Muscle fibre activation is unaffected by load and repetition duration when resistance exercise is performed to task failure

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KEY POINTS SUMMARY

- Performing resistance exercise with heavier loads is often proposed to be necessary for the recruitment of larger motor units and activation of type II muscle fibres, leading to type II fibre hypertrophy. Indirect measures (surface electromyography – EMG) have been used to support this thesis, but we propose that lighter loads lifted to task failure (i.e., volitional fatigue) result in similar activation of type II fibres.

- In this study we had participants perform resistance exercise to task failure with heavier and lighter loads with both a normal and longer repetition duration (i.e., time under tension).

- Type I and type II muscle fibre glycogen depletion was determined by neither load nor repetition duration during resistance exercise performed to task failure.

- Surface EMG amplitude was not related to muscle fibre glycogen depletion or anabolic signalling; however, muscle fibre glycogen depletion and anabolic signalling were related.

- Performing resistance exercise to task failure, regardless of load lifted or repetition duration, necessitates the activation of type II muscle fibres.

ABSTRACT

Heavier loads (>60% of maximal strength) are believed to be necessary during resistance exercise (RE) to activate and stimulate hypertrophy of type II fibres. Support for this proposition comes from observation of higher surface electromyography (EMG) amplitudes during RE when lifting heavier vs. lighter loads. We aimed to determine the effect of RE, to task failure, with heavier versus lighter
loads and shorter or longer repetition durations on: EMG-derived variables, muscle fibre activation, and anabolic signalling. Ten recreationally-trained young men performed four unilateral RE conditions randomly on two occasions (two conditions, one per leg per visit). Muscle biopsies were taken from the vastus lateralis before and one hour after RE. Broadly, total time under load, number of repetitions, exercise volume, EMG amplitude (at the beginning and end of each set), and total EMG activity were significantly different between conditions (P<0.05); however, neither glycogen depletion (in both type I and type II fibres) nor phosphorylation of relevant signalling proteins were different between conditions. We conclude that muscle fibre activation and subsequent anabolic signalling are independent of load, repetition duration, and surface EMG amplitude, when RE is performed to task failure. Our results provide evidence that type I and type II fibres are activated when heavier and lighter loads are lifted to task failure. We propose that our results explain why RE training with higher or lower loads, when loads are lifted to task failure, result in equivalent muscle hypertrophy and occurs in both type I and type II fibres.

INTRODUCTION

It has been proposed that performing resistance exercise (RE) with heavier loads (greater than 60% one repetition maximum [1RM] strength) is required to elicit muscle hypertrophy and to recruit and result in hypertrophy of type II muscle fibres (Ratamess et al., 2009; Grgic et al., 2018). In contrast, data shows that performing RE training with relatively lighter loads to task failure (i.e., volitional fatigue) results in hypertrophy of both type I and type II muscle fibres (Mitchell et al., 2012; Morton et al., 2016; Schoenfeld et al., 2017). Indeed, type II muscle fibre hypertrophy, even when lighter loads are lifted to task failure, is indicative of recurrent type II fibre activation (Mitchell et al., 2012; Morton et al., 2015; Morton et al., 2016). However, others have stated, on the basis of greater muscle surface electromyography (EMG) amplitude (Jenkins et al., 2015; Looney et al., 2016; Haun et al., 2017) or decomposition of the EMG signal (Muddle et al., 2018), that heavier loads are superior to lighter loads in terms of recruiting higher threshold motor units and thus the eventual hypertrophy of type II fibres (Grgic et al., 2018).

According to the size principle of motor unit recruitment, performing submaximal contractions results predominantly in the recruitment of smaller (i.e., lower threshold) motor units that innervate type I fibres, but increasing fatigue necessitates the recruitment of larger (i.e., higher threshold) motor units that innervate type II muscle fibres (Mendell, 2005). Accordingly, several
acute aerobic (Gollnick et al., 1973; Gollnick et al., 1974b; Vollestad et al., 1984; Vollestad & Blom, 1985; Prats et al., 2013; Kristensen et al., 2015) and resistance (Bell & Jacobs, 1989; Robergs et al., 1991; Koopman et al., 2006) exercise studies have shown that sustained submaximal contractions result in the substrate depletion (which is indicative of preceding depolarization or ‘activation’) of type II muscle fibres as fatigue ensues. Nonetheless, despite considerable debate on the ability of surface electromyography (EMG) to provide insight into motor unit recruitment during fatiguing contractions (Dideriksen et al., 2010; Dideriksen et al., 2011; Enoka & Duchateau, 2015; Vigotsky et al., 2016), the thesis that type II fibre activation is confined to, or is superior, with the lifting of heavier loads has been asserted.

The primary purpose of our study was to evaluate the effect of manipulating load and repetition duration (i.e., time under tension) during RE performed to task failure on muscle fibre activation, which we quantified via fibre type-specific glycogen depletion (Bell & Jacobs, 1989; Robergs et al., 1991; Koopman et al., 2006). In addition, we measured surface EMG to ascertain how well EMG amplitude aligned with muscle fibre type-specific glycogen depletion. Additionally, to obtain mechanistic insight into how muscle fibre activation would be translated, we examined the phosphorylation of select signalling proteins prominent in contraction-related anabolism. We hypothesized that performing RE to task failure, independent of any specific RE variable, would result in activation of type I and type II muscle fibres to an equivalent extent and comparable increases in anabolic signalling. In addition, we hypothesized that surface EMG would be a poor indicator of muscle fibre type-specific glycogen depletion (i.e., fibre activation) and that muscle fibre glycogen depletion and anabolic signalling would be related.

METHODS

Ethical Approval. All participants were informed of the purpose, methodology, and potential risks of the study before giving verbal and written informed consent. The study conformed to the standards set by the latest revision of the Declaration of Helsinki and to the most recent Canadian Tri-Council policy statement on the use of human participants in research (http://www.pre.ethics.gc.ca/eng/policy-politique/initiatives/tcps2-eptc2/Default/). The study was approved by the Hamilton Integrated Research Ethics Board (Project Number 0802) and was registered at clinicaltrials.gov (NCT03991117).
Study Participants. Ten recreationally-trained young men (22±3 y, 81.6±8.9 kg, 178±6 cm, mean±SD) volunteered to participate in this study. We defined ‘recreationally-trained’ as engaging in at least one to three RE sessions per week for at least two years.

Resistance exercise training conditions. Participants’ legs were assigned in randomized crossover fashion to perform one of four unilateral RE protocols. The four RE conditions varied in the repetition duration and load (percentage of single maximal voluntary isotonic strength: %1RM). The conditions were: 80 %1RM Regular (80R; 1s:1s:1s [eccentric:pause:concentric]), 80 %1RM Slow (80S; 3:1:3), 30 %1RM Regular (30R; 1:1:1) and 30 %1RM Slow (30S; 3:1:3). The rest between each set in each condition was 180 s. Repetition cadence was maintained by an in-ear metronome at 60 bpm; however, for greater accuracy, repetition duration was quantified with the rise and fall of vastus lateralis (VL) EMG activity. RE volume (kg) was calculated by multiplying the number of repetitions in all three sets by the load lifted per repetition. Total time under load (TUL; s) was calculated by multiplying repetition duration by the number of repetitions in all three sets determined from signal from the VL EMG. Finally, impulse (kg·s) was calculated by multiplying the load lifted per repetition by the repetition duration and by the number of repetitions in all three sets.

Study Design. Each participant came in for a familiarization session before the RE trials began, which was used to obtain an independent assessment of 1RM for each leg during knee extension (Atlantis, QC, Canada) and to familiarize them with performing isometric maximum voluntary contractions (MVC; leg curl and knee extension; Biodex dynamometer, System 3, Shirley, NY, USA). Using a unilateral within-subject crossover design, participants came in on two separate occasions (separated by at least 72 h) to perform two of the four RE conditions each day (one on each leg) in a randomized order (Figure 1). Briefly, on each of the two trial days, participants arrived following an overnight fast and a muscle biopsy was taken from their vastus lateralis (VL) under local anaesthesia (2% xylocaine) to serve as the baseline for both conditions performed that day. After the muscle biopsy, dry reusable electrodes (Biometrics SX230, Biometrics Ltd., Newport, UK) were placed on each participant’s VL, vastus medialis (VM), and semitendinosus (ST; in line with the direction of muscle action) along with a reference electrode and electronic joint goniometer (SG 150, Biometrics, Ltd; collected at 2048 Hz and low pass filtered at 8 Hz) on the head of the fibula and about their knee joint, respectively. When the electrodes were in place, each participant performed three isometric knee extensions with their leg...
positioned at 60° and isometric leg curls at 45° to record peak torque and maximum voluntary excitation (MVE; i.e., the highest EMG signal [knee extension and leg curls, respectively] (Mathiassen et al., 1995)) of the quadriceps and hamstrings, respectively. Afterwards, each participant performed two of the four conditions consecutively (one on each leg), which involved three sets to task failure (i.e., the participant was unable to complete another concentric muscle action) with three isometric knee extension MVCs between each condition’s set (~15 s delay between the knee extension machine and their first MVC). Exactly one hour following the last MVC in each condition, a muscle biopsy was taken from the VL (one each leg).

**Reduction in peak torque and EMG analyses.** Muscle fatigue was quantified in the knee extensors as the reduction in isometric peak torque relative to the pre-testing peak torque. Surface EMG was recorded on a Biometrics data logger (DataLOG MWX8, Biometrics Ltd., band-pass 20-450 Hz, input impedance ~10¹⁵ Ω, CMRR > 96 dB) and analyzed with LabVIEW (version 8.2; National Instruments, Austin, TX, USA). The raw EMG signals were sampled at 2048 Hz, full-wave rectified, and smoothed with a 6 Hz low pass filter. The skin was shaved and marked (with a dot from a permanent marker) prior to bipolar integral dry reusable electrode (Biometrics SX230, Biometrics Ltd., Newport, UK) placement with a fixed inter-electrode distance of 2 cm. Care was taken not to place electrodes directly over a biopsy site in the case that the biopsy-induced oedema impaired motor unit recruitment or EMG signal. The average for each phase of each repetition was modelled with a second order polynomial regression equation, and a fast Fourier transformation was performed on each 250 ms window to calculate mean power frequency (MnPF).

The peak EMG amplitude (EMG<sub>amp</sub>) of the second repetition of each set is referred to as the ‘initial EMG<sub>amp</sub>’. Similarly, the peak EMG<sub>amp</sub> of the last repetition of each set is referred to as ‘final EMG<sub>amp</sub>’. The integrated (or total) EMG is the area under the curve throughout each set. The initial EMG<sub>amp</sub>, final EMG<sub>amp</sub>, and integrated EMG are presented as %MVE for each muscle (VL, VM, and ST). MVE was measured each trial day during the initial isometric knee extension (VL and VM) and leg curl (ST) MVCs. MnPF and average EMG of each set’s second repetition (initial MnPF and initial average EMG) and each set’s last repetition (final MnPF and final average EMG) were also calculated.
Muscle glycogen and fibre-type histochemistry. Muscle tissue from each biopsy was mounted in optimal cutting temperature (OCT) media, frozen in liquid nitrogen-cooled isopentane, and stored in a -80°C freezer until analysis. Cross sections were cut 5 μm thick using a Microm HM550 Cyrostat (Thermo Fisher Scientific, Waltham, MA) with particular care taken not to expose samples to any freeze-thaw cycles (Fairchild & Fournier, 2004). Fibre type-specific glycogen depletion was quantified by combining a brightfield periodic acid-Schiff stain (PAS), as described previously (McManus, 1948; Gollnick et al., 1973; Gollnick et al., 1974a; Gollnick et al., 1974b; Vollestad et al., 1984; Vollestad & Blom, 1985; Robergs et al., 1991; Koopman et al., 2006; Cumming et al., 2014), with a immunofluorescent myosin heavy chain (MHC) stain (Bloemberg & Quadrilatero, 2012; Morton et al., 2016; Jakubowski et al., 2019) on single cross sections similar to the methodology described elsewhere (Schaart et al., 2004). Briefly, cross sections were fixed using 3.7% formaldehyde in PBS for 60 min, treated with 1% periodic acid in distilled water for 5 min (#3951, Sigma-Aldrich, Toronto, ON, Canada), rinsed in tap water, stained with Schiff’s reagent for 15 min (#3952016, Sigma-Aldrich, Toronto, ON, Canada), rinsed with distilled water, and then rinsed in PBS prior to fluorescent staining. For the fluorescent immunohistochemistry, antibodies raised against dystrophin (MANDYS1 [3B7]), MHC I (BA-F8), MHC IIA/X (SC-71), and MHC IIX (6H1; Developmental Studies Hybridoma Bank, Iowa, USA) were combined with secondary isotype-specific antibodies (488 [A-21131], 594 [A-21125], and 647 [A-21238]; Alexa Fluor, Invitrogen, Thermo Scientific) before they were mounted with Prolong Diamond Antifade Reagent (Life Technologies, Toronto, ON, Canada) (Bloemberg & Quadrilatero, 2012). Each slide included muscle sections from a single participant within a single day (e.g., slide 1: pre, 80R and 30R; slide 2: pre, 30S and 80S) and all staining was done within a period of two weeks in batches of three to five slides per day. One day after each stain cross sections were imaged (brightfield before fluorescent, similar to a previous publication (Schaart et al., 2004)) with a CoolSNAP HQ2 fluorescent camera (Nikon Instruments, Melville, NY, USA) at 20X magnification with the following exposure times: 400 ms (FITC), 100 ms (TRITC), and 200 ms (Cy5).

Muscle glycogen and fibre type analyses. Fibre type, cross sectional area, and glycogen content were determined by tracing the fibre dystrophin border in ImageJ (version 2). Each trace was converted to a region of interest (ROI) and saved before being superimposed to another image of interest (i.e., brightfield or another fluorescent channel). Quantification of PAS intensity was determined by first converting the image to a grayscale image and then calibrating the stain to 0.68 μm/pixel. In addition,
by setting thresholds for background vs. stain intensity, we excluded the quantification of freezing-induced artifact from each ROI on every channel. To quantify fibre type, the intensity of each colour within each ROI was exported alongside the brightfield data for objective quantification of type I and type II fibres. Only fibres with a circularity >0.85 were used for analyses and care was taken not to circle any fibres towards the outside of the cross-section. An average of 275±167 and 191±126 fibres per section (1322±400 and 896±350 fibres per participant) were used for the fibre type/PAS and cross-sectional area analysis, respectively. The tracer was blinded to both the participant and conditions during image analyses.

**Western Blot Analysis.** Muscle samples were homogenized using RIPA buffer (#R0278, Sigma Aldrich, Toronto, ON, Canada) and a bead homogenizer with protease and phosphatase inhibitors (#05892970001 and 04906837001, Roche, Sigma-Aldrich, Toronto, ON, Canada). A bicinchoninic acid assay (#23227, ThermoFisher Scientific, Waltham, MA, USA) was performed on the whole muscle homogenate to quantify the protein content of each sample. Samples were prepared in Laemmli buffer (#1610747, Biorad, Hercules, CA, USA) with beta-mercaptoethanol (M6250, Sigma Aldrich, Toronto, ON, Canada) and brought to equal concentrations of 20 µg/µl. SDS-PAGE was done on 7.5 µl/sample along with two 7.5 µl prestained protein standards (#1610375, Biorad, Hercules, CA, USA) and a calibration curve (2.5, 5, 7.5, and 10 µl of all post-training samples pooled) on 26-well gels (4-15% Criterion TGX Stain-Free, #5678085, Biorad, Hercules, CA, USA). As a quality check for protein separation along the gel, the gel was imaged by ultraviolet activation with the Chemidoc MP StainFree Imager (Biorad, Hercules, CA, USA) before it was transferred to a nitrocellulose membrane via a Trans-Blot Turbo Transfer System (Biorad, Hercules, CA, USA) at 100 V for 30 min in 4°C transfer buffer (25 mM Tris, 192 mM glycine, 0.1 % SDS and 20% methanol, pH 8.3). Transfer success was visualized with both ultraviolet activation of the gel and membrane via a Chemidoc MP StainFree Imager (BioRad, Hercules, CA, USA).

Nitrocellulose membranes were blocked in bovine serum albumin (BSA) for two hours, washed three times for five minutes with TBST, cut into specific sections according to the molecular weights of our protein targets, and incubated in primary antibodies at 4°C with the 5% BSA block at concentrations between 1:500 and 1:1500 (depending on the affinity of the primary antibody). The primary antibodies we used were total mTOR (#2972), phosphorylated mTOR (Ser2448, #5536), total p70 S6k (#9202), phosphorylated p70 S6k
(Thr389, #9205), total 4E-BP1 (#9452), phosphorylated 4E-BP1 (Thr37 and Thr46, #2855),
total S6 ribosomal protein (#2217), phosphorylated S6 ribosomal protein (Ser240 and Ser244,
#2211), total AkT (#4691), phosphorylated AkT (Ser473, #9271), total FAK (#13009),
phosphorylated FAK (Tyr397, #8556), total p44/42 MAPK ERK1/2 (#9102), and
phosphorylated p44/42 MAPK ERK1/2 (Thr202 and Tyr204, #9101), which were all from
Cell Signalling Technologies (Danvers, MA, USA). After an overnight incubation,
membranes were washed again three times for five minutes in TBST, incubated in secondary
antibody (1:20 000; Anti-rabbit, HRP-linked, #7074, Cell Signalling Technologies, Danvers,
MA, USA) for one hour at room temperature, washed another three times in TBST, rocked
for five minutes in ECL substrate (Clarity Max, #1705062, Biorad, Canada), and then imaged
on the ChemiDoc MP (Biorad, CA, USA). The ladder was imaged in colourmetric mode and
the proteins of interest were measured in chemiluminescence mode. All image analysis was
performed in ImageLab (Version 5.2.1., Biorad, Hercules, CA, USA). Each gel lane was
calibrated to the gel lanes of our calibration curve and each protein band was calibrated to the
protein bands of our calibration curve as detailed elsewhere (Murphy & Lamb, 2013;
MacInnis et al., 2017). Afterwards, the calibrated protein band was divided by the calibrated
gel lane to quantify absolute protein band intensity.

Statistical Analyses. When there was only one within-subject independent variable of interest (e.g.,
fibre type), one-way repeated measures analyses of variance (ANOVA) were used. When there were
two within-subject independent variables (e.g., time and condition) a two-way repeated measures
ANOVA was used. When there were three within-subject independent variables (e.g., repetition
duration, load, and initial vs. final repetitions) a three-way repeated measures ANOVA was used.
Whenever statistical significance was found with an ANOVA test, a Bonferroni post-hoc test was
used. Lastly, bivariate, two-tailed Pearson’s correlations were run to assess relatedness on select
variables. All statistical analyses were performed in SPSS (version 20; Chicago, IL, USA), statistical
significance was set at P<0.05, and values are presented as mean±standard deviation (SD) unless
indicated.
RESULTS

Resistance exercise training variables. All RE variables are presented in Table 1. There were significant differences between conditions for every RE variable (P<0.01). Specifically, post-hoc analyses revealed a significant difference between the load lifted per repetition (80R and 80S > 30R and 30S), repetition duration (80S and 30S > 80R and 30R), number of repetitions per set (30R > 30S > 80R > 80S), volume per session (80R and 30R > 80S and 30S), total TUL per session (30S > 30R > 80R > 80S), and impulse per session (80S and 30S > 80R and 30R; P<0.05; Table 1).

EMG and decline in peak torque. EMG results are presented for the VL as that was the muscle from which we took biopsies. Our analyses and conclusions would not change if we were to report VL and VM instead of only VL (data not shown).

Initial peak EMGamp was greater in higher-load conditions (80R: 66±15 and 80S: 65±15 %MVE) compared to lower-load conditions (30R: 46±21 and 30S: 45±15 %MVE; P<0.01; Figure 2: Panel A). Following an increase in peak EMGamp in each condition (P<0.01), final peak EMGamp remained higher in high-load conditions (80R: 90±27 and 80S: 88±26 %MVE) compared to lower-load conditions (30R: 79±38 and 30S: 64±25 %MVE; P=0.04; Figure 2: Panel B) with no time by condition interaction (P=0.34). Similarly, the initial average EMG (80R: 66±15; 80S: 64±15; 30R: 34±13; 30S: 39±12 %MVE, P<0.01) and final average EMG (80R: 76±18; 80S: 68±18; 30R: 48±13; 30S: 54±14 %MVE, P<0.01) were significantly greater in the higher load conditions (Figure 3); however, there was a greater increase in average EMG in the lighter-load conditions (30R: 14±4 and 30S: 14±8 %MVE) compared to the higher-load conditions (80R: 10±7 and 80S: 4±6 %MVE, P<0.01) and integrated EMG was significantly higher in lower-load conditions (30R: 29±13 and 30S: 24±13 %MVE·s) compared to higher-load conditions (80R: 16±7 and 80S: 17±5 %MVE·s; P<0.01, Figure 2: Panel C). In addition, there was a trend for higher initial MnPF in regular repetition duration conditions (80R: 90±10 and 30R: 91±15 Hz) compared to slower repetition duration conditions (80S: 83±11 and 30S: 88±8 Hz; P=0.06) and, after a significant decrease in each condition (P<0.01), a similar trend for higher MnPF in regular repetition duration conditions (80R: 81±7 and 30R: 78±12 Hz) compared to slower repetition duration conditions
conditions (80S: 77±13 and 30S: 73±5 Hz; P=0.07). Finally, the decrease in MnPF was greater in lighter-load conditions (30R: 14±11 and 30S: 15±10 Hz) compared to higher-load conditions (80R: 9±5 and 80S: 6±3 Hz, P=0.03).

Muscle peak torque data is presented in Figure 2, Panel D. The data are presented collapsed across conditions because there was a main effect for time (P<0.01) but no differences between conditions (P=0.83) or time by condition interaction (P=0.73). Post-hoc analyses revealed significant differences between each set (P<0.05) with the exception of peak torque measured after the second and third sets, which was not different (P=0.53).

Fibre size and distribution. There was no difference between conditions for fibre distribution or cross-sectional area (type I: 44±10 %, 5622±1291 µm² and type II: 57±9 %, 7460±1503 µm², respectively).

Fibre-specific glycogen depletion. Muscle glycogen data is presented in Figure 4. There was significantly greater glycogen content in type II versus type I muscle fibres types at rest (Figure 4: Panel A; P<0.01). Glycogen content decreased in each condition (P<0.01) with a significant time by fibre type interaction such that there was a greater decrease in glycogen in type II fibres (-0.06±0.05 AU, Figure 4: Panel B) than type I fibres (-0.04±0.05 AU, Figure 4: Panel C; P=0.02). However, there were neither main nor interaction effects for condition indicating no influence of load or repetition duration on muscle fibre activation (all P>0.20).

Western blot analysis. The ratio of phosphorylated/total protein expression data are presented in Figure 5. Phosphorylated corrected for total expression of S6 ribosomal protein, FAK, ERK1, and ERK2 changed following RE (P<0.05) but there were no main effects for condition with the exception of phosphorylated/total S6 ribosomal protein (P=0.04), which was significantly higher post-exercise in 30R than 30S (Figure 5: Panel C).

Correlational Analysis. There were no significant correlations between initial EMG<sub>amp</sub>, final EMG<sub>amp</sub>, integrated EMG, the change in EMG<sub>amp</sub>, the change in average EMG, or the change in MnPF with
type I, type II, or total (the sum of type I and type II) muscle fibre glycogen depletion (all $r<0.24$; $P>0.15$). However, there were weak to moderate correlations between type I and type II glycogen depletion and anabolic signalling (phosphorylation/total) and the reduction in peak torque (Table 2).

**DISCUSSION**

We found that performing RE to task failure with varying loads and conventional (1:1:1) or longer (3:1:3) contraction cadences (i.e., time under tension) resulted in no significant differences in fibre type-specific glycogen depletion, which is a direct measure of the ‘use’ (and preceding activation) of the fibres we assessed (Gollnick et al., 1973; Gollnick et al., 1974b; Vollestad et al., 1984; Vollestad & Blom, 1985). By manipulating load and repetition duration we were able to create substantial differences in a number of RE variables (i.e., number of repetitions, exercise volume, total TUL, and impulse; Table 1) allowing us to evaluate how such differences influenced surface EMG (Figure 2 and 3), force loss (Figure 2: Panel D), muscle fibre activation (Figure 4: Panels B and C), and anabolic signalling (Figure 5). Our main finding was, independent of load or repetition duration, that performing RE to task failure resulted in substrate depletion of (therefore activation and recruitment of motor neurons that innervate) both type I and type II fibres with no significant differences observed between conditions. In addition, we confirm that when RE is performed to task failure the maximal amplitude of surface EMG at the beginning or the end of a set is not related, as some have posited (Looney et al., 2016), to muscle fibre activation, reductions in peak torque, nor to signalling protein phosphorylation, which have been tied to protein synthesis and anabolism and to hypertrophy. Thus, as ourselves (Vigotsky et al., 2016; Vigotsky et al., 2017) and others (Farina et al., 2004; Dideriksen et al., 2010; Dideriksen et al., 2011; Enoka & Duchateau, 2015) have concluded, the current data suggests surface EMG does not accurately assess type I or type II muscle fibre activation during RE.

**Resistance training variables and muscle fibre activation**

Previous research has demonstrated that isokinetic MVC (Bell & Jacobs, 1989), heavier load RE (Robergs et al., 1991; Koopman et al., 2006) and lighter load RE (Robergs et al., 1991) all result in glycogen depletion in both type I and type II fibres. Nonetheless, some have hypothesized that type II muscle fibre activation is exclusive, or greater, when performing RE with heavier loads (Grgic et al., 2018). This thesis has been buoyed by measurements of greater surface EMG amplitude during resistance exercise (Jenkins et al., 2015; Looney et al., 2016; Haun et al., 2017) and isometric exercise with subsequent algorithmic decomposition of the EMG signal to track motor units (Muddle et al., 2018).
et al., 2018). We manipulated load and repetition duration to create three-fold differences in load, repetition duration, number of repetitions, exercise volume, total TUL, and impulse (Table 1), and nearly two-fold differences in EMG$_{amp}$ at the beginning of each set, EMG$_{amp}$ at the end of each set, and the integrated (or total) EMG between conditions (Figure 2). However, performing RE to task failure resulted in similar magnitudes of glycogen depletion (i.e., the use and therefore activation) in type I and type II muscle fibres (Figure 4) and similar levels of anabolic signalling protein phosphorylation (Figure 5). Thus, we conclude that neither load, repetition duration, nor the accompanying surface EMG$_{amp}$ align with muscle fibre type-specific activation when RE is performed to task failure.

**Surface EMG and muscle fibre activation**

Surface EMG records the electrical activity of numerous motor units, which can be decoded and modeled as an indirect measurement of individual neuron firing, individual neuron ‘drop out’, and individual neuron recycling (Enoka & Duchateau, 2015; Vigotsky et al., 2016; Muddle et al., 2018). However, the relationship between surface EMG$_{amp}$ and motor unit recruitment is not easily ascertained during sustained/fatiguing contractions (Dideriksen et al., 2010; Dideriksen et al., 2011), it may be convoluted by non-random motor unit distribution in the VL (Knight & Kamen, 2005), and it is preferential to superficial motor neurons (Muceli et al., 2015). Here, we demonstrate that performing RE to task failure with lower loads does not result in 100 %MVE (Figure 2: Panel B and Figure 3) but does result in the use/activation of type II muscle fibres that are part of larger, higher threshold motor units (Figure 4: Panel C). Indeed, larger motor units produce larger action potentials and experience greater reductions in firing rates with sustained contractions (Potvin & Fuglevand, 2017); thus, it is not surprising that the increase in EMG signal is attenuated in higher loads (Figure 3) and that lower-load contractions to task failure never reaches 100 %MVE (Figure 2: Panel B). Evidently, particularly during sustained or repeated isotonic contractions, caution is warranted regarding the efficacy of EMG$_{amp}$ to infer fibre type-specific motor unit recruitment (Farina et al., 2004; Dideriksen et al., 2010; Dideriksen et al., 2011; Enoka & Duchateau, 2015; Vigotsky et al., 2016).
Anabolic signalling and muscle fibre activation

We have shown that type II muscle fibre hypertrophy occurs with low-load RE when loads are lifted to fatigue, which would require type II muscle fibre activation (Mitchell et al., 2012; Morton et al., 2015; Morton et al., 2016). Here, we demonstrate that, independent of load or repetition duration, performing RE to task failure results in both type I and type II muscle fibre activation (Figure 4) and to an equivalent extent. We also observed equivalent increases in the phosphorylation of a number of canonical signalling proteins (Figure 5), and extend our findings to support the recommendation of submaximal loading in older or unhealthy populations (McLeod et al., 2019). Moreover, we found significant correlations between glycogen depletion versus reduction in peak torque and increase in anabolic signalling protein phosphorylation, and add that in some cases those proteins (e.g., p70 S6k) have established correlations with muscle protein synthesis (Burd et al., 2010) and changes in fibre cross-sectional area following RE training (Mitchell et al., 2013). Thus, we confirm our hypothesis that type II muscle fibre activation occurs when lighter loads are lifted to task failure and add that fibre-specific glycogen depletion is related to muscle fatigue and signalling protein activation in the VL, which underscores the subsequent type II muscle fibre hypertrophy previously reported (Mitchell et al., 2012; Morton et al., 2016).

Limitations

We quantified muscle fibre activation via glycogen depletion because it is the primary substrate used during RE (Koopman et al., 2006) and has been used extensively in studies to directly establish that fibres were activated and used (Gollnick et al., 1973; Gollnick et al., 1974b; Vollestad et al., 1984; Vollestad & Blom, 1985; Robergs et al., 1991; Koopman et al., 2006; Prats et al., 2013). Indeed, substrate depletion in a muscle fibre is indicative that the fibre was used and therefore activated; however, we acknowledge that the method may lack sensitivity as an indication of muscle fibre depolarization and that glycogen is not the only substrate used during fatiguing contractions (Koopman et al., 2006). In addition, unilateral RE induces a small increase in strength in the contralateral limb (Munn et al., 2004); however, the best evidence of an origin for the cross-limb education effect is in the central nervous system and not within the muscle itself (Carroll et al., 2006). Indeed, muscle activity is negligible in the non-contracting VL (Figure 6), which reinforces evidence that post-exercise rates of protein turnover are exclusive to the muscle group that is contracting (Wilkinson et al., 2014; Holwerda et al., 2018). Thus, we see no reason to hypothesize that
contracting one limb resulted in muscle fibre activation in the contralateral limb. Otherwise, we acknowledge that our intramuscular analyses is limited to ~275 muscle fibres in the VL per biopsy, which may not be representative of all muscle fibres in the VL or of surrounding muscles (Burke & Tsairis, 1974). Finally, we elected to take muscle biopsies one hour post-exercise to measure protein phosphorylation to avoid waiting so long that significant glycogen resynthesis would occur (Robergs et al., 1991; Koopman et al., 2006; Camera et al., 2012; Cumming et al., 2014); though, we acknowledge that measuring protein phosphorylation one hour post-exercise provides only a snapshot in the time course of protein signalling and that minimal glycogenesis may have resulted in an underestimation of type II fibre glycogen depletion (Vollestad et al., 1989).

**Conclusion**

We discovered that performing RE to task failure, independent of load or repetition duration, resulted in equivalent type I and type II muscle fibre glycogen depletion, which is indicative of preceding fibre activation. Moreover, by manipulating load and repetition duration, we demonstrate that no specific RE variable (e.g., number of repetitions, exercise volume per session, total TUL per session, or impulse) affected fibre type-specific glycogen depletion in the VL when RE was performed to task failure. Indeed, despite similar magnitudes of glycogen depletion, there were substantial and significant differences in EMG amplitude, average EMG, mean power frequency, and integrated (total) EMG in each condition. Thus, we also show that surface EMG amplitude, average EMG, mean power frequency, and integrated (total) EMG are not indicative of fibre type-specific glycogen depletion in the VL. In contrast, and similar to glycogen depletion, RE-induced anabolic signalling was independent of load and repetition duration. Therefore, we conclude that muscle fibre activation is aligned with reductions in peak torque and anabolic protein signalling, and that neither are determined by load or repetition duration when RE is performed to task failure.

**REFERENCES**


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**ADDITIONAL INFORMATION SECTION**

**Competing Interests**

The authors have no competing interests financial or otherwise to declare.

**Author Contributions**

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RWM, CM, JRP, and SMP designed the study, RWM, MWS, AFZ, AJ, and SMP performed the data collection, RWM, MWS, AFZ, and IYZM performed the data analysis, RWM and SMP drafted the manuscript, and all authors critically revised the manuscript and approved the final version.

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**AUTHOR’S TRANSLATIONAL PERSPECTIVE**

Elwood Henneman first described the ‘size principle’, which described the systematic recruitment of motor units based on their morphology, in a series of publications in and around 1965. Since, exercise physiologists have corroborated the size principle by showing that increased fatigue while lifting submaximal loads necessitates an increase in the recruitment of larger, higher threshold, motor units and their associated type II muscle fibres. Research in the realm of resistance exercise has found, contrary to wide held belief, that lifting relatively light loads to task failure (volitional fatigue) results in the hypertrophy of type II fibres. Thus, we hypothesized that when resistance exercise was performed to task failure, the inevitable muscular fatigue would necessitate the recruitment of larger motor units and subsequent activation (use) of type II muscle fibres, which would be independent of load or contraction cadence (or ‘time under tension’). Indeed, we confirm these hypotheses and provide evidence that is in agreement with the size principle as described by Henneman. Specifically, we show that performing resistance exercise to task failure, independent of load or contraction cadence, resulted in the activation of both type I and type II muscle fibres. Moreover, while surface EMG is an informative and accessible way to measure general muscular activity, we add to the existing literature that calls for caution against using EMG amplitude to infer the activation of individual motor units. Lastly, we illustrate here that muscle fibre activation and muscle anabolism

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are related, and that neither are affected by the load or contraction cadence used during resistance exercise when exercise is performed to task failure.

FIGURE LEGENDS

Figure 1. Study schematic representing one of the two trial days. The two arrows represent each of the participant’s legs.
Figure 2. EMG amplitude during the second (Panel A) and last (Panel B) repetitions of each set, integrated (or total) EMG throughout each set (Panel C), and peak torque before and after each set (Panel D). * and † indicate a significant difference between the other conditions or times of measurement (P<0.05). The data are presented as box and whisker plots with the median (line), mean (cross), inter-quartile range (box), and minimum and maximum values (tails).
**Figure 3.** The average EMG in the VL relative to knee angle during the second (dotted line) and final (solid line) repetition in each condition: 80R (Panel A), 80S (Panel B), 30R (Panel C), and 30S (Panel D). *Significant difference between 80R and 80S conditions (P<0.01).
Figure 4. Histochemical analysis of skeletal muscle glycogen content at rest (Panel A) and the change in glycogen content following each condition in type I (Panel B) and type II (Panel C) fibres. * Indicates significantly different from type I fibres (Panel A), or significantly different from rest (Panels B and C; P<0.01). The data are presented as box and whisker plots with the median (line), mean (cross), inter-quartile range (box), and minimum and maximum values (tails).
Figure 5. Phosphorylation/total expressions for 4E-BP1 (Panel A), p70s6k (Panel B), S6 ribosomal protein (Panel C), FAK (Panel D), ERK1 (Panel E), and ERK2 (Panel F). * Indicates a main effect for time and the different letters above individual bars indicate a significant time by condition interaction between those conditions (P<0.05). The data are presented as box and whisker plots with the median (line), mean (cross), inter-quartile range (box), and minimum and maximum values (tails).
Figure 6. A representative raw EMG signal during the 80R condition. Panel A is the raw EMG signal in the VL of the exercising leg and Panel B is the raw EMG signal in the VL of the non-exercising leg.
### Table 1. Resistance Exercise Training Variables

<table>
<thead>
<tr>
<th></th>
<th>80R</th>
<th>80S</th>
<th>30R</th>
<th>30S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Load per repetition (kg)</td>
<td>66±8ª</td>
<td>65±8ª</td>
<td>25±4ª</td>
<td>25±3ª</td>
</tr>
<tr>
<td>Repetition duration (s)</td>
<td>2.9±0.4ª</td>
<td>5.3±0.6ª</td>
<td>2.7±0.6ª</td>
<td>5.4±0.5ª</td>
</tr>
<tr>
<td>Repetitions per set</td>
<td>9±2ª</td>
<td>6±1ª</td>
<td>20±4ª</td>
<td>14±4ª</td>
</tr>
<tr>
<td>Volume (kg)</td>
<td>1788±574ª</td>
<td>1242±348ª</td>
<td>1532±400ª</td>
<td>1071±354ª</td>
</tr>
<tr>
<td>Total TUL (s)</td>
<td>76±20ª</td>
<td>99±17ª</td>
<td>158±19ª</td>
<td>225±52ª</td>
</tr>
<tr>
<td>Impulse (kg·s)</td>
<td>5055±1680ª</td>
<td>6518±1590ª</td>
<td>3938±603ª</td>
<td>5723±1639ª</td>
</tr>
</tbody>
</table>

Impulse (kg·s) is calculated by multiplying the load lifted per repetition, by the repetition duration, and by the number of repetitions in all sets. Significant differences identified via post-hoc analyses are indicated by letter superscript with means bearing dissimilar letters being significantly different (all P<0.05). Values are mean±SD.
Table 2. Correlations between anabolic signalling protein phosphorylation (relative to total protein), peak torque, and glycogen depletion

<table>
<thead>
<tr>
<th></th>
<th>Δ Type I Glycogen</th>
<th>Δ Type II Glycogen</th>
<th>Δ Total Glycogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ p-mTOR</td>
<td>0.13</td>
<td>0.13</td>
<td>0.10</td>
</tr>
<tr>
<td>Δ p-4E-BP1</td>
<td>0.15</td>
<td>0.12</td>
<td>0.18</td>
</tr>
<tr>
<td>Δ p-p70 S6k</td>
<td>0.41*</td>
<td>0.37*</td>
<td>0.33*</td>
</tr>
<tr>
<td>Δ p-S6</td>
<td>0.30</td>
<td>0.37*</td>
<td>0.29</td>
</tr>
<tr>
<td>Δ p-FAK</td>
<td>-0.03</td>
<td>0.01</td>
<td>-0.02</td>
</tr>
<tr>
<td>Δ p-ERK1</td>
<td>0.37*</td>
<td>0.33*</td>
<td>0.32*</td>
</tr>
<tr>
<td>Δ p-ERK2</td>
<td>0.32</td>
<td>0.31</td>
<td>0.30</td>
</tr>
<tr>
<td>Δ peak torque</td>
<td>0.23</td>
<td>0.25</td>
<td>0.32*</td>
</tr>
</tbody>
</table>

Values are Pearson r values. * indicates P<0.05.