| 1 | Title - Walking with increased step length variability increases the metabolic cost of walking |
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| 3 4 | Running Title - Metabolic Cost of Step Length Variability |
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| 9 | |
| 10 | Key Words: Energetics, Step frequency, Gait variability, Locomotion, Visually guided stepping |
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| 12 | Summary Statement - For every 1% increase in step length variability, there is an 0.7% |
| 13 | increase in the metabolic cost of walking. |
| 14 | |
| 15 | Abstract - Older adults and neurological populations tend to walk with slower speeds, more gait |
| 16 | variability, and a higher metabolic cost. This higher metabolic cost could be related to their |
| 17 | increased gait variability, but this relationship is still unclear. The purpose of this study was to |
| 18 | determine how increased step length variability affects the metabolic cost of waking. Eighteen |
| 19 | healthy young adults completed a set of 5-minute trials of treadmill walking at 1.20 m/s while we |
| 20 | manipulated their step length variability. Illuminated rectangles were projected onto the surface |
| 21 | of a treadmill to cue step length variabilities of 0, 5 and 10% (coefficient of variation). Actual |
| 22 | step lengths and their variability were tracked with reflective markers on the feet, while |
| 23 | metabolic cost was measured using indirect calorimetry. Changes in metabolic cost across |
| 24 | habitual walking (no projections) and the three variability conditions were analyzed using a |
| 25 | linear mixed effects model. Metabolic power was largest in the 10% condition (4.30 ± 0.23) |
| 26 | W/kg) compared to 0% (4.16 \pm 0.18 W/kg) and habitual (3.98 \pm 0.25 W/kg). The participant's |
| 27 | actual step length variability did not match projected conditions for 0% (3.10%) and 10% |
| 28 | (7.03%). For every 1% increase in step length variability, there is an 0.7% increase in metabolic |
| 29 | cost. Our results demonstrate an association between the metabolic cost of walking and gait step |
| 30 | length variability. This suggests that increased gait variability contributes to a portion of the |
| 31 | increased cost of walking seen in older adults and neurological populations. |

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32 INTRODUCTION:

33 With age and increased neurological impairment, people walk slower (Bohannon, 1997; 34 Steffen et al., 2002), with shorter steps (Osoba et al., 2019), greater gait variability (Owings & Grabiner, 2004) and increased metabolic energy demands (Christiansen et al., 2009; Martin et 35 al., 1992; Zamparo et al., 1995). Increased metabolic cost of walking has been mentioned as a 36 potential risk factor for reduced gait speed and mobility in older individuals (Schrack et al., 37 2012). Reduced gait speed is associated with mortality (Newman et al., 2006; Studenski et al., 38 2011), cardiovascular disease, and other adverse effects (Abellan van Kan et al., 2009; Szanton 39 40 et al., 2021). As outlined by Boyer et al., (2023) "... the significant societal, economic, and personal burdens associated with mobility limitations highlight the importance of understanding 41 42 the mechanisms for increased metabolic cost of walking...". While the association between reduced gait speed and increased metabolic cost is well studied (but not yet fully understood; 43 44 Boyer et al., 2023), less is known about the potential association between other gait changes, 45 such as increased gait variability, and the metabolic cost of walking.

Increasing stride frequency from habitual (+15 strides / minute) increases metabolic cost
by approximately 19% during walking (Holt et al., 1991). As step frequency increases, there is a
greater cost of moving the legs (Doke et al., 2005). Decreasing stride frequency from habitual (15 strides / minute) increases metabolic cost by almost 30% (Holt et al., 1991). This is likely due
to the increased metabolic cost of redirecting the center of mass between steps (Donelan et al.,
2002). Indeed, people habitually self-select a step frequency which minimizes metabolic cost
(Holt et al., 1995).

53 However, people do not walk with consistent step lengths in daily life because walking often occurs in short bouts (Seethapathi & Srinivasan, 2015), at variable speeds, and not on level 54 55 ground (Kowalsky et al., 2021; Voloshina et al., 2013). Varying step length can also be helpful 56 when encountering obstacles (Patla et al., 1991) or maintaining stability (Young & Dingwell, 57 2012). Even for a long walking bout, at a constant speed, on level ground, with one's optimal average step length, there will be small variabilities in step length around this average - a mix of 58 59 steps that are both shorter and longer than optimal (Owings & Grabiner, 2004), due to intrinsic sensory and neuromotor noise (Dean et al., 2007; Dingwell et al., 2017). Added variability can 60 61 be expected to be more metabolically costly (O'Connor et al., 2012), as each step deviates from 62 optimal step parameters (Holt et al., 1995).

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63 Few studies have tried to quantify the impact of step length variability on metabolic cost (O'Connor et al., 2012; Rock et al., 2018). O'Connor et al. (2012) used virtual visual flow field 64 65 perturbations to increase gait variability and evaluate metabolic cost. At a constant speed of 1.25 m/s, high frequency medio-lateral rotations of the virtual visual flow field induced the largest 66 increase in metabolic cost (5.9%). Participants walked with increased step length variability in 67 this condition, but the metabolic increase was most strongly coupled with increased step width 68 69 variability. More recently, Rock et al. (2018) evaluated metabolic cost of transport and step 70 length variability across a range of walking speeds. Although speed had the greatest impact on 71 metabolic cost, at a constant speed of 1.25 m/s, each 1% increase in step length variability was 72 also associated with a 5.9% increase in metabolic cost. In these studies, the observed increases in 73 metabolic cost with increased step length variability could not be separated from changes in step 74 width variability or walking speed, respectively, so the isolated effects of increased step length 75 variability on the metabolic cost of walking are still unknown.

76 Therefore, we set out to quantify the isolated effects of increased step length variability on the metabolic cost of walking. We used visual cues to increase step length variability by 77 78 projecting illuminated rectangles (stepping stones) on a treadmill progressing at the speed of the 79 belt (Hollands et al., 1995; Hoogkamer et al., 2015; Roerdink et al., 2009; Van Ooijen et al., 80 2015). The stepping stones were spaced to the participant's habitual step length, with increasing 81 levels of step length variability per condition. By directly targeting step length variability, using 82 projected stepping stones, we were able to evaluate the metabolic cost of walking with increased 83 step length variability independent from other gait changes that may arise when using 84 perturbations that indirectly affect step length variability. We hypothesized that increases in step 85 length variability would increase the metabolic cost of walking.

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87 MATERIALS AND METHODS:

88 **Participants**:

Eighteen healthy young adults (7F; 24.4 ± 3.7 years; 171.2 ± 17.2 cm; 70.5 ± 13.3 kg)
completed this study. Eligible participants were between the ages of 18 and 45 years old, had not
experienced lower extremity injuries or surgery within the past six months, and were free of any
existing orthopedic, cardiovascular or neuromuscular conditions. Written informed consent was

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93 obtained from each participant prior to the study. All procedures were approved by the

94 Institutional Review Board at the University of Massachusetts Amherst (#3002).

95 **Procedures:**

We provided each participant with a pair of standardized shoes in their size (Speed
Sutamina, PUMA SE, Herzogenaurach, Germany). We placed retroreflective markers on each
foot at the fifth metatarsal head and calcaneus, and a four-marker cluster on the sacrum.

99 Participants first walked at a speed of 1.20 m/s (Das Gupta et al., 2019) for five minutes 100 to familiarize themselves with the dual-belt treadmill (Bertec, Columbus, OH, USA) and the 101 indirect calorimetry mouthpiece. Next, they walked for three minutes at 1.20 m/s for which we 102 evaluated habitual step length during the final 30 seconds. An eight-camera Migus system 103 (Qualisys, Gothenburg, Sweden) recorded kinematic data at 100 Hz. We used kinematic data 104 from the left and right calcaneus to determine habitual step length using a custom Matlab script 105 (The MathWorks, Natick, MA, USA). Step length for each leg was calculated as the distance 106 between the anterior and posterior positions of the ipsilateral and contralateral calcaneus 107 markers, respectively, during the maximum anterior position of the calcaneus marker for each 108 step (Desailly et al., 2009). Mean step length and standard deviation were used to determine the 109 coefficient of variation, i.e., the mean step length divided by the standard deviation for each foot, 110 before averaging across feet.

Experimental conditions were: no projections (NP), 0%, 5% and 10% variability. Throughout we consider step length variability in terms of the coefficient of variation of the step length during a trial. At 0% variability, we projected stones with no variability in step lengths, whereas for the 10% condition we projected stones with 10% variability in step lengths across the entire trial. In a block randomized order, participants completed all four experimental conditions, before completing them again in reverse, for a total of eight trials (e.g., NP, 10%, 0%, 5%, 5%, 0%, 10%, NP).

The projected stepping stones were generated using a custom Matlab script. A vector of step lengths for the entire trial was created based on the participant's habitual step length, treadmill speed (1.20 m/s), and trial duration (5 minutes). A vector of corresponding step length perturbations was created using the *randn* function in Matlab, which generates a list of normally distributed random numbers with a mean of 0 and variance of 1. This step length perturbation vector was scaled by the desired step length variability (0, 5 or 10% coefficient of variation) and

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124 the habitual step length. A larger coefficient of variation increases the frequency of perturbed 125 steps relative to unperturbed, and of larger perturbations relative to smaller. To ensure that 126 differences in step length were perceivable by the participants, we discretized the perturbations 127 into bins that were 5% multiples of the participant's habitual step length. We limited 128 perturbations to a maximum of 15% in either direction, to prevent the possibility of unrealistic 129 perturbations at the tails of the normal distribution. Perturbations that fell within half of the 130 discretization interval above or below a perturbation target were reassigned to the target value 131 and applied. Thus, participants encountered distances that matched the habitual step length most 132 of the time, with less frequent longer and shorter perturbed steps (Fig. 1A). 133 We used an expired-gas analysis system (True One 2400, Parvo Medics, Salt Lake City,

134 UT, USA) to measure metabolic cost across the four experimental walking conditions. We added a 3D printed extension to the three-way valve mouthpiece (Hans Rudolf Mouthpiece, Shawnee, 135 136 KS, USA) that pitched forward (5 cm) and up (5 cm) at a 45-degree angle (Fig. 1B). The 137 participants were then able to see the approaching stepping stones. Each trial was five minutes 138 long and data across the last two minutes of each trial was used to evaluate metabolic power. We 139 calculated metabolic power (W/kg) using oxygen uptake, carbon-dioxide production, and the 140 Péronnet and Massicotte equation (Kipp et al., 2018; Péronnet & Massicotte, 1991). Metabolic 141 power was averaged across the two trials for each condition (Barrons et al., 2024).

142 Statistical Analysis:

143 We used linear mixed effects models to evaluate changes in metabolic cost with 144 increasing step length variability. We used the actual step length variability that participants 145 walked with for each condition, rather than the projected step length variability for that 146 condition. All statistical analysis was performed in R studio (4.2.2) with a linear mixed effects 147 regression (Wilkinson et al., 2023) using lme4 (1.1 - 32) and sistats (0.18.2) packages. A paired 148 t-test was used to compare metabolic power and step length variabilities between NP and 0%149 conditions. A linear model was used to investigate the impact of step length variability on 150 metabolic power during walking. Random intercepts were adjusted for each participant using 151 equation 1 where COV is the coefficient of variation of the step lengths during a trial. 152

153 Eqn 1: Metabolic Power $\sim COV + (1 | Participant)$

Metabolic power was then evaluated using the mixed effect model with a random

intercept for each participant and an independent slope corresponding to the participant specific

metabolic power response using equation 2.

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| 159 | Eqn 2: Metabolic Power ~ COV + (COV Participant) |
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| 161 | We used a likelihood ratio to test for significance between a model excluding step length |
| 162 | variability and an alternative model that did not. Chi-squared (χ^2) and significance values are |
| 163 | reported. For all statistical tests, significance was set at an alpha level of 0.05. |
| 164 | |
| 165 | RESULTS: |
| 166 | Metabolic power and step length variability were different between NP and 0% |
| 167 | conditions (3.98 \pm 0.25 vs. 4.16 \pm 0.18 W/kg; p = 0.0002 and 2.42 \pm 0.55% vs. 3.10 \pm 0.58%; p = |
| 168 | 0.001, respectively; Figure 2). Thus, when pooling the true step length variability across |
| 169 | conditions, we excluded the NP condition (i.e., we included only data from the 0% , 5% and 10% |
| 170 | step length variability trials in our model). Overall, metabolic power increased with increasing |
| 171 | step length variability ($\chi^2 = 9.41$; p = 0.002; Fig. 3). For every 1% unit increase in step length |
| 172 | variability, there was an increase in metabolic power of 0.03 ± 0.01 W/kg, or 0.7%. |
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| 174 | DISCUSSION: |
| 175 | In this study, we quantified the isolated effects of increased step length variability on the |
| 176 | metabolic cost of walking. In line with our hypothesis, increases in step length variability |
| 177 | resulted in increases in metabolic cost. Additionally, we observed a difference in metabolic cost |
| 178 | between walking without projections and with projections with no variability. Our results are |
| 179 | similar, but of smaller magnitude than observations from studies that indirectly increased step |
| 180 | length variability (O'Connor et al., 2012; Rock et al., 2018). |
| 181 | Our findings suggest a modest contribution of step length variability to the metabolic cost |
| 182 | of walking (0.7% increase in metabolic power per 1% increase in step length variability; Fig. 3) |
| 183 | at a common walking speed of 1.20 m/s. At this speed, every percent change in step length |
| 184 | variability increases metabolic cost by 0.03 W/kg. This increase is almost five times smaller than |
| 185 | the 0.14 W/kg increase in metabolic cost, for every 1% increase in variability, modeled by Rock |
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186 et al (2018). This difference is likely related to our direct manipulation of step length variability 187 at a single, constant speed, eliminating the metabolic penalty of walking slower than preferred 188 (Ralston, 1958). Additionally, the work of O'Connor et al. (2012), reported greater step width 189 variability (+65%), mean step width (+19%) and an increased metabolic cost (5.9%) while 190 walking with virtual visual flow perturbations (high frequency medio-lateral rotations). This 191 suggests that step width variability has a larger effect on metabolic cost than step length 192 variability. Indeed, walking with increased step width comes at a metabolic cost (Donelan et al., 193 2001).

194 Two mechanisms can be expected to play a role in elevating the metabolic cost of 195 walking with increased step length variability. First, adjusting to a closer stepping stone (i.e., 196 reducing step length), increases the metabolic cost of moving limbs at a higher rate (Doke et al., 197 2005). Second, an adjustment to a farther stepping stone (i.e., increasing step length) increases 198 the cost of lifting the center of mass over the point of collision (Donelan et al., 2001). An 199 additional possible mechanism, that relates to the need to step accurately on each stone, is an 200 increase in muscle co-contraction, commonly observed with increased accuracy demands 201 (Gribble et al., 2003). While some of the increased metabolic cost of walking could be attributed 202 to increased accuracy demands, an additional portion could be related to the cost of consistently 203 regulating steps across projected stones, degrading the contribution of passive dynamics to 204 walking (Wezenberg et al., 2011). These additional mechanisms could help explain the 205 differences in metabolic cost between walking with no projections (habitual) and with 0% step 206 length variability projections that closely matched habitual gait characteristics (Fig. 2).

207 Beyond slower gait with shorter steps and longer double support phases, older adults 208 have more gait variability and increased metabolic energy demands (Boyer et al., 2023; Schrack 209 et al., 2012). The larger gait variability in older adults and in those with neurological 210 impairments could contribute to their increased metabolic cost of walking as compared to young 211 (e.g., +8%; Martin et al., 1992) or neurologically healthy adults (+17-170%; Compagnat et al., 212 2020; Jeng et al., 2020; Rooney et al., 2022). At matched speeds, older adults walk with higher 213 step length variability than younger adults (Almarwani et al., 2016; Kang & Dingwell, 2008), 214 more so if the older adult has mobility impairments, with a reported 2.7% increase in step length 215 variability (James et al., 2020). Our data suggest that a 2.7% increase in step length variability 216 would increase the metabolic cost of walking by 1.7%. At their preferred speeds, adults with

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217 neurological impairments have been reported to walk with step length variabilities that are 218 approximately 2.5%, 3.0%, and 6.0% larger than neurologically healthy controls, for Parkinson's 219 disease, Multiple Sclerosis, and Cerebellar Ataxia, respectively (Buckley et al., 2018; Noh et al., 2020; Roemmich et al., 2012; Socie et al., 2013). Our data suggests that these increases in step 220 221 length variability will increase the metabolic cost of walking by 1.8, 2.1 and 4.2%, respectively. 222 The metabolic cost of walking with increased step length variability only contributes a small part 223 to the total increased metabolic cost (17-170%) of walking observed across these populations 224 (Compagnat et al., 2020; Jeng et al., 2020; Martin et al., 1992; Rooney et al., 2022).

225 Limitations and future directions:

226 To ensure that the step length variations were perceivable to participants, we binned step 227 lengths into 5% multiples, not exceeding 15% of their habitual step length. Normal walking does not contain such discretized step length variability. Although the perturbed conditions do not 228 229 perfectly reflect real-world gait variability, they provide an upper limit for the increases in 230 metabolic cost in young healthy individuals. Most participants were unable to exactly match the discretized step length variabilities that were projected; while the use of 5% bins is different than 231 232 real-world gait variability, participants still had trouble maintaining accuracy across the stepping 233 stones without additional feedback. We were limited in identifying how participants altered their 234 step length. In future research it could be insightful to evaluate whether participants took larger 235 or shorter steps when aiming for the stone targets, as well as the frequency and magnitude of 236 their errors. This would not change the relationship between step length variability and metabolic 237 cost, but it could help in relating task performance to step length variability within a condition. 238 While few participants were able to walk with 5 and 10% step length variability, 0% step length 239 variability was unachievable – the lowest value for a single participant was 2%. This is in line 240 with observations that walking inherently contains some variability in step length (Collins & 241 Kuo, 2013) which might also be distinct between participants.

Participants did not receive feedback on how well they were performing during a trial.
Feedback could have improved task performance (Shull et al., 2014) to better align with our
variability conditions. To investigate whether co-contraction, to maintain accuracy (Gribble et
al., 2003), contributes to the increased metabolic cost of walking with variable step lengths,
future studies should quantify foot placement accuracy and muscle activity across similar virtual
projections. Finally, to clarify the relationship between variability and gait deficiencies for

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populations already experiencing increased step length variability, further investigations shouldbe conducted with these populations specifically.

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251 Conclusion:

252 Older adults and those with neurological conditions walk with greater step length 253 variability and increased metabolic cost. In a population of healthy young adults, we found that 254 metabolic cost increases by approximately 0.7% (0.03 W/kg) for every 1% increase in step 255 length variability. Although most of our participants were unable to exactly match the projected 256 conditions, our virtual visual perturbation successfully increased step length variability and 257 metabolic cost across step variability conditions. The metabolic cost per unit of variability was 258 smaller than reported in previous work, indicating that step length variability plays a modest, 259 albeit significant role in the metabolic cost of walking.

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266

267 Competing interests

268 The authors declare no competing or financial interests.

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270 Author contributions

- 271 Conceptualization: M.E.W., W.H.; Methodology: A.B.G., M.E.W., W.H. Formal analysis:
- A.B.G., M.E.W., W.H. Investigation: A.B.G., M.E.W.; Resources: A.B.G., W.H.; Data curation:
- 273 A.B.G., M.E.W., W.H; Writing original draft: A.B.G., M.E.W., WH; Writing review &
- editing: A.B.G., D.N.M., W.H; Visualization: A.B.G; Supervision: D.N.M., W.H.; Project
- administration: W.H.; Funding acquisition: D.N.M., W.H
- 276

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Figures:

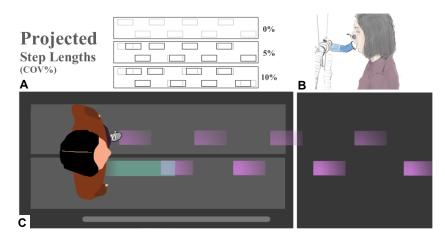


Figure 1. (A) Diagram of example step length conditions for 0, 5, and 10% variability. Grey rectangles are stepping stone targets projected at a participant's specific habitual step length. Black rectangles are the lengthwise deviations of perturbed stones generated randomly from discretized bins. (B) Illustration of indirect calorimetry including pitched 45-degree extension used to open participant field-of-view. (C) Top-down visual of a participant targeting projected stepping stones (purple; moving from right to the left) for 0% variability.



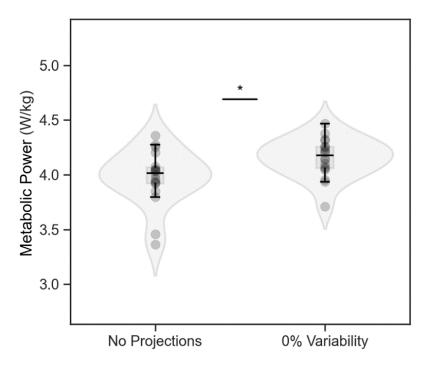


Figure 2. Metabolic power (n = 18) was lower for walking without any projected stepping stones than for walking over stepping stones projected without any step length variability. Values are mean \pm s.d., * p < 0.05

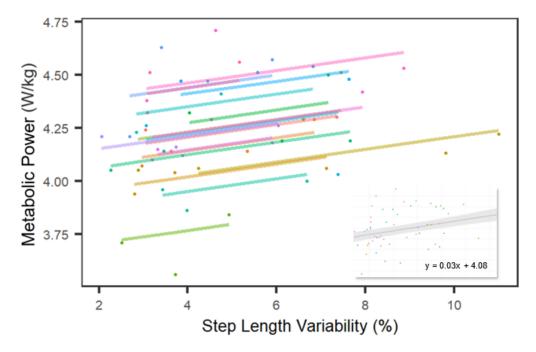


Figure 3: For every percentage increase in step length variability, there is a 0.03 W/kg (0.7%) increase in metabolic power, averaged between conditions and across participants (inset). Linear mixed effects models identified linear trends for each subject (n = 18; colored lines) across conditions.