and participants performed a neuromuscular evaluation, and rate of perceived exertion was recorded. Task failure was defined as a cadence below 37.5 rpm for 8 s.

2.2 Functional capacity assessment

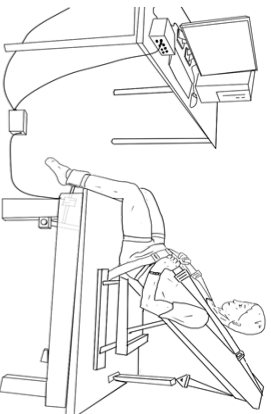
Functional capacity assessment included the Time-up-and-Go test (TUG, study 1 and study 3) and the 6-minute walk test (6MWT). For the TUG test, participants were required to stand up from a chair, walk a distance of three meters, turn around, return, and sit down on the chair (Mathias et al. 1986). The stopwatch was started when the back of the participants moved from the chair and stopped when the participants were seated on with their back on the back of the chair. For the 6MWT participants were instructed to cover the longest distance possible in 6 min on a corridor of 30 m (self-paced), and feedback on the time elapsed was provided every minute (Crapo et al. 2002).

3. ADDITIONAL DATA

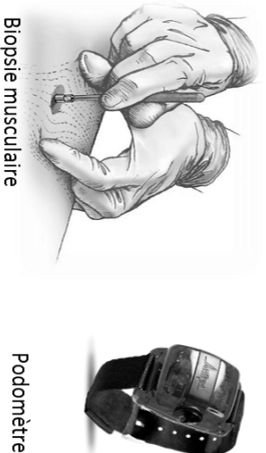
Additional data on muscle biopsies of the *vastus lateralis* were obtained from a collaboration with the Myology Unit of the University Hospital of Saint-Etienne, and the National Institute of Research for Agriculture, Nutrition and Environment (INRAE) of the University of Clermont Auvergne. We used those data that were already analyzed to compare muscle biological properties with the performance (e.g. maximal force, QIF test performance, etc.) and functional (e.g. 6-MWT, TUG test and physical activity) data that we measured on the same participants for study 1.

Résumé des méthodes

Etude 1



Dynamomètre isométrique



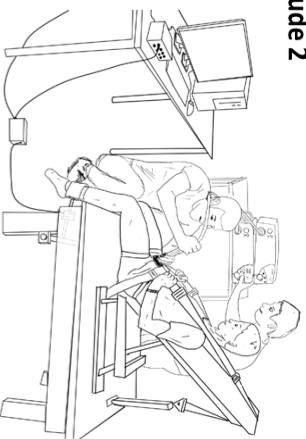
Biopsie musculaire

Podomètre

Comparer et évaluer l'association entre :

- La performance musculaire: la force, l'endurance musculaire, le contrôle musculaire.
- Echantillons de tissu musculaire: taille des fibres, capillarisation, activité enzymatique
- l'activité physique: nombre de pas par jour

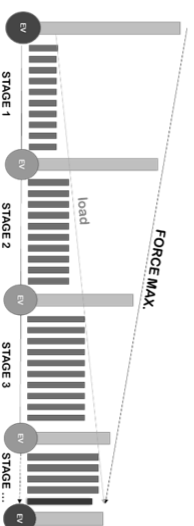
Etude 2



Dynamomètre isométrique



Stimulation magnétique périphérique

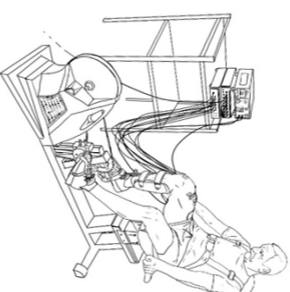


Étude de la performance et de la fatigue musculaire lors d'un test isométrique intermittent et incrémental du quadriceps (test QIF).
Une charge relative à la force maximale

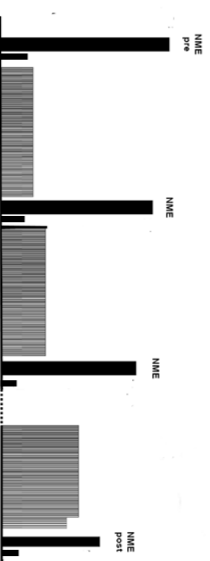
Etude 3



Dynamomètre isocinétique et stimulation électrique périphérique



Cycloergomètre innovant et stimulation électrique périphérique



Étude de la performance et de la fatigue musculaire dans trois tests intermittents et incrémentaux

- Isométrique
- Concentrique
- De Pédalage

Une charge relative au poids du corps

EXPERIMENTAL CONTRIBUTION

1. STUDY 1

Association between physical activity, quadriceps muscle performance and biological characteristics of very old men and women

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Jean-Claude Barthélémy, Frédéric Roche, Daniel Bechet, Léonard Féasson, Vianney Rozand

Published in The Journals of Gerontology: Series A; *in press*

Résumé :

L'objectif de cette étude était d'évaluer l'association entre l'activité physique, la performance des extenseurs du genou (KE) (force isométrique et fatigabilité) et les paramètres biologiques (propriétés structurales, microvasculaires et métaboliques du muscle) chez des hommes et des femmes très âgés en bonne santé. Trente adultes très âgés (82 ± 1 ans, 15 femmes) ont effectué un test de fatigue isométrique intermittent du quadriceps (QIF test) afin d'évaluer la force maximale des KE, le travail total (indice de performance absolue) et la fatigabilité. Des biopsies musculaires du muscle vaste latéral ont été effectuées pour évaluer la typologie et la morphologie des fibres musculaires, la microvasculature et l'activité enzymatique. Des analyses de corrélation ont été utilisées pour étudier les relations entre l'activité physique (pas/jour, actimétrie), la performance des KE et les données biologiques pour chaque sexe séparément. Les hommes, comparés aux femmes, ont montré un travail total plus important lors du QIF test ($44\,497 \pm 8\,629$ Ns vs. $26\,946 \pm 4\,707$ Ns ; $P < .001$). Les pas par jour étaient corrélés avec le travail total uniquement chez les femmes ($r = 0.73$, $P = 0.011$). Chez les hommes, le nombre de pas par jour était corrélé avec le pourcentage ($r = 0.57$, $P = 0.033$), le facteur de forme ($r = 0.75$, $P = 0.002$) et la tortuosité capillaire des fibres de type IIX ($r = 0.59$, $P = 0.035$). Aucune autre corrélation pertinente n'a été observée chez les hommes ou les femmes entre le nombre de pas par jour et les paramètres biologiques. Le niveau d'activité physique était positivement associé à la capacité des femmes très âgées à réaliser un test de fatigue, mais pas à la capacité de production de force maximale des KE. L'activité physique des hommes très âgés n'était pas corrélée à la performance musculaire. Nous suggérons que les femmes très âgées pourraient présenter un risque plus élevé de perte d'autonomie et que l'augmentation du nombre de pas par jour pourrait fournir un stimulus suffisant pour engendrer des adaptations chez les femmes moins actives.

Original Article

Association Between Physical Activity, Quadriceps Muscle Performance, and Biological Characteristics of Very Old Men and Women

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Abstract

The aim of the study was to evaluate the association between physical activity, knee extensors (KE) performance (ie, isometric strength and fatigability), and biological parameters (ie, muscle structural, microvascular, and metabolic properties) in healthy very old men and women. Thirty very old adults (82 ± 1 years, 15 women) performed an isometric Quadriceps Intermittent Fatigue (QIF) test for the assessment of KE maximal force, total work (index of absolute performance), and fatigability. Muscle biopsies from the *vastus lateralis* muscle were collected to assess muscle fibers type and morphology, microvasculature, and enzymes activity. Correlation analyses were used to investigate the relationships between physical activity (steps/day, actimetry), KE performance, and biological data for each sex separately. Men, compared to women, showed greater total work at the QIF test ($44\,497 \pm 8\,629$ Ns vs $26\,946 \pm 4\,707$ Ns; $p < .001$). Steps per day were correlated with total work only for women ($r = 0.73$, $p = .011$). In men, steps per day were correlated with the percentage ($r = 0.57$, $p = .033$), shape factor ($r = 0.75$, $p = .002$), and capillary tortuosity of type IIX fibers ($r = 0.59$, $p = .035$). No other relevant correlations were observed for men or women between steps per day and biological parameters. Physical activity level was positively associated with the capacity of very old women to perform a fatiguing test, but not maximal force production capacity of the KE. Physical activity of very old men was not correlated with muscle performance. We suggest that very old women could be at higher risk of autonomy loss and increasing the steps per day count could provide a sufficient stimulus for adaptations in less active women.

Keywords: Aging, Fatigue, Force steadiness, Myosin heavy chain I, Oldest old

Aging is usually accompanied by a loss in muscle mass quantity and quality, which considerably influences the quality of life in seniors (1). This loss gradually progresses across advanced age, with more rapid declines starting from the ninth decade (2,3). Consequently, people aged 80 years or older, usually defined as very old adults, could be particularly exposed to frailty, falls, and loss of autonomy. Habitual physical activity level is one important factor that could

contribute to lower the incidence of frailty in very old adults (4). For instance, the strength per unit of muscle has been reported to be similar among physically active individuals aged between 50 and 80 years, with an age-related decline observed only in inactive individuals (5). At the muscle tissue level, cross-sectional studies showed that old men and women who exercised regularly presented 20%–90% greater capillarization and aerobic enzyme activity than

nonexercisers (6). Yet, muscle fibers size remained similar between exercisers and nonexercisers (7). The effect of the level of physical activity on sarcopenia could be also mediated by sex-related differences. For instance, a longitudinal community-based study showed that physically active women presented a lower risk of being classified as sarcopenic than sedentary ones, although no association was observed in men (8). Furthermore, anaerobic enzyme activity has been suggested to potentially cause some of the reduction in muscle performance in older adults independently of physical activity that could however be related to muscle performance (9).

Surprisingly, despite the large body of studies evidencing the link between physical function and activity (10), physical activity is not systematically considered when investigating the effects of aging on physical function and skeletal muscle, which could explain some of the heterogeneous results in the literature (10). Yet, physical activity across the life span could be related to differences in muscle performance and influence muscle phenotype, capillarization, and enzymes activity in very old men and women (11), which could be determinant for maintaining autonomy.

The ability of individuals to perform the tasks of everyday life (eg, walking, regain balance, carrying weights) could be described as the capacity to (a) generate an adequate level of force, (b) maintain stable the level of target force, (c) be able to develop force rapidly, and (d) perform repeatedly the aforementioned capacities over time (endurance). This is particularly relevant when studying very old adults, for whom the age-related deteriorations in muscle function and structure are usually exacerbated (3). In addition to the reduction of the capacity to produce maximal force, especially in locomotor muscles like the knee extensors (KE), very old age is characterized by a decrease in rapid force production (12) and force steadiness (13) capacities, and more importantly, an increased fatigability compared with old adults (14). However, it remains unclear whether the level of daily physical activity influences KE function and fatigability in very old adults, quantified as a reduction in maximal force production capacity following a fatiguing exercise.

This study aimed to evaluate whether habitual physical activity is associated with KE performance (ie, isometric strength and fatigability) and biological parameters (muscle structural, microvascular, and metabolic properties) in healthy very old men and women. Fatigability was evaluated with an intermittent isometric incremental test, the Quadriceps Intermittent Fatigue (QIF) test (15). Biological parameters were assessed using muscle biopsy in the *vastus lateralis* muscle. We hypothesized that a greater physical activity level would be associated with higher capillarization, enzymatic activity and maintenance of type IIA and IIX fibers in both men and women, and lower KE fatigability. However, we hypothesized that there would be no association between physical activity and KE maximal force, according to previous evidence on old (>60 years) adults (16,17). Finally, we explored the association between biological and performance parameters and the sex-related differences in the analyzed parameters.

Method

Participants

Thirty active very old adults (82 ± 1 years; 15 females) volunteered to participate in the experiment and were clearly informed on the modality of the test prior to giving their written consent. Participants were recruited from the PROgnostic indicator OF cardiovascular

and cerebrovascular events (PROOF) cohort, a prospective longitudinal cohort of old adults started in 2001. Exclusion criteria for this study were body mass index less than 18.5 kg/m^2 (underweight) or more than 30 kg/m^2 (obesity) and psychological, musculoskeletal, or neurological disorders or disability. None of the very old adults recruited participated in competitive sports. Possible sarcopenic participants were identified following the guidelines of the European Working Group on Sarcopenia in Older People (18). Briefly, cutoff points were applied in hierarchical order as follows: (a) handgrip ($<27 \text{ kg}$ for men, $<16 \text{ kg}$ for women); (b) appendicular skeletal muscle mass (ASM; $<20 \text{ kg}$ for men, $<15 \text{ kg}$ for women) and ASM normalized by height² ($<7 \text{ kg/m}^2$ for men; $<6 \text{ kg/m}^2$ for women); (c) gait speed ($<0.8 \text{ m/s}$), 6-minute walking test (6MWT) distance covered ($<400 \text{ m}$), and Get Up and Go (GUG) test ($>20 \text{ s}$) (18). Before each visit, participants were asked to avoid exercise for 24 hours and alcohol consumption for 12 hours. All the experimental procedures were approved by the Comité de Protection des Personnes Sud-Est 1 (France), and the Agence Nationale Sanitaire et du Médicament (ANSM-France) and were performed in accordance with the declaration of Helsinki (ClinicalTrials.gov, Identifier: NCT02675192).

Experimental Procedures

Participants underwent 4 separate visits. The first 2 visits, separated by at least 72 hours, included the collection of blood samples and skeletal muscle biopsies (Visit 1) and the evaluation of the appendicular lean mass and total fat mass by dual-energy X-ray absorptiometry (iDXA, Lunar, France; Visit 2). Then, participants were recalled within the same year (10 ± 1 months apart, range 9–11 months) to participate in the last 2 visits, where neuromuscular performance was assessed. During the third visit, after the assessment of functional capacity (described below), participants were familiarized with the neuromuscular testing procedures and were given an accelerometer to wear for 7 days (5 weekdays and 2 weekend days) for physical activity assessment. Maximal voluntary isometric contractions (MVICs), force steadiness during 30-second contractions, rate of force development contractions, and the first 2 stages of the QIF test were repeated until the participants felt comfortable with the procedures and until the trials were judged (arbitrarily) satisfactory. This allowed participants to be familiarized with all the neuromuscular testing procedures for Session 4. Seven days later, on the fourth and final visits, KE strength evaluation and a fatiguing test were performed.

Functional Capacity Assessment

Functional capacity assessment included the GUG test, the 6MWT, and the handgrip test. For the GUG test, participants were required to stand up from a chair, walk a distance of 3 meters, turn around, return, and sit down on the chair (19). The stopwatch was started when the back of the participants moved from the chair and stopped when the participants were seated with their back on the back of the chair. The 6MWT consisted in covering the longest distance possible in 6 minutes on a corridor of 30 m (20). Handgrip strength was assessed using a hand dynamometer (Jamar, Chicago, IL) with an adjustable grip. The measure consisted in applying as much pressure as possible for at least 4 seconds while participants were standing upright with the arm along the body. Three trials were performed for each hand and only the maximal value among those collected for both limbs was recorded.

KE Strength and Fatiguing Test

Participants were seated upright on a custom-built chair with a knee angle set at 90° of flexion (180° corresponds to full extension) and a hip angle set at 120°. Extraneous movements of the upper body were minimized using straps across the thorax and waist. KE strength of the right leg was assessed using a force transducer (Omega Engineering Inc., Stamford, CT) firmly attached above the malleoli. Force was assessed at a sampling rate of 2 kHz, transmitted without analogical or digital filter using a Power-Lab data acquisition system (16/30-ML880/P; ADInstruments, Bella Vista, Australia), and recorded on a personal computer through LabChart software (v8; ADInstruments). Visual feedback was provided on a large screen positioned in front of the participant. The procedures on the isometric chair for Sessions 3 and 4 started with a standardized warm-up. The participants were asked to perform 3 contractions for ~ 3 seconds at intensities corresponding to 30%, 50%, and 70% of their perceived maximal force. After 1-minute rest, the maximal force was assessed with two 5-second MVICs separated by 1-minute rest, and a third MVIC was performed if the difference between the peak force values was more than 5%. Participants were instructed to push “as hard as possible” and strong verbal encouragements were provided. Only the best MVIC was retained for further analysis. Then, a 30-second constant contraction at 10% MVIC was performed for the measurement of force steadiness. After 2 minutes of recovery, 8 rapid contractions separated by 30-second rest were performed for the determination of the RFD. Participants were instructed to push “as fast as possible” and strong verbal encouragements were provided. If a countermovement was detected, or the peak force reached was less than 70% MVIC, the contraction was discarded and performed again. After a resting period of 5 minutes, a fatiguing task (QIF test (15)) was performed. Briefly, sets of 10 isometric intermittent contractions (5-second on/5-second off) were performed at a submaximal target force starting at 10% MVIC, with increments of 10% for each set. The target force level was displayed on the screen and the participants listened to a soundtrack indicating the contraction–relaxation rhythm. MVICs were performed before the QIF test and at exhaustion. A 30-second delay was respected between the end of the set and the start of the next one (5-second MVIC included). Exhaustion was defined as 2 consecutive contractions 10 N below the target force.

Neuromuscular Function Data Analysis

All neuromuscular data were analyzed offline using LabChart software (v8; ADInstruments). The maximal force was defined as the average of the highest 0.5-second window for each MVIC. Force steadiness was quantified using the coefficient of variation, calculated by the ratio between the standard deviation by the mean of the force during a 20-second period recorded in the middle of the constant 30-second contraction. RFD was averaged from the best 5 rapid contractions and calculated from the averaged slope of the 0–200 ms as previously described (12) and presented in both absolute and normalized (to maximal force and body weight [BW]) values. The onset was defined by a rise in force from the baseline over 2 Nm. Total number of contractions at the QIF test was used as an index of relative performance at the QIF test. Total work was calculated from the area under the curve of all the contractions of the QIF test and represented the absolute performance of the test. Fatigability was defined as the reduction in MVIC force after the QIF test.

Physical Activity

To objectively assess physical activity, participants wore an accelerometer on their wrist (wGT3X-BT; ActiGraph, Pensacola, FL) for at least 7 complete days (>8 hours of wear time per day), including one weekend (10,21). Physical activity (quantified as steps/day) and time spent in the light, moderate, vigorous, and very vigorous intensity domains data were analyzed using ActiLife software (v6.13.4; ActiGraph, Pensacola, FL). Participants were provided a log to complete and the conformity between the data from the self-reported activity log and accelerometer was checked. All the participants followed the instructions and were included in the further analyses. Daily energy expenditure was calculated using the Questionnaire d'Activité Physique Saint-Etienne, a validated self-administered physical activity questionnaire filled at the end of the second visit and represented retrospectively the activity done over a period of 1 week (22,23).

Muscle Biopsy and Blood Analyses

All participants had standard meals the day before the biopsy. Blood samples and muscle biopsy were collected in the morning after an overnight fast. In addition to standard medical examination, standard blood analyses (fasting glucose, triglycerides, high-density lipoprotein, low-density lipoprotein, and total cholesterol) and measurements of albumin, glycated hemoglobin, creatinine, creatine phosphokinase, thyroid-stimulating hormone, C-reactive protein, and fibrinogen were performed. For each participant, a single muscle biopsy was taken under local anesthesia from the superficial portion of the left vastus lateralis using a Weil-Blakesley conchotome (24) and a percutaneous technique (25). Each biopsy was divided into 2 samples that were either snap-frozen in liquid N₂ for enzymology or frozen in isopentane cooled on liquid N₂ and stored at –80°C for histology (26). Serial cross-sections (10 µm thick) were performed using a cryostat (Microm International, Walldorf, Germany) at –25°C. Detailed procedures for histological analysis (fiber type, cross-section area, and shape), capillary identification, and analysis and enzymatic activity analysis are described in [Supplementary Material S1](#).

Statistical Analysis

All variables are reported as mean ± standard deviation in the text, tables, and figures. Assumption of normality for all variables was investigated using the Shapiro–Wilk test. To measure the association between physical activity, performance (total work, number of contractions, maximal force, RFD, force steadiness, fatigability), and biological variables, correlation analyses were carried out separately for each sex group and evaluated through Pearson's correlation (*r*). To interpret the magnitude of correlation between the variables within each sex group, the following criteria were applied: trivial (*r* < 0.1), small (0.1 < *r* < 0.3), moderate (0.3 < *r* < 0.5), large (0.5 < *r* < 0.7), very large (0.7 < *r* < 0.9), almost perfect (*r* > 0.9), and perfect (*r* = 1). These data were also analyzed by means of a principal component analysis (PCA). This analysis was used to describe the reciprocal relationship among parameters (based on covariances), grouping and reducing the number of variables, to compare the differences between sexes for all the data obtained from muscle biopsies. Based on the correlation matrix, all the variables that showed a linear correlation of more than 0.4 with sex were selected for the PCA. Sampling adequacy and sphericity for PCA were checked using the Kaiser–Mayer–Olkin (KMO) test and the Bartlett test, respectively.

Table 1. Sex-Related Differences in Participant Characteristics

	Men (n = 15)	Women (n = 15)	<i>p</i>
Age (years)	82 ± 1	82 ± 1	1
Weight (kg)	72.6 ± 7.4	64.4 ± 8.1	.008
Height (m)	168.6 ± 5.4	160.8 ± 6.4	.001
BMI (kg/m ²)	26.1 ± 2.8	25.7 ± 2.8	.67
Fat mass (% total mass)	26.9 ± 3.8	38.6 ± 3.4	<.001
ALM (% total mass)	29.7 ± 2.8	23.2 ± 2.5	<.001
Upper limbs (% ALM)	26.8 ± 2.0	23.2 ± 1.9	<.001
Lower limbs (% ALM)	73.2 ± 2.1	76.8 ± 1.9	<.001
Bone mass (kg)	2.3 ± 0.4	1.8 ± 0.4	<.001
Handgrip strength (kg)	32.9 ± 8.6	20.6 ± 5	<.001
6-minute walking test (m)	431 ± 54	390 ± 57	.05
Time Up and Go test (s)	12.0 ± 1.6	11.9 ± 2.4	.83

Notes: BMI = body mass index; ALM = appendicular lean mass. *p* values refer to men–women comparisons. Bold values were used to differentiate a significant *p* value from a non significant.

The principal components among the output factors were selected for each PCA based on the scree-plot and an Eigenvalue of more than 1. Direct Oblimin (delta = 0) rotation was used with Kaiser normalization. Two different principal components were applied to the observations to determine which groups of histochemical, morphometric, and biochemical parameters are more importantly determined by sex. Differences between men and women were evaluated using Student's *t*-test for independent samples or Mann–Withney *U*-tests on all the variables that were available for both groups. Statistical significance was set at *p* ≤ .05, and all statistical analyses were conducted with SPSS v23 (SPSS Inc., Chicago, IL).

Results

Baseline Measurements

Table 1 summarizes the participants' characteristics and functional tests performance of men and women. Very old men were heavier, taller, and exhibited less body fat, but more bone and muscle mass than old women. Very old men also performed better at the handgrip strength and 6MWT than very old women.

Objective measure of physical activity using actimetry (mean wearing time: 15.5 ± 3.6 h/day; range 11–22 hours) showed similar number of steps per day for men (8 841 ± 2 042 steps/day) and women (9 312 ± 2 089 steps/day; *p* = .53). The time spent at light (men: 77.4 ± 6.6%; women: 73.8 ± 7.7% *p* = .09) and moderate intensities (men: 22.6 ± 6.6%; women: 26.2 ± 7.7%; *p* = .097) showed no significant differences. No vigorous and very vigorous intensities were detected.

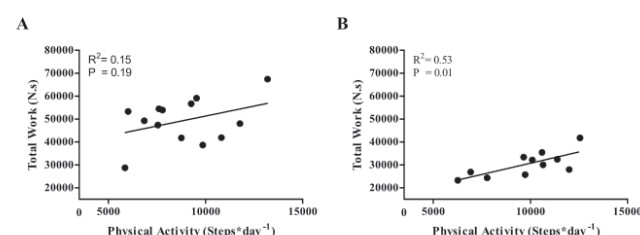
KE force characteristics are presented in Table 2. Very old men showed higher maximal force, RFD (absolute and normalized to BW), and force steadiness than very old women. However, no difference in daily energy expenditure was found between very old men and very old women (122.1 ± 12.2 vs 124.9 ± 10.9 kJ/kg/24 h; *p* = .51).

Of the 30 octogenarians, 24 successfully completed the QIF test (13 men and 11 women). The causes for not completing the QIF test were technical problems (2 women) and discomfort with the procedures, mainly attributed to knee pain that arose following repeated intermittent isometric contractions (2 men and 2 women). Absolute performance at the QIF test (total work) was greater for men (44 497 ± 8 629 Ns) than women (26 946 ± 4 707 Ns; *p* < .001), while number of contractions was similar between groups (men: 63 ± 10; women: 59 ± 5; *p* = .23). No significant difference was observed

Table 2. Sex-Related Differences in KE Force, Rate of Force Development, and Force Steadiness

	Men (n = 15)	Women (n = 15)	<i>p</i>
KE force (N)	418.8 ± 87.7	261.5 ± 57.9	<.001
KE force/BW (N/kg)	5.74 ± 0.90	4.17 ± 1.32	.001
KE force/tested leg ALM (N/kg)	55.6 ± 12.0	45.2 ± 11.7	.02
RFD (N/s)	1 712.2 ± 421.7	1 062.1 ± 269.7	<.001
RFD/KE force (/s)	4.09 ± 0.62	4.16 ± 1.05	.83
RFD/BW (N.kg/s)	23.54 ± 5.07	16.19 ± 5.24	.001
Force steadiness (%)	6.1 ± 2.4	9.3 ± 4.2	.02

Notes: KE = knee extensors; BW = body weight; ALM = appendicular lean mass; RFD = rate of force development. *p* values refer to men–women comparisons. Bold values were used to differentiate a significant *p* value from a non significant.

**Figure 1.** Relationship between physical activity and performance at the QIF test in men (A) and women (B). QIF = Quadriceps Intermittent Fatigue.

in the percentage of force loss at exhaustion (men: $-19.7 \pm 8\%$; women: $-25.7 \pm 7.5\%$; *p* = .07).

Among the participants, one man and one woman were identified as possible sarcopenic (Supplementary Material S3). Despite this very old man would be classified as sarcopenic according to the definition provided by Cruz-Jentoft et al. (18), this is questionable in the light of its KE maximal force (374 N) and total work (43 440 Ns) performed, which were within 1 SD from the group mean (418.8 ± 87.7 N and $44 497 \pm 8 629$ Ns, respectively). For the sarcopenic woman, it was not possible to perform the QIF test due to knee pain while performing repeated isometric contractions. However, maximal force (155 N) was below 1 SD for her group (261.5 ± 57.9 N), indicating the possible presence of sarcopenia.

Correlation Analysis Between Performance Parameters and Physical Activity

Men showed no correlation between physical activity and total work ($r = 0.38$, *p* = .19; Figure 1A), while a large significant correlation was observed in women ($r = 0.73$, *p* = .01; Figure 1B). Hence, total work at the test was positively associated with the number of steps per day performed by the very old women. Men, but not women, showed a large negative correlation between the number of steps per day and the duration of the GUG test ($r = -0.60$, *p* = .018). Therefore, more active men performed better at the GUG test. Physical activity was not correlated with other KE performance parameters such as fatigability, number of contractions, maximal force, RFD, or force steadiness for both sex groups. Finally, MVIC force was negatively correlated with fat mass in women ($r = -0.52$, *p* = .048), but not in men ($r = .03$, *p* = .91).

Muscle Biopsies and Blood Samples

Histochemical, morphometric, and biochemical parameters are presented in Figure 2. Because fibers classified as I-IIA were found only

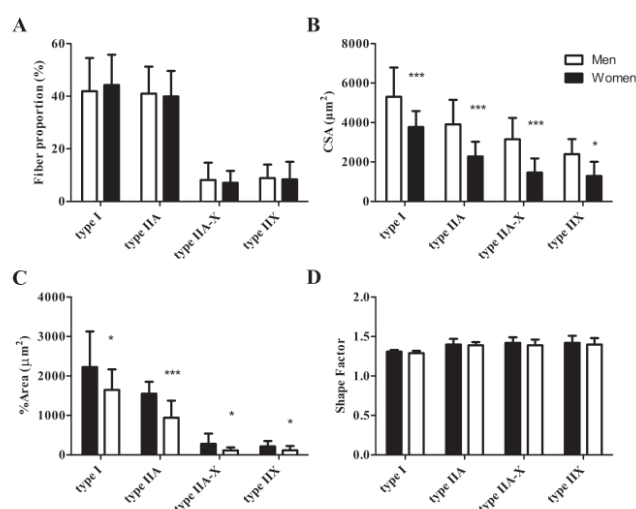


Figure 2. Comparison in fiber proportion (A), cross-sectional area (CSA) (B), percentage area (%A) (C), and shape factor (D) for different fiber types (I, IIA, IIA-X, and IIX) between very old men and women. *Significant difference between sexes: $p < .05$, *** $p < .001$.

for one very old man, this fiber type was discarded from further analysis. Men and women had similar percentages of type I, type IIA, type IIA-X, and type IIX muscle fibers (Figure 2). However, all cross-sectional areas per fiber type were greater for men than women, which resulted in higher percentage area (%A) for types I, type IIA, type IIA-X, and type IIX fibers for men than women (Figure 2). Men and women exhibited similar shape factors for types I, IIA, IIA-X, and IIX fibers.

Microvascular and enzymatic parameters are presented in Table 3. Data revealed higher capillary density (CD), capillary per fiber (C/F), and capillary area per fiber (CAF) for types I, IIA, and IIA-X fibers for men than women. Similarly, capillary tortuosity (LC/PF) was larger for type I fiber in men than women. β -hydroxyacyl CoA dehydrogenase (β -HAD) and citrate synthase were higher for men than women, while no sex-related differences were noticed for the other enzyme concentration (LDH, MK, CK, Complex II and IV).

Blood parameters are presented in Supplementary Material S2. In agreement with higher percentage of fat mass and lower percentage of muscle mass, women exhibited higher blood cholesterol and lower creatinine than men.

Correlation Analysis Between Performance and Biological Parameters

In men, physical activity was correlated with the percentage ($r = 0.57$, $p = .033$), shape factor (SF; $r = 0.75$, $p = .002$), and LC/PF of type IIX fibers ($r = 0.59$, $p = .035$). No other relevant correlations were observed for men or women between physical activity and biological parameters.

In women, maximal force was correlated with citrate synthase ($r = 0.56$, $p = .03$), CSA of type IIA fibers ($r = 0.56$, $p = .03$), capillary per fiber ($r = 0.59$, $p = .02$), CAF of type I fibers ($r = 0.60$, $p = .017$), CAF of type IIA fibers ($r = 0.55$, $p = .034$), and LC/PF of type I fibers ($r = 0.57$, $p = .028$). No other relevant correlations were observed for men or women between KE performance and biological variables.

Principal Component Analysis

The variables that respected the selection criteria and thus inserted in the PCA model were %A (I, IIA, IIA-X, IIX), CAF (I, IIA, IIA-X, IIX), β -HAD, citrate synthase, and SF (I, IIA, IIA-X, IIX). KMO value

Table 3. Sex-Related Differences for Muscle Capillary and Enzymatic Activity Parameters

	Men (n = 15)	Women (n = 15)	p
Capillary (CD31)			
Capillary density (cap/mm ²)	302.3 \pm 50.5	266.7 \pm 30.3	.028
Capillary/fiber	1.60 \pm 0.44	1.05 \pm 0.32	.001
CAF I	4.49 \pm 0.76	3.20 \pm 0.77	<.001
CAF IIA	3.28 \pm 0.55	2.33 \pm 0.6	<.001
CAF IIA-X	2.38 \pm 0.61	1.72 \pm 0.68	.021
CAF IIX	2.11 \pm 0.68	1.84 \pm 1.02	.43
LC/PF I (%)	16.0 \pm 2.5	12.3 \pm 2.1	<.001
LC/PF IIA (%)	12.5 \pm 2.6	10.6 \pm 2.7	.064
LC/PF IIA-X (%)	11.1 \pm 4.1	8.4 \pm 2.5	.062
LC/PF IIX (%)	10.4 \pm 4.6	12.6 \pm 10.10	.49
Enzymatic Activity			
β -HAD (UI/mg)	0.19 \pm 0.02	0.15 \pm 0.03	<.001
LDH (UI/mg)	0.45 \pm 0.07	0.45 \pm 0.06	.86
Citrate synthase (UI/mg)	0.05 \pm 0.01	0.04 \pm 0.01	.014
MK (UI/mg)	1.69 \pm 0.36	1.53 \pm 0.46	.30
CK (UI/mg)	8.07 \pm 0.56	7.65 \pm 1.16	.24
Complex II (UI/mg)	0.35 \pm 0.03	0.33 \pm 0.04	.29
Complex IV (UI/mg)	0.03 \pm 0.01	0.03 \pm 0.01	.15

Notes: CAF = capillary area per fiber; LC/PF = capillary tortuosity; β -HAD = β -hydroxyacyl CoA dehydrogenase; LDH = lactate dehydrogenase; MK = myokinase; CK = creatine kinase; Complex II = second respiratory chain complex; Complex IV = fourth respiratory chain complex. *p* values refer to men–women comparisons. Bold values were used to differentiate a significant *p* value from a non significant.

for the present analysis indicated good appropriateness (0.65), and the Bartlett test indicated that sphericity of the data was respected ($p < .001$). Four components with an Eigenvalue of more than 1 were extracted and most part of the variance (62%) could be explained by the first 2 components. Component 1 was called “muscle oxidative quality,” explaining 44% of the variance and was composed by the %A of type I (0.91) and IIA fibers (0.51), the CAF of type I (0.85) and IIA fibers (0.78), and the enzymatic activity of the β -HAD (0.90) and citrate synthase (0.85). The second component, “morphological fibers abnormalities,” explained 18% of the variance and was composed of SF I (0.75), SF IIA (0.90), SF IIA-X (0.84), and SF IIX (0.59). The third and the fourth components of the PCA were considered as not meaningful: Component 3 was composed by the %A of type IIA-X (0.94) and IIX fibers (0.75) and CAF IIA-X (0.56) and the fourth component by a mix of the other 3 components. Together, the third and fourth components explained only 18% of the total variance (11% for Component 3 and 7% for Component 4). Furthermore, as the relative weight of all the variables of the third and fourth factors was less than 0.5, those components were not judged relevant.

Successively, by performing correlation analysis between Components 1 and 2 (extracted from biological parameters) and the performance parameters, maximal force in very old women was correlated with the PCA Component 1 “muscle oxidative quality” ($R = 0.67$, $p = .017$).

Discussion

The aim of this study was to investigate whether there would be an association between physical activity and KE performance in active octogenarians, and if any biological factor could explain these differences among very old men and women. The main finding was

that total work performed during the QIF test was correlated with the level of daily physical activity in women only. This indicated that greater physical activity was linked to greater absolute performance of the fatiguing exercise. However, there was no correlation between physical activity and maximal force or biological (muscular and blood) parameters. Stronger women showed higher cross-sectional area, capillarization, and enzymatic activity of MHC I and IIA fibers than weaker women. In men, physical activity was not correlated with KE performance but was associated with higher percentage and capillary tortuosity of type IIX fibers. Furthermore, physical activity was correlated with performance at the GUG test.

Physical Activity and Muscle Function

Octogenarian women showed a large positive correlation between the total work performed during the QIF test and the amount of physical activity assessed as steps per day. Very old women are weaker than very old men and thus more exposed to the loss of autonomy, despite their higher life expectancy. This phenomenon is known as the “male–female health-survival paradox” (27). In this population, where the main physical activity consists of walking (28), large locomotor muscles such as the KE could be particularly affected by the level of physical activity. Walking may require greater relative force in octogenarian women than men, as suggested by the lower KE force normalized to BW (~27% lower), the higher percentage of fat mass, and the lower percentage of appendicular lean mass. Thus, daily walking activity may be intense enough to induce endurance-like adaptations in octogenarian women, due to their low level of maximal force. This could explain the relationship between physical activity and total work during the QIF test. Although this relationship was not observed in men, we cannot exclude a positive effect of physical activity on neuromuscular parameters for this sex group, as suggested by the correlation between the number of steps per day and the performance at the GUG test. Furthermore, as the sample of participants was composed of healthy and active very old adults, their physical activity was relatively high (29) and no particular abnormalities were evidenced by the blood profile of very old men and women. This selection bias may underestimate the positive effect of physical activity on neuromuscular performance in very old men and women (10). For instance, it has been previously shown a greater reinnervation capacity (index of resilience to muscle atrophy) in active women (master athletes) compared to prefrail/frail older women (30). Interestingly, in this study, men who were more active showed higher percentage and capillary tortuosity of type IIX fibers, but also greater shape factor for type IIX muscle fibers. The altered shape might represent the first signs of fiber disuse, cell death, or denervation as suggested by Purves-Smith et al. (31), or reorganization of the motor units in the skeletal muscle of older adults (32). Previously, our research group used the same index to study the deformation of muscle fibers in older adults with metabolic syndrome (26). This alteration was not observed in women, maybe due to the minimal percentage of type IIX fibers observed in very old women independent of age and physical activity (30). The results of this study could indicate that physical activity slowed down the deterioration process of type IIX fibers in octogenarian men.

The number of steps per day was not correlated with KE maximal force for both groups. This result is not surprising because recent studies on older lifelong exercisers showed that maximal force of both large (KE) and small muscles (tibialis anterior) involved in locomotion was not influenced by endurance training performed for long periods (>5 years (16,17,33)). However, strength has been

demonstrated as the best predictor of sarcopenia or functional limitation (34). Because walking was not associated with maximal force in this study, resistance training might represent an appropriate intervention to prevent strength loss and consequently reducing the prevalence of sarcopenia and autonomy loss in octogenarians (35). One possible limitation of the current approach is the quantification of physical activity as step count. It has been previously suggested that high-impact activities would have a greater influence on physical function (36). Future studies should try different approaches to investigate the relationships between physical activity and muscle function. However, despite the fact that none of our participants practiced sports or were engaged in training activities, the possibility that their physical activity habits would have changed across the year cannot be ruled out. This is an important aspect to underline as the delay between the physical activity assessment and the biopsy was not negligible and could partially explain the scarce associations found between physical activity and biological parameters or between neuromuscular and biological parameters (described below).

Relationship Between Neuromuscular and Biological Parameters

Correlation and PCAs performed in this study indicated that capillarization and size per type I and IIA fiber, together with the enzymatic activity of oxidative enzymes, were positively associated with KE maximal force for the very old women group. Considering that the intrinsic fiber contractile properties are not influenced by age (3,37), maintaining an elevated muscle capillarization and enzymatic oxidative capacity has been shown critical for strength maintenance in older adults (38). Indeed, preferential atrophy of the type IIX fibers (characterized by low capillarization) is counterbalanced by an increase in cross-sectional area of the more abundant type I and type IIA fibers (26). Similar cross-sectional areas were previously reported by our group for type IIA–IIX and type IIX fibers in old men muscle (26,39). Similar cross-sectional areas were also reported by other groups for type IIX fibers in old men and women (40) and even in young men (41). Furthermore, the activity of oxidative enzymes reflects mitochondrial function and capacity, which is correlated to better muscle quality in older adults (ie, muscle force normalized to muscle size (42)).

We did not find any other correlation between biological and neuromuscular parameters in very old men and women. The reduced number of associated biological and neuromuscular parameters could be explained by the heterogeneous deterioration of the neural and muscular tissue with aging among individuals, determined by a lifelong interaction between genetic and environmental factors. Moreover, neuromuscular parameters such as force, RFD, or steadiness are not only determined by muscle biological parameters but also by the efficiency of the central and peripheral nervous systems, for example, neural excitability and motor drive transmission (43). This leads to a large variability in performance and biological parameters, even in an apparently homogeneous group of active older adults free from pathology or disease (44,45).

Sex-Related Differences

Octogenarian women showed lower walking speed, maximal force, RFD, and force steadiness than men. Absolute performance at the QIF test was greater in men due to their greater maximal force. However, men and women displayed the same relative performance, quantified as the number of contractions during the test. Total muscle mass was greater in men than women, in accordance with previous

studies (37,46). Interestingly, octogenarian women presented a negative correlation between fat mass and maximal force. Increase in fat mass with aging is associated with increased muscular fat infiltration in older adults, compromising muscle contractile properties of the whole muscle (46), and may ultimately decrease the capacity to produce maximal force.

At the myocellular level, the lower cross-sectional area of muscle fibers and capillary tortuosity observed in women compared to men were accompanied by lower enzymatic oxidative enzymes activity (citrate synthase and β -HAD). Principal component analysis confirmed these findings, indicating that capillarization and dimension per type I and IIA muscle fibers, together with the enzymatic activity of oxidative enzymes, could properly discriminate between men and women. The present results reported a lower proportion of type IIX and hybrid fibers in both men and women compared to more oxidative fibers, that is, type I and IIA muscle fibers. The same result has been consistently found in the literature in both young and old adults in different muscles (26,45,47,48). In this study, the %A of I and IIA fibers was 8–14 times higher than the %A of type IIX fibers. The large percentage size of slow-twitch fibers may be explained by their greatest use in daily life activities of very old adults, which mainly consists in walking. Consequently, the underuse of type II muscle fibers could induce a progressive loss of their respective motor units by repeated cycles of denervation–reinnervation of the muscle fibers and a switch to more oxidative characteristics (47,49,50). Taken together, these results indicate that oxidative slow-twitch motor units constitute the greatest part of the muscle in octogenarian men and women and that the skeletal muscle has a high reliance on aerobic metabolism. A possible limitation to detect sex-related differences in this study is the limited number of participants who we were able to recruit. For example, fatigability was not different between sexes in this study ($p = .07$), and this may be different with a higher number of participants. As data obtained from very old adults present large between-subject variability (43), future studies evaluating larger sample sizes may be required.

Conclusions

The level of daily physical activity was positively associated with the performance of octogenarian women during a KE fatiguing test but not with maximal force production capacity. In men, physical activity level was not correlated with muscle performance, but to greater maintenance of type IIX muscle fibers and better performance to the GUG test. When evaluating the relationship between muscle function and biological parameters, KE strength was negatively associated with fat mass in octogenarian women and positively associated with muscle oxidative properties. These results were not observed for men. In conclusion, very old women sampled for this study could be at higher risk of autonomy loss than men and would benefit from increasing their physical activity level. It is also possible that the lack of significant correlations is due to the homogeneity in terms of physical activity level in the sample analyzed. Homogeneous samples could make it more difficult to investigate correlations between physical activity and biological parameters.

Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

Conflict of Interest

None declared.

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Author Contributions

G.V., T.L., J.-C.B., F.R., L.F., D.B., and V.R. conceived and designed research. G.V., C.C.-G., A.D., M.G., D.B., L.F., and V.R. conducted the experiments and collected the data. G.V., C.C.-G., A.D., and M.G. analyzed the data. G.V., T.L., D.B., L.F., and V.R. drafted the manuscript. All authors provided critical appraisal to the manuscript and approved the final version. All authors have read and approved the final version of the manuscript.

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SUPPLEMENTAL MATERIAL S1

Detailed procedures for histological analysis (Fiber type, cross section area and shape), capillary identification and analysis and enzymatic activity analysis

Fiber type, cross-section area (CSA) and shape.

Two serial cross-sections were labeled with monoclonal antibodies against myosin heavy-chain-I (MHC-I) (A4.951 from Enzo Life Sciences, Farmingdale, NY, United States) or MHC-IIA (N2.261, Enzo life Sciences), co-labeled with anti-laminin- α 1 (Sigma) to outline the fibers, and resolved with corresponding secondary antibodies conjugated to Alexa-Fluor 488 or 546 (Invitrogen). Images were captured with a high-resolution cooled digital DP-72 camera coupled to a BX-51 microscope (Olympus Life Science, Munich, Germany) at a resolution of 0.64 μ m/pixel. Four fields, each containing 80 ± 15 fibers, were analyzed per subject. The contractile Type (I, I-IIA, IIA, IIA-IIX or IIX), cross-sectional area (CSA) and perimeter were determined for each fiber using Visilog-6.9 (Noesis, Gif-sur-Yvette, France) according to (Meunier et al. 2010). Thresholding of MHC images distinguished either positive, negative, or intermediate MHC labeling. Type I fibers were positive only for MHC-I, and type IIA fibers positive only for MHC-IIa. Type X fibers were negative for both MHC-I and MHC-IIa. Type I-IIA hybrid fibers were intermediate for both MHC-I and MHCIIa, while type IIA-IIX hybrid fibers were only intermediate for MHC-IIa labeling. A shape factor [$\text{perimeter}^2 / (4\pi * \text{CSA})$] was calculated, with a value of 1.0 indicating a circle and a value > 1.0 an increasingly elongated ellipse (Kirkeby and Garbarsch 2000; Bruusgaard et al. 2006; Verdijk et al. 2007; Gueugneau et al. 2015). Finally, the area occupied by each fiber type for 100 fibers was obtained by multiplying the fiber type distribution to the fiber-type specific cross-sectional area (%A). For all participants, fiber type proportion and morphometry were assessed for an average of 300 fibers per individual.

Capillaries

The identification of capillaries was performed using the monoclonal anti-CD31 (M0823, Dako, Glostrup, Denmark), which recognizes platelet endothelial cell adhesion molecule (PECAM-1), a transmembrane glycoprotein strongly expressed by vascular endothelial cells (Charifi et al. 2004). Cross-sections were labelled with anti-CD31, co-labelled with anti-laminin- α 1, and resolved with corresponding secondary antibodies conjugated to Alexa-Fluor 488 or 546. CD31 and MHC images were matched and analyzed with ImageJ to analyze the capillary network as previously described (Gueugneau et al. 2016). A mean of 450 fibers and 800 capillaries were analyzed per subject. Capillary density (CD) was expressed as the number of capillaries counted per square mm. Capillary-to-fiber ratio (C/F) was calculated as the ratio between the number of capillaries and the number of fibers present in the same area. In addition to global indices (CD, C/F), individual fiber indices were also determined. For each fiber type, the mean number of capillaries around a single fiber (CAF) was calculated. The capillary-to-fiber interface was estimated using LC/PF index, which is the length of contact (LC) between capillaries and the fibers divided by perimeter of the same fiber (PF).

Enzymes activity

Approximately 20 mg of muscle were homogenized in 400 μ L of buffer (Manitol 225 mM, tris HCL 10 mM, saccharose 75 mM, EDTA 0.1 mM) with a tissue homogenizer (Ultra-Turrax T8 IKA-Werke, Staufen, Germany). Samples were centrifuged (20 min, 650 g, 4°C) and the supernatant was carefully collected. The pellet was re-homogenized in 2/3 of the previous buffer and centrifuged a second time (20 min, 650 g, 4°C) with the previously-collected supernatant. The protein concentration was determined with bovine serum albumin (BSA) as a standard (Pierce BCA Protein Assay Kit, Thermo Scientific, Waltham, MA) and the supernatants were [JT1] stored at -80°C until subsequent analyses were performed. Myokinase, creatine kinase, enolase, lactate dehydrogenase, citrate synthase, β -hydroxyacyl-CoA dehydrogenase and second and fourth respiratory chain complex maximal activities (UI/mg of protein) were determined by absorbance with a multi-label microplate reader

(CLARIOstar, BMG LABTECH GmbH, Germany) according to (Merlet et al. 2020). Maximal activities of myokinase (MK), creatine kinase (CK), enolase (ENO), lactate dehydrogenase (LDH), CS, β -HAD [JT2] and second (CII[Jt3]) and fourth (CIV) respiratory chain complexes (UI/mg of protein) were determined.

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SUPPLEMENTAL MATERIAL S2

Sex-related differences in blood parameters.

Blood samples	Men (n=15)	Women (n=15)	P-value
Fibrinogen (g/L)	3.22 ± 1.1	3.53 ± 0.5	0.33
Creatinine (micromole/L)	92.6 ± 18.2	66.2 ± 21.9	0.001
Glucose (mmol/L)	6.5 ± 2.7	5.3 ± 0.5	0.12
CRP (g/L)	1.32 ± 1.03	2.31 ± 1.84	0.08
CPK (g/L)	136.7 ± 81.1	111.9 ± 69.9	0.37
Albumin (g/L)	44.5 ± 2.3	42.8 ± 3.3	0.12
Total cholesterol (g/L)	2.0 ± 0.5	3.7 ± 2.2	0.01
HDL cholesterol (g/L)	0.31 ± 0.16	0.74 ± 0.16	0.04
LDL cholesterol (g/L)	1.04 ± 0.36	1.52 ± 0.34	0.001
Triglycerides (g/L)	1.16 ± 0.46	1.10 ± 0.34	0.68
TSH (mU/L)	1.69 ± 0.78	1.91 ± 0.80	0.45
Glycated hemoglobin (%)	5.98 ± 1.17	5.31 ± 1.49	0.18
Systolic pressure (mm HG)	140 ± 11.2	135 ± 11.6	0.24
Diastolic pressure (mm HG)	73 ± 6.2	76 ± 7.8	0.25

CRP, C-reactive protein; CPK, creatine phosphokinase; HDL, high density lipoprotein; LDL, low density lipoprotein; TSH, thyroid-stimulating hormone. P-values refers to men-women comparisons.

SUPPLEMENTAL MATERIAL S3.

Prevalence of sarcopenia determined according to Cruz-Jentoft et al. 2019 (the European Working Group on Sarcopenia in Older People, EWGSOP2019).

Participant	6-min Walking Test distance (m)	Gait Speed (m/s)	GUG (s)	Handgrip (Kg)	ASM (kg)	ASM/m ² (Kg/m ²)	Sarcopenic (EWGSOP2019)
Man1	430	1,2	11,8	27,1	18,0	6,99	sarcopenic
Man2	448	1,2	12,48	27,2	19,3	6,53	
Man3	325	0,9	13,06	22,7	22,1	8,43	
Man4	320	0,9	16,1	18,1	20,0	8,22	
Man5	439	1,2	9,28	44,5	21,7	7,70	
Man6	465	1,3	10,6	37,6	21,5	7,35	
Man7	387	1,1	14,1	38,6	24,2	7,90	
Man8	488	1,4	12,7	47,6	23,6	8,06	
Man9	419	1,2	11,8	38,6	21,0	7,70	
Man10	469	1,3	11,2	31,8	24,6	8,21	
Man11	501	1,4	11,4	28,1	19,7	6,80	
Man12	470	1,3	11,2	31,8	21,3	7,83	
Man13	430	1,2	11,3	22,7	16,4	6,42	
Man14	439	1,2	10,5	40,8	25,0	8,18	
Man15	430	1,2	13,1	36,3	26,7	9,75	
Woman1	436	1,2	10,8	24,9	13,4	5,56	sarcopenic
Woman2	360	1,0	12,6	15,9	17,3	5,88	
Woman3	340	0,9	10,2	22,7	16,4	6,72	
Woman4	520	1,4	9	27,2	14,0	5,48	
Woman5	405	1,1	11	17,2	12,0	5,33	
Woman6	415	1,2	7,6	18,1	15,0	6,56	
Woman7	328	0,9	14,9	9,1	15,1	6,78	
Woman8	431	1,2	10,2	24,9	15,2	5,80	
Woman9	350	1,0	14,8	19,5	12,8	4,96	
Woman10	290	0,8	12,5	18,1	15,6	5,90	
Woman11	452	1,3	9,6	21,8	14,0	5,72	
Woman12	360	1,0	16,2	19,5	13,7	5,47	
Woman13	368	1,0	13,1	18,1	16,2	6,03	
Woman14	390	1,1	11,5	22,7	16,5	7,15	
Woman15	400	1,1	14,2	29,5	17,1	6,26	

Summary of the study 1

The results of the present study showed that, in very old adults, physical activity is positively associated with the total work (absolute performance) performed at the QIF test for women, and greater maintenance of type IIX muscle fibers and performance at the Get-up-and-go test for men. Because the level of physical activity was relatively homogeneous across our sample, our results indicated that small variation in habitual physical activity level, quantified as steps.day^{-1} , could have an influence on important parameters such as knee extensors performance (women) and performance at a functional test usually adopted in clinical settings (Get-up-and-go test; men). This underlines the need to take into consideration physical activity in aging studies (Varesco et al. 2021b). Because of the homogeneity in physical activity level and because healthy and active very old individuals do not represent the population of octogenarians, the effects of physical activity found in the present study might be underestimated.

In study 1 we also explored the possible association between neuromuscular and biological parameters. Interestingly, in women, while maximal knee extensors isometric strength was negatively associated with fat mass, it was positively associated with muscle oxidative properties. No such association were observed for men, despite they possess greater maximal force and showed higher performance. This could result in different age-related differences in men and women following prolonged efforts (i.e. following a fatiguing task). Furthermore, no biological parameter was associated with performance and performance fatigability in very old adults. To further understand the mechanisms of performance and performance fatigability with aging, non-invasive techniques such as stimulations techniques could provide useful information into the etiology of performance fatigability.

In the second study, we aimed to investigate the age-related differences in performance and performance fatigability in healthy and active men and women using the same QIF test paired with stimulation techniques.

2. STUDY 2

Age-related differences in performance and fatigability during an isometric quadriceps intermittent fatigue test

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Résumé:

L'objectif de cette étude était d'examiner les différences liées à l'âge dans la fatigabilité induite par un test de fatigue isométrique intermittent du quadriceps chez des hommes et des femmes jeunes (<35 ans), âgés (>60 ans) et très âgés (>80 ans). La perte de force maximale, la fonction contractile et le niveau d'activation volontaire des muscles extenseurs du genou ont été évalués tout au long d'un test de fatigue isométrique à l'aide de stimulations magnétiques du nerf fémoral. Les personnes âgées ont effectué plus de contractions (indice de performance relative) que les jeunes ($P = 0.046$) et les personnes très âgées ($P = 0.007$), sans différence entre les personnes jeunes et très âgées. Le travail total (performance absolue) était plus important chez les jeunes et les personnes âgées par rapport aux personnes très âgées ($P < 0.001$), sans différence entre les personnes jeunes et âgées. À l'épuisement, la perte de force était plus importante chez les jeunes ($-28 \pm 9 \%$) que chez les personnes âgées ($-19 \pm 8 \%$), mais pas chez les personnes très âgées ($-23 \pm 8 \%$). La réponse à la stimulation du nerf fémoral a diminué de façon similaire à l'épuisement pour les trois groupes d'âge, indiquant une altération similaire de la fonction contractile avec l'âge. Aucune altération de l'activation volontaire n'a été observée. Les altérations des paramètres neuromusculaires étaient similaires chez les hommes et les femmes. Cette étude a montré que les personnes âgées étaient moins fatigables que les jeunes lors d'une tâche de fatigue isométrique intermittente des extenseurs du genou. Cette plus grande résistance à la fatigue n'était pas maintenue chez les personnes très âgées, indépendamment du sexe. La fatigabilité à l'épuisement était principalement due à des altérations de la fonction contractile pour les trois groupes d'âge.

Abstract (250 words)

The aim of the present study was to investigate age-related differences in fatigability induced by an isometric quadriceps intermittent fatiguing test in young (<35 years old), old (>60 years old) and very old (>80 years old) men and women. Maximal force loss, contractile function and voluntary activation of the knee extensors were evaluated throughout an isometric fatiguing test using femoral nerve magnetic stimulations. Older adults performed more contractions (index of relative performance) than young ($P = 0.046$) and very old adults ($P = 0.007$), without differences between young and very old adults. Total work (absolute performance) was greater for young and old adults compared to very old adults ($P < 0.001$), without differences between young and old adults. At exhaustion, force loss was greater for young ($-28 \pm 9\%$) compared to old adults ($-19 \pm 8\%$), but not very old adults ($-23 \pm 8\%$). The response to femoral nerve stimulation decreased similarly at exhaustion for the three age groups, indicating similar alteration in contractile function with age. No impairment in voluntary activation was observed. Impairments in neuromuscular parameters were similar for men and women. This study showed that older adults were less fatigable than young adults during an isometric intermittent fatiguing task of the knee extensors. This greater fatigue resistance was not maintained in very old adults independent of sex. Fatigability at exhaustion was likely due to impairments in contractile function for the three age groups.

Keywords: Very old; Fatigue; Magnetic stimulation; Contractile function; Knee extensors.

Introduction

Healthy ageing is usually accompanied by impairments in neuromuscular function and fatigability, leading to increased risk of autonomy loss and incidence of frailty (Hunter 2018). These impairments are not linear with age. For example, the decline of maximal isometric force accelerates in very old adults (i.e. >80 years) compared to old adults (i.e. >60 years) (Mitchell et al. 2012; Venturelli et al. 2018), with greater impairments in women compared to men (Haynes et al. 2020; Varesco et al. 2021a). In a recent meta-analysis, Krüger et al. (Krüger et al. 2018) reported an overall lower performance fatigability (exercise-induced maximal force loss) in older compared to young adults after isometric fatiguing tasks. However, these results are equivocal, with some studies reporting a large effect size (Hedges's $g > 2$) and others reporting no age-related difference or greater fatigability in older adults (Krüger et al. 2018). Importantly, studies investigating performance fatigability after isometric tasks in very old adults are limited. Allman and Rice (Allman and Rice 2001) compared performance fatigability after an intermittent isometric fatiguing task of the elbow flexors between young and very old adults, and reported similar values between the two age groups. However, the absence of a group of older adults did not allow the rate of decline in neuromuscular function between old and very old adults to be determined. Justice et al. (Justice et al. 2014) showed that time to exhaustion during an isometric sustained fatiguing task of the ankle dorsiflexors was longer for older adults (65-75 yr) compared to young adults (<35 yr), with similar performance between young and very old adults (75-90 yr). However, performance fatigability was not investigated in this study, nor the mechanisms explaining these age-related differences, *i.e.* above (voluntary activation) or below (contractile function) the neuromuscular junction.

In old adults, impairments in voluntary activation following single-limb exercises seem to be similar compared to young adults (Krüger et al. 2018). Conversely, impairments in contractile function with fatigue show inconsistent results, with similar or lower alterations in old compared to young adults after isometric fatiguing tasks (Krüger et al. 2018). Again, evidence for alterations in voluntary

activation and contractile function in very old adults are scarce. Sundberg et al. (Sundberg et al. 2018b) observed greater impairments in contractile function but not voluntary activation compared to young and old adults. Because variability increases with advancing age, standardized and validated testing procedures as well as assessments of physical activity levels are needed when comparing groups of different ages (Varesco et al. 2021b).

Thus, the present study aimed to investigate performance fatigability of the knee extensor (KE) muscles induced by the isometric quadriceps intermittent fatiguing (QIF) test in young, old and very old men and women. We expected similar performance fatigability in young and very old adults after the isometric intermittent fatiguing test, while older adults would show a lower performance fatigability. We further hypothesized lower impairments in contractile function in old adults than very old and young adults, without age-related differences in voluntary activation impairments.

Methods

Participants

Thirty young adults [15 men (YM); 15 women (YW)], 19 old adults [10 men (OM); 9 women (OW)] and 30 very old adults [15 men (VOM); 15 women (VOW)] participated in the study and were clearly informed on the experimental procedures prior giving their written consent. Participants were free from psychological, musculoskeletal and neurological disorders or disability, and were asked to avoid exercise and alcohol consumption for 24 h before each visit. All the procedures were approved by the Comité de Protection des Personnes and were performed in accordance with the declaration of Helsinki (2013; ClinicalTrials.gov identifier: NCT02675192). The University Hospital of Saint-Etienne (France) was the sponsor of this study.

Experimental protocol

Participants visited the laboratory on two separate occasions. During the first visit, all participants were familiarized with the neuromuscular testing procedures, and an accelerometer (wGT3X-BT;

ActiGraph, Pensacola, USA) was given to wear for 7 consecutive days to objectively assess physical activity (Varesco et al. 2021b). The old and very old adults also performed a 6-minutes-walk-test (6MWT), which consisted in covering the longest distance possible in 6 minutes in a corridor of 30 meters (Crapo et al. 2002). One week later during the second visit, KE function was evaluated using the isometric QIF test (Bachasson et al. 2013).

Experimental setup

Participants were seated upright on a custom-build chair with the hip angle set at 120° (180°= full extension) to facilitate coil placement over the femoral triangle (see below). Knee angle was fixed at 90°. The right leg was systematically evaluated other than for specific reasons (i.e. operated knee, unilateral pain). In these instances, the left leg was tested (1 OW, 2 VOM, 1 VOW). Extraneous movements of the upper body were minimized using belts across the waist and the thorax. Force of the KE was assessed using a force transducer (Omega Engineering Inc., Stamford, CT, USA) firmly attached over the malleoli, and digitized at a sampling rate of 2 kHz by PowerLab System (16/30-ML880/P, AD Instruments, Bella Vista, Australia).

Femoral nerve magnetic stimulation

Femoral nerve magnetic stimulation (FNMS) was performed with a 45-mm figure-of-eight coil powered by two linked Magstim 200 stimulators (peak magnetic field: 2.5 T, stimulation duration: 0.1 ms; Magstim, Whitland, UK). The coil was positioned above the femoral triangle to recruit the femoral nerve and all stimuli were given at 100% of the maximum stimulator output. Optimal stimulation site evoking the highest twitch force amplitude was determined with minor adjustments and marked on the skin. FNMS supramaximality was checked with decreasing stimulator power output (100%, 95%, and 90%). Because FNMS effectiveness in deliver supramaximal stimuli is altered when fat thickness under the coil increases (Tomazin et al. 2011), clear plateaus in maximal

twitch amplitude were not observed for all participants. Thus, only valid responses to FNMS were retained for analysis (YM: 15; OM: 8; VOM: 10; YW: 15; OW: 9; VOW: 8).

Isometric QIF test

The session started with a warm-up consisting of three ~3-s contractions at 30%, 50% and 70% of the perceived maximal force. Successively, two 5-s maximal voluntary isometric contractions (MVICs) were performed, separated by 1-min of rest. A third MVIC was performed if the difference between the peak force values was >5%. Participants were instructed to push “as hard as possible” and strong verbal encouragement was provided. Visual feedback of the force signal was displayed on a screen positioned in front of the participants. Then, a neuromuscular evaluation (NME) was performed. NME consisted of a 5-s MVIC during which a 100-Hz doublet ($Db_{100,s}$) was superimposed through FNMS, and a resting potentiated 100-Hz doublet (Db_{100}) delivered ~2 s after relaxation. Once the baseline measures were performed, the QIF test started after at least 1 min of recovery.

The QIF test consisted of incremental sets of 10 intermittent (5-s on / 5-s off) isometric contractions, starting at 10% MVIC for the first set, with increments of 10% MVIC per set until exhaustion (Bachasson et al. 2013). Visual feedback of the target force level was provided, and participants followed a soundtrack with the contraction-relaxation rhythm. A line of 4-N thickness indicated the target force (2 N below and 2 N above). Exhaustion was defined as two consecutive contractions where force decreased below the target force level for 2.5 s. NME was performed immediately (~2 s) at the end of each set and at task failure. The duration between the end of a set and the beginning of the following one was standardized at 25 s.

Data analysis

Physical activity data were analyzed using ActiLife software (v6.13.4, ActiGraph, Pensacola, USA) and quantified as steps·day⁻¹. All the remaining data were analyzed using Labchart software (v8, ADInstruments). MVIC was calculated as the average of the highest 0.5-s window of the contraction.

Performance fatigability was defined as the percentage of MVIC loss from baseline. Total work was calculated as the sum of the force–time integral of each contraction and indicated absolute performance at the QIF test. Total number of contractions performed indicated relative performance. Voluntary activation (VA) was calculated from the formula: $VA\% = Db_{100,s}/Db_{100} \times 100$. Impairments in contractile function were quantified as the change in Db_{100} from baseline.

Statistical Analysis

All variables are reported as mean \pm SD in the text, tables and figures. Generalized Linear Models were used to analyze the age- and sex-related differences in anthropometric (age, weight, height) and neuromuscular characteristics at baseline (absolute and normalized MVIC, Db_{100} , VA), and performance (total work, number of contractions). Generalized Estimating Equations analyses using an autoregressive (AR-1) structure were performed on data expressed relative to baseline to investigate the effects of age and sex across the QIF test on MVIC, Db_{100} and VA [stage (stages 1, 2, 3, 4 and exhaustion) \times age (young: YA vs. OA vs. VOA) \times sex (men, women)]. Because participants performed different numbers of stages, data were compared until the last stage performed by all participants (i.e. 4th stage). For all analyses, when significant effects were observed, Bonferroni correction was applied to post-hoc analyses. Significance was set at $P \leq 0.05$. Statistical analysis was conducted using SPSS software (Version 23.0, IBM, Chicago, IL).

Results

Baseline measurements

Table 1 summarizes the participants' characteristics. Young and old adults walked significantly more steps·day⁻¹ than very old adults (age effect, $P < 0.001$), with women being more active than men (sex effect, $P = 0.013$). Very old adults performed shorter distance at the 6MWT than old adults (age effect, $P < 0.001$) with women performing shorter distance than men (sex effect, $P < 0.001$).

Table 1. Characteristics of the participants

	YM	YW	OM	OW	VOM	VOW
Age (years)	24±3	23±3	68±4***	67±5***	82±1***,\$\$\$	82±1***,\$\$\$
Weight (kg)	76.7±12.2	58.5±10.0###	79.5±10.5	52.4±3.7###	72.6±7.5	64.5±8.1###,\$\$
Height (cm)	179.7±6.7	163.6±6.1###	176.9±5.7	161.9±4.9###	168.6±5.4***,\$\$	160.8±6.5##
Steps.day⁻¹	11,245±2,364	12,083±2,750	11,890±4,746	15,458±2,851	8,957±2,168	9,687±2,120
6MWT (m)			683.4±59.6	600.6±68.7###	430.7±52.3\$\$\$	389.7±57.1###,\$\$\$

YM: young men; YW: young women; OM: old men; OW: old women; VOM: very old men; VOW: very old women; 6MWT: 6-minutes Walk Test. # = significantly different from men of the same age group. ## = $P < 0.01$; ### = $P < 0.001$. * = significantly different from the young group of the same sex. *** = $P < 0.001$. \$ = significantly different from the old group of the same sex. \$\$ = $P < 0.01$; \$\$\$ = $P < 0.001$.

Men were stronger than women for all age groups. MVIC decreased with age (sex \times age interaction: $P < 0.001$; Table 2). When normalized to body weight, MVIC showed no sex \times age interaction ($P = 0.545$), but decreased with age (age effect, $P < 0.001$), being greater in men than women (sex effect, $P = 0.006$; Table 2). Absolute Db₁₀₀ values at baseline showed significant sex \times age interaction ($P = 0.001$). For men, Db₁₀₀ significantly decreased with age (all $P < 0.001$; Table 2). For women, significant differences were found between YW and VOW, and between OW and VOW (all $P < 0.001$). No difference was observed between YW and OW ($P = 0.407$; Table 2). Db₁₀₀ was different between sexes only in the young group ($P < 0.001$; Table 2). VA did not show main effects of age or sex ($P = 0.278$ and $P = 0.098$, respectively), nor age \times sex interaction ($P = 0.567$; Table 2).

Table 2. Neuromuscular characteristics of participants at baseline.

	YM	YW	OM	OW	VOM	VOW
MVIC (N)	690±94	468±91 ^{###}	532±141 ^{***}	343±43 ^{###,**}	356±79 ^{***, \$\$\$}	246±40 ^{###,***,\$}
MVIC/BW (N.kg⁻¹)	9.1±1.2	8.1±1.5	6.9±2.2	6.6±0.9	5.0±1.2	3.9±0.6
Db₁₀₀ (N)	233±45	168±42 ^{###}	146±93 ^{***}	140±25	43±25 ^{***, \$\$\$}	39±45 ^{***, \$\$\$}
VA (%)	93±6	96±3	90±11	95±5	90±15	89±10

YM: young men; YW: young women; OM: old men; OW: old women; VOM: very old men; VOW: very old women; MVIC: maximal voluntary isometric contraction force; BW: body weight; Db₁₀₀: amplitude of the doublet evoked at rest. VA: voluntary activation. [#]= significantly different from men of the same age group. ^{##}= $P<0.01$; ^{###}= $P<0.001$. ^{*}= significantly different from the young group of the same sex. ^{**}= $P<0.01$; ^{***}= $P<0.001$. ^{\$}= significantly different from the old group of the same sex. ^{\$}= $P<0.05$; ^{\$\$\$}= $P<0.001$.

Performance fatigability

Among all the participants, 2 VOM and 4 VOW did not perform the QIF test due to knee pain. Thus, performance data were analyzed for 13 VOM and 11 VOW. Number of contractions was similar across sexes ($P = 0.733$) but differed with age ($P = 0.008$). Post-hoc analysis indicated that older adults performed more contractions than young ($P = 0.046$) and very old adults ($P = 0.007$), without differences between young and very old adults ($P = 1.000$, Figure 1A). No significant age \times sex interaction was found for number of contractions ($P = 0.218$). Total work showed significant main effects of age ($P < 0.001$) and sex ($P < 0.001$), but no age \times sex interaction ($P = 0.949$). Post-hoc analyses showed greater total work for young and old adults compared to very old adults, without differences between young and old adults, and greater total work for men compared to women (Figure 1B).

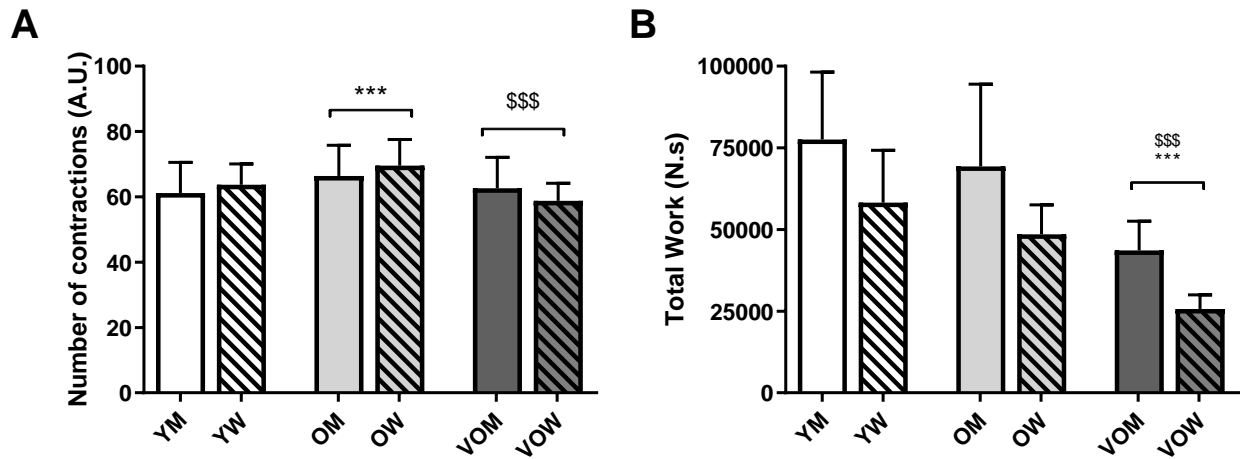


Figure 1. Total number of contractions (panel A) and total work performed at the quadriceps intermittent fatigue test (panel B) for young, old and very old men and women. Total work was greater for men than women in all age groups (all $P < 0.05$). Squared parentheses indicate significant main effect of age. YM: young men; YW: young women; OM: old men; OW: old women; VOM: very old men; VOW: very old women. ***= significantly different from the young group ($P < 0.001$). \$\$\$= significantly different from the old group ($P < 0.001$).

No main effect of sex nor sex \times stage, sex \times age or sex \times stage \times age interactions were found for the changes in MVIC, Db₁₀₀ and VA during and after the QIF test (all $P > 0.05$). Thus, performance fatigability data for men and women were pooled together.

A significant stage \times age interaction was observed for the decrease in MVIC relative to baseline ($P < 0.001$; Figure 2A). The decrease in MVIC was greater for very old adults than young adults at stages 1, 2 and 3. At exhaustion, MVIC loss was greater for young ($-28 \pm 9\%$) compared to old adults ($-19 \pm 8\%$), but not very old adults ($-23 \pm 8\%$; Figure 2A). Db₁₀₀ showed a significant effect of stage ($P < 0.001$; Figure 2B), indicating that the response to FNMS decreased throughout the test without age-related differences. Significant age \times stage interaction was found for VA ($P = 0.027$; Figure 2C), with a lower voluntary activation at stage 1 for very old adults compared to baseline and young adults.

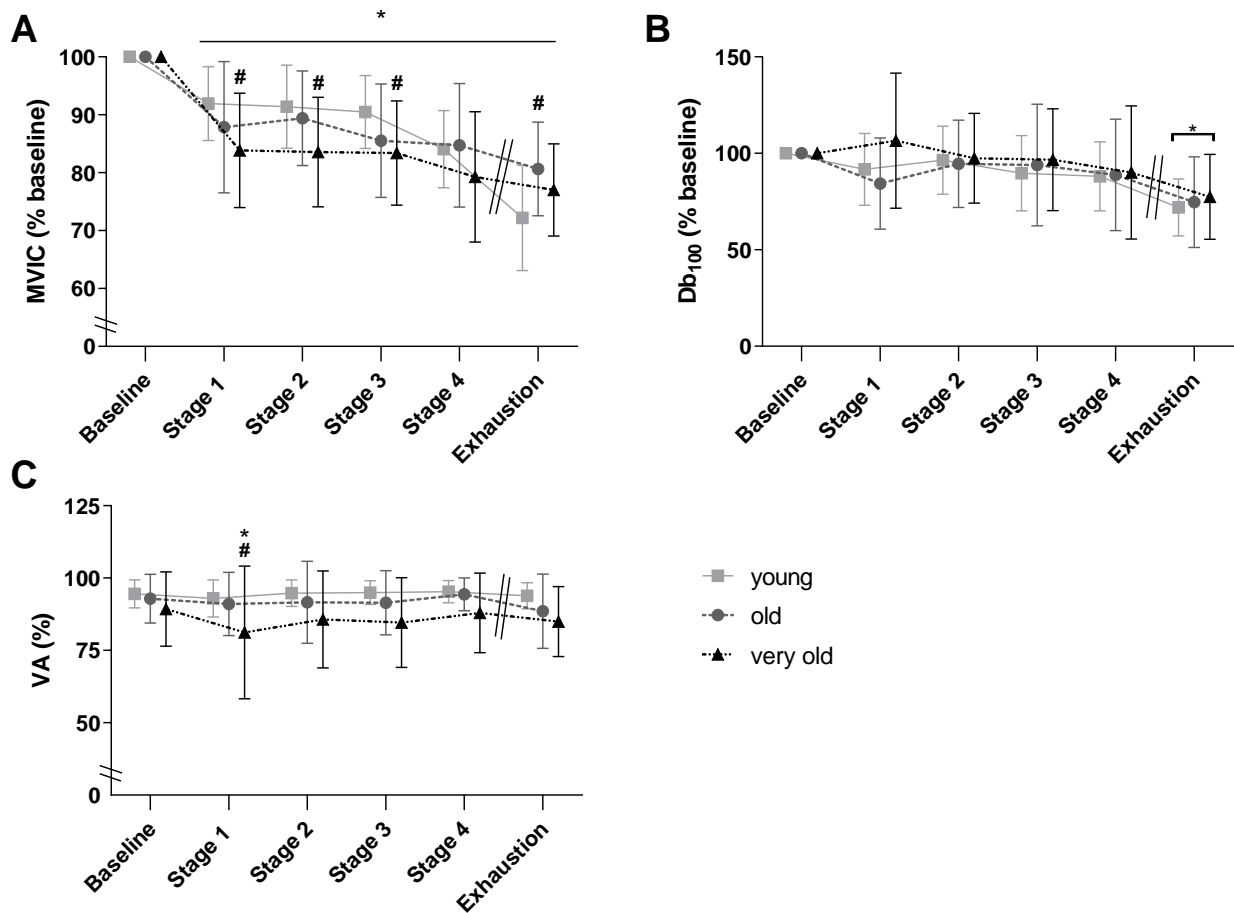


Figure 2. Maximal isometric force (MVIC, Panel A), doublet amplitude (Db_{100} , Panel B) and voluntary activation (VA, Panel C) during the QIF test for young, old and very old adults. Because no effect of sex was observed, men and women were pooled together. Squared parenthesis in panel B indicates significant effect of stage. *= significantly different from pre ($P < 0.05$). # = significantly different from young adults ($P < 0.05$).

Discussion

The present study investigated the age-related differences in performance and performance fatigability using a standardized isometric fatiguing test in men and women. Greater relative performance (number of contractions) was observed for old adults compared to young and very old adults, independent of sex. Similar total work was observed for young and old adults, with very old adults showing the lowest total work values. After the QIF test, force loss was greater for young

compared to old adults without differences with very old adults, independent of sex. Impairments in contractile function were similar across ages, with no changes in voluntary activation.

Neuromuscular characteristics at baseline

Maximal force production capacity (absolute and relative to body weight) decreased with age for both sexes. This could be explained by a greater fat mass accumulation with advancing age, lowering the force-body weight ratio, or by a decrease in muscle quality (strength per unit of muscle volume). Other than age, a decreased muscle quality could be induced by the lower physical activity of very old adults compared to the other age groups. Indeed, physical activity has a positive effect on the maintenance of muscle quality with ageing (Barbat-Artigas et al. 2014), and appears particularly important in maintaining muscle function and autonomy in very old adults (Varesco et al. 2021a). Furthermore, the force evoked at rest by FNMS decreased with age, in agreement with previous studies evaluating the KE muscles (Senefeld et al. 2013; Mcphee et al. 2014; Dalton et al. 2015; Solianik et al. 2017), evidencing a loss of muscle contractile tissue with age. VA at baseline was similar between age groups. With age, a moderate alteration in VA was previously observed, but results are heterogeneous between studies (Rozand et al. 2020). The absence of age-related differences in VA in the present study might be due to the relatively high physical activity level of the participants (Tudor-locke et al. 2011), which has been shown to preserve the capacity to activate the working muscles (Rozand et al. 2020; Varesco et al. 2021b). It is possible, however, that the effectiveness of FNMS was limited by the fat thickness of older and very old adults (Tomazin et al. 2011), limiting the interpretation of those results despite our precautions.

Performance and performance fatigability

Old adults performed more contractions compared to the other two groups. However, because young adults were stronger than old adults, total work performed (absolute performance) was similar between those two groups. MVIC loss at exhaustion was greater for young than for old adults, but

not very old adults. These results indicate greater fatigue resistance for the old adults (similar absolute performance with reduced force loss), but not very old adults (similar force loss, but lower absolute performance) compared to young adults. In older adults, the preferential loss in fast-twitch motor units is associated with an increase in slow-twitch motor units proportion and size with age, resulting in a relatively higher reliance on the oxidative metabolism (Lanza et al. 2005; Hunter et al. 2005; Krüger et al. 2018; McNeil and Rice 2018). This would lead to a greater fatigue resistance for old than young adults during isometric tasks at relative workloads (Hunter et al. 2005; Solianik et al. 2017; Krüger et al. 2018). Similar results were previously found on middle-age adults compared to young adults using a similar QIF test (Bachasson et al. 2016).

A novel finding of the present study is the loss of greater fatigue resistance in very old adults compared to young adults during isometric tasks of the KE. This agrees with previous evidences on the elbow flexors during an isometric intermittent time to exhaustion task at 60% MVIC (Allman and Rice 2001). These authors did not observed any differences in time to exhaustion or performance fatigability etiology between young and very old adults, despite the greater absolute workload in young adults (Allman and Rice 2001). In very old adults, muscle atrophy and loss of both slow- and fast-twitch motor units would decrease neuromuscular function compared to old adults (Purves-Smith et al. 2014; Hunter 2018). We reported greater force loss at low workloads (*i.e.* 10%, 20% and 30% of MVIC) for very old compared to young adults. At exhaustion, MVIC percentage loss of very old adults was between that of young and old adults, without significant difference from the latter two groups, as already reported for concentric tasks (Sundberg et al. 2018b).

No sex-related differences in performance fatigability were observed for the three age groups. Similar results were reported during intermittent isometric task of the plantar dorsiflexors (Kent-Braun et al. 2002) and concentric task of the KE (Sundberg et al. 2018b). Conversely, women showed lower performance fatigability compared to men after a sustained 120-s KE MVIC independent of age (Solianik et al. 2017), probably because they performed lower total work (Solianik et al. 2017; Hureau

et al. 2021). Taken together with the literature, the present results indicate that sex-related differences in performance fatigability are task-specific in young and older adults.

The significant drop in Db_{100} for the three age groups indicated impairments in contractile function throughout the QIF test, without age- or sex-related differences. Different results have been observed in studies comparing young adults with old (Krüger et al. 2018) and very old (Allman and Rice 2001; Sundberg et al. 2018b) adults, probably because of the different fatiguing protocols adopted (Thomas et al. 2018). In isometric mode, blood flow occlusion induced by sustained contractions impedes oxygenated blood to reach the capillaries (Moritani et al. 1992). This facilitates impairments in contractile function (Copithorne and Rice 2020), attributed to an alteration in excitation-contraction coupling linked with high ATP turnover, free phosphate and H^+ accumulation (Sundberg and Fitts 2019). Metabolites accumulation in the working fibers and the recruitment of fast-twitch motor units, composed by type II fibers [less oxygen dependent (Moritani et al. 1992)], could impair performance particularly in very old adults (Sundberg et al. 2019). Regarding central impairments, high levels of physical activity of our participants might prevented an observable alteration in VA, as previously reported comparing trained and untrained individuals (Bachasson et al. 2016). Nevertheless, differences in fatigability in absence of impairments in VA have already been observed in studies investigating sex- (Hunter et al. 2006) and age-related differences (Sundberg et al. 2018b).

Conclusions

The present study showed that the greater fatigue resistance of old adults during an isometric intermittent fatiguing task of the KE was not maintained at very old age compared to young adults. Performance fatigability at exhaustion was likely due to impairments in contractile function for the three age groups. Finally, performance fatigability and its etiology were similar between men and women independent of age.

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Summary of the study 2

In study 2 we observed similar total work (absolute performance) between young and old adults, with very old adults performing less total work. Young adults were stronger than the other two age groups, so for each contraction they performed more work. Older adults showed reduced performance fatigability at exhaustion and performed more contractions than young adults, which resulted in a similar absolute performance between those two age groups. Very old adults performed similar number of contraction than young adults but possessed lower strength, which resulted in lower total work compared to the other two age groups. Thus, this greater “fatigue resistance” observed in old adults was not maintained at very old age. This may be due to the important deteriorations at the skeletal muscle level, as very old adults presented similar impairments in contractile function than the others age groups despite lower total work produced, while no impairments in voluntary activation were observed. We did not find sex-related differences in performance or performance fatigability with age.

As we showed in study 1, activities such as walking could impact neuromuscular function with aging. Walking requires the dynamic contractions of multiple muscular groups to sustain a load relative to the individuals’ body weight. Thus, the results observed in study 2 might not be directly transferable to the activities of daily living. Indeed, when concentric tasks were used, performance fatigability has been shown to decrease with advancing age [e.g. (Sundberg et al. 2018b)]. When comparing young and old adults during a cycling task performed at similar total work, young adults presented lower performance fatigability, impairment in contractile function and no alterations in voluntary activation compared to the old adults (Weavil et al. 2018).

In study 3, we aimed to investigate performance and performance fatigability across age during isometric, concentric and cycling fatiguing tasks. To compare age groups in a more ecologically valid condition than in study 2, we set the workload as percentage of body weight.

STUDY 3

Effects of old and very old age on performance and fatigability during isometric, concentric and cycling fatiguing tasks in men

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To submit

This version of the paper has not been reviewed by all co-authors

Résumé:

L'objectif de cette étude était d'étudier la performance et la fatigabilité lors de tâches de fatigue isométrique, concentrique et de cyclisme chez des hommes jeunes, âgés et très âgés. Quinze hommes jeunes, 13 hommes âgés et 12 hommes très âgés ont effectué de manière aléatoire trois tâches de fatigue : un test incrémental de fatigue isométrique (ISO) et concentrique (CON) sur un dynamomètre isocinétique et un test similaire sur un cyclo-ergomètre (BIKE). L'ISO et le CON consistaient en des paliers incrémentaux de 75 contractions (0.8 s on/0.8 s off), avec une durée et un cycle d'utilisation similaires pour le BIKE (120 s à 37.5 rpm). Les augmentations étaient spécifiques à chaque appareil et définies en pourcentage du poids de corps. La stimulation électrique nerveuse percutanée a été utilisée pour évaluer l'activation volontaire (VA) et l'amplitude de la secousse musculaire au repos (Tw). La performance (nombre de paliers) était plus importante chez les jeunes adultes que chez les personnes âgées et très âgées pour les trois sessions, et plus importante chez les personnes âgées que très âgées pour le BIKE uniquement ($P < 0.001$). La diminution de la force maximale (fatigabilité) était plus importante chez les jeunes adultes que chez les personnes très âgées après ISO ($P < 0.001$), mais similaire dans tous les groupes d'âge après CON et BIKE. L'amplitude du Tw a diminué dans les trois sessions indépendamment de l'âge ($P < 0.001$). La VA a diminué uniquement chez les jeunes adultes après BIKE ($P < 0.05$). Ces résultats montrent que la performance diminue avec l'âge indépendamment de la tâche lorsque la charge est fixée par rapport au poids du corps individuel. La fatigabilité était plus importante chez les jeunes adultes que chez les personnes très âgées pendant la tâche isométrique, mais similaire entre les groupes d'âge pendant les tâches dynamiques. La fatigabilité était principalement due à des altérations de la fonction contractile.

Abstract (250 words)

The aim of the present study was to investigate performance and fatigability during isometric, concentric and cycling fatiguing tasks in young, old and very old men. Fifteen young, 13 old and 12 very old males randomly performed three fatiguing tasks: an isometric (ISO) and a concentric (CON) incremental fatigue tests on an isokinetic dynamometer and a similar test on a validated cycle ergometer (BIKE). ISO and CON consisted of stages of 75 contractions (0.8-s on/0.8-s off), and similar duration and duty cycle for BIKE (120-s at 37.5 rpm). Increments were device-specific and set as percentage of body weight. Percutaneous electrical nerve stimulation was used to evaluate voluntary activation (VA; i.e. interpolated twitch technique) and resting twitch amplitude (Tw) . Performance (number of stages) was greater for young than old and very old adults for the three sessions, and greater for old than very old adults for BIKE only ($P<0.001$). The decrease in maximal force (i.e. performance fatigability) was greater for young than very old adults after ISO ($P<0.001$), but similar across age-groups after CON and BIKE. Tw amplitude decreased in the three sessions independent of age ($P<0.001$). VA decreased only for young adults in BIKE ($P<0.05$). These results showed that performance decreased with advancing age independent of the task when the load is set relative to the individual body weight. Performance fatigability was greater for young than very old adults during the isometric task, but similar between age groups during the dynamic tasks. Performance fatigability was mainly driven by impairments in contractile function.

Introduction

The inherent deterioration in muscle structure and motor command with advancing age results in a progressive loss of strength, power and physical function, which are associated with reduced autonomy and quality of life (Van Roie et al. 2011; Buckinx and Aubertin-Leheudre 2019). These age-related alterations are exacerbated at very old age (i.e. >80 years old), increasing the risk of frailty, hospitalization and ultimately death (Newman et al. 2006). While it is critical to study age-related alterations in force production, further considering the ability to repeatedly perform muscle contractions as required during daily activities is crucial. These repeated actions are known to induce performance fatigability, i.e. decline in maximal strength or power.

The age-related alterations in strength and performance fatigability depend on the task characteristics, such as muscle groups involved or mode of contraction (Hunter et al. 2016; Hunter 2018; Krüger et al. 2018). It has been shown that the deterioration in muscle function would particularly affect the large muscle groups of the lower limbs, such as the knee extensors (Janssen et al. 2000; Hughes et al. 2001a; Ditroilo et al. 2010), whose function impacts locomotion and other activities of daily living (Hurley et al. 1998). In the literature, the workloads of the fatiguing tasks are usually set relatively to maximal force to account for differences in muscle strength between populations. After isometric tasks of the knee extensors, healthy old adults (60-80 years old) showed lower performance fatigability than young adults [e.g. (Callahan et al. 2009; Solianik et al. 2017; Varesco et al. 2021c)]. However, this lower performance fatigability during isometric tasks is not preserved in very old adults (Varesco et al. 2021c). Conversely, following a maximal-velocity concentric task, maximal power and force losses were larger for old compared to young adults, and even more pronounced in very old adults (Sundberg et al. 2018b).

These results suggest that the age-related differences in knee extensors performance fatigability vary across tasks. However, some of the results reported by previous studies may be explained by the lower total work (absolute performance) performed by the older adults. When young and old adults

performed a similar total work during single-limb concentric and cycling tasks, Weavil et al. (2018) showed lower performance fatigability in young compared to old adults. Greater alterations in performance might occur in very old adults, especially after cycling tasks that stress the cardiovascular system to a greater extent compared to single-limb tasks [e.g. (Weavil et al. 2018)]. Thus, results obtained during cycling exercises could be more transferable to the locomotor activities of daily living. Moreover, using more ecologically valid workloads would provide further insights into the changes in neuromuscular performance with age, as already suggested when studying sex-related differences (Hureau et al. 2021). For the lower limbs, the intensity of exercise can be set relatively to body weight, as recently done to study cancer-related fatigue (Brownstein et al. 2020). Indeed, most of the activities of daily living, such as walking, require the ability to carry its own body weight rather than a percentage of maximal capacity.

Finally, neuromuscular alterations associated to performance fatigability with aging vary across studies, but have been mainly attributed to impairments in contractile function, with minimal reduction in voluntary activation (Krüger et al. 2018). The extend of such impairments depends primarily on the contraction mode adopted and the intensity of exercise (Sundberg et al. 2018b; Weavil et al. 2018; Krüger et al. 2020; Varesco et al. 2021c), and could vary during the fatiguing tasks (Varesco et al. 2021c). For example, our research group found greater performance fatigability along an isometric task for very old compared to young adults, but no difference at exhaustion (Varesco et al. 2021c). Further studies evaluating performance fatigability kinetic along fatiguing tasks with different contraction mode (e.g. isometric, concentric and cycling tasks) would help to understand age-related differences in neuromuscular function.

Thus, the aim of the present study was to investigate performance and performance fatigability in young, old and very old men during isometric, isokinetic concentric, and cycling fatiguing tasks with workloads set as percentage of body weight. We hypothesized lower performance with advancing age independent of the task. We also hypothesized lower fatigability in old but not very old adults

after the isometric fatiguing task only, and greater fatigability with advancing age after the concentric and cycling fatiguing tasks.

Methods

Participants

Fifteen young, 13 old and 12 very old men participated in the study and were clearly informed on the experimental procedures prior giving their written consent. Participants were free from psychological, musculoskeletal and neurological disorders or disability. Participants were asked to avoid exercise and alcohol consumption for 24h before each visit. Very old adults were needed to score more than 20 at the mini-mental state examination [mean score = 28 ± 3 ; (Jacqmin-Gadda et al. 1997)]. All the procedures were approved by the Comité de Protection des Personnes and were performed in accordance with the declaration of Helsinki (ClinicalTrials.gov, Identifier: NCT04516538). The university hospital of Saint-Etienne was the sponsor of the present study.

Experimental protocol

Participants visited the laboratory on four occasions separated by 7 days. During the first visit, all participants were familiarized with the procedures used to assess neuromuscular function and fatigability, and an accelerometer (wGT3X-BT; ActiGraph, Pensacola, USA) was given to wear for 7 consecutive days to objectively assess physical activity (Varesco et al. 2021b). The old and very old adults also performed a 6-minutes-walk-test (6MWT), which consisted in covering the longest distance possible in 6 minutes on a corridor of 30 meters (Crapo et al. 2002). Following the 6MWT, old and very old men performed a Timed-Up-and-Go (TUG) test, in which participants were required to stand up from a chair, walk a distance of three meters, turn around, return, and sit down on the chair (Mathias et al. 1986). The stopwatch was started when the back of the participants moved from the chair and stopped when the participants were seated on with their back on the back of the chair.

During the successive experimental sessions, participants performed intermittent incremental tests either in isometric (ISO), concentric (CON) or cycling (BIKE) mode in a randomized order. ISO and CON derived from the so-called quadriceps intermittent fatigue (QIF) test initially developed in isometric (Bachasson et al. 2013) and concentric (Varesco et al. 2021d) contraction modes, respectively. In the present study, these tests were adapted to standardize the duty-cycle and duration of the stages across the three sessions.

The first part of the session was similar across visit 2, 3 and 4. First, the stimulation intensity for peripheral nerve electrical stimulation was identified. Successively, participants performed a standardized isometric warm-up that consisted of three contractions at 30%, 50% and 70% of the perceived maximal force. Then, participants performed two maximal voluntary contractions in both isometric (MVIC) and concentric (MVCC) modes [range of motion: 60° (90°-120°, knee full extension = 120°), velocity: 120°.s⁻¹]. MVCC could not be performed on the cycle-ergometer. An additional trial was performed if the difference between the peak force values was >5%. For MVIC, participants were asked to push “as hard as possible”, while for the MVCC they were instructed to push “as hard and fast as possible”.

After 2 min of recovery, two neuromuscular evaluations (NME) were performed, separated by 1 min of rest. NME was designed to evaluate performance fatigability and its etiology using evoked twitch at rest to assess impairments in contractile function and the interpolated twitch technique to calculate voluntary activation (Merton 1954). NME consisted of a 5-s MVIC during which a single electrical stimulation was superimposed (superimposed twitch, SIT). Then, another stimulation was delivered at rest (twitch, Tw) ~2 s after the MVIC. Visual feedback of the force (or torque) signal was displayed on a large screen positioned in front of the participants and strong verbal encouragement was provided. The fatiguing tasks (QIF tests) started at least 1 min after the second NME. A schematic representation of the experimental procedure is presented on Figure 1.

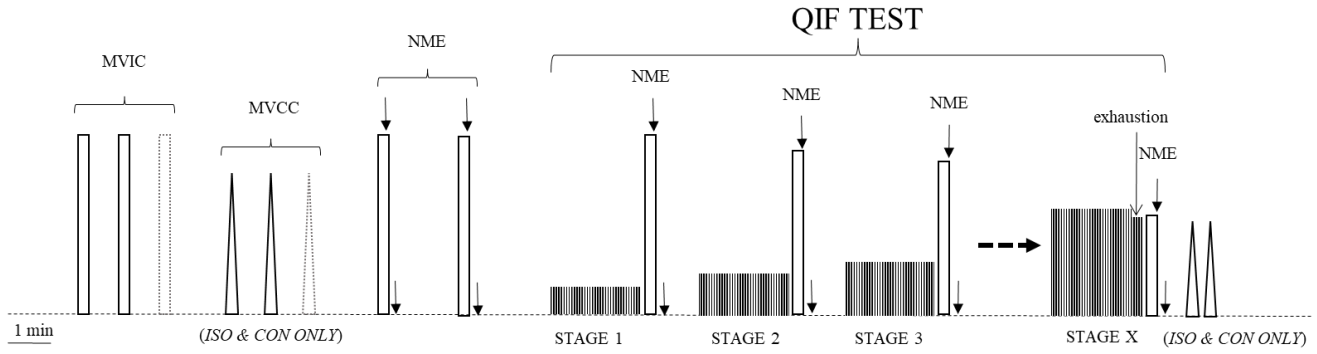


Figure 1. Experimental procedures for the three body-weight based quadriceps intermittent fatigue (QIF) tests. Filled arrows indicate single twitch electrical stimulation. Dotted contractions were performed only if the difference between peak force values of the previous contractions was >5%. ISO = isometric body-weight based QIF test; CON = concentric body-weight based QIF test. MVIC = maximal voluntary isometric contraction. MVCC = maximal voluntary concentric contraction. NME = neuromuscular evaluation

QIF tests

Both ISO and CON were performed on an isokinetic ergometer and consisted of 75-contraction stages (0.8 s on/0.8 s off; stage duration = 120 s) at increasing workloads until exhaustion. The workload of the first stage corresponded to 10% of the body weight and was calculated with the following formula:

$$\text{workload first stage (Nm)} = \frac{[\text{body weight (Kg)} \times 9,80665] \times \text{lever arm (m)}}{10}$$

Starting from the second stage, the increments were set at 5% of body weight. The target load was displayed on a large screen using a 2-Nm thick line (1Nm above – 1Nm below). For ISO, participants listened to a soundtrack with the contraction-relaxation rhythm. For CON, participants were instructed to start the contraction immediately after the machine passively returned the leg to the starting position (90° knee flexion). Stages were separated by 1 min. Immediately (~2 s) at the end of each stage and at task failure, NME was performed and the rate of perceived exertion (RPE) was scored on a 6-20 Borg scale (Borg 1982). Verbal encouragement were provided. Task failure was

defined as 5 consecutive contractions below the target line. After the last NME at task failure, 2 MVCC were performed.

BIKE was performed on a custom-made cycle-ergometer allowing to perform NME on the bike (Doyle-Baker et al. 2017) and consisted of 120-s stages at increasing workloads at a cadence of 37.5 rpm (75 pedal stroke) to match the duty-cycle with ISO and CON. Due to different configurations of the experimental setting, the workload was calculated differently than ISO and CON. After pilot studies, to obtain similar duration for BIKE compared to ISO and CON for very old adults, the workload was set at 0.6 W.kg^{-1} for the first stage and 0.3 W.kg^{-1} for the successive increments. Participants were provided visual feedback of the cadence and verbal encouragement. Immediately ($\sim 2 \text{ s}$) after each 120-s stage and at task failure, the pedals were blocked and participants performed NME, and RPE was scored. Task failure was defined when the cadence was below 37.5 rpm for 8 s, to match what was defined for ISO and CON.

Experimental setting

For both ISO and CON, participants were seated upright on an isokinetic dynamometer (Con-Trex MJ; CMV AG, Dubendorf, Switzerland) and were tested on the right leg. Hip and knee angles were set at 95° and 90° , respectively ($180^\circ = \text{full extension}$). Extraneous movements of the upper body were minimized using belts strapped across the thorax and waist. During the first visit, the position of each participant and the alignment between the dynamometer and the right knee joint rotational axis were registered and reproduced for the next visits. For BIKE, participants seated on a validated custom-made cycle-ergometer that allowed to lock the force-measuring pedals and reproduce the same position as for the isokinetic dynamometer [for further details, see (Doyle-Baker et al. 2017)]. Briefly, when the pedals were locked, the right knee and ankle angles were set at 90° , and hip angle was approximately 100° ($180^\circ = \text{full extension}$). Force was measured using instrumented pedals (Model PF1.0.0, Radlabor GmbH, Freiburg, Germany) that were located between the pedal and crank. Torque (ISO and CON) and force (BIKE) were sampled without analogical or digital filters (16/30-

ML880/P, ADInstruments, Bella Vista, Australia). Electromyographic (EMG) activity was recorded from the *vastus lateralis* (VL), *rectus femoris* (RF) and *vastus medialis* (VM) muscles of the right leg. Pairs of self-adhesive Ag/AgCl surface electrodes (recording diameter=10 mm, Kendall MediTrace 130 foam electrode, MA) were placed in bipolar configuration over the muscle belly following the SENIAM guidelines. The interelectrode (center to center) distance was 30 mm. A reference electrode was placed on the right patella. Finally, two electrodes were placed over the right clavicle and the tenth left rib, respectively, to measure heart rate (HR). The skin was shaved, abraded, and cleaned with alcohol swab before electrode placement. EMG signals were amplified (gain = 500) using an octal bio-amplifier (ML138, AD Instruments) with a bandwidth frequency range of 10–500 Hz and digitized by a Power-Lab System (16/30-ML880/P, AD Instruments). Torque (or force) and EMG signals were recorded simultaneously at 2 kHz on a computer using Labchart v8 (AD Instruments). Reliability and agreement for the specific ergometers and neuromuscular procedures adopted in the present study are satisfactory and can be found elsewhere [see (Maffiuletti et al. 2007; Bachasson et al. 2013; Doyle-Baker et al. 2017; Varesco et al. 2021d)].

Peripheral Nerve Electrical Stimulation

Square-wave stimulus of 2-ms duration (400 V) were delivered over the femoral nerve through a constant current stimulator (DS7R, Digitimer, Welwyn Garden City, UK). The cathode electrode (Kendall MediTrace) was placed on the inguinal triangle and the anode (50 × 90 mm Durastick Plus electrode; DJO Global, Vista, CA) was placed midway between the great trochanter and the lower border of the iliac crest (Piponnier et al. 2019). The stimulation intensity was progressively increased by 10 mA until torque or force and compound muscle action potential recorded through EMG (M-wave) reached a plateau. Stimulation intensity at the plateau was then increased by 30% to ensure supramaximality. Mean stimulation intensity was similar across sessions and groups and on average was 21.1 ± 6.5 mA.

Data analysis

All data were analyzed offline using Labchart software (v8, ADInstruments). Performance was quantified as total number of stages performed until exhaustion. In case of incomplete stage at exhaustion, the proportion of the last stage was also considered (e.g. 7.5 stages). MVIC was defined as the highest 0.5-s window of the contraction. MVIC prior the QIF tests was also normalized to body weight. MVCC was defined as the maximal value recorded for each MVCC. Performance fatigability was defined as the percentage of reduction in MVIC and MVCC (when applicable). Voluntary activation (VA) was calculated using the following equation:

$$VA(\%) = (1 - \frac{SIT}{Tw}) \times 100$$

where SIT is the amplitude of the superimposed twitch evoked by peripheral nerve stimulation during MVIC, and Tw is the amplitude of the twitch evoked at rest. Changes in Tw were used to identify impairments in muscle contractile function. To evaluate neuromuscular propagation, changes in M-wave amplitude (M_{max}) from baseline for the VL, RF and VM were analyzed from the EMG response to Tw.

Statistical Analysis

All variables are reported as mean \pm SD in the text, tables and figures. Normality of data was checked using the Shapiro-Wilk test. Because measures from the cycle-ergometer showed a systematic ~30% difference compared to classic dynamometers (Doyle-Baker et al. 2017), data at baseline were analyzed separately for each session. Unpaired *t*-test were used to analyze differences between old and very old adults in the performance at the 6MWT and TUG test. One-way ANOVAs were used to analyze age-related differences in participants' characteristics (age, weight, height, physical activity, workload increments as %MVIC and %MVCC), neuromuscular data at baseline (absolute and normalized MVIC, MVCC, Tw, VA), %MVCC loss and performance. HR at exhaustion was analyzed using a mixed-effect ANOVA with session as within-group factor and age as between-

subject factor. Generalized Estimating Equations (GEE) analyses using an autoregressive (AR-1) structure were performed on data expressed relative to baseline to investigate the effect of age on the evolution of MVIC, Tw, VA and RPE for each session. Because participants performed different numbers of stages, data were compared until the last stage performed by all participants [i.e. 3rd stage (ISO and BIKE) or 2nd stage (CON)] and then at exhaustion (Varesco et al. 2021a). For all ANOVAs and GEEs, when significant effects were observed, Bonferroni correction was applied to post-hoc analyses. Significance was set at $P \leq 0.05$. Statistical analysis was conducted using IBM SPSS software (Version 23.0, SPSS Inc., Chicago, IL).

Results

Table 1 summarizes the participants' characteristics. Physical activity was similar across the three age groups ($P = 0.209$). Old adults performed better at the 6MWT ($P < 0.001$) and the TUG test ($P = 0.001$) than very old adults.

Table 1. Characteristics of the participants

	Young	Old	Very old
Age (years)	24±3	70±4 ^{###}	86±1 ^{###, \$\$\$}
Weight (kg)	72.1±8.8	79.2±9.5	71.0±5.0
Height (cm)	178.0±6.9	173.8±5.4	164.7±3.7 ^{###, \$\$}
Steps.day⁻¹	10,477±2,818	11,647±2,399	9,366±3,127
6MWT (m)		669.7±97.9	452.1±92.2 ^{\$\$\$}
TUG test (s)		4.1±0.7	6.3±1.9 ^{\$\$}

6MWT: 6-minutes Walk Test. TUG test: Timed Up and Go test. [#]= significantly different from young adults. ^{##}= $P < 0.01$; ^{###}= $P < 0.001$. ^{\$}= significantly different from the old group. ^{\$\$}= $P < 0.01$; ^{\$\$\$}= $P < 0.001$.

Three old adults and one very old adult dropped the study due to pain during the study. Thus, eleven old adults completed ISO, 10 completed CON and 13 completed BIKE. Thirteen very old adults completed ISO and BIKE, and twelve completed CON.

Neuromuscular properties at baseline are presented in Table 2. MVIC, MVCC and Tw decreased with age, while no differences in voluntary activation were observed (ISO: $P = 0.113$; CON: $P = 0.050$; BIKE: $P = 0.199$). The increments of 5% of body weight for ISO corresponded to $6 \pm 1\%$, $9 \pm 3\%$ and $12 \pm 3\%$ MVIC for young, old and very old, respectively, and was significantly different between all groups (all $P < 0.05$). The increments of 5% of body weight for CON corresponded to $15 \pm 3\%$, $25 \pm 9\%$ and $31 \pm 8\%$ MVCC for young, old and very old, respectively. Young adults had lower increments (%MVCC) compared to old adults ($P = 0.005$) and very old adults ($P < 0.001$), with no difference between old and very old adults ($P = 0.130$).

Table 2. Neuromuscular properties of the participants at baseline

	Young	Old	Very old
MVIC (N)			
ISO	627±169	461±98 ^{##}	324±72 ^{###,\$}
CON	590±124	440±74 ^{##}	306±73 ^{###,\$}
BIKE	408±89	295±46 ^{###}	212±52 ^{###,\$}
MVIC / Body weight (N/kg)			
ISO	8.6±1.5	5.9±1.6 ^{###}	4.5±0.9 ^{###,\$}
CON	8.2±1.1	5.5±1.0 ^{###}	4.3±0.9 ^{###,\$}
BIKE	5.6±0.8	3.8±0.9 ^{###}	3.0±0.6 ^{###,\$}
MVCC (N)			
ISO	483±110	333±61 ^{###}	241±49 ^{###,\$}
CON	485±119	334±66 ^{##}	235±49 ^{###,\$}
Tw (N)			
ISO	176±44	135±29 [#]	106±17 ^{###}
CON	170±49	124±26 [#]	106±17 ^{###}
BIKE	143±29	93±25 ^{###}	74±18 ^{###}
VA (%)			
ISO	95±4	97±5	92±7
CON	96±3	97±4	92±7
BIKE	96±4	97±4	93±7

MVIC: maximal voluntary isometric contraction force; MVCC: maximal voluntary concentric contraction force; Tw: resting twitch; VA: voluntary activation. [#]= significantly different from young adults. [#]= $P<0.05$; ^{##}= $P<0.01$; ^{###}= $P<0.001$. ^{\$}= significantly different from old adults. ^{\$}= $P<0.05$.

Performance and fatigability at the QIF tests

Performance, expressed as number of stages performed, showed a significant stage \times age interaction ($P < 0.001$). Number of stages was lower in old and very old adults compared to young adults for the three sessions (Figure 2A). For BIKE, a significant difference was also found between old and very old adults.

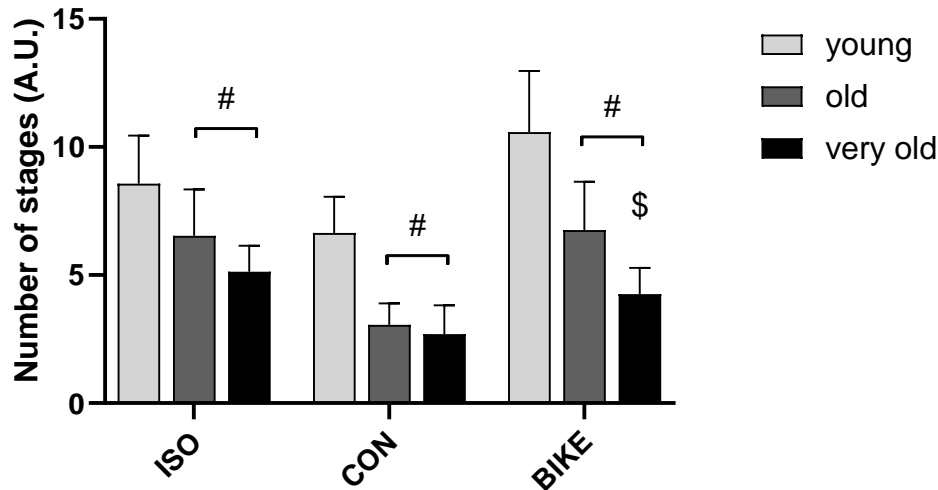


Figure 2. Number of stages performed by the young, old and very old adults at the isometric (ISO), concentric (CON) and cycling (BIKE) QIF tests (Panel A). # = significant difference from young; \$ = significant difference from old. Significance was set as $P < 0.05$.

The loss in MVIC during the QIF test showed significant stage \times age interaction for all the sessions (all $P < 0.001$). For ISO, MVIC decreased from baseline starting from stage 1 for old and very old adults, and from stage 2 for young adults (Figure 3A). At stage 3, the reduction in MVIC was significantly lower for the young compared to very old adults, while the opposite was found at exhaustion (Figure 3A). For CON, MVIC decreased in the three age groups starting from stage 1 ($P < 0.001$), without age-related differences ($P = 0.079$; Figure 3B). For BIKE, the decrease in MVIC was significant in old and very old adults starting from stage 1, while the decrease was significant only at exhaustion for the young adults (Figure 3C). The loss in MVCC for ISO was significant reduced at exhaustion ($P < 0.001$) without groups difference ($P = 0.947$), being $-24 \pm 11\%$ for young, $-23 \pm 10\%$ for old and $-22 \pm 11\%$ for very old. For CON, MVCC at exhaustion was reduced for the three age groups (young: $-23 \pm 16\%$; old: $-21 \pm 13\%$; very old: $-26 \pm 11\%$; all $P < 0.01$). No differences between groups were found at exhaustion (all $P > 0.05$).

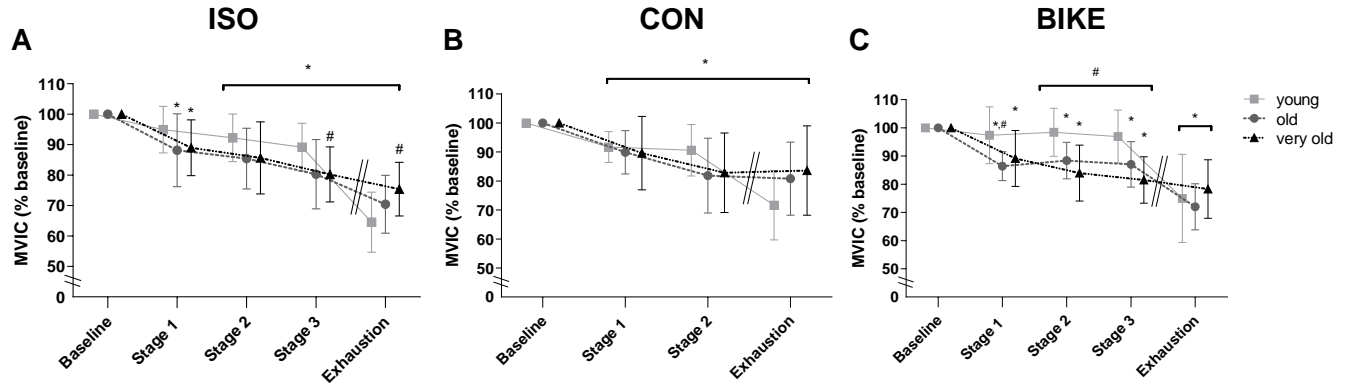


Figure 3. Maximal voluntary isometric contraction force (MVIC) of the three age groups across the isometric (ISO; panel A), concentric (CON; panel B) and cycling (BIKE; panel C) body weight based QIF tests. The last common stage was stage 3 for ISO and BIKE and stage 2 for CON. *=significant difference from baseline ($P < 0.05$). # = significant difference from the young adults ($P < 0.05$).

A significant stage \times age interaction was found for all sessions for Tw (all $P < 0.001$). For the three sessions, the reduction of Tw occurred earlier for old and very old adults, but the reduction at exhaustion was similar in the three age groups (Figure 4). For VA, a significant stage \times age interaction was found only for BIKE ($P = 0.008$), with a reduced VA at exhaustion for the young adults only. No main effects were observed for ISO and CON (all $P > 0.05$; Figure 5).

VL M_{\max} showed a significant stage \times age interaction for BIKE only ($P < 0.001$), while no significant differences across stages or age groups were found for the other sessions and for the RF and VM (all $P > 0.05$, data not presented). VL M_{\max} increased from stage 2 until exhaustion for very old adults (all $P < 0.05$). At exhaustion, VL M_{\max} was also increased for young adults ($P = 0.043$). VL M_{\max} was greater for very old compared to young adults at stages 1, 2 and 3 (all $P < 0.05$). Amplitude of the M-wave for VL, RF and VM is presented in Supplemental Materials 1.

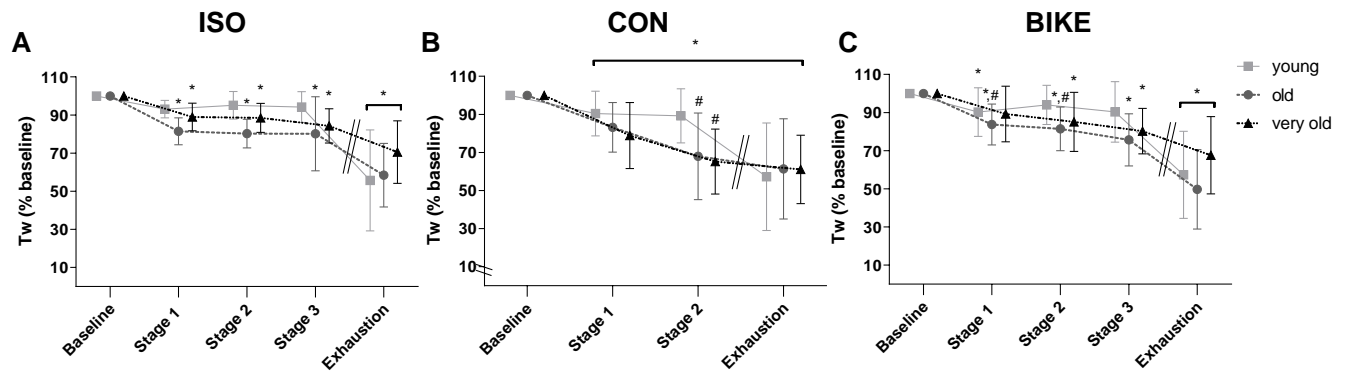


Figure 4. Twitch amplitude (Tw) of the three age groups across the isometric (ISO; panel A), concentric (CON; panel B) and cycling (BIKE; panel C) body weight based QIF test. The last common stage was stage 3 for ISO and BIKE and stage 2 for CON. *=significant difference from baseline ($P < 0.05$). # = significant difference from the young adults ($P < 0.05$).

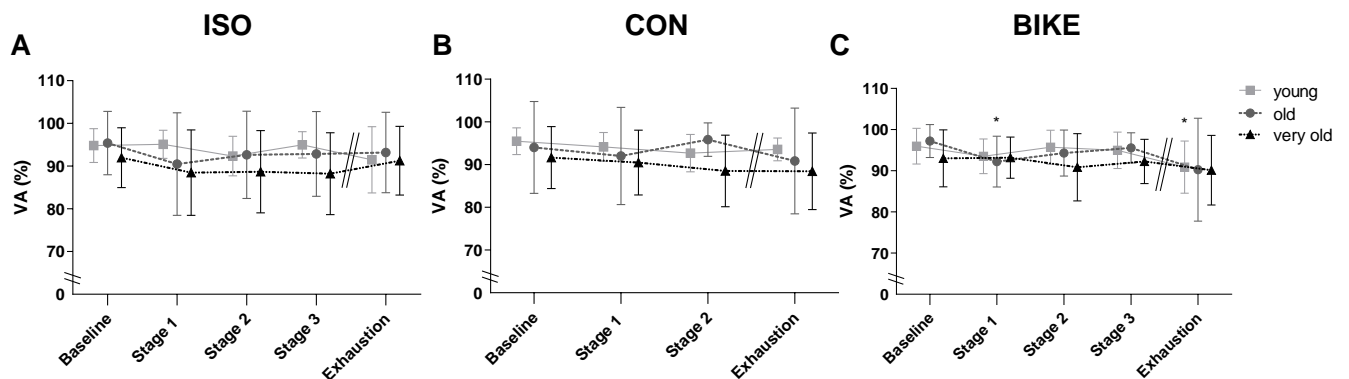


Figure 5. Voluntary activation (VA) of the three age groups across the isometric (ISO; panel A), concentric (CON; panel B) and cycling (BIKE; panel C) body weight based QIF test. The last common stage was stage 3 for ISO and BIKE and stage 2 for CON. *=significant difference from baseline ($P < 0.05$).

Perceived exertion and heart rate

For all sessions, RPE showed significant stage \times age interactions (all $P < 0.001$). For the three sessions, RPE was lower for young adults in the early stages, but higher at exhaustion compared to

old and very old adults, except for CON for which young and old adults were not significantly different at exhaustion. RPE data are presented on supplemental material 2.

For HR, significant session ($P < 0.001$) and age ($P < 0.001$) effects were found, with no significant interaction ($P = 0.493$). For all age groups, ISO and CON showed significant lower values compared to BIKE (all $P < 0.001$), without differences between ISO and CON ($P = 1.000$). For the three sessions, young adults (ISO: 132 ± 21 bpm, CON: 128 ± 16 bpm; BIKE: 159 ± 21 bpm) showed significantly higher HR at exhaustion compared to old (ISO: 105 ± 14 bpm; CON: 106 ± 7 bpm; BIKE: 139 ± 17 bpm, $P = 0.002$) and very old adults (ISO: 94 ± 15 bpm; CON: 95 ± 23 bpm; BIKE: 114 ± 17 bpm; $P < 0.001$), with higher values for old compared to very old adults ($P = 0.038$).

Discussion

The present study aimed to investigate age-related differences in performance and fatigability during isometric, isokinetic concentric, and cycling fatiguing tasks. The load was set relative to the body weight of the participants to simulate everyday life conditions. We observed an overall better performance for young adults compared to old and very old adults for the three fatiguing tasks. Old adults performed better than very old adults in the multiarticular cycling task (BIKE) only, which was accompanied by a better performance at the functional tests (i.e. 6MWT and TUG test). For BIKE, maximal force at the last common stage decreased for the old and very old adults but not young adults. Maximal force loss at exhaustion in ISO was greater for young compared to very old adults only, with similar performance fatigability across age groups for CON and BIKE, contrary to our hypothesis. The large decrease in Tw amplitude in the three sessions independent of age indicated that performance fatigability was mainly due to impairments in contractile function. Voluntary activation was impaired at exhaustion only for the young adults in BIKE.

Functional and neuromuscular characteristics at baseline

All participants could be defined as active based on their number of steps.day⁻¹ (Tudor-locke et al. 2011). Old adults performed better at the 6MWT and TUG test than very old adults. Because physical activity was relatively high and similar across age groups in the present study, we assumed that these age-related differences in physical function and neuromuscular performance were due to the effect of healthy aging rather than physical inactivity (Lazarus and Harridge 2018). Maximal isometric and concentric force decreased with age, with similar values than that observed in the literature on isokinetic dynamometers (Sundberg et al. 2018b) or cycle-ergometer (Krüger et al. 2020). Evoked responses (Tw) were reduced in old and very old adults compared to young adults. This would be mainly due to loss of muscle mass and fat infiltration within the muscle contractile tissue that impede an effective contraction, reducing the maximal force and power output in older adults (Harridge and Lazarus 2017). Despite lower values with advancing age, we did not observe significant differences in the evoked responses between old and very old adults, contrary to Sundberg et al. (2018). This discrepancy was probably due to differences in physical activity of very old adults between studies (4590 ± 3606 steps.day⁻¹ in Sundberg et al. (2018) vs. 9366 ± 3127 steps.day⁻¹ in the present study). Indeed, physical activity has been shown to potentially preserve muscle function and performance, as well as improve voluntary activation, even in very old adults (Barbat-Artigas et al. 2014; Rozand et al. 2020; Varesco et al. 2021b, a). This might also explain the absence of age-related differences in voluntary activation, as already suggested by a recent meta-analysis (Rozand et al. 2020).

Fatigability at similar stages performed

For the common stages performed at the isometric QIF test, performance fatigability occurred earlier in old and very old adults compared to young adults. These results were most likely due to the greater absolute load performed relatively to their maximal force production capacity, which corresponded to ~6%, ~9 % and ~12% of MVIC for young, old and very old adults, respectively. This was confirmed by the lower perceived exertion in young compared to older adults for all common stages, and lead to significant impairments in Tw starting from the first stages for old and very old adults but

no impairment in voluntary activation. However, even if very old adults performed at higher absolute workload compared to old adults, no differences between those two groups were found in performance fatigability or perceived exertion for all common stages. Previously, it was proposed that the slowing in muscle contractile properties with advancing age would lead to lower MU firing rates, resulting in a reduced cost of contraction for the isometric mode (Kent-Braun 2009; Tevald et al. 2010). The lower cost of contraction in very old compared to old adults might have counterbalanced the difference in absolute workload between groups, resulting in similar performance fatigability.

For CON, no age-related differences in performance fatigability were observed at the two common stages. This may be due to the reduced number of stages performed by old and very old adults limiting the development of greater level of performance fatigability (Thomas et al. 2018), and the higher variability observed in these groups. Old and very old adults showed lower Tw compared to young adults at the last common stage. The greater workload increments in old (~25% MVCC) and very old (~31% MVCC) adults compared to young adults (~15% MVCC) probably induced a faster rise in metabolite accumulation (Sundberg et al. 2019). These results were supported by the greater perceived exertion observed for old and very old adults compared to young adults at all common stages.

For BIKE, performance fatigability and decrease in Tw occurred earlier in old and very old adults, similarly to previous observations when young and old adults were matched for total work during a cycling task (Weavil et al. 2018). The different rate of performance fatigability development observed in BIKE between young and older adults could be due to the reduction in cycling efficiency (Sacchetti et al. 2010) and anaerobic capacity, because of the preferential atrophy of fast-twitch fibers with aging and the higher reliance on the aerobic metabolism (Hunter et al. 2016; Allen et al. 2021). The decrease in maximal heart rate and cardiac output with aging (Wilson and Tanaka 2000; Poole et al. 2016; Krüger et al. 2020) could induce lower oxygen delivery to the muscles during cycling (Weavil et al.

2018) and greater metabolite accumulation inducing impairments in contractile function, as shown by the drop in T_w for old and very old. This was also supported by the greater perceived exertion for old and very old adults compared to young adults. No impairments were observed for voluntary activation in the three age groups.

Performance and performance fatigability

Isometric task. In agreement with our first hypothesis, performance was lower for old and very old adults compared to young adults during ISO with no difference between old and very old adults. Contrary to our second hypothesis, similar performance fatigability was observed between groups at exhaustion. Previously, our research group found similar total work but lower fatigability for old compared to young adults after an isometric QIF test performed with increasing percentages of MVIC (Varesco et al. 2021c). The discrepancy in the results is likely due to differences in the workload (% body weight vs. % MVIC). Another potential explanation could be the shorter duty cycle used in the present study (0.8 s on/0.8 s off) compared to previous studies (≥ 5 s on/5 s off) (Callahan et al. 2009; Solianik et al. 2017; Varesco et al. 2021c). Indeed, sustained contractions induce greater blood flow occlusion and metabolite accumulation in young adults compared to old adults, because of their larger muscle mass and intramuscular pressure generated during isometric contractions (Russ and Kent-Braun 2003). Short contractions might prevent significant or prolonged blood flow occlusion. The lower cost of contraction in isometric mode with advancing age (Tevald et al. 2010) could partially explain the similar performance, RPE and performance fatigability in old and very old adults, despite the latter exercised at higher percentage of their MVIC.

Concentric task. In CON, old and very old adults showed lower performance compared to young adults with no difference between old and very old adults. However, performance fatigability was not different across age, contrary to our hypothesis. The age-related impairments in performance fatigability depend on total work performed (Weavil et al. 2018) but also contraction velocity (Callahan and Kent-Braun 2011; Dalton et al. 2012; Hunter et al. 2016; Senefeld et al. 2017; Sundberg

et al. 2018b, 2019; Weavil et al. 2018). The results of the present study are in agreement with previous studies using similar contraction velocity (80 to 180°.s⁻¹), even if the workload was set relatively to maximal power or strength (Callahan and Kent-Braun 2011; Dalton et al. 2012). Similar performance and performance fatigability were observed between old and very old adults. Previously, some evidence suggested that contraction efficiency decreased in concentric mode with advancing age (Layec et al. 2018), associated to greater metabolite accumulation (P_i, H⁺ and dihydrogen phosphate) within the working muscles of old adults (Sundberg et al. 2019). However, despite contraction efficiency could have influenced performance in young and older adults, this does not explain the similar performance observed between old and very old adults. For CON, the similar workload (relatively to MVCC) between old and very old adults and the high variability combined with the short duration of exercise (due to the high workload as %MVCC) could have masked some of the age-related differences between old and very old adults (Hunter et al. 2016).

Cycling task. During BIKE, we observed a decrease in performance with advancing age. This was the only session in which very old adults showed a lower performance than old adults. However, performance fatigability was similar across groups. As previously mentioned, greater involvement of the cardiovascular system for this session could explain some of the age-related differences observed in performance, and the similar performance fatigability across groups. The greater muscle mass involved during BIKE than ISO and CON, combined with decreased cycling efficiency (Sacchetti et al. 2010) and anaerobic capacity with age, could have increased the oxygen deficit. This lower oxygen availability would result in higher utilization of the anaerobic metabolism, rate of metabolite accumulation and energetic turnover, with consequent relative oxygen deficit and cardiovascular stress. This would explain the lower performance of very old adults compared to the other two groups, because this population possesses lower cardiovascular and anaerobic capacities, relying more on oxidative metabolism (Russ and Kent-Braun 2004; Varesco et al. 2021a). A rise in the cardiovascular

stress combined with performance fatigability for all the muscle masses involved increased perceived exertion and induced exercise cessation for old and especially very old adults.

The reduced contribution of the cardiovascular system in ISO and CON compared to BIKE partially explain the age-related differences in performance across sessions. However, despite the similar duty cycle used in the three fatiguing tasks, the different workload used with the isokinetic dynamometer (ISO and CON) and the cycle-ergometer (BIKE) as well as the different muscle masses involved do not allow a statistical comparison of performance and performance fatigability between the three sessions. Because of its characteristics, BIKE is likely more ecologically valid than ISO and CON, and evidenced impairments in performance in very old adults compared to young and old adults for the activities of daily living, independent of physical activity.

Contractile function and voluntary activation

At exhaustion, no alterations in voluntary activation were observed in the three sessions except for young adults after BIKE. However, large impairments in contractile function were found after the three fatiguing tasks independent of age, suggesting peripheral alterations were the main responsible of performance fatigability, as previously reported in aging studies (Sundberg et al. 2018b; Krüger et al. 2018; Weavil et al. 2018). The concentrations of metabolites accumulated within the different muscles involved in cycling could be particularly important for young adults, who performed for longer duration and at higher intensities during BIKE, and could significantly activate the chemoreceptors of the groups III/IV afferents, causing impairments in voluntary activation, despite similar reductions in T_w across groups (Bigland-Ritchie and Woods 1984; Taylor et al. 2016b). For BIKE, significant alteration in the *vastus lateralis* M-wave amplitude were also found at exhaustion for young and very old adults. For young adults it is possible that changes in sarcolemmal excitability occurred concurrently to impairments in voluntary activation because of the great work performed. For very old adults, lower neuromuscular junction stability with advancing age (Hepple and Rice 2016) could have played a role in altering the amplitude of M-wave despite the lower total work

performed compared to the other groups. Similar impairments in contractile function at task failure for the three age groups suggest similar mechanisms or muscular limiting factor to the performance, such as actin-myosin cross-bridge formation, calcium phosphorylation, ATP depletion or drop in pH (Allen et al. 2008b, a).

Conclusion

In the present study, we showed that young men performed better than old and very old men during isometric and dynamic (single-limb and cycling) fatiguing tasks when the load was set relative to body weight. Old and very old adults performed similarly at ISO and CON. During the cycling task, very old adults were less performant than old adults. However, performance fatigability, i.e. loss in maximal force, was greater in young adults than very old adults during the isometric fatiguing task, but similar between age groups during dynamic and cycling tasks. Performance fatigability was mainly driven by impairments in contractile function independent of age, with only young adults showing modest impairments in voluntary activation following the cycling task.

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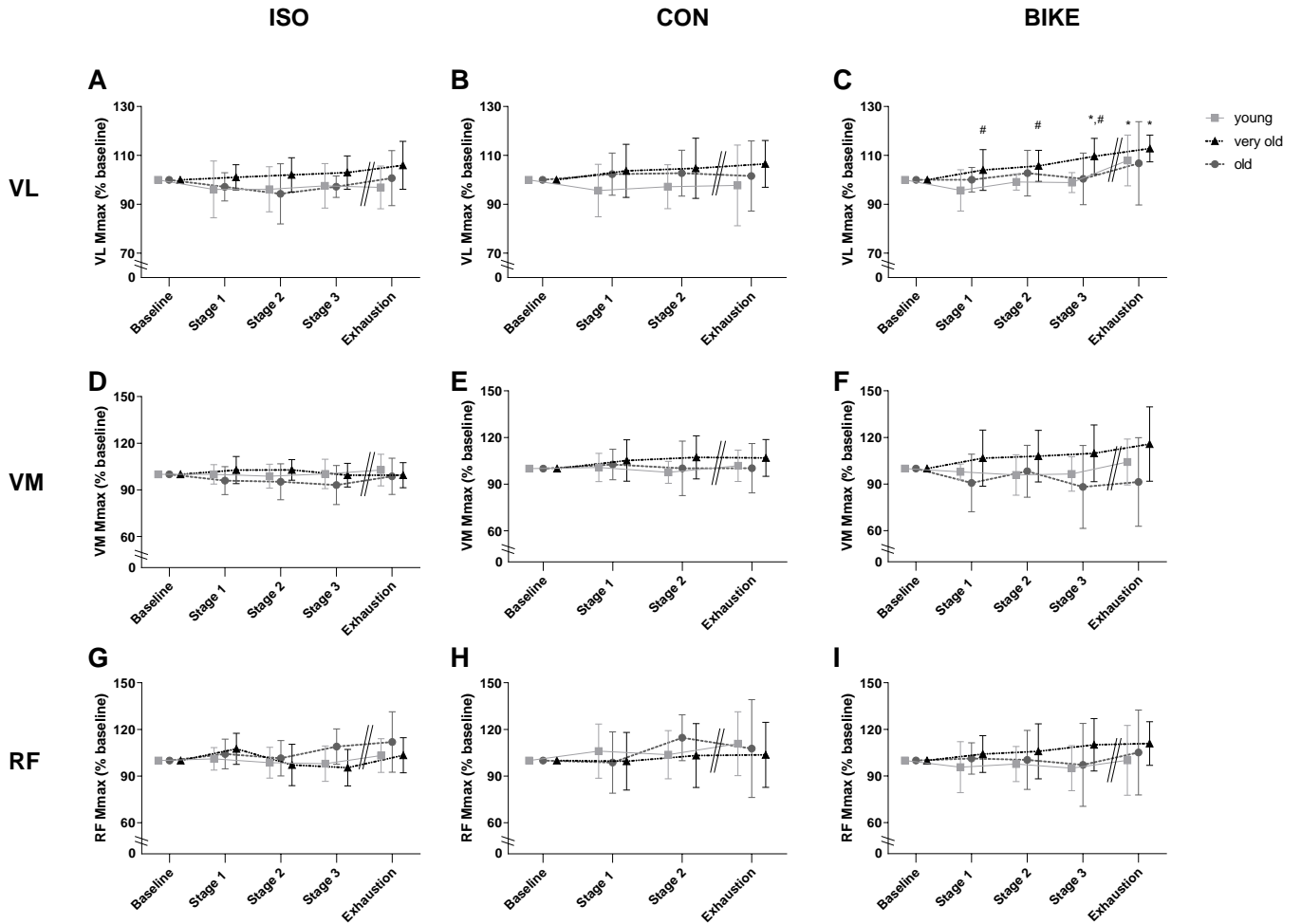
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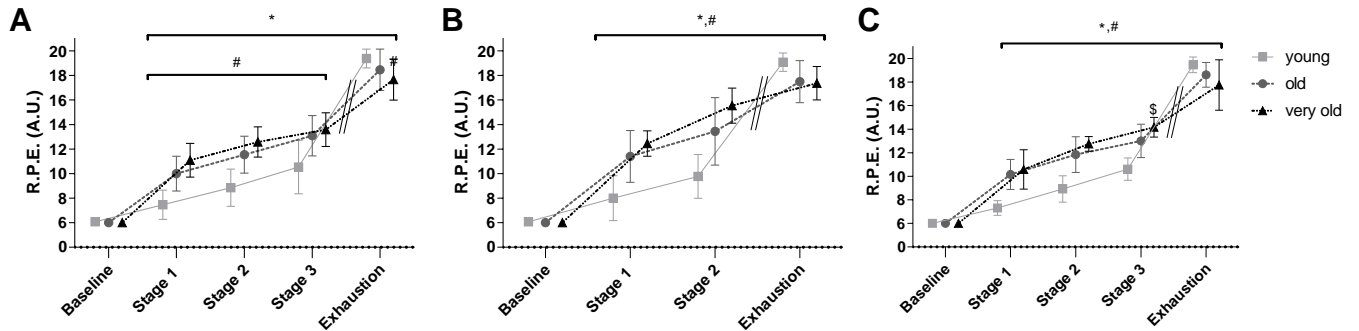
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SUPPLEMENTAL MATERIAL 1



M -wave amplitude (M_{max}) at the isometric (ISO), concentric (CON) and cycling (BIKE) quadriceps intermittent fatigue test for the three muscles evaluated: the vastus lateralis (VL; panels A, B, C), vastus medialis (VM; panels D, E, F) and rectus femoris (RF; G, H, I). *=significant difference from baseline ($P < 0.05$). # = significant difference from the young adults ($P < 0.05$).

SUPPLEMENTAL MATERIAL 2



Rate of perceived exertion of the three age groups across the isometric (ISO; panel A), concentric (CON; panel B) and cycling (BIKE; panel C) body weight based QIF test. The last common stage was stage 3 for ISO and BIKE and stage 2 for CON. *=significant difference from baseline ($P < 0.05$). # = significant difference from the young adults ($P < 0.05$). \$ = significant difference from the old adults ($P < 0.05$).

Summary of the study 3

In study 3, we observed a better performance for young adults at the three fatiguing tasks compared to old and very old adults. Old adults performed better than very old adults in the cycling task only. Performance fatigability was similar at exhaustion for CON, whereas very old adults had a lower decrease in maximal force than young adults after ISO. Performance fatigability was similar across groups for the cycling task at exhaustion, but force loss of old and very old adults was greater than young adults at the early stages.

Performance fatigability was mainly driven by impairments in contractile function for the three conditions independent of age. In active older adults, voluntary activation might be preserved by physical activity and don't play a key role in the age-related differences in performance and performance fatigability at the QIF tests.

Because we showed that the age-related differences in performance and performance fatigability vary when the intensity is set relative to maximal force production capacity or body weight, future studies should consider studying the neuromuscular alterations with aging in more ecologically valid conditions.

DISCUSSION AND PERSPECTIVES

The present work aimed to evaluate the age-related changes in performance and fatigability of the knee extensors in young, old, and very old adults. In this area, studies including very old adults are scarce (Krüger et al. 2018), and the neuromuscular characteristics of this population are not well understood (Hunter et al. 2016). The age-related alterations might also be influenced by the physical activity level of the individuals (Varesco et al. 2021b). Furthermore, the age-related impairments in neuromuscular function are greater for the lower limbs compared to the upper limbs, with the knee extensors function associated with the completion of the activities of daily living, risk of falls, and frailty (Hurley et al. 1998; De Rekeneire et al. 2003; Pijnappels et al. 2008). Finally, because most of the studies in the literature used single-limb tasks performed at intensities relative to maximal force or power (e.g. (Lanza et al. 2004; Callahan et al. 2009; Solianik et al. 2017; Sundberg et al. 2019), and given the task-specificity of the changes in performance and fatigability with age (Hunter et al. 2016; Krüger et al. 2018), it is important to investigate the age-related changes across different contractions modes and to use more ecologically valid conditions, such as setting the workload as a percentage as body weight.

In this context, the present work included only healthy and active individuals, and we tested the knee extensors performance and fatigability across isometric and dynamic conditions, with workload set relative to maximal force production capacity and relative to body weight. To address the main objective, we separated the experimental work into three parts. The first one aimed to investigate the neuromuscular properties of very old men and women. In particular, we evaluated the association between neuromuscular, biological, and functional parameters in association with the habitual level of physical activity. The second study evaluated the differences across young, old, and very old men and women in performance, performance fatigability, and its etiology during an isometric fatiguing task, as this kind of exercise is usually adopted to compare different populations in a laboratory setting. Finally, the third study aimed to understand the age-related alterations across different

fatiguing tasks and setting the workload as a percentage of body weight to simulate a more ecologically valid investigation of performance fatigability.

In the first study, we observed a significant positive association between the level of habitual physical activity and knee extensors absolute performance at the QIF test in very old women only. Furthermore, women who possessed greater strength showed lower fat mass, greater cross-sectional area, capillarization, and enzymatic activity of fibers type I and IIA.

Firstly, we evidenced how variations in physical activity in a relatively homogeneous group of very old adults could influence important parameters such as absolute performance at the QIF test (women), performance at the time-up-and-go test (men), or even maintenance of type IIX fibers (men). Thus, we provided further evidence on the important effects of physical activity in very old adults, suggesting also that it is necessary to consider physical activity when performing aging studies (Varesco et al. 2021b).

Secondly, study 1 evidenced important sex-related differences in neuromuscular performance, biological and functional parameters. Those factors could, in theory, affect performance fatigability. However, we found that absolute performance or performance fatigability were not related to maximal strength, structural maintenance, oxidative capacity, or enzymatic activity in both very old men and women. Indeed, in study 2, no differences were noticed between men and women in the evolution of fatigability with age, as previously observed during concentric tasks (Sundberg et al. 2018b).

Those results indicated also that, in very old adults, maximal strength of the knee extensors is not related to isometric performance and performance fatigability. Furthermore, the results obtained from study 1 also suggested that better structural maintenance, greater oxidative capacity, or higher enzymatic activity were not associated with better performance or lower performance fatigability in healthy and active very old adults. While muscle biopsies are sampled by a restricted area of a single muscle fascicle, performance fatigability is a more complex phenomenon that is determined by

multiple aspects. Biological parameters obtained from muscle biopsies might represent only a small part of these factors or not well represent the whole muscle function. Thus, the association between biological parameters and performance fatigability is not obvious.

With this premise, in study 2 we wanted to investigate the changes in performance fatigability of the knee extensors in healthy active young, old, and very old men and women during a standardized isometric intermittent task. We reported similar absolute performance but lower performance fatigability for old adults compared to young and very old adults. We observed different results after the isometric fatiguing test of study 3, with a lower performance in old adults compared to young adults, and similar performance fatigability across age. This difference could be explained by the greater relative workload for old and very old adults than young adults in study 3, as it was set as a percentage of body weight. Indeed, young and older adults had a similar weight, but young adults were stronger than older adults. The greater fatigue resistance in old adults compared to young adults in study 2 may be explained by the lower blood flow occlusion and metabolite accumulation (Russ and Kent-Braun 2003) or lower cost of contraction for older adults in isometric mode (Tevald et al. 2010). In the isometric task of study 3, we used a similar protocol but with shorter contractions (0.8 s) that might prevent significant blood flow occlusion. Previously, by occluding blood flow in young and old adults when performing an isometric task of the ankle dorsiflexors, Chung et al. (2007) did not find a main effect of ischemia on the age-related differences. These authors excluded ischemia as the main factor explaining different fatigue resistance with aging (Chung et al. 2007). However, given the high density of type I muscle fibers (Holmback et al. 2003) and the relatively small age-related impairments in the ankle dorsiflexors function until very old age (McNeil et al. 2005), these results might not be valid for the knee extensors and deserve further investigations.

In study 2 it was also observed that fatigue resistance during the isometric task was not maintained at very old age. We defined fatigue resistance as a lower performance fatigability associated with a greater or at least similar performance between groups. Indeed, while performance was maintained

in old adults possibly because of lower performance fatigability, very old adults showed lower absolute performance and similar fatigability compared to the other two age groups. Similar results were previously reported comparing very old and young adults after an isometric task on the upper limbs (Allman and Rice 2001). In study 2, we suggested that the important atrophy and accelerated decline in motor function could reduce the knee extensors performance and fatigability at very old age (Purves-Smith et al. 2014; Hunter 2018). It is possible that, in study 2, maximal force influenced the age-related differences in performance, as the workload was set in percentage of MVIC, while in study 3 the workload was set in percentage of body weight. In study 3, very old adults performed at higher absolute workload than old adults, because of the decreased MVIC/body weight ratio. In study 3 we observed similar performance and performance fatigability for old and very old adults after the isometric task, with significantly lower performance fatigability in very old than young adults. Previously, it was proposed that during isometric contractions the cost of contractions would be reduced with advancing age, because of the progressive slowing in muscle contractile properties and the lower MU firing rates (Connelly et al. 1999; Tevald et al. 2010). Although speculative, the greater economy of contraction in the isometric mode for very old adults could have partially compensated for the greater absolute workload performed in study 3 compared to old adults. However, the main cause is possibly attributable to the great variability in physiological responses typical of older adults that masked some of the age-related differences in performance and fatigability (Hunter et al. 2016). This means that during the isometric task of study 3, variability overcame a possible difference between old and very old adults. For example, in study 1 we showed large variability across various biological and functional parameters in a homogeneous group of healthy very old adults in terms of age and physical activity, with slight changes in step count influencing both biological and functional characteristics. Taken together, the results obtained in studies 2 and 3 suggest that contraction rhythm and workload influenced the difference in performance and fatigability between old and very old adults in isometric mode.

In study 2, central and peripheral alterations were investigated using magnetic stimulations. We did not observe impairments in voluntary activation, while contractile function was similarly impaired in all three age groups. The extend of those impairments could vary across different fatiguing protocols adopted (Thomas et al. 2018). Similar to what was observed in the present study, previous investigations found impairments in contractile function, with limited impairments in voluntary activation (Callahan et al. 2009; Solianik et al. 2017). We confirmed those results during an incremental intermittent fatiguing task and extended those findings to very old adults. The ischemia caused by isometric contractions impedes oxygenated blood to reach the capillaries (Moritani et al. 1992), promoting impairments in contractile function (Copithorne and Rice 2020). The main impairments are due to mechanisms such as reduction in excitation-contraction coupling linked to high rates of ATP turnover, and accumulation of free phosphate and H^+ (Sundberg and Fitts 2019). Because in very old adults the percentage of type I fibers was not related to performance or performance fatigability, similar loss in type I and type II muscle fibers, metabolites accumulation in the working fibers, and the recruitment of fast-twitch motor units could impair performance in this age group (Sundberg et al. 2019).

In study 3, participants performed a concentric and a cycling fatiguing task in addition to the isometric task. During and after the concentric task, similar performance fatigability was found across groups, while performance was greater for young than old and very old adults. The earlier impairments in contractile function during the task for old and very old adults compared to young adults suggest greater metabolite accumulation (P_i , H^+ and dihydrogen phosphate) within the working muscles of old adults (Sundberg et al. 2019), which may be induced by lower contraction efficiency in concentric mode with advancing age (Layec et al. 2018) and the greater workload relative to their MVCC. This rapid rise in contractile function impairment would have led to quickly reach task failure. The variability in performance fatigability could have masked some of the age-related impairments in maximal torque loss (Hunter et al. 2016), which were evidenced by previous studies (Callahan and

Kent-Braun 2011; Dalton et al. 2012; Sundberg et al. 2018b). This short duration of the concentric task, combined with great variability in physiological outcome with advancing age (Hunter et al. 2016), could also explain the similar performance observed between old and very old adults for the concentric task.

The cycling fatiguing task was performed to investigate performance and performance fatigability in a laboratory setting, but getting closer to the physical demands of the activities of daily living. In particular, locomotor activities usually requires the coordination and co-contraction of multiple muscle groups and sustain a load proportional to the individuals' body weight. The characteristics of the task (contraction rhythm, stage duration) were as similar as possible to the single-limb tasks, although we recognized that direct comparison across tasks was not possible. The results obtained in the cycling session showed reduced performance with advancing age but, surprisingly, performance fatigability was similar across the three age groups. We argued that the large muscle mass involved and the decreased cycling efficiency with age (Sacchetti et al. 2010) raised the rate of metabolite accumulation, energetic turnover, leading to oxygen deficit and increased, *in fine*, cardiovascular stress. Lower cardiovascular performance is usually observed with advancing age (Tanaka et al. 2001) but, at the same time, old and especially very old adults rely mainly on the oxidative metabolism for muscular work (Russ and Kent-Braun 2004; Varesco et al. 2021a). This greater cardiovascular stress, combined with performance fatigability from all the muscle mass involved increased perceived exertion, leading to task failure. Theoretically, this cascade of mechanisms would have occurred first in the population who possess the lower cardiovascular and muscular performance, i.e. very old adults. This did not occurred, as old and very old showed alterations in performance fatigability at the same stages. It is possible that during the first stages, cardiovascular stress was not great enough or the large variability could masked some age-related impairments. While performance fatigability was similar across age for the knee extensors, other muscles involved in the cycling task [i.e. knee

flexors, hip extensors, plantarflexors etc. (Ericson et al. 1986)] may show a different behaviour, limiting performance.

The task was performed at a constrained rpm (37.5), which lead to the development of similar performance fatigability in the three groups, associated with similar reduction in contractile function at exhaustion. Young adults performed for a longer duration and thus reached a higher workload, leading to greater absolute metabolite accumulation but similar relative performance fatigability compared to the other groups. This large metabolite accumulation across the different muscles involved in the task could also explain the impairments in voluntary activation observed only for this group at exhaustion. Indeed, the group III/IV afferents chemoreceptors respond to great metabolite accumulation, and it has been reported as the main mechanism leading to the decrease in voluntary activation (Bigland-Ritchie and Woods 1984; Taylor et al. 2016b). Although speculative, single-limbs intermittent tasks might not be sufficient to increase the concentration of metabolite and to induce impairments in voluntary activation in studies 2 and 3 and previous investigations (Sundberg et al. 2018b; Krüger et al. 2018), even if other studies suggest these alteration may be task-dependent (Solianik et al. 2017; Weavil et al. 2018).

CONCLUSION

Taken together, the results of the present work showed that in healthy and active individuals the age-related differences in performance fatigability vary across contraction modes, workload selected and age groups evaluated. The association observed between physical activity level and knee extensors performance (women) or functional tests (men) suggest that walking may be a sufficient stimulus to promote adaptations in very old adults. However, differences in performance or fatigability were not directly associated with specific biological characteristics. By comparing populations of young, old and very old adults, we observed lower performance fatigability in old adults compared to young adults independent of sex after an isometric fatiguing test with a workload set relatively to maximal force production capacity. The absence of difference between young and very old adults suggests that

the greater fatigue resistance of old adults is not maintained in very old adults. Finally, when the workload was set relative to individual body weight, young men showed better performance than old and very old men during isometric, concentric and cycling fatiguing tasks, and differences between old and very old men in the cycling task only. However, performance fatigability was similar between groups for the three conditions. The results of the cycling task and the specific workload used to simulate ecologically valid conditions suggest that very old adults would be more affected during activities of daily living. Finally, performance fatigability was mainly driven by impairments in contractile function independent of age, sex and contraction mode.

PERSPECTIVES

The present work offers different future perspectives on the investigation of the age-related differences between old and very old adults. Age could be used as a continuous variable to evaluate the association between age and performance fatigability. This type of analysis was not relevant in our studies because of the homogeneity in the age of our participants, especially in very old adults who were recruited from a cohort with a maximum of 3-years difference between participants. Furthermore, our sample of healthy and active very old adults could represent the fittest part of this population, and represents only a limited portion of the general population of very old adults. Consequently, the results observed in those individuals might not be extended to the whole population of octogenarians.

The participants of all our studies were considered as physically active based on their number of steps per day. Despite the homogeneity in age and physical activity in the very old population, we were able to observe that even slight variation in physical activity could impact neuromuscular performance (women) and other functional parameters (men) in very old adults. However, we were not able to capture the real effect of physical activity on the muscular characteristics of very old adults as we did not recruit sedentary participants. Future studies should exhaustively compare differences

in physiological function between active and inactive older individuals to investigate the effects of inherent aging and those of physical inactivity.

The evaluation of old and very old adults in a laboratory setting does not represent an ecologically valid condition and the transferability of the results to the activities of daily living may be limited. Performing fatiguing tasks with workloads set in percentage of body weight could be more representative of these activities but is not sufficient. Evaluating the association between such tests and performance at the activities of daily living along the whole day would inform on the transferability of these parameters on the real-life setting.

Finally, future studies could investigate performance fatigability after eccentric contractions in very old adults. Indeed, many activities of daily living involve eccentric actions like going down the stairs, sitting, putting down objects and controlling the speed of movement, etc.. It has been shown that eccentric strength was better preserved than isometric and concentric strength with age, but little is known about very old adults. Furthermore, eccentric exercise involves the cardiovascular system at a lower extent than concentric exercise, with a greater force developed. Thus, eccentric training could be an interesting opportunity for very old adults for which the alterations in the cardiovascular system induce early fatigue occurrence and could be a limiting factor for training efficiency.

SYNTHÈSE

Dans l'étude 1, nous avons montré que l'activité physique était associée à la performance lors d'un test de fatigue des muscles extenseurs du genou chez les femmes très âgées uniquement. Chez les hommes très âgés, l'activité physique était associée à la performance lors d'un test fonctionnel habituellement réalisé en milieu clinique. Ces résultats indiquent que même une petite variation de l'activité physique peut avoir un impact sur les capacités physiques et fonctionnelles des personnes très âgées, et que la marche suffit à induire ces changements. Etant donné que ni la force ni les paramètres biologiques ne pouvaient expliquer les différences de performance et de fatigue entre les sujets, nous avons cherché dans la deuxième étude à évaluer les différentes altérations de la performance et de la fatigue liées à l'âge chez des adultes jeunes, âgés et très âgés en bonne santé.

Dans l'étude 2, nous avons observé une plus grande résistance à la fatigue (moindre diminution de force maximale volontaire) chez les personnes âgées par rapport aux personnes jeunes et très âgées lors d'un test incrémental isométrique. Malgré une force maximale plus faible, les personnes âgées se fatiguaient moins que les jeunes et pouvaient donc effectuer plus de contractions, atteignant ainsi un travail total similaire. Cette résistance à la fatigue n'était cependant pas maintenue chez les personnes très âgées, qui montraient une fatigue similaire aux personnes jeunes. Enfin, nous n'avons pas observé de différence de fatigue entre les sexes.

Les activités de la vie quotidienne telles que la marche requièrent différents types de contractions incluant des contractions dynamiques, impliquent plusieurs groupes musculaires, et nécessitent le maintien d'une charge relative au poids de corps des individus. Ainsi, les résultats obtenus dans l'étude 2 pourraient ne pas être transférables aux conditions de la vie réelle. Dans l'étude 3, nous avons étudié les altérations liées à l'âge dans des conditions plus écologiques, en fixant la charge de travail en pourcentage du poids de corps et en étudiant différents modes de contraction. Pour ce faire, les participants ont effectué une tâche de fatigue isométrique, concentrique et sur vélo. Nous avons constaté que les performances étaient inférieures chez les personnes âgées et très âgées par rapport

aux jeunes adultes pour les trois tâches effectuées. Seule l'exercice sur vélo a induit une différence de performance entre les personnes âgées et les personnes très âgées, ces dernières présentant des performances inférieures. Pour les trois tâches effectuées, la fatigue induite était similaire quel que soit l'âge. La différence de résultat entre les études 2 et 3 indiquent que la réalisation d'une tâche avec une charge relative à la force maximale plutôt qu'au poids de corps des individus pourrait masquer certaines altérations liées à l'âge dans les activités de la vie quotidienne. Étant donné que nous avons montré une détérioration croissante liée à l'âge dans des conditions plus proches des activités de la vie quotidienne (exercice sur vélo effectué en pourcentage du poids corporel), les études futures devraient envisager d'étudier les altérations neuromusculaires avec le vieillissement dans des conditions plus écologiques.

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ANNEXE 1

Rate of force development and rapid muscle activation characteristics of knee extensors in very old men

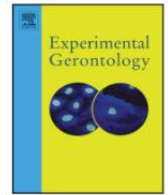
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Rate of force development and rapid muscle activation characteristics of knee extensors in very old men

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ABSTRACT

The age-related decrease in neuromuscular performance is usually accentuated in very old age (> 80 yr) as evidenced by a marked reduction in maximal force production. However, little is known about the ability to rapidly produce force, which limits daily activities and increase the risk of falling. We aimed to assess rapid force production characteristics and rate of muscle activation of the knee extensors in 15 very old (82 ± 1 yr) vs 12 young (24 ± 4 yr) men. Maximal force (F_{\max}) and maximal rate of force development (RFD_{\max}) were determined during separated specific isometric contractions. EMG from the *vastus lateralis* was analyzed to assess the rate of EMG rise (RER). Finally, RFD and RER were examined at time intervals of 0–50, 50–100, 100–200, 0–200 ms and 0–30, 0–50, 0–75 ms, respectively. We reported lower F_{\max} (414 ± 91 N vs. 661 ± 139 N) and absolute RFD_{\max} (8720 ± 2056 N*s⁻¹ vs. 5700 ± 2474 N*s⁻¹) in the very old men compared to young men ($P < 0.01$). When normalized to F_{\max} , RFD_{\max} was similar between groups. Normalized RFD at 0–50 and 0–200 ms were lower (–34% and –46%, $P = 0.04$) for the very old men, while no difference was observed at 50–100 ms and 100–200 ms. RER values were higher (~346%, $P < 0.01$) for the young men at every time interval. These data suggest that the decline in RFD_{\max} is associated with the decrease in F_{\max} . Impairments in RFD were accentuated in the first phase of the contraction, which has been associated in the literature with impairments in neural factors associated with aging.

1. Introduction

The ability to quickly produce a high amount of force is strongly related to the completion of daily activities and can be further associated with the risk of falling in older adults (Thompson et al., 2013; Klass et al., 2008; Aagaard et al., 2007). A useful tool to assess the capacity to rapidly increase force is the rate of force development (RFD), considered to be more sensitive than maximal isometric force (F_{\max}) to detect changes in neuromuscular function in clinical and old populations (Maffiuletti et al., 2016; Rodríguez-Rosell et al., 2017). Another advantage of RFD is the possibility to differentiate various phases of the force-time curve to better detect performance changes after a training protocol or in clinical vs healthy populations (Maffiuletti et al., 2016; Andersen and Aagaard, 2006; Folland et al., 2014). This analysis can be further implemented by studying the rise in the EMG

signal. The rate of EMG rise (RER) has been used to evaluate the rate of muscle activation concurrent to the rise in force in order to account for the neural factors that contribute to RFD (Aagaard et al., 2002; Clark et al., 2011; Thompson et al., 2014).

Healthy older adults present lower peak RFD than young adults for various upper and lower limb muscle groups, including the knee extensors (Izquierdo et al., 1999; Mau-Moeller et al., 2013), plantar flexors (Thompson et al., 2014), dorsiflexors (Klass et al., 2008) and elbow extensors (Bellumori et al., 2013). Conflicting results were reported when RFD was normalized to F_{\max} : while some studies showed greater values in young vs older adults (Klass et al., 2008; Thompson et al., 2014; Mau-Moeller et al., 2013; Korhonen et al., 2006), others did not (Thompson et al., 2013; Thelen et al., 1996). The discrepancy in the results could be due to different time intervals examined and different techniques used to normalize RFD by F_{\max} (e.g. some author calculated

Abbreviations: KE, knee extensors; F_{\max} , maximal force; RFD, rate of force development; RFD_{\max} , maximal rate of force development; $tRFD_{\max}$, time to maximal rate of force development; RMS, Root Mean Square; RER, rate of EMG rise; MVC, maximal voluntary contraction; VL, vastus lateralis; CV, coefficient of variation

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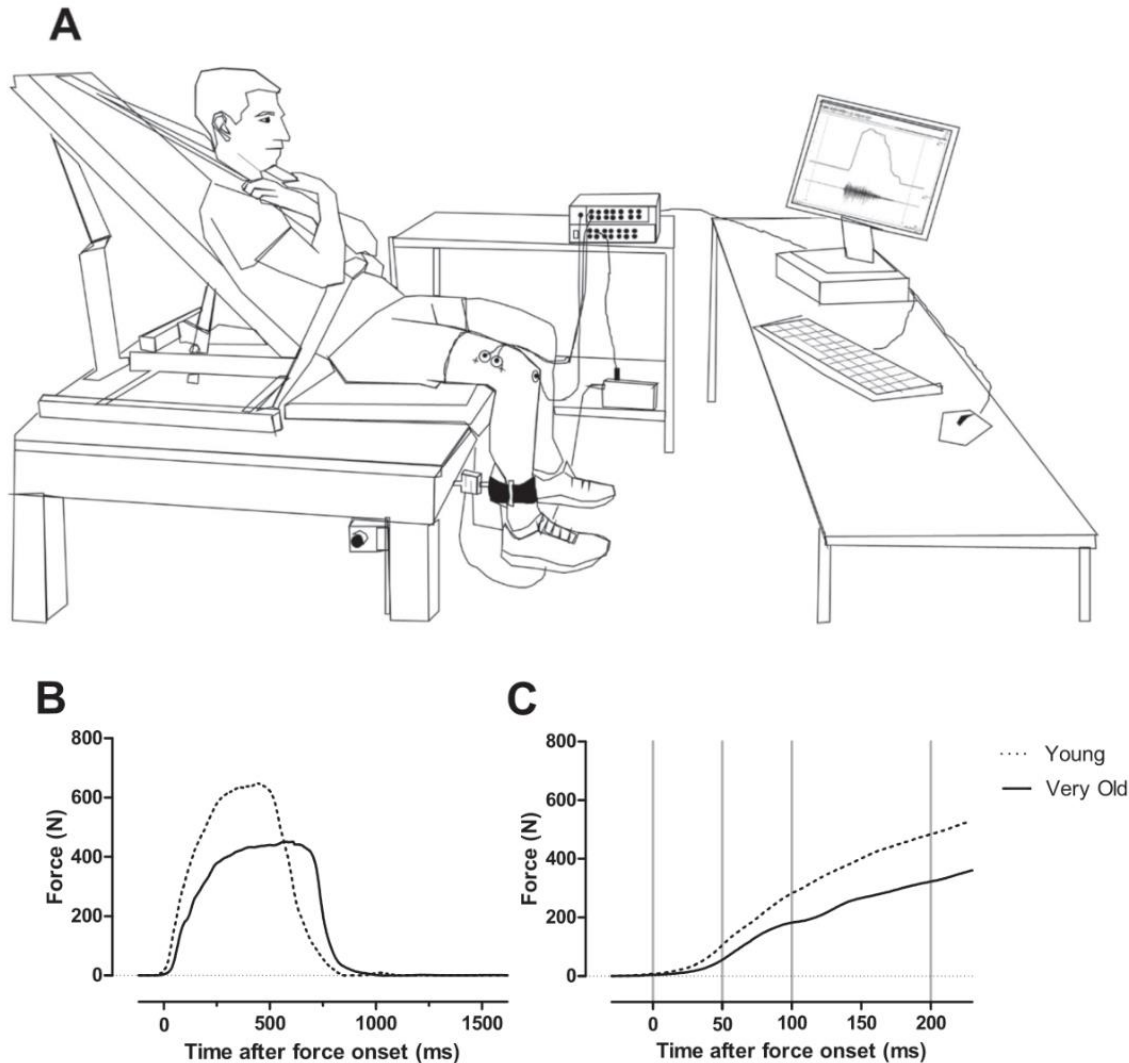


Fig. 1. Example of the force recording during the isometric impulsive contraction for two representative participants. A: Schematic representation of the experimental setup.

B: Rapid force development of the knee extensors between young (dotted line) and very old men (solid line). C: Rapid force development at different time intervals: 0–50 ms, 50–100 ms, 100–200 ms and 0–200 ms.

RFD on the force-time curve, while others used %MVC on the x-axis and measured the RFD value at the point when the force reached 10%, 30% or 50% of the MVC (Thompson et al., 2014)). However, it remains unclear whether the age-related decrease in RFD capacity is only due to the decrease in F_{max} . For instance, RFD may be influenced more than F_{max} by changes at the motor units level (i.e. loss of fast-twitch motor units (Hepple and Rice, 2016)), or neural impairments (i.e. motor unit discharge rate and doublets (Klass et al., 2008)).

Regarding the methodology, it has to be noted that a large part of the available literature measured RFD during maximal isometric voluntary contractions (MVC), asking the participants to exert isometric contractions as strong and as fast as possible (e.g. Thompson et al., 2014; Izquierdo et al., 1999; Mau-Moeller et al., 2013). Instead, recent recommendations suggest to perform specific impulsive isometric contractions as fast as possible to investigate RFD separately from F_{max} (Maffiuletti et al., 2016). The rigor in the instructions is essential, as Sahaly et al. (Sahaly et al., 2001) showed a significant improvement in peak RFD when participants were instructed to push “fast” rather than “hard and fast”. Consequently, RFD analyzed during MVC could be suboptimal and may not represent the maximal capacity to rapidly increase force. Furthermore, considering that older adults exhibit greater

variability in force measurements compared to young adults (Hunter et al., 2016), measuring RFD during an MVC may lead to inconsistent results and a misinterpretation of the age-related changes in RFD.

In this scenario, also the age group of participants is determinant: several cross-sectional and longitudinal studies focusing on the advanced stages of human life documented a sudden acceleration in the decline of neuromuscular function starting from the ninth decade (Mitchell et al., 2012; Venturelli et al., 2018). However, RFD has been mainly studied in individuals aged between 60 and 80 years and only one study focused on the RFD performance in octogenarians (Bellumori et al., 2013). In their study, Bellumori et al. (Bellumori et al., 2013) analyzed the absolute peak RFD from MVC of the elbow extensors and index finger abductors in very old adults and observed a ~50% reduction in RFD compared to young adults. Considering that the age-related impairments in neuromuscular performance are typically greater in the lower limb compared to upper limb muscles (Mitchell et al., 2012; Frontera et al., 2000) with functional repercussions on balance and autonomy (McGregor et al., 2014), investigating the capacity to rapidly increase force of the lower limb muscles is particularly relevant in very old adults. Identifying the mechanisms of the possible impairment in RFD may help to design specific interventions to

preserve the neuromuscular function in very old age.

In the present study, we aimed to investigate the age-related changes in RFD characteristics and RER of knee extensors (KE) between healthy young (< 30 years) and active very old men (> 80 years). We hypothesized that 1) both absolute and relative (to F_{\max}) RFD characteristics of the KE would be considerably reduced in the octogenarians compared to the young adults during specific impulsive contractions and 2) RER of the *vastus lateralis* would be lower in very old adults.

2. Methods

2.1. Participants

Twelve recreationally active males of 24 ± 4 years (range 20–33 yr, 182 ± 6 cm, 76 ± 8 kg) and fifteen active very old men of 82 ± 1 years (range 80–83 yr, 168 ± 6 cm, 71 ± 9 kg) volunteered to participate in the experiment and gave their written consent. Very old participants were selected from the PROgnostic indicator OF cardiovascular and cerebrovascular events (PROOF) cohort, which is a prospective longitudinal cohort of old adults started in 2001 (Barthélémy et al., 2007). Individual physical activity of the participants from this cohort is regularly assessed using a wrist wearable accelerometer (wGT3X-BT, ActiGraph, Pensacola, USA) and analyzed using ActiLife software (v6.13.4, ActiGraph, Pensacola, USA). Considering those data, all the very old men were considered as physically active (8840 ± 1972 steps.day⁻¹) (Tudor-Locke et al., 2006). All participants were free from musculoskeletal or neurological disorders. Before the experimental session, participants were asked to avoid strenuous exercises for at least 24 h. All the experimental procedures were approved by the Comité de Protection des Personnes Sud-Est 1 (France), and the Agence Nationale Sanitaire et du Médicament (ANSM-France) and was performed in accordance with the Declaration of Helsinki (ClinicalTrials.gov, Identifier: NCT02675192).

2.2. Mechanical recording and electromyography

Participants were seated upright on a custom-built chair with hip angle set at 120° (180° corresponds to full extension) and knee angles set at 90° of flexion (Fig. 1A). Knee extensors force of the right leg was assessed using a calibrated force transducer (Omega Engineering Inc., Stamford, CT, USA). Extraneous movements of the upper body were minimized by three belts across the thorax and waist. The force transducer was firmly attached above the malleoli. Force was sampled at 2 kHz and recorded without analogical or digital filter using a PowerLab data acquisition system (16/30-ML880/P, ADInstruments, Bella Vista, Australia). Passive resting force was subtracted from the signal so that the baseline was set at 0 N. Electromyographic (EMG) activity was recorded from the *vastus lateralis* (VL) muscle of the right leg using pairs of self-adhesive Ag/AgCl surface electrodes (recording diameter = 10 mm, Kendall MediTrace foam electrode, MA) placed in bipolar configuration over the muscle belly with an interelectrode (center to center) distance of 30 mm. Electrodes were placed following the SENIAM project recommendations. A reference electrode was placed on the right patella. Prior to electrode placement, the skin was shaved, abraded and cleaned with alcohol swab to lower the impedance (< 5 k Ω , 87v, Fluke Corp., Everett, WA, USA). EMG and force signals were digitized simultaneously at the same sampling rate (2 kHz) by PowerLab System (16/30-ML880/P, AD Instruments, Bella Vista, Australia). Force and EMG data were analyzed offline using Labchart 8 software (ADInstruments). EMG signals were amplified (gain = 500) using an octal bio-amplifier (ML138, AD Instruments) with a bandwidth frequency range of 10–500 Hz obtained through a band-pass filter. Prior to analysis, data were rectified and smoothed using a moving root-mean-square (RMS) with a time constant interval of 50 ms (Aagaard et al., 2002).

2.3. Experimental protocol

All the participants were clearly informed on the modality of the test, and an experimenter provided practical demonstration if required. Participants performed a standardized warm-up consisting in 10 isometric contractions of the knee extensors at increasing intensities until reaching 90% of their perceived F_{\max} . Warm-up was followed by familiarization with the experimental procedures: participants were instructed to extend their knee (in isometric condition) “as fast as possible” in order to assess the RFD variables (Maffiuletti et al., 2016). Familiarization consisted in 5 to 10 non-successive impulsive contractions, allowing participant to feel comfortable with the procedures. Successively, 5-min of rest were allowed before asking participants to perform two 5-s MVC separated by at least 60 s of rest. If the difference between the two MVCs was > 5%, participants performed a third MVC. In order to perform MVC, participants were asked to contract “as hard as possible”, strong verbal encouragements and visual feedback were provided. After a 3-min rest, participants performed a series of eight isometric contractions (0.5–1.5 s; Fig. 1B) separated by 30 s of rest. Strong verbal encouragement was provided. In case of counter-movement, i.e. quick flexion prior to the knee extension, determined from a force drop of 2 N below the baseline, or if the force attained was < 70% of the F_{\max} (Folland et al., 2014; De Ruiter et al., 2004), the trial was repeated again in order to obtain eight reliable contractions.

2.4. Data analysis

All data were analyzed offline using Labchart 8 Software (ADInstruments). The F_{\max} and associated RMS-EMG signal (EMG_{\max}) were defined as the average of the highest 0.5-s window among the best MVC. Repeatability of MVC was tested using a bivariate correlation.

The maximal rate of force development (RFD_{\max}) was determined as the maximal value of the force-time derivative curve for each RFD contraction. Absolute RFD_{\max} was used to identify the best five impulsive contractions retained and then averaged for further analysis, while the others were discarded (Fig. 1B). To assess the influence of within-subject variability among young and very old adults, the coefficient of variation (CV) was calculated for each participant and compared between groups.

Time to RFD_{\max} ($tRFD_{\max}$) and force level at 25, 50, 100 and 200 ms from the onset of the force (see below) were identified. Then, the mean RFD was calculated at three time intervals of 0–50 ms (RFD_{0-50}), 50–100 ms (RFD_{50-100}), 100–200 ms ($RFD_{100-200}$) and 0–200 ms (RFD_{0-200}) from the linear slope of the force-time curve (Aagaard et al., 2002) (Fig. 1C). The onset of the force development was defined automatically as the point at which the level of force exceeded three standard deviations over the average resting baseline (De Ruiter et al., 2004). This method has been chosen because it provides a valid and reliable method to determine the onset of the contraction when low-noise dynamometers (~1 N) are used (Maffiuletti et al., 2016). All RFD parameters are presented in both absolute values and expressed as percentage of F_{\max} .

The rate of EMG rise (RER) was calculated as the linear slope of the RMS EMG-time curve from two different onsets: the first onset was set 70 ms prior to the force onset as suggested by Aagaard et al. (Aagaard et al., 2002), and the second onset was visually identified from the rise in the RMS EMG-time curve. RER was analyzed at time intervals of 0–30 ms (RER30), 0–50 ms (RER50) and 0–75 ms (RER75). All EMG signals were normalized to EMG_{\max} (Thompson et al., 2014). Electro-mechanical delay was measured from the RMS EMG onset visually identified to the force signal onset.

2.5. Statistical analysis

Data are reported as mean \pm SD in the text, the table and the figures. Assumption of normality (Shapiro-Wilk test) was tested for all

variables and outliers were determined using a step of $1.5 \times$ Interquartile range from the Turkey's Hinges. Intraclass correlation coefficient (ICC, Two-way mixed model), standard error of the measurement (SEM), average CV and repeated-measure analysis of variance (ANOVA) were used to assess reliability of RFD measures (Weir, 2005) on both groups pooled together and separated. Independent samples *t*-tests were used to compare F_{\max} , EMG_{\max} , and CV between groups. Mann-Whitney *U* Tests were used to compare time to RFD_{\max} , absolute and relative RFD_{\max} . Two-way ANOVAs were performed with age as between-subjects factor and time as within-subjects factor for all the remaining force and EMG-derived variables. Sphericity (Mauchly test) were tested for all dependent variables. If the assumption of sphericity was violated, the correction of Greenhouse-Geisser was applied. When significant main effects were observed, Bonferroni's test was used for post-hoc analysis. Significance was set as $P < 0.05$ for all tests. Statistical analysis was conducted using IBM SPSS software (Version 23.0, SPSS, Inc., Chicago, IL).

3. Results

Due to technical issues, the EMG signals for two very old men were unusable and thus discarded. Thus, EMG parameters are presented for 13 very old men. Absolute values for F_{\max} , EMG_{\max} , RFD, RFD_{\max} and $tRFD_{\max}$ are presented in Table 1. Reliability of RFD measurement was excellent either when both groups were pooled together (ICC = 0.98, CI = 0.97–0.99; SEM = $893.87 \text{ N} \cdot \text{s}^{-1}$; CV = $11 \pm 6\%$; ANOVA: $F_{(4,104)} = 0.84$, $P = 0.50$) and when the young (ICC = 0.96, CI = 0.91–0.98; SEM = $998.78 \text{ N} \cdot \text{s}^{-1}$; CV = $12 \pm 6\%$; ANOVA: $F_{(4,44)} = 2.10$, $P = 0.10$) and very old men (ICC = 0.98, CI = 0.96–0.99; SEM = $810.80 \text{ N} \cdot \text{s}^{-1}$; CV = $9 \pm 6\%$; ANOVA: $F_{(4,56)} = 0.27$, $P = 0.90$) were analyzed separately. F_{\max} was consistent between measures ($r = 0.98$, $P < 0.001$) and significantly lower (37%) for the very old men compared to the young men ($P < 0.001$). The associated EMG_{\max} was also lower for the older adults (27%, $P = 0.42$). Absolute RFD_{\max} was greater in the young men (35%, $P < 0.001$), however the age-related difference was not observed when normalized to the maximal force (Fig. 2A, $P = 0.78$). Normalized RFD was not influenced by the between-subject variability, as CV was similar between young ($9 \pm 6\%$) and very old men ($12 \pm 6\%$, $P = 0.21$).

It can be noticed on Fig. 2A that two outliers from the very old group may have influenced the mean. Nevertheless, the statistical analysis was also performed without these two participants and the results remained unchanged. Finally, no age-related differences were observed for the $tRFD_{\max}$ ($P = 0.25$).

Of those two outliers, one very old man showed higher F_{\max} (554 N) and RFD ($12,409 \text{ N} \cdot \text{s}^{-1}$) than some young men.

From the onset of the contraction, there were significant age-related differences in the absolute force level at every time point and were $23.1 \pm 8.5 \text{ N}$ vs $12.6 \pm 10.5 \text{ N}$ (25-ms, $P = 0.01$), $135.2 \pm 43.8 \text{ N}$ vs

$53.8 \pm 38.4 \text{ N}$ (50-ms, $P < 0.001$), $340.7 \pm 59.6 \text{ N}$ vs $152.4 \pm 73.5 \text{ N}$ (100-ms, $P < 0.001$) and $533.3 \pm 91.1 \text{ N}$ vs $258.9 \pm 84.2 \text{ N}$ (200-ms, $P < 0.001$) for young and very old men, respectively (Fig. 3A). When normalized to F_{\max} , the differences between groups were less pronounced. Normalized force values were $3.6 \pm 1.3\%$ vs $3.1 \pm 2.3\%$ (25-ms, $P = 0.49$), $20.7 \pm 5.8\%$ vs $13.3 \pm 9.3\%$ (50-ms, $P = 0.02$), $52.1 \pm 5.5\%$ vs $36.9 \pm 17.3\%$ (100-ms, $P = 0.007$) and $81.5 \pm 6.7\%$ vs $62.2 \pm 15.6\%$ (200-ms, $P = 0.001$) for young vs very old men, respectively (Fig. 3B).

Average RFDs were greater for the young than the very old men at every time interval (Table 1). When normalized to the F_{\max} , average RFDs were $3.8 \pm 1.2\%$ (0–50 ms), $6.1 \pm 0.9\%$ (50–100 ms) and $3.0 \pm 0.5\%$ (100–200 ms) for young men and $2.5 \pm 1.7\%$ (0–50 ms), $4.8 \pm 2.7\%$ (50–100 ms) and $2.5 \pm 1.0\%$ (100–200 ms) for very old men (Fig. 2B). Significance was found only at the 0–50 ms time interval ($P = 0.04$), while no differences were observed at 50–100 ms ($P = 0.16$) and 100–200 ms ($P = 0.20$). Finally, significant difference was found at the 0–200 ms time interval between young ($4.2 \pm 0.7\%$) and very old men ($3.5 \pm 0.9\%$; $P = 0.04$).

RER of the VL, expressed as % EMG_{\max} , exhibited different results depending on the onset considered (Fig. 4). Data obtained with the RER onset 70 ms prior to the force onset showed significantly lower RER during the 0–30 ms time interval in the young men ($P = 0.04$), while no age-related differences were observed during the 0–50 ms ($P = 0.26$) and 0–75 ms time intervals ($P = 0.36$; Fig. 4A). The opposite trend was observed when RER onset was visually set at the point of RMS EMG rise, RER was greater for the young than the very old men for every time interval (RER30: $P = 0.003$; RER50: $P < 0.001$; RER75: $P = 0.007$; Fig. 4B). Electromechanical delay was longer in the very old men ($76.8 \pm 27.5 \text{ ms}$) than in the young men ($37.4 \pm 0.9 \text{ ms}$; $P < 0.001$).

4. Discussion

The aim of the present study was to assess the age-related impairments in rapid force production capacity and rate of muscle activation of the KE. We reported reduced F_{\max} and absolute RFD_{\max} in active octogenarians compared to young men. For every time interval of the force-time curve, absolute force and RFD values were considerably lower in very old men, as initially hypothesized. Contrary to our hypothesis, no age-related difference in RFD_{\max} was observed when normalized to F_{\max} . However, normalized RFD values in the early phase of the contractions (0–50 ms) were lower in very old compared to young men. This age-related difference disappeared in the successive phases of the contraction. Regarding RER, when the onset was defined manually on the RMS EMG-time curve, RER was significantly lower for very old men at every time intervals.

Although investigating the capacities of the KE is functionally relevant in old and very old age (Mitchell et al., 2012; Laukkanen et al., 1995), the literature regarding RFD performance with aging for this

Table 1
Absolute force, RFD and EMG data.

	Young men	Range (min.–max.)	Very old men	Range (min.–max.)
F_{\max} (N)	660.9 ± 139.1	455.0–911.0	$414.3 \pm 90.8^{\#}$	268.0–554.0
EMG_{\max} (mV)	0.62 ± 0.22	0.24–1.09	$0.45 \pm 0.16^{\#}$	0.26–0.80
RFD_{\max} ($\text{N} \cdot \text{s}^{-1}$)	8719.7 ± 2055.9	6278.0–11,625.0	$5700.5 \pm 2473.9^{\#}$	3262.0–12,409.0 ^a
$tRFD_{\max}$ (s)	70.3 ± 36.1	40.9–168.5	85.8 ± 33.1	45.0–127.0
RFD 0–50 ms ($\text{N} \cdot \text{s}^{-1}$)	2494.7 ± 877.7	1543.0–4684.0 ^a	$1034.4 \pm 738.4^{\#}$	222.0–2470.0
RFD 50–100 ms ($\text{N} \cdot \text{s}^{-1}$)	3992.7 ± 929.8	2885.0–6033.0	$2040.2 \pm 1258.7^{\#}$	568.0–4035.0
RFD 100–200 ms ($\text{N} \cdot \text{s}^{-1}$)	1986.6 ± 513	1283.0–3071.0 ^a	$1101.6 \pm 544.2^{\#}$	379.0–1925.0
RFD 0–200 ms ($\text{N} \cdot \text{s}^{-1}$)	2725.2 ± 410.8	2097.7–3441.85	$1470.5 \pm 503.3^{\#}$	618.38–2241.95

F_{\max} = maximal force; EMG_{\max} : maximal EMG; RFD = rate of force development; RFD_{\max} = maximal rate of force development; $tRFD_{\max}$ = time to the maximal rate of force development.

^a From an outlier.

^{*} $P < 0.05$.

[#] $P < 0.01$.

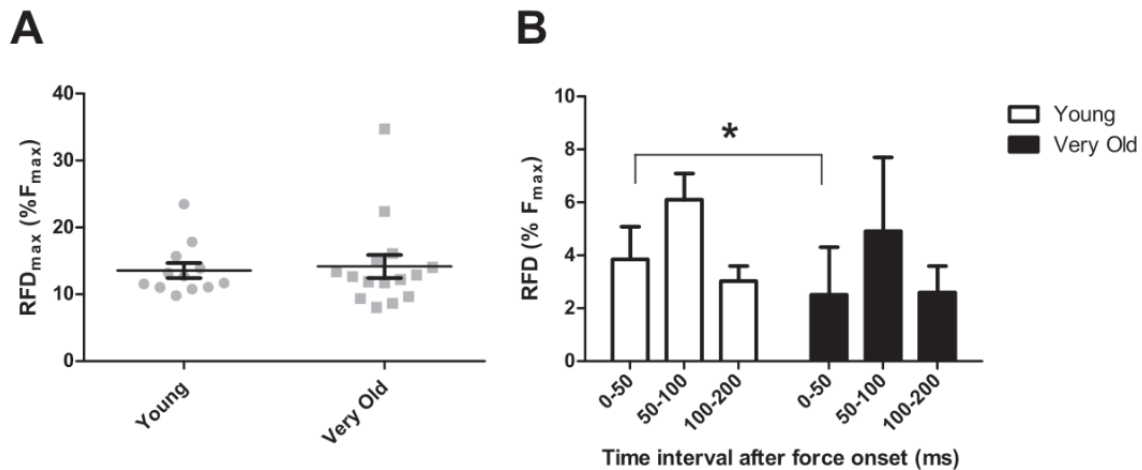


Fig. 2. Normalized RFD performance of young vs very old adults. A: Differences in the RFD_{max} normalized to the F_{max}. B: Ratio of the RFD and F_{max} at three time intervals of 0–50 ms, 50–100 ms and 100–200 ms after the force onset between young men (white) and very old men (black). * = $P < 0.05$.

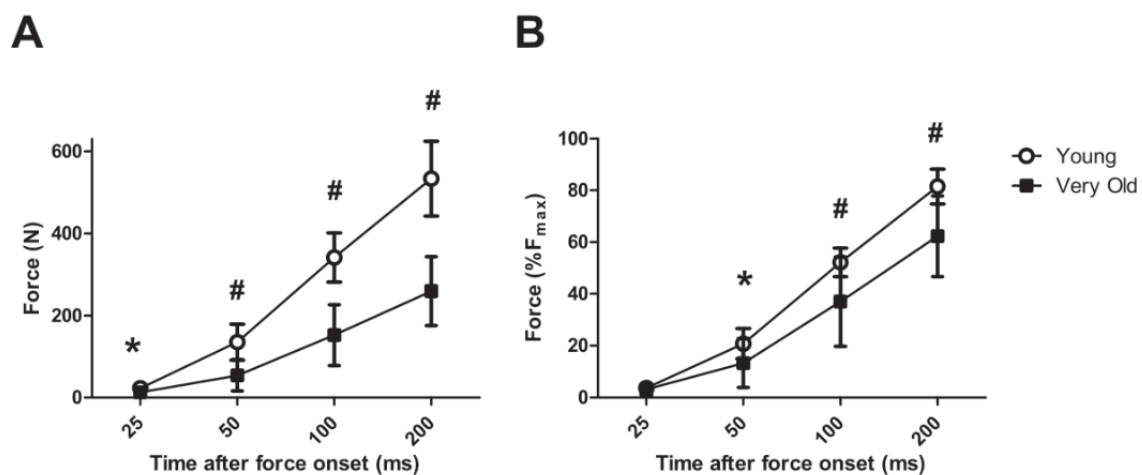


Fig. 3. Differences between young men (empty circles) and very old men (black squares) at four time points after the force onset. A: Difference in absolute force level. B: Difference in force level normalized to the F_{max}. * = $P < 0.05$; # = $P < 0.01$.

muscle group is limited. Thompson and colleagues (Thompson et al., 2013) observed ~28% lower F_{max} and absolute RFD_{max} measured during isometric KE MVC in old (67 yr) compared to young adults (25 yr), while Mau Moeller et al. (Mau-Moeller et al., 2013) reported

even greater age-related impairments (F_{max}, ~40%; RFD_{max}, ~65%) between young (25 yr) and old (70 yr) men during the same task. In agreement with the previous studies, we reported a ~36% lower absolute F_{max} and RFD_{max} in very old men compared to young men.

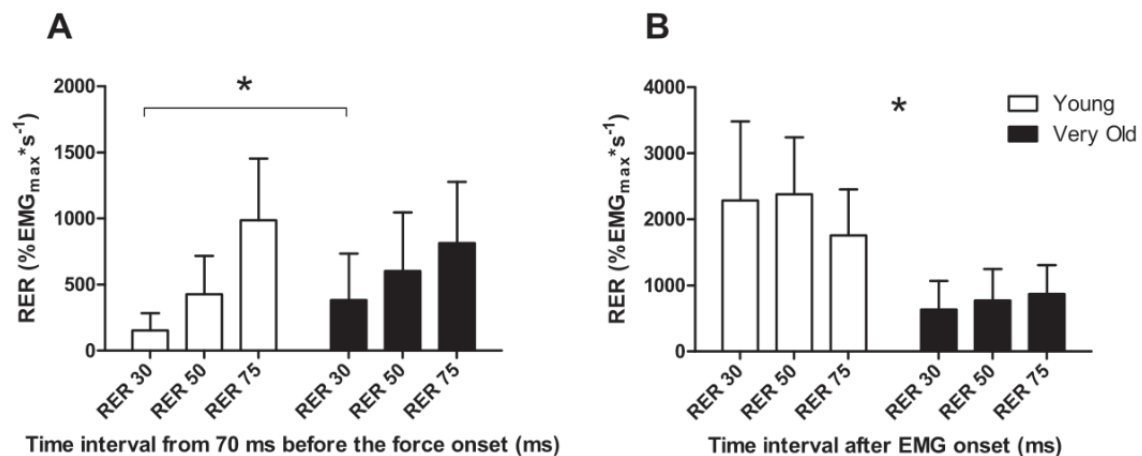


Fig. 4. Comparison between young (white) and very old (black) men in the RER normalized to the maximal EMG at three time intervals of 0–30 ms, 0–50 ms and 0–75 ms. A: onset was automatically set 70 ms prior to the force onset. B: onset was visually set at the rise in RMS EMG-time curve. * = $P < 0.05$.

During the rapid isometric contractions, the absolute force level of very old men was significantly lower than the young men at every time point, and this difference persisted when normalized to F_{\max} . These results confirm the marked impairment in the capacity to rapidly increase the level of force with very old age that accompany the decline in maximal force production capacity (Venturelli et al., 2018).

Interestingly, no age-related differences were observed in RFD_{\max} when normalized to F_{\max} , which could indicate similar factors are responsible for the decline of those two parameters. Maximal force and rapid force development are both affected by sarcopenia and the consequent muscle remodeling process with aging (Gueugneau et al., 2015). Only one very old man was more performant in both F_{\max} and RFD_{\max} than some young men. This is in agreement with previous investigations showing no age-related change in normalized RFD in plantar flexors (Thelen et al., 1996), dorsiflexors (Thelen et al., 1996), leg extensors (Thompson et al., 2013) and leg flexors (Thompson et al., 2013). However, other studies reported a reduction in the relative RFD_{\max} of plantar flexors (Thompson et al., 2014), dorsiflexors (Klass et al., 2008) and leg extensors (Mau-Moeller et al., 2013; Korhonen et al., 2006). In contrast with the previous investigations, in the present study F_{\max} and RFD were obtained during different contractions and giving specific instructions, according to the most recent recommendations (Maffiuletti et al., 2016; Rodríguez-Rosell et al., 2017), obtaining good within-subject reliability of the RFD measures in both groups. Consequently, different muscles involved, data analysis and protocols adopted may partially explain the discrepancies in the literature. The majority of the studies analyzed RFD during MVC, while we used specific impulsive isometric contractions. As we asked our participants to push “as hard as possible” during MVC rather than “hard and fast”, we could not compare RFD during MVC and the impulsive contractions. Other than methodological factors, the high level of physical activity of our participants may influenced age-related differences in RFD. In the present study, the very old participants were healthy community-dwelling volunteers, and classified as “active” according to the number of steps/day measured (threshold defined at 7000 steps/day between active and sedentary very old men (Tudorlocke et al., 2011)). These healthy individuals may not represent the overall population over 80 years, but rather those with greater lifespan, physical activity behaviors and/or with biological superiority (Porter et al., 1995). The natural selection bias of healthy participants over 80 years (alive, autonomous, without motor or neural impairments, etc. ...) could also explain why the age-related declines in performance observed in previous cross-sectional and longitudinal studies were less important than what expected in the ninth decade from linear extrapolation models (Aniansson et al., 1986; Greig et al., 1993). Moreover, physical activity has important implications in the age-related changes in RFD. For example, Aagaard et al. (Aagaard et al., 2007) showed that chronic exposure to strength training preserves RFD_{\max} at a similar level in young and old individuals, and Caserotti et al. (Caserotti et al., 2008) reported similar performances after 12-weeks of heavy-resistance training in old (60–65 years) and very old women (80–89 years). Further investigations should compare normalized RFD of very old men with different levels of physical activity to clarify the relative contribution of maximal force decline, aging and physical activity level in RFD performance. The normalized 0–200 ms time interval was used to provide a more representative indicator of the age-related RFD changes, and was significantly lower in the very old men. This reflects an overall impairment in the capacity of rapid force generation with aging that has been evidenced at every time point on the force-time curve. To better illustrate RFD differences between groups, different time epochs were analyzed and normalized to F_{\max} .

The average normalized RFD values were significantly lower only in the early phase of the contraction (0–50 ms), but not in the successive time intervals (50–100 ms and 100–200 ms), contrary to the absolute RFD values and the normalized force values. The non-significant difference in the middle time epochs regarding RFD data could be

explained by the higher between-subject variability in very old men. Despite higher variability, the difference at 0–50 ms was great enough to reach statistical significance. In other words, the force increment in this short time period (50 ms) was greater for the young men, which determined a difference in the relative RFD between groups. This could suggest that different neural strategies could be adopted during aging to compensate the progressive loss in RFD capacity, still evident in the 0–50 ms time interval. This could be partially supported by our results on time to reach RFD_{\max} , which was similar between groups. Similar results were obtained by Thompson et al. (Thompson et al., 2014) and Mau-Moeller et al. (Mau-Moeller et al., 2013). Thompson et al. (Thompson et al., 2014) found a difference between young and old adults in the plantar flexors at time intervals of 0–50 ms and 100–200 ms. However, these authors didn't report the time interval 50–100 ms. Thus, it seems that the early phase of the contraction is determinant for the age-related impairment in absolute RFD capacity of the leg extensors. Previous investigations associated the first phase of the contraction (0–50 ms) with the rate of muscle activation. For instance, a recent study of Gerstner et al. (Gerstner et al., 2017) on the plantar flexors found similar rate of torque development capacity between young and old men (~69 yr) in the firsts 50 ms of contraction, while it was reduced in the late interval (100–200 ms). These authors suggested that while factors like poorer muscle quality, the inability to sustain a high rate of muscle activation and reduced level of physical activity could influence the late interval of contraction in old men, muscle activation could reflect changes in the early time interval. To provide further insight, we quantified muscle activation using the rate of EMG rise (RER). We set two different RMS EMG onset for the RER analysis. With the EMG onset fixed 70 ms prior to force onset (Aagaard et al., 2002), RER was significantly higher in very old compared to young men only in the early phase of the contraction (0–30 ms), while no age-related differences were observed at 0–50 ms and 0–75 ms. However, the electromechanical delay was significantly different between groups, and only the electromechanical delay of the very old men was close to the fixed value of 70 ms suggested by Aagaard et al. (Aagaard et al., 2002). Thus, setting the onset visually on the RMS EMG-time curve seems the best option when two different populations are compared. In the present study, when the RMS EMG onset was set manually, RER was significantly lower for very old men for all the time intervals considered. The longer electromechanical delay in the very old group compared to the young, despite in small part dependent from the electrode placement, indicates a deficit in the transmission and conversion of the voluntary drive to force. The reduced capacity to rapidly activate the muscles suggests that impairments occur also in the voluntary drive, e.g. the generation of doublet discharge, the decrease in discharge frequency and/or the inability to maintain high discharge frequencies, leading to different or less efficient activation pattern with aging (Klass et al., 2008; Mitchell et al., 2011). To interpret those results, it has been shown that the age-related slowing of muscle contractile kinetics, the progressive reduction in type II muscle fibers (Mitchell et al., 2012) and changes in the muscle architecture (Gerstner et al., 2017) are muscle dependent and could influence the EMG signal. Limiting our analysis to the VL and recognizing the limitations of surface EMG measurements (Farina et al., 2004), further evidences are needed to clarify the specific role of neural vs contractile mechanisms involved in the loss of RFD across different muscles.

5. Conclusions

The present study demonstrated that rapid force development capacity is impaired in active octogenarians. However, the age-related difference is minimized when RFD is normalized to the maximal force of each individual. The results suggest that muscle force and RFD have common mechanisms determining neuromuscular changes associated with healthy aging. The impairments in RFD are accentuated in the early phase of the contraction, which has been associated in the

literature with the rate of muscle activation. Further studies are needed to establish whether similar levels of Fmax or RFDmax are related to similar motor function in very old age.

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Declaration of Competing Interest

No conflicts of interests, financial or otherwise are declared by the authors.

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ANNEXE 2

Physical activity and aging research: opportunities abound

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Physical activity and aging research: opportunities abound

G. Varesco, S.K. Hunter, and V. Rozand

Abstract: Aging is associated with large between-subjects variability in motor function among older adults, which can compromise identifying the mechanisms for age-related reductions in motor performance. This variability is in part explained by differences among older adults in habitual physical activity. Quantifying and accounting for physical activity levels of the participants in aging studies will help differentiate those changes in motor function associated with biological aging rather than those induced by inactivity.

Novelty:

- Quantification of physical activity levels in studies with older participants will help differentiate the effects of aging rather than physical inactivity.

Key words: aging, physical activity, variability, motor function.

Résumé : Le vieillissement est associé à une grande variabilité inter-sujets de la fonction motrice chez les personnes âgées et peut compromettre l'identification des mécanismes de réduction de la performance motrice liés à l'âge. Cette variabilité s'explique en partie par les différences d'habitudes en matière d'activité physique entre les personnes âgées. La quantification et la prise en compte des niveaux d'activité physique des participants dans les études sur le vieillissement aideront à différencier les changements de la fonction motrice associés au vieillissement biologique plutôt que ceux induits par l'inactivité. [Traduit par la Rédaction]

Nouveauté :

- La quantification des niveaux d'activité physique dans les études avec des participants âgés aidera à différencier les effets du vieillissement plutôt que ceux issus de l'inactivité physique.

Mots-clés : vieillissement, activité physique, variabilité, fonction motrice.

Introduction

The progressive age-related deterioration in neuromuscular and cardiovascular systems is accompanied by larger variability between older adults than young, which increases even further after 80 years. Factors that may contribute to the between-subjects variability within a group include genetics, circulating hormones, systemic inflammation, nutrition and physical activity (Hunter et al. 2016). Physical activity is an important modifiable factor influencing the varying rates of muscle deconditioning with age and sarcopenia (age-related decline in muscle mass and strength) (Hunter et al. 2016). Surprisingly, physical activity is rarely recognized or considered a differentiating parameter such as sex or age, despite its highly impactful influence on physiology and function in both young and older adults. Even when the average level of physical activity of the participants is reported, it is seldom treated as a source of variability in the statistical approach. Pooling participants with different levels of habitual physical activity increases the variability, potentially masking the age-related differences between young and older adults in their physiological outcomes and function.

Aging is associated with marked declines in activity levels in both males and females in most countries (data available at www.cdc.gov/physicalactivity/data).

To better understand the changes in function due to aging, rather than changes in activity levels, future studies need to minimize the potential large confounding effects of habitual exercise. This can be done by quantifying and controlling for the quantity and type of physical activity of the participants. Such an approach is particularly important for cross-sectional studies when young and older cohorts are compared, and the young adults are typically more active than the older adults (Tudor-Locke et al. 2011). In such cases, the young adults tend to be a more homogeneous group and show lower data dispersion for many physiological variables and function compared with old adults (Hunter et al. 2016). In this commentary, we (1) summarize the recent findings on several of the physiological differences that distinguish active and inactive older adults, (2) highlight the importance of assessing physical activity, and (3) provide recommendations on how to address the confounding effects of differences in activity levels with aging for future research studies.

Physical activity and aging

In the general population, physical activity may play a key role in differentiating physiological characteristics and function among old, and, even more so, among very old (>80 years) adults.

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Older adults who were compared for physical activity levels (active vs. inactive), differed in various characteristics and function in the neuromuscular system including corticospinal excitability (Hassanlouei et al. 2017; Rozand et al. 2019), skeletal muscle mitochondrial density (Distefano et al. 2018), skeletal muscle lipid content (St-Jean-Pelletier et al. 2017) and muscle quality (Barbat-Artigas et al. 2014). Muscle quality (strength per unit of muscle volume), for example, was shown to decrease with age (Barbat-Artigas et al. 2014); however, there were no differences in muscle quality among physically active individuals aged 50 and 80 years (Barbat-Artigas et al. 2014). Thus, while active old adults exhibit preserved strength per unit of muscle, inactive older adults exhibited large declines with advanced age. Similarly, corticospinal excitability of the knee extensor muscles, quantified by eliciting motor evoked potentials with cortical stimulation, was similar in young and older adults (Hassanlouei et al. 2017). When participants were separated based on their physical activity level, highly active participants exhibited reduced corticospinal excitability compared with the low active participants, independent of age. This physical activity-related effect was specific to the locomotor muscles, because corticospinal excitability was not different between active and inactive older adults in upper limb muscles (Rozand et al. 2019). Thus, the greater preservation of the physical and physiological function in active vs. inactive older adults could be muscle dependent, or may indicate that the upper limb differences in activity between active and inactive older adults (when activity is characterized based on steps) are minimal.

In the last decade, several studies have shown that the type of activity and exercise training (e.g., endurance vs. resistance exercises) can affect physiological systems differently with aging. For example, older lifelong endurance exercisers (LEE) possess superior maximal aerobic capacity (Carrick-Ranson et al. 2020; Gries et al. 2018), left ventricular mass (Carrick-Ranson et al. 2020), blood volume ($\dot{V}O_{2\max}$) (Carrick-Ranson et al. 2020), capillarization and aerobic enzyme activity (Gries et al. 2018) compared with inactive older adults. Such differences in adaptations between the cohorts were not surprising given the central role of these variables in endurance activity. Further, the benefits of lifelong exercise were found at the neuromuscular level, with more stable neuromuscular transmission (Power et al. 2016) and greater power of the slow twitch muscle fibers (Gries et al. 2019). However, LEE did not have preserved or increased muscle strength compared with controls because maximal force capacity did not differ between older adults who exercised and non-exercisers (Chambers et al. 2020). Motor unit preservation in LEE is still controversial, with greater preservation observed in the tibialis anterior by Power et al. (2010), although not by others (Piasecki et al. 2016). There is also minimal preservation of estimated motor units number in the vastus lateralis (Piasecki et al. 2019) and the biceps brachii (Power et al. 2012) muscles. According to the absence of strength preservation with endurance exercise, the most recent World Health Organization's (WHO) recommendations for optimal exercise in older adults include 2 sessions of strength training in addition to the 150 min of moderate intensity physical activity (guidelines available from www.who.int/dietphysicalactivity/factsheet_recommendations/en).

Lifelong strength-trained older adults typically have preserved muscle strength and power (less of an age-related decline) when compared with inactive older adults (Mckendry et al. 2018). This relative preservation of strength and power is associated with greater limb muscle mass and neural drive to the contracting muscles than inactive older adults (Unhjem et al. 2016), counteracting the sarcopenia and reduced neural drive that could occur with advanced aging (Rozand et al. 2020). Taken together, these results indicate that the type of physical activity (endurance vs. resistance exercises) and quantity (active vs. non-active adults) need to be carefully evaluated and quantified when studying age-related changes in physiological function.

Quantification of physical activity in older adults

Two primary challenges to accurately quantify activity levels in older adults are the method of measurement of activity and the definition of active and inactive participants. Physical activity level can be quantified using questionnaires and logs or direct quantification of activity with devices such as accelerometers (Strath et al. 2013). Questionnaires and logs of activity are commonly used (Strath et al. 2013): they are not expensive and can be self-administered, making them suitable for studying large samples over long periods of time (months or years), and provide qualitative information about the type and modality of physical activity performed. However, in the old and very old populations, where the primary physical activity involves walking, questionnaires showed weak correlations to objective measurements such as accelerometry, because the sensitivity of questionnaires to light activity is limited (Strath et al. 2013).

Objective measurements of physical activity in older adults are usually performed using motion sensors such as accelerometers, which provide quantitative data to determine activity levels and consequently categorize active and inactive older adults. To obtain consistent measurements, it is recommended to wear the accelerometers either on the hip or wrist for 7 complete days (including 1 weekend), and data should be validated for at least 4 days over 8 hours (Ekelund et al. 2017). However, accelerometers are limited in their ability to record (1) the intensity of activity, e.g., walking uphill vs. on flat ground where the metabolic demand varies considerably; and (2) the type of activity performed, e.g., strength exercises, cycling or water activities, although recent advances in technology are making inroads (e.g., smartphone apps). The combined use of activity logs and accelerometry could potentially address some of those limitations and at present is considered best practice.

A considerable challenge is determining a threshold to categorize active and inactive individuals when comparing these groups. Although the latest WHO recommendations for physical activity are time based with a combination of duration and intensity [150 min/week in moderate intensity or 75 min/week in vigorous intensity physical activity (www.who.int/dietphysicalactivity/factsheet_recommendations/en)], physical activity is usually reported as steps-day⁻¹ (Tudor-Locke et al. 2011). Although governmental guidelines (e.g., the U.S. Department of Health & Human Services) adopted the 10 000 steps-day⁻¹ as the daily activity recommendation, this number has been questioned because it is an arbitrary threshold rather than an evidence-based choice (Tudor-Locke et al. 2011). For example, older adults are considered to meet the WHO recommendations for physical activity if they accrue more than 7000 steps-day⁻¹ (Tudor-Locke et al. 2011). Although debatable, arbitrary thresholds remain a standard practice due to the lack of normative data for specific populations. However, setting a single arbitrary threshold may not always be appropriate, because the attributed group of the participants above and under the threshold may vary from 1 week to another. One possible solution involves using 2 cut-off points separated by a gap in the step count to account for the between-subjects variability in physical activity of the older population. For example, the inclusion criteria could allow the testing of only older adults walking >7000 steps-day⁻¹ (active) and <6000 steps-day⁻¹ (sedentary) as a block-design study. However, the lack of empirical evidence precludes the determination of specific step count-based thresholds. Additional studies are needed to provide further insight into determining differences in motor impairments between physical activity and healthy aging using this approach.

Addressing variability induced by physical activity in aging studies

One method to address variability and improve statistical power in aging studies is to increase the sample size. However,

well-designed experiments can limit the need of large sample sizes, which are not always possible to obtain, by reducing both within- and between-subject variability. Within-subject variability usually depends on biological or instrument-dependent oscillations in the studied outcome and can be minimized by using validated methods and reliable instrumentation. Between-subject variability can be reduced by choosing a study design such as the block design, which involves allocating participants to discrete groups that share similar characteristics (e.g., active vs. inactive). Sufficient sample size for each group is needed to guarantee adequate statistical power. In this case, narrowing the boundaries of the groups (e.g., 7000 to 10 000 steps-day⁻¹ for the active people) will lower between-subject variability and likely require smaller sample sizes to obtain adequate statistical power. It is also recommended to control for physical activity within a group by covarying for it when performing statistical analysis such as the block-design experiments.

This approach to reduce variability in aging studies can be extended to other types of activity such as resistance training. For example, resistance training can be quantified as a continuous variable by evaluating intensity, duration and/or volume (intensity \times duration) of training via questionnaires, logs, or wearable devices. Consequently, the effects of resistance training on the outcome measure can be controlled by either implementing a block-design study, covarying for activity, or both.

Conclusion

Activity status has a large impact on physiology and the functional outcomes in older adults and the variability between older adults. For aging studies, the large variation in activity levels between older adults can present as a confounding factor when not controlled or considered. Consequently, opportunities for high impact studies abound across many areas of motor and cognitive function by controlling for and categorizing participants according to activity levels. Controlling for activity levels with aging will improve the reproducibility of the results between studies, account for the variability in aging studies in human physiology, and more clearly highlight the age-related changes in physiology and function rather than those due to activity.

Take-home messages

- Large between-subjects variability in aging studies can limit the understanding of the mechanisms of the aging process.
- Physical activity is a primary modifiable factor explaining large variability among older adults and with human aging.
- Active older adults have superior maintenance of their neuromuscular and cardiorespiratory systems than inactive older adults.
- Quantification of physical activity levels in studies with older participants will help differentiate the effects of aging rather than physical inactivity.
- Opportunities for high-impact studies to understand the effects aging and benefits of activity abound by differentiating participants based on their physical activity level.

Conflict of interest statement

The authors report that there are no conflicts of interest.

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FATIGABILITY AND NEUROMUSCULAR PERFORMANCE OF OLD AND VERY OLD HEALTHY ADULTS

ABSTRACT:

The objectives of this thesis were to evaluate neuromuscular function and performance fatigability (loss of maximal strength with fatigue) in young (<35 yr), old (>60 yr) and very old adults (>80 yr) across different fatiguing protocols. To do so, we first characterized neuromuscular function of very old adults in relationship with daily physical activity and biological parameters obtained via muscle biopsies. The results of this work showed that differences in muscle performance were sex dependent and related to the level of physical activity in very old women only. Successively, we compared performance and performance fatigability in young, old and very old adults using an intermittent isometric fatiguing task performed relatively to maximal isometric force. We observed greater fatigue resistance for old adults compared to young adults that was not maintained at very old age. No sex-related differences were observed. We finally tested the age-related differences in a more ecologically valid condition. Thus, we studied the age-related differences during an isometric, a concentric isokinetic and a cycling fatiguing task. The workload was based on the individual body weight. The results showed an overall greater performance for young adults compared to the older adults. Similar performance was observed for old and very old adults at the isometric and concentric tasks, while lower performance was observed for very old adults at the cycling task. No differences in performance fatigability were observed between old and very old for this latter session suggesting that the greater involvement of the cardiovascular system might induced very old participants to stop the exercise.

We showed that the age-related differences in performance and performance fatigability vary across tasks and when the intensity is set relative to maximal force production capacity or body weight.

FATIGUE ET PERFORMANCES NEUROMUSCULAIRES D'ADULTES SAINS ÂGÉS ET TRÈS ÂGÉS

RESUME :

Les objectifs de cette thèse étaient d'évaluer la fonction neuromusculaire et la fatigabilité (perte de la force maximale avec la fatigue) chez des personnes jeunes (<35 ans), âgées (>60 ans) très âgées (>80 ans) à travers différents protocoles de fatigue. Pour ce faire, nous avons d'abord caractérisé la fonction neuromusculaire d'adultes très âgés en relation avec l'activité physique journalière et les paramètres biologiques obtenus par biopsies musculaires. Les résultats de ce travail ont montré que les différences de performance musculaire étaient dépendantes du sexe et liées au niveau d'activité physique chez les femmes très âgées uniquement. Par la suite, nous avons comparé les performances et la fatigabilité chez des personnes jeunes, âgées et très âgées en utilisant une tâche de fatigue isométrique intermittente avec une charge relative à la force maximale isométrique. Nous avons observé une plus grande résistance à la fatigue chez les personnes âgées par rapport aux jeunes, résistance qui ne s'était pas maintenue chez les personnes très âgées. Aucune différence liée au sexe n'a été observée. L'étape suivante consistait à tester les effets de l'âge dans des conditions plus écologiques. Ainsi, nous avons étudié les différences liées à l'âge lors d'une tâche de fatigue isométrique, isocinétique concentrique et de pédalage. La charge de travail était basée sur le poids du corps. Les résultats ont montré une performance globale supérieure chez les jeunes par rapport aux personnes plus âgées. La performance était similaire chez les personnes âgées et les très âgées pour les tâches isométriques et concentriques, tandis que des performances inférieures ont été observées chez les personnes très âgées pour la tâche de pédalage. Aucune différence dans la fatigabilité n'a été observée entre les personnes âgées et très âgées pour cette dernière session, ce qui suggère la plus grande implication du système cardiovasculaire pourrait conduire les personnes très âgées à arrêter l'exercice. Nous avons montré que les différences liées à l'âge dans la performance et la fatigabilité varient selon les tâches et la façon de sélectionner l'intensité d'exercice.