

## SUPPLEMENTARY METHODS

### Motor Controller:

The motor controller used in this study was programmed in Simulink (Mathworks Inc., Natick, MA, USA), and simultaneously commanded muscle stimulation and environment dynamics while recording muscle-tendon unit (MTU) and muscle fascicle (contractile element, CE) force/displacement using a dSpace DS1103 control board (dSpace Inc., Paderborn, DE). Tendon/aponeurosis (series elastic element, SEE) displacement was assumed to adhere to the following relationship:

$$\Delta L_{SEE} = \Delta L_{MTU} - \Delta L_{CE}$$

The controller used direct measurements of biological MTU force as an input, and simulated interaction with a virtual environment consisting of a modeled extension spring in parallel with the biological MTU interacting with mass in gravity across a fixed mechanical advantage (see Free Body Diagram in **fig. 1**). The motor controller was programmed to adhere to the following relationship:

$$(F_{Exo} + F_{MTU})l_{in} = F_{load}l_{out}$$

Where  $F_{Exo}$  was the simulated elastic exoskeleton (Exo) force,  $F_{MTU}$  was the measured MTU force,  $F_{load}$  was the force applied to the virtual mass, and  $l_{in}/l_{out}$  were moment arm lengths for the MTU+Exo and mass respectively. By rearranging this equation, we could express net force on the virtual mass ( $F_{net}$ ) as follows:

$$F_{net} = M\ddot{x}_{load} = \left( l_{in}/l_{out} \right) (F_{Exo} + F_{MTU}) - Mg$$

Where  $M$  was the mass of the virtual inertial/gravitational load,  $\ddot{x}_{load}$  was the acceleration of the virtual mass, and  $g$  was the gravitational acceleration ( $9.8\text{m/s}^2$ ). By solving for  $\ddot{x}_{load}$ , and integrating twice, we could compute a time-step displacement for the mass ( $dx_{load}$ ). Motor arm displacement at each time-step ( $dx_{MTU}$ ), adhered to the following relationship:

$$l_{in}dx_{MTU} = -l_{out}dx_{load}$$

Integration of the equations was performed using a fixed-step, 4<sup>th</sup> order runge-kutta solver (ode45) at each time step, with a sampling rate of 10 kHz (**fig. 1A**).

### **Exoskeleton Model:**

Every experimental condition receiving elastic exoskeleton (Exo) assistance was assigned an Exo ‘slack length’ ( $l_{slack}$ ), or a length below which there was no tension in the virtual parallel spring. Springs were also modeled so as to not generate compressive loads for lengths  $< l_{slack}$ . Each trial began with the biological MTU under 1 N of passive tension, and this initial absolute motor position ( $l_{slack}$ ) was stored at the onset of the first stimulus pulse. Force from the virtual Exo was computed as follows:

$$F_{Exo}(l_{Exo}) = \begin{cases} k_{Exo}(l_{Exo} - l_{slack}), & l_{Exo} > l_{slack} \\ 0, & l_{Exo} \leq l_{slack} \end{cases}$$

Where  $l_{Exo}$  is absolute exoskeleton length, and  $F_{Exo}$  is exoskeleton force.

### **Experimental Metrics:**

*Muscle Properties for Normalization* - All values of peak force ( $F_{peak}$ ) reported were normalized to a measured  $F_{max}$  for each muscle preparation. Once  $F_{max}$  was determined for each prep, this value was used in conjunction with absolute muscle fascicle (CE) length data from passive pluck conditions to estimate  $l_0$ . This was done using equations from (Azizi and Roberts, 2010) to perform a least-squared error fit to experimental data. To estimate maximum muscle shortening velocity ( $v_{max}$ ), the following relationship was assumed (Sawicki et al., 2015):

$$v_{max} = -13.8l_0 \cdot s^{-1}$$

Where  $s$  is the unit of time, seconds.

*Length/Velocity Metrics* - To determine how muscle fascicle (CE) mechanical state (*i.e.*, length and velocity,  $l_{CE}$  and  $v_{CE}$  respectively) influenced force production capability, we report normalized strain and velocity at  $F_{peak}$ . We also report peak CE shortening velocity over a cycle of stimulation, as this (along with force) heavily influenced the amount of positive muscle work performed over a cycle of stimulation.

*Muscle Activation/Deactivation Time Constants* - To estimate muscle active state and metabolic energy consumption from a known stimulus pulse, it was necessary to determine muscle activation/deactivation time constants ( $\tau_{act}$  and  $\tau_{deact}$  respectively). Values reported here are based on a brute-force least squared error fit of equations describing stimulation/activation coupling from Zajac (Zajac, 1989) to stimulation/force data from our initial 300ms maximal contractions. To do this, we swept a range of possible  $\tau_{act}$  and  $\tau_{deact}$  values (0-0.2s), modeled

resultant activation from the known stimulus pulse, and identified the combination which minimized mean-squared error between observed normalized force and modeled normalized active state.

*Instantaneous and Average Positive Mechanical Power* - Instantaneous mechanical power ( $P_{mech}(t)$ ) for all components of the muscle-tendon unit (MTU) (*i.e.*, MTU, CE and SEE) were computed as follows:

$$P_{mech}(t) = -F(t) * v(t)$$

Where  $F(t)$  and  $v(t)$  are instantaneous force and velocity of whatever system component power is being computed for (*e.g.*, MTU, CE or SEE).  $F(t)$  was made negative here to ensure muscle shortening corresponded to positive power output per convention from previous work (Josephson, 1999). To compute average net mechanical power ( $\bar{P}_{mech}^{net}$ ), or the average rate of work over a stimulation cycle, we integrated instantaneous power and normalized by cycle period ( $T_{Drive} = \omega_{Drive}^{-1}$ ) as follows:

$$\bar{P}_{mech}^{net} = \frac{1}{T_{Drive}} \int_{t=0}^{T_{Drive}} P_{mech}(t) dt$$

To determine average rates of positive and negative work ( $\bar{P}_{mech}^{+}$  and  $\bar{P}_{mech}^{-}$  respectively) we used this same approach but only integrated values during shortening (positive) or lengthening (negative), with values of opposite sign set to zero. All mechanical power output data reported in this manuscript was scaled by subject muscle mass to allow for between-prep comparisons (**table 1**).

*Estimates of Metabolic Cost and Apparent Efficiency* - To estimate instantaneous metabolic cost we used a non-dimensional model parameterized in terms of normalized muscle fascicle (CE) velocity,  $p_{met}(v_{CE}/v_{max})$  (Alexander, 1997). To provide values of instantaneous metabolic cost in watts ( $P_{met}(t)$ ),  $p_{met}$  was scaled by the physiological constant  $F_{max}$ , and muscle active state  $\alpha(t)$  as follows from (Krishnaswamy et al., 2011):

$$P_{met}(t) = F_{max} * \alpha(t) * p_{met}(t)$$

To determine average metabolic rate ( $\bar{P}_{met}$ ), we took the same approach to integrating  $P_{met}$  that was used for mechanical power. All reported values of  $\bar{P}_{met}$  are scaled by subject muscle mass to allow for between-prep comparisons.  $\alpha(t)$  was modeled using a stimulus pulse of known duration, previously determined  $\tau_{act}$  and  $\tau_{deact}$  time constants, and equations from Zajac (Zajac, 1989). The magnitude of our normalized stimulus pulse was scaled by the relative activation level in each condition. Activation dynamics for 100%, 80%, and 60% *Stim* trials were modeled using stimulus pulses of magnitude 1, 0.8, and 0.6 respectively.

To estimate CE, MTU, and MTU+Exo apparent efficiency ( $\epsilon_{app}$ ) we simply divided  $\bar{P}_{met}$  by  $\bar{P}_{mech}$  from whichever system component (e.g., MTU, CE, or MTU+Exo) was of interest. Because dynamics observed here were generally cyclic (*i.e.*,  $\bar{P}_{mech}^{net} \sim 0$ ), positive power was used in all efficiency calculations as follows:

$$\epsilon_{app} = \bar{P}_{mech}^+ / \bar{P}_{met}$$

## REFERENCES

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