Intrinsic motor neurone excitability is increased after resistance training in older adults

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ABSTRACT

This study investigated the effects of high-intensity resistance training on estimates of the motor neurone persistent inward current (PIC) in older adults. Seventeen participants (68.5±2.8 years) completed a 2-week non-exercise control period followed by 6 weeks of resistance training. Surface electromyographic signals were collected using two 32-channel electrodes placed over *soleus* to investigate motor unit discharge rates. Paired-motor unit analysis was used to calculate delta frequency (ΔF) as an estimate of PIC amplitudes during (a) triangular-shaped contractions to 20% of maximum torque capacity, and (b) trapezoidal- and triangular-shaped contractions to 20% and 40% of maximum torque capacity, respectively, to understand their ability to modulate PICs as contraction intensity increases. Maximal strength and functional capacity tests were also assessed. For the 20% triangular-shaped contractions, ΔF (0.58-0.87 pps; $p \leq 0.015$) and peak discharge rates (0.78-0.99 pps; $p \leq 0.005$) increased after training, indicating increased PIC amplitude. PIC modulation also improved after training. During the control period, mean ΔF differences between 20% trapezoidal- and 40% triangular-shaped contractions were 0.09-0.18 pps ($p=0.448$ and 0.109, respectively), which increased to 0.44 pps ($p<0.001$) after training. Also, moderate-to-very large correlations ($r=0.39$-0.82) were observed between changes in 20% triangular-shaped contraction ΔF and changes in peak discharge rates and broad measures of motor function. Our findings indicate, for the first time, that increased motor neurone excitability is a potential mechanism underpinning training-induced improvements in motor neurone discharge rate, strength, and motor function in older adults. This increased excitability is likely mediated by enhanced PIC amplitudes, which are larger at higher contraction intensities.

Key words: persistent inward currents, motor unit, strength training, aging.

INTRODUCTION

Ageing is accompanied by a diminished ability to produce muscular force and power (Suetta et al., 2019), leading to impairments in physical function and increasing falls risk (Alcazar et al., 2021; Lanza et al., 2021; L. B. da R. Orssatto et al., 2020). Age-related alterations within the nervous system, including in the motor neurone, could partially underpin these dysfunctions (Hepple & Rice, 2015; Manini et al., 2013; L. B. da R. Orssatto et al., 2018; L. B. R. Orssatto et al., 2022). Motor neurones are chiefly responsible for integrating and amplifying synaptic input into motor neurone discharge of the innervated muscle fibres (Heckman & Enoka, 2012). In the presence of monoaminergic input, motor neurone voltage-sensitive sodium and calcium channels, located mainly on the dendrites, can produce ongoing depolarising currents known as persistent inward currents (PICs) (Heckman et al., 2005; Lee & Heckman, 1999a, 2000). PICs generate a non-linear input-output relation between motor neurone synaptic input and discharging output (Binder et al., 2020; Johnson & Heckman, 2014) that is strongly influenced by the level of serotonergic and noradrenergic input received at the motor neurones. Thus, PIC amplification may vary according to the task's demand, allowing discharge rate and hence voluntary drive and force adjustment (Heckman, 1994; Heckman & Binder, 1991; Huh et al., 2017; Johnson & Heckman, 2014; Naufel et al., 2019; L. B. R. Orssatto, Mackay, et al., 2021; Powers et al., 2008; Powers & Heckman, 2015). This process works as a variable gain mechanism in which greater monoaminergic input would enhance PIC strength when greater force levels were required (L. B. R. Orssatto, Mackay, et al., 2021). Indeed, PICs are essential for motor neuron discharge rate modulation and thus strongly influence motor control. Recently, reductions in PIC amplitudes have been detected in the motor neurones of older adults (Hassan et al., 2021; L. B. R. Orssatto, Borg, et al., 2021), with recent evidence indicating that the lower motor neuronal discharge rates and reduced motor control ability older adults is linked to this loss of PIC function (L. B. R. Orssatto, Borg, et al., 2021; L. B. R. Orssatto, Mesquita, et al., 2021). As PICs may play such a vital role in motor control as well as the maintenance of motor function in ageing, strategies that might mitigate the weakening of PICs and thus counteract the losses of discharge rate and minimise the loss of motor function with ageing are highly sought after (Latella, 2021; L. B. R. Orssatto, Mesquita, et al., 2021).

Resistance training evokes significant improvements in maximal muscular strength and power, functional capacity, and muscle size in older adults (Byrne et al., 2016; Csapo & Alegre, 2016; da Rosa Orssatto et al., 2019; Peterson et al., 2010, 2011). At the motor neurone level, short-term isometric resistance training has been observed to increase maximal discharge rates (Christie & Kamen, 2010; Kamen & Knight, 2004) and reduce the action potential afterhyperpolarisation duration (Christie & Kamen, 2010) in older adults. These findings are relevant because increases in motor neurone discharge rates are known to mediate the increases in force following short-term resistance training (Del Vecchio et al., 2019). However, it is still uncertain if these resistance training adaptations could be facilitated by an increase in PIC amplitudes. Evidence from rat studies shows that motor neurones needed lower input currents for motor unit activation and to achieve rhythmic discharge, discharged at higher rates per amount of current injection (i.e., increased frequency-current relationship slopes, and thus gain), and produced higher steady-state discharge rates after a block of resistance training, implying that enhancements in PIC amplitudes were triggered by the training (Krutki et al., 2017). Therefore, resistance exercise may be a promising strategy to enhance PIC amplitudes, which may then promote improvements in motor neurone discharge rates and counteract the neuromuscular system deterioration observed with ageing (Arnold & Bautmans, 2014; Walker, 2021). However, this hypothesis remains to be explicitly tested.

To our knowledge, evidence for increased PICs after exercise training or other forms of muscle loading derived primarily from either invasive animal experiments or computational modelling (P. Gardiner et al., 2006; Ge & Dai, 2020; Krutki et al., 2015, 2017). However, PIC amplitudes can be estimated non-invasively in human motor neurones by pairing the discharge profiles of a higher-threshold with a lower-threshold motor unit obtained during slowly increasing and decreasing triangular- or trapezoidal-shaped contractions (Gorassini et al., 2002b; Powers et al., 2008; Stephenson & Maluf, 2011). The difference in discharge rate of the control unit at the time of recruitment and de-recruitment of the test unit is described as the change in (Δ) frequency (ΔF) (Gorassini et al., 2002b; Powers et al., 2008; Stephenson & Maluf, 2011), which is understood to be proportional to PIC amplitude (10, 13). Thus, changes in ΔF following exercise training should reflect changes in PIC amplitudes. Moreover, the PIC-related variable gain system is modulated by the level of monoaminergic input onto the motor neurones according to the increase of the level of voluntary descending drive (Lee & Heckman, 1999a, 2000; Naufel et al., 2019; L. B. R. Orssatto, Mackay, et al., 2021). This can be estimated in humans by measuring ΔF for the same motor neurones at different contraction intensities with matched rates of force rise and fall and contraction duration (L. B. R. Orssatto, Mackay, et al., 2021). Increased ΔF at higher contraction intensities will indicate a greater monoaminergic input onto motor neurones (L. B. R. Orssatto, Mackay, et al., 2021). In the present study, the effects of a 6-week resistance training in older adults on estimates of PIC amplitude (i.e., ΔF) and its modulation according to the level of voluntary descending drive (i.e., its changes at different contraction intensities) was examined. Additionally, we explored the relationship between changes in ΔF and peak discharge rates, maximum muscular strength, and performances in complex motor function tests. We therefore tested the hypotheses that ΔF would increase after resistance training in older adults, that its increase would be greater at higher contraction intensities, and that its changes would be correlated with changes in muscle strength and motor function.

METHODS

Study design and participants

This study adopted a single-arm trial design in which all eligible participants were allocated into a single intervention group. They completed a familiarisation session in which triangular- and trapezoidal-shaped contractions and maximal plantar flexion isometric contractions, countermovement jump, sit-to-stand, and timed up-and-go assessments were practiced. At least one week later, they performed a testing session (i.e., *-2 weeks*), followed by a 2-week no-training control period after which the testing session was repeated (*0 weeks*). During the control period, participants were requested to maintain their current physical activity and diet routines. After the control period, the participants performed a 6-week resistance training program before completing a third testing session (*6 weeks*) 72 – 96 h after the final training session.

To be accepted into the study the participants had to be: a) aged 65 years or older; b) not taking medications that could influence the monoaminergic system, such as serotonin or noradrenaline (e.g., beta-blockers and serotonin reuptake inhibitors); c) free from any lower body musculoskeletal disorders that could influence testing outcomes or resistance training performance; d) not undertaking resistance training practice in the last year; and e) willing to avoid caffeinated drinks 24 h prior to each testing session. This study was approved by the University Human Research Ethics Committee, and all participants gave written informed consent before participating. Data collection was conducted during the COVID-19 pandemic and all safety procedures followed the local state government policies.

Outcome testing procedures and analyses

At *-2 weeks*, *0 weeks*, and *+6 weeks*, *soleus*, *gastrocnemius medialis* and *gastrocnemius lateralis* muscle size, body composition, plantar flexion torque with high-density electromyography recordings were conducted followed by the functional capacity assessments, in the respective order. The leg press 1-repetition maximum (1-RM) test was conducted before the beginning of sessions 2 and 12 of the training period as training session 1 acted as a familiarisation to this exercise. Standardised instructions and loud verbal encouragement were given to all participants in all testing sessions.

Torque data acquisition and analysis

The participants were seated upright in the chair of an isokinetic dynamometer (Biodex System 4, Biodex Medical system, Shirley, NY) with the knee fully extended (0˚) and ankle in the anatomical position (0˚). A warm-up consisting of six 5-s submaximal voluntary isometric plantar flexion contractions (2 × 30%, 2 × 60%, and 2 × 80% of perceived maximal effort) was performed, followed by a 3-min rest. Subsequently, three maximal voluntary plantar flexion contractions lasting ~3-s each with 60-s rest intervals were performed, from which the peak torque was recorded. The participants then performed 3-5 plantar flexion practice trials of the triangular-shaped contractions to 20% of their peak torque. They followed the torque path provided in real time on a 58-cm computer monitor during each contraction. Data collection commenced 5 min after the end of practice contractions, during which the participants then performed four triangular-shaped contractions to 20% of their peak torque with a rate of torque rise and decline of 2%/s, and with 60-s rest intervals. Thereafter, participants performed two trapezoidal-shaped contractions to 20% and two triangular-shaped contractions to 40% of their maximal torque capacity. Both trapezoidal- and triangular-shaped contraction had a duration of 30 s and a rate of torque increase and decrease of 20% per 7.5 s. Contraction duration and rate of torque increase and decrease were identical between contraction intensities because: a) longer muscle contractions result in a spike frequency adaptation, making motor units discharge at lower frequencies for a given force, causing larger ΔF (Powers & Heckman, 2015; Vandenberk & Kalmar, 2014); and b) faster increases can reduce ΔF through spike-threshold accommodation (Powers & Heckman, 2015; Vandenberk & Kalmar, 2014). This strategic identification of contraction intensity-dependent ΔF modulation in young adults has been reported in a previous study (L. B. R. Orssatto, Mackay, et al., 2021). When an abrupt torque increase or decrease was observed (i.e., the torque trajectory was not closely followed) for any of the triangular- or trapezoidal contractions, the trial was excluded and repeated. The order of the 20% trapezoidal- and 40% triangular-shaped contractions was randomised. During offline analysis, torque data were low-pass filtered with a 15-Hz cut-off frequency using a fourth-order zero-lag Butterworth filter (Blazevich et al., 2007). The peak torque was determined as the highest point in the torque-time curve.

Surface electromyography acquisition and analysis

Surface electromyograms (sEMG) were recorded during the 20% and 40% triangular-shaped and 20% trapezoidal-shaped contractions using two semi-disposable 32-channel electrode grids with a 10-mm interelectrode distance (ELSCH032NM6, OTBioelettronica, Torino, Italy). After shaving, abrading, and cleaning the skin with 70% isopropyl alcohol, two electrode grids were placed over the medial and lateral portions of *soleus* (either side of the Achilles tendon) using a bi-adhesive foam layer and conductive paste (Ten20, Weaver and Company, Colorado, USA). A strap electrode (WS2, OTBioelettronica, Torino, Italy) was dampened and positioned around the ankle joint as a ground electrode. To ensure the same electrode positioning across all assessments timepoints, anatomic landmarks and skin marks were traced onto individual acetate sheets during the first assessment session. The sEMG signals were acquired in monopolar mode, amplified (256×), band-pass filtered (10–500 Hz), and converted to a digital signal at 2048 Hz by a 16-bit wireless amplifier (Sessantaquattro, OTBioelettronica, Torino, Italy) using OTBioLab+ software (version 1.3.0., OTBioelettronica, Torino, Italy) before being stored for offline analysis.

The recorded data were processed offline using the DEMUSE software (Holobar & Zazula, 2007). For each contraction, only the triangular or trapezoidal contractions yielding the lowest deviation from the torque trajectory were analysed. If both contractions presented a similar torque trajectory, the contraction with the highest number of identified motor units was analysed. High-density sEMG signals were band-pass filtered (20-500 Hz) with a second-order, zero-lag Butterworth filter. Thereafter, a blind source separation method, the convolutive kernel compensation method, was used for signal decomposition (Holobar et al., 2014; Holobar & Zazula, 2007) from each triangular contraction. Convolutive kernel compensation yields the filters of individual motor units (so-called motor unit filters) that, when applied to high-density sEMG signals, estimate the motor unit spike trains (Holobar et al., 2014; Holobar & Zazula, 2007). To identify the same motor unit at each time point (i.e., *-2 weeks*, *0 week*, and *+6 weeks*), motor unit filters identified by convolutive kernel compensation at individual contractions on each time point were applied to the concatenated high-density sEMG signals recorded at other time points. Afterwards, motor unit filters identified from each time point were applied to the concatenated recordings (Francic & Holobar, 2021) yielding the motor unit spike trains of all the identified motor units across all the concatenated time points. After removing motor unit duplicates simultaneously identified from two or more time points a trained investigator manually inspected motor unit spike trains and edited the discharge patterns of the motor units. Only the motor units with a pulse-to-noise ratio equal to or greater than 30 dB were kept for further analysis (Holobar et al., 2014).

For the motor unit discharge rate analyses, the observed discharge events for each motor unit were converted into instantaneous discharge rates and fitted with a 5th-order polynomial function. The maximum value obtained from the polynomial curve was considered the peak discharge rate. Thereafter, PIC amplitude was estimated using the paired motor unit analysis (Gorassini et al., 2002a). Motor units with a low recruitment threshold (i.e., control units) were paired with higher recruitment threshold motor units (i.e., test units). ΔF was calculated as the change in discharge rates of the control motor unit from the moment of recruitment to the moment of de-recruitment of the test unit (Gorassini et al., 2002a; Heckman et al., 2005). In order to produce motor unit pairs, the following criteria were adopted: 1) rate-rate correlations between the smoothed discharge rate polynomials of the test and control units was $r \geq 0.7$; 2) test units were recruited at least 1.0 s after the control units; and 3) the control unit did not show discharge rate saturation after the moment of test unit recruitment (>0.5 pps) (Binder et al., 2020; Gorassini et al., 2002a; Hassan et al., 2020; Udina et al., 2010; Vandenberk & Kalmar, 2014). Figure 1 shows an example of a paired motor unit analysis using two motor units tracked across *-2 weeks* and *+6 weeks*.

Motor units from the 20% triangular contractions were analysed in two ways:

a) Unmatched motor units: all the motor unit pairs identified on each time point were included in the analysis. This method allows inclusion of a greater number of motor units; however, it does not inform whether the same motor units were compared across time points.

b) Matched motor units: only pairs of motor units identified in at least two time points were included. This method ensures that the discharge rates of the same motor unit is compared between time points. However, fewer than 5% of the total motor units and pairs could be tracked and identified at all the three time points (i.e., *-2 weeks*, *0 week*, and *+6 weeks*) using this method because of the method's limitations. Therefore, we first compared motor units tracked across the Control period (*-2 weeks* to *0 week*). Thereafter, we merged the data from *-2 weeks* and *0 weeks* (named as "*Control period*") to increase the number of motor units tracked to *+6 weeks*. In this case, motor units identified at either *-2 weeks* or *0 week*, or both, as well as *+6 weeks*, were included. Data from motor units identified at *-2 weeks* and *0 weeks* were averaged before compared to *+6 weeks*.

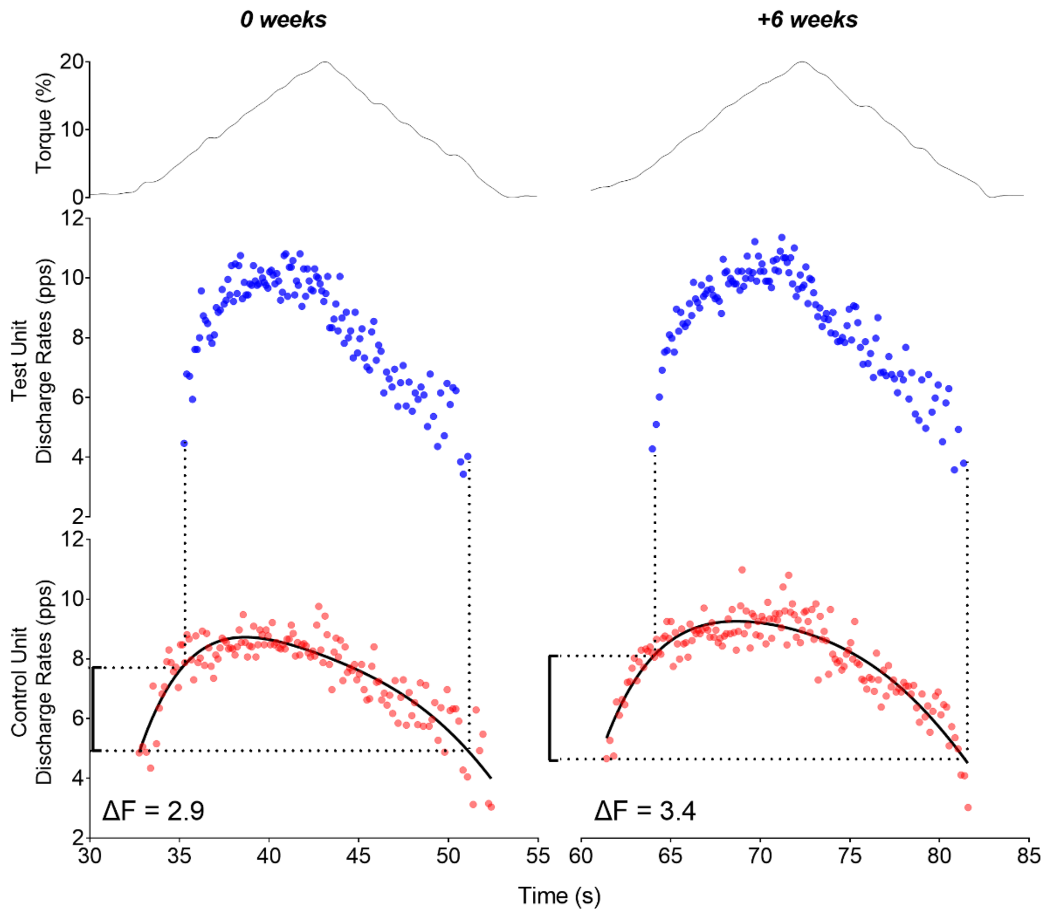

Figure 1. Data illustrating the delta frequency (ΔF) calculation from a single participant for triangular-shaped contractions to 20% of their peak torque. The same pair of motor units was identified at *0 weeks* (displayed in the left panels) and *+6 weeks* (in the right panels). Panels on the first row show the torque traces for contractions with 20% of the participant's peak torque. The participants' control units are displayed on the bottom row (red motor units) and test units on the middle rows (blue motor unit). Brackets indicate the ΔF values. The black continuous lines are the 5th-order polynomial fits for the control units.

Motor units from the 20% trapezoidal and 40% trapezoidal contractions were tracked between intensities within each time point. Therefore, the motor units included in this analysis have a recruitment threshold ranging from 0 to 20% of each participant's peak torque. This strategy was adopted because the main goal of the present analysis was to quantify the modulation of ΔF with increases in neural drive (i.e., changes in ΔF with increased contraction intensity) (L. B. R. Orssatto, Mackay, et al., 2021). In addition, motor units were not tracked across time points because it would significantly reduce the number of motor units included in the analysis. Figure 2 illustrates an example of two distinct paired motor unit analyses, before and after the resistance training.

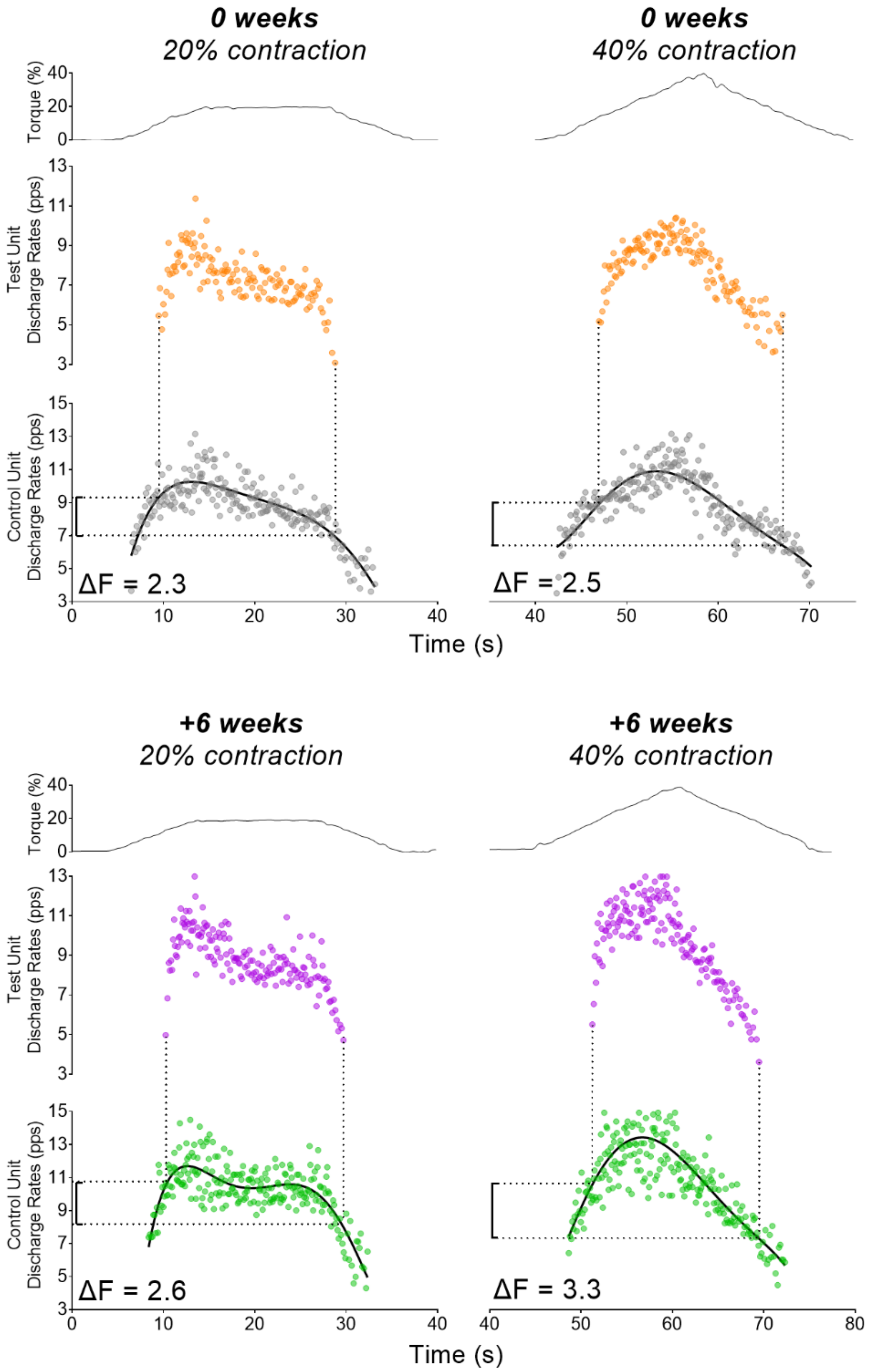

Figure 2. Data illustrating the delta frequency (ΔF) calculation from a single participant for triangular- and trapezoidal-shaped contractions to 20 and 40% of their peak torque, respectively. Different colours indicate the discharge rates for different motor units. Note

that the same pairs of motor units were identified at the 20% (displayed in the left panels) and 40% contractions (in the right panels). But motor units have not been tracked across time points. Therefore, different pairs of motor units are displayed at *0 weeks* (upper panels) and *+6 weeks* (lower panels). Panels on the first rows for *0 weeks* and *+6 weeks* show the torque traces for contractions with 20% and 40% of the participant's peak torque. The participants' control units are displayed on the bottom row (green and grey motor units) and test units on the middle rows (orange and purple motor units) for *0 weeks* and *+6 weeks*. Brackets indicate the ΔF values. The black continuous lines are the 5th-order polynomial fits for the control units.

Functional capacity

The timed up-and-go, 5-x sit-to-stand, and 30-s sit-to-stand were recorded with a 120-Hz iPad Air camera (3^{rd} generation, Apple Inc., California, USA) and stored for *a posteriori* offline analyses. Tests were analysed with Kinovea Software (version 0.8.27) in order to reduce the measurement errors that can exist when functional tests are timed with a stopwatch (da Silva et al., 2018). For the timed up-and-go test, a chair (seat 46 cm high) was placed against the wall and a cone was placed 3 m away from the chair. The participants stood, walked around the cone, and returned to the seated position as quickly as possible without running or using their hands at any time. During offline analysis, the starting time was selected when the first trunk flexion movement was visually detected and the end time when the trunk completely leaned towards the backrest. The quickest of three attempts (30-s rest) was selected for the analysis (da Silva et al., 2018). For the 5-x sit-to-stand and 30-s sit-to-stand tests, participants stood then re-sat on a chair as quickly as possible. They kept their arms crossed during the entire test and leant their backs on the backrest of the chair after each repetition. The time taken to perform 5 repetitions and the number of repetitions performed in 30 s were used for the 5-x sit-to-stand and 30-s sit-to-stand tests analysis, respectively (da Silva et al., 2018). One participant could not perform the functional capacity tests properly (i.e., without using his hands to stand from the chair) and therefore was excluded from analysis.

The countermovement jump test was performed on a force place (2000 Hz; Kistler Instrument Corp., Amherst, NY, USA). Participants performed 3 jump trials interspersed by a 60-s rest interval. If participants did not use the correct technique (Dias et al., 2011) or lost balance during the jump or landing, up to two additional attempts were allowed. The highest jump achieved was used for analysis and jump height was calculated based on the flight time equation (Dias et al., 2011). Participants received the command “3,2,1, jump!”, and then performed the squat movement followed by the maximum vertical jump, with a fast transition between squatting and jumping. Both hands were kept on the hips during the jump and legs and feet were maintained in full extension during the flight and initial landing phase.

1-repetition maximum leg press

The horizontal leg press 1-RM load was performed after conducting a general warm-up with bodyweight exercises, followed by 10 and 6 repetitions of the leg press using moderate perceived loads. The 1-RM load was determined with no more than 4 attempts, interspersed by rest intervals of 3-5 min (the longer rest was given when 2 reps were completed near to maximum load). Participants were requested to perform two repetitions with any given load and the lift was considered successful when only one repetition was fully performed (Brown & Weir, 2001). The load was increased if two repetitions were completed or decreased if no repetition was performed. An excellent reliability is observed for 1-RM leg press strength testing regardless of participant age (Grgic et al., 2020).

Muscle cross-sectional area and thickness, and body composition

Changes in muscle size were assessed to determine whether such change might have been associated with any changes in motor performance, or whether any changes in force output were rather more attributable to changes within the nervous system. All measurements were conducted using B-mode ultrasonography with a linear transducer probe (47 mm, 6 - 43 MHz, L4-15, ESAOTE, MyLabSeven. Genova, Italy), coated with a water-soluble transmission gel to provide acoustic contact without depressing the dermal surface. G*astrocnemius lateralis* and *medialis* cross-sectional areas images were recorded using the extended field-of-view method on the site with the greater circumference (most bulky area). It was not possible to obtain good quality and reliable cross-section area images for *soleus* using the extended field-of-view method for some participants. This is because of a greater skin-probe angle derived from their *soleus* small circumference. Therefore, *soleus* thickness still images were assessed at 30% of the distance between the medial condyle of the tibia and the medial malleolus of the fibula (Perkisas et al., 2018). The anatomical sites where the probe was positioned were traced with anatomic landmarks and skin marks onto individual acetate sheets during the first assessment session. This method was used to ensure that all measurements were acquired from the same sites across all the neuromuscular assessments timepoints. Ultrasound images were evaluated using ImageJ software (National Institutes of Health, Bethesda, Maryland, USA). Three images were obtained from each site and the average of them was analysed.

Body composition was assessed with a multi-frequency bioelectrical impedance device (MC-780, Tanita, Japan), following the manufacturer's manual. Body fat percentage and lower-body appendicular skeletal muscle mass data were analysed. This method has been shown to provide similar results to dual-energy X-ray absorptiometry when assessing changes in fat-free mass or body fat after nutritional and resistance training over a period of 4-12 weeks (Antonio et al., 2019; Schoenfeld et al., 2020).

Food intake

At the end of the *-2 weeks* testing session, participants were asked to provide a 24-h food recall, which was recorded by a certified dietitian. 48 h prior *0 weeks* and *+6 weeks* testing sessions, each participant received a food intake report with the list of foods they consumed before the *-2 weeks* session and were asked to replicate it before subsequent sessions. This strategy was adopted to standardise testing macronutrient consumption and to avoid potential effects of food intake on serotonin and noradrenaline concentrations, which could potentially influence intrinsic motor neurone excitability (L. B. R. Orssatto, Mesquita, et al., 2021).

Resistance training program

A 6-week, "power-oriented" resistance training program was completed using horizontal leg press, calf raise (Cal Gym, Australia), assisted vertical countermovement jump, and assisted ankle hop (on a step) exercises, followed by chest press and lat pulldown exercises (Cal Gym, Australia). The power-oriented exercises were characterised by the intention to perform the concentric contraction as fast as possible (da Rosa Orssatto et al., 2019). All sessions and exercises were performed with a 1:1 supervision ratio, twice a week (at least 48-h interval between sessions), with 3 sets and 6 repetitions per set, followed by 3-min rest intervals.

In week 1, participants were familiarised with the exercises and the OMNI-RES, which is a scale developed to obtain the subjective perceived effort during resistance training sets (Gearhart et al., 2009; Naclerio et al., 2011). They were requested to perform each exercise at a subjective “moderate” velocity and the load was adjusted to a subjective perception of 3-4 on the OMNI-RES scale. Familiarisation to the “assisted countermovement jumps” and “assisted ankle hops on a step” were performed with moderate velocity and avoiding any jump (i.e., bodyweight squats and calf raises). The horizontal leg press and ankle hops on a step were also performed at moderate velocity with ~2-s concentric and ~2-s eccentric phases.

In week 2, the training load was determined based on the OMNI-RES scale and participants were asked to move at a fast concentric velocity, assisted by visual velocity feedback using the Gym Aware device and software (GymAware, Australia). Loads were based on each participant's subjective perception at 6-7 on the OMNI-RES scale. Also, during this week, they increased the concentric velocity between training sessions, without performing at maximal velocity, for all exercises, however jumps were still avoided in the countermovement jump and ankle hops on a step exercises.

From weeks 3 to 6, maximal concentric effort resistance training was performed. Training loads ranged 6-7 on the OMNI-RES scale; if participants reported a perceived effort lower than 6 or higher than 7 for at least 2 sets, the load was increased or reduced, respectively, for the next set. The jump exercises were performed using their own body mass, and participants were requested to jump as fast and as high as possible on each repetition, with a 2-5-s rest interval between jumps for balance reestablishment and to observe the velocity feedback. Participants performed all the exercises with maximal intended concentric velocity, with eccentric phases lasting ~2-s eccentric for machine resistance exercises and <1-s for the plyometric exercises. During week 5, a 3-day lockdown was ordered by the local government due to COVID-19. Therefore, one session from week 5 was replaced with home-based plyometric training where participants were requested to perform 6 sets of 6 repetitions for the countermovement jump and ankle hops on a step.

Real-time concentric velocity feedback was provided during the horizontal leg press, calf raise, assisted countermovement jump, and assisted ankle hop exercises using Gym Aware software and device. Concentric mean velocity was displayed on an iPad (Air, 3rd generation, Apple Inc., California, USA) screen after each repetition. The maximum velocity performed in the previous set was subsequently used as the threshold, making their next velocity feedback red coloured when performed below the threshold or yellow when above the threshold. All participants received verbal feedback after each repetition encouraging them to increase their velocity compared to the target velocity from the previous set. This strategy was adopted because previous research shows that higher velocities are reached and maintained when setting a target velocity than simply the instruction to move as fast as possible (Hirsch & Frost, 2021).

Data analyses

Separate linear mixed-effect models were used to compare ΔF values, peak discharge rates, and recruitment thresholds over time as a fixed factor (Yu et al., 2021). For the unmatched motor unit analysis, a random intercept was included for each participant in the study to account for the correlation between repeated observations on each individual. For the matched motor unit analysis, single motor units were nested according to each participant and a random intercept was included. Linear mixed-effect models were also used to compare ΔF values obtained at different contraction intensities (20% vs 40% of peak torque) over time. Single motor units were nested according to each participant for the factor ‘contraction intensity’, and a random intercept was included for each participant for the factors ‘contraction intensity’ and ‘time’. Separate one-way repeated-measures analyses of variances were adopted to compare the effects on physical function and body composition over time. When a significant effect was observed, Bonferroni *post-hoc* correction was adopted for pairwise comparison. The effect sizes derived from the F ratios were calculated with the omega squared (ω^2) method (0–0.01, very small; 0.01–0.06, small; 0.06–0.14, moderate; and >0.14, large) (Lakens, 2013).

Repeated-measures Bland–Altman within-subject correlations were used to determine the association between changes in ΔFs with changes in motor unit discharge rates and physical function variables across *-2 weeks*, *0 week*, and *+6 weeks* for unmatched motor units, and across *-2 weeks + 0 weeks* (control period) and *+6 weeks* for matched motor units (Bakdash & Marusich, 2017). Correlation magnitude was interpreted based on Cohen's (Cohen, 1988) criteria: trivial, $r < 0.1$; small, $r = 0.1 – 0.3$; moderate, $r = 0.3 – 0.5$; large, $r = 0.5 – 0.7$; very large, $r = 0.7 – 0.9$; and nearly perfect, $r > 0.9$.

All analyses were completed in R (version 4.0.5) using the RStudio environment (version 1.4.1717). Linear mixed-effects models were fitted using the *lmerTest* package (Kuznetsova et al., 2017). Estimated marginal mean differences and 95% confidence intervals between time points were determined using the *emmeans* package (Lenth et al., 2021). The standardised difference (Cohen's *d*) between time points was also calculated using the population standard deviation from each respective linear mixed-effects model as the denominator (Lenth et al., 2021). Cohen's *d* was interpreted based on Rhea's criteria for when investigating the effects of resistance training in untrained individuals: trivial, < 0.50; small, 0.50 – 1.25; moderate, 1.25 – 1.9; large, > 2.0. The repeated-measures correlation coefficients were computed with the *rmcorr* package (Bakdash & Marusich, 2017). Significant difference was accepted at $p \leq 0.05$. All descriptive data are presented as mean (95% confidence interval lower and upper limits), unless indicated differently. The dataset and R code can be found at *https://github.com/orssatto/RT-PICs-ageing*.

RESULTS

Participants

Sixty-two older adults volunteered for the study, including 45 women and 17 men. After eligibility screening, 32 volunteers dropped out due to logistic limitations or unavailability to attend the resistance training sessions. Ten participants were excluded due to meeting some exclusion criteria (i.e., current resistance training practice, n = 3; total knee replacement, n = 3; antidepressant ingestion, n = 2; and coronary bypass surgery, n = 2). 20 participants (women n = 13, and men n = 7) were eligible to participate and completed the initial assessments. Three participants dropped out before exercise program commencement (n = 1, domestic unintentional injury, and n = 2, no reason provided). Ultimately, 17 participants [12 women and 5 men, age = 68.5 (67.1, 69.8) years, body mass = 80.0 (69.6, 90.5) kg, and height: 1.66 (1.62, 1.70)] both started and concluded the exercise program, with no further dropout.

ΔF, peak discharge rates and recruitment threshold

Unmatched and matched motor unit's ΔF values, peak discharge rates and recruitment thresholds, obtained during the triangular-shaped contractions at 20% of each participant peak torque, showed no change between *-2 weeks* and *0 weeks* sessions but increased after resistance training. Figure 3 presents ΔF individual data points and estimated marginal means for the unmatched motor units (A) and matched motor units (B). Table 1 presents the estimated marginal mean and mean differences for ΔF, peak discharge rates and recruitment thresholds.

There was an effect of time ($F = 8.39$; $\omega^2 = 0.09$, $p <0.001$) on ΔF when comparing unmatched motor units. ΔF was similar between *-2 weeks* and *0 weeks* ($p = 1.000$; $d = 0.16$) but higher at *+6 weeks* compared to *-2 weeks* ($p < 0.001$; $d = 0.76$) and *0 weeks* ($p = 0.015$; $d = 0.60$). When comparing matched motor units, ΔF was similar between *-2 weeks* and *0 weeks* ($p = 0.726$; $d = -0.12$) but higher at *+6 weeks* when compared to *-2 weeks* + *0 weeks* (control period; $p < 0.001$; $d = 1.02$).

There was an effect of time ($F = 9.34$; $\omega^2 =0.10$, $p < 0.001$) on peak discharge rates when comparing unmatched motor units. Peak discharge rate was not different between *-2 weeks* and *0 weeks* ($p = 0.863$; $d = 0.11$) but higher at *+6 weeks* compared to *-2 week* ($p < 0.001$; $d = 0.78$) and *0 weeks* ($p = 0.005$; $d = 0.68$). When comparing matched motor units, peak discharge rates were not different between *-2 weeks* and 0 weeks ($p = 0.974$; $d = -0.01$) but higher at *+6 weeks* when compared to *-2 weeks* + *0 weeks* (control period; $p < 0.001$; $d = 1.42$).

There was an effect of time ($F = 7.214$; $\omega^2 = 0.09$, $p = 0.001$) on recruitment threshold when comparing unmatched motor units. Recruitment threshold was not different between *-2 week* and *0 weeks* ($p = 0.702$; $d = -0.20$) but higher at *+6 week* compared to *-2 week* ($p = 0.018$; $d = 0.61$) and *0 weeks* ($p = 0.002$; $d = 0.81$). When comparing matched motor units, recruitment threshold was not different between *-2 weeks* and *0 weeks* ($p = 0.960$; $d = 0.02$) but higher at *+6 weeks* when compared to *-2 weeks* + *0 weeks* (control period; $p = 0.001$; $d = 1.00$).

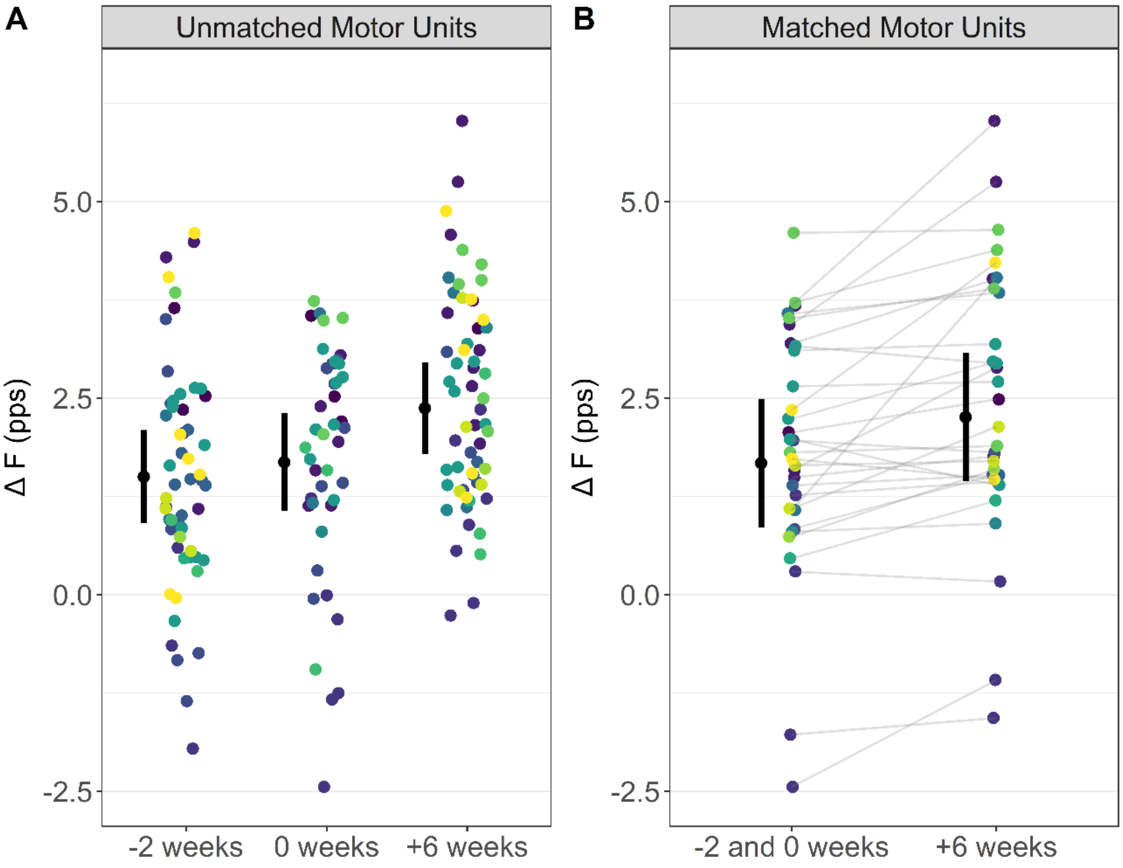

Figure 3. ΔF data derived from the analyses with all the unmatched motor units (A) and from the matched motor units only (B) over time. On panel A, *-2 weeks* = *0 week*, *+6 weeks* > *-2 weeks*, and *+6 weeks* > *0 week*. On panel B, *+6 weeks* > *-2 weeks* + *0 weeks* (Control period). The mean (black circle) and 95% confidence interval are offset to the left, with individual data points coloured by participants. pps = peaks per second.

Table 1. Estimated marginal mean and mean differences (95% confidence interval lower and upper limits) for ΔF, peak discharge rates, and recruitment threshold from the analyses with unmatched and matched motor units at *-2 weeks*, *0 week*, and *+6 weeks*.

	Estimated marginal mean (95% CI)			Estimated marginal mean difference (95% CI)		
Unmatched motor units						
Time	*-2 weeks*	*0 week*	*+6 weeks*	*0 weeks – -2 weeks*	*+6 weeks – -2 weeks*	*+6 weeks – 0 weeks*
ΔF (pps)	1.50 (0.91, 2.09)	1.69 (1.06, 2.31)	2.37 (1.79, 2.95)	0.19 (-0.41, 0.78)	**0.87 (0.34, 1.41)**	**0.69 (0.12, 1.25)**
Peak discharge rates (pps)	8.34 (7.59, 9.08)	8.48 (7.71, 9.25)	9.33 (8.59, 10.06)	0.14 (-0.51, 0.80)	**0.99 (0.40, 1.58)**	**0.85 (0.22, 1.47)**
Recruitment threshold (% of peak torque)	6.24 (4.75, 7.72)	5.70 (4.14, 7.27)	7.85 (6.41, 9.29)	-0.54 (-2.12, 1.05)	**1.61 (0.23, 3.00)**	**2.15 (0.68, 3.62)**
Matched motor units						
Time	*Control period* (*-2 weeks + 0 week*)		*+6 weeks*	*0 weeks – -2 weeks*		*+6 weeks – Control period*
ΔF (pps)	1.67 (9.85, 2.49)		2.26 (1.44, 3.08)	-0.08 (-0.57, 0.41)		**0.58 (0.29, 0.88)**
Peak discharge rates (pps)	8.76 (8.09, 9.44)		9.54 (8.86, 10.21)	0.01 (-0.57, 0.59)		**0.78 (0.49, 1.06)**
Recruitment threshold (% of peak torque)	5.04 (3.37, 6.71)		7.19 (5.52, 8.86)	0.05 (-2.07, 2.17)		**2.15 (0.92, 3.38)**

For the matched motor unit analysis, note that motor units matched between *-2 weeks* and *0 weeks* were analysed separately and they are not matched with *+6 weeks*. In addition, motor units identified at *+6 weeks* were matched with motor units identified in at least one control period time point (i.e., *-2 weeks* or *0 week*), which were merged and named as "*-2 weeks + 0 week*". Bolded estimated marginal mean differences indicate statistical significance ($p \leq 0.05$).

ΔF modulation between 20% and 40% contraction intensities

ΔF values from motor units recruited up to 20% of each participant's peak torque increased for both the 20% trapezoidal contractions and 40% triangular contractions. It was observed a greater magnitude of increase for ΔF values during the 40% triangular contractions compared to the 20% trapezoidal contractions, which consequently increased the between-contraction-intensity mean differences (i.e., increased ΔF modulation). Figure 3A presents ΔF individual data points and estimated marginal means for the unmatched motor units (A) and matched motor units (B). Table 1 presents the estimated marginal mean and mean differences for ΔF, peak discharge rates and recruitment thresholds.

There was an intensity by time effect (F = 3.024; $\omega^2 = 0.03$, p = 0.052) on ΔF. ΔF at 20% and 40% were not different at *-2 weeks* (p = 0.448; *d* = 0.18) and *0 weeks* (p = 0.109; *d* = 0.38); but ΔF at 40% was greater than at 20% at *+6 weeks* (p < 0.001; *d* = 0.90). ΔF at 20% was similar between *-2 weeks* and *0 weeks* (p = 0.866; *d* = -0.46), and between *-2 weeks* and *+6 weeks* (p = 0.171; *d* = 0.79) but was higher at *+6 weeks* compared to *0 weeks* (p = 0.004; *d* = 1.24). ΔF at 40% was similar between *-2 weeks* and *0 weeks* (p = 0.999; *d* = -0.26) but was higher at *+6 weeks* compared to *-2 weeks* (p = 0.001; *d* = 1.51) and *0 weeks* (p < 0.001; *d* = 1.77).

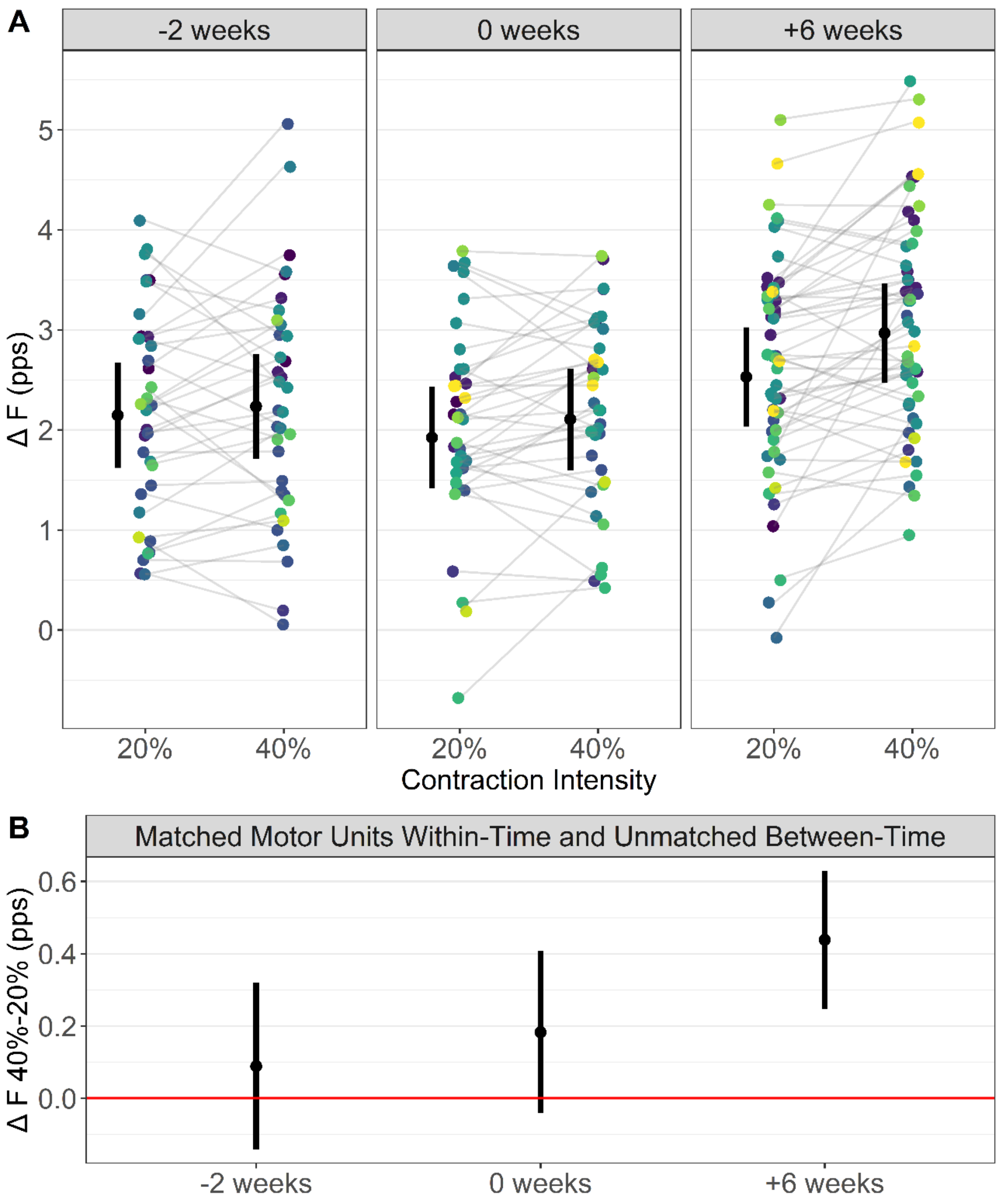

Figure 4. ΔF data derived from the analysis comparing contraction intensities at 20% and 40% of each participant peak torque. On panel A, data from *-2 weeks* (left panel), *0 weeks* (middle panel), and *+6 weeks* (right panel) are presented with motor units matched within each time point. On panel B, ΔF estimated marginal mean differences between 40 and 20% contractions from *-2 weeks*, *0 week*, and *+6 weeks* are presented. Note that ΔF increased from *0 weeks* to *+6 weeks* at 20%, and in a higher magnitude from *-2 weeks* and *0 weeks* to *+6 weeks* at 40% contractions (Panel A). As a result of the greater increases in ΔF at 40% contractions, there was an increase in the ΔF difference between 20% and 40% contractions at *+6 weeks* compared to *-2 weeks* and *0 weeks* (Panel B).

Motor unit identification

For the 20% triangular contractions, no motor unit could be identified for three participants, and therefore, they were not included in this analysis. The total number of motor units identified in the remaining 14 participants were 108 at *-2 weeks*, 105 at *0 week*, and 135 at *+6 weeks*. For the Unmatched motor unit analyses, it was possible to obtain ΔF values from 55 test motor units at *-2 weeks*, 43 at *0 week*, and 59 at *+6 weeks*. For the matched motor unit analyses, it was possible to obtain ΔF values from 18 tracked motor units across *-2 weeks* and *0 weeks* (from 7 participants), and from 31 tracked motor units across *-2 weeks* and/or *0 weeks* and *+6 weeks* (from 13 participants).

For the 20% trapezoidal and 40% triangular contractions, no motor unit could be identified for 4 participants. The total number of motor units identified in the remaining 13 participants on the 20% trapezoidal contractions were 126 at *-2 weeks*, 142 at *0 week*, and 146 at *+6 weeks*. On the 40% triangular contractions, 135 motor units were identified at *-2 weeks*, 151 at *0 week*, and 154 at *+6 weeks*. ΔF values were obtained from 35 test motor units tracked across intensities at *-2 weeks*, 38 at *0 week*, and 51 at *+6 weeks*, from those 13 participants.

Peak torque, countermovement jump, and functional capacity

The plantar flexors isometric peak torque, countermovement jump height, and all the functional capacity tests (i.e., timed-up-and-go, 5-times sit-to-stand, and 30-s sit-to-stand) showed no change between *-2 weeks* and *0 weeks* sessions but increased after resistance training. Leg press 1-RM increased from *0 weeks* to *+6 weeks*. The estimated marginal means and estimated marginal mean differences for the abovementioned variables are presented in Table 2.

There was an effect of time (F = 29.196; ω^2 = 0.62; p < 0.001) on peak torque, which was not different between *-2 weeks* and *0 weeks* (p = 0.813; *d* = -0.38) but was higher at *+6 weeks* compared to *-2 weeks* (p < 0.001; *d* = 2.05) and *0 weeks* (p < 0.001; *d* = 2.44). There was an effect of time (F = 16.279; ω^2 = 0.47, p < 0.001) on countermovement jump height. The height was not different between *-2 weeks* and *0 weeks* (p = 1.000; *d* = 0.27) but higher at *+6 weeks* compared to *-2 weeks* (p < 0.001; d = 1.81) and *0 weeks* (p < 0.001; *d* = 1.54). There was an effect of time (F = 30.039; ω^2 =

0.64, $p < 0.001$) on timed-up-and-go. Timed-up-and-go was not different between *-2 weeks* and *0 weeks* ($p = 0.637$; $d = 0.45$) but quicker at *+6 weeks* compared to *-2 weeks* ($p < 0.001$; $d = -2.12$) and *0 weeks* ($p < 0.001$; $d = -2.57$). There was an effect of time ($F = 18.090$; $\omega^2 = 0.51$, $p < 0.001$) on the 5-times sit-to-stand. 5-times sit-to-stand was not different between *-2 weeks* and *0 weeks* ($p = 0.420$; d = 0.15) but quicker at *+6 weeks* compared to *-2 weeks* ($p < 0.001$; $d = -1.91$) and *0 weeks* ($p < 0.001$; $d = -1.76$). There was an effect of time ($F = 57.241$; $\omega^2 = 0.77$, $p < 0.001$) on the 30-s sit-to-stand. 30-s sit-to-stand was not different between *-2 weeks* and *0 weeks* ($p = 0.541$; $d = 0.49$) but higher at *+6 weeks* compared to *-2 weeks* ($p < 0.001$; $d = 3.49$) and *0 weeks* ($p < 0.001$; $d = 3.01$). There was an effect of time ($F = 113.64$; $\omega^2 = 0.86$, $p < 0.001$) on the 1-RM, which was higher at *+6 weeks* compared to *0 weeks* ($p < 0.001$; $d = 3.66$).

Body composition

Soleus muscle thickness, *gastrocnemius medialis* and *lateralis* cross-sectional areas, lower-limbs skeletal muscle mass, and body fat percentage did not change at any time points. The estimated marginal means and estimated marginal mean differences for the abovementioned variables are presented in Table 2. There was no effect of time on *soleus* muscle thickness ($F = 2.890$; $\omega^2 = 0.10$, $p = 0.071$), *gastrocnemius medialis* cross-sectional area ($F = 2.757$; $\omega^2 = 0.11$; $p = 0.082$), *gastrocnemius lateralis* cross-sectional area ($F = 1.080$; $\omega^2 < 0.01$, $p = 0.352$), lower-body skeletal muscle mass ($F = 0.029$; $\omega^2 = -0.06$, $p = 0.972$), or body fat percentage ($F = 2.593$; $\omega^2 = 0.08$, $p = 0.090$).

Table 2. Estimated marginal mean and mean differences (95% confidence interval lower and upper limits) for peak torque, countermovement jump height, timed-up-and-go, 5-time sit-to-stand, 30-s sit-to-stand, 1-RM, muscle thickness, cross-sectional area, lower-limbs muscle mass, and body fat percentage at *-2 weeks*, *0 week*, and *+6 weeks*.

	Estimated marginal mean (95% CI)			Estimated marginal mean difference (95% CI)		
Time	*-2 weeks*	*0 week*	*+6 weeks*	*-2 weeks – 0 week*	*-2 weeks – +6 weeks*	*0 weeks – +6 weeks*
Peak torque (N·m)	93.4 (79.6, 107.1)	89.8 (76.0, 103.6)	112.4 (98.7, 126.2)	-3.6 (-11.4, 4.3)	**19.1 (11.2, 26.9)**	**22.7 (14.8, 30.5)**
CMJ height (cm)	9.49 (7.02, 11.95)	9.80 (7.34, 12.26)	11.57 (9.11, 14.03)	0.31 (-0.66, 1.28)	**2.09 (1.12, 3.06)**	**1.78 (0.81, 2.75)**
Timed-up-and-go (s)	6.39 (5.97, 6.81)	6.52 (6.10, 6.94)	5.77 (5.35, 6.19)	0.13 (-0.12, 0.39)	**-0.62 (-0.88, -0.36)**	**-0.75 (-1.01, -0.50)**
5-time sit-to-stand (s)	12.09 (10.82, 13.35)	11.95 (10.69, 13.22)	10.35 (9.09. 11.62)	-0.14 (-0.93, 0.65)	**-1.73 (-2.53, -0.94)**	**-1.60 (-2.39, -0.81)**
30-s sit-to-stand (reps)	12.44 (11.03, 13.85)	12.75 (11.34, 14.16)	14.69 (13.28, 16.10)	0.31 (-0.25, 0.87)	**2.25 (1.69, 2.81)**	**1.94 (1.38, 2.50)**
Leg press 1-RM (kg)	-	107.4 (90.7, 124.0)	138.5 (121.9, 155.2)	-	-	**31.2 (25.0, 37.4)**
Soleus thickness (cm)	3.56 (3.29, 3.83)	3.41 (3.14, 3.67)	3.36 (3.10, 3.63)	-0.15 (-0.36, 0.06)	-0.20 (-0.41, 0.02)	-0.04 (-0.25, 0.16)
Gastrocnemius lateralis CSA (cm^2)	6.74 (5.39, 8.09)	6.51 (5.17, 7.86)	6.50 (5.16, 7.85)	-0.23 (-0.67, 0.22)	-0.24 (-0.69, 0.22)	-0.01 (-0.46, 0.45)
Gastrocnemius medialis CSA (cm^2)	11.90 (10.05, 13.76)	11.36 (9.51, 13.21)	11.67 (9.82, 13.52)	-0.55 (-1.14, 0.04)	-0.24 (-0.82, 0.35)	0.31 (-0.24, 0.87)
Lower-limb skeletal muscle mass (kg)	15.99 (13.74, 18.24)	15.99 (13.75, 18.24)	15.97 (13.72, 18.22)	0.01 (-0.24, 0.26)	-0.02 (-0.27, 0.23)	-0.02 (-0.27, 0.23)
Body fat (%)	35.1 (31.0, 39.2)	35.2 (31.1, 39.2)	34.43 (30.4, 38.5)	0.1 (-0.8, 1.0)	-0.7 (-1.6, 0.2)	-0.7 (-1.6, 0.1)

CI, confidence interval; CMJ, countermovement jump; 1-RM, 1-repetition maximum; CSA, cross-sectional area. Bolded estimated marginal mean differences indicate statistical significance ($p \leq 0.05$).

Repeated-measures correlations

The repeated-measures correlations indicated that changes in ΔF from both matched and unmatched motor units are associated with the changes in motor unit peak discharge rate, plantar flexors peak torque, leg press 1-RM, countermovement jump height, and functional capacity tests (i.e., timed-up-and-go, 5-times sit-to-stand, and 30-s sit-to-stand). Table 3 presents the repeated-measures correlation coefficients, interpretation, and respective 95% confidence intervals and interpretations.

Table 3. Repeated-measures correlation coefficients (95% confidence intervals lower and upper limits) and interpretation for ΔF from unmatched and matched motor units from 20% triangular contractions correlated with motor unit peak discharge rates, plantar flexor peak torque, countermovement jump height, and functional capacity tests.

	ΔF Unmatched motor units	ΔF Matched motor units
Peak discharge rate	0.62 (0.26, 0.83) Large	0.54 (0.22, 0.75) Large
Peak torque	0.61 (0.24, 0.82) Large	0.54 (-0.04, 0.85) Large
Leg press 1-RM	0.82 (0.27, 0.97) Very large	0.77 (0.35, 0.93) Very large
Countermovement jump height	0.39 (-0.05, 0.70) Moderate	0.49 (-0.12, 0.83) Moderate
Timed-up-and-go	-0.46 (-0.75, -0.03) Moderate	-0.78 (-0.94, -0.35) Very large
5-time sit-to-stand	-0.54 (-0.79, -0.13) Large	-0.67 (-0.91, -0.12) Large
30-s sit-to-stand	0.64 (0.28, 0.85) Large	0.82 (0.43, 0.95) Very large

1-RM, 1-repetition maximum. Correlation coefficients (i.e., r) are interpreted as: trivial, $r < 0.1$; small, $r = 0.1 – 0.3$; moderate, $r = 0.3 – 0.5$; large, $r = 0.5 – 0.7$; very large, $r = 0.7 – 0.9$; and nearly perfect, $r > 0.9$.

DISCUSSION

The primary finding of this study was that ΔF values increased after 6 weeks of resistance training in older adults and that this occurred alongside an increased ability to modulate ΔF values (and presumedly PIC strength) as increases in neural drive were required during contraction. The results indicate not only that PIC amplitudes can increase in response to several weeks of resistance training in older adults, but that the ability to vary the input-output gain to increase muscle force production might enhanced. Moreover, our exploratory analysis showed that increases in ΔF were statistically associated with increases in motor neurone peak discharge rates during the submaximal contractions, maximal strength capacity in plantar flexion and whole-leg exercises, and functional capacity in a range of tasks requiring jumping, chair sit-to-stand, or walking. This evidence suggests that increased motor neuronal PICs may be a neural mechanism partly underpinning the improvements in peak discharge rates, strength, and functional capacity following resistance training in older adults.

Estimates of persistent inward current strength (ΔF)

The increased ΔF values observed after the training period provides foundational evidence that the adaptive increases in motor neurone discharge output following resistance training in older adults may be partly mediated by adaptations in intrinsic motor neurone excitability. It is important to note that although the increases in ΔF values are significant, the values at *+6 weeks* remain smaller than previously obtained in *soleus* of young adults when tested during similar triangular-shaped contractions with a 2%/s force increase–decrease rate (Kim et al., 2020; L. B. R. Orssatto, Borg, et al., 2021; Trajano et al., 2020). Although our findings show a partial restoration of PIC amplitude following resistance training in this population, these increases may theoretically contribute to a reduction in age-related loss of strength and function. It is of subsequent interest to quantify changes in PICs after longer training periods to determine whether values closer to young adults can be obtained, and to determine whether PIC changes remain after training cessation or whether they are rather a response that requires continued physical exertion to retain.

Specific investigation of the effects of resistance training on estimates of motor neuronal PICs has not previously been conducted in humans. However, a series of studies showing evidence of adaptations in some motor neurone properties following resistance training supports the present findings. In one study, adaptations that could be explained by increases in PICs, including smaller intracellular currents required to evoked rhythmic discharging, increased presence of fast discharging rates at the same level of intracellular depolarisation, and faster maximum discharge rates during repetitive discharging, were observed after 5 weeks of resistance training in rats (Krutki et al., 2017). In another study, overload provided by tenotomy of synergists (*gastrocnemius lateralis*, *soleus*, and *plantaris*) to produce plantar flexor muscle overload resulted in similar changes in fast (i.e., presumably higher-threshold motor units), but not slow (i.e., lower-threshold motor units), muscle fibres of adults rats (Krutki et al., 2015). Together, these studies show a marked adaptability of intrinsic motor neuronal excitability in response to muscle overload when tested in animal models. In humans, no previous study has explicitly investigated the effects of resistance training on PICs. Nevertheless, modifications in motor neurone properties that might be explained by enhanced PICs have been described. For example, increases in maximal discharge rates and decreases in afterhyperpolarisation duration after 2 weeks of maximal dorsiflexion isometric contractions resistance training in young and older adults (Christie & Kamen, 2010). Nonetheless, although Del Vecchio et al. (2019) found increased peak discharge rates at different target forces (i.e., 35, 50, and 70% of peak force contractions) after 4 weeks of explosive and sustained isometric contractions in young adults, the authors argued that the absence of changes in motor unit derecruitment threshold relative to force produced indicates a lack of increase in motor unit hysteresis, and thus that a change in ΔF should not be seen if it had been measured. However, the interrogation of discharging characteristics of a lower-threshold motor unit, not the muscle force or joint torque generated, is the only validated method to estimate the effective synaptic input (drive) to the motor units, and thus to estimate current-frequency hysteresis or input-output hysteresis (Gorassini et al., 2002a; Powers et al., 2008). Notably, our study provides novel evidence of increased PIC strength in humans in accordance with findings from animal models.

The mechanisms underpinning the increased ΔF observed in our study remain unknown. Although speculative, it is reasonable to suggest that adaptations within the monoaminergic system may have played a role. The magnitudes of serotonergic and noradrenergic input onto the motor neurones are responsible for adjustment of the PIC-related input-output gain (Lee & Heckman, 1998, 2000). PICs can act as a variable gain control system by non-linearly amplifying the net excitatory inputs up to 5-fold, proportional to the monoaminergic input (Heckman & Binder, 1991; Johnson & Heckman, 2014). This mechanism allows motor neurones to achieve appropriate discharge outputs to generate force across a diverse range of contraction intensities (Binder et al., 2020; Huh et al., 2017; Johnson & Heckman, 2014; Naufel et al., 2019; L.

B. R. Orssatto, Mackay, et al., 2021). Based on this, age-related reductions in serotonin and noradrenaline release onto motor neurones (Ko et al., 1997; Liu et al., 2019, 2020; Michaud et al., 2013; Míguez et al., 1999; Shibata et al., 2006) is suggested to explain the lower estimated PIC amplitudes in this population (Hassan et al., 2021; L. B. R. Orssatto, Borg, et al., 2021). Hypothetically, a relative restoration of the aged monoaminergic system following resistance training could speculatively contribute to counteract the reductions in PICs with ageing. Supporting this hypothesis, increases in serotonin immunoreactivity in the hypoglossal nucleus was observed after tongue resistance training in old rats (Behan et al., 2012), suggestive of an increased serotonergic input to the hypoglossal nucleus in the region of motor neurones innervating the genioglossus muscle of the tongue. Therefore, improved monoaminergic system function is one potential mechanism for ΔF enhancement in our study.

An important finding of the present study is the enhanced ability of the motor neurones to modulate ΔF in proportion to motor output, and presumably to the level of descending drive, following resistance training (Figure 4). Before resistance training (i.e., *-2* and *0 weeks*), the older adults showed small, but non-significant, increases on ΔF between contraction intensities of 20% and 40% of maximal voluntary contraction torque. After training, however, the ΔF difference between contraction intensities increased significantly, consistent with an enhanced capacity to modulate ΔF in proportion to motor output. This is of special interest because substantial ΔF modulation has been observed in *soleus* in young adults at contraction intensities ranging 10 to 30% of maximal voluntary contraction torque when controlling contraction for rate and duration of torque rise and decline (L. B. R. Orssatto, Mackay, et al., 2021). As discussed above, the PIC-related variable gain control system is capable of amplifying the net excitatory input in accordance with levels of serotonergic and noradrenergic input onto the motor neurones (Lee & Heckman, 1999a, 2000). Thus, the lesser capacity to modulate ΔF at higher contraction intensities at pre-training supports the hypothesis monoaminergic system dysfunction may affect PICs in this population, as discussed previously (Hassan et al., 2021; L. B. R. Orssatto, Borg, et al., 2021). Moreover, the improvement in ΔF modulation by *+6 weeks* might feasibly reflect an increased serotonergic and/or noradrenergic input onto the motor neurones after training. These findings are consistent with the hypothesis that monoaminergic system function is a key factor influencing PIC modulation in older adults; this hypothesis should be explicitly tested in future studies.

Another mechanism that might speculatively underpin the increased ΔF after training is a change in the density, number, composition, or function of voltage-gated calcium channels on the motor neurone (P. F. Gardiner, 2006). Motor neuronal PICs are generated by somato-dendritic calcium and sodium L-type channels (Lee & Heckman, 1999b; Li et al., 2004). Sodium channels are rapidly activated by membrane depolarisation (Catterall, 1992) and generate transient, rapidly inactivating sodium currents, responsible for the rising phase of the action potential (Hodgkin & Huxley, 1952; Kuo et al., 2006), although they also generate a longer acting PIC during prolonged membrane depolarisation that supports sustained repetitive firing (Kuo et al., 2006). On the other hand, voltage- and calcium-gated calcium channels contribute to relatively slow PIC activation (Lee & Heckman, 1998) and are facilitated by repeated activation (Binder et al., 2020). It is not possible to measure channel properties in humans, however evidence of plasticity in motor neurone biophysical properties following resistance training in rats (Krutki et al., 2017) may reflect alterations in the voltage-gated channels. For example, decreases in the threshold-to-peak spike rise time as well as the minimal current amplitude required for depolarisation (Krutki et al., 2017) could indicate enhanced sodium channel function. Also, increased steady-state firing frequency, suggestive of increased discharge rates for a given amount electrical current injection onto the motor neurone (i.e., increased f-I slope) (Krutki et al., 2017), could indicate calcium channel modification. The hypothesis that increased voltage-gated calcium channels density, number, composition, and function could contribute to the enhanced ΔF observed in the present study should be explicitly tested in the future.

Peak discharge rates and recruitment threshold

The repeated-measures correlation results (Table 3) indicate that increases in ΔF may have partly contributed to the faster peak discharge rates observed in ours and other studies (testing *tibialis anterior* and *vastus lateralis)* following resistance training in older adults (Christie & Kamen, 2010; Kamen & Knight, 2004). Consequently, a greater motor neurone discharge rate could theoretically underpin the increased voluntary activation after resistance training in older adults (Arnold & Bautmans, 2014). Indeed, voluntary activation is typically reduced in this population (Rozand et al., 2020); thus, these improvements would explain the increases in maximal strength underpinned by neural adaptation. This is supported by the large and very large associations between increases in ΔF and peak plantar flexor torque and leg press 1-RM (i.e., maximal strength), respectively. Enhanced PIC strength in older adults would amplify the voluntary supraspinal drive received at the motor neurone, enhancing the motor neuronal discharge rates and increasing force output.

The increases in motor neurone recruitment thresholds during ramped contractions observed in our study are not consistent with findings from resistance training studies in young adults. Reduced recruitment threshold was observed in *tibialis anterior* motor units of young adults after 4 weeks of isometric resistance training (Del Vecchio et al., 2019) and 12 weeks of ballistic-type training (Van Cutsem et al., 1998). A reduced recruitment threshold could feasibly result from changes in motor neuronal biophysical properties such as an increased intrinsic excitability by the strengthening of PICs. However, these contrasting results could indicate that young and older adults might use different motor unit activation strategies to increase the produced force during ramped contractions before and after resistance training. Previous data from our group and others have shown lower recruitment thresholds in untrained older adults (Erim et al., 1999; Fling et al., 2009; Klass et al., 2008; L. B. R. Orssatto, Borg, et al., 2021; Pascoe et al., 2011). As discharge rate modulation appears to be limited in aged motor neurones (e.g., rate coding is compressed) (Barry et al., 2007), older individuals would need an earlier recruitment of additional motor units to achieve an increase in motor output (Barry et al., 2007). This compressed discharge rate modulation may be a compensatory strategy to an impaired ability to amplify excitatory synaptic input through reduced PIC activation (Hassan et al., 2021; L. B. R. Orssatto, Borg, et al., 2021). Therefore, restoring PIC-related discharge rate modulation in older adults following resistance training could increase the ability of motor units to discharge at higher rates and, consequently, reduce the number of units required to initiate and then increase force production during submaximal ramped contractions.

Physical function and muscle hypertrophy

The observed improvements in maximal strength (i.e., isometric peak torque and 1-RM), countermovement jump, and both walking and chair standing capacities after the training period are unsurprising as they are consistent with the extant literature (Borde et al., 2015; Byrne et al., 2016; Csapo & Alegre, 2016; da Rosa Orssatto et al., 2019; Peterson et al., 2010; Steib et al., 2010). However, little is known about the neuromuscular mechanisms underpinning these adaptations in older adults. Our study provides novel evidence that increased motor neuronal PIC strength may partly underpin these changes following resistance training because of the moderate-to-large within-subject correlations observed between the improvements in our strength and functional capacity tests and changes in ΔF. Nevertheless, as these correlations cannot prove causation, further research should be done to assess the effects of PIC alterations on physical function, particularly in elderly cohorts.

Of note, we did not observe any indication of lower-limb muscle hypertrophy, as measured using both bioelectrical impedance and ultrasonographic methods. These results contrast a recent meta-analysis showing power-oriented resistance training to be an effective method to induce muscle hypertrophy in older adults (L. B. R. Orssatto et al., 2020). However, the present 6-week training duration was shorter than the 10 to 36 week studies included in that analysis. Also, the session volume reported in these studies was typically greater than imposed in ours, which might also influence hypertrophy (Figueiredo et al., 2017; Peterson et al., 2011). Nonetheless, the absence of detectible muscle hypertrophy ensures that the observed improvements in motor output would have resulted mostly from adaptations within the nervous system, which is expected in the early phase of adaptation to resistance training (Pearcey et al., 2021) and allowed a better assessment of the impact of neurological adaptation on strength and functional capacity.

Strengths and Limitations

The main strength of our study was the use of a non-invasive, validated (Gorassini et al., 2002a; Powers et al., 2008), and widely used method to estimate PIC amplitude in humans (Hassan et al., 2019, 2021; L. B. R. Orssatto, Borg, et al., 2021; L. B. R. Orssatto, Mackay, et al., 2021; Trajano et al., 2020; Udina et al., 2010). However, ΔF values need to be interpreted with caution as they can be affected by spike frequency adaptation, spike frequency accommodation, and the proportion of sub-threshold to supra-threshold PICs (Afsharipour et al., 2020; Gorassini et al., 2002a; L. B. R. Orssatto, Mackay, et al., 2021; Vandenberk & Kalmar, 2014). We attempted to control spike frequency adaptation and spike frequency accommodation by standardising the rate of torque rise and contraction duration for the triangular and trapezoidal contractions on their respective comparisons. Nevertheless, the proportion of sub-threshold PICs could not be estimated and controlled with the respective methods. In addition, it is possible that different strategies might have been used to accomplish the trapezoidal contractions at 20% vs. triangular contractions at 40% of maximal voluntary contraction torque.

An additional strength of the present study was the analysis of data from both unmatched and matched motor units across time. The unmatched motor unit analysis allowed the inclusion of a greater number of motor units in the statistical models. However, this method does not ensure that the compared motor units received the same synaptic input across time points, which was addressed by inclusion of the matched motor unit analysis. Tracking motor units across each time point substantially reduced the total number of motor units available for the paired motor unit analysis. Nonetheless, both the unmatched and matched motor unit analyses produced similar results, providing more confidence in our results.

ΔF values obtained in our study are derived from motor units recruited between 0 and 20% of peak torque at low contraction intensities (20% and 40% of peak torque). Therefore, our data might not provide information in relation to PIC behaviour of higher threshold motor neurones or modulation at higher contraction intensities. This is of particular interest because the function and structure of higher threshold motor units are more affected by ageing than lower threshold units (Hepple & Rice, 2015; Manini et al., 2013). Also, as discussed above, greater adaptations are observed in higher-threshold motor neurones in rats after both resistance training and synergist tenotomy (Krutki et al., 2015, 2017). It is therefore likely that resistance training provides additional benefit to higher-threshold motor neurones and thus neural responses at higher contraction intensities. It is important to note that the motor unit decomposition of high-density surface electromyograms during high-intensity contractions is difficult due to technical and methodological limitations. For example, fewer motor units are usually decomposed from signals obtained in higher intensity contractions because of overlap of action potentials from multiple motor units. Also, higher intensity contractions are known to induce bias towards the identification of higher-threshold motor units (Hassan et al., 2019). From a physiological standpoint, longer contraction durations (>30 s) would be required as contraction intensity increases, resulting in fatigue that might then influence motor neurone discharge behaviour and, consequently, PIC estimation. We recommend that future studies attempt to address these technical limitations, thus allowing more detailed investigation of the effects of exercise on PICs during higher intensity contractions.

Lastly, our study did not include a concurrent control group during the intervention period. Alternatively, we adopted a study design in which the same participants undertook a non-exercise control period before the training intervention. The advantage of our design is that all participants received the treatment and benefited from the outcomes from the exercise intervention. Additionally, repeated measurements on the same individuals helps to reduce variability associated with between-subject (between-group) comparisons. It is notable that none of the tested variables were altered during the control period, and that statistical relationships were observed between changes in ΔF and changes in strength and functional capacity, suggesting that the changes observed after the training period resulted from the training protocol itself. Nonetheless, the current results provide the necessary proof-in-principle support for larger, randomised, controlled trials to be conducted in the future.

This study provides novel evidence of increased intrinsic lower-threshold motor neurone excitability, likely mediated by stronger PICs, after resistance training in older adults. This was accompanied by an increased ability to modulate PIC strength along with increases in neural drive during higher intensity contractions. These results indicate that not only is PIC strength enhanced but also the ability to vary the motor neurone input-output gain to increase muscle force production is improved. Moreover, we found strong associations between changes in PIC strength and increases in peak discharge rates, maximal strength, and functional capacity following the resistance training. These data contribute to our understanding of the effects of exercise on motor neurone excitability, which is a potential mechanism underpinning the increased motor neurone discharge rate output, strength, and motor function in older adults.

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