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RESEARCH ARTICLE

Early deactivation of slower muscle fibres at high movement frequencies

Ollie M. Blake* and James M. Wakeling

ABSTRACT

Animals produce rapid movements using fast cyclical muscle contractions. These types of movements are better suited to faster muscle fibres within muscles of mixed fibre types as they can shorten at faster velocities and achieve higher activation–deactivation rates than their slower counterparts. Preferential recruitment of faster muscle fibres has previously been shown during high velocity contractions. Additionally, muscle deactivation takes longer than activation and therefore may pose a limitation to fast cyclical contractions. It has been speculated that slower fibres may be deactivated before faster fibres to accommodate their longer deactivation time. This study aimed to test whether shifts in muscle fibre recruitment occur with derecruitment of slow fibres before faster fibres at high cycle frequencies. Electromyographic (EMG) signals were collected from the medial gastrocnemius at an extreme range of cycle frequencies and workloads. Wavelets were used to resolve the EMG signals into time and frequency space and the primary sources of variability within the EMG frequency spectra were identified through principal component analysis. Early derecruitment of slower fibres was evident at the end of muscle excitation at higher cycle frequencies, as determined by reduced low-frequency EMG content, and additional slower fibre recruitment was present at the highest cycle frequency. The duration of muscle excitation reached a minimum of about 150 ms and did not change for the three highest cycle frequencies, suggesting a duration limit for the medial gastrocnemius. This study provides further evidence of modifications of muscle fibre recruitment strategies to meet the mechanical demands of movement.

KEY WORDS: Electromyography, Preferential recruitment, Activation, Wavelets, Cycling

INTRODUCTION

Fast movements that involve high cycle frequencies require rapid muscle activation–deactivation times. Faster muscle fibres and faster muscles are better suited to achieve these high cycle frequencies. Deactivation is longer than activation (Buchthal et al., 1973; Burke et al., 1973; Lee et al., 2011) and may pose a limitation to maximal contraction cycle frequencies. However, the size principle of motor unit recruitment (Henneman et al., 1965a; Henneman et al., 1965b) dictates that slower fibres, with longer relaxation times, are deactivated last. While the size principle adequately describes many recruitment strategies, alternative strategies may also exist (Gillespie et al., 1974; Gollnick et al., 1974; Grimby and Hannerz, 1977; Hoffer et al., 1981; Citterio and Agostoni, 1984; Wakeling et al., 2006; Hodson-Tole and Wakeling, 2008a) when the demands exceed the abilities of the slowest muscle fibres.

The cat paw shake provided an early example of preferential recruitment of fast muscle during high-frequency contractions (Smith et al., 1980). The predominantly slow-fibred soleus muscle was inactive while the predominantly fast-fibred lateral gastrocnemius was active during the paw shake, which was identified using electromyography (EMG). The lateral gastrocnemius is a muscle with mixed fibre types, yet it was not possible to distinguish the contribution of slow muscle fibres in the muscle at the time. However, it was suggested that recruitment of the slower fibres in the lateral gastrocnemius would be counterproductive to the cyclical action (Smith et al., 1980). In contrast, Josephson and Edman (Josephson and Edman, 1988) proposed that complete inhibition of slow fibres may be detrimental to the power production for very fast contractions, as slow fibres may provide resistance to whole muscle shortening. Indeed, recent evidence suggests that slow fibres within mixed muscle help the muscle reach higher shortening velocities (Holt et al., 2014).

More recently, techniques to distinguish the activity of different motor unit types from the EMG signal have been developed (von Tscharner, 2000; Wakeling et al., 2002; Wakeling and Rozitis, 2004). Wakeling and colleagues (Wakeling et al., 2002) qualitatively showed the presence of preferential recruitment of faster muscle fibres during the cat paw shake in a single muscle (medial gastrocnemius), but was unable to provide quantitative evidence through statistical methods. In a subsequent study, a statistically significant shift towards preferential recruitment of faster muscle fibres was displayed in the human medial gastrocnemius at high cycle frequencies independent of the muscle fascicle strain (Wakeling et al., 2006); however, this shift was identified in the middle of the bursts of muscle excitation and was correlated with the muscle fibre strain rate. Similar findings were subsequently repeated in the rat (Hodson-Tole and Wakeling, 2008b) and the goat (Lee et al., 2013).

With the inclusion of force measurements, Roberts and Gabaldón (Roberts and Gabaldón, 2008) showed shorter muscle relaxation times during running compared with walking in the lateral gastrocnemius of the turkey, independent of strain rate. It was speculated that alterations in muscle fibre recruitment during running, such that faster fibres remain active after deactivation of slow fibres, might explain the shorter relaxation times as they should not decrease if dominated by slow fibres as dictated by the size principle (Henneman et al., 1965a; Henneman et al., 1965b). Studies have qualitatively indicated such a recruitment pattern in humans (Wakeling, 2004) and rats (Hodson-Tole and Wakeling, 2008a) running at various speeds, while Lee and co-workers (Lee et al., 2013) were unable to confirm a relationship between relaxation rates and muscle fibre recruitment in goats. Despite having been repeatedly suggested, an early derecruitment of slower fibres during muscle relaxation at high cycle frequencies has not yet been

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demonstrated. The purpose of this study was to investigate whether slow muscle fibres are derecruited before fast fibres at the end of muscle excitation during high-frequency contractions, as demonstrated by shifts in the frequency content of the EMG signal.

RESULTS

The experimental subjects were able to complete all cycling conditions (pedal cadences between 40 and 180 rpm, at power outputs of 100–400 W) and EMG signals were recorded and frequency and timing data were identified. Principal components determined from wavelet analysis displayed distinct features in both frequency and timing of the EMG intensities during muscle excitation (Fig. 1A). In order to identify the influence of alterations in frequency, the data were normalized to unit intensity for each pedal cycle, and the mean across all subjects was subtracted prior to the principal component (PC) analysis, with the resulting PCs describing the variance from the mean. The first 20 PCs accounted for almost 70% of the variance, with the first two components explaining over 24%. The first and second PCs displayed distinct shifts in both frequency and timing of the peak EMG intensity within each burst of muscle excitation (Fig. 1A), and loading scores for these components identified the relative amounts of these shifts. The first PC showed a decrease in EMG intensity at high frequencies (peak ~170 Hz) earlier in the muscle excitation (~29% of the duration) and an increase in EMG intensity at low frequencies (peak ~92 Hz) later in the muscle excitation (~55% of the duration). Conversely, the second PC displayed decreased EMG intensity at low frequency (peak ~62 Hz) earlier (35% muscle excitation duration) in the muscle excitation and increased EMG intensity at high frequency later (56% muscle excitation duration).

There was a significant effect of pedalling cadence and no significant effect of crank power output on the EMG intensity. In addition, there was a significant correlation between EMG intensity and cadence (r=0.85) and decreasing differences in EMG intensity for a given cadence at increasing power outputs (Fig. 2). For example, EMG intensity was significantly different at 40 rpm and not significantly different at 180 rpm for all power outputs (Fig. 2). EMG intensity for each cadence–power combination showed a monotonic increase from 60 to 120 rpm, while the cadences above 120 rpm also displayed a linear increase at a significantly steeper slope (paired t-test: P<0.01; Fig. 2).

There was a significant effect of pedalling cadence on the loading scores for the first two PCs (PC1 and PC2; Fig. 1B) and 13 of the first 20 PCs, and a significant effect of power output on the loading

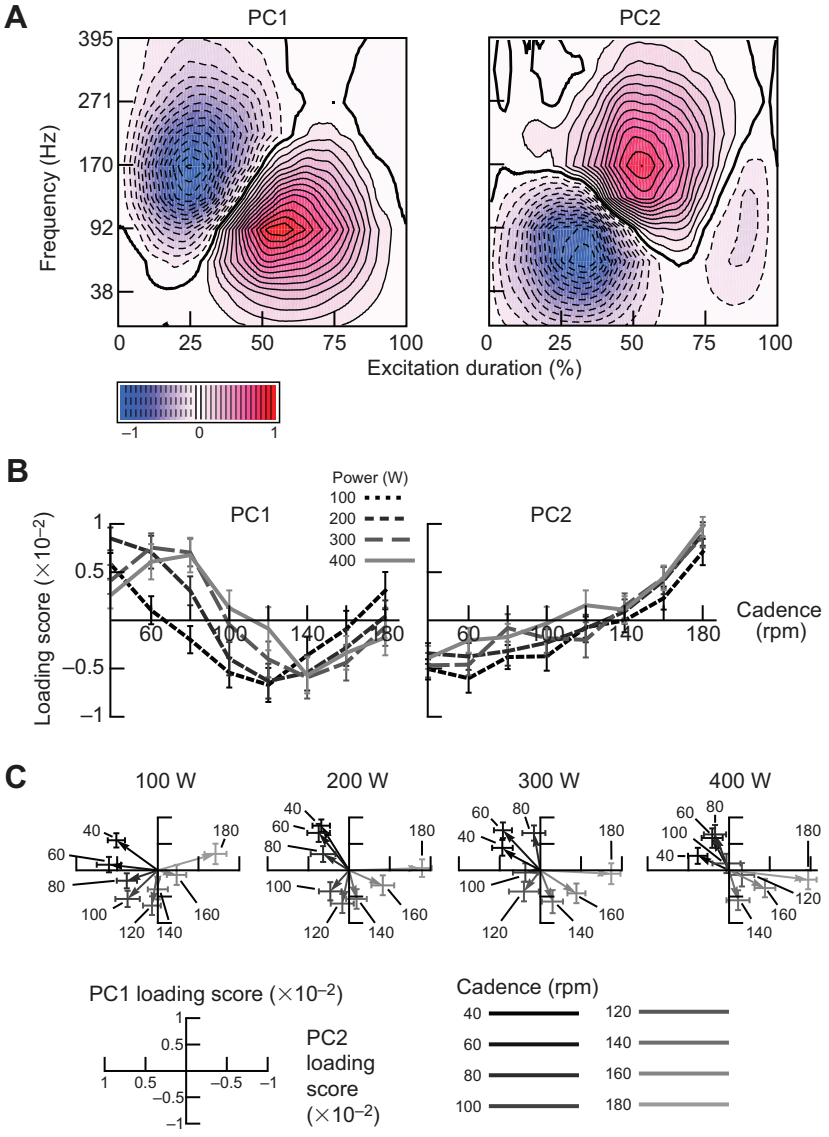

Fig. 1. Principal component (PC) representations of the time–frequency patterns of the EMG intensity and PC loading scores for each cadence–power output condition. (A) PC weightings for PC1 and PC2 showing the variance from the mean EMG frequency spectra for medial gastrocnemius during muscle excitation. The EMG intensities are shown relative to the time within each muscle burst (% excitation duration), and their frequency content. Intensities have been normalized to the absolute maximum for each PC and show both increases (positive values: red with continuous contour lines) and decreases (negative values: blue with interrupted contour lines) in frequency at specific times during excitation. (B) Mean ± s.e.m. loading scores for PC1 and PC2 at each cadence–power output combination. (C) Mean ± s.e.m. loading scores PC2 versus PC1 at each cadence–power output combination. This shows the general pattern of increasing vector angles with increasing cadence at the lowest workloads and negative PC2 and positive PC1 loading scores at low cadences and the opposite at high cadences at the highest workloads.

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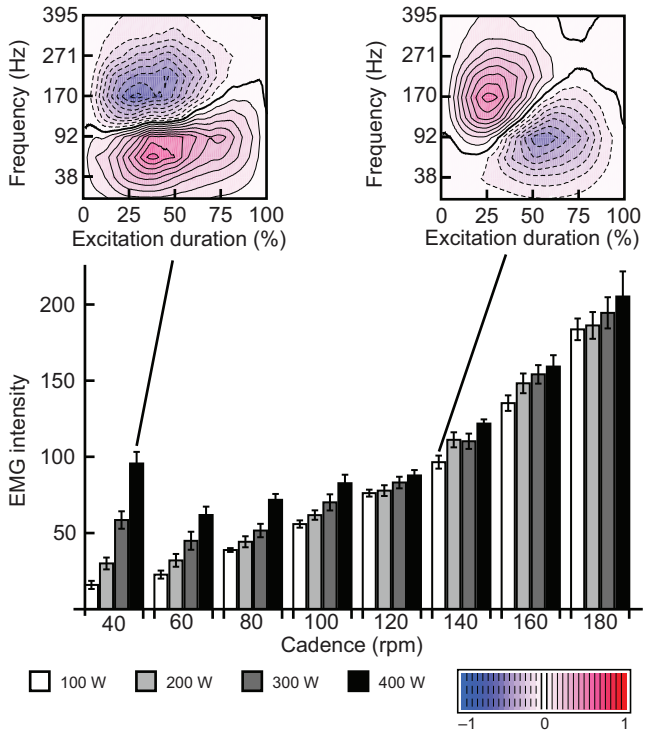

Fig. 2. EMG intensity for each cadence–power output combination and an example of two principal component representations showing different recruitment patterns. Mean ± s.e.m. EMG intensity at each cadence–power output combination. The contour plots show reconstructions of the variance from the mean EMG frequency spectra across the muscle excitation duration and have been normalized to the absolute maximum. The 140 rpm at 100 W (right contour plot) condition shows an example of preferential recruitment of faster muscle fibres when compared with the 40 rpm at 400 W condition (left contour plot) due to its increased high frequencies (red with continuous contour lines), decreased low frequencies (blue with interrupted contour lines) and no significant difference in EMG intensity (bar graph).

scores for PC2, but not PC1, and six of the first 20 PCs. Vectors created between the first two PC loading scores revealed a circular pattern with increasing cadence at the lowest workloads (Fig. 1C). In general, low cadences had positive PC1 and negative PC2 loading scores, which were reversed at high cadences (Fig. 1C). Reconstructions of the signal variance of the EMG intensity using the first two PCs for each cadence–power combination can be seen in Fig. 3 and showed changes in frequency across the burst of muscle excitation. Across all workloads at 40 and 60 rpm the reconstructions showed an increase in EMG intensity at low frequencies (centred on 62–92 Hz) and a decrease in the EMG intensity at high frequencies (centred on 170 Hz). At 80, 100, 120 and 140 rpm there was a distinct shift of the EMG intensity earlier in the burst duration. At 140, 160 and 180 rpm there was an increase in the high-frequency and subsequent decrease in the low-frequency component of the EMG intensity, with the increased high frequency occurring later in the muscle burst at 180 rpm Additional low-frequency components (>62 Hz) appeared at 180 rpm at the same time of muscle excitation (~60–65%) as the increased high frequencies (Figs 3, 5).

The absolute duration of the bursts of muscle excitation decreased from 753.35±50.39 ms at 40 rpm and 400 W to 151.68±5.31 ms at 180 rpm and 300 W (Fig. 4). There was a general decreasing trend in duration of muscle excitation with increasing cadence, with the difference between successive cadences also decreasing (Fig. 4). At all power outputs there was a significant difference between 40, 60 and 80 rpm, a mixture of significant and non-significant differences between 80 and 140 rpm, and no significant difference in burst duration between 140, 160 and 180 rpm, while pedal cycle duration decreased. Also, there was no significant difference in burst duration between power outputs for a given cadence from 100 to 180 rpm.

DISCUSSION

Preferential recruitment of faster muscle fibres during rapid contractions has been shown in various animal studies, including humans (Gillespie et al., 1974; Gollnick et al., 1974; Grimby and Hannerz, 1977; Hoffer et al., 1981; Citterio and Agostoni, 1984; Wakeling et al., 2006; Hodson-Tole and Wakeling, 2008a; Lee et al., 2013). It has been suggested that complete inhibition of slow muscle fibres in a muscle with mixed fibre types would provide resistance to whole muscle shortening and therefore be detrimental to power production for very fast contractions (Josephson and Edman, 1988). Therefore, it has been speculated that slow muscle fibres are activated, but deactivated before faster muscle fibres (Hodson-Tole and Wakeling, 2008a; Roberts and Gabaldón, 2008; Lee et al., 2013). This is to account for their longer deactivation times, as the time between the end of excitation and the end of muscle shortening represents an increasing portion of the muscle contraction–relaxation cycle at increasing cycle frequencies. Qualitative evidence of this has been shown in humans (Wakeling, 2004) and rats (Hodson-Tole and Wakeling, 2008a), but never quantitatively and statistically. These previous studies indicated graphical evidence for an increase in faster fibre and decrease in slower fibre excitation at the end of a running stride, in contrast to predictions made by the size principle (Henneman et al., 1965a; Henneman et al., 1965b). In the current study we put the muscle through an extreme range of conditions to push the recruitment strategies to their limits, in order to quantitatively and statistically test whether shifts in muscle fibre recruitment occur with derecruitment of slow fibres before fast fibres at the highest cycle frequencies, as seen through EMG frequency analysis.

The distinction of muscle fibre type from the spectral properties of the EMG signal has been controversial (Farina, 2008; von Tscharner and Nigg, 2008) and poses a limitation to the interpretation of the frequency changes. However, progressive developments in EMG decomposition have made it possible to distinguish faster and slower muscle fibres based on the signal frequency with fast and slow muscle fibres producing high and low EMG frequencies, respectively (Gerdle et al., 1988; Solomonow et al., 1990; Elert et al., 1992; Kupa et al., 1995; von Tscharner, 2000; Wakeling and Rozitis, 2004; Wakeling, 2009; Wakeling and Horn, 2009). In addition, by using wavelet analysis it is possible to retain both time and frequency information of the EMG signal to evaluate the changes in frequency with respect to muscle excitation duration (von Tscharner, 2000; Wakeling et al., 2002; Wakeling and Rozitis, 2004). In this study we hypothesized that the early derecruitment of slower muscle fibres during muscle relaxation would occur at the highest cycle frequencies, and this would be demonstrated by a relative reduction in the low-frequency component (60–90 Hz) of the EMG intensity at the end of the burst of EMG excitation (Wakeling et al., 2001; Wakeling and Horn, 2009).

The data revealed a shift of the EMG intensity from low to high frequencies similar to previous findings (Wakeling et al., 2006), a shift of these frequencies relative to the duration of muscle excitation (Fig. 1A, Fig. 3), and the addition of low-frequency components at the highest pedalling rates (Figs 3, 5). Changes in EMG frequency between different conditions can be attributed to

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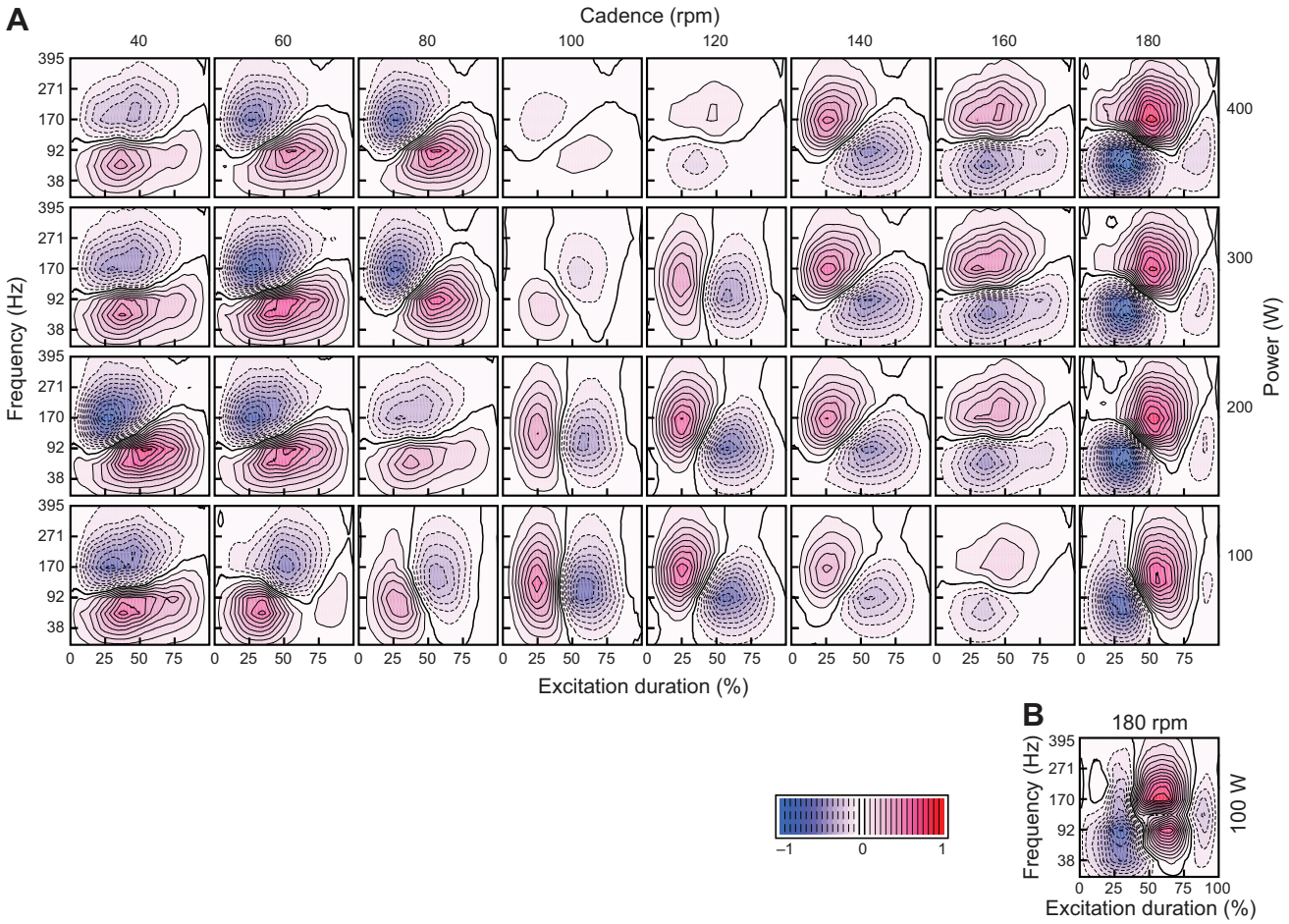

Fig. 3. Principal component reconstructions of the variance from the mean EMG frequency spectra across the muscle excitation duration. (A) Each time–frequency plot was constructed from the first two PCs at each cadence–power output combination. Values have been normalized to the absolute maximum across all reconstructions and show both increases (positive values: red with continuous contour lines) and decreases (negative values: blue with interrupted contour lines) in EMG intensity at specific frequencies and at specific times during excitation. This shows the shift in frequency emphasis from low frequencies at the lowest cadences to high frequencies at the highest cadences and finally the addition of low frequencies at 180 rpm. This also shows how the frequency emphasis moves with respect to the time within the burst of muscle excitation. (B) Reconstruction using the first 20 PCs at 180 rpm at 100 W showing the additional low frequency in a distinct band centred on ~92 Hz with both the high and low frequencies later in the muscle excitation duration.

shifts in muscle fibre recruitment when there is no significant difference in EMG intensity between conditions (Wakeling et al., 2006). For example, this can be seen between the following conditions: 40 rpm at 400 W and 140 rpm at 100 W (Fig. 2). The first and second PCs represented a large portion of the signal variance and displayed both a shift in frequency and timing (Fig. 1A) and were significantly affected by cadence (Fig. 1B). When the first PC loading scores were negative and the second PC loading scores were positive, there was a decrease and increase in low- and high-frequency components of the EMG signal, indicating both a decrease and increase in the contributions of slower and faster muscle fibres, respectively, at the end of muscle excitation (Fig. 1A). This occurred at 140, 160 and 180 rpm at most workloads (Fig. 1C), providing evidence of an early derecruitment of slower muscle fibres at the end of muscle excitation, thereby supporting the hypothesis. This increase in high-frequency and decrease in low-frequency components of the EMG could clearly be seen at 65, 75 and 85% of muscle excitation duration at the highest cycle frequencies (lighter lines in Fig. 5). This is in contrast to the increase in intensity for the lower frequencies and decrease in intensity for the higher frequencies at the lowest cadences (darker lines in Fig. 5).

The principal components determined from the EMG frequency spectra differ from those established previously (Wakeling, 2004; Wakeling and Rozitis, 2004; Wakeling et al., 2006; Hodson-Tole and Wakeling, 2008a; Wakeling, 2009; Lee et al., 2011; Lee et al., 2013) as time was retained and the mean was subtracted. In these previous studies the mean was not subtracted in order to evaluate and reconstruct the entire signal, whereas subtraction of the mean in the current study results in the principal components representing variance of the time-varying frequency spectra (Ramsay and Silverman, 2005) and highlight the major shifts in frequency away from the mean. In our previous studies that have not subtracted the mean, PC1 resembles the mean signal and PC2 and PC3 describe the major sources of variation from PC1. Thus the percentages of the signal explained by PC1 and PC2 from the previous studies should not be compared with the percentage of variance explained by PC1 and PC2 in the present study. PC1 and PC2 in the current study would therefore more closely resemble PC2 and PC3 without the mean subtracted.

The excitation duration reached a plateau beyond 140 rpm with the shortest duration of 151.68±20.60 ms (Fig. 4). This may indicate that these cycle frequencies reached or were approaching the

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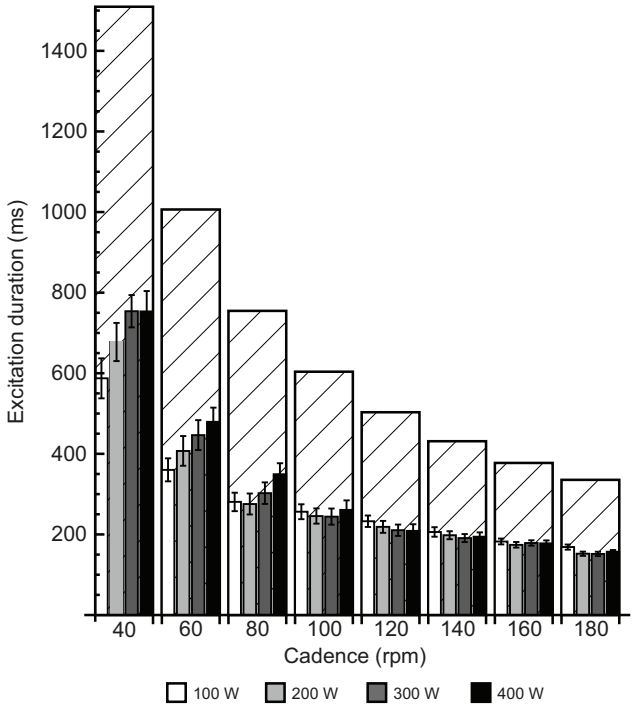

Fig. 4. Muscle excitation duration at each cadence–power output combination. Values show means ± s.e.m. as well as the cycle duration (striped bars). There was no significant difference in muscle excitation duration between 140, 160 and 180 rpm, which may represent the minimum duration of the medial gastrocnemius. Therefore muscle excitation duration was an increasing proportion of the cycle duration at faster cadences.

minimum muscle excitation duration for the medial gastrocnemius. It has been speculated that pedalling cadences greater than 165 rpm would surpass the maximum shortening velocity of the slow muscle fibres (Sargeant and Beelen, 1993) and as deactivation is longer than activation, during the activation–deactivation cycle of muscle contraction (Buchthal et al., 1973; Burke et al., 1973; Lee et al., 2011) the highest cycle frequencies in this study may exceed the relaxation capabilities of the slowest muscle fibres. The short excitation durations can partially be explained by the ability of the muscle to reach higher cycle frequencies because active shortening accelerates the relaxation dynamics expected from isometric twitches (Caiozzo and Baldwin, 1997; Askew and Marsh, 1998). As noted, the EMG frequency analysis indicated that there was also some manipulation of the timing of muscle fibre recruitment relative to muscle excitation that may have helped enable the slow fibres to participate at such high cycle frequencies. As speculated (Wakeling, 2004; Hodson-Tole and Wakeling, 2008a) this could be accomplished through recurrent inhibition of the slower fibres by spinal interneurons. One possible source of such inhibition would be the Renshaw cells: the fastest motoneurons have been shown to provide the most stimulus to the Renshaw cells, which in turn inhibit motoneuron activity with the greatest inhibitory influence on the slowest motoneurons (Friedman et al., 1981). The functional contribution of Renshaw cells to recurrent inhibition *in vivo* is still under debate; however, their potential role remains plausible given the evidence (for a review, see Hultborn et al., 2004).

With positive loading scores for the first PC (Fig. 1B), the 180 rpm conditions at 100 and 200 W displayed an increase in low-frequency components of the EMG intensity (Fig. 3A, Fig. 5) contrary to the pattern found at 140 and 160 rpm (evident at 75% muscle excitation duration in Fig. 5). The inclusion of the first 20 PCs, to account for a greater proportion of the signal variance, in a further reconstruction, provided added clarity and showed that the increase in frequency later in the muscle excitation duration was in two distinct bands (centred on ~92 and 170 Hz; Fig. 3B). The addition of lower frequency components, the plateau in excitation duration and the non-linear increase in EMG intensity at 180 rpm indicate that there was greater excitation of the muscle, including slow muscle fibres, to achieve the highest cycle frequency. Previous research has shown that mixed muscles can reach higher maximal shortening velocities than those predicted from extrapolation from a Hill-type (Hill, 1938) model (Close and Luff, 1974; Josephson and Edman, 1988). Indeed, recent evidence in rats suggests that the involvement of slow fibres during contractions of mixed fibre-type muscles helps the muscle reach higher shortening velocities (Holt et al., 2014), which is supported here.

This study provides new evidence to support previous research showing early derecruitment of slower muscle fibres during high-frequency cyclical contractions. In addition, the study provides new evidence of the contribution of slow muscle fibres in order to achieve the highest cycle frequencies. In summary, changes to the EMG signal, in both frequency and time, provide evidence of modifications of muscle fibre recruitment strategies depending upon the mechanical demands of a movement.

MATERIALS AND METHODS

Protocol and data collection

Eleven males volunteered to participate in the study. All participants gave their informed written consent to participate before participation in the study. All procedures were approved by the ethics committee in accordance with the Office of Research Ethics at Simon Fraser University. Due to the extreme cycle frequencies required, the participants were trained competitive cyclists (means ± s.e.m.: age 33.9±3.1 years, mass 72.8±2.1 kg, height 179.1±1.9 cm, cycling distance per year 10,773±1575 km).

After a 10 min warm-up consisting of 5 min at 100 W followed by 5 min increasing 20 W every minute, the participants cycled at 40, 60, 80, 100, 120, 140, 160 and 180 rpm at each of 100, 200, 300 and 400 W. Each trial was 30 s in duration followed by 90 s rest, and the trials were completed in a randomized block design. The conditions were first randomized into blocks by cadence, and power output was then randomized within each block. Completing all four power outputs at a single cadence helped facilitate better compliance of the desired cadence. The participants rode an indoor cycle trainer (SRM, Julich, Germany) that was configured as closely as possible to their own racing bicycle, and used their own clipless pedals and shoes.

Muscle excitation of the medial gastrocnemius was monitored continuously using bipolar Ag/AgCl surface EMG electrodes (10 mm diameter and 21 mm spacing; Norotrode; Myotronics, Kent, WA, USA) and sampled at 2000 Hz through a 16-bit analog-to-digital converter (USB-6210; National Instruments, Austin, TX, USA). This muscle was selected as it has previously been used to identify preferential recruitment of faster muscle fibres in response to increasing muscle fascicle strain rates, independent of the level of muscle excitation and muscle fascicle strain (Wakeling et al., 2006). The electrode sites were cleaned with isopropyl and shaved prior to application and the electrodes were secured using stretchable adhesive bandages and tubular net bandages to help reduce movement artifacts. Normal and tangential forces applied to each crank arm, at the pedal–crank arm interface, as well as cycle frequency, were measured (Powerforce, Radlabor, Freiburg, Germany) and recorded at 2000 Hz through the same 16-bit analog-to-digital converter, and subsequently used to calculate power output.

Data analysis

The EMG signals were resolved into intensities in both time and frequency space using wavelets (j=0 to 10) (von Tscharner, 2000) and divided into

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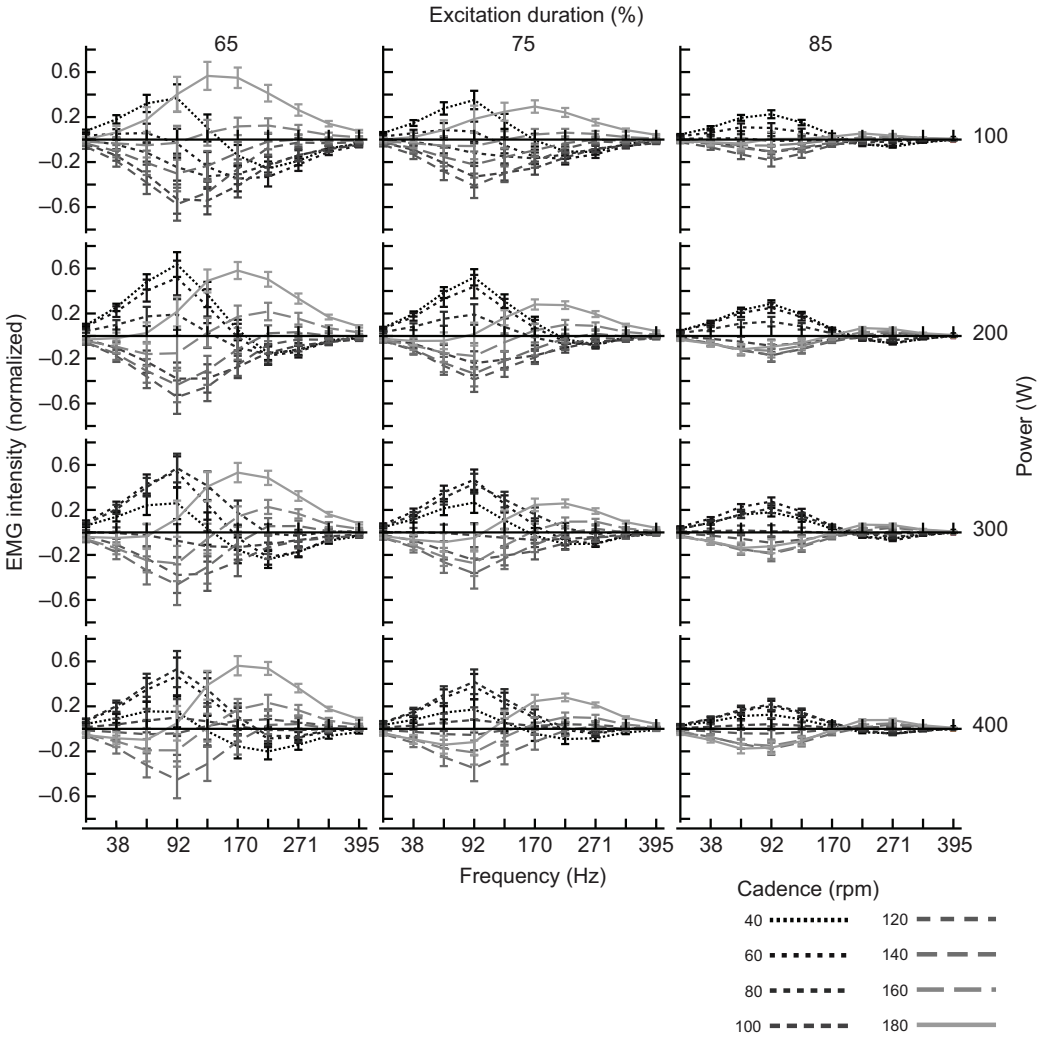

Fig. 5. EMG intensity across the frequency spectra at 65, 75 and 85% muscle excitation duration for each cadence at each power output. This shows evidence of early derecruitment of slower muscle fibres at the end of muscle excitation at the highest cycle frequencies. This is shown in the decrease in intensity for lower frequencies and increase in intensity for higher frequencies at the highest cadences (lighter lines), which is in contrast to the increase in intensity for the lower frequencies and decrease in intensity for the higher frequencies at the lowest cadences (darker lines). At 180 rpm (continuous line) there is also an increase in lower frequencies when compared with 140 and 160 rpm, which is particularly evident at 75% duration. Values shown are means ± s.e.m.

individual pedal cycles. If the intensity of the first wavelet (centre frequency 6.90 Hz) was the greatest of all wavelets for a particular pedal cycle it was considered to have too much noise from movement artifact and was not analysed further. For all subsequent analyses wavelet j=0 was excluded leaving 10 wavelets acting as a band-pass filter between ~11 and 432 Hz. Of these 'clean' pedal cycles, for each subject and each condition, all cycles within 5 rpm of the desired cadence were selected and the middle five steady-state cycles were retained. Total EMG intensity per pedal cycle was calculated as the sum of intensities across all wavelets.

In order to evaluate the frequency components during the primary portion of muscle excitation, an onset/offset threshold of 5% of the difference between the minimum and maximum EMG intensity was selected. The EMG intensities between the onset and offset were used to determine muscle excitation duration and subsequently interpolated to 100 points per wavelet per pedal cycle for all further analyses. The EMG intensities for each pedal cycle were normalized to unit intensity across the entire spectra and the mean was subtracted (100 points per wavelet per pedal cycle) in order to maximize the variance from the mean frequency spectra (Ramsay and Silverman, 2005) and analyse the impact of this frequency shift.

PC analysis was used to identify the primary sources of variability within the frequency spectra across all subjects (Wakeling and Rozitis, 2004). A $p \times N$ matrix **A** was constructed with p=1000 (100 points per wavelet × 10 wavelets) and N=number of pedal cycles. The eigenvector–eigenvalue pairs of the covariance matrix of **A** were calculated where the eigenvectors represented the PC weightings and the eigenvalues represented the amount of the frequency spectra explained by each PC. The product of the transpose of the matrix composed of the PC weightings (eigenvectors) and the original matrix **A** produced the loading scores of each pedal cycle on each PC.

The first two PCs explained a significant portion of the variance from the mean and contained polarized positive and negative aspects that displayed shifts in the time and frequency content of the EMG intensity (Fig. 1A). To visualize the impact of these PCs on each condition the variance from the mean was reconstructed using the vector product of these PC weightings and loading scores (Fig. 3). It was expected that at the highest cycle frequencies and lowest power outputs there would be an increase in high-frequency and decrease in low-frequency components at the end of the burst of muscle excitation, signifying earlier offset of low frequencies relative to high frequencies.

Statistics

General linear model analysis of variance (ANOVA) was used to identify the effect of power output and cadence as well as the level of interaction between power output and cadence on EMG intensity with subject as a random factor. Tukey's *post hoc* test was performed to determine significant differences between power outputs for a given cadence with respect to EMG intensity. In addition, a one-way ANOVA was used to evaluate the effect of condition (cadence–power combination) on EMG intensity with Tukey's *post hoc* test to compare EMG intensities across all conditions. Pearson correlation coefficients were determined between all combinations of total EMG intensity, power output, cadence and the first 20 PC loading scores.

General linear model ANOVAs were also used to determine the effects of cadence and power output on the loading scores for each of the first 20 PCs, as well as the muscle excitation duration. Tukey's *post hoc* tests were performed to identify differences between these durations across all cadences for a given power output and across each power output for a given cadence. In order to test the effects of cadence and power output on the PCs,

loading scores for each PC were tested individually as a dependent variable with subject as a random factor and cadence and power output as fixed factors using subject, cadence and power output in a full factorial design.

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Competing interests
The authors declare no competing financial interests.

Author contributions
O.M.B. and J.M.W. participated in the conception and design of the study as well as the data collection, analysis and interpretation. O.M.B. was the primary author of the manuscript and J.M.W. revised it. Both authors read and approved the final manuscript.

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