

Figure S1. Design details of the set-up to add effective mass to the rat plantaris muscles.

The carbon fibre rod rested on a cone-shaped component to reduce the area of contact between the component and the rod and minimize the effects of friction. We inserted a pin through the rod and cone to support and allow for passive rotation of the movement arm. We secured this set-up to a micromanipulator on a stand so that we could make fine adjustments to the position of the pin when inserting the pin into the rat plantaris muscles.

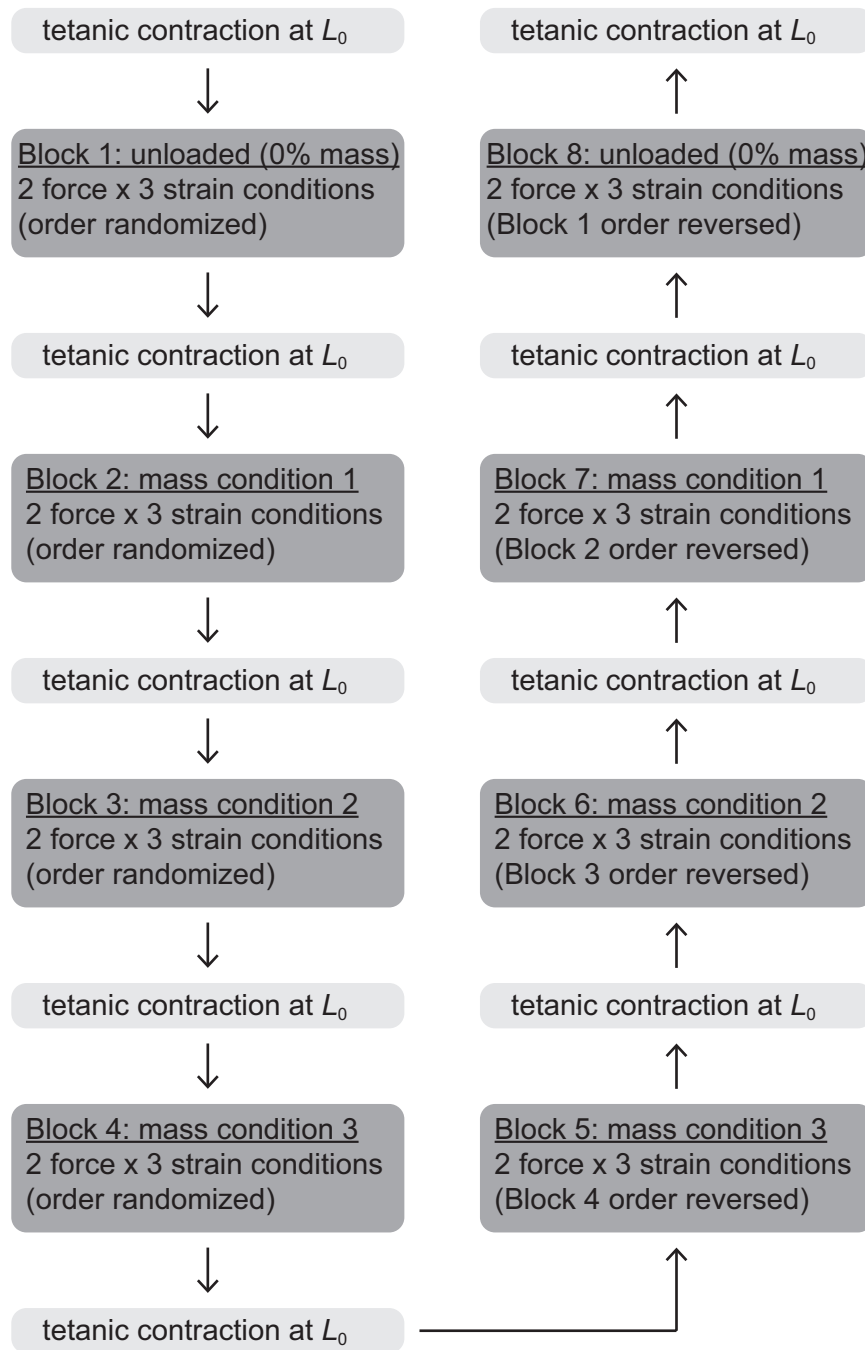


Figure S2. Flow diagram of the experimental trials. Each muscle underwent all combinations of the two force, three strain, and three mass conditions twice for a total of 36 work-loops. We blocked the work-loop trials by mass condition to reduce damage to the muscle from adjusting the pin attached to the movement arm. We randomized the order of the conditions within each trial and the order of the blocks within each experiment, except for the unloaded condition which always occurred first to avoid disturbing the pin once it was placed in the muscle (Block 1). We repeated Blocks 1-4 in reverse order to further control for order and fatigue effects (Blocks 5-8), and also conducted maximal tetanic contractions at optimal length L_0 to monitor the extent of muscle force drop over the duration of the experiments.

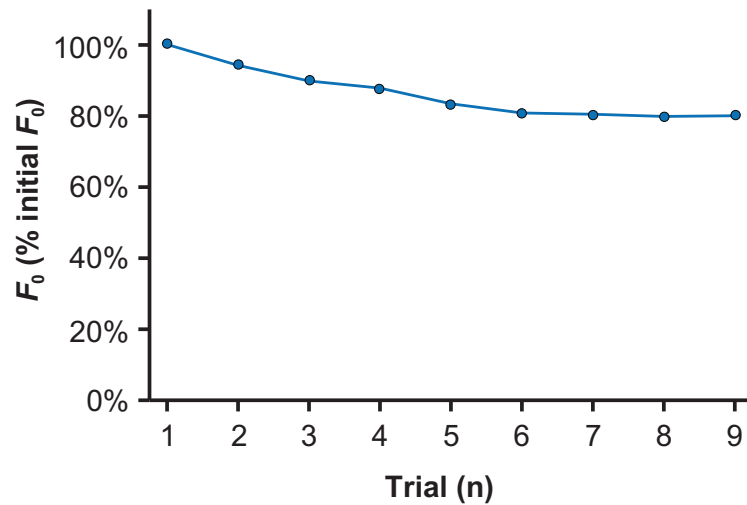


Figure S3. Reduction in maximum isometric muscle force F_0 over the duration of a representative experiment. We determined F_0 for each maximum tetanic contraction at optimal length that we conducted between each block of work-loops. F_0 is plotted against trial number and is expressed as a percentage of the initial F_0 at Trial 1.

Table S1. Muscle model parameter definitions, values, and sources.

Parameter	Value	Source
Muscle mass	$5.71 \times 10^{-4} \text{ kg } (\pm 5.49 \times 10^{-5} \text{ kg})$	Mean (s.d.) of experimental data
Optimal length, L_0	$4.03 \times 10^{-2} \text{ m } (\pm 2.10 \times 10^{-3} \text{ m})$	Mean (s.d.) of experimental data
Maximum isometric force, F_0	$4.89 \text{ N } (\pm 1.70 \text{ N})$	Mean (s.d.) of mean of first and last tetanic control contractions at L_0 for each rat
Activation rate constant, τ_{act}	$3.30 \times 10^{-2} \text{ s } (\pm 5.74 \times 10^{-3} \text{ s})$	Mean (s.d.) of values determined by fitting excitation-activation transfer function from Zajac (1989) to experimental twitches
Ratio between τ_{act} and deactivation rate constant, β	0.6	Dick et al. (2017)
Maximum intrinsic shortening velocity, v_0	5 s^{-1}	approximated from Swoap et al. (1997) (4.48 s^{-1})
Location of added mass in lumped added mass simulations	9 th point mass from fixed end (56% of muscle length)	Closest point mass to mean of experimental data

Table S2. Mechanical work data for the experimental work-loop trials. The first column contains the rat identification number, the second column contains the force condition, the third column contains the added mass condition expressed as a mean percentage of the muscle mass across all rats, and the fourth column contains the strain amplitude expressed as the change in muscle length (ΔL_m) relative to optimal length (L_0). The final column contains the mean work values calculated over the last three cycles per trial and the two repeated trials (see Fig. S2). The mean work is dimensionless and normalized to L_0 for each rat, as well as the trial-varying maximum isometric force F_0 and passive force at L_0 for the active and passive force trials, respectively. We treated the rat identification number, force condition, added mass condition, and strain amplitude as categorical predictor variables in the statistical analysis, and we treated the mechanical work as a continuous response variable.

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