Validation of Hill-type muscle models in relation to neuromuscular recruitment and force-velocity properties: predicting patterns of \textit{in vivo} muscle force

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Manuscript: (7138 words total; 5455 text body) includes 6 figures and 0 tables.
Abstract

We review here the use and reliability of Hill-type muscle models to predict muscle performance under varying conditions, ranging from *in situ* production of isometric force to *in vivo* dynamics of muscle length change and force in response to activation. Muscle models are frequently used in musculoskeletal simulations of movement, particularly when applied to studies of human motor performance in which surgically implanted transducers have limited use. Musculoskeletal simulations of different animal species also are being developed to evaluate comparative and evolutionary aspects of locomotor performance. However, such models are rarely validated against direct measures of fascicle strain or recordings of muscle-tendon force. Historically, Hill-type models simplify properties of whole muscle by scaling salient properties of single fibers to whole muscles, typically accounting for a muscle’s architecture and series elasticity. Activation of the model’s single contractile element (assigned the properties of homogenous fibers) is also simplified and is often based on temporal features of myoelectric activation (EMG) recorded from the muscle.

Comparison of standard one-element models with a novel two-element model and with *in situ* and *in vivo* measures of EMG, fascicle strain, and force recorded from the gastrocnemius muscles of goats shows that a two-element Hill-type model, which allows independent recruitment of slow and fast units, better predicts temporal patterns of *in situ* and *in vivo* force. Recruitment patterns of slow/fast units based on wavelet decomposition of EMG activity in frequency-time space are generally correlated with the intensity spectra of the EMG signals, the strain rates of the fascicles, and the muscle-tendon forces measured *in vivo*, with faster units linked to greater strain rates.
and to more rapid forces. Using direct measures of muscle performance to further test
Hill-type models, whether traditional or more complex, remains critical for establishing
their accuracy and essential for verifying their applicability to scientific and clinical
studies of musculoskeletal function.
Over the prior three decades, Hill-type muscle models have gained broad use for assessing the functions of muscles across different movements and for analyzing changes that occur, for example, with aging, neuromuscular-related diseases, or rehabilitation from injury (Anderson and Pandy, 2003; Fox et al., 2009; Hall et al., 2011; Hamner et al., 2010; Peterson et al., 2011; Thelen, 2003). In large part this reflects their use in studies of human motor performance, for which surgically-implanted transducers to assess muscle function are challenging and have limited use.

Consequently, despite considerable progress incorporating Hill-type models into simulations of walking, running, and other dynamic movements (Delp et al., 2007), the accuracy of in vivo muscle forces predicted by these models largely remains unknown. Musculoskeletal simulations that rely on Hill-type models are also being developed to evaluate comparative and evolutionary aspects of the locomotor performances of different animal species (Full and Ahn, 1994; Kargo et al., 2001; and see Rankin et al., 2014 in a related symposium paper) as well as to infer the locomotor capability of fossil taxa (Hutchinson and Garcia, 2002). We review here past work that has attempted to validate Hill-type muscle models and the recruitment patterns of slow versus fast motor units derived from wavelet-decomposition techniques (von Tscharner, 2000; Wakeling and Rozitis, 2004; Wakeling and Syme, 2002) in relation to muscle force and work. We also review our own efforts to develop improved Hill-type models validated against forces and fascicle strains measured from the lateral (LG) and medial (MG) gastrocnemius muscles of goats across differing locomotor conditions.

Validation of Hill-type models against in vivo measurements is critical for improving their predictive accuracy and for establishing the reliability of
simulations that depend on them for studies relevant to human health and comparative locomotive performance. Hill-type models characterize the force generated by a fiber as a function of its activation and its instantaneous length and velocity. The force-length (F-L) and force-velocity (F-V) properties of the muscle often are based on data combined from studies of different muscles in different animal species. Limited, and largely indirect, data are available for human muscles. In cases for which F-L and F-V data are obtained from ectothermic species (e.g. frog or toad), the properties must be adjusted by estimates of thermal effects on contractile performance of muscles in endothermic species. To represent the whole muscle, the properties of a single fiber are scaled to represent the whole muscle, typically accounting for the muscle’s architecture and series elasticity. Despite these simplifying limitations, Hill-type models are widely adopted for clinical applications due to their computational efficiency and relative simplicity. However, traditional formulations of Hill-type models have limitations that diminish their accuracy, particularly when assessing in vivo motor tasks that involve time-varying, submaximal contractions.

A few previous in situ studies have examined the accuracy of time-varying muscle forces predicted by Hill-type models. For example, Sandercock and Heckman (1997) examined whether a traditional Hill-type model could predict the forces generated by the cat soleus in situ, imposing length changes and near-tetanic stimulation corresponding to slow-speed locomotion. Their model reproduced measurements of in vivo force to within 10% of maximal tension when force was rising but overestimated force by 30% during relaxation. In a related experiment,
Perreault et al. (2003) evaluated a Hill-type model of the same muscle via the cross-extension reflex pathway, also imposing length changes corresponding to locomotion. This study revealed root-mean-square errors (RMSE) in force that were greater than 50% when motor unit firing rates were low (10-20 Hz), the rates most relevant to natural movements. These and other studies, e.g. van Ingen Schenau et al. (1988), including our own work (e.g. Lee et al., 2013b; Wakeling et al., 2012), have confirmed that Hill-type models are sensitive to assumptions about activation and the implications of these assumptions. Intrinsic properties, in particular force-velocity properties, contribute greatly to the errors in force predicted by these models (Perreault et al., 2003).

The work we review here is directed toward improving the accuracy with which a muscle’s force and work output (force x fascicle length change) during movement can be estimated both for clinical applications and for studies directed at understanding the comparative and evolutionary significance of variations in muscle architecture and musculoskeletal organization. Our work tests the hypothesis that a two-element Hill-type model, which allows for independent and time-varying recruitment of slow and fast contractile elements, predicts the time course of force rise, the peak force, and the time course of force relaxation more accurately than traditional one-element models across varying locomotor conditions.

Our work also seeks to evaluate whether the force-velocity properties of a muscle are affected by submaximal recruitment of either slow-fiber or fast-fiber fractions of the muscle.
Materials and Methods

Six African pygmy goats (*Capra hircus* L; 3 males, 3 females; mass 25.85 ± 6.20 kg) were tested at Harvard University’s Concord Field Station. The experimental protocol involved four main steps over a three-day period: surgical implantation of transducers, *in vivo* testing, surgical implantation of nerve cuffs, and *in situ* testing. Details of these protocols are described elsewhere (Lee et al., 2011; Wakeling et al., 2012; Lee et al., 2013a) and are reviewed here in brief.

Collection of experimental data

Goats were trained to walk, trot, and gallop on a treadmill on level and inclined grades. Varying the speed of the treadmill during level locomotion elicited the most pronounced differences in recruitment (Lee et al., 2013a). Thus, trials in which goats walked, trotted, and galloped on the level grade were used to test the different models. Prior to testing, the MG and LG muscles were each instrumented with three pairs of fine-wire offset twist silver EMG electrodes (California Fine Wire, Inc.) to measure excitation (Lee et al., 2011) and a pair of 2.0 mm sonomicrometry crystals (Sonometrics, Inc.) to measure fascicle length at mid-belly (resolution = 0.3 µm; Gillis et al., 2005). A custom-fabricated “E”-shaped buckle force transducer (Biewener and Baudinette, 1995; McGuigan et al., 2009) was used to measure force at the Achilles tendon (post-mortem calibrations yielded $r^2 > 0.99$). Lead wires from all transducers were fed through a subcutaneous tunnel to a connector that was sutured to the skin proximal to the hip. Animals received post-operative analgesia (buprenorphine 0.1 to 0.5 mg/kg, subcutaneously) during a 24-hour recovery
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No animals showed signs of post-operative infection (no antibiotics were administered).

In vivo lateral and medial tendon forces were estimated from the total in vivo tendon force, measured by the single force buckle on the tendon, using the ratio of the maximum lateral and medial forces measured from isolated in situ recordings from two tendon buckles (see below). Tendon forces were normalized by the maximum force, $F_{\text{max}}$, recorded during trotting on the incline, consistent with previous analyses (Lee et al., 2013b).

In vivo data were recorded at 5000 Hz while goats walked, trotted, and galloped on the treadmill (Lee et al., 2013b). EMG signals were amplified (gain of 100-1000) and recorded with minimal filtering (bandpass 30-3000 Hz, notch at 60 Hz using P511J amplifiers, Grass Technologies). Between 15 and 20 strides were recorded for each condition. Sufficient rest was given between trials to ensure that the goats could perform steadily at each speed and gait.

Following completion of the in vivo experiments, the goats were deeply anesthetized (isoflurane to effect) and the tibial component of the sciatic nerve exposed and isolated deep to the biceps femoris muscle. Tripolar nerve cuff electrodes (constructed of silver wire and silastic tubing) were then placed on isolated bundles of the tibial nerve innervating the LG and on those innervating the MG. The electrodes were constructed to provide both stimulating and blocking impulses to the nerve (see Wakeling and Syme [2002] and Lee et al. [2011] for further details). The blocking electrode was located distal to the stimulating electrode, and a 1000 Hz blocking stimulus used to preferentially excite slower
motor units and to block fast units. The peroneal nerve was cut to prevent reflex feedback. An additional force transducer was surgically attached on the medial portion of the Achilles tendon.

To carry out in situ recordings of the muscles’ forces and lengths under twitch and tetanic conditions, each animal was placed on its right side while its left hind limb was secured in a customized stereotactic frame (80/20 Inc.) using stainless steel bone pins inserted into the femur and tibia. The animal and muscles were kept warm with a heating pad and heat lamp, with temperatures recorded by a thermistor and maintained at \( 33 \pm 2 \, ^\circ\text{C} \). In situ recordings were made to measure the muscles’ active and passive force-length relationships using tetanic stimulation (supramaximal: 1.5x the voltage required to elicit maximal isometric force) for a range of ankle angles. The tibial nerve was stimulated under computer control (Labview 7.1, National Instruments Corp; A320R, World Precision Instruments). A series of different nerve-stimulation protocols were used to elicit twitches from different populations of motor units, thereby enabling identification of the twitch profiles for both slow and fast motor units (see Lee et al. [2011] for details).

**EMG analysis of motor recruitment**

To extract information about the motor units recruited during walking, trotting, and galloping, the spectral properties of the EMG signals corresponding to signals from slow motor units and from fast motor units were identified using wavelet analysis (von Tscharner, 2000; Wakeling and Rozitis, 2004; Lee et al., 2011); this technique allowed different frequency bands (101 to 1857 Hz)
represented in the EMG signals to be identified as functions of time. Major features of the EMG intensity spectra associated with recordings from the goats’ LG and MG muscles were identified using principal components (PC) analysis (Fig. 1).

Reconstruction of the spectra from the PC weightings and their loading scores showed that the majority (75-97%) of the EMG signal for any given spectrum could be reconstructed using the first two principal components (I and II). PCI loading scores provide a measure of the total EMG intensity, while PCII loading scores relative to PCI loading scores provide a measure of the relative frequency content within the signal (Hodson-Tole and Wakeling, 2007; Wakeling, 2004).

To quantify the contributions of high-frequency versus low-frequency content within the EMG signal, we calculated the angle (θ), formed by plotting the PCI loading score against the PCII loading score (Fig. 1; Wakeling, 2004; Wakeling and Rozitis, 2004; Hodson-Tole and Wakeling, 2007). Small values of θ have a positive PCII contribution and indicate a relatively large high-frequency content, whereas large values of θ have a negative PCII contribution and indicate a relatively large low-frequency content (Fig. 1B). On this basis, we used the angle θ to assess patterns of motor unit recruitment. To identify recruitment patterns directly from the raw EMG signals, optimized wavelets were derived, yielding center frequencies for slow (242.3 Hz) and faster motor units (532.7 Hz) (Lee et al., 2011; Wakeling, 2004; Hodson-Tole and Wakeling, 2008).

Evaluation of Hill-type Muscle Models

The muscles’ activations derived from the EMG recordings were used to test...
the predictive accuracy of a novel, two-element Hill-type model against in vivo
measurements (see Fig. 2) of muscle force and fascicle strain (normalized by
optimal fascicle length). Three simpler one-element models were also examined: (i)
a homogenous model, which estimates muscle force using average properties of the
fibers corresponding to slow and fast motor units; (ii) a hybrid model, which
estimates muscle force using the maximum shortening velocity of the fast motor
units and a force-velocity curvature that is weighted based on the proportion of
slow and fast fibers; and (iii) an orderly recruitment model, which assumes that the
active muscle displays intrinsic properties of progressively faster motor units as
activation levels increase. In this model, active fibers are assigned the intrinsic
properties of slow fibers at low activation. This model is based on classical studies of
orderly recruitment during steady stretch reflexes (Henneman et al., 1974) and
follows previous modeling approaches (Umberger et al., 2003; van Soest and
Bobbert, 1993).

The inputs to each model included the time-varying fascicle lengths, which
were also used to calculated fiber contractile velocity and pennation angle, and the
activations states. To drive the muscle models, the EMG intensities were used as
excitations for a series of coupled first-order differential equations, constituting
transfer functions (Lee et al., 2011) that enabled estimation of the activation level of
each muscle. Activation levels were normalized to the maximum activation during
incline trotting, consistent with our procedure for normalizing the measured tendon
forces. Transfer functions were derived for the whole muscle, the slower motor
units, and the faster motor units using in situ data pooled from the six goats; see Lee
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et al. (2011). For further details regarding muscle architecture and fascicle strain inputs to the models, and the means by which slow and fast motor units where characterized, see Lee et al. (2013). All simulations were run using Mathematic (Wolfram Research Inc., Champaign, IL).

For the three one-element models (homogenous, hybrid, orderly), the active component of the muscle fiber force was given by:

\[ F_f = a(t) F_a(l) F(v) \]  

(1)

where \( a(t) \) is the time-varying level of activation (Lee et al., 2011), normalized to the maximum activation measured during the in vivo experiments (Lee et al., 2013b), and \( F_a(l) \) is the active force-length relationship, normalized to a maximum of 1 and determined from in situ tetanic F-L measurements. Fiber length \( (l) \) and contractile velocity \( (v) \) also varied with time \( (t) \).

The two-element model incorporated independently-activated slow and fast contractile elements in parallel (Wakeling et al., 2012). The EMG intensities at the low-frequency and high-frequency bands (determined from the EMG signals and optimized wavelets) were used with the transfer functions to estimate the activation levels for the slow and fast elements, \( a_{\text{slow}}(t) \) and \( a_{\text{fast}}(t) \), respectively. The active component of the total muscle fiber force was thus given by:

\[ F_{f,\text{tot}} = F_{f,\text{slow}} + F_{f,\text{fast}} \]  

(2)

where \( F_{f,\text{slow}} \) and \( F_{f,\text{fast}} \) are the normalized forces for slow and fast fibers, respectively, as determined from Equation 1 using fiber-specific values of \( a(t), v_0 \) (the maximum unloaded shortening velocity), and F-V curvature (see Lee et al., 2013b for further details).
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For the one-element models, whole muscle force was estimated from the active fiber force as follows:

\[ F_m = c[F_f + F_p(l)] \cos \alpha \] (3)

where \( F_f \) is the active component of the fiber force (Eqn. 1) and \( F_p \) is the passive component of force as a function of fiber length \( l \), as measured in situ. Constant \( c \) (peak isometric force measured for each muscle) and pennation angle \( \alpha \) scaled the fiber force to the muscle. By comparison, whole muscle force generated by the 2-element model was estimated from the active fiber forces generated by the independently-activated slow and fast contractile elements:

\[ F_m = c[F_{f,\text{slow}} + F_{f,\text{fast}} + F_p(l)] \cos \alpha \] (4)

Pennation angle was calculated at each time step from the resting pennation angle and the fascicle length, assuming that the thickness of the muscle remained constant (Zajac, 1989; Millard and Delp, 2012). The inputs to each model included the time-varying fascicle lengths measured by sonomicrometry, which were also used to calculate the fiber contractile velocity, the pennation angle, and the activation levels.

Because muscle models are generally sensitive to the maximum unloaded shortening velocity (Wakeling et al., 2012), two sets of values for \( v_0 \) were tested: one set used values (2.74 and 3.59 \( l_{\text{opt}} \) s\(^{-1}\) for slower and faster fibers) estimated from in situ measures of activation rates scaled by literature values across a range of species (Lee et al., 2013b); the other set used values (5 and 10 \( l_{\text{opt}} \) s\(^{-1}\) for the slow and fast fibers) based on previous models of larger species (Wakeling et al., 2012). The force-velocity relationship was characterized by its curvature, \( k \), which depended on fiber type (Otten, 1987). In the two-element model, we used curvature...
values of 0.18 and 0.29 for slow fibers and fast fibers, respectively (Wakeling et al. 2012). In the one-element models, we used an intermediate value of $k$ to represent muscles with mixed fiber types. Values for all parameters in the models are given elsewhere (Lee et al., 2011 & 2013b).

**Statistics**

Simulations were run for the different model formulations (4), goats (6), muscles (2), EMG electrodes (3), gaits (3), assumed fiber-type proportions (2), and sets of $v_0$ values (2) using custom software (Mathematica Wolfram Research Inc., Champaign, IL). Differences between the predicted forces and the measured forces (normalized to the maximum force measured *in vivo*, for each goat, during trotting on the incline) were characterized by the coefficient of determination ($r^2$) and by the root mean-square error (RMSE). To determine if $r^2$ and RMSE differed between goats, models, LG and MG muscles, type of gait, fiber-type proportion, and $v_0$, we used a General linear model repeated-measures multi-factor Analysis of Variance with the following independent variables: goat (random), models, muscles, gait, fiber-type proportion, and $v_0$. Statistical differences were considered significant at the $\alpha = 0.05$ level. Tukey *post-hoc* analyses were conducted to identify significant differences between levels within a factor, as appropriate. All statistical tests were conducted using Minitab Statistical Software (Minitab Inc, State College, PA, US).

**Results**

The LG and MG showed similar patterns of EMG activity in relation to
measurements of fascicle strain and muscle-tendon force across different gaits, speeds, and grades (Fig. 2). In both muscles, EMG intensity increased significantly with increased speed on the level and was greatest during trotting on the incline (Lee et al., 2013a). LG and MG forces paralleled the changes in EMG intensity, increasing with locomotor speed and grade and indicating a correlated increase in muscle force with increased motor recruitment. LG fascicles and MG fascicles both shortened to produce work for most of limb support during walking, trotting, and galloping. Normalized shortening rates of LG fascicles ranged from an average of -0.98 l_{opt} s^{-1} during level walking to -2.02 l_{opt} s^{-1} during uphill trotting over the concentric phase of muscle force production; shortening rates of MG fascicles similarly ranged from -1.09 l_{opt} s^{-1} during level walking to -1.67 l_{opt} s^{-1} during uphill trotting (Lee et al., 2013a).

Measurements of motor unit recruitment, derived from wavelet decomposition of the EMG recordings in frequency-time space (Fig. 3A), showed that the LG was preferentially recruited at faster speeds on the level surface, particularly at a gallop, whereas MG was recruited more strongly on the inclined surface (Fig. 3B). Additionally, the LG showed greater recruitment of fast units (lower \theta) compared with the MG, consistent with its greater percentage of fast versus slow fibers (myosin I: 31.0 \pm 2.6%; myosin IIa: 69.0 \pm 2.6%) compared with the MG (myosin I: 49.4 \pm 4.7%; myosin IIa: 50.6 \pm 4.7%; n=5)(Fig. 4). Neither muscle expressed myosin IIb or IIx fibers (unpublished results, Carr, Miara & Biewener). The frequency content of the EMG signals recorded from the LG during locomotion on the level was significantly associated with the fascicle shortening
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velocity, the rate of force rise, and the rate of force relaxation (Lee et al., 2013b).

We tested the ability of a novel two-element Hill-type model, which allows independent activation of slow and fast contractile elements over time, to predict the patterns of force rise and relaxation that we measured in vivo. We also tested three traditional one-element models (homogenous, hybrid, and orderly). The three one-element models predicted force magnitudes and rates of rise and relaxation that were not significantly different (p>0.05 for all comparisons). Consequently, the following results focus on a comparison of the 1-element orderly recruitment model with the 2-element model.

The two-element model and (all of) the one-element models that we tested generally captured the detailed changes in MG and LG forces measured in vivo (Fig. 5) during walking, trotting, and galloping. However, all models exhibited errors that included inaccurate estimates of the peak forces developed by the muscles and, in particular, insufficient rates of force rise and decline. Across all models, the RMSE (as a percentage of the maximum in vivo force measured during trotting on the incline) ranged from 17.0% to 32.2% for the LG and from 9.6% to 15.5% for the MG (Fig. 6).

Across the different gaits examined, the two-element model generally performed better than the one-element models. In particular, the two-element model predicted time-varying LG forces with statistically higher $r^2$ (better correlation) and lower RMSE (less error) than did the orderly one-element model, both during galloping ($r^2$, $p = 0.03$; RMSE, $p = 0.001$; Tukey post hoc test) and during trotting (RMSE, $p < 0.001$; Fig. 6). The two-element model also predicted time-
varying MG forces with significantly lower RMSE than did the orderly one-element model during trotting and galloping (p = 0.001 and p = 0.01; Fig. 6). No differences were observed in terms of \( r^2 \) or RMSE for walking.

ANOVA revealed that the accuracy of predicted \textit{in vivo} force was dependent on the muscle, the gait, and the choice of \( v_0 \) for the models. The models generally predicted force more accurately for the MG than for the LG, with higher \( r^2 \) and lower RMSE for walking and galloping (p < 0.001). Adjusting \( v_0 \) significantly influenced the accuracy of the predicted LG forces (\( r^2 \) p < 0.001), with the faster values of 5 and 10 \( l_{opt} \text{ s}^{-1} \) providing a better fit (mean \( r^2 = 0.37 \)) than the experimentally-determined set of \( v_0 \) of 2.74 and 3.59 \( l_{opt} \text{ s}^{-1} \) (mean \( r^2 = 0.34 \)). The performance of the models also differed significantly across the different gaits (\( r^2 \), p < 0.001; RMSE, p < 0.001; Fig. 6).

**Discussion**

The work reviewed here provides the first attempt to validate Hill-type muscle models against directly measured \textit{in vivo} muscle forces and extends earlier work (Perreault et al., 2003; Sandercock and Heckman, 1997) to test Hill-type models using measurements of \textit{in situ} force. Comparisons of force patterns predicted from the models and measured \textit{in vivo} show generally good agreement in the overall patterns and magnitudes of goats’ MG and LG forces across different gaits. However, errors in peak force and, in particular, errors in the rates of force rise and decline are apparent in the forces predicted from the models relative to those measured \textit{in vivo}. Interestingly, the predicted rise in force matches the
measured in vivo force more accurately during locomotion at slower speeds (walking), whereas the predicted decline in force matches the in vivo force more accurately during locomotion at faster speeds (e.g., galloping (Fig. 5)). Such inaccuracies in force rise and decline, relative to the length changes of muscle fibers, will affect calculations of work and power produced by a muscle over the course of a stride. Our past work (Wakeling et al., 2012) of testing these models against in situ data also indicates that the accuracy of a Hill-type model’s predictions is particularly susceptible to values of shortening velocity used in the model.
that achieve an accuracy with RMS errors, as observed here, ranging from 17.0 to 32.2\% for the goat LG and from 9.6 to 15.5\% for the goat MG (Fig. 6).

Although the three different one-element models all provide reasonably accurate fits to time-varying force, our two-element model that allows independent activation of slow and fast contractile elements shows an improved fit to time-varying force with RMS errors that were significantly diminished during trotting and galloping (but not during walking) (Fig. 6). Nevertheless, both a two-element model that considers independent recruitment of slow and fast motor units and the more traditional one-element models tested retain significant error in the rates of force development and relaxation that they predict (Fig. 5). Such errors likely can be reduced by refining measurements of the contractile properties (intrinsic shortening velocity, F-V curvature, and history-dependent F-L effects) used in the models. A key limitation of our studies was our reliance on in situ measurements of twitch force rise and relaxation rates, rather than determining F-V properties and $v_o$ directly for goat MG and LG muscles. Our past work (Wakeling et al., 2012) indicates that the accuracy of a Hill-type model’s predictions is particularly susceptible to values of $v_o$. When validating our muscle models against in vivo data, we obtained better fits when we used higher values of $v_o$ than when we used those values estimated from our in situ twitch data (Lee et al., 2013b).

Additionally, in recent studies that analyzed the effects of differential, submaximal recruitment of slow versus fast motor units of the rat plantaris muscle in situ (Holt et al., 2014), we found that the muscle’s F-V properties were most strongly affected by the fraction of motor units activated, rather than by whether
the activated fraction of the muscle was comprised of mainly slow or mainly fast motor units. The maximum shortening velocity of the muscle as well as its F-V curvature were greatest when the entire muscle was activated; these attributes were reduced significantly when either the muscle’s faster motor units or slower motor units were activated alone. Force rise time also was significantly longer when the whole muscle was activated than when only the slow fraction or the fast fraction was activated. These results indicate that significant inertial, viscous, and elastic effects operate, in addition to motor unit recruitment, and influence the output of the whole muscle. Recent studies of frog and turkey muscles also show important interactions between the contractile and series elastic elements of a muscle-tendon unit (Azizi et al., 2008; Azizi and Roberts, 2010) that affect the operating length, velocity, and net power output of the muscle’s fibers.

A more rapid rate of force development but slower rate of muscle shortening, as evidenced by our studies of the rat plantaris when fractional portions of the muscle (whether slow or fast) are recruited (Holt et al., 2014), suggests that such effects likely underlie the limitations of our models of goat muscles to predict accurately in vivo force rise and relaxation rates. Under most in vivo conditions, muscles are submaximally activated. Thus, although inertial, viscous, and elastic effects are challenging to assess, such effects in addition to time-varying recruitment patterns of slow versus fast motor units will need to be incorporated into future phenomenological Hill-type muscle models. Additionally, more accurate and realistic architectural features of muscles, particularly those that allow for muscle bulging and that account for muscle gearing (Azizi et al., 2008; Wakeling et al.,
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...will need to be incorporated, as these features will help to model the contractile behavior of whole muscle based on scaled properties of slow and fast muscle fibers. Finally, further validation of muscle models against direct measures of muscle performance in vivo will remain critical for evaluating their accuracy and their reliability for application to studies of musculoskeletal function.

Differences in architecture, fiber composition, and intrinsic motor unit properties of the LG and MG muscles (Wakeling et al., 2011; Maganaris et al., 1998; Lee et al., 2013a,b) may explain why the models, on average, predicted force more accurately for the MG than for the LG. The MG and LG are thought to have different motor unit twitch profiles (Lee et al., 2011; Vandervoort and McComas, 1983), and these differences also may explain why the 2-element model predicted force more accurately for the MG than for the LG.

This review summarizes the main findings from a multifaceted and integrated set of studies involving in vivo and in situ experiments. Each of the different facets is subject to specific assumptions and limitations that should be considered when evaluating the global accuracy, validity and usefulness of these findings. The models presented here are driven in a forward-dynamics manner using the EMG signals to drive the activation levels of the muscles. The PC analysis for the EMG explained 76% of the signal for PC I and II. In a companion in situ and isometric study using the same animals and EMG placements, PC I and II explained 87% of the signal (Lee et al. 2011): the unexplained component of signal would have included signal noise, and the fact that the spectral components of each motor unit action potential for indwelling EMG studies are very dependent on the location
and orientation of the electrodes relative to the active motor units. The additional 11% of the signal unexplained in the in vivo data reported here likely contained movement artifacts, and contributions from the muscle length, temperature, and fatigue that fluctuate during each recording session (Wakeling et al. 2001). However, the advantage of the use of PC analysis to decode the EMG signals is that it extracts major components of the signal and effectively filters out these lower order effects (Wakeling, 2009), which are not accommodated by the models under evaluation. The activation levels of the muscles, as calculated from the EMG intensities, used to drive the models (whether the total intensity, or the fast- or slow- components) are all derived from the same raw signals and, thus, would be expected to show similar onset and offset times with similar time-courses for the predicted forces. However, where the EMG intensities largely differ is in their relative magnitude, and this is the important feature that drives the differences in the model performances.

The reviewed studies show that 2-element models are more effective at predicting muscle forces when they are driven by the EMG signal-derived active states. What is remaining to uncover is how such models would respond, or even be implemented, in inverse-dynamics simulations of muscle force. Simulations can be used to predict the muscle excitations based on driving forces and dynamics (Crowninshield and Brand, 1981; Thelen et al. 2003; Delp et al. 2007). However, the introduction of multi-element muscles results in the possibility that each muscle is underdetermined with no unique set of fast- and slow- activations for the contractile elements. Predicted activations would thus need to be determined by
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Optimization; however, the appropriate physiological cost functions that determine motor unit recruitment patterns may be multifactorial and have yet to be fully established (Hodson-Tole and Wakeling, 2009). This is clearly an area for future research, to establish an appropriate framework to evaluate multi-element muscle models within inverse-dynamic simulations.

Acknowledgements.

Support for participation in this symposium was provided by the Company of Biologists, and the Society for Integrative and Comparative Biology (Divisions of Vertebrate Morphology, Comparative Biomechanics, Neurobiology, and Animal Behavior), in addition to NIH grant R01AR055648, which funded the work reviewed here. We thank Drs. Jennifer Carr, Maria Miara, and Natalie Holt for their expert assistance with the experiments that were carried out at Harvard University and Pedro Ramirez for care of the animals.

Figure Legends

Figure 1. Schematic showing (A) principal component (I and II) weightings across the frequency spectra of a wavelet-decomposed EMG signal and (B) PCI loading score plotted against PCII loading score. The example vectors show PCI versus PCII loading scores when slow motor units are predominantly recruited (large $\theta$, negative PCII loading) and when fast motor units are primarily recruited (low $\theta$, positive PCII loading). (C) Center frequencies of the optimized wavelets derived from EMG recordings (Lee et. al., 2011). (D) Using these optimized wavelets, raw
EMG signals (gray) recorded from the MG muscles of goats during walking on the incline and galloping on the level, for example, can be decomposed to show that faster motor units are recruited at higher intensities during galloping than during walking. In each plot, the data corresponding to slower (red) and faster (blue) motor units are differentiated by color.

**Figure 2.** Representative *in vivo* patterns of total rectified EMG intensity, force, and fascicle strain (normalized by optimal length) for the LG (A-C) and MG (D-F) of goats across different locomotor tasks, normalized over a full stride. Gait and grade are denoted by color, as indicated. These patterns match those recorded previously from goats during *in vivo* locomotor activities (McGuigan et al., 2009).

**Figure 3.** (A) Principal component (I and II) loading scores obtained from our analysis, following wavelet decomposition, of EMG signals recorded from goats’ LG and MG muscles during locomotion. In combination, PCI and PCII explain about 76% of the EMG signals recorded from the muscles. (B) PCI loading score plotted against PCII loading score for the MG (solid) and LG (dashed) during the stance phase of different locomotor tasks, as indicated by the different symbols. Angle θ quantifies the contribution of low-frequency versus high-frequency content in the EMG signal (smaller values of θ indicate higher-frequency content). Results are from Lee et al. (2013a).

**Figure 4.** Light micrograph (400x mag.) of portions of the goat LG and MG muscles
stained immunohistochemically for type IIa (fast oxidative-glycolytic) myosin-specific antibody. Light fibers express type I (slow-oxidative) myosin. No fibers in either the LG or MG of goats stained for myosin type IIx or type IIb. From Carr, Miara and Biewener (unpublished).

**Figure 5.** Normalized forces recorded *in vivo* from goats’ LG and MG across different gaits (walking, trotting, and galloping) compared with those predicted by an orderly recruitment 1-element model and by a 2-element model. These are representative data with $r^2$ and RMSE values that are similar to the mean $r^2$ and RMSE values of all the simulations. Muscle-tendon forces were normalized relative to those recorded during trotting on a slope. Average changes in force over time are shown. Original data reported by Lee et al. (2013b.)

**Figure 6.** Root mean-square errors (RMSE) of the LG and MG forces predicted by the one-element and two-element models (expressed as a percentage of the maximum *in vivo* force measured during trotting on an incline). Values are the mean ± s.e.m. (n=6 goats). The two-element model was assigned $v_o$ values of 5 and 10 $l_{opt}$ s$^{-1}$ for the slow and fast fibers, respectively, and a proportion of 75% fast fibers. The one-element and two-element models showed significant differences (denoted by horizontal bars with the indicated $p$-value) as determined by *post hoc* Tukey tests. Original data reported by Lee et al. (2013b.)
Developing and validating muscle models against in vivo data

References


