Comparison of the validity of Hill and Huxley muscle tendon complex models using experimental data obtained from rat m. Soleus \textit{in situ}

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Abstract

The relationship between mechanical and metabolic behaviour in the widely used Hill muscle-tendon complex (MTC) model is not straightforward, while this is an integral part of the Huxley model. In this study we assessed to what extent Huxley and Hill type MTC models yield adequate predictions of mechanical muscle behaviour during stretch-shortening cycles (SSC). In fully anaesthetized male Wistar rats (N=3), m. Soleus was dissected completely free, except for the insertion. Cuff electrodes were placed over the n. Ischiadicus. The distal end of the tendon was connected to a servo motor, via a force transducer. The setup allowed for full control over muscle stimulation and length, while force was measured. Quick release and isovelocity contractions (part 1), and SSC (part 2) were imposed. Simulations of part 2 were made with both a Hill and a Huxley MTC model, using parameter values determined from part 1. A modification to the classic two-state Huxley model was made to incorporate series elasticity, activation dynamics and active and passive force-length relations. Results were similar for all rats. Fitting of the free parameters to data of part 1 was near perfect ($R^2 > .97$). During SSC, predicted peak force and force during relaxation deviated from the experimental data, for both models. Overall, both models yielded similarly adequate predictions of the experimental data. We conclude that Huxley and Hill MTC models are equally valid with respect to mechanical behaviour.

Introduction

All human locomotor tasks (e.g. walking, cycling, rowing, swimming) involve periodic movements. Both mechanical behaviour and metabolic energy expenditure are important variables in the study of such movements. A musculoskeletal model that can accurately predict both is therefore an essential tool in biomechanics research. Currently, the Hill muscle model (Hill, 1938) is most used in musculoskeletal modelling (e.g. Delp et al., 2007; Lee et al., 2013; Lai et al., 2014; Biewener et al., 2014; Lee et al., 2013). Musculoskeletal models using the Hill model have been successfully applied to study phenomena in which only mechanical behaviour is considered (e.g. Van Soest et al., 1993; Bobbert, 2012; Azizi and Roberts, 2010; Lai et al., 2014). However, in the Hill model a direct relation between metabolic and mechanical behaviour is absent. Attempts have been made to establish such a relation (Anderson and Pandy, 2001; Bhargava et al., 2004; Lichtwark and Wilson, 2005a), but these involve adding extra, phenomenological relations to the model, which introduces extra parameters, the values of which are not straightforward to determine. Moreover, it is unclear whether metabolic cost models based on heat terms obtained in stylized experiments, can be generalized to real life stretch-shortening-contractions (SSC). This problem may (partially) explain the lack of success in predicting whole body metabolic energy expenditure in human walking using such musculoskeletal models (Anderson and Pandy, 2001). An alternative to the Hill muscle model is the Huxley model (Huxley, 1957), which models the dynamics of cross-bridge cycling and as such, does entail a direct relation between metabolic and mechanical behaviour. The original Huxley model and multi-state variations thereof have been widely studied, but mainly to address fast time scale phenomena on the single fibre level (e.g. muscle’s transient response to a quick release or stretch), which could not be explained by the Hill model (Huxley and Simmons, 1971; Eisenberg and Hill, 1978; Tözeren, 1985; Campbell, 2009; Stoecker et al., 2009). In explaining the latter phenomena, series elasticity and/or submaximal activation are not modelled. However, both tendons (Alexander, 2002; Roberts, 2002; Lichtwark and Barclay, 2010; Lai et al., 2014) and the time course of muscle activation (Van Soest and Casius, 2000; Lichtwark and Wilson, 2005b) play a crucial role in the functioning of human muscle-tendon complexes (MTC) in vivo. Thus, if the Huxley model is to be used in
musculoskeletal modelling, it should be implemented as a MTC model, which includes modelling of series elastic structures and activation dynamics. Although several attempts have been made (Comincioli et al., 1984; Julian, 1969; Williams, 2011; Stoecker et al., 2009), the latter is mathematically complicated, and difficult to formulate in a generic way, such that the MTC model can be incorporated in a larger musculoskeletal model. Moreover, the Huxley model was previously considered to be computationally too demanding for use in musculoskeletal modelling (Van den Bogert and Cole, 1998; Winters and Stark, 1987; Zahalak, 1981; Cadova et al., 2012). Zahalak (1981) mitigated the computational issues by introducing the simplifying assumption that the distribution of the fraction of attached cross-bridges always follows a gaussian curve: the distribution moment (DM) approximation. Many authors aiming to describe macroscopic muscle behaviour using the Huxley model have adopted the DM approach (Cadova et al., 2012; Winters and Stark, 1987; Cholewicki and McGill, 1995; Gielen et al., 2000; El Makssoud et al., 2011). Notwithstanding the important contribution the DM approach has made to the usage of cross bridge models in MTC modelling, it is problematic that, in many applications, it is impossible to establish to what extent the assumption on the distribution affects the predicted muscle behaviour (Wu et al., 1997; Tözeren, 1985). Moreover, the computational issues mentioned above are no longer relevant at present. In the present study, the original two-state Huxley model was extended to include both activation dynamics and parallel/series elasticity, and was formulated as a generic state-space model. As an important step towards implementation of the Huxley model in musculoskeletal modelling, the aim of the present study was to assess the validity of the latter model, with respect to mechanical behaviour. To evaluate the relative merit of the Huxley MTC model in the context of large scale musculoskeletal modelling, its validity was compared to that of the Hill model. Although widely used in musculoskeletal modelling, there are only a few studies in which the Hill model is validated for realistic muscle contractions and in which parameters were estimated from data independent of the data used to validate the model (Krylow and Sandercock, 1997; Sandercock and Heckman, 1997; Williams et al., 1998; Lee et al., 2013). Therefore, as recently pointed out by Biewener et al. (2014), there is an ongoing need for validation of the Hill model. As such, a secondary aim of the present study was to contribute to previous work in which the Hill model was validated for realistic muscle contractions. To these ends, data was collected from rat m. Soleus (SOL) in situ. The experimental data was divided in two parts. Data from the first part was used for the estimation of parameter values for both models. Data from the second part, consisting of realistic stretch shortening cycles, was used to evaluate model performance, by comparing the experimental data to model predictions thereof.

Methods and materials

Model formulation

Both the Hill and the Huxley models consisted of an active, contractile element (CE) and a parallel elastic element (PEE), which were both aligned in series to a series elastic element (SEE), as depicted in Fig. 1. The following relations can be readily deduced from Fig. 1:

\[ l_{CE} + l_{SEE} = l_{MTC} \]  

\[ F_{CE} + F_{PEE} = F_{SEE} \]

with \( l_{CE} \) and \( F_{SEE} \) denoting CE length and SEE force, respectively. Note that in the present paper, velocities of muscle components are defined as the time derivatives of their lengths. Consequently, muscle velocity during shortening is defined as negative velocity. Both PEE and SEE are modelled as quadratic springs, similar to Blümel
\[ F_{EE} = \begin{cases} \epsilon_{EE}(l_{EE} - l_{EE}^0)^2, & \text{if } l_{EE} \geq l_{EE}^0 \\ 0, & \text{otherwise} \end{cases} \]  

(3)

with \( \epsilon_{EE} \) and \( l_{EE}^0 \) the Elastic Element shape parameter and slack length, respectively. For both models, the normalized CE force-length relation is modelled as a 4th order polynomial:

\[ F_{CE}^{\text{isom}} = \epsilon_{CE}^{\text{norm}} \left( \frac{b_{CE}}{l_{CE} - l_{CE}^0} - 1 \right)^4 + 1 \]  

(4)

with \( F_{CE}^{\text{isom}} \), \( \epsilon_{CE}^{\text{norm}} \) and \( b_{CE} \), CE isometric force normalized by maximal CE isometric force at optimal CE length, the CE normalized shape parameter and CE length normalized to CE optimum length, respectively. For both models, activation dynamics, governing the relation between muscle stimulation and the fraction of cross-bridges participating in the contractile process (active state, \( q \)), were incorporated similar to Curtin et al. (1998). Active state is dependent on the free Ca\(^{2+}\) concentration in the sarcoplasmic reticulum (\( \gamma \)), according to a saturating, sigmoid relation:

\[ q = \left( 1 + \kappa^n \right) \frac{\gamma^n}{\gamma^n + \kappa^n} \]  

(5)

with \( \kappa \) equal to the value of \( \gamma \) when 50% of the cross-bridges is participating in the contractile process and \( n \) the coefficient expressing the cooperativity of binding. Note that, in comparison to the model by Curtin et al. (1998) the first factor in Eq. (5) is added to ensure \( q(1) = 1 \), and that \( q = \max(q, q_{\text{min}}) \) with \( q_{\text{min}} \) the minimum level of activation. The dependency of the free Ca\(^{2+}\) concentration on stimulation (\( \text{stim} \)) is modelled as a first order dynamical system:

\[ \dot{\gamma} = \begin{cases} \frac{\text{stim} - \gamma}{\tau_{\text{act}}}, & \text{if } \text{stim} \geq \gamma \\ \frac{\text{stim} - \gamma}{\tau_{\text{deact}}}, & \text{otherwise} \end{cases} \]  

(6)

with \( \tau_{\text{act}} \) and \( \tau_{\text{deact}} \) the activation and deactivation time constant, respectively, and the superimposed dot representing differentiation with respect to time.

**Hill model** The concentric part of the Hill force-velocity relation is modelled according to Van Soest et al. (1993):

\[ \dot{b}_{CE}^{\text{rel}} = \frac{b_{CE}^{\text{rel}} \left( F_{CE}^{\text{rel}} - q \cdot F_{CE}^{\text{isom}} \right)}{F_{CE}^{\text{rel}} + q \cdot a_{CE}^{\text{rel}}} \]  

(7)

with \( a_{CE}^{\text{rel}} \) and \( b_{CE}^{\text{rel}} \) the Hill constants, defining the shape of the hyperbola and \( F_{CE}^{\text{rel}} \) the CE force normalized by maximal CE isometric force at optimal CE length. At low values of \( q \), maximal CE shortening velocity decreased with decreasing \( q \), the so called ‘orderly recruitment’ model (cf Van Soest et al., 1993; Camilleri and Hull, 2005) by scaling \( b_{CE}^{\text{rel}} \) with:

\[ b_{\text{scale}}^{\text{rel}} = \left( 1 - \left( 1 - b_{\text{min}}^{\text{scale}} \right) \left( \frac{q - q_{\text{crit}}}{q_{\text{min}} - q_{\text{crit}}} \right) \right) \]

if \( q \leq q_{\text{crit}} \), with \( b_{\text{min}}^{\text{scale}} \) the minimal value of the scale factor at \( q = q_{\text{min}} \). To ensure maximum CE contraction velocity is not affected by CE length at CE lengths above optimum, \( a_{CE}^{\text{rel}} \) was scaled by the isometric force from Eq. (4) if \( l_{CE}^{\text{rel}} \geq l_{CE}^{\text{opt}} \).
The eccentric part of the Hill force-velocity curve is modelled as a hyperbola with an oblique asymptote. The hyperbola is defined by the ratio between the concentric and eccentric slope in the isometric point (r_slope), and the intercept of the asymptote with the ordinate (F_asympt). The slope of the asymptote is equal to the slope of the concentric part at q = q_min and l_CE = (1 + 0.95) · l_CE_opt.

Huxley model  The Huxley MTC model used in the present study is a modified version of the classic two-state model (Huxley, 1957), in which the time course of the distribution of the fraction of attached cross-bridges (n), over their bond length (x) is modelled:

\[ \frac{\partial n}{\partial t} - u \frac{\partial n}{\partial x} = f(x) - (f(x) + g(x)) n \]  

with f(x) and g(x) the attachment and detachment rate functions, respectively. The input variable u is the relative velocity of the actin and myosin filaments, and is linearly dependent on CE velocity according to:

\[ u = \frac{s}{2h \cdot l_CE} \hat{l}_CE \]  

with h the maximum bond length at which the myosin head can attach and s the sarcomere length. In order to make the the model suitable for simulation in a larger, musculoskeletal framework, Eq. (8) is transformed to a set of ordinary differential equations by method of characteristics (Zahalak, 1981):

\[ \frac{dt}{d\lambda} = 1 \]
\[ \frac{dn}{d\lambda} = f(x) - [(x) - f(x)] n \]
\[ \frac{dx}{d\lambda} = u \cdot 1 \]

with bold font denoting vector notation and 1 a vector of size x with all entry’s equal to 1. The top equation can be integrated to obtain \( \lambda = t \), resulting in:

\[ \frac{dn}{dt} = f(x) - [(x) - f(x)] n \]
\[ \frac{dx}{dt} = u \cdot 1 \]  

Assuming that each attached cross-bridge exerts a force proportional to its excursion x, CE force is proportional to the first order moment of the distribution \( n(x, t) \), according to:

\[ F_{CE} = \frac{F_{CE \max}}{I_{ss}^0} \cdot \int_{-\infty}^{\infty} n(x, t)x \, dx \]  

with \( I_{ss}^0 \) the first order moment of the steady state solution of Eq.’s (10), evaluated at \( u = 0 \). The effects of the force length relation can be incorporated in the CE model as follows. Consider an isometric CE contraction. Following a step in CE length, x will change instantly, which will result in a step in force, according to eq. (11). However, the new distribution \( n(x, t) \) cannot reach steady state instantaneously, as the attachment and detachment of cross-bridges is a dynamic process. As such, it is appropriate to include the force length relation in Eq.’s (10) as a scale factor of the steady state solution. Similar reasoning can be applied to the inclusion of active state: assuming muscle active state represents the fraction of M-sites available for binding, a step in q will not result in a
step in CE force, but rather in a dynamic response of \( n(x, t) \) to a new steady state. Therefore, in contrast to other recent studies in which activation dynamics were included in the Huxley model (Campbell, 2009; Stoecker et al., 2009; Zahalak and Motabarzadeh, 1997; Cadova et al., 2012), both active state and the force length relationship are included in the present Huxley CE model as a scale factor to the steady state solution of Eq.’s (10), resulting in:

\[
\begin{align*}
\frac{dn}{dt} &= q \cdot F_{CE}^{isom} \cdot f(x) - [g(x) - f(x)] n \\
\frac{dx}{dt} &= u \cdot 1
\end{align*}
\]

which constitutes the equations governing the dynamics of the distribution \( n(x, t) \) in the present study. For the rate functions \( f(x) \) and \( g(x) \) Huxley chose piecewise linear functions, in which the probability of attachment increased with bond length. The motivation for this choice was purely mathematical. In the present study we also chose a simple form of the rate functions, which reflects intuitive assumptions one might have about their form; \( f(x) \) is modelled as a piecewise constant function:

\[
f(x) = \begin{cases} 
    f_1, & \text{if } 0 \leq x \leq h \\
    0, & \text{otherwise}
\end{cases}
\]

with \( f_1 \) the attachment rate parameter. \( g(x) \) is modelled as two quadratic functions with both their extreme values and shape parameters determined by a single parameter:

\[
g(x) = \begin{cases} 
    g_2 x^2 + g_2, & \text{if } x \leq 0 \\
    g_3 (x-h)^2 + g_3, & \text{if } x \geq h \\
    0, & \text{otherwise}
\end{cases}
\]

with \( g_2 \) and \( g_3 \) the detachment rate parameters. Note that \( g(x) = 0 \) in the region where cross-bridges can attach (i.e. Huxley’s \( g_1 = 0 \), our notation thus agrees with Zahalak (1981)), which is normally not the case since this would result in zero energy expenditure during steady state isometric contraction. However, in the present study only mechanical behaviour is considered and it was found that the force velocity curve can be adequately modelled while constraining \( g_1 \) to be zero (see Fig. 5D). Allowing the value of \( g_1 \) to be determined in the optimization aimed at describing the force-velocity data was found not to improve the quality of the fit. We emphasize that, if data on energy consumption would be available, \( g_1 \) would have been made a tunable parameter, used to fit the additional data regarding metabolic energy expenditure. This would have resulted in values for the other rate parameters that are different from those reported here.

The stepwise nature of Eq.’s (13) and (14) required a small integration step size during simulations. Therefore, to improve numerical tractability, the rate functions were smoothed using a sine function in an interval of \( 0.1 \)h around the transition points, resulting in rate functions with a continuous first derivative, as depicted in Fig. 2. It was found that the smoothing did not materially change model behaviour.

Assuming equidistantly spaced \( x \), a given CE length completely determines \( x \), according to Eq. (9). Therefore, for given \( l_{MTC} \), \( n \) and \( \gamma \), a corresponding value for CE length can be found such that the resulting distribution \( n(x) \) satisfies Eq.’s (1) and (2). Moreover, once \( l_{CE} \) is known, the first equation of Eq.’s (12) can be evaluated. For inputs \( l_{MTC}(t) \) and \( stim(t) \), \( [n \ \gamma]^T \) is thus a state vector of the Huxley MTC model. However, finding \( l_{CE} \) which satisfies Eq.’s (1) and (2) involves numerically estimating the root of a nonlinear function. As the latter will have to be done at each integration step during a simulation, this is computationally highly inefficient. Therefore, in
In the present study, we chose to include $l_{CE}$ in the (now quasi-)state vector: $[n \hspace{1mm} l_{CE} \hspace{1mm} \gamma]^T$. However, as shown above, Eq.'s (1) and (2) can already be satisfied from $[n \hspace{1mm} l_{CE} \hspace{1mm} \gamma]^T$, and cannot be used to compute $\dot{l}_{CE}$, as is done in the Hill model. Instead, as was also conceived by Stoecker et al. (2009), we consider the question: if, at the current state, Eq. (2) is satisfied, which value of $\dot{l}_{CE}$ ensures that Eq. (2) is still satisfied in the next instance. In other words, the time derivative of Eq. (2) should be satisfied:

$$\dot{F}_{CE} + \dot{F}_{PEE} = \dot{F}_{SEE} \tag{15}$$

The time derivative of SEE and PEE force is:

$$\dot{F}_{EE} = \begin{cases} \frac{k_{EE}(l_{EE}) \cdot \dot{l}_{EE}}{l_{EE}^0}, & \text{if } l_{EE} \geq l_{EE}^0 \\ 0, & \text{otherwise} \end{cases} \tag{16}$$

with $l_{PEE} = l_{CE}$, $l_{SEE} = l_{MTC} - l_{CE}$ and $k_{EE}(l_{EE}) = 2c_{EE}(l_{EE} - l_{EE}^0)$. Let us further define:

$$I_{hx} = \frac{F_{max}^{CE} \cdot \int_{-\infty}^{\infty} \dot{n}(x, t) x \, dx}{I_{ss}^{CE}}$$

$$I_{n} = \frac{F_{max}^{CE} \cdot \int_{-\infty}^{\infty} n(x, t) \, dx}{I_{ss}^{CE}}$$

The time derivative of CE force then equals:

$$\dot{F}_{CE} = I_{hx} + I_{n} \cdot \frac{s}{2h \cdot l_{opt}^{CE}} \cdot \dot{l}_{CE} \tag{17}$$

Substituting Eq.'s (16) and (17) in Eq. (15), and solving the resulting equation for $\dot{l}_{CE}$ yields:

$$\dot{l}_{CE} = \frac{I_{n} \cdot \frac{s}{2h \cdot l_{opt}^{CE}} \cdot \dot{l}_{MTC} - I_{hx}^{CE} \cdot k_{SEE}(l_{SEE}) \cdot \dot{l}_{SEE}}{I_{n} \cdot \frac{s}{2h \cdot l_{opt}^{CE}} + k_{PEE}(l_{PEE}) + k_{SEE}(l_{SEE})} \tag{18}$$

Note that with $[n \hspace{1mm} l_{CE} \hspace{1mm} \gamma]^T$ as a quasi state, combinations of $n$ and $l_{CE}$ can occur that violate Eq. (2). Thus, the initial conditions must be chosen such that Eq. (2) is satisfied at $t = 0$, and the difference between CE and SEE force must be monitored throughout the simulation. In all simulation results presented here, the difference between CE and SEE force remained below $10^{-8}$ N.

**Experiment**

**Surgery and setup** All experiments, animal handling and procedures were approved by the animal ethics committee of the VU University Amsterdam (protocol number FBW 12-03). Data was collected from male, Wistar rat m. Soleus (SOL) (n=3, see Table 1 for rat and muscle mass). We chose SOL for this study because it is a parallel-fibred, homogeneous, fatigue-resistant muscle, which means ignoring these respective properties in our modelling efforts will have minimal influence on our results. After anaesthetization, which consisted of two doses of 6 mL urethane per 1 kg bodyweight, 15 minutes apart, the hind body of the rat was shaved bare and the skin opened and partly removed. Subsequently, SOL was exposed by dissecting surrounding muscles. Care was taken to maximally separate SOL from all its surrounding tissue, except for the origin and the neurovascular tract, which passes through the m. Gastrocnemius Lateralis (GL). Specifically, the n. Plantaris was removed,
and all connections to the m. Plantaris (PL) and m. Gastrocnemius Medialis (GM) were severed. The complete Achilles tendon was dissected free and at its insertion a small part of the calcaneus was cut and left attached to the tendon, leaving the complete Soleus MTC intact. The operation technique proved crucial for the success of the experiments, as exemplified in Fig. S1. After dissection of the SOL, the distal end of the Achilles tendon was tightly knotted to a steel rod, using the leftover part of the calcaneus. The femur was made accessible (blunt dissection) and attached to a screw terminal. Finally, a cuff electrode was placed over the n. Ischiadicus and connected to a direct current stimulus isolator (Digitimer Limited, Hertfordshire, UK, model DS3). Hereafter, the rat was fixated on the experiment table by securing the screw terminal and fixating the foot. The steel rod was connected to a servo controlled, linear motor (custom made, VU University, Amsterdam, The Netherlands), via a force transducer (miniature strain gauge transducer, Honeywell International Inc., Morrice Township, New Jersey, USA). In Fig. 3 an overview of the setup is depicted. The resolution of the entire setup was 10 mN and 1.0 µm, for force measurement and length manipulation, respectively. All data was collected at 1000 Hz. The laboratory was climate-controlled and kept at a constant temperature of 24 °C and humidity of 80%.

<table>
<thead>
<tr>
<th>Table 1. Rat and m. Soleus mass.</th>
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<tr>
<td>Rat mass (g)</td>
</tr>
<tr>
<td>m. Soleus mass (g)</td>
</tr>
</tbody>
</table>

**Measurement protocol**  At the start of each trial, the MTC was brought to its desired initial length by a manually operated micromanipulator (analogue resolution of position control 0.1 mm). After each trial the muscle was shortened below slack length, by the same means. During all measurements, the muscle was kept moist by applying saline after each trial. In order to prevent fatigue, time between trials was at least 2 minutes. First, supramaximal current amplitude was determined at a length close to MTC optimum length, by increasing the amplitude until peak force during a tetanic stimulation no longer increased. During the experiment, maximal isometric force was monitored to ensure the chosen current amplitude still resulted in supramaximal stimulation. Supramaximal current amplitude was approximately 0.5 mA in all experiments. Stimulation frequency and pulse width were 100 Hz and 10 µs in all experiments. MTC optimum length was found iteratively in a series of trials, by applying tetanic stimulation at increasing MTC length (1 mm per step) until maximum force decreased. Next, the muscle was alternately stimulated isometrically at optimum length and at short length, until forces at both lengths stabilized.

For part 1 of the experiment, the applied contraction protocols were based on assumptions following the Hill MTC model. To collect data pertaining to the force-length relations of SEE, PEE and CE, 8 to 10 contractions with a quick release protocol were applied, similar to Blümel et al. (2012), at different MTC lengths ranging from active slack length to 1 mm over optimum MTC length, in 1 mm steps. The quick release protocol started with an isometric phase until force levelled off, followed by a rapid 0.1 mm MTC length decrease. Stimulation ceased shortly thereafter (Fig. 4A). Next, to collect data pertaining to the concentric part of the CE force-velocity relation, a series (15-20) of step-ramp contractions were applied, similar to Blümel et al. (2012) and Curtin et al. (1998). Each step-ramp started with an isometric phase at MTC optimum length, followed by a step decrease in length and immediately thereafter a ramp shortening (Fig. 4B), at a velocity intended to result in constant force during the ramp. The protocol was applied at different combinations of step sizes and ramp slopes, such that the forces measured during the ramp covered the force range from close to 0 to maximal isometric force. To collect data
pertaining to the eccentric part of the CE force-velocity relation, a series (4-6) of isokinetic, eccentric contractions were applied. Each contraction started isometrically at MTC optimum length followed by an eccentric ramp lengthening (Fig. 4C). Note that the eccentric contraction protocol was applied (contrary to the order in which it is listed here) at the very end of the experiment, to minimize the influence of any adverse effects of the lengthening contractions. As we did not have access to biochemical measures in the present study, the parameters pertaining to the activation dynamics were estimated by fitting them on behavioural data, similar to Curtin et al. (1998). These data included several trials of the previous protocols and a single isokinetic release trial, which consisted of an isometric force build up, followed by isovelocity shortening, at a velocity close to the maximum MTC velocity encountered during the stretch-shortening cycles (SSC) of part 2 of the experiment (see below). Stimulation was ceased at onset of the shortening phase. In part 2 of the experiment, (SSC) were imposed on the muscle. Each SSC consisted of 5 quasi-sinusoidal movement cycles (frequency 1 Hz, amplitude 1 mm) in which stimulation was applied in the middle three cycles, during shortening (Fig. 6, top inset, note that in the figure only the final two cycles during which stimulation was present, are shown). The stimulation timing was chosen such that net positive mechanical work performed by the muscle was large. After the measurements, SOL was dissected, weighted and stored in fixation fluid (75% tridest, 4% formaldehyde, 15% ethanol, water and 1.7 g · L⁻¹ Thymol). After removal of SOL, the animal was terminated by an injection of an overdose pentobarbital (euthanasaat).

Data analysis

Data analysis was based on assumptions pertaining to the Hill version of the model depicted in Fig. 1. For both models, parameters were intentionally estimated as much as possible from behavioural data, in order to allow for a comparison between models that was not compromised by different levels of uncertainty in each models’ parameter values. Table 2 shows the values of all parameters that were not fitted on the data, and lists there respective literature sources.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>symbol [unit]</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normalized CE shape</td>
<td>$c_{\text{CE}}^{\text{norm}}$</td>
<td>-16</td>
<td>Burkholder and Lieber (2001)</td>
</tr>
<tr>
<td>Cross-bridge binding cooperativity</td>
<td>$n$</td>
<td>2</td>
<td>cf Curtin et al. (1998); Brown et al. (1999)</td>
</tr>
<tr>
<td>50% Calcium saturation point</td>
<td>$\kappa$</td>
<td>0.35</td>
<td>cf Curtin et al. (1998); Brown et al. (1999)</td>
</tr>
<tr>
<td>Minimum active state</td>
<td>$q_{\text{min}}$</td>
<td>1e-6</td>
<td>arbitrary small value</td>
</tr>
<tr>
<td>Minimal scale factor of $b_{\text{rel}}$</td>
<td>$b_{\text{scale}}^{\text{rel}}$</td>
<td>0.1</td>
<td>Camilleri and Hull (2005)</td>
</tr>
<tr>
<td>Value of $q$ at which $b_{\text{rel}}$ is scaled</td>
<td>$q_{\text{crit}}$</td>
<td>0.3</td>
<td>Camilleri and Hull (2005)</td>
</tr>
<tr>
<td>Maximum attachment bond length</td>
<td>$h$ [m]</td>
<td>1e-8</td>
<td>Huxley (1957); Zahalak (1981)</td>
</tr>
<tr>
<td>Rat sarcomere length</td>
<td>$s$ [m]</td>
<td>2.6e-6</td>
<td>Burkholder and Lieber (2001)</td>
</tr>
</tbody>
</table>

For each quick-release trial, first the length change ($\Delta l$) and the force change ($\Delta F$) during the quick-release was determined as the difference between the average of 8 samples, immediately prior and after the quick-release. Maximum (SEE) force $F_{\text{max}}$ was obtained and the average force level during the step ($F_{\text{avg}}$) was determined as

\[
F_{\text{avg}} = \frac{\sum F_{\text{i}}}{n}
\]

where $F_{\text{i}}$ is the force level of each sample and $n$ is the number of samples.
the average of the force prior and after the quick-release. Combined, this provided a measure of SEE compliance as a function of SEE force, which was integrated with respect to force to obtain SEE elongation as a function of force:

$$\Delta l_{\text{SEE}}(F_{\text{SEE}}) = \int \frac{\Delta l}{\Delta F}(F_{\text{avg}}) \, dF$$

After inverting the latter relation, Eq. (3) was fitted to it to obtain a value for $c_{\text{SEE}}$ (Fig. 4A). Next, (SEE) force prior to contraction, ($F_{\text{pass}}$) was determined for each trial and expressed as a function of $l_{\text{MTC}} - \Delta l_{\text{SEE}}$, by making use of Eq. (3) for SEE. To these data points, Eq. (3) was fitted again (Fig. 4B), yielding a value for $c_{\text{PEE}}$ and an offset value for PEE slack length: $l_{\text{offset}}_{\text{PEE}} = l_{\text{0,PEE}} - l_{\text{0,SEE}}$. At this point Eq. (1)-(3) could be used to transform $F_{\text{max}}(l_{\text{MTC}})$ to $F_{\text{CE}}(l_{\text{MTC}} - \Delta l_{\text{SEE}})$. The non-normalized version of Eq. (4),

$$F_{\text{CE}} = c_{\text{CE}}(l_{\text{CE}} - l_{\text{offset}}_{\text{CE}})^4 + F_{\text{max}}^{\text{CE}},$$

was then fitted to the latter relation (Fig. 5C), which readily yielded an estimate of $F_{\text{CE}}$ and $c_{\text{CE}}$. The value of CE optimum length ($l^{\text{opt}}_{\text{CE}}$) could be found by assuming that $l_{\text{CE}}$ at the first zero crossing of the polynomial equals $0.5 \cdot l^{\text{opt}}_{\text{CE}}$ (cf Fig. 2 in Burkholder and Lieber, 2001):

$$l^{\text{opt}}_{\text{CE}} = 2 \cdot \left( \frac{-F_{\text{max}}^{\text{CE}}}{c_{\text{CE}}} \right) \frac{1}{4}$$

After $l^{\text{opt}}_{\text{CE}}$ was found, SEE slack length could be determined as: $l^{0}_{\text{SEE}} = l^{\text{offset}}_{\text{CE}} - l^{\text{opt}}_{\text{CE}}$. Subsequently, PEE slack length could be determined as: $l^{0}_{\text{PEE}} = l^{\text{offset}}_{\text{PEE}} - l^{0}_{\text{SEE}}$. Parameters pertaining to the SEE, PEE and CE force length relations were assumed to be equal for both models.

Data from the step-ramp and isokinetic eccentric trials were treated similarly. First, $l_{\text{CE}}(t)$ and $F_{\text{CE}}(t)$ were determined by applying Eq.’s (1)-(3) with the now known parameters on the experimental force and length traces. Next, the 20 consecutive samples of the force trace for which their sum of squared difference from their average was smallest, were selected from the set of samples (after the step) for which stimulation was present and MTC velocity was constant. After normalization, the average CE force and the slope of the best fit straight line through $l_{\text{CE}}(t)$ of these 20 samples, provided one data point in the force velocity curve (Fig. 5D). For the Hill model, the inverse of Eq. (7), with $q = F^{\text{isom}}_{\text{CE}} = 1$, was fitted to these data (i.e. the difference between model and data CE force was minimized in the fitting procedure).

For the treatment of the data from the quick-release trials it is assumed that the step happens instantaneously. In practice, this is obviously not the case. At this point in the analysis, the violation of this assumption could be corrected for, by first computing the CE displacement during the step as the product of the velocity corresponding to the average force level ($\Delta F$) and the duration of the step ($\sim 6$ ms). Next, the estimated CE displacement was subtracted from the length change ($\Delta l$) and the analysis of the quick-release trials was repeated, yielding corrected values for the parameters pertaining to the force-length relations. The latter parameters were also used in the treatment of the trials pertaining to the force-velocity relation, which could thus also be re-analysed. The above procedure was iterated until the difference between the parameter estimates in consecutive loops was smaller than some arbitrary small value. For all rats, the iteration procedure required no more than 15 iterations, and yielded marginally changed parameter values.

The Huxley model rate parameters were estimated on the previously found force-velocity data points. Huxley model CE force was computed from the steady state solution to Eq.’s (12), evaluated with $q = F^{\text{isom}}_{\text{CE}} = 1$ at each CE velocity. The rate parameters were then found by minimizing the difference between CE model and data force.
For both models separately, the time constants in Eq. (6) were estimated by fitting simulation results to 4 different, experimentally obtained force traces, simultaneously. The 4 trials were (i) a quick-release trial around optimum length (Fig. 4A); (ii) a step-ramp trial with the constant shortening velocity similar to the maximum MTC shortening velocity encountered during part 2 of the experiment (Fig. 4B); (iii) an eccentric isokinetic trial, at a velocity where both the Hill and the Huxley model accurately fitted the corresponding data point in the force-velocity relation (Fig. 4C); and (iv) an isokinetic release trial, which consisted of an isometric phase followed by isokinetic shortening with stimulation ending at the onset of the shortening phase, at a shortening velocity at or around maximum MTC shortening velocity encountered during part 2 of the experiment (Fig. 4D). The latter 4 trials represent a wide range of contraction conditions. We therefore expected the parameter set resulting from this fit to generalize well to other contraction types.

Results

The statements made in this section hold for all three rats, unless specified otherwise. The muscles remained metabolically stable throughout the experiment. Maximum isometric force at optimum MTC length at the end of the experiment did not differ by more than 10% from that at the beginning of the experiment.

Part 1: parameter estimation

The contraction protocols from part 1 of the experiment resulted in measured force traces (Fig. 4A-C) from which data pertaining to both the force-length relations and the concentric and eccentric force-velocity relations, could be accurately extracted. Fig. 5A and 5B show typical examples (rat 3) of the fit of Eq. (3) to respectively the SEE and PEE data obtained from the quick release trials. For both relations, the model described the data well (PEE: $R^2 > 0.97$, SEE: $R^2 > 0.99$). The data from the CE force-length relation resembled a parabolic form. First, the curve shows a steep ascending limb, which was followed by a plateau and a decrease in force at CE lengths larger than optimum CE length (Fig. 5C). The proposed model for the CE force length relation (Eq. (4)) described these data well ($R^2 > 0.99$). Data pertaining to the concentric part of the force-velocity curve followed a typical hyperbolic shape (Fig. 5D), which was described well by both the Hill and Huxley model. Fewer data points were collected on the eccentric part of the force-velocity curve, and these data showed a more irregular pattern for each rat, which was described similarly well by both models. For the force-velocity relation we found $R^2 > 0.98$ for both models.

Results of simulations of data trials used to estimate the force-length relations of CE, SEE and PEE, matched the observed data closely (Fig. 4A), particularly those parts from which the data in Fig. 5A-C, was derived. This is also the case for the force-velocity relation, for both the Hill and the Huxley model, in equal measure (Fig. 4B and 4C). For both models, the fit of the activation dynamics parameters yielded parameter values with which all four contractions could be adequately described (Fig. 4A-D), resulting in low root mean squared (RMS) differences between model predictions of force and experimental data (Table 3, part 1).

For both models, all parameter values estimated from the experimental data obtained in part 1 of the experiment are provided in Table 4. Note that the parameter values of rat 1 and 3 are similar, whilst the values of rat 2 deviate from the latter two rats. Note also that the only between-model difference in parameter values concerns the activation dynamics parameters, since the parameters pertaining to the CE, SEE and PEE force-length characteristics are assumed equal for both models.
Table 3. Root mean squared (RMS) differences between predicted and experimental force traces. Values are in [mN]. Data presented in this table corresponds to the force traces shown in Fig. 4 and Fig. 6 for part 1 (RMS averaged over all 4 trials) and part 2, respectively.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Rat 1</th>
<th>Rat 2</th>
<th>Rat 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Part 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Data-Hill</td>
<td>85</td>
<td>79</td>
<td>45</td>
</tr>
<tr>
<td>Data-Huxley</td>
<td>77</td>
<td>81</td>
<td>63</td>
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<td>Hill-Huxley</td>
<td>23</td>
<td>31</td>
<td>40</td>
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<td></td>
<td>Part 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Data-Hill</td>
<td>86</td>
<td>64</td>
<td>75</td>
</tr>
<tr>
<td>Data-Huxley</td>
<td>75</td>
<td>60</td>
<td>52</td>
</tr>
<tr>
<td>Hill-Huxley</td>
<td>13</td>
<td>16</td>
<td>28</td>
</tr>
</tbody>
</table>

Table 4. Model parameter values. See text for explanation of variable symbols. Units are given in brackets behind the parameter symbol.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>symbol [unit]</th>
<th>rat 1</th>
<th>rat 2</th>
<th>rat 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both models</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum isometric CE Force</td>
<td>$F_{\text{CE}}^\text{max}$ [N]</td>
<td>1.27</td>
<td>1.25</td>
<td>1.26</td>
</tr>
<tr>
<td>Optimum CE length</td>
<td>$l_{\text{CE}}^{\text{opt}}$ [mm]</td>
<td>17.1</td>
<td>21.6</td>
<td>18.6</td>
</tr>
<tr>
<td>SEE shape</td>
<td>$c_{\text{SEE}}$ [kN m$^{-2}$]</td>
<td>1.30</td>
<td>0.85</td>
<td>1.5</td>
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<td>SEE slack length</td>
<td>$l_{\text{SEE}}^{0}$ [mm]</td>
<td>19.5</td>
<td>13.3</td>
<td>18.6</td>
</tr>
<tr>
<td>PEE shape</td>
<td>$c_{\text{PEE}}$ [kN m$^{-2}$]</td>
<td>0.87</td>
<td>1.21</td>
<td>0.68</td>
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<td>PEE slack length</td>
<td>$l_{\text{PEE}}^{0}$ [mm]</td>
<td>9.8</td>
<td>12.4</td>
<td>9.7</td>
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<td>Hill model</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hill constant</td>
<td>$a_{\text{rel}}$ [$F_{\text{CE}}^\text{max}$]</td>
<td>0.25</td>
<td>0.072</td>
<td>0.20</td>
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<tr>
<td>Hill constant</td>
<td>$b_{\text{rel}}$ [$l_{\text{CE}}^{\text{opt}}^{\text{rel}}$]</td>
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<td>0.24</td>
<td>0.40</td>
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<td>Isometric force slope ratio</td>
<td>$r_{\text{slope}}$ [ ]</td>
<td>1.45</td>
<td>0.38</td>
<td>2.89</td>
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<td>Ecc. CE force asymptote</td>
<td>$F_{\text{asympt}}$ [N]</td>
<td>3.91</td>
<td>3.44</td>
<td>2.02</td>
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<td>$\tau_{\text{act}}$ [ms]</td>
<td>97</td>
<td>20</td>
<td>90</td>
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<tr>
<td>Deactivation time constant</td>
<td>$\tau_{\text{deact}}$ [ms]</td>
<td>133</td>
<td>97</td>
<td>121</td>
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<tr>
<td>Huxley model</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Attachment rate parameter</td>
<td>$f_1$ [s$^{-1}$]</td>
<td>99</td>
<td>66</td>
<td>101</td>
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<tr>
<td>Con. detachment rate parameter</td>
<td>$g_2$ [s$^{-1}$]</td>
<td>370.0</td>
<td>785</td>
<td>479</td>
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<tr>
<td>Ecc. detachment rate parameter</td>
<td>$g_3$ [s$^{-1}$]</td>
<td>62</td>
<td>108</td>
<td>57</td>
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<tr>
<td>Activation time constant</td>
<td>$\tau_{\text{act}}$ [ms]</td>
<td>86</td>
<td>9</td>
<td>79</td>
</tr>
<tr>
<td>Deactivation time constant</td>
<td>$\tau_{\text{deact}}$ [ms]</td>
<td>126</td>
<td>89</td>
<td>105</td>
</tr>
</tbody>
</table>
Part 2: stretch-shortening cycles

The stretch shortening protocol applied during part 2 of the experiment resulted in considerable force production during shortening (Fig. 6). The observed force traces were similar for the three SSC within a trial (difference between lowest and highest observed peak force, averaged over animals < .03 N). As can be appreciated from Fig. 6, both models yielded similar predictions of the experimental data. During simulations with both models, CE started contracting eccentrically while significant force was still present, as indicated by the crosses in the force traces in Fig. 6. Predicted peak force was lower, for both models in equal measure. Forces predicted by both models deviated from experimental data during relaxation, both during the concentric and eccentric phase, in equal measure. Table 3, part 2 lists the RMS differences between the measured force traces and force traces pertaining to simulations with the Hill and Huxley models, corresponding to the data in Fig. 6. Absolute RMS differences between model predictions and experimental data were similar to those found for the trials used to fit the activation dynamics time constants (Table 3, part 1). As can be appreciated from Table 3, the difference between simulated and measured force traces was similar for both models. Furthermore, the difference between the two models was smaller than the difference between each of the models and the data. In all simulation results of SSC, CE length varied between $0.8 \cdot l_{opt}^{CE}$ and $1 \cdot l_{opt}^{CE}$ and CE velocity varied between $-1.2 \cdot l_{opt}^{CE} \cdot s^{-1}$ and $0.4 \cdot l_{opt}^{CE} \cdot s^{-1}$. Following the similarity in force traces (given equal SEE characteristics), simulated CE length and velocity traces were similar for both the models.

Discussion

The aim of this study was to evaluate and compare the validity of the Huxley and Hill MTC models, with respect to mechanical behaviour, during SSC in which the muscle was primarily active during MTC shortening. Data was collected from rat SOL in situ. Importantly, the data used to fit the parameters of both models (part 1) was not used to evaluate model performance (part 2). Furthermore, to minimize bias in the comparison between the Hill and the Huxley MTC models, parameters were estimated from the same behavioural data as much as possible, for both models. For both models, the fitting of the model parameters on data from part 1 of the experiment was exceptionally good (Fig. 4 and 5). Comparison of simulation results to data from part 2 of the experiment (Fig. 6) shows that both models predict experimental data reasonably well during realistic stretch shortening cycles. Moreover, comparison between the simulation results of the Hill and the Huxley model (Fig.s 4 and 6), indicates that both models behave similarly, in a wide range of contraction conditions.

Limitations and methodological issues

The number of animals included in this study is modest (n=3). However, given the exploratory nature of this work, and the similarity of the results between rats, we deemed the number of animals included sufficient.

The between-animal variability in the parameter values warrants attention. Specifically, rat 2 differs from rats 1 and 3 (Table 4). These differences reflect the progressive interdependency in the parameter estimation procedure, i.e. the parameters estimated after the force-length relations are influenced by the previously estimated parameter values. Therefore, the relatively small differences between rats in parameters pertaining to the force-length relations, are amplified in the estimates of the force velocity parameter values, for both models. Any differences between model behaviour and data are subsequently ‘corrected’ for in the estimates of the activation dynamics parameters. Therefore, although the behaviour during the SSC was similar for all three rats, estimated parameter
values differed. Note however, that it is not uncommon to find large variety in animal specific parameter estimates, as illustrated by Blümel et al. (2013). Thus the large variability in parameter values found here probably reflects a combination of (small) between-animal biological variance and amplification of differences arising from the estimation procedure. We feel that the methodological issues discussed above do not jeopardize the validity of the results and do not compromise the possibility to draw conclusions regarding the key questions of this study.

**Parameter estimation procedure was successful**

The present experiments regarding the model parameter estimation (part 1) were similar to those previously reported by Blümel et al. (2012) (stick insect), Curtin et al. (1998); Williams et al. (1998) (lamprey) and Krylow and Sandercock (1997) (rat SOL). The experimental data obtained in the present study agrees with data obtained in the latter studies. The fitting of parameters to these data was successful, for both models in equal measure. For the Hill model, the quality of the fits were similar to those presented by Blümel et al. (2012). The Hill model parameters presented in the current study agree with previously reported values in rat SOL, near optimum length (cf table 1 in Krylow and Sandercock, 1997). The latter suggests that the measurements and procedures for estimating the parameters pertaining to the force-length and force-velocity curves employed in the current study are reliable and valid.

For the activation dynamics, we obtained the parameter values pertaining to the steady state relation between free calcium concentration and active state (Eq. (5)) from literature. The time constants of the (de)activation dynamics were estimated on four representative trials (Fig. 4), an approach similar to Curtin et al. (1998). For reasons mentioned previously, these values differed between rats. Note that for all rats, both the activation and deactivation time constants were smaller for the Huxley model than for the Hill model (Table 4). This is not surprising: the Huxley model contains an additional dynamical step in the transformation of stimulation to force, as compared to the Hill model. During an isometric contraction of the CE, a stepwise change in active state, would result in a stepwise change in force in the Hill model, but leads to a dynamic response in the Huxley model, as it takes time for the cross-bridges to attach or detach and for the distribution \(n(x,t)\) to settle on its new steady state value (see also methods section). Thus to arrive at a given force level within a given time limit, the activation dynamics time constants must be smaller in the Huxley model compared to the Hill model.

An important point to note here is that the estimated parameter values pertaining to the force-length and force-velocity relations were consistent for both models. The parameter estimation procedure applied to simulations of the experimental data yields similar parameter values, as particularly those parts of the force traces from which the data was extracted to form the force-length and force-velocity relations, were adequately simulated (Fig. 4). For the Hill model, this was not surprising, as the assumptions on which the data analysis was based, were tailored to the Hill model. However, for the Huxley model this was not obvious, since cross-bridge force at any time does not only depend on the state at that time, but also on its history. Yet, a prominent feature of the simulation results presented in Fig’s 4 and 6, is the similarity between the model predictions of the Hill and the Huxley model, over a wide range of contraction conditions. It might be that this similarity arises because the dynamics of cross-bridge cycling are so fast, that the distribution \(n(x,t)\) is similar to the steady state distribution at the instantaneous velocity, at all time points during a simulation. If this would be the case, the macroscopic behaviour of the Huxley model would effectively be the same as the Hill model, given the similarity in the shape of the Hill and Huxley (steady state) force-velocity curves (Fig. 5D). To investigate this possibility, the distribution \(n(x)\) during a simulation of a SSC, was compared to the steady state distribution at the same instantaneous velocity, activation level and relative CE length, at 4 different time points (Fig. 7). As can be appreciated from Fig. 7, the
two distributions differed at $t = 0.1$ s and $t = 0.34$ s and were similar at $t = 0.22$ s and $t = 0.46$ s. Thus, the similarity between the behaviour of the Hill and Huxley models is not trivially caused by a large difference in time scales between microscopic behaviour and macroscopic behaviour of the Huxley model, which would render modelling the cross-bridge distribution superfluous.

**Hill and Huxley MTC models yield similar predictions of experimental data during stretch-shortening cycles**

The experimental data of the SSC’s obtained in the present study agree well with similar experiments performed previously on mammalian muscle (Lichtwark and Barclay, 2010). The level of agreement between Hill model predictions and experimental data found in the current study, agrees with previous studies in which a similar approach was adopted (Biewener et al., 2014; Williams et al., 1998; Krylow and Sandercock, 1997; Sandercock and Heckman, 1997). As model predictions were similar for both models (see Table 3 and Fig. 6), we will discuss the results for both the models simultaneously. For both models, predictions of the SSC deviated from the data, both during relaxation and, to a lesser degree, during stimulation (Fig. 6). Note that in Table 3, the absolute RMS differences between part 1 and part 2 are not large, but the RMS differences relative to the peak force attained during the trial is larger for the simulations of part 2. Below, we discuss possible causes for this discrepancy between model predictions and experimental data.

With respect to the activation phase, the main difference between the trials on which the activation parameters were fitted on and the SSC was, that during the former activation always occurred isometrically (on MTC level), whilst during the latter it occurred during shortening of the MTC. The latter observation suggests that the concentric part of the force velocity relation is dependent on the time course of activation, in a way that is not modelled here.

The deviation during relaxation can possibly be attributed to inadequate extrapolation of the eccentric part of the force-velocity curve, of which we have collected only limited data. During relaxation, MTC velocity was close to zero whilst muscle force was decreasing, thus SEE was shortening and CE was probably contracting eccentrically. Although the latter was indeed the case, the force is already deviating well before the CE starts contracting eccentrically (see crosses in Fig. 6). Moreover, during the part where significant force was present, eccentric velocities did not exceed the range of values for which experimental data was collected. Therefore, in our view, inadequate extrapolation of the eccentric force velocity curve cannot explain the discrepancy between experimental data and model predictions, during relaxation.

There are two well-known phenomena in muscle physiology, that were not modelled in the current study and that should also be considered as a possible explanation of the discrepancy mentioned. These are: (i) muscle inhomogeneities, either stemming from inhomogeneous fibre type distributions or serial sarcomere strain inhomogeneity (Palmer et al., 2011) and (ii) contraction history effects (e.g. potentiation; Krarup 1981 and shortening induced force depression; Meijer et al. 1997; Rassier and Herzog 2004). With respect to the first option, Lee et al. (2013) have recently shown that explicit modelling of fibre type distribution can improve Hill model predictions of goat m. Gastrocnemius mechanical behaviour in vivo. However, rat SOL consists for the most part of slow twitch, type I fibers ($\sim 85\%$, cf. Delp and Duan, 1996; Pousson et al., 1991). It is therefore unlikely that lumping all fibre types has had a large influence on our results. Serial sarcomere strain inhomogeneity has been shown experimentally (Palmer et al., 2011), and was also pointed out by Curtin et al. (1998) to explain the discrepancy between model predictions and experimental data, during the relaxation phase of SSC. In a Huxley modelling framework, it has been shown that modelling sarcomere strain inhomogeneity can account for the stretch response of muscle (Campbell, 2009) and can result in emergent behaviour that cannot be explained by
a lumped fibre model (Stoecker et al., 2009). Whilst not ruling out the possibility of such a mechanism being at work here, we have no grounds to assume this mechanism would work differently during the contractions in part 1 as compared to part 2 and consequently we have no grounds to assume that this mechanism explains the discrepancy between experimental data and simulation results during relaxation. With respect to the second option it is noted that the force traces pertaining to each sinusoid were nearly equal, which indicates that effects of history dependence were minimal on the time scale of the sinusoid’s period time. However, within one sinusoid, the muscle is passively stretched prior to active shortening. This stretch may have altered the excitability of calcium gated potassium channels (Mallouk and Allard, 2000), caused the release of extra calcium (Ji et al., 2002), or altered cross bridge contraction dynamics (Haugen, 1991); these processes were not modelled in the current study. However, if these processes would result in increased force during the relaxation phase of the shortening contraction, an increased force at maximum length should also be expected. As the latter was not observed we consider it unlikely that any of these processes underlies the observed discrepancies.

Another possible explanation may be related to the duration of stimulation: > 0.5 s during part 1 vs < .25 s during part 2. The duration of stimulation may affect the calcium saturation characteristic Eq. (5). This could lead to over-saturation of the model during part 2, which may explain the overestimation of the force during the last part of relaxation. Although, in this study we did not fit the parameters in Eq. (5) on the data in part 1, but based them on (Curtin et al., 1998), it is still possible that these parameter resulted in more adequate prediction of the data in part 1, as compared to part 2. In short, we believe that the discrepancy between model predictions and experimental data is caused by inadequate modelling of physiological effects that arise due to the differences in the contraction protocols between part 1 as compared to part 2.

For the Hill model, the level of agreement between predicted and experimental data is not perfect, which is in line with previous work (Lee et al., 2013; Krylow and Sandercock, 1997; Williams et al., 1998; Sandercock and Heckman, 1997). The latter suggests caution when applying the model in situations in which accurate quantification of muscle force is required (e.g. in patient-specific modelling). However, Hill models have been successfully applied in musculoskeletal models aimed at addressing conceptual questions regarding design and control of the motor system (Van Soest et al., 1993; Bobbert, 2012; Azizi and Roberts, 2010; Richards and Clemente, 2012; Kistemaker et al., 2007; Lichtwark and Wilson, 2007). Given the similarity in predicted behaviour of the Hill and Huxley models MTC in the current study, it stands to reason that the Huxley model could at least be applied in a similar fashion. The level of agreement between predicted and experimental data displayed in the current study is deemed adequate, for the latter type of applications.

The present study is the first to rigorously validate a Huxley MTC model and compare it to the Hill MTC model, in the context of physiologically relevant stretch shortening cycles. It is concluded that, with respect to mechanical behaviour, the current Huxley MTC model predicts the experimental data as well as the Hill MTC model. As such, large scale musculoskeletal modelling using the current Huxley MTC model is a viable option. It remains to be determined whether the Huxley model can adequately describe both mechanical behaviour and metabolic energy consumption, from a unified framework. In future research we will investigate the validity of the present Huxley model in terms of metabolic energy consumption and the relation of the latter to mechanical behaviour.

Acknowledgments

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Competing Interests

The authors declare no competing interests.

Author Contributions

Conception and design: all authors. Performed experiments: GCB and KKL. Performed data analysis: KKL, RTJ and AJKvS. Drafting of the manuscript: all authors.

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References


Figure 1. Schematic of muscle-tendon complex model. CE: contractile element, represents the muscle fibers. PEE: parallel elastic element, represents all elastic tissue parallel to muscle fibers. SEE: series elastic element, represents all elastic tissue in series to the muscle fibers. Both the Hill and Huxley model follow the schematic depicted in this figure.

Figure 2. Rate functions of the Huxley model. Rate of attachment \( f(x) \), solid line) and detachment \( g(x) \), dashed line) of cross-bridges as a function of normalized bond length \( x \), according to Eq.’s (13) and (14). The graph corresponds to parameter values from rat 3 (see Table 4).
Figure 3. Schematic of the experimental setup. The hind limb of the rat was fixated on the experiment table by securing the femur and the foot. The distal end of the Achilles tendon was attached to a servo controlled motor arm, via a force transducer. Cuff electrodes were placed over the n. Ischeadicus, which innervates SOL. The setup allowed for full control over efferent muscle stimulation and MTC length, while muscle force was measured.

ff: foot/femur fixation
fd: force transducer
la: lever arm
SOL: m. Soleus
GL: m. Gastrocnemius Lateralis
cu: cuff electrode
Figure 4. Typical examples (rat 3) of data and simulation results of each experimental protocol of part 1 of the experiment. All panels: the inset displays the experimental protocol. The solid, black lines represent MTC length and the thick black bars indicate times during which the muscle was stimulated. The bottom graphs show measured force as a function of time (solid black lines) and model fits with the Hill (solid red lines) and Huxley (dashed blue lines) models. For all simulations, model input was equal to the experimental protocol depicted. MTC length at t=0 was equal to optimum length for all examples shown in this figure. Panel A: quick-release protocol. This protocol was imposed at different initial MTC lengths. Data from the quick-release trials provided the basis for the data displayed in Fig. 5A-C. Panel B: step-ramp protocol. Different combinations of step sizes and shortening velocities were imposed. Data from these trials provided the basis for the concentric part of the force-velocity curve (Fig. 5D). Panel C: isokinetic, eccentric contraction. The protocol was imposed at different lengthening velocities. Data from these trials provided the basis for the eccentric part of the force-velocity relation (Fig. 5D). Panel D: isokinetic, concentric contraction. The activation dynamics parameters of both Hill and Huxley models were estimated by fitting simulation results to the data of all four panels shown in this figure, simultaneously.
Figure 5. Typical examples (rat 3) of experimental data and model fits obtained from part 1 of the experiment. In panels A-C, solid black lines are the model fit to the data (open circles). In panel D, the solid red line and the dashed blue line are the Hill and Huxley model fit to the data, respectively. Data in panels A-C was obtained from quick-release trials (Fig. 4A). Data in panel D was obtained from step-ramp and eccentric isokinetic release trials (Fig. 4B-C). Panel A: SEE force-length relation. Panel B: PEE force-length relation. Panel C: CE force-length relation. Panel D: CE force-velocity relation. $R^2 > .99$ for all relations depicted in this figure.
Figure 6. Experimental data and simulation results of the stretch-shortening cycles (part 2). The final two periods of the SSC trials during which stimulation was present are depicted here. The top inset represents MTC length as a function of time, which was equal for all three rats (panels A-C). Stimulation is indicated by thick black bars. All panels depict measured force (solid black line) and predicted force from Hill (solid red line) and Huxley (blue, dashed line) models. The blue and red crosses mark the time were simulated CE velocity became eccentric, during the simulations of the Hill and Huxley model, respectively.
Figure 7. Comparison of the distribution $n(x)$ simulated during stretch-shortening cycles, and the steady state $n(x)$. Each panel depicts the distribution $n(x)$ at one point in time, as obtained from simulation of a SSC (dashed line) and the steady state solution to eq Eq.’s (12), evaluated at the instantaneous CE velocity, CE length and activation level (solid line). Time, normalized CE velocity, activation level, normalized CE isometric force and simulated SEE force are listed below for each panel (units in square brackets, where applicable): $(t[s], l_{rel}^{CE}[s], q, F_{isom}^{CE}, F_{SEE}[N])$. Panel A: (0.1 -0.25 0.73 1.00 0.64). Panel B: (0.22 -0.35 0.94 1.00 0.57). Panel C: (.34 -0.22 0.31 0.99 0.37). Panel D: (0.46 0.020 0.098 0.99 0.14). Note: eccentric contraction phase, $l_{rel}^{CE} \leq 0$. The data in this figure correspond to Fig. 6C. Note the different scales on the abscissa at each panel.