

Evaluating the “cost of generating force” hypothesis across frequency in human running and hopping

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Abstract:

The volume of active muscle and duration of extensor muscle force well-explain the associated metabolic energy expenditure across body mass and speed during level-ground running and hopping. However, if these parameters fundamentally drive metabolic energy expenditure, then they should pertain to multiple modes of locomotion and provide a simple framework for relating biomechanics to metabolic energy expenditure in bouncing gaits. Therefore, we evaluated the ability of the ‘cost of generating force’ hypothesis to link biomechanics and metabolic energy expenditure during human running and hopping across step frequencies. We asked participants to run and hop at 0%, $\pm 8\%$ and $\pm 15\%$ of preferred step frequency. We calculated changes in active muscle volume, force duration, and metabolic energy expenditure. Overall, as step frequency increased, active muscle volume decreased due to postural changes via effective mechanical advantage (EMA) or duty factor. Accounting for changes in EMA and muscle volume better related to metabolic energy expenditure during running and hopping at different step frequencies than assuming a constant EMA and muscle volume. Thus, to ultimately develop muscle mechanics models that can explain metabolic energy expenditure across different modes of locomotion, we suggest more precise measures of muscle force production that include the effects of EMA.

Keywords: Locomotion, Energetics, Biomechanics, Spring-mass model

1 Introduction

2 For decades, biomechanists and physiologists have sought to link the mechanics of running and
3 hopping with the corresponding metabolic energy expenditure. One prevailing approach is the ‘cost of
4 generating force’ hypothesis, which was proposed by Taylor and colleagues (Kram and Taylor, 1990;
5 Taylor, 1994; Taylor et al., 1980) and posits that the primary determinant of metabolic energy expenditure
6 required for running and hopping is the cost of generating muscle force to support body weight. This
7 hypothesis is predicated on the fact that animals produce stride-average vertical ground reaction forces
8 equal to body weight when running or hopping on level ground. Previous studies have demonstrated that
9 metabolic energy expenditure depends on animal size, and that metabolic energy expenditure increases in
10 almost direct proportion to the total weight of a running animal (Taylor et al., 1980). Further, per unit of
11 body mass, it is more metabolically costly for smaller animals (e.g., mouse) to generate a unit of force than
12 larger animals (e.g., horse) (Taylor, 1985), because small animals take more frequent strides and use less
13 economical muscle fibers to produce force quickly (Heglund and Taylor, 1988). Thus, the metabolic energy
14 expenditure during running and hopping varies with size and may depend on the number of strides taken
15 per second, or stride frequency.

16 Kram and Taylor (Kram and Taylor, 1990) expanded the ‘cost of generating force’ hypothesis to
17 explain why metabolic energy expenditure increases near linearly when running or hopping at faster
18 velocities. They reasoned that the rate of force generation (i.e., the rate of cross bridge cycling) could be
19 approximated by the inverse of ground contact time and formally proposed that the rate of metabolic energy
20 expenditure (\dot{E}_{met} in Watts) during running equals an animal’s body weight (F_{BW}) multiplied by the inverse
21 of ground contact time (t_c^{-1}) and a metabolic cost coefficient (c) (Eqn. 1).

$$\dot{E}_{met} = F_{BW} \cdot t_c^{-1} \cdot c \quad [1]$$

22 To produce the force needed to support body weight over each stride, animals need to activate a volume of
23 muscle (i.e., the number of active actin-myosin crossbridges), which is primarily influenced by body weight
24 and the leg’s effective mechanical advantage (EMA). EMA is the ratio of the ground reaction force moment
25 arm to the muscle tendon moment arm. Kram and Taylor assumed that active muscle volume and EMA
26 were independent of velocity (Biewener, 1989), which is why they simplified the equation to use force in
27 units of body weight. Using this assumption, equation 1 well-described the increase in metabolic energy
28 expenditure for a 10-fold increase in velocity and 4500-fold increase in body weight during forward
29 hopping, trotting, and running animals (Kram and Taylor, 1990; Roberts et al., 1998a).

30 Since Kram and Taylor (Kram and Taylor, 1990), multiple studies have shown that active muscle
31 volume and EMA change across running velocity and limb morphology (Kipp et al., 2018b; Roberts et al.,
32 1998b; Wright and Weyand, 2001). Notably, Roberts et al. (Roberts et al., 1998b) demonstrated that running
33 bipeds have a greater EMA than size-matched quadrupeds due to their upright posture, which influences
34 active muscle volume and metabolic energy expenditure. Thus, the authors proposed a refined version of
35 the ‘cost of generating force’ hypothesis to account for changes in active muscle volume where the rate of
36 metabolic energy expenditure equals the product of active muscle volume (V_m), the inverse of ground
37 contact time, and a new cost coefficient (k) (Eqn. 2)

$$\dot{E}_{met} = V_m \cdot t_c^{-1} \cdot k \quad [2]$$

38 Kipp et al. (Kipp et al., 2018b) applied this refined version of the ‘cost of generating force’ hypothesis (Eqn.
39 2) to human running and found that humans decrease their EMA and increase active muscle volume by as
40 much as 53% from 2.2 m·s⁻¹ to 5.0 m·s⁻¹. Thus, the authors concluded that the curvilinear increase in

41 metabolic energy expenditure with running velocity (Batliner et al., 2018) results from an increase in active
42 muscle volume and an increase in the rate of force production due to shorter ground contact times.

43 Though the rate of force generation and active muscle volume well-explain metabolic energy
44 expenditure across different running and hopping velocities, it is unknown if these biomechanical variables
45 explain changes in metabolic energy expenditure across different stride and step frequencies, where a step
46 equals ground contact and the subsequent aerial time and two steps comprise a stride. Previous studies have
47 shown that humans have a preferred step frequency for running and hopping that minimizes metabolic
48 energy expenditure, and deviating from the preferred step frequency increases metabolic energy
49 expenditure (Allen and Grabowski, 2019; Cavagna et al., 1988; Cavanagh and Williams, 1982; Farris and
50 Sawicki, 2012; Grabowski and Herr, 2009; Högberg, 1952; Raburn et al., 2011; Swinnen et al., 2021); thus
51 there is a U-shaped relationship between metabolic energy expenditure and step frequency (Doke and Kuo,
52 2007; Snyder and Farley, 2011; Swinnen et al., 2021). When considering the ‘cost of generating force’
53 hypothesis, Gutmann and Bertram (Gutmann and Bertram, 2017a; Gutmann and Bertram, 2017b) suggest
54 that the rate of force production alone (Eqn. 1) cannot fully account for the U-shaped changes in metabolic
55 energy expenditure with hopping frequency. Instead, accounting for changes in active muscle volume and
56 the rate of force production (Eqn. 2) may better explain this U-shaped relationship. Previous studies have
57 suggested that the U-shaped relationship is due to simultaneous increasing and decreasing metabolic costs
58 (Doke and Kuo, 2007; Snyder and Farley, 2011; Swinnen et al., 2021) where ground contact time decreases
59 with increased step frequency during human running and hopping, which implies that humans must produce
60 forces at a faster rate and increase metabolic cost (Farley et al., 1991). Simultaneously, increased step
61 frequencies are accompanied by shorter steps during running and less center of mass displacement during
62 running and hopping, both of which may increase EMA and reduce active muscle volume and decrease
63 metabolic cost (Monte et al., 2021). Thus, accounting for changes in the rate of force production and active
64 muscle volume through EMA may better describe metabolic energy expenditure across step frequencies
65 than the cost of generating force alone. We hypothesized that accounting for changes in active muscle
66 volume and the rate of force production (Eqn. 2) better explains changes in metabolic energy expenditure
67 across step frequencies compared to the original “cost of generating force” equation, which estimates active
68 muscle volume from body weight (Eqn. 1) for both running and hopping. Further, we hypothesize that
69 active muscle volume decreases as step frequency increases in running and hopping due to increased EMA.

70

71 **Materials and Methods**

72 *1. Participants*

73 Ten healthy runners (6F, 4M; average \pm s.d., mass: 60.7 ± 8.9 kg, height: 1.72 ± 0.09 m, age: 24.5
74 ± 3.4 years) with no reported cardiovascular, neurological, or musculoskeletal impairments participated in
75 the study. All participants reported running for exercise at least 30 minutes per day, three times per week,
76 for at least 6 months. Each participant provided written informed consent to participate in the study
77 according to the University of Colorado Boulder Institutional Review Board.

78

79 *2. Experimental Protocol*

80 Over two separate days, participants performed a series of running trials on a force-measuring
81 treadmill (Treadmetrix, Park City, UT; 1000 Hz) and stationary, two-legged hopping trials on force plates
82 (Bertec, Columbus, OH; 1000 Hz) while we simultaneously measured ground reaction forces, lower limb
83 kinematics, and metabolic energy expenditure throughout each trial. On the first day, participants performed
84 six 5-min running trials at $3 \text{ m}\cdot\text{s}^{-1}$. During the first trial, we determined each participant’s preferred step

85 frequency (PSF). We collected ground reaction forces (GRFs) for 15-sec during the third and fifth minute
86 of the first trial and determined average PSF from ground contact events identified by a 20 N vertical GRF
87 threshold. We then instructed participants to complete the remaining running trials while matching their
88 step frequency to the timing of an audible metronome. The metronome was set to 85%, 92% 100%, 108%
89 and 115% of their PSF, similar to previous studies (Snyder and Farley, 2011; Swinnen et al., 2021), and the
90 order of the trials was randomized.

91 On the second day, participants performed five, 5-min stationary hopping trials, on both feet. To
92 account for the effects of frequency on metabolic energy expenditure and given the similarity of frequencies
93 that minimize metabolic energy expenditure during hopping and running (Allen and Grabowski, 2019;
94 Cavagna et al., 1997; Farris and Sawicki, 2012; Grabowski and Herr, 2009; Kaneko et al., 1987), we
95 instructed participants to hop in place while matching their step frequency to the audible metronome set to
96 85%, 92% 100%, 108% and 115% of their PSF from day 1. The order of the hopping trials was randomized,
97 and we did not determine preferred hopping frequency.

98

99 3. *Metabolic Energy Expenditure*

100 We measured participants' rates of oxygen consumption and carbon dioxide production via indirect
101 calorimetry (TrueOne 2400, ParvoMedics, Sandy, UT) throughout each running and hopping trial. We
102 instructed participants to refrain from exercising before each experimental session or ingesting caffeine
103 four hours before each experimental session to minimize day-to-day variability in metabolic rates.
104 Additionally, participants were instructed to be at least two hours postprandial at the start of each
105 experimental session to mitigate potential effects of diet on metabolic measurements. Further, each
106 experimental session was performed at the same time each day and separated by at least 24 hours to
107 eliminate any potential effects of day-to-day variability or fatigue. We calculated gross steady-state
108 metabolic power from the average metabolic rates during the last two minutes of each 5-min trial using a
109 standard equation (Kipp et al., 2018a; Péronnet and Massicotte, 1991).

110

111 4. *Kinematic and Kinetics*

112 We positioned 40 reflective markers bilaterally on both legs and the pelvis. Markers on the ankles
113 and knees were used to define joint centers and clusters of 3-4 markers were placed on each segment prior
114 to experimental trials. We collected lower limb kinematic data for 15-sec during the last minute of each
115 trial using 3-dimensional motion capture (Vicon Nexus 2.3, Oxford, UK; 200 Hz) simultaneously with
116 GRFs. We analyzed 20 steps from each trial and used a 4th order low-pass Butterworth filter with a 20 Hz
117 cut-off to process analog GRF signals and marker trajectories (Alcantara, 2019; Mai and Willwacher, 2019).
118 We determined ground contact using a 20 N vertical GRF threshold for both running and hopping and
119 calculated the rate of force production as the inverse of ground contact time (t_c^{-1}).

120 To calculate EMA and V_m , we estimated the average extensor muscle-tendon unit force (F_{mtu}) about
121 each joint assuming a constant muscle-tendon moment arm (r) for each muscle group and using
122 instantaneous ankle, knee, and hip sagittal joint moments from Visual 3D (Visual 3D, C-Motion Inc.,
123 Germantown, MD, USA) (Biewener et al., 2004; Kipp et al., 2018b). We only included joint moment values
124 that exceeded 25% of the maximum extensor moment due to the inherently noisy center of pressure
125 measurements caused by low force values at the beginning and end of the ground contact phase (Biewener
126 et al., 2004; Griffin et al., 2003; Kipp et al., 2018b). Because the net joint moments of the knee and hip
127 include flexion moments from bi-articular muscles, we accounted for forces in bi-articular muscles by
128 assuming F_{mtu} was proportional to physiological cross-sectional area of active muscle fibers (Eq. 3-5).

$$M_{ankle} = r_{ankle} \cdot F_{mtu,ankle} \quad [3]$$

$$M_{knee} = r_{knee} \cdot F_{mtu,knee} - \left(r_{BF} \cdot F_{mtu,hip} \frac{PCSA_{BF}}{\sum PCSA_{hip}} \right) - \left(r_{GAS} \cdot F_{mtu,ankle} \frac{PCSA_{GAS}}{\sum PCSA_{ankle}} \right) \quad [4]$$

$$M_{hip} = r_{hip} \cdot F_{mtu,hip} - \left(r_{RF} \cdot F_{mtu,knee} \frac{PCSA_{RF}}{\sum PCSA_{knee}} \right) \quad [5]$$

129 where M is the net joint moment, r is a weighted-average muscle-tendon moment arm, and $PCSA$ is the
 130 physiological cross-sectional area. GAS , BF , and RF represent the properties of the gastrocnemius, biceps
 131 femoris, and rectus femoris muscles, respectively. We calculated $F_{mtu,ankle}$ from Eqn. 3, and solved Eqn. 4
 132 and 5 simultaneously due to the two unknown quantities of $F_{mtu,knee}$ and $F_{mtu,hip}$. We considered moments
 133 that extend joints to be positive. Values for r and $PCSA$ were taken from the anthropometric data of four
 134 male human cadavers reported in Biewener et al. (Biewener et al., 2004) and previously used in Kipp et al.
 135 (Kipp et al., 2018b). We then used the quotient of the average sagittal plane resultant GRF magnitude and
 136 F_{mtu} at each joint during ground contact to calculate EMA, which equals the quotient of r and the GRF
 137 moment arm (R).

$$EMA = \frac{GRF}{F_{mtu}} = \frac{r}{R} \quad [6]$$

138 We calculated V_m separately for each joint (Eqn. 7) and then summed them to estimate the total
 139 average V_m per leg. To do this, we assumed the muscles produced force isometrically with a constant stress
 140 ($\sigma = 20 \text{ N}\cdot\text{cm}^{-2}$) (Perry et al., 1988) and combined this with our estimates of F_{mtu} and weighted-average
 141 fascicle length (L) from Biewener et al. (Biewener et al., 2004),

$$V_m = \frac{F_{mtu} \cdot L}{\sigma} \quad [7]$$

142

143 5. Estimating cost-coefficients and metabolic energy expenditure

144 We calculated the metabolic cost-coefficients, c and k , for each trial during running and hopping
 145 using Eqns. 1 and 2. We averaged each cost-coefficient across the range of frequencies (separately for
 146 running and hopping). Then we implemented the respective cost-coefficient averages in addition to V_m , t_c^{-1} ,
 147 l , and F_{BW} to predict metabolic power for each step frequency during running and hopping using Eqns. 1
 148 and 2.

149

150 6. Statistics

151 To evaluate the agreement between measured metabolic power and predicted metabolic power from
 152 Eqns. 1 & 2, we performed limits of agreement analyses (Bland-Altman) for each target step frequency and
 153 calculated the systematic bias (mean differences) and 95% limits of agreement. We also constructed linear
 154 mixed-effects models ($\alpha = 0.05$) to determine the effect of measured step frequency relative to PSF on t_c^{-1} ,
 155 c , k , EMA , V_m , average joint extensor moment, and average sagittal plane resultant GRF magnitude. In each
 156 linear mixed-effects model, we considered measured step frequency relative to PSF as a fixed effect and
 157 participant as a random effect. Model coefficients are reported alongside their p-values and represent the

158 change in the dependent variable per a 1% change in measured step frequency relative to PSF. We
159 performed all statistical analyses in R (version 3.6.3) (R Core Team, 2020) using custom scripts and
160 packages (Datta, 2017; Pinheiro et al., 2020; Revelle, 2019; Wickham, 2016).

161

162 **Results**

163 We removed data for one participant at the 85% PSF and 92% PSF running trials because they were
164 >3% off of the target step frequencies.

165

166 *1. Running*

167 On average, measured metabolic power was minimized when participants ran at their PSF (Fig.
168 1A), which was a step frequency of (avg. \pm s.d.) 2.90 ± 0.09 Hz (Table 1). As participants deviated from
169 their PSF, average measured metabolic power increased by 17% and 9% when running at 85% of PSF and
170 115% of PSF, respectively (Fig. 1A). Overall, metabolic power estimated with Eqn. 1 underestimated
171 average metabolic power for step frequencies slower than PSF (up to 13% at 85% PSF) but overestimated
172 average metabolic power for step frequencies equal to or greater than PSF (up to 9.5% greater at 108%
173 PSF) (Fig. 2A & 3A). Limits of agreement analysis show metabolic power estimated with Eqn. 2 had a bias
174 closer to zero and lower than Eqn. 1 at each step frequency, however, the magnitude of the upper and lower
175 limits of agreement for Eqn. 2 were greater than those of Eqn. 1 due to increased variability (Fig. 2A & 3A)
176 during running.

177 The linear mixed-effects model showed that total V_m decreased by 20.54 cm^3 for every 1% increase
178 in step frequency relative to PSF ($p < 0.001$; Fig. 4; Table 2). Specifically, participants decreased ankle,
179 knee, and hip V_m by 3.68 cm^3 , 10.34 cm^3 , and 5.54 cm^3 , respectively, for every 1% increase in step frequency
180 ($p < 0.001$ for each; Fig. 4; Table 2). Despite the reduction in joint-specific V_m , we did not detect significant
181 changes in ankle, knee, or hip EMA across step frequency ($p = 0.66$; $p = 0.05$; $p = 0.59$, respectively). Average
182 (\pm s.d.) EMA across step frequencies for the ankle, knee, and hip was 0.314 ± 0.017 , 0.393 ± 0.084 , and
183 0.714 ± 0.117 , respectively (Fig. 5, Table 2). Rather, the changes in joint-specific V_m may have been due to
184 the decrease in average ankle, knee, and hip extensor moments as step frequency increased. Average ankle,
185 knee, and hip extensor moments decreased by $0.66 \text{ N}\cdot\text{m}$ ($p < 0.001$), $1.3 \text{ N}\cdot\text{m}$ ($p < 0.001$), and $0.31 \text{ N}\cdot\text{m}$
186 ($p < 0.01$), respectively, for every 1% increase in step frequency (Table 1). Finally, t_c^{-1} increased by 0.02 s^{-1}
187 for every 1% increase in step frequency relative to PSF during running ($p < 0.001$; Fig. 6A). We used these
188 variables to solve for the cost-coefficient and found that c decreased by $0.003 \text{ J}\cdot\text{N}^{-1}$ for every 1% increase
189 in step frequency ($p < 0.001$; Fig. 7A), but k did not change across step frequency, and averaged (\pm s.d.)
190 $0.087 \pm 0.003 \text{ J}\cdot\text{cm}^{-3}$ ($p = 0.18$; Fig. 7A).

191

192 *2. Hopping*

193 On average, measured metabolic power numerically increased by 10% and 2% when hopping at
194 85% of PSF and 115% of PSF, respectively, relative to 100% PSF ($p > 0.9$; Fig. 1B). On average, metabolic
195 power estimated with Eqn. 1 underestimated metabolic power for step frequencies slower than PSF (up to
196 16% at 85% PSF) but overestimated metabolic power for step frequencies greater than PSF (up to 17% at
197 115% PSF) (Fig. 2B & 3B). Metabolic power estimated with Eqn. 2 had a bias closer to zero and lower
198 than Eqn. 1 at each step frequency (Fig. 2B & 3B). The magnitude of the upper and lower limits of
199 agreement for Eqn. 2 were greater than those of Eqn. 1 due to increased variability during hopping (Fig. 2B
200 & 3B).

201 The linear mixed-effects models showed that total V_m decreased by 21 cm³ for every 1% increase
202 in step frequency relative to PSF ($p < 0.001$; Fig. 4; Table 2). Participants decreased joint-specific V_m at the
203 knee and hip by 18.0 cm³ and 3.7 cm³ for every 1% increase in step frequency ($p < 0.001$ and $p = 0.008$,
204 respectively; Fig. 4), whereas ankle V_m did not change across step frequency and averaged (\pm s.d.) $729 \pm$
205 176 cm³ ($p = 0.32$; Fig. 4). We found that knee EMA increased by 0.008 for every 1% increase in step
206 frequency ($p < 0.001$; Fig. 5, Table 2). However, ankle and hip EMA did not change across step frequency
207 and averaged (\pm s.d.) 0.35 ± 0.04 ($p = 0.06$) and 0.90 ± 0.37 ($p = 0.35$), respectively (Fig. 5; Table 2).
208 Similarly, participants decreased average knee extensor moment by 2.6 N·m for every 1% increase in step
209 frequency ($p < 0.001$; Table 1). However, average ankle and hip extensor moments did not change across
210 step frequency and averaged (\pm s.d.) 129 ± 27 N·m ($p = 0.32$) and 56 ± 13.9 N·m ($p = 0.77$), respectively.
211 Finally, t_c^{-1} increased by 0.04 s⁻¹ for every 1% increase in step frequency relative to PSF during stationary
212 hopping ($p < 0.001$; Fig 6B). We used these variables to solve for the cost-coefficients and found that c
213 decreased by 0.0022 J·N⁻¹ for every 1% increase in step frequency ($p < 0.001$; Fig. 7B), but k did not change
214 across step frequency and averaged (\pm s.d.) 0.054 ± 0.002 J·cm⁻³ ($p = 0.20$; Fig. 7B).

215

216 Discussion

217 Our data support our first hypothesis that accounting for changes in active muscle volume (V_m) and
218 the rate of force production (t_c^{-1}) (Eqn. 2) better explain changes in metabolic energy expenditure across
219 step frequencies compared to the original “cost of generating force” equation, which estimates V_m through
220 body weight (Eqn. 1). We also found that accounting for changes in V_m and t_c^{-1} (Eqn. 2) results in a constant
221 cost-coefficient, k (Fig. 7), across step frequencies for running and hopping. The average values for k (0.087
222 J·cm⁻³ and 0.056 J·cm⁻³ for running and hopping, respectively) are in line with previous values reported for
223 human running (0.079 J·cm⁻³) at different velocities (Kipp et al., 2018b). Our data also support our second
224 hypothesis, that V_m is reduced as step frequency increases in human running and stationary hopping. When
225 step frequency increased from 85% PSF to 115% PSF, we found that V_m decreased by 18% and 26% during
226 running and hopping, respectively. This reduction predominantly occurred due to changes at the knee in
227 both running and hopping, with smaller or non-significant contributions from the ankle and hip during both
228 tasks (Fig. 4; Table 2). We found that the knee accounted for ~55% and ~87% of the change in total V_m
229 during running and hopping, respectively, whereas, when humans run at faster velocities from 2.2 – 5.0
230 m·s⁻¹, the ankle, knee, and hip account for ~39%, ~20%, and ~41% of the change in total active muscle
231 volume, respectively (Kipp et al., 2018b). Our data, along with previous studies, support the general
232 hypothesis that the metabolic cost of bouncing gaits is related to V_m recruited to generate force and the rate
233 that the force is produced (Heglund and Taylor, 1988; Kipp et al., 2018b; Roberts et al., 1998b; Taylor et
234 al., 1980; Wright and Weyand, 2001).

235 The mechanism by which total V_m decreased with step frequency differed between running and
236 hopping. We found that joint-specific effective mechanical advantage (EMA) was independent of step
237 frequency during running (Fig. 4; Table 2). Therefore, the reductions in total V_m during running were likely
238 due to greater duty factors, which resulted in reduced stance-average resultant ground reaction forces
239 (GRFs) and the corresponding joint moments (Table 1). In comparison, during hopping, EMA at the knee
240 increased by 82% when step frequency increased from 85% to 115% PSF, while the magnitude of stance-
241 average resultant GRF did not change (Fig. 5; Table 1 & 2). This might imply that participants decreased
242 total V_m during hopping by altering their lower limb position to hop with a straighter leg and extended knee
243 as step frequency increased. When taken together, these results suggest that humans may utilize two
244 different mechanisms to alter total V_m during bouncing gaits, duty factor (Beck et al., 2020) and EMA.

245 Previously, Kipp et al. (Kipp et al., 2018b) demonstrated that humans utilize both mechanisms
246 simultaneously to increase total V_m when running at different velocities. They found that runners increased
247 total V_m by 53% with faster running velocities from 2.2 – 5.0 m·s⁻¹ due to a concurrent decrease in duty
248 factor and decrease in hip EMA, which is likely due to the increased step frequency that accompanies faster
249 running velocity (Heglund and Taylor, 1988).

250 Our measures of knee and ankle EMA during two-legged, stationary hopping conflict with those
251 of Monte et al. (Monte et al., 2021), who suggest that knee EMA is independent of step frequency (2.0 –
252 3.5 Hz). Our data may differ from those of Monte et al. due to a difference in methodology. We calculated
253 joint-specific average EMA during the stance phase when joint moments exceeded 25% of their peak value,
254 whereas Monte et al. separated stance into two phases and included EMA values obtained when GRF and
255 center of pressure are small (near ground contact or toe off), which increases variability in EMA and may
256 obscure changes that occur with step frequency (Griffin et al., 2003). There may have also been difference
257 in inter-participant hopping strategies between studies, where participants may have kept their knees
258 “locked” or “unlocked”. While our average knee EMA data suggest that participants straighten their legs
259 to hop at faster step frequencies, three of our participants did not appreciably change their knee EMA across
260 step frequency (Fig. 5). This may suggest that some of our participants choose a “locked” knee strategy and
261 that the difference in participants and possibly strategies between the two studies could have been due to
262 chance. Further research is warranted to determine if a difference in hopping strategy could explain the
263 difference in knee EMA between studies.

264 The “cost of generating force” hypothesis originally put forth by Kram & Taylor (Kram and Taylor,
265 1990) provides a simple equation (Eqn. 1) that links biomechanics to metabolic energy expenditure across
266 running velocities. The equation assumes that across running speeds, animals employ a constant EMA and
267 muscles operate at similar shortening velocities. Each of these assumptions affects metabolic cost (Taylor,
268 1994), but do not detract from the elegance of a simple equation to well predict the metabolic cost of running
269 in different sized animals across velocities. By addressing these assumptions, the accuracy for predicting
270 metabolic energy expenditure for running and hopping at different step frequencies could be improved
271 (Kipp et al., 2018b; Roberts et al., 1998b; Wright and Weyand, 2001).

272 The cost-coefficients, encompass factors that influence muscle metabolic energy expenditure per
273 unit active muscle volume (J·cm³). As such, the values of these coefficients change when unaccounted
274 factors that affect metabolic energy expenditure change. For example, changes in muscle force-length-
275 velocity affect metabolic energy expenditure per unit of force production but are not accounted for in
276 equations 1 & 2. In the present study, we show that accounting for changes in V_m (through EMA) and t_c^{-1}
277 (Eqn. 2) yields a constant cost-coefficient, k , across step-frequency (Fig. 7). Thus, by incorporating EMA
278 into Eqn. 2, the metabolic energy expenditure per unit active muscle volume is consistent across step
279 frequencies, unlike in the simpler Eqn. 1 (Fig. 7). It may be possible for future studies, using ultrasound or
280 modeling approaches to account for additional assumptions, such as muscle fiber shortening velocity and
281 length to further refine the “cost of generating force” hypothesis.

282 Changes in muscle contractile dynamics (i.e., muscle shortening velocity and average operating
283 length) likely influence total active muscle volume. In a recent study, Beck et al (Beck et al., 2020)
284 demonstrated that producing the same cycle-average forces with a decreasing duty factor (the product of
285 contact time and frequency) during cyclic soleus contractions requires greater peak muscle force, a decrease
286 in fascicle operating length, and a general increase in active muscle volume and metabolic energy
287 expenditure. The authors proposed that accounting for duty factor may improve the calculation of active

288 muscle volume by providing a surrogate for muscle contractile dynamics; thereby addressing one of the
289 assumptions of the “cost of generating force” hypothesis.

290 A potential limitation of our study is the use of static internal muscle-tendon moment arms, fascicle
291 lengths, and pennation angles to estimate active muscle volume. We intentionally did this to allow a direct
292 comparison of our results to those of previous studies (Biewener et al., 2004; Kipp et al., 2018a) that account
293 for active muscle volume changes during human locomotion. We found that accounting for active muscle
294 volume increases inter-participant variability of predicted metabolic energy expenditure compared to
295 assuming constant active muscle volume (Fig. 2 & 3). This increase in variability may be due to the
296 assumption of fixed-length, muscle moment arms at each joint. Inter-participant variability in total active
297 muscle volume and predicted metabolic energy expenditure using Eqn. 2 might be reduced by accounting
298 for changes in muscle moment arms during the stance phase. Previous studies have shown that muscle-
299 tendon moment arms change with joint angle (Arnold et al., 2010; Hoy et al., 1990; Rasske et al., 2017).
300 Thus, using variable muscle-tendon moment arms that change with joint angle could further improve the
301 estimate of active muscle volume and metabolic energy expenditure.

302

303 **Conclusion**

304 In this study, we evaluated the “cost of generating force” hypothesis for predicting metabolic
305 energy expenditure across different step frequencies during running and hopping. We found that accounting
306 for changes in effective mechanical advantage to compute active muscle volume resulted in a near-constant
307 cost-coefficient, k , and improved the estimation of participant metabolic energy expenditure across step
308 frequencies.

List of symbols and abbreviations

c	cost-coefficient
EMA	effective mechanical advantage
\dot{E}_{met}	metabolic power
F_{BW}	force in units of body weight
F_{mtu}	muscle-tendon force
GRF	ground reaction force
k	cost-coefficient
L	fascicle length
M	joint moment
PCSA	physiological cross-sectional area
PSF	preferred step frequency
r	muscle-tendon moment arm
R	GRF moment arm
t_c	ground contact time
t_c^{-1}	rate of muscle force production
V_m	active muscle volume
σ	muscle stress

Competing interests

No competing interests declared.

Author Contributions

Conceptualization: S.P.A., O.N.B., A.M.G; Methodology: S.P.A., O.N.B., A.M.G; Investigation: S.P.A., O.N.B; Formal analysis: S.P.A; Writing – original draft: S.P.A; Writing – review & editing: S.P.A., O.N.B., A.M.G; Visualization: S.P.A; Supervision: A.M.G.

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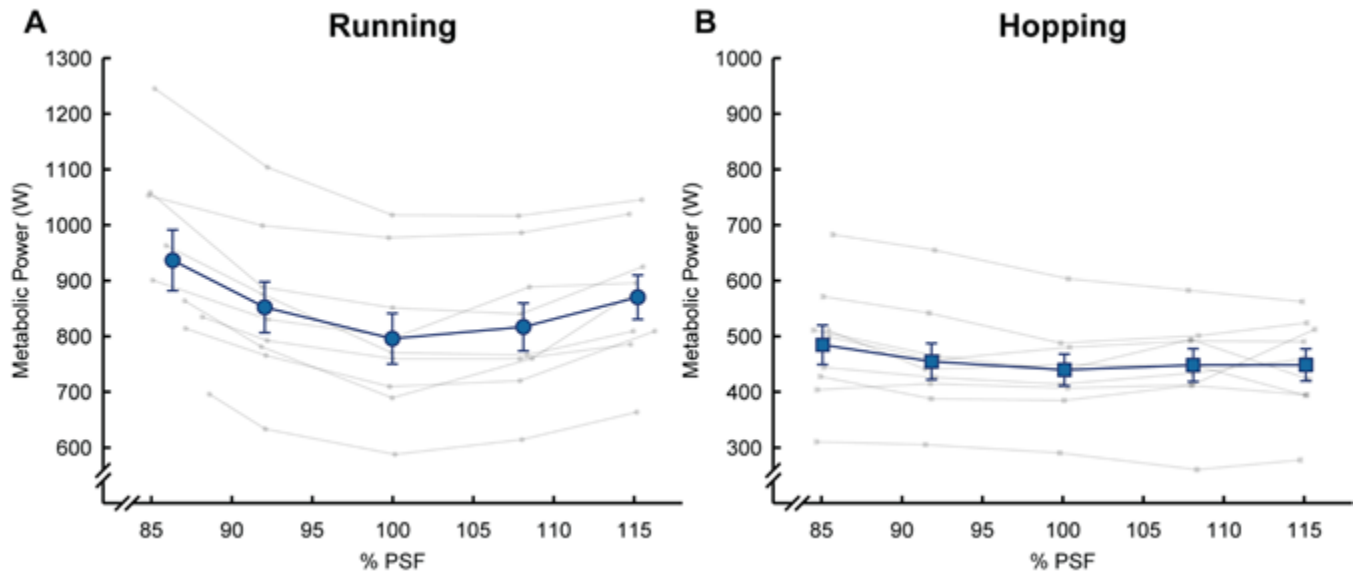


Figure 1: Gross metabolic power across percentage of preferred step frequency. Average \pm s.e.m. metabolic power (large, blue symbols) and values from individual subjects (small, grey symbols) versus the percentage of running preferred step frequency (% PSF) in A) running and B) hopping. Vertical and horizontal error bars may not be visible behind data points.

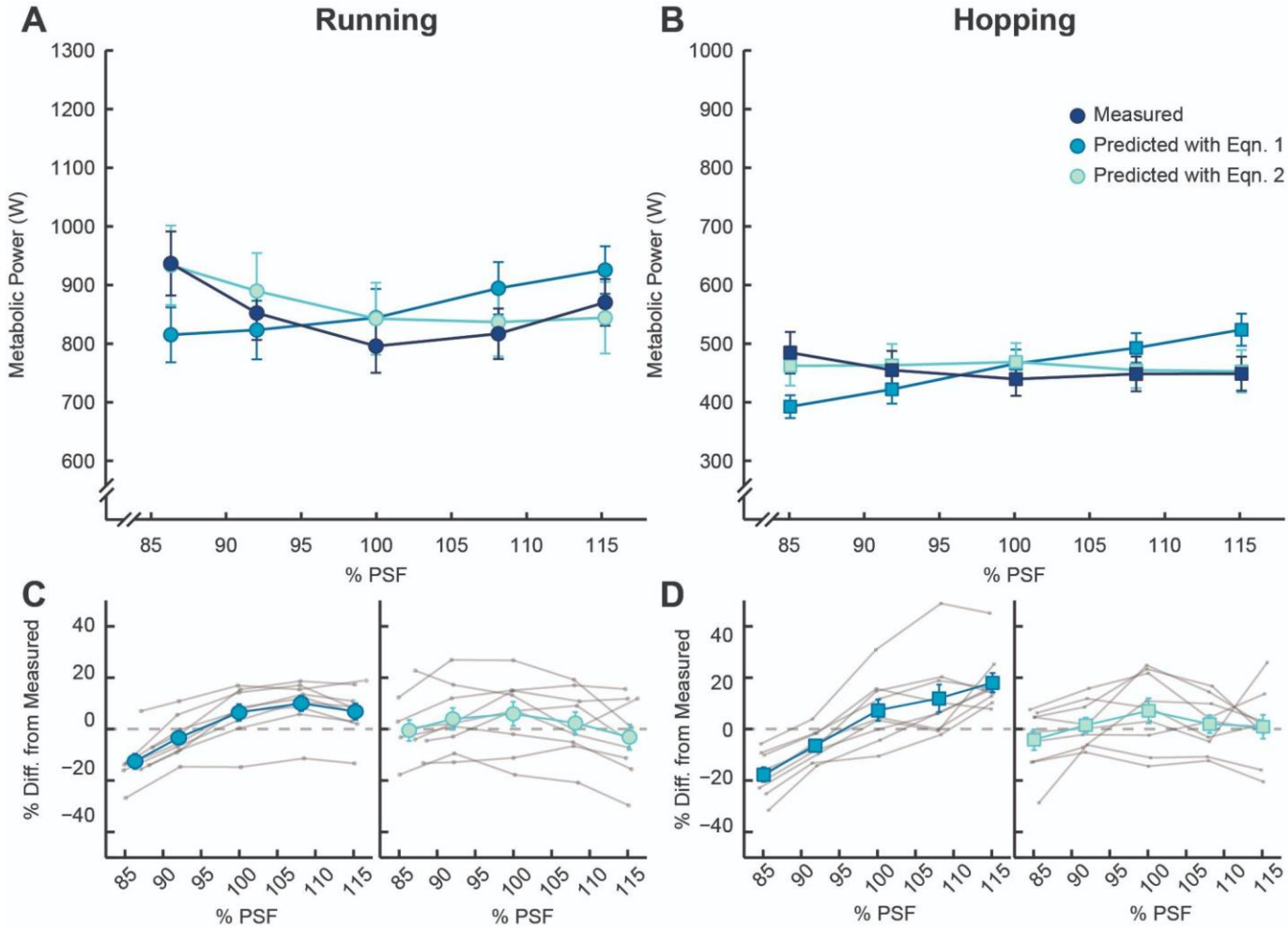


Figure 2: Predicted gross metabolic power across percentage of preferred step frequency. Average \pm s.e.m. (large, colored symbols) gross metabolic power for measured (dark blue) and predicted values using Eq.1 (blue) and Eqn. 2 (light blue) versus the percentage of running preferred step frequency (% PSF) in A) running and B) hopping. Vertical and horizontal error bars may not be visible behind the data points. C) Running and D) hopping percent difference between each equation and measured metabolic power for average \pm s.e.m. and participants (small, grey symbols).

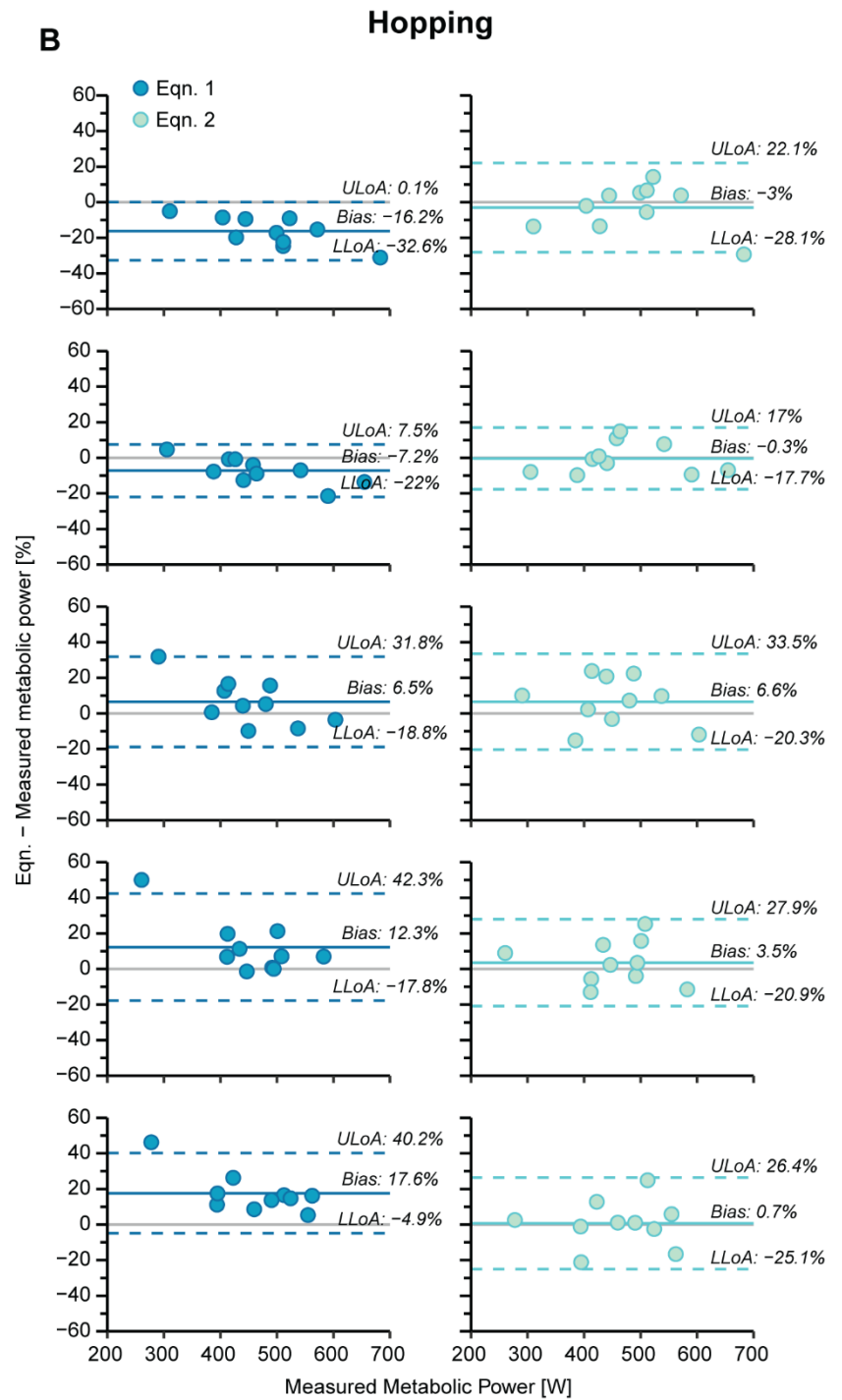
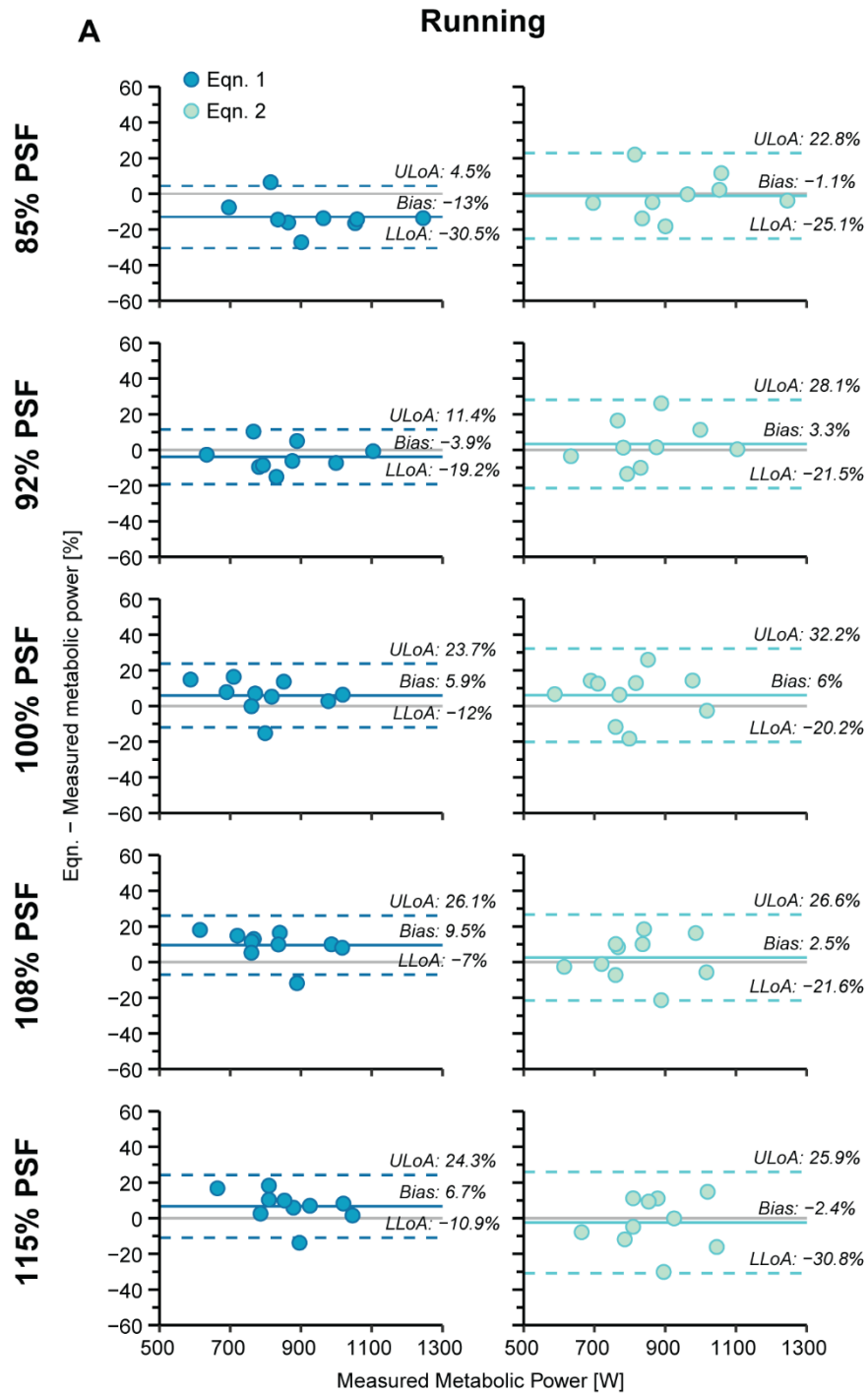


Figure 3: Limits of Agreement (Bland-Altman). Limits of agreement analysis comparing the percent difference between Eqn. 1 (blue symbols) or Eqn. 2 (light blue symbols) and gross metabolic power measured via indirect calorimetry. Mean differences (Bias) are indicated by the solid-colored lines, while the lower and upper limits of agreement (LLoA/ULoA) are denoted by dashed-colored lines. LLoA/ULoA were calculated using 1.96 SD.

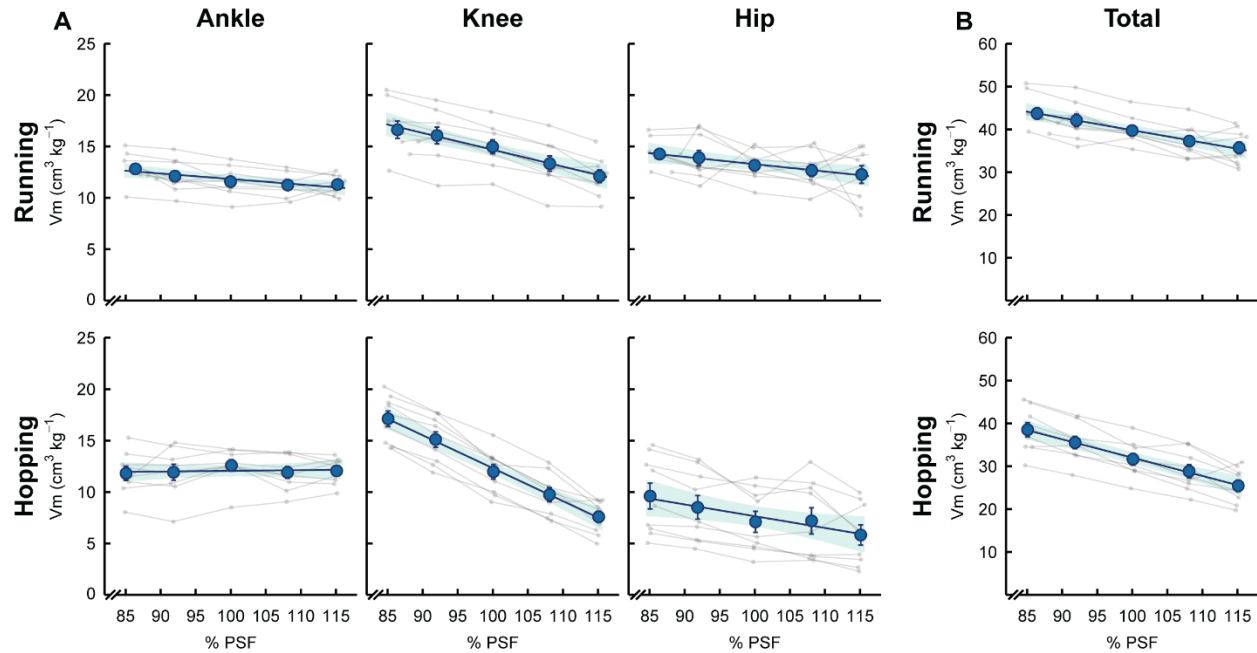


Figure 4: Active muscle volume across percentage of preferred step frequency. Average \pm s.e.m. active muscle volume (V_m) of the leg extensors during ground contact (large, blue symbols) and values from individual subjects (small, grey symbols) versus the percentage of preferred running step frequency (% PSF) for running and hopping. A) V_m of the muscles surrounding the ankle, knee, and hip joints during running and hopping, and B) is the summed total of the ankle, knee, and hip joint V_m . The dark lines represent the results of linear mixed-effects models, and the shaded regions represent the model's 95% confidence intervals. Coefficients and intercepts for each of the linear mixed-effects models are presented in Table 2. Vertical and horizontal error bars may not be visible behind data points.

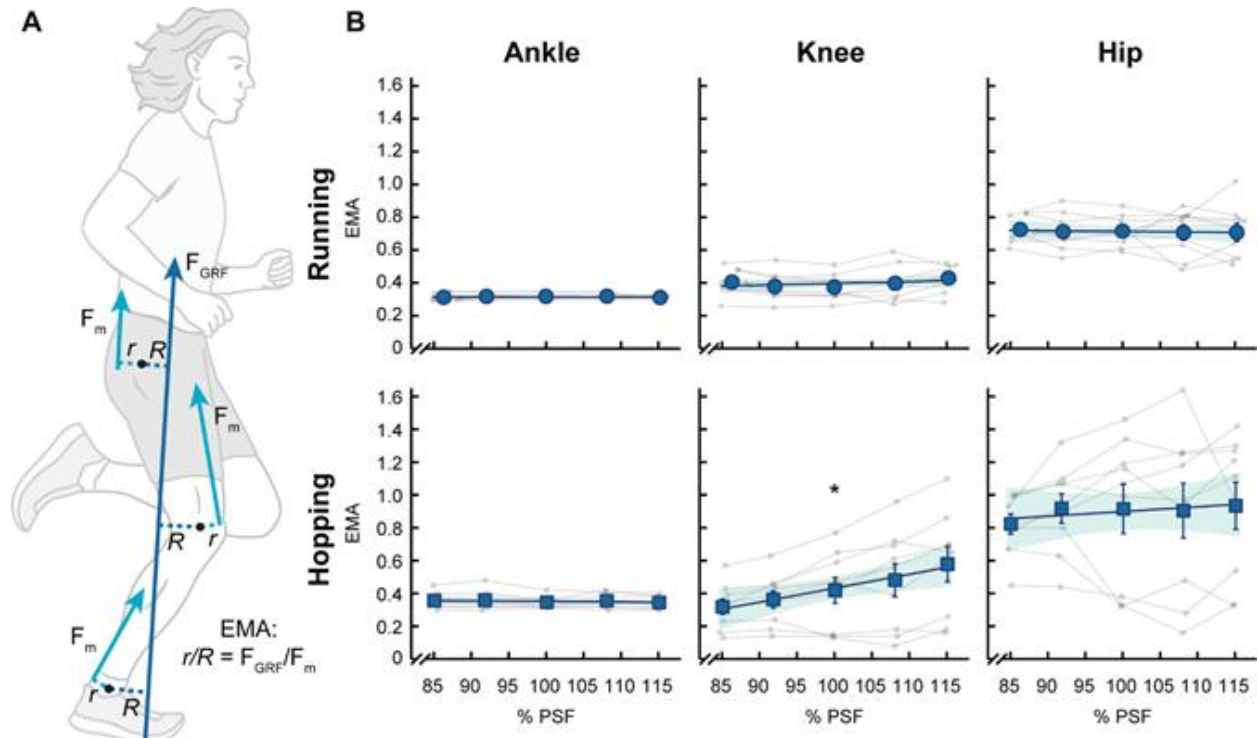


Figure 5: Effective mechanical advantage (EMA) across percentage of preferred step frequency. A) Illustration of EMA during running, which equals the ratio of the muscle-tendon moment arm (r) and the external resultant ground reaction force moment arm (R) or the ratio of resultant ground reaction force (F_{GRF}) and muscle force (F_m). B) Average \pm s.e.m. EMA for the ankle, knee, and hip joints (large, blue symbols) with values for individual subjects (small, grey symbols) versus the percentage of preferred running step frequency (% PSF). The dark lines represent the results of linear mixed-effects models and the shaded regions represent the model's 95% confidence intervals. Coefficients and intercepts for each of the linear mixed-effects models are presented in Table 2. * indicates if the model slope is significantly different from zero. Vertical and horizontal error bars may not be visible behind data points.

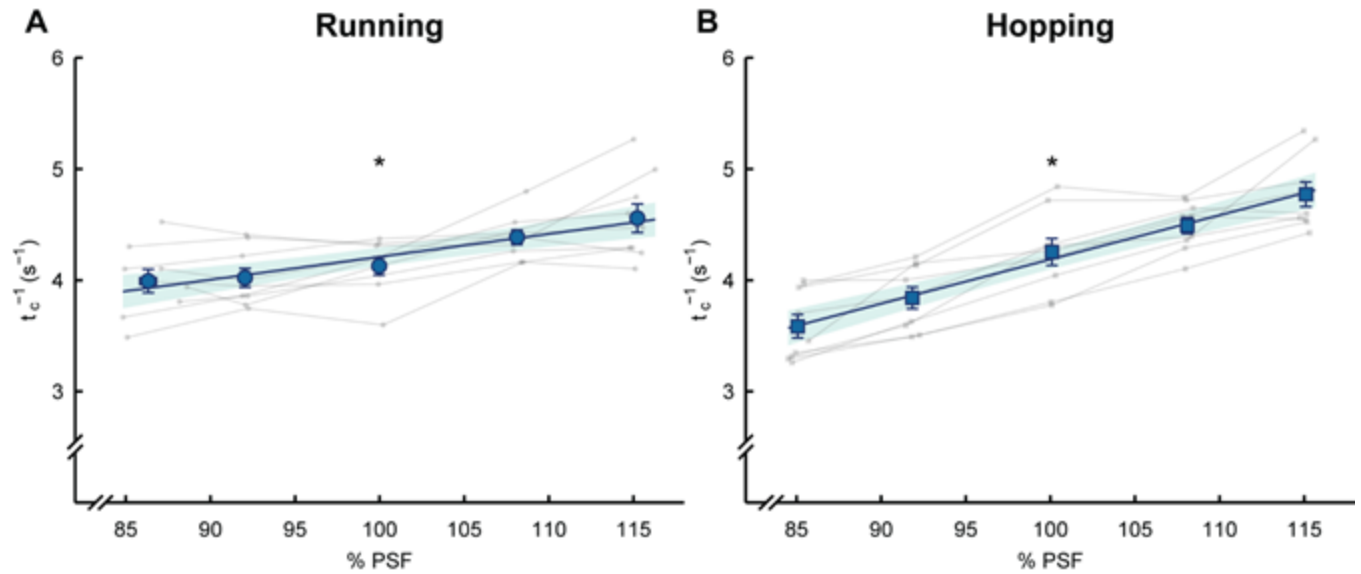


Figure 6: Rate of force production across percentage of preferred step frequency. Average \pm s.e.m. rate of force production (t_c^{-1} ; large, blue symbols) and values from individual subjects (small, grey symbols) versus the percentage of running preferred step frequency (% PSF) for A) running and B) hopping. The dark lines represent the model prediction across percentage of preferred step frequency (running: $t_c^{-1} = 0.021 \cdot \text{PSF} + 2.160$, hopping: $t_c^{-1} = 0.040 \cdot \text{PSF} + 0.216$) and the shaded areas represent the 95% confidence interval. * indicates if the model slope is significantly different from zero. Vertical and horizontal error bars may not be visible behind data points.

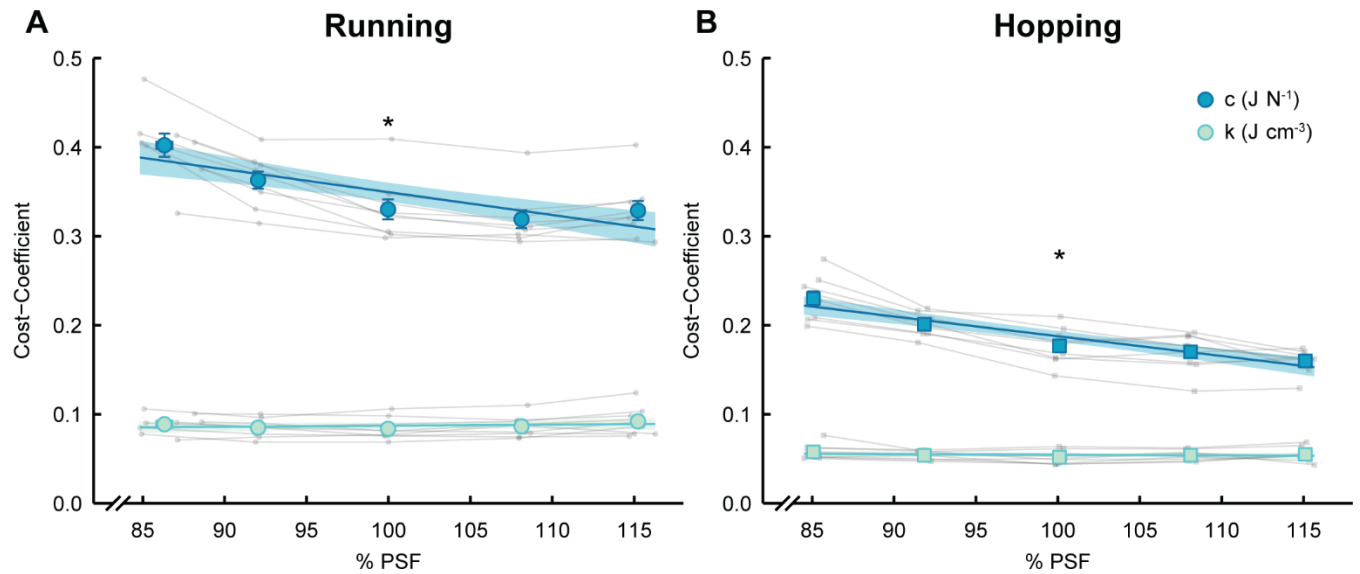


Figure 7: Cost-coefficient across percentage of preferred step frequency. Average \pm s.e.m. cost coefficients (c – large, dark blue symbols and k – large, light blue symbols) and values from individual subjects (small, grey symbols) versus the percentage of running preferred step frequency (% PSF) in A) running and B) hopping. The lines represent the results of the linear mixed-effects model where $c = -0.003 \cdot \text{PSF} + 0.601$ ($p < 0.001$) and $k = 1.16 \times 10^{-4} \cdot \text{PSF} + 0.075$ ($p = 0.18$) for running, and $c = -0.0022 \cdot \text{PSF} + 0.407$ ($p < 0.001$) and $k = 0.001 \cdot \text{PSF} + 0.045$ ($p = 0.20$) for hopping. The p-values indicate if the slope is significantly different than zero. The dark lines represent the results of linear mixed-effects models, and the shaded regions represent the model's 95% confidence intervals. Vertical and horizontal error bars may not be visible behind data points.

Table 1. Biomechanical variables for running ($3 \text{ m} \cdot \text{s}^{-1}$) and hopping in place at different percentages of preferred running step frequency.

	Target % PSF	Achieved % PSF	Achieved step frequency (Hz)	Stance Avg. resultant GRF (N)	Avg. extension moment (N·m)		
					Ankle	Knee	Hip
Running	85	86.3 ± 1.5	2.50 ± 0.1	939 ± 165	142 ± 32	106 ± 30	64 ± 14
	92	92.0 ± 0.2	2.67 ± 0.1	890 ± 154	134 ± 33	103 ± 26	58 ± 12
	100	100.0 ± 0.1	2.90 ± 0.1	848 ± 137	127 ± 27	95 ± 23	52 ± 12
	108	108.1 ± 0.3	3.13 ± 0.1	831 ± 117	123 ± 25	81 ± 24	52 ± 14
	115	115.2 ± 0.5	3.34 ± 0.1	807 ± 102	122 ± 17	70 ± 20	54 ± 16
Hopping	85	85.1 ± 0.4	2.46 ± 0.1	889 ± 134	128 ± 30	118 ± 28	37 ± 15
	92	91.8 ± 0.3	2.66 ± 0.1	877 ± 139	130 ± 39	102 ± 27	35 ± 12
	100	100.1 ± 0.2	2.90 ± 0.1	883 ± 121	137 ± 33	75 ± 24	34 ± 11
	108	108.1 ± 0.2	3.13 ± 0.1	870 ± 127	130 ± 33	57 ± 18	37 ± 12
	115	115.1 ± 0.3	3.33 ± 0.1	868 ± 131	132 ± 30	41 ± 16	37 ± 13

Average \pm s.d.; PSF, preferred running step frequency; GRF, ground reaction force; Avg. joint moment is defined when extension moments are greater than 25% of the peak joint moment.

Table 2. Linear mixed-effects model results for effective mechanical advantage (Fig. 5) and active muscle volume (Fig. 4) at the ankle, knee, hip, and summed total while running and hopping at different percentages of preferred running step frequency.

		EMA			V_m		
		Intercept	Slope	p-value	Intercept	Slope	p-value
Running	Ankle	0.32	-0.24·10 ⁻⁵	0.87	1095.6	-3.8	<0.001
	Knee	0.29	1.0·10 ⁻³	0.07	1896.3	-10.2	<0.001
	Hip	0.76	-4.9·10 ⁻⁴	0.67	1354.6	-5.8	<0.001
	Total	-	-	-	4345.2	-19.7	<0.001
Hopping	Ankle	0.39	-3.6·10 ⁻⁴	0.06	655.1	0.7	0.32
	Knee	-0.40	8.4·10 ⁻³	<0.001	2565.3	-18.0	<0.001
	Hip	0.63	2.7·10 ⁻³	0.35	917.8	-3.8	<0.001
	Total	-	-	-	4138.8	-21.0	0.008

EMA: effective mechanical advantage; V_m : active muscle volume; p-value < 0.05 indicates a slope significantly different from zero with respect to normalized stride frequency. Linear mixed-effects model is in the form of $Y = \text{Intercept} + \%PSF \cdot \text{Slope}$.