

Supporting Information

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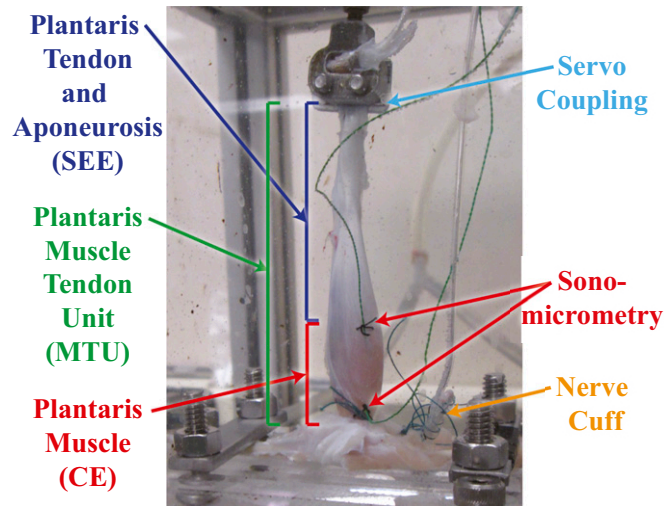


Fig. S1. An annotated photograph of an experimental preparation. Colors used correspond to those of the figure schematic in Fig. 1A to allow for easy comparisons between components of the MTU.

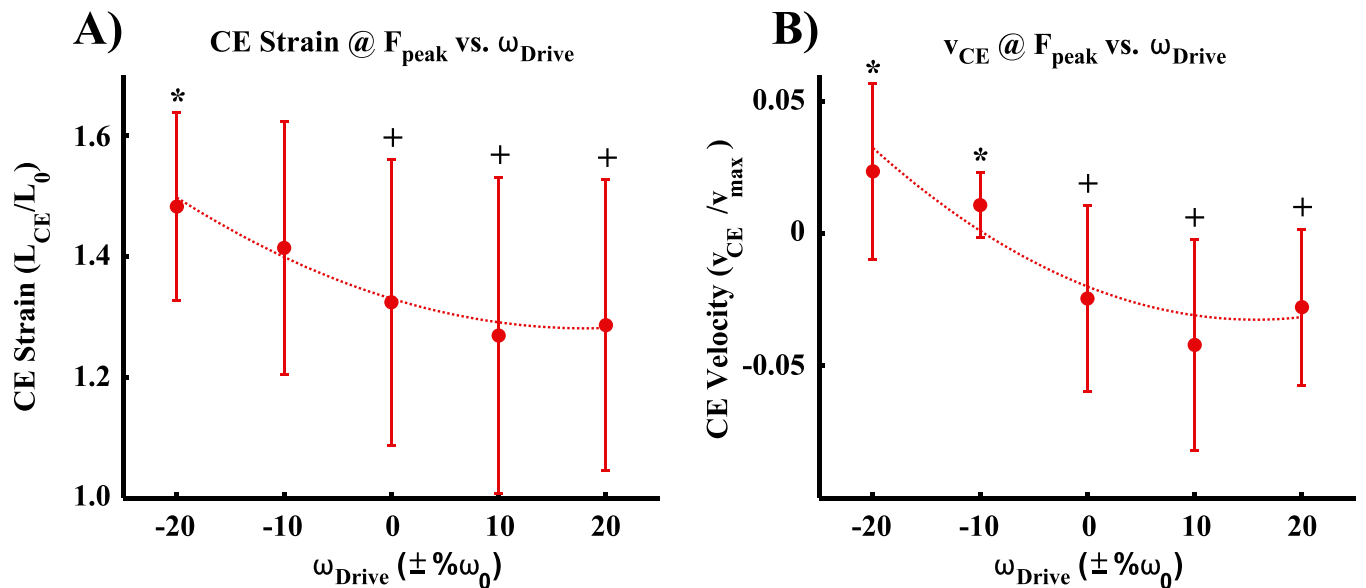


Fig. S2. Mean \pm SD values of (A) normalized CE strain and (B) normalized CE velocity at peak force. In all figures, statistically significant pairwise comparisons ($P < 0.05$) to the ω_0 condition are annotated with an asterisk. In both plots, the global maximum mean value was not the ω_0 condition, so pairwise comparisons to the global maximum were also performed. Conditions found to be significantly different from the global maximum are indicated by a plus sign.

Table S1. Table of experimental metrics, *P* values from one-way repeated-measures ANOVA (factor: normalized driving frequency, ω_{Drive}/ω_0), whether pairwise comparisons are to maximum or minimum value, and condition where max/min value was observed for all reported metrics

Metric	ANOVA <i>P</i> value	Max/min comparison	Max/min condition	Post hoc <i>t</i> test w.r.t. max/min	Post hoc <i>t</i> test w.r.t. ω_0
F_{peak}/F_{max}	0.032	Max	-10% ω_0	-20%, +20%	+20%
%Phase F_{peak}	0.009	Max	-10% ω_0	+10%, +20%	+20%
%Phase Stim. On	0.004	Min	ω_0	—	-20%
MTU \bar{P}_{mech}^+	<i>0.053</i>	—	—	—	—
MTU \bar{P}_{mech}^-	0.001	Min	-10% ω_0	-20%, +20%	+20%
MTU \bar{P}_{mech}^{net}	<i>0.64</i>	—	—	—	—
% \bar{P}_{mech}^+ CE	<0.0001	Min	ω_0	—	-20%, -10%, +20%
% \bar{P}_{mech}^+ SEE	<0.0001	Max	ω_0	—	-20%, -10%, +20%
\bar{P}_{met}	0.004	Min	ω_0	—	-20%
MTU ϵ_{app}	<i>0.075</i>	—	—	—	—
CE Strain @ F_{peak}	0.003	Max	-20% ω_0	0%, +10%, +20%	-20%
CE v @ F_{max}	0.035	Max	-20% ω_0	0%, +10%, +20%	-20%, -10%
CE \bar{P}_{mech}^+	<i>0.61</i>	—	—	—	—
CE \bar{P}_{mech}^-	<i>0.78</i>	—	—	—	—
CE \bar{P}_{mech}^{net}	<i>0.064</i>	—	—	—	—
SEE \bar{P}_{mech}^+	<i>0.088</i>	—	—	—	—
SEE \bar{P}_{mech}^-	0.025	Min	+10% ω_0	-20%	-20%
SEE \bar{P}_{mech}^{net}	<i>0.44</i>	—	—	—	—

A *P* value with italic text indicates a nonsignificant trend with respect to stimulation driving frequency, ω_{Drive} . The final two columns list conditions found to be significantly different from min/max or the ω_0 condition when subjected to a post hoc *t* test. Dashes indicate either no basis for pairwise comparison due to nonsignificant ANOVA (italicized values), or pairwise comparisons which yielded no significant results despite significant ANOVA.

Table S2. Table of regression fit order, equation, R^2 , and *P* value for all reported metrics

Metric	Fit order	Fit equation	R^2	<i>P</i> value	Figure
F_{peak}/F_{max}	Second	$0.90 - 0.14\omega - 4.73\omega^2$	0.26	0.037	3A
%Phase F_{peak}	First	$48.10 - 18.47\omega$	0.47	0.0002	3B
%Phase Stim. on	Second	$26.60 - 48.80\omega + 479.19\omega^2$	0.46	0.012	3B
% \bar{P}_{mech}^+ CE	Second	$21.40 - 41.13\omega + 408.64\omega^2$	0.71	<0.0001	3C
% \bar{P}_{mech}^+ SEE	Second	$78.60 + 41.13\omega - 408.64\omega^2$	0.71	<0.0001	3C
MTU \bar{P}_{mech}^+	Second	$32.93 - 23.20\omega - 240.35\omega^2$	0.21	<i>0.076</i>	3D
MTU \bar{P}_{mech}^-	Second	$-30.64 + 19.66\omega + 249.87\omega^2$	0.27	0.030	3D
MTU \bar{P}_{mech}^{net}	First	$2.48 - 3.54\omega$	0.03	<i>0.43</i>	3D
CE Strain @ F_{peak}	First	$1.33 - 0.54\omega + 1.48\omega^2$	0.12	<i>0.084</i>	S2A
CE v @ F_{max}	Second	$-0.02 - .16\omega + .51\omega^2$	0.40	0.0039	S2B
\bar{P}_{met}	Second	$113.56 - 64.46\omega + 472.46\omega^2$	0.09	<i>0.36</i>	S3G
MTU ϵ_{app}	Second	$0.33 - 0.09\omega - 4.03\omega^2$	0.22	<i>0.064</i>	S3I

A *P* value with italic text indicates a nonsignificant trend with respect to stimulation driving frequency, ω_{Drive} . For inputs to regression equations, ω_{Drive} is entered as a normalized percentage change between ω_{Drive} and ω_0 (e.g., -10% $\omega_0 = -0.1$, $\omega_0 = 0$, etc.).

Table S3. Mean \pm SD values of average positive (\bar{P}_{mech}^+), negative (\bar{P}_{mech}^-), and net (\bar{P}_{mech}^{net}) mechanical power for the components (CE and SEE) of the MTU over a cycle of muscle stimulation for all frequencies

Metric	ANOVA <i>P</i> value	-20% ω_0	-10% ω_0	ω_0	+10% ω_0	+20% ω_0
CE \bar{P}_{mech}^+	<i>0.61</i>	13.81 \pm 8.18	11.81 \pm 2.6	9.39 \pm 6.35	10.44 \pm 8.77	10.51 \pm 6.90
CE \bar{P}_{mech}^-	<i>0.78</i>	-9.85 \pm 3.83	-5.36 \pm 2.25	-7.71 \pm 8.02	-9.69 \pm 11.22	-8.90 \pm 6.51
CE \bar{P}_{mech}^{net}	<i>0.064</i>	3.96 \pm 4.84	6.45 \pm 3.28	1.68 \pm 2.97	0.75 \pm 6.30	1.60 \pm 3.98
SEE \bar{P}_{mech}^+	<i>0.088</i>	15.65 \pm 6.48 ^{*,†}	29.28 \pm 8.03	32.59 \pm 16.39	33.52 \pm 19.08	25.64 \pm 16.51
SEE \bar{P}_{mech}^-	0.025	-16.72 \pm 7.52 ^{*,†}	-31.97 \pm 9.58	-32.17 \pm 13.87	-33.01 \pm 16.6	-24.87 \pm 15.08
SEE \bar{P}_{mech}^{net}	<i>0.44</i>	-1.07 \pm 3.27	-2.68 \pm 3.86	0.42 \pm 3.22	0.51 \pm 4.86	0.77 \pm 1.57

Conditions significantly different (post hoc paired *t* test, $P < 0.05$) from ω_0 and/or the global observed maximum/minimum (bold) are indicated by a superscript of * and †, respectively, for these metrics. All values reported are in units of Watts per kilogram of muscle mass. Nonsignificant *P* values are indicated with italic.

Table S4. Mean \pm SD values of measured and estimated physiological constants from all experimental preparations ($n = 5$)

Physiological parameter	Mean \pm SD
ω_0	2.34 \pm 0.11 Hz
I_{in}	1
I_{out}	21
M	0.085 kg
F_{max}	43.41 \pm 10.31 N
τ_{act}	0.066 \pm 0.011 s
τ_{deact}	0.100 \pm 0.020 s
I_0	10.74 \pm 2.89
V_{max}	-148 \pm 40 mm/s
Muscle mass	4.79 \pm 0.98 g
Animal mass	374.3 \pm 50.3
Fatigue %	76 \pm 16.9%