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Abstract

There is currently a limited amount of literature investigating the age-related changes in eccentric muscle function *in vitro*. The present study uniquely uses the work loop technique, to better replicate *in vivo* muscle function, in the assessment of the age and muscle-specific changes in acute and sustained concentric and eccentric power and recovery. Whole soleus or EDL muscles were isolated from 10-week and 78-week old mice, and acute and sustained concentric and eccentric work loop power assessed. Despite an age-related increase in body and muscle mass, peak absolute power for both muscles was unaffected by age. Peak concentric power normalised to muscle mass declined significantly for each muscle, whilst peak normalised eccentric power declined only for soleus. Fatigue resistance and recovery for the soleus did not differ between age or contraction type. Older EDL was less resistant to concentric fatigue, but was better able to withstand sustained eccentric activity than young EDL. We have shown that age-related changes in muscle quality are more limited for eccentric function than concentric function. A greater bodily inertia is likely to further reduce *in vivo* locomotor performance in older animals.

Key Words: fatigue, muscles, sarcopenia, dynapenia, CD-1 mice

Introduction

The age-related decline in force (1), power (2) and fatigue resistance (3) has been associated with reduced mobility, quality of life and greater mortality in older adults (4). *In vitro* (5) and *in situ* (6) studies that allow for the examination of contractile and morphological characteristics of muscle have been valuable in allowing the examination of the muscle and fibre type specific ageing response (1,5,7). Given that ageing has been shown to affect neural recruitment (8)**,** assessment of isolated muscle performance can be made, independent of the central nervous system and motivational effects, allowing true maximal force and power output (PO) to be measured. Furthermore, an isolated skeletal muscle approach allows for an accurate measurement of performance relative to muscle size (muscle quality). Assessments of muscle quality using *in vivo* may be confounded by complications in accurately measuring lean tissue mass and intramuscular adipose tissue mass (9). Finally, the effects of muscle endurance cannot be accurately determined *in vivo* given the likely elevation in body mass in older adults. Therefore, larger, older adults would have to generate greater force to overcome greater inertia of the moving limb (10).

Previous isolated muscle work has demonstrated that increasing age is associated with a significant decline in peak absolute force and isometric stress (force relative to muscle cross-sectional area) (11–13), and concentric PO (14). Moreover, Tallis *et al*. (14) indicated that at the skeletal muscle level, fatigue resistance is age and muscle-specific, rationalising previous ambiguous findings examining the effects of ageing on fatigue (7,13,15). By contrast, there is limited evidence that examine age-related changes in eccentric power in isolated muscle models, especially given the important role of eccentric activity in older adults during locomotion and everyday tasks such as balance, moving from a standing to seated position and stair descent (16,17) without undue damage or fatigue (18).

A small number of *in vitro* and *in situ* studies have assessed the effects of age on contraction-induced damage caused by eccentric muscle activity, whereby muscles are activated during substantial increases in muscle length. Such studies have demonstrated that skeletal muscles in older rodents are more susceptible to contraction-induced damage (13,19–21), but produce the same (19) or greater force (21) than younger animals. The strains used in previous animal models are substantially greater than those that would occur *in vivo* (22,23), ranging from 10% to 50% of mean fibre length (13,18,19), and as such further work is needed to establish the effect of eccentric muscle activity using smaller strains that more closely approximates the *in vivo* function of skeletal muscle.

The present study uses the work loop (WL) technique to better replicate *in vivo* contractile dynamics to examine the age-related changes in muscle PO (24–26). Ageing affects isometric force production (5,11–13,15,21,27) and eccentric force production during isovelocity lengthening of skeletal muscles (13,19–21,28), however these contraction types rarely occur *in vivo* (25,29). Estimation of muscle power derived from isokinetic assessments of muscular strength assume that the muscle activates and relaxes instantaneously, and fails to consider dynamic muscle length changes (25). As such, isometric and isovelocity methods have been shown to poorly estimate muscle power compared with the WL technique (25,29). By using sinusoidal waveforms and stimulation parameters that more closely replicate *in vivo* conditions, the WL technique considers the muscle force production during dynamic activity, by considering simultaneous changes in force, muscle length and activation level (24,25,30). Previous studies that have used the WL typically use strains of 0.10 (±5% of optimal length) to ascertain maximal WL power of isolated mouse soleus and EDL (14,30–32). The current work uniquely assesses the age-related changes in peak and sustained concentric and eccentric power using a strain that is more representative of the agonist-antagonist co-activation of skeletal muscles *in vivo* (22,23,33).

The aim of this study was to examine the age-related changes in concentric and eccentric muscle function of mouse soleus (predominantly slow-twitch) and EDL (predominantly fast-twitch) using parameters that better represent *in vivo* dynamic muscle activity, and to determine whether such parameters lead to an age-specific change in the fatigue resistance and subsequent recovery of muscle power following a protocol of repeated maximal concentric and eccentric activation. Furthermore, the present work looked to establish differences between the absolute performance and performance normalised to muscle size derived from isometric contractions and acute measures of concentric and eccentric power, with the absolute performance providing insight into the real wold function of the muscle and the normalised measures providing valuable information with respect to changes in muscle quality.

Methods

Animal Information

Following approval from the Coventry University ethics committee, female CD-1 mice were purchased at 8 weeks of age (Charles River, Harlow, UK) and allowed to mature in-house at Coventry University. Animals were housed in groups of 8-10 and kept in 12:12 hour light:dark cycles at 50% relative humidity. Mice were provided with *ad libitum* access to food (CRM(P); SDS/Dietex International Ltd, Whitham, UK) and water.

Mice were aged to either 10 weeks (n=40) and 78 weeks (n=40) to represent young and old animals respectively. A 10-week age group was utilised as previous research has identified that peak contractile function occurs at this age in the CD-1 strain (14). Previous work has demonstrated that a significant reduction in contractile function occurs by 50 weeks of age for the CD-1 mouse (14), and that mice of this age have an estimated survival rate of 85%, with mortality rate ~50% by 78 weeks of age (34). An older 78-week old group was chosen as it was anticipated that more substantial ageing would have occurred and that this age group would be more reportative of the more advanced stages of ageing that have yet to be examined in this strain. The CD-1 strain was specifically chosen due to the outbred nature of this strain aligning more closely to the heterogeneous variability of human populations than the more commonly used C57Bl6/J strain (7,11,12,19,21,28,29).

Each age group was further split into: Young Concentric (YC), Young Eccentric (YE), Old Concentric (OE) and Old Eccentric (OE) and underwent either the repeated concentric (YC & OC) or eccentric (YE & OC) protocol (n=10 per muscle per protocol).

Muscle Preparation

Animals were sacrificed via cervical dislocation in accordance with the British Home Office Animals (Scientific Procedures) Act 1986, Schedule 1. Animals were weighed to determine body mass, with whole soleus or EDL rapidly isolated from the right hindlimb in frequently changed, oxygenated (95% O_2; 5% CO_2), chilled (~5°C) Krebs-Henseleit solution of composition (mM) NaCl 118; KCl 4.75; $MgSO_4$ 1.18; $NaHCO_3$ 24.8; KH_2PO_4 1.18; glucose 10; CaCl 2.54; pH 7.55 at room temperature (32). Aluminium foil t-clips were wrapped around the distal tendons of each preparation to prevent tendon slippage during the experimental protocol. At the proximal end, a small piece of bone was left intact to allow for attachment to the mechanics rig.

Experimental Set-Up

Each muscle was placed in a flow through chamber filled with circulated oxygenated Krebs-Henseleit solution with a reservoir of solution heated, via an external heater/cooler water bath (Grant LTD6G, Grant Instruments, Shepreth, UK), to 37.0±0.2°C. The temperature within the bath was continuously monitored using a digital thermometer (Checktemp C, Harvard Apparatus, Cambridge, UK). Each muscle was attached, using crocodile clips, to a force transducer (UF1, Pioden Controls Ltd, Henwood Ashford, UK) at one end, and a motor arm (V201, Ling Dynamic Systems, Royston, UK) at the other. The motor arm position was detected by a Linear Variable Displacement Transducer (DFG5.0, Solartron Metrology, Bognor Regis, UK). The muscle was activated through electrical stimulation of the surrounding solution via parallel platinum electrodes, with electrical currents provided by a table top power supply (PL320, Thurlby Thandar Instruments, Huntingdon, UK). Stimulation and length change parameters were controlled manually using custom written software (CEC TestPoint, Measurement Computing, Norton, MA) via a D/A board (KPCI3108, Keithley Instruments, Cleveland, USA) on a standard desktop PC. Once in position, each preparation was allowed to

stabilise for 10-minutes prior to performing isometric contractions. Once stabilised, all preparations were tested for isometric and concentric WL properties prior to their pre-assigned fatigue protocol, with the methodological approach concurrent with previously published protocols (10,14,25,32).

Isometric Contractions

All preparations were optimised for length and stimulation parameters (14-18V for EDL, 12-16V for soleus; fixed stimulation amplitude of 160mA and pulse width of 1.2ms) through a series of isometric twitch contractions, with each parameter individually altered until peak twitch force was attained, as determined by measuring force via a digital storage oscilloscope (2211, Tektronix, Marlow, UK). The muscle length that corresponded to maximal twitch force was measured, using an eyepiece graticule, and defined as L_0. Estimated fibre length for the EDL and soleus was calculated as 75% and 85% of L_0 respectively (23). Maximal isometric force was measured by provision of tetanic stimulations to the preparation. The EDL received a 250ms burst of electrical stimulation and the soleus a 350ms burst of electrical stimulation. The frequency at which the stimulations were provided was altered until peak isometric force was achieved. This was typically 200-220Hz for EDL and 120-140Hz for soleus for both ages. The durations of muscle activation and relaxation were measured as time to half-peak tetanus (THPT) and last stimulus to half tetanus relaxation (LSHR) respectively. A rest period of 5-minutes was imposed between each tetanic stimulation to allow for sufficient recovery.

Assessment of Concentric Work Loop PO

Each muscle was held at the previously determined L_0 and the stimulation amplitude and stimulation frequency that resulted in maximal isometric force were implemented. In the first instance, maximal concentric PO for all experimental groups was determined.

Each preparation was subjected to four sinusoidal length change cycles per set of activity. A cycle frequency, which describes the rate at which the WL's were performed, of 5Hz for soleus and 10Hz for EDL was used as these cycle frequencies typically elicited maximal concentric PO in young (10,14,30–32) and old mice (14). A phase shift of -10ms and -2ms were utilised for the soleus and EDL respectively to elicit peak power (14,32). These phase shifts dictated that the stimulation started 10ms (for soleus) and 2ms (for EDL) before the muscle reached its maximal length. Phasic bursts of electrical stimulation were provided during muscle shortening for durations initially of 50ms and 65ms to the soleus and EDL respectively. This ensured that the muscles were stimulated primarily during shortening and largely inactive during lengthening. Each set of WLs' was performed every 5-minutes to allow for sufficient recovery. For each WL cycle, a plot of muscle force against muscle length was generated by the Testpoint software, where the area of the WL equated to positive work during active shortening and negative work during passive lengthening (24). The strain (typically 0.08 – 0.10 for all muscles and ages) and burst duration for each muscle was altered to ensure maximal concentric PO was achieved (31).

Assessment of Eccentric Work Loop PO

Eccentric power was not initially assessed for the YE and OE groups as optimisation of the muscle to achieve maximal eccentric PO may damage the muscle. Instead, the second WL of the eccentric fatigue protocol was taken to calculate eccentric PO.

Repeated Concentric and Eccentric Work Loop Protocols

The ability to sustain power during repeated concentric and eccentric muscle activity for each experimental group was determined by imposing fifty consecutive WL's on each muscle. For the YC and OC groups, the strain and stimulation parameters which elicited maximal concentric PO were maintained.

For the YE and OE groups, a cycle frequency of 5Hz and 10Hz was maintained for soleus and EDL respectively. A strain of -0.10 was used for all muscles to ensure the muscle passively shortened, followed by stimulation through lengthening. A stimulation phase shift of -10ms and -5ms for the soleus and EDL muscles respectively was maintained to ensure the stimulation was provided before the shortest muscle length. A burst duration of 72ms and 55ms was used for the soleus and EDL respectively to ensure the muscle was sufficiently stimulated throughout the lengthening phase.

Time to fatigue of the experimental groups was measured as the time taken for power to drop to 50% of the maximum concentric or eccentric PO.

Recovery Protocol

The ability for each muscle to recover concentric power following the concentric and eccentric protocols was monitored for 30-minutes. The YC and OC groups were stimulated every 10-minutes as performed in our previous work (14,32), whilst the YE and OE groups were stimulated every 5-minutes to more closely examine recovery. The recovery of concentric PO after 30-minutes was expressed as a percentage relative to the pre-protocol maximal concentric PO for each group.

Reassessment of Maximal Force and Stress

Following 5-minutes recovery after the final WL of the recovery protocol, maximal absolute force and isometric stress was reassessed in the YE and OE groups as a further means of assessing potential contraction-induced damage, with the severity of the damage determined by the magnitude of the deficit in force and stress compared to pre-fatigue measurements (20,26). This was not performed with muscles of the YC and OC groups as repeated concentric WL's do not significantly impair the recovery of power in the soleus and EDL following the same fatigue protocol used in previous studies (14,32).

Each experiment lasted for approximately 105 minutes from the moment of cervical dislocation through to the final stimulation.

Muscle Mass Dimensions and Calculations

After the final stimulation, muscles were removed from the rig, and bone, aluminium clips, and tendons removed leaving the skeletal muscle intact. The muscles were blotted lightly with tissue paper to remove excess fluid and weighed to determine wet muscle mass (TL-64, Denver Instrument Company, Arvada, USA). The mean muscle cross-sectional area (CSA) was calculated from L_0, muscle mass and an assumed muscle density of 1060kg.m^{-3} (35). Maximal isometric stress (kN.m^2) was calculated as peak tetanic force divided by mean muscle CSA. Absolute PO (Watts) was calculated as the net work of a WL (i.e. the work done by the muscle during shortening minus the work done on the muscle during lengthening) multiplied by cycle frequency. Normalised PO (W.kg^{-1} muscle mass) was calculated as absolute PO divided by muscle mass.

Statistical Analyses

All data are presented as mean ± standard error of mean (S.E.M). All data was normally distributed and showed homogeneity of variance, so parametric analyses were employed. The data for animal and muscle morphology, isometric contractile properties, maximal concentric and eccentric WL force and power were pooled for age for each muscle and analysed using independent samples T-Tests (Excel 2016, Microsoft).

A two-factor analysis of variance (ANOVA) was performed in SPSS (SPSS, IL, USA) to determine if repeated WL's caused a significant reduction in muscle PO and whether this was age-specific. Independent samples T-test were used to determine significant differences in time to fatigue between each age group for both soleus and EDL.

Recovery was assessed by a two-factor ANOVA with power and age as the main factors. An independent samples t-test was used to determine whether there was a significant difference between age groups in PO normalised to muscle mass after 30-minutes of recovery.

A repeated measures ANOVA was used to determine whether post-fatigue maximal absolute force and isometric stress of the muscles that underwent the eccentric protocol were significantly affected by age. Level of significance was set at $P<0.05$ for all analyses.

Results

Morphological and Isometric Contractile Properties

Ageing resulted in a significant increase in animal body mass (56%), soleus and EDL muscle mass (30% and 12% respectively) and CSA (32% and 9% respectively) with no change in muscle length (Table 1). Maximal isometric stress (absolute force divided by muscle cross-sectional area) for the 10-week old soleus and EDL are in line with previously reported values for the CD-1 strain (Table 2): 189kN.m^2 to 267kN.m^2 for soleus (32,36), 250kN.m^2 to 300kN.m^2 for EDL (14,36,37). There was a significant age-related reduction in maximal isometric twitch and tetanus stress for the both muscles, whilst absolute twitch and tetanus force declined significantly for the EDL only (Table 2). THPT was significantly longer for older soleus but not older EDL whilst LSHR was significantly prolonged for older EDL but not for soleus (Table 2).

Maximal Concentric and Eccentric Work Loop PO and Peak Force

Power normalised to muscle mass for 10-week old animals are similar to previously reported values for the soleus, ranging from 31.7W.kg^{-1} to 33.0W.kg^{-1} for the soleus (32,36),: 59.8W.kg^{-1} to 99.0W.kg^{-1} for EDL (14,36,37) (Table 2). Absolute concentric and eccentric PO generated by soleus and EDL was not significantly affected by age ($P>0.24$ for all) though when normalised to muscle mass, the maximal concentric power of both the soleus and EDL was significantly lower in the OC groups by 21% and 16% for soleus and EDL respectively (Table 2). Maximal eccentric PO normalised to muscle mass was not significantly altered by age for the EDL ($P>0.77$) but declined significantly for older soleus by 27% (Table 2).

Peak concentric force of the OC EDL was 23% lower than the peak concentric force achieved by the YC EDL muscles ($P<0.002$), though there were no differences in peak concentric force for young and old soleus (Table 2, $P>0.41$). Likewise, there were no

significant differences in the peak eccentric force achieved both muscles of each age (Table 2, P>0.84 for both).

Fatigue Resistance to Repeated Concentric and Eccentric Contractions

Fifty consecutive concentric contractions resulted in significant reductions in relative power over time for both muscles (Figure 1 A&B; P<0.001). Age did not affect fatigue of soleus (Figure 1A; P=0.87). Typical WL shapes in showed that the YC soleus had slightly more pronounced negative work in the early part of relengthening than OC soleus as demonstrated by increased passive force through relengthening back to L_0 with each WL, though this has little effect on fatigue resistance (Figures 2 C&D). No differences in concentric fatigue were observed for the first 1.2 seconds for the EDL, but fatigued significantly faster thereafter compared to the YC EDL group, with a 10% decrease in time to 50% of pre-protocol maximum power (Figure 1B; P<0.05). Negative work of the OC EDL was greater than YC EDL during the relengthening phase of the WL as shown by an increase in passive force through relengthening from the shortest muscle length with each WL, and therefore had poorer fatigue resistance (Figure 2 A&B).

Fifty repeated eccentric WL's did not elicit a significant reduction in relative PO over time for YE or OE soleus (Figure 1C; P=0.88). WL shapes indicated OE soleus have smaller WL areas at WL 2 due to absorbing less net work than YE soleus. By WL 18, YE soleus produces slightly less force during muscle lengthening, but this has no impact on muscle fatigability (Figure 3 C&D). By contrast, the EDL was unable to sustain eccentric power over time for both the YE and OE groups (Figure 1D; P<0.05). The muscles of the YE EDL lost power significantly faster than the OE EDL group (Figure 1D; P<0.001), with a 29% difference in the time to 50% of relative maximum PO. Positive work during the re-shortening phase of

the WL is likely to be greater for the OE EDL than the YE EDL due to a downward shift in the right-hand portion of the YE EDL WL shape (Figure 3 A&B).

Recovery of Concentric Power

There was no significant difference in recovery of concentric power between young and old soleus following the concentric protocol (P=0.38), nor in recovery over time (Figure 4A; P=0.47). There was a tendency for age to affect recovery of power for YE and OE soleus (P=0.08) but there was a significant recovery over time (Figure 4C; P<0.001). The soleus from each age had fully recovered by 30-minutes (YE 98±2%; OE 98±1%).

The EDL of the OC group recovered concentric power to a greater magnitude than the YC group following the concentric protocol (Figure 4B; P=0.03, 59±4% vs. 43±5% respectively after 30-minutes), with significant recovery over time (P=0.03), though there was no age*time interaction (P=0.96). OE EDL had a significantly higher relative PO than YE EDL following the eccentric protocol (P<0.001), with a significant reduction in YE EDL relative PO over time (Figure 4D; P<0.001). There was a tendency for an interaction between age and time indicating a potential for older EDL to lose less power during the recovery period (P=0.08).

Recovery of Absolute Force and isometric Stress

Maximal absolute force and isometric stress of the soleus remained unchanged for both ages (Figure 5 A&B; P>0.36 for both). Absolute force and isometric stress declined significantly for the EDL, but to a greater magnitude in YE EDL, with force declining by 59% and 40% for YE and OE EDL respectively and stress by 57% and 38% for YE and OE EDL respectively (Figure 5A&B; P<0.001 for all).

Discussion

The present work is the first to compare the age and muscle-specific changes in acute and sustained concentric and eccentric PO by utilising the WL technique as a better representation of real world muscle function. An age-related reduction in acute and sustained concentric PO for the soleus and EDL did not mirror those observed for eccentric muscle activity, which was largely unaffected for the older skeletal muscles. Moreover, age did not affect the ability for the soleus to sustain concentric and eccentric power. The most significant finding pertains to the ability of older EDL to better withstand the damaging effects of a sustained bout of eccentric muscle activity than younger EDL, using a strain that better replicates *in vivo* muscle activity. Consequently, the reduction in eccentric contraction-induced force and power loss following sustained eccentric activity of the older EDL may indicate a reduced susceptibility to eccentric muscle damage with increasing age.

Age-Related Changes in Skeletal Muscle Contractility

Despite a significantly larger animal body mass and muscle mass (Table 1), absolute force and power remained unchanged in older soleus and EDL, whilst isometric stress (absolute force divided by cross-sectional area) and concentric power normalised to muscle mass was significantly reduced in the older age group (Table 2). In line with previous work examining the effects of ageing on muscle quality, there is a marked reduction in isometric stress and normalised WL power in the older skeletal muscles of this study (32,36,37), and this is more pronounced in the present study using 78-week old mice compared to Tallis *et al*. (14) using younger, 50-week old mice of the same strain. The greater animal body mass may act as a training stimulus due to the elevated body mass creating a loading effect on the lower limbs, hence the maintenance of absolute force and absolute concentric power (10).

Similar to previous work examining the effect of ageing on isolated skeletal muscle (13,14,28), these results infer that substantial muscle ageing can occur without the muscle wasting that defines sarcopenia. The reduction in stress and normalised concentric power indicate an age-related reduction in muscle quality. Larger muscle of poorer quality adds further to an already elevated bodily inertia (10) and may further contribute to poorer *in vivo* locomotor capacity. A loss in muscle quality is not consistent for all contraction types as eccentric power normalised to muscle mass was well maintained in old EDL, though normalised eccentric power for the soleus declined with age. This muscle-specific reduction in acute eccentric power may partially explain the ambiguity surrounding eccentric contractile properties in older human populations, where some have reported an age-related decline in eccentric force of lower limbs (38,39) whilst others report no change (40,41).

The age-related decline in muscle function may relate to an increase in dysfunctional Ca^{2+} handling proteins particularly given the age-related increase in soleus activation time and EDL relaxation time (Table 2). Previous literature has observed an age-related dysfunction in sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) activity in slow-twitch (42) and fast-twitch (14) muscle fibres, likely causing leakage of sarcoplasmic reticulum (SR) Ca^{2+} from ryanodine receptors into the cytoplasm (43). Excitation-contraction uncoupling and reduced SERCA activity could explain why normalised concentric and eccentric power declines to a greater magnitude in older soleus than EDL (Table 2) given that slow-twitch fibres experience greater excitation-contraction uncoupling than fast-twitch fibres (44).

Increasing age has been shown to have an effect on the interaction of the contractile proteins needed for cross bridge formation (27). Single fiber experiments, independent of calcium kinetics and non-contractile elements, demonstrate significant reductions in isometric force production with age, resulting in fewer actin-myosin binding sites maintaining a strong-binding structure (27). By contrast, eccentric force production of single permeabilised EDL

fibres of 27 month old mice has been shown to be significantly higher than those of younger animals, but were not different in whole muscles (21). Peak eccentric force was unaffected by age, but was significantly lower for older EDL during eccentric WL's (Table 2) indicating age-related alterations in cross-bridge kinetics are likely to be dependent on the mode of muscle activity. Additionally, intramyocellular lipid accumulation occurs with progressive ageing (45) which have been further associated with a reduction in muscle quality (10).

Fatigue Response During Repeated Concentric Activity

There was no difference in concentric fatigue resistance between soleus muscles from young and old mice (Figure 1A) though this was not the case for older EDL (Figure 1C). WL shapes indicate older EDL produced more negative work during muscle relengthening as the fatigue protocol progressed compared with YC EDL (Figure 2A&B). Relaxation time increases with each WL (14,31,32) indicating that Ca^{2+} has not been fully reabsorbed by the SR prior to the next contraction, therefore the muscle is still partially active through relengthening resulting in the progressive absorption of negative work leading to increased fatigability that is more pronounced in older EDL. Whilst there is a lack of evidence observing sustained concentric power using mammalian tissues, it appears 78-week old EDL muscles are able to sustain concentric power for a longer period than the 50-week old animals during the same fatigue protocol (14). Whilst absolute power is well maintained, it is expected that the older individuals will fatigue faster *in vivo* when working at the same relative intensities due to an elevated body mass (7).

Fatigue Response During Repeated Eccentric Activity

The fatigue response to the eccentric protocol was age and muscle-specific. For soleus muscle, the reduction in power during sustained eccentric activity was not significantly affected

by age, and as such there was very little difference in the WL shapes (Figure 3 C&D). As the maximal eccentric power of the older group was substantially lower, this would result in a reduced amount of relative power over the duration of the fatiguing protocol (Figure 1C).

Conversely, sustained power following the eccentric protocol was significantly reduced in young EDL compared to old EDL (Figure 1D), despite maximal eccentric power production being unaffected by age (Table 2). Typical WL shapes indicated that the force produced during lengthening decreased much more in young EDL, than old EDL, over the series of WL's (Figure 3A).

Repeated eccentric activity may work to sustain locomotor performance *in vivo* given the elevated body mass. Older muscles generated the same concentric and eccentric absolute PO and peak WL force, yet body mass of older mice increased by 56%. Therefore, the older mice would have to generate greater power to overcome the bodily inertia during locomotion or braking motions at the same speed as younger mice (14).

Recovery of Concentric Power

Recovery of concentric power (Figure 4 A&C), and recovery of absolute force and isometric stress (Figure 5 A&B) of the soleus following repeated concentric and eccentric activity was unaffected by age. Given that there was no age-related change in fatigue resistance of the soleus following each protocol, the consequent ability to recover concentric power indicates no undue damage or fatigue.

By contrast, the recovery of the EDL of the older age group was significantly greater following the concentric protocol when compared to the young group (Figure 4B), likely due to the ability of slower muscle to recover faster following repetitive stimulation (32), which would also explain the near full recovery of the soleus for both ages and contraction types. Tallis *et al*. (14) observed no differences between the 10-week and 50-week old EDL in the

recovery of concentric power, with the OC EDL group of this study recovering to a greater extent than the 50-week old animals.

The current study used a smaller strain as a closer representation of length changes which occur more regularly *in vivo* (22,23), although this smaller strain may have still caused damage in the EDL for both ages as demonstrated by a consistent reduction in post eccentric protocol power (Figure 2D) and reduced absolute force and isometric stress (Figures 5 A&B). Given that high eccentric force is associated with greater muscle damage (18), this may account for the difference in recovery observed between the EDL and soleus. Interestingly, recovery of the young EDL was impaired to a greater extent, following the eccentric protocol, than the older EDL (Figure 4D), which could be due to greater structural damage in the younger group. An age-related increase in structural damage of the skeletal muscles has been previously attributed to greater impairment of contractile function following eccentric muscle activity in older EDL. Zerba *et al*. (20) found that the tetanic force deficit following 75 lengthening actions at a total mean fiber length change of 25% was significantly greater in older mouse EDL muscles compared to young and adult mice. Additionally, the relative loss of isometric force following single stretches of single permeabilised of 27-34-month-old rat EDL fibres was greater than that of 5-6-month-old rats at a strain of 10% and 20% of mean fiber length, but was not different at 5% (19), highlighting that larger strains are required to significantly damage older muscles during acute and sustained eccentric activity.

Implications

Given the increase in muscle mass and body mass, skeletal muscles *in vivo* would need to generate greater power to overcome the greater bodily inertia. Coupled with a reduction in concentric muscle quality and fatigue, older adults are likely to fatigue faster during activities involving concentric activation of skeletal muscles compared to eccentric actions, which are relatively well preserved in isolated muscle. Focus should be placed on interventions for managing weight status and improving eccentric function relative to body size to improve locomotor function. However, it should still be recognised that eccentric activity is associated with greater damage, such that recovery of muscle following this mode of exercise may be hindered in older adults due to the age-related reduction in myogenesis (46).

Limitations

The current study provides a simplified approach to assessing the type of dynamic muscle activity that occurs *in vivo*. A sinusoidal waveform was used as an approximation of the otherwise complex cyclical length change patterns that occur during *in vivo* locomotion (16). Fibre recruitment and length change waveform are likely to be manipulated throughout the activity *in vivo* (47). During locomotion, the pattern of activation is likely to frequently change to maximise positive work and minimise negative work during concentric activity, and *vice versa* during eccentric activity, to maximise the muscle's ability to sustain PO for longer durations.

Whilst reporting isometric stress and normalised WL power provides a measure of muscle quality, the reduction in stress with aging is likely to be primarily caused by a reduction in the performance of contractile tissues rather than the increase in non-contractile mass (10). In older muscles, it is expected that a smaller proportion of the muscle mass is contractile proteins due to a greater non-contractile mass, such as increased intramuscular fat deposition, meaning the density of the muscle is likely to decrease. As we assumed a constant muscle density with age, the muscle CSA may be underestimated in the older skeletal muscles due to an overestimated muscle density (14). This may mean that the isometric stress is likely to be an overestimate in older muscles, meaning the magnitude of the decline in normalised force may be greater than reported. It would be useful in future studies to analyse skeletal muscle protein and contractile protein across a similar age range to provide valuable insight into the underpinning mechanisms for observed changes in contractile function relative to the increase in muscle mass in older animals.

Conclusion

This study demonstrates that ageing is not uniform for all contraction types and muscles, which may have complex consequences for *in vivo* locomotor function in older adults. The loss of force and power relative to muscle size in the present study, as opposed to the apparent reductions in absolute force and power as observed in humans, appears to be the greatest factor in alterations of contractile function in this study, offering further support to the dynapenic mechanism of muscle ageing. The observed general reduction in muscle quality, coupled with an age-related increase in body mass observed in the present study, could be a key factor in the reduced locomotor function of older adults. However, older EDL muscles are capable of withstanding repeated eccentric muscle activity to a greater extent than younger muscles and appear to sustain less damage than younger EDL muscles. This could be important for exercise prescription, where eccentric exercises can be incorporated into training regimens to improve eccentric muscular function. Thus, locomotor capabilities and physical activity levels could be increased and overall quality of life improved.

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References

1. Lauretani F, Russo CR, Bandinelli S, et al. Age-associated changes in skeletal muscles and their effect on mobility: an operational diagnosis of sarcopenia. *J Appl Physiol*. 2003;95(5):1851-1860. doi:10.1152/japplphysiol.00246.2003.

2. Reid KF, Fielding RA. Skeletal muscle power: a critical determinant of physical functioning in older adults. *Exerc Sport Sci Rev*. 2012;40(1):4-12. doi:10.1097/JES.0b013e31823b5f13.

3. Sunnerhagen KS, Hedberg M, Henning GB, Cider A, Svantesson U. Muscle performance in an urban population sample of 40- to 79-year-old men and women. *Scand J Rehabil Med*. 2000;32(4):159-167.

4. Rizzoli R, Reginster JY, Arnal JF, et al. Quality of life in sarcopenia and frailty. *Calcif Tissue Int*. 2013;93(2):101-120. doi:10.1007/s00223-013-9758-y.

5. Ballak SB, Degens H, de Haan A, Jaspers RT. Aging related changes in determinants of muscle force generating capacity: a comparison of muscle aging in men and male rodents. *Ageing Res Rev*. 2014;14:43-55. doi:10.1016/j.arr.2014.01.005.

6. Warren GL, Ingalls CP, Lowe DA, Armstrong RB. Excitation-contraction uncoupling: major role in contraction-induced muscle injury. *Exerc Sport Sci Rev*. 2001;29(2):82-87.

7. Pagala MK, Ravindran K, Namba T, Grob D. Skeletal muscle fatigue and physical endurance of young and old mice. *Muscle Nerve*. 1998;21(12):1729-1739. doi:10.1002/(SICI)1097-4598(199812)21:12<1729::AID-MUS16>3.0.CO;2-V.

8. Hepple RT, Rice CL. Innervation and neuromuscular control in ageing skeletal muscle. *J Physiol*. 2016;594(8):1965-1978. doi:10.1113/JP270561.

9. Fragala MS, Kenny AM, Kuchel GA. Muscle quality in aging: a multi-dimensional approach to muscle functioning with applications for treatment. *Sports Med*. 2015;45(5):641-658. doi:10.1007/s40279-015-0305-z.

10. Tallis J, Hill C, James RS, Cox VM, Seebacher F. The effect of obesity on the contractile performance of isolated mouse soleus, EDL, and diaphragm muscles. *J Appl Physiol*. 2017;122(1):170-181. doi:10.1152/japplphysiol.00836.2016.

11. Moran AL, Warren GL, Lowe DA. Soleus and EDL muscle contractility across the lifespan of female C57BL/6 mice. *Exp Geron*. 2005;40(12):966-975. doi:10.1016/j.exger.2005.09.005.

12. Graber TG, Kim JH, Grange RW, McLoon LK, Thompson LV. C57BL/6 life span study: age-related declines in muscle power production and contractile velocity. *Age Dordr Neth*. 2015;37(3):9773-015-9773-1. Epub 2015 Apr 17. doi:10.1007/s11357-015-9773-1.

13. Chan S, Head SI. Age- and gender-related changes in contractile properties of non-atrophied EDL muscle. *PLoS ONE*. 2010;5(8):e12345. doi:10.1371/journal.pone.0012345.

14. Tallis J, James RS, Little AG, Cox VM, Duncan MJ, Seebacher F. Early effects of ageing on the mechanical performance of isolated locomotory (EDL) and respiratory (diaphragm) skeletal muscle using the work-loop technique. *Am J Physiol Regul Integr Comp Physiol*. 2014;307(6):R670-84. doi:10.1152/ajpregu.00115.2014.

15. González E, Delbono O. Age-dependent fatigue in single intact fast- and slow fibers from mouse EDL and soleus skeletal muscles. *Mech Ageing Dev*. 2001;122(10):1019-1032. doi:10.1016/S0047-6374(01)00229-9.

16. Dickinson MH, Farley CT, Full RJ, Koehl MA, Kram R, Lehman S. How animals move: an integrative view. *Science*. 2000;288(5463):100-106. doi:10.1126/science.288.5463.100.

17. LaStayo PC, Woolf JM, Lewek MD, Snyder-Mackler L, Reich T, Lindstedt SL. Eccentric muscle contractions: their contribution to injury, prevention, rehabilitation, and sport. *J Orthop Sports Phys Ther*. 2003;33(10):557-571. doi:10.2519/jospt.2003.33.10.557.

18. Lovering RM, Brooks SV. Eccentric exercise in aging and diseased skeletal muscle: good or bad? *J Appl Physiol*. 2014;116(11):1439-1445. doi:10.1152/japplphysiol.00174.2013.

19. Brooks SV, Faulkner JA. The magnitude of the initial injury induced by stretches of maximally activated muscle fibres of mice and rats increases in old age. *J Physiol*. 1996;497 (Pt 2)(Pt 2):573-580. doi:10.1113/jphysiol.1996.sp021790.

20. Zerba E, Komorowski TE, Faulkner JA. Free radical injury to skeletal muscles of young, adult, and old mice. *Am J Physiol*. 1990;258(3 Pt 1):C429-35.

21. Brooks SV, Faulkner JA. Isometric, shortening, and lengthening contractions of muscle fiber segments from adult and old mice. *Am J Physiol - Cell Physiol*. 1994;267(2):C507-C513.

22. Butterfield TA. Effect of altering starting length and activation timing of muscle on fiber strain and muscle damage. *J Appl Physiol*. 2006;100(5):1489-1498. doi:10.1152/japplphysiol.00524.2005.

23. Hoyt DF, Wickler SJ, Biewener AA, Cogger EA, De La Paz KL. In vivo muscle function vs speed. I. Muscle strain in relation to length change of the muscle-tendon unit. *J Exp Biol*. 2005;208(Pt 6):1175-1190. doi:10.1242/jeb.01486.

24. Josephson RK. Mechanical power output from striated muscle during cyclic contraction. *J Exp Biol*. 1985;114(1):493-512.

25. James RS, Young IS, Cox VM, Goldspink DF, Altringham JD. Isometric and isotonic muscle properties as determinants of work loop power output. *Pflüg Arch*. 1996;432(5):767-774. doi:10.1007/s004240050197.

26. Choi SJ, Widrick JJ. Combined effects of fatigue and eccentric damage on muscle power. *J Appl Physiol*. 2009;107(4):1156-1164. doi:10.1152/japplphysiol.00403.2009.

27. Lowe DA, Thomas DD, Thompson LV. Force generation, but not myosin ATPase activity, declines with age in rat muscle fibers. *Am J Physiol Cell Physiol*. 2002;283(1):C187-92. doi:10.1152/ajpcell.00008.2002.

28. Phillips SK, Bruce SA, Woledge RC. In mice, the muscle weakness due to age is absent during stretching. *J Physiol*. 1991;437:63-70. doi:10.1113/jphysiol.1991.sp018583.

29. Lynch GS, Hinkle RT, Chamberlain JS, Brooks SV, Faulkner JA. Force and power output of fast and slow skeletal muscles from mdx mice 6-28 months old. *J Physiol*. 2001;535(2):591-600. doi:10.1111/j.1469-7793.2001.00591.x.

30. James RS, Altringham JD, Goldspink DF. The mechanical properties of fast and slow skeletal muscles of the mouse in relation to their locomotory function. *J Exp Biol*. 1995;198(2):491-502.

31. Askew GN, Young IS, Altringham JD. Fatigue of mouse soleus muscle, using the work loop technique. *J Exp Biol*. 1997;200(22):2907-2912.

32. Tallis J, James RS, Cox VM, Duncan MJ. The effect of a physiological concentration of caffeine on the endurance of maximally and submaximally stimulated mouse soleus muscle. *J Physiol Sci*. 2013;63(2):125-132. doi:10.1007/s12576-012-0247-2.

33. Hortobágyi T, DeVita P. Mechanisms responsible for the age-associated increase in coactivation of antagonist muscles. *Exerc Sport Sci Rev*. 2006;34(1):29-35. doi:10.1097/00003677-200601000-00007.

34. Lang PL, White WJ. *Growth, Development, Life Span and Select Lesion Incidence in the Aging CD-1 Mouse.* Wilmington, MA: Charles Rivers Laboratories; 2005.

35. Méndez J, Keys A. Density and composition of mammalian muscle. *Metabolism*. 1960;9:184-188.

36. Tallis J, James RS, Cox VM, Duncan MJ. The effect of physiological concentrations of caffeine on the power output of maximally and submaximally stimulated mouse EDL (fast) and soleus (slow) muscle. *J Appl Physiol Bethesda Md 1985*. 2012;112(1):64-71. doi:10.1152/japplphysiol.00801.2011.

37. James RS, Kohlsdorf T, Cox VM, Navas CA. 70 microM caffeine treatment enhances in vitro force and power output during cyclic activities in mouse extensor digitorum longus muscle. *Eur J Appl Physiol*. 2005;95(1):74-82. doi:10.1007/s00421-005-1396-2.

38. Lindle RS, Metter EJ, Lynch NA, et al. Age and gender comparisons of muscle strength in 654 women and men aged 20-93 yr. *J Appl Physiol*. 1997;83(5):1581-1587.

39. Delbaere K, Bourgois J, Witvrouw EE, Willems TM, Cambier DC. Age-related changes in concentric and eccentric muscle strength in the lower and upper extremity: A cross-sectional study. *Isokinet Exerc Sci*. 2003;11(3):145-151.

40. Perry MC, Carville SF, Smith IC, Rutherford OM, Newham DJ. Strength, power output and symmetry of leg muscles: effect of age and history of falling. *Eur J Appl Physiol*. 2007;100(5):553-561. doi:10.1007/s00421-006-0247-0.

41. Poulin MJ, Vandervoort AA, Paterson DH, Kramer JF, Cunningham DA. Eccentric and concentric torques of knee and elbow extension in young and older men. *Can J Sport Sci*. 1992;17(1):3-7.

42. Lamboley CR, Wyckelsma VL, McKenna MJ, Murphy RM, Lamb GD. Ca(2+) leakage out of the sarcoplasmic reticulum is increased in type I skeletal muscle fibres in aged humans. *J Physiol*. 2016;594(2):469-481. doi:10.1113/JP271382.

43. Andersson DC, Betzenhauser MJ, Reiken S, et al. Ryanodine receptor oxidation causes intracellular calcium leak and muscle weakness in aging. *Cell Metab*. 2011;14(2):196-207. doi:10.1016/j.cmet.2011.05.014.

44. Ryan M, Ohlendieck K. Excitation-Contraction Uncoupling and Sarcopenia. *Basic Appl Myol*. 2004;14(3):141-154.

45. Goodpaster BH, Carlson CL, Visser M, et al. Attenuation of skeletal muscle and strength in the elderly: The Health ABC Study. *J Appl Physiol 1985*. 2001;90(6):2157-2165.

46. Le Grand F, Rudnicki MA. Skeletal muscle satellite cells and adult myogenesis. *Curr Opin Cell Biol*. 2007;19(6):628-633. doi:10.1016/j.ceb.2007.09.012.

47. Wakeling J, Rozitis A. Motor unit recruitment during vertebrate locomotion. *Anim Biol*. 2005;55(1):41-58. doi:10.1163/1570756053276880.

Table 1 - Comparisons of pooled animal and muscle morphological measurements for each group.

Animal Morphology	**Young (10 weeks)**		**Old (78 weeks)**	
Body mass (g)	29.7 ± 0.5		46.4 ± 2.0*	
Muscle Morphology	**Soleus**		**EDL**	
	Young	**Old**	**Young**	**Old**
Muscle length (mm)	9.4 ± 0.1	9.3 ± 0.1	9.0 ± 0.1	9.2 ± 0.1
Muscle mass (mg)	7.6 ± 0.3	9.9 ± 0.3*	10.9 ± 0.3	12.0 ± 0.3*
Muscle CSA (m^2)	$7.65x10^{-7} \pm 2.76x10^{-8}$	$1.01x10^{-6} \pm 3.16x10^{-8}$*	$1.14x10^{-6} \pm 2.59x10^{-8}$	$1.24x10^{-6} \pm 2.98x10^{-8}$*

Values presented as mean ± S.E.M.

* denotes significant ($P<0.05$) difference between age groups.

Animal morphology; n=40 per age. Muscle morphology; n=20 per muscle per age.

CSA = cross-sectional area.

Table 2 - Pooled isometric and work loop contractile properties of young (10 weeks) and old (78 weeks) soleus and EDL muscles.

	Soleus			EDL		
Contractile Measure	**Young**	**Old**	**% change vs. young**	**Young**	**Old**	**% change vs. young**
Maximal twitch force (mN)	28 ± 1	28 ± 1	1%	64 ± 2	56 ± 3	-12%*
Maximal tetanus force (mN)	212 ± 9	224 ± 6	6%	376 ± 12	331 ± 15	-12%*
Maximal twitch stress ($kN.m^2$)	37 ± 2	29 ± 1	-23%*	57 ± 2	45 ± 2	-20%*
Maximal tetanus stress ($kN.m^2$)	280 ± 10	225 ± 7	-20%*	332 ± 9	269 ± 13	-19%*
Time to half peak tetanus (ms)	34 ± 1	39 ± 1	13%*	15 ± 1	16 ± 1	9%
Last stimulus to half relaxation (ms)	44 ± 2	48 ± 2	9%	13 ± 1	16 ± 1	29%*
Maximal concentric PO (Watts)	237 ± 12	246 ± 12	4%	1066 ± 43	1007 ± 57	-6%
Maximal concentric PO ($W.kg^{-1}$ muscle mass)	31 ± 1	25 ± 1	-21%*	99 ± 5	83 ± 4	-16%*
Peak concentric force (mN)	86 ±6	80 ± 4	-7%	198 ±10	152 ±7	-23%*
Maximal eccentric PO (Watts)†	-1038 ± 53	-1052 ± 48	-1%	-2682 ± 150	-2990 ± 214	-10%
Maximal eccentric PO ($W.kg^{-1}$ muscle mass)†	-146 ± 10	-115 ± 6	27%*	-260 ± 21	-253 ± 17	3%
Peak eccentric force (mN)†	294 ± 15	298 ± 9	1%	485 ± 17	484 ± 19	0.3%

Values presented as mean ± S.E.M.

* denotes significant (P<0.05) differences between each age group.

n=20 for all muscles and ages except for where † is placed, indicating n=10 per muscle per age.

PO = Power output. Stress = force ÷ muscle cross-sectional area.

Figures

Figure 1 - Time-course of sustained power production of young (●) and old (■) skeletal muscles relative to the pre-protocol maximum concentric power during fifty repeated concentric contractions (A, soleus; B, EDL), and eccentric power relative to the maximum eccentric power during fifty repeated eccentric muscle actions (C, soleus; D, EDL). Values presented as mean ± S.E.M. * significant differences between age groups for time to fatigue.

Figure 2 - Effect of age on typical work loop shapes following concentric fatigue for the YC and OC EDL (A & B) and YC and OC soleus (C & D). Figures plotted as force against strain (%L_0). Work loops 2, 10, and 18 of the fatigue protocol are shown for each group. Work loops to be interpreted in the anti-clockwise (right-to-left) direction from L_0, indicated by a downwards arrow for each figure.

Figure 3 - Effect of age on typical work loop shapes following eccentric fatigue at 10Hz cycle frequency for YE EDL (A) and soleus (C) and OE EDL (B) and soleus (D). Figures plotted as force against strain (%L_0). Work loops 2, 10, and 18 of the fatigue protocol are shown for each group. Work loops to be interpreted in the clockwise (left-to-right) direction from L_0, indicated by a downwards arrow for each figure.

Figure 4 - Time-course of recovery of concentric power output relative to the pre-protocol maximum power for young (●) and old (■) skeletal muscles every 10-minutes following repeated concentric contractions (A, soleus; B, EDL) and every 5-minutes following repeated eccentric muscle activity (C, soleus; D, EDL). Values presented as mean ± S.E.M. * significant difference between age groups at each time point.

Figure 5 – Age-related changes in maximal absolute force (A) and maximal isometric stress (B) prior to fatigue (SOL-Pre & EDL-Pre, n=20 for both muscles and ages). Absolute force (A) and isometric stress (B) were reassessed following the final work loop of the recovery protocol in the YE & OE groups (SOL-Post & EDL-Post; n=10 for both muscles and ages). Values presented as mean ± S.E.M. * significant ($P<0.05$) changes in maximal absolute force or isometric stress with age. • significant ($P<0.05$) reductions in pre vs. post assessments of maximal absolute force or isometric stress. Stress = force ÷ muscle cross-sectional area.

Tables

Table 1 - Comparisons of pooled animal and muscle morphological measurements for each group.

Table 2 - Pooled isometric and work loop contractile properties of young (10 weeks) and old (78 weeks) soleus and EDL muscles.