

1 **Title:** Relatively Shorter Muscle Lengths Increase
2 the Metabolic Rate of Cyclic Force Production

3
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21 **Abstract**

22 During animal locomotion, force-producing leg muscles are almost exclusively responsible for
23 the whole-body's metabolic energy expenditure. Animals can change the length of these leg
24 muscles by altering body posture (*e.g.*, joint angles), kinetics (*e.g.*, body weight), or the structural
25 properties of their biological tissues (*e.g.*, tendon stiffness). Currently, it is uncertain whether
26 relative muscle fascicle operating length has a measurable effect on the metabolic energy
27 expenditure of cyclic locomotion-like contractions. To address this uncertainty, we measured the
28 metabolic energy expenditure of human participants as they cyclically produce two distinct ankle
29 moments at three separate ankle angles (90°, 105°, 120°) on a fixed-position dynamometer
30 exclusively using their soleus. Overall, increasing participant ankle angle from 90° to 120° (more
31 plantar flexion) reduced minimum soleus fascicle length by 17% (both moment levels, $p < 0.001$)
32 and increased metabolic energy expenditure by an average of 208% (both $p < 0.001$). Across both
33 moment levels, the increased metabolic energy expenditure was not driven by greater fascicle
34 positive mechanical work (higher moment level, $p = 0.591$), fascicle force rate (both $p \geq 0.235$), or
35 active muscle volume (both $p \geq 0.122$); but it was correlated with average relative soleus fascicle
36 length ($r = -0.179$, $p = 0.002$) and activation ($r = 0.51$, $p < 0.001$). Therefore, the metabolic energy
37 expended during locomotion can likely be reduced by lengthening active muscles that operate on
38 the ascending-limb of their force-length relationship.

39 **Key Words:** Energetic, Economy, Efficiency, Dynamometer, Soleus

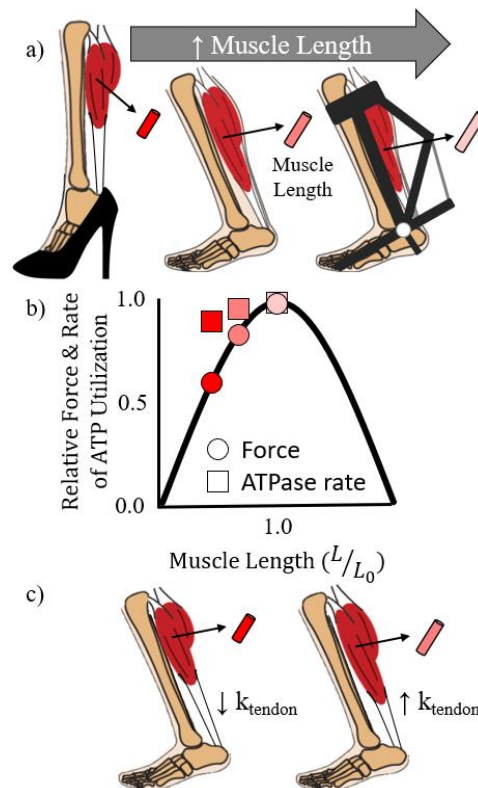
40 **Introduction**

41 Leg muscles govern walking and running performance. During the stance phase of walking and
42 running, leg extensor muscles produce force in part to support and accelerate the body into the
43 subsequent step. Concurrently, these force-producing leg muscles are almost exclusively
44 responsible for the whole-body's metabolic energy expenditure (Poole *et al.*, 1992; Griffin *et al.*,
45 2003; Marsh & Ellerby, 2006). Further, reducing an animal's metabolic energy expenditure to
46 perform a locomotor task increases how far they can travel at a given speed (Beck *et al.*, 2018)
47 and how fast they can cover a fixed distance (Hoogkamer *et al.*, 2016). Thus, reducing the
48 metabolic energy expenditure of an animal's leg muscles while they continue fulfilling the
49 physical requirements to sustain locomotion should improve locomotor performance.

50

51 Simply increasing the operating length of leg extensor muscles may decrease whole-body
52 metabolic energy expenditure and improve locomotor performance. After all, the leg extensor
53 muscles of many walking and running animals operate at shorter lengths than optimal (on the
54 ascending limb of the force-length relationship) during ground contact (Roberts *et al.*, 1997;
55 Biewener & Corning, 2001; Burkholder & Lieber, 2001; Daley & Biewener, 2003; Rubenson *et*
56 *al.*, 2012; Bohm *et al.*, 2019). This is notable because muscles utilize more adenosine
57 triphosphate (ATP) per unit of force production when they are maximally activated at shorter
58 lengths than optimal (less economical force production) (Fig. 1) (Elzinga *et al.*, 1984;
59 Stephenson *et al.*, 1989; Kentish & Stienen, 1994; Hilber *et al.*, 2001). Numerically, Hilber and
60 colleagues (Hilber *et al.*, 2001) reported that compared to maximally producing force at an
61 optimal length, sarcomere force production is 127% and 177% less economical at 0.8 and 0.6 of
62 its optimal length, respectively. To envision the implications of these values during locomotion,

63 the largest human ankle extensor muscle (soleus) is reported to produce force at 0.65 to 0.99 of
64 its maximal voluntary contraction's optimal length during the ground contact of walking and
65 running (Rubenson *et al.*, 2012). These *in vivo* muscle lengths (*i.e.*, 0.65 to 0.99) may be
66 overestimated due an inverse relationship between muscle activation and optimal fascicle length
67 (Holt & Azizi, 2014; Hessel *et al.*, 2020). Furthermore, at shorter muscle lengths than optimal,
68 active force production is reduced per unit of activation (Hill, 1953; Gordon *et al.*, 1966). Hence,
69 for muscles to keep producing the force required to sustain locomotion, operating at shorter
70 lengths than optimal requires the body to activate more muscle fibers and/or increase rate coding,
71 both of which increase metabolic energy expenditure (Christie *et al.*, 2016; Beck *et al.*, 2019).
72 Altogether, increasing the length of active leg muscles that operate at shorter lengths than
73 optimal during stance likely decreases whole-body metabolic energy expenditure during
74 locomotion.



75

76 **Figure 1.** Illustrations of triceps surae fascicle lengths during the mid-stance of walking a) in
77 high-heeled shoes (Csapo *et al.*, 2010; Cronin *et al.*, 2012), barefoot, with an ankle exoskeleton
78 (Nuckols *et al.*, 2020), as well as c) barefoot with a more compliant and stiffer Achilles tendon
79 (k_{tendon}). b) Conceptual graph showing isometric muscle fascicle force production and adenosine
80 triphosphate (ATP) utilization relative to optimal muscle operating length (L_0) (Hilber *et al.*,
81 2001).

82

83 Despite the aforementioned rationale, muscle operating lengths are not often considered to have
84 a *measurable* effect on whole-body metabolic energy expenditure during locomotion (Taylor,
85 1994; Minetti & Alexander, 1997; Pontzer, 2016; Kipp *et al.*, 2018; van der Zee & Kuo, 2020).
86 This omission may be because the metabolic influence of producing force at different muscle
87 lengths is typically studied during isometric contractions (Elzinga *et al.*, 1984; Stephenson *et al.*,
88 1989; Kentish & Stienen, 1994; Hilber *et al.*, 2001), rather than during cyclic length changing
89 contractions that better mimic locomotion muscle mechanics. Another reason is that it is difficult
90 to separate the metabolic effect of muscle operating lengths from other biomechanical
91 parameters during locomotor-like contractions. For instance, when force-producing muscles
92 shorten they perform mechanical work, and the further that a force-producing muscle shortens
93 the more metabolic energy it expends (Fenn, 1924; Ortega *et al.*, 2015). Scientists commonly
94 attribute this increased metabolic energy expenditure to greater muscle mechanical work (Fenn,
95 1924; Ortega *et al.*, 2015) rather than the muscle producing force at less economical lengths.
96 While it is nearly impossible to experimentally disentangle the metabolic effect of muscle
97 operating lengths from other metabolically-relevant biomechanical parameters during
98 locomotion, such as force and work, a controlled experiment that emulates aspects of locomotion
99 may be capable of accomplishing the task.

100

101 To help link walking and running biomechanics to metabolic energy expenditure, our goal was to
102 determine the metabolic influence of cyclically producing force at different muscle fascicle
103 lengths. To accomplish this goal, we quantified the fascicle mechanics and metabolic energy
104 expenditure of human soleus muscles as they cyclically produced force at different relative
105 lengths. Based on the notion that producing a given force at relatively shorter fascicle lengths
106 increases metabolic energy expenditure (Elzinga *et al.*, 1984; Stephenson *et al.*, 1989; Kentish &
107 Stienen, 1994; Hilber *et al.*, 2001; Beck *et al.*, 2019), we hypothesized that cyclically producing
108 the same average force at relatively shorter fascicle lengths would increase metabolic energy
109 expenditure.

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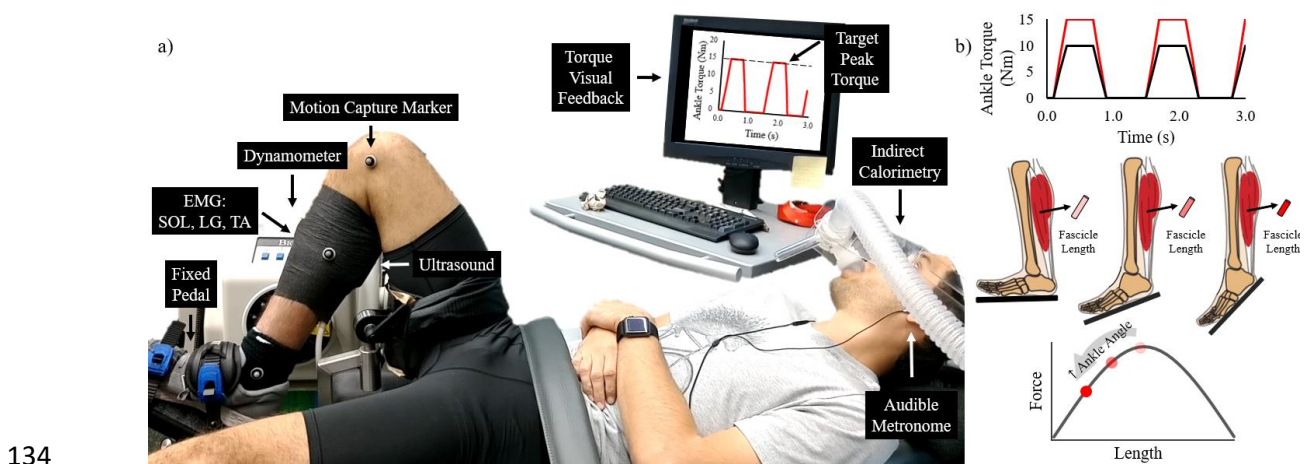
111 **Methods**

112 *Participants.* Nine volunteers completed the protocol (average \pm SD; age: 26.3 ± 2.6 years;
113 standing height: 1.77 ± 0.07 m; mass: 74.9 ± 11.4 kg; resting metabolic power 87 ± 12 W;
114 Achilles tendon moment arm during barefoot standing: 4.9 ± 0.4 cm; resting soleus fascicle
115 length: 4.1 ± 0.6 cm; estimated maximum soleus fascicle shortening velocity: 182 ± 25 mm/s
116 (Bohm *et al.*, 2019)). Prior to the study, each participant gave informed written consent in
117 accordance with the Georgia Institute of Technology Central Institutional Review Board.

118

119 *Protocol.* Participants arrived to the laboratory in the morning following an overnight fast. Upon
120 arrival, participants laid supine on a dynamometer with custom attachments that supported their
121 legs in the testing position: right knee and ankle supported at 50° and 90° , respectively (Fig. 2).
122 90° indicates perpendicular segments and more acute angles indicates joint (dorsi)flexion. In this

123 position, participants rested for 10 minutes while breathing into a mouth piece that channeled
124 expired air to a metabolic cart (TrueOne 2400, ParvoMedic, Sandy, UT, USA). Next, we shaved
125 participant leg hair and used electrode preparation gel to lightly abrade the skin superficial to
126 their right soleus, lateral gastrocnemius, and tibialis anterior (NuPrep, Weaver and Co., Aurora,
127 CO). Subsequently, we placed a bipolar surface electrode over the skin superficial to each
128 respective muscle belly and in the same orientation as the muscle fascicles (Delsys Inc., Natick,
129 MA). We secured a linear-array B-mode ultrasound probe to the skin superficial of each
130 participant's right medial soleus (Teleded, Vilnius, Lithuania). We placed reflective markers on
131 the dynamometer at its axis of rotation, 10 cm above the axis of rotation, as well as on the
132 participant's skin/clothes superficial to their right leg's medial knee-joint center, medial
133 malleolus, and first metatarsal head (Fig. 2).



135 **Figure 2.** a) Experimental setup of a participant cyclically generating soleus muscle force to
136 produce a plantar flexor moment that exerts an external torque on a fixed dynamometer pedal
137 following the cues of an audible metronome and visual feedback. EMG, electromyography;
138 SOL, soleus; LG, lateral gastrocnemius; TA, tibialis anterior. b) Illustrations of the two target
139 torque levels (peak torque: 10 Nm and 15 Nm), three ankle angles (90°, 105°, and 120°) with the
140 corresponding hypothetical minimum soleus fascicle operating lengths and their respective
141 location on a muscle force-length relationship.

142

143 Next, participants performed four maximum voluntary contractions (MVCs) with their ankle
144 joint center in-line with the dynamometer's axis of rotation (Biodex Medical Systems Inc.,
145 USA): three plantar flexion MVCs and one dorsiflexion MVC. In a random order, participants
146 conducted plantar flexor MVCs with their right knee at $\sim 70^\circ$, 60° and 50° and their ankle at 90°
147 (Rubenson *et al.*, 2012). Because MVC ankle moment did not increase with more extended knee
148 angles, we deemed the contribution of the bi-articular gastrocnemius on ankle moment to be
149 negligible (Rubenson *et al.*, 2012). Next, participants performed a dorsiflexion MVC with their
150 right knee at 50° and ankle at 90° to maximally activate their tibialis anterior. At least two
151 minutes of rest preceded each MVC to mitigate fatigue (Kawakami *et al.*, 2000).

152

153 Participants then performed six, five-minute trials with their knee at 50° separated by at least five
154 minutes of rest. These trials consisted of each participant repeatedly producing plantar flexor
155 moments on a fixed-position dynamometer foot-pedal at the downbeat of an audible metronome
156 and then relaxing at the subsequent upbeat (metronome played at 1.5 Hz; Fig. 2). To guide ankle
157 plantar flexor moments throughout each trial, participants watched a computer screen that
158 displayed the trial's target maximum dynamometer torque and the recorded dynamometer torque
159 profile over the previous 5-10 s. Participants performed trials at each of the two dynamometer
160 torque levels (10 Nm and 15 Nm) at the following ankle angles: 90° , 105° , and 120° . We
161 randomized the trial order and collected rates of oxygen uptake and carbon dioxide production,
162 dynamometer torque data (100 Hz), motion capture data (200 Hz) (Vicon Motion Systems, UK),
163 soleus fascicle length and orientation (100 Hz), as well as the surface electromyography of the
164 soleus, tibialis anterior, and lateral gastrocnemius (1000 Hz) (Fig. 2).

165

166 *Soleus fascicle mechanics.* To determine soleus fascicle kinematics, we recorded B-mode
167 ultrasound images containing the posterior-medial soleus. We recorded soleus fascicle images
168 during 20 seconds in the last two minutes of the metabolic trials. Within these 20 s, we post-
169 processed soleus fascicle lengths and pennation angles throughout six consecutive moment
170 generation cycles using a semi-automated tracking software (Farris & Lichtwark, 2016). For
171 semi-automated images that did not accurately track the respective soleus fascicle's position, we
172 manually redefined the desired fascicle. We filtered soleus fascicle angle and length using a
173 fourth-order low-pass Butterworth filter (6 Hz) and took the derivative of fascicle length with
174 respect to time to determine fascicle velocity.

175

176 To quantify soleus kinetics, we used a custom Matlab script (Mathworks Inc., Natick, MA, USA)
177 that filtered motion capture data using a fourth-order low-pass Butterworth filter (6 Hz) and
178 subtracted the resting dynamometer torque from the corresponding trial torque. We computed net
179 dynamometer torque from 12 consecutive moment generation cycles that encompassed the
180 analyzed fascicle kinematic data. Due to small fluctuations in dynamometer torque, we
181 implemented a 1 Nm dynamometer torque threshold to decipher the duration of active force
182 production. Using anthropometric measures and filtered data, we calculated the net ankle
183 moment using dynamometer torque and the position of the ankle's axis of rotation relative to the
184 dynamometer's axis of rotation, then we estimated the change in soleus muscle-tendon moment
185 arm lengths at each ankle angle (Bobbert *et al.*, 1986). In turn, we divided net ankle moment
186 (m_{ank}) by the Achilles tendon moment arm length (r_{AT}) to calculate muscle-tendon force. Next,
187 we divided muscle-tendon force by the cosine of fascicle pennation angle (θ_p) to calculate active
188 soleus fascicle force (F_{sol}).

189
$$F_{sol} = \frac{m_{ank}}{r_{AT}} / \cos(\theta_p) \quad \text{Eq. 1}$$

190 We assumed that passive muscle fascicle forces are negligible and we omitted the data from one
191 five-minute metabolic trial because the participant achieved an average maximum ankle moment
192 that was >5 Nm more than targeted. Further, we deemed optimal soleus fascicle length to be
193 consistent across muscle activation magnitudes (de Brito Fontana & Herzog, 2016) and the same
194 value that we measured during resting at a 90° ankle angle (Beck *et al.*, 2020). We set maximum
195 fascicle shortening velocity to 4.4 resting lengths per second (Bohm *et al.*, 2019) based on the
196 notion that only slow oxidative soleus fibers are active during sustained metabolic trials (Beck *et*
197 *al.*, 2020).

198

199 *Biomechanical models.* Recently, two studies performed similar experimental protocols and
200 well-linked the mechanics of muscle fascicles cyclically producing force to metabolic energy
201 expenditure. One study indicated that the combination of muscle fascicle force rate (\dot{F}), positive
202 mechanical work (W_+), and force-time integral ($\int F dt$), scaled by corresponding cost
203 coefficients (a , b , c), well-explains muscle metabolic energy expenditure (Eq. 2) (van der Zee &
204 Kuo, 2020). The other study suggested that active muscle volume (V_{act}) well-explains metabolic
205 energy expenditure (\dot{E}_{met}) during cyclic contractions that varied in duty factor (Beck *et al.*,
206 2020). Briefly, active muscle volume is calculated using active muscle fascicle force production
207 (F_{act}), optimal fascicle length (l_0), stress (σ), and the fascicle's force-length and force-velocity
208 force potential (FL and FV , respectively) (Eq. 3) (Beck *et al.*, 2019). Due to the similarity of
209 these previous studies to the present one, as a secondary objective of this paper we tested

210 whether those muscle mechanics models could explain the present study's metabolic data (Eq. 2
211 and 3).

$$212 \quad \dot{E}_{met} = (a)\dot{F} + (b)W_+ + (c) \int F dt \quad \text{Eq. 2}$$

$$213 \quad \dot{E}_{met} \propto V_{act} = \frac{F_{act} \cdot l_0}{\sigma \cdot FL \cdot FV} \quad \text{Eq. 3}$$

214 *Muscle activation.* We band-pass filtered the raw soleus, lateral gastrocnemius, and tibialis
215 anterior, electromyography signals between 20 and 450 Hz from the same 12 consecutive torque
216 generation cycles that we used to assess net ankle moment. We full-wave rectified the filtered
217 electromyography signals and calculated the root mean square of the rectified signals using a 40-
218 millisecond moving window. Due to technical issues, we were unable to collect one participant's
219 tibialis anterior activation during the metabolic trials.

220

221 *Metabolic energy expenditure.* During the resting trial and each cyclic force-production trial, we
222 used open-circuit expired gas analysis to record the participant's rates of oxygen uptake (\dot{V}_{O_2})
223 and carbon dioxide production (\dot{V}_{CO_2}). We averaged \dot{V}_{O_2} and \dot{V}_{CO_2} over the last minute of each
224 trial and used a standard equation to calculate metabolic power (W) (Peronnet & Massicotte,
225 1991). Next, we subtracted each participant's resting metabolic power from their experimental
226 values to yield net metabolic power. We removed three metabolic data values (of 54) from our
227 analyses because the corresponding respiratory exchange ratio did not reflect a respiratory
228 quotient value that was indicative of fat and/or carbohydrate oxidation (Peronnet & Massicotte,
229 1991).

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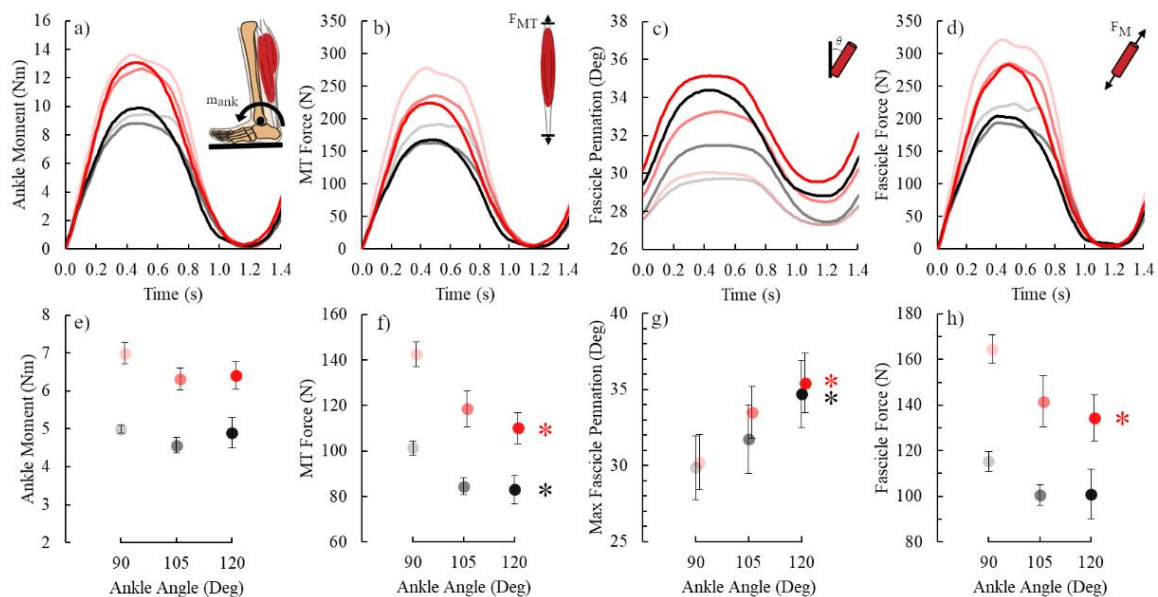
231 *Statistical analyses.* Unless otherwise specified, we performed all statistical tests within the
232 targeted lower and higher ankle moment trials independently. We performed t-tests to determine
233 whether the targeted lower and higher cycle-average torque trials elicited different average ankle
234 moments. We performed linear mixed models to determine the influence of ankle angle on the
235 duration of active force production, force production cycle frequency, average ankle moment,
236 average muscle-tendon force, average and maximum soleus fascicle pennation angle, average
237 fascicle force, fascicle force-time integral, positive fascicle mechanical work, fascicle force rate,
238 average and minimum fascicle operating lengths, maximal fascicle shortening velocity, average
239 fascicle Hill-type force-length-velocity force potential, average soleus active muscle volume,
240 soleus activation, lateral gastrocnemius activation, tibialis anterior activation, and net metabolic
241 power. We also performed linear mixed models with two independent variables (average muscle
242 fascicle length and positive mechanical work) and one dependent variable (net metabolic power).
243 We performed independent linear regressions to determine the correlation between 1) average
244 relative muscle fascicle length and 2) average soleus muscle activation on net metabolic power.
245 We set the significance level ($\alpha = 0.05$) and performed statistical analyses using RSTUDIO
246 software (RSTUDIO, Inc., Boston, MA, USA).

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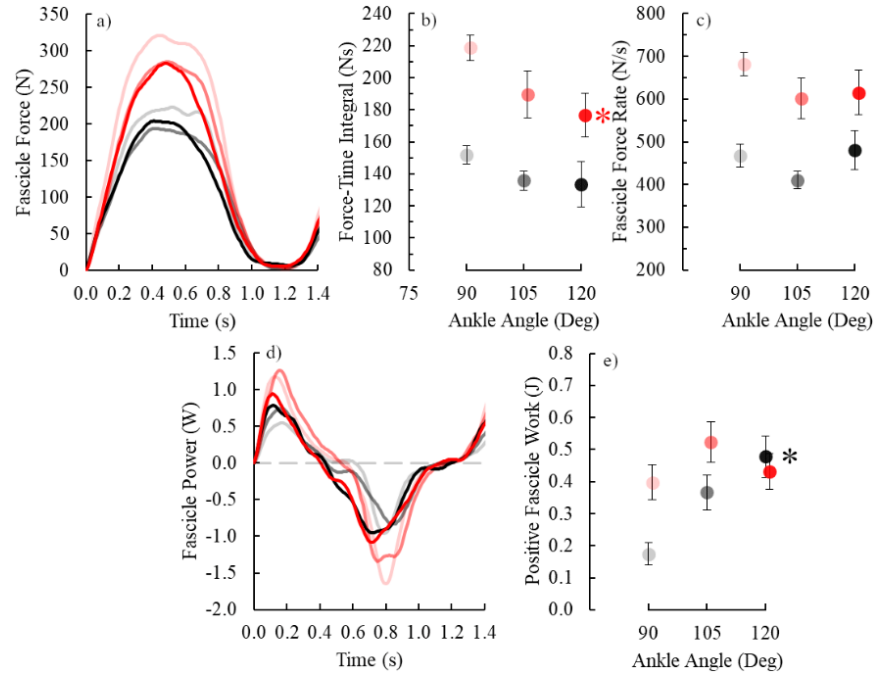
248 **Results**

249 Consistent with the study design, participants produced two distinct cycle average \pm SD ankle
250 moment levels: 4.85 ± 0.72 Nm and 6.58 ± 0.94 Nm ($p < 0.001$) (Fig. 3). Within each moment
251 level, the duration of active force production (both $p \geq 0.158$), force production cycle frequency
252 (both $p \geq 0.375$), and cycle average ankle moment (both $p \geq 0.678$) each remained constant across
253 ankle angles. However, not all metrics remained constant across ankle angles. Increasing ankle

254 angle 30° lengthened participant Achilles tendon moment arms by 0.93 cm (Bobbert *et al.*,
 255 1986), thereby decreasing average soleus muscle-tendon force (both $p \leq 0.002$) (Fig. 3). Greater
 256 ankle angles also increased average and maximum soleus fascicle pennation angles (both
 257 $p \leq 0.001$) (Fig. 3), which helped yield statistically similar cycle average soleus fascicle force
 258 production across ankle angles for the low ankle moment level ($p=0.063$) but not the higher
 259 ankle moment level ($p=0.003$) (Fig. 3). Similarly, at the lower ankle moment level, soleus
 260 fascicle force-time integral was independent of ankle angle ($p=0.070$), but it decreased by 19%
 261 due to increasing ankle angle from 90° to 120° within the higher moment level ($p=0.003$) (Fig.
 262 4).



263
 264 **Figure 3.** Top row: time-series plots of average a) ankle moment (m_{ank}), b) muscle-tendon force
 265 (F_{MT}), c) soleus fascicle pennation angle, and d) soleus fascicle force (F_M). Bottom row: average
 266 \pm SE e) average ankle moment, f) average MT force, g) maximum fascicle pennation angle, and
 267 h) average soleus fascicle force versus ankle angle. Black and red symbols are offset for clarity
 268 and indicate the lower and higher ankle moment levels, respectively. Lighter to darker colors
 269 indicate more dorsiflexed to plantar flexed ankle angles per moment level. Black and red
 270 asterisks (*) indicate that the corresponding moment level's ankle angle affects the indicated
 271 dependent variable ($p < 0.05$).



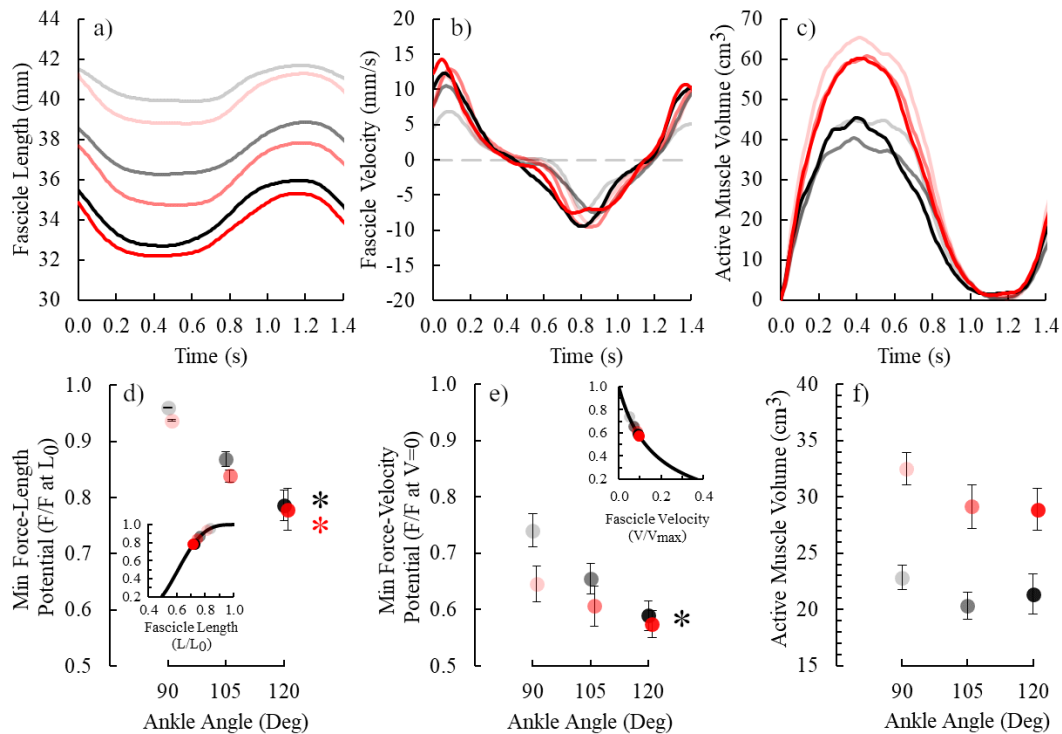
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273 **Figure 4.** Time-series plots of average soleus fascicle a) force and d) power, as well as average \pm
 274 SE soleus fascicle b) total force-time integral, c) force rate, d) and positive mechanical work.
 275 Black and red symbols are offset for clarity and indicate the lower and higher ankle moment
 276 levels, respectively. Lighter to darker colors indicate more dorsiflexed to plantar flexed ankle
 277 angles per moment level. Black and red asterisks (*) indicate that the corresponding moment
 278 level's ankle angle affects the indicated dependent variable ($p < 0.05$).

279

280 Despite systematically shortening soleus fascicle lengths, increasing ankle angle did not alter
 281 many biomechanical parameters that previous dynamometer studies well-linked to net metabolic
 282 power. Specifically, both average and minimal soleus fascicle operating lengths decreased with
 283 increasing ankle angle (both $p < 0.001$). These shorter fascicle operating lengths reduced the
 284 average soleus fascicle force-length potential by 7-8% across ankle moment levels ($p < 0.001$)
 285 (Fig. 5). Further, greater ankle angles yielded faster maximum soleus fascicle shortening
 286 velocities at the lower ankle moment level ($p < 0.001$), but not the higher ankle moment level
 287 ($p = 0.099$). Combining cycle average fascicle force production and force-length-velocity
 288 potential (Beck *et al.*, 2019), ankle angle did not affect estimated cycle average soleus active

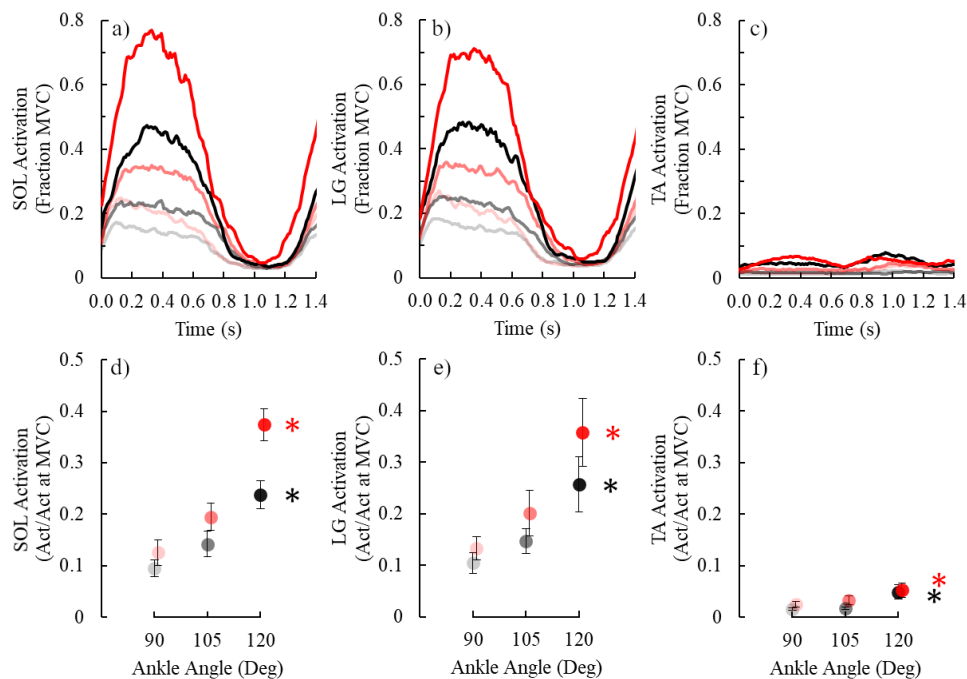
289 muscle volume (both $p \geq 0.122$) (Fig. 5). Additionally, as aforementioned soleus fascicle force-
 290 time integral remained constant or slightly decreased with increased ankle angles (Fig. 3).
 291 Regarding the other metrics from equations 2 and 3 (Beck *et al.*, 2019; van der Zee & Kuo,
 292 2020), soleus fascicle force-rate was independent of ankle angle (both $p \geq 0.235$) (Fig. 4), and
 293 positive soleus fascicle work increased across ankle angles within the lower ankle moment level
 294 ($p < 0.001$), but not within the higher ankle moment level ($p = 0.591$) (Fig. 4)..



295
 296 **Figure 5.** Top row: time-series plots of average soleus a) fascicle length, b) fascicle velocity, and
 297 c) active muscle volume. Bottom row: average \pm SE d) minimum Hill-type force-length
 298 potential, e) minimum Hill-type force-velocity potential, and average f) active muscle volume
 299 versus ankle angle. Within panels d) and e) are the respective force-potentials plotted on the
 300 force-length and force-velocity curves, respectively. Black and red symbols are offset for clarity
 301 and indicate the lower and higher ankle moment levels, respectively. Lighter to darker colors
 302 indicate more dorsiflexed to plantar flexed ankle angles per moment level. Black and red
 303 asterisks (*) indicate that the corresponding moment level's ankle angle affects the indicated
 304 dependent variable ($p < 0.05$).

305

306 Cyclically producing force at different ankle angles altered plantar flexor muscle activation. Both
307 soleus and lateral gastrocnemius muscle activation increased by 140-200% with increasing ankle
308 angle within each moment level (all $p < 0.001$) (Fig. 6). Even though tibialis anterior activation
309 statistically increased at greater ankle angles (both $p \leq 0.027$), we considered its influence on net
310 metabolic power to be trivial because its cycle average activation was merely 2-5% of its MVC
311 value across conditions.



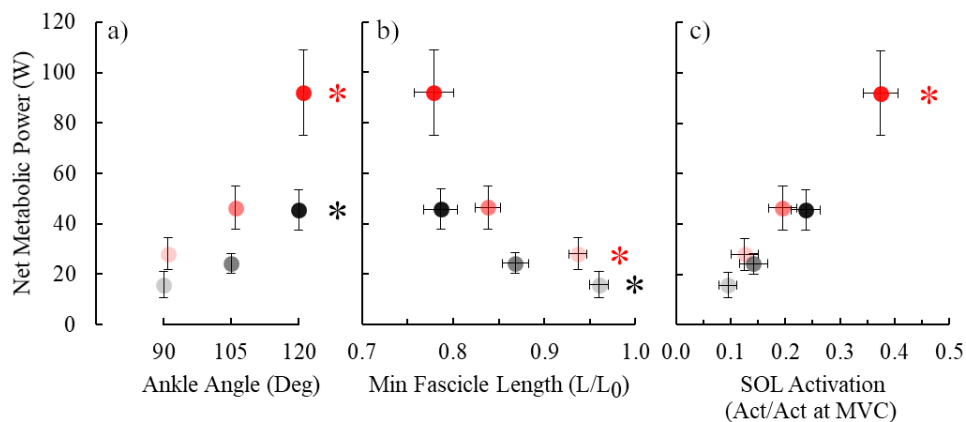
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313 **Figure 6.** Top row: time-series plots of average a) soleus (SOL) activation (Act), b) lateral
314 gastrocnemius (LG) activation, and c) tibialis anterior (TA) activation. Bottom row: average \pm
315 SE d) SOL activation, e) LG activation, and f) TA activation versus ankle angle. MVC is
316 maximum voluntary contraction. Black and red symbols are offset for clarity and indicate the
317 lower and higher ankle moment levels, respectively. Lighter to darker colors indicate more
318 dorsiflexed to plantar flexed ankle angles per moment level. Black and red asterisks (*) indicate
319 that the corresponding moment level's ankle angle affects the indicated dependent variable
320 ($p < 0.05$).

321

322 Ankle angle affected the metabolic power of cyclic force production. Changing ankle angle from
323 90° to 120° increased net metabolic power by 189% and 228% within the lower and higher ankle

324 moment levels, respectively (both $p < 0.001$) (Fig. 7). Unlike previous dynamometer studies
325 (Beck *et al.*, 2020; van der Zee & Kuo, 2020), neither the combined cost of muscle force-time
326 integral, positive mechanical work, and force rate (Eq. 2); nor active muscle volume (Eq. 3)
327 could explain the metabolic data (Suppl. Fig. 1). This is especially evident within the higher
328 moment level where net metabolic power increased by 228% across ankle angles, but all of the
329 following variables were either unchanged or decreased with increasing ankle angle (Suppl. Fig.
330 1): force-time integral (Fig. 4), force rate (Fig. 4), positive mechanical work (Fig. 4), and active
331 muscle volume (Fig. 5). Further, within each moment level, positive mechanical work did not
332 relate to net metabolic power while controlling for the influence of average fascicle length
333 ($p \geq 0.405$). On the contrary, while controlling for positive mechanical work, decreasing average
334 fascicle length was associated with an increased net metabolic power (both $\beta = -1.4$ to -3.1 ;
335 $p \leq 0.047$). Pooled across ankle moment levels and participants, without controlling for other
336 mechanical parameters, average relative muscle fascicle operating length inversely correlated
337 with net metabolic power (linear regression: $r = -0.179$, $p = 0.002$). Additionally, average soleus
338 activation positively correlated with net metabolic power across ankle moment levels and
339 participants ($r = 0.51$, $p < 0.001$) (Fig. 7).



340

341 **Figure 7.** Average \pm SE net metabolic power versus a) ankle angle, b) minimum fascicle length,
342 and c) average soleus activation. Black and red symbols are offset for clarity and indicate the
343 lower and higher ankle moment levels, respectively. Lighter to darker colors indicate more
344 dorsiflexed to plantar flexed ankle angles per moment level. Black and red asterisks (*) indicate
345 that the corresponding moment level's ankle angle affects the indicated dependent variable ($p <$
346 0.05).

347

348 **Discussion**

349 During locomotion, relative muscle fascicle operating lengths depend on the body's posture,
350 kinetics, and structural properties. In the present study, we controlled for participant limb-joint
351 kinetics (constant cycle average ankle moment), structural properties (within participant design),
352 and independently altered muscle fascicle operating lengths via postural changes (changing ankle
353 angle). Using this protocol, we found that reduced muscle fascicle operating lengths *per se*
354 increased metabolic energy expenditure during cyclic force production – supporting our
355 hypothesis.

356

357 Why does cyclically producing force at relatively shorter muscle fascicle lengths increase
358 metabolic energy expenditure? Muscle metabolic energy expenditure increases by either
359 activating a greater volume of muscle or by expending more metabolic energy per unit active
360 muscle volume (increased metabolic power density, W/cm^3) (Beck *et al.*, 2019). Our results
361 suggest that relative fascicle lengths primarily affect the latter. To explain, within the higher
362 ankle moment level, changing ankle angle from 90° to 120° shortened average muscle fascicle
363 operating lengths by 17% and decreased fascicle force-potential by 8% (Hill, 1953; Gordon *et*
364 *al.*, 1966). To keep producing the same force profile, active muscle volume could theoretically
365 increase by 8% (Hill, 1953; Gordon *et al.*, 1966). We would predict this change to elicit a mere

366 8% greater net metabolic power (Beck *et al.*, 2019), which is a far cry from the observed 228%
367 increased metabolic energy expenditure. Therefore, even if there is a small increase in active
368 muscle volume due to operating at shorter muscle fascicle lengths (*i.e.*, 8%), which we did not
369 detect in our data, metabolic power density would need to increase by ~200% across ankle
370 angles to match the net metabolic power data. In other words, producing force at relatively
371 shorter fascicle lengths is likely metabolically expensive because muscles utilize ATP at faster
372 rates, not because of the recruitment of more muscle fibers.

373

374 An increased metabolic power density from producing force at relatively shorter muscle lengths
375 may be attributed to at least two factors. First, when sarcomeres produce force at shorter lengths
376 than optimal, force production decreases faster than the corresponding ATP utilization (Fig. 1)
377 (Elzinga *et al.*, 1984; Stephenson *et al.*, 1989; Kentish & Stienen, 1994; Hilber *et al.*, 2001). One
378 proposed mechanism is that the net force produced by relatively shorter sarcomeres is mitigated
379 by opposing forces that arise from compressed myofibrillar proteins. Another contributing factor
380 that could help explain both greater muscle activation and net metabolic power at relatively
381 shorter fascicle lengths is increased rate-coding (Enoka, 2015; Christie *et al.*, 2016). To continue
382 producing a similar force magnitude at shorter fascicle lengths, participants may have increased
383 the discharge rate of their soleus motor units, which would cycle ATP utilizing actin-myosin
384 cross-bridges faster - thereby increasing metabolic power density.

385

386 It is important to recognize the difference between absolute and relative muscle fascicle
387 operating length since they affect metabolic energy expenditure in different directions. Across

388 vertebrates, the dimensions and quantity of actin-myosin cross-bridges per sarcomere are
389 generally constant (Taylor, 1994; Burkholder & Lieber, 2001). This means that animals with
390 optimally longer muscle fascicles typically have more sarcomeres in-series than animals with
391 optimally shorter fascicles. Because whole-muscle force production depends on a muscle's
392 cross-sectional area and not its length, while considering other factors, absolutely longer muscle
393 fascicles yield less economical force production due to more ATP consuming cross-bridges per
394 unit force production (Taylor, 1994; Roberts *et al.*, 1998). However, shortening a muscle fascicle
395 by a fixed distance (*e.g.*, 10 mm), shifts sarcomeres further down their ascending limb in an
396 optimally shorter versus longer fascicle (*e.g.*, 40 mm versus 50 mm). Based on our data,
397 decreasing a muscle fascicle's length by a fixed distance likely increases metabolic energy
398 expenditure more in an optimally shorter versus longer muscle fascicle. Hence, accounting for
399 both absolute and relative muscle fascicle lengths may help explain the metabolic differences
400 across participants with different optimal muscle fascicle lengths – such as in individuals pre-
401 and post-injury (Williams & Goldspink, 1978; Hullfish *et al.*, 2019).

402

403 Accounting for both absolute and relative muscle fascicle lengths during locomotion may also
404 help explain the differences in metabolic energy expenditure across animal species. For example,
405 per unit body mass, smaller animals (*e.g.*, mice) have shorter muscle fascicles (Alexander *et al.*,
406 1981; Bennett, 1996) and expend more metabolic energy during locomotion than larger animals
407 (*e.g.*, elephants) (Taylor *et al.*, 1970; Taylor *et al.*, 1982; Pontzer, 2007; Rubenson *et al.*, 2007).
408 Based on the metabolic energy expenditure of producing force isometrically at an optimal
409 fascicle length, the characteristically shorter muscle fascicles in smaller animals is
410 conventionally viewed as an economical trait (Kram & Taylor, 1990; Roberts *et al.*, 1998; Kram,

411 2000). However, since many muscles in walking and running animals produce force while on the
412 ascending limb of their force-length curve (Roberts *et al.*, 1997; Biewener & Corning, 2001;
413 Burkholder & Lieber, 2001; Daley & Biewener, 2003; Rubenson *et al.*, 2012; Bohm *et al.*,
414 2019), optimally shorter muscle fascicle lengths may not necessarily translate to more
415 economical locomotion. This may be especially true regarding comparisons across animals sizes,
416 considering that smaller animals have relatively greater extensor muscle forces (Biewener, 1989)
417 and more compliant tendons (Biewener, 2000) than larger animals; since both factors contribute
418 to shorter muscle fascicles operating lengths. Hence, future studies that consider relative, in
419 addition to absolute, muscle fascicle length during locomotion may better link walking and
420 running biomechanics to metabolic energy expenditure across species.

421

422 There are many assumptions that may limit the findings of this study. First, consistent with
423 previous studies (Beck *et al.*, 2020; van der Zee & Kuo, 2020), we assumed that the metabolic
424 contribution of co-activating leg muscles was negligible. This assumption is based on the notion
425 that there was likely slack in the biarticular gastrocnemius muscle-tendons (Rubenson *et al.*,
426 2012) and that the tibialis anterior muscle activation was trivial (Fig. 6). Second, we assumed
427 that the soleus is primarily comprised of homogeneous muscle fibers (Johnson *et al.*, 1973), and
428 that these fibers are exclusively recruited during the present study's submaximal metabolic trials
429 (Henneman, 1957). Hence, we deemed all active muscle fascicles in the soleus to have the same
430 maximum shortening velocity, which we estimated to be 4.4 optimal lengths per second (Bohm
431 *et al.*, 2019). As mentioned, we assumed that the soleus had uniform fascicle mechanics across
432 the whole muscle, which is oversimplifies the complex architecture of the human soleus
433 (Bolsterlee *et al.*, 2018). Nonetheless, passively changing muscle-tendon length alters soleus

434 fascicle lengths and pennation angles change in the same direction across soleus muscle
435 compartments (Bolsterlee *et al.*, 2018). Despite our many limitations and non-locomotor
436 experiment, we find assurance when comparing our results to the most analogous locomotion
437 experiment - walking in footwear with different heel heights. Similar to our study, increasing
438 footwear heel height elicits postural changes that reduce triceps surae muscle fascicle forces
439 (Simonsen *et al.*, 2012), relative muscle fascicle operating lengths (Csapo *et al.*, 2010; Cronin *et*
440 *al.*, 2012), and increase whole-body metabolic energy expenditure during walking and running
441 compared baseline conditions (*i.e.*, barefoot or in flats) (Ebbeling *et al.*, 1994; Gu & Li, 2013).

442

443 In conclusion, our results suggest that operating further down the ascending limb of a muscle's
444 force-length curve may have a measurable influence on the metabolic energy expenditure during
445 locomotion. Implications may help resolve why locomotion economy differs across and within
446 animal species, in addition to informing biomechanical interventions that reduce user metabolic
447 energy expenditure and consequently augment locomotor performance.

448

449 *Authors' contributions.* O.N.B. contributed to the conception and design of the study, acquisition
450 of data, the analysis and interpretation of data, as well as the drafting of the article. J.N.S. &
451 L.H.T. contributed to acquisition of data and the revising of the article. J.R.F. contributed to the
452 conception of the study, interpretation of data, as well as the drafting of the article. G.S.S.
453 contributed to the conception and design of the study, the analysis and interpretation of data, as
454 well as the drafting of the article. All authors approve of the manuscript and agree to be held

455 accountable for all aspects of the work in ensuring that questions related to the accuracy or
456 integrity of any part of the work are appropriately investigated and resolved.

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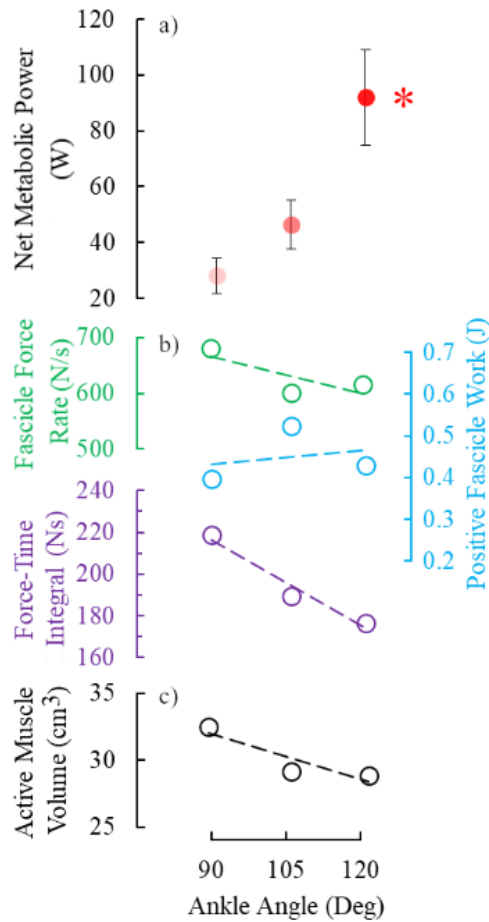
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654

655

Supplementary Figures



656

657 **Supplementary Figure 1.** Net metabolic power is independent from the cost of 1) the sum of
658 soleus fascicle force rate, positive fascicle work, and force-time integral, and 2) active muscle
659 volume at the high ankle moment level (cycle average ankle moment: 6.57 Nm). a) Average \pm
660 SE net metabolic power versus ankle angle. b) Average soleus fascicle force rate, positive
661 fascicle work, force-time integral, and c) active muscle volume versus ankle angle. Dashed lines
662 indicate linear regression. For panels b and c, symbol color corresponds to the respective colored
663 y-axis.