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Motor unit discharge rate and the estimated synaptic input to the vasti muscles is higher in open compared to closed kinetic chain exercise

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ABSTRACT

Purpose. Conflicting results have been reported on whether closed kinetic chain exercises (such as a leg press) may induce more balanced activation of vastus medialis (VM) and lateralis (VL) muscles compared to open kinetic chain exercise (such as pure knee extension). This study aimed to 1) compare between-vasti motor unit activity and 2) analyze the combined motor unit behavior from both muscles between open and closed kinetic chain exercises.

Methods. Thirteen participants (four women, mean±SD age: 27±5 years) performed isometric knee extension and leg press at 10, 30, 50, 70% of the maximum voluntary torque. High density surface EMG signals were recorded from the VM and VL and motor unit firings were automatically identified by convolutive blind source separation. We estimated the total synaptic input received by the two muscles by analyzing the difference in discharge rate from recruitment to target torque for motor units matched by recruitment threshold.

Results. When controlling for recruitment threshold and discharge rate at recruitment, the motor unit discharge rates were higher for knee extension compared to the leg press exercise at 50% (estimate = 1.2 pps, standard error (SE) = 0.3 pps, $P = 0.0138$) and 70% (estimate = 2.0 pps, SE = 0.3 pps, $P = 0.0001$) of maximal torque. However, no difference between the vasti muscles were detected in both exercises. The estimates of synaptic input to the muscles confirmed these results.

Conclusion. The estimated synaptic input received by VM and VL was similar within and across exercises. However, both muscles had higher firing rates and estimated synaptic input at the highest torque levels during knee extension. Taken together, the results show that knee-extension is more suitable than leg-press exercise at increasing the concurrent activation of the vasti muscles.

Key Words

motor unit; discharge rate; single-joint; multi-joint; kinetic chain

New and noteworthy

There is a significant debate on whether open kinetic chain, single-joint knee extension exercise can influence the individual and combined activity of the vasti muscles compared to closed kinetic chain, multi-joint leg press exercise. Here we show that attempting to change the contribution of either the VM or VL via different forms of exercise, does not seem to be a viable

strategy. However, the adoption of open kinetic chain knee extension induces greater discharge rate and estimated synaptic input to both vasti muscles compared to the leg press.

INTRODUCTION

An imbalance in the activation of vastus medialis (VM) and vastus lateralis (VL) has been associated with the development of patellofemoral pain syndrome (15, 27); one cause of anterior knee pain (33). The possibility that an exercise could allow one synergistic muscle to be preferentially activated with respect to another, has therefore been of longstanding clinical interest.

In the selection of an exercise regime, a distinction between the so-called open kinetic chain and closed kinetic chain exercises has been made. Nevertheless, it is difficult to identify pure “open” or “closed” kinetic chain exercises. Open kinetic chain exercises, such as knee extension, are usually considered to be single-joint movements that are performed in non-weight bearing with a free distal extremity (21). In contrast, closed kinetic chain exercises, such as the leg press, are multi-joint movements performed in weight bearing or simulated weight bearing with a fixed distal extremity (21). Beyond the biomechanical differences between the two exercises, previous studies have reported that the muscles of the quadriceps femoris are not homogeneously activated during such exercises (4). To date, surface electromyography (EMG) has been used to evaluate differences in quadriceps femoris activation between these exercise tasks. Earlier studies suggested a more balanced activation (31), defined as a ratio between the EMG amplitude of VM and VL close to 1, in a leg press exercise compared to open kinetic chain knee extension. For instance, Irish and colleagues (11) showed that the ratio between the activation of VM with respect to VL was greater during closed kinetic chain (e.g. squat and lunge) than in open kinetic chain exercises (e.g. knee extension). Conversely, Spairani et al. (29) did not find any difference between knee extension and leg press in the relative activation of VM and VL.

Recent work has confirmed that high-density EMG (HDEMG) can be decomposed to identify and assess a large number of motor units over a wide range of torques (5, 18, 25), providing more direct evidence on the strategies used by the central nervous system to control muscle force/torque (13) and overcome the limitations of global surface EMG measurements (19). Indeed, when the firings of a large number of motor units are recorded, it is possible to extract reliable information about the synaptic organization of motor commands to the motoneurons (7). However, to date there have been no studies directly evaluating differences in the synaptic input received by the vasti muscles between open versus closed kinetic chain knee exercises.

In this study, we applied state-of-the-art direct measures of vasti motor unit behaviour during submaximal contractions over a wide range of torques (from 10 to 70% of the maximum voluntary torque, MVT) when performing isometric knee extension and leg press exercises. The first aim of this study was to identify possible differences in the contribution between VM and VL across the exercise tasks. Since recent work revealed that the vasti muscles receive a similar amount of synaptic input (19), we hypothesized that these muscles will show similar discharge rates between the exercises. The second aim of the study was to compare the vasti net activation (the combined motor unit activity of both VM and VL) between knee extension and leg press, since single joint exercise are anecdotally adopted to increase muscle activation.

METHODS

Participants

Thirteen healthy and physically active participants (four women) (mean±SD age: 27±5 years, height: 174±9 cm, body weight: 69±9 kg) took part in the study. All participants were right leg dominant (determined by asking which leg they would use to naturally kick a ball). Exclusion criteria included any neuromuscular disorders, current or previous history of knee pain which warranted treatment from a health care practitioner and age > 18 or < 35 years. Participants were asked to avoid any strenuous activity 24 h prior to the measurements. Data were collected between April and July 2017 and at a laboratory within the Centre of Precision Rehabilitation for Spinal Pain (CPR Spine). The study was conducted according to the Declaration of Helsinki (2004) and the ethics committee of the School of Sport, Exercise and Rehabilitation Sciences (University of Birmingham) approved the study (approval code CM09/03/17-1). All participants gave their written, informed consent. The study is reported according to the STROBE guidelines.

Experimental protocol

Participants attended the laboratory on two occasions, separated by 48 hours, at the same time of the day. Experimental procedures were the same on the two occasions, with the only difference being the exercise type performed (knee extension versus leg press) which were assigned in a randomised balanced order. All measurements were conducted on the right lower limb. In both sessions, the setup was arranged so that participants could see the feedback of the exerted torque on a monitor mounted 1.5 m in front of their eyes.

For the open kinetic chain knee extension exercise, participants were comfortably seated on an isokinetic dynamometer (Biodex System 3, Biodex Medical Systems Inc., Shirley, NY, USA) in an adjustable chair. The trunk was vertical and the hip, knee, and ankle joint angles were 90° in order to keep the thigh in a horizontal position. The rotational axis of the dynamometer was aligned with the right lateral femoral epicondyle while the lower leg was secured to the dynamometer lever arm above the lateral malleolus.

For the leg press exercise, participants were in supine with their hip, knee, and ankle joint angles in 90° in order to keep the tibia in a horizontal position. The rearfoot was fixed on the lever of the dynamometer through a custom-built board. They were requested to push in a horizontal direction against the board. At the beginning of each session, the subjects performed three maximum voluntary contractions each over a period of 5 s, with 2 min of rest between trials. The highest MVT was used as a reference for the definition of the submaximal torque levels. In each of the experimental sessions, the submaximal torques were expressed as a percent of the MVT measured during the same session. Five minutes of rest was provided after the MVT measurement. Then, following a few familiarization trials at low torque levels, the participants performed two sets of submaximal isometric knee extension contractions at 10, 30, 50 and 70% MVT in a randomized order. The randomization order of these contractions was kept constant for each subject in the two sessions to minimize the possible influence of cumulative fatigue on the results. The contractions at 10-30% were sustained for 30 s, while the contractions at 50 and 70% MVT were maintained for 15 s. In each trial, the subjects were instructed to keep the torque exertion as stable as possible during the hold-phase. To this aim, they received visual feedback of the torque exerted, which was displayed as a trapezoidal path, with hold-phase durations as specified above. The rate of change of torque in ramp phases was kept constant in all contractions (10% of the MVT per second), thus the ascending and descending ramps lasted 1 s for 10%, 3 s for 30%, 5 s for 50%, and 7 s for 70% of MVT.

Data acquisition

EMG signals were acquired from the VM and VL, biceps femoris (BF) and semitendinosus (ST) muscles during the maximal and submaximal isometric contractions. For VM and VL, surface EMG was recorded in a monopolar montage with two-dimensional adhesive grids (SPES Medica, Salerno, Italy) of 13 × 5 equally spaced electrodes (each of 1 mm diameter, with an inter-electrode distance of 8 mm), with one electrode absent from the upper right corner. The electrode grids were positioned as described previously (14, 18). The area of skin where the grids were to be located was firstly slightly abraded with abrasive paste and then cleaned with water. The electrode cavities were filled with conductive paste (SPES

Medica, Salerno, Italy) and the electrode grid was positioned over the distal region of the VM and VL muscles. The electrode columns (comprising 13 electrodes) were oriented along the muscle fibers. Signals from the BF and ST were recorded in bipolar mode with Ag–AgCl electrodes (Ambu Neuroline 720, Ballerup, Denmark; conductive area 28 mm^2, interelectrode distance 2 cm) and were positioned according to guidelines (1). Reference electrodes were positioned around the right wrist and ankle. The location of the EMG electrodes was marked on the participant's skin using a permanent ink marker, allowing similar electrode placement across the experimental sessions.

Torque and EMG signals were sampled at 2048 Hz and converted to digital data by a 16-bit analog-to-digital converter (Quattrocento, 400-channel EMG amplifier, OT Bioelettronica, Torino, Italy, 3dB, bandwidth 10-500 Hz). EMG signals were amplified by a factor of 150 and were bandpass-filtered (bidirectional, 4th order, zero lag Butterworth, bandwidth 10-500 Hz). All data were stored on a computer hard disk and analyzed with Matlab (v. 2018b, The Mathworks Inc., Natick, Massachusetts, USA). Finally, before decomposition, the 64-monopolar EMG channels were re-referenced offline to form 59 bipolar derivations, as the differences between adjacent electrodes in the column direction.

Signal processing

Torque. The torque signal was low-pass filtered offline with an averaging moving window of 0.5 s. During the submaximal contractions, the stable torque region was visually identified by an operator blinded to the condition. The standard deviation (SD) and coefficient of variation (CoV) of torque (SD torque/mean torque) were calculated from the stable torque region.

EMG amplitude. The average rectified value (ARV) was computed over epochs of 1 s and averaged over all HDEMG channels to increase the repeatability between sessions (9, 16). These values were extracted from the first 15 s of stable torque region of the contractions. ARV was normalized for the ARV recorded during the MVT, in order to compensate for peripheral differences between the two muscles (3). Indeed, a number of confounding factors affects the difference in EMG amplitude between the two muscles (6) and therefore normalizing the EMG amplitude relative to that recorded during the MVT may partially overcome this drawback (3). The level of antagonist activation was quantified as the mean ARV values of BF and ST.

Motor unit decomposition and analysis. The EMG signals recorded during the submaximal isometric contractions (from 10% to 70% MVT) were decomposed offline with a method that has been extensively validated (25). The signals were decomposed throughout the entire duration of the submaximal contractions, and the discharge times of the identified motor units were converted in binary spike trains (18). The accuracy of the decomposition was tested with the silhouette measure, which was set to 0.90. The mean discharge rate and the discharge rate variability (CoV of the interspike interval [CoV_{isi}] see below for details) were calculated during the stable plateau region of the torque signal. Recruitment thresholds for each motor unit were defined as the torque (%MVT) at the times when the motor unit began discharging action potentials. Discharge rate at recruitment was calculated from the first six motor unit discharges. Discharges that were separated from the next by <33.3 or >250 ms (30 and 4 pps, respectively) (18) were corrected and edited manually by an experienced operator using a custom algorithm.

Motor unit tracking.

A motor unit tracking procedure was adopted to increase the robustness of the comparison between the two exercise. Motor units were tracked across the two sessions (knee extension and leg press) with the approach described in Martinez-Valdes et al. (20). Briefly, after the full blind HDEMG decomposition was performed on the data from the first session, we applied a semi-blind procedure on the data from the second session, focusing on motor unit action potential profiles similar to the ones extracted from the first session. The cross-correlation threshold for the two-dimensional spatial representation of motor unit action potentials was set to 0.8. This procedure was successfully applied for the VM and VL for at least 8 out of 13 participants, depending on torque level.

Estimates of synaptic input. The amount of synaptic input received by the vasti muscles was investigated with a method previously suggested by Martinez-Valdes et al. (19). Here, the total synaptic input received by the vasti muscles (which is reflected by changes in motor unit firing properties) represents the sum of all sources of input to motor neurons, such an increase in descending drive from supra-spinal centers (26), as well as afferent Ia input (23), among others. A difference in synaptic input received by the motor neuron pools of the two muscles can be estimated by the difference in the relative rate of increase in discharge rate between motor units in the two muscles. Hence, the discharge rate of motor units with the same recruitment thresholds (i.e., with a difference in threshold 0.5% MVC) in the two muscles was used as a measure to compare the synaptic inputs received by the pools of motor neurons. This measure corresponds to the increase in discharge rate from recruitment to the target torque relative to the increase in torque from the recruitment threshold [target torque (10, 30, 50, and 70% MVC) minus recruitment threshold torque].

Statistical analysis

Statistical analysis was performed in R (ver 3.5.2, R Development Core Team, 2009). To analyse motor units behaviour, we performed a multilevel mixed linear regression analysis through the package *lme4* Version 1.1.19 (2). Linear mixed effects models are particularly suitable in this experimental design since: 1) they allow the whole sample of extracted motor units to be analyzed and not just the mean observations for each subject and condition. This allows a better evaluation of data variations than conventional ANOVA statistics; 2) they account for the non-independence of observations (e.g. observations from the same subjects) with correlated error. This is particularly useful in such a repeated-measure study because it has been demonstrated that motor unit discharge data is correlated within a subject even across testing days (32), 3) they separately treat the effects caused by the experimental manipulation (fixed effects) and those that were not (random effects).

Torque

MVT achieved in the two exercise tasks was compared using a paired student t-test. COV of torque was analyzed with a generalized linear mixed effects model, with the within-subject fixed effects exercise and torque, as test variables and the random slope of exercise and torque over participants as random factors.

EMG amplitude

ARV was analyzed with a linear mixed effects model, with the within-subject fixed effects muscle, exercise and torque, as test variables and the random slope of muscle and exercise over participants as random factors.

Motor unit rate coding

Mean discharge rate of motor units was analysed with a linear mixed effects model, using the within-subject fixed effects muscle, exercise, and mean torque, as test variables, and the discharge rate at recruitment and recruitment threshold, as control variables. In such a way it is possible to characterize the discharge rate during the stable part of the contraction (i.e. at ≈ 10, 30, 50, and 70% MVT) controlling for the discharge rate at recruitment and motor unit recruitment threshold. We considered the random intercept over participants and the random slope of exercise, muscle, and torque over participants as random factors. Each likelihood ratio tests showed that random slope models (subject-specific slopes for the fixed effects exercise, muscle, and torque) significantly improved the model, so we constructed random slope models. Statistical significance of fixed effects was determined using type III Wald *F* tests with

Kenward–Roger degrees of freedom and the ANOVA function from R's *car* package (ver. 3.0.3).

discharge rate ~ muscle × exercise × torque × (exercise + muscle + torque| subject) + discharge rate at recruitment + recruitment threshold

After running the model, the residuals were checked for normality using the Shapiro–Wilk test. When the assumption of normality was violated the residual outliers were removed with the Cook's distance method (using a distance of 4 times the standard deviations) as previously suggested (32). *Post hoc* pairwise comparisons (with Tukey correction) were performed using least squares contrasts, as employed in R's *lsmeans* package (ver. 2.30.0). The post hoc tests were evaluated at 10, 30, 50, and 70% of the continuous variable torque. The post hoc results were reported with mean estimate (M) and standard error (SE).

Motor unit recruitment threshold, discharge rate at recruitment, and CoV_{isi} were analyzed with a linear mixed effects model, with the within-subject fixed effects muscle, exercise and torque, as test variables and the random slope of muscle and exercise over participants as random factors. We could not include the random slope of torque in these cases because of singular fit violation (i.e. multiple collinearity).

Task-related differences in firing rate and estimated synaptic input

Linear regression was used to characterize the association for each motor unit between the differences in discharge rate at the target torque (mean discharge rate at ≈ 10, 30, 50, and 70% MVT) and at recruitment and between the torque achieved during the stable part of the contraction (i.e. ≈ 10, 30, 50, and 70% MVT) and motor unit recruitment threshold. The slopes of these linear regressions were compared between the two muscles by analysis of covariance as done previously (19).

RESULTS

Torque

The torque exerted during the MVT was lower in the knee extension exercise (188±35 Nm) compared to the leg press (263±88 Nm, P = 0.007). The amount of torque fluctuations was similar between the two tasks. Indeed, the coefficient of variation of torque was not different

(P = 0.259) between the knee extension exercise (M = 3.2%, SE = 0.2%) and leg press (M = 2.9%, SE = 0.2%) and across torque levels (P = 0.358).

Normalized EMG amplitude

A representative example of the EMG signals recorded from the VL is reported in Figure 1A. The estimates of normalized ARV for VM and VL are reported in Figure 2A and 2B, respectively. As expected, normalized ARV increased with increasing torque (F = 3817.3, P < 0.0001). In general, the knee extension exercise was associated with greater normalized ARV at high torque levels, without any difference between muscles. Indeed, there was an exercise × torque interaction (F = 82.1, P < 0.0001), indicating that the knee extension exercise induced greater overall vasti activation (i.e. combining VM and VL ARV) than the leg press exercise at 50 (M = 0.11, SE = 0.01, P = 0.0003) and 70% MVT (M = 0.17, SE = 0.20, P < 0.0001) but not at lower torque levels. However, no differences between muscles were found (F = 1.8, P = 0.179).

--- Figure 2 about here ---

The level of antagonist activation was not different between exercise tasks (F = 0.3, P = 0.573). However, the level of antagonist activation increased at increasing torque and on average was 3.8 µV (SE = 1.3 µV), 11.0 µV (SE = 1.1 µV), 18.2 µV (SE = 1.2 µV), 25.4 µV (SE = 1.4 µV), at 10, 30, 50, and 70% of MVT, respectively.

Motor unit population data

The total number of decomposed motor units across the different torque levels and sessions was between 1059 and 1172, for the VM and VL, respectively. Thus, for each subject and torque level, an average of 10±3 and 11±4 motor units were extracted for VM and VL, respectively. A representative example of the results of motor unit decomposition is reported in Fig. 1A and 1B.

Recruitment threshold. The recruitment threshold descriptive statistics are reported in Table 1. Recruitment threshold increased with increasing torque (F = 14046, P < 0.0001). At high torque levels the recruitment threshold was higher for knee extension compared to the leg press: this difference was more pronounced in VM than in VL. This was indicated by the muscle × exercise × torque interaction (F = 4.6, P < 0.031). Post hoc tests showed that for the VM, higher recruitment thresholds were recorded during knee extension compared to the leg press at 50% (knee extension – leg press: M = 4.6 %, SE = 0.7%, $P < 0.0001$) and 70% (knee extension – leg press: M = 7.5 %, SE = 0.7 %, $P < 0.0001$). Likewise, the knee extension exercise was associated with higher VL recruitment thresholds compared to the leg press, but the magnitude of difference was smaller both at 50% (knee extension – leg press: M = 3.3 %, SE = 0.5 %, $P < 0.0011$) and 70% (knee extension – leg press: M = 5.2 %, SE = 0.7 %, $P < 0.0001$).

--- Table 1 about here ---

Motor unit discharge rate. The estimates of the motor unit discharge rate described by the model are reported in Figure 3A and 3B for VM and VL, respectively. As expected, when controlling for discharge rate at recruitment and recruitment threshold, the mean motor unit discharge rate increased with increasing torque ($F = 567.5$, $P < 0.0001$). In general, motor unit discharge rates were influenced by the exercise type but were not different between muscles. The difference between the two exercises emerged only at high torque levels, as indicated by the exercise × torque interaction ($F = 272.9$, $P < 0.0001$). Since there was no difference between muscles ($F = 0.4$, $P = 0.50$), the post hoc tests are reported by merging the data from VM and VL. When controlling for recruitment threshold and discharge rate at recruitment, higher motor unit discharge rates were recorded during the knee extension exercise compared to the leg press at 50% (M = 1.2 pps, SE = 0.3 pps, $P = 0.0138$) and 70% (M = 2.0 pps, SE = 0.3 pps, $P = 0.0001$) of MVT. The control variables of recruitment threshold ($F = 2617.2$, $P < 0.0001$) and discharge rate at recruitment ($F = 871.0$, $P <0.0001$) significantly affected motor unit discharge rates.

--- Figure 3 about here ---

COV of interspike interval. The COV_{isi} increased with torque ($F = 221.1$, $P < 0.0001$): being 12.1%, SE = 0.5%; 13.4%, SE = 0.5%; 14.5%, SE = 0.5%; 15.7%, SE = 0.5%; for 10, 30, 50, and 70% of MVT, respectively. No other difference for muscle or exercise type emerged (all P values > 0.18).

Tracked motor unit data

The number of tracked motor units across testing sessions was between 165 and 101 for VM and VL, respectively. Thus, for each subject and condition an average of 3.1±1.0 and 1.9±0.7 motor units were tracked for VM and VL, respectively. The cross-correlation values from the projecting vectors of the tracked motor units was 0.84±0.04 and 0.80±0.04 for VM and VL respectively. The results of tracked motor units confirmed the results from the group level analysis. When controlling for discharge rate at recruitment and recruitment threshold, the mean motor unit discharge rate increased with increasing torque (F = 951.9, P < 0.0001). Similar to the group level findings, when controlling for recruitment threshold and discharge rate at recruitment, the motor unit discharge rates were higher during the knee extension exercise compared to the leg press at torque levels ≥ 50% of MVT as indicated by the exercise × torque interaction (F = 272.9, P < 0.0001). Since there was no difference between muscles (F = 0.4, P = 0.50), the post hoc tests are reported on the merged data from VM and VL. When controlling for recruitment threshold and discharge rate at recruitment, the knee extension exercise showed higher motor unit discharge rates compared to the leg press at 50% (M = 1.1 pps, SE = 0.3 pps, P = 0.0318) and 70% (M = 1.7 pps, SE = 0.3 pps, P = 0.0007) of MVT. The control variables recruitment threshold (F = 571.4, P < 0.0001) and discharge rate at recruitment (F = 204.9, P <0.0001) significantly affected the discharge rates of the tracked motor units.

COV of interspike interval. The COV_{isi} of the tracked motor units increased with torque (F = 30.7, P < 0.0001) and on average was 12.5%, SE = 0.7%; 13.6%, SE = 0.5%; 13.8%, SE = 0.5%; 14.8%, SE = 0.8%; for 10, 30, 50, and 70% of MVT, respectively. No other difference for muscle or exercise emerged (all P values > 0.11).

Estimate of synaptic input

Comparison between muscles. For each subject and exercise, an average of 5, 6, 6, and 3 motor units were matched (by recruitment threshold) between VM and VL at 10, 30, 50, and 70% of MVT, respectively. The linear regressions between the increase in discharge rate from recruitment to the target torque relative to the increase in torque from the recruitment threshold are reported in Figure 4. At 10% MVT (Figure 4A and 4E) both muscles showed a regression non-different from constant value (both muscles and exercises P > 0.123). For all other contraction levels (except for leg press at 70% MVT, VM: P = 0.834, VL: P = 0.481, see Figure 4H) both vasti muscles showed a regression line which was different from the constant value (all P values < 0.021, see Figure 4B, 4C, 4D, 4F and 4G). However, the intercept (all P values > 0.291) and slope (all P values > 0.302) were not different between muscles for either exercise at any of the contraction levels.

Comparison between exercises. At 10% MVT, both exercises showed a regression non-different from constant value (both muscles and exercises P > 0.329, see Figure 5A). For all other contraction levels (except for the leg press exercise at 70% MVT, P = 0.530, see Figure 5B, 5C and 5D), both exercises showed regression line different from constant value (all P values < 0.012). Nonetheless, the intercept was different only at 30% (P = 0.016, see Figure 5B); the slope was steeper in knee extension than leg press at 50% (P = 0.023, Figure 5C) and 70% (P = 0.038, Figure 5D) of MVT.

DISCUSSION

This study uniquely compared knee extensor motor unit rate coding between open kinetic chain knee extension and closed kinetic chain leg press exercise using HDEMG. When controlling for recruitment threshold and discharge rate at recruitment, mean motor unit firing rates at target torque were similar between VM and VL in both exercise types suggesting that the amount of synaptic input received by the two muscles was similar and their relative contribution did not differ with exercise type. These findings refute the value of using the leg press exercise over open kinetic chain knee extension exercises for the selective activation of the VM. When comparing the overall vasti activation, the motor unit discharge rates were higher during the knee extension exercise compared to the leg press exercise when performed at 50% and 70% of MVT. Collectively these findings indicate that the synaptic input to the vasti muscles was higher during the knee extension exercise compared to the leg press.

Differences between the vastus medialis and lateralis

Previously, the ratio between the activation (i.e. the EMG amplitude) of the VM and VL has been used to assess differences in the contribution of each muscle in different exercises (28). This approach has led to conflicting results (28), with some studies showing greater relative activation of VM compared to VL during closed kinetic chain exercises (e.g. squat and lunge) compared to open kinetic chain exercises (e.g. knee extension) (11, 31) but with others showing no difference (29, 30). While the protocols adopted in these studies may differ from each other for some aspects (namely, subject position, knee angle, etc.), we suggest that these conflicting results are mainly due to limitations of classic bipolar surface EMG methods. Indeed, bipolar surface EMG can be unreliable and influenced by many factors including electrode positioning, thereby reducing the accuracy of amplitude estimates to effectively infer changes in synaptic input (22). Bipolar recordings may under- or over-estimate EMG amplitude because of the uneven distribution of action potentials within the muscle volume (8). In contrast, the HDEMG used in this study provides a superior representation of muscle activation compared to bipolar EMG since the greater number of EMG channels (59 bipolar EMG channels) provides a more representative estimate of muscle activity, increasing the reliability and sensitivity of EMG amplitude parameters. Using this approach, we found very little difference in VM and VL behaviour between the two exercise types (Figure 2). These findings suggest that the activation of the VM and VL did not differ between the two exercises. Nevertheless, analysis of EMG amplitude between the VM and VL cannot be used to infer the synaptic input received by the two muscles (19). For these reasons, the analysis of motor unit firing properties is fundamental to investigate the synaptic input received by muscles.

The motor unit discharge rate at a given torque depends on discharge rate at recruitment and recruitment threshold (10). Hence, the mere analysis of motor unit firing rates, without taking into account these variables, does not provide a suitable estimate of the input received by the motoneurons. Conversely, controlling for the discharge rate at recruitment and recruitment threshold provides a robust estimate of the synaptic input received by the motor neuron pools since discharge rates indicate the nonlinear transformation of synaptic input into motor neuron outputs (13). When controlling for recruitment threshold and discharge rate at recruitment, the discharge rate of VM and VL motor units were similar for both exercise types, see Figure 3. This suggest that the net excitatory synaptic input to the pool of motor neurons of the vasti was similar. This was furthermore confirmed by the analysis of regression between delta discharge rate and delta torque which was previously adopted as a way to estimate synaptic input (19). In addition, this analysis, which is based on the same assumptions, clearly showed no difference between the synaptic input received by VM and VL at all torque levels in both exercises (Figure 4). These results are in line with the recent finding that the vasti muscles share most of their synaptic input (14, 19). Taken together, these findings strongly suggest that the vasti muscles were controlled in a similar way by the central nervous system in leg extension (open kinetic chain) and leg press (closed kinetic chain) tasks. Thus, attempting to selectively activate either the VM or VL via different knee extension exercises does not seem to be a viable strategy in rehabilitation settings.

Knee extension vs. leg press

The two tasks investigated in this study constitute the isometric version of two popular exercises in clinical and sport settings. They are intrinsically different from many points of view. The knee extension task is a single-joint exercise involving a relatively small amount of muscle mass (mainly the knee extensors) while the leg press is a multi-joint exercise involving more muscles, such as the hip extensors. From the standpoint of torque-vector direction, in the knee extension exericse the torque is directed perpendicularly to the tibia, while in leg press the torque is directed parallel to the tibia. For this reason, the leg press tends to produce lower shear forces and higher compression forces at the knee. Finally, the knee extension is considered an open kinetic chain exercise, while the leg press is a closed kinetic chain exercise. Anecdotally, single-joint/open kinetic chain exercises are thought to induce higher muscle activation compared to multi-joint/closed kinetic chain exercises (21). While it seems reasonable that targeting a specific muscle with a single-joint exercise may result in higher activation, the available literature on this topic is conflicting. While some studies have reported higher vasti EMG amplitude during single-joint compared to multi-joint tasks (11, 29) others studies reported no difference (30, 31). As mentioned above, the most likely cause of such conflicting results are the methodological drawbacks of interference EMG analysis.

Since the level of hamstring muscle activity was not different between the two exercises, the greater vasti activation in the pure knee extension task cannot be explained by higher coactivation of antagonist muscles. However, in the leg press the load is shared between knee extensors and hip extensors muscles, hence the greater involvement of hip extensors at the expense of knee extensors cannot be excluded. In any case, the addition of motor unit decomposition in this study allowed us to directly clarify the amount of synaptic input delivered to the vasti muscles.

When controlling for discharge rate at recruitment and recruitment threshold, the average motor unit discharge rate was greater in knee extension exercise than the leg press at 50 and 70% of MVT (Figure 3). The possibility to track the motor units between the two sessions allowed us to monitor the behaviour of individual motor units across the two exercises. This analysis confirmed that motor unit discharge rate was higher in knee extension than the leg press at 50 and 70% of MVT. The same finding come from the analysis of the synaptic input (Figure 5): the regression lines between delta discharge rate and delta torque showed significantly steeper slope in the knee extension exercise compared to the leg press at 50 and 70% MVT. Together, these findings suggested that the synaptic input received by the motor unit pool was greater in the knee extension exercise. A reduction in net synaptic input in the leg press exercise could be attributed to a decrease in excitatory input and/or an increase in inhibitory input to motoneurons (13). On the one hand, a greater antagonist activation may induce an inhibition of agonist muscles, but this seems not to be the case since the activity of the hamstrings did not differ between tasks. However, it is difficult to exclude potential inhibition on the sole basis of the EMG amplitude of the antagonist muscles. In any case, multi-joint exercise implies a larger muscle mass acting to accomplish the task and therefore the load is shared between knee extensors and hip extensors which may reduce the demand on the knee extensors. On the other hand, the higher synaptic input to vasti muscles may be explained by the fact that the torque-vector for knee extension may be more favourable to the activation of the vasti muscles compared to that of the leg press (4). Indeed, the muscle contributions in multi-joint tasks are directionally tuned and combined to produce the movement in the desired direction (24). Thus, in a leg press the activation of the vasti may be modulated in favour of the hip extensors. The observed difference between the exercises emerged at the higher torque levels only which suggests that an increased synaptic input mostly affected high threshold motor units. This confirms the necessity to investigate the motor unit rate coding across the whole range of submaximal contractions since some changes may not be observed for the lower threshold motor units (Martinez-Valdes 2017).

Limitations

The current findings should be considered in light of some limitations. First, the relative intensity between the two exercises was controlled by normalizing the requested torque by MVT. However, there remains a possible inter-exercise difference in the torque produced by the vasti due to different torque-vector directions. Second, due to small shifts in skin displacement between the two sessions, the tracking of motor units across sessions was not possible in some subjects at high torque levels (50 and 70% of MVT). However, in the subset of conditions where the tracking was possible, the tracking confirmed the observed results from the full motor unit pool. Because of the limitations of surface EMG, the present results could be influenced by the more superficial motor units which seem to be associated with fast-twitch type II fibers (12). These units tend to have larger action potentials (17, 19) and are therefore easier to identify by the decomposition algorithm in comparison to deeper motor units (25). Furthermore, while all participants were physically active and they were familiar with exercises typically adopted in the gym, they may not be accustomed with both exercises at the same extent. This may potentially lead to MVT underestimation with less practiced exercise or with the more complex exercise, in this case the leg press. Finally, in this study we adopted isometric contractions because currently the motor unit decomposition algorithms are best suited for this specific condition. For this reason, the applicability of the present findings to dynamic conditions should be considered with caution.

Conclusions

The synaptic input received by VM and VL was similar and their relative contribution was not affected by exercise type. Hence, attempting to change the contribution of either the VM or VL via exercise selection does not seem to be a viable strategy. However, open kinetic chain knee extension was associated with overall greater synaptic input to vasti muscles. This finding suggests a single-joint knee extension is more suitable than a multi-joint leg press exercise to increase the activation of the vasti muscles.

REFERENCES

1. **Barbero M, Merletti R, and Rainoldi A**. *Atlas of muscle innervation zones*. Springer, 2012.
2. **Bates D, Mächler M, Bolker B, and Walker S**. Fitting Linear Mixed-Effects Models Using lme4. *2015* 67: 48, 2015.
3. **Burden A**. How should we normalize electromyograms obtained from healthy participants? What we have learned from over 25 years of research. *J Electromyogr Kinesiol* 20: 1023-1035, 2010.
4. **Ema R, Sakaguchi M, Akagi R, and Kawakami Y**. Unique activation of the quadriceps femoris during single- and multi-joint exercises. *Eur J Appl Physiol* 116: 1031-1041, 2016.
5. **Farina D, and Holobar A**. Characterization of Human Motor Units From Surface EMG Decomposition. *P Ieee* 104: 353-373, 2016.
6. **Farina D, Merletti R, and Enoka RM**. The extraction of neural strategies from the surface EMG: an update. *J Appl Physiol (1985)* 117: 1215-1230, 2014.
7. **Farina D, Negro F, Muceli S, and Enoka RM**. Principles of Motor Unit Physiology Evolve With Advances in Technology. *Physiology (Bethesda)* 31: 83-94, 2016.
8. **Gallina A, Merletti R, and Gazzoni M**. Uneven spatial distribution of surface EMG: what does it mean? *Eur J Appl Physiol* 113: 887-894, 2013.
9. **Gallina A, Pollock CL, Vieira TM, Ivanova TD, and Garland SJ**. Between-day reliability of triceps surae responses to standing perturbations in people post-stroke and healthy controls: A high-density surface EMG investigation. *Gait Posture* 44: 103-109, 2016.
10. **Heckman CJ, and Enoka RM**. Motor unit. *Compr Physiol* 2: 2629-2682, 2012.
11. **Irish SE, Millward AJ, Wride J, Haas BM, and Shum GL**. The effect of closed-kinetic chain exercises and open-kinetic chain exercise on the muscle activity of vastus medialis oblique and vastus lateralis. *J Strength Cond Res* 24: 1256-1262, 2010.
12. **Johnson MA, Polgar J, Weightman D, and Appleton D**. Data on the distribution of fibre types in thirty-six human muscles. An autopsy study. *J Neurol Sci* 18: 111-129, 1973.
13. **Johnson MD, Thompson CK, Tysseling VM, Powers RK, and Heckman CJ**. The potential for understanding the synaptic organization of human motor commands via the firing patterns of motoneurons. *J Neurophysiol* 118: 520-531, 2017.

14. **Laine CM, Martinez-Valdes E, Falla D, Mayer F, and Farina D**. Motor Neuron Pools of Synergistic Thigh Muscles Share Most of Their Synaptic Input. *J Neurosci* 35: 12207-12216, 2015.

15. **Makhsous M, Lin F, Koh JL, Nuber GW, and Zhang LQ**. In vivo and noninvasive load sharing among the vasti in patellar malalignment. *Med Sci Sports Exerc* 36: 1768-1775, 2004.

16. **Martinez-Valdes E, Falla D, Negro F, Mayer F, and Farina D**. Differential Motor Unit Changes after Endurance or High-Intensity Interval Training. *Med Sci Sports Exerc* 49: 1126-1136, 2017.

17. **Martinez-Valdes E, Farina D, Negro F, Del Vecchio A, and Falla D**. Early Motor Unit Conduction Velocity Changes to High-Intensity Interval Training versus Continuous Training. *Med Sci Sports Exerc* 50: 2339-2350, 2018.

18. **Martinez-Valdes E, Laine CM, Falla D, Mayer F, and Farina D**. High-density surface electromyography provides reliable estimates of motor unit behavior. *Clin Neurophysiol* 127: 2534-2541, 2016.

19. **Martinez-Valdes E, Negro F, Falla D, De Nunzio AM, and Farina D**. Surface electromyographic amplitude does not identify differences in neural drive to synergistic muscles. *J Appl Physiol (1985)* 124: 1071-1079, 2018.

20. **Martinez-Valdes E, Negro F, Laine CM, Falla D, Mayer F, and Farina D**. Tracking motor units longitudinally across experimental sessions with high-density surface electromyography. *J Physiol* 595: 1479-1496, 2017.

21. **Mayer F, Schlumberger A, van Cingel R, Henrotin Y, Laube W, and Schmidtbleicher D**. Training and testing in open versus closed kinetic chain. *Isokinet Exerc Sci* 11: 181-187, 2003.

22. **Mesin L, Merletti R, and Rainoldi A**. Surface EMG: the issue of electrode location. *J Electromyogr Kinesiol* 19: 719-726, 2009.

23. **Miles TS, Turker KS, and Le TH**. Ia reflexes and EPSPs in human soleus motor neurones. *Exp Brain Res* 77: 628-636, 1989.

24. **Muceli S, Boye AT, d'Avella A, and Farina D**. Identifying representative synergy matrices for describing muscular activation patterns during multidirectional reaching in the horizontal plane. *J Neurophysiol* 103: 1532-1542, 2010.

25. **Negro F, Muceli S, Castronovo AM, Holobar A, and Farina D**. Multi-channel intramuscular and surface EMG decomposition by convolutive blind source separation. *Journal of neural engineering* 13: 026027, 2016.

26. **Olivier E, Bawa P, and Lemon RN**. Excitability of human upper limb motoneurones during rhythmic discharge tested with transcranial magnetic stimulation. *J Physiol* 485 (Pt 1): 257-269, 1995.

27. **Petersen W, Ellermann A, Gosele-Koppenburg A, Best R, Rembitzki IV, Bruggemann GP, and Liebau C**. Patellofemoral pain syndrome. *Knee Surg Sports Traumatol Arthrosc* 22: 2264-2274, 2014.

28. **Smith TO, Bowyer D, Dixon J, Stephenson R, Chester R, and Donell ST**. Can vastus medialis oblique be preferentially activated? A systematic review of electromyographic studies. *Physiother Theory Pract* 25: 69-98, 2009.

29. **Spairani L, Barbero M, Cescon C, Combi F, Gemelli T, Giovanetti G, Magnani B, and D'Antona G**. An electromyographic study of the vastii muscles during open and closed kinetic chain submaximal isometric exercises. *International journal of sports physical therapy* 7: 617-626, 2012.

30. **Stensdotter AK, Hodges P, Ohberg F, and Hager-Ross C**. Quadriceps EMG in open and closed kinetic chain tasks in women with patellofemoral pain. *J Mot Behav* 39: 194-202, 2007.

31. **Stensdotter AK, Hodges PW, Mellor R, Sundelin G, and Hager-Ross C**. Quadriceps activation in closed and in open kinetic chain exercise. *Med Sci Sports Exerc* 35: 2043-2047, 2003.

32. **Tenan MS, Marti CN, and Griffin L**. Motor unit discharge rate is correlated within individuals: a case for multilevel model statistical analysis. *J Electromyogr Kinesiol* 24: 917-922, 2014.

33. **Witvrouw E, Lysens R, Bellemans J, Cambier D, and Vanderstraeten G**. Intrinsic risk factors for the development of anterior knee pain in an athletic population. A two-year prospective study. *Am J Sports Med* 28: 480-489, 2000.

Table 1 – Descriptive statistics of motor units recruitment threshold, expressed as % of MVT, for each muscle and exercise. Data are reported as mean±SD (range)

	Knee extension		Leg press	
Contraction level (% MVT)	Vastus Medialis	Vastus Lateralis	Vastus Medialis	Vastus Lateralis
10%	8.3±2.7 (1.42 – 13.6)	8.57±3.10 (0.2 – 15.4)	8.4±2.5 (2.4 –13.9)	8.3±3.1 (1.5 – 14.0)
30%	23.6±6.3 (6.3 – 34.8)	22.9±6.7 (4.4 – 37.4)	23.1±5.8 (6.3 – 35.0)	22.4±5.7 (4.11 – 35.5)
50%	34.4±7.6 (20.3 – 53.2)	36.2±8.9 (14.0 – 52.5)	34.6±7.9 (11.6 – 50.0)	34.9±7.8 (8.7 – 49.2)
70%	53.8±10.2 (27.1 – 72.2)	52.2±10.3 (21.8 – 71.4)	45.0±9.2 (16.9 – 70.4)	44.3±9.5 (18.2 – 75.6)

Legend
MVT, Maximal Voluntary Torque

Captions

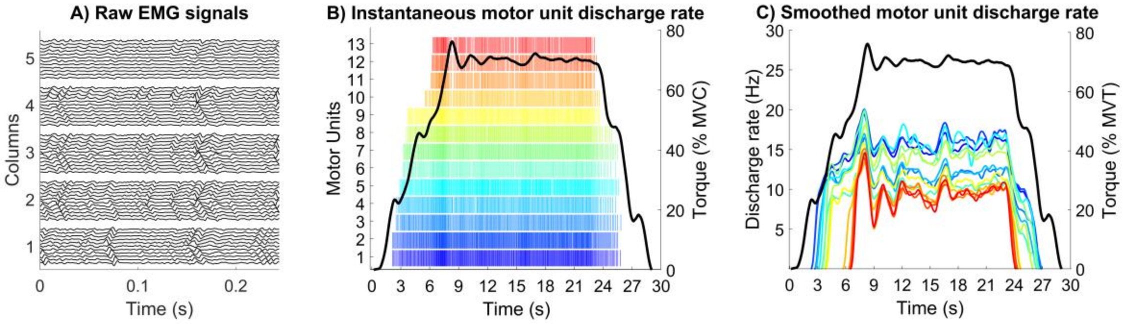

Figure 1 – A) Representative examples of raw electromyographic (EMG) signals (5 columns and 12 lines) recorded from the vastus lateralis at 70% of maximal voluntary contraction (MVT). B) Instantaneous discharges of 13 motor units are reported as vertical lines. The torque signal is reported as the black line. C) Smoothed discharge rates (smoothed with a Hanning window of 1 s) are reported for the same 13 motor units. Note that the late recruited motor units (represented in orange and red) are those with the lower discharge rate in the plateau phase of the contraction. Note also that the shape of the discharge rate profiles of motor units are similar to the shape of torque signal.

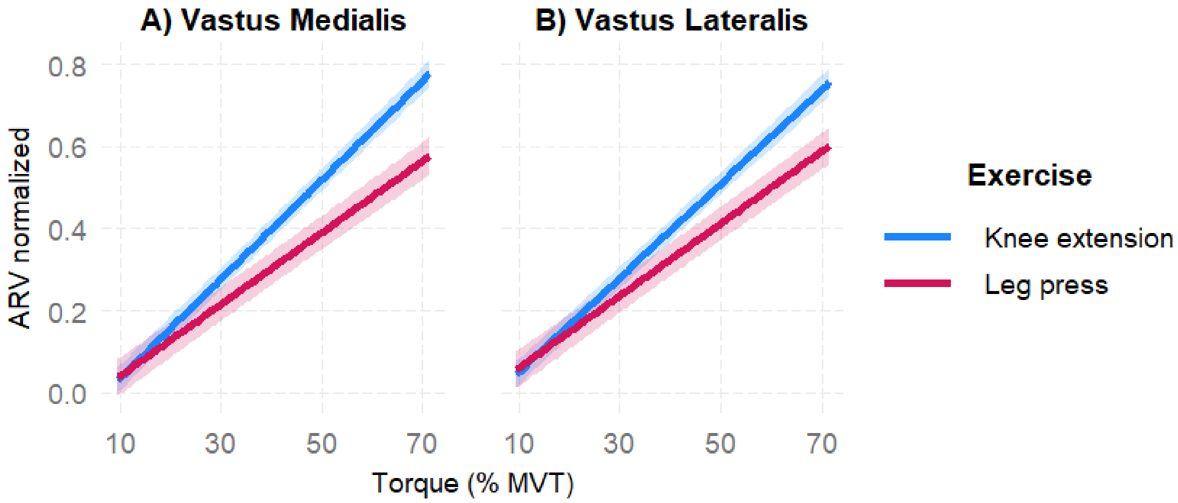

Figure 2. Estimates (with 95% confidence intervals) of EMG amplitude (average rectified value, ARV) normalized for ARV in maximal voluntary contraction across torque levels are reported for A) vastus medialis (VM) and B) vastus lateralis (VL).

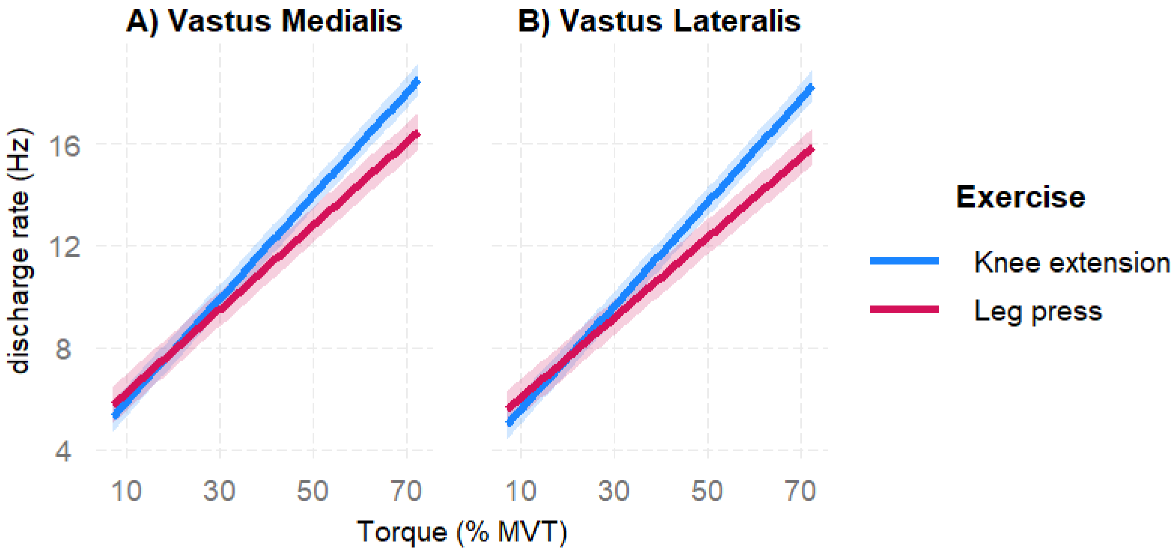

Figure 3. Estimates (with 95% confidence intervals) of motor unit discharge rates are reported for A) vastus medialis (VM) and B) vastus lateralis (VL) muscles. The estimates are calculated from the motor units population (a total of 1059 and 1172 motor units for VM and VL respectively), adjusted for motor unit recruitment threshold and discharge rate at recruitment. The linear mixed model adopted to obtain these estimates included random slope (i.e. subject specific variation) of the factor muscle, torque level and exercise.

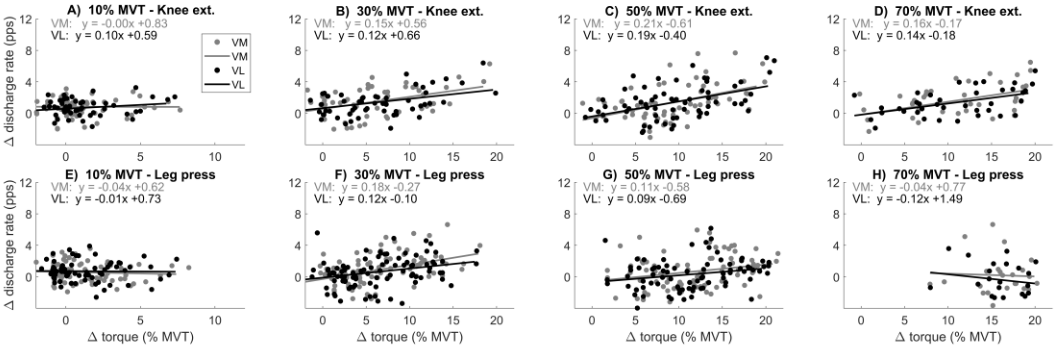

Figure 4. Linear regression analysis of the difference between vastus medialis (VM, in grey) and vastus lateralis (VL, in black) mean discharge rate at target torque and discharge rate at recruitment (y-axis) and the difference between target torque [10, 30, 50, and 70% maximal voluntary torque (MVT)] and motor unit recruitment threshold (x-axis) at 10%, 30%, 50%, and 70% of MVT. The motor units were matched between VM and VL for recruitment threshold. Linear regression equations are shown in the figure. None of the regression lines (slopes and intercepts) differed significantly between muscles.

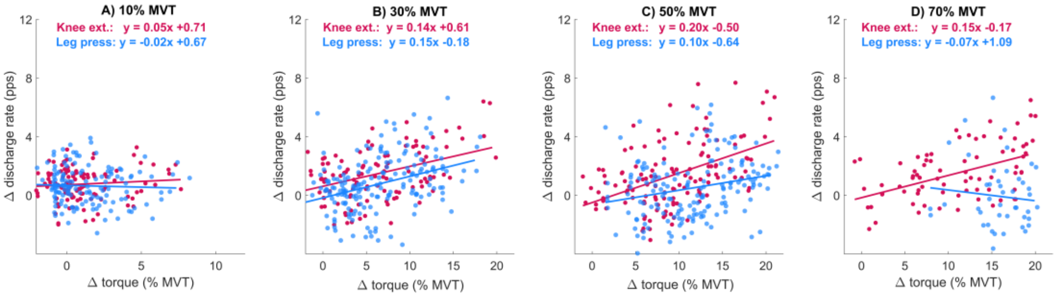

Figure 5. Linear regression analysis of the difference between knee extension (red) and leg press (blue) mean discharge rate at target torque and discharge rate at recruitment (y-axis) and the difference between target torque [10, 30, 50, and 70% maximal voluntary torque (MVT)] and motor unit recruitment threshold (x-axis) at 10%, 30%, 50%, and 70% of MVT. Since there was no difference between muscles, the vastus medialis and lateralis data are merged. Linear regression equations are shown in the figure. The slope of the regression lines was significantly steeper in knee extension than leg press at 50% and 70% of MVT, see results section.