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Neuromuscular fatigue and recovery after a high-intensity intermittent exercise in young and older men

Master Thesis in Physiotherapy

(Kinesiology and Biomechanics)

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CONTENTS

LIST OF ABBREVIATIONS

ANOVA	– analysis of variance
BMI	– body mass index
DEXA	– dual-energy x-ray absorptiometry
ES	– electrical stimulation
HIIE	– high-intensity intermittent exercise
HR	– heart rate
LFF	– low-frequency fatigue
LSD	– least significant difference
MVC	– maximal voluntary contraction
OM	– older men
P20	– peak torque at 20 Hz electrostimulation
P100	– peak torque at 100 Hz electrostimulation
PPO	– peak power output
QF	– quadriceps femoris
RPE	– rating of perceived exertion
RPPO	– relative peak power output
RyR1	– ryanodine receptor
SD	– standard deviation
SE	– standard error
VO_{2peak}	– peak oxygen consumption
YM	– young men

ABSTRACT

Aim: The aim of the present study was to evaluate neuromuscular fatigue and recovery after a single session of high-intensity intermittent exercise (HIIE) on a cycle ergometer in young and older men.

Methods: Ten young (age 25.9 ± 3.5 years, total body lean mass 61.3 ± 7.6 kg) and 11 older men (age 63.7 ± 8.3 years, total body lean mass 60.0 ± 4.3 kg) participated in the present study. In this study, anthropometry (height, mass, body mass index), dual-energy x-ray absorptiometry, incremental test, maximal voluntary contraction, and low- and high-frequency electrical stimulation of quadriceps femoris (QF) muscle, and Borg's rating of perceived exertion scale were used. After warm-up, the participants performed 6x30 s of HIIE on cycle ergometer interspersed by 4 min recovery period (Wingate test). For statistical analysis, programs Excel 2016 and SPSS 20 were used.

Results: Peak oxygen consumption was higher ($p<0.01$) in young as compared to older men (45.7 ± 6.5 ml/kg/min, 35.3 ± 6.6 l/kg/min, respectively). At the end of 6^{th} bout of HIIE, relative peak power output of QF muscle decreased ($p<0.001$) in young and older men (45% and 57%, respectively). 5 min after HIIE, maximal voluntary contraction torque of QF muscle decreased ($p<0.001$) in young and older men (25% and 15%, respectively), as well as one h after HIIE in young ($p<0.05$, 12%) and older men ($p<0.01$, 11%). 5 min and one h after HIIE, the peak torque of QF muscle at 20 Hz (P20) electrical stimulation decreased ($p<0.001$) in young (49% and 51%, respectively) and older men (47% and 37%, respectively), whereas 24 h after HIIE, the decline in P20 remained only in older men ($p<0.05$, 13%). 5 min and one h after HIIE, the peak torque of QF muscle at 100 Hz (P100) electrical stimulation decreased ($p<0.001$) in young (37% and 42%, respectively) and older men (28% and 29%, respectively). One h and 24 h after HIIE, the P20/P100 ratio decreased ($p<0.05$) in young (10% and 13%, respectively) and older men (10% and 7%, respectively).

Conclusion: A single session of HIIE on a cycle ergometer induced a marked peripheral fatigue in QF muscle in young and older men. Low-frequency fatigue was induced that did not recover completely 24 h after HIIE in both groups, whereas subjectively perceived fatigue level recovered completely.

Keywords: Wingate test, neuromuscular fatigue, older and young men, electrical stimulation

LÜHIÜLEVAADE

Eesmärk: Käesoleva uuringu eesmärgiks oli hinnata neuromuskulaarset väsimust ja taastumist pärast ühekordset kõrge intensiivsusega kordustööd (HIIE) veloergomeetril noortel ja vanemaealistel meestel.

Metoodika: Kümme noort (vanus 25.9 ± 3.5 eluaastat, kogu keha lihasmass 61.3 ± 7.6 kg) ja 11 vanemaealist (vanus 63.7 ± 8.3 eluaastat, kogu keha lihasmass 60.0 ± 4.3 kg) meest osalesid käesolevas uuringus. Uuringus kasutati antropomeetria (keha pikkuse, keha massi, kehamassi indeksi), kahe energiatasemega röntgen-absorptsiomeetria (DEXA), koormustesti, maksimaalse tahtelise lihaskontraktsiooni määramist, madala ja kõrge sagedusega elektrilist stimulatsiooni (ES) ning Borgi tajutud pingutuse hindamise skaalat. Pärast soojendust sooritasid osalejad 6 kordust 30 s HIIE't veloergomeetril puhkeintervalliga 4 min (Wingate test). Andmete statistiliseks analüüsiks kasutati programme Excel 2016 ja SPSS 20.

Tulemused: Maksimaalne hapnikutarbimisvõime oli kõrgem ($p < 0.01$) noortel võrreldes vanemaealiste meestega (vastavalt 45.7 ± 6.5 ml / kg / min, 35.3 ± 6.6 l / kg / min). HIIE kuuenda korduse lõpuks langes ($p < 0.001$) suhteline maksimaalne võimsus reie-nelipealihases (QF) noortel ja vanemaealistel meestel (vastavalt 45% ja 57%). 5 min pärast HIIE't langes ($p < 0.001$) maksimaalse tahtelise lihaskontraktsiooni (MVC) jõumoment QF nii noortel kui ka vanemaealistel meestel (vastavalt 25% ja 15%), sarnane muutus oli üks tund pärast HIIE't noortel ($p < 0.05$, 12%) ja vanemaealistel meestel ($p < 0.01$, 11%). 5 min ja üks tund pärast HIIE't langes ($p < 0.001$) maksimaalne QF jõumoment saadud 20 Hz (P20) elektrostimulatsioonil noortel (vastavalt 49% ja 51%) ja vanemaealistel (vastavalt 47% ja 37%,), samas 24 tundi pärast HIIE't jäi P20 langus püsima vaid vanemaealistel meestel ($p < 0.05$, 13%). 5 min ja üks tund pärast HIIE't langes ($p < 0.001$) maksimaalne QF jõumoment saadud 100 Hz (P100) elektrostimulatsioonil noortel (vastavalt 37% ja 42%) ja vanemaealistel meestel (28% and 29%, vastavalt). Üks tund ja 24 tundi pärast HIIE't langes ($p < 0.05$) P20/P100 suhe noortel (vastavalt 10% ja 13%) ja vanematel meestel (vastavalt 10% ja 7%).

Kokkuvõte: Ühekordne HIIE veloergomeetril põhjustas QF märkimisväärset perifeerset väsimust noortel ja vanemaealistel meestel. Tekkis madalsageduslik väsimus, mis ei taastunud 24 tundi pärast HIIE't kummaski grupis, kuid aga tajutava väsimuse tase taastus täielikult.

Märksõnad: Wingate test, neuromuskulaarne väsimus, vanemaealised ja noored mehed, elektriline stimulatsioon

1. LITERATURE REVIEW

1.1 Physiological effects of high-intensity intermittent exercise

High-intensity intermittent exercise (HIIE) involves lower training volume which makes it a time-efficient strategy (Buchheit and Laursen, 2013). HIIE increases aerobic capacity and metabolic health in young and older adults (Adamson et al., 2014; Buchheit and Laursen, 2013), and improves physical performance in athletes of several kinds of sports (Buchheit and Laursen, 2013; Fiorenza et al., 2019). Different HIIE protocols are used in high-intensity interval trainings, that are characterized by repeated short (10-45 s) to long (2–4 min) bouts of maximal or submaximal (90% -100% of maximal heart rate (HR)) work efforts, interspersed by recovery phases of rest or low-intensity exercise (60% of maximal HR) (Buchheit and Laursen, 2013). The most utilized protocol is Wingate test or bouts of all-out work effort on a stationary cycle ergometer in combination with rest pauses (Bar-Or, 1987; Bell et al., 2015); sprint combined with jogging as a recovery phase (Lattier et al., 2004; Škof and Strojnik, 2005) or sports specific HIIE combined with jogging as a recovery phase (Fajrin et al., 2018). HIIE is a hard type of exercise that demands high motivation and tolerance to occuring discomfort (Fiorenza et al., 2019).

HIIE protocols used as high-intensity interval training are an efficient way to improve sport specific skills in both athletic and untrained healthy population. It is proved to show variable effects on body's cellular functions even after short training programs. Recent study showed that chronic responses to HIIE training improve explosive power, speed, and agility in young athletes (Fajrin et al., 2018). According to a study by Adamson and colleagues (2014), HIIE protocol performed twice a week is sufficient to ensure a significant change in peak oxygen consumption (VO_{2peak}), functional capacity and metabolic health in an untrained middle-aged population (Adamson et al., 2014). Chronic responses to HIIE training helps to promote mitochondrial biogenesis and inhibit the physiological changes associated with aging (Little et al., 2010), as well as increases sarcoplasmic and myofibrillar protein synthetic rate in older men (Bell et al., 2015).

In the past several decades, scientists showed that HIIE has benefits and is a valuable tool in the creating sets of exercises for patients with a chronic diseases (Ross et al., 2016). It was found that chronic responses to HIIE training lead to a rapid enhancement in skeletal muscle oxidative capacity, as reflected by the increased mitochondrial density and function after short-term training program in healthy, overweight/obese, coronary disease, and type II diabetes individuals (Gillen and Gibala, 2014). Moholdt et al. (2014) while investigating different HIIE protocols in patients with coronary heart disease found that higher exercise intensities of the work interval provides improvement in VO_{2peak} more efficiently. Also the effects of strength and HIIE training were evaluated in older men, aged 84-87 years, suffering from chronic obstructive pulmonary disease (Guadalupe-Grau et al., 2017). Regular HIIE training combined with strength training was well tolerated by the older patients with chronic obstructive pulmonary disease and their upper- and lower-body maximal dynamic and isometric muscle strength, agility, walking speed, and aerobic capacity were improved without any noticeable adverse effects (Guadalupe-Grau et al., 2017). HIIE training is an effective exercise strategy for improving time to exhaustion and peak power output (PPO), and decreasing fat mass in overweight/obese population aged 33-46 years with body mass index (BMI) 32-38 kg/m^2 (Smith-Ryan et al., 2016).

According to the study of adaptation mechanisms of skeletal muscle in the context of HIIE training, oxidative stress occurs as a natural and necessary process, which results in different structural degradations (Bellinger et al., 2008). Among other factors, the oxidation and degradation of ryanodine receptor (RyR1) occur (Bellinger et al., 2008). Regular and repeated HIIE causes type I RyR1 modification, which results in a decreased Ca^{2+} ions release from the sarcoplasmic reticulum into myoplasm during muscle contraction, as well as in increased Ca^{2+} ions leak from sarcoplasmic reticulum during resting state (Bellinger et al., 2008), which decrease muscle fatigue resistance (Westerblad et al., 2010). The manifestation of this acute adaptation is the worsening of characteristics of muscle contractile properties 24 h after a single session of HIIE (Place et al., 2015). However regular HIIE training improves muscle contractile properties as a result of chronic adaptation (Little et al., 2010).

1.2 Neuromuscular fatigue and recovery during high-intensity intermittent exercise

Repeated and intense use of muscles affects physiological processes throughout the neuromuscular system and leads to muscle fatigue or even exhaustion (Gandevia, 2001). Neuromuscular fatigue is a complex phenomenon, that can be defined as a reduction in maximal force-generating capacity of skeletal muscles and occurs due to processes (metabolic and ionic disturbances) distal to the neuromuscular junction (peripheral fatigue) (Allen et al., 2008), but also when the central nervous system fails to drive the motoneurons adequately (central fatigue) (Gandevia, 2001).

The type of muscular contraction performed during exercise, as well as exercise duration and intensity determines the level of strength reduction (Millet and Lepers, 2004). As neuromuscular fatigue depends also on the type of muscle contraction, difference between concentric and eccentric work has been registered. Generally, the strength loss is lower in concentric than in isometric contractions, while greatest decrease and muscular damage exists after eccentric contractions - it can be confirmed that fatigue is task dependent (Millet and Lepers, 2004). Voluntary and electrically elicited contractions combined with surface electromyography have been mostly used to assess muscle fatigue and recovery with the same apparatus during exercise protocol. Peripheral fatigue can be demonstrated by a decrease in the twitch or tetanic force elicited by peripheral nerve stimulation, while the muscle is at rest. Central fatigue refers to more proximal processes and is defined as exercise-induced failure of voluntary activation of the muscle (Gandevia, 2001). During and after physical exercise fatigue level can be investigated not only by using objective measures but also by applying subjective ones. It was noted that Borg's rating of perceived exertion (RPE) scale is a helpful tool in assessing individual's perceived fatigue level during physical effort (Borg, 1985).

An acute HIIE designed as cycling to exhaustion at 80% of maximal power output (6 min loading and 4 min rest) leads to a peripheral fatigue that develops early during constant-load intense cycling, while central fatigue appears to be present at the end of the exercise (Decorte et al., 2012; Hureau et al., 2016). Combination of peripheral and central fatigue factors limits the capacity to keep PPO with repeated HIIE. Whereas Fernandez-del-Olmo et al. (2013) found that central mechanisms are the main reason of the force reduction, while the peripheral fatigue is associated with an intramuscular impairment after two bouts of maximal cycling test (Wingate test). Lattier et al. (2004) explored the contribution of central and peripheral fatigue factors after another type of HIIE - high-intensity interval uphill running exercise. They found that the reason of muscle fatigue following running HIIE was connected with changes in excitation-contraction coupling, but not with central fatigue factors or alterations at the cross-bridge level. Despite, it is suggested that cycling exercise induces less muscular stress as it involves mainly concentric contractions (Place et al., 2015) and therefore causes lower muscular damage compared to running (Millet and Lepers, 2004). Thus central fatigue is more likely to occur after prolonged running than after cycling exercises (Millet and Lepers, 2004).

1.3 Ageing-related changes in neuromuscular system

Ageing is a physiological process, which includes a gradual decrease in skeletal muscle mass and function (sarcopenia), strength (dynapenia) and endurance (Degens and Alway, 2006). Deficit in muscle strength and contractile properties of skeletal muscle, muscle fiber type switching, loss of regenerative capacity (Degens and Alway, 2006) and increase in fat mass (obesity) can also occur (Buckinx et al., 2018). Seene et al. (2012) reviewed that ageing is related to a decreased synthesis rate of muscle proteins and particularly myofibrillar proteins. Average reduction of quadriceps femoris (QF) muscle by 10% by the age of 50 years and by 40% by the age of 80 years is explained by the decrease in fiber number and size that occur in fast-twitch fibers (Lexell et al., 1988). In addition to altered muscle volume, structural changes in myelinated neurons in both central and peripheral nervous system may affect an individual's ability to conduct and transmit neural signals (McKinnon et al., 2017). This means that neurons are less able to effectively transmit both motor and sensory commands to the muscles (McKinnon et al., 2017). Taken together, it is apparent that ageing is accompanied by reduced function of the musculoskeletal and nervous system.

Secondary to ageing-related physiological changes, lifestyle and physical activity are important factors to notice. It is well established that physical inactivity and sedentary lifestyle cause negative age-associated changes in muscle function and metabolism (Distefano and Goodpaster, 2018). Physical inactivity is strictly correlated with poor quality of life and risk of disease and mortality (Fielding et al., 2011). Exercise and physical activity can significantly reduce, or in some cases prevent declines in muscle function (Distefano and Goodpaster, 2018). Exercise interventions provide positive changes in multiple parameters related to dynapenia or obesity, fat mass, as well as functional and aerobic capacity in elderly (Buckinx et al., 2018). Nowadays very little is known how a single session of HIIE affects neuromuscular system.

2. AIM AND OBJECTIVES

The aim of the present study was to evaluate neuromuscular fatigue and recovery after a single session of HIIE on a cycle ergometer in young and older men.

Objectives of the study:

1. To estimate changes in relative peak power output during HIIE on a cycle ergometer.
2. To assess maximal voluntary contraction torque of QF muscle before, and 5 min, one h, and 24 h after HIIE.
3. To estimate electrically elicited tetanic contraction torque characteristics of QF muscle before, and 5 min, one h, and 24 h after HIIE.
4. To evaluate subjectively perceived fatigue level before, immediately, and 24 h after HIIE.

3. METHODS

3.1 Participants

A total 24 men volunteered to participate in this study. Before baseline measures, each participant visited the laboratory to read and sign a written informed consent form prior to the study. After that, they were familiarized with all testing and exercising procedures. Three of the participants were excluded from the study due to high arterial blood pressure (older men, n=2) and a knee pain (young men, n=1). A total of 21 healthy men took part in the study. They were distributed into two groups according to the age. The group of young men (YM) included participants aged 25-29 years (n=10), and the group of older men (OM) included participants aged 63-72 years (n=11). The data of the participants are presented in Table 1.

Table 1. Descriptive data of the participants (mean ± SD)

Characteristics	Young men (n= 10)	Older men (n= 11)
Age (years)	25.9 ± 3.5	63.7 ± 8.3
Body height (cm)	177.8 ± 5.9	177.5 ± 5.0
Body mass (kg)	82.2 ± 13.3	83.6 ± 8.6
BMI (kg/m^2)	25.9 ± 3.6	27 ± 2.1
VO_{2peak} (ml/kg/min)	45.7 ± 6.5	35.3 ± 6.6**
Total body fat mass (kg)	16.0 ± 5.7	18.7 ± 5.0
Total body lean mass (kg)	61.3 ± 7.6	60.0 ± 4.3
Total body fat (%)	19.3 ± 4.0	22.6 ± 3.8
Legs lean mass (kg)	21.4 ± 3.0	19.6 ± 5.8
Legs fat mass (kg)	5.5 ± 2.0	5.6 ± 1.6

n- number of participants in the group, BMI- body mass index, VO_{2peak}- peak oxygen consumption.
**p<0.01 as compared to young men.

All participants were nonsmokers, with no history of chronic or acute diseases (cardiovascular, pulmonary, nephrological, or neuromuscular), with no use of medications or nutritional supplements. They did not have any lower body musculoskeletal injuries in the previous 6 months and were physically active, but none of the participants was undertaking HIIE-type training. Different types of physical activity were presented in both groups: for example, for YM 30% (3/10) of the participants did football, 20% (2/10) went to the gym. For OM fitness training was the most popular within the group, which formed 45% (5/11) of the participants. Participants were excluded if they were being overweight or underweight (defined as having a BMI more than 30 kg/m^2 and less than 18.5 kg/m^2, respectively), high arterial blood pressure (more than 140/90 mmHg), acute and chronic inflammatory injuries, sensitivity to electrical impulses, the presence of a cardiac stimulator, endoprosthesis or bone metal plates for fracture repair.

All experimental procedures were approved by the Research Ethics Committee of the University of Tartu (prot. nr. 267/T-9, 20.02.2017).

3.2 Study design

Present master's thesis is based on data of international project "Adaptive changes in skeletal muscles during high-intensity interval training in young and older men", which was conducted at the Laboratory of Kinesiology and Biomechanics (Ujula 4, Tartu) and the Laboratory of Kinanthropometry (Jakobi 5, Tartu) of the University of Tartu during the period of autumn 2017 - spring 2018.

The role of the author of this thesis was to enter anthropometric, MVC torque data, physical activity, and life quality assessment questionnaires' quantitative indicators into Microsoft Excel (2016) tables, as well as statistical processing of the data and comparison of the data between the groups. In addition, the author participated in preparation process for muscle electrostimulation and voluntary contraction (cleaning the participants' skin, applying the electrode gel to the contact surface, and attaching the stimulating electrodes to the participants' skin during testing and exercising procedures), and HIIE by motivating the participants verbally during efforts.

In order to make measurements, a definite study scheme for time and procedures was designed (Figure 1). Initially the participants performed anthropometric, body composition and cardiovascular fitness test. One week later, the participants were familiarized with Wingate test, MVC torque, electrical stimulation (ES) tests at 20 Hz (P20) and 100 Hz (P100).

In the present study, before, and 5 min, one h, and 24 h after HIIE, the following parameters were determined: MVC torque and electrically elicited tetanic contraction characteristic (P20 and P100). The relative peak power output (RPPO) was determined during HIIE bouts. Subjectively perceived fatigue level was assessed before, immediately after, and 24 h after HIIE**.**

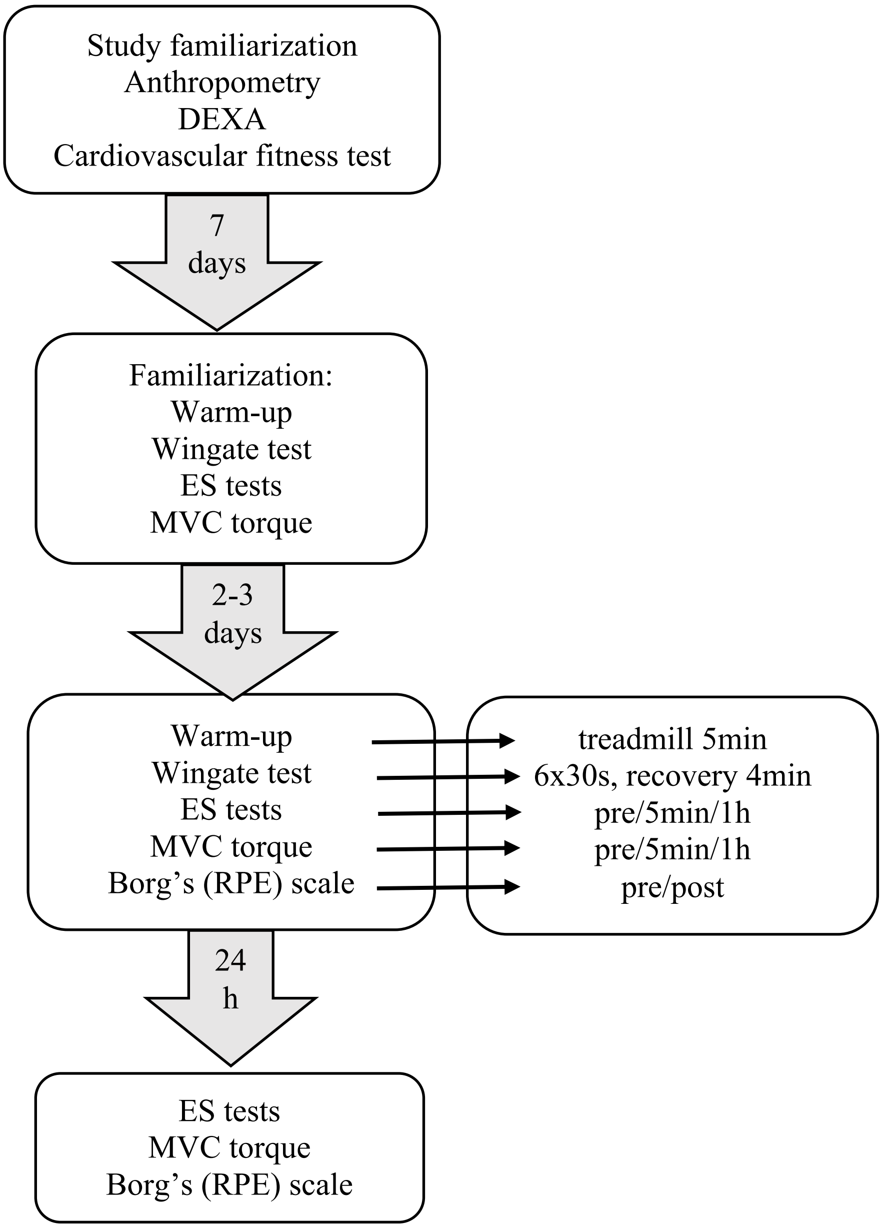


Figure 1. Study design. DEXA- dual-energy x-ray absorptiometry; MVC- maximal voluntary contraction and ES- electrical stimulation of quadriceps femoris muscle; RPE- rating of perceived exertion.

The participants were asked to refrain from caffeine for 24 h before each test and avoid participation in any sports activity 24 h before tests. As earlier reported, caffeine has a direct ergogenic effect on skeletal muscle (Tarnopolsky, 2008) and may cause dose-dependent increases in force production and duration of the contraction of skeletal muscle (Olorunshola and Achie, 2011). All tests were carried out in the same conditions (room temperature 22C°) for each participant and by the same research staff. Participants performed HIIE wearing a T-shirt, shorts and training shoes. HIIE day was set to the participants' preference (days of the week and time of day).

3.3 Measurements

3.3.1 Anthropometry

All participants were measured once before the study. Height was measured with a standard anthropometer (Soehnle professional, Germany) ± 0.1 cm. Body mass was determined using standard electronic scale (in light clothing and with no footwear) to the nearest ± 0.1 kg. BMI was calculated by dividing body mass (kg) by height (m) squared. Anthropometric data of the participants are presented in Table 1.

3.3.2 Body composition

A dual-energy x-ray absorptiometry (DEXA) scanner (Hologic Discovery QDR Series, USA) was used to determine the composition of the whole body by passing low-radiation x-ray beams through tissues. Before measurement, the participants were informed about the method and preparation for this procedure. During the measurement the participants of the study were scanned in a supine position according to standard method guidelines (Madsen et al., 1997). Total body fat percentage and mass, total body lean mass, and fat and lean mass of the lower limbs were measured (Table 1).

3.3.3 Cardiovascular fitness test

To assess aerobic capacity and cardiovascular fitness participant performed incremental test with electrocardiogram on a cycle ergometer (Lode Corrival Ergometer, LODE Holding Co, Groningen, The Netherlands). The test began with 2 min of cycling at 40 W, after which the intensity increased steadily by 20 W every one min. Oxygen consumption during loads was determined (Cortex Metamax 3B, Germany). For VO_{2peak}, the highest level of oxygen consumption was determined at the last load within 30 s (Andersen, 1995). Data of the participants' VO_{2peak} are presented in Table 1.

3.3.4 High-intensity intermittent exercise

HIIE was performed on a cycle ergometer (Monark 834E, Sweden). The participants were trained one-on-one under the supervision of the trained research staff. The optimal seat height was adjusted for each participant on the familiarization day. HIIE protocol was designed as Wingate test (Bar-Or, 1987).

Before HIIE, a warm-up was performed on a treadmill (speed up to 6 km/h) for 5 min at the beginning of exercising protocol. After warm-up, the participants were allowed 5 s unloaded pedaling to reach 100 rate per min and were instructed to maintain maximal pedal speed. In exercise phase, the participants began to pedal 6 repetitions of 30 s bouts and they were instructed to work as hard as they could during this phase once the appropriate resistance was applied. The load was applied to the flywheel by adding a predetermined amount of weight to the load tray of the cycle ergometer. In the present study, the load was 7.5% of participant's body mass. Recovery intervals between exercise phases were 4 min. During the recovery, the participants could get off the cycle ergometer and were allowed to drink water, sit on the chair, or to lie if needed. HR was tracked by chest strap HR monitor and watch (Polar RS300X, Finland) to monitor HR during exercise. The participants were motivated verbally during the exercise (using words "Go-go", "Continue"). During the HIIE bouts, the PPO values were registered on a personal computer and relative peak power output (RPPO) was calculated, using formula RPPO (W/kg) = PPO (W)/ body weight (kg), respectively.

3.3.5 Isometric maximal voluntary contraction torque measurement

Voluntary force-generation capacity of QF muscle of the dominant leg was estimated by isometric MVC torque. Before the test, leg dominance was determined by asking the participants which leg they would use to kick a soccer ball (English et al., 2006). In this study two older and one young men had left leg as a dominant, all other were right leg dominant. During the measurement, the participants sat in a custom-made dynamometric chair with the knee and hip angles equal to 120° and 110°, respectively. The body position of the participant was secured by three Velcro belts placed over the chest and hip to avoid co-activation of other muscles. The dominant leg of participant was fixed to transducer 3 cm above the apex of lateral malleolus and the unilateral knee extension force was recorded. Length of calf segment (L) was measured. Measurement range of the strain-gauge transducer was 10 to 2000 N (1778 DST-02, Russia). Force signals were sampled at the frequency of 1 kHz and stored on a hard disk of a computer using software WSport Lab (Urania, Estonia). The participant was asked to develop and hold maximal force for 1-2 s. Three trials were performed with rest periods of 30 s between the measurements. The attempt with the highest MVC torque force was taken for further analysis. In this study MVC torque (N·m) was calculated by formula **(1)**: MVC force (N) · L (m). MVC force measurement were performed before, and 5 min, one h, and 24 h after HIIE.

3.3.6 Electrically elicited tetanic contraction torque measurement

For measuring isometric tetanic contraction torque of QF muscle participants sat in a custom-made dynamometric chair as described before in part 3.3.5. Before the stimulation, the participants were instructed to relax their muscles. A high voltage stimulator (Digimer, DS7A4, United Kingdom) was used.

Electrical stimuli to the QF muscle were delivered through three self-adhesive-stimulating electrodes (8 x 12 cm). One stimulation electrode was placed laterally over the vastus lateralis muscle and the other electrode was placed over the vastus medialis muscle in the distal part of the thigh. The third ground electrode was placed in the proximal third of the thigh. Prior to attaching the stimulating electrodes, the underlying skin was prepared with isopropyl alcohol, and electrode gel was applied to the contact surface. The electrical stimulation was delivered in sequences of square wave pulses of 1 ms duration. The highest stimulation voltage possible was chosen in order to recruit the greatest number of fibres. The following data were measured: isometric tetanic contraction torque (N·m) of QF muscle, aroused by electrical stimulation at 20 Hz (P20) and 100 Hz (P100) frequencies and appropriate peak torque was calculated by formula **(1)** for P20 and P100. The duration of each electrical stimuli was 1 s. Changes in the P20/P100 ratio after HIIE were calculated in percentages to evaluate low-frequency fatigue (LFF) (Jones, 1996). The measurement in P20 and P100 was performed before, and 5 min, one h, and 24 h after HIIE.

3.3.7 Subjectively perceived fatigue level

In the present study the Borg's rating of perceived exertion (RPE) scale was used to assess the participants' subjectively perceived fatigue level. The participants were asked to rate their level of fatigue on the Borg's RPE scale before, immediately after, and 24 h after HIIE. Borg's RPE scale ranges from 6 meaning "no exertion at all", to 20 meaning "maximal exertion" (Borg, 1985; Appendix 1)

3.4 Statistical analysis

Data are presented as means, and standard deviation (± SD), and standard errors (± SE). Data analysis was performed using Microsoft Excel (2016) and SPSS 20. Repeatedly measured variables in groups were used to assess the significance of deviation by paired Student t-test. To determine statistically significant differences between the means of groups the one-way analysis of variance (ANOVA) was used, followed by the least significant difference (LSD) test. The significance level was set to $p<0.05$.

4. RESULTS

4.1 Descriptive data of the participants

Anthropometry, body composition and cardiovascular fitness data are presented in Table 1. VO_{2peak} was higher ($p<0.01$) in YM as compared to OM. Body composition and anthropometry parameters did not differ significantly ($p>0.05$) between YM and OM.

4.2 Relative peak power output during high-intensity intermittent exercise

The RPPO value during HIIE are presented in Figure 2. The RPPO value at the beginning of 1st bout of HIIE did not differ significantly ($p>0.05$) in YM and OM (8.29 ± 0.36 W/kg and 8.23 ± 0.31 W/kg, respectively). RPPO value attained at the end of 1st and at the end of 6th bouts of HIIE was higher ($p<0.001$ and $p<0.01$, respectively) in YM as compared to OM.

In YM, RPPO value decreased ($p<0.001$) at the end of the 1st and 6th bouts of HIIE as compared to initial level at the beginning of 1st bout of HIIE. The respective values for the OM also decreased ($p<0.001$) as compared to HIIE 1st bout beginning value.

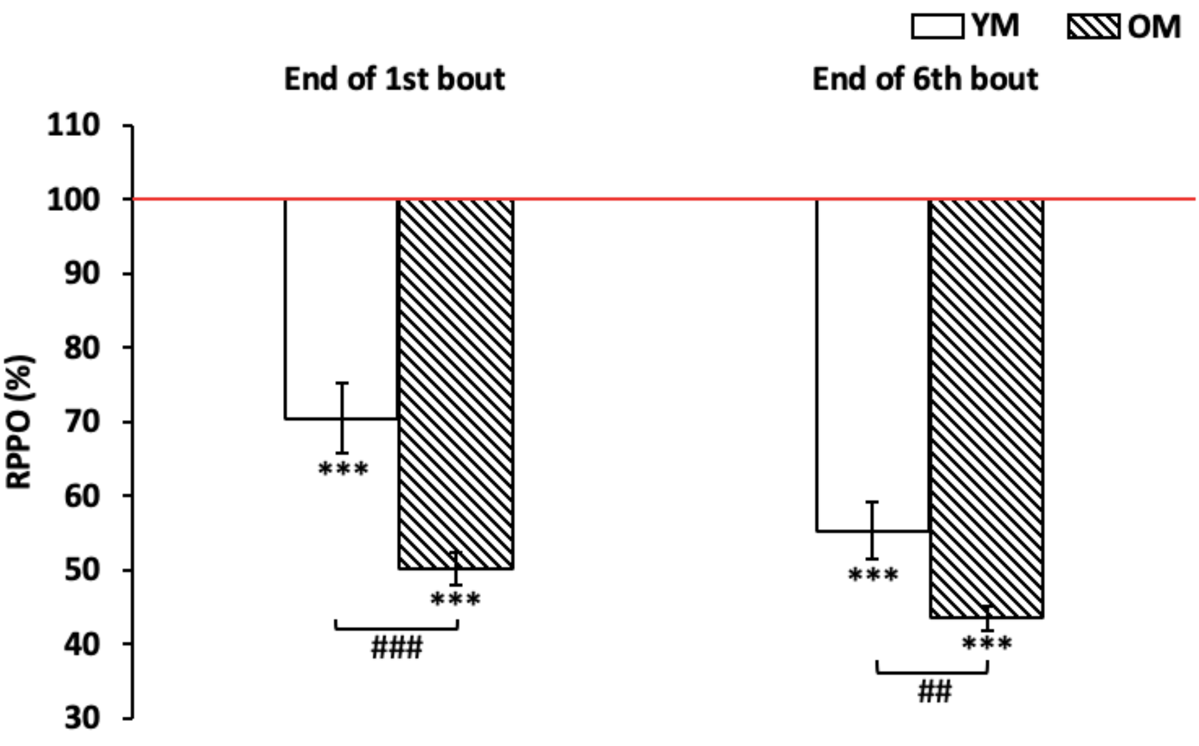


Figure 2. Relative peak power output (RPPO, %) at the end of the 1st and 6th bouts of HIIE in young and older men. Data expressed as percent of the initial level at the beginning of the 1st bout of HIIE (mean±SE). $^{***}p<0.001$ as compared to the initial level at the beginning of the 1st

bout of HIIE. ##p<0.01, ###p<0.001 as compared to young men. YM- young men, OM- older men.

4.3 Maximal voluntary contraction torque of quadriceps femoris muscle

MVC torque value of QF muscle before and after HIIE are presented in Figure 3. Pre-HIIE the MVC torque value did not differ in YM as compared to OM (305.3 ± 22.0 N·m and 265.4 ± 11.8 N·m, respectively). MVC torque attained 5 min, one h and 24 h after HIIE was consistently higher in YM than in OM, however, these differences between the groups were not significant (p>0.05).

In YM, 5 min and one h after HIIE, the MVC torque decreased (p<0.001 and p<0.05, respectively) as compared to MVC torque pre-HIIE value but recovered completely by 24 h after HIIE. In OM, the respective values 5 min and one h after HIIE also decreased (p<0.001 and p<0.01, respectively) as compared to MVC torque pre-HIIE value.

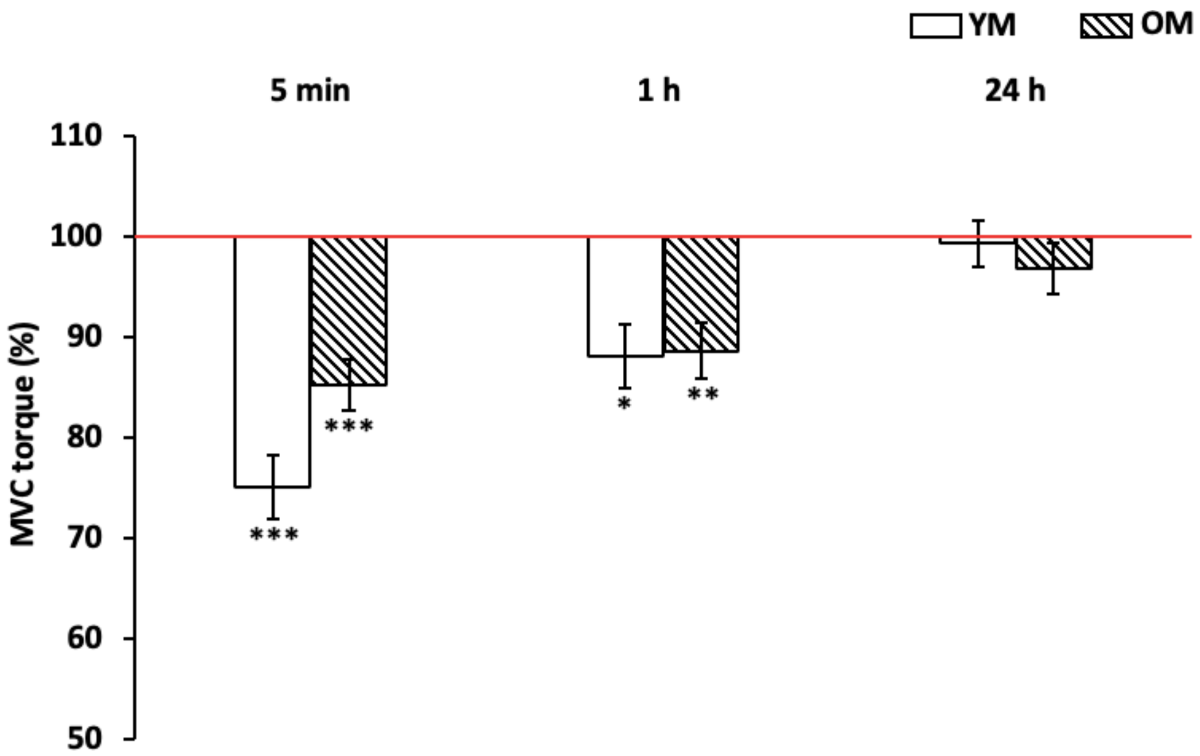
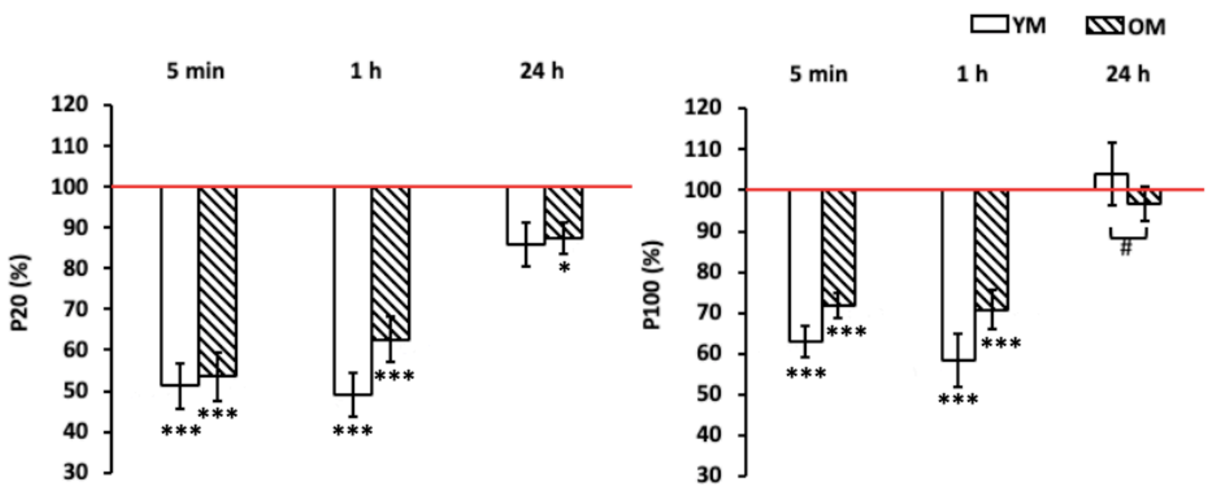


Figure 3. Recovery of QF muscle maximal voluntary contraction (MVC, %) torque 5 min, one and 24 h after HIIE in young and older men. Data expressed as percent of pre-HIIE value (mean±SE). *p<0.05, **p<0.01, ***p<0.001 as compared to pre-HIIE value. YM- young men, OM- older men.

4.4 Electrically elicited quadriceps femoris muscle contraction torque

P20 values of QF muscle before and after HIIE are presented in Figure 4a. Pre-HIIE P20 value did not differ ($p>0.05$) in YM as compared to OM (130.00 ± 12.14 N·m and 102.41 ± 7.05 N·m, respectively). There was no significant ($p>0.05$) difference in P20 value attained 5 min, one h, and 24 h after HIIE in YM and OM.

In YM and OM, 5 min and one h after HIIE P20 value decreased ($p<0.001$) as compared to pre-HIIE value. Furthermore, P20 value did not recover completely by 24 h after HIIE in OM ($p<0.05$) as compared to pre-HIIE value. Whereas the respective value for the YM recovered to pre-HIIE level.



(a) (b)

Figure 4. Recovery of electrically elicited peak torque of QF muscle at 20 Hz stimulation (P20, %) (a) and at 100 Hz stimulation (P100, %) (b) 5 min, one h and 24 h after HIIE in young and older men. Data expressed as percent of pre-HIIE value (mean±SE). $^{*}p<0.05$, $^{***}p<0.001$ as compared to pre-HIIE value. $^{\#}p<0.05$ as compared to young men. YM- young men, OM- older men.

P100 value before and after HIIE are presented in Figure 4b. P100 pre-HIIE value was greater ($p<0.05$) in YM as compared to OM (159.07 ± 9.02 N·m and 126.19 ± 7.48 N·m, respectively).

In YM and OM, the P100 decreased ($p<0.001$) 5 min and one h after HIIE as compared to pre-HIIE value. 24 h after HIIE the P100 value was greater ($p<0.05$) in YM as compared to OM, though the complete recovery was observed in both groups.

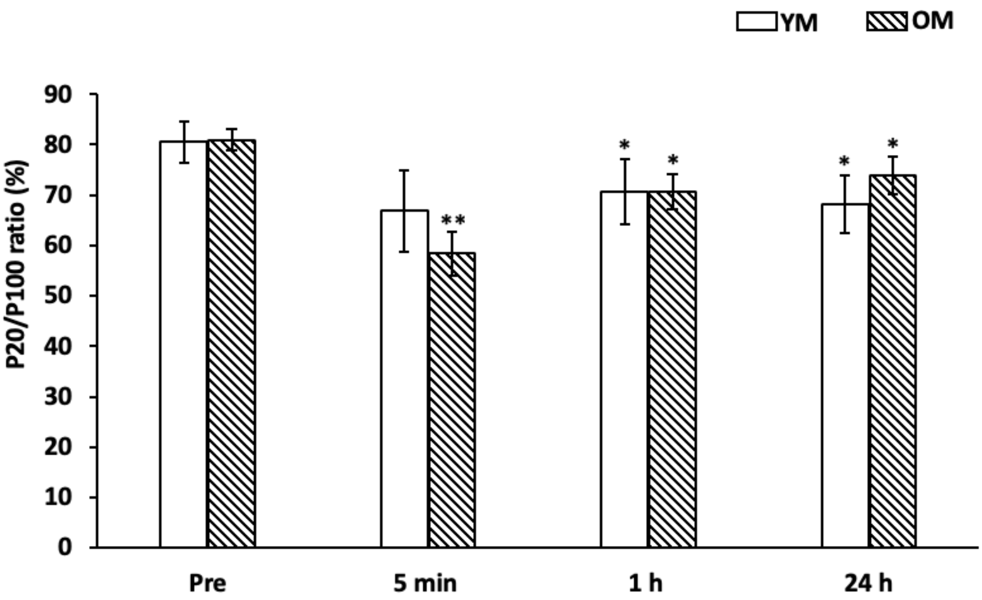


Figure 5. Ratio of electrically elicited peak torque of QF muscle at 20 Hz and at 100 Hz (P20/P100 ratio, %) before, 5 min, one h and 24 h after HIIE in young and older men. $^{*}p<0.05$, $^{**}p<0.01$ compared to pre-HIIE (Pre) value (mean±SE). YM- young men, OM- older men.

P20/P100 ratio value before and after HIIE are presented in Figure 5. Pre-HIIE P20/P100 ratio value did not differ ($p>0.05$) in YM as compared to OM (81% and 81%, respectively).

In YM, one h after HIIE P20/P100 ratio value decreased ($p<0.05$), as compared to pre-HIIE value. In OM, the respective value 5 min and one h after HIIE decreased ($p<0.01$ and $p<0.05$, respectively) as compared to pre-HIIE value. Furthermore, 24 h after HIIE decline ($p<0.05$) in P20/P100 ratio remained without complete recovery in both groups.

4.5 Subjectively perceived fatigue level

Borg's RPE values before and after HIIE are presented in Table 2. Before HIIE Borg's RPE values did not differ ($p>0.05$) in YM and OM. Immediately after HIIE, Borg's RPE value increased ($p<0.001$) in YM and OM, as compared to pre-HIIE values. Borg's RPE value immediately after HIIE was higher ($p<0.01$) in YM as compared to OM. 24 h after HIIE Borg's RPE value in OM was higher ($p<0.05$) as compared to YM. Though 24 h after HIIE Borg's RPE value recovered completely in YM and OM.

Table 2. Rating of perceived exertion level before and after HIIE by The Borg's scale (6-20) in young and older men (mean ±SE)

	Borg's RPE scale points	
Estimation time	**Young men**	**Older men**
Pre-HIIE	8.0 ± 0.7	9.4 ± 0.6
Immediately after HIIE	17.2 ± 0.4***	15.4 ± 0.5***[##]
24 h after HIIE	7.7 ± 0.6	10.0 ± 0.8[#]

***$p<0.001$ as compared to pre-HIIE value. [#]$p<0.05$, [##]$p<0.01$ as compared to young men.

5. DISCUSSION

The main findings of this study show that HIIE on a cycle ergometer caused: (1) a prolonged (>24 h) decrease in P20/P100 ratio, that induced a LFF in young and older men; and (2) subjectively perceived fatigue level after 24 h of HIIE was significantly higher in older men as compared to young men, though the complete recovery of subjectively perceived fatigue level was observed in both groups as compared to pre-HIIE data.

In the present study, the results show a reduction in voluntary and electrically elicited force-generation capacity of the QF muscle in young and older men after HIIE, which is the main feature of neuromuscular fatigue.

Electrically elicited muscular contractions are locally conducted and are independent from the central nervous system. The results of the present study show that in both groups the decline of the peak torque produced by electrically elicited muscle contraction (P20, P100) was greater in comparison to MVC torque decline after HIIE. This demonstrates that fatigue after HIIE occurs mainly due to peripheral mechanisms. Such predominant influence of peripheral fatigue was also demonstrated by Škof and Strojnik (2005), who explored neuromuscular fatigue and recovery dynamics after intensive anaerobic interval running exercise of 5x300 m runs (with a 1-min active recovery interval of 100 m easy run between them) in well-trained young men.

Futhermore, the results obtained from electrical stimulation at 20 Hz and 100 Hz frequencies show that significant declines in QF muscle peak torques were present for both stimulations in both groups. However the decline in P20 was greater for young and older men. Moreover a complete recovery did not occur 24 h after HIIE in older men. Based on this, it can be suggested that HIIE caused LFF. Induced LFF after HIIE is also supported by the fact of significant decrease in P20/P100 ratio for young and older men after 24 h of HIIE. This confirms that LFF is charactarised by a proportionately greater loss of force in response to low- versus high-frequency muscle stimulation. LFF is long-lasting, taking hours or even several days for a full recovery and plays a significant role in the reduction in the force-generation capacities of skeletal muscle (Keeton and Binder-Macleod, 2006). LFF has been suggested to occur due to muscle fibre damage or impairment in excitation-contraction coupling mechanism of muscle activation (Jones, 1996). It has been shown by earlier studies that changes in contractile properties of QF muscle after running HIIE are related to excitation-contraction coupling disturbances, having predominant LFF, but not central fatigue (Lattier et al., 2004; Škof and Strojnik, 2005). The recent study by Krusnauskas et al. (2018)

also demonstrated that 3x60 s cycling HIIE (with 4 min of comfortable and minimal resistance cycling as a rest period between the repetitions) induced a significantly prolonged LFF in young and older women aged 19-20 years and 65-68 years, respectively, due to disturbances in excitation-contraction coupling in skeletal muscle. This is consistent with the results of the present study and indicates that present LFF, the decline in force-generating capacity, occurs due to reduced Ca^{2+} release from sarcoplasmic reticulum, which leads to decrease in free myoplasmic Ca^{2+} ions and in myofibrillar Ca^{2+} sensitivity (Balnave and Allen, 1995). In contrast, Place et al. (2015) found no changes in myofibrillar Ca^{2+} sensitivity and no impairments in contractile function of the myofibrillar proteins after one session of HIIE, 3-6x30 s of all-out cycling and 4-min rest periods, suggesting fatigue mechanism can occur due to defects in sarcoplasmic reticulum Ca^{2+} release. These changes within Ca^{2+} release can be explained by acute metabolic stress in skeletal muscle fibres, which is followed by an increase in the release of reactive nitrogen/oxygen species. In case of chronic HIIE training the release of reactive nitrogen/oxygen species may influence mitochondrial biogenesis by stimulating it and increasing endurance (Bellinger et al., 2008; Lanner et al., 2012; Place et al., 2015). In our study we observed excitation-contraction coupling disturbances that continued for at least 24 h after 6x30 s of all-out cycling exercise in young and older men. This finding may suggest that this type of HIIE conducted regularly and repeatedly may be able to improve muscle endurance by activating cellular signalling pathways.

Futhermore during exercise the decline in RPPO was significantly high and progressive in both groups. For the young men, RPPO decreased 30% (end of 1st bout) and progressed to 45% (end of 6th bout) during HIIE. The respective RPPO decrease in older men was from 50% to 57%, confirming the fact of peripheral fatigue development, as it is known that peripheral fatigue develops progressively during the exercise (Gandevia, 2001). A significantly higher decrease in RPPO in older as compared to young men may be due to ageing-related decline in functioning of the musculoskeletal and nervous system. Also, we observed a sharper decrease in RPPO during HIIE in young men (15%) when compairing to older men (7%). This may be explained by the fact of ageing-related changes in muscle fibres, namely a decrease in the number of fast-twitch fibers and increase in slow-twitch fibers grouping in older men (Lexell et al., 1988). It could happen due to the degeneration of fast-twitch fibers and becoming slow-twitch fibers as the result of reinnervation (Lexell et al., 1988), but could not be measured under our study conditions. Although, we can hypothesize that older men, who participated in the present study, had more slow-twitch fibers which slow force production, but are more resistant to fatigue during prolonged exercise. However, no significant differences in total body and legs lean mass were observed between groups in our study. Therefore, we cannot state that there was lack of muscle mass in older men, though, we can think of changes in qualitative aspect of muscle contractile properties that are related to ageing.

In this study we also observed significant decline in MVC torque 5 min and one h after HIIE in both groups. The respective results were 25% and 12% for young men and 15% and 11% for older men. This difference between groups might also be related to ageing-related changes in muscle fibers. Moreover, MVC torque recovered 24 h after HIIE in both groups. This supports the fact of recovery from peripheral fatigue after all-out cycling HIIE.

Some intrinsic group differences within repeatedly measured variables were more prominent than differences between the groups. As Figure 5. shows, the mean value of P20/P100 ratio 5 min after HIIE was lower than one h later. Though, a statistical difference can be noted only after one h of recovery in young men. The reason is overly large SE value in 5 min measurement, showing there was a large variation in the young men population and a large difference in the sample means.

Recent studies recommend that HIIE protocol work- and recovery ratio should be tailored for each individual´s need, to maintain high efficiency in accordance with low risk for health (Ross et al., 2016). In our study, the work and recovery durations were chosen from a standard Wingate protocol, which is considered a gold standard for anaerobic testing (Bar-Or, 1987). These 6 x 30 s work sessions separated by 4 min recovery periods are frequently used for healthy young or older adults as a modality for VO_2 (Little et al., 2010) and PPO improvement (Fernandez-del-Olmo et al., 2013). As seen from our data older men did have a significantly higher perceived fatigue level by Borg's RPE scale 24 h after HIIE as compared to young men. Since satisfaction from training results in more commitment to training protocol, the overall fatigue lasting more than 24 h can have a negative effect on protocol completion. Different modalities and work-recovery durations below Wingate as 6s:60s (work:recovery) (Adamson et al., 2014) and 8s:12s (Kong et al., 2016) have also showed a significant VO_{2peak} improvement in young and older people. A significant improvement in VO_{2peak} after 5-week regular HIIE training was noted in physically moderate-active young women aged 19-20 years from pre-training 34.1 ± 5.7 ml/kg/min to post-training 36.6 ± 6.6 ml/kg/min (Kong et al., 2016). Adamson et al. (2014) demonstrated a significant increase in VO_{2peak} after HIIE training of eight weeks in untrained men and women aged 43-51 years from pre-training 27.2 ± 7 ml/kg/min to post-training 29.9 ± 7 ml/kg/min. In our study, the initial level of aerobic capacity was significantly greater in young men as compared to older men 45.7 ± 6.5 ml/kg/min and 35.3 ± 6.6 ml/kg/min, respectively, therefore we can suggest that both participant groups consisted of physically active men, who are subjected to regular physical load.

Low-volume high-intensity exercises have resulted in higher enjoyment than moderate intensity continuous exercises (Thum et al., 2017). So, in order to maintain high motivation among elderly patients shorter modalities of HIIE could be used. Also, depending on the health condition and preferences not only cycling can be used for HIIE. High-intensity interval training protocols are found to be safe for overweight/obese people (Smith-Ryan et al., 2016), individuals with type 2 diabetes (Gillen and Gibala, 2014), cardiovascular and pulmonary diseases (Guadalupe-Grau et al., 2017; Moholdt et al., 2014; Ross et al., 2016).

Current study paper shows an effective, low volume and time-efficient training method to produce high amplitude acute changes in physiological parameters both in young and older men. However, when prescribing HIIE as a training modality for chronic patients, precautions are needed in order to avoid health complications. Definitely "low risk" patients who are younger and have less complex cardiovascular disease characteristics are safer to be involved into training (Wewege et al., 2018). Patients with unstable symptoms, relatively lower baseline level of aerobic fitness and no recent history of performing regular moderate or vigorous physical activity should remain cautious, however, there is no current research to indicate HIIE training unsafety for any specific cardiovascular disease patients (Wewege et al., 2018).

One limitation of the present study is the small number of participants. Definitely a bigger number of participants would improve the statistical power of the study. One more limitation is that subjectively perceived fatigue level was assessed only twice after HIIE (immediately and 24 h after). It would have been informative to see changes in Borg's RPE also one h after HIIE.

Therefore, future research is necessary to adjust HIIE protocols for older age groups with an effect on physiological changes and fulfilled enjoyment.

6. CONCLUSIONS

1. The decline in relative peak power output of QF muscle during HIIE on a cycle ergometer was greater in older men as compared to young men.
2. The decrease in tetanic contraction torque elicited at low-frequency electrical stimulation was noted 5 min and one h after HIIE in young and older men, whereas in older men this parameter did not recover completely 24 h after HIIE.
3. The decrease in tetanic contraction torque elicited at high-frequency electrical stimulation and in MVC torque was noted 5 min and one h after HIIE in young and older men, but these parameters recovered completely 24 h after HIIE in both groups.
4. The increase in subjectively perceived fatigue level was noted immediately after HIIE with greater extent in young as compared to older men, and this level recovered completely 24 h after HIIE in both groups.
5. A single session of HIIE induced a marked low-frequency fatigue evaluated by ratio of tetanic contraction torques in low- and high-frequency electrical stimulation that did not recover completely 24 h after HIIE.

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APPENDIX 1. Borg's rating of perceived exertion (RPE) scale (6-20)

6	No exertion at all
7	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard (heavy)
16	
17	Very hard
18	
19	Extremely hard
20	Maximal exertion

(Borg, 1985)

APPENDIX 2. Lihtlitsents lõputöö reprodutseerimiseks ja üldsusele kättesaadavaks tegemiseks

Mina, Anna Ivanova (sünnikuupäev 22.06.1995)

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