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Title of the thesis:

## "Effects of muscle disuse/hospitalization and physical training/retraining on the neuromuscular function in healthy older adults: iso-inertial resistance exercise training as a potential countermeasure form"

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# Abstract:

Population ageing is a global phenomenon and it is causing many concerns regarding the economic, social and health consequences that it might bring to individual countries. Ageing is associated with the decline of the physical performance and the greater risk for the occurrence of physical disabilities. Everyday tasks require good levels of muscle performance, such as muscle force and power abilities. Hence, older people, due to their reduced physical capability levels, might have difficulties in performing these daily actions. Even the walking ability might result impaired in this population which might be associated with a loss in physical independence and, in extreme cases, death. Physical performance deterioration depends on primary ageing processes and lifestyle habits, as well. Within the lifestyle factors, periods of inactivity (i.e. defined as periods where muscles are not stimulated adequately, muscle disuse) are frequent in older people and this might exacerbate performance deterioration in this population. Indeed, muscle disuse causes further impairments in muscle force and power abilities which, added to the intrinsic alterations brought about by ageing processes, might influence everyday tasks to a greater extent. However, influences of muscle disuse on the walking ability in older people are not well studied. Weather muscle disuse can produce negative consequences to this well established and natural motor action in older people is investigated in this thesis. Muscle disuse effects on muscle power and force of the lower limb in older people are studied as well in the current work. Furthermore, to better understand how muscle performance changes with muscle disuse, investigations on the motor control, muscle volume and muscle fibers characteristics accompany the previous analyses. Information about how muscle disuse influences the neuromuscular function of older people might be of clinical importance. Indeed, muscle disuse might be associated to hospitalization periods due to health problems. Interventions that preserve the deterioration of muscle performance before and even after hospitalization might be required to rapidly reintegrate older patients into society. Thus, studying the effects of potential countermeasures, that might be involved before and after hospitalization (or before and after disuse events), on the neuromuscular function of older individuals, is another theme that the current thesis faces. In particular, physical training has been chosen as the promising countermeasure that might help older people to recover from hospitalization or even other muscle disuse periods. As muscle power performance decreases to a greater extent compared to muscle force due to both ageing and muscle disuse conditions, attention is given to a particular form of resistance training: the flywheel iso-inertial resistance training. Although this training has been shown to call for greater muscle power improvements, compared to traditional gravity dependent trainings, its effects on other neuromuscular function features (i.e. muscle force, architecture, etc.) and on the walking ability are

not studied in depth. Hence, the current thesis provides new evidence on this topic by further compared it with traditional resistance training adaptations.

# List of Publications

1) Effects of 14 days of bed rest and following physical training on metabolic cost, mechanical work, and efficiency during walking in older and young healthy males.
**Floreani M.**, Rejc E., Taboga P., Ganzini A., Pišot R., Šimunič B., Biolo G., Reggiani C., Passaro A., Narici M., Rittweger J., di Prampero P.E., Lazzer S.
PLoS One. 2018 Mar 12;13(3): e0194291. doi: 10.1371/journal.pone.0194291. eCollection 2018.

2) Loss of maximal explosive power of lower limbs after 2 weeks of disuse and incomplete recovery after retraining in older adults.
Rejc E., **Floreani M.**, Taboga P., Botter A., Toniolo L., Cancellara L., Narici M., Šimunič B., Pišot R., Biolo G., Passaro A., Rittweger J., Reggiani C., Lazzer S.
J Physiol. 2018 Feb 15;596(4):647-665. doi: 10.1113/JP274772. Epub 2018 Jan 19.

3) Eight weeks of iso-inertial resistance training improve maximal explosive power of lower limbs in healthy older adults.
**Floreani M**., Rejc E., Gambin S., Vavassori L., Lazzer S.
(working in progress)

4) Short-term effects of rolling massage on energy cost of running and power of the lower limbs.
Giovanelli N., Vaccari F., **Floreani M.**, Rejc E., Copetti J., Garra M., Biasutti L., Lazzer S.
Int J Sports Physiol Perform. 2018 May 10:1-23. doi: 10.1123/ijspp.2018-0142.

5) Effects of endurance training on neuromuscular fatigue in healthy active men. Part I: Strength loss and muscle fatigue.
Mira J., Aboodarda SJ., **Floreani M.**, Jaswal R., Moon S.J., Amery K., Rupp T., Millet G.Y.
Eur J Appl Physiol. 2018 Nov;118(11):2281-2293. doi: 10.1007/s00421-018-3950-8. Epub 2018 Aug 18.

6) Effects of endurance cycling training on neuromuscular fatigue in healthy active men. Part II: Corticospinal excitability and voluntary activation.
Aboodarda S.J., Mira J., **Floreani M.**, Jaswal R., Moon S.J., Amery K., Rupp T., Millet G.Y.
Eur J Appl Physiol. 2018 Nov;118(11):2295-2305. doi: 10.1007/s00421-018-3951-7. Epub 2018 Aug 20.

# Introduction

The current thesis is composed of two parts. The first part represents the main field of study of the three years PhD experience. In this section the following themes are introduced, described and discussed: muscle disuse, age-related physical deterioration and physical training intervention in older people. The present PhD student scientific contribution related to these themes and produced during his PhD experience is reported: two published papers and a work, whose scientific approval is still pending, are included in this first section. The second and last part of the thesis deals with other themes that the PhD student has worked on during the previous three years. Effects of muscle fatigue on the neuromuscular function after an endurance cycling training and effects of self-myofascial release on the running performance of young healthy people are examined in this part. The PhD student scientific contribution related to these topics is presented and it includes a total of three works.

# SECTION ONE

## 1.1 General overview

The world is facing a demographic transformation. Each year the percentage of old people (age > 60 years) within the global population is increasing dramatically. It is estimated that in the next 30 years this number will rise with greater extent especially in the western societies (Nations, 2012). By 2050 the percentage of people with this characteristic will reach almost the 20% of the total global population (Nations, 2012); in Europe this number will be even greater going beyond the value of the 30% of European citizens; Italy will not be excluded by this global phenomenon and its population will be composed by almost 40% of people over 60 years old by the half of this century. As written by Mazzola and colleagues (Mazzola *et al.*, 2016), population aging causes many concerns in regard to economic growth, sustainability of effective health care and pension system and the well-being of elderly people. Among these, the last issue might be extremely challenging because it necessitates resources to preserve the remaining physical independence of older and elderly people and reduce public health care costs. Indeed, old people are more prone to develop disabilities and physical limitations than individuals with less than 60 years of age (Rimmer, 1994). As a person gets older it is possible to assist to a decrease in their physical performance. This is the result of the muscle mass loss that characterized the ageing process, defined as sarcopenia (Narici & Maffulli, 2010). Consequently, difficulties in successfully executing common motor tasks might arise from daily living actions (e.g. raising from a chair, climbing the stairs, etc.) (Aagaard *et al.*, 2010; Reid & Fielding, 2012). Interestingly, this age-associated deterioration in performance might be due to both the intrinsic process of ageing and life-style habits (i.e. reduced physical activity levels, inactivity, ect.), as suggested by Souminen (Suominen, 2011). Suetta and colleagues (Suetta *et al.*, 2007) reported that muscle disuse or inactivity periods are frequent in elderly people. They might be associated to hospitalization periods or disease states. However only few scientific works have focused on studying the effects of muscle disuse on the aged neuromuscular function. The current evidence suggests that disuse might be responsible for exacerbating the decrease in muscle strength and power ability, already impaired in elderly people due to the intrinsic sarcopenic process (Hoenig & Rubenstein, 1991; Creditor, 1993). Reduced levels of physical capabilities might be the consequences of the decrease in muscle force and power performance and they might cause a greater risk for the incidence of falls and injuries (Wall *et al.*, 2013). Hence, older people might require even greater efforts to perform the same daily routines achieved before experiencing inactivity. The result of this chain of physiological events might bring older individuals to lose their physical independence with an associated increased mortality rate (Wall *et al.*, 2013). Moreover, muscle force and power changes due to inactivity periods might alter other features of physical functioning. Indeed, previous works have suggested that muscle weakness and lower extremity strength loss might influence

specific descriptors of a spontaneous and intrinsically well-established motor action like human walking (Aboutorabi *et al.*, 2016). These results might rise concerns about the integrity of this motor task especially when older people are subjected to periods of disuse. Thus, potential perturbations in muscle force and power abilities might undermine the work performed by muscles and might require greater energy demands (i.e. low economy) during walking (Mian *et al.*, 2006). An impaired walking ability in elderly, due to muscle disuse, might lead these people to further reduce their physical activity levels with severe consequences on the achievement of their daily tasks (Kortebein *et al.*, 2008). A deeper comprehension of these physiological perturbations related to muscle disuse in elderly might require efforts that go beyond the evaluation of the ability of the muscle to generate force and power. An investigation that can put together information regarding the motor control of walking, the work performed, and the energy spent by muscles during its execution might help to understand at what extent muscle disuse influences the performance of this motor action in elderly people. Such investigations are lacking in the current scientific literature.

Studying the effects of muscle disuse on the neuromuscular function of older individuals might be of clinical importance. Indeed, the understanding of the consequences of these alterations might help to organize well-designed counter-measures (i.e. physical training, nutritional interventions, ect.) that can help this population to preserve their physical independence and a good quality of life after periods of inactivity. Among all the counter-measures proposed in literature, physical training has shown to be a promising one. Resistance exercise training, in particular, has been observed to produce positive results on the neuromuscular system by increasing the muscle mass both in young (Welle *et al.*, 1996) and in older subjects (Peterson *et al.*, 2011). Furthermore, researchers have found that even muscle performance (i.e. muscle force and power) is retained with this type of intervention (Izquierdo *et al.*, 2001; Caserotti *et al.*, 2008; Peterson *et al.*, 2010). Iso-inertial resistance training, in particular, have resulted to promote even greater muscle power enhancements than the ones obtained by traditional gravity-dependent resistance training (i.e. weight stuck, etc.) (Onambele *et al.*, 2008; Naczk *et al.*, 2014; Maroto-Izquierdo *et al.*, 2017a). This training modality has been studied since its first introduction (Berg & Tesch, 1994), about twenty-five years ago, but only recently it has received more consideration. Indeed, still little is known regarding the exercise intensity and volume that should be prescribed with this type of training. Moreover, further researches are needed that might help to understand which of the neuromuscular adaptations could be evoked by the iso-inertial training and if it should be consider a better training method to improve specific muscle function characteristics compared with traditional gravity-dependent resistance training. Anyway, the age of the participants, involved in a resistance training program, might mitigate the muscle adaptations and the functional outcomes that the physical intervention can produce. In fact, muscle protein synthesis

and anabolic signaling in an aged muscle have been shown to behave differently compared with a young one after being exposed to traditional high intensity resistance exercise (Kumar *et al.*, 2009). A similar response has been observed in term of force gain when a resistance training program showed to promote a greater increase in maximal muscle strength in young than older adults (Lemmer *et al.*, 2000). Therefore, young and old people might respond to the resistance training stimuli to a different extent. In agreement with this observation researchers have found age-related differences in recovery of the neuromuscular function when strength training was proposed to both young and older people after a short period of muscle disuse (Hvid *et al.*, 2010). Precisely, retraining fully restored muscle mechanical function and muscle fiber area in young adults only. These data suggest that older individuals might require longer time to recover from muscle function alterations caused by muscle disuse.

Based on the previous results it emerges that muscle disuse might produce deleterious effects on muscle performance especially in older people. However, physical training might help them to recover the muscle health loss during those periods of inactivity. Physical training, though, might be important not only immediately after muscle disuse, but it might play an even greater role when it is proposed as a preventive measure. If studies show that muscle force in elderly people can decrease by a certain entity because of a muscle disuse period, a physical training program, organized before it, should be able to induce improvements in muscle function of at least the same extent. Either as a preventive measure or as a rehabilitation strategy, physical training in elderly should be optimized and efficient. Inadequate physical interventions might expose them to the deleterious effects of ageing where the risk for the occurrence of physical disabilities and loss of independence is always present. Hence, further research, focused on understanding the effects of various types of physical training on muscle health and functional ability in the elderly population, should be encouraged.

### 1.1.1 Population ageing

In agreement with *World Population Prospects: the 2017 Revision* (United Nations, 2017b) world population reached 7.6 billion units in the mid 2017. Compared to 1993 and 2005 situations global population increased by 2 billion and 1 billion units, respectively. In 2017 the percentage of people aged 5 or below was 9% compared to the total global population; the 26% were under age 15; the 13% aged 60 or over and the 2% were 80 or over. Based on the indexes of fertility, mortality, migration and the age distribution profile of each country in the world, the report estimates that global population will keep growing at least until 2050. Statistical projections, presented in the revision document, indicate that there is a 95% probability that global population will reach values between 8.4 and 8.7 billion of units by 2030. By 2050 the total amount of global population will be very close to 10 billion of units. Indeed, although in recent years fertility index has declined in all regions of the world the net value is still positive with some specific countries that are contributing to a greater extent in keeping this index high over 0. Africa, with a population of 1.2 billion of units and 41% of people aged 14 or below has currently the highest fertility levels of any other region in the world (4.7 births per women in 2010-2015). Europe, on the other hand, is an exception to this trend. It has a far lower fertility index compared to Africa but instead its index has increased from 1.4 in 2000-2005 to 1.6 in 2010-2015.

However, fertility and longevity indexes are expected to decrease and increase, respectively, in the next 30 years in almost all the region of the world. This prospect might lead to population ageing. Indeed, the percentage of people aged 60 or over is expected to double by 2050, shifting from 962 million of units in 2017 to nearly 2.1 billion of units. Similar trend has been prospected to happen for the percentage of people aged 80 or over. Precisely, this age class of population will experiment to grow even faster than the number of older persons overall (age 60 or over). They will rise from 137 million of units in 2017 to almost 425 million of units in 2050. Europe has currently 25% of people aged 60 or over and projections suggest that this number will rise to reach 35% by 2050. Similar trends are expected to involve other regions of the world: Latin America, the Caribbean, Asia, North America, Oceania and eventually Africa. Interestingly, the *World Population Ageing 2017* report (United Nations, 2017a) shows that in 2050 it is expected that nearly 8 in 10 of the world's older persons will be living in the developing regions.

Hence, as written on the United Nation website, population ageing might have all the right characteristics to become one of the most significant social transformations of the twenty-first century. It might have implications in many fields of nowadays society: from labour to financial markets and from healthcare to pension system. It is therefore important to organise a plan of action and start to face this global issue right away.

### 1.1.2 Sarcopenia

In this paragraph the thesis explores one of the most troubling consequences of ageing in humans: the progressive and inevitable reduction of muscle mass. As will be seen below, this phenomenon heavily contributes to the decline in the physical functioning of older people (> 65 years of age). It might lead to the development of physical limitations, loss of independence, reduction in the quality of life and eventually death. This paragraph together with the previous one might help the reader to understand the importance of the challenge that the nowadays society will face in the next 20-30 years. As the number of old people will increase in the world population so it will do the probability of this people developing physical function limitations and movement diseases. It results that the economic, healthcare and pension systems of each nation will be severely stressed with serious consequences at the social level.

Age-related muscle mass loss is called sarcopenia. This term was first proposed by Rosemberg (Rosenberg, 1989). It is composed by the Greek words *sarcos* and *penia* which mean *flesh* and *a lack of*, respectively. A lot of studies in the scientific literature have attempted to quantify the loss of muscle mass as a function of advancing age (see for review (Mitchell *et al.*, 2012)). However, there is still little consensus in establishing what mathematical relationship better describes the behaviour of the two variables. The use of different techniques to evaluate muscle mass in humans might explain part of the discrepancies found between studies. It is estimated that decrements in muscle mass go from 8% to 49% between the second and the eighth decades of life. It has been calculated that the median of the values of the rate of muscle mass loss, reported across different studies, is 0.37% each year for women and 0.47% for men (Mitchell *et al.*, 2012).

Sarcopenia is a condition that might not influence each muscle in the same way. Postural muscles are more vulnerable to age-related muscle mass loss than non-postural ones. In particular a study finding shows that the lower limbs are prone to lose their muscle tissue more than the upper limbs and this happens both in men and women without distinction (Janssen *et al.*, 2000). As written by Narici and Maffulli (Narici & Maffulli, 2010), this last point might not seem so reasonable to happen given the importance that has the locomotion in everyday life. Indeed, the constant use of lower limbs muscles in order to move human body in the surrounding space should keep these muscles better trained compared to the upper limb ones. Nevertheless, the greater muscle mass loss experienced by the former might be explained by lifestyle (a reduced level of physical activity) and/or neurophysiological changes (specific motor units loss that involves lower limb muscles to a greater extent).

The loss of muscle mass which characterized sarcopenia occurs partially because changes at the level of the single muscle fiber are involved. With ageing it has been shown that there is a decrease in muscle fiber size and number (i.e. hypoplasia). Size alterations might be fiber type specific: type II

fibers have been observed to go through greater atrophic processes (i.e. becoming thinner) compared to type I fibers (Larsson *et al.*, 1978; Klitgaard *et al.*, 1990; Brunner *et al.*, 2007; Murgia *et al.*, 2017). The current view regarding the occurrence of hypoplasia is that the total number of muscle fibers might decrease with increasing age and it might concern type I and type II fibers indistinctively (Lexell *et al.*, 1988; Klein *et al.*, 2003; Brunner *et al.*, 2007). Muscle fibers are also classified by their myosin heavy chain (MHC) isoforms content. MHC isoforms are elements of the muscle fiber that are responsible for its power generation capacity. There are 3 MHC isoforms in humans: MHC-I, MHC-IIa, MHC-IIx. A single muscle fiber can express more than an isoform simultaneously (i.e. there are fibers that contain both MHC-I and MHC-IIa, and fibers that contain both MHC-I and MHC-IIx). Fibers expressing MHC-I only have lower shortening velocity, rate constant tension raise and tension cost values than fibers containing MHC-IIx. MHC-IIa fibers have intermediate properties (Canepari *et al.*, 2010). Ageing might cause a greater co-expression of different MHC isoforms within muscle fibers (Andersen *et al.*, 1999; D'Antona *et al.*, 2003). Indeed Andersen and colleagues found that more than 50% of the 2264 muscle fibers extracted from vastus lateralis muscle of very old people (average age: 88 years), co-expressed more than one MHC isoforms (Andersen *et al.*, 1999). Rowan and colleagues have suggested that denervation processes might be responsible for the manifestation of increased co-expression of MHC isoforms and muscle fibers atrophy in advance age (Rowan *et al.*, 2012). Cycles of denervation and reinnervation of muscle fibers occur continuously during the life of the individual. Precisely muscle fibers are characterized by a temporary disconnection from their original motor neuron. Subsequently reinnervation processes take place and a new contact might be built between rather the denervated muscle fiber and its original motor neuron or the fiber and a motor neuron that belongs to another motor unit (Hepple & Rice, 2016). With ageing though, these mechanisms might become impaired. Due to alteration that concern the neuromuscular junction components a fraction of the denervated muscle fibers might not be reinnervated again and they progressively become atrophied (Hepple & Rice, 2016).

Sarcopenia develops at different velocity during ageing. Muscle mass loss is accelerated at ages beyond the 80% survival rate in humans (>70 or 80 years) (Lexell *et al.*, 1988). This acceleration might be the consequence of an increased loss of motor neurons and motor units that have been shown to happen in old adults aged 80 or over (McNeil *et al.*, 2005). However, the development of sarcopenia is not only due to neuropathic changes although these are probably the most important causes. The aetiology of sarcopenia involves several other factors such as hormonal, immunological, nutritional and lifestyle (i.e. a reduced level of physical activity) changes (Narici & Maffulli, 2010). The result of all these modifications is a physical performance decrease that is linked to a reduction in the muscle ability to produce force and power. Like the progression of muscle loss with ageing the

physical functioning declines with a non-linear pattern during human life. It accelerates after 50 years of age (Wilmore, 1991) and it becomes further impaired after the 8$^{th}$ decade of life in response to the greater muscle atrophy shown to exist at this stage. It has been previously reported that the reduction in muscle force occurs prior to remarkable muscle mass loss (Goodpaster *et al.*, 2006). Even when muscle loss is significant muscle force declines to greater extent compared to the degree of muscle atrophy alone (Goodpaster *et al.*, 2006). These observations might be explained by the presence of impairments at the level of the nervous system and support the view that neuropathic alterations might play an important role in determining physical performance especially in older adults. The greater decline in muscle force once muscle atrophy becomes evident might result in a depression of the specific muscle tension (i.e. force divided by the muscle cross-sectional area). Findings from previous studies reveal that older people have a lower specific tension compared with young individuals (Jubrias *et al.*, 1997; Macaluso *et al.*, 2002). Nevertheless, beyond the neurological factors there are others that might contribute to produce this decline in specific tension: force alterations at the level of the single muscle fiber (Larsson *et al.*, 1997) and mechanical changes in tendon properties (Narici & Maganaris, 2007) are the most plausible ones.

Together with muscle force also muscle power decrease with advancing age. Indeed Power and colleagues report that contractile speeds, rates of force development and muscle relaxation are slowed in older adults compared with young individuals (Power *et al.*, 2013). Precisely ageing causes muscle fibers fascicle to be shorter in elderly men when young controls matched for height, body mass and physical activity were taken into account in the experiment (Narici *et al.*, 2003). Similar result has been found for pennation angle (i.e. the angle of insertion through which the fibers connect to the tendon aponeurosis). Older adults have a lower pennation angle compared with young counterparts (Narici *et al.*, 2003). A decrease in fascicle length might be the consequence of a loss of sarcomeres in series reflecting an impairment of muscle shortening velocity. On the other hand, the reduction in pennation angle might be due to a loss of sarcomeres in parallel that might produce a diminished muscle force. These findings are in agreement with the fact the power decreases with advancing age, since it is the product of muscle force and muscle shortening velocity. Although quadriceps muscle force has been demonstrated to be a stronger predictor of mortality in older adults than muscle mass (Newman *et al.*, 2006), lower limb muscle power might be considered a more critical determinant of physical functioning than muscle strength in this population (Reid & Fielding, 2012). For instance, Suzuki and colleagues have found that compared with muscle strength, muscle power of the ankle plantar and dorsi flexors better predicted the chair rise and stair climb performances (i.e. motor tasks involved in everyday activities), respectively, in a sample of community-dwelling women (ages: 65 – 84 years) (Suzuki *et al.*, 2001). Hence it might be of clinical relevance to note that lower limb

muscle power impairments (3.5% / year) occurs more rapidly than muscle force ones (1-2% / year) (Skelton *et al.*, 1994). All these findings make the lower limb muscle power an important index that needs to be constantly monitored during ageing to evaluate the physical health of older people. A lot of everyday activities require the individual to not only express muscle force but especially velocity of contraction, and so power (i.e. walking, stair climbing, rising from a chair, ecc.). Muscle power requirement might become extremely important in maintaining balance and locomotion. Indeed, by using neuromechanical simulations of human walking Song and Geyer (Song & Geyer, 2018) have found that the loss of muscle strength and muscle contraction speed, due to sarcopenia, largely contribute to the reduced walking economy (Martin *et al.*, 1992; Mian *et al.*, 2006) and speed (Himann *et al.*, 1988) observed in the older population. Muscle power requirements are also important in falls prevention which are common among older adults (Suzuki *et al.*, 1992). In a fall event the capacity of an individual to quickly restore the perturbated balance might lie primarily on their ability to generate force and contraction speed (Schultz *et al.*, 1997). Hence the progressive muscle power reduction that characterized older people might threaten their ability to safety walk, increasing the risk for the occurrence of falls and fall-related injuries that might eventually lead to lose their independence.

### 1.1.3 Ageing and walking performance

Human walking is generally defined as an automatic process which requires, beyond the activation of the musculoskeletal system, the participation of either cortical or subcortical and spinal nervous structures (Malone & Bastian, 2010; Pearson, 2013). Conscious control of walking might become necessary in the initiation of the movement, when task perturbations occur (i.e. challenges from the surrounding environment), when the individual needs to face new physical challenges, and in the case of injuries (Takakusaki, 2013). To sustain walking energy must be spent. The metabolic cost (Cw) of walking is the amount of energy required by the individual to move 1kg of body mass over a unit distance (i.e. 1 meter) and is determined by several factors such as: generating force to support body weight, performing work to redirect and accelerate the centre of mass from step to step and swinging the limbs and maintaining stability (Grabowski *et al.*, 2005). Precisely, the energy spent during resting is not considered in the calculation of Cw. The activation and contraction of skeletal muscles move the limbs and produce work. The total amount of work done by the muscles divided by the metabolic cost represents the efficiency of walking (Cavagna & Kaneko, 1977). Hence efficiency, metabolic cost and mechanical work are all indexes of the walking performance. Ageing leads to a deterioration of these indexes. Indeed, there is evidence that shows the presence of a greater metabolic cost in older adults compared with young ones (Martin *et al.*, 1992; Mian *et al.*, 2006). Nevertheless, the causes behind this alteration are still not well-understood. Different experiments have been made to investigate what factors might dominantly explain the greater cost of transport (i.e. metabolic cost) observed in the old population during walking. These research findings have suggested that lateral stabilization cost (Ortega *et al.*, 2008), basal metabolism and mechanical work requirements (Mian *et al.*, 2006) might not play a crucial role in determining this phenomenon. Mian and colleagues (Mian *et al.*, 2006) have found though, that muscle activation patterns and especially co-contraction strategies of agonist-antagonist muscles, developed as adaptations to safely walk, might contribute to decrease the economy of walking in the older population. Other causes that can explain the decreased walking performance with ageing might be the loss in muscle mass, force and power. From researches conducted on cerebral palsy and stroke patients it emerges that muscle force might be an important prerequisite for the preservation of the walking capacity (Desloovere *et al.*, 2006; Bohannon, 2007; Ross & Engsberg, 2007). Although still debated studying the effects of the diminished muscle force capacity on the walking performance in humans might be extremely difficult. As suggested by Song and Geyer (Song & Geyer, 2018) this is because in human experiments it is impossible to analyse independently the influence of each single age-related alteration on walking features. To overcome this problem simulation models of human walking have been implemented in some recent studies (van der Krogt *et al.*, 2012; Song & Geyer, 2018). Their results reveal that the decline in the capacity

of the muscle to produce force, especially if localized at the level of lower limb muscles, might explain well the greater metabolic cost of walking observed in older people.

The quest for the causes of the deterioration of walking performance in the old population is still vivid as demonstrated by recent studies on this topic. Although multifactorial there might be major determinant elements that lead to the decline in the walking capacity such as a decreased in muscle force and increased in co-contraction activity of agonist antagonist muscles. Interventions orientated to alter these last features might further help to understand their contribution in the determination of walking performance in older people. They might also serve to understand what elements need to be strengthened for rehabilitation purposes.

### 1.1.4 Ageing and skeletal muscle disuse

Periods of inactivity (i.e. muscle disuse) are common in older population (Suetta *et al.*, 2007). The occurrence of these periods might be due to lifestyle habits or other situations such as episodes of illness, disease or injury (English & Paddon-Jones, 2010). The reduced physical activity that characterized muscle disuse events with ageing leads to a further loss of muscle mass and a decrease in physical performance which might threaten the normal execution of activity of daily living. Alteration in the performance of motor tasks such as walking, climbing the stairs or rising from a chair might contribute to decline the quality of life of older people. Such actions might progressively require an increased physical effort and the individual might experience fatigue rapidly. Based on these premises older people, who underwent to disuse periods, might become more prone to interrupt the execution of the ongoing activity or they even might abstain from performing easy motor tasks. This situation might drive them to enter a vicious circle (Kortebein *et al.*, 2008) where physical activity results more and more depressed. As motor activity reduces so it does the physical capacity of the individual. If these physical characteristics deteriorate beyond specific muscle force and cardiorespiratory fitness levels the individual might lose their motor independence (Shephard, 2009). The progress of this vicious circle might result in an increased risk of experiencing injuries and physical complications (i.e. increased risk for falls and fractures), which might require hospitalization and eventually the admission to special care structures (i.e. institutionalization) (Covinsky *et al.*, 2003). The severity of the consequences of muscle disuse especially in the older people might be exacerbated by the fact that they hardly regain all the muscle mass lost during periods of inactivity (Suetta *et al.*, 2013). The accumulation of muscle disuse events during advanced age might significantly contribute to the etiology and development of sarcopenia (English & Paddon-Jones, 2010; Wall *et al.*, 2013).

Muscle disuse has been studied in older and young people by using specific lab settings which include bed rest and limb immobilization experiments. While several researches have examined the effects of muscle inactivity on young people only few studies have investigated the combined effects of muscle disuse and ageing on healthy organisms. No matter who are the subjects studied (i.e. young or old) it emerges that the longer is the muscle disuse event the more severe are the alteration in the muscle tissue and physical capacity of the individual (Narici & de Boer, 2011). Indeed, it has been observed that long bed rest trials (i.e. > 20 days) might cause lower limb muscle volume to decrease up to 30% of the value collected before the experiment took place (LeBlanc *et al.*, 1988; LeBlanc *et al.*, 1992; Ferretti *et al.*, 2001). On the other hand, short bed rest experimentations (i.e. < 20 days) might induce a reduction in muscle volume of only 3% to 12% (Ferrando *et al.*, 1995; Kubo *et al.*, 2004). The atrophic response to muscle disuse, though, might be age-dependent. Suetta and

colleagues (Suetta *et al.*, 2009) have shown that a greater decline in lower limb muscle volume occurred in young compared with older people after 2 weeks of limb immobilization (-8.9% vs -5.2% in the young and the old group, respectively). On the contrary Tanner and colleagues found that leg lean mass and strength decreased in older but not in younger adults after 5 days of bed rest challenge (Tanner *et al.*, 2015). Interestingly researchers agree that lower limb muscles are more vulnerable to disuse-induced atrophy than upper limb ones and this phenomenon might occur in young and older population without distinction (Desplanches *et al.*, 1998; Narici & de Boer, 2011).

The reduction in muscle mass and volume that characterized inactivity might reflect single muscle fiber atrophy. Muscle fibers decrease in size but not in number (Roy *et al.*, 1987). Scientific evidences suggest that the atrophic response to muscle disuse might be fiber type specific. In particular the slow type I fibers might be more prone to develop size alteration than the fast ones (Hortobagyi *et al.*, 2000; Haus *et al.*, 2007). However, these findings are still debated because significant size changes have been reported to occur also in fast II A fibers (Jaweed, 1994).

It is interesting to note that the major drivers of disuse atrophy are related to muscle protein turnover. Despite conflicting results regarding the role of muscle protein breakdown during inactivity periods, several research findings support the fact that blunted post absorptive and post prandial muscle protein synthesis characterize muscle disuse events (Tanner *et al.*, 2015; Rudrappa *et al.*, 2016). Hence muscle protein synthesis rather than muscle protein breakdown appears to be the major determinant of the atrophic disuse-related response. Alteration in muscle protein turnover might also explain why older adults seem to be more susceptible than young persons to muscle loss after short-term bed rest (Tanner *et al.*, 2015).

Parallel to the decrease in muscle mass and volume there might be a reduction in the ability of the muscle to produce force and power. Similar force decrements have been observed after 2 weeks of leg immobilization protocol between young and old subjects (-15.7% vs -19.8% in old and young respectively) (Suetta *et al.*, 2009). However, this deterioration in muscle performance might even be more severe than the atrophic response observed after inactivity. For instance, Kubo and colleagues found that while knee extensor muscles volume declined by only 10% after 20 days of bed rest their force ability resulted depressed by 20% (Kubo *et al.*, 2004). This phenomenon might be associated with a decrease in muscle specific force that have been reported to happen after muscle disuse periods both in young and older people (Suetta *et al.*, 2009). As in ageing muscle specific force results depressed because of several factors (i.e. alteration at the single fiber level, changes in neural drive and in tendon characteristics). At the level of the single muscle fiber the specific tension might be impaired due to a decrease in myosin concentration (i.e. a disproportionate loss of myosin content with respect to cross sectional area of muscle fibers) (D'Antona *et al.*, 2003). The fact that neural

factors might also play a role in decreasing muscle specific force has been shown to occur by some research groups (Kawakami *et al.*, 2001; Clark *et al.*, 2006). Maximal voluntary activation of the knee extensor muscles has been found to decrease after 20 days of bed rest. Central activation changes have also been reported by Clark and colleagues (Clark *et al.*, 2006). These authors suggested that the deficits in central activation observed after 4 weeks of unilateral lower limb suspension (i.e. muscle disuse protocol) might contribute significantly to the disuse-related force deterioration.

All these observations and findings show that disuse periods induce deleterious effects on the neuromuscular system of both young and older adults. Among these two populations the latter is more susceptible to the severe consequences of inactivity. This because older adults might develop sarcopenic condition which, as seen previously, represents a threat for their physical functioning. Hence disuse events combined with concurrent sarcopenic processes might further deteriorate the physical capabilities of the older individuals. They exacerbate the risk for the occurrence of injuries and physical complications which lead to the loss motor independence and, in some circumstances, premature death.

### 1.1.5 Ageing and strength training

Physical exercise has been shown to be a promising countermeasure to attenuate physical performance deterioration with advancing age. Resistance exercise training, in particular, has been observed to produce positive results in preserving and even improving muscle mass (Peterson *et al.*, 2011), force (Peterson *et al.*, 2010) and power (Izquierdo *et al.*, 2001; Caserotti *et al.*, 2008) in older people, slowing down the progression of the sarcopenic process (described in previous paragraphs). Researchers have shown that 10 to 14 weeks of strength training using heavy loads ( > 70% 1RM) have lead to modest increases (+5% - 12%) in muscle cross sectional area (i.e. CSA) and volume in elderly individuals (Frontera *et al.*, 1988; Reeves *et al.*, 2004a; Suetta *et al.*, 2004). On the contrary Aniansson and co-workers (Aniansson *et al.*, 1986) have found that low-intensity resistance training produced more limited results in a similar population. This last finding suggests that elderly might have a lower capacity to respond to strength training compared with young individuals. In support of this observation Kumar and colleagues have found more depressed muscle protein synthesis and anabolic signaling responses in older that in young people when both the groups were exposed to the same exercise training stimuli (Kumar *et al.*, 2009). Although the effects of resistance training might be blunted with advancing age some researchers have also demonstrated that the capacity for strength improvement might be preserved even in extremely old persons (i.e. age > 90) (Fiatarone *et al.*, 1990). Strength training might induce changes at the level of the single muscle fiber area and in muscle architecture in old subjects. Indeed, Frontera and colleagues reported that both type I and type II fibers increased their area by 33.5% and 27.6% respectively, after 12 weeks of heavy strength training (Frontera *et al.*, 1988). Muscle architecture changes include gains in fiber pennation angles. These improvements in muscle architecture configuration have occurred both in vastus lateralis and gastrocnemius muscles (Reeves *et al.*, 2004b; Morse *et al.*, 2007). Similar increments have been observed in young individuals (Aagaard *et al.*, 2001) but they took a shorter time to be manifested compared with older people (Suetta *et al.*, 2009).

Strength training have shown to produce neural changes with advancing age. It improved the rapid force production capacity (RFD) (Hakkinen *et al.*, 1998; Hakkinen *et al.*, 2001) and the ability of the central nervous system to recruit and activate muscles (Morse *et al.*, 2007) during maximal isometric contraction tasks. In particular RDF gains might be due to the increase in motoneuron firing frequency observed in older people after training (Kamen & Knight, 2004).

Changes in motor control strategies occur with ageing. The most evident age-related alteration in motor control is the amount of agonist/antagonist muscle co-contraction required during the execution of motor tasks. High levels of antagonist co-contraction is interpreted as compensatory mechanisms to increase joint stiffness and stability (Hortobagyi & DeVita, 2000) and it might contribute to

increase the metabolic demand of a motor action (Mian *et al.*, 2006). Elderly adults might have an elevated antagonist muscle co-contraction during simple (i.e. leg flexion) (Hakkinen *et al.*, 2001) and complex movements (i.e. walking, stair climbing, etc.) (Hortobagyi & DeVita, 2000). Strength training has been reported to decrease antagonist muscle co-contraction in simple task only (Hakkinen *et al.*, 2001). Nevertheless, complex daily motor actions might result unaffected by resistance training.

Among the different types of resistance training isoinertial flywheel resistance training program (FW) has recently received increasing attention. Introduced firstly by Berg and Tesch (Berg & Tesch, 1994), more or less than twenty five years ago, as a device to counter act the adverse consequences of the exposure of the neuromuscular system to microgravity in astronauts during spaceflights, it has become popular in different sport and movement fields: athletic performance (Gual *et al.*, 2016), rehabilitation (Greenwood *et al.*, 2007) and ageing (Onambele *et al.*, 2008). The reason why this device has having such success is manifold: compared to the traditional isotonic exercise (i.e. done using free weights or weight stack machines, GD) i) it allows the subject to perform a maximal concentric force throughout the range of motion and it requires them to generate an eccentric force greater than the concentric one, provided a maximal effort during the execution of the exercise and an appropriated technique (i.e. delayed breaking action during the eccentric phase); ii) it ensures accommodated resistance; iii) it permits the subject to generate maximal force since the beginning of the exercise leading progressively to a decrease in the force developed (Tesch *et al.*, 2017). Although these results might favour the prescription of FW resistance training by physical experts more than the GD, recent reviews on this topic ended up to divergent conclusions. While the review of Maroto-Izquierdo and colleagues (Maroto-Izquierdo *et al.*, 2017b) concluded that FW resistance training is superior to GD exercise in promoting muscle strength, power and size gains in healthy subjects and athletes, Vicens-Bordas and his team (Vicens-Bordas *et al.*, 2018) pointed out that the two trainings had similar outcomes in term of strength improvements. The comparison between these two types of training regarding their efficacy to produce muscle adaptation and functional enhancement might be of clinical interest in elderly population.

## 1.2 Aims of section one

Based on the premises described in the introduction, the aims of the current thesis are the following:

1) to investigate the effects of a 14-day bed rest period (i.e. muscle disuse) and the subsequent 2-week physical training on the metabolic cost (Cw), the mechanical work, the efficiency and the co-contraction time of agonist-antagonist lower limb muscles during walking in older and young healthy adults.

2) to investigate the effects of a 14-day bed rest period and the subsequent 2-week physical training on the maximal force and power production of lower limb muscles in older and young healthy adults with the intent to examine different factors that contribute to the occurrence of these alterations and their related recovery.

3) to study the differences between young and older adults due to bed rest and the subsequent physical training period in: i) the metabolic cost, mechanical work, efficiency and co-contraction time of agonist-antagonist lower limb muscles during walking, ii) maximal muscle force and power production, iii) muscle volume, muscle fiber properties and motor control

4) to study the effects of two different types of resistance training programs (8-week period of Iso-inertial vs gravity-dependent) on maximal strength, maximal explosive power and muscle architecture in healthy older subjects only; to further investigate the effects of these two types of training on the metabolic cost and motor control during walking in older subjects; to find evidence that might help to understand if one training differentiates from the other in producing neuromuscular adaptations that might protect against the deleterious effects of muscle disuse periods in older people.

## 1.3 Main results of section one

The main results from the works collected in the present thesis are the following:

1) 14 days of bed rest and the following physical training did not influence metabolic cost, mechanical work, efficiency and co-contraction time during walking at different speeds both in the older and young groups. Human walking is recognized to be an automatic and well optimized process regulated by subcortical and spinal structures rather than cortical ones. Due to this intrinsic nature walking resulted to be preserved after a prolonged period (i.e. 14 days) of muscle disuse independently from the age od the individuals. Although muscle power and force decreased in both groups because of the inactivity (see below), walking performance appeared to be more resistant to the disuse stimulus. From these findings it can be suggested that walking on flat terrain might be proposed and encouraged immediately after prolonged muscle inactivity events (i.e. up to 14 days) both in young and in old adults. From a rehabilitation point of view this result might represent a meaningful basis to work on. Firstly, the preservation of normal walking means that the individual is still physical independent, and this is extremely important with advancing age. In addition, walking stimulates dynamic balance, motor control and, in appropriate conditions (i.e. from flat to steeper surfaces), intense cardiorespiratory and muscle force/power responses. Hence it might be proposed also as a form of specific training to enhance the general fitness of the individual after muscle disuse experiences.

2) 14 days of bed rest induced a significant decrement of the maximal explosive power both in the older and young groups. The subsequent 2-weeks physical training intervention restored muscle power in the young group only.
Muscle disuse influenced the ability of the lower limb muscles to generate force and power in both young and old adults. Similar findings have already been reported in the scientific literature. Particular attention on the re-training intervention though has been dedicated by the present study. Indeed, while young adults were able to regain completely the muscle power and force capacity lost during the prolonged disuse condition, old adults presented an incomplete recovery in function (i.e. maximal explosive power not fully recovered). Although the retraining intervention proposed in this study was not specific for power enhancement young adults still restored completely their physical function (i.e. maximal muscle force and power). Hence from these results it might be postulated that old individuals require a more specific and intense training stimulus compared with young adults aimed at exclusively developing muscle power. Thus, resistance training (i.e. isotonic weight bearing

machine, isoinertial systems) seems to play an important role in regaining the physical function lost during prolonged disuse events in old people.

3) before, after the 14 days of bed rest and after the 2-week physical training program the older group showed higher metabolic cost and lower efficiency values of walking compared with the young one; furthermore, co-contraction time of proximal and distal muscles were higher in the older than in young sample across the different walking speeds. These results support the view that agonist/antagonist co-contraction levels of lower limb muscles might contribute to the increased metabolic cost of walking observed with ageing. In particular, while most of the previous studies have examined the causes of ageing-induced increase in the metabolic cost of walking in elderly individuals (i.e. > 65 years) we have widened this investigation investigating the same causes in old adults (i.e. 55 to 64 years).

4) i) the young group generated a greater maximal explosive power than the older one across the three time periods analysed (before, after bed rest and after the physical training); ii) compared with the young sample, the older one showed a greater abundance of slow fibres and thinner and less abundance of fast fibers in vastus lateralis muscle; iii) similar levels of co-contraction at the knee and ankle joints was observed in both groups during the execution of maximal explosive power task. These findings show that specific maximal explosive power generated with lower limb muscles is greater in young compared with older adults independently from the protocol condition (before, after BR and after the physical re-training). The fiber type distribution (i.e. the abundance of the different fibers within the muscle) and the selected atrophy of type II fibers in older people might have played a role in determining the differences in the specific power between the young and old adults.

4) Eight weeks of iso-inertial training induced slightly higher improvements in maximal power gains of the lower limbs than the traditional gravity-dependent training. Both the trainings equally contribute to produce improvements in maximal muscle strength and physical fitness levels. None of the trainings promoted changes of muscle architecture. Furthermore, no effect of the training intervention, regardless of the exercise modality, was observed on the metabolic cost and co-contraction time of agonist-antagonist lower limb muscles during habitual walking speeds. These findings suggest that compared with traditional isotonic training iso-inertial conditioning might be safe and recommended for older people to improve the ability of the muscles to generate power. Nevertheless, neither the iso-inertial nor the isotonic trainings might be able to induce remarkable changes in habitual walking performance of older adults.

## 1.4 Publications of section one

1) Effects of 14 days of bed rest and following physical training on metabolic cost, mechanical work, and efficiency during walking in older and young healthy males.
**Floreani M.**, Rejc E., Taboga P., Ganzini A., Pišot R., Šimunič B., Biolo G., Reggiani C., Passaro A., Narici M., Rittweger J., di Prampero P.E., Lazzer S.
PLoS One. 2018 Mar 12;13(3): e0194291. doi: 10.1371/journal.pone.0194291. eCollection 2018.

2) Loss of maximal explosive power of lower limbs after 2 weeks of disuse and incomplete recovery after retraining in older adults.
Rejc E., **Floreani M.**, Taboga P., Botter A., Toniolo L., Cancellara L., Narici M., Šimunič B., Pišot R., Biolo G., Passaro A., Rittweger J., Reggiani C., Lazzer S.
J Physiol. 2018 Feb 15;596(4):647-665. doi: 10.1113/JP274772. Epub 2018 Jan 19.

3) Eight weeks of iso-inertial resistance training improve maximal explosive power of lower limbs in healthy older adults.
**Floreani M**., Rejc E., Gambin S., Vavassori L., Lazzer S.
(working in progress)

PLOS ONE

RESEARCH ARTICLE

# Effects of 14 days of bed rest and following physical training on metabolic cost, mechanical work, and efficiency during walking in older and young healthy males

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## Abstract

In this study, we investigated: i) the effects of bed rest and a subsequent physical training program on metabolic cost (Cw), mechanical work and efficiency during walking in older and young men; ii) the mechanisms underlying the higher Cw observed in older than young men.Twenty-three healthy male subjects (N = 16 older adults, age 59.6±3.4 years; N = 7 young, age: 23.1±2.9 years) participated in this study. The subjects underwent 14 days of bed rest followed by two weeks of physical training (6 sessions). Cw, mechanical work, efficiency, and co-contraction time of proximal muscles (vastus lateralis and biceps femoris) and distal muscles (gastrocnemius medialis and tibialis anterior) were measured during walking at 0.83, 1.11, 1.39, 1.67 $m \cdot s^{-1}$ before bed rest (pre-BR), after bed rest (post-BR) and after physical training (post-PT).No effects of bed rest and physical training were observed on the analysed parameters in either group. Older men showed higher Cw and lower efficiency at each speed (average +25.1 and -20.5%, P<0.001, respectively) compared to young. Co-contraction time of proximal and distal muscles were higher in older than in young men across the different walking speeds (average +30.0 and +110.3%, P<0.05, respectively).The lack of bed rest and physical training effects on the parameters analyzed in this study may be explained by the healthy status of both young and older men, which could have mitigated the effects of these interventions on walking motor function. On the other hand, the fact that older adults showed greater Cw, overall higher co-contraction time of antagonist lower limb muscles, and lower efficiency compared to the young cohort

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throughout a wide range of walking speed may suggest that older adults sacrificed economy of walking to improve stability.

## Introduction

Walking is generally defined as an automatic process that is predominantly controlled by subcortical structures, and normally requires conscious control only in case of a challenging environment or perturbation [1]. Walking can be characterized by its efficiency, which is defined as the ratio between mechanical work performed by muscles and metabolic cost of walking (Cw) [2]. The metabolic cost of walking (Cw), expressed as the amount of energy spent above resting to transport 1 kg body mass over a distance of 1 m, is determined by several factors such as: generating force to support body weight, performing work to redirect and accelerate the centre of mass from step to step, swinging the limbs and maintaining stability [3].

Ageing is characterized by the developing of sarcopenia [4], which is directly responsible for loss of muscle strength, functional impairment, increased risk of falling, loss of autonomy, physical disability and poor quality of life [5–7]. In particular, loss of muscle strength in elderly people has detrimental effects on walking pattern, reducing freely chosen speed, step length and swing phase compared to young people [8–13]. Furthermore, elderly adults show impaired balance and proprioceptive abilities and reduced joint range of motion [14–16]. Although no consistent findings related to the effects of these neuromuscular alterations on the mechanical work requirements of walking in older adults have been shown [17], the interest on this topic is still vivid as reported by a recent review of Aboutorabi and colleagues [18]. Indeed, these authors have commented on a series of studies that found several differences in gait parameters between old and young individuals. In particular, it emerged that muscle weakness and lower extremity strength loss, due to sarcopenia, might influence muscle activation, stiffness and power at the level of single joints. Increased hip flexor power for compensating the reduced plantar flexor power might be an example of these sarcopenia-induced neuromuscular adaptation [11]. Hence, complex motor control mechanisms that may be adopted by older people might also alter the trajectory of their body centre of mass in walking, so leading to potential changes in their mechanical work requirements. This could be one of the reasons why Aboutorabi and colleagues stated that future studies oriented on analysing the differences in gait parameters between old and young individuals should be focused on investigating the effects of aging on the centre of mass displacement in elderly subjects [18]. Ageing-related neuromuscular adaptations may also lead to simultaneous greater activity of agonist and antagonist leg muscles (co-contraction) in the gait phases [17, 19]. These negative adaptations may play a role in determining the lower efficiency and higher metabolic cost of walking observed in older individuals [17, 20].

Disuse (i.e. bed rest) further increases the detrimental effects of ageing on metabolism and muscle protein turnover [21–24]. It is also well known that disuse induces skeletal muscle atrophy with consequent loss of force production [25]. In particular, postural muscles (i.e. knee extensors and ankle plantar flexors) are more susceptible to atrophy than non-postural ones in response to disuse and unloading [26–28]. The duration of disuse plays an important role in determining the amount of muscle atrophy: in fact, shorter bed rest (7 days) induced a relatively low decrease in thigh muscle volume (about 3%) [29], while longer bed rest (20 days) promoted a 12% and 10% decrement in plantar flexor and knee extensor muscle volumes, respectively [30]. Along this line, a 29% and a 18% decreases in triceps surae and quadriceps muscles volume, respectively, were found after 89 days of bed rest [31].

Physical inactivity or bed rest during hospitalization has been proposed as a primary factor contributing to the functional decline in elderly hospitalized patients [22, 32]. Decline in muscle force related to unloading condition may have negative effects on gait descriptors and walking motor control [21], leading to a decrease in the walking economy. Importantly, lower walking economy after disuse can further contribute to the reduced daily activity in the older population [33], leading to an inactive lifestyle and its deleterious adaptations in an already frail population.

An effective intervention for mitigating ageing-induced impairment of muscle function and physical functioning is represented by physical training. For example, isotonic and isoinertial resistance training improved lower limb muscle strength as well as functional balance during standing [34–36]. However, training interventions that did not require specific resistance training equipment were also found to be effective for improving physical functioning (i.e. stair climbing) [24, 37, 38].

Then, the main purpose of the present study was to investigate the effects of a 14-day bed rest and a subsequent 2-week physical training on Cw, mechanical work, and efficiency during walking in older and young healthy subjects. We hypothesized that the neuromuscular deconditioning induced by bed rest would have negatively influenced Cw, efficiency and mechanical work, and that these negative effects would have been greater in the older subjects. We also hypothesized that physical training performed afterward would have been sufficient for the reconditioning of these parameters. The secondary aim of this study was to investigate the difference in walking pattern between young and older adults (i.e. 55 to 64 years), as most of the studies examining the causes of ageing-induced increase in Cw are based on data collected from elderly individuals (i.e. > 65 years). We hypothesized that the higher Cw observed in older adults would coincide with higher mechanical work and higher co-contraction of representative thigh and leg antagonist muscles.

## Materials and methods

### Subjects

Sixteen older adult males (Older) and seven young males (Young) participated in this study (Table 1). All subjects had a full medical history and physical examination, with the routine haematology and biochemistry screens. None of the subjects experienced any significant disease and none was taking medications regularly or made use of any medication known to influence physical performance. The study was approved by the Slovenian National Committee for Medical Ethics at the Ministry of Health (Republic of Slovenia) and conformed to the Declaration of Helsinki. The purpose and objectives were carefully explained to the subjects and written informed consent was obtained from all of them.

**Table 1. Physical characteristics of older (N = 16) and young (N = 7) subjects before bed rest (Pre-BR), after bed rest (Post-BR), and after 2 weeks of physical training (Post-PT).**

| | Older (n = 16) | | | Young (n = 7) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Pre-BR | Post-BR | Post-PT | Pre-BR | Post-BR | Post-PT | G | T | G x T |
| Age (y) | 59.6 ± 3.4 | | | 23.1 ± 2.9 | | | 0.001 | | |
| Stature (m) | 1.73 ± 0.05 | | | 1.77 ± 0.07 | | | 0.192 | | |
| Body mass (kg) | 79.9 ± 12.3 | 77.5 ± 11.7 | 79.3 ± 11.6 | 74.8 ± 8.8 | 71.6 ± 8.3 | 74.4 ± 8.1 | 0.310 | 0.001 | 0.352 |
| Body mass index ($kg \cdot m^{-2}$) | 26.6 ±4.4 | 25.8 ± 4.1 | 26.5 ± 4.2 | 24.0 ± 2.4 | 22.9 ± 2.1 | 23.8 ± 2.2 | 0.142 | 0.018 | 0.284 |

Results are in mean ± SD. Significance by GLM of the main effects of Group (Older *vs* Young), Time (Pre-BR *vs* Post-BR *vs* Post-PT) and Group x Time interaction (G x T).

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## Study protocol

The experimental bed rest campaign was conducted at the Valdoltra Orthopaedic Hospital, Ankaran (Slovenia). The subjects spent 19 consecutive days at the hospital, including 14 days of bed rest [24]. During the 14-day bed rest, eight randomly selected older adults underwent daily 45 minutes of computerized cognitive training by navigating through virtual mazes with the use of a joystick and computer. The same eight older subjects also received a nutritional support based on 0.4 g whey protein/kg body weight/day at breakfast during the first 14 days of rehabilitation period. Since no significant effects of both cognitive and nutritional interventions were observed on reported parameters [39], the two older groups were pooled for statistical analysis [24]. During the whole bed rest procedure, constant surveillance and 24-hour medical care was provided and all subjects received an individually controlled normo-caloric diet [40] and passive physical therapy to avoid cardiovascular disorders. Subjects performed all daily activities in bed, were allowed to freely communicate, watch television and listen to the radio, read, use computer and to receive visitors. After bed rest, subjects underwent physical training, which was conducted in the same facility.

Full testing sessions were conducted one day before the beginning of bed rest (pre-BR) and the day after the end of the 14-day bed rest (post-BR). Each of them consisted in two parts: the first one concerned the assessment of anthropometric characteristics and body composition, while the second one focused on the evaluation of metabolic cost (Cw), mechanical work and muscular activation during walking at different speeds. Subjects walked constantly on a motor-driven treadmill (Zebris Medical GmbH, Isny, Germany) at the following speeds: 0.83, 1.11, 1.39, and 1.67 $m \cdot s^{-1}$. Each speed was maintained for 4 minutes, and there was no recovery between walking speed trials. All subjects were familiarized with walking on treadmill 2 days before the beginning of bed rest. They experienced walking at all speeds that were subsequently tested. The familiarization duration lasted about 15–20 minutes [17]. Subjects returned to the laboratory 2 weeks after the end of bed rest in order to perform the same testing sessions. During these 2 weeks, subjects underwent the physical training program reported here below.

## Physical training program

Subjects began a 28-day physical training program the second day after the end of BR; however, only the initial 14 days of physical training were considered in this study, i.e the same duration of the disuse period. Physical training consisted of 6 sessions in total (3sessions/week); each session, which lasted about 65 minutes, was followed by 1 or 2 days of routine daily activity. Physical training was aimed at reconditioning both the neuromuscular and aerobic systems, proposing a series of exercises that did not require specific training equipment so that they could be translated to any home and community environment. The first 12 minutes of each training session were devoted to warm-up; subjects performed 6 minutes of Nordic walking, its speed being determined from a 2-km walking test performed before BR, and 6 minutes of active stretching (10 exercises). Then, subjects performed 20 minutes of strength and balance exercises. This section started with half squat (1 set; 10 repetitions; overload: from no overload to a 6 kg ball held with both hands), and continued with a circuit training (30 seconds of exercise followed by 30 seconds of rest) comprised of 8 motor tasks. In particular, the following strength exercises focused on lower limbs were proposed: frontal and sagittal plane lunge, double leg heel raise with elastic resistance; hip extension with elastic resistance. Also, balance exercises mainly consisted of dynamic standing balance activities (i.e. standing on toes; standing on balance foams) and functional movements that involved reaching and passing objects. Strength and balance exercises were followed by 30 minutes of aerobic exercise (e.g. Nordic walking; brisk walking, running). The last 3 minutes were devoted to cool down

(relaxation and breathing exercises). Subjects' heart rate was preventively monitored during each training session. Training was conducted at the hospital and in the gym near the hospital and supervised by 6 physical trainers who instructed the subjects to properly perform the different exercises. All subjects performed all planned training sessions.

## Anthropometric characteristics

Body mass was measured to the nearest 0.1 kg with a manual weighing scale (Seca 709, Hamburg, Germany) with the subject dressed only in light underwear and without shoes. Stature was measured to the nearest 0.5 cm on a standardized wall-mounted height board.

## Metabolic cost of walking

In each testing session, measurements of resting oxygen consumption ($V'O_2$), carbon dioxide ($V'CO_2$) production and heart rate (HR) were carried out by using a metabolic unit (Quark-$b^2$, Cosmed, Italy). Ventilatory and gas exchange responses were measured continuously. The volume and gas analysers were calibrated using a 3-liter calibration syringe and standard calibration gases (16.00% $O_2$; 4.00% $CO_2$). Each participant stood quietly relaxed for five minutes whilst metabolic measures were being collected breath by breath. Real-time plots of $V'O_2$, heart rate, and respiratory exchange ratio were closely monitored during the last minute of each walking speed to ensure that metabolic steady state was reached. Data post processing included the calculation of mean values of $V'O_2$, $V'CO_2$ and HR over the fourth (and last) minute of each walking speed, which were considered for further analysis.

In addition, respiratory exchange ratio (RER) was monitored to ensure that it remained under the specific threshold of 1.0. All these precautions were required to indicate that metabolism was essentially oxidative. The metabolic cost of walking ($C_W$, $J\cdot(kg\cdot m)^{-1}$) was calculated by dividing net energy expenditure (obtained by subtracting pre-exercise standing $V'O_2$ from gross $V'O_2$ and converted to joules according to the formula given by Garby and Astrup [41], which accounts for the RER-dependent variation of $O_2$ energy equivalence) by speed and body mass.

## Mechanical work

Three-dimensional kinematic information was collected using two digital cameras (Basler—Pilot, Ahrensburg, Germany) sampling at 210Hz [42–44]. The cameras were positioned symmetrically 5 m behind the treadmill, spaced about 6 m one from the other with an angle between the respective optical axes of about 65°. Two cubic 1x1x1m metal boxes with markers at every edge were used for calibration purposes and for the setting of a laboratory frame of reference. The position of every marker was precisely measured so that the measurement error was less than 0.5 mm. The calibration plane was placed between the cameras locations and the subject so that it resulted precisely within the calibrated volume throughout the walking tests. A calibration video was recorded. Digitalization of the images from this video provided us with the coefficients required to perform the direct linear transformation (DLT) technique included in the video analysis software (see below). DLT technique has been proven to lead to very good results in reconstructing the three-dimensional data from photographic observations of either objects or human bodies engaged in locomotion [45–47]. Kinematic analysis of the following body segments was performed: head-trunk (ear lobe, anterior to tragus of ear-iliac crest), thigh (great trochanter-lateral epicondyle of femur), shank (lateral epicondyle of femur- lateral malleolus), foot (calcaneus-$5^{th}$ metatarsal head), upper arm (shoulder-elbow), and forearm (elbow-wrist) [17, 48]. Reflective markers (0.75 cm radius plastic ball) that contrasted the environmental lights and colors were positioned on the body reference points

mentioned above. A 3D software (SIMI motion 3D) was used to track and reconstruct the three-dimensional position of each marker. The tracked data were filtered through a moving-average filter (radius = 1) [49]. Appropriate regression equations [50] that considered anthropometric data of the 11 rigid segments (head-trunk, upper arms, forearms, thighs, shanks and feet) were then used to compute the position of the segments, the body center of mass ($COM_{wb}$), and mechanical work. This analysis was performed by using a custom-made Matlab routine (v6.3, Mathworks, Inc, USA).

Total mechanical work ($W_{TOT}$) performed during walking is described as the sum of two separate entities: the mechanical external work ($W_{EXT}$) and the mechanical internal work ($W_{INT}$) [2, 51]. $W_{EXT}$ represents the work done to raise and accelerate the $COM_{wb}$ within the environment [51], whilst $W_{INT}$ is the work necessary to accelerate the limbs with respect to the $COM_{wb}$ [2, 51]. We calculated $W_{INT}$ as the sum of the positive increments in the kinetic energy of each limb according to the procedure described by Mian and colleagues [17]. $W_{EXT}$ was calculated as the sum of positive increments in the total mechanical energy of $COM_{wb}$ in agreement with the procedure described by Mian and colleagues [17], which was already used also by our research group [49]. $W_{TOT}$, $W_{INT}$ and $W_{EXT}$ were obtained from ten consecutive representative strides (i.e. without anomalous movements of limbs, torsion of the head or trunk, etc.). We calculated the interchange of the mechanical energies of the centre of mass (pendulum-like mechanism) using the recovery index (R) as adopted by Cavagna and colleagues [52] and presented in the later work of Mian's group [17]. We determined the efficiency (EFF) of the total positive work produced during walking of each subject dividing $W_{TOT}$ by $C_{W.}$ We determined also the walking stride frequency (SF, expressed as strides·min$^{-1}$) by calculating the total number of peak vertical displacements of a marker positioned at the level of the calcaneus in the right foot, over each minute of walking.

## Surface electromyography recordings

Surface electromyography (EMG) was collected from four muscles of the right lower limb: *vastus lateralis* (VL), *biceps femoris* (BF), *gastrocnemius medialis* (GM) and *tibialis anterior* (TA). Pre-gelled surface EMG electrodes (circular contact area of 1 cm diameter, BIOPAC Systems, Inc., USA) were placed (inter electrode distance equal to 20 mm) at the following locations [53]: a) for VL at two-third on the line from the anterior superior iliac spine to the lateral side of the patella; b) for BF midway between the ischial tuberosity and the lateral epicondyle of the tibia; c) for GM on the most prominent bulge of the muscle; d) for TA at one third on the line between the tip of the fibula and the tip of the medial malleolus. In order to ensure a good electrode-skin interface, prior to the application of the electrodes, the subject's skin was shaved, rubbed with an abrasive paste, cleaned with an alcohol solution, and dry-cleaned with gauze. EMG data were sampled at a frequency of 2 kHz, and recorded by a four-channel electromyography system (EMG100C, BIOPAC Systems, Inc., USA; Band-pass Filter: 10–500 Hz; RMS Noise Voltage: 0.2 μV; Input impedance: 2 MΩ; Common Mode Rejection Ratio: 110 dB). In order to place electrodes in the same anatomical location during the three different experimental sessions, electrodes position was marked on acetate paper using moles and small angiomas (which may be assumed to maintain a fixed position) as reference points. The EMG electrodes were fixed at the beginning of each experimental session and were not removed between walking tests.

EMG raw signal recorded between the third and fourth minute of each walking bout performed by the subject was full-wave rectified and then low-pass filtered with a cut-off frequency of 10 Hz. The determination of EMG onset and offset activity of each muscle was achieved by using a computer-automated procedure [17]. Visual inspection of EMG activity

was added in order to monitor the suitability of the algorithm used. In particular, we calculated the amount of stride duration (% of stride duration) in which two representative antagonist muscles were active at the same time (co-contracted). Eight representative strides for each walking speed were considered to calculate the average co-contraction value for both proximal, thigh muscles (VL and BF) and distal, leg muscles (GM and TA).

### Statistical analysis

Statistical analyses were performed using PASW Statistic 18 (SPSS Inc., IL, USA) with significance set at $P<0.05$. All results are expressed as means ± SD. Normal distribution of the data was tested using the Shapiro-Wilk test. Sphericity (homogeneity of covariance) was verified by the Mauchly's test. When the assumption of sphericity was not met, the significance of the F-ratios was adjusted according to the Greenhouse-Geisser procedure.

Differences in anthropometric characteristics and body composition of older and young subjects, before (pre-BR) at the end (post-BR) and 14 days after physical training (post-PT), were studied with General Linear Model repeated measures with two factors considering ANOVA of the main effects of group (G: Older *vs* Young), time (T: pre-BR *vs* post-BR *vs* post-PT) and group x time interaction.

Changes of Cw, mechanical and electromyographic recordings, were studied with General Linear Model repeated measures with three factors considering groups (G: Older *vs* Young), time (T: pre-BR *vs* post-BR *vs* post-PT) and speeds (considering four different speeds, S: 0.83, 1.11, 1.39, 1.67 $m \cdot s^{-1}$). When no difference was found across time on Cw, mechanical and electromyographic outcomes, the values of pre-BR, post-BR and post-PT were averaged and compared between groups (G: Older *vs* Young) as a function of speeds (S: 0.83, 1.11, 1.39, 1.67 $m \cdot s^{-1}$).

Multiple comparisons were made with the Tukey HSD post hoc test when the Greenhouse-Geisser epsilon correction factor was $P > 0.50$ or with the Bonferroni post hoc test when the epsilon was $P < 0.05$.

## Results

### Physical characteristics of subjects

Baseline values of stature, body mass and body mass index (BMI) were not significantly different between groups at baseline (Table 1). Bed rest induced significant body mass decrease ($P<0.001$) in Older (-3.1%) and Young (-4.4%). Physical training performed after bed rest increased body mass in both groups (+2.4% and +3.9%, $P<0.001$, in Older and Young, respectively).

### Metabolic measurements

No effects of bed rest and physical training were observed on metabolic cost of walking (Cw), respiratory exchange ratio (RER) and heart rate (HR) in both groups (Fig 1, Table 2).

Mean values of $C_W$ were significantly higher in Older than in Young at each speed by an overall mean of 25.1% ($P<0.001$, Fig 1A, Table 2). Furthermore, $C_W$ changed with speed in both groups and was significantly lower at 1.11 and 1.39 $m \cdot s^{-1}$ than at 0.83 and 1.67 $m \cdot s^{-1}$ (average: -11.5%, $P<0.001$, for Older; -15.5%, $P<0.001$, for Young).

Mean values of respiratory exchange ratio (RER) were significantly higher in Older than in Young at each speed by an overall mean of 4.0% ($P<0.001$, Fig 1B, Table 2) and remained lower than 1 for each speed in Older and in Young. RER increased significantly with speed in both groups. RER at 1.11, 1.39 and 1.67 $m \cdot s^{-1}$ were significantly higher than RER measured at

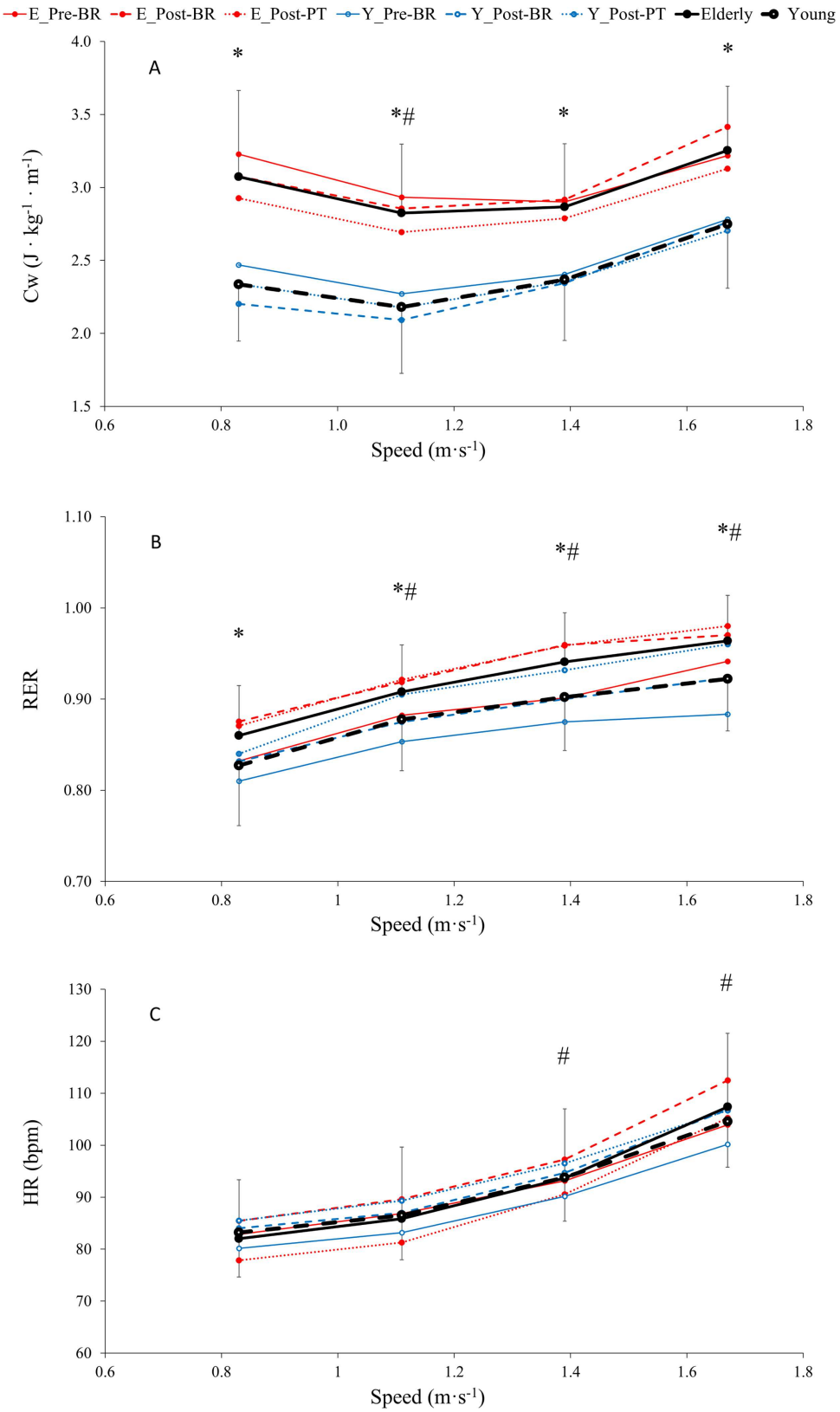


**Fig 1. Averaged values of metabolic cost of walking (Cw, A), respiratory exchange ratio (RER, B) and heart rate (HR, C) across time (pre-BR, post-BR and post-PT) as a function of speed, in older (-●-) and young (- o -) subjects**

**(see statistics paragraph).** *(The lines represent mean values obtained before (solid line) and after (dashed line) bed rest, and 14 days after physical training (dotted line) in older (red full circle) and young (blue open circle) subjects).* Results are in mean ± SD. *: $P < 0.001$, Older group is significantly different than Young at a given speed. #: $P < 0.001$, values at given speeds are significantly different than at 0.83 m · $s^{-1}$. GLM results reported in main text.

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0.83 m·$s^{-1}$ in Older (by 5.6, 9.4 and 12.1%, respectively, P<0.001, Table 2) and in Young (by 6.1, 9.1 and 11.5%, respectively, P<0.001, Table 2).

Mean values of heart rate (HR) were not significantly different between groups at each speed (Fig 1C, Table 2). Then, HR was significantly higher at speeds 1.39 and 1.67 m·$s^{-1}$ (by 14.2 and 30.9% for Older and by 12.7 and 25.6% for Young, P<0.001, Table 2) than at speed 0.83 m·$s^{-1}$.

## Mechanical measurements

No effects of bed rest and physical training were observed on total work ($W_{TOT}$), recovery index (R), stride frequency (SF) and efficiency (EFF) (Fig 2, Table 2).

Mean values of $W_{EXT}$ and $W_{TOT}$ at each point were significantly higher in Older than in Young (Fig 2A and 2C, Table 2) at speed 1.67 m·$s^{-1}$ (by 17.5 and 7.8%, respectively, P<0.05). $W_{EXT}$ and $W_{TOT}$ increased significantly as a function of speed (Fig 2A and 2C, Table 2). $W_{EXT}$ and $W_{TOT}$ were significantly higher at speeds 1.39 m·$s^{-1}$ (by 29.1 and 20. 8%, respectively, for Older; and by 22.1 and 10.1%, respectively, for Young; P<0.001) and 1.67 m·$s^{-1}$ (by 64.9 and 47.2%, respectively, for Older; and by 39.1 and 27.6%, respectively, for Young, P<0.001) than at 0.83 m·$s^{-1}$. $W_{INT}$ values were not significantly different between the two groups across all walking speeds. At speeds of 1.11, 1.39 and 1.67 m·$s^{-1}$, mean $W_{INT}$ values of Older and Young tended to be greater than those observed at 0.83 m·$s^{-1}$ although not statistically significant.

Mean values of R were significantly lower in Older than in Young (Fig 2D, Table 2) at speeds 1.39 and 1.67 m·$s^{-1}$ (by 7.3 and 13.9%, respectively, P<0.05). Moreover, R decreased significantly in Older as a function of speed and was 12.4% lower at 1.67 m·$s^{-1}$ than at 0.83 m·$s^{-1}$ although not statistically significant.

Mean values of SF (Fig 2E, Table 2) were significantly higher in Older both at 0.83 and 1.11 m·$s^{-1}$ by 11.8 and 8.9%, respectively (P<0.05). Moreover, SF increased as a function of speed

**Table 2. Metabolic, mechanical work and electromyography recordings results of General Linear Model repeated measures with three factors considering group (G: Older vs young), time (T: Pre-BR *vs* Post-BR *vs* Post-PT), speeds (considering four different speeds, S: 0.83 *vs* 1.11 *vs* 1.39 *vs* 1.67 m·$s^{-1}$) and interaction (G x T x S).**

| | Group | Time | Speeds | G x T x S |
|---|---|---|---|---|
| ***Metabolic*** | | | | |
| Metabolic cost of walking | 0.001 | 0.141 | 0.001 | 0.849 |
| Respiratory exchange ratio | 0.001 | 0.141 | 0.001 | 0.344 |
| Heart rate | 0.146 | 0.302 | 0.001 | 0.658 |
| ***Mechanical work*** | | | | |
| External work | 0.004 | 0.370 | 0.001 | 0.628 |
| Internal work | 0.687 | 0.328 | 0.001 | 0.582 |
| Total work | 0.016 | 0.981 | 0.001 | 0.650 |
| Recovery | 0.017 | 0.836 | 0.001 | 0.977 |
| Stride Frequency | 0.042 | 0.911 | 0.001 | 0.123 |
| Efficiency | 0.001 | 0.734 | 0.001 | 0.498 |
| ***Electromyography*** | | | | |
| Proximal co-contraction time | 0.038 | 0.797 | 0.001 | 0.310 |
| Distal co-contraction time | 0.043 | 0.707 | 0.001 | 0.238 |

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and was significantly higher at 1.11, 1.39 and 1.67 $m \cdot s^{-1}$ than at 0.83 $m \cdot s^{-1}$ in both groups (by 4.6, 9.7 and 17.2% in Older, $P<0.05$; and 7.5, 18.3 and 26.2% in Young, $P<0.05$).

Mean values of EFF (Fig 2F, Table 2) were significantly lower in Older among all speeds by an overall mean of -20.5% ($P<0.001$). Additionally, EFF increased as a function of speed and was significantly higher at 1.39 and 1.67 $m \cdot s^{-1}$ than at 0.83 $m \cdot s^{-1}$ in both groups (by mean 29.0% in Older and by 16.6 in Young, $P<0.001$).

## Electromyographic measurements

No effects of bed rest and physical training were observed on co-contraction time of proximal and distal muscles in Older and Young (Fig 3, Tab. 2).

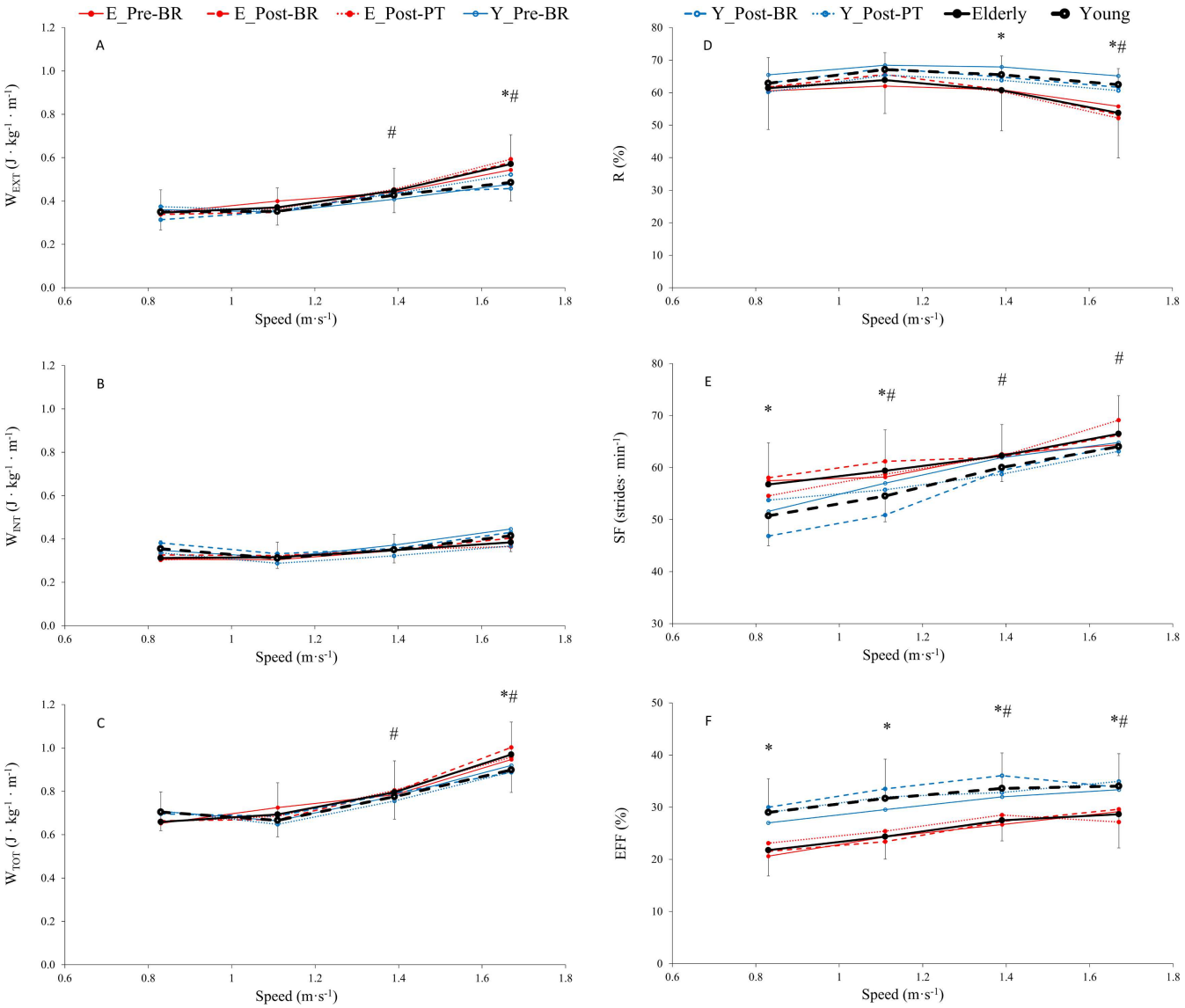


**Fig 2. Averaged values of external work ($W_{EXT}$, A), internal work ($W_{INT}$, B), total work ($W_{TOT}$, C), recovery (R, D), stride frequency (SF, E) and efficiency (EFF, F) across time (pre-BR, post-BR and post-PT) as a function of speed, in older (-●-) and young (- o -) subjects (see statistics paragraph).** *(The lines represent mean values obtained before (solid line) and after (dashed line) bed rest, and 14 days after physical training (dotted line) in older (red full circle) and young (blue open circle) subjects).* Results are in mean ± SD. *: $P < 0.05$, Older group is significantly different than Young at a given speed. #: $P < 0.001$, values at given speeds are significantly different than at 0.83 $m \cdot s^{-1}$. GLM results reported in main text.

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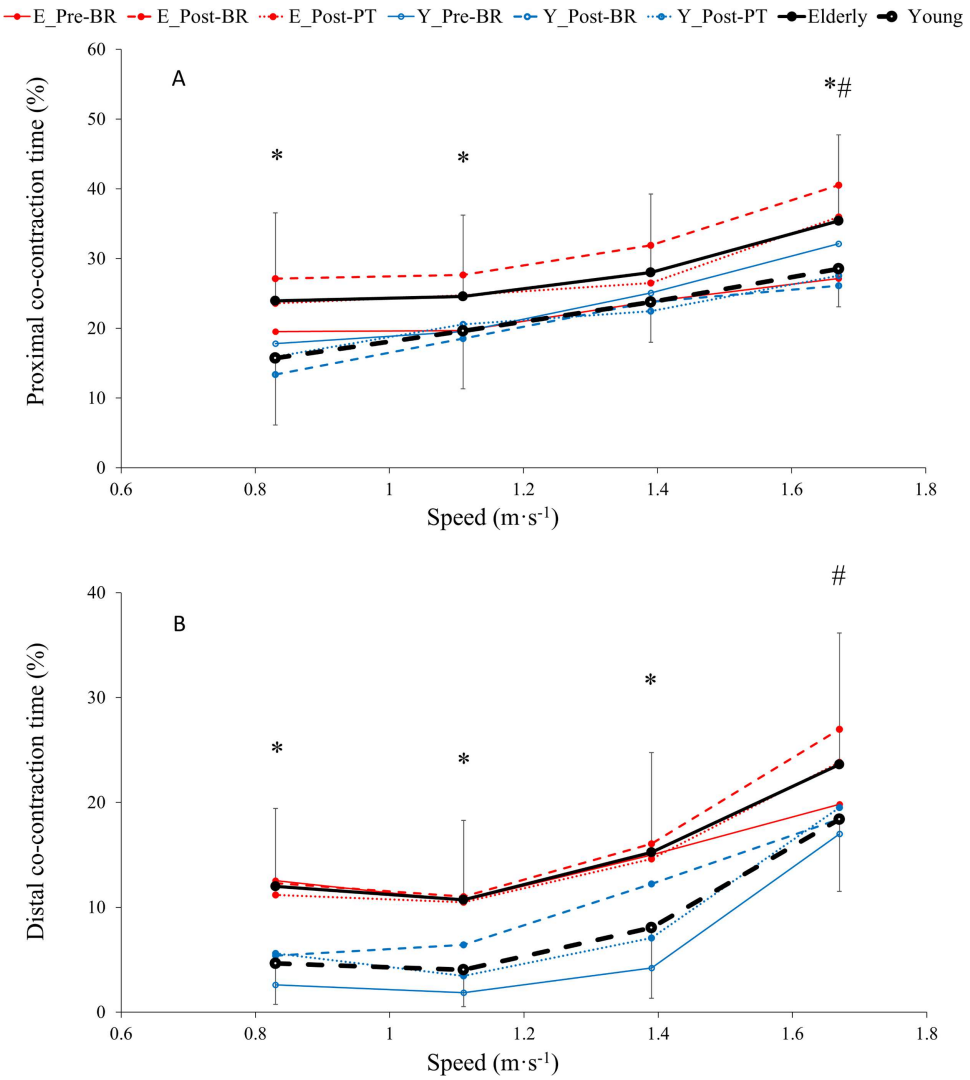


**Fig 3. Averaged values of proximal (*vastus lateralis* and *biceps femoris*, A) and distal (*gastrocnemius medialis* and *tibialis*, B) co-contraction time across time (pre-BR, post-BR and post-PT) as a function of speed, in older (-●-) and young (- o -) subjects (see statistics paragraph).** *(The lines represent mean values obtained before (solid line) and after (dashed line) bed rest, and 14 days after physical training (dotted line) in older (red full circle) and young (blue open circle) subjects).* Results are in mean ± SD. *: $P < 0.05$, Older group is significantly different than Young at a given speed. #: $P < 0.001$, values at given speeds are significantly different than at 0.83 m · $s^{-1}$. GLM results reported in main text.

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Mean co-contraction time values of proximal muscles (VL-BF, Fig 3A, Table 2) were significantly higher in Older than in Young at speeds 0.83, 1.11 and 1.67 m·$s^{-1}$ (average 52.3, 25.2 and 24.2%, respectively, $P<0.05$). Mean co-contraction time of distal muscles (GM-TA, Fig 3B, Table 2) were significantly higher in older than in young subjects at speeds equal to 0.83, 1.11 and 1.39 m·$s^{-1}$ (average 157.7, 165.7 and 89.1%, respectively, $P<0.05$).

Co-contraction time increased as a function of speed for proximal muscles and was significantly higher at 1.67 m·$s^{-1}$ compared to 0.83 m·$s^{-1}$ in Older as in Young (by 48.0 and 81.4%, respectively, $P<0.001$). Similarly, co-contraction time of distal muscles was higher at 1.67 m·$s^{-1}$ compared to 0.83 m·$s^{-1}$ (by 97.0 and 295.2% in Older and Young, respectively, $P<0.001$).

## Discussion

The main results of the present study showed that: 1) 14 days of bed rest and the following physical training did not influence Cw, mechanical work and co-contraction time during walking at different speeds in both older and young subjects; 2) before, after bed rest and after physical training, older subjects showed higher Cw, SF and lower R and EFF than young subjects, and 3) co-contraction time of proximal and distal muscles were higher in Older than in Young across the different walking speeds.

As reported in our previous work [24], for the same population examined in the present study, bed rest induced several undesirable consequences in both older and young subjects; also, partial recovery was observed after physical training. In particular, bed rest caused a significant reduction in total lean mass and quadriceps muscle volume as well as a significant increase in the percentage of body fat. These changes occurred in concert with decreased strength (i.e., maximal voluntary isometric force and explosive power of lower limb) and fitness level (i.e., $V'O_2$peak) [24]. While all parameters describing muscle volume and function showed a complete recovery at the end of 14 days of physical training in young subjects, an incomplete recovery of quadriceps volume, explosive power of lower limbs and $V'O_2$peak was observed in the older group [24].

In spite of the abovementioned adaptations brought about by bed rest and physical training, no changes in Cw, mechanical work and co-contraction time during walking were induced by these two interventions. This unexpected finding was likely related to the healthy status of older individuals, which could have mitigated the negative effects of bed rest on walking motor function. The age of older subjects (mean 60 years; range: 53–65 years) may have also played an important role, as a normal gait pattern is retained in 85% of individuals aged 60 and only in 18% of individuals aged 85 [54]. Furthermore, bed rest-induced adaptations on walking speed and functional parameters were reported in elderly subjects whose mean age was between 7 and 10 years higher than the group of older adults investigated in the present study [21, 33]. Our results can be also explained by the fact that walking is a relatively basic and automatic motor task that is primarily controlled at the spinal level with the contribution of sensory information derived from the lower limbs [55]. Walking is also an optimized human gait, as for example changes in gravity ranging between 1 g (Earth) and 0.17 g (Moon) seem to have limited effects on Cw, while the cost of transportation of other types of locomotion (i.e. running; hopping) is affected to a much greater extent by this change in gravity [56]. A previous study by our group also supports the view that bed rest-induced neuromuscular adaptations may not affect some motor patterns substantially controlled by the spinal cord, as bilateral power deficit of lower limbs and co-contraction between knee extensors and flexors assessed during explosive efforts were not altered after 35 days of bed rest in young healthy volunteers [57]. Hence, it seems plausible that, in healthy older adults, 14 days of bed rest may not be sufficient for disrupting walking pattern, even if lower limb muscle and cardiovascular performance impaired [24]. From a rehabilitative perspective, walking on flat, even surfaces seems a physical activity that can be proposed immediately after prolonged periods of disuse (i.e. 2 weeks of hospitalization) in older adults that have an otherwise healthy neuromuscular system, as mechanics and economy of walking remain similar as they were prior to disuse. This could help preventing the vicious circle often observed in older individuals who further reduce their daily physical activity after hospitalization [33].

In the present study, Cw was about 25.1% higher in older adults than in young subjects. This finding is in agreement with previous studies that observed an increased Cw related to ageing [17, 58–61]. Decreased strength, metabolic rate, and maximal oxygen consumption are some of the age-related consequences that can influence Cw. In older and elderly adults, subtle

changes in the pattern and neuromuscular control of locomotion may result in altered trajectories of the body centre of mass and the limbs, and changes in posture such as increasing trunk flexion. These adaptations may lead to an increased mechanical work, thus contributing to an increase in $V'O_2$ [18]. However, in the present study, Older and Young performed similar $W_{INT}$, $W_{EXT}$ and $W_{TOT}$ to lift and accelerate the centre of mass during walking, even though Older showed higher Cw than Young. Moreover, only at 1.67 $m \cdot s^{-1}$ Older showed higher $W_{EXT}$, $W_{TOT}$ and lower R than Young. It is interesting to note that the literature presents a variety of $W_{INT}$ values that tend to be either greater [2], smaller [62] or more similar [17] to those reported in the present study. Also, $W_{INT}$ values observed in this study tend to increase as a function of walking speed, even if this trend is not significant and less marked compared to the data previously reported [2, 17, 48, 62]. The peculiar trend of data reported in the present manuscript can be due to the filtering procedure used for the 3D coordinate analysis and to the motion capture technique, which is slightly different compared to the multi-camera systems presently available in the market. These observations have been already reported in literature. In fact, Nardello and colleagues [62] concluded that the adopted filtering protocol, among all the other methodological differences, can be responsible for the $W_{INT}$ discrepancies presented in different papers.

The differences in walking pattern between young and older individuals found in this study do not always agree with those found in other studies. For example, Mian and co-workers reported greater $W_{INT}$ values in elderly compared to young subjects [17]. This discrepancy may be explained by the fact that age of the older group is lower in the present study compared to the cited reference (i.e. mean age = 74 ± 3 years in [18]).

However, the fact that $W_{INT}$, $W_{EXT}$ and $W_{TOT}$ were overall not significantly different between the two groups of the present study suggests that other mechanical factors, such as changes in the metabolic cost of stabilizing the body, the efficacy of muscular system and/or the amount of co-contraction, may have contributed to the greater Cw observed in Older. Also, the fact that visual and vestibular functions are generally impaired with ageing [63, 64] may favour novel strategies for stabilizing the body during walking [65], and consequently increasing Cw. However, metabolically expensive strategies to improve stability such as increased co-contraction of antagonist muscles may not be detected by mechanical analyses and still contribute to the greater Cw in older individuals [17, 66–68]. In the present study, EMG recordings were helpful to detect alterations in co-contraction pattern of lower limb muscles during walking. In fact, we detected longer co-contraction times both in proximal and distal muscles in Older compared to Young. Interestingly, similar results were obtained by Mian and co-workers for the co-contraction time of thigh muscles [17]. Increased co-contraction is interpreted as compensatory mechanism to increase joint stiffness and stability, and it is associated with normal, healthy ageing [66, 69]. Despite the beneficial role that this mechanism might play in older and elderly adults to promote safer walking, it might be also disadvantageous because it can increase the cost of locomotion [17, 69, 70]. Our results are in agreement with these studies and support the view that older adults tend to adapt walking pattern increasing co-contractions of antagonist muscles to conceivably improve stability and safety, even if this leads to sacrifice walking economy. Another ageing-related factor that could contribute to the greater Cw in older subjects is the decreased efficiency of the muscle itself, which would then require more metabolic energy to perform a given amount of mechanical work [17, 71].

In conclusion, 14 days of bed rest and the following physical training did not induce any adaptation on Cw, mechanical work, efficiency and co-contraction of antagonist lower limb muscles during walking in healthy young and older individuals. In addition, older adults presented higher Cw, stride frequency, co-contraction time of proximal and distal antagonist muscles and lower efficiency compared to young subjects.

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# Loss of maximal explosive power of lower limbs after 2 weeks of disuse and incomplete recovery after retraining in older adults

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**Key points**

- Disuse in older adults can critically decrease lower limb muscle power, leading to compromised mobility and overall quality of life.
- We studied how muscle power and its determinants (muscle mass, single muscle fibre properties and motor control) adapted to 2 weeks of disuse and subsequent 2 weeks of physical training in young and older people.
- Disuse decreased lower limb muscle power in both groups; however, different adaptations in single muscle fibre properties and co-contraction of leg muscles were observed between young and older individuals.
- Six physical training sessions performed after disuse promoted the recovery of muscle mass and power. However, they were not sufficient to restore muscle power to pre-disuse values in older individuals, suggesting that further countermeasures are required to counteract the disuse-induced loss of muscle power in older adults.

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**Abstract** Disuse-induced loss of muscle power can be detrimental in older individuals, seriously impairing functional capacity. In this study, we examined the changes in maximal explosive power (MEP) of lower limbs induced by a 14-day disuse (bed-rest, BR) and a subsequent 14-day retraining, to assess whether the impact of disuse was greater in older than in young men, and to analyse the causes of such adaptations. Sixteen older adults (Old: 55–65 years) and seven Young (18–30 years) individuals participated in this study. In a subgroup of eight Old subjects, countermeasures based on cognitive training and protein supplementation were applied. MEP was measured with an explosive ergometer, muscle mass was determined by magnetic resonance, motor control was studied by EMG, and single muscle fibres were analysed in vastus lateralis biopsy samples. MEP was ∼33% lower in Old than in Young individuals, and remained significantly lower (−19%) when normalized by muscle volume. BR significantly affected MEP in Old (−15%) but not in Young. Retraining tended to increase MEP; however, this intervention was not sufficient to restore pre-BR values in Old. Ankle co-contraction increased after BR in Old only, and remained elevated after retraining (+30%). Significant atrophy occurred in slow fibres in Old, and in fast fibres in Young. After retraining, the recovery of muscle fibre thickness was partial. The proposed countermeasures were not sufficient to affect muscle mass and power. The greater impact of disuse and smaller retraining-induced recovery observed in Old highlight the importance of designing suitable rehabilitation protocols for older individuals.

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## Introduction

People above the age of 60 years represent the fastest growing segment of the population in developed countries, and their quality of life can be dramatically compromised by reduced mobility (McPhee *et al.* 2013). Epidemiological research has associated reduced mobility with loss of muscle mass and muscular weakness (Janssen, 2006; Hairi *et al.* 2010). In particular, sarcopenia is a condition that can result from different factors, and can lead to outcomes characterized by progressive and generalized loss of skeletal muscle mass and function (strength and physical performance) (see Cruz-Jentoft *et al.* 2010). The loss of muscle mass leads to a decrease in muscle strength; additionally, older people also experience a loss of strength per unit of muscle mass (Rutherford & Jones, 1992; Goodpaster *et al.* 2006), which may explain why muscle weakness in the elderly is a better predictor of mortality than muscle mass alone (Newman *et al.* 2006). Interestingly, skeletal muscle power has been proposed as a more critical determinant of physical functioning in the elderly population than muscle strength (Reid & Fielding, 2012) or size (Runge *et al.* 2004). Muscle power exerted by knee and lower limb extensors has been shown to decline earlier and more rapidly than muscle strength with advancing age (Aagaard *et al.* 2010), and it is considered a better performance predictor of various motor tasks (i.e. rising from a chair, climbing stairs) as compared to muscle strength in mobility-limited elderly people (Bassey *et al.* 1992; Foldvari *et al.* 2000; Reid & Fielding, 2012).

Besides ageing, disuse is an important cause of muscle deterioration. Periods of skeletal muscle disuse or unloading can occur in healthy people as a consequence of injury or illness. The physiological effects of disuse and unloading have been widely studied by bed rest (BR), which is a recognized experimental model to induce substantial muscle dysfunction within a few weeks, remarkably compromising the generation of muscle power. Young healthy males decrease their ability to generate maximal muscular power of the lower limbs by about 24–30% after 35–90 days of BR without countermeasures (Ferretti, 1997; Rittweger *et al.* 2007; Rejc *et al.* 2015). The loss of muscle power after disuse is closely correlated to muscle atrophy. BR studies on young healthy males showed that disuse-induced muscle atrophy is unevenly distributed, being larger in postural than non-postural muscles, larger in the extensors than in the other thigh muscle groups, and greater in the calf muscles than in the other leg muscle groups (Ferretti *et al.* 2001; Alkner & Tesch, 2004; de Boer *et al.* 2008; Belavy *et al.* 2009). Atrophic response to BR can be rapid; for example, 7 days of BR induced a 3% decrease of thigh muscle mass (Ferrando *et al.* 1995). Longer BR induced greater atrophic effects, with a 12% and 8% decrement in gastrocnemius medialis and vastus lateralis muscle thickness after a 35-day BR, respectively (de Boer *et al.* 2008), and a reduction of quadriceps and calf muscle mass by about 30% after 90–120 days of BR (Alkner & Tesch, 2004; Shackelford *et al.* 2004). Other important components of the loss of muscle power in relation to disuse are the decreased ability in motor unit recruitment (Lambertz *et al.* 2001; Clark *et al.* 2006) and the reduction of muscle intrinsic force (force/cross sectional area) (Pavy-Le Traon *et al.* 2007; Narici & de Boer, 2011).

In spite of the vast amount of literature focused on the separate effects of either ageing or disuse, there are rather few studies that have investigated the combined effects of these two factors (ageing and disuse) on neuromuscular function. However, the occurrence of disuse periods in the elderly population is frequent (Suetta *et al.* 2007) and can lead to serious consequences such as further reduction of daily physical activity (Kortebein *et al.* 2008), functional decline (Hoenig & Rubenstein, 1991; Creditor, 1993), greater risks for falls and consequent hip fractures, which have long-lasting negative effects on quality of life and a strong association with mortality (Wall *et al.* 2013). In healthy elderly individuals, neuromuscular function seems differently affected by short-term disuse than in the young population. In particular, 2 weeks of immobilization by unilateral, whole leg casting reduced quadriceps femoris activation in old but not young men, while the decline in quadriceps volume was smaller in old compared to young (Suetta *et al.* 2009). Under the same experimental design, elderly individuals also showed a greater decrease in rapid force capacity (Hvid *et al.* 2010). Similarly, Deschenes *et al.* (2008) observed that muscle performance during faster contractions of the quadriceps femoris was more impaired in elderly than young individuals after 1 week of lower limb suspension. Shorter periods of disuse (i.e. 4 days of one-leg immobilization) also have a greater impact on the neuromuscular system in old than in young subjects (Hvid *et al.* 2013, 2014). Interestingly, elderly individuals also showed reduced recovery of neuromuscular function as compared to young subjects when physical retraining was proposed after immobilization. For example, 4 weeks of physical training subsequent to 2 weeks of immobilization induced increments in quadriceps femoris volume that were smaller in elderly than in young males (Suetta *et al.* 2009), and promoted full recovery of muscle fibre area and rapid force capacity in young but not in elderly subjects (Hvid *et al.* 2010). Age-related differences in the recovery of neuromuscular function seem to increase with shorter immobilization and retraining periods, as 7 days of recovery subsequent to 4 days of immobilization were not sufficient to restore isometric and dynamic muscle strength as well as rapid muscle force capacity in elderly, while these parameters were fully recovered in young subjects (Hvid *et al.* 2013, 2014).

The differential impact of a period of disuse on bone metabolism (Buehlmeier *et al.* 2017), protein synthesis (Biolo *et al.* 2017), plasma brain-derived neurotrophic factor (BDNF) levels (Soavi *et al.* 2016) and adipokine values (Jurdana *et al.* 2015) in older adults compared to young people has been investigated by our group in a recent BR study. Also, we have examined the combined effects of ageing and disuse, followed by physical retraining, on muscle mass and performance (Pisot *et al.* 2016). Because ageing can exacerbate the negative effects of disuse on different systems, specific countermeasures (cognitive training during BR and protein supplementation during physical retraining) were proposed to an experimental subgroup of older adult individuals. Computerized cognitive training during BR improved executive/attention ability as well as processing speed (Marusic *et al.* 2018), and significantly modulated plasma BDNF levels (Passaro *et al.* 2017). On the other hand, Pisot *et al.* (2016) noted that these two countermeasures did not promote significant effects on muscle mass and performance in the experimental subgroup of older adults as compared to control older adults who did not receive any countermeasure. Among the combined effects of age and disuse, the loss of lower limb extensors power has particular relevance with regard to everyday life movements such as rising from a chair or climbing stairs (Bassey *et al.* 1992). Importantly, Pisot *et al.* (2016) observed that in older adults, the reduction of maximal explosive power (MEP) of lower limb extensors induced by the 14-day BR was higher (−15.2%) than expected from the reduction of muscle mass (−8.3%). This prompted us to further examine different factors that may contribute to such MEP decrease. In this study, we re-examined the issue by investigating how BR affected muscle mass, single muscle fibre properties and motor control, and whether these factors played a similar role on the BR-induced loss of MEP in young and older subjects. In addition, these parameters were also analysed in both young and older individuals after a period of physical retraining to gain insight into how ageing affects muscle function recovery. Finally, the results were also analysed separately for the two subgroups of older adults (with and without countermeasures) to corroborate the previous statements related to the lack of positive effects of cognitive training and protein supplementation on these parameters.

## Methods

### Ethical approval

The present study was conducted according to the standards set by the latest revision of the Declaration of Helsinki except for registration in a database, and was approved by the National Ethical Committee of the Slovenian Ministry of Health on 17 April 2012, under the acronym IR-aging 1200. The purposes and objectives of this study were carefully explained to the subjects and written informed consent was obtained from all of them.

### Subjects

Sixteen healthy older adult males (Old; age: 59.6 ± 3.4 years) and seven healthy young males (Young; age: 23.1 ± 2.9 years) participated in this study. Before the start of the study, all subjects filled out a physical activity-related questionnaire (Craig *et al.* 2003), had

a full medical history and physical examination that also included routine haematology and biochemistry screens, and underwent a fitness battery test. Exclusion criteria were: smoking; regular alcohol consumption; ferromagnetic implants; history of deep vein thrombosis with D-dimer $> 500\ \mu g\ L^{-1}$; acute or chronic skeletal, neuromuscular, metabolic and cardiovascular disease conditions; pulmonary embolism; or a short physical performance battery score <9 (Guralnik *et al.* 1994). None of the subjects experienced any significant disease and none was taking medications regularly or made use of any medication known to influence physical performance.

## Study protocol

The subjects spent 19 consecutive days at the Orthopedic Hospital of Valdoltra (Ankaran, Slovenia), including 3 days of familiarization to study environment and diet, baseline data collection, and 14 days of BR. Immediately after BR, subjects underwent supervised physical training, which was conducted at the same facility and at a nearby gym. To explore possible interventions aimed at mitigating the negative disuse-induced adaptations and enhancing physical retraining effects, a subgroup of eight randomly selected older adults (Old_Int) underwent daily 45 min of computerized cognitive training by navigating through virtual mazes with the use of a joystick and computer during the 14-day BR. The same eight subjects also received a nutritional support based on 0.4 g whey protein kg body weight$^{-1}$ day$^{-1}$ at breakfast during the initial 14 days of physical training. By contrast, the eight older adults included in the control subgroup (Old_Ctrl) did not receive any additional countermeasure throughout the study.

Anthropometric characteristics, body composition and MEP of the lower limbs were measured 1 day before the BR (Pre-BR), the day after the 14-day BR (Post-BR) and after 2 weeks of physical training (R + 14). Quadriceps muscle volume was assessed after 12 h of BR initiation (Pre-BR), on the evening of the last day of BR (Post-BR) and following 12 h of horizontal position after 2 weeks of physical training (R + 14).

## Bed rest

The participants were housed in standard air-conditioned hospital rooms and were under constant visual surveillance with 24-h medical care. During BR, the subjects performed all daily activities in bed with no deviations from the lying position permitted, and both exercise and muscle contraction tests were not allowed. All participants received hospital meals three times a day and followed an individually controlled eucaloric diet during the BR period. Dietary energy requirements were designed for each subject multiplying resting energy expenditure by factors 1.2 and 1.4 in the bed rest and ambulatory periods, respectively (Biolo *et al.* 2008). The macronutrient food content was set at 60% carbohydrates, 25% fat and 15% proteins. Energy balance was checked weekly by fat mass assessment.

During BR, all subjects received passive physiotherapy treatments (i.e. joint mobility and stretching, relief massage in the presence of acute back pain) three times per week. To prevent thrombosis, a D-dimer test was repeated on Day 7 of bed rest where participants reached elevated values but scored $<500\ \mu g\ L^{-1}$; however, additional Doppler ultrasound check-up was conducted in all of them and all wore compression socks.

## Physical training programme

Subjects began a 28-day physical training programme on the second day after the end of BR; however, only the initial 14 days of physical training were considered in this study, i.e. the same duration of the disuse period. Physical training consisted of six sessions in total (three per week); each session, which lasted about 65 min, was followed by 1 or 2 days of routine daily activity. Physical training was aimed at reconditioning both the neuromuscular and the aerobic systems, proposing a series of exercises that did not require specific training equipment so that they could be translated to any home and community environment. The first 12 min of each training session was devoted to warm-up; subjects performed 6 min of Nordic walking, its speed being determined from a 2-km walking test performed before BR, and 6 min of active stretching (10 exercises). Subjects then performed 20 min of strength and balance exercises. This section started with half squat (one set; 10 repetitions; overload: from no overload to a 6 kg ball held with both hands), and continued with a circuit training (30 s of exercise followed by 30 s of rest) consisting of eight motor tasks. In particular, the following strength exercises focused on lower limbs were proposed: frontal and sagittal plane lunge, double leg heel raise with elastic resistance; hip extension with elastic resistance. Also, balance exercises mainly consisted of dynamic standing balance activities (i.e. standing on toes; standing on balance foams) and functional movements that involved reaching and passing objects. Strength and balance exercises were followed by 30 min of aerobic exercise (e.g. Nordic walking, brisk walking, running). The last 3 min were devoted to cool down (relaxation and breathing exercises). Subjects' heart rate was preventively monitored during each training session. Training was conducted at the hospital and in the gym near the hospital and supervised by six physical trainers who instructed the subjects to properly perform the different exercises. All subjects performed all planned training sessions.

## Measurements

**Anthropometric characteristics and body composition.** Body mass (BM) was measured to the nearest 0.1 kg with a manual weighing scale (Seca 709, Hamburg, Germany) with the subject dressed only in light underwear and no shoes. Stature was measured to the nearest 0.5 cm on a standardized wall-mounted height board.

Body composition was measured by using bioelectrical impedance analysis with a tetra-polar impedance-meter (BIA101, Akern, Florence, Italy), according to an accepted method (Lukaski *et al.* 1986). Body composition [fat-free mass (FFM) and fat mass (FM)] was obtained from the software provided by the manufacturer. This method has already been utilized and validated to investigate changes in body composition during BR and in clinical settings (Birch & Fisher, 1998; Kyle *et al.* 2004).

**Magnetic resonance imaging.** Quadriceps femoris muscle volume of the right leg was measured from turbo spin-echo, T1-weighted, magnetic resonance images (MRI) obtained with a 1.5 T Magnetom Avanto device (Siemens Medical Solution, Erlangen, Germany). On each MRI slice, contours corresponding to the quadriceps muscles were delineated by an MRI expert, using the image processing tools OsiriX (Pixmeo Sarl, v.4.1.2). Quadriceps muscle volume was then derived by summation of a series of evenly spaced truncated cones between each of two axial images, a process that included an average of 25 images (range 23–28) and covered the entire length of the quadriceps.

**Maximal explosive power of the lower limbs.** The biomechanical parameters of the explosive efforts were studied by means of an Explosive Ergometer (EXER), described previously in detail (Lazzer *et al.* 2009). Briefly, the EXER consists of a metal frame supporting one rail, which was inclined by 20 deg. A seat, fixed on a carriage, was free to move on the rail, its velocity along the direction of motion being continuously recorded by a wire tachometer (LIKA SGI, Vicenza, Italy). The subject was able to accelerate him/herself and the carriage seat backward by pushing on two force platforms (LAUMAS PA 300, Parma, Italy) positioned perpendicular to the rail. The total moving mass of the EXER (seat and carriage together) was equal to 31.6 kg. Force and velocity analog outputs were sampled at 2000 Hz using a data acquisition system (MP100; BIOPAC Systems, Inc., Goleta, CA, USA). The instantaneous power was calculated from the product of instantaneous force and velocity values.

The subject was seated on the carriage seat, secured by a safety harness tightened around the shoulders and abdomen, with their arms on the handlebars. Two mechanical blocks were used to set the distance between the seat and the force platforms, so that the knee angle at rest was 110 deg. The blocks also prevented any countermovement and, consequently, any recovery of elastic energy during the pushing phase. After a brief familiarization session with the laboratory equipment, the subjects performed four maximal explosive efforts, the duration of which was about 400 ms. After each push, subjects rested for 2 min with their feet placed on a support. The attempt with the greatest peak power (MEP) was taken into account for further analysis.

**Surface electromyography recordings.** Surface electromyography (EMG) data were collected from four muscles of the right lower limb: vastus lateralis (VL), biceps femoris (BF), gastrocnemius medialis (GM) and tibialis anterior (TA). Pre-gelled surface EMG electrodes (circular contact area of 1 cm diameter, BIOPAC Systems) were placed (inter-electrode distance 20 mm) at the following locations (Hermens *et al.* 2000): (a) for VL at two-thirds on the line from the anterior superior iliac spine to the lateral side of the patella; (b) for BF midway between the ischial tuberosity and the lateral epicondyle of the tibia; (c) for GM on the most prominent bulge of the muscle; (d) for TA at one-third on the line between the tip of the fibula and the tip of the medial malleolus. To ensure a good electrode–skin interface, prior to the application of the electrodes, the subject's skin was shaved, rubbed with an abrasive paste, cleaned with an alcohol solution and dry-cleaned with gauze.

EMG data were sampled at a frequency of 2 kHz, and recorded by a four-channel EMG system (EMG100C, BIOPAC Systems; Band-pass Filter: 10–500 Hz; RMS noise voltage: 0.2 $\mu$V; input impedance: 2 M$\Omega$; common mode rejection ratio: 110 dB). To place electrodes in the same anatomical location during the three different experimental sessions, the position of electrodes was marked on acetate paper using moles and small angiomas (which may be assumed to maintain a fixed position) as reference points. The EMG electrodes were fixed at the beginning of each experimental session and were not removed between explosive efforts and isometric contractions (see below) of the lower limbs.

**Maximal voluntary contractions.** To normalize EMG signal recorded from the four analysed muscles (VL, BF, GM and TA) during explosive efforts, maximal voluntary isometric contractions (MVCs) of the right lower limb were performed either on a special chair (a) or on an adapted examination bed (b):

a) The subject was seated with his legs hanging vertically down. A strap, connected in series to a force sensor (TSD121C, BIOPAC Systems), was tightened around the subject's right ankle. The force sensor was fixed in series to a steel frame. The position of this frame was set prior the execution of isometric knee

extension and knee flexion to obtain a knee angle of 110 deg.

b) The subject lay prone on an examination bed. His right foot was tightened around a custom made attachment connected to an isometric dynamometer. The anterior part of the foot sole was placed against the attachment in a flat standardized position, to obtain an ankle angle of 90 deg.

Force and EMG exerted during MVCs were recorded at a frequency of 2 kHz using a data acquisition system (MP100, BIOPAC Systems). Subjects were asked to perform three MVCs of 4–5 s for each isometric effort. To prevent fatigue, after each contraction subjects rested for 2 min.

**EMG data analysis.** The EMG activity defined in a 500-ms window centred on maximal force exerted during MVC was analysed: EMG raw signal was processed using a 5-ms running-window root mean square, and its mean value was considered as 100% MVC.

EMG raw signal recorded during the push (i.e. throughout the period of force development) of the explosive efforts was processed using a 5-ms running-window root mean square to obtain its mean value throughout each push. This was then expressed as percentage of the value obtained during MVC. To investigate a co-contraction feature during the push phase of the explosive efforts at the knee and ankle joints, the ratio between EMG amplitude of BF (%MVC) and VL (%MVC) (knee co-contraction) and the ratio between EMG amplitude of TA (%MVC) and GM (%MVC) (ankle co-contraction) were calculated. The greater the value of these indexes the greater the level of co-contraction.

**Single muscle fibre experiments.** Single muscle fibre analysis was performed on samples obtained from the mid-region of the left vastus lateralis muscle. Biopsy was done after anaesthesia of the skin, subcutaneous fat tissue, and muscle fascia with 2 mL of lidocaine (2%). A small incision was then made to penetrate skin and fascia, and the tissue sample was harvested with a purpose-built rongeur (Zepf Instruments, Tuttlingen, Germany). A fragment of the sample, used for single fibre analysis, was quickly stored in skinning solution with 50% glycerol at −20°C, while another fragment was frozen in isopentane cooled with liquid nitrogen. The solutions used had the following composition (mM): skinning solution: potassium propionate 150, magnesium acetate 5, Na-ATP 5, EGTA 5 and $KH_2PO_4$ 5; relaxing solution: KCl 100, imidazole 20, $MgCl_2$ 5, Na-ATP 5 and EGTA 5; preactivating solution had a similar composition except that EGTA concentration was reduced to 0.5 mM, and 25 mM creatine phosphate and 300 U mL$^{-1}$ creatine phosphokinase were added; activating solution (pCa 4.6) was also similar to relaxing solution with the addition of 5 mM $CaCl_2$, 25 mM creatine phosphate and 300 U mL$^{-1}$ creatine phosphokinase. The pH of all solutions was adjusted to 7.0 at the temperature at which solutions were used (12°C). Protease inhibitors (E64 10 $\mu$M and leupeptine 40 $\mu$M) were present in all solutions.

On the day of the experiment, single muscle fibre segments were dissected from the samples stored and mounted in a drop of relaxing solution between the force transducer (AME-801 SensorOne, Sausalito, CA, USA) and the electromagnetic puller (Scientific Instruments, Heidelberg, Germany) equipped with a displacement transducer. The signals from the force and displacement transducers were fed and stored in a computer after A/D conversion (interface CED 1401 plus, CED, Cambridge, UK). For data storage, recall and analysis the software Spike 2 (CED) was used. The experimental setup consisted of an inverted microscope (Axiovert 25, Zeiss, Oberkochen, Germany) with a movable and thermo-regulated aluminium plate on the stage. On the aluminium plate, three drops (70 $\mu$l volume) of relaxing, pre-activating and activating solution, respectively, were kept under a coverslip connected with a movable arm. Under each solution drop, an opening through the aluminium plate made the fibre segment visible via the objective piece of the inverted microscope. A stereomicroscope (Konus Diamond, Konus, Italy) placed above the inverted microscope was used for manipulation of the fibre segment. A digital camera (Optikam B5, Optika, Bergamo, Italy) allowed measurements of sarcomere length with a custom-designed software in at least two regions of interest covering approximately 20 sarcomeres each with resolution of 19.5 pixels $\mu$m$^{-1}$. Further details of the setup are reported elsewhere (Doria *et al.* 2011). Once mounted in the setup, fibre segments were gently elongated in the relaxing solution to 120% of the slack length, which corresponded to a sarcomere length of 2.685 ± 0.011 $\mu$m (mean ± SE, $n = 710$). The segments then transferred to the pre-activating solution for at least 1 min and finally maximally activated by immersion in the activating solution. During maximal activation, isometric force ($F_o$) was measured in several consecutive contractions and unloaded shortening velocity ($V_o$) was determined according to the slack test procedure. To this end, five instantaneous length changes (<1 ms) were imposed with amplitudes ranging from 5 to 15% of the resting length. Unloaded shortening velocity was obtained from the slope of the linear regression between the time required to take up the slack and the amount of shortening imposed and was expressed in fibre length per second. Cross-sectional area (CSA) was calculated from the measurements of three fibre diameters, assuming a circular shape of the fibre section, while the fibre was immersed in relaxing solution. Furthermore, specific force $P_o = F_o/\text{CSA}$ was also calculated.

**Table 1. Baseline characteristics of the Control (Old_Ctrl) and Interventions (Old_Int) groups of older adults and Young subjects**

| | Old_Ctrl ($n = 8$) | Old_Int ($n = 8$) | Young ($n = 7$) | $P$ |
|---|---|---|---|---|
| Age (years) | 59.9 ± 3.3* | 59.4 ± 3.6* | 23.1 ± 2.9 | <0.001 |
| Stature (m) | 1.73 ± 0.04 | 1.74 ± 0.06 | 1.77 ± 0.07 | 0.358 |
| Body mass (kg) | 79.6 ± 10.5 | 80.3 ± 14.7 | 74.8 ± 8.8 | 0.633 |
| BMI (kg m$^{-2}$) | 26.8 ± 4.2 | 26.4 ± 4.8 | 24.0 ± 2.4 | 0.342 |
| Physical activity (min week$^{-1}$) | 874 ± 549 | 922 ± 587 | 597 ± 238 | 0.354 |
| SPPB score | 11.6 ± 0.2 | 12.2 ± 0.3 | 12.0 ± 0.0 | 0.625 |
| Self-assessed general health | 3.9 ± 0.9 | 3.5 ± 0.9 | 4.3 ± 0.8 | 0.215 |
| Self-assessed quality of life | 3.2 ± 1.0 | 3.1 ± 1.0 | 3.7 ± 1.1 | 0.312 |

All values are means ± SD.
*P*: significance by general linear model of the main effects of Group (Old_Ctrl *vs.* Old_Int *vs.* Young).
*Significantly different from Young.
BMI: body mass index; SPPB: short physical performance battery score (Guralnik *et al.* 1994).

The composition in Myosin Heavy Chains (MyHC) isoforms of each fibre segment was determined on 8% polyacrylamide slab gels after denaturation in SDS (SDS-PAGE) as described by Doria *et al.* (2011). Gels were silver stained and three bands were separated in the region of 200 kDa, corresponding (in order of migration from the fastest to the slowest) to MyHC-1, MyHC-2A and MyHC-2X. The same protocol was followed to separate MyHC isoforms in the frozen fragment of the biopsy, with Coomassie Blue staining of the gels. The relative proportions of MyHC isoforms were obtained from the measurements of the brightness area product (BAP, i.e. the product of the area of the band by the average brightness subtracted local background after black–white inversion) after scanning the gels to an accuracy of 600 d.p.i. For each sample the electrophoretic separation and the densitometry measurements were repeated three times.

**Statistical analyses.** Statistical analyses were performed using SPSS 19.0 software (IBM, Chicago, MI, USA), with significance set at $P < 0.05$. Normal distribution of the data was tested using the Shapiro–Wilk test. Sphericity was verified by Mauchly's test. When the assumption of sphericity was not met, the significance of the F-ratios was adjusted according to the Greenhouse–Geisser procedure. Differences in baseline data of body mass, stature and body mass index among groups were analysed by a general linear model of the main effect of group (Old_Int *vs.* Old_Ctrl *vs.* Young). Changes of anthropometric characteristics, body composition, quadriceps femoris volume (QF), MEP, MVC, EMG data and per cent distribution of MyHC isoforms were analysed with general linear model repeated measures with two factors considering three groups (Old_Int *vs.* Old_Ctrl *vs.* Young) and time (Pre-BR *vs.* Post-BR *vs.* R + 14). When significant differences were found, a Bonferroni *post hoc* test was used to determine the exact location of the difference. Changes of muscle fibres characteristics (CSA, $F_o$, $P_o$ and $V_o$) were analysed with a generalized linear mixed model, which accounts for fixed effects due to group (Old_Int *vs.* Old_Ctrl *vs.* Young) and time (Pre-BR *vs.* Post-BR *vs.* R + 14) taking into account the correlation of the data. The same analyses were also performed pooling together the two subgroups of older adults (Old group) to strengthen the focus of the present study on ageing, disuse and physical training.

## Results

### Physical characteristics of subjects

All participants were able to comply with the study protocol. There was no dropout, and no medical complications occurred beside transient hypotension during the 10 min orthostatic tolerance testing at the end of BR in seven participants (three in Old and four in Young). Baseline values of body mass, stature, body mass index, body composition and lifestyle were not different among the two groups of older adults and the Young group (Table 1). However, quadriceps femoris muscle volume was significantly greater in Young compared to Old (+15.3%, $P = 0.038$, Table 2), while it was similar between Old_Int and Old_Ctrl ($P = 0.757$).

Body mass, total body fat-free mass and quadriceps femoris muscle volume were similar between Old_Ctrl and Old_Int both after BR and after physical training ($P$ values ranging from 0.394 to 0.971; Table 2).

BR induced significant decreases in body mass (−3.1 and −4.4%, $P < 0.001$, in Old and Young, respectively) and in total body fat-free mass (−6.2 and −7.6%, $P < 0.005$, in Old and Young, respectively) in both Old and Young (Table 2). Similarly, a significant decrease in muscle volume of quadriceps femoris occurred in Old (−8.3%, $P < 0.001$), and the same trend was observed in Young (−6.1%, $P = 0.052$). Physical training performed after BR increased body mass in both groups (+2.4 and +3.9%, $P < 0.001$, in Old and Young, respectively);

**Table 2. Effects of 2 weeks of bed rest and 2 weeks of physical retraining on physical characteristics of the Control group (Old_Ctrl) and Interventions group (Old_Int) of older adults and Young subjects**

| | Old_Ctrl ($n = 8$) | | | Old_Int ($n = 8$) | | | Young ($n = 7$) | | | Significance | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre-BR | Post-BR | R + 14 | Pre-BR | Post-BR | R + 14 | Pre-BR | Post-BR | R + 14 | Group | Time | G × T |
| BM (kg) | 79.6 ± 10.5 | 77.6 ± 10.4 | 79.5 ± 10.1 | 80.3 ± 14.7 | 77.35 ± 13.6 | 79.2 ± 13.7 | 74.8 ± 8.8 | 71.6 ± 8.3 | 74.4 ± 8.1 | 0.590 | <0.001 | 0.243 |
| FFM (kg) | 59.8 ± 6.9 | 56.7 ± 6.8 | 60.4 ± 6.9 | 63.8 ± 11.1 | 59.3 ± 7.9 | 60.6 ± 10.4 | 60.9 ± 3.9 | 56.3 ± 3.8 | 57.8 ± 4.4 | 0.709 | <0.001 | 0.131 |
| FM (kg) | 19.9 ± 4.9 | 20.9 ± 4.8 | 19.1 ± 4.6 | 16.4 ± 6.9 | 18.1 ± 8.8 | 18.6 ± 7.2 | 14.0 ± 6.2 | 15.3 ± 7.0 | 16.6 ± 6.6 | 0.370 | 0.066 | 0.173 |
| QF ($cm^3$) | 1663 ± 173 | 1524 ± 197 | 1613 ± 157 | 1702 ± 298 | 1561 ± 246 | 1648 ± 233 | 1988 ± 270 | 1867 ± 204 | 1954 ± 211 | 0.016 | <0.001 | 0.981 |

All values are means ± SD.
Pre-BR: before bed rest; Post-BR: after bed rest; R + 14: after physical retraining; BM: body mass; FFM: fat-free mass; FM: fat mass; QF: quadriceps femoris muscle volume.
Significance by general linear model of the main effects of Group (Old_Ctrl *vs.* Old_Int *vs.* Young), Time (Pre-BR *vs.* Post-BR *vs.* R + 14) and Group × Time interaction (G × T).

however, the increment in total body fat-free mass and quadriceps femoris muscle volume was significant in Old only (+4.4%, $P < 0.006$, and +5.7%, $P < 0.001$, respectively). It is important to note that physical training tended to restore quadriceps femoris muscle volume to Pre-BR values in Old (−3.1%, $P = 0.048$), and to fully restore it in Young (−1.7%, $P = 0.428$) (Table 2).

## MEP and MVCs of the lower limbs

MEP, peak force, peak velocity and specific MEP values were similar between the two groups of older adults (Old_Ctrl and Old_Int) at all three investigated time points ($P$ values ranging from 0.561 to 0.982; Fig. 1 and Table 3).

Young subjects consistently exerted higher MEP than Old (∼ +33%, $P < 0.001$) across the three investigated time points (Fig. 1*A*). Accordingly, greater peak force and velocity (∼ +16% for both measurements, $P < 0.005$) were found in Young compared to Old (Table 3). Importantly, MEP normalized to quadriceps femoris muscle volume (specific MEP) was also consistently higher in Young than in Old (∼ +19%, $P < 0.005$) at Pre-BR, Post-BR and R + 14 (Fig. 1*B*).

BR caused a decrease in MEP (−15.2%, $P < 0.001$, Fig. 1*A*) as well as peak force and velocity (Table 3) in Old; a similar trend was observed in Young, as MEP decreased by 10.4% ($P = 0.067$) after BR. The subsequent physical retraining significantly increased MEP only in Old (+8.1%, $P = 0.018$; Fig. 1*A*). However, MEP developed at R + 14 was still significantly lower than before BR for Old (−8.3%, $P = 0.011$) but not for Young (−5.7%, $P = 0.370$). In Old, this lack of complete MEP recovery at R + 14 was accompanied by a significantly lower peak force (−8.1%, $P < 0.001$) compared to Pre-BR (Table 3). Specific MEP did not change significantly across the three experimental time points in both Old and Young groups (Fig. 1*B*). However, it might be of note that in Old specific MEP tended to decrease after BR (−7.7%, $P = 0.133$) and to remain lower than at Pre-BR (−7.0%, $P = 0.148$) also after the completion of physical training.

To examine the changes in contractile force related to disuse and retraining, the force developed during MVCs of quadriceps femoris muscle was analysed. As seen in Fig. 2*A*, MVC values were lower in Old compared to Young (by approximately −20%) at any time point, and showed a significant decrease at the end of BR only in older adults (Old: −13%, $P < 0.001$; Old_Ctrl: −15%, $P = 0.012$; Old_Int: −11%, $P = 0.006$). MVC values recorded at R + 14 were comparable to those collected before BR for both Old and Young. No significant differences were observed between MVC values exerted by Old_Ctrl and Old_Int at any time point (difference ranging from 6 to 11%; $P$ values ranging from 0.204 to 0.521). MVC normalized by quadriceps femoris muscle volume (specific MVC; Fig. 2*B*) was not significantly different among Old_Ctrl, Old_Int

and Young, and did not change significantly across the three experimental time points; however, it tended to follow the trend observed for MVC.

### Single muscle fibre variations with age and bed rest

To assess whether the structural and functional characteristics of single muscle fibres could contribute to the difference between older adults and young individuals and to the changes of lower limb muscle force and power following BR and physical retraining, large populations of fibres were dissected from biopsy samples taken from vastus lateralis and analysed as described in the Methods.

The total populations analysed with single fibre experiments comprised 233 fibres (84 in Old_Ctrl; 77 in Old_Int; 72 in Young) in Pre-BR biopsy sampling, 243 fibres (84 in Old_Ctrl; 86 in Old_Int; 73 in Young) in Post-BR and 241 fibres (84 in Old_Ctrl; 87 in Old_Int; 70 in Young) in post physical retraining sampling. When grouped according to their myosin isoform composition, which was adopted as a molecular marker of fibre type, slow fibres represented 27–28% in the biopsies of Young subjects and 33–39% in the biopsies of Old, suggesting an age-related fibre type transition. Fast 2A fibres represented 35–44% in the biopsy samples of Young and 24–31% in the biopsy samples of Old. The remaining fraction comprised

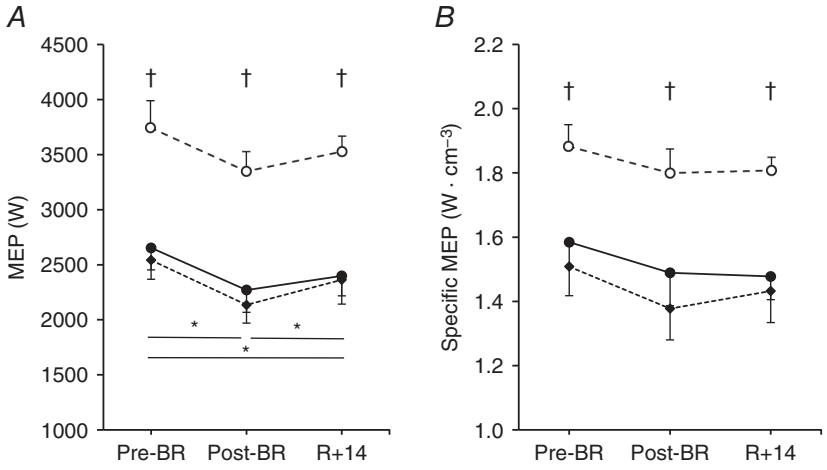


**Figure 1. Effects of bed rest and physical retraining on maximal explosive power of the lower limbs in older adults enrolled in the Control group, Interventions group and Young subjects**
Values are mean ± SE. Maximal explosive power (MEP, *A*) and MEP normalized per unit of quadriceps femoris muscle volume (Specific MEP, *B*) exerted before bed rest (Pre-BR), after bed rest (Post-BR) and after physical retraining (R + 14). Control group (Old_Ctrl, ●), Interventions group (Old_Int, ♦) and Young subjects (○). Differences in MEP and specific MEP were tested using general linear model and following *post hoc* analysis with Bonferroni corrections. *Difference between periods in the two Old groups; †difference between the two Old groups and Young.

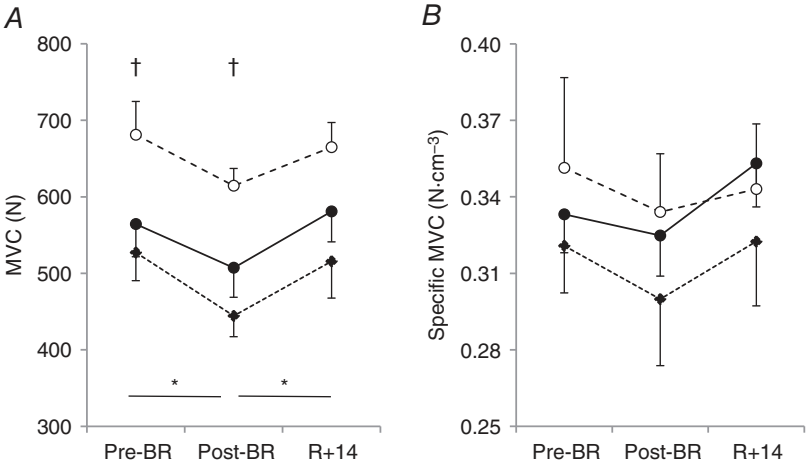


**Figure 2. Effects of bed rest and physical retraining on maximal voluntary isometric contractions of the right quadriceps femoris muscle in older adults enrolled in the Control group, Interventions group and Young subjects**
Values are mean ± SE. Force exerted during maximal voluntary isometric contractions (MVC, *A*), and MVC normalized per unit of quadriceps femoris muscle volume (Specific MVC, *B*). Control group (Old_Ctrl, ●), Interventions group (Old_Int, ♦) and Young subjects (○). Pre-BR: before bed rest; Post-BR: after bed rest; R + 14: after physical retraining. Differences in force were tested using general linear model and following *post hoc* analysis with Bonferroni corrections. *Difference between periods in the two Old groups; †difference between the two Old groups and Young.

**Table 3. Effects of 2 weeks of bed rest and 2 weeks of physical retraining on peak force and velocity during maximal explosive efforts of the Control (Old_Ctrl) and Interventions (Old_CT) groups of older adults and Young subjects**

| | Old_Ctrl ($n = 8$) | | | Old_Int ($n = 8$) | | | Young ($n = 7$) | | | Significance | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre-BR | Post-BR | R + 14 | Pre-BR | Post-BR | R + 14 | Pre-BR | Post-BR | R + 14 | Group | Time | G × T |
| $F$ (N) | 1457 ± 185 | 1297 ± 191 | 1319 ± 176 | 1466 ± 185 | 1299 ± 160 | 1367 ± 221 | 1693 ± 210 | 1588 ± 190 | 1649 ± 112 | 0.009 | < 0.001 | 0.364 |
| $v$ (m·s$^{-1}$) | 2.02 ± 0.22 | 1.94 ± 0.29 | 1.99 ± 0.24 | 2.03 ± 0.19 | 1.87 ± 0.21 | 1.98 ± 0.19 | 2.44 ± 0.16 | 2.31 ± 0.15 | 2.32 ± 0.11 | 0.001 | 0.002 | 0.645 |

All values are means ± SD.
Pre-BR: before bed rest; Post-BR: after bed rest; R + 14: after physical retraining; $F$: peak force; $v$: peak velocity.
Significance by general linear model of the main effects of Group (Old_Ctrl *vs.* Old_Int *vs.* Young), Time (Pre-BR *vs.* Post-BR *vs.* R + 14) and Group × Time interaction (G × T).

hybrid fibres (slow-fast 2A and fast 2A-2X) and in Young subjects a minor group of pure 2X fibres (see also Table 4). No difference was detectable in electrophoretic separation of MyHC isoforms on muscle homogenates (Table 5).

The variations of CSA and of isometric force developed during maximal activation ($F_o$) are reported in Fig. 3 together with their ratio, $P_o = F_o$/CSA, and the value of unloaded shortening velocity ($V_o$). Only the most abundant fibre types, slow, fast 2A and fast 2A-2X, are considered, because for the other types (hybrid slow-fast 2A and pure 2X) there were too few fibres to produce reliable measurements and statistical comparisons. Interestingly, the response to disuse was different in slow and fast 2A between Young and Old subjects, while the two subgroups of older adults (Old_Ctrl and Old_Int) showed very similar results. In baseline sampling, slow fibres showed similar CSA in Young and Old, while fast 2A fibres showed significantly lower CSA values in Old (−27%, $P < 0.05$) compared to Young subjects (Table 4 and Fig. 3*A* and *E*). BR caused a significant reduction of CSA, i.e. atrophy at the single fibre level, in fast 2A fibres of Young subjects (−28%, Fig. 3*E*) and in slow fibres of Old subjects (−19%, Fig. 3*A*), clearly pointing to a differential sensitivity to disuse. By contrast, CSA values after BR were similar between Old_Ctrl and Old_Int both in slow, in fast 2A and in fast 2A2X fibres ($P$ values ranging from 0.512 to 0.881, Fig. 3*A*, *E* and *I*). The determination of isometric force ($F_o$, see Fig. 3*B*) revealed a significant decrease in slow fibres of both Young (−42%) and older adults (−22% in Old_Ctrl; −29% in Old_Int) at the end of BR and this resulted in a reduction in specific force ($P_o$) in the slow fibres of the Young (−44%, Fig. 3*C*) subjects. No significant difference was observed in unloaded shortening velocity comparing Pre-BR, Post-BR and R + 14 (Fig. 3*D*, *H* and *N*). In both Young and Old, fast 2A-2X fibres showed a trend to a decrease in $F_o$ and $P_o$ after BR, which remained below statistical significance. Importantly, at R + 14 CSA values of slow and fast 2A fibres and $F_o$ values of slow fibres were lower than before BR in the older adult subjects, indicating an incomplete structural and functional recovery (Fig. 3*A*, *B* and *E*). Moreover, fast 2A-2X fibres of Young showed lower values of $F_o$ and $P_o$ at R + 14 than before BR (Fig. 3*L* and *M*).

## Knee and ankle co-contraction

Finally, to gain insight into the contribution of motor control to the variations in MEP, we recorded the EMG activity of knee and ankle flexor and extensor muscles to calculate an index of co-contraction between antagonist muscles (see Methods). As shown in Fig. 4, the level of knee and ankle co-contraction during explosive extensions of the lower limbs was not significantly different among Old_Ctrl, Old_Int and Young at any of the three investigated time points. Also, knee co-contraction did not

**Table 4. Baseline data of single fibres analysed from biopsy samples collected prior to the beginning of bed rest; in addition to the three groups of fibres reported in the table, hybrid slow-2A ($n$ = 16 in Old_Ctrl; $n$ = 14 in Old_Int; $n$ = 7 in Young) and few pure fast 2X ($n$ = 2 in Old_Ctrl; $n$ = 0 in Old_Int; $n$ = 3 in Young) fibres were also found and characterized**

| | Slow/1 | | | | Fast 2A | | | | 2A-2X | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Old_Ctrl | Old_Int | Young | $P$ | Old_Ctrl | Old_Int | Young | $P$ | Old_Ctrl | Old_Int | Young | $P$ |
| Fibre (number) | 29 | 36 | 19 | | 24 | 17 | 32 | | 13 | 10 | 11 | |
| CSA ($\mu m^2$) | 6426 ± 2768 | 6335 ± 3233 | 6256 ± 3918 | 0.984 | 5607 ± 1820* | 5601 ± 2363* | 7662 ± 3388 | 0.009 | 5338 ± 2731 | 4561 ± 1289 | 7282 ± 3131 | 0.053 |
| $F_o$ (mN) | 0.683 ± 0.389 | 0.732 ± 0.320 | 0.864 ± 0.583 | 0.333 | 0.565 ± 0.338* | 0.650 ± 0.239* | 0.962 ± 0.611 | 0.005 | 0.897 ± 0.459* | 0.555 ± 0.252* | 1.447 ± 1.060 | 0.017 |
| $P_o$ (mN mm$^{-2}$) | 116 ± 69 | 133 ± 76 | 144 ± 78 | 0.445 | 103 ± 54 | 135 ± 86 | 132 ± 54 | 0.179 | 177 ± 89 | 130 ± 66 | 188 ± 96 | 0.265 |
| $V_o$ (L s$^{-1}$) | 0.543 ± 0.681 | 0.854 ± 0.820 | 0.911 ± 0.798 | 0.268 | 2.873 ± 2.970 | 1.795 ± 1.277 | 1.771 ± 1.715 | 0.168 | 2.924 ± 2.440 | 2.939 ± 2.132 | 3.417 ± 2.658 | 0.907 |

All values are means ± SD.
CSA: cross sectional area; $F_o$: isometric force; $P_o$: specific force; $V_o$: unloaded shortening velocity.
$P$: significance by general linear mixed model of the main effect of group (Old_Ctrl *vs.* Old_Int *vs.* Young).
*Significantly different from Young.

change significantly across the investigated time points in all three groups. In Young, ankle co-contraction was also not affected by BR and physical retraining. Conversely, in both older adult groups, ankle co-contraction showed an increase after BR (Old: +27.6%, $P = 0.035$), and remained greater than at Pre-BR also at R + 14 (Old: +30.4%, $P = 0.017$) (Fig. 4).

## Discussion

In this study, we showed that the MEP of lower limbs in healthy older adult males (mean age: 60 years) was about 30% lower than in young males (mean age: 23 years). MEP normalized by quadriceps femoris muscle volume was also substantially lower (−19%) in older adults. Fourteen days of BR induced a significant decrement of MEP in Old, and a similar trend was observed in Young. A period of physical retraining that had the same duration of BR (2 weeks) tended to increase MEP in both groups; however, this intervention was not sufficient to restore muscle power to Pre-BR values in older adults. Additional interventions (cognitive training and protein supplementation) tested on one subgroup of older adults (Old_Int) did not mitigate BR-induced muscle atrophy and MEP decrement and did not enhance physical training-induced adaptations.

### Lower limb muscle function was affected by age

Before any intervention, MEP developed by a group of older adult men was about 30% lower than that exerted by a group of young subjects with similar life style, body weight and stature (Fig. 1*A*). Note that the difference in MEP as well as in other parameters was clearly detectable even if the age of the older group was not very advanced (approximately 60 years). The rate of decline of muscle function is relatively slow from 20 to 50 years of age, and becomes marked after 50 years of age (Wilmore, 1991). Previous studies found that muscle power decreases over the age range 65–89 years by about 3.5% every year (Skelton *et al.* 1994), with longitudinal observations indicating a 6% annual loss of muscle power over 3 years among adults aged 70–85 years (Clark *et al.* 2013). Muscle mass of the lower limbs, and in particular of the knee extensors (quadriceps femoris), directly affects the level of muscle power that can be exerted by lower limbs (Ferretti *et al.* 2001). As expected, our results indicated that quadriceps muscle volume was lower (−15.3%; Table 2) in older adults (Rosenberg, 1997; McPhee *et al.* 2013), contributing to explain their lower MEP. However, when MEP was normalized per unit of quadriceps muscle volume (specific MEP), it was still substantially lower (∼−19%) in Old than in Young (Fig. 1*B*). This difference in specific MEP generated during a fast

**Table 5. Per cent distribution of MyHC isoforms in the Control (Old_Ctrl) and Interventions (Old_Int) groups of older adults and Young subjects before bed rest, after bed rest and after physical retraining**

| | Old_Ctrl ($n = 8$) | | | Old_Int ($n = 8$) | | | Young ($n = 7$) | | | Significance | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre-BR | Post-BR | R + 14 | Pre-BR | Post-BR | R + 14 | Pre-BR | Post-BR | R + 14 | Group | Time | G × T |
| MyHC 1 | 47.4 ± 13.0 | 43.8 ± 10.8 | 44.0 ± 8.7 | 41.6 ± 7.7 | 41.8 ± 8.1 | 41.0 ± 9.7 | 35.7 ± 7.5 | 38.0 ± 8.4 | 35.9 ± 13.4 | 0.095 | 0.623 | 0.871 |
| MyHC 2A | 36.3 ± 10.2 | 35.0 ± 7.5 | 38.2 ± 16.5 | 37.1 ± 8.6 | 38.6 ± 9.1 | 40.3 ± 13.6 | 45.2 ± 13.5 | 48.3 ± 6.6 | 47.7 ± 12.3 | 0.084 | 0.536 | 0.921 |
| MyHC 2X | 15.8 ± 11.8 | 21.2 ± 9.3 | 17.1 ± 14.8 | 18.1 ± 11.2 | 19.6 ± 9.4 | 18.3 ± 15.9 | 19.1 ± 12.6 | 15.2 ± 8.8 | 16.4 ± 12.7 | 0.889 | 0.963 | 0.679 |

All values are means ± SD.
Pre-BR: before bed rest; Post-BR: after bed rest; R + 14: after physical retraining; *F*: peak force; *v*: peak velocity.
Significance by general linear model of the main effects of Group (Old_Ctrl *vs.* Old_Int *vs.* Young), Time (Pre-BR *vs.* Post-BR *vs.* R + 14) and Group × Time interaction (G × T).

(∼400 ms) and relatively complex movement that involves three joints and the interaction of uni- and multi-articulate muscle–tendon units was conceivably due to different components. One of them can be related to different single muscle fibre characteristics between Young and Old. Single muscle fibre analysis showed a greater abundance of slow fibres in Old (≈ 40%) compared to Young (≈ 28%) (Table 4). These data are in agreement with previous observations (Lexell *et al.* 1988; Klitgaard *et al.* 1990*b*), but are not supported by electrophoresis and densitometry of MyHC, which accounts not only for fibre abundance but also for fibre size. Slow fibres develop less force and shorten at lower speed (see Table 4) and this implies a lower peak power output. In addition, fast 2A fibres were not only less abundant but also thinner (see Table 4) in Old compared to Young. An age-related selective atrophy of fast fibres in knee extensor muscles was observed for the first time by Klitgaard *et al.* (1990a) and confirmed by more recent studies (e.g. Brunner *et al.* 2007; Murgia *et al.* 2017). Thus, quantitative variations in muscle fibre type distribution and size, together with different levels of intramuscular fat and fibrosis, would be expected to play a role in age-related loss of specific power (Marcus *et al.* 2012; McGregor *et al.* 2014).

In addition, differences in muscle architecture and tendon properties between young and older adults have been previously found, making these factors potential contributors to the age-related decline in muscle power and mobility (Stenroth *et al.* 2015). In particular, muscle fascicle length affects muscle power production capacity according to force–length and force–velocity relationships (Narici & Maganaris, 2007). Also, the lower tendon stiffness shown by elderly subjects (Stenroth *et al.* 2012) conceivably impairs the tendon roles of "energy re-distributor" and "power amplifier" during explosive movements (Hof *et al.* 1983; Fukashiro *et al.* 2006; Cormie *et al.* 2011).

Finally, motor control is an important component for the multi-articular development of muscle power, and its deterioration has been recently indicated as one of the relevant mechanisms responsible for age-related loss of muscle function (Venturelli *et al.* 2015). In particular, ageing leads to the loss of spinal motor neurons and changes in maximal motor neuron firing frequency, activation capacity, co-contraction of antagonist muscles and spinal inhibitory circuitry (Klass *et al.* 2008; Aagaard *et al.* 2010). Our results indicated that the level of co-contraction at the knee and ankle joints was similar between Old and Young (Fig. 4); hence, it can be assumed that this neuromuscular feature was not responsible for the MEP difference between groups. However, activation capacity was not examined in the present study, and its age-related decrement (Onambele *et al.* 2007) could have played a role in the lower absolute and specific MEP exerted by Old individuals.

### Disuse-induced loss of lower limb muscle power and volume

Our results showed that 14 days of BR impaired MEP development in Old (−15.2%), and that the same trend was observed in Young (−10.4%) (Fig. 1*A*). This was accompanied by lower values of peak force and velocity in Old (Table 3). The amount of MEP lost in the present study was similar to that observed after a 10-day BR in healthy elderly individuals (−14%) (Kortebein *et al.* 2008), and is lower than that observed after longer periods of disuse without countermeasures in young participants [about 24–30% after 35–90 days of BR (Ferretti, 1997; Rittweger *et al.* 2007; Rejc *et al.* 2015)]. It is worth noting that, in the present study, explosive lower limb extensions were performed under simulated microgravity (i.e. against an after-load equal to about 48% body mass), allowing fast lower limb extension movements. Interestingly, Deschenes *et al.* (2008) found that, at slower isokinetic knee extensions, muscle power was similarly

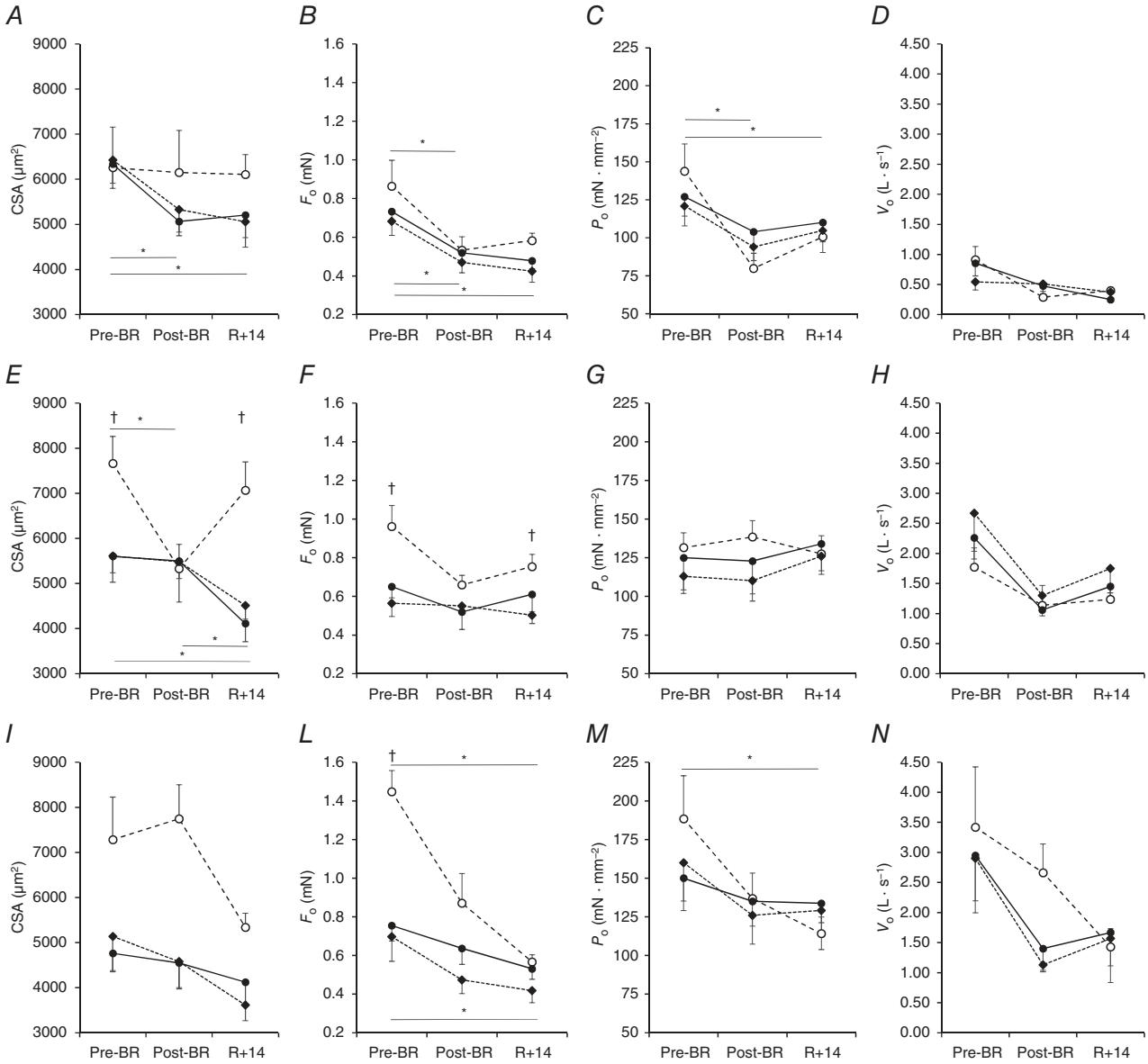


**Figure 3. Effects of bed rest and physical retraining on single fibre parameters**
Cross sectional area (CSA: *A*, *E*, *I*), isometric force during maximal activation ($F_o$: *B*, *F*, *L*), specific force or tension ($P_o$: *C*, *G*, *M*) and single fibre shortening velocity ($V_o$: *D*, *H*, *N*) in slow or type 1 fibres (*A–D*), fast 2A (*E–H*) and fast 2A2X (*I–N*) in the older adult Control group (Old_Ctrl, ●), Interventions group (Old_Int, ♦) and in Young subjects (○). Values are mean ± SE. Differences in CSA, $F_o$, $P_o$ and $V_o$ were tested using general linear mixed model and are indicated in the lower part of each panel for Old and the upper part for Young. *Difference between periods; †difference between the two Old groups and Young.

affected in elderly and young individuals (∼ −11%) after 1 week of unilateral lower limb suspension. However, at higher contraction speeds, disuse significantly affected muscle power only in elderly. In addition, other studies showed that, after a period of short-term immobilization, rapid muscle force capacity was more affected in elderly than young men (Hvid *et al.* 2010, 2014). These findings might suggest that disuse degrades the functional capacity of fast-twitch muscle tissue in older adults more than in young. However, the present study does not support the hypothesis that BR-induced muscle atrophy targeted primarily fast fibres in older adults; in fact, atrophy was more pronounced in slow fibres of Old and in fast 2A fibres in Young (see below). Hence, it seems plausible that BR accelerated ageing-related neural impairments that preferentially affect fast motor neurons (i.e. firing frequency and activation capacity) (Deschenes *et al.* 2008; Aagaard *et al.* 2010), thus inhibiting the capacity of older adults to activate fast-twitch muscle tissue during fast muscle contractions. In addition, we found that co-contraction between representative plantar flexors and dorsi flexors increased only in older adults after BR. This can be considered an additional disuse-induced negative neural adaptation that was amplified by ageing (Aagaard *et al.* 2010), as greater levels of co-contraction generally impair power exertion (Reeves *et al.* 2006*a*). The view that BR may have accelerated ageing-related neural impairments is also consistent with the fact that specific MEP tended to be more affected in older adults (−7.8%) than in young (−4.3%) (Fig. 1*B*). In fact, neural adaptations are one of the factors that can affect muscle-specific power (see discussion above and Narici & de Boer, 2011). From a functional perspective, the substantial disuse-induced loss of muscle power in older adults and elderly individuals can be deleterious, as their lower limb muscle power is already closer to the critical threshold required for independent mobility, physical functioning and lower risk of falling (Reid & Fielding, 2012).

Muscle atrophy is another factor that contributed to the loss of lower limb muscle power after disuse. BR-induced muscle atrophy is larger in postural than non-postural muscles, larger in the extensors than in the other thigh muscle groups, and larger in the calf muscles than in the other leg muscle groups (Ferretti *et al.* 2001; Alkner & Tesch, 2004; de Boer *et al.* 2008; Belavy *et al.* 2009). In particular, Ferretti *et al.* (2001) indicated that the loss of lower limb muscle power after 42 days of BR was primarily explained by the reduction of quadriceps femoris CSA, and that other factors such as impaired neural activation or fibre-specific tension might have accounted for only 5%. In the present study, total-body fat free mass was not affected by BR in the two groups. Conversely, quadriceps femoris muscle volume decreased by a similar extent in Old (−8.3%) and Young (−6.1%); in both cases, the muscle volume decrement was smaller than the decrease of MEP (−15.2% in Old and −10.4% in Young). A comparable overall amount of muscle atrophy was reported by Suetta *et al.* (2009) after 2 weeks of immobilization by unilateral, whole leg casting; however, these authors reported that quadriceps muscle volume decreased more in young (−8.9%) than in elderly males (−5.2%). This last finding is not in agreement with the similar muscle atrophy between young and older adults that was found in the present study. Data on the combined effect of ageing and disuse on muscle atrophy seem to be not consistent (Suetta *et al.* 2009), as also shown by the greater adductor pollicis muscle atrophy found in elderly compared to young individuals after 2 weeks of immobilization (Urso *et al.* 2006). The differences observed between the present study and the two above mentioned studies could be at least partially explained by the different experimental conditions (i.e. BR *vs.* immobilization; larger *vs.* smaller muscle group; age: older adults *vs.* elderly individuals).

It is important to note that, when analysed at single fibre level, the impact of 2 weeks of disuse is more pronounced in slow fibres of Old and in fast 2A fibres in Young. Fast 2A-2X fibres are also responsive to BR in Young. This

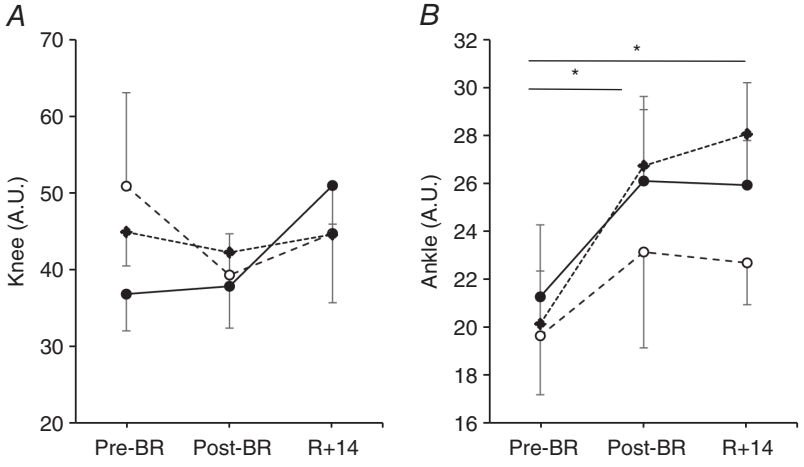


**Figure 4. Effects of bed rest and following physical retraining on the level of co-contraction at the knee and ankle joint in older adults enrolled in the Control group, Interventions group and in Young subjects**
Values are mean ± SE. Control group (Old_Ctrl, ●), Interventions group (Old_Int, ♦) and Young subjects (○). Level of co-contraction between biceps femoris and vastus lateralis (Knee, *A*) and between tibialis anterior and gastrocnemius medialis (Ankle, *B*) recorded during explosive efforts of the lower limbs before bed rest (Pre), after bed rest (Post-BR) and after physical retraining (R + 14). Differences in the level of Knee and Ankle co-contraction were tested using general linear model and following *post hoc* analysis with Bonferroni corrections. *Difference between periods in the two Old groups.

differential response suggests a different functional role of the two major fibre types in the habitual postural and locomotor activities which are suppressed during BR. A greater sensitivity to disuse in fast fibres has been reported in previous studies (e.g. Hvid *et al.* 2011). In that study, however, the decrease in force and specific force was not different between young and elderly subjects. A possible explanation can be found in the different protocols, as Hvid and co-workers induced not only disuse (as in BR), but also immobilization by applying a cast on the legs.

### Six sessions of physical training performed after disuse were not sufficient to restore lower limb muscle power in older adults

In the present study, a period of physical retraining after BR of the same duration as the BR promoted incomplete MEP recovery in older adults, while it restored MEP to pre-BR values in young individuals (Fig. 1*A*). Similarly, specific MEP tended to remain lower than at Pre-BR in older adults (−6.5%). These findings are in agreement with previous studies that investigated similar periods of disuse and retraining, showing incomplete recovery of rapid force capacity in elderly but not young individuals (Hvid *et al.* 2010, 2014). It is plausible that negative adaptations induced by disuse and ageing on the neural system were not fully counteracted by physical retraining in the elderly, playing a role in the incomplete recovery of muscle function during fast muscle contraction. In particular, after physical retraining, co-contraction of ankle muscles remained similar to that at Post-BR and greater than at Pre-BR in older adults (Fig. 4), conceivably contributing to the impaired muscle power exertion (Reeves *et al.* 2006*a*). In addition, other potential ageing- and disuse-induced neural adaptations not tested in the present study [i.e. loss of spinal motor neurons; impaired maximal motor neuron firing frequency and activation capacity; altered motor unit recruitment pattern (Klass *et al.* 2008; Aagaard *et al.* 2010; Narici & de Boer, 2011)] may not have been restored by physical retraining, contributing as well to the impaired muscle power output observed in the elderly during fast muscle contractions. Interestingly, quadriceps muscle volume did not completely recover Pre-BR values in older adults (−3.1%), and an incomplete recovery of contractile function was also observed in single muscle fibres of the older participants in partial agreement with previous observations (Suetta *et al.* 2009; Hvid *et al.* 2014). Also, the present study further supports the view that recovery of muscle function in older adults is particularly impaired during fast muscle contractions, because muscle strength exerted during isometric MVC of the knee extensors was fully restored after physical retraining in both older adults and young subjects (Fig. 2*A* and *B*). Complete recovery of muscle strength during isometric MVC in the elderly was also found after 4 weeks of retraining, which were subsequent to 2 weeks of immobilization (Suetta *et al.* 2009). By contrast, shorter retraining (1 week) did not lead to the same positive outcome, even if the immobilization period was shorter (4 days) (Hvid *et al.* 2014).

These findings seem to highlight the role of training volume as an important determinant for the recovery of muscle function in the elderly. Along this line, most of the studies that reported positive neuromuscular adaptations in the elderly involved a greater number of training sessions (i.e. ∼20–45 sessions) than the above mentioned studies (Reeves *et al.* 2006*a*; Reid & Fielding, 2012).

While discussing the lower limb muscle function recovery observed in the present study, it is also important to note that the proposed physical training was not primarily focused on the increment of lower limb muscle power, but rather to a more global physical improvement. In fact, more than half of each training session was devoted to aerobic exercise, and no explosive or high-speed movements were included during strength exercises.

Interestingly, training interventions that emphasized explosive power focusing on higher movement speed, even without requiring specific resistance training equipment, were safe and effective for improving lower limb muscle power and physical functioning (i.e. stair climbing) in the elderly population (Bean *et al.* 2002, 2004; Reid & Fielding, 2012). Similarly, physical training performed with isotonic weight-resistance machines or inertial load has the potential to increase lower limb muscle strength and power in elderly individuals (Reeves *et al.* 2006*a*,*b*; Onambele *et al.* 2008). Hence, further studies focused on the retraining of muscle power after a period of disuse should include higher-speed, power-orientated exercises. The improvement and recovery of muscle power after disuse in older adults and elderly individuals is of particular interest in view of its relevance for independent mobility and quality of life in the elderly population (Reid & Fielding, 2012). In fact, several studies identified lower limb muscle power as a significant predictor of functional performance in older adults (reviewed by Reid & Fielding, 2012).

### Cognitive training and protein supplementation did not significantly affect muscle mass and power in older adults

Two distinct countermeasures, cognitive training and protein supplementation, were adopted to attenuate the impact of bed rest and improve recovery, respectively, in a subgroup of older adult participants. Cognitive training by navigating through virtual mazes might activate the same neural systems involved in mobility and this might produce, in turn, subliminal muscle contractions. An improved executive/attention ability and processing speed (Marusic *et al.* 2018) as well as suppression of the increase in plasma BDNF concentration (Passaro *et al.* 2017) have been observed after bed rest in the older

individuals enrolled in the present study who were exposed to cognitive training. However, cognitive training did not lead to any mitigation of BR-induced loss of muscle mass and power, as also reported by Passaro *et al.* (2017), who additionally analysed the correlation between variations of explosive power and BDNF plasma levels.

The usefulness of protein supplementation in BR experiments has been considered in many studies with mixed results (see Stein & Blanc, 2011), possibly in relation to the amount and the timing of administration. In the present BR study, development of insulin resistance (Pisot *et al.* 2016) and anabolic resistance (Biolo *et al.* 2017) were detected. The protein supplementation was restricted to the physical retraining phase, thus starting from a condition in which anabolic resistance was present. No significant effect of protein supplementation on the recovery of muscle mass and power was found; however, we cannot rule out the possibility that the lack of effect was just due to the insufficient amount of protein.

### Limitations of the study

Two main outcomes of this study are that (1) the loss of lower limb muscle power due to disuse was more pronounced in older adults than in young individuals; and (2) physical retraining that had the same duration as disuse was not sufficient to fully recover muscle power and volume in older adults. However, from a statistical standpoint, these messages are weakened by the small number of participants enrolled in this study; this is a consequence of logistical limitations that are intrinsic to this type of studies.

Similar limitations are also related to the uneven sample size of the groups. Also, the fact that only male subjects were enrolled limits the applicability of the findings reported in the present study. It should be noted that women, and particularly post-menopausal women, can count on lower muscle mass than matched male subjects, and thus may be more responsive to disuse than men. Finally, bioelectrical impedance analysis has some intrinsic limitation for body composition analysis; however, this methodology was used in the present study only to describe anthropometric characteristics of the population, while a more sensitive technique (MRI) was used for assessing the changes in muscle volume of the representative lower limb extensor examined (quadriceps femoris).

### Conclusions

Two weeks of disuse decreased the lower limb muscle power in both young and older individuals. Six physical training sessions performed in the 2 weeks subsequent to disuse promoted the recovery of muscle mass and power; however, they were not sufficient to restore muscle function to pre-disuse values in older individuals. Also, countermeasures based on cognitive training and protein supplementation were not effective for reducing the impact of disuse and improving physical retraining in older adults. Taken together, these findings indicate that susceptibility to the impact of disuse increases with ageing, and suggest that a greater number of training sessions, the inclusion of power-orientated exercises and more effective countermeasures are required to restore muscle mass and power after 2 weeks of disuse in older individuals.

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## Additional information

### Competing interests

None declared

### Author contributions

E.R., P.T., M.N., B.S., R.P., G.B., A.P., J.R., C.R. and S.L. were responsible for study conception and experimental design; E.R., M.F., P.T., A.B., L.T., L.C., J. R. and S.L. performed the experiments; E.R., M.F., P.T., B.S., R.P., C.R. and S.L. analysed and interpreted the data; E.R., M.F., P.T., C.R. and S.L. drafted the article; and E.R., M.F., P.T., M.N., B.S., R.P., G.B., A.P., J.R., C.R. and S.L. revised the article critically for important intellectual content. All authors have approved the final version of the manuscript and agreed to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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**Eight weeks of iso-inertial resistance trainings improve maximal explosive power of lower limbs in healthy older adults**

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## INTRODUCTION

Each year the percentage of people, within the world population, that ages sixties or above is increasing dramatically. By 2050 this fraction might even reach the 22% of the world population (Nations, 2012). As an individual gets older it may increase their incidence to experience physical performance limitation which is the consequence of several factors: biological, lifestyle and psychosocial ones (Tieland *et al.*, 2018). Within the biological field the alterations in muscle skeletal function, due to the ageing process, play a crucial role (Tieland *et al.*, 2018). The decline in the muscle skeletal performance that a person experiences as they age might be the consequence of sarcopenia, which is described as a physiological age-related reduction in skeletal muscle mass beyond a specific threshold (Janssen *et al.*, 2002). Precisely motor neuron loss, neuromuscular junction instability and repeated cycles of denervation and reinnervation of muscle fibers might lay upstream of the age-related loss of skeletal muscle mass and functionally they might lead to reduction in muscle force and power abilities, impaired coordination and eventually falls and frailty (Hepple & Rice, 2016). Because of these last findings together with the fact that the ability of the muscle to generate power is even more impaired than the ability to produce muscle force (Aagaard *et al.*, 2010; Reid & Fielding, 2012) with ageing, old people may progressively reduce their ability to perform everyday tasks (i.e. rising from a chair, climbing the stairs, etc.). Even the walking ability is subjected to changes due to the detrimental effects of the ageing process. Indeed, new neuromuscular strategies such as an increased co-contraction time of agonist-antagonist lower limbs muscles have been shown to take place during usual walking in this population (Mian *et al.*, 2006; Floreani *et al.*, 2018): this strategy plays an important role in increasing the metabolic cost of walking and might serve as a way to increase dynamic stability by sacrificing walking economy. Based on these premises it should be hypothesised that without appropriate counter measurements the quality of life of older people may necessarily decrease ending up to the loss of physical independence (Janssen *et al.*, 2002; Goodpaster *et al.*, 2006; Delmonico *et al.*, 2007). Hence, in order to prevent frailty and physical disability, which are associated with increased care needs and costs (Wolff *et al.*, 2002), some counter measurements are required. Physical exercise has been shown to be a promising one. Resistance exercise training, in particular, has been observed to produce positive results in preserving and even improving muscle mass (Peterson *et al.*, 2011), force (Peterson *et al.*, 2010) and power (Izquierdo *et al.*, 2001; Caserotti *et al.*, 2008) in elderly people, slowing down the progression of the sarcopenic process. Among the different types of resistance training isoinertial flywheel resistance training program (FW) has recently received increasing attention. Introduced firstly by Berg and Tesch (Berg & Tesch, 1994), more or less than twenty five years ago, as a device to counter act the adverse consequences of the exposure of the neuromuscular system to microgravity in astronauts during spaceflights, it has

become popular in different sport and movement fields: athletic performance (Gual *et al.*, 2016), rehabilitation (Greenwood *et al.*, 2007) and ageing (Onambele *et al.*, 2008). The reason why this device has having such success is manifold: compared to the traditional isotonic exercise (i.e. done using free weights or weight stack machines, GD) i) it allows the subject to perform a maximal concentric force throughout the range of motion and it requires them to generate an eccentric force greater than the concentric one, provided a maximal effort during the execution of the exercise and an appropriated technique (i.e. delayed breaking action during the eccentric phase); ii) it ensures accommodated resistance; iii) it permits the subject to generate maximal force since the beginning of the exercise leading progressively to a decrease in the force developed (Tesch *et al.*, 2017). Although these results might favour the prescription of FW resistance training by physical experts more than the GD, recent reviews on this topic ended up to divergent conclusions. While the review of Maroto-Izquierdo and colleagues (Maroto-Izquierdo *et al.*, 2017) concluded that FW resistance training is superior to GD exercise in promoting muscle strength, power and size gains in healthy subjects and athletes, Vicens-Bordas and his team (Vicens-Bordas *et al.*, 2018) pointed out that the two trainings had similar outcomes in term of strength improvements. The comparison between these two types of training regarding their efficacy to produce muscle adaptation and functional enhancement might be of clinical interest in elderly population. Indeed, older people might need a physical intervention that should be capable of maximizing their physical benefits in really short time in order to speed up re-education path after hospitalization. Same necessities may be required even before hospitalization when they need to increase their physical status to avoid movement complications once hospitalization period is terminated. Hence the present study aims to investigate the effects of two different types of resistance training (FW vs GD) across a period of 8 weeks on maximal strength, explosive power and muscle architecture in healthy older subjects. Because of all the benefits that FW resistance training might bring to people involved in the program, as suggested by some researches and recalled previously, we hypothesize that FW training would be superior compared with GD one in promoting force, power and muscle adaptation. Furthermore, due to poor evidence in literature regarding the effects of FW training on physical functional measurements on elderly population, the second purpose of this study is to investigate the influence of this training on the metabolic cost (CW), agonist-antagonist leg muscles co-contraction time (CCT) during habitual walking speeds and more general measures of physical fitness (i.e. 6-minute walking and time up and go, TUG, exercises). The comparison between the influences derived by FW and GD trainings on these last measurements is investigated within the second aim purpose.

## SUBJECTS AND METHODS

### *Subjects*

Twenty-nine healthy older Caucasian subjects (15 males and 14 females) participated in this research study but only twenty-four of them (13 males and 11 females) completed the whole experiment (see Results). All the characteristics of the subjects are reported in Table I. Prior to the beginning of the study, all subjects filled out two physical activity-related questionnaires (IPAQ-SF (Craig *et al.*, 2003), SF12 (Ware *et al.*, 1996)), had a full medical history and physical examination in order to test their eligibility to the study protocol. Exclusion criteria were the following: age of the participant below and over 60 and 75 years respectively, presence of mobility limitation, neurological diseases (i.e. disk herniation, spinal nerve lesions, etc.) and/or frailty linked to bone health problems (i.e. osteoporosis or severe osteoporosis, etc.). The purposes and objectives of this study were carefully explained to the subjects and written informed consent was obtained from all of them. The study protocol was approved by the ethics committee of the University of Udine (Italy) and was conducted according to the ethical standards of the Helsinki Declaration.

### *Study protocol*

All the subjects were asked to complete 8 weeks of personalized progressive physical training program interposed between two batteries of tests. The whole group of subjects was randomly split into two groups (experimental and control). The Control group (6 male and 6 female), CTRL, underwent a traditional resistance training protocol where exercises were performed on a horizontal gravity dependent leg press machine. The Experimental group (7 male and 5 female), EXP, followed a resistance training program whose exercises were done on an isoinertial machine. The battery of tests included: anthropometric and body composition measurements, maximal voluntary isometric force and maximal explosive power tests, metabolic cost of walking measurements and physical performance evaluations (see the following paragraphs). In addition, physical capacities were monitored weekly to adjust physical activities individually.

### *Physical training program*

The training program was performed twice a week for a period of 8 weeks (15 ± 2 sessions in total). Training was conducted at the exercise physiology laboratory of the University of Udine and supervised by 3 physical trainers who instructed the subjects to properly perform the different exercises. The research assistant and the physical trainers were responsible for verifying that each subject participated in each training session, performed exercises correctly, and completed at least 80-90 % of the exercise session.

Each training session, independently from group membership, lasted about 60 minutes and consisted of 3 parts: warm up and postural exercises, resistance training and cool down exercises.

The warm up and postural exercises were 20 minutes long and included stretching and flexibility exercises for the main muscle groups, from the neck muscles to the shank ones.

The resistance training was group specific. The CTRL group was asked to perform 3 to 5 series of 10 to 12 repetitions of leg press exercise. The EXP group, on the contrary, carried out the same amount of volume but on the isoinertial machine. In order to compare the two resistance training exercises the following expedients were taken into account: i) the angle displacement of the knee and ankle joints during the execution of the two exercises was close to 100° ii) the training load was set so that the single participant was able to perform between 12 to 14 repetitions with good form independently from the type of exercise considered, adapted from previous works (Norrbrand *et al.*, 2008; Norrbrand *et al.*, 2010). The first 4 sessions of the training intervention were dedicated to make the participant familiar with the specific resistance exercises.

The CON subjects, on leg press (Technogym, Cesena, Italy), push with the legs flexed (80-90° at the level of the knee) against the external resistance provided by the loads. The aim of the leg press exercise was to extend the knees to approximately 170° (avoiding full extension), bend them resisting to the descent of the loads and repeat the extension/flexion cycle again till reaching the amount of repetitions set for the exercise session. The concentric and eccentric phases of this exercise were executed at comfortable movement speed (i.e. 2 -3 seconds of duration for each single phase).

The Experimental subjects performed the training on an isoinertial machine (Twister Pulley, Sport Tech & Tools S.L.U., Cádiz, Spain) where workloads were provided by the inertia of a rotating mass. Changes in the inertia were accomplished by using increments of more or less than 0.018 $kg*m^2$ (range of inertia employed: from 0.060 to 0.160 $kg*m^2$). These increments were set by connecting single loads to the rotating mass. The participant worn a harness at the level of the hip and pelvis which was connected by a special rope to the rotating mass (a flywheel disk). Precisely through the use of a couple of pulleys the rope transmitted the force downwards and perpendicular to the participant's position on the machine. The exercise started from a squatting position where the knees where flexed at 90°. Participants were asked to start the exercise by pushing upward with a knee extension movement. This action generated the force necessary to put the rope under tension, rotate the flywheel connected to the rope and let the latter start to wrap around the former. Once participants reached almost full standing position, the rope, already wrapped up round upon the flywheel, unrolled in the other direction pulling the body downward. We required each member of the EXP group to perform the upward phase as fast as they could whilst for the downward phase they were asked to resist gently for the first part of the movement and then slow down till the body reached the starting

position and repeat the whole cycle again. Each two weeks working loads for both the training exercises were increased by the amount that made the participant able to perform 13 to 15 repetitions with good form. Training volume was increase gradually either for both groups: from 3 series of 10 to 12 repetitions for the first three weeks of intervention to 5 series of 10 to 12 repetitions for the remaining weeks. Training sessions were interposed by a period of at least 48 hours where participants were asked to keep their usual physical practises without exceeding in doing strenuous activities.
The last part of each training session was dedicated to breathing (2 min. of diaphragmatic belly breathing and 2. min of diaphragmatic rib cage breathing) and lower back mobility exercises (2x10 "cat & camel", 2x10 "press up").

**Measurements**

***Anthropometric characteristics, body composition and morphological properties of the thigh muscles***

Body mass (BM) was measured to the nearest 0.1 kg with a manual weighing scale (Seca 709, Hamburg, Germany) with the subject dressed only in light underwear and no shoes. Stature was measured to the nearest 0.5 cm on a standardized wall-mounted height board.
Body composition was measured by using bioelectrical impedance analysis with a tetra-polar impedance-meter (BIA101, Akern, Florence, Italy), according to accepted method (Lukaski *et al.*, 1986). Body composition (fat-free mass, FFM, and fat mass, FM) was obtained from the software provided by the manufacturer. This method has been already utilized and validated to investigate changes in body composition in the elderly (Birch & Fisher, 1998; Kyle *et al.*, 2004).
The lean (fat-free) volume of the right thigh (TMM) was estimated by thigh length, circumference and skinfold measurements, following the Jones and Pearson's method (Jones & Pearson, 1969), corrected by the equation provided by Layec and colleagues' work (Layec *et al.*, 2014).
The vastus lateralis fiber fascicle length (L), pennation angle (Pa) and muscle thickness (VL MT) together with rectus femoris muscle thickness (RF MT) were measured using B-mode ultrasound probe (Esaote Biomedica, AU3Partner, Florence, Italy) on all subjects laid supine. VL and RF images were taken at 50% of the thigh length, the distance between the great trochanter and the lateral knee joint space, by ultrasound probe (7.5-MHz linear-array transducer) as suggested by Narici and colleagues (Narici *et al.*, 1989). The head of the probe was held perpendicular to the dermal surface and both longitudinal and transverse images were collected from the muscles of interest. At each scan site probe location was reported on an acetate paper whose purpose was to ensure proper placement of the probe across repeated scans among different experimental conditions (before and after the training program). Ultrasound scans were analysed offline with digitizing software (ImageJ 1.44p,

National Institute of Health, USA). Muscle thickness was measured following Weiss's method (Weiss, 1984). Pennation angle was measured as the angle of fascicle insertion into the deep aponeurosis. L was defined as the length of the fascicle between the deep and superficial aponeuroses (Narici *et al.*, 1996).

**Maximal explosive power of the lower limbs**

The biomechanical parameters of the explosive efforts were studied by means of the Explosive Ergometer (EXER), described previously in details (Lazzer *et al.*, 2009). Briefly, EXER consists of a metal frame supporting one rail, which was inclined by 20 degrees. A seat, fixed on a carriage, was free to move on the rail, its velocity along the direction of motion being continuously recorded by a wire tachometer (LIKA SGI, Vicenza, Italy). The subject was able to accelerate himself and the carriage seat backward pushing on two force platforms (LAUMAS PA 300, Parma, Italy) positioned perpendicular to the rail. The total moving mass of the EXER (seat and carriage together) was equal to 31.6 kg. Force and velocity analog outputs were sampled at 2000 Hz using a data acquisition system (MP100; BIOPAC Systems, Inc., Goleta, CA, USA). The instantaneous power was calculated from the product of instantaneous force and velocity values.

The subject was seated on the carriage seat, secured by a safety harness tightened around the shoulders and abdomen, with his arms on handlebars. Two mechanical blocks were used to set the distance between the seat and the force platforms, so that the knee angle at rest was 110 degrees. The blocks also prevented any countermovement and, consequently, any recovery of elastic energy during the pushing phase. After a brief familiarization session with the laboratory equipment, the subjects performed four maximal explosive efforts, the duration of which was about 400 ms. After each push, subjects rested for two minutes with their feet placed on a support. The attempt with the greatest peak power (maximal explosive power, MEP, obtained from the product of velocity, V, by force, F, generated during the effort) was taken into account for further analysis.

**Maximal Voluntary Contractions**

Maximal voluntary isometric contractions (MVCs) of the right lower limb was performed on a special chair. The subject was seated with the legs hanging vertically down. A strap, connected in series to a force sensor (TSD121C, BIOPAC Systems, Inc., Goleta, CA), was tightened around the subject's right ankle. The force sensor was fixed in series to a steel frame. The position of this frame was set prior the execution of isometric knee extension and knee flexion in order to obtain a knee angle of 110 degrees.

Force exerted during MVCs were recorded at a frequency of 2 kHz using a data acquisition system (MP100, BIOPAC Systems, Inc., Goleta, CA). Subjects were asked to perform three MVCs of four – five seconds for each isometric effort. To prevent fatigue, after each contraction subjects rested for two minutes. Before MVCs a series of 5 to 10 warm up contractions (two to three seconds long each) at different intensities were performed.

**Metabolic cost of walking**

Subjects walked constantly on a motor-driven treadmill (Saturn, HP Cosmos, Germany) at 0.83 and 1.11 $m \cdot s^{-1}$. These speeds have been chosen because they are associated with the lower metabolic cost of walking in elderly people (Mian *et al.*, 2006). Each speed was maintained for at least six minutes (from 6 to 8 minutes), and there were four minutes of recovery between walking speed trials, until $V'O_2$ and heart rate reached the resting values. All subjects were familiarized with walking on treadmill two days before the tests. The familiarization phase lasted about 15-20 minutes (Mian *et al.*, 2006). In each testing session, measurements of oxygen consumption ($V'O_2$), carbon dioxide ($V'CO_2$) production and heart rate (HR) were carried out by using a metabolic unit (Quark-$b^2$, Cosmed, Italy). Ventilatory and gas exchange responses were measured continuously. The volume and gas analysers were calibrated using a 3-liter calibration syringe and standard calibration gases (16.00% $O_2$; 4.00% $CO_2$). Each participant stood quietly relaxed for five minutes whilst resting metabolic measures were being collected breath by breath. Real-time plots of $V'O_2$, heart rate, and respiratory exchange ratio were closely monitored during the two minutes of each walking speed to ensure that metabolic steady state was reached. Data post processing included the calculation of mean values of $V'O_2$, $V'CO_2$ and HR over the last two minutes of each walking speed, which were considered for further analysis. In addition, respiratory exchange ratio (RER) was monitored to ensure that it remained under the specific threshold of 1.0. All these precautions were required to indicate that metabolism was essentially oxidative. The metabolic cost of walking ($C_W$, $J \cdot (kg \cdot m)^{-1}$) was calculated by dividing net energy expenditure (obtained by subtracting pre-exercise standing $V'O_2$ from gross $V'O_2$ and converted to joules according to the formula given by Garby and Astrup (Garby & Astrup, 1987), which accounts for the RER-dependent variation of $O_2$ energy equivalence) by speed and body mass.

**Surface electromyography recordings**

Surface electromyography (EMG) was collected from four muscles of the right lower limb: vastus lateralis (VL), biceps femoris (BF), gastrocnemius medialis (GM) and tibialis anterior (TA). Pre-gelled surface EMG electrodes (circular contact area of 1 cm diameter, BIOPAC Systems, Inc., USA)

were placed (inter electrode distance equal to 20 mm) at the following locations (Hermens *et al.*, 2000): a) for VL at two-third on the line from the anterior superior iliac spine to the lateral side of the patella; b) for BF midway between the ischial tuberosity and the lateral epicondyle of the tibia; c) for GM on the most prominent bulge of the muscle; d) for TA at one third on the line between the tip of the fibula and the tip of the medial malleolus. In order to ensure a good electrode-skin interface, prior to the application of the electrodes, the subject's skin was shaved, rubbed with an abrasive paste, cleaned with an alcohol solution, and dry-cleaned with gauze. EMG data were sampled at a frequency of 2 kHz and recorded by a four-channel electromyography system (EMG100C, BIOPAC Systems, Inc., USA; Band-pass Filter: 10-500 Hz; RMS Noise Voltage: 0.2 μV; Input impedance: 2 MΩ; Common Mode Rejection Ratio: 110 dB). In order to place electrodes in the same anatomical location during the three different experimental sessions (before and after the training program), electrodes position was marked on acetate paper using moles and small angiomas (which may be assumed to maintain a fixed position) as reference points. The EMG electrodes were fixed at the beginning of each experimental session and were not removed between walking tests.

EMG raw signal recorded between the last two minutes of each walking bout performed by the subject was full-wave rectified and then low-pass filtered with a cut-off frequency of 10 Hz. The determination of EMG onset and offset activity of each muscle was achieved by using a computer-automated procedure (Mian *et al.*, 2006). Visual inspection of EMG activity was added in order to monitor the suitability of the algorithm used. In particular, we calculated the amount of stride duration (% of stride duration) in which two representative antagonist muscles were active at the same time (co-contracted). Eight representative strides for each walking speed were considered to calculate the average co-contraction value for both proximal, thigh muscles (VL and BF) and distal, leg muscles (GM and TA).

**Physical performance evaluations**

The 6 minutes walking test (6MWT) is a practical simple test that it is safer, better tolerated and it better reflects activities of daily living than other walk tests (e.g. shuttle walk tests) (Enright, 2003). Each participant was asked to walk for 6 minutes on a flat and hard surface at their preferred speed with the only aim of covering as much distance as possible. The test was performed in a long corridor located inside our lab facility. The 6MWT evaluates the global and integrated responses of all the systems involved during exercise, including the pulmonary and cardiovascular systems, systemic circulation, peripheral circulation, blood, neuromuscular units, and muscle metabolism (Solway *et al.*, 2001). It does not provide specific information on the function of each of the different organs and systems involved in exercise or the mechanism of exercise limitation, as is possible with maximal

cardiopulmonary exercise testing. It assesses the submaximal level of functional capacity. Because most activities of daily living are performed at submaximal levels of exertion, the 6MWT performance may better reflect the functional exercise level for daily physical activities.

The Timed "Up & Go" (TUG) measures, in seconds, the time taken by an individual to stand up from a standard chair (approximate seat height of 46 cm), walk a distance of 3 meters, turn, walk back to the chair, and sit down again (Podsiadlo & Richardson, 1991). The subject was asked to wear his regular footwear. No physical assistance was given. They started with their back against the chair and their arms resting on their knees. Once they heard the "go" signal, they got up and walked at a comfortable and safe pace to a line on the floor 3 meters away, turned, returned to the chair, and sat down again. A familiarization trial and three official ones were performed by each subject.

**Statistical analyses**

Statistical analysis was performed with R software (R Development Core Team (2008), R foundation for Statistical Computing, Vienna, Austria) together with R studio IDE (RStudio Team (2015), RStudio: Integrated Development for R, RStudio, Inc., Boston, MA). All results are expressed as means ± SD. Normal distribution of the data was tested by Shapiro-Wilk test. The assumption of homogeneity of the variances was then verified by Levene's test. Once the previous two assumptions were respected differences in baseline data of physical anthropometric characteristics, specific muscle forces, maximal power abilities together with physical conditions of the participants (6MWT and TUG performances) among the two groups, were analysed by Welch's t-tests. Differences in the data among groups due to the eight weeks of intervention were analysed by general linear mixed model with 3 main predictors variables in the case of the metabolic cost and co-contraction data (group, time, measure and their interactions) and 2 main predictors for the rest of them (group, time and their interactions). General linear mixed model requires the respect of the assumption of sphericity hence Mauchly's test was performed with this attempt. No post hoc tests was performed when no high order effects (i.e. interactions of predictors variables) were observed.

**RESULTS**

***Measurements at baseline***

Of the twenty-nine participants that were recruited twenty-four completed the whole study protocol. The dropouts were due to personal problems not related to the study.

Baseline values of age, stature, body mass, body mass index, body composition, thigh muscle volume, life style level (IPAQ-SF and SF12 scores), force and power were not different among the two groups

(Table 1). However, the 6MWT score was significantly greater in EXP compared to CTRL subjects (+ 13.3 %, P = 0.007, Table 1).

***Measurements after the training intervention***

Eight weeks of physical training program did not change anthropometric characteristics, body composition, thigh muscle volume and morphological characteristics of vastus lateralis and rectus femoris muscles in both groups (Table 2). Although no interaction effect of group by time was found, physical training produced an increase in MEP in EXP group by +10.8% and in CTRL by +0.31% (Fig. 1, Table 3, p: 0.056). Pf, and pv values were similar between the two groups of subjects before and after the training period (P values ranging from 0.136 to 0.412; Figure 1 and Table 3).

Fig. 2 and Table 3 show the changes in MVC related to training in both groups. It can be observed that MVC values increased in EXP and CTRL (+11.1 and +13.5% in EXP and CTRL groups, $p<0.001$, respectively); however no specific effect of type of training was appreciated.

Analysis of the metabolic data collected during walking revealed no effect of physical training on Cw, RER and HR in both groups (Fig 3, Table 4, P values ranging from 0.199 to 0.508). Independently from group membership and time, RER and HR values were significantly greater at 4km/h than those observed at 3km/h (Table 4, $P < 0.001$). Inverse results were found for Cw which, independently from the group membership and time, was greater at the walking speed of 3km/h than at 4km/h by 12.6% (Table 4, $P < 0.001$).

As shown in the Table 4, physical training did not change CCT values of both proximal (VL-BF) and distal (GM-TA) muscles in both groups. Furthermore, CCT levels of lower limb muscles were similar at each walking speed between the two groups before and after the training intervention (Table 4). Independently from group and time there was no effect of Speed on CTT. Interestingly CCT of distal muscles differed from that observed in proximal muscles (+66%, $P < 0.001$ pooling together data from the walking speeds and the time points: data not shown).

Global physical performances were assessed by using 6MWT and TUG test. The results of these tests are reported in Table 5. Eight weeks of physical training increased 6MWT performance in both groups without differences in score gains between the two trainings (+5.2 % and +5.6 %, $P < 0.05$, in CTRL and EXP respectively). No specific effects on TUG scores were found.

**DISCUSSION**

The results of the present study show that an 8-weeks flywheel isoinertial resistance training in healthy older subjects, induced 1) slightly higher improvements of maximal power output with lower limb muscles, 2) similar improvements of maximal strength gain and physical fitness than traditional

gravity-dependent resistance training. The two types of resistance training did not induce changes on muscle architecture, metabolic cost and co-contraction time of agonist antagonist lower limb muscles during habitual walking.

The comparison between these two exercise modalities, gravity-dependent vs flywheel isoinertial, in evoking muscular adaptations and muscle strength and power gains is not new in scientific literature. As Vicens-Bordas and his team underlined in their last review article (Vicens-Bordas *et al.*, 2018), the current literature available on this topic is made off very few randomized controlled trial studies. Even more important a wide range of the participants age exists across all these studies. The age of the participants, involved in a resistance training program, might mitigate the results that the physical intervention can produce (Lemmer *et al.*, 2000; Kumar *et al.*, 2009). Hence, to contextualize the findings of the present study in the current literature the age of the participants to resistance training programs need to be considered.

We found that the elderly participants who followed the FW training increased MEP values by 10.8%. On the contrary, GD members increased MEP by only 0.31%, in agreement with the study of Onambele and colleagues (Onambele *et al.*, 2008). These researchers found that the group trained with the FW mode increased the peak isokinetic power by 28% while the one that trained with the GD regime increased by only 4%. These results, could be due to the fact that the flywheel isoinertial training has been shown to result in greater eccentric peak force and power compared with traditional weight stack or gravity dependent exercise mode during similar exercise movements (Norrbrand *et al.*, 2010; Tesch *et al.*, 2017). However, disagreements with the present results have also been observed in literature. Although not specifically related to elderly people, Greenwood and colleagues (Greenwood *et al.*, 2007) have found that both the exercise modalities equally contribute to increase the muscle power output of quadriceps muscles in middle age men after knee injury. As they suggested training interventions that last more than few months (i.e. > 3months) might show wider differences in muscle performance enhancements between the exercise modalities.

Our results, further, show that MVC increased after the physical training independently from exercise modality. Specifically, GD group increased MVC values by 13.5% while for FW group the increment was around 11%. Slightly different results were found by Onambele's group (+8% and +17% for GD and FW respectively). Differences in the standardization of the training loads between the two exercise modalities might have contributed to produce the contrasting results observed in literature in term of force and power adaptations. By choosing the load corresponding to the maximal power output, exclusively for their FW group, Onambele and colleagues might have emphasized the enhancement in muscle power in this group only at the expense of muscle force development. Although this approach might be the appropriate choice when working with elderly people, due to

their vulnerability to muscle power losses as they age, their load standardization method between FW and GD exercise might not guarantee equal working efforts. Hence, we preferred to use a different standardization strategy, adopted by and modified from the work of Norrbrand and colleagues (Norrbrand *et al.*, 2008). This method focused on performing the exercises (FW or GD) with a training load that produced a specific target: voluntary failure or impaired movement execution (see "Subjects and Methods").

The fact that our results show an increase in muscle force without a parallel increase in muscle thickness (see Table 2 and 3) might suggest that hypertrophic processes have not prevailed at the end of the training intervention. We speculate that the muscle force gain might have resulted especially from neural adaptations. In the early phase of a resistance training these neural adaptations predominate (Sale, 1988; Bemben & Murphy, 2001), independently from age (Bemben & Murphy, 2001), and as the training goes on the hypertrophic process might become more important. Thus, the short duration of the present training protocol together with the low-to-moderate training load intensity and the low exercise volume might have favoured the development of neural adaptations by delaying the anabolic muscle response.

In addition, we have found that the Cw and CCT of lower limb muscles during walking at the speeds of 0.83 and 1.11 m/s (i.e. habitual walking speeds in elderly population (Oberg *et al.*, 1993)) are not influenced by training intervention, independently from the exercise modality. Interventions to improve walking have historically been multifactorial (i.e. strengthening, endurance and flexibility programs), as written by Brach and colleagues (Brach & Vanswearingen, 2013). Though, all these interventions have resulted in only modest improvements in walking (Sauvage *et al.*, 1992; Judge *et al.*, 1993; Topp *et al.*, 1993), as further supported by the small 6-MWT score gains observed in the present study. As suggested by Branch and colleagues we think that more specific exercise training, task oriented, with the ability to reproduce and at the same time challenge the habitual walking movements, might eventually improve walking dynamics and its energy demands.

Flywheel isoinertial and gravity-dependent resistance trainings are both important exercise programs that might serve to increase the physical functioning in healthy adults by counteracting the adverse effects of ageing. However, still poor evidence exists that might help to choose which one should be preferred for its useful benefits on the aged neuromuscular system. The current evidence suggests that both the trainings are equally capable of generating muscle force improvements, independently from the age of the participants. Nonetheless there are still little data in literature to draw firm conclusions about muscle performance enhancements other than muscle strength. The present results i) provide new evidence to support the hypothesis that isoinertial is superior to gravity-dependent training in generating muscle power improvements in elderly people; ii) confirm the lack of

differences between the two training modalities in producing muscle force enhancements.; iii) yield new information regarding the effects of flywheel resistance training on metabolic and neuromuscular characteristics of walking in elderly people.

**Limitations of the study**

The results observed in this study are related to the training protocol employed. This training intervention is not without limitations. In this section we provide a detailed list of some of these limitations that might have played a role in determining the present results.

i) *Training loads employed*. The training loads chosen for the FW and GD groups might have been influenced by individual motivation and coordination in the performance of the task. The problem of the standardization of the working load and volume between FW and GD resistance trainings has risen some concerns in literature (Maroto-Izquierdo *et al.*, 2017, 2018; Vicens-Bordas *et al.*, 2018) and it might require further research;

ii) *The short duration of the training intervention.* The reason why we chose to constrain the training intervention to a 8 weeks period was link to rehabilitation and prevention purposes. We have tried to optimise the benefits of an organized physical training program by minimizing the time and the energy demands for healthy older people. Physical interventions with these features might be economically more accessible by single individuals because of the limited number of exercise sessions. Even if short, the present training intervention respected the minimal exercise dose suggested by previous guidelines (i.e. resistance exercise for older adults: frequency, at least 2 d/wk; intensity, moderate) (Nelson *et al.*, 2007; American College of Sports *et al.*, 2009). Indeed, such training programs might be required before and even after hospitalization in order to speed up rehabilitation processes or physically prepare the patient to surgical procedures, avoiding dramatical physical function deficits once hospitalization ends. Interestingly, Rejc and colleagues (Rejc et al., 2017) have found that after a period of 14 days of bed rest older people decreased MEP values by 15% and quadriceps muscle volume by 8.3%. This would mean that if both our training groups had been hospitalized for a similar amount of days, immediately after the current physical training intervention, they would probably have lost more muscle power than the one gained by this physical exercise program itself. In addition, both the present trainings would not have adequately prevented the muscle mass from being lost during the hospitalization period. Reduced hospitalization time might result in smoothed muscle volume and functional decrements (Ferrando et al., 1995). Thus, to better maximize the benefits of our exercise intervention, we should have probably asked the participant to train with a greater exercise volume (i.e. the number of training sessions per week) and/or training intensity (i.e. the percentage of 1-repetition maximum).

iii) *No effect of gender was investigated.* Although we recognised that gender might influence the effects of a resistance training program on muscle functions in healthy elderly adults (Beneka *et al.*, 2005), we were interested in investigating the effect of each of the two trainings (FW and GD) on muscle performance in random samples of elderly people independently from gender characterization.

## ADDITIONAL INFORMATION

### Competing interests

None declared

Table 1. Baseline physical and anthropometric characteristics in the two groups (CTRL vs EXP)

| | CTRL GROUP (n:12) | EXP GROUP (n:12) | SIGNIFICANCE |
|---|---|---|---|
| Age [years] | 68.3 ± 3.0 | 67.1 ± 3.8 | 0.415 |
| Stature [cm] | 166.0 ± 9.5 | 167.4 ± 9.0 | 0.711 |
| Body mass [kg] | 73.2 ± 14.8 | 72.9 ± 13.7 | 0.966 |
| BMI [cm/kg$^2$] | 26.0 ± 4.2 | 26.3 ± 3.8 | 0.839 |
| FFM [kg] | 47.6 ± 8.3 | 48.5 ± 8.9 | 0.790 |
| FM [kg] | 25.6 ± 7.6 | 24.4 ± 8.4 | 0.722 |
| TMM [cm$^3$] | 2912 ± 926 | 3222 ± 1036 | 0.447 |
| IPAQ-SF | 3129 ± 1034 | 2312 ± 1560 | 0.314 |
| SF12 physical | 47.9 ± 8.2 | 51.6 ± 5.3 | 0.298 |
| SF12 mental | 51.7 ± 8.1 | 55.0 ± 9.9 | 0.474 |
| MVC [Nm] | 156 ± 47 | 198 ± 57 | 0.073 |
| MEP [W] | 2552 ± 965 | 2988 ± 1227 | 0.381 |
| TUG [sec] | 8.2 ± 2.0 | 7.9 ± 1.0 | 0.681 |
| 6MWT [m] | 518 ± 49 | 587 ± 62 | 0.007 |

All values are expressed as means ± SD.

Significance of P value: <0.05

BMI: body mass index; FFM: fat free mass; FM: fat mass; TMM: thigh muscle mass; IPAQ-SF: international physical activity questionnaire short version; SF12: 12-item short form health survey; MVC: maximal voluntary isometric contraction of the knee extension muscles; MEP: maximal explosive power; TUG: time up and go test; 6MWT: 6-minute walking test.

Table 2. Effects of the physical training on anthropometric characteristics and muscle architecture in the two groups (CTRL vs EXP)

| | CTRL GROUP (n:12) | | EXP GROUP (n:12) | | SIGNIFICANCE | | |
|---|---|---|---|---|---|---|---|
| | Pre | Post | Pre | Post | G | T | G x T |
| Body mass [kg] | 73.2 ± 14.8 | 73.2 ± 15.4 | 72.9 ± 13.7 | 71.2 ± 12.9 | 0.846 | 0.999 | 0.101 |
| BMI [cm/kg$^2$] | 26.0 ± 4.2 | 26.0 ± 4.3 | 26.3 ± 3.8 | 25.7 ± 3.7 | 0.972 | 0.999 | 0.123 |
| FFM [kg] | 47.6 ± 8.3 | 48.2 ± 9.0 | 48.5 ± 8.9 | 49.1 ± 8.8 | 0.798 | 0.126 | 0.931 |
| FM [kg] | 25.6 ± 7.6 | 24.9 ± 7.9 | 24.4 ± 8.4 | 22.0 ± 8.6 | 0.539 | 0.460 | 0.169 |
| TMM [cm$^3$] | 2912 ± 926 | 2915 ± 969 | 3222 ± 1036 | 3267 ± 1030 | 0.417 | 0.974 | 0.714 |
| MT VL [cm] | 1.88 ± 0.37 | 1.92 ± 0.38 | 1.93 ± 0.37 | 2.03 ± 0.35 | 0.591 | 0.142 | 0.253 |
| Pa VL [°] | 11.1 ± 2.0 | 11.2 ± 1.6 | 11.6 ± 1.7 | 12.0 ± 1.5 | 0.362 | 0.781 | 0.144 |
| L VL [cm] | 9.8 ± 1.5 | 9.9 ± 1.6 | 9.7 ± 1.8 | 9.8 ± 1.8 | 0.897 | 0.542 | 0.919 |
| MT RF [cm] | 1.46 ± 0.27 | 1.50 ± 0.26 | 1.57 ± 0.22 | 1.60 ± 0.22 | 0.317 | 0.141 | 0.980 |

All values are expressed as means ± SD.

Significance of P value: <0.05

BMI: body mass index; FFM: fat free mass; FM: fat mass; TMM: thigh muscle mass; MT: muscle thickness; Pa: pennation angle; L: fascicule length.

General linear model mixed design: effect of Group (G); effect of Time (T); effect of Group x Time (G x T)

Table 3. Effects of the physical training on maximal knee extensor muscles force and maximal lower limb muscles power in the two groups (CTRL vs EXP)

| | CTRL GROUP (n:12) | | EXP GROUP (n:12) | | SIGNIFICANCE | | |
|---|---|---|---|---|---|---|---|
| | Pre | Post | Pre | Post | G | T | G x T |
| MVC [N*m] | 156 ± 47 | 177 ± 48 | 198 ± 57 | 220 ± 52 | 0.062 | < 0.001 | 0.886 |
| MEP [W] | 2552 ± 965 | 2560 ± 1008 | 2988 ± 1227 | 3310 ± 1429 | 0.260 | < 0.05 | 0.056 |
| F [N] | 1076 ± 275 | 1084 ± 301 | 1165 ± 357 | 1234 ± 361 | 0.412 | 0.052 | 0.113 |
| V [m/s] | 2.30 ± 0.40 | 2.29 ± 0.37 | 2.51 ± 0.41 | 2.63 ± 0.42 | 0.136 | 0.175 | 0.111 |

All values are expressed as means ± SD.

Significance of P value: <0.05

MVC: maximal voluntary isometric contraction of the knee extension muscles; MEP: maximal explosive power; F: peak force; V: velocity.

General linear model mixed design: effect of Group (G); effect of Time (T); effect of Group x Time (G x T)

Table 4. Effects of the physical training on heart rate (HR), respiratory exchange ratio (RER), metabolic cost (Cw) and co-contraction time (CCT) of proximal (VL-BF) and distal (GM-TA) muscles during walking at 3 and 4 km/h in the EXP and CTRL groups.

| | CTRL GROUP (n:12) | | EXP GROUP (n:12) | | SIGNIFICANCE | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre | Post | Pre | Post | G | T | S | GxT | GxS | TxS | GxTxS |
| HR 0.83 m/s (bpm) | 81 ± 12 | 82 ± 11 | 86 ± 9 | 83 ± 5 | 0.719 | 0.221 | <0.001 | 0.947 | 0.052 | 0.603 | 0.070 |
| HR 1.11 m/s (bpm) | 91 ± 6 | 87 ± 10 | 88 ± 8 | 88 ± 5 | | | | | | | |
| RER 0.83 m/s | 0.83 ± 0.039 | 0.82 ± 0.054 | 0.82 ± 0.045 | 0.79 ± 0.048 | 0.251 | 0.199 | <0.001 | 0.241 | 0.480 | 0.201 | 0.618 |
| RER 1.11 m/s | 0.85 ± 0.032 | 0.85 ± 0.041 | 0.85 ± 0.039 | 0.83 ± 0.054 | | | | | | | |
| Cw 0.83 m/s [J/(kg*m)] | 3.131 ± 0.440 | 3.242 ± 0.655 | 2.917 ± 0.521 | 3.036 ± 0.513 | 0.389 | 0.508 | <0.001 | 0.828 | 0.341 | 0.098 | 0.592 |
| Cw 1.11 m/s [J/(kg*m)] | 2.786 ± 0.408 | 2.809 ± 0.505 | 2.703 ± 0.581 | 2.652 ± 0.463 | | | | | | | |
| CCT VL-BF 0.83 m/s [%] | 33.1 ± 4.1 | 30.9 ± 2.3 | 31.0 ± 8.9 | 28.8 ± 10.5 | 0.316 | 0.092 | 0.087 | 0.686 | 0.154 | 0.816 | 0.398 |
| CCT VL-BF 1.11 m/s [%] | 33.0 ± 5.1 | 30.2 ± 2.1 | 26.7 ± 8.9 | 25.7 ± 7.0 | | | | | | | |
| CCT GM-TA 0.83 m/s [%] | 19.1 ± 7.9 | 18.8 ± 8.9 | 17.5 ± 7.9 | 16.2 ± 6.7 | 0.464 | 0.294 | 0.675 | 0.306 | 0.171 | 0.739 | 0.740 |
| CCT GM-TA 1.11 m/s [%] | 20.2 ± 7.7 | 20.5 ± 7.7 | 16.7 ± 6.3 | 15.4 ± 6.4 | | | | | | | |

All values are expressed as means ± SD.

Significance of P value: <0.05

HR: heart rate; RER: respiratory exchange ratio; Cw: metabolic cost of walking; CCT: co-contraction time.

General linear model mixed design: effect of Group (G); effect of Time (T); effect of walking Speed (S); effect of Group x Time (G x T); effect of Group x Speed (G x S); effect of Time x Speed (T x S); effect of Group x Time x Speed (G x T x S).

Table 5. Effects of the physical training on 6MWT and TUG performances in the EXP and CTRL groups.

| | CTRL GROUP (n:12) | | EXP GROUP (n:12) | | SIGNIFICANCE | | |
|---|---|---|---|---|---|---|---|
| | Pre | Post | Pre | Post | G | T | G x T |
| 6MWT [m] | 518 ± 49 | 545 ± 52 | 587 ± 62 | 619 ± 57 | 0.002 | 0.049 | 0.790 |
| TUG [sec] | 8.2 ± 2.0 | 7.7 ± 0.8 | 7.9 ± 1.0 | 7.7 ± 1.1 | 0.762 | 0.175 | 0.638 |

All values are expressed as means ± SD.

Significance of P value: <0.05

6MWT: 6-minute walking test; TUG: time up and go test.

General linear model mixed design: effect of Group (G); effect of Time (T); effect of Group x Time (G x T).

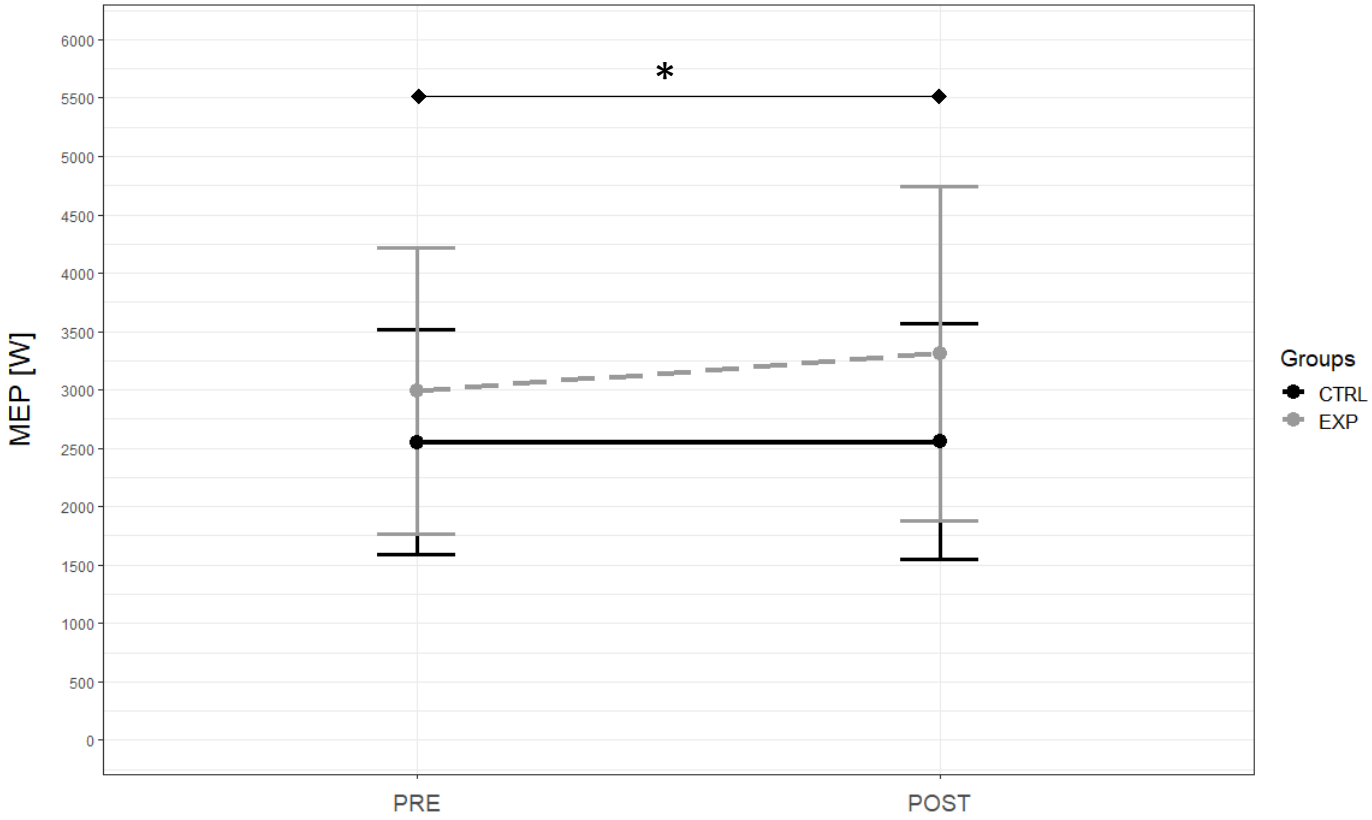


Figure 1. Effects of the physical training on MEP in EXP (- -●- -) and CTRL (-●-) groups as a function of Time. Values are means ± SD. Differences in MEP were tested using general linear mixed model.

* Difference between time points (PRE vs POST): P <0.05.

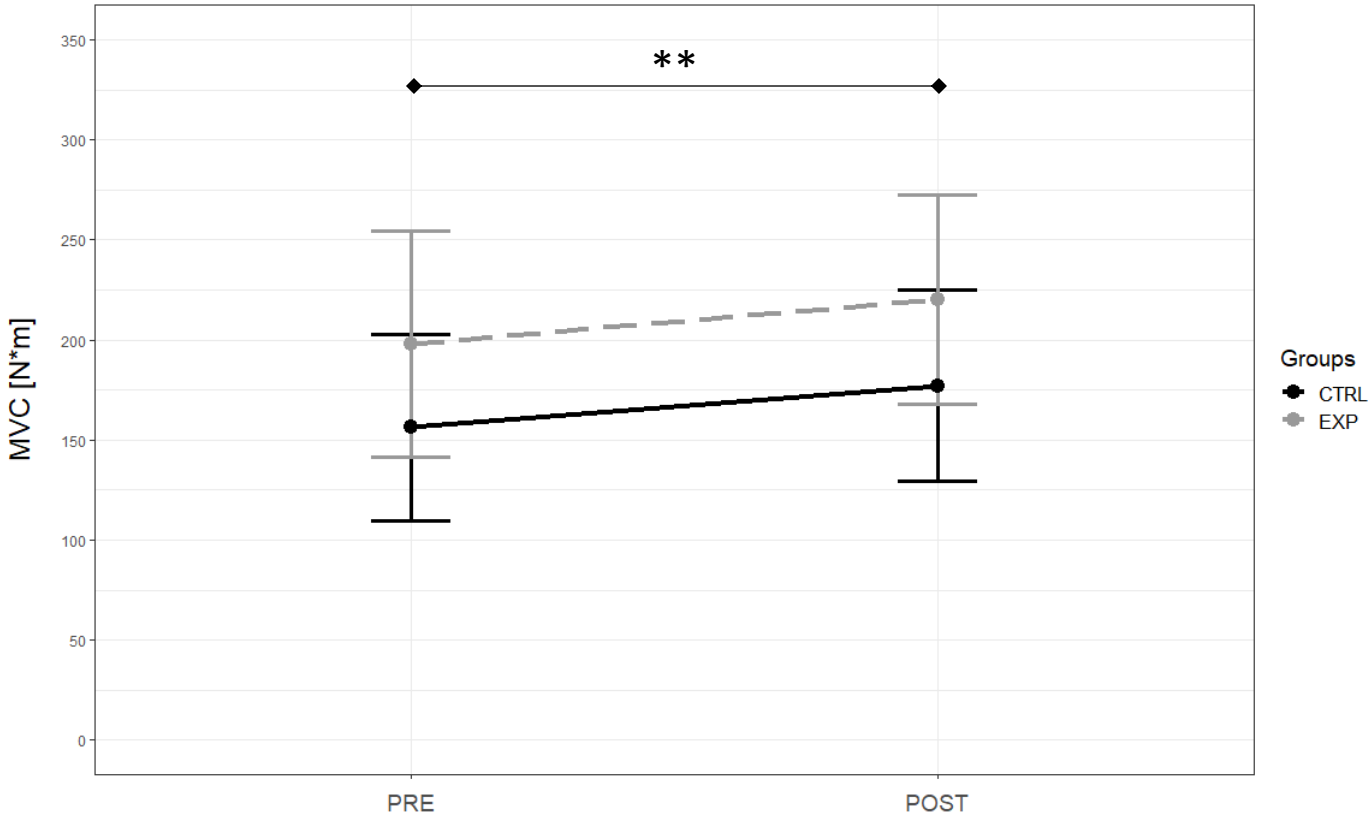


Figure 2. Effects of the physical training on MVC in EXP (- -●- -) and CTRL (-●-) groups as a function of Time. Values are means ± SD. Differences in MVC were tested using general linear mixed model.

** Difference between time points (PRE vs POST): P <0.001.

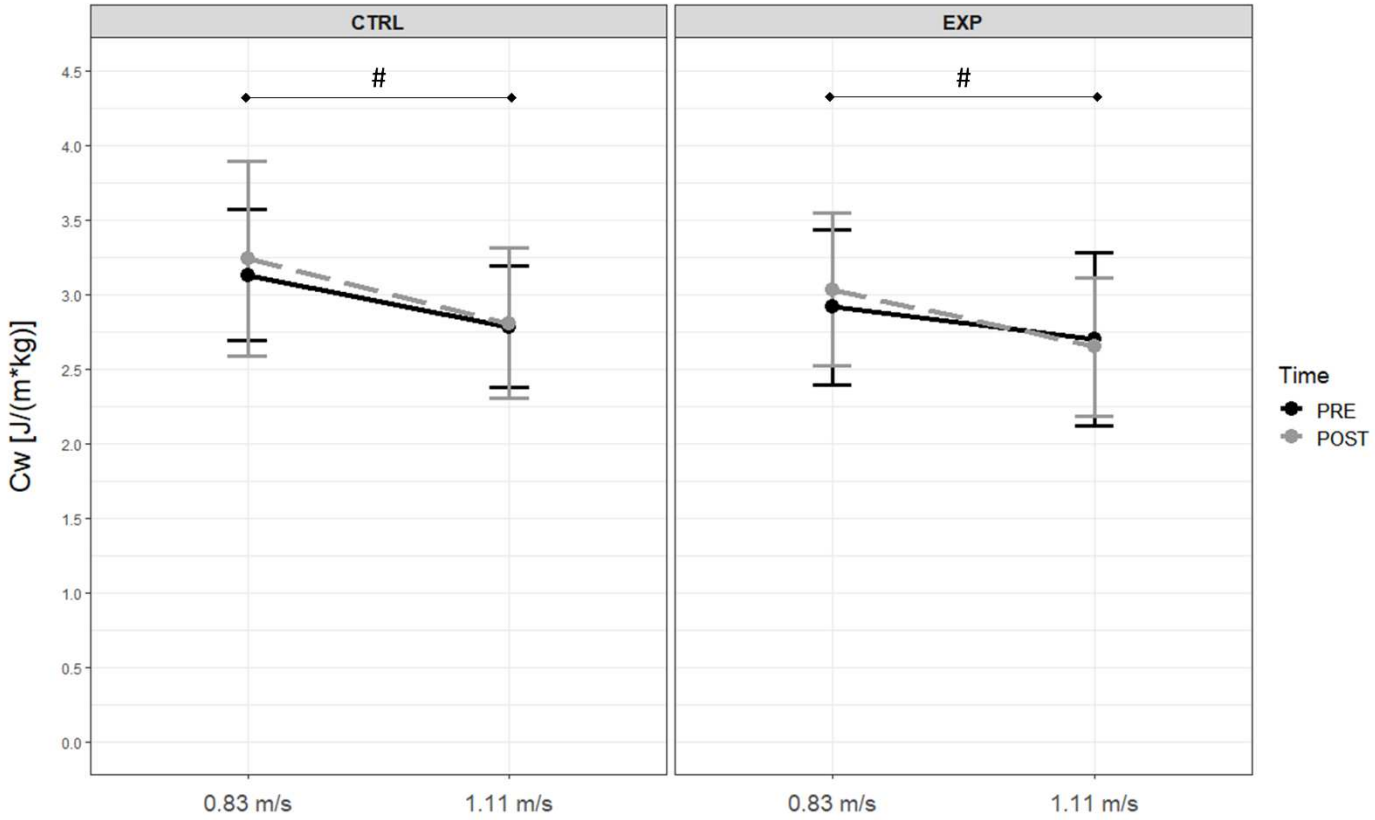


Figure 3. Effects of the physical training experimental manipulation on Cw in CTRL (A) and EXP (B) groups as a function of walking Speed, before (-●-) and after (- -●- -) the physical training period. Values are means ± SD. Differences in Cw were tested using general linear mixed model.

# Difference between walking speeds (0.83 m/s vs 1.11 m/s): P <0.001.

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# SECTION TWO

## 2.1 Aims of section two

1) The first work related to this section of the thesis investigated the effects of a single bout of self-myofascial release treatment on the running performance and muscle power abilities of lower limbs in young healthy subjects.

2) The other two works in this section investigated the effects of an eight-weeks endurance cycling training program on

i) the time course of fatigue development,

ii) the peripheral contributions and

iii) the central contributions (i.e. corticospinal adaptations)

of the deterioration of neuromuscular function during a fatiguing exercise test conducted till voluntary exhaustion in young healthy male subjects.

## 2.2 Main results of section two

1) A single bout of self-myofascial release treatment acutely i) improved maximal power ability of lower limb muscles only when dynamic explosive movements required elastic energy to be stored by muscle-tendinous structure and ii) increased the metabolic cost of running in healthy young people.

2) At the same workload as in pre-training session fatigue is attenuated during a fatiguing exercise test after endurance cycling training in healthy young male subjects. Furthermore, no effects of the training intervention was found on the voluntary activation values (i.e. central contributions).

## 2.3 Publications of section two

4) Short-term effects of rolling massage on energy cost of running and power of the lower limbs.
Giovanelli N., Vaccari F., **Floreani M.**, Rejc E., Copetti J., Garra M., Biasutti L., Lazzer S.
Int J Sports Physiol Perform. 2018 May 10:1-23. doi: 10.1123/ijspp.2018-0142.

5) Effects of endurance training on neuromuscular fatigue in healthy active men. Part I: Strength loss and muscle fatigue.
Mira J., Aboodarda SJ., **Floreani M.**, Jaswal R., Moon S.J., Amery K., Rupp T., Millet G.Y.
Eur J Appl Physiol. 2018 Nov;118(11):2281-2293. doi: 10.1007/s00421-018-3950-8. Epub 2018 Aug 18.

6) Effects of endurance cycling training on neuromuscular fatigue in healthy active men. Part II: Corticospinal excitability and voluntary activation.
Aboodarda S.J., Mira J., **Floreani M.**, Jaswal R., Moon S.J., Amery K., Rupp T., Millet G.Y.
Eur J Appl Physiol. 2018 Nov;118(11):2295-2305. doi: 10.1007/s00421-018-3951-7. Epub 2018 Aug 20.

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**Section:** Original Investigation

**Article Title:** Short-Term Effects of Rolling Massage on Energy Cost of Running and Power of the Lower Limbs

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# Short-term effects of rolling massage on energy cost of running and power of the lower limbs

**Original Investigation**

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**Running head:** Self-massage effects on running and jumping

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## Abstract

**Purpose:** Self-myofascial release (SMFR) is a type of self-massage that is becoming popular among athletes. However, SMFR effects on running performance have not been investigated yet. The aim of the present study was to evaluate the effects of SMFR on cost of running (Cr). In addition, we evaluated the effects of SMFR on lower limbs muscle power. **Methods:** The measurement of Cr and lower limb muscle power during squat jump (SJ) and counter movement jump (CMJ) were performed before (PRE), immediately after (POST) and 3hours after (POST 3h) a SMFR protocol (experimental condition). In the "control condition" testing session, the same measurements were performed without undergoing the SMFR protocol. Experimental and control conditions were tested in a randomized order. **Results:** Cr at POST trended to increase as compared to PRE (+6.2±8.3%, $p$=0.052), while at POST 3h Cr was restored to PRE values (+0.28±9.5%, $p$=0.950). In the experimental condition, no significant "Time" effect was observed for maximal power exerted during SJ. On the other hand, maximal power exerted during CMJ at POST and POST 3h was significantly higher than that observed at PRE (+7.9±6.3%, $p$=0.002; and +10.0±8.7%, $p$=0.004, respectively). The rate of force development measured during CMJ also increased after SMFR, reaching statistical significance at 200 ms from force onset at POST 3h (+38.9%, $p$=0.024). **Conclusions:** an acute use of foam roller for SMFR performed immediately prior to running may negatively affect the endurance running performance, while its use should be added before explosive motor performances that include stretch-shortening cycles.

**Keywords**: mechanics; energetics; stiffness; jump; foam roller; self-myofascial release

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## Introduction

Many athletes commonly use different strategies (i.e. dynamic or static stretching, massages, self-myofascial release) before and/or after competitions or training sessions in order to improve flexibility, accelerating recovery time and decreasing injury risk.[1] However, in the literature there are conflicting results about the effects of these strategies on performance.[1] Indeed, static stretching, which is one of the most common used, improves flexibility but it can negatively affects the leg press one repetition maximum, muscle strength endurance, 20-m sprint performance and vertical jump height.[2-5] Also, static stretching does not seem effective for promoting shorter recovery time.[6] However, the negative effects of static stretching on neuromuscular performance may be minimized if static stretching is followed by a dynamic activity.[7] On the other hand, dynamic stretching improves flexibility along with increasing torque during an isokinetic exercise and it seems to be more suitable for improving performance.[2]

Self-myofascial release (SMFR) is a type of self-massage that can be performed by the subject himself rather than by a clinician.[8] The most common devices used for SMFR are foam roller and roller massager. SMFR can promote short-term flexibility improvement, and it does not seem to have negative effects on performance.[2,3,9-11] In fact, no differences in maximal force and power were detected after a SMFR protocol.[2,12] Moreover, SMFR has been shown effective for reducing delay onset muscle soreness (DOMS), accelerating the recovery after intense and eccentric training sessions.[9] However, Casanova et al.[13] showed that rolling massage treatment does not limit the negative effects of exercise-induced muscle damage caused by five sets of twenty unilateral calf rises. SMFR application time seems important for its effect on muscle function. Short application time (<30 s) seems to have no significant effects on performance.[12] Thus, for SMFR longer applications are suggested, particularly for wide muscles.[14] The mechanisms underlying SMFR-induced adaptations on muscle function are not completely

understood; however, they conceivably include biomechanical, physiological, neurological and psychological components.[8] In particular, SMFR may influence the connective fascia characteristics, and specifically its water content and stiffness.[15] In fact, the traction and compression of the fascia leads to a loss of water, which is followed by re-hydration that reaches its peak about 3-4 hours after the mechanical stress application.[15] This "sponge-effect" was reported while examining an *in vitro* model; thus, the effects of traction and compression might be different compared to those obtained *in vivo*. Also, another study suggested that heavy rolling massage and manual massage over tender points can increase the pain threshold by acting both at peripheral level and at central level.[16]

In spite of the increasing number of studies that have examined the influence of SMFR on sport performance,[8] to the best of our knowledge the effects of SMFR on running performance have not been investigated yet. Energy cost of running (Cr) plays a relevant role in determining the performance among middle and long distance runners along with the maximal oxygen uptake and the fraction of its maintained during the effort.[17] In turn, our research group has also observed that Cr is affected by muscle power of lower limb extensors.[17] Hence, the primary objective of the present study was to evaluate the effects of SMFR treatment on Cr. In addition, we evaluated the effects of SMFR on lower limbs muscle power. Our hypothesis is that Cr and lower limb muscle power would be impaired immediately after SMFR, possibly because of the muscle stiffness alteration caused by the loss of water content in the fascia. On the other hand, Cr and lower limb muscle power may be improved 3 hours after SMFR treatment, possibly because fascia water content and consequently muscle stiffness might be higher than before treatment.[15]

## Methods

### Subjects

Thirteen active sport students (mean age: 26.3±5.3 years) were enrolled in the study (Table 1). Participants reported to practice different sports (i.e. soccer, track and field, mountain running, parkour) for 9.9±3.5 hours·week$^{-1}$ on average. They were informed about the study protocol, read and signed an informed consent before starting the measurements. The study was approved by the local Institutional Review Board.

### Experimental Design

In this randomized crossover design study, participants visited the laboratory three times. During the first day, we collected informed consent, stature, body mass and body composition. Then, the subjects familiarized with all the testing procedures (treadmill running, squat jump (SJ), counter movement jump (CMJ) and SMFR treatment). During the "experimental condition" testing session, subjects ran ten minutes on a treadmill at self-selected speed and Cr was calculated for the last two minutes of the trial. Immediately after the treadmill run, participants performed three SJ and three CMJ on an Explosive Ergometer (EXER). The rest period between each jump was 3 minutes. The trial with the highest peak power exerted during SJ and CMJ was considered for further analysis. After the lower limb muscle power assessment, subjects underwent a sixteen-minute SMFR treatment with the assistance of a therapist. Immediately after the SMFR treatment and 3 hours after the treatment, participants repeated the running assessment at the same speed that was self-selected during the first trial, SJ and CMJ. During the "control condition" testing session, subjects performed the same procedures except for the SMFR treatment (Figure 1). The first testing session occurred two or three days after the initial visit to the laboratory, while the second testing session five or six days after the initial visit to the laboratory.

*Anthropometric measurements.* Body mass was measured with a manual weighing scale (Seca, Germany) and the stature on a standardized wall-mounted height board. We measured body composition by bioelectrical impedance analysis (Akern, Italy) using the software provided by the manufacturer (Bodygram,1.31).

*Energy cost of running.* We measured ventilation, oxygen consumption ($V'O_2$) and carbon dioxide production ($V'CO_2$) with a metabolic unit (K5, Cosmed, Italy) during the 10-minute running trial on a motorized treadmill (Saturn, HP Cosmos, Germany). Volume and gas analysers were calibrated before every trial as previously described.[17] Heart rate was measured with a dedicated device (Garmin, USA). Running trials that presented respiratory exchange ratio (RER) equal or higher than 1.0 would have been excluded from data analysis. However, none of the participants achieved RER values higher than 1.0 during the test. To compute the energy cost of running (in $J \cdot kg^{-1} \cdot m^{-1}$), data of the last two minutes of each trial were averaged. The Cr was then calculated from the ratio of the $V'O_2$ to the speed and then multiplied for an energy equivalent from 19.62 to 21.13, depending on the RER.[18]

*Perceived Exertion.* During the last minute of each running trial we asked the subjects to evaluate perceived exertion by using the Borg CR-10 Scale with the 0 value meaning "nothing at all" to 10 value meaning "extremely strong".[19] Similarly, we collected the pain perception during each SMFR exercise by asking to the subject to evaluate the perceived pain from 0 (no pain) to 10 (maximum pain).[20]

*Maximal power and rate of force development during explosive lower limb extension.* Peak power of the lower limbs was assessed during SJ and during CMJ by means of the Explosive-Ergometer (EXER), which was previously described elsewhere.[17] Briefly, the subject, sitting on a seat that is fixed to a carriage that is free to move on a rail, accelerates himself and the carriage seat backward by pushing on two force platforms (PA 300, Laumas, Parma, Italy). The velocity along the direction of motion is continuously recorded by a wire

tachometer (SGI, Lika Electronic, Vicenza, Italy). The analog outputs of the force and velocity transducers are digitized and recorded by a data acquisition system (MP 100, Biopac). Power was obtained from the instantaneous product of the developed force (F, N) and the sledge velocity (*v*, m/s). We asked the participants to perform three SJ and three CMJ; the rest period between each jump was 3 minutes. The SJ and CMJ attempts with the highest peak power were considered for further analysis. In particular, we analysed the rate of force development (RFD) during the SJ and CMJ by assessing the force level every 50 ms from the onset of force development for 200 ms, expressing the force values as percentage of the maximal force exerted.[21] The onset of force development was determined by visual inspection of the force traces as well as by determining the moment in which the force value exceeded the mean baseline value plus 3 standard deviations.

*SMFR treatment.* The device used in this study to perform SMFR was BLACKROLL® Standard (BLACKROLL, Germany), with dimensions of 30 cm x 15 cm. This device was selected because of its smaller size, which makes transportation to training camps and competitions easier and it is largely used among professional athletes (as informally reported by an Olympic athlete). SMFR was applied on the following eight muscle groups of both limbs: plantar fascia, gastrocnemius, tibialis anterior, anterior thigh with extended knee, anterior thigh with flexed knee, posterior thigh, gluteus, fasciae latae. SMFR was performed under supervision of an expert physiotherapist. Each muscle group was treated for one minute;[14] the change in body position to treat a different muscle group took about 10 seconds. The pressure applied to the foam roller was self-selected; however, we instructed the participant to apply as much body mass as tolerable on the foam roller.[22] The application frequency was about 0.5 Hz (i.e. each rolling cycle lasted about 2 seconds).[23]

*Statistical Analyses.*

We analysed the data using PASW Statistic 18 (SPSS Inc., IL, USA) with significance set at $p \leq 0.05$. All results are reported as mean ± standard deviation (SD). Differences in Cr, lower limb muscle power and rate of force development collected at PRE, POST and POST 3h were studied with General Linear Model repeated measures with two factors considering ANOVA of the main effects of Condition (C: experimental condition *vs* control condition), Time (T: PRE *vs.* POST *vs.* POST 3h) and Condition x Time interaction. When significant differences were found, a Bonferroni post hoc test was used to determine the exact location of the difference.

## RESULTS

*Energy cost of running*. Cr determined at PRE was very similar between the control and experimental conditions (6.55±1.52 and 6.32±1.61 J $kg^{-1}$ $m^{-1}$, respectively, $p$=0.110). Also, in both conditions, the "Time" factor did not affect significantly Cr (Figure 2). However, in the experimental condition, Cr at POST tended to increase as compared to PRE (+6.2±8.3%, $p$=0.052), while at POST 3h Cr was restored to PRE values (+0.28±9.5%, $p$=0.950).

*Maximal power of lower limb extensors and rate of force development.* At PRE, the maximal power of lower limb extensors detected during SJ in the control condition was similar to that observed in the experimental condition (57.0± 10.2 W/kg and 54.9±14.6 W/kg, respectively, $p$= 0.471). Similarly, the maximal power measured during CMJ in the control condition was not different than that observed in the experimental condition (62.1±11.1 W/kg and 58.9±15.7 W/kg, respectively, $p$= 0.251). At PRE, in both conditions, the maximal power exerted during CMJ was higher than that exerted during SJ (+8.4±9.2%; $p<0.001$). Maximal power detected at POST and POST 3h in the control condition was not different than that observed at PRE for both SJ ($p$=0.741 and $p$=0.392, respectively, Figure 3A) and CMJ ($p$=0.750 and $p$=0.139, respectively, Figure 3C). In the experimental condition, no significant "Time"

effect was observed for maximal power exerted during SJ, as power values similar to those assessed at PRE were found at POST (+4.5±7.8%, $p$=0.102) and POST 3h (+5.8±11.2%, $p$=0.139) (Figure 3B). On the other hand, maximal power exerted during CMJ at POST and POST 3h was significantly higher than that observed at PRE (+7.9±6.3%, $p$=0.002; and +10.0±8.7%, $p$=0.004, respectively) (Figure 3D).

The RFD measured during SJ was not different across PRE, POST and POST 3h time points in both conditions ($p$>0.05; Figure 4A and 4B). Similarly, peak force during SJ was not different between the control and experimental conditions (p=0.469; p=0.829 and p=0.907 at PRE, POST and POST 3h, respectively). On the other hand, RFD assessed during CMJ tended to increase right after the SMFR treatment (p=0.073 at 200 ms from the onset of force exertion), reaching a significant difference between PRE and POST 3h at 200 ms from the onset of force exertion (+38.9%, $p$=0.024, Figure 4D). These changes in RFD after SMFR treatment coincided with significant increments in peak force, which was 1819±362 N at PRE, 1925±548 N at POST and 1972±461 N at POST 3h (p= 0.177 between PRE and POST, and p=0.011 between PRE and POST 3h).

*Perceived Exertion.* In the control conditions the perceived exertion during the running trial at PRE was 2.7±1.2 and it was not different from the values registered at POST (2.8±1.1; $p$=0.723) and at POST 3h (2.8±1.1*;* $p$=0.586).

In the experimental condition, perceived exertion during the running trial at PRE was 2.6±1.1. This value was similar to those collected at POST (2.7±1.0, $p$=0.720), while it tended to be larger compared to that observed at POST 3h (2.2±0.9, $p$=0.054).

During SMFR treatment, the greatest pain was reported at the fascia latae (7.6±1.9), and the least pain at the plantar fascia (2.9±1.2). In addition, the other treated areas were scored as follow: gastrocnemius 4.5±1.6, tibialis anterior 5.5±1.2, anterior thigh with extended knee 5.1±2.1, anterior thigh with flexed knee 4.2±1.4, posterior thigh 3.5±1.8, gluteus 4.5±1.8.

## Discussion

The present study showed that a 16-min SMFR treatment can acutely promote different motor output adaptations depending on the characteristics of the tested motor tasks. In particular, this intervention did not modify the maximal lower limb power exertion during explosive efforts without storage of elastic energy (SJ), improved the maximal power exertion during explosive efforts characterized by storage of elastic energy (CMJ), and tended to impair the energy cost of running (Cr) immediately after the intervention. The present study did not find any effect of SMFR on peak power during SJ, which can be considered a maximal explosive effort without storage of elastic energy involved. In particular, EXER does not allow any storage of elastic energy during SJ, as two mechanical blocks prevent any countermovement. Peak power exerted during SJ is primarily determined by the mass of lower limb extensor muscle chain, and particularly knee extensors, as well as by the muscle activation pattern.[24] It was previously shown that SMFR can induce neural adaptations, possibly inhibiting motor pools activation and altering the motor recruitment pattern, in response to pain receptors activation.[25] Seen as maximal power during SJ was not affected by SMFR, it is possible that neural-induced adaptations were limited in the present study, and/or that different motor pools were affected differently by SMFR according to the different pain level recorded across muscles (see Results). Hence, SMFR-mediated activation pattern adaptations may have counterbalanced neural inhibition and led to an overall lack of SMFR influence on power exertion during SJ.

CMJ was performed on the EXER removing the blocks, and thus allowing the carriage seat to move along the rail without restriction. Power output exerted during CMJ is influenced by the same physiological variables as SJ, with the addition of the elastic energy stored during the transition between eccentric and concentric phase. In the present study, we observed that peak power exerted during CMJ as well as RFD tended to increase immediately after SMFR

application, and they further increased after three hours from SMFR application. The muscle mass, which affects lower limbs muscle power, is not altered by SMFR; hence, other physiological variables should be responsible for the changes observed in CMJ power output after SMFR. As reported above, it has been shown that SMFR can induce neural adaptations, possibly inhibiting motor pools activation and altering the motor recruitment pattern, in response to pain receptors activation.[25] Hodgson et al. reported that CMJ performance was not impaired after treatments that included the use of foam roller (by itself or in combination with static stretching), whereas it was negatively affected when the subjects underwent only static stretching.[3] It was then suggested that foam rolling may counterbalance the negative effects of static stretching on explosive performance.[3] The improvement in CMJ that we reported in the present study may be due to an improved storage and/or utilization of elastic energy, as a similar outcome was reported, for example, by Wilson et al. while investigating the effects of a flexibility training on rebound bench press vs. purely concentric bench press.[26] Also, Bradbury-squires et al. reported better efficiency while performing a lunge after foam roller treatment, suggesting that the same workload was performed with lower EMG activity because of a roller treatment-induced suppression of the H-reflexes.[14,25] Moreover, SMFR treatment-related nociceptive sensory input may have modified the muscle activation pattern, possibly improving the agonist-antagonist coordination and/or activation ratio during CMJ. Also, the increased RFD may be related to a better synchronization of motor units.[27] However, further studies that include the assessment of EMG activity should be performed in order to investigate neural-related adaptations due to SMFR during explosive efforts that include stretch-shortening cycles.

SMFR treatment can potentially also lead to an increased muscle compliance (i.e. lower muscle stiffness), and this adaptation can enhance the ability of the musculotendinous unit to store elastic energy.[28] SMFR can acutely alter viscoelasticity properties of the fascia (e.g.

shifting the balance between viscous and elastic proprieties more towards the latter) due to the prolonged rolling (one minute for each muscle) and the heat induced by the treatment may affect the muscle and tendon stiffness.[14] Indeed, the mechanical pressure and the heat following the roller massage may affect the fascia in two ways: 1) making the tissues soften and reducing their viscosity. And 2) remobilizing the fascia back to its gel-like state.[29] This last adaptation, however, could be maintained up to four hours from SMFR application.[15] It is plausible that these SMFR-induced adaptations on different tissues of the lower limb also contributed to an overall improvement in storage of elastic energy during maximal, explosive efforts of lower limbs that included a countermovement.

The energy cost of running tended to increase immediately after SMFR application ($p = 0.052$), thus impairing running performance.[30] It is plausible that this adaptation was related to the loss of water in the fascia, which could reduce the musculotendinous stiffness.[15] This decline in stiffness may affect the ability to store and release elastic energy during running.[31] In addition, the higher flexibility promoted by a SMFR treatment may have also negatively affected Cr, leading to greater energy expenditure for muscle stabilization.[6,9-11] However, the negative effects of SMFR treatment on Cr were not present after three hours form the treatment, as Cr returned to its initial value. It seems important to comment on the opposite acute effect that SMFR had on Cr and CMJ, as both running and CMJ performance are based, at least partially, on the storage of elastic energy. The stance phase, which comprises both eccentric and concentric phases, lasted on average ~300 ms, considering the mean speed equal to 11 km/h that the subjects maintained on average throughout the test.[32] Also, the knee angle during the stance phase ranged between about -15 and -45 deg (considering 0 deg the completed extension).[33] Conversely, CMJ push phase lasted between 500 and 600 ms, and the knee angle ranged between -80 and 0 deg. Also, we did not measure ground reaction forces during running; however, from the literature we can estimate peak forces during running of about 1.8 times the

body weight (i.e. 1200 N).[32,34] On the other hand, peak forces exerted during CMJ were much higher (about 1900 N, see results). Finally, it is worth noting that CMJ was performed on a sledge ergometer, and hence the balance/stabilization component was negligible in this task. It seems possible that the acute effects of SMFR on gel-like state of the fascia and on the tissue viscosity, which conceivably leads to lower musculotendinous stiffness, may differently affect the recovery of elastic energy based on the amount of forces generated by the lower limb and on the movement duration and/or range of motion, favoring the storage of elastic energy when higher forces come into play. It is also interesting to note that both Cr and CMJ power exertion tended to improve at POST 3h compared to POST. This may suggest that rehydration of fascia and consequent increase in stiffness are of benefit for both high- and low-force motor tasks that involve storage of elastic energy.[15]

It is also important to note that the present findings are related to a single session of SMFR. It is possible that long-term SMFR application could affect Cr and lower limb power output in a different manner. For example, data related to static stretching showed that athletes who practiced stretching chronically obtained higher flexibility without affecting the energy cost of running.[35] Conversely, other authors reported that acute stretching can increase Cr because of a reduction of the mechanical efficiency of the lower body through the reduction of musculotendinous stiffness.[36]

*Critique of methods*

We acknowledge that our study has some limitations. First, the pressure that each subject applied to the foam roller was self-selected. Also, we did not directly measure the stiffness or water content of muscle and tendon tissues; thus, further studies are required to examine the effects of SMFR on these tissues. Finally, the lack of EMG activity recordings did not allow us to investigate in detail the effects of SMFR on neuromuscular activation characteristics.

## Practical applications

The results of the present study suggest that an acute use of foam roller for SMFR performed immediately prior to running may negatively affect the endurance running performance. Conversely, performing SMFR treatment 3 hours before the running performance could be valuable because it would not alter Cr while promoting increased muscle power, which, in turn, may positively affect the running performance.[17] Also, conversely to other methodologies such as static stretching, SMFR may enhance the performance in athletic sports that include a high degree of elastic storage capacity (such as CMJ).

## Conclusions

In conclusion, the findings of the present study demonstrate that an acute bout of foam rolling impairs the cost of running but it increases the power of the lower limbs when elastic energy is involved (CMJ). Athletes and coaches have to be aware of these results in order to use this tool when it is appropriate.

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**FIGURE 1.** Experimental design.

SMFR: self-myofascial release. Test Pre and Test Post: 10' running test + squat jump + counter movement jump

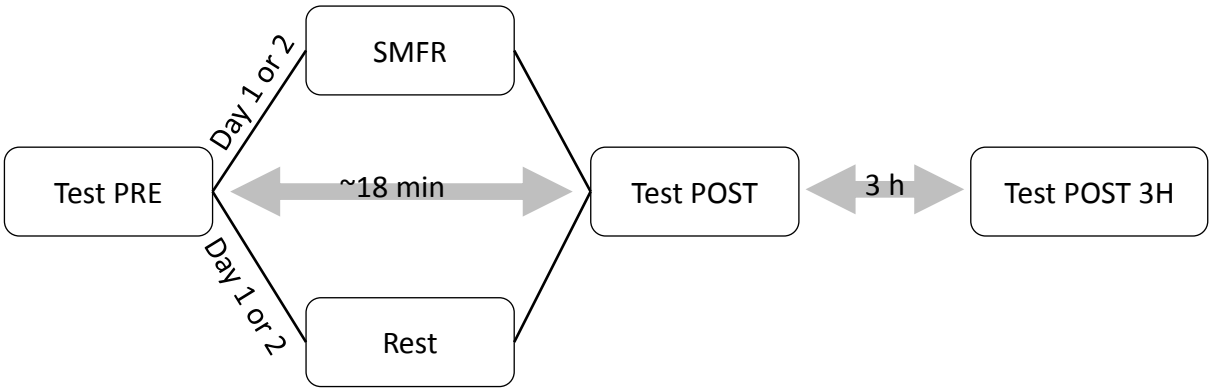
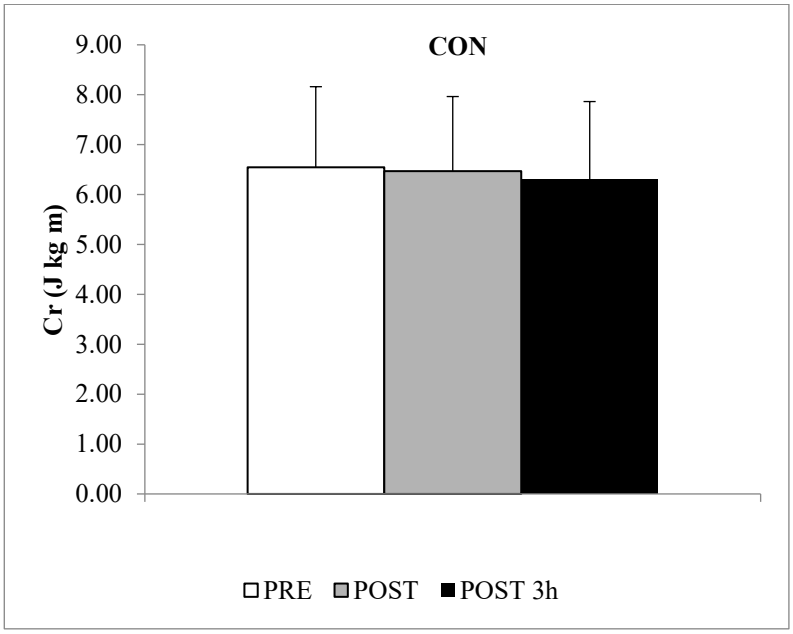


**FIGURE 2.** Energy cost of running ($J \cdot kg^{-1} \cdot m^{-1}$) measured before (PRE, white column), immediately after (POST, grey column) and 3h after the treatment period (POST 3h, black column) in control (CON, A) and experimental (EXP, B) condition.

A.

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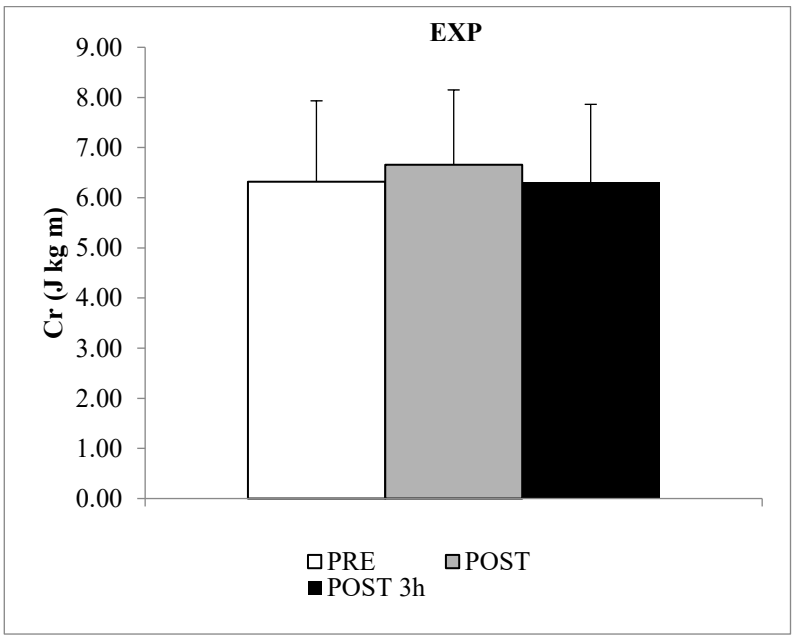
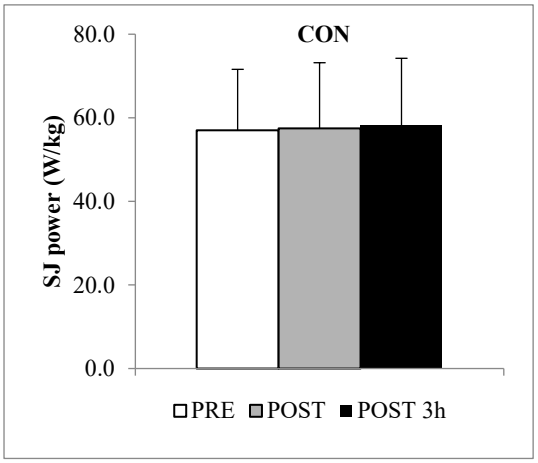
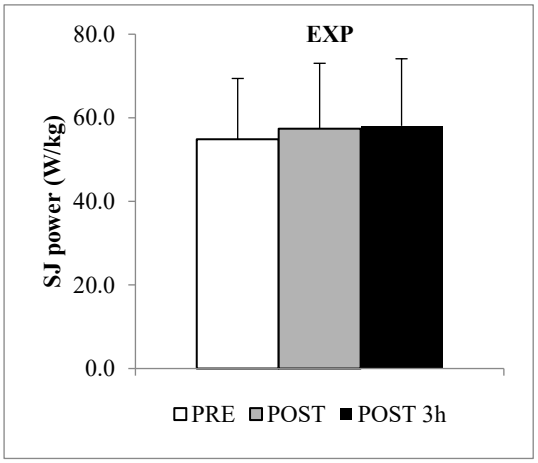


**FIGURE 3.** Squat jump peak power (SJ, $W \cdot kg^{-1}$, Figure A and $B^{-1}$) and counter movement jump peak power (CMJ, $W \cdot kg^{-1}$, Figure C and D) measured before (PRE, white column), immediately after (POST, grey column) and 3h after the treatment period (POST 3h, black column) in control (CON, A and C) and experimental (EXP, B and D) condition. *P<0.05

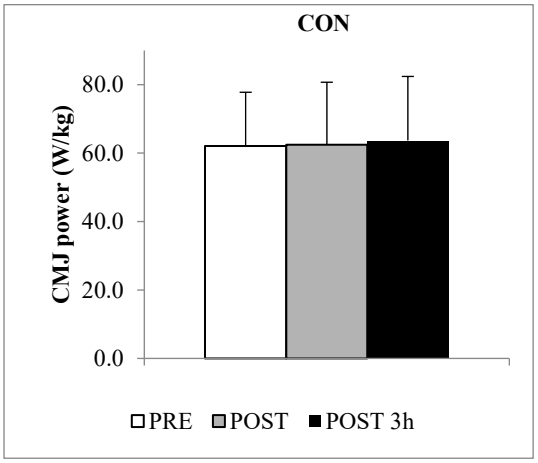
A.




B.




C.




D.

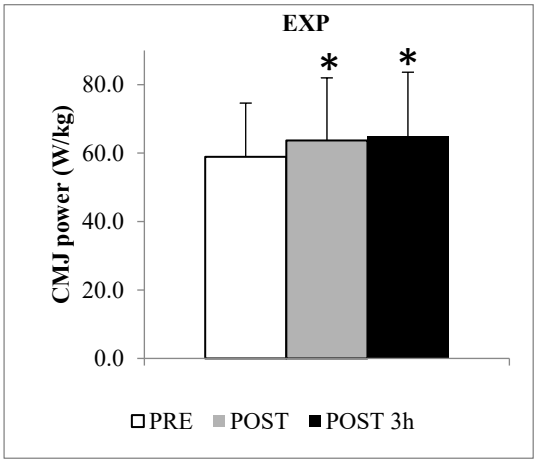
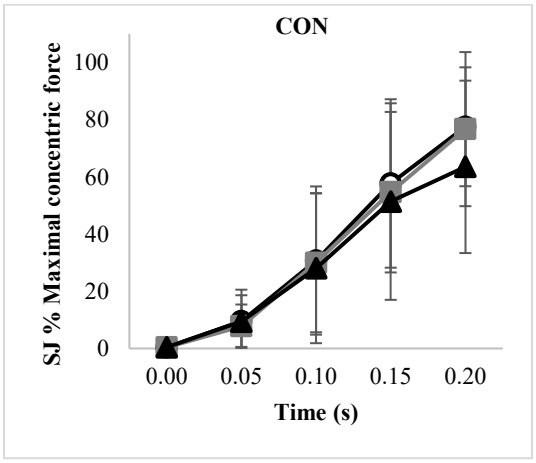


**FIGURE 4.** Rate of force development during explosive squat jump (SJ, in % maximal concentric force, Panels A and B) and counter movement jump (CMJ, in % maximal concentric force, Panels C and D) measured before (white circles) immediately after (POST, grey squares) and 3h after the treatment period (POST 3h, black triangle) in control (CON, A and C) and experimental (EXP, B and D) condition. *P<0.05

A.

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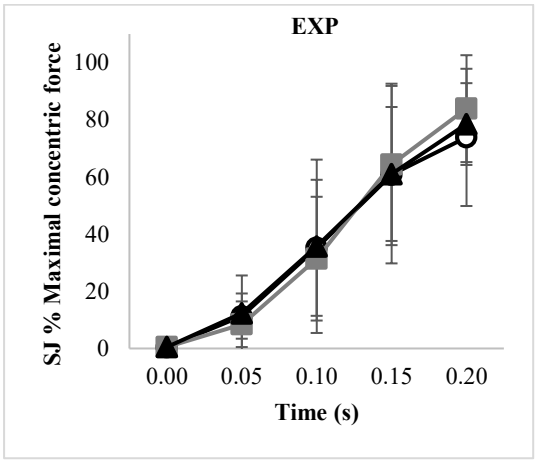
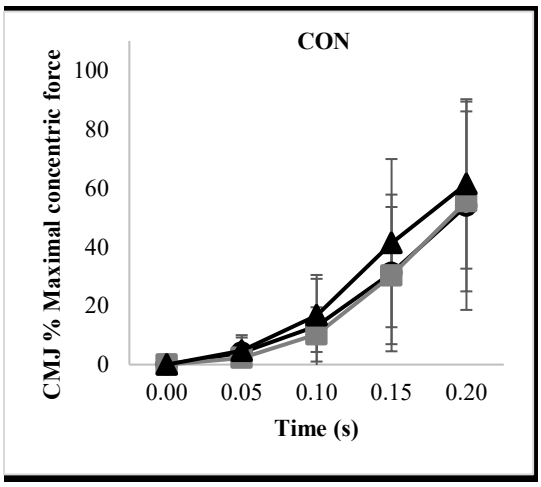



C.

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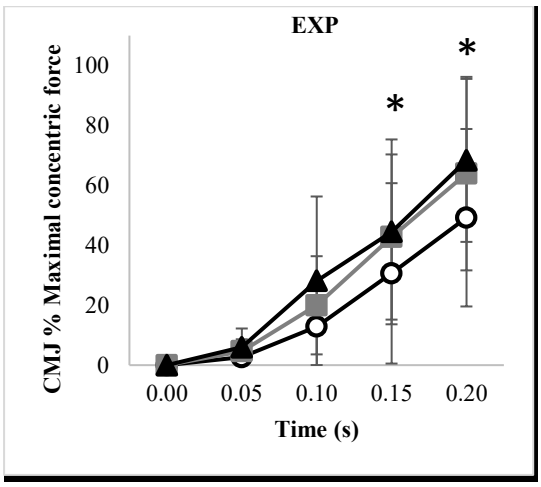

**TABLE 1.** Physiological characteristics of the subjects (n=13) and training status.

| | |
|---|---|
| Age (y) | 26.3 ± 5.3 |
| Stature (m) | 1.80 ± 0.06 |
| Body mass (kg) | 69.0 ± 10.3 |
| Fat mass (%) | 16.5 ± 7.7 |
| Training status (hh/week) | 9.9 ± 3.5 |

All values are mean±SD.

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ORIGINAL ARTICLE

# Effects of endurance training on neuromuscular fatigue in healthy active men. Part I: Strength loss and muscle fatigue

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## Abstract

**Purpose** The adaptations induced by endurance training on the neuromuscular function remain under investigation and, for methodological reasons, unclear. This study investigates the effects of cycling training on neuromuscular fatigue and its peripheral contribution measured during and immediately after cycling exercise.

**Methods** Fourteen healthy men performed a fatigue test before a 9-week cycling program (PRE) and two tests after training: at the same absolute power output as PRE ($POST_{ABS}$) and based on the post-training maximal aerobic power ($POST_{REL}$). Throughout the tests and at exhaustion (EXH), maximal voluntary contraction (MVC) and peripheral fatigue were assessed in the quadriceps muscle by electrical nerve stimulation [single twitch (Pt); high-frequency doublet ($Db_{100}$) and low-to-high-frequency ratio ($Db_{10:100}$)].

**Results** Time to EXH was longer in $POST_{ABS}$ than PRE (34 ± 5 vs. 27 ± 4 min, $P < 0.001$), and $POST_{REL}$ tended to be longer than PRE (30 ± 6 min, $P = 0.053$). MVC and peripheral fatigue were overall less depressed in $POST_{ABS}$ than PRE at isotime. At EXH, MVC and $Db_{10:100}$ were similarly reduced in all sessions (–37 to −42% and −30 to −37%, respectively). $Db_{100}$ tended to be less depressed in $POST_{ABS}$ than PRE (–40 ± 9 vs. −48 ± 16%, $P = 0.050$) and in $POST_{REL}$ than PRE (–39 ± 9%, $P = 0.071$). Pt decreased similarly in $POST_{ABS}$ and PRE (–52 ± 16 vs. −54 ± 16%), but $POST_{REL}$ tended to be less depressed than PRE (–48 ± 14%, $P = 0.075$).

**Conclusions** This study confirms fatigue attenuation at isotime after training. Yet lower or similar fatigue at EXH indicates that, unlike previously suggested, fatigue tolerance may not be upregulated after 9 weeks of cycling training.

**Keywords** Aerobic training · Excitation–contraction coupling failure · Neuromuscular function · Peripheral fatigue

## Abbreviations

| | |
|---|---|
| CST | Constant-load submaximal training |
| $Db_{10}$ | Low-frequency doublet |
| $Db_{10:100}$ | Low-frequency fatigue |
| $Db_{100}$ | High-frequency doublet |
| ECC | Excitation–contraction coupling |
| EXH | Exhaustion |
| GET | Gas exchange threshold |
| HIIT | High-intensity interval training |
| HR | Heart rate |
| La | Lactate |
| MVC | Maximal voluntary contraction |
| NMF | Neuromuscular fatigue |
| $POST_{ABS}$ | Fatigue session based on the same absolute power output as before training |
| $POST_{REL}$ | Fatigue session based on the same relative intensity as before training |
| PRE | Initial fatigue test |
| Pt | Peak twitch |
| RCP | Respiratory compensation point |
| RPE | Rate of perceived exertion |
| SERCA | Sarco(endo)plasmic reticulum $Ca^{2+}$ ATPase |
| TMS | Transcranial magnetic stimulation |
| TTE | Time to exhaustion |
| $TTE_{PRE}$ | TTE of PRE |

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| | |
|---|---|
| $\dot{V}CO_2$ | $CO_2$ output |
| $\dot{V}E$ | Minute ventilation |
| $\dot{V}E/\dot{V}CO_2$ | Ventilatory equivalent of $VCO_2$ |
| $\dot{V}O_{2max}$ | Maximal oxygen uptake |
| $\dot{V}O_{2peak}$ | Peak $\dot{V}O_2$ |
| $W_{max}$ | Maximal aerobic power output |

## Introduction

Endurance training is an effective method to improve aerobic fitness. Indeed, greater maximal oxygen uptake ($\dot{V}O_{2max}$) and improved lactate threshold have been documented after high-intensity interval training [HIIT, (Astorino and Schubert 2014)] or constant-load submaximal training [CST, (Gunnarsson et al. 2013)]. A combination of both training modalities (Milanović et al. 2015) has shown to be effective and may actually be more suitable for populations with no previous cycling experience. Mechanisms such as increase in stroke volume (Warburton et al. 2004; Daussin et al. 2008), blood and plasma volume (Warburton et al. 2004), muscle-oxidative capacity (Daussin et al. 2008), and mitochondrial content (MacInnis et al. 2017) have been widely documented. Although endurance training is known to increase performance, as evidenced by the post-training increase in time to exhaustion (TTE) during single limb (Vila-Chã et al. 2010, 2012a, b; Zghal et al. 2015) and whole-body fatigue tasks (O'Leary et al. 2017), TTE (or other type of performance indices) does not provide information regarding neural and muscular contributions to the improved resistance to fatigue. Neuromuscular fatigue (NMF) is defined as any exercise-induced change in the central nervous system and/or muscles, resulting in a force output that is less than anticipated for a given voluntary contraction or stimulation (MacIntosh and Rassier 2002). The sites of failure may be originated at any level of the motor pathway and are usually divided into central and peripheral. Central fatigue describes the reduction in voluntary activation or neural drive of the central nervous system (Goodall et al. 2012). Peripheral fatigue may occur at or distal to the neuromuscular junction and is characterized by a reduction in the electrically evoked responses on relaxed muscles.

While the relative contributions of central and peripheral factors to fatigue have been extensively studied in different exercise modes (Lepers et al. 2002; Gruet et al. 2014), intensities and durations (Temesi et al. 2014), and environmental conditions (Goodall et al. 2012), much less is known regarding the effects of training on NMF or even neuromuscular function in general. Vila-Chã's group showed that, following endurance training, the excitability of the H reflex pathway was increased (Vila-Chã et al. 2012a) and the rate of decline of motor unit conduction velocity was lowered (Vila-Chã et al. 2012b). Zghal et al. (2015) observed greater peripheral fatigue at EXH after an 8-week knee extensor endurance training program.

Whilst these studies provide some evidence on neuromuscular adaptations after endurance training, single limb fatiguing task (Vila-Chã et al. 2010, 2012a, b; Zghal et al. 2015) and/or single limb training protocol (Zghal et al. 2015) have little transfer to whole-body exercise. O'Leary et al. (2017) was the first study, and the only one so far to our knowledge, investigating the effects of cycling endurance training on neuromuscular fatigue measured in specific conditions, i.e., after a cycling fatigue exercise. This recent study was particularly interesting as (i) two training programs were compared (HIIT vs. CST) and (ii) for each of them, two fatigue tests were carried out after training: one at the same absolute power output as before training ($POST_{ABS}$), and one at the same relative intensity as before training ($POST_{REL}$). Central and peripheral fatigues were significantly depressed at isotime. In $POST_{REL}$, the authors observed greater peripheral fatigue at EXH in HIIT when compared to pre-training, with no changes in CST. Although speculative, a training-induced upregulation in the inhibitory III/IV afferent feedback might have increased the tolerance to metabolic disturbance in the working muscles, as also suggested by Zghal et al. (2015). Alternatively, adaptations at a supraspinal level may take place such that greater peripheral fatigue would be tolerated by the central nervous system (Millet 2011).

Although O'Leary et al. (2017) attempted to assess fatigue at EXH as quickly as possible, the time required to move subjects from the cycle ergometer to the isometric chair for neuromuscular assessment might have significantly affected exercise-induced fatigue interpretation. Indeed, previous research has shown that fatigue recovers within the first seconds after exercise (Mira et al. 2017). To circumvent the methodological limitations of previous literature, we developed and validated a cycle ergometer that allows NMF evaluation on itself in isometric mode within 1 s (Doyle-Baker et al. 2017). This device presents clear advantages. First, it allows NMF assessment in cycling, which is specific to the exercise performed during the training intervention. Second, it permits the assessment of neuromuscular fatigue right at EXH, without having to move the subject to an isometric ergometer and thus avoiding fatigue misinterpretation. Third, the lack of delay enables the accurate investigation of the time course of fatigue development and etiology during the fatigue test.

The aim of the present study was thus to investigate the effects of an 9-week endurance cycling training program on the time course of fatigue development and the peripheral contributions at both relative ($POST_{REL}$) and absolute intensities ($POST_{ABS}$). It was hypothesized that: (i) fatigue would be attenuated for any given workload (isotime) in $POST_{ABS}$,

and; (ii) greater levels of fatigue at EXH would be tolerated in $POST_{REL}$ when measured for the first time with no delay.

## Methods

### Subjects

Fourteen healthy men (mean ± standard deviation (SD), age 26 ± 6 years, height 178 ± 6 cm, mass 77 ± 10 kg, $\dot{V}O_{2peak}$ 45 ± 3 ml·min$^{-1}$ kg$^{-1}$) participated in the study. Subjects provided written informed consent prior to their participation. The study was performed in accordance with the latest edition of the Declaration of Helsinki and was approved by the ethics board of the University of Calgary (REB 15-2566). All subjects were recreationally active (i.e., 3 ± 1 sessions of exercise per week, each lasting 54 ± 26 min) and had no signs or symptoms of neurological, cardiovascular, circulatory or orthopedic disorders. They continued their sporting activities during the training period. Subjects refrained from any type of physical exercise at least 2 days before testing and did not drink any beverages with alcohol and caffeine on test days.

### Experimental design

Figure 1a illustrates the experimental protocol. All testing sessions were performed on a customized electromagnetically braked recumbent cycle ergometer (Doyle-Baker et al. 2017) at a similar time of the day. Prior to training, subjects completed a familiarization and an experimental trial, which were separated by at least 48 h. The familiarization trial comprised of a maximal incremental test to set the exercise intensity of the initial fatigue test (i.e., PRE, Fig. 1a) and familiarization to NMF evaluations on the customized cycle ergometer. PRE consisted of a fatigue test to EXH (see below) with an objective of assessing TTE and neuromuscular function before, during and immediately after cycling. Then, subjects initiated a 9-week training protocol, which comprised a combination of CST and HIIT sessions (Fig. 1a). All training sessions were completed on a cycle ergometer (Ergoline Ergoselect 200 P, Ergoline GmbH, Germany). At least 48 h after the last training session, a new maximal incremental test was performed to determine peak $\dot{V}O_2$ ($\dot{V}O_{2peak}$) and maximal aerobic power output ($W_{max}$). Finally, two fatigue tests were completed in random order at least 48 h after the incremental test (Fig. 1a) and interspersed with at least 72 h: (i) $POST_{ABS}$, performed up to EXH at the same absolute power output as PRE and (ii) $POST_{REL}$, performed up to EXH at the same percentage of $W_{max}$ determined post-training.

### Procedures

#### Maximal incremental test and familiarization

During the first visit, subjects completed a maximal incremental test to determine $\dot{V}O_{2peak}$ and $W_{max}$. The test started at 90 W and comprised 15-W increments every minute until volitional EXH. Subjects cycled at a self-selected pace, which was used thereafter in all testing and training sessions (± 10 rpm). Upon test cessation, a familiarization of NMF testing procedures was conducted. For that purpose, maximal and submaximal isometric voluntary contractions of the knee extensors were carried out on the customized cycle ergometer with and without femoral nerve stimulation and transcranial magnetic stimulation (TMS, see companion papers for further details). Once the subjects were able to perform these procedures consistently, they were used to perform the same evaluation right after cycling, i.e., after braking the pedals. After the 9-week training period, the maximal incremental test was repeated to determine the new $\dot{V}O_{2peak}$ and $W_{max}$. A familiarization session was quickly performed to remind subjects of the NMF evaluation on the customized cycle ergometer.

#### Fatigue test

Each fatigue test (i.e., PRE, $POST_{ABS}$ and $POST_{REL}$) started with a warm-up consisting of 3 min of cycling at 40% of pre-training $W_{max}$. Between the end of warm-up and the actual test, 2 min of rest were allowed. The fatiguing protocol then comprised 5-min stages with power output increments at the start of each stage until the subject reached volitional EXH or could no longer cycle at the predetermined pace (± 10 rpm) (Fig. 1b). In PRE and $POST_{ABS}$, the starting power output was set at 55% of pre-training $W_{max}$ and then increased by 5% of pre-training $W_{max}$ at the start of every stage. $POST_{REL}$ was similar, except that the power output was set based on the post-training $W_{max}$ (i.e., starting power output set at 55% of post-training $W_{max}$; increments of 5% of post-training $W_{max}$). Three subjects did not improve their $W_{max}$ enough (improvement < 5 W) to justify performing a $POST_{REL}$ session. Thus, for these subjects, the sole post-training session they performed was considered both $POST_{ABS}$ and $POST_{REL}$ for analysis purposes. A fourth subject did not perform $POST_{REL}$ because he left the country right after $POST_{ABS}$. At the end of each stage and at EXH, pedals were locked for NMF evaluation. A standardized period of 40 s was devoted for each NMF evaluation (Fig. 1c), after which the pedals were unlocked to allow the subject to resume cycling until next evaluation or EXH.

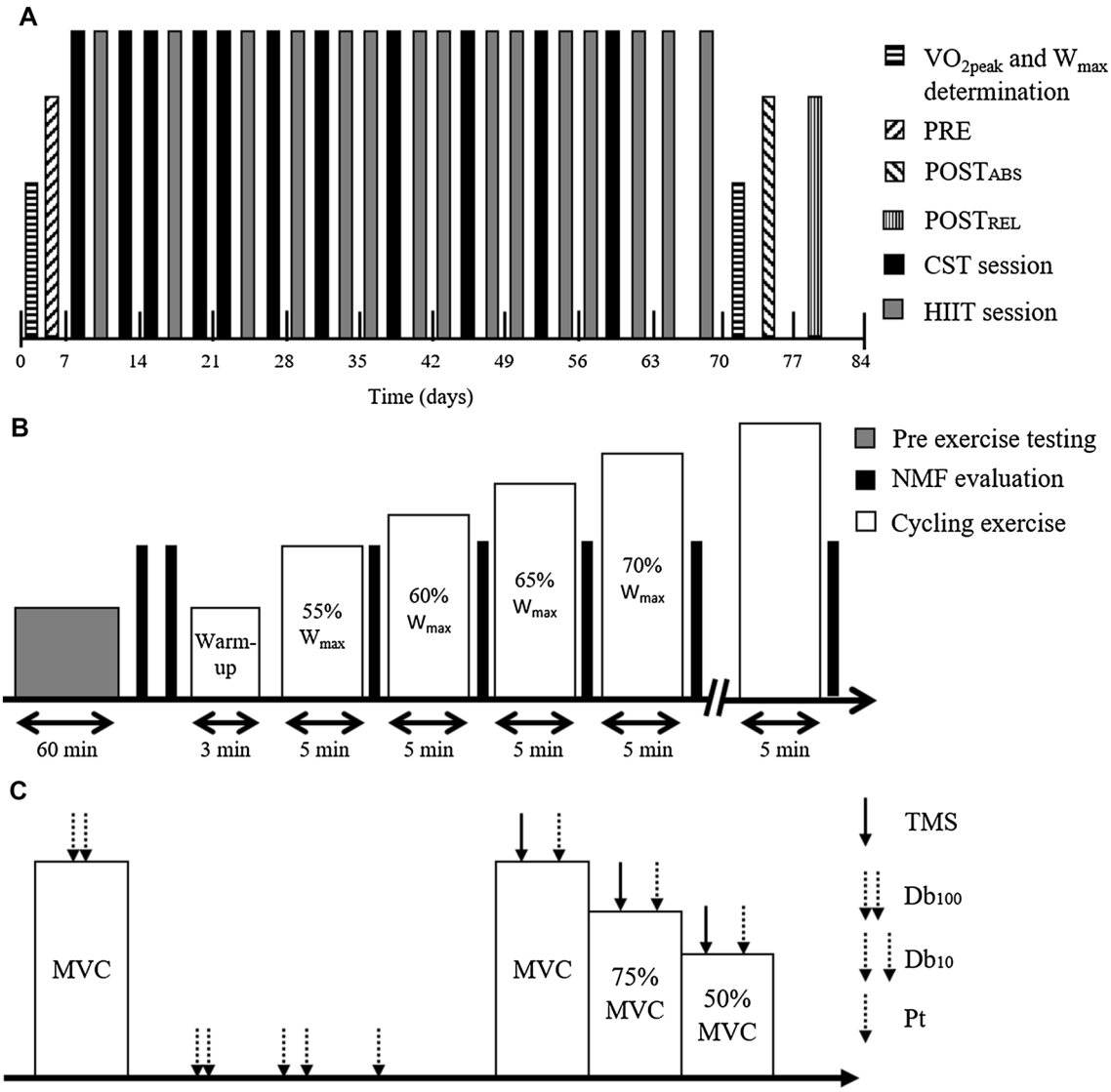


**Fig. 1** **a** Shows the experimental design, which was composed of two sessions for $VO_{2max}$ and $W_{max}$ determination, 9 weeks of cycling training, and three fatigue sessions. **b** Illustrates a typical fatigue session. Pre-exercise testing included electrically evoked responses to elicit the highest M-wave and twitch response in the unfatigued state, warm-up followed by 2×4-s MVCs, determination of optimal TMS site, and determination of optimal TMS intensity. **c** Shows the measurements carried out in each neuromuscular function assessment. *CST* constant-load submaximal training, $Db_{10}$ low-frequency doublet, $Db_{100}$ high-frequency doublet, *HIIT* high-intensity interval training, *MVC* maximal voluntary contraction, *NMF* neuromuscular fatigue, $POST_{ABS}$ post-training fatigue session at same absolute power output as PRE, $POST_{REL}$ post-training fatigue session at same relative intensity as PRE, *PRE* fatigue session before training, *Pt* peak twitch, *TMS* transcranial magnetic stimulation, $VO_{2peak}$ peak oxygen consumption, $W_{max}$ maximal aerobic power output

### NMF evaluation

Measurements of NMF were carried out before, during, and right at EXH of the fatigue tests (Fig. 1b). Before cycling, subjects completed two NMF evaluations interspersed with 2 min of rest. Immediately at the end of each cycling stage and at EXH, the pedals were locked at fixed position (approximately 90° angle at the ankle and knee joint, and 100° at the hip joint) and the NMF evaluation was performed within 1 s. Every NMF evaluation comprised one 5-s MVC of the right knee extensors with one superimposed high-frequency doublet (femoral nerve electrical stimulation, 100 Hz) delivered on the plateau, and was followed by 3 stimuli (100- and 10-Hz paired pulses and single pulse) on the relaxed muscle separated by 3 s. 2–3 s after the single pulse, subjects were asked to perform another 5-s MVC followed by a voluntary adjustment in force output to match 75% MVC then 50% MVC of the preceding MVC with no recovery between force levels [(Mira et al. 2017), Fig. 1c]. TMS was delivered at MVC, 75% MVC and 50%

MVC. A detailed description of the TMS procedures can be found in the companion manuscript. Each NMF evaluation lasted ~ 35–40 s.

### Training

Subjects completed 26 training sessions over a period of 9 weeks (8 training weeks and 1 tapering week). Details on weekly sessions are described in Table 1. In the first 3 weeks, 2 CST sessions and 1 HIIT were performed per week. From week 4 to 8, training comprised 1 CST session and 2 HIIT sessions. In week 9, subjects completed a tapering period that consisted in only 2 low-volume HIIT sessions. The power output of the training sessions was initially set as $\%W_{max}$, which was associated to a given heart rate (HR) and rate of perceived exertion (RPE). However, this power output would inevitably become easier to sustain and be progressively associated to lower HR and RPE responses due to training effects. To ensure target HR and RPE (see below) were attained, one of the investigators recorded these two parameters at the end of each work and rest interval during HIIT sessions and every 5 min during CST sessions. If HR and/or RPE were falling outside of the target intervals (± 5 bpm for HR and ± 1 for RPE), power output was readjusted. In CST sessions, HR should correspond to that of 60–70%$W_{max}$ and RPE should be 6–7 on a 1–10 scale [modified Borg's scale (Noble et al. 1983)]. In HIIT sessions, HR should correspond to that of 90–100%$W_{max}$ and RPE should be 10 by the last 2 work intervals.

## Equipment and measurements

### Innovative cycle ergometer and force assessment

All fatigue sessions were carried out on a custom-built recumbent cycling ergometer with electromagnetically braked Velotron system (Racermate Inc, Seattle, WA, USA) and powered by Velotron Coaching Software (Racermate Inc). This innovative cycle ergometer features a special locking system, which enables to lock the pedals instantly in a

**Table 1** Overview of the training protocol

| | # of sessions | Work duration (min) | Work intensity | Recovery duration (min) | Recovery intensity | # of sets |
|---|---|---|---|---|---|---|
| Week 1 | | | | | | |
| CST | 2 | 30 | 70%$W_{max}$ | | | |
| HIIT | 1 | 1 | 90–100%$W_{max}$ | 1 | 30%$W_{max}$ | 8 |
| Week 2 | | | | | | |
| CST | 2 | 30 | 70%$W_{max}$ | | | |
| HIIT | 1 | 1 | 90–100%$W_{max}$ | 1 | 30%$W_{max}$ | 8 |
| Week 3 | | | | | | |
| CST | 2 | 35 | 70%$W_{max}$ | | | |
| HIIT | 1 | 1 | 90–100%$W_{max}$ | 1 | 30%$W_{max}$ | 8 |
| Week 4 | | | | | | |
| CST | 1 | 35 | 70%$W_{max}$ | | | |
| HIIT | 2 | 1.5 | 90–100%$W_{max}$ | 1 | 30%$W_{max}$ | 8 |
| Week 5 | | | | | | |
| CST | 1 | 40 | 70%$W_{max}$ | | | |
| HIIT | 2 | 1.5 | 90–100%$W_{max}$ | 1 | 30%$W_{max}$ | 8 |
| Week 6 | | | | | | |
| CST | 1 | 40 | 70%$W_{max}$ | | | |
| HIIT | 2 | 1.5 | 90–100%$W_{max}$ | 1 | 30%$W_{max}$ | 8 |
| Week 7 | | | | | | |
| CST | 1 | 45 | 70%$W_{max}$ | | | |
| HIIT | 2 | 2 | 90–100%$W_{max}$ | 1 | 30%$W_{max}$ | 8 |
| Week 8 | | | | | | |
| CST | 1 | 45 | 70%$W_{max}$ | | | |
| HIIT | 2 | 2 | 90–100%$W_{max}x$ | 1 | 30%$W_{max}$ | 8 |
| Week 9 | | | | | | |
| CST | 0 | 0 | / | / | / | / |
| HIIT | 2 | 2 | 90–100%$W_{max}$ | 1 | 30%$W_{max}$ | 4 |

fixed position. During the first visit, cycle ergometer seat position (height and fore-aft position) was adjusted to allow the subject to cycle comfortably and to ensure the isometric position adopted while performing the NMF evaluation was as close as possible to that usually held on an isometric chair (i.e., 90° angle at the knee and ankle joint and a 100° angle at the hip joint). This position was kept constant in all testing sessions. To limit body movement, subjects were firmly secured to the seat with non-compliant straps over the hip and chest. The cycle ergometer enabled the measurement of isometric force via a previously validated (Stapelfeldt et al. 2007) wireless pedal force analysis system (Model PowerForce 1.0.0, Radlabor GmbH, Freiburg, Germany) located between the pedal and the crank. This allowed to conduct NMF evaluations directly on the cycle ergometer, without having to move subjects to the isometric chair and thus minimizing fatigue misinterpretation. Force was sampled at 500 Hz and recorded using Imago Record software (version 8.50, Radlabor GmbH). When the pedals were locked in a fixed position, the cranks were parallel to the ground. During the NMF evaluations, subjects were asked to lock their ankle and push their foot forward to preferably recruit the knee extensors and force was measured in line with the crank. To provide real-time visual force feedback, the PowerForce signal was transmitted to a PowerLab system (16/35, ADInstruments, Bella Vista, Australia) using a National Instruments 16-bit A/D card (NI PCI-6229, National Instruments, Austin, TX, USA) and connector block (BNC-2111, National Instruments) and displayed on a computer monitor placed in front of the subject. The data recorded using Imago Record were analyzed offline using Labchart 8 software (ADInstruments).

### Femoral nerve stimulation

Percutaneous electrical stimulation was delivered to the femoral nerve via a cathode electrode (10-mm stimulating diameter; Meditrace 100, Covidien) secured with tape in the inguinal triangle. The anode, a $50 \times 90$ mm rectangular electrode (Durastick Plus, DJO Global, Vista, CA, USA), was placed in the gluteal fold. A constant current stimulator (DS7A, Digitimer, Welwyn Garden City, Hertfordshire, UK) was used to deliver a square-wave stimulus of 1-ms duration. To determine optimal stimulus intensity at the start of each fatigue test, single-pulse stimulation was delivered to the femoral nerve until both maximal peak twitch (Pt) and maximal M-wave amplitude were obtained. The stimulating intensity ($157 \pm 47$, $130 \pm 52$, $111 \pm 44$ mA in PRE, $POST_{ABS}$ and $POST_{REL}$, respectively) was supramaximal (i.e., 130% of optimal intensity) and held constant throughout the protocol.

### Oxygen uptake

During the maximal incremental tests used to determine $\dot{V}O_{2peak}$ and $W_{max}$, pulmonary gas exchange and ventilation were measured breath-by-breath with a metabolic cart (Quark CPET, COSMED, Rome, Italy). Before each test, calibration of the gas analyzer was completed according to manufacturer's instructions. HR was continuously recorded with a HR monitor (Garmin International, Schaffhausen, Switzerland), which was synchronized with the metabolic cart. Gas exchange threshold (GET) was visually identified as the $\dot{V}O_2$ at which (i) there was a first disproportional increase in $CO_2$ output ($\dot{V}CO_2$) relative to $\dot{V}O_2$, (ii) minute ventilation ($\dot{V}E$) systematically rose in relation to $\dot{V}O_2$ and end-tidal $O_2$ and (iii) the ventilatory equivalent of $\dot{V}CO_2$ ($\dot{V}E/\dot{V}CO_2$) and partial pressure of end-tidal $CO_2$ were stable (Beaver et al. 2012). To identify the respiratory compensation point (RCP), the $\dot{V}O_2$ at which both partial pressure of end-tidal $CO_2$ began to decrease after a period of isocapnic buffering (Whipp et al. 1989) and the second breakpoint in the $\dot{V}E-\dot{V}O_2$ relationship occurred were determined. Then, RCP was confirmed as the $\dot{V}O_2$ at which there was a systematic increase in $\dot{V}E/\dot{V}CO_2$. Two different investigators who were blinded to the identity of the subjects analyzed GET and RCP. The means of these values were used for analysis. If there was a discrepancy of either GET or RCP of $> 100$ mL min$^{-1}$, the threshold was determined by investigator agreement. GET and RCP were then expressed as a percentage of $\dot{V}O_{2peak}$. All data editing, processing, and modeling were performed using OriginLab (version 9.2, OriginLab, Northampton, MA, USA). During training, HR was recorded with a HR monitor (A110, Bion, Taipei, Taiwan) 10 s before the end of each work/rest bout in HIIT sessions, while it was recorded every 5 min in CST sessions.

### Blood lactate

In each fatigue test session, fingertip blood withdrawal was performed to measure blood lactate concentration ([La]) in a 2-µl capillary sample with a portable lactate analyzer (Lactate Scout, SensLab Gmb, Lepzig, Germany). This procedure was carried out at rest as well as 1 and 3 min after EXH.

### RPE

To determine subjective perception of effort, RPE was obtained using the modified Borg's scale [0–10, Noble et al. (1983)]. RPE was recorded in the last minute of each stage during PRE, $POST_{ABS}$ and $POST_{REL}$. During training, RPE was recorded 10 s before the end of each work/rest bout in

HIIT sessions, while it was recorded every 5 min in CST sessions.

### Data analysis

In the maximal incremental tests, $\dot{V}O_{2peak}$ was considered as the highest 30-s $\dot{V}O_2$ average and maximal HR was the highest recorded HR during the test.

Training responses (power output, HR, and RPE) were compared between the first session of week 1 and last session of week 8 of both CST and HIIT. For that purpose, data from the final minute of CST sessions and from the last high intensity set of HIIT sessions were considered for analysis.

Regarding the fatigue tests, the first MVC of each NMF evaluation (i.e., the one during which a 100-Hz doublet was delivered) was the one used to quantify voluntary force as it was the MVC performed with no recovery. Since two NMF evaluations were performed before cycling, only the best response of each parameter (MVC, high-frequency doublet, low-frequency doublet, peak twitch, and M-wave) was considered for further analysis.

To assess peripheral fatigue, the amplitudes of the high-frequency doublet ($Db_{100}$), low-frequency doublet ($Db_{10}$), and peak twitch (Pt) were measured. Low-frequency fatigue was quantified by calculating the ratio between $Db_{10}$ and $Db_{100}$ ($Db_{10:100}$). M-wave was quantified as peak-to-peak amplitude and area of the vastus lateralis.

Since there was intra-individual and inter-individual variability in the number of completed stages of fatigue tests, interpolation was used to present the data. For that purpose, data were interpolated between stages and EXH so intermediate data points would be attributed to each second of the total TTE. Then, data were expressed as function of TTE of PRE ($TTE_{PRE}$). In other words, if subject A's $TTE_{PRE}$ was 22 min, all data of the three fatigue tests would be expressed in percentage of this duration. For statistical purposes, the duration was then converted to percentage of $TTE_{PRE}$ so it would be possible to analyze data at fixed time points (i.e., isotime, before cycling, at 25, 50, 75 and 100% of $TTE_{PRE}$). The actual EXH values of the three fatigue tests were considered in a separate analysis.

### Statistical analysis

Data are reported as mean ± SD. Shapiro–Wilk test was performed to check if data were normally distributed and homogeneity of variances was determined with the Levene's test. If sphericity was violated, Greenhouse-Geisser corrections were applied.

Dependent samples t tests were performed to compare (i) power output, HR, and RPE between the first session of week 1 and last session of week 8 of both CST and HIIT; (ii) $W_{max,}$ GET, RCP, and $\dot{V}O_{2peak}$ of the maximal incremental tests pre- and post-training; (iii) power output at the start and at EXH of the fatigue tests between PRE vs. $POST_{ABS}$ and PRE *vs*. $POST_{REL}$.

To determine the effect of training on blood [La], two separate two-way ANOVAs (condition × time) with repeated measures were performed on two conditions (PRE vs. $POST_{ABS}$ or PRE vs. $POST_{REL}$) and three time points (before exercise, 1 and 3 min after EXH).

To determine the training effect on the rate of fatigue and its etiology, two separate two-way ANOVAs (condition × time) with repeated measures were performed on two conditions (PRE vs. $POST_{ABS}$ or PRE vs. $POST_{REL}$) and five time points: baseline, 25, 50, 75 and 100% of $TTE_{PRE}$. Where the ANOVA revealed significant main effects or interactions, data were further explored using post hoc comparisons with a Bonferroni correction. To compare fatigue at EXH, two *t* tests with dependent samples were performed (PRE vs. $POST_{ABS}$ or PRE vs. $POST_{REL}$) on the % change from baseline to EXH. For all statistical analyses, an alpha level of $P = 0.05$ was used as the cut-off for significance.

## Results

### Training program

All subjects completed the 9 weeks of training with a remarkably high adherence of 96% (~ 1 session missed out of 26 in average for each subject). Training characteristics of the first vs. last HIIT and CST sessions are described in Table 2. Although power output and HR were not different between the first and last session of neither CST nor

**Table 2** Training characteristics to the first and last CST and HIT sessions

| | CST | HIT |
|---|---|---|
| First session | | |
| Protocol | 30 min | 8 × 1 min |
| Power Output (W) | 156 ± 29 | 223 ± 40 |
| Total work (kJ) | 280 ± 53 | 107 ± 19 |
| $\%W_{max}$ | 61 ± 5 | 92 ± 7 |
| HR (b $min^{-1}$) | 162 ± 12 | 180 ± 13 |
| RPE (1–10) | 7 ± 1 | 9 ± 1 |
| Last session | | |
| Protocol | 45 min | 8 × 2 min |
| Power output (W) | 161 ± 30 | 231 ± 45 |
| Total work (kJ) | 435 ± 80*** | 222 ± 43*** |
| HR (b $min^{-1}$) | 156 ± 12 | 184 ± 8 |
| RPE (1–10) | 6 ± 2 | 10 ± 0* |

Significantly different from first session: *$P < 0.05$, ***$P < 0.001$

**Table 3** Cardiorespiratory responses to maximal incremental test before (pre-training) and after training (post-training)

| | Pre-training | Post-training |
|---|---|---|
| $W_{max}$ (W) | 249 ± 38 | 260 ± 35* |
| $VO_{2peak}$ (ml min$^{-1}$ kg$^{-1}$) | 45 ± 3 | 49 ± 3*** |
| GET (ml min$^{-1}$ kg$^{-1}$) | 34 ± 3 | 39 ± 4*** |
| GET (%$VO_{2peak}$) | 77 ± 5 | 80 ± 6 |
| RCP (ml min$^{-1}$ kg$^{-1}$) | 41 ± 3 | 45 ± 4*** |
| RCP (% $VO_{2peak}$) | 91 ± 4 | 93 ± 5 |
| $HR_{max}$ (b min$^{-1}$) | 188 ± 8 | 186 ± 11 |

$W_{max}$ maximal aerobic power output, *$VO_{2peak}$* peak oxygen consumption, *GET* gas exchange threshold, *RCP* respiratory compensation point, *$HR_{max}$* maximal heart rate

Significantly different from pre-training: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

HIIT, total work increased significantly from the first to the last session in both CST (+ 156 ± 12%, $P < 0.001$) and HIIT (+ 208 ± 13%, $P < 0.001$), as sessions were progressively longer from 30 to 45 min in CST and from 8 × 1- to 8 × 2-min work intervals in HIIT.

## Maximal incremental test

The effects of training on $W_{max}$, $\dot{V}O_{2peak}$, GET, RCP, and $HR_{max}$ are shown in Table 3. Training induced a significant increase in $\dot{V}O_{2peak}$ (+ 9 ± 6%, $P < 0.001$), $W_{max}$ (+ 5 ± 7%, $P = 0.025$) and in power output at which appeared GET (190 ± 29 *vs.* 207 ± 19 W, $P = 0.008$) and RCP (226 ± 32 vs. 240 ± 30 W, $P = 0.007$). However, the %$\dot{V}O_{2peak}$ at which GET and RCP occurred were not different between pre and post-training ($P = 0.087$ and $P = 0.308$, respectively). In addition, maximal HR remained unaltered as a result of training ($P = 0.511$).

## Physiological variables during the fatigue test

TTE was significantly longer in $POST_{ABS}$ than PRE (34 ± 5 vs. 27 ± 4 min, respectively, $P < 0.001$), and $POST_{REL}$ tended to be longer than PRE (30 ± 6 min, $P = 0.053$). Power output at the beginning of the cycling test and at EXH are presented in Table 4. Blood [La] at rest as well as 1 and 3 min after EXH is also shown in Table 4. There were no [La] differences between the three fatigue tests 3 min after EXH. Heart rate significantly rose to a similar extent in all sessions ($P < 0.001$). There was also a significant increase in RPE in all sessions ($P < 0.001$). RPE was lower in $POST_{ABS}$ than in PRE at 75% (8 ± 1 vs. 9 ± 1, respectively, $P < 0.001$) and 100% of $TTE_{PRE}$ (9 ± 1 vs. 10 ± 0, respectively, $P = 0.009$).

**Table 4** Physiological variables during the fatigue tests

| | PRE | $POST_{ABS}$ | $POST_{REL}$ |
|---|---|---|---|
| Initial power output (W) | 137 ± 21 | 137 ± 21 | 143 ± 19$^{\#}$ |
| Power output at EXH (W) | 197 ± 34 | 212 ± 35$^{\&}$ | 216 ± 33$^{\#}$ |
| Baseline [La] (mmol L$^{-1}$) | 1.5 ± 0.4 | 1.4 ± 0.4 | 1.4 ± 0.4 |
| [La] Post 1 min (mmol L$^{-1}$) | 10.8 ± 2.6* | 13.3 ± 3.1*$^{\&}$ | 12.7 ± 4.0* |
| [La] Post 3 min (mmol L$^{-1}$) | 10.3 ± 2.5* | 11.1 ± 2.7* | 11.3 ± 2.8* |

*EXH*, exhaustion, *[La]* blood lactate concentration

Significantly different from baseline (main effect of time): *$P < 0.05$; significantly different from the same time point between PRE vs. $POST_{ABS}$: $^{\&}P < 0.05$; significantly different from the same time point between PRE *vs.* $POST_{REL}$: $^{\#}P < 0.05$

## Neuromuscular variables before, during and after the fatigue test

At baseline, no significant changes in MVC, $Db_{10:100}$, Pt and M-wave were induced by training. However, $Db_{100}$ was significantly higher for $POST_{ABS}$ (166 ± 39 N, $P = 0.003$) and $POST_{REL}$ when compared to PRE (178 ± 43 vs. 150 ± 29 N, respectively, $P < 0.001$).

MVC was significantly reduced throughout all fatigue sessions ($P < 0.001$, Fig. 2a). In addition, an interaction effect was observed between PRE *vs.* $POST_{ABS}$, i.e., a lower MVC depression was found in $POST_{ABS}$ than in PRE at 75% (– 19 ± 9 vs. − 28 ± 1 3%, $P < 0.001$) and at 100% of $TTE_{PRE}$ (–25 ± 10 vs. − 42 ± 14%, $P < 0.001$). At EXH, MVC reduction from baseline did not differ between fatigue tests (PRE = − 42 ± 14%, $POST_{ABS}$ = − 37 ± 8%, $POST_{REL}$ = − 37 ± 12%).

The three fatigue tests resulted in peripheral fatigue as evidenced by the significant drop in Pt, $Db_{100}$, and $Db_{10:100}$ throughout exercise. A lower reduction of Pt and $Db_{100}$ (Fig. 2b, c) occurred in $POST_{ABS}$ than in PRE from 25% $TTE_{PRE}$. No interaction effect was observed for $Db_{10:100}$ (Fig. 2d). A lower depression was also found for $Db_{100}$ in $POST_{REL}$ than in PRE from 25% $TTE_{PRE}$. There was a tendency for a lower reduction of $Db_{100}$ at actual EXH in $POST_{ABS}$ *vs.* PRE (− 40 ± 9 vs. − 48 ± 16%, respectively, $P = 0.050$) and in $POST_{REL}$ vs. PRE (− 39 ± 9 vs. − 45 ± 12%, respectively, $P = 0.071$). Additionally, Pt tended to be less depressed at EXH in $POST_{REL}$ compared to PRE (− 48 ± 14 vs. − 54 ± 16%, $P = 0.075$). M-wave remained unchanged during exercise and at EXH in all fatigue sessions (Table 5).

# Discussion

The aim of the present study was to determine the effects of cycling training on the magnitude and etiology of fatigue during and immediately after a cycling fatigue test to EXH using an innovative ergometer that allows NMF

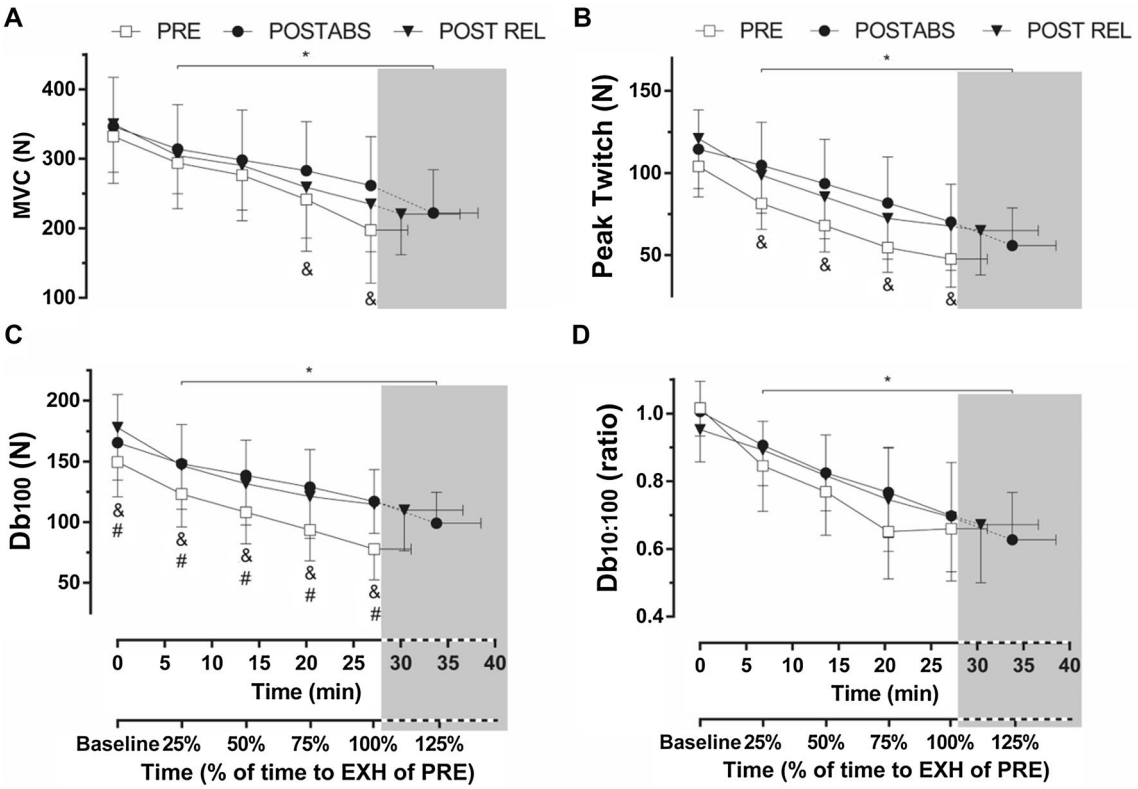


**Fig. 2** Changes in maximal voluntary force (MVC, **a**); peak twitch (Pt, **b**); high-frequency doublet ($Db_{100}$, **c**) and low-frequency fatigue ($Db_{10:100}$, **d**) during the cycling test. All panels show data at baseline as well as 25, 50, 75 and 100% of time to EXH of PRE ($TTE_{PRE}$). Shaded area indicates values at EXH for $POST_{ABS}$ and $POST_{REL}$. The first *x* axis represents the actual times to EXH (min) of the 3 sessions. Data are mean (SD). Significantly different from baseline (main effect of time): $^{*}P < 0.05$; significantly different from the same time point between PRE vs. $POST_{ABS}$: $^{\&}P < 0.05$; significantly different from the same time-point between PRE vs. $POST_{REL}$: $^{\#}P < 0.05$

**Table 5** M-wave areas (μV s) of the vastus lateralis during the fatigue tests

| | PRE | $POST_{ABS}$ | $POST_{REL}$ |
|---|---|---|---|
| Baseline | 91 ± 30 | 101 ± 26 | 107 ± 27 |
| 25% | 91 ± 30 | 96 ± 25 | 98 ± 22 |
| 50% | 94 ± 29 | 96 ± 25 | 98 ± 21 |
| 75% | 94 ± 30 | 96 ± 26 | 101 ± 20 |
| 100% | 98 ± 31 | 99 ± 27 | 105 ± 26 |
| EXH | 98 ± 31 | 97 ± 29 | 107 ± 27 |

*EXH* exhaustion

measurements with no delay. We hypothesized that (i) for a given workload, NMF would be lower in $POST_{ABS}$ than in PRE and (ii) longer TTE and greater peripheral fatigue would be observed at EXH in $POST_{REL}$ than in PRE. Although our findings corroborate the first hypothesis and $POST_{REL}$ tended to be longer than PRE, peripheral fatigue was lower at EXH in $POST_{REL}$ than in PRE, which contradicts previous research on the topic. By allowing NMF assessment directly on the cycle ergometer, this study provides the first evidence about the effects of endurance training on the development of NMF throughout a cycling fatigue test as well as right at EXH, thus minimizing fatigue underestimation and misinterpretation.

## Training responses and time to exhaustion

In agreement with previous research (Berger et al. 2006; Jenkins and Quigley 1992; Daussin et al. 2008; O'Leary et al. 2017), our training protocol has proven to be effective in improving several parameters of subjects' aerobic fitness (i.e., GET, RCP, and $\dot{V}O_{2peak}$). The training protocol used in this study included CST sessions performed at 65% $W_{max}$. This intensity was chosen based on previous research, which showed that 3–4 weekly sessions of cycling exercise performed at 60–65% $W_{max}$ over a period of 6–24 weeks leads to significant improvements in exercise performance and $\dot{V}O_{2max}$ (Warburton et al. 2004; Berger et al. 2006; Daussin et al. 2008). Since most of our subjects were not engaged in cycling activities prior to training, including CST sessions (especially in the early stages) was an effective approach to gradually familiarize subjects with cycling

while simultaneously ensuring training adaptations. We also believe that this prevented any drop out from our study. Although CST shows clear improvements in aerobic fitness, HIIT sessions are usually recommended to optimize benefits (Laursen and Jenkins 2002). HIIT designs similar to the one used in our study have been shown to be effective in improving $\dot{V}O_{2max}$ (Warburton et al. 2004; Berger et al. 2006). Although used less frequently in research settings, training protocols with a combination of HIIT and CST are widely used in the field by athletes and are also capable of increasing $\dot{V}O_{2max}$ (McKenzie et al. 2000).

On average, subjects performed an additional 6 min of cycling in $POST_{ABS}$ than in PRE. Our findings are consistent with the previous literature (Daussin et al. 2008; Seiler et al. 2013; O'Leary et al. 2017) and likely resulted from the increase in aerobic fitness. TTE tended to be longer in $POST_{REL}$ than in PRE, which is somehow in agreement with our hypothesis. Following a low-force knee extensor training, Zghal et al. (2015) reported a longer TTE after training when subjects were asked to maintain an isometric knee extensor contraction at 15% MVC. Interestingly, O'Leary et al. (2017) observed an increase in TTE after HIIT. The high physiological stress that typically characterize HIIT sessions might have translated towards nearly significant longer TTE even at the same relative intensity.

## Neuromuscular function at rest

At baseline, high-frequency doublet was higher in $POST_{ABS}$ and $POST_{REL}$ when compared to PRE. Although not significantly, MVC and Pt also showed higher values at rest post-training. A reduction in $Ca^{2+}$ reuptake may increase cytosol [$Ca^{2+}$], which in turn would augment $Ca^{2+}$ available for cross-bridge formation and thus enable stronger contractions. This has been shown to occur after 5 weeks of cycling training (4 × week), as lower $Ca^{2+}$ reuptake was confirmed by a decrease in sarco(endo)plasmic reticulum $Ca^{2+}$ ATPase (SERCA) content in the vastus lateralis muscle (Majerczak et al. 2008). Although speculative, decreased $Ca^{2+}$ reuptake as a result of training is likely to have occurred in our study, which may explain why $Db_{100}$ was increased in the unfatigued state in $POST_{ABS}$ and $POST_{REL}$. Alternatively, or in addition, the high loads during HIIT might have triggered an increase in muscle mass, which might have resulted in post-training higher voluntary contraction and evoked responses.

## Neuromuscular fatigue during the fatigue sessions

MVC was less depressed at a given workload (75 and 100% $TTE_{PRE}$) in $POST_{ABS}$ compared to PRE. Additionally, $Db_{100}$ was less depressed after training (both in $POST_{ABS}$ and $POST_{REL}$). Since this was accompanied by unchanged M-wave, training adaptations likely occurred beyond the sarcolemma. Pt was less depressed in $POST_{ABS}$ than in PRE from 25% $TTE_{PRE}$, which suggests attenuation of excitation–contraction coupling (ECC) failure. $Db_{10:100}$ also displayed a lower depression in $POST_{ABS}$ vs. PRE, although this did not reach statistical significance (interaction effect, $P = 0.093$). ECC failure is generally attributed to decreased calcium release and reuptake by the sarcoplasmic reticulum, reduced calcium sensitivity, possibly related to accumulation in inorganic phosphate concentration (Allen et al. 2008). The lower decrease in MVC, $Db_{100}$ and Pt in $POST_{ABS}$ throughout the session confirms a training-induced attenuation of total and peripheral fatigue for a given time at a given work load, which is likely linked to the increase in aerobic fitness. Although not tested in the present study, improved $\dot{V}O_2$ kinetics, which is a well-known training-induced adaptation (Murias et al. 2016), might have also played a role (Temesi et al. 2017). As a result of training, it seems plausible to admit that every time there is an increment in power output, a steady state in $\dot{V}O_2$ will be more quickly attained (Murias et al. 2016). This would thus result in lower contribution of the anaerobic metabolism to obtain energy. Since anaerobic metabolism is associated to net accumulation of metabolites and impairment of muscle function, reducing its contribution to obtain energy likely results in less peripheral fatigue accumulation. Indeed, greater peripheral fatigue has been reported in subjects with slow $\dot{V}O_2$ kinetics (Temesi et al. 2017).

The similar total and peripheral fatigue (except for $Db_{100}$) in $POST_{REL}$ and PRE is likely related to the slightly higher intensity of $POST_{REL}$. The possible improved $\dot{V}O_2$ kinetics might have not been enough to counteract the responses typically seen in non-steady exercise such as recruitment of additional type-II fibers (Burnley et al. 2012) and disruption of intramuscular homeostasis (Jones et al. 2008). Thus, despite the improvement in performance, total and peripheral fatigue were not different between the two sessions.

## Neuromuscular fatigue at exhaustion

MVC was depressed to a similar extent in $POST_{ABS}$ and PRE. This is in accordance with O'Leary et al. (2017), despite the greater reduction in our study (–37–42 vs. − 16–22%), probably due to the immediate measurement of MVC with our innovative ergometer. The lack of differences in MVC reduction between $POST_{REL}$ and PRE is also in agreement with previous research (Zghal et al. 2015; O'Leary et al. 2017). Lower ($Db_{100}$) or similar (Pt and $Db_{10:100}$) peripheral fatigue were observed in $POST_{ABS}$ compared to PRE. Since this was accompanied by unchanged M-wave, training adaptations likely occurred beyond the sarcolemma and evidence on blood [La] may help to explain our peripheral fatigue results. It may be that the improved transport capacity of lactate/$H^+$ from the muscle to blood (Pilegaard et al.

2012) increased blood [La] (confirmed by the higher blood [La] observed in our study at EXH in $POST_{ABS}$) and possibly lowered muscle [La] and $H^+$. Indeed, reduced muscle [La] and higher blood [La] have been observed after training (Pilegaard et al. 2012). Thus, training might have resulted in decreased muscle metabolite concentration, which in turn blunted peripheral fatigue at EXH in $POST_{ABS}$. Therefore, peripheral fatigue was either lower (tendency for $Db_{100}$) or not different at EXH between $POST_{ABS}$ and PRE.

$Db_{100}$ and Pt tended to be less depressed in $POST_{REL}$ than PRE. As TTE also only tended to be prolonged in $POST_{REL}$, this suggests that training tended to attenuate peripheral fatigue at EXH for a slightly higher power output and exercise duration. In contrast with our findings, Zghal et al. (2015) and O'Leary et al. (2017) found greater peripheral fatigue at EXH in $POST_{REL}$. The authors speculated that III/IV fibers, which discharge as a result of mechanical stimulus and accumulation of metabolites, might have been upregulated such that their threshold level was increased (Sinoway 1996) and higher levels of metabolic disturbance in the muscle would be tolerated. The authors also suggested that there may be greater tolerance to the level of peripheral fatigue by the central nervous system. The contrasting results between these two studies and our study might be explained by factors such as different exercise training and testing. Indeed, Zghal et al. (2015) used single-limb exercise training and testing and O'Leary et al. (2017) used either CST or HIIT for training and constant-load cycling exercise for testing. Our findings are thus likely to be explained by other mechanisms than those suggested in the previous literature. The improved $VO_2$ kinetics (Murias et al. 2016) suggested before may help to explain why the tendency for longer TTE in $POST_{REL}$ was accompanied by lower peripheral fatigue at EXH in $POST_{REL}$ compared to PRE. The unchanged blood [La] in $POST_{REL}$ as opposed to the increased [La] in $POST_{ABS}$ might be related to exercise duration. Indeed, subjects performed about one more stage (i.e., 5 min) in $POST_{ABS}$, which resulted in one more increment in power output and could increase the contribution of the anaerobic metabolism due to greater ischemia, thus resulting in higher blood lactate in $POST_{ABS}$ vs. $POST_{REL}$. The fact that no differences were observed in RPE at EXH between the three sessions supports the idea that similar perceived exertion was attained regardless of the TTE and confirms this variable is of key importance (i.e., lower perceptual stress for a given power output after training) in exercise regulation (Marcora and Staiano 2010; O'Leary et al. 2017).

### Methodological considerations

Our results should be interpreted bearing in mind the following limitations. The fatigue assessment between the stages and at exhaustion lasted 35–40 s. Since the NMF evaluation comprised several voluntary and evoked contractions, it is possible that the evaluation itself might have resulted in some degree of fatigue. Thus, the fatigue might have been influenced, even if minimally, by the NMF evaluations. This question could be addressed in future studies by including a control group solely performing NMF evaluations, with no cycling exercise. We also acknowledge that the lack of a control group whereby subjects would not undergo any exercise intervention might limit the conclusions drawn upon our results. In addition, prescribing exercise based on the exercise intensity domains rather than a percentage of $VO_{2max}$ could have been more appropriate to further individualize the training loads. By doing this, we would ensure more similar ventilatory and metabolic responses between the subjects to each type of training (i.e., CST and HIIT). Furthermore, it is important to bear in mind that subjects who underwent our training intervention were healthy active men. Different outcomes could have been observed with different populations (i.e., women, older adults, patients, athletes, etc). Lastly, starting an intervention study with only 14 subjects is a risk due to the variability of the measures of electrical and magnetic stimulation and the difficulty to commit to 9 weeks of intense training. Indeed, a larger sample should have been included to account for possible drop outs.

## Conclusion

The present study described for the first time the effects of a combined endurance training on fatigue kinetics during and immediately after a fatigue test. We confirmed that at the same power output as in PRE, fatigue is attenuated during a fatigue test after training. However, our results also showed that training induces either unchanged or lower peripheral fatigue at EXH in $POST_{ABS}$ and $POST_{REL}$ when compared to PRE, which suggests that longer post-training TTE are not due to an upregulation of III/IV fibers and greater tolerance to peripheral fatigue as previously suggested. Further studies are needed to investigate improved transport capacity of lactate/$H^+$ from the working muscle to blood and faster $\dot{V}O_2$ kinetics as potential mechanisms affecting fatigue accumulation.

**Author contribution statement** TR, GYM, SJA, and JM conceived and designed the research. SJA, JM, MF, RJ, SJM, and KA conducted the experiment and analyzed data. JM wrote the manuscript. All authors read and approved the manuscript.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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ORIGINAL ARTICLE

# Effects of endurance cycling training on neuromuscular fatigue in healthy active men. Part II: Corticospinal excitability and voluntary activation

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## Abstract

This study investigated the effects of 9-week endurance cycling training on central fatigability and corticomotor excitability of the locomotor muscles. Fourteen healthy participants undertook three incremental fatiguing cycling tests to volitional exhaustion (EXH): (i) before training (PRE), (ii) after training at the same absolute power output as PRE ($POST_{ABS}$) and (iii) after training at the same percentage of $\dot{V}O_{2max}$ as PRE ($POST_{REL}$). At baseline (i.e. before cycling), every 5 min during cycling and immediately at EXH, a neuromuscular evaluation including a series of 5-s knee extensions at 100, 75 and 50% of maximal voluntary knee extension (MVC) was performed. During each contraction, transcranial magnetic and peripheral nerve stimuli were elicited to obtain motor evoked potential (MEP), silent period (SP) and compound muscle action potential (Mmax) and to calculate voluntary activation (VA). The MEP·$Mmax^{-1}$ ratio recorded from vastus lateralis at 100 and 50% MVC did not show any difference between conditions. At 75% MVC, MEP exhibited significantly lower values in $POST_{ABS}$ and $POST_{REL}$ compared to PRE at baseline ($P=0.022$ and $P=0.011$, respectively) as well as at 25% of time to EXH of PRE ($P=0.022$) for $POST_{REL}$. No adaptations, either at baseline or during cycling, were observed for VA and SPs. In conclusion, endurance training may result in some adaptations in the corticomotor responses when measured at rest or with low level of fatigue, yet these adaptations do not translate into attenuation of central fatigue at a similar cycling workload or at exhaustion.

**Keywords** Brain · Central adaptation · Electromyography · Locomotor muscles · Transcranial magnetic stimulation

## Abbreviations

| | |
|---|---|
| BF | Biceps femoris |
| CST | Constant-load submaximal training |
| Db100 | High-frequency doublet |
| EMG | Electromyography |
| EXH | Exhaustion |
| HIIT | High-intensity interval training |
| HR | Heart rate |
| MEP | Motor evoked potential |
| $MEP_{100}$, $MEP_{75}$, $MEP_{50}$ | MEP·$Mmax^{-1}$ ratio (i.e. MEP is expressed as a % of Mmax recorded at 100, 75 and 50% of MVC, respectively) |
| $SP_{100}$, $SP_{75}$, $SP_{50}$ | Silent period recorded at 100, 75 and 50% of MVC, respectively |
| MVC | Maximal voluntary contraction |
| NMF | Neuromuscular fatigue |
| PNS | Peripheral motor nerve stimulation |
| $POST_{ABS}$ | Fatigue session based on the same absolute power output as before training |
| $POST_{REL}$ | Fatigue session based on the same relative intensity as before training |
| PRE | Initial fatigue test |
| RMS | Root mean square |

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|---|---|
| $RMS_{100}{\cdot}Mmax_{100}{}^{-1}$, $RMS_{75}{\cdot}Mmax_{75}{}^{-1}$ $RMS_{50}{\cdot}Mmax_{50}{}^{-1}$ | Root mean square is expressed as a % of Mmax amplitude recorded at 100, 75 and 50% of MVC, respectively |
| RPE | Rate of perceived exertion |
| SIT | Superimposed twitch |
| SP | Silent period |
| TMS | Transcranial magnetic stimulation |
| TTE | Time to exhaustion |
| $VA_{PNS}$ | Voluntary activation (using peripheral nerve stimulation) |
| $VA_{TMS}$ | Voluntary activation (using transcranial magnetic stimulation) |
| VL | Vastus lateralis |
| $\dot{V}O_{2max}$ | Maximal oxygen uptake |
| $\dot{V}O_{2peak}$ | Peak $\dot{V}O2$ |
| $W_{max}$ | Maximal aerobic power output |

## Introduction

As discussed in Part I of this companion article, endurance training is an effective method to enhance cardiovascular fitness parameters such as maximal oxygen uptake ($\dot{V}O_{2max}$), lactate threshold and muscle oxidative capacity (Perot et al. 1991; Daussin et al. 2008; Murias et al. 2010a, b, 2011). Besides these adaptations, there is empirical evidence indicating that endurance training can change the plasticity of neuromuscular processes which determine the magnitude of central neural drive and excitability of the corticospinal pathway innervating the exercised muscles (Behrens et al. 2015; Zghal et al. 2015; O'Leary et al. 2017). Yet the neural changes induced by endurance training are clearly an under-investigated field.

A practical approach to study training adaptations in the neuromuscular system is to measure the adaptations of the processes at peripheral (at or distal to the neuromuscular junction) and central (proximal to the neuromuscular junction) levels, at baseline and then during exercise. Although the magnitude of force and power output is ultimately determined by the contractile machinery of the skeletal muscle (Taylor et al. 2016), it is critical to assess the central motor responses because modulation of corticospinal pathway efficiency in transmission of the motor cortical signals is a determining factor in the development of central drive (Taylor et al. 2006). Transcranial magnetic stimulation (TMS) of the cerebral cortex, in combination with electrical stimulation of the peripheral motor nerve (PNS), is a technique which is extensively used to investigate the responsiveness of the entire motor pathway innervating the locomotor muscles following acute exercise (Sidhu et al. 2009; Jubeau et al. 2014; Weavil et al. 2016) or different modes of training (Jensen et al. 2005; Carroll et al. 2009; Zghal et al. 2015). Regarding the neural changes, TMS is used to elicit both excitatory (motor evoked potential, MEP) and inhibitory (silent period, SP) responses in the corticomotor pathway, which are detectable from electromyography (EMG) signals at the level of muscle. TMS and PNS also evoke twitch responses in muscles which are used to quantify cortical ($VA_{TMS}$) and central ($VA_{PNS}$) voluntary activations, respectively (Todd et al. 2003, 2004).

Several studies have explored the influence of acute (single session) fatiguing cycling exercises on modulations of the corticospinal pathway driving the leg muscles (Sidhu et al. 2009, 2017; Goodall et al. 2012; Gruet et al. 2013; Weavil et al. 2016). Multiple lines of research are indicating that activation of fatigue-sensitive group III and IV muscle afferents could suppress the excitatory effect of increasing muscle activity during cycling exercise (Weavil et al. 2016; Sidhu et al. 2009, 2017). Considering that training may upregulate the firing threshold of group III and IV afferents (Sinoway 1996) and may enhance the ability of the central nervous system to tolerate a greater peripheral fatigue (Zghal et al. 2015; O'Leary et al. 2017), it is of interest to explore the potential adaptations that an endurance training can cause in neuromuscular fatigue development during a fatiguing cycling exercise.

Surprisingly, only a couple of studies have addressed this issue. Zghal et al. (2015) demonstrated that 8 weeks of low-force isometric and concentric knee extensions significantly delayed central fatigability quantified by $VA_{TMS}$ and $VA_{PNS}$ while did not change the excitability of the corticospinal pathway. O'Leary and colleagues (2017) investigated the influence of 6-week high-intensity interval or moderate-intensity continuous cycling protocols and quantified the post-training adaptations during both absolute (based on the pre-training $\dot{V}O_{2max}$, $POST_{ABS}$) and relative cycling intensities (based on post-training $\dot{V}O_{2max}$, $POST_{REL}$). These investigators found an attenuation of the exercise-induced reduction in $VA_{PNS}$ at $POST_{REL}$ session compared to PRE but did not find any adaptation in corticospinal excitability (monitored in the size of MEP) and inhibition (monitored in the duration of SP) or in the $VA_{TMS}$. A limitation in the O'Leary et al. (2017) study is that participants needed to move from the cycle ergometer to the isometric chair for the post-exercise assessments. This process results in a time delay in neuromuscular assessment, which could possibly have influenced the magnitude of corticospinal response (Mira et al. 2017). Furthermore, due to this time delay, investigators were not able to perform neuromuscular assessments within the time course of cycling exercise. To resolve these issues, we have developed an instrumented cycle ergometer that allows the execution of isometric knee

extensions within a second from interception of cycling task (Doyle-Baker et al. 2018).

This article, which is Part II of a companion paper, focuses on the effects of 9 weeks of endurance training on (i) etiology of central fatigue development (i.e. $VA_{TMS}$ and $VA_{PNS}$) during cycling and at volitional exhaustion (EXH) and (ii) plasticity and adaptations of the corticospinal pathway excitability and inhibition during fatiguing cycling and at EXH. It was hypothesized that (i) training would result in an attenuation of cycling-induced reduction in $VA_{TMS}$ and $VA_{PNS}$ and an increase in corticospinal excitability when measurements are performed at similar workloads during cycling at PRE vs. $POST_{ABS}$, (ii) training would also result in an attenuation, although of less magnitude, of cycling-induced reduction in $VA_{TMS}$ and $VA_{PNS}$ and an increase in corticospinal excitability when measurements are performed at EXH following PRE vs. $POST_{ABS}$, and (iii) no change would be observed in MEP, $VA_{TMS}$ and $VA_{PNS}$ when comparing PRE vs. $POST_{REL}$, i.e. for a similar exercise duration and at EXH.

## Methods

The current experiment included cardiovascular fitness measurements quantified during maximal incremental tests (i.e. $\dot{V}O_{2max}$). In separate session, the experiment also included measurements of neuromuscular function assessments at rest and during a fatiguing cycling exercise. The peripheral (i.e. evoked muscle contractile property and muscle membrane excitability) and central (i.e. corticospinal excitability and inhibition, EMG, $VA_{PNS}$ and $VA_{TMS}$) fatigue indices were assessed during incremental cycling tests. The current article (Part II) focuses on central adaptations; thus, the experimental design and procedure of measurements associated with central fatigue and corticospinal excitability will be presented in more detail. More information regarding other measurements (including aerobic fitness and peripheral adaptations) can be found in the companion article (Part I).

## Participants

The same group of individuals studied in the companion article (Part I) including fourteen recreationally active (i.e. $3 \pm 1$ sessions of exercise per week, each lasting $54 \pm 26$ min) participants volunteered to participate in this study. After having been informed of the experimental procedures and possible risks, all participants completed the TMS safety checklist (Rossi et al. 2011). Two participants did not undergo TMS because both had the history of concussion. Thus, twelve male participants (mean ± SD, $25 \pm 7$ years, $179 \pm 11$ cm, $76 \pm 11$ kg) completed neuromuscular evaluations including TMS. $VA_{PNS}$ was computed for all fourteen participants. Participants were instructed to refrain from rigorous physical activity 2 days before testing sessions and drinking alcohol and caffeine on days of experiment. The procedures were conducted in accordance with declaration of Helsinki and approved by the Health Research Ethics Authority of University of Calgary (#REB15-2566).

## Experimental design

All testing sessions were performed on a customized electromagnetically braked recumbent cycle ergometer (Doyle-Baker et al. 2018) at a similar time of the day. Prior to training, participants completed a familiarization session that comprised of a maximal incremental test to determine the exercise intensity of the fatigue test and familiarization to NMF evaluations. Pre-training testing session (PRE) consisted of a fatigue test to EXH with NMF measurements performed before, during and after an incremental cycling exercise (see NMF evaluation below). Then, subjects initiated a 9-week training protocol followed by 1 week of tapering. Training comprised of a combination of high-intensity interval training (HIIT) and submaximal constant-load training (CST) sessions. At least 48 h (and no more than 72 h) after the last training session, a new maximal incremental test was performed to determine peak $\dot{V}O_2$ ($\dot{V}O_{2peak}$) and maximal aerobic power output ($W_{max}$). Additionally, two fatigue tests (or only one test in two subjects who did not improve their $W_{max}$ by more than 5 W, i.e. not enough to justify performing a $POST_{REL}$ session) were completed in random order at least 48 h after the incremental test to EXH and interspersed with at least 72 h: (i) $POST_{ABS}$, performed at the same absolute power output as PRE and (ii) $POST_{REL}$, performed at the same percentage of $W_{max}$ determined at post-training.

## Procedures

### Incremental fatigue test and NMF evaluation

Each NMF testing session (i.e. PRE, $POST_{ABS}$ and $POST_{REL}$) comprised of 5-min stages with power output increments at the start of each stage until the subject reached EXH. At baseline, at the end of each stage and at EXH, pedals were locked allowing NMF evaluation on the cycle ergometer. After each NMF evaluation (total duration: ~40 s) the pedals were unlocked to allow the subject to resume cycling. In PRE and $POST_{ABS}$, power output was set at 55% of pre-training $W_{max}$ and then increased by 5% of pre-training $W_{max}$ at the start of every stage. The same approach was used during $POST_{REL}$, except that the initial power output and 5% increment were set based on the post-training $W_{max}$. Every NMF

evaluation included one 5-s MVC with one superimposed high-frequency doublet (100 Hz) delivered on the force plateau, followed by three stimuli (100-Hz and 10-Hz paired pulses and single pulse) on the relaxed muscle separated by 3 s. Two to three seconds after the single pulse, subjects were asked to perform another 5-s MVC followed by a voluntary reduction in force output to match 75% and then 50% MVC of the preceding 100% contraction with no recovery between force levels (Mira et al. 2017). Single-pulse TMS and PNS were delivered at MVC, 75 and 50% MVC contractions with ~2 s TMS–PNS interstimulus intervals.

### Electromyography (EMG)

At the beginning of each NMF testing session, participants were equipped with electromyography (EMG) and stimulating electrodes. Pairs of self-adhesive Ag/AgCl surface electrodes (Kendall MediTrace foam electrodes, MA) were located on the muscle belly of the right vastus lateralis (VL) and biceps femoris (BF) (proximity 20 mm center to center). Before the placement of electrodes, the area of skin was shaved, abraded with sandpaper and cleansed with alcohol swab to decrease skin resistance. The ground electrode was placed on the patella bone. An inter-electrode impedance of $<5$ kΩ was obtained prior to recording to ensure an adequate signal-to-noise ratio. All EMG signals were digitized at a sampling rate of 2000 Hz by PowerLab system (16/30-ML880/P, ADInstruments, Bella Vista, Australia) and amplified with an octal bio-amplifier (ML138, ADInstruments). EMG signals were bandpass filtered (5–500 Hz) and all data were analyzed offline using Labchart 8 software (ADInstruments).

### Transcranial magnetic stimulation (TMS)

Single TMS pulses were manually delivered to the left motor cortex via a concave double-cone coil (110-mm, maximum output of 1.4 T) connected to a magnetic stimulator (Magstim 200$^2$, The Magstim Company Ltd., Whitland, UK). The optimal location of the coil was searched on the scalp for every centimeter from 2 cm anterior and 1 cm to the left of the vertex (i.e. the midpoint from nasal–inion and tragi). Single pulses at 50% of TMS maximum stimulator output were delivered during brief (2–3 s) knee extension contractions at 20% MVC and the location which evoked the highest MEP amplitude for VL (with a small MEP for BF) was marked on a latex swim cap worn by participants (Temesi et al. 2014). The optimal intensity of the magnetic stimulator was determined via calculating the VL, BF and SIT stimulus–response curve (Temesi et al. 2014). Participants performed brief contractions at 20% MVC and TMS was delivered at 20, 30, 40, 50, 60, 70 and 80% of the maximal stimulator output in random order. Four contractions were performed at each intensity with a 15-s interval between contractions. The intensity which showed the highest SIT (with largest MEP for VL and smallest MEP for BF) was chosen as TMS intensity for the rest of the testing session. The stimulating intensity was $65 \pm 10$, $67 \pm 7$ and $65 \pm 11$% of the maximal stimulator output at PRE, $POST_{ABS}$ and $POST_{REL}$, respectively.

### Femoral nerve stimulation

Percutaneous electrical stimulation was delivered to the femoral nerve via a cathode electrode (Kendall MediTrace foam electrodes, MA) secured with tape in the inguinal triangle. The anode, a $50 \times 90$-mm rectangular electrode (Durastick Plus, DJO Global, Vista, CA), was placed in the gluteal fold. A constant current stimulator (DS7A, Digitimer, Welwyn Garden City, Hertfordshire, UK) was used to deliver a square-wave stimulus of 1-ms duration. The supramaximal stimulation (i.e. 130% of optimal intensity) was calculated and held constant throughout the protocol. The stimulating intensity was $156 \pm 40$, $132 \pm 55$ and $112 \pm 45$ mA in PRE, $POST_{ABS}$ and $POST_{REL}$, respectively.

### Training

Subjects completed 26 training sessions over a period of 9 weeks (8 training weeks and 1 tapering week). In the first 3 weeks, two CST and one HIIT sessions were performed per week. From week 4 to week 8, training comprised of one CST session and two HIIT sessions. In week 9, subjects completed a tapering period that consisted of only two low-volume HIIT sessions. The power output of the training sessions was initially set as $\%W_{max}$, which was associated with a given heart rate (HR) and rate of perceived exertion (RPE). In CST sessions, HR was set at 65% $W_{max}$ and RPE at 7. In HIIT sessions, HR was set at 90–100% $W_{max}$ and RPE should be 10 by the last 2 work intervals. Since training adaptations would reduce HR and RPE responses to a cycling exercise with a constant power output, the investigators recorded these two parameters during each session. If HR and/or RPE were falling outside of the target intervals ($\pm 5$ bpm for HR and $\pm 1$ for RPE), power output was readjusted.

### Data analysis

The MEP and compound muscle action potentials (Mmax) areas recorded during voluntary contractions were measured from the initial deflection of EMG signal from baseline to the second crossing of the horizontal axis. The MEP responses were normalized to the subsequent Mmax recorded during the same contraction (100, 75 or 50% of MVC) to obtain $MEP \cdot Mmax^{-1}$ ratio (i.e. $MEP_{100}$, $MEP_{75}$ and $MEP_{50}$ expressed in % of Mmax). The duration of the

silent period (SP) was assessed during 100, 75 and 50% of MVC contractions ($SP_{100}$, $SP_{75}$, $SP_{50}$) as the interval from the MEP stimulus artifact to the return of the continuous EMG by visual inspection (Schnitzler and Benecke 1994) by an experienced investigator from our lab (Jubeau et al. 2014). The background EMG (root mean square; RMS) of the VL was quantified over 500 ms duration prior to the point of each stimulus (TMS and PNS) at each target force. To evaluate the central drive during contractions, the RMS values were normalized to the peak-to-peak amplitude of Mmax recorded at each contraction to obtain RMS·Mmax$^{-1}$ ratio (i.e. $RMS_{100}{\cdot}Mmax_{100}{}^{-1}$, $RMS_{75}{\cdot}Mmax_{75}{}^{-1}$, $RMS_{50}{\cdot}Mmax_{50}{}^{-1}$).

$VA_{PNS}$ was calculated with the twitch interpolation technique by quantifying the evoked responses to electrical stimulation of the femoral nerve (in this study, the 100-Hz doublet) during a MVC and 2 s after the MVC on relaxed muscle. The modified version of the $VA_{PNS}$ formula recommended by Strojnik and Komi (1998) was used as in some cases superimposed doublet (Db100) was applied while subject was not able to maintain a perfect MVC plateau. The formula was as follows:

$$\mathrm{VA}\ (\%)\ =\ 100 - D \times \left(\mathrm{Fb}/F_{\mathrm{MAX}}\right)/F_{\mathrm{PT}} \times 100,$$

where $D$ is the difference between the voluntary force output just before the superimposed Db100 (i.e. Fb) and the maximum force evoked by $Db_{100}$. $F_{MAX}$ is the MVC force output and $F_{PT}$ is the potentiated twitch ($Db_{100}$) force evoked at rest.

Given that $VA_{PNS}$ can be modulated by changes in functional efficiency of motor axons at supraspinal or spinal level, TMS has been used to assess the cortical voluntary activation (Todd et al. 2003, 2004). We attempted to quantify $VA_{TMS}$ based on the supraspinal interpolated twitch technique. Thus, the amplitude of estimated resting twitch was calculated based on the size of TMS-evoked superimposed twitches at MVC, 75 and 50% MVCs. Although the size of the voluntary force output and the evoked twitches during the contractions demonstrated a linear regression line at baseline ($r^2 > 0.9$), as participants approached the volitional exhaustion, more non-linear regressions were found. The reason for this issue is not clear. It could be speculated that since neuromuscular assessments were performed immediately after the fatiguing task, unlike delayed assessments in all previous experiments with whole-body dynamic exercises, participants might have had poor motor control during fatigue state, preventing them from showing acceptable regressions. As a result, the $VA_{TMS}$ data were excluded from the results. Nonetheless, the size of TMS-induced superimposed twitches at MVC was calculated and compared between different time points and testing sessions (Gandevia et al. 1996; Hunter et al. 2008).

Since the number of completed stages during the fatigue tests was different between subjects and from PRE to POST testing sessions, data were interpolated as a function of time to EXH of PRE ($TTE_{PRE}$). For instance, if subject A's $TTE_{PRE}$ was 22 min, all data of the three fatigue tests would be expressed as a percentage of this duration (see Part I). Accordingly, the duration of cycling for the $POST_{ABS}$ and $POST_{REL}$ sessions was converted to a percentage of $TTE_{PRE}$ and data were analyzed at fixed time points (i.e. baseline, 25, 50, 75, 100% of $TTE_{PRE}$). The total duration of cycling to EXH for the three fatigue tests was also considered for analysis.

### Statistical analysis

Data are reported as mean ± standard deviation (SD). Shapiro–Wilk test was performed to check if data were normally distributed and homogeneity of variances was determined with the Levene's test. If sphericity was violated, Greenhouse–Geisser corrections were applied. To determine the effect of the endurance training on etiology of central fatigue and corticospinal measures, two separate two-way (condition × time) ANOVAs with repeated measures were performed on two conditions (PRE vs. $POST_{ABS}$ or PRE *vs*. $POST_{REL}$) and five time points: baseline, 25, 50, 75, and 100% of $TTE_{PRE}$. Where the ANOVA revealed significant main effects or interactions, data were further explored using pairwise comparisons with a Bonferroni correction. To compare fatigue at EXH, two *t* tests with dependent samples were performed (PRE vs. $POST_{ABS}$ or PRE vs. $POST_{REL}$) on the percentage change from baseline to EXH. For all statistical analyses, an alpha level of 0.05 was used as the cutoff for significance.

## Results

From the 12 participants who undertook TMS, 2 participants did not improve their $W_{max}$ enough to justify performing a $POST_{REL}$ session so the TMS parameters are reported for 10 participants.

### Voluntary activation and EMG indices

$VA_{PNS}$ demonstrated a significant time effect for PRE vs. $POST_{ABS}$ ($F_{4,44} = 86.62$, $P < 0.001$) and PRE vs. $POST_{REL}$ ($F_{4,36} = 6.31$, $P = 0.011$) (Table 1). This measure showed smaller values at 75 and 100% of $TTE_{PRE}$ compared to baseline (all $P = 0.05$). There was no difference between testing sessions during cycling or at EXH. In addition, the amplitude of superimposed twitches evoked by TMS at MVCs did not demonstrate any significant time, condition or interaction effect (Fig. 1).

In all testing sessions, $RMS_{100}{\cdot}Mmax_{100}{}^{-1}$ exhibited lower values at 100% of $TTE_{PRE}$ compared to baseline

**Table 1** Group data (mean and SD) for variables measured during the three testing sessions

| | Baseline | T25 | T50 | T75 | T100 | EXH |
|---|---|---|---|---|---|---|
| VA PNS (%)*† | | | | | | |
| PRE | 94.9 (3.9) | 95.7 (2.4) | 94.0 (5.1) | 92.8 (4.7) | 92.3 (5.4) | – |
| $POST_{ABS}$ | 95.6 (3.3) | 93.7 (5.5) | 92.7 (5.9) | 90.8 (5.9) | 89.3 (6.2) | 86.1 (9.1) |
| $POST_{REL}$ | 95.6 (3.1) | 92.2 (5.5) | 91.7 (5.6) | 89.9 (5.1) | 88.0 (7.0) | 88.5 (7.9) |
| $RMS_{100}{\cdot}Mmax_{100}{}^{-1}$ $ | | | | | | |
| PRE | 0.059 (0.027) | 0.057 (0.020) | 0.051 (0.016) | 0.046 (0.015) | 0.042 (0.023) | – |
| $POST_{ABS}$ | 0.052 (0.027) | 0.056 (0.033) | 0.050 (0.021) | 0.049 (0.023) | 0.047 (0.026) | 0.044 (0.013) |
| $POST_{REL}$ | 0.046 (0.015) | 0.047 (0.020) | 0.042 (0.010) | 0.044 (0.010) | 0.039 (0.013) | 0.037 (0.011) |
| $RMS_{75}{\cdot}Mmax_{75}{}^{-1}$ | | | | | | |
| PRE | 0.031 (0.011) | 0.038 (0.014) | 0.037 (0.013) | 0.036 (0.014) | 0.035 (0.017) | – |
| $POST_{ABS}$ | 0.032 (0.010) | 0.033 (0.008) | 0.033 (0.007) | 0.034 (0.007) | 0.034 (0.007) | 0.034 (0.010) |
| $POST_{REL}$ | 0.033 (0.010) | 0.034 (0.010) | 0.036 (0.008) | 0.040 (0.010) | 0.035 (0.009) | 0.036 (0.008) |
| $RMS_{50}{\cdot}Mmax_{50}{}^{-1}$ | | | | | | |
| PRE | 0.020 (0.007) | 0.024 (0.007) | 0.023 (0.008) | 0.024 (0.008) | 0.018 (0.008) | – |
| $POST_{ABS}$ | 0.021 (0.004) | 0.024 (0.008) | 0.025 (0.006) | 0.026 (0.008) | 0.022 (0.007) | 0.027 (0.014) |
| $POST_{REL}$ | 0.020 (0.006) | 0.024 (0.008) | 0.025 (0.011) | 0.023 (0.009) | 0.023 (0.010) | 0.020 (0.009) |
| $SP_{100}$ (ms) | | | | | | |
| PRE | 295 (70) | 287 (74) | 290 (84) | 287 (79) | 282 (86) | – |
| $POST_{ABS}$ | 291 (81) | 293 (69) | 293 (70) | 278 (70) | 283 (73) | 288 (73) |
| $POST_{REL}$ | 281 (89) | 262 (97) | 256 (94) | 258 (81) | 267 (83) | 265 (89) |
| $SP_{75}$ (ms) | | | | | | |
| PRE | 346 (84) | 322 (83) | 311 (82) | 302 (82) | 307 (71) | – |
| $POST_{ABS}$ | 343 (88) | 344 (84) | 333 (77) | 323 (75) | 316 (72) | 320 (81) |
| $POST_{REL}$ | 322 (94) | 306 (95) | 291 (98) | 292 (93) | 292 (86) | 295 (82) |
| $SP_{50}$ (ms) | | | | | | |
| PRE | 358 (92) | 336 (76) | 327 (82) | 310 (78) | 317 (85) | – |
| $POST_{ABS}$ | 376 (70) | 347 (82) | 346 (81) | 331 (73) | 326 (90) | 327 (54) |
| $POST_{REL}$ | 337 (82) | 310 (90) | 304 (98) | 323 (94) | 310 (96) | 310 (43) |

Voluntary activation quantified using peripheral nerve stimulation ($VA_{PNS}$) showed a significant time effect with smaller values at 75 (*) and 100% (†) of $TTE_{PRE}$ compared to baseline. The VL root mean square EMG normalized to Mmax amplitude was quantified for each contraction (i.e. $RMS_{100}{\cdot}Mmax_{100}{}^{-1}$, $RMS_{75}{\cdot}Mmax_{75}{}^{-1}$ and $RMS_{50}{\cdot}Mmax_{50}{}^{-1}$). The $RMS_{100}{\cdot}Mmax_{100}{}^{-1}$ demonstrated a significant time effect with lower values at 100% of $TTE_{PRE}$ ($) of PRE compared to baseline ($P<0.05$). No significant difference was observed for silent period (SP) measured at different contraction intensities and conditions

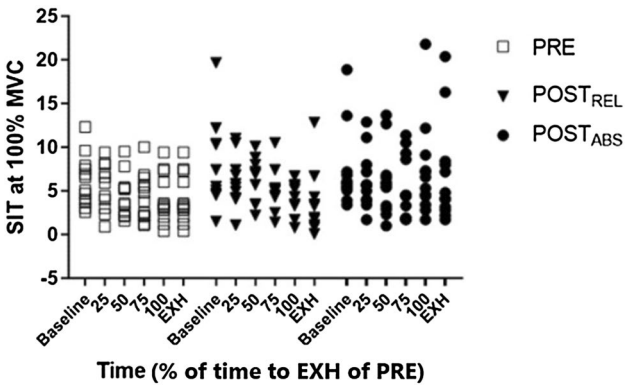


**Fig. 1** Individual TMS-induced superimposed twitches (SIT) at MVC for the three testing sessions. There was no difference between testing sessions at different time points of cycling or at EXH

($P<0.05$). $RMS_{100}{\cdot}Mmax_{100}{}^{-1}$ of the VL demonstrated a significant drop across the cycling task (time effect: $F_{4,36}=4.98$, $P=0.003$). There was no difference between the three sessions either throughout cycling or at EXH (Table 1). No significant main or interaction effect was observed for $RMS_{75}{\cdot}Mmax_{75}{}^{-1}$ and $RMS_{50}{\cdot}Mmax_{50}{}^{-1}$.

## Corticospinal excitability

Normalized $MEP_{100}$ and $MEP_{50}$ did not show any difference in $POST_{ABS}$ and $POST_{REL}$ compared to PRE either during cycling or at EXH (Fig. 2a). $MEP_{75}$ showed a significant interaction effect between PRE vs. $POST_{ABS}$ ($F_{4,44}=4.65$, $P=0.003$) as well as PRE vs. $POST_{REL}$ ($F_{4,36}=4.98$, $P=0.003$). Pairwise comparisons indicated that at baseline, this parameter was significantly

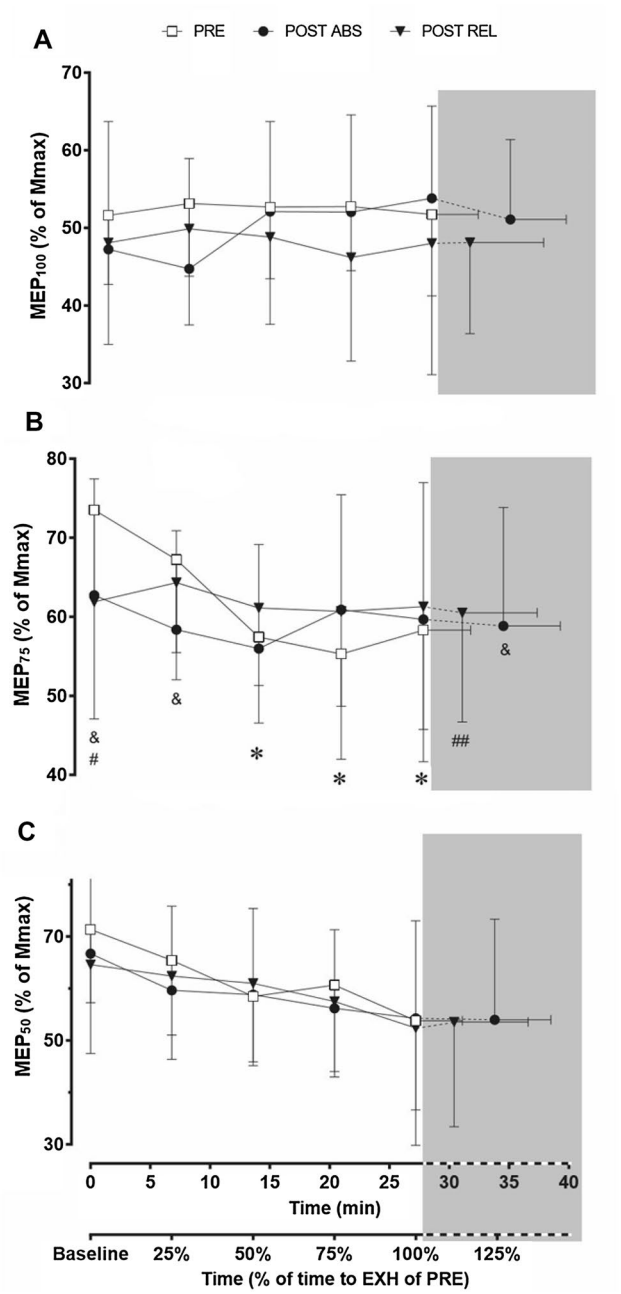


**Fig. 2** MEP·Mmax$^{-1}$ ratio recorded from VL at MVC ($MEP_{100}$, **A**), 75% ($MEP_{75}$, **B**) and 50% MVC ($MEP_{50}$, **C**). There was no difference between testing sessions during cycling or at EXH for $MEP_{100}$ and $MEP_{50}$. *&* Denotes significant difference between PRE and $POST_{ABS}$. *#* Denotes significant difference between PRE and $POST_{REL}$. * Denotes significantly smaller value than baseline (only for PRE)

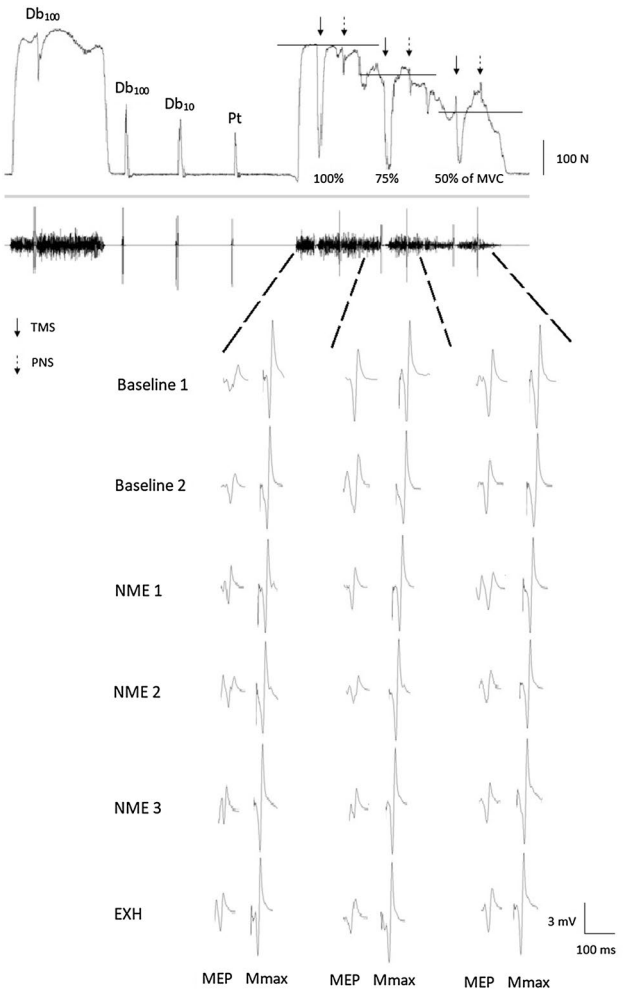


**Fig. 3** A single-subject raw data traces of force output and EMG signals at 100, 75, and 50% MVC recorded at PRE. The transcranial magnetic stimulation (TMS) and electrical stimulation of the peripheral motor nerve (PNS) were used to evoke motor evoked potential (MEP) and compound muscle action potential (Mmax) in the vastus lateralis muscle. The measurements were performed at two baselines, during three neuromuscular evaluations (NME 1–3) and at exhaustion (EXH)

lower in $POST_{ABS}$ ($p = 0.022$) and $POST_{REL}$ ($P = 0.011$) compared to the PRE (Fig. 2b). Furthermore, $MEP_{75}$ exhibited lower value at $POST_{ABS}$ than PRE at 25% of $TTE_{PRE}$ ($P = 0.022$). $MEP_{75}$ declined significantly from baseline to 50% of $TTE_{PRE}$ ($P = 0.037$) at PRE and then showed a steady-state pattern until EXH (Fig. 3). Accordingly, $MEP_{75}$ at EXH demonstrated a significantly greater decrease from baseline at PRE compared to $POST_{ABS}$ ($P = 0.040$) and $POST_{REL}$ ($P = 0.006$). It is worth noting that the VL Mmax area demonstrated no difference between PRE vs. $POST_{ABS}$ and $POST_{REL}$ at any contraction intensity (100, 75 and 50% MVC) either during cycling or at EXH.

The data presented in Table 2 indicate that BF MEP area (mV.s) declined across the cycling task (time effect: $F_{4,36} = 3.91$, $P = 0.010$). No significant condition or interaction effect was observed between PRE, $POST_{ABS}$ and $POST_{REL}$ sessions at different contraction intensities.

**Table 2** Group data (mean and SD) for biceps femoris (BF) MEP area (mV.s) recorded during the three testing sessions

| | Baseline | T25 | T50 | T75 | T100 | EXH |
|---|---|---|---|---|---|---|
| $MEP_{100}$ (μV.s)*†$ | | | | | | |
| PRE | 11.2 (9.6) | 11.4 (8.8) | 11.8 (7.7) | 10.4 (7.2) | 8.8 (8.1) | – |
| $POST_{ABS}$ | 12.4 (13.1) | 12.3 (12.5) | 9.6 (6.1) | 11.2 (10.2) | 8.8 (5.0) | 8.7 (5.1) |
| $POST_{REL}$ | 13.3 (8.7) | 12.5 (6.5) | 8.5 (6.8) | 10.6 (6.8) | 5.4 (5.7) | 5.7 (6.3) |
| $MEP_{75}$ (μV.s)*†$ | | | | | | |
| PRE | 9.3 (7.6) | 9.4 (8.6) | 8.3 (6.7) | 8.2 (7.3) | 7.2 (5.8) | – |
| $POST_{ABS}$ | 11.3 (13.4) | 6.6 (5.4) | 7.4 (5.6) | 7.5 (5.7) | 6.6 (5.2) | 5.5 (4.2) |
| $POST_{REL}$ | 9.2 (5.5) | 5.8 (4.1) | 5.9 (5.1) | 5.9 (5.2) | 5.2 (6.5) | 4.1 (5.1) |
| $MEP_{50}$ (μV.s)*†$ | | | | | | |
| PRE | 8.7 (7.1) | 7.4 (6.6) | 7.2 (6.7) | 7.9 (7.4) | 7.8 (6.2) | – |
| $POST_{ABS}$ | 9.5 (11.5) | 6.2 (4.3) | 6.4 (5.5) | 5.6 (4.2) | 6.1 (5.6) | 4.6 (4.4) |
| $POST_{REL}$ | 8.3 (5.6) | 4.4 (3.7) | 5.3 (5.5) | 4.8 (5.9) | 5.8 (5.8) | 4.7 (3.9) |

BF $MEP_{100}$, $MEP_{75}$ and $MEP_{50}$ showed a significant time effect with smaller values at 75 (*) and 100% (†) of $TTE_{PRE}$ as well as at task failure (EXH) ($) compared to baseline. No significant difference was observed between PRE and $POST_{ABS}$ and $POST_{REL}$ sessions

### Silent periods

The duration of SP recorded from VL at 100, 75 and 50% MVC did not show any time, condition or interaction effects. There was also no difference between the three sessions at EXH (Table 1).

## Discussion

We investigated the influence of a combination of HIIT and CST endurance training protocol on the time course of central fatigue development and adaptations of the corticospinal excitability and inhibition during a fatiguing cycling task to EXH. The main findings were that (i) the VL corticospinal excitability, at 75% MVC only, demonstrated significantly lower values post-training at baseline and low levels of fatigue (i.e. at 25% of $TTE_{PRE}$), (ii) there was no effect of training on the corticospinal inhibition (i.e. no change in SP) either at rest or during exercise and (iii) $RMS_{100}{\cdot}Mmax_{100}{}^{-1}$and $VA_{PNS}$ were reduced with fatigue yet no adaptation due to training was evidenced. These findings suggest that although 9 weeks of HIIT and CST cycling training may result in some adaptations in corticospinal responses in healthy individuals particularly with no or low levels of fatigue, these adaptations do not translate into facilitation of corticospinal excitability when fatigue occurs or attenuation of central fatigue at a similar workload or at EXH.

### Central fatigability

Measurements of VA and EMG activity normalized to Mmax ($RMS_{100}{\cdot}Mmax_{100}{}^{-1}$) are conventional tools to explore the contribution of central fatigue in the loss of voluntary force output during locomotion exercise. There are only two training studies that have measured the influence of endurance training on changes in central fatigability. In line with our results that only showed reduction in $VA_{PNS}$ across cycling task without any adaptations from pre- to post-training sessions, O'Leary et al. (2017) demonstrated that endurance training did not significantly alter the amplitude of $VA_{PNS}$ and $VA_{TMS}$ reductions from before to after a cycling task in $POST_{ABS}$. They found less central fatigue ($VA_{PNS}$) measure at EXH at $POST_{REL}$ session for the group that undertook HIIT training. Zghal et al. (2015) also found that central fatigue was attenuated when the post-training fatiguing task was performed at similar duration and intensity compared to the pre-training. These investigators attributed the results to the potential reduction in inhibitory feedbacks originated from activation of group III and IV afferents located in exercising muscles since a lower peripheral fatigue was observed at post-training. We also found an attenuation in peripheral fatigue at $POST_{ABS}$ compared to PRE when measurements were performed at the similar power output (see the companion article); however, surprisingly our participants did not show any adaptation in $VA_{PNS}$ or $RMS_{100}{\cdot}Mmax_{100}{}^{-1}$ from pre- to post-training sessions. Similarly, no adaptation was observed in the size of SITs induced by TMS during MVCs. The reason for this discrepancy in findings is unclear and we can only speculate about differences in training (concentric and isometric knee extensions in Zghal et al. (2015), HIIT or CST cycling in O'Leary et al. (2017) vs. combination of HIIT and CST in our study) or assessment protocols. The rationale behind using both HIIT and CST training in our experiment resided in the fact that while the CST had been shown to improve both central and peripheral components of the aerobic fitness (Jones and Carter 2000; Gunnarsson et al. 2013), the HIIT sessions had been demonstrated to provide further adaptations in terms

of muscle buffer capacity (Edge et al. 2006) and $VO_2$max (Laursen and Jenkins 2002; Warburton et al. 2004). The participants did not have the experience of regular endurance exercise, thus starting off the training protocol with CST sessions and gradually introducing the HIIT sessions could be an effective approach to familiarize subjects with cycling and simultaneously ensuring training adaptations. Regarding the assessment protocol, it is worth noting that our investigation is the first endurance training study that has quantified neuromuscular fatigue during cycling and at EHX within a second from cessation of the task (Doyle-Baker et al. 2018).

## Corticospinal excitability and inhibition

Previous experiments that measured the influence of endurance training on corticospinal excitability of the locomotor muscles did not find any adaptations following training. Zghal et al. (2015) reported no adaptation following 8 weeks of isometric and concentric knee extensions and O'Leary et al. (2017) found similar results after 6 weeks of high-intensity interval or moderate-intensity continuous cycling protocols. Contrary to these findings, the results in our study indicate that the magnitude and pattern of change in corticospinal adaptations following endurance training may vary based on the intensity of contractions performed during neuromuscular assessments. More specifically, although the $MEP_{100}$ and $MEP_{50}$ did not demonstrated any adaptation from pre- to post-training sessions, the $MEP_{75}$ demonstrated significantly lower values at baseline for both post-training sessions compared to PRE (Fig. 2). Considering that at baseline the corticospinal measures might not be affected by any reflex response induced by the fatiguing cycling task, the reduced $MEP_{75}$ observed at both $POST_{ABS}$ and $POST_{REL}$ compared to PRE could be an indication of potential long-term corticospinal plasticity to endurance training. The reason for this adaptation is unclear; however, it is worth noting that a reduction in corticomotor excitability has been observed following strength training. For instance, in two separate studies, Carroll et al. (2002, 2009) demonstrated that 4 weeks of resistance training of first dorsal interosseous and extensor carpi radialis muscles reduced the MEP amplitudes recorded from these muscles during submaximal contractions. Falvo et al. (2010) also demonstrated that 3 weeks of unilateral leg extensions could reduce movement-related cortical potentials during submaximal contractions. These authors suggested that lower level of neuronal excitability was required to complete submaximal leg extension following resistance training. These observations were interpreted as potential enhancements in movement efficiency and specificity of the brain activity during execution of submaximal contractions (Falvo et al. 2010). We can only speculate why we failed to observe a reduction in $MEP_{100}$ and $MEP_{50}$. The lack of changes at MVC could be attributed to the necessity of mobilizing all mental effort (and consequently generating motor cortical output) to perform the maximal contractions (Nybo and Secher 2004). On the contrary, the intensity of contractions at 50% of MVC might be quite low to necessitate any modulation in corticospinal excitability from pre- to post-training trials.

Our analysis further indicated that the $MEP_{75}$ gradually declined during the first half of cycling test at PRE (from baseline to 50% of $TTE_{PRE}$) and then showed a steady-state pattern until volitional EXH (Fig. 3). For the $POST_{ABS}$ and $POST_{REL}$ sessions, a plateau from baseline to EXH was observed across cycling time points (Fig. 2). A potential explanation for the reduction in corticospinal excitability at PRE could be the inhibitory role that group III and IV afferents might have played, inducing a disfacilitation of the corticomotor excitability during the fatiguing cycling task. Weavil et al. (2016) and Sidhu et al. (2017) suggested that activation of group III and IV muscle afferents could suppress/disfacilitate the excitability of the corticomotor pathway during a cycling task to EXH, although there are still several lines of evidence indicating that the corticospinal excitability increases after fatiguing tasks (Gruet et al. 2013). The reduction in MEP size has also been reported when pain was induced artificially by injecting hypertonic saline to increase the activity of group III and IV afferents during single-joint contractions (Le Pera et al. 2001; Svensson et al. 2003). Therefore, it could be speculated that, while accumulation of fatigue metabolites could suppress the corticomotor pathway excitability at PRE, reduction in discharge frequency of group III and IV afferents could have prevented the reduction of central excitability across the cycling task at post-training sessions. Indeed, participants in the present study demonstrated less peripheral fatigue at EXH following endurance training which was attributed to improvement in transport capacity of lactate/$H^+$ from the muscle to blood (for more details, see Part I).

In the present study, the corticospinal inhibition, quantified by assessing the SP duration, did not show any chronic adaptation from pre- to post-training sessions. This result is partially in concert with those of Zghal et al. (2015) who did not find any adaptation in the corticomotor inhibition following endurance training when SP was measured following sustained contraction until EXH. These investigators, however, found a significant reduction in duration of SP when comparing exercises of similar duration (i.e. isotime). They suggested that endurance training could have increased the fatigue resistance of muscle fibers and consequently reduced inhibitory feedback from the III and IV afferent fibers to the central nervous system. Divergence of their results from those of the present study might have been due to differences in the experimental protocols to induce fatigue during neuromuscular evaluations (i.e. sustained isometric contraction at 15% of MVC vs. cycling). On the other hand, although the

SP duration often increases with the development of fatigue induced by sustained isometric contractions, this measure does not change during whole-body exercises (Goodall et al. 2012; Jubeau et al. 2014; Sidhu et al. 2009, 2017). Therefore, although our findings suggest the idea that short-term cycling training may not alter corticospinal inhibition, further studies are required to scrutinize the influence of different training protocols on excitability of intracortical inhibitory interneurones via assessment of techniques such as short- and long-interval intracortical inhibition.

### Methodological considerations

A methodological consideration of the present study is that the sequence of contractions at neuromuscular evaluations (100% then 75% then 50% MVC) was consistent throughout the experiment. It remains unclear how corticospinal responses at 75 and 50% MVC could have been modulated with the preceding MVC. In addition, the current experiment is lacking a control group; thus, further studies are required to include another group of individuals attending neuromuscular evaluation sessions alone, without any cycling exercise. The MEP responses monitor the excitability of the entire corticospinal pathway (above the neuromuscular junction) including the motor cortical and spinal motoneurones (Gandevia et al. 1999; Taylor et al. 2002). Thus, the adaptations observed for $MEP_{75}$ do not specify whether the changes occurred at the cortical and/or spinal motoneurone level.

## Conclusion

Contrary to our primary hypotheses, 9 weeks of HIIT and CST cycling training did not result in any significant change in the voluntary activation ($VA_{PNS}$) when measurements were performed during cycling or at EXH. In addition, a reduction was observed in corticospinal excitability when MEP was measured at 75% of MVC at the baseline level in $POST_{ABS}$ and $POST_{REL}$ session compared to PRE. These findings suggest that endurance training may provide some long-term corticospinal adaptations in execution of submaximal contractions; however, these adaptations may not necessarily be translated into any significant change in central fatigue at a given workload or at EXH.

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