Original Research

Central and peripheral quadriceps fatigue in young and middle-aged untrained and endurance-trained men: A comparative study

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Abstract

This study aimed to compare quadriceps function (i.e. strength, endurance, central, and peripheral fatigue) of young (Young-UnTr) and middle-aged (MidAge-UnTr) untrained men and young endurance-trained men (Young-Tr). Twenty-four male subjects (eight Young-UnTr (26 ± 4 yr), eight Young-Tr (29 ± 3 yr), and eight MidAge-UnTr (56 ± 4 yr) performed a maximal cycling test to assess their fitness level. On a separate visit, subjects performed sets of 10 intermittent (5-s on/5-s off) isometric contractions starting at 10% maximum voluntary contraction (MVC), with 10% MVC increments from one set to another until exhaustion. Electrophysiological and mechanical (e.g. twitch) evoked responses elicited with magnetic femoral nerve stimulation in the relaxed muscle and during MVC (i.e. estimation of voluntary activation using the interpolated twitch technique) were measured at baseline and after each set to assess peripheral and central fatigue, respectively. Endurance (= total number of contractions) was also evaluated. Young-UnTr exhibited larger reductions in evoked quadriceps mechanical responses than MidAge-UnTr and Young-Tr after identical standardized muscle loading (e.g. after the 50% MVC set, reduction in single potentiated twitch was −36 ± 9%, −21±16%, and −2 ± 4%, respectively). At both 50% MVC set and exhaustion, MidAge-UnTr exhibited similar reduction in maximal voluntary activation and displayed similar endurance compared to Young-UnTr. Young-Tr exhibited greater endurance than Young-UnTr without significant changes in maximal voluntary activation throughout the test. This study provides robust comparative data regarding the influence of chronic exposure to endurance training and middle-aged on central and peripheral quadriceps fatigability and endurance. Endurance-trained subjects showed smaller level of peripheral fatigue and displayed no significant central fatigue, even at exhaustion and despite greater endurance performance. Our findings also demonstrate that men in the sixth decade exhibit significant alterations in quadriceps function typically observed in much older subjects. These data emphasize the need for developing normative data for both central and peripheral quadriceps fatigability.

Keywords: Muscle, endurance, maximal strength, fatigue, endurance training, aging, magnetic stimulation, femoral nerve

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Introduction

Chronic exposure to exercise training and increasing age critically influence neuromuscular performance.[1] Due to its functional importance and involvement in numerous chronic diseases (e.g. COPD, chronic heart failure, metabolic disorders), quadriceps function is increasingly explored in humans. However, studies providing robust comparative data regarding the influence of training status and aging on quadriceps function (i.e. strength, endurance, central and peripheral fatigability) using identical methodology are lacking.

Basic effects of increase or decrease use of the neuromuscular system have been widely studied.[2,3] Endurance training induces multiple adaptations both at the muscle level (e.g. increased mitochondrial density, decreased insulin resistance, metabolic shift toward increase lipid utilization, improved perfusion, myofibers hypertrophy, fast-to-slow shift in myosin isoform expression) and neural level (e.g. improved motor unit recruitment and firing patterns).[1,4] These adaptations translate into reduced contractile fatigability and greater endurance as previously demonstrated by longitudinal studies.[5] However, studies

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comparing neuromuscular fatigue and endurance in highly trained and untrained individuals are surprisingly scarce and focused on the effects of resistance training.[6,7] Regarding central fatigue, only one study reported lower reduction in voluntary activation (VA) at exhaustion in the plantar flexors of strength-trained compared to untrained men.[6] Furthermore, methodology used in the latter studies exhibited several shortcomings (e.g. only pre- to post-maximal exercise differences were measured, thus time-course of fatigue was not reported; muscle loading was dependent on subject cooperation to reach maximal achievement). In addition, contractile fatigability was not quantified using standard methods such as reduction in motor responses elicited with peripheral nerve stimulation.[8] Therefore, the influence of chronic exposure to endurance training on peripheral and central fatigue both for standardized amount of work and at task failure in comparison with untrained individuals needs to be specifically investigated.

Changes in neuromuscular apparatus associated with aging (e.g. muscle mass loss, motor unit and neuromuscular junction remodeling, altered metabolic capacity[9]) and their effects on performance have been deeply investigated.[10,11] Older individuals have been shown to develop less peripheral fatigue during isometric loading; however, this advantage is lost during dynamic contractions (i.e. particularly when using power reduction as an index of fatigue[12,13]). In most of these studies, the fitness level of participants is unknown and although investigators aimed to match physical activity levels in young and old study groups, the confounding effect related to training status cannot be ruled out.[10,13,14] Another important aspect is that participants recruited in these studies were mostly >65 years and data regarding the early effect of aging on neuromuscular fatigability are lacking.[13,15,16] Age-related changes in the central nervous system might also impair VA capacities before, during, and after a fatiguing task,[15,17,18] but available studies provide controversial results.[19–22]

Therefore, the current work aims to compare fatigue and endurance in young and middle-aged untrained and young endurance-trained men using a recently developed standardized procedure.[23] Based on previous findings aforementioned, we hypothesized that: (i) Young endurance-trained subjects may exhibit smaller peripheral fatigue after identical standardized muscle loading, smaller central fatigue, and greater endurance compared to young untrained subjects; (ii) Middle-aged subjects may exhibit smaller peripheral fatigue after identical standardized muscle loading, larger central fatigue, and greater endurance compared to young subjects with similar fitness level.

Table 1 Subjects' characteristics and results of the maximal cycling test

	Young-UnTr	Young-Tr	MidAge-UnTr
Subjects characteristics			
Age (y)	26 ± 4	29 ± 3	56 ± 4
Height (cm)	175 ± 7	175 ± 5	176 ± 7
Weight (kg)	71 ± 10	68 ± 3	83 ± 11*
BMI ($kg \cdot m^{-2}$)	23.3 ± 2.8	22.2 ± 0.9	26.4 ± 2.5*
Maximal cycling test			
W_{peak} (W)	271 ± 42	365 ± 18††	220 ± 33*
$VO_{2,peak}$ ($l \cdot min^{-1}$)	3.13 ± 0.51	4.99 ± 0.27††	2.95 ± 0.44
$VO_{2,peak}$ ($ml \cdot min^{-1} \cdot kg^{-1}$)	48 ± 6	74 ± 4†††	36 ± 6***
$VO_{2,peak}$ (% Pred)	105 ± 10	168 ± 6†††	109 ± 14
HR_{max} (% predicted)	97 ± 3	94 ± 4	99 ± 5
$[La]_{max}$ ($mmol \cdot L^{-1}$)	12.2 ± 4.9	11.6 ± 3.2	9.5 ± 1.5***
VT1 (% $VO_{2,max}$)	59 ± 4	68 ± 6†	62 ± 5
VT2 (% $VO_{2,max}$)	83 ± 3	87 ± 7	82 ± 3

Note: Mean values ± SD; Young-UnTr, young untrained subjects; Young-Tr, young trained subjects; MidAge-UnTr, middle-aged untrained subjects; BMI, body mass index; $VO_{2,peak}$, peak oxygen consumption; W_{peak}, peak workload; HR_{max}, maximal heart rate; $[La]_{max}$, blood lactate concentration 3 min after exhaustion; VT1, first ventilatory threshold; VT2, second ventilatory threshold.
†Significant difference between Young-UnTr and Young-Tr.
*Significant difference between Young-UnTr and MidAge-UnTr
($P < 0.05$, †† or **$P < 0.01$, ††† or ***$P < 0.001$).

Material and methods

Participants

Twenty-four men were enrolled in this study and gave written informed consent: eight young endurance-trained (Young-Tr, age range 23–32 years, $VO_{2,peak}$ range 87–120% of predicted value), eight young untrained (Young-UnTr, age range 27–33 years, $VO_{2,peak}$ range 159–173% of predicted value), and eight middle-aged untrained (MidAge-UnTr, age range 50–59 years, $VO_{2,peak}$ range 89–127% of predicted value). Young-Tr subjects were triathletes (regional or national levels) who trained at least 12 h per week. Young-UnTr and MidAge-UnTr were not involved in structured physical activity for more than 3 h per week and none had physically demanding professions (i.e. students or office workers). Their main characteristics are displayed in Table 1. All subjects were free from any neuromuscular, metabolic, and cardiorespiratory disorders. The study was conducted according to the Declaration of Helsinki and was approved by the local Committee on Human Research (Comité de protection des personnes Sud-EST V).

Study design

After inclusion, subjects performed two exercise test sessions. During the first visit, subjects underwent clinical examination, anthropometric measurements, and performed a maximal incremental exercise test on a cycle ergometer. On the second session, quadriceps function was assessed (see below).

Maximal cycling test

Subjects performed a standard maximal incremental exercise test on a computer-controlled electrically braked cycle ergometer (Ergometrics 800, Ergoline, Bitz, Germany) with breath-by-breath gas analysis and electrocardiogram (Medisoft, Dinant, Belgium) for the determination of peak workload (W_{peak} i.e. workload at last achieved set) and peak oxygen consumption ($VO_{2,peak}$). Based on usual recommendations, i.e. initial power and increments chosen to obtain an exercise duration ranging from 8 to 12 min,[24] exercise protocols were defined as follows: Initial power was set at 40 W for 2 min in Young-UnTr and MidAge-UnTr and 80 W in Young-Tr. Then, power output was increased by $15\,\mathrm{Wmin}^{-1}$ in Young-UnTr and MidAge-UnTr and $25\,\mathrm{Wmin}^{-1}$ in Young-Tr. A fingertip blood sample was obtained 3 min after exhaustion and analyzed for lactate concentration (NOVA+, Nova Biomedical Corporation, Waltham MA). The first and second ventilatory thresholds were determined from the time-course curves of minute ventilation (VE), VE/VO_2, and VE/VCO_2 (i.e. carbon dioxide production) by one independent operator. The first ventilatory threshold corresponded to the last point before a first non-linear increase in both VE and VE/VO_2. The second ventilatory threshold corresponded to the last point before a second non-linear increase in both VE and VE/VO_2, accompanied by a non-linear increase in VE/VCO_2.[25]

Quadriceps function

Quadriceps function was assessed using a recently designed procedure (i) allowing the combined assessment of quadriceps strength, endurance, and fatigability assessment in a single session, (ii) limiting the influence of psychological and motivational confounding factors using progressive loading, (iii) assessing central and peripheral fatigue kinetics after identical standardized muscle loading rather than at exhaustion only and using single and doublets magnetic nerve stimulations limiting the confounding effect of uncomfortable electrical stimulation trains.[23]

Experimental setup. Measurements were conducted on the right limb for all subjects. Subjects laid reclined on a customized chair. The knee was flexed to 90° and the hip angle was 130° to facilitate coil placement in the femoral triangle. Voluntary strength and evoked responses to magnetic femoral nerve stimulation were measured with a strain gauge (SBB 200 kg Tempo Technologies, Taipei, Taiwan) connected to an inextensible ankle strap. Compensatory movement of the upper body was limited by two belts across the thorax and abdomen. Subjects were instructed to keep their hands on their abdomen at all times. Visual feedback of both the force produced and the target force levels (see below) was provided to the subjects.

Magnetic stimulation. Magnetic femoral nerve stimulation was performed with a 45-mm figure-eight coil powered by two Magstim 200 stimulators (peak magnetic field 2.5 T, stimulation duration 0.1 ms; Magstim, Whitland, United Kingdom) linked with a Bistim Module (Magstim). We used single stimulations (twitch) and paired stimulations (10 Hz and 100 Hz doublets) given at maximum stimulator output. The coil was positioned high in the femoral triangle in regard to the femoral nerve. After 20 min of rest, optimal stimulation site allowing maximal unpotentiated quadriceps twitch as well as maximum *vastus lateralis* M-wave amplitude (M_{ampl}) (see below) was determined and marked on the skin. To overcome confounding effects related to exercise-induced axonal hyperpolarization[26] or minor changes in coil location, the supramaximality of magnetic femoral nerve stimulation was assessed with decreasing stimulator power output (100%, 95%, 90%, 85%, and 80%). Supramaximality was confirmed since unpotentiated quadriceps twitch and M_{ampl} were not significantly reduced until 80% of the maximal power output in all subjects as previously reported.[27] Quadriceps surface EMG signal was recorded from the *vastus lateralis* as a surrogate for the whole quadriceps.[28] EMG signals were amplified (BioAmp, ADInstruments, Sydney, Australia) and 5 to 500 Hz filter was used. EMG and force signals were digitized (Powerlab, ADInstruments) at a sampling frequency of 2000 Hz and recorded (Labchart; ADInstruments).

Quadriceps intermittent fatigue test. After a standardized warm up, subjects performed first three 5-s maximal voluntary contractions (MVC) with 1 min of rest between each MVC. Following these MVCs allowing full muscle potentiation,[29] the baseline neuromuscular assessment was performed. It consisted of a 5-s MVC superimposed with 100-Hz doublet followed 2 s later (i.e. in relaxed muscle) by two potentiated doublets at 100 Hz (Db_{100}) and 10 Hz (Db_{10}) delivered 4 s apart. Fifteen seconds later the subject performed a second MVC followed after 2 s by one potentiated single twitch (Tw_p). During all MVCs, the experimenter vigorously encouraged subjects. Potentiated evoked high- and low-frequency paired stimuli allow assessment of both high- and low-frequency peripheral fatigue[27,29] and high-frequency superimposed stimuli provide optimal resolution for central activation assessment.[28]

Following the baseline assessment, sets of 10 intermittent (5-s on / 5-s off) isometric contractions at submaximal target forces were performed, starting at 10% MVC for the first set and increasing by 10% MVC for each set until task failure. Subjects were given visual feedback of the target force level and listened to a soundtrack with the contraction-relaxation rhythm. The range used for the target force level was defined as $\pm$2.5% of MVC. Task failure was defined as two consecutive contractions below the target force level for more than 2.5 s. Five seconds after the end of each 10-contraction set, at exhaustion and after 30 min of recovery (Post 30), neuromuscular assessments similar to baseline assessments were performed. Right before the neuromuscular assessment at Post 30, subjects performed two MVCs 30 s apart in order to fully potentiate the quadriceps response to magnetic femoral nerve stimulation.[29]

Data analysis

The following parameters were calculated from the mechanical responses to stimulation: peak force for Tw_p, Db_{100}, Db_{10}, and the ratio Db_{10}:Db_{100} ($Db_{10:100}$ as an index of low-frequency peripheral fatigue[27]) to characterize peripheral mechanisms of neuromuscular function. Peak force during superimposed Db_{100} was used to calculate voluntary activation (VA, characterizing central mechanisms of the neuromuscular function, see below). Peak-to-peak M-wave amplitude, area, and latency (from stimulation to first M-wave peak) were calculated from Tw_p to assess possible alterations of action potential propagation.[30] Maximal rates of force development (MRFD) and relaxation (MRFR) were calculated from Tw_p to provide further insights into muscle contractility. VA was calculated from the equation $[(1-(\text{Superimposed } Db_{100}/Db_{100}))\times 100]$. A correction was applied to the original equation when the superimposed stimulation was administrated before or after the maximal MVC force.[31] The following parameters were calculated from submaximal contractions: mean force, force-time product, 4-s root mean square from EMG signal normalized with M_{ampl} (RMS/M). We expressed the increase of RMS/M during each 10-contraction set as percentage increase between the mean three first and the mean three last contractions of each set.

Statistical analysis

All variables are reported as mean ± standard deviation. Normal distribution and homogeneity of variances analysis were confirmed using the Kolmogorov–Smirnov and Skewness test, respectively. One-way ANOVAs were conducted to compare Young-UnTr versus Young-Tr and Young-UnTr versus MidAge-UnTr for subject characteristics and neuromuscular function at Baseline. To compare changes in variables during the QIF test, we used repeated measures ANOVAs within each group since the number of sets completed by Young-UnTr, MidAge-UnTr, and Young-Tr subjects was different. Two-way ANOVAs (time × group) were used to compare changes over the sets completed by all groups. T-tests with Bonferroni's corrections were used for *post hoc* analysis for all ANOVAs conducted. The alpha level was set at 0.05 for all tests. Statistical analysis was performed with a statistical software package (Statistica, Statsoft, Inc., Tulsa, OK, USA).

Results

Maximal cycling test

Main results from the maximal cycling test are shown in Table 1. As expected, Young-Tr had higher W_{peak} and $VO_{2,peak}$ than Young-UnTr subjects and Young-UnTr had higher W_{peak} and $VO_{2,peak}$ than MidAge-UnTr. The first ventilatory threshold occurred at a greater percentage of $VO_{2,peak}$ in trained compared to untrained young participants and no other significant difference between groups was found for the first and the second ventilatory threshold. Blood lactate concentration 3 min after exhaustion was ≥ 8 mmol.l^{-1} and maximum heart rate was ≥ 90% of the theoretical value in all subjects confirming maximal incremental cycling test.

Quadriceps function at baseline

Quadriceps neuromuscular function is shown in Table 2. Volitional and evoked strength were greater in Young-Tr and smaller in MidAge-UnTr compared to Young-UnTr either as absolute values or normalized to body weight. Young-Tr had higher Tw_p MRFD compared to Young-UnTr but this difference did not persist when normalized to Tw_p (data not shown; all $P > 0.05$). Lower Tw_p MRFD and MRFR were observed in MidAge-UnTr compared to Young-UnTr and these differences remained significant when normalized to Tw_p (10.6 ± 1.8 vs. 8.8 ± 1.3 for MRFD and − 4.06 ± 0.6 vs. −3.2 ± 0.8 for MRFR in Young-UnTr and MidAge-UnTr, respectively). MidAge-UnTr showed smaller M-wave amplitude and area and similar duration compared to Young-UnTr. $Db_{10:100}$ was higher in Young-Tr and similar in MidAge-UnTr compared to Young-UnTr. VA was higher in Young-Tr and similar in MidAge-UnTr compared to Young-UnTr.

Table 2 Quadriceps neuromuscular function at Baseline

	Young-UnTr	Young-Tr	MidAge-UnTr
Voluntary strength			
MVC (Nm)	270 ± 35	342 ± 53†††	213 ± 67*
MVC/body weight (Nm·kg^{-1})	3.9 ± 0.6	5.0 ± 07†††	2.6 ± 0.6***
Evoked responses			
Potentiated single twitch			
Tw_p (Nm)	75 ± 8	100 ± 15†††	62 ± 14*
Tw/body weight (Nm·kg^{-1})	1.08 ± 0.21	1.47 ± 0.18†††	0.75 ± 0.07***
Tw_p contraction time (ms)	72 ± 11	75 ± 25	76 ± 12
Tw_p MRFD (Nm·s^{-1})	794 ± 146	1049 ± 189†††	540 ± 119*
Tw_p MRFR (Nm·s^{-1})	−305 ± 58	−361 ± 69	−195 ± 54**
M-wave amplitude (mV)	12.5 ± 2.4	12.4 ± 6.1	7.0 ± 3.5**
M-wave duration (ms)	10.4 ± 2.4	9.6 ± 3.4	11.9 ± 0.8
M-wave area (mV·ms)	0.14 ± 0.03	0.10 ± 0.04	0.08 ± 0.03**
Potentiated doublets			
Db_{100} (Nm)	113 ± 12	144 ± 19†††	94 ± 20*
Db_{100}/body weight (Nm·kg^{-1})	1.64 ± 0.27	2.12 ± 0.23†††	1.13 ± 0.13***
$Db_{10:100}$	0.96 ± 0.11	1.07 ± 0.06†	0.89 ± 0.08
Central parameters			
VA (%)	91.2 ± 3.4	94.2 ± 0.9†	91.7 ± 3.6

Note: Mean values ± SD; Young-UnTr, young untrained subjects; Young-Tr, young trained subjects; MidAge-UnTr, middle-aged untrained subjects; MVC, maximum voluntary contraction; Db_{100}, peak potentiated 100 Hz doublet; Tw_p, peak potentiated single twitch; MRFD, maximal rate of force development; MRFR, maximal rate of force relaxation; $Db_{10:100}$, ratio of the peak potentiated doublets at 10 over 100 Hz; VA, voluntary activation level.
†Significant difference between Young-UnTr and Young-Tr
*Significant difference between Young-UnTr and MidAge-UnTr ($P < 0.05$, †† or **$P < 0.01$, ††† or ***$P < 0.001$).

Quadriceps fatigue

Endurance. The total number of submaximal contractions was greater in Young-Tr (81 ± 8; $P < 0.001$) and similar in MidAge-UnTr (59 ± 11; $P = 0.95$) compared to Young-UnTr (59 ± 6). The ratio of the last submaximal contraction (i.e. at exhaustion) and the first following MVC at exhaustion was similar in Young-UnTr (0.99 ± 0.13), Young-Tr (1.00 ± 0.06, $P = 0.78$), and MidAge-UnTr (0.95 ± 0.08, $P = 0.55$).

MVCs and evoked responses. Representative recordings of MVC, EMG, and responses elicited with magnetic femoral nerve stimulation are displayed in Figure 1. Changes in MVC and evoked muscular responses (Tw_p, Db_{100}, $Db_{10:100}$)

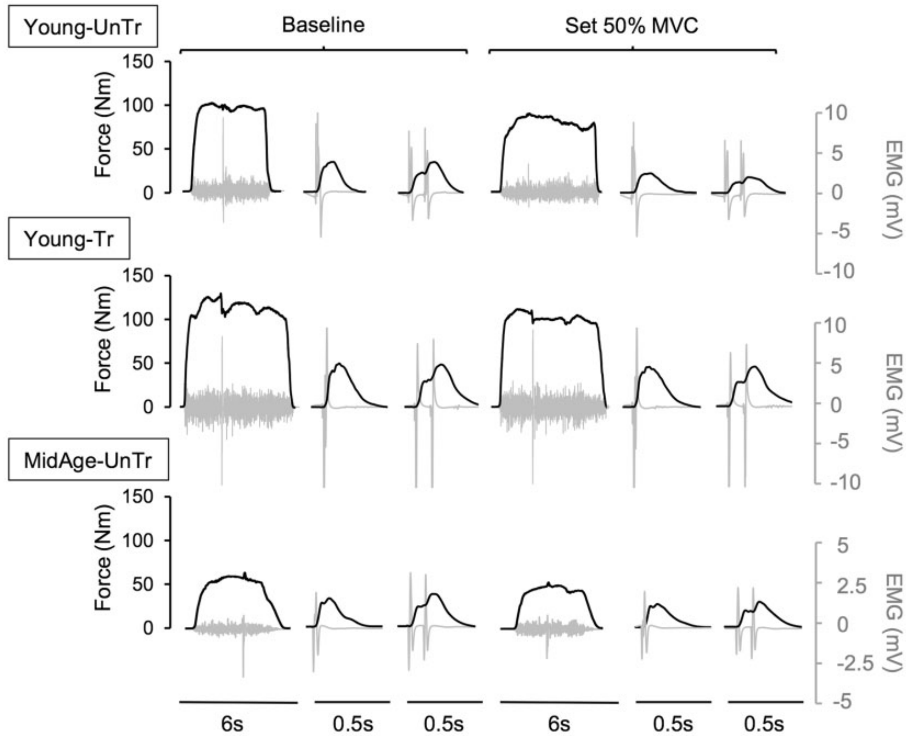

Figure 1 Representative recordings of maximal voluntary strength (MVC), electromyography data (EMG), and responses to magnetic femoral nerve stimulation in young untrained (Young-UnTr), young trained (Young-Tr) and middle-aged untrained (MidAge-UnTr) subjects. Force and EMG signals recorded during MVC, 100 Hz and 10 Hz doublets are shown at baseline and after the 50% MVC set, respectively. Note stimulation artifacts on the EMG signal indicating magnetic femoral nerve stimulations

during the quadriceps fatigue test are displayed in Figures 2 and 3. Young-UnTr exhibited greater reduction in MVC, Tw_p, and Db_{100} compared to Young-Tr (time × group interaction: all $P < 0.001$) and MidAge-UnTr (time × group interaction: all $P < 0.01$). No significant change over time was found in M-wave characteristics in all groups (all $P > 0.05$; data not shown). Young-UnTr exhibits greater and earlier rise in RMS/M during sets than Young-Tr and MidAge-UnTr (ANOVA interaction effect, $P < 0.01$; Figure 4). From exhaustion to Post 30, Young-UnTr showed a significant recovery in evoked responses, while these parameters remained stable in Young-Tr and MidAge-UnTr (see Figure 3).

VA. Changes in VA are shown in Figure 2. Young-UnTr and MidAge-UnTr had a significant reduction in VA at the end of the test, while Young-Tr subjects did not (main time effect: $P = 0.35$). No significant differences were found for VA between Young-UnTr and Young-Tr (time × group interaction: $P = 0.84$) and between Young-UnTr and MidAge-UnTr (time × group interaction: $P = 0.81$).

Discussion

The current study investigated the impact of fitness level and age (young vs. middle-aged men) on quadriceps strength, endurance, and fatigability using isometric intermittent muscle loading and magnetic femoral nerve stimulation as recently proposed by our group. The main results are: (1) compared to Young-UnTr, Young-Tr showed smaller decrease in MVC and evoked responses both after identical standardized muscle loading (with better endurance as anticipated) and at task failure, (2) Young-Tr was the only group to show no significant decreases in VA; (3) MidAge-UnTr subjects exhibited smaller decrease in MVC and evoked responses after identical standardized muscle loading and at task failure than Young-UnTr subjects but similar endurance and central fatigue.

Fitness level-related differences

Quadriceps function at baseline. Evoked strength either absolute or normalized to body weight was ~20% greater and VA was slightly larger in Young-Tr compared to Young-UnTr. These results illustrate that greater maximal voluntary strength production in highly trained subjects relies on both muscular (e.g. increased muscle mass, myofibers hypertrophy) and neural (e.g. supraspinal/spinal circuitry adaptations, enhanced motor unit firing/recruitment) adaptations induced by chronic exposure to exercise.[1,4,32] These results are also in line with previous studies reporting greater soleus activation in strength-trained compared to untrained individuals.[6] Greater $Db_{10:100}$ in Young-Tr compared to Young-UnTr might be interpreted as a left force-frequency shift induced by endurance training previously demonstrated in longitudinal studies.[33] However, in some individuals, $Db_{10:100}$ values were above 1 suggesting lower temporal summation during stimulation at 100 Hz compared to 10 Hz. Although changes in $Db_{10:100}$ have been shown to provide similar estimation of high- versus

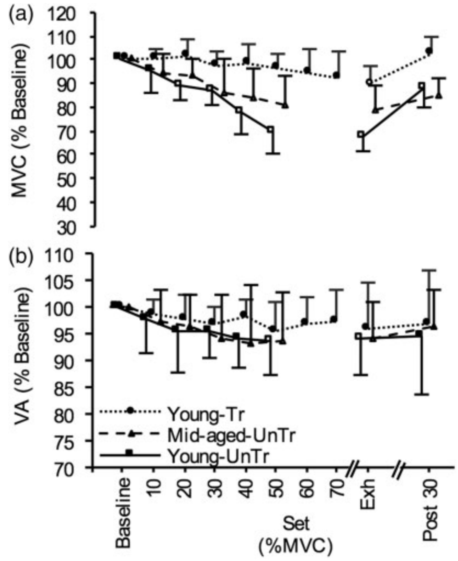

Figure 2 Maximal voluntary strength (MVC, Panel A) and voluntary activation (VA, Panel B) during the quadriceps intermittent fatigue test (QIF) in young untrained (Young-UnTr), young trained (Young-Tr) and middle-aged untrained (MidAge-UnTr) subjects. Baseline, initial measurement; 10–70, measurements after sets of 10 contractions corresponding to 10-70% of MVC; Exh, measurement immediately after exhaustion; Post 30, measurement 30 min after exhaustion; White-filled markers indicate significant difference from Baseline for each group ($P < 0.05$); †significant difference between Young-UnTr and Young-Tr; *significant difference between Young-UnTr and MidAge-UnTr ($P < 0.05$)

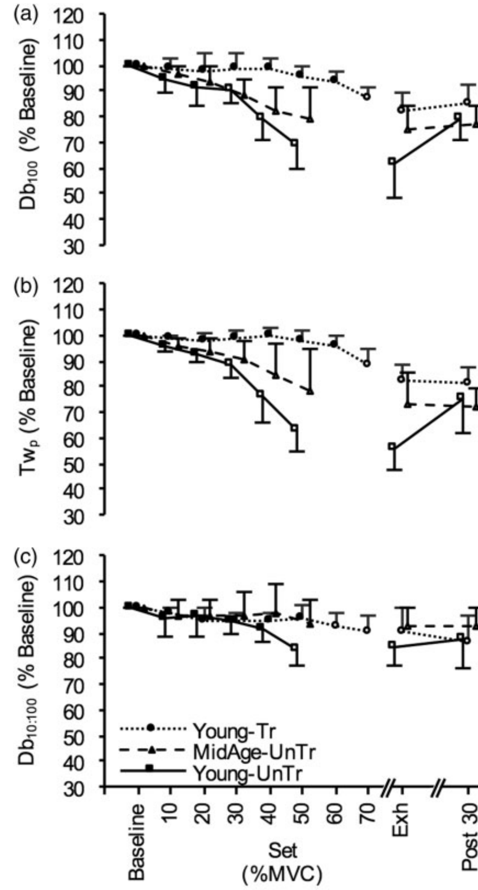

Figure 3 Potentiated peak 100 Hz doublets (Db_{100}, Panel A), potentiated peak twitch (Tw_p, Panel B) and ratio of potentiated 10 Hz over 100 Hz doublets ($Db_{10:100}$, Panel C) during the quadriceps intermittent fatigue test in young untrained (Young-UnTr), young trained (Young-Tr) and middle-aged untrained (MidAge-UnTr) subjects. Baseline, initial measurement; 10–70, measurements after sets of 10 contractions corresponding to 10-70% of MVC; Exh, measurement immediately after exhaustion; Post 30, measurement 30 min after exhaustion; White-filled markers indicate significant difference from Baseline for each group ($P < 0.05$); †significant difference between Young-UnTr and Young-Tr; *significant difference between Young-UnTr and MidAge-UnTr ($P < 0.05$)

low-frequency fatigue compared to ratio obtained with tetanic stimulation trains,[27] this variable should not be used to describe muscle contractile properties in the unfatigued state.

Quadriceps function during the fatigue test. Delayed and smaller reductions in both volitional and evoked muscular responses in highly trained compared to untrained subjects illustrate muscle adaptations induced by endurance training previously demonstrated by interventional studies (e.g. enhanced oxidative and glycolytic enzymatic activities, increased blood flow capacity, fast-to-slow shift in myosin isoform expression).[34] Accordingly, delayed and depressed increase in RMS/M demonstrates lower compensation for peripheral fatigue in Young-Tr (Figure 4). Young-Tr exhibited greater endurance, which is consistent with reduced contractile fatigability. Young-Tr did not exhibit a significant decrease in VA during the fatiguing task suggesting a reduced propensity to develop central fatigue. This is in line with delayed reduction in VA during exercise previously reported in strength-trained subjects in a cross-sectional study[6] and following endurance training after identical muscle loading.[5] Lack of detection of significant central fatigue at the group level at task failure in Young-Tr was, however, surprising. One potential explanation may be that the progressive exercise paradigm and assessment procedures employed in the current study may have induced a minimal amount of central fatigue in these highly trained individuals that the twitch interpolation technique failed to detect.[35,36] The use of additional measurements (e.g. using

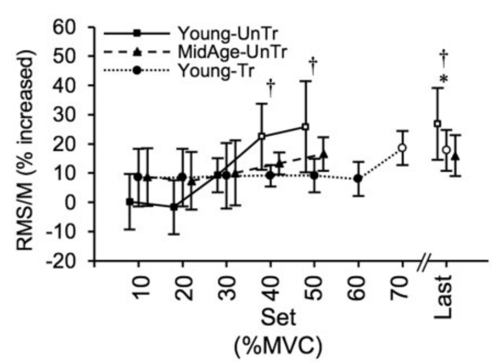

Figure 4 Percentage increase of root mean square normalized to M-wave amplitude (RMS/M) during submaximal contractions sets in young untrained (Young-UnTr), young trained (Young-Tr) and middle-aged untrained (MidAge-UnTr) subjects. Last, last ended set; White-filled markers indicate significant difference from Baseline for each group ($P < 0.05$); †significant difference between Young-UnTr and Young-Tr; *significant difference between Young-UnTr and MidAge-UnTr ($P < 0.05$)

transcranial and cervicomedullary stimulations[37]) may be useful to gain more insights into spinal and supraspinal factors responsible for task failure in these subjects. Conversely, the low level of contractile fatigue at exhaustion in Young-Tr (i.e. ~10 % reduction in Tw_p and Db_{100}) supports the contribution of central factors responsible for task failure (e.g. inability to repeatedly produce very high central drive, induction of muscle or joint discomfort[35,38]). The influence of chronic exposure to endurance training on factors responsible for task failure should be further investigated using different types of fatiguing task inducing variable amounts of central and/or peripheral fatigue (low- vs. high-intensity; short- vs. long-duration; sustained vs. intermittent).

Age-related differences

Young-UnTr and MidAge-UnTr had $VO_{2,peak}$ values corresponding to similar percentages of their age-predicted $VO_{2,peak}$ (~108 vs. 109%, respectively; see Table 1) supporting that differences observed in quadriceps strength and fatigability can mainly be attributed to aging processes and not to differences in fitness level.

Quadriceps function at baseline. Volitional and evoked strength either absolute or normalized to body weight was ~30% smaller in MidAge-UnTr compared to Young-UnTr. MidAge-UnTr exhibited similar ability to reach high VA levels (i.e. >90%) compared to Young-UnTr subjects. Similar results on the quadriceps were reported in much older (~80 years) subjects,[22] supporting the limited contribution of VA deficit to impaired maximal strength with increasing age.[18,39] Therefore, lower volitional and evoked strength in older subjects may be mainly attributed to a reduction in the number/size of type II myofibers and to a lesser extent type I myofibers,[40,41] and to motor unit remodeling.[42] Although it was not assessed in the present work, greater antagonist muscle activation during MVC may also contribute to reduced strength in older participants.[43] MidAge-UnTr subjects exhibit lower MRFD and MRFR either as absolute values or normalized to Tw_p amplitude, indicating slower muscle contractile properties supporting reduced type II myofibers size/number.[10] Neuromuscular junction remodeling and decreased efficiency of Ca^{2+} handling may also contribute to slowing contractility.[44,45] In line with previous reports,[46] MidAge-UnTr also exhibited smaller M-wave amplitude and area that may be due, at least in part, to smaller muscle size and excitation-contraction uncoupling.[42]

Quadriceps function during the fatigue test. After identical standardized muscle loading, MidAge-UnTr exhibited lower reduction in MVC indicating lower quadriceps fatigability compared to Young-UnTr (see Figure 2). Lower decrease in evoked strength (i.e. Db_{100}, Db_{10}, Tw_p) demonstrated lower contractile fatigability in older participants, while smaller reduction in $Db_{10:100}$ confirmed smaller sensitivity to low-frequency fatigue (See Figure 3). The absence of significant alteration of M-wave characteristics in both groups excludes the involvement of neuromuscular propagation failure. Lower rise in RMS/M in MidAge-UnTr compared to Young-UnTr during the last submaximal contraction set is consistent with the lower amount of induced-peripheral fatigue in MidAge-UnTr. These findings are in line with findings from a previous meta-analysis that demonstrated reduced contractile fatigability, at least during isometric loading, in subject above 70 years of age.[13] This fatigue-resistant phenotype may be related to both baseline (e.g. slower muscle phenotype induced by reduction in type II myofibers size/number[47]) and within-exercise (e.g. metabolic economy, increased force production for a given motor unit discharge rate[48]) alterations in the aged neuromuscular system. The current work therefore demonstrates that men in the sixth decade exhibit similar reduction in quadriceps fatigability as observed in much older subjects. The question whether endurance-trained middle-aged men would exhibit less marked changes in peripheral fatigability remains to be addressed.

Regarding central factors, similar VA reductions in both groups suggest that, in contrast to peripheral fatigability, central fatigability was similar in MidAge-UnTr compared to Young-UnTr. This supports findings from Kent-Braun *et al.*[20] who reported no effects of aging on VA reduction of ankle dorsiflexors during an incremental isometric task. The effects of aging on central fatigue have been assessed in various other muscle groups (e.g. tibialis anterior,[49] plantar flexor,[50] elbow flexor[21]) with contrasted results probably due to the variety of stimulation protocols, the nature of the fatiguing task, the age range, and the fitness level of participants. In a recent study, Yoon *et al.*[51] reported no effect of age on central fatigue assessed by transcranial magnetic stimulation during both isometric and dynamic loading, which supports our findings. Interestingly, MidAge-UnTr exhibited similar endurance (i.e. the total number of submaximal contractions) compared to Young-UnTr while greater endurance may have been expected considering the lower peripheral fatigability in older subjects. These results contrast with previous studies reporting longer endurance time in older compared with younger adults[13] and could be attributable to the incremental muscle loading used in the current study. Similar reduction of VA at exhaustion and comparable ratio of the last submaximal contraction and the first following MVC suggest that alterations in voluntary drive cannot explain these results (i.e. similar endurance performance with less peripheral fatigue). Alterations in sensory information processing associated with aging, including increased nociceptive sensitivity may also contribute to the reduced ability to maintain high-intensity isometric contractions despite lower levels of peripheral.[52,53] A fatiguing task inducing larger amounts of central fatigue may also help to address this issue as well as alternative measurements of central fatigue in order to overcome limitations related to the twitch interpolation technique.[54] Our results also showed that Young-UnTr exhibited greater recovery in evoked responses from exhaustion to Post 30 compared to MidAge-UnTr. This may be partly attributed to the smaller level of peripheral fatigue in MidAge-UnTr at exhaustion.[55] Conversely, similar VA recovery in Young-UnTr and MidAge-UnTr does not corroborate delayed recovery of neural centers with aging previously reported probably due to the limited amount of central fatigue induced.[17,21,56] Although Young-UnTr and MidAge-UnTr had similar endurance, it is important to note that measurements performed 30 min after task failure were not performed after strictly identical standardized muscle loading and should thus be interpreted with caution.

The present study provides a robust comparison of quadriceps function (i.e. strength, endurance, peripheral and central fatigability) between young and middle-aged untrained men and young men chronically exposed to endurance training. Endurance-trained subjects showed smaller level of peripheral fatigue and displayed no significant central fatigue, even at exhaustion and despite greater endurance performance. Our findings also demonstrate that men in the sixth decade exhibit significant alterations in quadriceps function that are typically observed in much older subjects. These data highlight the importance of using identical neuromuscular assessment procedures when comparing populations and emphasize the need for developing normative data for both central and peripheral quadriceps fatigability.

Authors' contribution: All authors contributed to the conception and design of the experiments. DB and ND collected and analyzed the data. DB and SV drafted the original manuscript. All authors contributed to data interpretation and critical revision of the manuscript and all authors approved the final version of the manuscript.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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