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**Sex Differences in Quadriceps Alternating Muscle Activation Patterns
During Fatigue**

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Sex Differences in Quadriceps Alternating Muscle Activation Patterns During Fatigue

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Synergistic alternating muscle activation (AMA) consists of a period of co-activation (Co-A) and a period of trade-off (TO). Together they form a load-sharing cycle which is a neuromuscular control strategy that attenuates fatigue. However, the structure of AMA interactions of synergistic muscles has only been investigated during low-level contractions of 2.5-10% maximal voluntary contraction (MVC) and has yet to be investigated at moderate force levels ($\geq 20\%$ of MVC) and differentiated between the sexes. The purpose of this study was to quantify the activation relationship between pairs of synergistic quadriceps muscles to further understand the patterns (durations of Co-A and TO and frequency of AMA cycles). Surface electromyographic (EMG) data was collected from 16 individuals (8 male, 8 female) from the rectus femoris (RF), vastus lateralis (VL), vastus medialis (VM), and vastus medialis oblique (VMO) during a fatiguing contraction at 20% MVC. Synergistic muscle pairs (VL-RF, VL-VM, VL-VMO, RF-VM, RF-VMO, VM-VMO) were analyzed for Co-A, TO, and AMA frequency during 3 phases of the fatiguing contraction. The synergistic pairs were in Co-A significantly longer than TO during all fatigue phases. Some muscle pairs differed significantly from each other in time spent in each state (Co-A or TO) during the final contraction phase. There was no significant difference in AMA patterns within individual muscle pairs between fatigue phases. There were strong positive and negative correlations between endurance time and Co-A and TO durations respectively for every muscle pair in males during the final two fatigue phases. For the same measures in females, only the RF-VL, RF-VMO, and VL-VM muscle pairs demonstrated a significant negative and positive correlation in the middle fatigue phase for Co-A and TO respectively. AMA was present in both male and female EMG data, but contrary to expectations that AMA cycle frequency would produce significant differences throughout the contraction, the endurance time correlations were where significant differences were present.

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Introduction

Alternating muscle activation (AMA) is a strategy of the motor control system to improve task performance. An AMA involves a synergistic pair of muscles that co-activate (Co-A), where two or more muscles are increasing or decreasing in EMG activity in-phase, or 'trade off' (TO) in which muscle activation levels in the muscle pairs or groups are increasing or decreasing in EMG activity out-of-phase during a contraction (39,50). AMA helps to attenuate fatigue to maximize endurance time (19, 33, 55).

Synergistic muscles that serve the same joint typically have several different mechanical features, including cross sectional area, muscle fiber type composition, muscle fiber architecture and some span more than one joint (8, 11, 37). This results in individual muscles having different fatigue rates, rates of force development, and maximum force capacity, which result in individual muscles in a synergistic group or pair having different contributions to task performance. AMA has been found primarily in synergistic muscle groups that are used for postural control such as the triceps surae (38, 49), back extensors (35), and trapezius (56) at low-level contractions between 2.5% and 10% MVC to fatigue. AMA has also been found in the quadriceps femoris during intensities ranging from 2.5% to 10% of MVC across varying contraction types. However, AMA has only been found to be present at intensities between 2.5% and 5% of MVC during sustained isometric contractions (4, 31, 32).

TO is not the complete inactivity of a muscle in a synergistic pair, it is the negative correlation of EMG amplitudes between two synergistic muscles, i.e., EMG activity in one muscle decreases while the synergist EMG activity increases (50). Few studies have investigated TO; it occurs predominantly when muscles are at their optimum length and the load on the muscle is sub-maximal (50). TO allows a muscle in a synergistic pair a brief recovery period when one of the synergists in the pair is active the other is minimally active (39).

During knee extension of 2.5% MVC, there is a strong negative correlation between the frequency of AMA of the rectus femoris (RF) -vastus lateralis (VL) and RF-vastus medialis (VM) pairs and the reduction in force production in men (32). This indicates that increasing AMA frequency is likely a strategy used by the central nervous system to combat fatigue.

Males and females fatigue at different rates depending on the type of contraction, intensity of contraction, and muscles involved in the contraction (1, 11, 27). At 20% isometric MVC of the quadriceps femoris to failure men fatigued faster than women and that fatigue was attributed to peripheral factors via shear modulus increase in the VL and changes in evoked torque across quadriceps muscles(1). This is supported in the literature in that women have been found to have longer endurance times when performing low intensity sustained isometric contraction tasks when the intensity is equal across sexes (27). Examining potential sex differences in AMA measures could reveal differences in neuromuscular control strategies used to combat fatigue.

The purpose of this study was to quantify the behavior of pairs of quadriceps muscles, [VL-RF, VL-VM, VL-VMO, RF-VM, RF-VMO, VM-vastus medialis oblique (VMO)] in the form of load-sharing frequency (number AMA cycles) and Co-A and TO durations throughout a sustained moderate-intensity (20% MVC) isometric contraction. We hypothesized that AMA frequency would increase with fatigue and that co-activation duration would increase while trade-off duration would decrease. In order to investigate this, we used a novel data analysis metric in the form of sinusoidal shaped correlations extracted from the EMG recordings.

Methodology

All data collection procedures were approved by the Institutional Review Board of the University of Texas at Austin. All participants signed a written consent form prior to participation in the study. Eight females and eight males participated in the study (age of 23.1 ± 2.4 years). All participants were free of lower limb disorders and prior surgeries.

Experimental procedures

Participants were seated on a customized chair. Their hips were secured to the seat with a strap. The legs were positioned at 90° and the ankle of the dominant leg (leg preferred for kicking a ball) was fixed to a load cell (High-Accuracy Miniature Universal Load Cell, LC203-100, OMEGA) with a cuff placed around the ankle that was connected to a bridge amplifier signal conditioner (DMD4059, OMEGA). The conditioner was adjusted via shunt calibration for accuracy and precision.

The sites for electrode placement were cleaned with 70% isopropyl alcohol and then shaved with a disposable shaver. Ag/AgCl electrodes (Dimension: $3/4'' \times 1\ 1/8''$, Danlee Medical Products, Inc., NY, US) were used for EMG recording. The electrodes for the VMO were placed superomedial and proximal to the patella with an orientation angle of 55° relative to the long axis of the femur. Electrodes for the VL were placed superlaterally relative to the patella with an orientation angle of 15° . Electrodes for the VM were placed above the VMO electrodes over the VM muscle belly at an angle of 15° relative to the line between the anterior spina iliaca superior and the joint space in front of the anterior border of the medial ligament. Electrodes for the RF were placed at 50% on the line from the anterior spina iliaca superior to the superior part of the patella. Ground electrodes were placed over the ipsilateral patella (26).

The participant's first task was to perform three 3s MVCs of knee extension. The highest plateau in the force data was selected as the pre-fatigue MVC. After a 5-min

recovery period, the participant performed a sustained isometric contraction of 20% MVC until endurance limit. Endurance time was determined to be the time at which the tremor exceeded $\pm 5\%$ MVC or fell below 15% MVC. The participant was provided with visual feedback of the force on a computer monitor in front of them.

Data and Analysis

EMG analog signals of the VMO, VL, VM, and RF were amplified ($\times 1,000$) (Coulbourn Instruments, LLC., PA, U.S.), band-pass analog filtered (8-1 kHz), and then digitalized at a sampling rate of 1 kHz. The digital signal was further band-pass (20-450 Hz, 4th order Butterworth) and notch (60 Hz) filtered.

The EMG signals were A/D converted by a data acquisition unit (Micro 1401, Cambridge Electronic Design, Cambridge, England), and incorporated within the data analyses software Spike 2 (version 7.20, Cambridge Electronic Design, England). The built-in algorithms of Spike 2 were used for mathematical processing of the EMG signals. The root mean square (RMS) of the EMG of the VMO, VL, VM and RF were calculated with a moving average window of 10 ms.

Once the RMS was calculated the data was transferred to MATLAB (R2020b). In MATLAB, each individual signal was then linearly de-trended as a whole timeseries without a moving window. After that the muscles were put into pairs (RF-VM, RF-VMO, RF-VL, VL-VM, VL-VMO, VM-VMO) and Pearson correlations were run for each pair with 100ms moving windows. This produced a time series of Pearson correlation values that demonstrated sinusoidal patterns fluctuating between ± 1 . All sinusoidal correlation patterns consisted of positive and negative correlation phases (Figure 1) that continue to occur throughout the fatiguing contraction. A positive correlation indicated that the muscle activity between pairs of synergistic muscles were increasing or decreasing in parallel (Co-A) whereas the negative correlation phase indicated that the muscle activity was increasing or decreasing out of phase (TO).

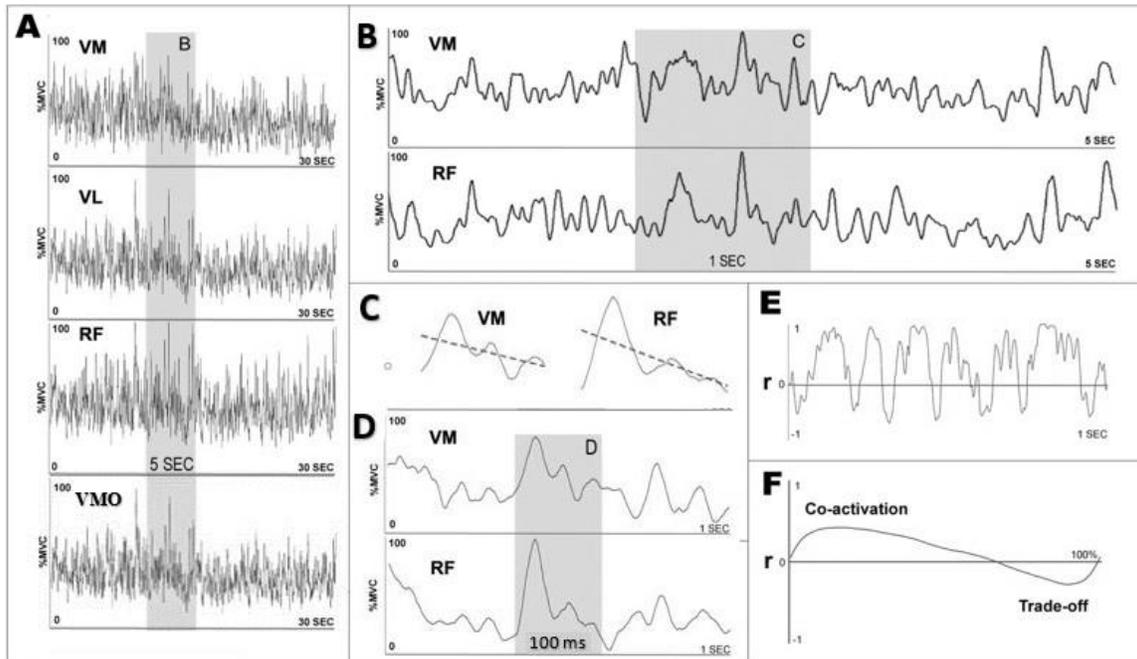


Figure 1. A) Shows the raw EMG data from each quadriceps muscle. B) Shows the RMS of the EMG data. C) Shows an example of a trend line that was removed to make the detrended EMG measures. D) Shows the 100 ms moving window used for the Pearson correlation time series. E) Shows the results of the correlation and illustrates what multiple AMA cycles look like. This is how AMA cycle frequency is shown with this analysis structure. F) Shows the areas that are considered Co-activation and Trade off in each load sharing cycle. The positive r values are Co-A and the negative r values are TO.

Then each of these timeseries values was broken into three 15 sec bins from the initiation of the contraction, the middle of the contraction, and the end (task failure) of the contraction. From this data alternating muscle activation cycle frequency, the Co-A and the TO durations were calculated. Alternating muscle activation cycle frequency is the amount of times a muscle pair completes one “cycle” of Co-A and TO which is the zero cross event at the onset of a positive correlation phase to the zero cross event at

the end of a negative correlation phase divided by 15 seconds. Co-A is the amount of time a pair is in positive correlation values during the contraction which is found via the difference between the temporal locations of the zero cross event at the onset and offset of a positive correlation phase. TO is the amount of time a pair is in negative correlation values during the contraction which is found via difference between the temporal locations of the zero cross event at the onset and offset of a negative correlation phase. This can be seen in part F of Figure 1, the entire graph would be considered one load sharing cycle, the positive values are Co-A, and the negative values are TO.

Statistical Analysis

AMA cycle frequencies and Co-A and TO durations from the sustained contractions were contrasted with a three-way (muscle pair, contraction phase, and sex) repeated measures ANOVA with Tukey's post-hoc analyses.

Pearson correlations were run for endurance time and Co-A and TO durations, and AMA cycle frequency in each fatigue phase for each sex. MVC is presented in terms of force produced and used as the value to determine the 20% of MVC contraction value.

Statistical significance was accepted at $p < 0.05$ and data are presented as mean \pm standard error.

Results

Maximum Voluntary Contraction Values

The MVC values are presented below. The male and female values were significantly different via a t-test($p < 0.05$). Males had an average MVC of 100.78 ± 5.24 newtons, females had an average MVC of 67.24 ± 3.56 newtons, and when pooled the average MVC was 84.01 ± 5.25 newtons.

Alternating Muscle Activation Cycle Frequency

The AMA cycle frequency (number of Co-A + TO cycles) results did not demonstrate statistical significance across contraction phase, muscle pair, or sex.

Muscle Pair	AMA Frequency (# of Cycles/s) (Mean \pm se)		
	First Phase	Second Phase	Third Phase
RF-VL	5.66 ± 0.12	5.58 ± 0.16	5.54 ± 0.15
RF-VM	5.72 ± 0.13	5.48 ± 0.19	5.61 ± 0.11
RF-VMO	5.46 ± 0.12	5.59 ± 0.16	5.31 ± 0.14
VL-VM	5.35 ± 0.12	5.21 ± 0.23	5.53 ± 0.14
VL-VMO	5.56 ± 0.13	5.07 ± 0.27	5.27 ± 0.14
VM-VMO	5.32 ± 0.15	5.12 ± 0.29	5.26 ± 0.16

Table 1. Illustrates the AMA frequency for all muscle pairs and contraction phases.

Muscle Pair Differences

There was a significant difference between Co-A and TO durations within muscle pairs in each contraction phase ($p < 0.005$ for contraction phase x muscle pair). Each pair exhibited differences between Co-A and TO durations in the first, second, and third 15 sec phase of the contraction. There were no sex differences for this measure ($p > 0.05$). Data are shown in Figure 2.

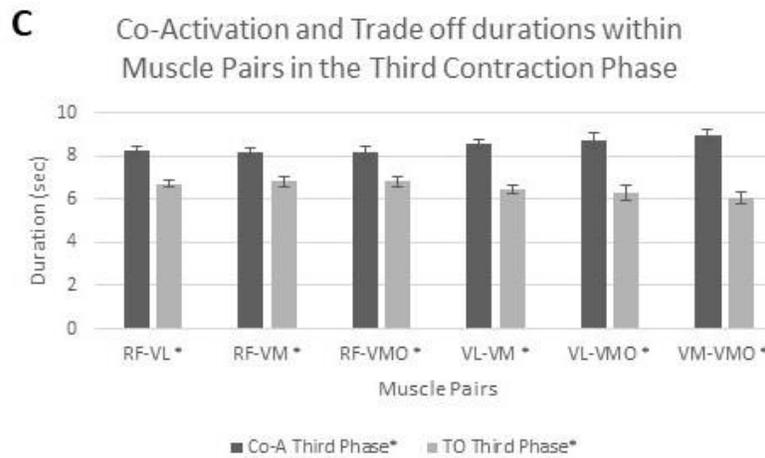
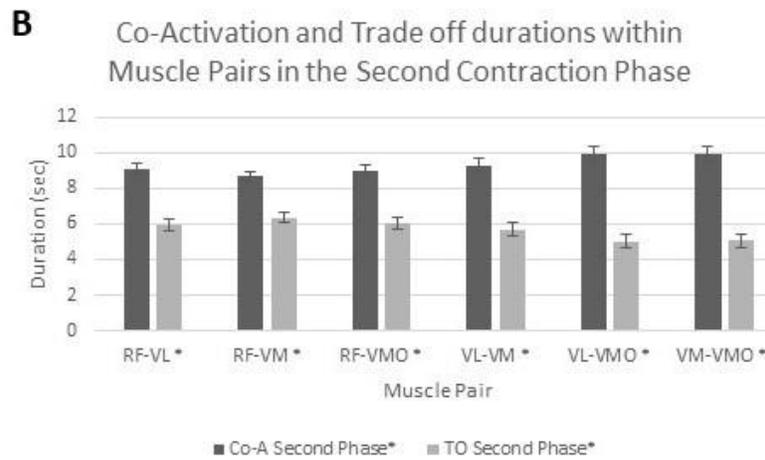
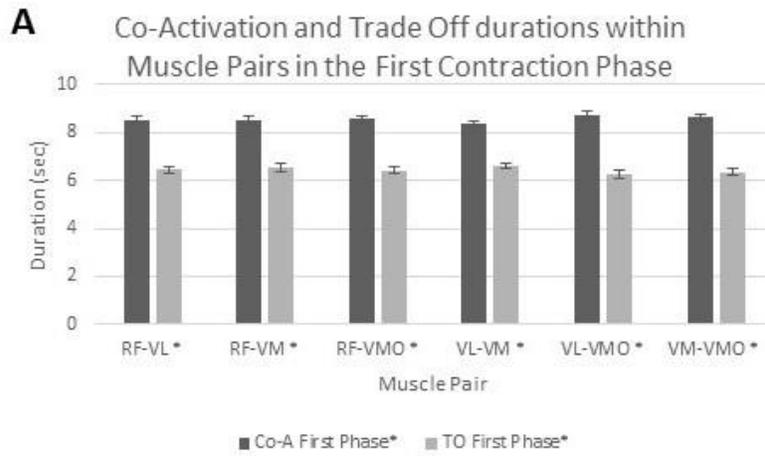


Figure 2. A) Illustrates the statistically significant differences between Co-A and TO durations within muscle pairs during the first contraction phase. B) Illustrates the statistically significant differences between Co-A and TO durations within muscle pairs during the second contraction phase. C) Illustrates the statistically significant differences between Co-A and TO durations within muscle pairs during the third contraction phase.

Final Fatigue Phase Differences

There was also a significant difference between muscle pairs' Co-A and TO durations during the last 15 second phase of the contraction ($p < 0.005$ for contraction phase x muscle pair). The only muscle pair that did not demonstrate any significant difference when comparing a muscle pair's Co-A and TO durations to other pairs' Co-A and TO durations was the VL-VM pair. However there were significant differences between the durations of: Co-A of the RF-VL pair and Co-A the VL-VMO and VM-VMO pairs, TO of RF-VL and TO the VL-VMO and VM-VMO pairs, Co-A of the RF-VM pair and the Co-A of VL-VMO and VM-VMO pairs, TO of the RF-VM pair and the TO of VL-VMO and VM-VMO pairs, Co-A of the RF-VMO and the VM-VMO pair, and finally the TO of the RF-VMO and the TO of VL-VMO and VM-VMO pairs. There were no sex differences for this measure ($p < 0.05$).

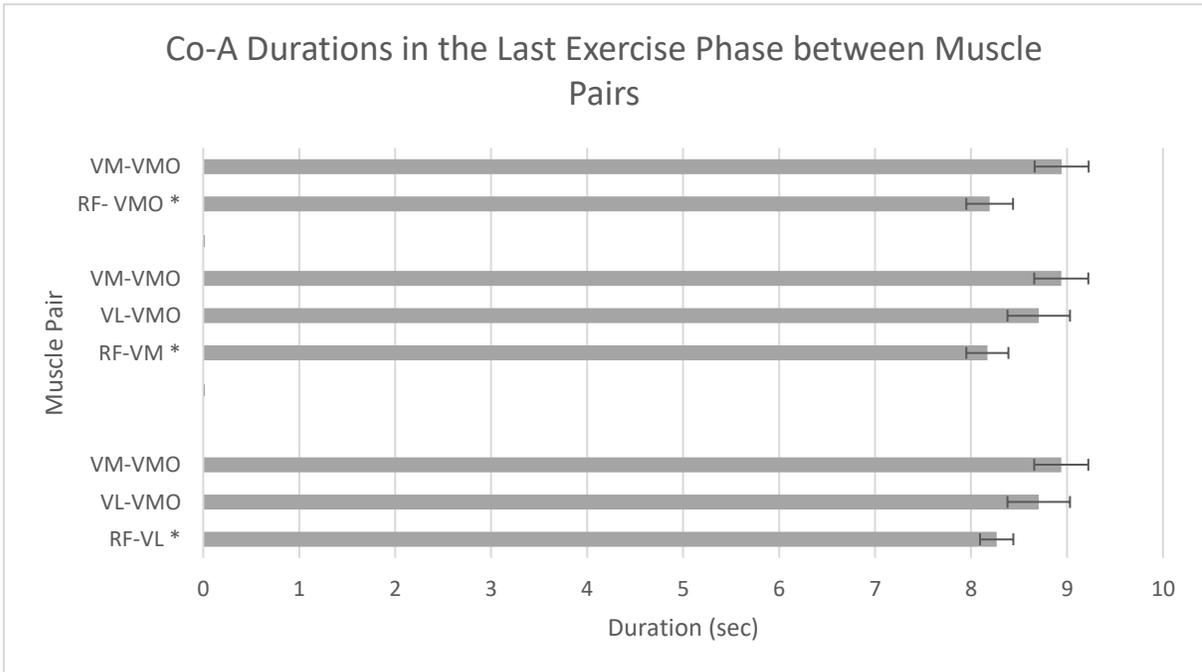


Figure 3. Illustrates the significant difference in Co-A durations between muscle pairs in the final phase of the contraction as denoted by *.

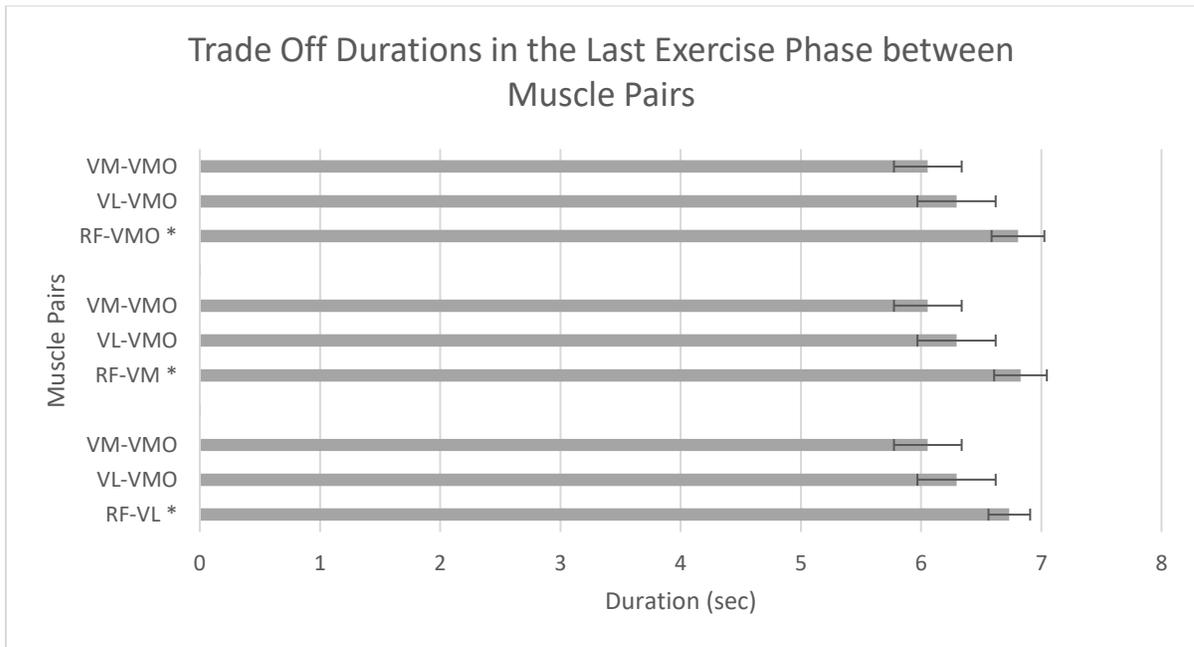
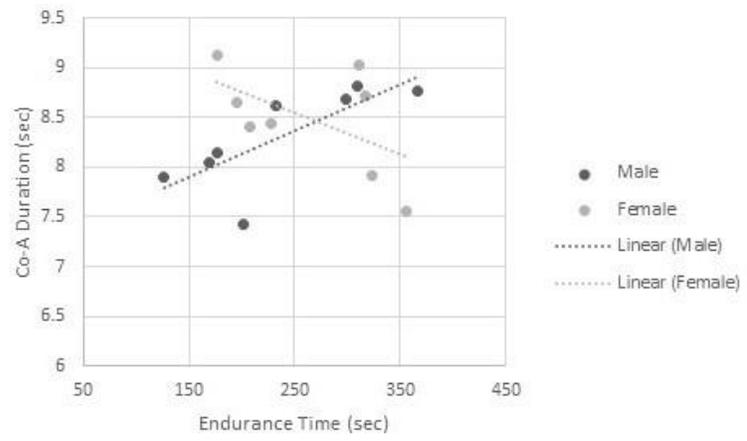


Figure 4. Illustrates the significant difference in TO durations between muscle pairs in the final phase of the contraction as denoted by *.

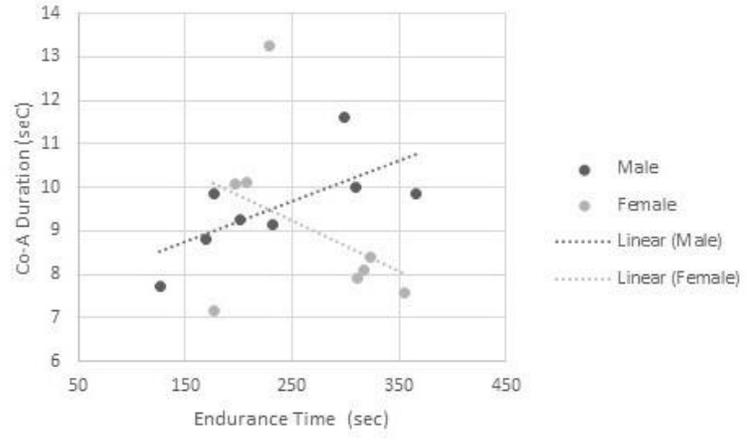
Endurance time correlations

When comparing endurance time to Co-A and TO there were strong correlations when the sexes were divided. An example of the relationship between endurance time and Co-A and endurance time and TO durations is presented in figures 5 and 6 below for the VL-VM pair.

A



B



C

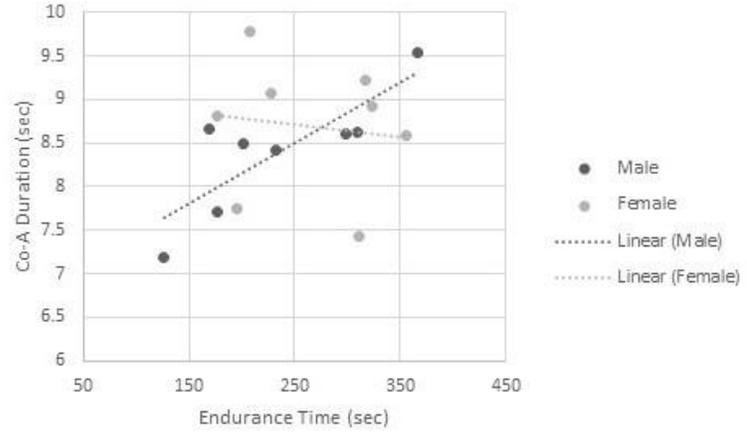


Figure 5. A) Scatterplot illustrating the correlation between Co-A and Endurance time with linear regressions for the VL-VM pair in the first contraction phase. B) Scatterplot illustrating the correlation between Co-A and Endurance time with linear regressions for the VL-VM pair in the second contraction phase. C) Scatterplot illustrating the correlation between Co-A and Endurance time with linear regressions for the VL-VM pair in the third contraction phase.

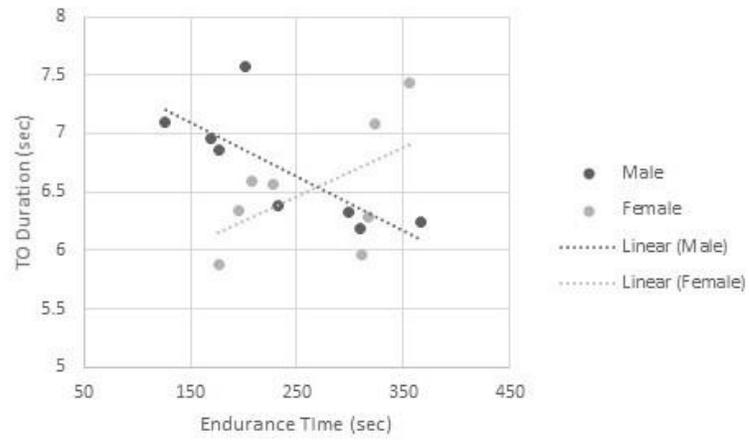
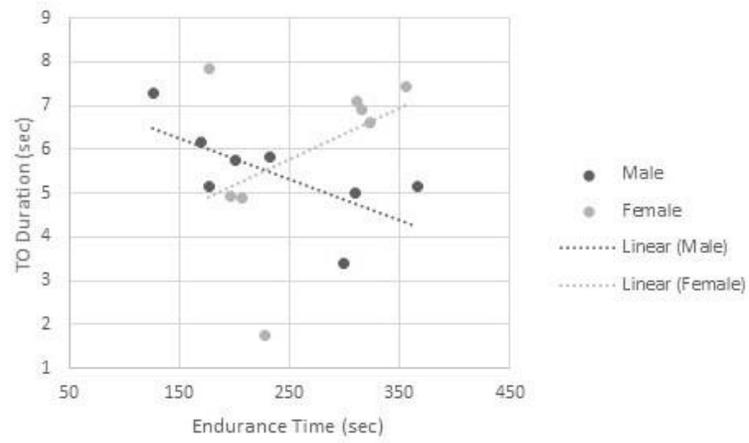
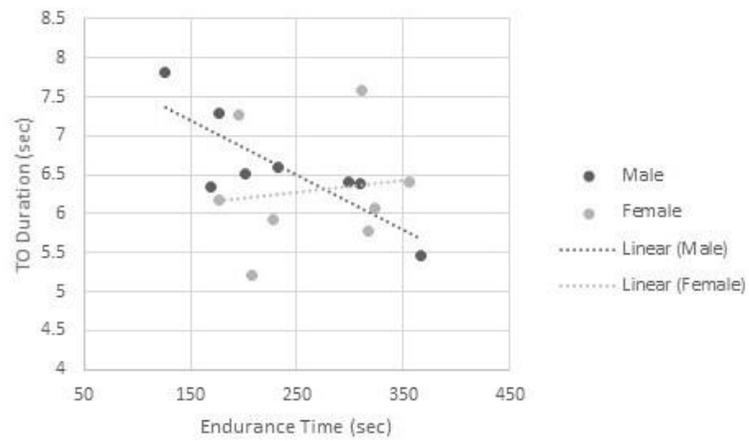
A**B****C**

Figure 6. A) Scatterplot illustrating the correlation between TO and Endurance time with linear regressions for the VL-VM pair in the first contraction phase. B) Scatterplot illustrating the correlation between TO and Endurance time with linear regressions for the VL-VM pair in the second contraction phase. C) Scatterplot illustrating the correlation between TO and Endurance time with linear regressions for the VL-VM pair in the third contraction phase.

The rest of the endurance time and Co-A and TO duration correlation coefficients are presented in the tables below.

Muscle Pair	Male Correlation Coefficients					
	First Phase		Second Phase		Third Phase	
	Co-A	TO	Co-A	TO	Co-A	TO
RF-VL	0.44	-0.44	0.68*	-0.68*	0.74*	-0.74*
RF-VM	0.03	-0.03	0.91*	-0.91*	0.82*	-0.82*
RF-VMO	0.08	-0.08	0.68*	-0.68*	0.5	-0.5
VL-VM	0.77*	-0.77*	0.69*	-0.69*	0.81*	-0.81*
VL-VMO	0.52*	-0.52*	0.85*	-0.85*	0.83*	-0.83*
VM-VMO	0.54*	-0.54*	0.75*	-0.75*	0.69*	-0.69*

Table 2. Illustrates the male r values from the correlation between endurance time and Co-A and TO durations. Significant r values are denoted with *.

Muscle Pair	Female Correlation Coefficients					
	First Phase		Second Phase		Third Phase	
	Co-A	TO	Co-A	TO	Co-A	TO
RF-VL	-0.66*	0.66*	-0.21	0.21	-0.26	0.26
RF-VM	0.21	-0.21	-0.35	0.35	-0.17	0.17
RF-VMO	-0.35	0.35	-0.5	0.5	-0.28	0.28
VL-VM	-0.54*	0.54*	-0.4	0.4	-0.13	0.13
VL-VMO	-0.18	0.18	-0.41	0.41	0.17	-0.17
VM-VMO	-0.41	0.41	-0.35	0.35	0.003	-0.003

Table 3. Illustrates the female r values from the correlation between endurance time and Co-A and TO durations. Significant r values are denoted with *.

There was no correlation between endurance time and load sharing frequency for either sex or when the data was pooled ($p > 0.05$ for all).

Discussion

The primary findings from this study were that AMA patterns occurred between all pairs of quadriceps muscles during isometric knee extension at 20% of MVC effort. The basic structure of an AMA cycle was stable but the temporal relationships between co-activation and trade-off did not change as much as expected to successfully meet the demands of the task. Despite not finding significant difference for AMA cycles the muscle pairs still demonstrated the AMA patterns of Co-A and TO throughout all three phases of the fatiguing contraction. However, the Co-A durations were significantly longer than the TO durations for all the muscle pairs meaning that the muscle pairs did not cycle through their phases as rapidly or frequently as expected to manage fatigue. This would mean that either the muscles did not experience fatigue which is untrue as measures of fatigue were significant in the last phase of exercise or that in a healthy population at a 20% MVC load sharing strategies are not utilized to the extent that they would register as significant. This is potentially due to the intensity of MVC that the subjects were tested at. It has been found that differences in AMA frequency and Co-A and TO durations are much more apparent at lower intensity (< 20% of MVC) contractions than at higher intensity contractions (39).

The endurance time correlations revealed that men and women potentially have different strategies to attenuate fatigue. The men had positively correlated values with Co-A for endurance time meaning that they utilize co-activation of the synergistic quadriceps muscles to attenuate fatigue. The female subjects showed the opposite, though not for all muscle pairs. For the RF-VL, RF-VMO, and VL-VM they utilized trade off to attenuate fatigue and extend endurance time.

The occurrence of AMA patterns has been suggested to be limited between muscles that either have different fiber type ratios, innervation sources or number of spanned articulations (5, 30, 49, 52). Investigations of quadriceps muscle activation patterns during sustained contractions have shown that recruitment or activation level

of the bi-articular rectus femoris does not necessarily coincide with that of the mono-articular vastus medialis or vastus lateralis muscles (3, 4, 27, 30, 49). The concept that the rectus femoris has been found to be either more or less active than the vasti muscles during a sustained contraction has led researchers to the conclusion that the quadriceps muscles are independently controlled (6) and only trade-off between bi- and mono-articular muscles (31). The results of the current study found that AMA patterns were present between bi- and mono-articular muscles, and in mono-articular synergistic muscles. A control scheme that allows AMA between all synergistic muscles across different contraction intensities may be beneficial to the fatiguing motor system as it may distribute activation levels across synergistic muscles that have different characteristics (ie., fiber type ratios, cross sectional area). Although limited experimental evidence exists in humans, it is plausible to suggest that the mono articular vasti muscles are linked via excitatory interneurons (41) whereas inhibitory interneurons link the mono articular vasti muscles and the bi articular rectus femoris muscle (41, 55). Therefore the wide range of synergistic muscle activation patterns noted in the literature may not stem from independent central command signals sent to each agonist muscle, but rather by one common excitatory drive to which the agonist muscles within a spinal module may either respond similarly or differently based on the balance of excitatory and inhibitory neural interactions, thus managing fatigue.

Synergistic muscle control can also be habitual rather than optimal as shown during a study that simulated paralyzing a wrist muscle. The participants simply increased activation in all muscles rather than recruiting only the useful muscles (16). This goes against the optimal controller model that is typically described in the literature and instead suggests that the brain may store and recall motor recruitment patterns that the lower sensorimotor system deems appropriate. It was found that synergy can be learned or entrained at the supraspinal level and affect local spinal circuits so that the CNS “learns” new synergistic or more efficient ways of accomplishing tasks (34). The idea of a Central Pattern Generator (CPG) is to quickly execute a movement and reduce

the degrees of freedom presented by the system. While this occurs in healthy individuals, problems arise when an individual has some sort of dysfunction or pain. It has been shown that when the internal joint stresses presented in the knee occur the neuromuscular control patterns of the quadriceps change to try and attenuate the imbalance of lateral joint stresses (6). When these issues are corrected the new patterns are entrained at the supraspinal level and can solve the dysfunction or pain. This avenue of synergy for AMA means that individuals who experience neuromuscular dysfunction could be trained out of it resulting in new potential rehabilitation efforts.

One interesting aspect of the current study is the lack of significant difference in the last contraction phase of the VL-VM pair for Co-A and TO durations when compared to other pairs. The VL and VM operate to stabilize the patella during knee extension tasks (6, 41) and a lack of stability due to imbalanced VL-VM control can cause pain and mal-tracking of the patella, which manifests itself as patellofemoral pain syndrome (PFPS) (10, 21, 43, 54). Given that the participants had no knee pain this result is consistent with the literature. However, another common predictor of PFPS is VMO activation, specifically onset time, relative to the VL (10, 54). This would imply that in the present study there should not have been any significant difference in the final fatigue phase for the VMO-VL pair compared to the other muscle pairs but there was significant difference. While the measure in the present study is not onset time it is total time in relative activation to another muscle, in this case the VL. This could be used as a predictor for PFPS like other studies have suggested (10, 54) or could simply be that the VMO is not preferentially activated in this specific knee extension task for patellar stability. This also creates new avenues of research to look at these same measures across larger groups of individuals across different age ranges and with differing levels of training or dysfunction to examine the relationships between alternating muscle activity variability and Co-A and TO durations of these different muscle pairs.

Future studies should focus on dysfunction so that the data can be used in rehabilitation measures to try and resolve knee pain issues that stem from neuromuscular control. Knee pain is often linked to dysfunctional neuromuscular control (7, 28). Post ACL reconstruction is another avenue to examine as there is typically a reduction in muscle cross sectional area in the affected limb and reduced motor neuron recruitment or motor-unit firing frequency which can contribute to pain and dysfunction of the affected limb (29, 44). Knee osteoarthritis is also a common issue in aging populations and individuals who have knee OA demonstrate reduced neuromuscular activation of the quadriceps and increased fatiguability of the quads (37, 48, 51). The largest potential impact of this research on rehabilitation is on PFPS. It has been established that activation imbalances between the VM and VL can cause patellar mal-tracking and induce anterior/retropatellar knee pain which is the main symptom of PFPS (10, 21, 43, 54). The VL and VM activation relationship's primary function is to regulate internal joint stresses of the patellofemoral joint and not simply to reduce the degrees of freedom that the CNS could take to perform a task (6). Therefore the lack of difference between the VL and VM in the present study would imply that the subjects do not have knee pain or PFPS which was consistent with the screening protocol. If PFPS was present it would be expected that the relationship between the VL and VM would change progressively as the subject moved through fatigue phases during the 20% of MVC contraction.

The limitations of this study are the small number of participants and that the data used was part of a larger collection process for a larger study meaning that the participants may have been fatigued or not focused on the specific 20% MVC to failure. They were also all healthy young subjects at varying training levels which would return very normal or expected data versus looking at a dysfunctional group or differing ageing populations.

Conclusion

Alternating muscle activation is evident in young healthy individuals during a 20% of MVC fatiguing contraction but there is no significant difference in AMA cycle frequencies of the muscle pairs, sexes, or contraction phases. However, there are significant differences in Co-A and TO durations between phases and pairs meaning that as fatigue occurs Co-A and TO durations between muscle groups and phases changes in response to fatigue. There were also interesting correlations between Co-A and TO durations when compared to endurance time that may indicate more specific strategies between the sexes as to how they attenuate fatigue. While this new avenue of analysis for EMG is novel its validity should be tested again across more studies and larger subject groups. Combining it with other measures to ensure that it is showing the appropriate results is the next step. The data analysis in this paper shows that AMA does in fact change across the timeline of a fatiguing contraction but only in regard to endurance time and Co-A and TO durations and not AMA cycles as expected. Changing the percentage of MVC used for the contraction or increasing the number of subjects could elucidate further differences. More research should be conducted on this topic, specifically with differing age groups, sexes, and individuals with knee pain or dysfunction.

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