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Modeling age-related changes in muscle-tendon dynamics during cyclical contractions in the rat gastrocnemius

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Running head: Gastrocnemius MTU dynamics with aging

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Abstract

Efficient muscle-tendon performance during cyclical tasks is dependent on both active and passive mechanical tissue properties. Here we examine whether age-related changes in the properties of muscle-tendon units (MTUs) compromise their ability to do work and utilize elastic energy storage. We empirically quantified passive and active properties of the medial gastrocnemius muscle and material properties of the Achilles tendon in young (~6 mos) and old (~32 mos) rats. We then used these properties in computer simulations of a Hill-type muscle model operating in series with a Hookean spring. The modeled MTU was driven through sinusoidal length changes and activated at a phase that optimized muscle-tendon tuning to assess the relative contributions of active and passive elements to the force and work in each cycle. In physiologically realistic simulations where young and old MTUs started at similar passive forces and developed similar active forces, the capacity of old MTUs to store elastic energy and produce positive work was compromised. These results suggest that the observed increase in the metabolic cost of locomotion with aging may be in part due to the recruitment of additional muscles to compensate for the reduced work at the primary MTU. Furthermore, the age-related increases in passive stiffness coupled with a reduced active force capacity in the muscle can lead to shifts in the force-length and force-velocity operating range that may significantly impact mechanical and metabolic performance. Our study emphasizes the importance of the interplay between muscle and tendon mechanical properties in shaping MTU performance during cyclical contractions.

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New & Noteworthy: The age-related increase in muscle and tendon tissue stiffness and reduction in active force capacity of the muscle compromise elastic energy utilization and positive work production, which may require the recruitment of additional muscle volume potentially contributing to the increased cost of locomotion observed in older individuals.

keywords: aging, elastic energy, cost of locomotion, fibrosis, ankle joint work

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1 INTRODUCTION

Locomotion is more energetically costly in older individuals. A comparison of healthy young men (~27yo) and healthy older men (~74yo) walking at varying speeds found that the metabolic cost of walking increased by an average of 31% in older men (39). Similarly, during running, older individuals have decreased overall efficiency, requiring muscle to consume significantly more metabolic energy per unit mechanical work with each step (15). While this may be partially due to a decreased efficiency of muscle contraction (16), a reduction in effective energy storage could also contribute to an increase in the metabolic cost of locomotion.

The dynamic function of tendon is integral to reducing the metabolic cost of locomotion. These energetic savings may occur by changing where muscles operate on their force-length and force-velocity curves (35), by reducing muscle work (6) or by reducing active muscle volume (24). Regardless of the mechanism by which tendons reduce the metabolic cost of muscle contraction, changes in the active and passive mechanical properties of muscles and tendons could reduce the ability of a muscle-tendon unit to effectively use these cost saving mechanisms.

The mechanical properties of tendon are critical in determining tendon dynamics and function (8). However, there is little consensus on how aging affects tendon properties. Changes in modulus and cross sectional area can alter the functional stiffness of tendon thereby changing tendon dynamics during

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77 cyclical tasks such as walking and running. Additionally, changes in the tendon
78 stiffness will lead to changes in the natural frequency of muscle-tendon units and
79 limbs, potentially compromising the effective use of resonance during such tasks
80 (56). Increases in tendon cross-sectional area (37, 54) and collagen cross-linking
81 due to an accumulation of advanced glycation end products (32), have been
82 demonstrated with age. These changes are likely to increase tendon stiffness
83 (48). The majority of experimental data comparing young and old connective
84 tissues in various animal systems support this and suggest an increase in elastic
85 modulus with age (1,13, 14, 18, 21, 54). Some studies, however, especially *in*
86 *vivo* studies on humans, have shown a decrease in the Young's modulus of
87 tendons (9, 29, 30, 33, 43, 44, 59).The lack of consensus in the literature
88 suggests that the effect of aging on the material properties of tendon may vary
89 based on preparation, the tendon being studied, the study species and the age
90 cohorts being compared. This lack of consensus has made it difficult to relate
91 changes in tendon properties to the metabolic cost of movement.
92 Age-related reduction in the force capacity of older muscles is well
93 established. A reduction in maximum isometric force can be as high as 2% per
94 year (55) and is often associated with dimensional changes in the muscles and
95 an overall loss of muscle mass due to atrophy (60). However, a reduction in
96 muscle mass or cross-sectional area (CSA) does not fully explain the decline in
97 force output in the elderly. Maximum isometric stress (force/CSA) also decreases
98 significantly with age. This has been attributed to age-related increases in non-
99 contractile tissues such as fat and collagen (21, 31, 47), changes in muscle

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100 architecture (42, 57), and increased stiffness of intramuscular connective tissues
101 (31). The age related reduction in muscle force can decrease the ability of a
102 muscle to stretch its external tendon, and compromise the storage and return of
103 elastic energy.

104 The purpose of this study was to examine the age-related changes in
105 muscle-tendon unit function. We use a well-established animal model of aging,
106 the F344xBN rat (5,25). The use of an animal model provides a more controlled
107 system than the cross sectional studies of humans, which are often fraught with
108 significant inter-individual variation and a more practical system than longitudinal
109 studies of human subjects that can extend over several decades. In addition, the
110 use of an animal model allows us to study both isolated components of the
111 musculoskeletal system as well as the integrated and intact system in order to
112 better understand the specific structures responsible for functional decrements.
113 Finally, the rat strain used in the study (developed by the National Institute of
114 Aging) is well suited for studies of healthy aging without the confounding
115 pathologies common in other model systems. In this study we first measured the
116 contractile, morphological and mechanical properties of the medial
117 gastrocnemius muscle and tendon. We then used these experimental variables
118 as inputs to a muscle-tendon model (Hill-type model operating with an in-series
119 spring) to simulate cyclic contractions (*i.e.,* work loops) and test: 1) whether old
120 MTU's can perform as much mechanical work as young ones, and 2) whether
121 elastic energy storage and return is compromised in old MTUs as a result of non-
122 optimal interaction between active and passive MTU properties.

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2 MATERIALS AND METHODS

2.1 Experimentally measured muscle-tendon properties

Active and passive muscle-tendon unit properties (Table 1) were measured *in situ* in young (n=8, age 5-9 months, body mass 362±32 g) and old (n=8, age 33-34 months, body mass 489±19 g) male Brown Norway x F344 F_1 hybrid rats, *Rattus norwegicus*, from the National Institutes of Aging (F344BN; National Institute on Aging, Bethesda, MD). The animal ages were chosen to ensure that the effect we documented was one of senescence and not developmental maturation (41). The medial gastrocnemius (MG) was identified as a good muscle model because of its size and accessibility, and its significant contribution to ankle power. All experimental procedures involving animals were approved by the Institutional Animal Care and Use Committee at the University of California, Irvine.

The passive and active force-length properties of the intact muscle were first determined *in situ* as previously described in (23). The rats were anaesthetized using 2% isoflurane, maintained on a closed system anesthesia machine (Parkland Scientific, Coral Springs, FL) and placed, prone, on a heat mat. The sciatic nerve was exposed via a small incision running from the caudal midline of the hind limb towards the base of the tail. A nerve cuff containing a stimulus and a ground electrode was placed around the nerve and the nerve was severed proximally. The area around the electrode was filled with warmed

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146 mineral oil and sutured closed. The Achilles tendon was then exposed and all
147 tendons, except that of the gastrocnemius, were severed. The calcaneus was cut
148 leaving a small amount of bone attached to the MG tendon. This bone and
149 tendon were secured in a custom-made clamp, as close to the end of the muscle
150 fibers as possible. An incision was made on the lateral aspect of the thigh and
151 the femur clamped into a stereotaxic frame. The distal clamp was connected to
152 the lever arm of an ergometer (310 B-LR, Aurora Scientific Inc., Ontario, Canada)
153 using steel cable (10 cm). Care was taken to minimize the compliance of the
154 setup. The muscle was wrapped in saline-moistened gauze and Saran wrap and
155 muscle temperature was maintained at 37 °C using a heat lamp.

156 Isometric, fixed end, twitch contractions were elicited, by applying a single
157 stimulus pulse to the sciatic nerve, at a range of lengths to produce a twich force-
158 length curve (Grass S48 stimulator, Grass medical instruments, Quincy, MA,
159 USA). This allowed us to quantify the optimal length of the muscle. Force and
160 ergometer position data were collected at 1000 Hz using a National Instruments
161 AD board (NI USB-6212) and recorded using Igor Pro 6.31 software
162 (Wavemetrics Inc., Lake Oswego, OR, USA). A single tetanic contraction was
163 elicited at the optimal muscle length ($L_{0\ muscle}$, Table 1) by applying a train of 0.2
164 ms square wave pulses at 100 Hz for 400 ms (F_{max}, Table 1). Because the lateral
165 gastrocnemius (LG) was still attached to the MG and was also stimulated by the
166 sciatic nerve, force values (F_{max}) were corrected based on the relative size of the
167 two muscles.

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After-loaded isotonic tetanic contractions were used to determine the force-velocity relationship of the muscle with the same stimulation protocol used for isometric tetanic contractions (23). Force was allowed to rise to a defined level (10, 30, 50, 70 or 90% of F_{max}) and the muscle allowed to shorten to maintain a constant force. The order of force levels was randomized and a 5 minute rest period allowed for recovery between contractions. Another isometric contraction was performed after the series of isometric contractions to monitor muscle fatigue and health; muscle force never fell below 90% of its original value. V_{max} was obtained by fitting the Hill equation to the pooled data for each age group. Activation (t_{act}) and deactivation (t_{deact}) time constants were defined based on activation equations from (61) and were chosen to match the force profile of a representative tetanus for each age group.

Passive stiffness of the muscle (k_{muscle}, Table 1) was measured during a passive stretch and calculated from the slope of a linear fit to passive force *vs.* muscle strain (relative to $L_{0\ muscle}$) in Igor Pro 6.31 (Wavemetrics Inc., OR, USA) (Fig. 1). The length of the contralateral MG tendon ($L_{0\ tendon}$) was measured *in situ* using calipers with the knee and ankle at 90°. The tendon was harvested immediately following euthanasia and frozen in physiological saline for up to 1 year before testing. Tendon stiffness (k_{tendon}) was measured as follows. One end of the tendon was attached by Kevlar thread to an ergometer (model 360C, Aurora Scientific Inc., Ontario, Canada) and the other was held in place with a stationary clamp. Each tendon was repeatedly stretched to 2% and 5% of its resting length for five cycles at two frequencies, 2.5Hz (2 sec) and 5Hz (1 sec).

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191 The strain profiles selected encompassed the predicted tendon strains during
192 walking and running (25), reached linear stress-strain profile and avoided plastic
193 deformation. Tendon stiffness (N/mm) was calculated as the slope of the linear
194 region of the third stretching cycle.
195
196 **2.2 Computational muscle-tendon model**
197 We used Simulink (MathWorks®, Natick, MA) to develop a computational
198 model (50) and ran simulations using empirical data from either young or old
199 muscles. Briefly, the models consisted of a Hill-type muscle model with nonlinear
200 F-L and F-V properties in series with a linearly elastic tendon. Muscle activation
201 was modeled after Zajac (61). The young and old models were validated by
202 comparing the force profiles of tetanic contractions measured *in situ* in young and
203 old muscles with the simulated output force profile of the models (Fig. 1).
204 Once validated, young and old muscle tendon unit models were oscillated
205 through 3 Hz stretch-shorten cycles with an amplitude that corresponded to 25%
206 of their resting length (muscle L_0 plus tendon slack length, $L_{slack\ tendon}$; 13.5mm for
207 young) starting at $1.1L_0$. In length matched simulations, old muscle tendon unit
208 models were also oscillated through 3 Hz stretch-shorten cycles with an
209 amplitude that corresponded to 25% of their resting length (muscle L_0 plus
210 tendon slack length, $L_{slack\ tendon}$; 11.2mm). However, the differences in the
211 empirically derived passive stiffness of the young and old muscles (Table 1)
212 meant that while these simulations provided a length matched comparison
213 between young and old muscle, they did not provide a reasonable force matched

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comparison. In order to achieve a force-matched simulation, we also cycled old muscle through a 3 Hz stretch-shorten cycle with a starting length of $1.0L_0$ and an amplitude corresponding to 12% MTU strain. This resulted in more comparable passive forces both at the beginning of the cycle and throughout the stretch-shorten cycle.

In all simulations, the muscle was stimulated for 10% of the cycle. The stimulus was applied at 12.5% intervals to find the timing of stimulation that minimized net muscle work. In all simulations the optimal phase, resulting in the least net work was 37.5% where 0% is the beginning of lengthening (Fig. 2).

3 RESULTS

Our empirical measurements are largely consistent with previous studies describing age-related changes to muscle and tendon. We found that aging results in a decrease in both maximum isometric force (F_{max}) and maximum shortening velocity (V_{max}) (Table 1). The passive stiffness of the muscle was higher and passive tension developed at shorter lengths ($1.0L_0$) in old muscles, compared to young muscles ($1.1L_0$) (Table 1). We also found that the stiffness of the series elastic tendon was higher in old compared to young muscles (Table 1). Activation time constant (t_{act}) was not different between young and old muscles, but old muscles took longer to deactivate (t_{deact}; Table 1) as has been observed elsewhere (10).

To validate the outputs of the muscle-tendon model, we compared an empirical tetanic contraction from an *in situ* preparation with the predicted force

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237 profile from a model simulation (Fig. 1). To simulate the removal of the tendon
238 from the *in situ* preparation we set tendon stiffness to an unrealistically high value
239 prior to our validation trials. The model predicted the path of force rise and decay
240 reasonably well, with the most obvious difference lying in the rate of force decay.
241 In the empirical tetanic contractions, force dropped at a faster rate than in the
242 computational model (Fig. 1).

243 First we compared young and old MTUs under strain-matched conditions
244 where the length trajectories imposed on the virtual MTUs were identical. Both
245 young and old muscles start the work loop cycle at $1.0L_0$ and undergo sinusoidal
246 length changes with 25% MTU strain (Fig. 2A, B). The young contractile element
247 (CE; Fig. 2A) initially shortens internally against the tendon while the old CE
248 produces force nearly isometrically. Additionally, in the young MTU the CE
249 makes up nearly twice as much of the total MTU strain as the SEE, but a larger
250 proportion of MTU strain is due to the stretch of the tendon (SEE) in the old MTU
251 (Fig. 2B). On the other hand, as the MTU is being passively stretched prior to
252 stimulation, the increased stiffness of the old muscle and tendon result in higher
253 initial and maximum passive forces in the old MTU (Fig. 2B). Under these strain-
254 matched conditions, old MTUs seemed better tuned for elastic energy utilization
255 than young MTUs. The old CE performed less active work and the SEE cycled
256 nearly 100% of the elastic energy stored in it (Fig. 3A, B).

257 Second, we compared young and old MTUs under force-matched
258 conditions, where the length trajectories imposed on the virtual MTU were
259 adjusted to produce similar passive forces prior to activation. This resulted in a

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passive force of 3.5N for the young MTU and 2.7N in the old MTU under force matched conditions, compared to a passive force prior to stimulation of 8.8N in the old MTU under strain-matched conditions. To match passive force profiles, old work loops were performed with shorter initial muscle length (1.0 *vs*. 1.1 $L_{0\ muscle}$) and at nearly half the amplitude (12% *vs.* 25% MTU strain) when compared to young (Fig. 2C). Under these conditions, muscle and tendon took up equal amounts of the imposed MTU strain prior to stimulation. At stimulation the muscle shortened against the tendon but produced less active force than the young muscle (Fig. 2C). Under these force-matched conditions positive MTU work was reduced to nearly a third of young MTU work (Fig. 3C)

We also compared the absolute and relative contributions of active and passive forces to total force and work (Fig. 4). Young MTUs produced a maximum force of 6N while strain-matched old MTUs produced nearly twice as much force (11.1N). However, 80% of the total force came from passive tissues in the old MTU, compared to 55% in the young MTU (Fig. 4A). Under force-matched conditions, the proportion of passive to total force in old MTUs dropped to 53%, similar to young MTUs. We also calculated the amount of positive work done by the muscle (active) and series elastic elements (passive) and the proportion of total positive work (CE + SEE) done by each element (Fig. 4B). Although the proportion of elastic potential energy, calculated as the product of total force and SEE length change, was similar in all three simulation conditions (44% to 50%) the absolute magnitudes of these differed greatly, with old MTUs

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under strain-matched conditions producing the most work but old MTUs in the force-matched model producing the least.

To examine whether age-related changes affected where muscles operated on the force-length and force-velocity curves we plotted the active muscle lengths and shortening velocities on empirically derived force-length and force-velocity curves (Fig. 5). The operating length of the muscle was largely determined by the initial length of the MTU and prescribed strain patterns. Therefore, during strain-matched simulations young and old muscles operated over similar regions of the force-length curve (I and II, Fig. 5A). However, during force-matched simulations old MTUs operated on the plateau and underwent less length change (III, Fig. 5A). Young MTUs underwent significant periods of active shortening (positive V/V_{max}) and also operated eccentrically near F_{max} for part of the cycle (I, Fig. 5B). In the strain-matched condition, old MTUs never shortened and operated closer to F_{max} (II, Fig. 5B). In force-matched conditions however, they behave more like the young MTU, undergoing both con- and eccentric contractions albeit at lower average lengthening velocities (III, Fig. 5B).

4 DISCUSSION

We used empirically informed simulations to assess how age-related changes in the mechanical properties of an MTU affect how mechanical work is distributed between the muscle and the series elastic elements during cyclical contractions and how such changes affect muscle operating lengths and velocities.

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305

306 **4.1 Changes in MTU Mechanical Properties with Age**

307 We found that in the rat gastrocnemius both the muscle and tendon
308 increased in stiffness with advanced aging, similar to what has been reported in
309 other studies (1,13, 14, 18, 21, 54) (Table 1). Muscle fibrosis and increased
310 deposition of collagen in the extracellular matrix (ECM) is common amongst
311 neuromuscular pathologies, atrophy, and aging (36). During aging, muscle stem
312 cells disproportionately shift from a myogenic (forming muscle cells) fate to a
313 fibrogenic fate and cause a relative increase in collagen content, leading to a
314 "fibrotic" muscle phenotype (1, 21, 32). Fibrotic muscles have increased passive
315 stiffness and develop passive tension at shorter relative lengths (Table 1; 36).

316 Despite numerous studies that have measured the tensile properties of
317 tendons, there has been no consensus on whether advanced aging increases
318 (13, 54), decreases (9, 30, 59) or has no effect (29, 40) on tendon stiffness or
319 modulus. This is surprising given the observed decrease in the crimp angle of
320 collagen and increased fiber cross-linking with aging (18), which suggest that
321 structural changes should lead to an increase in Young's modulus (18, 58).
322 However, none of these studies concerned the Achilles tendon of rats. Tendons
323 have been shown to have varying structural and material properties depending
324 on their *in vivo* function (8, 54). Therefore, it is worth conducting future studies to
325 explore how age-related effects might differ among muscle-tendon groups with
326 varying functions.

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We also documented a reduction of maximum isometric muscle force with aging (Table 1) similar to numerous other studies. However, our results suggest that at longer MTU lengths passive forces may compensate for the loss of active force capacity in old muscles (Fig. 2). This result may help explain previous findings that have shown that the age-related loss of force is more pronounced during concentric or isometric tasks compared to eccentric tasks where passive forces are more likely to contribute (27).

4.2 MTU strain and work

In the old MTU, we matched either total MTU strain (strain-matched simulation) or total MTU passive force (force-matched simulation) to the values seen in young MTUs. The reduction in active force capacity coupled with the increased stiffness of the muscle and series elastic elements meant that when young and old MTUs undergo the same strain cycle, passive forces contributed much more to the total MTU force and positive work in old MTUs. However the passive stretch of the old muscle requires more force production, either by the contraction of antagonists or by the generation of increased inertial loads, both of which are likely to bear an energetic burden. The strain-matched conditions are highly unlikely under physiologically realistic conditions. Firstly, antagonist muscles are likely to show a similar decline in active force capacity and therefore may not be able to generate sufficient forces to stretch muscles to such high passive tensions. Similarly, given that body mass is unlikely to increases in old animals and vertical velocity during walking is actually reduced (25, 45), the

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350 inertial/gravitational forces loading the muscle-tendons would not be high enough
351 to stretch old muscle to lengths that correspond to significant passive force. The
352 reduction in MTU amplitude from 25% to 12% in the force-matched simulations is
353 likely still an over estimate of the strain antagonist muscles would be able to
354 impose given the high passive stiffness of gastrocnemius MTU and the 50%
355 reduction in force producing capacity in old rats (Table 1).

356 The force-matched simulations are therefore more likely to represent the
357 realistic conditions to compare under the young and old MTU. It has been
358 previously shown that variation in the passive stiffness of a muscle shifts the
359 operating length such that muscles are recruited at similar passive forces rather
360 than similar lengths (4). The results of the force-matched model potentially
361 explain some of the distinct gait changes that occur with aging in a diversity of
362 animal groups including humans (25, 53). Under such conditions an old MTU
363 performs about one third of the work per stride compared to young MTUs (Fig.
364 4A). This result is consistent with the reduction in preferred walking speed with
365 age in humans (38) and the lower stride length observed in older humans and
366 animals (25, 53). Therefore, a scenario of muscles operating at shorter lengths
367 and undergoing lower muscle excursions may be more representative of *in vivo*
368 muscle conditions in older animals and humans.

369 The reduction in operating lengths and strain amplitude suggested by our
370 simulations (Fig. 5A) may also protect old muscle from damage. Operating on the
371 descending limb of the force-length relationship and undergoing larger
372 lengthening strains are associated with an increase in the likelihood of muscle

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damage (22). Avoiding such longer lengths may be particularly beneficial as fibers from old muscles are more prone to being damaged (12), and less efficient at recovering from injury (11).

Elastic energy storage was not always compromised in old MTUs. From the strain-matched models we could see that if there was enough force available to stretch the MTU to similar strains in the aged animals elastic energy storage would increase due to the increased stiffness of the muscle and tendon (Fig. 4). Although the absolute amount of elastic energy stored is much reduced in the force-matched model, the proportion of total work done by the passive elements is actually slightly increased. Hence, whilst the total work of the system may decrease with age, the contribution of the tendon does not necessarily change.

4.3 Energetic implications of changes in MTU properties due to aging

The physiologically more relevant force-matched simulations of the old MTU may have some potential implications for the energetic cost of locomotion. The reduced work production by the gastrocnemius in this condition will reduce the range of motion at the ankle, the primary joint where work is done during push off (19). This will require more work to be done by the more proximal muscles around the knee and hip joints (26). This shift in joint work results in a shift in the architecture of the muscles used from short fibered pennate muscles with long tendons in the distal limb to long fibered parallel muscles with little external tendon in the proximal limb. This shift in the architecture of muscles used may increase the cost of generating mechanical work (34, 49). Smaller

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396 muscle moment arms during crouched postures (7, 25) further contribute to a
397 reduction in ankle work. Similar kinematic and energetic changes were observed
398 when ankle excursion and work were limited by a rigid ankle orthotic prosthesis
399 in humans (28).

400 Additionally, there is a well-documented increase in both synergist and
401 antagonist muscle co-contraction with aging (20, 39, 46). It has been
402 hypothesized that co-contraction of antagonists may provide additional joint
403 stability to support the body weight (39) given the reduced capacity of each
404 muscle to produce force. Furthermore, the results of our force-matched
405 simulations show that co-contraction of synergistic muscles is a means of
406 compensating for reduced work due to the changes in active and passive
407 mechanical properties of the MTU. If the age-related changes we observe in rats
408 are similar in humans then the recruitment of additional muscles may contribute
409 to the additional metabolic cost of locomotion in the elderly; co-contraction of
410 knee extensors and flexors alone can account for 28-52% of the increased
411 metabolic cost of walking (39).

412 Changes in the stiffness of an MTU also alter its natural frequency.
413 Resonance occurs when a system is driven at its natural frequency, and has
414 been shown to significantly affect both MTU dynamics (51) and the metabolic
415 efficiency of locomotion (17). However, in our analyses we used a single driving
416 frequency for two different mechanical systems. This could explain why we
417 observed no shift in the stimulation onset that minimized active muscle work,
418 similar to what has been observed in the onset of gastrocnemius activity in a

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speed-matched study of young and old men walking (39). In an unconstrained work loop experiment it was shown that when MTUs of different mechanical properties were cycled at their natural frequency, stimulation onset shifted relative to length changes to tune MTUs for maximum force and elastic energy storage (51). By constraining the driving frequency of the MTU or during speed-matched *in vivo* studies we are likely forcing energy inefficient dynamics onto systems whose stiffness differs by nearly two-fold.

4.4 Potential Limitations

There were a number of simplifications we made to the structural morphology of our MTU model that are worth addressing. First, our model is a one dimensional muscle model that can account for the functional effects of certain morphological changes along the line of action of the free tendon (e.g. reduction in the cross-sectional area of muscles and tendon) but cannot address off-axis shape changes that occur during *in vivo* contractions. For example, our simulations do not account for three-dimensional effects such as muscle gearing (2) that have been shown to be significantly affected by age (23). In addition, our simulations used a series elastic element with a linear stress-strain relationship and a constant stiffness. The stress-strain curve of tendons has a non-linear stiffness at lower strains (toe region), which may have some small impact on MTU dynamics at the lowest force levels. In addition, aponeuroses have been shown to deform biaxially during stretch-shorten cycles and to function as variable stiffness springs (3). Thus, it is possible that the effects of aging on the

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442 dynamic changes in aponeurosis stiffness may also alter the length trajectory of
443 an MTU.

444 Aspects of our work loop protocol may also deviate from capturing all of
445 the features of *in vivo* MTU dynamics. Work loops that strictly enforce MTU
446 length change patterns and muscle stimulation phase provide a highly controlled
447 experimental framework that is useful for gaining initial insights (52). However,
448 while our imposed length trajectories were symmetrical sine waves, the actual
449 length trajectories of MTUs like the gastrocnemius may be more accurately
450 characterized as asymmetrical (51). In addition, we note that cyclic contractions
451 in freely moving animals are more likely the result of the dynamic interaction
452 between MTU force output, the load of the body and the dynamics of the
453 environment (51). Finally, any extrapolation from our results to the mechanics
454 and energetics of human locomotion are based on the assumption that
455 mechanical changes that occur with age are shared between our rodent model
456 and humans. Despite these limitations, our study is the first to use empirically
457 informed simulations to compare the dynamics of old and young MTUs and
458 provides fundamental insight into the mechanical interactions of muscles and
459 tendons.
460

461 **4.5 Conclusions**

462 In this study we used an empirically driven simulation of muscle-tendon unit
463 dynamics to explore the effect of age-related disruptions to active and passive
464 properties on the exchanges in energy during cyclical contractions. Our results

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suggest that the ability to store and return energy in tendons is not always compromised with age as passive forces compensate for a decline in active force production. However, age-related increases in passive stiffness are likely to reduce MTU strain, and therefore mechanical work. This reduced work capacity is likely to significantly impact energetic performance as more muscle mass may be required to retain comparable joint dynamics.

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Disclosures

The authors declare no conflicts of interest.

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663 **Figure Captions**

664 **Table 1**. Empirically derived model parameters (mean± S.D.) for young and old
665 rat medial gastrocnemius (MG) muscle-tendon unit.

666

667 **Figure 1.** Simulated and experimental tetanic contractions. Experimental data
668 (solid lines) consists of a single tetanic contraction of the intact medial
669 gastrocnemius, at L_0, with a 450 ms stimulation. Computational simulations
670 (dotted lines) match experimental data reasonably well in both the young (A) and
671 old (B) cases, with the most obvious difference being a slower rate of force decay
672 in the modeled data. F_{max} is the maximum isometric force.

673

674 **Figure 2.** Time series of length change, force development and power during
675 simulations (3Hz with 10% duty stimulation starting at 37.5%) for the three
676 conditions tested. The period of stimulation is indicated by the shaded grey
677 region. Total MTU (muscle-tendon unit) length change is different for young and
678 old MTUs (A and B) because resting MTU length differed in the two age groups
679 (54mm for young *vs.* 44.6mm for old MTU when measured with the ankle and
680 knee at 90°). During strain-matched simulations when the starting length and
681 strain amplitudes are the same (A and B), the old MTU develops high passive
682 forces prior to activation. During force-matched simulations when initial passive
683 force and total maximum force are similar (A and C), old MTU develops
684 significantly less power and store less energy in series elastic elements. CE is
685 the contractile element and SEE is the series elastic element.

686

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687 **Figure 3.** Muscle-tendon unit (MTU) and contractile element (CE) work loops
688 from simulations of MTU at 3Hz with 10% duty stimulation starting at 37.5% of
689 the cycle (with respect to the beginning of shortening). When young and old
690 MTUs are cycled under the same conditions (A, B) old MTUs start at a higher
691 passive force and, when stimulated, contract nearly isometrically performing less
692 positive work than young muscles (CE). However, if the initial passive force and
693 the maximum total force is matched between young and old MTUs, old MTUs are
694 stretched by only 12% of their resting length and the muscle starts contracting at
695 $1.0L_0$ compared to $1.1L_0$ of the young CE. Bolded regions of the work loops
696 represent periods of active force production.
697

698 **Figure 4.** Contributions of passive (SEE) and active (CE) elements to total force
699 and work production, under each of the three simulation conditions. A) When old
700 MTUs were cycled with the same conditions as young MTUs the peak total force
701 doubled due to a large increase in passive force and a reduction in active muscle
702 force. When old MTUs were allowed to begin cycling at shorter lengths and with
703 smaller strain excursions, both the absolute and relative passive and active peak
704 force patterns resembled that of young MTUs. B) In a young MTUs, CE and SEE
705 both contribute 50% of the work in a work loop. The relative values are similar in
706 old MTUs cycled under the same conditions, but the absolute value of work is
707 more than doubled. However, when the peak muscle force is kept at realistic
708 values for old MTUs (initial muscle length at $1L_0$ and 12% MTU strain) the whole

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709 MTU performs less than a third of the work that a young MTU does under
710 physiological conditions.
711

712 **Figure 5.** Operating range of young and old muscles under three simulation
713 conditions, mapped on experimentally derived force-length and force-velocity
714 curves: **I**. Young; **II**. Old, strain-matched conditions; **III.** Old, force-matched
715 conditions. Data are shown only for the active portion of the cycle. A) Young and
716 old muscle acting the same strain stretch-shorten conditions (muscle $L_{initial}$=1.1L_0,
717 25% MTU strain) operate on the descending limb of the force-length curve.
718 However, old muscle acting under conditions that match that MTU passive force
719 produced during stretching operate on the plateau, nearer to L_0. B) Young
720 muscle operates over a broader range of velocities than old muscle under either
721 strain-matched or force-matched condition.
722

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Table 1. Empirically derived model parameters (mean± SD) for young and old rat medial gastrocnemius (MG) muscle-tendon unit.

	Young	**Old**
Active parameters		
F_{max} (N)[1]	14.7±2.77 (n=8)	7.1± 1.41 (n=8)
V_{max} (m/s)[2]	0.122 (n=8)	0.107 (n=8)
t_{act} (s)[3]	0.062	0.062
t_{deact} (s)[3]	0.071	0.081
Passive parameters		
$L_{0\ muscle}$ (m)	0.039± 0.022 (n=11)	0.033± 0.0086 (n=3)
L_r ($L_{0\ muscle}$)[4]	1.1	1.0
k_{muscle} (N/m)	420± 87.3 (n=7)	970± 160.9 (n=5)
E_{muscle} (kPa)	0.79± 0.40 (n=5)	1.74± 0.87 (n=7)
$L_{slack\ tendon}$ (m)	0.0153± 0.018 (n=5)	0.0115± 0.013 (n=5)
k_{tendon} (N/m)	1102± 313 (n=5)	1505± 333 (n=5)
E_{tendon} (GPa)[5]	6.1± 1.78 (n=5)	10.2± 1.11 (n=5)

[1] Mean peak isometric stress multiplied by mean *triceps surae* cross-sectional area and corrected for the MG proportion of complex. (see Methods)

[2] Value was estimated by fitting a single curve to the compiled data for each age group.

[3] Selected to match the force rise (t_{act}) and force decay (t_{deact}) of a representative *in situ* tetanic contraction at L_0.

[4] Length at which muscle develops passive force.

[5] Calculated for 5% strain and 5Hz cycling

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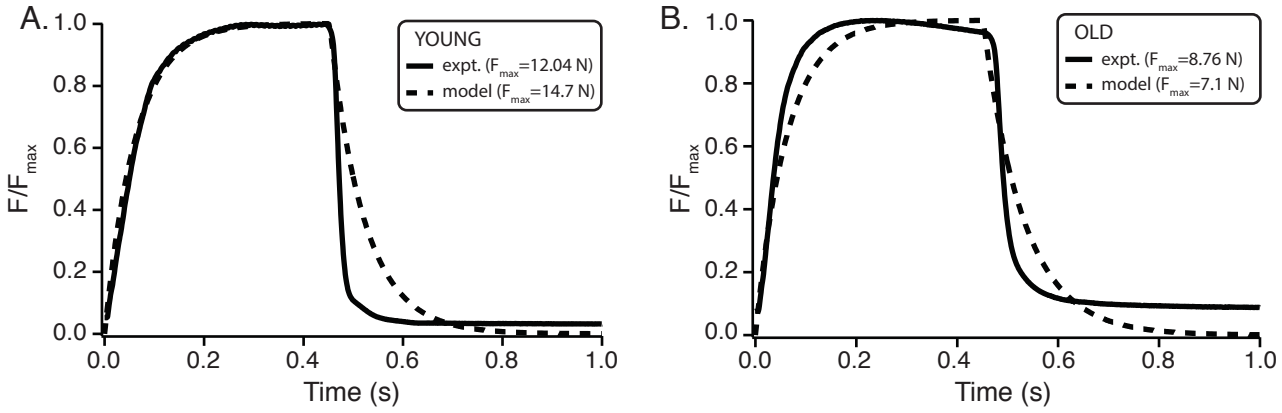

Figure 1
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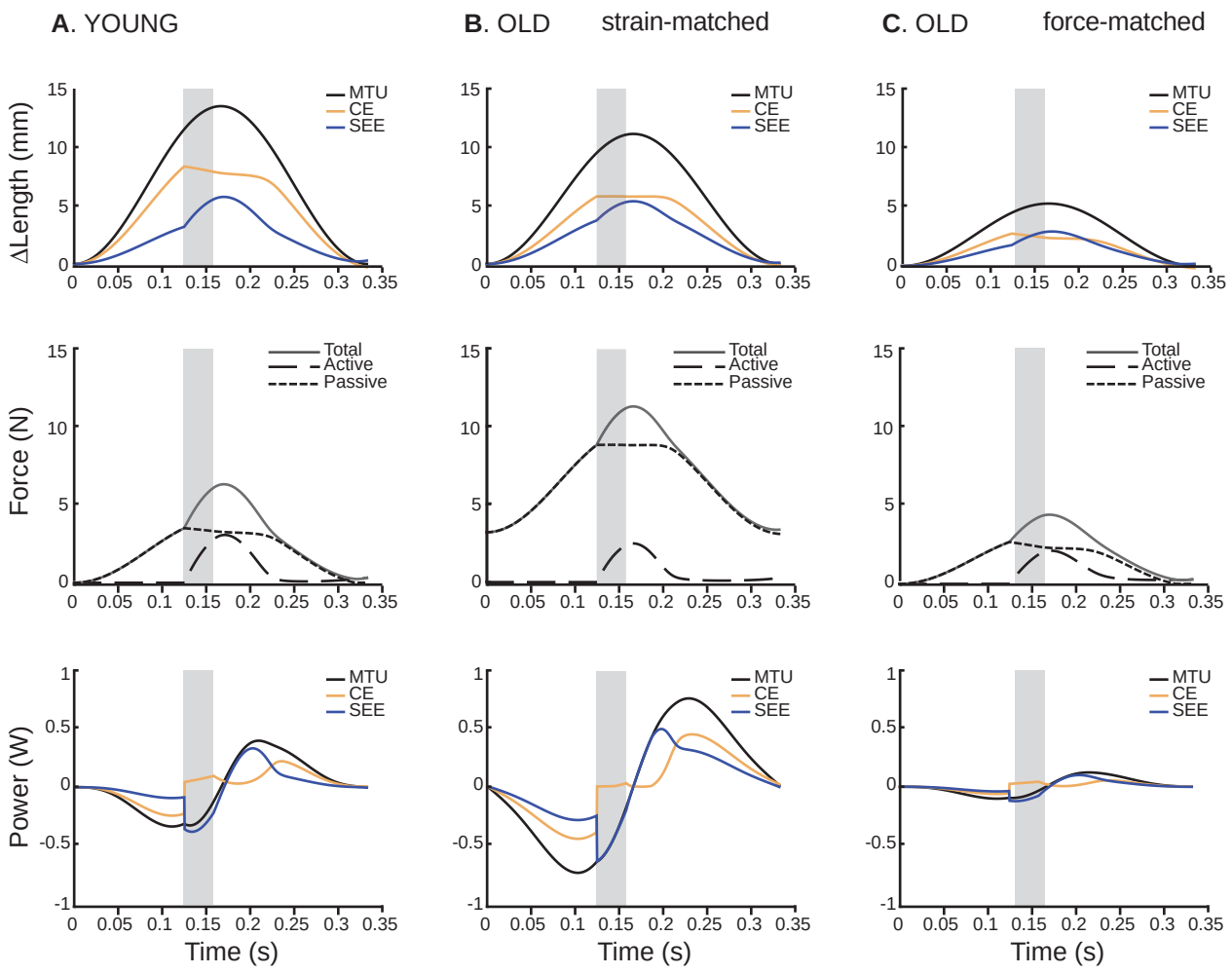

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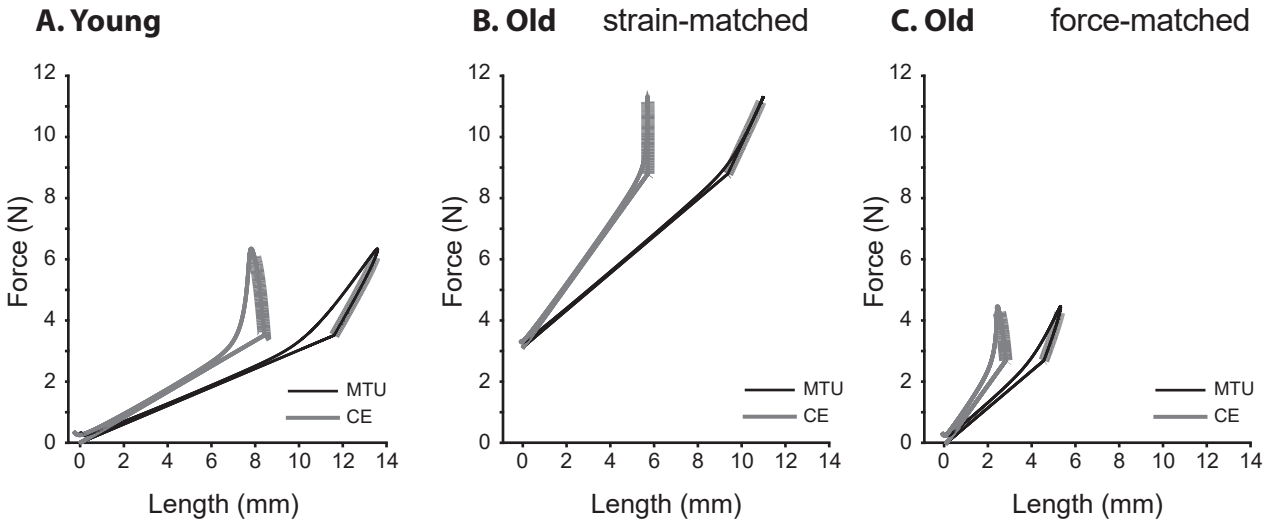

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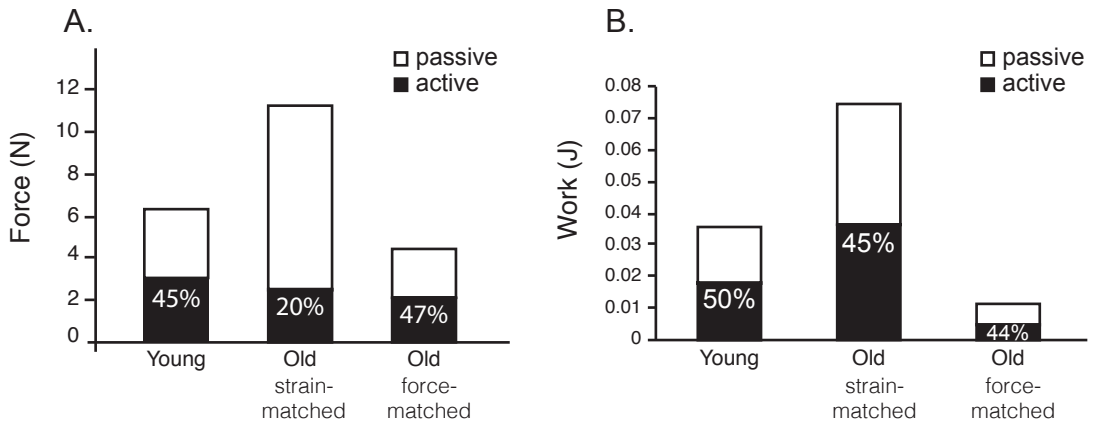

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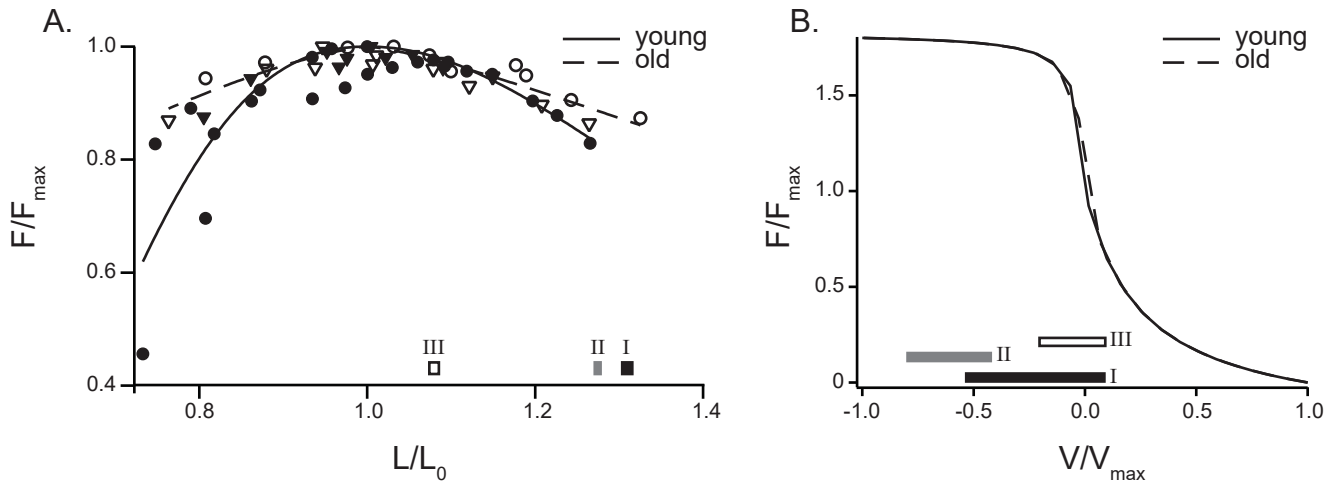

Figure 5
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