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RESEARCH ARTICLE

The influence of training-induced sarcomerogenesis on the history dependence of force

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ABSTRACT

The increase or decrease in isometric force following active muscle lengthening or shortening, relative to a reference isometric contraction at the same muscle length and level of activation, are referred to as residual force enhancement (rFE) and residual force depression (rFD), respectively. The purpose of these experiments was to investigate the trainability of rFE and rFD on the basis of serial sarcomere number (SSN) alterations to history-dependent force properties. Maximal rFE/rFD measures from the soleus and extensor digitorum longus (EDL) of rats were compared after 4 weeks of uphill or downhill running with a no-running control. SSN adapted to the training: soleus SSN was greater with downhill compared with uphill running, while EDL demonstrated a trend towards more SSN for downhill compared with no running. In contrast, rFE and rFD did not differ across training groups for either muscle. As such, it appears that training-induced SSN adaptations do not modify rFE or rFD at the whole-muscle level.

KEY WORDS: Fascicle, Muscle, Residual force enhancement, Residual force depression, Sarcomere, Eccentric, Concentric, Uphill running, Downhill running

INTRODUCTION

The history dependence of force is an intrinsic property of skeletal muscle that has been investigated in individual sarcomeres (Joumaa and Herzog, 2010; Joumaa et al., 2008; Johnston et al., 2016; Leonard et al., 2010) to the whole-human level (Seiberl et al., 2015; Chapman et al., 2018; Chen and Power, 2019), and is fundamental to a complete understanding of muscle contraction. Residual force enhancement (rFE) and residual force depression (rFD) are two history-dependent force phenomena, characterized by increases and decreases in isometric force following active lengthening and shortening, respectively, in comparison to a reference isometric contraction at the same muscle length and level of activation (Abbott and Aubert, 1952; Chapman et al., 2018; Herzog, 2004; Rassier and Herzog, 2004b; Seiberl et al., 2015). The magnitude of rFE is shown to be dependent on the amplitude of muscle lengthening and largely independent of lengthening velocity (Abbott and Aubert, 1952; Edman et al., 1978; Herzog and Leonard, 2002, 2005; Julian and Morgan, 1979; Fukutani et al., 2019), while rFD appears to be strongly and positively related to the work (i.e. product of force×length change) of muscle shortening (Herzog and Leonard, 1997; Herzog et al., 2000; Lee et al., 2000). Suggested mechanisms include contributions from non-contractile elements (i.e. titin) during lengthening for rFE (Herzog et al., 1998; Labeit et al., 2003; Joumaa and Herzog, 2014; Nishikawa, 2016; Nishikawa et al., 2012; Noble, 1992; Rode et al., 2009), and stress-induced angular actin deformations that impair cross-bridge attachments during shortening for rFD (Joumaa et al., 2018; Maréchal and Plaghki, 1979). Given that rFE and rFD are likely to be intertwined in everyday movements (e.g. during stretch-shortening cycles) (Herzog and Leonard, 2000), a means of maximizing rFE and/or minimizing rFD – the former being associated with increased neuromuscular economy (i.e. more force per unit of activation) (Jones et al., 2016; Paquin and Power, 2018) and reduced adenosine triphosphate usage (Joumaa and Herzog, 2013) – would be desirable to improving motor efficiency.

While there have been recent investigations into the plasticity of the history dependence of force in states of altered contractile capacity (Dargeviciute et al., 2013; Power et al., 2012a,b, 2013, 2014a,b; Ramsey et al., 2010; de Ruiter et al., 2000; de Ruiter and de Haan, 2003), there is still little work looking into its trainability (Chen and Power, 2019; Siebert et al., 2016), especially with a focus at the sarcomere level. Prior studies have demonstrated contraction-type-dependent morphological adaptations with training, whereby eccentric loading leads to an increase (Butterfield et al., 2005; Franchi et al., 2014, 2017; Lynn and Morgan, 1994; Lynn et al., 1998; Reeves et al., 2009; Timmins et al., 2016) and concentric loading leads to a decrease (Butterfield et al., 2005; Lynn and Morgan, 1994; Lynn et al., 1998; Timmins et al., 2016; Morais et al., 2020) in fascicle lengths/serial sarcomere numbers. Considering that rFE and rFD are length-dependent properties (i.e. the amplitude of force response is dependent on the length tested and muscle excursion), eccentric- and concentric-biased training could potentially modify rFE and rFD through respective increases and decreases to the number of sarcomeres in series that modulate the amplitude of sarcomere lengthening and shortening experienced for a given muscle length change. A study by Chen and Power (2019) found that rFE (but not rFD) was differentially increased by chronic (4 week) concentric resistance training, and decreased by chronic eccentric resistance, owing to a likely combination of mechanical and neurological factors. While there was ultrasound evidence of fascicle length adaptations, the data were obtained from a small subset of the participants. As such, it remains to be elucidated: (i) what mechanical factors were responsible for the alterations to history-dependent force properties, and (ii) whether these alterations would persist after accounting for supposed serial sarcomere number differences.

Therefore, the purpose of these experiments was to investigate the trainability of rFE and rFD, on the basis of serial sarcomere number (SSN) alterations. We employed a model of uphill- and downhill-running rats to allow for an isolated (*in vitro*) approach to investigate

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muscle contractile properties and sarcomerogenesis. Considering that rFE and rFD are dependent on the amplitude of sarcomere length changes, it was hypothesized that for a fixed muscle length change, rFE and rFD would increase following concentric training and decrease following eccentric training, owing to a respective reduction and addition of sarcomeres in series that modify the magnitude of sarcomere lengthening/shortening. However, should differences in rFE/rFD persist after normalizing for presumed serial sarcomere number discrepancies in a relative muscle length change, mechanisms other than lengthening/shortening amplitudes must contribute to the modifiability of the history dependence of force following chronic incline/decline running. As such, data from both fixed and relative length change protocols were presented in this study to elucidate the influence of sarcomerogenesis on the history dependence of force.

MATERIALS AND METHODS

Animals

Thirty-one male CD® Sprague-Dawley IGS rats were obtained (Charles River Laboratories, Senneville, QC, Canada) for study. Rats were housed in groups of three, with a maximum of two groups at any given time, and free fed a Teklad global 18% protein rodent diet (Envigo, Huntingdon, UK) and water. After a week of acclimation to the new housing conditions, each rat was familiarized with running and assigned to one of three experimental groups: uphill running, downhill running, and sedentary control (i.e. no running intervention). Following 20 days of exercise, rats recovered for 72 h before euthanisation via CO_2 asphyxiation followed by cervical dislocation and weighed (age: 18.8±0.28 weeks, mass: 523.6±11.0 g) before experimental testing. All procedures were approved by the Animal Care Committee of the University of Guelph.

Training protocol

The training approach was modelled after Butterfield et al. (2005), which observed significant serial sarcomere number differences after 10 days of exercise. We opted for 20 days to better ensure sufficient training stimuli for functional adaptations. One week prior to training, rats were familiarized with treadmill running (on a 0 deg gradient). Rats in the exercise intervention groups (i.e. uphill/downhill running) ran 5 consecutive days week^{-1} on an EXER 3/6 animal treadmill (Columbus Instruments, Columbus, OH, USA) set to a 15 deg incline or decline for 20 training days, over a 4 week period. Training sessions lasted 15 min on the first day and the daily duration was increased by 5 min day^{-1}, up to the target 35 min (by the fifth training day) for the remainder of the training period. At the start of each training session, rats were first introduced to a walking speed of 10 m min^{-1}, which was gradually increased at a rate of 1 m min^{-1} to the 16 m min^{-1} target running speed, roughly corresponding to 50% of the animal's $\dot{V}_{O_2,max}$ (Høydal et al., 2007). Rats were provided with 2 min of rest after each 5 min bout. Introduction into the training protocol was staggered so that the subsequent group could begin training once the previous group was halfway through their training period.

Experimental set up

Following sacrifice, the soleus (SOL) and extensor digitorum longus (EDL) muscles were carefully harvested from the right hind limbs (Ma and Irving, 2019). Silk-braided sutures (USP 2-0, metric 3) were tied along the musculotendinous junctions (MTJs) and mounted to the force–length controller/transducers in the 806D Rat Apparatus (Aurora Scientific, Aurora, ON, Canada). The muscles were bathed in a ∼25°C Tyrode solution with a pH of ∼7.4 (121 mmol l^{-1} NaCl, 24 mmol l^{-1} $NaHCO_3$, 5.5 mmol l^{-1} D-Glucose, 5 mmol l^{-1} KCl, 1.8 mmol l^{-1} $CaCl_2$, 0.5 mmol l^{-1} $MgCl_2$, 0.4 mmol l^{-1} NaH_2PO_4, 0.1 mmol l^{-1} EDTA) that was bubbled (Bonetto et al., 2015; Cheng and Westerblad, 2017) with a 95% O_2/5% CO_2 gas mixture (Praxair Canada Inc., Kitchener, ON, Canada). A 701C High-Powered, Bi-Phase Stimulator (Aurora Scientific, Aurora, ON, Canada) was used to evoke all contractions via two parallel platinum electrodes, submerged in the solution, situated on either side of the muscle. Force, length and stimulus trigger data were all sampled at 10,000 Hz with a 605A Dynamic Muscle Data Acquisition and Analysis System (Aurora Scientific, Aurora, ON, Canada). All data were analyzed with the 615A Dynamic Muscle Control and Analysis High Throughput (DMC/DMA-HT) software suite (Aurora Scientific, Aurora, ON, Canada).

Experimental procedures

An experimental schematic is depicted in Fig. 1. Testing with each muscle proceeded in order from protocol A to G, with the order of fixed (i.e. 4 mm length change) and relative (i.e. 10% optimal length change) protocols being randomized within the rFD and rFE conditions. All rFD and rFE protocols consisted of an rFD/rFE trial and a reference isometric (ISO) trial. The rFD trials (in protocols C and D) always preceded the ISO trials so that the comparatively lower forces in the rFD trials could be attributed to shortening-induced deficits, as opposed to fatigue. Likewise, the ISO trials (in protocols E and F) always preceded the rFE trials so that the comparatively higher forces in the rFE trials could be attributed to lengthening-induced enhancements, rather than fatigue. Contractions in protocols C to G were separated by 5 min intervals to minimize muscle fatigue. Prior to the start of the protocols, the muscle was passively set to a taut length that exerted ∼0.075 N of resting tension, as a starting point for approximating optimal length.

Protocol A: Twitch current

Single 1.25 ms pulses were incremented by 0.5 mA (starting at 1 mA) until a current suitable to elicit peak twitch force was determined.

Protocol B: Optimal length

A maximal tetanic stimulation (pulse width: 0.3 ms, duration: 1 s, frequency: 100 Hz) was delivered before the muscle was passively shortened or lengthened by 2 mm and a subsequent stimulation was delivered 1.5 min later. This was repeated until peak tetanic force was obtained and the corresponding muscle length at rest was taken as optimal length (L_o), measured from tie to tie (i.e. MTJ to MTJ) using 150 mm (resolution: 0.01 mm) electronic digital calipers (Marathon, Vaughan, ON, Canada). Once L_o was roughly determined, 1 mm and 0.5 mm increments were further used to more accurately establish L_o.

Protocol C: Fixed rFD

The fixed rFD trial was comprised of 3 distinct yet continuous phases: a 1 s pre-activation at L_o+2 mm, a −2 mm s^{-1} isokinetic shortening to L_o−2 mm, and a 3 s isometric at L_o−2 mm. The subsequent ISO.S trial consisted of a 6 s isometric at L_o−2 mm.

Protocol D: Relative rFD

The relative rFD trial was comprised of 3 distinct yet continuous phases: a 1 s pre-activation at 1.05L_o, a −2 mm s^{-1} isokinetic

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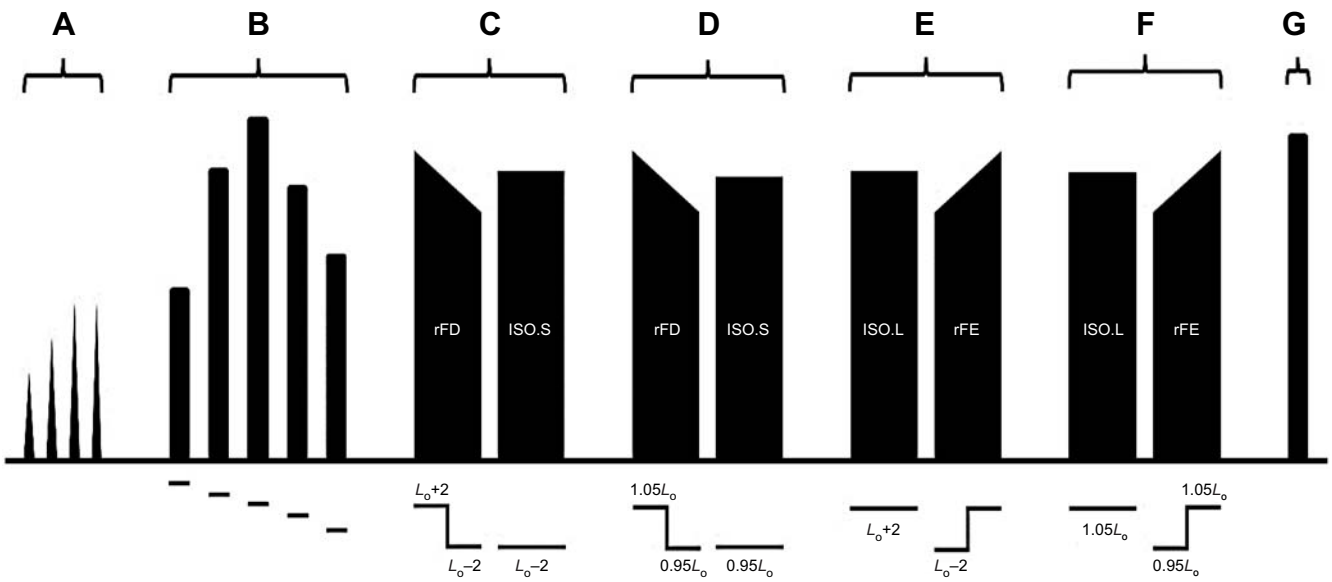

Fig. 1. Schematic experimental timeline. (A) Determination of twitch current. (B) Determination of optimal muscle length for isometric force production (L_o). (C) Fixed residual force depression (rFD) protocol (–4 mm). (D) Relative rFD protocol (–0.1L_o). (E) Fixed residual force enhancement (rFE) protocol (+4 mm). (F) Relative rFE protocol (+0.1 L_o). (G) Final muscle assessment. The fixed and relative protocols were randomized. ISO.S, short reference isometric; ISO.L, long reference isometric.

shortening to 0.95L_o and a 3 s isometric at 0.95L_o. The subsequent ISO.S trial consisted of a 6 s isometric at 0.95L_o.

Protocol E: Fixed rFE

The fixed rFE trial was comprised of 3 distinct yet continuous phases: a 1 s pre-activation at L_o−2 mm, a +2 mm s^{-1} isokinetic lengthening to L_o+2 mm, and a 3 s isometric at L_o+2 mm. The preceding ISO.L trial consisted of a 6 s isometric at L_o+2 mm.

Protocol F: Relative rFE

The relative rFE trial was comprised of 3 distinct yet continuous phases: a 1 s pre-activation at 0.95L_o, a +2 mm s^{-1} isokinetic lengthening to 1.05L_o, and a 3 s isometric at 1.05L_o. The preceding ISO.L trial consisted of a 6 s isometric at 1.05L_o.

Protocol G: Final muscle assessment

A final tetanic contraction was performed at L_o to assess decreases in isometric force-generating capacity.

Serial sarcomere number estimations

Following mechanical testing, muscles were removed from the bath, passively stretched to L_o (determined from protocol B), tied to wooden sticks, and fixed in 10% phosphate-buffered formalin for 48 h, rinsed with phosphate-buffered saline, and digested in 30% nitric acid for at least 6 h to remove connective tissue and allow individual muscles fascicles to be teased out (Butterfield et al., 2005). To obtain a global measure of SSN, six fascicles were carefully obtained from deep and superficial regions of each muscle. Fascicle lengths (FL) were measured using 150 mm (resolution: 0.01 mm) electronic digital calipers. Sarcomere length (SL) measurements were taken over six different regions across the length of each fascicle via laser diffraction (Coherent, Santa Clara, CA, USA) with a 5 mW diode laser (beam diameter: 500 µm, wavelength: 635 nm) and custom LabVIEW program (Version 2011, National Instruments, Austin, TX, USA) (Lieber et al., 1984), for a total of 36 SL measurements per muscle. Serial sarcomere numbers (SSN) were calculated as:

$$\mathrm{SSN} = \frac{\mathrm{FL}}{\text{mean SL}}. \quad (1)$$

Data analyses

Steady-state isometric force values were calculated by subtracting passive force (resting baseline, pre-activation) from active force 2 s and 1 s after the end of the active length change for the SOL and EDL, respectively. Timing of 'steady-state' values were based on previous analysis of the rate of tension decay after stretch (Ramsey et al., 2010) and kept standard across all muscles/contractions. Absolute rFE and rFD values (expressed in N cm^{-2}) were calculated as the increase and decrease in isometric force following active lengthening and shortening, respectively, relative to the ISO contraction at the same time point into the active contraction (i.e. 5 s and 4 s into the 6 s contraction for the SOL and EDL, respectively). Percent rFE and rFD values (expressed as %ISO) were calculated as the percent difference in steady-state isometric force, relative to the ISO contraction, at the same time point. Similarly, passive force enhancement (pFE) was calculated as the absolute and percent increase in passive force following active lengthening, relative to passive force (at rest, post-activation) of the ISO contraction. Peak eccentric force values were taken as the highest total (active+passive) force achieved during the active lengthening phase. Work of shortening values were calculated as the force-displacement integral for the active shortening phase of the rFD trials. In addition to raw values, all force and work measures were normalized to physiological cross-sectional area (PCSA, in cm^2) to obtain specific values (expressed in units cm^{-2}). PCSA was calculated as:

$$\mathrm{PCSA} = \frac{M_{\mathrm{muscle}}}{D_{\mathrm{muscle}} \times \text{normalized FL}}, \quad (2)$$

where M_{muscle} is muscle mass (g), muscle density (D_{muscle}) was assumed to be 1.112 g cm^{-3} (Ward and Lieber, 2005) and normalized

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Table 1. Force and work values across the various training groups and protocols for the SOL and EDL (*n*=31 male rats)

	Soleus (SOL)			Extensor digitorum longus (EDL)		
	Control	Uphill	Downhill	Control	Uphill	Downhill
F_o (N)	1.39±0.07	1.21±0.15	1.23±0.17	1.10±0.08	1.21±0.08	1.15±0.13
F_o (N cm^{-2})*	9.51±0.87	7.95±1.47	9.03±1.31	6.41±0.66	6.95±0.87	6.43±0.89
Fixed protocol						
Eccentric force (N)*	2.44±0.12	2.25±0.25	2.29±0.19	1.22±0.14	1.47±0.12	1.51±0.20
Eccentric force (N cm^{-2})*	16.78±1.55	14.38±2.23	16.90±1.73	7.01±0.91	8.32±0.99	8.46±1.31
Con. work (mJ)*,‡	3.34±0.22	2.99±0.41	3.00±0.43	1.96±0.18	2.19±0.19	2.23±0.28
Con. work (mJ cm^{-2})*	23.10±2.46	19.82±3.89	22.06±3.48	11.37±1.31	12.68±1.90	12.59±1.88
Relative protocol						
Eccentric force (N)*	2.23±0.14	2.12±0.29	2.07±0.21	1.26±0.16	1.39±0.12	1.52±0.22
Eccentric force (N cm^{-2})*	15.48±1.71	13.82±2.62	15.37±1.91	7.32±1.12	7.86±1.04	8.54±1.46
Con. work (mJ)*,‡	2.76±0.19	2.35±0.39	2.47±3.57	1.85±0.17	2.12±0.27	2.09±0.32
Con. work (mJ cm^{-2})*	19.07±2.00	15.69±3.63	18.19±2.88	10.77±1.29	12.34±2.20	11.92±2.04

Raw isometric force was not significantly different across muscles (P=0.203) or training groups (P=0.905). Raw peak eccentric force was 61% greater for the SOL than EDL. Raw work of shortening was 37% greater for the SOL than EDL (P<0.001) and 15% greater for the fixed compared with relative protocol (P=0.044). Specific values of isometric force, eccentric force and work of shortening were 34%, 96% and 65% greater for the SOL than EDL, respectively (P<0.05). Values are means±s.e.m. *P<0.05, significant effect of muscle; ‡P<0.05, significant effect of protocol. F_o, isometric force at optimal muscle length; Eccentric force, peak eccentric force; Con. work, work of shortening.

fascicle length was calculated as:

$$\text{Normalized FL} = \text{FL} \times \left(\frac{\text{SL}_o}{\text{measured SL}}\right), \quad (3)$$

where optimal sarcomere length (SL_o) was assumed to be 2.72 µm (based on the average of all measurements). Normalized FL were used to calculate PCSA in order to account for inconsistencies in L_o that arose during the muscle fixation-digestion process.

A two-tailed paired *t*-test was used to compare force values between the history-dependent and ISO trials to ascertain the presence of rFE, rFD and pFE. A two-way ANOVA with a Holm–Šidák analysis for all pairwise comparisons was used to compare SSN, SL, FL, L_o, and force and specific force values across muscles (SOL, EDL) and training groups (control, uphill, downhill). A three-way ANOVA with a Holm–Šidák analysis for all pairwise comparisons was used to compare peak eccentric force, work of shortening, rFD, rFE and pFE values across muscles (SOL, EDL), training groups (control, uphill, downhill), and protocols (fixed, relative). Significance was set to α=0.05. All data are reported as means±s.e.m.

RESULTS

Muscle force and work values across groups, muscles and protocols

There was no effect of muscle ($F_{1,56}$=1.661, P=0.203) or training ($F_{1,56}$=0.100, P=0.905) for raw isometric force at optimal length (Table 1). There was a main effect of muscle ($F_{1,108}$=62.378, P<0.001) for raw peak eccentric force, but no effect of training ($F_{1,108}$=0.126, P=0.882) or protocol ($F_{1,108}$=0.895, P=0.346), whereby the SOL produced 61% higher eccentric force than the EDL (Table 1). Furthermore, there was a main effect of muscle ($F_{1,108}$=19.043, P<0.001) and protocol ($F_{1,108}$=4.167, P=0.044) for raw work of shortening, but no muscle×protocol interaction ($F_{1,108}$=1.988, P=0.161) or effect of training ($F_{1,108}$=0.0522, P=0.949), such that work performed during shortening was 37% greater for the SOL than EDL and 15% greater for the fixed compared to relative protocol (Table 1).

In contrast, when force was normalized to PCSA and expressed as specific force, there was a main effect of muscle for specific isometric force at optimal length ($F_{1,56}$=6.941, P=0.011), specific peak eccentric force ($F_{1,108}$=65.911, P<0.001) and specific work of shortening ($F_{1,108}$=27.885, P<0.001), but no effect of training (P≥0.05) or protocol (P≥0.05). Specifically, the SOL exhibited 34% higher specific isometric force, 96% higher specific eccentric force, and performed 65% more specific work during shortening than the EDL across training groups and protocols (Table 1).

Muscle architectural adaptation to training

For SSN there was an interaction of muscle×training ($F_{2,371}$=3.237, P=0.040), in addition to main effects of muscle ($F_{1,371}$=119.567, P<0.001) and training ($F_{2,371}$=5.732, P=0.004). There was significantly greater SSN for the SOL than EDL (P=0.001) across training groups and protocols, and the downhill group had a significantly greater SSN than both the uphill (P=0.005) and control (P=0.017) groups across muscles and protocols (Fig. 2). Within

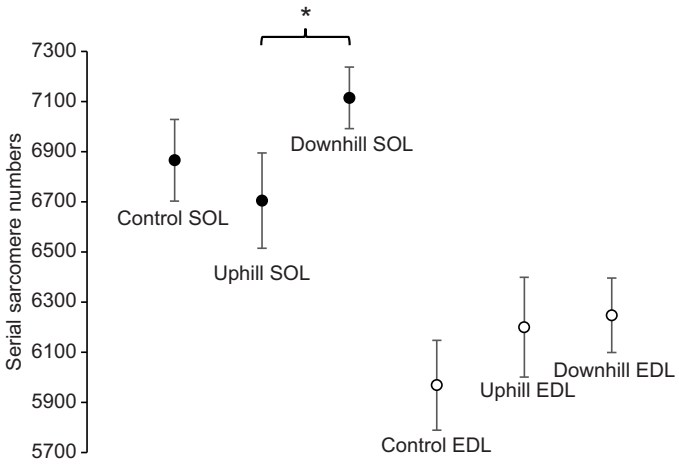

Fig. 2. Serial sarcomere numbers in uphill- and downhill-running rats. Mean±95% confidence intervals for serial sarcomere numbers (SSN) (*n*=31 male rats). There was an interaction of muscle×training (P<0.001) for SSN, in addition to main effects of muscle (P<0.001) and training (P=0.004). SSN within SOL were 6% greater for the downhill compared to uphill group (P=0.003), and within the EDL there was a trend (P=0.056) towards 4% more SSN for the downhill compared with the control group. *P<0.05. SOL, soleus; EDL, extensor digitorum longus.

SOL, downhill running (95% CI=6992.2, 7237.1) resulted in a 6% greater SSN (P=0.003) than uphill running (95% CI=6515.0, 6895.0), while SSN for the EDL did not significantly differ across training groups (P≥0.05) (Fig. 2). Moreover, there was a main effect of muscle for fascicle length ($F_{1,56}$=39.358, P<0.001) and muscle length ($F_{1,52}$=604.518, P<0.001), but no effect of training (P≥0.05) for either dependent variable, such that the SOL had a 17% shorter muscle but 13% longer fascicles compared with the EDL (Table 2). In contrast, there was no effect of either muscle ($F_{1,55}$=0.734, P=0.395) or training ($F_{2,55}$=0.182, P=0.834) on sarcomere lengths (Table 2).

History dependence of force response to training

Residual force depression

There was a main effect of muscle ($F_{1,108}$=11.892, P<0.001) for absolute rFD (expressed in N cm^{-2}), but no effect of training ($F_{2,108}$=0.440, P=0.645) or protocol ($F_{1,108}$=2.793, P=0.098) (Fig. 3A). Absolute rFD was present across all training groups and protocols (P<0.05) for both muscles, with 45% greater absolute rFD for the SOL than EDL (Fig. 3B). Absolute rFD was positively and linearly associated with specific work of shortening (Fig. 3D) for the SOL (R^2=0.71; $F_{1,29}$=70.292, P<0.001) and EDL (R^2=0.61; $F_{1,29}$=45.362, P<0.001). In contrast, there was no significant relationship between absolute rFD and SSN (SOL: R^2=0.051, P=0.228; EDL: R^2=0.107, P=0.073), fascicle length (SOL: R^2=0.012, P=0.565; EDL: R^2=0.104, P=0.077), or muscle length (SOL: R^2=0.0004, P=0.914; EDL: R^2=0.113, P=0.074) for either muscle.

Likewise, there was a main effect of muscle ($F_{1,108}$=67.200, P<0.001) for percent rFD (expressed as %ISO.S), but no effect of training ($F_{2,108}$=0.295, P=0.745) or protocol ($F_{1,108}$=1.526, P=0.219). Percent rFD was present across all training groups and protocols (P<0.05) for both muscles, with 2.1× greater percent rFD for the EDL than SOL (Fig. 3C). There was no significant relationship between percent rFD and specific work of shortening (SOL: R^2=0.028, P=0.364; EDL: R^2=0.026, P=0.386), SSN (SOL: R^2=0.087, P=0.113; EDL: R^2=0.001, P=0.845), fascicle length (SOL: R^2=0.051, P=0.223; EDL: R^2=0.001, P=0.845), or muscle length (SOL: R^2=0.001, P=0.851; EDL: R^2=0.113, P=0.074) for either muscle.

Residual force enhancement

There was a main effect of muscle ($F_{1,108}$=30.138, P<0.001) and protocol ($F_{1,2}$=0.0736, P=0.004) for absolute rFE (expressed in N cm^{-2}), but no significant muscle×protocol interaction ($F_{2,108}$=3.908, P=0.051) or effect of training ($F_{2,108}$=0.0736, P=0.929) (Fig. 4A). Absolute rFE was present across all training groups and protocols (P<0.05) for both muscles (Fig. 4B), wherein absolute rFE was 2.3× greater for the SOL than EDL across training groups and protocols, and 56% greater for the fixed compared with relative protocol across muscles and training groups. Absolute rFE was positively and linearly associated with specific eccentric force (Fig. 4D) for the SOL (R^2=0.57; $F_{1,29}$=39.108, P<0.001) and EDL (R^2=0.72; $F_{1,29}$=76.100, P<0.001). Whereas, there was no significant relationship between absolute rFE and SSN (SOL: R^2=0.0004, P=0.913; EDL: R^2=0.086, P=0.110), fascicle length (SOL: R^2=0.004, P=0.744; EDL: R^2=0.079, P=0.127) or muscle length (SOL: R^2=0.012, P=0.573; EDL: R^2=0.001, P=0.856) for either muscle.

In contrast, there was a main effect of protocol ($F_{1,108}$=7.014, P=0.009) for percent rFE (expressed as %ISO.L), but no effect of muscle ($F_{1,108}$=0.759, P=0.386) or training ($F_{2,108}$=0.805, P=0.450). Percent rFE was present across all training groups (P<0.05) for both muscles, with 43% greater percent rFE for the fixed compared with relative protocol (Fig. 4C). There was no significant relationship between percent rFE and specific eccentric force (SOL: R^2=0.026; $F_{1,29}$=0.852, P=0.364; EDL: R^2=0.022; $F_{1,29}$=0.641, P=0.430), SSN (SOL: R^2=0.054, P=0.216; EDL: R^2=0.039, P=0.284), fascicle length (SOL: R^2=0.040, P=0.280; EDL: R^2=0.040, P=0.280) or muscle length (SOL: R^2=0.004, P=0.747; EDL: R^2=0.006, P=0.689) for either muscle.

Passive force enhancement

There was a main effect of muscle ($F_{1,107}$=24.172, P<0.001) for absolute pFE (expressed in N cm^{-2}), but no effect of training ($F_{1,107}$=1.151, P=0.320) or protocol ($F_{1,107}$=1.1491, P=0.225). Absolute pFE was present across all training groups and protocols (P<0.05) for both muscles, with 2.2× greater absolute pFE for the SOL than EDL (Fig. 5A). Absolute pFE was positively and linearly associated with absolute rFE (Fig. 5C) for the SOL (R^2=0.41; $F_{1,29}$=19.897, P<0.001) and EDL (R^2=0.69; $F_{1,29}$=65.279, P<0.001), amounting to 29% (0.71±0.10 N cm^{-2}) and 37% (0.30±0.047 N cm^{-2}) of the absolute rFE observed for the SOL and EDL, respectively. Furthermore, absolute pFE was positively and linearly associated with specific eccentric force (Fig. 5D) for the SOL (R^2=0.49; $F_{1,29}$=28.212, P<0.001) and EDL (R^2=0.65; $F_{1,29}$=53.236, P<0.001). Meanwhile, for the SOL, there was no significant relationship between absolute pFE and SSN (R^2=0.010, P=0.596), fascicle length (R^2=0.032, P=0.341), or muscle length (R^2=0.124, P=0.061). Whereas for the EDL, there was a weak but significant relationship between absolute pFE versus SSN (R^2=0.158; $F_{1,29}$=5.458; P=0.027) and absolute pFE versus fascicle length (R^2=0.150; $F_{1,29}$=5.124, P=0.031), but not between absolute pFE and muscle length (R^2=0.003, P=0.768).

Similarly, there was a main effect of muscle ($F_{1,107}$=31.979, P<0.001) for percent pFE (expressed as %ISO.L), but no effect of training ($F_{1,107}$=1.152, P=0.320) or protocol ($F_{1,107}$=0.149, P=0.700). Percent pFE was present across all training groups and protocols (P<0.05) for both muscles, with 3.3× greater percent pFE for the SOL than EDL (Fig. 5B). There was no significant relationship between percent pFE and percent rFE for the SOL (R^2=0.028, P=0.367) but a significant, albeit weak, relationship for

Table 2. Muscle length measures across the various training groups for the SOL and EDL (n=30 male rats)

	Soleus (SOL)			Extensor Digitorum Longus (EDL)		
	Control	Uphill	Downhill	Control	Uphill	Downhill
SL (µm)	2.75±0.11	2.76±0.14	2.77±0.09	2.75±0.05	2.62±0.11	2.69±0.11
FL (mm)*	18.60±0.32	18.53±0.51	19.33±0.26	16.27±0.51	16.89±0.54	16.94±0.26
L_o (mm)*	30.75±0.86	30.62±0.73	31.08±0.51	36.88±0.67	37.27±0.86	37.70±0.63

The SOL had a 17% shorter muscle (*P<0.001) but 13% longer fascicles (*P<0.001) compared with the EDL. However, there was no effect of training for either muscle length or fascicle length (P≥0.05).
Values are means±s.e.m. SL, average sarcomere length; FL, fascicle length; L_o, optimal muscle length.

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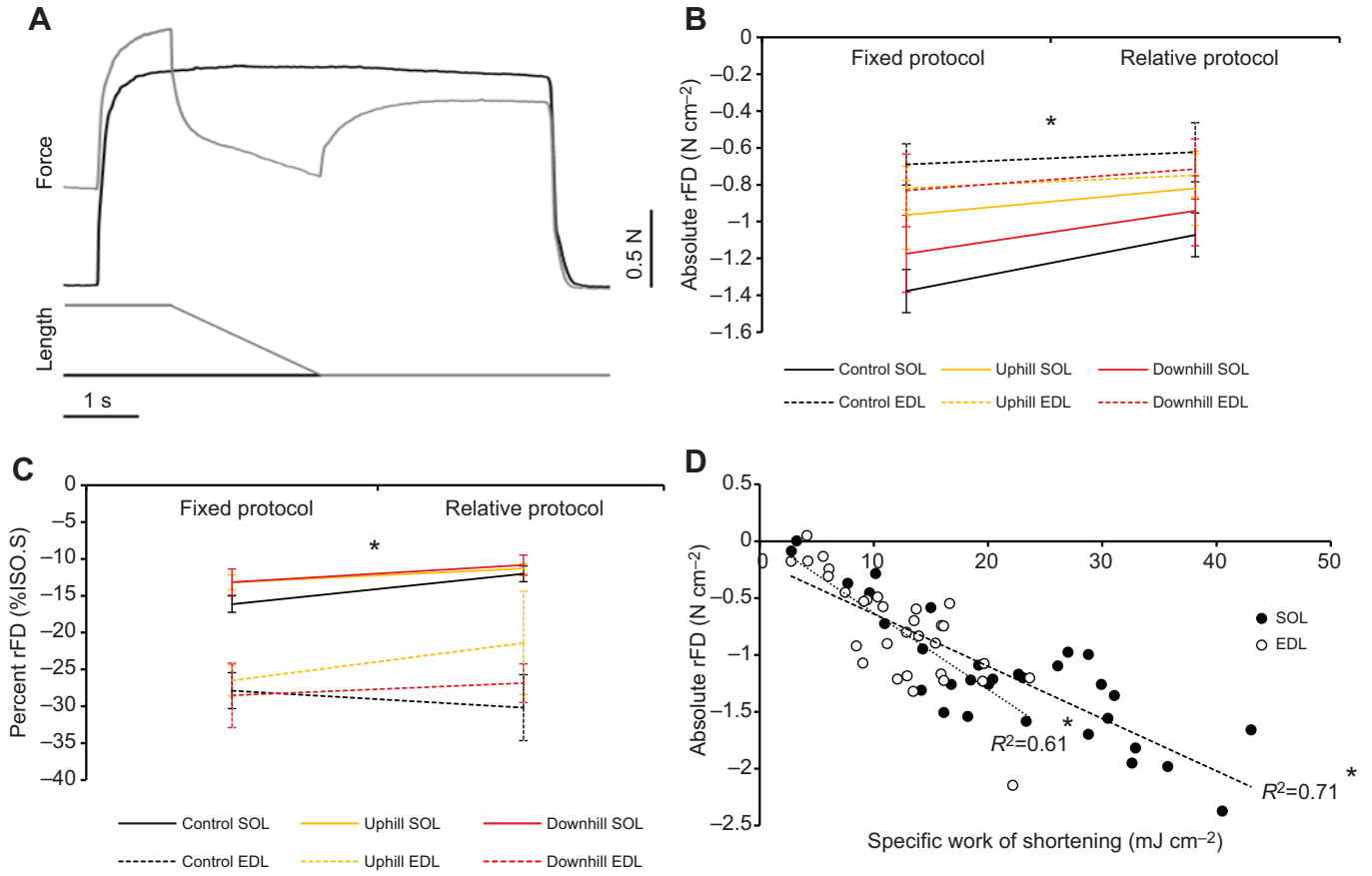

Fig. 3. Residual force depression. (A) Experimental data traces from the soleus, with the rFD contraction in grey and ISO.S contraction in black. Note the comparatively lower isometric force in the rFD condition for the same muscle length and level of activation. (B) Absolute rFD values across the various training groups and protocols for the SOL and EDL (n=31 male rats). Absolute rFD was 45% greater for the SOL than EDL (*P<0.001) but was not significantly different across training groups (P=0.645) or protocols (P=0.098). (C) Percent rFD values across the various training groups and protocols for the SOL and EDL (n=30 male rats). Percent rFD was 2.1× greater (*P<0.001) for the EDL than SOL but was not significantly different across training groups (P=0.745) or protocols (P=0.219) (n=31 male rats). (D) Relationship between specific work of shortening and absolute rFD values across training groups during the fixed protocol for the SOL (black) and EDL (white) (n=31 male rats). Specific work of shortening was positively and linearly associated with absolute rFD for both the SOL (R^2=0.71, *P<0.001) and EDL (R^2=0.61, *P<0.001). Values are means±s.e.m. *Indicates an effect of muscle (P<0.05) in B and C and a significant relationship (P<0.05) in D.

the EDL (R^2=0.152; $F_{1,29}$=5.195, P=0.030). Additionally, there was a weak but significant relationship between percent pFE and specific eccentric force for the SOL (R^2=0.180; $F_{1,29}$=6.377, P=0.017) and EDL (R^2=0.223; $F_{1,29}$=8.300, P=0.007). Finally, there was no significant relationship between percent pFE and SSN (SOL: R^2=0.0003, P=0.927; EDL: R^2=0.050, P=0.225), fascicle length (SOL: R^2=3.644×10^{-5}, P=0.974; EDL: R^2=0.047, P=0.240) or muscle length (SOL: R^2=0.036, P=0.327; EDL: R^2=0.009, P=0.617) for either muscle.

Summary

The downhill training group had more sarcomeres in series compared with the uphill group for the SOL but not EDL. Additionally, within the EDL, there was a trend towards more SSN for the downhill compared to control group. The SOL was shorter than the EDL; however, the SOL had longer fascicles. There was no effect of training on fascicle length or sarcomere length. There was no effect of training on any parameter of force/work production. Upon normalization to PSCA, the SOL produced more isometric force, peak eccentric force, and work of shortening as compared with the EDL across groups. Finally, while there were clear adaptations in SSN to training, rFD and rFE were unaltered. The work of shortening and pFE was strongly related to the magnitude of rFD and rFE in both muscles, respectively.

DISCUSSION

The purpose of these experiments was to determine whether the history dependence of force could be modified by contraction-type-dependent SSN adaptations. Serial sarcomere numbers and history-dependent forces for the SOL and EDL muscles of rats were compared across training groups following 4 weeks of uphill and downhill running. In accordance with our hypothesis, there was a greater SSN for the SOL with downhill compared with uphill running, and a trend towards a greater SSN for the EDL with downhill running in comparison to the control group. While SSN appeared to differ with training there was no concomitant adaptation to history-dependent forces. Together, these results indicate that training-induced SSN adaptations do not modify whole-muscle, *in-vitro* measures of rFD, rFE and pFE in the lower hind limb muscles of rats.

Serial sarcomere number adaptations

In the present study, SSN (Fig. 2) was consistent with the ∼7500 count for the vastus lateralis (VL) (Butterfield et al., 2005), with

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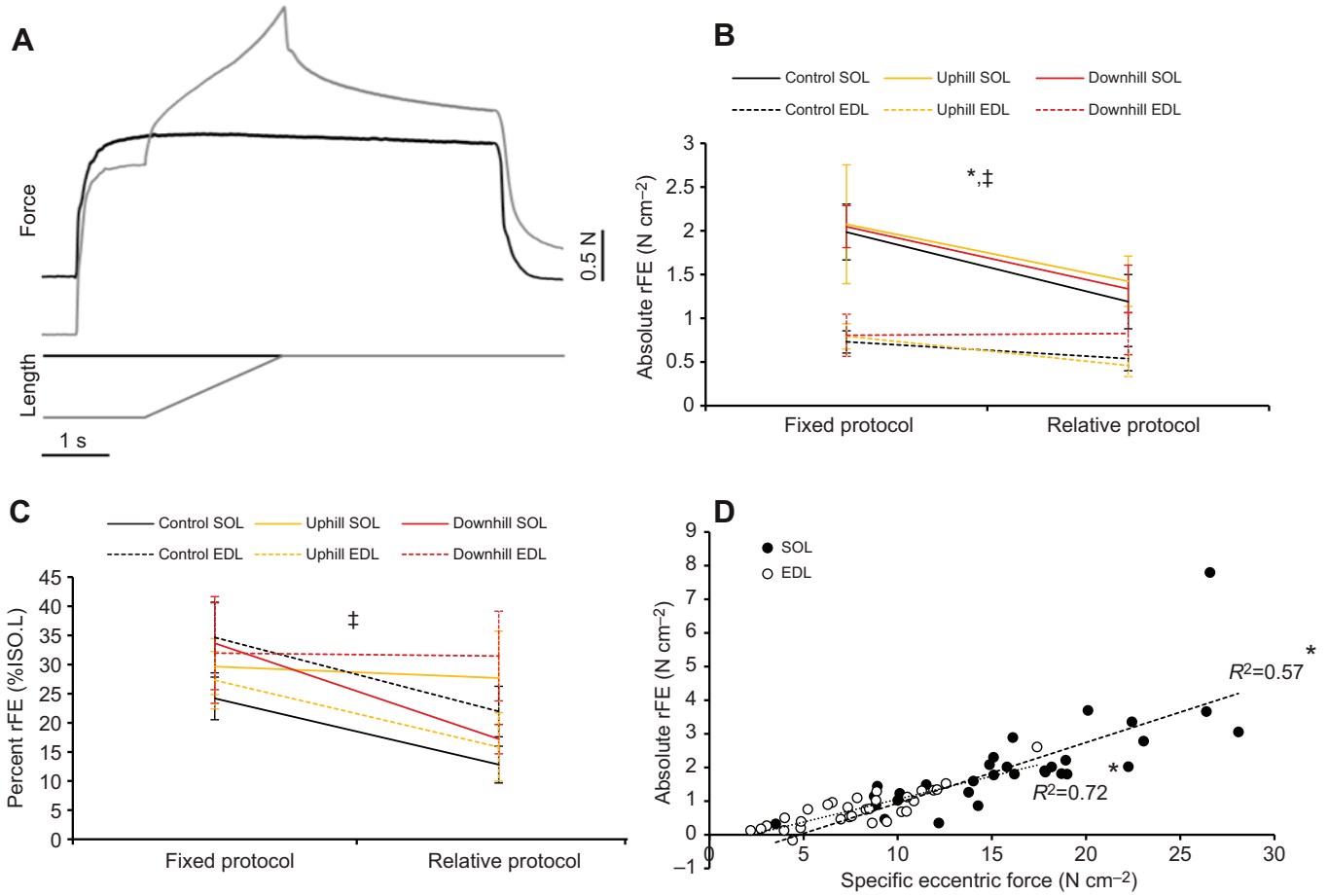

Fig. 4. Residual force enhancement. (A) Experimental data traces from the soleus, with the rFE contraction in grey and ISO.L contraction in black. Note the comparatively higher isometric force in the rFE condition (and comparatively higher passive force in the pFE condition) for the same muscle length and level of activation. (B) Absolute rFE values across the various training groups and protocols for the SOL and EDL (n=31 male rats). Absolute rFE was 2.3× greater for the SOL than EDL (*P<0.001) and 56% greater for the fixed compared to relative protocol (‡P=0.004), with no significant difference across training groups (P=0.929). (C) Percent rFE values across the various training groups and protocols for the SOL and EDL (n=30 male rats). Percent rFE was 43% greater for the fixed compared to relative protocol (‡P=0.009) but was not significantly different across muscles (P=0.386) or training groups (P=0.450). (D) Relationship between specific eccentric force and absolute rFE values across training groups during the fixed protocol for the SOL (black) and EDL (white) (n=31 male rats). Specific eccentric force was positively and linearly associated with absolute rFE for both the SOL (R^2=0.57, *P<0.001) and EDL (R^2=0.72, *P<0.001). Values are means±s.e.m. *Indicates an effect of muscle (P<0.05) in B and a significant relationship (P<0.05) in D. ‡Indicates an effect of protocol (P<0.05).

values across muscles ranging from ∼3400 for the vastus intermedius (VI) of rats (Lynn and Morgan, 1994; Lynn et al., 1998; Butterfield et al., 2005) to ∼21,000 for the tibialis anterior (TA) of rabbits (Butterfield and Herzog, 2006). While our measured 2.72 µm SL average was towards the higher end of the typical 2.4–2.8 µm range (Stephenson and Williams, 1982), values from this study were obtained from muscles passively fixed at their optimal length for force production, as opposed to given joint angle or skinned fibre. As a result, the compliance of the in-series connective tissues would result in a shortening of sarcomeres upon activation and lengthening upon deactivation, and subsequently, an overestimation of SL when measured at a passive length.

In line with previous findings (Lynn and Morgan, 1994; Lynn et al., 1998; Butterfield et al., 2005; Morais et al., 2020), downhill running resulted in 6% greater SSN for the SOL when compared with uphill running, but not a sedentary control group. Moreover, while SSN were not significantly different across training groups for the EDL, there was a trend (P=0.056) towards 4% greater SSN for the downhill versus control group, indicating a potential stimulus for sarcomerogenesis with downhill running. Given that strain dynamics were not measured during training, insight into the actions of the SOL and EDL during uphill and downhill running have to be inferred based on previous observations. In a similar treadmill training programme as the current study, Morais et al. (2020) showed that in mice, concentric training led to a decrease in SSN for the VL and TA while eccentric training had no effect. Additionally, there was no training-induced sarcomerogenesis effect for the medial gastrocnemius (MG) and vastus medialis (VM). However, when inferring SSN adaptations, it is important to consider the specific muscle fibre excursion during locomotion (Hu et al., 2017). It is possible those muscles which did not show sarcomerogenesis did not experience a minimal threshold to warrant adaptations.

During rat locomotion, soleus activity begins just prior to foot contact and ends immediately before foot lift-off, whereas EDL activity begins just after foot lift-off and ends immediately after foot contact (Nicolopoulos-Stournaras and Iles, 1984). This would imply that the SOL is loaded eccentrically during downhill running and concentrically during uphill running, which would support the findings of increased SSN with eccentric training and decreased SSN with concentric training (Butterfield et al., 2005; Butterfield and Herzog, 2006), as first predicted by Morgan (1990). While not statistically significant, the greater apparent number of sarcomeres

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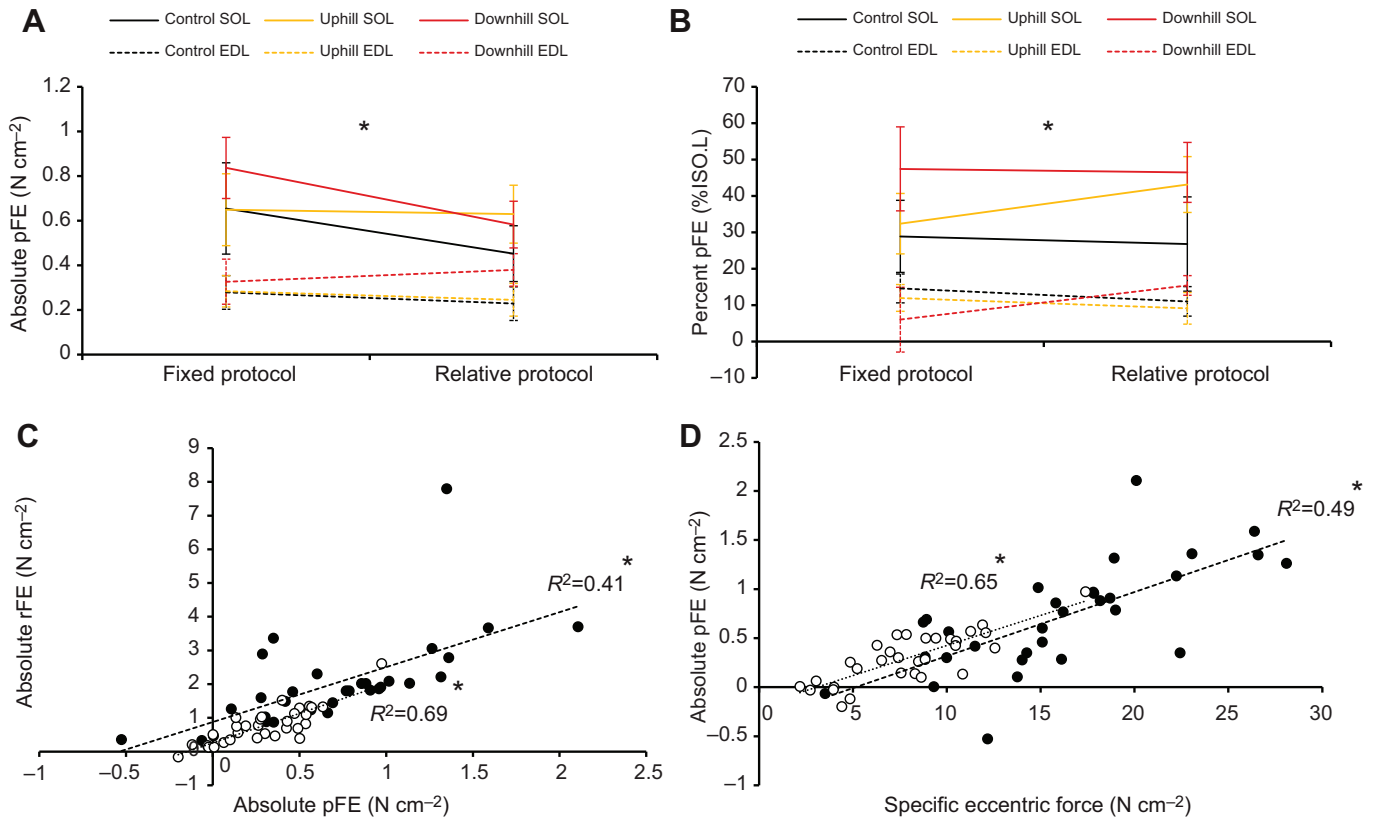

Fig. 5. Passive force enhancement. (A) Absolute pFE values across the various training groups and protocols for the SOL and EDL (n=31 male rats). Absolute pFE was 2.2× greater for the SOL than EDL (*P<0.001) but was not significantly different across training groups (P=0.320) or protocols (P=0.225). (B) Percent pFE values across the various training groups and protocols for the SOL and EDL (n=30 male rats). Percent pFE was 3.3× greater for the SOL than EDL (*P<0.001) but was not significantly different across training groups (P=0.320) or protocols (P=0.700). (C) Relationship between absolute pFE and absolute rFE values across training groups during the fixed protocol for the SOL (black) and EDL (white) (n=31 male rats). Absolute pFE was positively and linearly associated with absolute rFE for both the SOL (R^2=0.41, *P<0.001) and EDL (R^2=0.69, *P<0.001). (D) Relationship between specific eccentric force and absolute pFE values across training groups during the fixed protocol for the SOL (black) and EDL (white) (n=31 male rats). Specific eccentric force was positively and linearly associated with absolute pFE for both the SOL (R^2=0.49, *P<0.001) and EDL (R^2=0.65, *P<0.001). Values are means±s.e.m. *Indicates an effect of muscle (P<0.05) in A and B and a significant relationship (P<0.05) in C and D.

for both training groups in comparison to the sedentary control suggests that the EDL performed eccentric contractions during downhill and uphill running, which may be explained by eccentric braking actions at the onset of foot contact.

Strength measures

Perhaps contrary to expectations, training did not alter isometric forces, eccentric forces, and work of shortening for either muscle. However, unlike the strength-oriented resistance training employed by Chen and Power (2019), the main goal of our incline/decline-running model was to induce differential SSN adaptations, which has previously been demonstrated for the quadriceps muscles of rats (Lynn and Morgan, 1994; Lynn et al., 1998; Butterfield et al., 2005) and is partially supported by the present findings for the lower hind limb muscles. Yet, SSN increases were not mirrored by increases in muscle length (Table 2) or force/work performed (Table 1). However, architectural adaptations at the level of the fascicle do not always translate to the whole muscle (Sharifnezhard et al., 2014), owing to factors such as pennation angles and connective tissue compliance, which mediate the transfer of forces across functional scales. Furthermore, there is the ever-present issue of 'testing specificity'; assuming that neuromuscular adaptations are geared towards specific environmental perturbations, testing the muscle under different physiological/contractile conditions (i.e. *in vitro*) could likely wash out potential *in vivo* responses (e.g. strain of specific muscle fibre regions during locomotion). Additionally, while SSN was significantly higher for the SOL following downhill versus uphill running, from a functional standpoint, a 6% increase might not be sufficient to elicit prominent changes in muscle force, as evidenced by the current results.

With respect to the rFE and rFD contractions, during a 4 mm muscle length change the 6% SSN difference for the SOL would roughly equate to an average sarcomere length change of 0.563 µm for the downhill group and 0.596 µm for the uphill group. However, this 0.033 µm difference in sarcomere length does not account for confounding variables such as pennation angle, curvature of the fascicle, connective tissue compliance, and sarcomere length non-uniformities. Therefore, irrespective of whether SSN adaptations also took place for the EDL, it is likely that SSN differences would not be large enough to promote differences in force at the whole-muscle level. While it could be argued that longer training periods might lead to greater adaptations, Lynn and Morgan (1994) found that SSN counts peaked after just 1 week of incline/decline running, with no further changes leading up to the end of the 3 week training period. In a similar treadmill training programme to the current study, Morais et al. (2020) showed that, in mice, concentric training led to a decrease in SSN for the VL and TA, while eccentric training

had no effect. Additionally, there was no training-induced sarcomerogenesis effect for the MG and VM. However, it remains to be determined how steeper inclines/declines, different loading protocols, or specific active lengthening/shortening of a target muscle might affect training adaptations.

Residual force depression

In line with the 8–72% rFD previously reported *in vitro* (Abbott and Aubert, 1952; Edman, 1975; Herzog and Leonard, 1997; Herzog et al., 1998; Joumaa and Herzog, 2010; Joumaa et al., 2012; Maréchal and Plaghki, 1979; Meijer, 2002; Morgan et al., 2000; Pun et al., 2010; Sugi and Tsuchiya, 1988), steady-state isometric force values following active shortening were 13% and 28% lower than the ISO for the SOL and EDL, respectively. Percent rFD was 2.1× greater for the EDL (95–100% type II fibres) than the SOL (75–80% type I fibres) (Eng et al., 2008; Ranatunga and Thomas, 1990; Wigston and English, 1991), and is consistent with findings of 1.8× greater percent rFD for type II compared with type I fibres from rabbit psoas and soleus muscle (Joumaa et al., 2015). On the other hand, absolute rFD was 45% greater for the SOL than EDL, contrary to the observations for percent rFD. The discrepancy between relative and absolute values of rFD in the present study is most likely due to reduced contractile capacity (i.e. possibly fatigue) of the predominantly fast-twitch EDL muscle (Brooks and Faulkner, 1991), as evidenced by the comparatively lower overall strength measures (Table 1). Given that one of the underlying factors contributing to rFD is a stress-induced impairment of cross-bridge attachments during active shortening, it is not surprising then that a reduced capacity to perform work during shortening would result in lower absolute values of rFD (Fig. 3D). However, after accounting for differences in isometric strength (i.e. lower contractile capacity), percent rFD was greater for the EDL, suggesting greater intrinsic rFD for fast-twitch muscles, owing to distinct force-velocity relationships (i.e. a higher V_{max} for fast-twitch muscles) that facilitate the capacity to generate more work and/or sarcomere length non-uniformities than slow-twitch muscles for the same shortening velocity (Joumaa et al., 2015; Lee and Herzog, 2003; Morgan et al., 2000; Pinnell et al., 2019).

Contrary to our hypothesis, absolute and percent rFD values were not different following uphill and downhill training for either muscle, despite differences in SSN for the SOL, and possibly, EDL. As a result, it is possible that SSN adaptations were not prominent enough or specifically geared to evoke functional differences under our *in vitro* contractile conditions. The lack of training-induced alterations to rFD in the present study is consistent with findings from Chen and Power (2019), suggesting that neither SSN adaptations (in the present study) nor potential neurological adaptations (Chen and Power, 2019) serve to modify rFD at the whole-muscle level.

Residual force enhancement

In line with the 8–52% rFE previously reported *in vitro* (Abbott and Aubert, 1952; Edman et al., 1982; Meijer, 2002; Pun et al., 2010; Rassier and Pavlov, 2012; Sugi and Tsuchiya, 1988), steady-state isometric force values following active lengthening were 24% and 27% higher than the ISO for the SOL and EDL, respectively. Absolute rFE was 2.3× greater for the SOL than EDL while percent rFE was not significantly different between muscles, contrary to observations from Ramsey et al. (2010), who found ∼55% greater percent rFE for the EDL compared with SOL muscle of rats. In argument against reports that absolute rFE is preserved in conditions of reduced contractile force (Fukutani and Herzog, 2018; Rassier and Herzog, 2004a), we show that absolute rFE is strongly and positively associated with the force established during the active lengthening phase of the rFE contraction (Fig. 4D), suggesting a strength-dependent component of rFE.

Although rFE is typically attributed to non-contractile elements (i.e. titin), contractile contributions (e.g. decreased cross-bridge detachment) cannot be discounted (Rassier and Herzog, 2004a; Lee et al., 2007; Pinnell et al., 2019). Furthermore, cross-bridge cycling is necessary for the engagement of both contractile and non-contractile elements involved in rFE (Fukutani et al., 2019; Lee et al., 2007; Pinnell et al., 2019; Rassier and Herzog, 2005, 2004a; Rassier et al., 2003; Herzog and Leonard, 2002). Therefore, with reduced contractile capacity, cross-bridge kinetics would become impaired, leading to hypothetical decreases in cross-bridge- and/or titin-mediated force enhancement. As such, the lower absolute rFE for the EDL (relative to the SOL) is attributed to a lower contractile capacity of muscle (Brooks and Faulkner, 1991), as reflected by the comparatively lower overall strength measures for the EDL (Table 1). Conversely, after accounting for differences in isometric force (i.e. strength/weakness), percent rFE was not different between the SOL and EDL, suggesting a lack of intrinsic difference for rFE between slow-twitch and fast-twitch muscles. While in contrast to Ramsey et al. (2010), and reports of stiffer titin isoforms for predominantly fast-twitch muscles (Horowits et al., 1986; Prado et al., 2005), observations from Fukutani and Herzog (2018) of similar/greater absolute and percent rFE values for the soleus (94.5% type I fibres) compared with psoas (100% type IIa fibres) muscle of rabbits (Prado et al., 2005) are in line with our findings.

Contrary to our hypothesis, absolute and percent rFE values were not different following uphill and downhill training for either muscle, despite differences in SSN for the SOL, and possibly, EDL. In the same context as rFD, it is possible that the SSN adaptations were not prominent enough or specifically geared to evoke functional differences under our *in vitro* contractile conditions. The lack of training-induced alterations to rFE in the present study is in agreement with Siebert et al. (2016), but in contrast to findings from Chen and Power (2019), which reported increases to rFE with concentric training and decreases to rFE with eccentric training. Discrepancies between this study and the one by Chen and Power (2019) may be due to differences in training protocols, neurological components, and study designs. Training in Chen and Power (2019) was predominantly load-focused, with *in vivo* torque measurements performed in the presence of an intact neurological system, the latter of which is primarily responsible for strength adaptations (e.g. increased motor unit activation/decreased neural inhibition) during the early phases (i.e. first 3–4 weeks) of resistance training (Douglas et al., 2017; Hahn, 2018). In contrast, the current training protocol involved a large aerobic component, with *in vitro* force measurements performed independent of neurological contributions. Additionally, Chen and Power (2019) looked at within-individual comparisons (pre- versus post-training), whereas this study and the one by Siebert et al. (2016) compared values in a cross-sectional manner, which could have masked more minute responses. Ultimately though, the results suggest that alterations to history-dependent properties via SSN adaptations do not serve to modify rFE (or rFD) at the whole-muscle level.

Passive force enhancement

In line with the ∼3–54% pFE previously observed in the literature (Herzog and Leonard, 2002, 2005; Lee and Herzog, 2002; Lee et al., 2007; Herzog, 2019), resting forces following active lengthening

were 37% and 11% higher than those from the ISO for the SOL and EDL, respectively. Additionally, pFE contributed to 29% and 37% of the rFE for the SOL and EDL, respectively, which is consistent with the ∼8–84% pFE-to-rFE ratio reported in the literature (Herzog and Leonard, 2002, 2005; Lee and Herzog, 2002; Lee et al., 2007) and highlights a common history-dependent mechanism. Considering that pFE is strongly and positively associated with rFE (Fig. 5C), the simultaneously greater absolute and percent pFE values for the SOL compared with EDL is in contrast to the greater absolute but similar percent rFE observed. Disparity between percent values of rFE and pFE is most likely due to differences between the isometric reference contractions, wherein active force (for rFE) is diminished but passive tension (for pFE) is preserved with lower contractile capacity. In other words, strength-related impairments to cross-bridge cycling would lead to concurrent decreases in absolute rFE and pFE for the EDL (as illustrated by the strong, positive relationships to eccentric force in Fig. 4D and Fig. 5D), but normalization to preserved resting forces would not amplify pFE values like smaller ISO forces would to rFE. As a consequence, comparisons cannot be made regarding intrinsic differences in pFE between the SOL and EDL from this study.

Although absolute values of pFE were not associated with SSN or fascicle lengths for the SOL, there were significant, albeit weak, relationships for the EDL, which highlight the length-dependence of pFE for fast-twitch muscles (shown to possess stiffer titin isoforms). Yet, the fact that absolute and percent pFE values were not different following uphill and downhill training for either muscle in the present study only further reinforces the notion that SSN adaptations were ineffective at inducing alterations to history-dependent forces at the whole-muscle, *in vitro* level.

This is the first study investigating the trainability of the history dependence of force in relation to the addition and subtraction of sarcomeres in series. We decided to perform active muscle lengthening and shortening about the plateau region where force is optimal. Without carefully constructing a length-tension relationship for each muscle we cannot, with certainty, state the lengthening and shortening occurred only on the plateau region, and thus cannot rule out part of the dynamic phase falling on the descending or ascending limb. Performing a length–tension relationship would have confirmed this and provided important insight into the passive tension of individual sarcomeres. Moreover, inherent to these history-dependent force experiments is the assumption that force reaches an isometric steady-state following the dissipation of force transients (i.e. returning to a 'steady-state' isometric force following the active lengthening and shortening dynamic phase of the contraction). We did not track force decay over time, thus used standard timing to assume a steady-state was reached. Additionally, in the context of the present study, the question is if these force transients change with training, thus, the exact time points measured are less important, as long as the protocol is standardized. Finally, there is the possibility that pFE could have been overestimated as passive force transients (following a passive stretch of the muscle) were not accounted for.

Conclusions

This is the first study to demonstrate SSN differences for the soleus, and possibly EDL, muscle of uphill- and downhill-running rats, which had previously only been reported for the rat VI, VL and TA, and recently the VL and TA of mice (Morais et al., 2020). Consequently, it appears that rFD, rFE, and pFE are not differentially modifiable at the whole-muscle, *in vitro* level, on the basis of training-induced alterations to SSN, as a means of altering sarcomere shortening/lengthening amplitudes. However, these findings highlight the relevance of reporting both absolute and relative history-dependent force values, which suggest that absolute values of rFD, rFE and pFE are not maintained during conditions of reduced contractile force, which is most likely due to impaired cross-bridge engagement. In addition to furthering our understanding of muscle contractions, from a practical standpoint, increases to rFE or decreases to rFD could serve to improve the neuromuscular economy of functional movements, particularly during stretch–shortening cycles, wherein rFE appears to attenuate rFD in an amplitude-dependent manner. As such, increases to rFE could benefit force production during both lengthening- and shortening-induced conditions. Considering how intrinsic cross-bridge dynamics are to history-dependent force properties (and vice versa), future studies investigating the trainability of the history dependence of force should look to improve the contractile capability of muscle through strength-focused training protocols.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: J.C., G.A.P.; Methodology: J.C., P.M., S.F., M.V., S.E., A.M.N., S.H.B., G.A.P.; Formal analysis: J.C., S.F., S.E., G.A.P.; Investigation: J.C., P.M., S.E., A.M.N., G.A.P.; Resources: S.H.B., G.A.P.; Data curation: J.C., S.F., S.E., G.A.P.; Writing - original draft: J.C.; Writing - review & editing: J.C., P.M., S.F., M.V., S.E., A.M.N., S.H.B., G.A.P.; Supervision: S.H.B., G.A.P.; Project administration: J.C.; Funding acquisition: S.H.B., G.A.P.

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