ABSTRACT: We have suggested previously that motor units recruited reflexively contribute to torque produced during neuromuscular electrical stimulation (NMES), but this has not been tested directly. The current experiments were designed to quantify the contributions to twitch torque made by motor units recruited at M-wave and H-reflex latencies. The relationship between M-wave amplitude and torque was not linear. Rather, increases in M-wave amplitude caused the largest torque increases when M-waves were small. In addition, the torque contributions made by motor units recruited at M-wave and H-reflex latencies did not sum linearly, as changes in H-reflex amplitude only caused significant changes in torque when M-waves were small ($<18\%$ M_{max}). This nonlinear summation of torque can be explained by the different latencies of twitches evoked by M-waves and H-reflexes. Large M-waves produce strong contractions at a short latency, possibly introducing slack into adjacent muscle fibers and reducing the ability of motor units recruited reflexively to generate torque.

Muscle Nerve **40:** 221–230, 2009

NONLINEAR TWITCH TORQUE SUMMATION BY MOTOR UNITS ACTIVATED AT M-WAVE AND H-REFLEX LATENCIES

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Accepted 1 January 2009

Neuromuscular electrical stimulation (NMES) is traditionally thought to elicit muscle contractions through the direct activation of motor axons.[20] Recently, Collins and colleagues have shown that, during NMES, motor units can also be recruited through a reflex pathway, by which the activation of sensory axons delivers an excitatory input to spinal motor neurons.[3,4,8,13] Directly activating motor axons produces a synchronized burst in the electromyographic (EMG) signal at a relatively short latency (∽5 ms), referred to as the motor wave, or M-wave. Activating large-diameter sensory axons provides excitatory input to neurons in the spinal cord that can evoke a synchronized reflex discharge of EMG activity at a longer latency (∽35 ms), known as the Hoffmann reflex, or H-reflex. At a constant muscle length, the amplitude of the M-wave or H-reflex is monotonically related to the number of motor units recruited through these distinct pathways, as recruiting additional motor units produces larger EMG bursts. The presence of H-reflexes during NMES has been cited as evidence that motor units are recruited reflexively and contribute to torque,[13] although the idea that they contribute to torque has not been tested experimentally. The present experiments were designed to quantify the reflex contribution to the torque produced by single pulses of electrical stimulation, a step toward understanding the importance of a reflex pathway in generating torque during NMES.

The relative amplitudes of M-waves and H-reflexes are influenced by stimulation pulse width, as wider pulses tend to recruit a greater proportion of sensory axons over motor axons.[15] Therefore, for a given M-wave amplitude, wider pulses produce larger H-reflexes.[14,17] Presently, we utilize this relationship between pulse width and H-reflex amplitude, along with the natural variability in H-reflex amplitude, to quantify the contribution to plantarflexion torque made by soleus motor units recruited at M-wave or H-reflex latencies (hereby termed the M-wave or H-reflex contribution to torque). We de-

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Abbreviations: ANOVA, analysis of variance; EMG, electromyographic; H-reflex, Hoffmann reflex; LG, lateral gastrocnemius; MG, medial gastrocnemius; M_{max}, maximum peak-to-peak motor wave amplitude; M-wave, motor wave; NMES, neuromuscular electrical stimulation; TA, tibialis anterior; T_{max}, maximum peak twitch torque

Key words: EMG; motor unit recruitment; muscle contraction; muscle fiber architecture; reflexes

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livered stimulation pulses to the tibial nerve using several pulse widths to generate recruitment curves with a range of H-reflex amplitudes for each M-wave amplitude. This permitted a comparison to be made in which the effect of H-reflex amplitude on twitch torque was isolated.

Earlier work by Maffiuletti and colleagues suggested that the reflex contribution to torque can be predicted from H-reflex amplitude.[16] However, their method requires two assumptions that may not be realistic. The first assumption is that there is a linear relationship between both M-wave and H-reflex amplitude and torque. Although there is an approximately linear relationship between H-reflex amplitude and peak twitch torque for the small range of H-reflex amplitudes that can be elicited below motor threshold[12] (when M-waves are not present), it is not known that this relationship holds true across the entire amplitude range. Presently, there is no direct evidence that the relationship between M-wave amplitude and peak twitch torque is linear. The amplitude of M-waves and H-reflexes depends on the number of muscle fibers activated as well as the muscle architecture, with muscle fibers closer to the recording electrodes having a larger effect. Torque that develops during a muscle twitch depends on the number and type of muscle fibers recruited. These effects of muscle architecture and fiber type may introduce nonlinearities into the relationship between M-wave and H-reflex amplitude and torque.

The second assumption of Maffiuletti's method is that the peak twitch torques produced by the separate motor unit populations (i.e., those recruited at M-wave and H-reflex latencies) summate linearly. In reality, stimulation pulses that only elicit an M-wave generate twitch contractions at shorter latencies and with faster rise times than twitch contractions associated with an H-reflex alone.[2,21] This is a function of the length of each pathway and the types of motor units recruited. Therefore, the peak torques of the twitches associated with M-waves and H-reflexes occur at different times, which we would expect to result in less than linear summation of peak twitch torque. The earlier onset and faster rise time of the contractions produced by the muscle fibers activated at an M-wave latency may also introduce slack into adjacent fibers and reduce their ability to produce torque if they are then activated at an H-reflex latency.

In the present experiments, we quantified the relationship between soleus M-wave and H-reflex amplitude and plantarflexion twitch torque. We hypothesized that information about H-reflex amplitude would not be sufficient to predict the H-reflex contribution to torque due to the complicating effects of muscle architecture, muscle fiber type, and variations in twitch time course. Rather, we hypothesized that the torque contributions of M-waves and H-reflexes would not sum linearly and that the H-reflex amplitude would have a larger effect on twitch torque when M-waves were relatively small.

METHODS

Experiments were conducted on 12 volunteer healthy adult subjects (8 males, 4 females), ranging in age from 21 to 43 years. All subjects gave written informed consent before participation in the study. The experiments were approved by the University of Alberta Health Research Ethics Board and were conducted in accordance with the Declaration of Helsinki.

Experimental Setup. Subjects were seated with their right hip flexed to 110°, right knee flexed to 90°, and right ankle at 90°. The subjects' trunks were at an approximate angle of 20° reclined from the vertical. Flexing the knee to 90° reduces the torque-generating capacity of the two-joint gastrocnemius muscle[5,9,11,19] and allows us to more accurately quantify the relationship between the soleus M-wave, H-reflex, and torque. Knee flexion also reduces the amplitude of gastrocnemius EMG activity,[5,9] lessening the potential impact of cross-talk between muscles. Surface EMG was collected using self-adhesive bipolar electrodes (1′/Ag/AgCl; Disposable A10043 Gel Electrodes; Vermed, Inc., Bells Falls, Vermont) placed over the soleus and tibialis anterior (TA). In 4 of the 12 subjects, surface EMG was also collected from the lateral gastrocnemius (LG) and medial gastrocnemius (MG). Soleus electrodes were placed on the belly of the muscle, aligned with the midline between the LG and MG, approximately 4 cm distal from the gastrocnemius insertion point. TA electrodes were placed over the widest part of the muscle, approximately one third of the distance from the tip of the fibula to the medial malleolus. LG and MG electrodes were placed over the most prominent bulge of the muscle bellies. All electrode pairs were placed to align as closely as possible with the direction of the underlying muscle fibers.

EMG data were amplified by a factor of 1000 and bandpass filtered between 30 and 3000 Hz using P611 AC amplifiers (Grass Instruments, AstroMed, West Warwick, Rhode Island). Isometric plantarflexion torque was measured using a dynamometer (System 3; Biodex Medical Systems, Inc, Shirley, New York), with the lateral malleolus aligned with the dynamometer axis and the foot strapped firmly to the Biodex footplate. Torque data were low-pass filtered at 100 Hz. The EMG and torque data were sampled at 10,000 Hz.

Tibial Nerve Stimulation. A constant current stimulator (DS7A; Digitimer, Inc., Welwyn Garden City, Hertfordshire, UK) was used to deliver rectangular electrical pulses to the tibial nerve. The stimulating electrodes (Ag/AgCl; Disposable A10043 Gel Electrodes; Vermed, Inc.) were placed on the skin of the popliteal fossa along the approximate path of the tibial nerve, with the cathode distal to the anode. TA contractions were minimized by moving the stimulating electrodes if any contractions were observed through visual inspection and palpation during stimulation. The maximal soleus M-wave amplitude (M_{max}) was first determined by increasing the current until the M-wave amplitude reached a plateau. We then applied stimulation pulses with a current that was varied randomly, ranging from sub-threshold to supramaximal, to generate M-wave and H-reflex recruitment curves for the soleus. Stimulation pulses were separated by a random time interval of between 5 and 8 seconds. Soleus M-wave amplitudes were measured in real time following each pulse and normalized by M_{max}.

During data collection, data were sorted online according to the normalized M-wave amplitudes and divided into 50 equally sized bins that spanned the range from 0% to 100% M_{max}. Therefore, each bin had a width of 2% M_{max}, and data with an M-wave of 0–2% of M_{max} were placed in the first bin, data with an M-wave of 2–4% of M_{max} were placed in the second bin, and so on. For a complete recruitment curve, the trial was ended after each bin contained at least one response, thereby requiring a minimum of 50 stimulation pulses. In practice, each recruitment curve included approximately 100 pulses, with some bins containing more than one twitch response (median per bin = 2). In order to produce a range of H-reflex amplitudes for a given M-wave size, we collected recruitment curves using pulse widths of 50 μs, 200 μs, 500 μs, 1 ms, and 2 ms. The order in which these recruitment curve trials were collected was varied randomly. Data collected using all pulse widths were combined for analysis, so for each subject each bin contained a minimum of 5 twitch responses (median per bin = 10). The data collection session was completed in 90 minutes for all subjects.

Data Analysis and Statistics. After data collection was complete, we measured soleus M-wave amplitude, H-reflex amplitude, and peak twitch torque for each stimulation pulse. We also measured the TA M-wave amplitude in all 12 subjects, and the LG and MG M-wave amplitude in the 4 subjects for whom these data were collected. M-waves and H-reflexes were measured peak-to-peak and normalized to M_{max} for that muscle in each subject. Peak torque was normalized to the maximal peak torque (T_{max}) generated across all trials and pulse widths for each subject.

Contributors to Peak Twitch Torque. During the course of each experiment, a given soleus M-wave response amplitude was accompanied by a range of soleus H-reflex, TA M-wave, LG M-wave, and MG M-wave amplitudes. This variability was caused by our use of several stimulation pulse widths as well as normal trial-to-trial changes in EMG responses.

Our initial goal was to determine whether each of these independent measures (soleus H-reflex, TA M-wave, LG M-wave, and MG M-wave amplitude) made a significant contribution to the peak twitch torque. We tested this by sorting each subject's data into 50 bins based on the soleus M-wave amplitude, similar to the process described previously. Each bin contained data points with a soleus M-wave amplitude within a 2% M_{max} range. Therefore, for this analysis, the M-wave was approximately the same amplitude (within 2% M_{max}), but the amplitude of the independent measure (such as soleus H-reflex) varied. We quantified the relationship between each independent measure and peak twitch torque by performing a linear regression for the data in each 2% M_{max} bin. Although the relationship between these independent measures and peak twitch torque may be nonlinear, we used this linear regression analysis as the simplest and most appropriate method of quantifying the expected monotonic relationship. Therefore, for each 2% M_{max} bin, the contribution of each independent measure to peak twitch torque was quantified as the slope of the best linear fit, with steeper slopes indicating that the amplitude of this EMG response had a stronger influence on torque.

To determine whether each of these independent measures had a significant effect on torque, we combined the data from all of the subjects and tested whether these slope values were different than zero. This was done by grouping the calculated slope values across all 50 bins and all subjects and then testing whether these values were significantly different than zero using Wilcoxon's signed-rank test. Statistical significance was accepted at $P < 0.05$. For the purpose of clarity, in what follows we describe the analyses performed on soleus H-reflex amplitude,

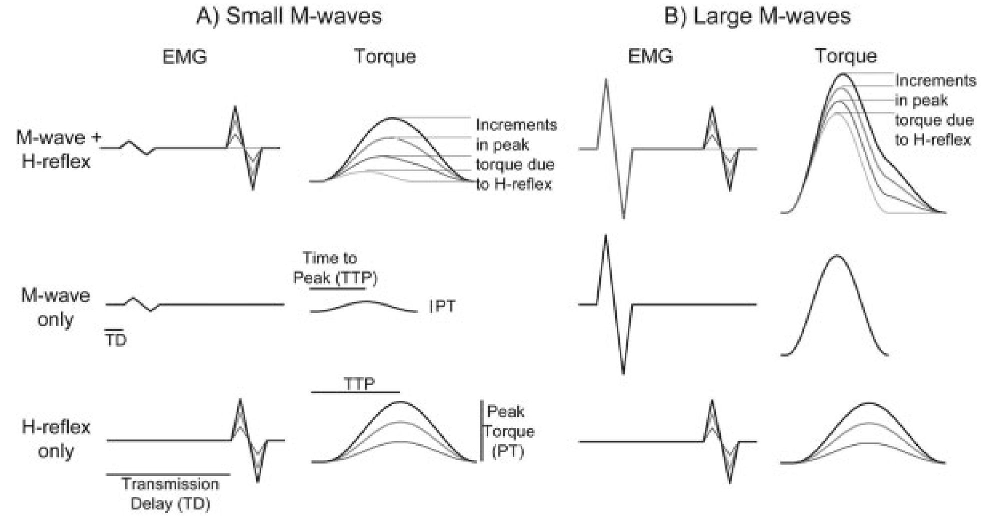

FIGURE 1. Theoretical linear summation of torque produced by M-waves and H-reflexes during twitch contractions. **(A)** Sample EMG and twitch torque traces for stimulation pulses that elicit small M-waves. When both M-waves and H-reflexes are present, larger H-reflexes are associated with higher peak twitch torques (top panel). The theoretical H-reflex contribution to twitch torque can be calculated by subtracting the torque due to the M-wave (middle panel). This subtraction results in the torque profile due to the H-reflex only, or *H-twitch* (bottom panel). **(B)** Sample EMG and twitch torque traces for stimulation pulses that elicit large M-waves. Although the H-reflex and H-twitch amplitudes are identical to those in **(A)**, the effect on peak twitch torque is slightly smaller due to differences in the twitch time course.

which was the only independent measure found to have a significant effect on torque (see Results).

Soleus M-Wave and H-Reflex Contributions to Peak Twitch Torque. The soleus M-wave contribution to peak twitch torque was quantified as the torque produced independent of soleus H-reflex amplitude (the constant term or y-intercept of the linear regression equation; see Results). We subsequently sought to determine whether the contribution of the soleus H-reflex to torque was influenced by the size of the soleus M-wave. We used Friedman's test to determine whether the H-reflex contribution to torque (quantified as the slope value described earlier) varied significantly across the M-wave amplitude bins. If a significant effect was found ($P < 0.05$), Tukey's post hoc test was performed, where appropriate, to determine for which M-wave amplitude bins the H-reflex amplitude significantly influenced peak twitch torque.

For a comparison with the method proposed by Maffiuletti and colleagues,[16] we also performed a linear regression on each subject's data with peak twitch torque as the dependent variable and soleus M-wave and H-reflex amplitudes as the independent variables.

Soleus H-Reflex Contribution to Overall Twitch Torque Profile. The soleus H-reflex contribution to torque for the entire duration of the twitch contraction, rather than just the peak twitch torque, was also quantified. A twitch contraction evoked in association with both a soleus M-wave and an H-reflex may have a torque response that is simply a linear sum of the torque produced by the muscle fibers recruited at an M-wave latency and the torque produced by fibers recruited at an H-reflex latency. Based on this assumption, we can calculate the H-reflex contribution to twitch torque, hereby termed the *H-twitch*, even when we do not have data in which only an H-reflex is evoked. As shown in Figure 1, the torque contributions of H-reflexes with various amplitudes (bottom panel) were calculated by subtracting the torque due to M-waves alone (middle panel) from that due to M-waves and H-reflexes (top panel). In the hypothetical situation shown in the top panels of Figure 1, even though the torque responses to M-waves and H-reflexes sum linearly, identical H-twitch responses can have an effect on peak twitch torque that is dependent, albeit only weakly, upon the size of the twitch produced by the M-wave. In all subjects, for each 2% M_{max} bin we performed a linear regression between H-reflex amplitude and H-twitch peak torque and quantified this relationship as the slope between the two variables.

These individual results were then combined for group analysis. We first performed Wilcoxon's signed-rank test to determine if the slope values were significantly ($P < 0.05$) different than zero. If significance was found, we performed Friedman's test to determine whether the relationship between H-reflex amplitude and H-twitch peak torque varied depending on the M-wave amplitude. Finally, if Friedman's test revealed significance, we performed a Tukey post hoc test to determine for which M-wave amplitudes this relationship was significantly greater than zero.

Maximal Soleus M-Wave Amplitude. For each recruitment curve trial, the maximal amplitude soleus M-wave response was recorded. In order to determine whether the M-wave amplitude changed as a result of the repetitive stimulation delivered over the course of this experiment,[6] we performed a repeated-measures analysis of variance (ANOVA) comparing maximal M-wave amplitude across trial number for the grouped data.

Soleus H-Reflex Responses across Range of Soleus M-Wave Amplitudes. For each subject, we calculated the mean H-reflex amplitude elicited for the twitch responses in each of the 2% M_{max} bins. These data were then combined across subjects to perform group analysis. We performed Friedman's test to determine whether H-reflex amplitude varied significantly ($P < 0.05$) across M-wave amplitude. If a significant effect was found, we determined for which bins the H-reflex amplitude had significantly declined from its peak using Tukey's post hoc test. This analysis was performed to test whether the H-reflex contribution to twitch torque was solely limited by decreases in H-reflex amplitude at higher stimulation currents, as the antidromic block associated with relatively large M-waves can prevent motor units from firing at an H-reflex latency.[10,18]

RESULTS

Soleus H-reflex amplitude had a significant effect on plantarflexion peak twitch torque. Conversely, TA M-wave amplitude, LG M-wave amplitude, and MG M-wave amplitude each did not have a significant effect on peak twitch torque. Using data from all 12 subjects, the calculated slopes between soleus H-reflex amplitude and peak twitch torque were significantly greater than zero ($P = 0.003$), whereas the slopes between TA M-wave amplitude and peak twitch torque were not significantly different than zero ($P = 0.809$). For the subset of 4 subjects for whom gastrocnemius EMG data were collected, the slopes between soleus H-reflex amplitude and peak twitch torque were significantly greater than zero ($P = 0.011$), whereas the slopes between LG and MG M-wave and peak twitch torque were not significantly different than zero ($P = 0.870$ and $P = 0.274$, respectively).

The slope of the relationship between H-reflex amplitude and torque varied depending on the amplitude of the M-wave. For a single subject, Figure 2, shows data from a bin in which M-waves were small (10–12% M_{max}). For this subject, this was associated with H-reflex amplitudes ranging in amplitude from 5% to 17% M_{max}. As can be seen in Figure 2A and B, with these small M-waves, larger H-reflexes were associated with larger peak torques. Similarly, the peak amplitude of the H-twitch torque (the reflex contribution to the overall torque profile calculated by the subtraction method described in Methods and Fig. 1) increased with larger H-reflexes (Fig. 2C). For these data, the relationship between H-reflex amplitude and peak twitch torque had a positive slope (Fig. 2D) (slope = $1.4\%T_{max}/\%M_{max}$). The M-wave contribution was quantified as the y-intercept (y-intercept = 40% T_{max}), because this represents the torque produced that is independent of H-reflex amplitude. There was also a positive slope between H-reflex amplitude and H-twitch peak torque (Fig. 2E) (slope = $1.1\%T_{max}/\%M_{max}$).

For larger M-waves, H-reflex amplitude did not influence torque. This is illustrated in Figure 3 using data from the same subject as for Figure 2. Figure 3 shows data for the bin in which M-wave amplitudes were between 26% and 28% M_{max}. In this case H-reflex amplitudes ranged from 5% to 13% M_{max}. As shown in Figure 3A and B, changes in H-reflex amplitude did not influence peak twitch torque. In addition, the calculated H-twitches did not demonstrate the consistent rising-then-falling torque profile that we would expect from a twitch contraction produced by motor units recruited at H-reflex latency (Fig. 3C). For these data associated with relatively large M-waves, the slope between H-reflex amplitude and peak twitch torque was close to zero (Fig. 3D). Similarly, there was no clear non-zero slope between H-reflex amplitude and H-twitch peak torque (Fig. 3E).

Across subjects, the calculated M-wave and H-reflex torque contributions varied depending upon the amplitude of the M-wave. Larger M-waves (independent of H-reflex amplitude) produced larger peak twitch torques (Fig. 4A). The M-wave contribution to twitch torque increased nonlinearly over the full range of M-wave amplitudes (Fig. 4B), as increases in M-wave amplitude had a larger influence on torque when M-waves were small. In addition, the contribution of H-reflex amplitude to peak twitch torque decreased as M-wave amplitude increased (Fig. 4C). With M-waves >18% M_{max}, H-reflexes did

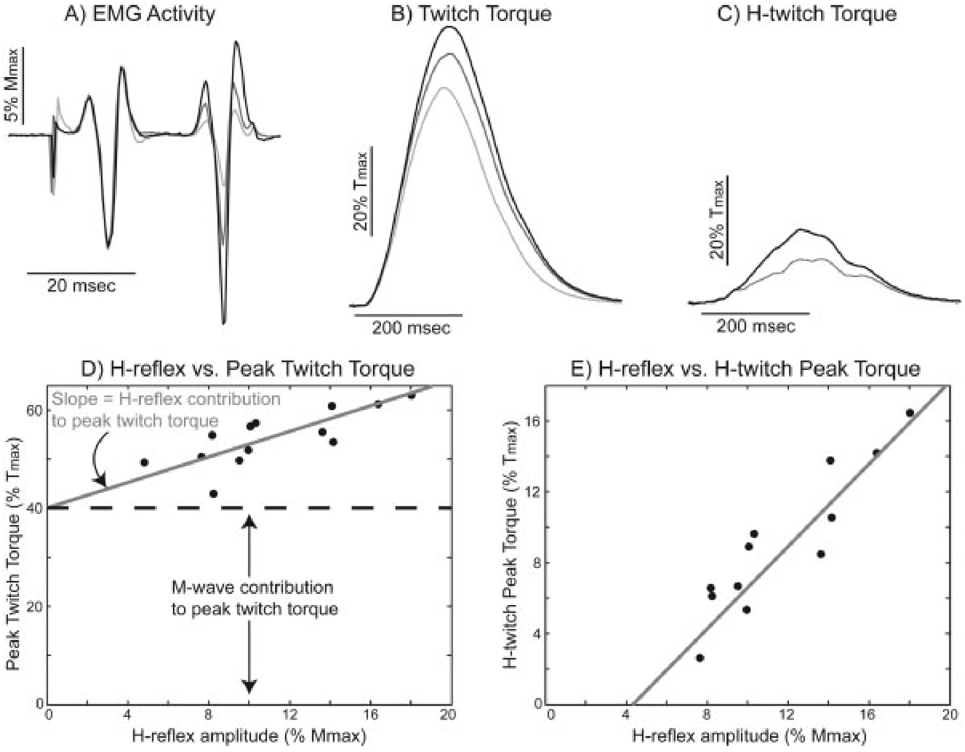

FIGURE 2. For small M-waves, the amplitude of the H-reflex had a significant effect on peak twitch torque and the H-twitch torque. Shown are data from a single subject for the bin in which M-waves were 10–12% M_{max}. **(A)** H-reflex amplitudes ranged from 5% to 17% M_{max}. **(B)** Larger H-reflexes were associated with larger peak twitch torques. **(C)** Larger H-reflexes were also associated with larger H-twitch torques. **(D)** The relationship between H-reflex amplitude and peak twitch torque had a positive slope. **(E)** The relationship between H-reflex amplitude and with peak H-twitch torque also had a positive slope.

not contribute significantly to the peak twitch torque. The relationship between H-reflex amplitude and H-twitch peak torque also was dependent upon M-wave amplitude, as the calculated slope was not significantly different from zero when the M-waves were >14% M_{max} (Fig. 4D).

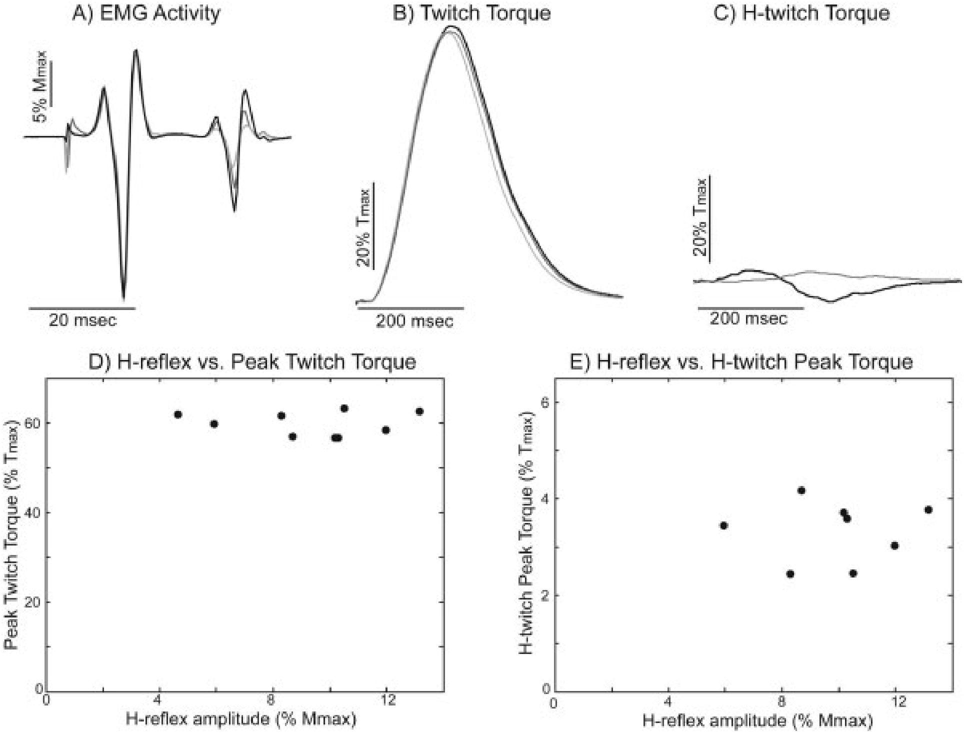

FIGURE 3. For large M-waves, the amplitude of the H-reflex did not have a significant effect on twitch torque. Shown are data from a single subject for the bin in which M-waves were 26–28% M_{max}. **(A)** H-reflex amplitudes ranged from 5% to 13% M_{max}. **(B)** The peak twitch torque was approximately constant despite the different H-reflex amplitudes. **(C)** The calculated H-twitches did not demonstrate a typical twitch torque response. **(D)** The slope between H-reflex amplitude and peak twitch torque was approximately zero. **(E)** The slope between H-reflex amplitude and H-twitch peak torque was also approximately zero.

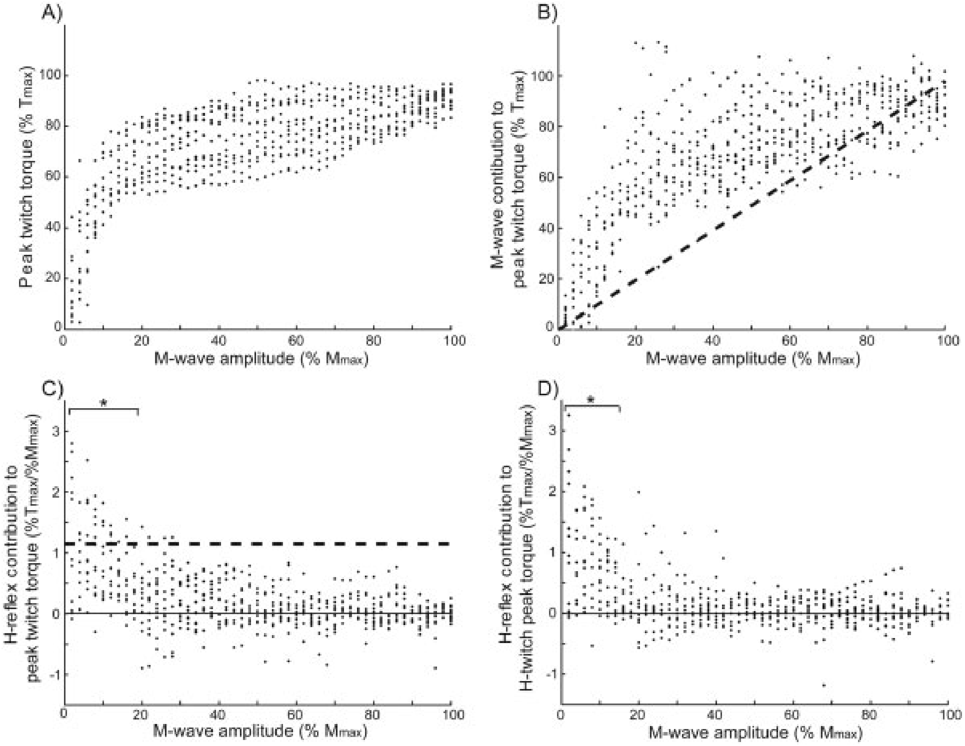

FIGURE 4. M-wave and H-reflex contributions to torque varied with M-wave amplitude. Shown are data calculated for each subject, across the full range of stimulus intensities. **(A)** Peak twitch torque increased with M-wave amplitude. **(B)** The contribution of M-waves to torque increased with larger M-waves. **(C)** The contribution of H-reflexes to torque decreased with larger M-waves. The asterisk shows the region over which H-reflexes made a significant contribution to peak twitch torque (when M-waves were $<18\%$ M_{max}). **(D)** The relationship between H-reflex amplitude and H-twitch peak torque decreased in strength with larger M-waves. The asterisk shows the region over which H-reflexes made a significant contribution to H-twitch peak torque (when M-waves were $<14\%$ M_{max}). The dashed lines in panels (B) and (D) represent the linear regression best fit.

The M-wave and H-reflex contributions to torque can be predicted by a linear regression of peak twitch torque to M-wave and H-reflex amplitudes.[16] This method provided good fits for the data collected in the present study ($R^2 = 0.75$–0.98). Fitting data with a linear regression assumes that both M-wave and H-reflex amplitude are related to peak twitch torque in a linear fashion. The linear regression predictions of M-wave and H-reflex torque contribution across the range of M-wave amplitudes are shown as dashed lines in Figure 4B and C. These predictions largely underestimated the M-wave contribution to torque and overestimated the H-reflex contribution to torque.

There was substantial variation in the maximal M-wave amplitude across subjects, but there was no significant change due to recruitment curve trial number ($P = 0.42$). From the first to fifth trials, the maximal M-wave amplitudes were 6.54 ± 3.55 V, 6.44 ± 3.85 V, 6.36 ± 3.79 V, 6.40 ± 3.95 V, and 6.31 ± 3.82 V, respectively.

Across subjects, the mean H-reflex amplitude recorded had the largest median value when the M-wave amplitude was 14–15% M_{max} (Fig. 5). H-reflex amplitude decreased at larger M-wave amplitudes. However, this decline did not become significant until the M-wave amplitude reached 64% M_{max}. Therefore, we would expect that the H-reflex was of sufficient amplitude to have an effect on peak twitch torque throughout this range of M-wave values.

DISCUSSION

NMES can recruit separate motor unit populations through direct motor axon activation and through a

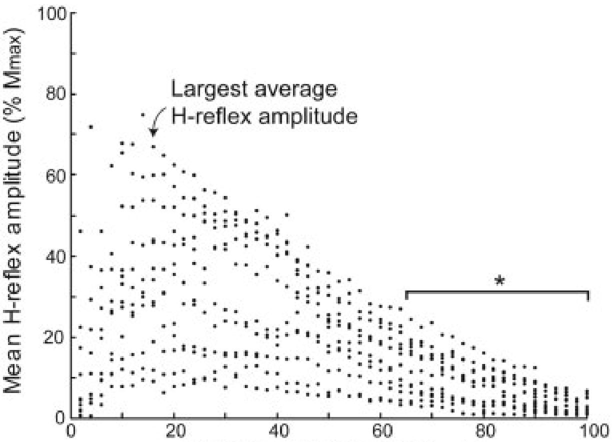

FIGURE 5. Data for each subject showing the average H-reflex amplitude for each bin across the full range of stimulus intensities. The asterisk shows the region (when M-wave amplitudes are $>64\%$ M_{max}) over which the H-reflexes were significantly smaller than those produced with an M-wave of 14–16% M_{max}.

reflex pathway. These populations can be distinguished by the latency at which they fire following each stimulation pulse. By delivering electrical stimulation with varying pulse widths and taking advantage of the inherent trial-to-trial variability in H-reflex amplitude, we evoked soleus H-reflexes with a range of amplitudes for each soleus M-wave amplitude. This enabled us to assess the extent to which transmission along each of these pathways contributes to the torque developed during twitch contractions evoked by single stimulation pulses. We found that there was not a linear relationship between M-wave amplitude and torque. In addition, the H-reflex contribution to twitch torque was significant only when M-waves were relatively small. The torque contributions of M-waves and H-reflexes did not summate linearly, indicating that the reflex contribution to torque during NMES cannot be predicted simply from H-reflex amplitude.

Soleus M-wave amplitude was not linearly related to torque, in contrast to the suggestions of earlier work.[12,16] We found that a given increase in M-wave amplitude was associated with larger increases in torque when the M-waves were small. Therefore, at low currents, direct motor axon activation recruited motor units that had either a relatively small effect on M-wave amplitude (due to a small number of innervated muscle fibers, a deep location of these fibers, etc.), a relatively large effect on peak torque (due to a high torque-generating capacity of the innervated fibers), or both. This nonlinear relationship between M-wave amplitude and torque is likely to be related to the recruitment order of motor units by the direct activation of motor axons, although quantifying that order was beyond the scope of the present experiments.

The finding that the soleus H-reflex contribution to peak twitch torque decreased as soleus M-wave amplitude increased may seem obvious, as antidromic block in motor neurons progressively reduces the amplitude of H-reflexes, whereas M-wave amplitude increases.[10,18] However, increases in M-wave amplitude were associated with decreases in the H-reflex contribution to torque even when relatively large H-reflexes were present. H-reflexes did not contribute significantly to peak twitch torque when the M-wave amplitude was 18–20% M_{max}, despite the fact that relatively large H-reflexes were still present. Therefore, the lack of an H-reflex contribution to torque with large M-waves was not due simply to decreasing H-reflex amplitude.

Increasing soleus M-wave amplitude decreased the influence of soleus H-reflex amplitude on peak twitch torque. In theory, this could occur even if there is linear summation between the torques produced by the muscle fibers recruited at M-wave and H-reflex latencies, simply because the peak torques produced by these two fiber populations do not occur at the same latency after a stimulation pulse. This is illustrated in Figure 1, as an increase in M-wave amplitude caused a slight decrease in the H-reflex contribution to peak torque. If the difference in latency to peak torque were solely responsible for the nonlinearity presently observed, H-reflex amplitude would still influence the overall twitch profile, with a torque contribution quantified in this study as H-twitch. In reality, we found that with large M-waves ($>14\%$ M_{max}) the H-twitch effectively disappeared, as the twitch torque was similar over its entire time course, regardless of H-reflex size. This indicates that with larger M-waves, the muscle fibers recruited at an H-reflex latency were ineffective in producing torque at any point during the twitch contraction. Therefore, the difference in time to peak torque for a twitch produced by an M-wave or H-reflex is not the dominant factor in producing the nonlinear relationship between H-reflex amplitude and twitch torque.

One possible explanation for our primary finding is that, because soleus muscle fibers activated at M-wave and H-reflex latencies are organized in parallel within the muscle, the fibers that contract earlier in time as M-waves may shorten the muscle and introduce slack in fibers that are inactive. Therefore, fibers recruited at an H-reflex latency would first be required to take up this slack before they can produce torque. For small M-waves, we would expect this effect to be minimal, and thus the contribution of H-reflex amplitude to be largest; with larger M-waves, the introduction of slack could eventually result in the muscle fibers recruited at an H-reflex latency not contributing to twitch torque at all. This explanation is consistent with the lack of a significant relationship between H-reflex amplitude and torque, and the disappearance of H-twitches when M-waves were large. The introduction of slack into adjacent fibers by M-waves may be even more prevalent in a muscle with more variation in muscle fiber type than the soleus, which is a relatively homogeneous slow muscle.[1]

The torque generated by soleus M-waves and H-reflexes did not simply increase linearly with their amplitudes, in contrast to one of the assumptions of Maffiuletti and colleagues.[16] For our results, a linear regression method generally underestimated the torque contribution of M-waves and overestimated the contribution of H-reflexes. Despite the nonlinearities in the relationship, a simple linear regression between M-wave and H-reflex amplitude and peak twitch torque can be used to fit the data. For the subjects tested in this study, experimental peak twitch torque and peak twitch torque predicted from a linear regression were related, with R^2 values that ranged from 0.75 to 0.98. Therefore, for the purposes of predicting twitch torque from the EMG activity elicited after the delivery of a single stimulation pulse, Maffiuletti and colleagues' method[16] is effective. However, their method does not address nonlinearities in the relationship that may be important in predicting the relative torque contributions of the direct and reflex pathways.

To assess the relationship between soleus M-wave and H-reflex amplitude and twitch torque, we assumed that all of the torque was due to synchronous activation of soleus motor units. The activation of other muscles, such as gastrocnemius and TA, may have also influenced the measured torque. To address this, subjects' knees were placed at 90° of flexion, shortening the bi-articular gastrocnemius muscle and reducing its torque-generating capacity.[5,9,11,19] We also placed the stimulation electrodes to avoid activating the TA via current spread to the common peroneal nerve. Despite these efforts, it is possible that the contraction of muscles other than the soleus could have affected the measured plantarflexion torque. However, EMG activity in the TA, LG, and MG did not have a significant effect on peak twitch torque, indicating that this effect was relatively minor, and it was not responsible for our results.

Another possible source of error in these experiments is cross-talk between muscles of the lower leg.[7] However, recent work has suggested that cross-talk from the gastrocnemius to the soleus is minimal when bipolar electrodes are used to record soleus EMG data,[22,23] as in these experiments. In addition, if cross-talk from the TA or gastrocnemius had a large effect on the EMG activity recorded from the soleus, we would likely expect the size of TA or gastrocnemius M-waves to significantly influence the peak twitch torque produced in each 2% M_{max} bin. As this was not the case, cross-talk does not appear to be a major contributor to the current results.

We quantified the contributions of direct motor axon activation and synaptic recruitment through a reflex pathway to the torque produced during a muscle twitch. We have previously proposed that motor units activated through this reflex pathway contribute to the torque produced during NMES.[3,13] The current work was a first step toward quantifying the reflex torque contribution. Presently, we propose that, with larger M-waves, H-reflex amplitude does not contribute to peak twitch torque because of the introduction of slack into the reflex-recruited muscle fibers. With tetanic stimulation this may be less of an issue, as continuous activation could take up this slack when a muscle contraction is sustained. Nevertheless, the results from the present experiments indicate that the torque produced due to the reflex recruitment of motor units cannot be predicted simply from the associated H-reflex amplitude. Any prediction must take into account nonlinearities in the relationship between EMG and torque.

This work was supported by the Alberta Heritage Foundation for Medical Research, the Canadian Institutes of Health Research, and the Natural Sciences and Engineering Research Council of Canada.

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