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Running title: Maximal M-wave and resistance training

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Key words: Muscle excitability, M-wave, Strength training, Surface Electromyography, Sarcolemmal excitability

ABSTRACT

This study compared elbow flexor (EF; Experiment 1) and knee extensor (KE; Experiment 2) maximal compound action potential (M_{max}) amplitude between long-term resistance trained (LTRT; n=15 and n=14, 6±3 and 4±1 years of training) and untrained (UT; n=14 and n=49) men; and examined the effect of normalising electromyography (EMG) during maximal voluntary torque (MVT) production to M_{max} amplitude on differences between LTRT and UT. EMG was recorded from multiple sites and muscles of EF and KE, M_{max} was evoked with percutaneous nerve stimulation, and muscle size was assessed with ultrasonography (thickness, EF) and magnetic resonance imaging (cross-sectional area, KE). Muscle-electrode distance (MED) was measured to account for the effect of adipose tissue on EMG and M_{max}. LTRT displayed greater MVT (+66-71%, $p<0.001$), muscle size (+54-56%, $p<0.001$), and M_{max} amplitudes (+29-60%, $p\leq0.010$) even when corrected for MED ($p\leq0.045$). M_{max} was associated with the size of both muscle groups ($r\geq0.466$, $p\leq0.011$). Compared to UT, LTRT had higher absolute voluntary EMG amplitude for the KE ($p<0.001$), but not the EF ($p=0.195$), and these differences/similarities were maintained after correction for MED; however, M_{max} normalisation resulted in no differences between LTRT and UT for any muscle and/or muscle group ($p\geq0.652$). The positive association between M_{max} and muscle size, and no differences when accounting for peripheral electrophysiological properties (EMG/M_{max}), indicates the greater absolute voluntary EMG amplitude of LTRT might be confounded by muscle morphology, rather than provide a discrete measure of central neural activity. This study therefore suggests limited agonist neural adaptation after LTRT.

New & Noteworthy

In a large sample of long-term resistance-trained individuals we showed greater maximal M-wave amplitude of the elbow flexors and knee extensors compared to untrained, which appears to be at least partially mediated by differences in muscle size. The lack of group differences in voluntary EMG amplitude when normalised to maximal M-wave suggests that differences in muscle morphology might impair interpretation of voluntary EMG as an index of central neural activity.

INTRODUCTION

Resistance training is known to increase maximal force generating capacity of muscle when performed regularly (27). The initial (<2-4 weeks) increases in muscle force production following resistance training are thought to be primarily underpinned by neural factors (60), followed by adaptation in muscle morphology (>5-8 weeks; Ref. 27). It is largely unclear however, whether neural factors contribute to the substantial increases in force production with long-term resistance training (LTRT; > several months or years).

Owing to logistical issues associated with long-term resistance training research, only limited data concerning neural changes exist from medium-term longitudinal studies. Studies employing surface electromyography (EMG) recordings during maximal voluntary isometric contractions have shown either no change (49) or an increase in signal amplitude (33). Cross-sectional studies have demonstrated greater EMG activity of LTRT individuals during a maximal voluntary isometric contraction compared to untrained (UT) controls (6, 22). However, greater absolute EMG amplitude with LTRT does not necessarily represent modifications of neural properties (19, 25, 36, 47). Indeed, absolute surface EMG amplitude is subject to alterations by various peripheral electrophysiological properties distinct from neural drive. These include muscle propagation of action potentials from the neuromuscular junction to the sarcolemma (e.g. muscle membrane properties, fibre size; Ref. 25), and volume conduction of signals from the sarcolemma through the intermediate tissues to the electrode on the skin surface (e.g. subcutaneous adipose tissue, Ref. 14). To account for the influence of subcutaneous adipose tissue, which may differ between LTRT and UT individuals, the EMG signal amplitude can be corrected for the muscle-electrode distance (MED; primarily adipose tissue, Ref. 42). Such an approach has also

revealed greater maximal EMG activity between LTRT and UT individuals (6). However, correction for MED does not account for differences in muscle propagation, specifically muscle morphology (44), and muscle membrane properties (18) that would be expected to influence the size of single fibre action potentials (30, 32). To account for the aforementioned factors, normalisation to maximal compound action potential is required (maximal M-wave, M_{max}; 38, 45), particularly in the case of maximal voluntary contractions, where other possible reference values (e.g. EMG during maximal voluntary torque, MVT; Ref. 8) are invalid. Comparing voluntary EMG amplitude corrected for MED to normalisation to maximal M-wave could therefore allow the distinction between the influence of adipose tissue and other peripheral properties on the amplitude of the signal, both of which could differ between LTRT and UT individuals.

Given the M_{max} may be useful for normalising voluntary EMG activity during maximal contractions, it is important to consider the potential impact of long-term resistance training on maximal M-wave amplitude. The maximal M-wave represents the summated electrical activity of motor units within the recording volume following depolarisation of their axons by a supramaximal electrical stimulus (58), and facilitates the assessment of peripheral electrophysiological properties of the neuromuscular system (58). For example, the maximal M-wave is influenced by, amongst other factors, changes in muscle morphology and muscle membrane properties (e.g., motor unit conduction velocity and the amplitude of transmembrane action potentials; Ref. 57). These factors are known to change with resistance training; for example, the greater muscle size of LTRT individuals (44) that is primarily due to enhanced muscle fibre size (43) may increase the size of single fibre action potentials (32) and thus also the amplitude of M_{max}. Indeed, a strong relationship between muscle size and M_{max}

amplitude has been shown in clinical populations (1); however, this relationship remains unexplored in the context of resistance training. A clear relationship between M_{max} and muscle size could indicate a confounding effect of muscle size on the amplitude of absolute EMG, and support the necessity for M_{max} normalisation of voluntary EMG, especially when comparing individuals and/or groups with distinct muscle sizes. Furthermore, LTRT individuals demonstrate increased motor unit conduction velocity (18, 48). The greater motor unit conduction velocity would theoretically lead to greater synchronisation of the constituent motor unit action potentials of an M-wave (37, 57), thereby increasing its amplitude, particularly in the propagating phase of the potential (58).

Data concerning M_{max} amplitude in LTRT individuals are equivocal; with reports of either greater amplitude (22) or no difference (40, 53) in biceps brachii M_{max} compared to controls. However, differences in joint configurations (52), and EMG recordings from single unspecified sites (5), may have contributed to these divergent findings. Furthermore, we are not aware of any data regarding M_{max} amplitude of LTRT individuals in lower limb muscles (e.g., knee extensors). For example, the knee extensors compared to elbow flexors, have a significantly different geometry and spread of the innervation zones, which might lead to differences in the amplitude of maximal M-wave between muscle groups (58) and affect the comparison between LTRT and UT individuals.

The purpose of the current investigation was to 1) compare M_{max} amplitudes between LTRT (i.e., multiple years of resistance training exposure) and UT individuals for both upper- (i.e., elbow flexors; Experiment 1) and lower- (i.e., knee extensors; Experiment 2) body muscles; 2) assess the relationship between M_{max} and muscle size; and 3) contrast the absolute voluntary EMG amplitude with that normalised to both MED and

M_{max} between LTRT and UT individuals. It was hypothesised that, due to expected larger muscle mass, M_{max} amplitude will be greater in LTRT compared to UT individuals. Furthermore, it was hypothesised that normalisation to M_{max} will eliminate any between-group difference in voluntary EMG amplitude.

MATERIALS AND METHODS

Participants

Two separate cohorts were tested in this study as part of a series of investigations assessing elbow flexor (Experiment 1; see Ref. 45) and knee extensor (Experiment 2; see Ref. 44) neuromuscular function of LTRT individuals. The experimental procedures were approved by the Loughborough University Ethical Advisory committee in accordance with Declaration of Helsinki and participants gave written informed consent prior to their participation. Physical activity levels were also assessed at the start of the study using the International Physical Activity Questionnaire (IPAQ; Ref. 13). In Experiment 1, a total of 29 participants were recruited for elbow flexor measurements, 15 LTRT (mean ± SD, age: 22 ± 4 years; stature: 1.79 ± 0.07 m; mass: 89 ± 11 kg; IPAQ: 6518 ± 1748 metabolic equivalent min/week) and 14 UT men (22 ± 3 years, 1.76 ± 0.11 m, 68 ± 10 kg, 1042 ± 464 metabolic equivalent min/week). Untrained individuals were of similar height (independent samples t-test, $p = 0.440$) and age ($p = 0.917$), but were lighter compared to LTRT ($p < 0.001$) and had lower levels of physical activity ($p < 0.001$). In Experiment 2, 63 men were recruited for knee extensor measurements, of which 14 were LTRT (22 ± 2 years, 1.84 ± 0.06 m, 92 ± 10 kg, 5568 ± 1457 metabolic equivalent min/week), whereas 49 were UT (25 ± 2 years, 1.76 ± 0.07 m, 73 ± 9 kg, 2326 ± 1337 metabolic

158 equivalent min/week). Untrained participants in the knee extensor cohort were older,
159 shorter, lighter and had lower levels of physical activity (independent samples t-test, p
160 < 0.001 for all). All participants were asymptomatic at the time of testing and reported
161 no major injuries within the last 3 months. Untrained participants were not engaged in
162 any systematic training and had not performed lower- or upper-body resistance
163 training for >18 months. The LTRT groups reported (via a detailed questionnaire and
164 follow-up oral discussion) regular, systematic, progressive heavy resistance training
165 for ≥3 years either of the elbow flexors (≥2 × per week; 6 ± 3 [range 3 – 16] years) or
166 knee extensors (≥ 2 × per week; 4 ± 1 [range of 3 – 5] years) with the primary aim of
167 developing maximal strength. Individuals were excluded from participation if they
168 reported the use of androgenic-anabolic steroids. Long-term resistance-trained
169 individuals commonly reported the use of nutritional supplements (e.g., whey protein
170 and creatine).

171

172 **Experimental overview**

173 The procedures for the two experiments were similar with participants visiting the
174 laboratory four times in total, with each visit 7 to 10 days apart. All measures were
175 conducted on the dominant limb. The first session involved habituation with the
176 procedures (including stimulations) and practice performing isometric maximal
177 voluntary contractions. Participants then completed two duplicate neuromuscular
178 assessments at a consistent time of day to avoid diurnal variation in neuromuscular
179 function. These sessions involved isometric dynamometry for recording contractile
180 forces and surface EMG during evoked contractions and maximal voluntary isometric
181 contractions of the elbow flexors or knee extensors. The last visit involved assessment
182 of muscle size using B-mode ultrasonography (Experiment 1) or 1.5-T magnetic

resonance imaging (MRI) scans (Experiment 2). Additionally, B-mode ultrasonography was performed in both experiments to measure MED.

Experimental procedures

Neuromuscular assessment

Neuromuscular assessment procedures were similar between elbow flexion (Experiment 1) and knee extensor (Experiment 2) cohorts. Following skin preparation and EMG electrode placement, participants performed a standardised warm-up consisting of 5-second isometric contractions at 50 (× 3), 75 (× 3) and 90% (× 1) of perceived MVT with 15-30 seconds of rest given between efforts. Following warm-up, three supramaximal twitches were evoked with percutaneous nerve stimulation (see below for details). After that, participants performed 3-4 maximal voluntary isometric contractions, and were instructed to “pull/push as hard as possible” for 3-5 seconds with ≥30 seconds of rest between efforts. Visual feedback of the force production was provided along with verbal encouragement, and the greatest force obtained during that session was displayed to facilitate maximal effort.

Torque and EMG recording

Neuromuscular assessments were performed with participants seated in rigid custom-made isometric dynamometers. In Experiment 1, participants were seated in an elbow flexion dynamometer (23) with the shoulder and elbow at 90 and 80°, respectively, the shoulder in slight horizontal abduction (~10°), and the forearm half-supinated (~45°) position (0° = anatomical position). The wrist was tightly strapped to a brace in series with a calibrated S-beam strain gauge (Force Logic, Swallowfield, UK). Additionally,

participants were tightly fastened across the pelvis and chest to prevent extraneous movement. In Experiment 2, participants were seated in a knee extension dynamometer (46) with knee and hip flexed at 115 and 126° (180° = full extension). To prevent extraneous movements, straps were tightly fastened across the participant's pelvis and shoulders. An ankle strap (35-mm-width reinforced canvas webbing) was positioned at ~15% of tibial length (lateral malleolus to the knee joint centre), above the malleoli, and in series with a calibrated S-beam strain gauge (Force Logic, Swallowfield, UK). We have previously shown that the aforementioned positions minimise joint angle changes during maximal isometric efforts (≤4° compared to 10-20° changes commonly observed with commercial dynamometers; Ref. 28), and maximise torque production and therefore reduce any confounding influence of the torque-angle relationship (41).

The analogue force signal was amplified (× 370) and sampled at 2 kHz (Micro 1401; Cambridge Electronics Design Ltd., Cambridge, UK). During the off-line analysis, force data were low pass filtered (500 Hz, zero-lag fourth-order Butterworth; Ref. 46), gravity corrected (subtraction of baseline force) and converted to torque (multiplied by lever length; the distance between the knee/elbow joint and the centre of the restraining strap). The greatest instantaneous torque achieved during maximal voluntary isometric contractions was taken as MVT.

Surface EMG (Trigno system; Delsys, Boston, MA) was recorded from superficial elbow flexor (biceps brachii long head, BBL; and biceps brachii short head, BBS) and knee extensors (vastus medialis, VM; vastus lateralis, VL; and rectus femoris, RF) muscles, after skin preparation (shaving, abrading, and cleansing with 70% ethanol), using wireless sensors (fixed 1-centimetre inter-electrode distance; Trigno Standard EMG sensors, Delsys, Boston MA). Specifically, two sensors were placed over the

biceps brachii at set percentages of the length between medial acromion and cubital fossa (BBL: 67%, BBS: 67%). For the knee extensors, six discrete sensors (two per superficial quadriceps muscle) were placed at set percentages of thigh length above the superior border of patella (VM: 35 and 30%, VL: 60 and 55%, RF: 65 and 55%), in parallel with presumed fibre orientation. Multiple rather than single site recordings were performed to minimise the error in amplitude estimation, which is higher in single site recordings due to implicit assumption that the amplitude of the signal scales proportionally with excitation across the whole motor pool (62). Averaging from multiple sites therefore likely provides a more comprehensive assessment of motor unit responsiveness to voluntary and evoked stimulation. Furthermore, we have previously shown that multiple site- and/or muscle recordings and subsequent averaging of data significantly improves the reliability of voluntary and evoked EMG activity and is thus favourable when assessing larger muscle groups (5).

The EMG signals were initially amplified and band-pass filtered at source (×300; 20-450 Hz) before further amplification (total of ×909) and sampled at 2 (knee extensors) and 4 (elbow flexors) kHz using the same A/D converter and software as for the force signal, thus allowing synchronisation. Due to the inherent delay in the EMG system (48 ms; Trigno EMG system), EMG signals were first temporally corrected during off-line analysis before additional band-pass filtering (6-500 Hz, zero-lag fourth-order Butterworth). EMG activity was quantified as root mean square (RMS) of the 500 ms epoch around MVT (250 ms either side of MVT). For individual knee extensor muscles, RMS EMG was first averaged across the two independent recording sites (e.g., for VM activity was averaged between the sensors placed at 35 and 30% of thigh length). After that, averaging across muscles was performed to quantify whole elbow flexor (BBL and BBS) or knee extensor (VM, VL and RF) EMG activity. Data were expressed

in absolute EMG values, normalised to M_{max}, and as absolute values corrected for muscle-electrode distance (see below). Normalisation to M_{max} was first performed for each corresponding measurement site before averaging within constituent muscles, and then for the whole muscle group.

Percutaneous nerve stimulation

Percutaneous stimulation (single 200 µs square-wave pulse; DS7AH, Digitimer Ltd., Welwyn Garden City, UK) of the brachial plexus (elbow flexors) or femoral nerve (knee extensors) was delivered to evoke M_{max}. The brachial plexus was stimulated with a securely taped cathode probe (1-centimetre diameter, Electro-Medical Supplies, Wantage, UK) and a gel-coated anode electrode placed over the deltoid (7 × 10 cm rubber electrode; Electro-Medical Supplies, Wantage, UK). The femoral nerve was stimulated with an identical, securely taped, cathode placed in the femoral triangle and the same anode placed over the greater trochanter. The optimal cathode position was determined in the beginning of the trial as the spot corresponding to the greatest M_{max} peak-to-peak amplitude at a constant submaximal current intensity. The current intensity was then progressively increased until there was a plateau in M_{max} peak-to-peak amplitude, after which it was increased by 30% to ensure supramaximal stimulus intensity. Three supramaximal stimuli were then delivered separated by 15 seconds. From those trials, peak-to-peak amplitude of M_{max} were calculated and averaged. Example traces from one participant of each group in the knee extensors and elbow flexors are depicted in Figure 1. In some cases of elbow flexion measurements, negative and/or positive peak values of M_{max} exceeded the maximum range of the recordings. This was the case for 21.8% (LTRT: 30.0%, UT: 13.1%) and 28.7% (LTRT: 38.9%, UT: 17.9%) of all trials, and occurred in 31.0% (LTRT: 40.0%, UT: 21.4%) and

34.5% (LTRT: 46.7%, UT: 21.4%) of the sample population in BBL and BBS, respectively. In such cases, clipped parts of M-waves were interpolated by fitting the M-wave response of the unclipped parts to the 6th order polynomial curves (R^2 = 0.98 – 1.00) to obtain the peak values. To test the validity of this approach, a random sample (n = 23) of unclipped trials were retrospectively clipped (i.e., a 10 ms epoch of data around the positive and negative peak was deleted) in order to compare the actual/original measured M_{max} amplitude (i.e., from unclipped recording) to M_{max} estimated from the clipped version with interpolation of the missing data by the 6th order polynomial fit. Comparison of M_{max} amplitude between the original, unclipped and the clipped, interpolated measurements revealed excellent agreement ($ICC_{3,1}$: 0.998 [0.996 – 0.999], Figure 1C), confirming the robustness of the approach.

Muscle size

Biceps brachii muscle thickness was assessed using B-mode ultrasonography (EUB-8500; Hitachi Medical Systems UK Ltd., Northamptonshire, UK) with participants positioned in the isometric elbow flexion dynamometer. Longitudinal images of the biceps brachii were recorded with the ultrasound probe (9.2 centimetre linear-array transducer, EUP-L53L; sampling rate 32 Hz, coated with water soluble transmission gel) placed perpendicular to the skin surface with the centre of the probe at positions corresponding to EMG electrodes location over the long and short head of the biceps brachii. Muscle thickness of the elbow flexors was quantified as the distance between the subcutaneous adipose tissue-muscle interface and muscle-bone interface at the centre of images using a public domain image analysis software (https://physlets.org/tracker/: Tracker, version 4.97). Values from the two images (of

306 the long and short head of biceps brachii) were averaged to provide a mean elbow
307 flexor value.

308 Quadriceps anatomical cross-sectional area (ACSA) was assessed with a 1.5-T MRI
309 scan of the dominant thigh. A receiver eight-channel whole-body coil (Signa HDxt; GE)
310 was used to acquire T1-weighted axial slices (5 mm thick, 0 mm gap) between anterior
311 superior iliac spine and the knee joint space in two overlapping blocks whilst
312 participants laid supine with the knee joint angle of ~163°. The alignment of the blocks
313 of slices was facilitated by oil-filled capsules placed on the lateral side of each
314 participants' thigh. The quadriceps muscles (VM, VL, RF and vastus intermedius) were
315 manually outlined in every third image (every 15 mm) starting from the most proximal
316 image in which the muscle appeared (OsiriX software, version 6.0; Pixmeo, Geneva,
317 Switzerland). For each constituent quadriceps muscle the image with the largest
318 ACSA was taken as its maximum ACSA, and the values from all four constituents were
319 summed for quadriceps ACSA (QACSA).

320 Due to resource limitations, measures of muscle size were performed with different
321 methodologies in the two experiments. Whilst muscle thickness is reportedly an
322 acceptable proxy of ACSA (29), we wanted to ensure this was the case in our
323 experiment. For this purpose, muscle thickness of the quadriceps was also assessed
324 by recording longitudinal images of quadriceps muscle in the UT group of Experiment
325 2 only. Images were recorded at set percentages of thigh length above the superior
326 border of patella that approximated the maximal ACSA for each constituent muscle
327 (VM = 20%, VL and vastus intermedius = 50%, RF = 75%). Muscle thickness was
328 quantified as the mean of the distance between deep and superficial aponeurosis at
329 each end, and the middle of each image. Muscle thickness for each constituent muscle
330 was then summed to quantify quadriceps muscle thickness. This analysis resulted in

mean quadriceps muscle thickness of 92.7 ± 10.8 cm, and significant associations with QACSA (Pearson's $r = 0.519$, $p < 0.001$).

Muscle-electrode distance (MED) and MED corrected voluntary EMG amplitude

Using a B-mode ultrasound probe placed perpendicular to the surface of the muscle, images of the distance between the skin surface and peripheral surface of the muscle were obtained at each of the sites where EMG electrodes were placed over the elbow flexor and knee extensor muscles. MED was measured by one trained investigator (Tracker version 4.92). Using the quadratic relationship between EMG and M_{max} amplitude and MED at the specific measurement site, EMG and M_{max} amplitude was corrected for MED as described previously (42). Briefly, an individual's residual EMG and M_{max} amplitude (i.e., measured vs expected/predicted according to the cohort relationship of EMG and M_{max} amplitude with MED) was summated with the pooled group mean of absolute EMG and M_{max} amplitude. Whole corrected EMG and M_{max} amplitude for each muscle group was then calculated by averaging corrected EMG and M_{max} amplitudes across the recording sites.

Data analysis and statistics

The data from duplicate sessions were averaged prior to further statistical analyses. All analyses were performed in SPSS (version 24: IBM, Armonk, NY). All data are presented as mean ± SD (with individual participant data also plotted). Significance was set at an alpha level of 0.05. Normality of data was assessed with the Shapiro-Wilk test. Data were distributed normally; thus, independent samples t-tests were performed to assess the differences in evoked and voluntary force and EMG variables

between LTRT and UT individuals. Effect sizes (Cohen's *d*) were estimated for absolute difference and were classified as trivial, small, moderate and large when <0.20, 0.20-0.50, 0.50-0.80 and >0.80, respectively(4). To assess the possible relationship between muscle size and M_{max}, bivariate correlation and linear regression were performed between muscle thickness and M_{max}, and QACSA and M_{max} for elbow flexors and knee extensors, respectively.

Using values obtained during the two duplicate neuromuscular assessments, variability and reliability were assessed using within-participant coefficient variation (CV; SD/mean × 100) and intraclass correlation coefficient ($ICC_{3,1}$; Ref. 9), respectively. A paired-samples t-test was used to calculate bias. The ICC values were defined as poor, moderate, good and excellent when <0.50, 0.50-0.75, 0.75-0.90 and >0.90, respectively(38). The CV values were considered acceptable, intermediate and unacceptable when <12%, 12-20% and >20%, respectively (6).

RESULTS

Between-test session reliability and variability

Reliability data is presented in Supplemental Table S1 [DOI: https://doi.org/10.6084/m9.figshare.13797674]. Maximal voluntary torque demonstrated excellent reliability and acceptable variability. Whole muscle group (knee extensor and elbow flexor) EMG variables had higher reliability and lower variability than for individual constituent muscles. Specifically, M_{max} and absolute voluntary EMG activity exhibited good and moderate (elbow flexors), and excellent and good (knee extensors) reliability, respectively, and variability was intermediate to acceptable for both muscle groups. When M_{max} was corrected for MED, reliability was

379 good (elbow flexors) and excellent (knee extensors), and variability was acceptable.
380 Voluntary EMG activity normalised to M_{max} exhibited poor and good reliability, and
381 variability intermediate and acceptable for the elbow flexors and knee extensors,
382 respectively. Voluntary EMG corrected for MED displayed intermediate-to-acceptable
383 variability and good reliability.

384

385 *Experiment 1 – Elbow flexors*

386 Long-term resistance-trained individuals produced 71% greater elbow flexor MVT (t_{27}
387 = -9.045, $p < 0.001$; Figure 2A), and this was accompanied by 56% greater muscle
388 thickness (t_{27} = -7.588, $p < 0.001$; Table 1) compared to UT.

389 Elbow flexor M_{max} was 29% greater in LTRT compared to UT individuals (t_{27} = -2.412,
390 $p = 0.010$; Figure 3A). This reflected a greater M_{max} in LTRT compared to UT for the
391 short head of biceps brachii (35%; t_{27} = -2.477, $p = 0.020$), but not for the long head
392 (t_{27} = -1.789, $p = 0.085$). When corrected for MED, elbow flexor M_{max} was still greater
393 in LTRT compared to UT (22%; t_{27} = -2.10, $p = 0.045$; Figure 3C), and this was also
394 the case for the short (31%; t_{27} = -2.432, $p = 0.022$), but not the long head of the biceps
395 brachii (t_{27} = -1.092, $p = 0.285$). Elbow flexor M_{max} was associated with biceps brachii
396 thickness ($r = 0.466$, $p = 0.011$; Figure 4), and this was also the case for the short (r =
397 0.489, $p = 0.007$), but not the long head of biceps brachii ($r = 0.249$, $p = 0.193$).

398 No differences were demonstrated between groups for elbow flexor voluntary EMG
399 activity ($t_{18.0}$ = -1.346, $p = 0.195$), and this was also the case for the long head of the
400 biceps brachii ($t_{15.9}$ = -0.336, $p = 0.741$). However, voluntary EMG activity of the short
401 head of biceps brachii was 26% greater in LTRT compared to UT individuals (t_{27} = -
402 2.149, $p = 0.041$; Figure 5A). There were no differences between LTRT and UT when

403 elbow flexor EMG activity was normalised to M_{max} (whole elbow flexor: $t_{27} = 0.456$, p
404 = 0.652; BBL: $t_{27} = 0.507$, p = 0.616, BBS: $t_{27} = 0.333$, p = 0.742; Figure 5B). When
405 corrected for MED, EMG activity of the elbow flexors ($t_{19.5} = -0.997$, p = 0.331) and the
406 long head of biceps brachii ($t_{15.3} = 0.268$, p = 0.793) was similar between LTRT and
407 UT. However, the EMG activity of the short head of biceps brachii when corrected for
408 MED was still greater by 21% in LTRT compared to UT controls ($t_{27} = -2.252$, p =
409 0.033, Figure 5C).

410

411 *Experiment 2 – Knee extensors*

412 Compared to UT, LTRT individuals produced 66% greater knee extension MVT ($t_{17.0}$
413 = -9.007, p < 0.001; Figure 2B). Muscle size, specifically QACSA, was 54% greater
414 for LTRT than UT ($t_{61} = -12.953$, p < 0.001; Table 2).

415 Knee extensor M_{max}, averaged across six recording sites, was 60% greater in LTRT
416 compared to UT individuals ($t_{17.6} = -3.774$, p = 0.001), with similar differences noted in
417 VM (+67%; $t_{61} = -4.227$, p < 0.001), VL (+62%; $t_{61} = -3.527$, p = 0.001) and RF (+45%;
418 $t_{16.7} = -2.612$, p = 0.018; Figure 3B). Correction for MED maintained the difference
419 between LTRT and UT in the knee extensor M_{max} (45%; $t_{16.768} = -3.781$, p = 0.002;
420 Figure 3D), as well as for VM (69%; $t_{61} = -5.985$, p < 0.001), with a tendency for a
421 difference in RF ($t_{16.782} = -2.090$, p = 0.052), but not VL ($t_{61} = -1.293$, p = 0.201). Knee
422 extensor M_{max} was associated with QACSA (r = 0.501, p < 0.001; Figure 4), and a
423 significant relationship was also observed for each of the constituent muscles (VM: r
424 = 0.430, p < 0.001; VL: r = 0.369, p = 0.003; RF: r = 0.419, p = 0.001).

425 Voluntary EMG activity of the knee extensors during MVT production was 64% greater
426 in LTRT compared to UT ($t_{61} = -4.853$, p < 0.001) with differences observed across all

427 muscles; VM (+66%; t_{61} = -4.853, $p < 0.001$), VL (+67%; t_{61} = -4.140, $p < 0.001$) and
428 RF (+58%; t_{61} = -3.726, $p < 0.001$; Figure 5D). When normalised to M_{max}, no
429 differences were observed between LTRT and UT individuals in whole knee extensor
430 EMG activity (t_{61} = 0.444, $p = 0.659$; Figure 5E), or for the individual muscles
431 investigated (VM: t_{61} = -1.664, $p = 0.601$; VL: t_{61} = -1.049, $p = 0.298$; RF: t_{61} = -1.025,
432 $p = 0.310$).

433 Correction for MED resulted in 42% greater EMG activity of the knee extensors LTRT
434 compared to UT (t_{61} = -5.959, $p < 0.001$; Figure 5F). The corrected EMG activity was
435 63% greater in LTRT compared to untrained in VM ($t_{17.0}$ = -5.973, $p < 0.001$), but not
436 in VL ($t_{16.2}$ = -1.755, $p = 0.098$) and RF ($t_{14.9}$ = -2.035, $p = 0.060$).

437

438 **DISCUSSION**

439 The present study examined differences in M_{max} and surface EMG activity during
440 maximal isometric voluntary contractions between LTRT and UT individuals in upper-
441 and lower limb muscles. As expected, LTRT individuals were stronger and had a
442 greater muscle size compared to UT (6, 40, 44, 53). This superior muscle strength and
443 size were accompanied by greater M_{max} amplitude of both muscle groups in LTRT
444 individuals, even when corrected for the confounding influence of muscle-electrode
445 distance. Furthermore, M_{max} was found to be associated with muscle size of both
446 muscle groups, confirming findings of a previous investigation in clinical populations
447 (1), but presenting a novel finding in the context of LTRT and UT individuals. Absolute
448 voluntary EMG activity at MVT was greater only in the knee extensors of LTRT, but
449 not the elbow flexors, and these between group differences/similarities were
450 maintained for voluntary EMG corrected for muscle-electrode distance. However,

normalisation of voluntary EMG to M_{max} amplitude removed any differences between the groups for both muscles. The dependence of differences in EMG activity between LTRT and UT individuals according to the normalising procedure, the physiological inferences that stem from these observations, as well as differences in M_{max} amplitude are discussed below.

Long-term resistance-trained individuals exhibit greater maximal compound action potential amplitude

In agreement with our hypothesis, LTRT individuals exhibited greater M_{max} amplitudes compared to untrained individuals for both the elbow flexor and knee extensor muscle groups. Previous studies of the elbow flexors found either greater (22) or similar (40, 53) M_{max} amplitude in LTRT individuals compared to controls, and no studies had examined the knee extensors. Compared to previous studies reporting no difference in M_{max}, the present investigation tested responses on a significantly larger sample population, and measured surface EMG signals from multiple constituent muscles of each muscle group (and, in the case of knee extensors, from multiple sites per muscle), which could have contributed to the differences between the studies. Indeed, multi-site recordings and averaging of EMG amplitudes across multiple sites and, where possible, muscles have been shown to be more reliable both for M_{max} and voluntary EMG amplitudes (Ref. 5; see also Supplemental Table S1 [DOI: https://doi.org/10.6084/m9.figshare.13797674]), and likely provides a more comprehensive assessment of motor unit responsiveness to voluntary and evoked stimulation.

The observation that M_{max} was greater in LTRT individuals was consistent for both muscle groups investigated and across individual muscles, suggesting the findings are robust. There are many possible mechanisms underpinning the observed differences including differences in the major processes of muscle propagation, from the neuromuscular junction to the sarcolemma, and volume conduction from the sarcolemma through the intermediate tissues to the electrode on the skin surface (37). Since many factors within these processes change concurrently with long-term resistance training, the current experiment was not able to discern a specific mechanism. Differences in adipose tissue, which may impact volume conduction, were unlikely responsible for a large between-group difference in M_{max} amplitude as the differences were maintained when responses were corrected for muscle-electrode distance. As expected (44), LTRT individuals had greater muscle size (biceps brachii thickness and QACSA, respectively). For both elbow flexors and knee extensors, we showed the size of the muscle was positively associated with M_{max} amplitude, a novel finding in the context of resistance training. Therefore, it seems likely that differences in muscle size contribute to the greater M_{max} amplitude of LTRT individuals compared to UT. The positive relationship between muscle size and M_{max} amplitude is likely the result of greater single fibre action potentials of larger muscle fibres (32, 39), leading to greater M_{max} amplitude in LTRT compared to UT individuals.

Increased conduction velocity of motor units and/or muscle fibres would theoretically increase synchronisation of the individual motor unit action potentials that constitute M_{max}, thus increasing its amplitude (37, 57), and could potentially also contribute to the greater M_{max} of LTRT individuals we have found. Indeed, motor unit conduction velocity has been shown to be greater in LTRT individuals (18, 48). However, M_{max} has also been shown to remain unchanged following short-term resistance training (≤7

499 weeks; Refs. 3, 17, 21, 51), despite a study of similar duration showing increases in
500 conduction velocity (12), suggesting that increased conduction velocity of motor units
501 might not necessarily be related to increased M_{max} amplitude in the context of
502 resistance training.

503 M_{max} amplitude may also increase through Na^+/K^+ pump-induced hyperpolarisation of
504 the sarcolemmal membrane (35) leading to increased single fibre action potential
505 amplitude. Changes in Na^+/K^+ pump activity have been shown with resistance training
506 (20, 31), and thus the association between M_{max} and muscle size could merely be an
507 artefact of other peripheral changes (e.g. augmented transmembrane potentials)
508 following resistance training. However, it seems unlikely that the greater muscle size
509 of LTRT individuals is not the result of greater fibre size (43, 59), which leads to greater
510 single fibre action potentials (32, 39). Therefore, the greater M_{max} amplitudes of LTRT
511 individuals compared to UT are likely the result of greater single fibre action potential
512 amplitudes, which would be expected to also affect the voluntary EMG amplitude (37).

513

514 *Comparison of voluntary EMG amplitude between long-term resistance-trained and*
515 *untrained individuals and the effect of signal normalisation*

516 Absolute voluntary EMG activity was greater for all the knee extensor muscles in LTRT
517 individuals compared to UT. These findings are in agreement with a study that
518 recorded absolute voluntary EMG activity of the knee extensors muscles of LTRT
519 individuals and interpreted it as greater agonist activation compared to untrained (6).
520 In contrast to the knee extensors, absolute voluntary EMG of the whole elbow flexors
521 did not differ between LTRT and UT individuals, though differences between groups
522 were noted for the short head of the biceps brachii. The similarity of whole elbow flexor

amplitude in the current study was in contrast to a previous experiment (22), although that involved measurements from only one unspecified head of the biceps brachii and maximal voluntary contractions whilst restrained by a hand rather than by a dynamometer that precluded measurement of functional differences between their groups.

The whole muscle group findings were largely unaffected once voluntary EMG was corrected for MED (i.e., greater in LTRT for the knee extensors, but similar for the elbow flexors) although the magnitude of the knee extensor differences was somewhat moderated (+42% for MED corrected EMG vs +64% for absolute EMG, and one rather than three constituent muscles showing differences). Thus, the observed effects were not fundamentally influenced by any differences in adipose tissue between the groups. These contrasting findings for the two muscle groups could be due to the suggestion that neural adaptations following resistance training might be limited in the elbow flexors (10) due to a high baseline activation level (2) that may be higher than that of the knee extensors (7). This possibility is supported by the lack of changes in elbow flexor EMG activity following short-term resistance training (3 weeks; Refs. 10, 22).

Critically, however, when EMG activity was normalised to M_{max}, a recommended procedure to account for the peripheral electrophysiological properties of the signal (including muscle propagation and volume conduction) and attempt to isolate central neural activation (42), there were no differences between LTRT or UT groups for either the elbow flexors or knee extensors, or any of their constituent muscles. The marked differences in M_{max} between groups and the clear association of muscle size with M_{max} quantitatively demonstrates the confounding influence of peripheral electrophysiological properties on the EMG signal amplitude. Therefore, this study provides original evidence to reinforce the theoretical basis for M_{max} normalisation.

548 Based on these findings voluntary EMG normalised to M_{max}, as opposed to absolute
549 voluntary EMG or voluntary EMG corrected for MED, appears to provide the best index
550 of central neural activation. These findings also indicate that caution is warranted when
551 interpreting absolute EMG amplitude, particularly when comparing individuals and/or
552 groups displaying differences in muscle morphology (e.g., ageing, disuse, resistance
553 training and athletic performance), due to the confounding influence of muscle size.

554 Despite the chronic strength training exposure (≥3 years) and markedly greater
555 strength of our LTRT groups, we found no evidence for greater neural activation in two
556 separate experiments with different muscle groups. Whilst this finding conflicts with a
557 medium-term study (33), it agrees with another (49), and indirectly supports a previous
558 supposition that neural adaptations might be maximised in the early stages of
559 resistance training (6). Overall, the similar EMG activity normalised to M_{max} of LTRT
560 individuals for both muscle groups suggests that the contribution of agonist neural
561 activity to the substantially greater force production capacity of LTRT individuals (+66-
562 71%) is minor compared to muscle size (+54-56%).

563 Specific to the knee extensors, the similarity of voluntary EMG activity when
564 normalised to M_{max} suggests the difference in absolute EMG activity between groups
565 may have been the result of peripheral adaptation to long-term resistance training (e.g.
566 enhanced single fibre action potential amplitude due to hypertrophy; Ref. 30), rather
567 than changes in central neural properties. The knee extensor results of the present
568 study contrast with some (11, 61), but not all (54) short-term training studies that found
569 augmented EMG activity when normalised to maximal M-wave. This contrast may
570 reflect the greater sensitivity of repeated measures longitudinal studies to detect
571 relatively subtle differences compared to the current cross-sectional study.

572

Study limitations and future considerations

Whilst the present study provides novel insight into neuromuscular adaptations with long-term resistance training in both upper- and lower-limb muscle groups in a large cohort, it is important to acknowledge the study limitations. The cross-sectional study design precludes control of training variables in the long-term resistance-trained groups, and knowledge of their baseline neuromuscular function (i.e., prior to engaging in training), which might be innately high. However, in the absence of a longitudinal training intervention of several years, which is logistically very challenging, cross-sectional studies can highlight the unique characteristics of LTRT individuals and de-emphasise any similar characteristics that are unlikely to be responsive to adaptation.

The observation that EMG activity, when normalised to M_{max}, was not different between LTRT and UT individuals does not necessarily exclude the influence of neural adaptations on strength increases with long-term resistance training. Indeed, interference EMG is only a crude indicator of neural drive to the agonist muscle(s) (19, 25, 47), largely due to the influence of amplitude cancellation on the signal amplitude (36), which might have prevented detection of modifications in neural strategies of LTRT individuals in the present investigation. Future studies using emerging techniques such as advanced EMG decomposition (24, 26) are needed to discern potential changes in motor unit properties with long-term resistance training. The current study also only assessed agonist muscle EMG, whilst there is extensive evidence for decreased antagonist activity (6, 61) and tentative evidence for increased stabiliser activity (10) after resistance training, both of which may contribute to the greater strength of LTRT individuals. It should also be noted that the recordings of knee extensors involved muscles that exclusively extend the knee (except for RF, which is also a hip flexor, but given the hip position in this study likely acts as primarily

598 a knee extensor). Conversely, the elbow flexors recordings involved the two heads of
599 biceps brachii which both flex the elbow and supinate the forearm, which might have
600 contributed to differences (or lack of them) between LTRT and UT in elbow flexors
601 compared to knee extensors.

602 Whilst the use of multiple site recordings is beneficial in terms of minimising error when
603 estimating activity across the motor pool and improved reliability, it has the potential
604 to introduce crosstalk between sensors. To minimise the potential for crosstalk, we
605 used sensors with short inter-electrode distance (10 mm; Ref. 15), and spatially
606 separated them in proximo-distal and medio-lateral directions. As reported previously
607 (42), the distance between individual sensors was a minimum of 3.5 centimetres (and
608 typically >4 centimetres), which is consistent with estimations that crosstalk in such an
609 electrode setup would account for only ~4% of the signal (63). Therefore, some small,
610 limited crosstalk might still have been present between sensors, although there is
611 currently no accepted analytical approach to assess the extent of crosstalk within an
612 inferential EMG signal (25).

613 A bipolar (single differential) electrode configuration was used in the present study to
614 record EMG signals. This configuration type is most commonly used in exercise
615 science studies and clinical fields because of its ability to minimise noise and cross-
616 talk (16) and is thus recommended when quantifying voluntary interference EMG
617 amplitude (34). However, whilst quantifying the amplitude of a maximal M-wave is valid
618 with bipolar configuration, examining the shape of the signal is problematic due to
619 inherent loses in the signal as a result of amplitude cancellation (56). Analysing the
620 shape of the signal potentially allows greater mechanistic insight (37) as it may
621 distinguish between factors contributing to the propagating (e.g. sarcolemmal
622 excitability) and non-propagating phases of the potential (e.g. muscle architecture)

623 which do not necessarily change concurrently in response to interventions (58). Future
624 studies should consider the analytical approach of separating maximal M-wave
625 phases recorded with monopolar configuration, to potentially gain greater insight into
626 the mechanisms augmenting maximal M-wave amplitude with long-term resistance
627 training.

628 Lastly, the present experiments were conducted on a male only population, therefore,
629 these data may only be generalised to males. Whilst presumably the physiological
630 differences between LTRT and UT individuals are likely to be similar regardless of sex
631 (55), further investigation is required to confirm whether similar findings would be
632 obtained in a female population.

633

634 *Conclusions*

635 The present investigation showed that LTRT men exhibit greater maximal compound
636 action potential amplitude in the elbow flexors and knee extensors compared to UT
637 controls, which, based on the positive association between M_{max} and muscle size,
638 appears to be partially mediated by the differences in muscle morphology between
639 groups. This indicates that absolute voluntary EMG signal amplitudes may be
640 confounded by peripheral muscle morphology, rather than providing a discrete
641 measurement of central neural activity. Some differences were observed in absolute
642 voluntary EMG amplitude for the knee extensors, but not elbow flexors between LTRT
643 individuals and UT that were maintained even after correction for MED. Subsequently,
644 however, when voluntary EMG amplitude was normalised to M_{max}, to account for the
645 peripheral electrophysiological properties of the EMG signal (and potential
646 confounders such as muscle size) there were no differences between LTRT and UT

647 individuals for any muscle group or individual muscles. Therefore, this study provides
648 no evidence for a difference in central neural activity between the groups and thus
649 agonist neural adaptation during maximal isometric muscle contractions in LTRT men.

Grants

Part of this study was supported by a grant (reference 20194) awarded to Prof Jonathan Folland from the Versus Arthritis Centre for Sport, Exercise and Osteoarthritis. Dr Sumiaki Maeo was supported by a grant (reference 18K17837) from the Japan Society for the Promotion of Science.

Conflict of interest

None to declare.

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LEGENDS TO FIGURES

Figure 1. Typical evoked responses to percutaneous nerve stimulation in the knee extensor (A) and elbow flexor (B) muscles in a long-term resistance trained (LTRT; blue) and an untrained (UT; red) individual. In knee extensors, recordings were made from two sites per muscle. Traces show the three evoked maximal M-waves overlaid in black with the mean response displayed in colour. In some cases of elbow flexion measurements, negative and/or positive peak values of maximal M-wave exceeded the maximum input range of EMG sensors. In such cases, clipped parts of M-waves were interpolated with 6^{th} order polynomials curves. To test the validity of this approach, a random sample (n = 23) of unclipped trials were retrospectively clipped (i.e., a 10 ms epoch of data around the positive and negative peak was deleted) in order to compare the actual/original measured M_{max} amplitude (i.e., from the unclipped recording to M_{max} estimated from the clipped version with interpolation of the missing data. The comparison showed excellent agreement as displayed in the Bland-Altman plot (C).

Figure 2. Elbow flexor (A) and knee extensor (B) maximal voluntary torque of long-term resistance-trained (LTRT; elbow flexors; n = 15; knee extensors, n = 14) individuals compared to untrained controls (UT; elbow flexors, n = 14; knee extensors, n = 49). ***$p < 0.001$ between groups determined from independent samples t-tests.

Figure 3. Absolute (A, B) and muscle-electrode distance corrected (C, D) M_{max} peak-to-peak amplitude of elbow flexors (A, C) and knee extensors (B, D) of long-term resistance-trained individuals (LTRT; elbow flexors; n = 15; knee extensors, n = 14) compared to untrained controls (UT; elbow flexors, n = 14; knee extensors, n = 49). EF, whole elbow flexor measurement, mean of the individual elbow flexor muscles;

877 BBL, biceps brachii long head; BBS, biceps brachii short head; KE, whole knee
878 extensor measurement mean of individual knee extensor muscles; VM, vastus
879 medialis; VL, vastus lateralis; RF, rectus femoris. Symbols denote a significant
880 difference between groups determined from independent samples t-tests as follows:
881 ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.

882 **Figure 4**. Maximal M-wave plotted as a function of muscle size (muscle thickness for
883 elbow flexors and anatomical cross-sectional area for knee extensors) in long-term
884 resistance-trained (LTRT, blue circles; elbow flexors; n = 15; knee extensors, n = 14)
885 and untrained controls (UT, red diamonds; elbow flexors, n = 14; knee extensors, n =
886 49). The dashed trend line denotes a non-significant relationship ($p = 0.193$).

887 **Figure 5**. Voluntary absolute EMG during maximal voluntary torque (MVT) production
888 (A and D), voluntary EMG during MVT normalised to M_{max} (B and E), and voluntary
889 EMG during MVT corrected for the confounding influence of muscle-electrode distance
890 (C and F) of long-term resistance-trained individuals (LTRT; elbow flexors; n = 15;
891 knee extensors, n = 14) compared to untrained controls (UT; elbow flexors, n = 14;
892 knee extensors, n = 49). EF, whole elbow flexor measurement, mean of individual
893 elbow flexor muscles; BBL, biceps brachii long head; BBS, biceps brachii short head;
894 KE, whole knee extensor measurement, mean of individual knee extensor muscles;
895 VM, vastus medialis; VL, vastus lateralis; RF, rectus femoris. Symbols denote a
896 significant difference between groups determined from independent samples t-tests
897 as follows: ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.