

## RESEARCH ARTICLE

# Interaction between step-to-step variability and metabolic cost of transport during human walking

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## ABSTRACT

Minimizing the metabolic cost of transport can affect selection of the preferred walking speed. While many factors can affect metabolic cost of transport during human walking, its interaction with step-to-step variability is unclear. Here, we aimed to determine the interaction between metabolic cost of transport and step length variability during human walking at different speeds. In particular, two aspects of step length variability were analyzed: the amount of variations ('variations') and the organization of the step-to-step fluctuations ('fluctuations'). Ten healthy, young participants walked on a treadmill at five speeds, ranging from 0.75 to 1.75 m s<sup>-1</sup>. Metabolic cost of transport, step length variations (coefficient of variation) and step length fluctuations (quantified via detrended fluctuation analysis) were calculated. A mixed-model ANOVA revealed that variations and walking speed were strong predictors of metabolic cost of transport ( $R^2=0.917$ ,  $P<0.001$ ), whereas fluctuations were not. Preferred walking speed (1.05±0.20 m s<sup>-1</sup>) was not significantly different from the speed at which metabolic cost of transport was minimized (1.04±0.05 m s<sup>-1</sup>;  $P=0.792$ ), nor from the speed at which fluctuations were most persistent (1.00±0.41 m s<sup>-1</sup>;  $P=0.698$ ). The minimization of variations occurred at a faster speed (1.56±0.17 m s<sup>-1</sup>) than the preferred walking speed ( $P<0.001$ ). Step length variations likely affect metabolic cost of transport because greater variations are indicative of suboptimal, mechanically inefficient steps. Fluctuations have little or no effect on metabolic cost of transport, but still may relate to preferred walking speed.

**KEY WORDS:** Locomotion, Energy, Fractal, Persistence, Step length

## INTRODUCTION

Every movement that a person makes requires muscles to act, and each muscle action requires the consumption of metabolic energy. As such, humans tend to prefer to walk in a way that makes economical use of metabolic energy (Alexander, 2002; Selinger et al., 2015). This typically takes the form of a minimization in the metabolic cost per unit distance, referred to as the metabolic cost of transport (MCOT; Bastien et al., 2005; Ralston, 1958). The minimization of MCOT results from a reduction of the force and work requirements of the muscles (Bastien et al., 2005; Dean and Kuo, 2011; Kim and Collins, 2015), and requires the regulation of a variety of gait characteristics. For example, walking speed is a major determinant of MCOT (Alexander, 2002; Browning and Kram,

2005; Ralston, 1958) in that there is an optimal speed that results in a minimized MCOT. While not all of the determinants of MCOT have been identified, sustained deviation in preferred step length or frequency (Minetti et al., 1995), step width (Donelan et al., 2001) and step variability (O'Connor et al., 2012) result in increased MCOT.

MCOT has been largely attributed to the force production of the muscles (Alexander, 2002). This may explain why changes in walking speed, step length or frequency and other gait characteristics affect MCOT. For example, consider step length during walking at a constant speed: very short steps require a high step frequency, which in turn requires fast muscle contractions that do not provide as much force as slower contractions (Hill, 1938). Furthermore, higher step frequencies require greater work to accelerate the limbs relative to the center of mass (Cavagna and Franzetti, 1986). As such, short and frequent steps are metabolically costly. Long steps are also suboptimal, as they require a larger vertical displacement of the center of mass and therefore more work is done to accelerate the center of mass (Cavagna and Franzetti, 1986), which necessitates increased force output from the muscles (Minetti et al., 1995).

In addition to changes in the mean step length, the amount of variation in step length (i.e. 'variations', assessed by the coefficient of variation) can affect MCOT. Studies that increase step length variations via visual perturbations (O'Connor et al., 2012) or by varying terrain (Voloshina and Ferris, 2013) have shown a proportional increase in the metabolic rate within a given walking speed, and so we would expect MCOT to follow the same trend. Increased step length variations are indicative of a larger proportion of metabolically suboptimal step lengths, and thereby increase MCOT.

When considering step-to-step variability as a determinant of MCOT, a complementary aspect to variations is the organization of the step-to-step fluctuations (i.e. 'fluctuations'; Hausdorff et al., 1996). Fluctuations are measured by analyzing the 'statistical persistence' of a series of steps. By measuring step length over hundreds of consecutive steps, it is possible to investigate changes in the level of statistical persistence of step length fluctuations (Hausdorff et al., 1995, 1996). Fluctuations that result in similar step-to-step changes (e.g. a long step is likely to be followed by more long steps) are said to have high persistence, and fluctuations that have high step-to-step dissimilarity (e.g. a long step is likely to be followed by a short step) are said to have low persistence.

Studies on the statistical persistence of fluctuations have revealed new information about locomotor control (Hausdorff et al., 1997; Marmelat et al., 2014a; Nakayama et al., 2010). For example, young healthy adults exhibit highly persistent fluctuations. This level of persistence is reduced when people are instructed to walk to the beat of a metronome (Roerdink et al., 2015) or by targeting specific step lengths during walking (Bohnsack-McLagan et al., 2016), indicating that fluctuations are affected by the level of direct

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neural control (i.e. attention to foot placement) as well as the level of feedback (i.e. error-based correction). In addition, fluctuations are affected by changes in walking speed (Chien et al., 2015; Jordan et al., 2007), where an 'optimal' level may be observed at preferred walking speed (PWS). This convergence of both fluctuations and MCOT at the PWS indicates that the two may be linked.

In order to investigate how step length fluctuations may be related to MCOT, let us explore how a series of step lengths may be arranged. If all steps are identical in length, the pendulum mechanics of walking will be readily maintained, where each limb will act like an inverted pendulum (Donelan et al., 2002). Deceleration will occur when the leading limb contacts the ground and acceleration will be provided by the trailing limb to continue forward propulsion. However, if consecutive steps are highly dissimilar (e.g. long–short–long), there will be altered transition phases from step to step. When transitioning from long to short, extra muscle action will be required to decelerate the body (Kuo et al., 2005). When transitioning from short to long, excess/abnormal acceleration will be required, which will also put a higher demand on the muscles (Donelan et al., 2002). If this suboptimal pattern continued for hundreds or thousands of steps, it could incur a greater MCOT due to altered step-to-step transitions. Additionally, such suboptimal patterns could lead to an asymmetrical step pattern, which would also contribute to an increased MCOT (Ellis et al., 2013). As such, it would seem that maintaining a step pattern of similar, symmetrical steps (i.e. more statistically persistent fluctuations) would be metabolically favorable. However, research investigating the link between fluctuations and MCOT is lacking.

The aim of this study was to determine the interaction between MCOT and step-to-step variability. By manipulating walking speed, we perturbed MCOT and observed the resulting changes in step length coefficient of variation (i.e. 'variations') and in the persistence of step length fluctuations (i.e. 'fluctuations'). Our primary hypothesis was that increases in MCOT would be associated with increases in variations and with decreases in fluctuations. Due to the established relationship between variations and metabolic rate (O'Connor et al., 2012; Voloshina and Ferris, 2013), we also hypothesized that variations would be a stronger determinant of MCOT than fluctuations. Lastly, we hypothesized that the minima (across various walking speeds) of MCOT and variations, as well as the highest persistence in fluctuations, would occur near the PWS.

## MATERIALS AND METHODS

### Subjects

Ten participants (5 female and 5 male) ranging in age from 21 to 33 years (means±s.d.; age: 24.2±3.4 years; height: 1.75±0.09 m;

mass: 80.0±15.7 kg) took part in this study (Table 1). Participants did not present with conditions that limited walking activity. Participants with a history of cardiac or neurological problems were also excluded. Informed consent was obtained from each participant. All procedures were approved by the Institutional Review Board at the University of Nebraska Medical Center, NE, USA.

### Procedure

Each participant attended a single session, which included all experimental procedures. Upon arrival, the participant was fitted with a tight-fitting suit to facilitate proper marker placement. For all procedures, participants wore their own footwear. Retroreflective markers, in conjunction with an infrared camera system (100 Hz; Motion Analysis, Santa Rosa, CA, USA), provided information about the participant's gait kinematics: specifically, pelvis and foot positions. The 12 cameras were positioned at the perimeter of the room, with the treadmill (Pro Tread AC 7600, Star Trac, Irvine, CA, USA) placed at the center of the capture volume. The 13 markers corresponded to the following anatomical landmarks, from proximal to distal: anterior and posterior iliac spines, sacrum, heel, lateral heel, fifth metatarsal head, and second metatarsal head. After fitting the participant with the markers, a recording of the static posture was taken with the participant standing, for use as a model in software for later processing.

A gas exchange system (TrueOne 2400, ParvoMedics, Sandy, UT, USA) was fitted to the participant in order to measure their oxygen and carbon dioxide inhalation/exhalation levels. In order to determine the participant's standing metabolic rate, measured in Watts per kg ( $W\ kg^{-1}$ ), a trial was recorded prior to walking trials where he/she stood for 5 min while gas exchange was measured. This metabolic rate was later subtracted from the metabolic rate recorded during each trial.

The PWS was determined prior to experimental trials using a typical incremental protocol (Marmelat et al., 2014b). This involved the participant walking on the treadmill at a slow speed ( $1.6\ km\ h^{-1}$ ;  $0.44\ m\ s^{-1}$ ), which was increased every 10 s in intervals of  $0.1\ km\ h^{-1}$  ( $0.028\ m\ s^{-1}$ ). This was continued until the participant indicated that a comfortable speed had been reached. The speed was then further increased until the participant indicated that the speed was uncomfortable, at which point the speed was incrementally decreased until a comfortable speed was indicated by the participant. The speed was adjusted according to participant feedback until the most comfortable speed was determined. Participants were blinded to the speed of the treadmill during the PWS determination. Participants had at least 3 min of rest after the PWS determination.

Participants completed five walking trials on a treadmill lasting 15 min each. Each walking trial was undertaken at a different speed ( $0.75, 1.00, 1.25, 1.50$  or  $1.75\ m\ s^{-1}$ ; randomized order), and participants were blinded to the exact speed. Set walking speeds were chosen, as opposed to relative speeds (e.g.  $PWS\pm 10\%$ ), to match previous research on MCOT (Browning and Kram, 2005; Griffin et al., 2002; Malatesta et al., 2003), as well as to better allow for the proper estimation of variable-specific minima/maxima. Participants rested for at least 3 min between each trial.

### Data processing

The gas exchange from the final 10 min of each trial was used to calculate mean metabolic rate, in order to ensure subjects were in a steady metabolic state, which takes an average of 3 min at moderate exercise (Poole and Richardson, 1997). Metabolic rate was calculated

**Table 1. Participant characteristics**

Subject	Sex	Age (years)	Mass (kg)	Height (m)	RMR ( $W\ kg^{-1}$ )	PWS ( $m\ s^{-1}$ )
1	M	22	80.29	1.83	1.48	1.08
2	M	23	68.04	1.79	1.55	1.14
3	F	24	72.57	1.65	0.90	0.92
4	F	22	72.12	1.78	1.24	1.06
5	F	33	55.34	1.57	1.26	1.08
6	F	25	88.90	1.71	1.13	1.31
7	M	21	90.72	1.89	1.48	1.22
8	M	23	89.81	1.74	1.26	0.58
9	F	23	70.76	1.69	1.17	1.06
10	M	26	111.13	1.81	1.05	1.08

RMR, resting metabolic rate recorded during a 5-min standing trial; PWS, preferred walking speed; M, male; F, female.

using the Brockway equation, which uses the volumes of oxygen consumed and carbon dioxide produced to calculate metabolic power in terms of Watts, or Joules per second ( $\text{J s}^{-1}$ ; Brockway, 1987), which was then normalized to body mass. After subtracting the standing metabolic rate, the value was divided by speed to obtain net MCOT, which is reported in units of Joules per kilogram of body mass per meter ( $\text{J kg}^{-1} \text{m}^{-1}$ ) (Peterson and Martin, 2010).

Step variables were determined using Cortex (Motion Analysis, Santa Rosa, CA, USA), which was used to record and track the data, and Visual3D (C-motion, Germantown, MD, USA) and MATLAB (The MathWorks Inc., Natick, MA, USA), which were used to process the tracked data and calculate mean step length, step length variations and step length fluctuations. Timing of heel strike and toe off were determined using the foot velocity with respect to the pelvis (Zeni et al., 2008). Step time was calculated as the time between contralateral heel strikes. Step length was calculated using the following equation:

$$\text{Step length}_n = (tv_n \times \text{step time}_n) - (\text{HSx}_{n+1} - \text{HSx}_n), \quad (1)$$

where  $\text{HSx}_n$  is the anteroposterior position of the heel marker at the  $n$ th heel strike, and  $\text{HSx}_{n+1}$  is the anteroposterior position of the contralateral heel marker at the next heel strike. The difference between these positions represents the participant's forward or backward movement relative to the treadmill. Treadmill velocity ( $tv$ ) was calculated for each step using the average velocity of the stance foot center of mass during the portion of stance when the foot was flat on the treadmill. It should be noted that this method of treadmill velocity calculation is not as robust as if we had directly measured the instantaneous treadmill belt speed, but appeared to be a reliable estimation, evidenced by the low standard deviation within a speed (s.d.=0.0098  $\text{m s}^{-1}$  at the slowest speed, 0.0154  $\text{m s}^{-1}$  at the highest speed). Treadmill velocity was then multiplied by the time duration of the step, and combined with the difference in consecutive heel strikes to obtain the full step length. After calculating the gait events and foot marker positions in Visual3D, they were exported to MATLAB for analysis, which was used to calculate step length mean, standard deviation and coefficient of variation (i.e. 'variations'). Coefficient of variation was chosen as the measure of step length variability magnitude because it is normalized to the mean step length for each condition and has been shown to be speed dependent (Jordan et al., 2007). Other measures of variability (such as s.d. of step length, step width and stance time) are reported in Table S1.

Detrended fluctuation analysis (DFA; Hausdorff et al., 1995) was used to quantify the level of statistical persistence in the step length time series (i.e. fluctuations). Briefly, DFA was performed via four procedural steps: (1) the time series was detrended so that the mean value was zero. To do this, the mean was subtracted from each value in the series. (2) The detrended time series was integrated, resulting in a new time series. (3) The integrated series was separated into non-overlapping boxes of equal size,  $n$ . The average root mean squared fluctuation error ( $F$ ) from a linear fit to the data was calculated within each box, and averaged for different box sizes ( $n$ ), ranging in size from 10 to  $N/4$  data points, where  $N$  is the length of the series. (4) The root mean squared fluctuation [ $F(n)$ ] was then plotted against the box size  $n$  in log–log coordinates. The presence of a linear least-squares line of best fit suggests the presence of a power-law relationship, i.e.  $F(n) \sim n^\alpha$ , where  $\alpha$  represents the scaling exponent estimated from the slope. A slope (i.e. DFA- $\alpha$ ) at or above 1.0 would indicate high statistical persistence, with decreases indicating lower persistence. The results from DFA have also been

referred to as level of 'self similarity' (Chien et al., 2015), 'fractal fluctuations' (Marmelat and Delignières, 2011) or degree of '1/f scaling' (Harrison and Stergiou, 2015). For examples and figures on the DFA calculation, please see Damouras et al., 2010 and Francis et al., 2002.

In order to keep inter-subject values comparable, the same number of data points was used for each subject and for each trial. Because DFA is sensitive to the number of data points (Damouras et al., 2010; Delignières et al., 2006), the number of data points used was maximized. The number of data points was determined by the participant who took the fewest steps (1150 steps) in any of the conditions. The first 350 steps (at least 3 min) were removed to avoid the possible adaptation period at the beginning of the trial, which has been shown to be very short in young, healthy populations (Van De Putte et al., 2006). As such, the final 900 steps of each trial were assumed to represent the steady-state behavior of the participants and were analyzed to calculate DFA- $\alpha$  and coefficient of variation.

### Statistical analyses

#### Effect of speed on net MCOT and step-to-step variability

In order to determine whether walking speed had an effect on each dependent variable, one-factor (five levels of speed) repeated-measures ANOVAs were used with Greenhouse–Geisser corrections when necessary (IBM SPSS Statistics, IBM, Armonk, NY, USA).

#### Prediction of net MCOT based on speed and step-to-step variability measures

A mixed-model ANOVA was used to determine the effect of variations and fluctuations on net MCOT. Because it has been shown that speed has a non-linear effect on MCOT (e.g. Griffin et al., 2002), speed and the square of speed were included in the analysis. The analysis conducted was a mixed-model, five-factor ANOVA (random effect: subject; fixed effects: speed, square of speed, step length coefficient of variation, and step length DFA- $\alpha$ ) with net MCOT as the outcome measure (MATLAB).

#### Preferred walking speed and maxima/minima predictions of net MCOT, variations and fluctuations

First, the minima of net MCOT and step length variations, as well as the maximum of step length fluctuations, were determined by fitting a second order polynomial to each subject's data as a function of speed using MATLAB. The minimum/maximum of these parabolae represented the predicted speed at which each variable was minimized/maximized. A one-factor repeated-measures ANOVA was used to determine whether the predicted speeds were different from the measured PWS. A least significant difference *post hoc* test was used to compare the measured PWS to the predicted speeds at which each variable (MCOT, variations and fluctuations) were minimized/maximized ( $\alpha=0.05$ ; IBM SPSS Statistics, IBM, Armonk, NY, USA).

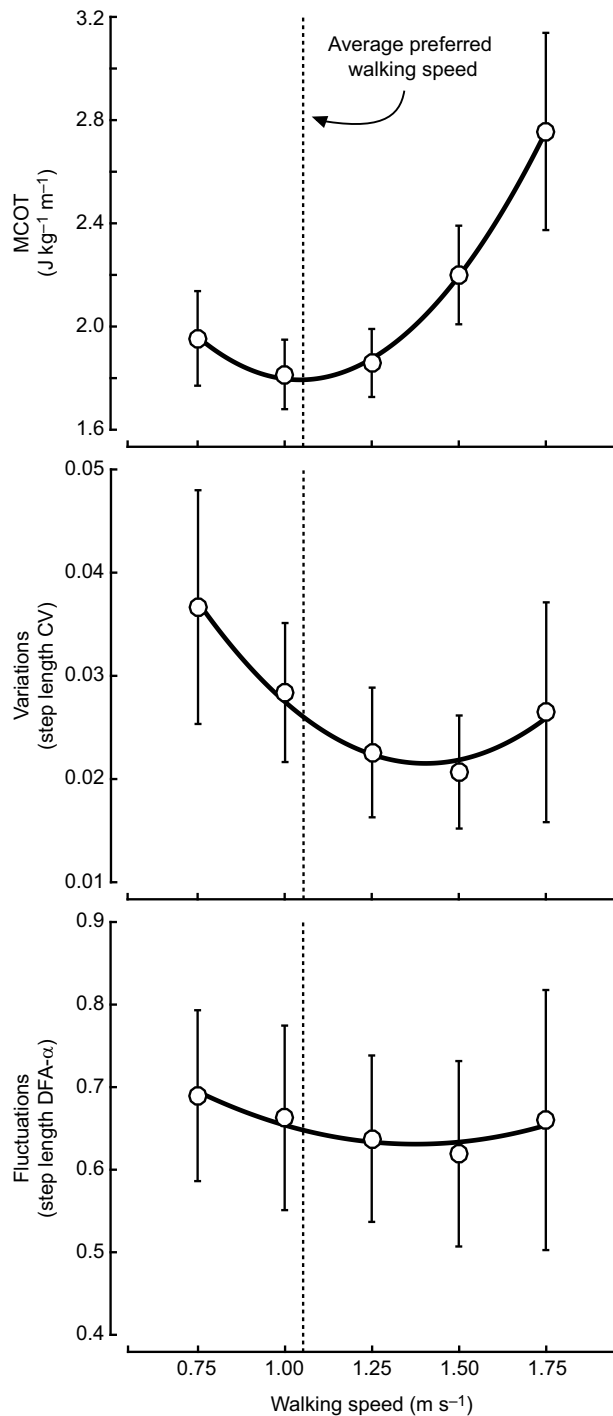
### RESULTS

#### Effect of speed on net MCOT, variations and fluctuations

For net MCOT, Mauchly's test,  $\chi^2_3=37.4$ , indicated a violation of the assumption of sphericity ( $P<0.001$ ); therefore, degrees of freedom were corrected using Greenhouse–Geisser estimates of sphericity ( $\epsilon=0.338$ ). A main effect of speed was found on net MCOT [ $F_{1,351,9}=77.5$ ,  $P<0.001$ ,  $\eta^2_p=0.896$  (Fig. 1)].

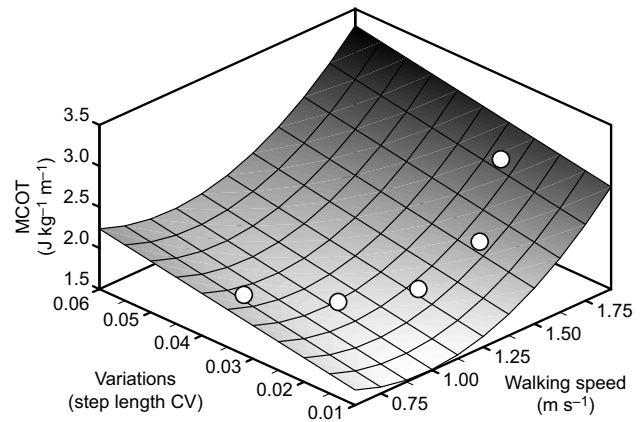
For step length variations, Mauchly's test,  $\chi^2_3=33.0$ , indicated a violation of the assumption of sphericity ( $P<0.001$ ); therefore,





**Fig. 1. Effect of speed on net metabolic cost of transport (MCOT), variations (step length coefficient of variation) and fluctuations (step length DFA- $\alpha$ ).** Mean values for each speed condition were calculated (circles, error bars= $\pm 1$  s.d.) and fitted with a second order polynomial. Significant effects of speed were observed on net MCOT ( $P < 0.001$ ) and on variations ( $P = 0.002$ ). No effect of speed was observed on fluctuations ( $P = 0.342$ ). The average preferred walking speed (PWS) across participants (dashed vertical line) was  $1.05 \pm 0.20$  m s $^{-1}$ . Subject-specific figures are shown in Fig. S2. CV, coefficient of variation; DFA, detrended fluctuation analysis.

degrees of freedom were corrected using Greenhouse–Geisser estimates of sphericity ( $\epsilon = 0.389$ ). A main effect of speed was found on step length variations [ $F_{1.557,9} = 11.3$ ,  $P = 0.002$ ,  $\eta_p^2 = 0.556$  (Fig. 1)].



**Fig. 2. Estimated net MCOT values as a function of variations (step length CV) and walking speed according to the model.** Circles represent the measured mean net MCOT for each speed condition. A strong relationship was observed between the observed net MCOT values and the values estimated by the model ( $R^2 = 0.917$ ). CV, coefficient of variation.

In testing the effect of speed on step length fluctuations, Mauchly's test,  $\chi^2_5 = 4.149$ , indicated no violation of sphericity ( $P = 0.904$ ). A main effect of speed was not found on step length fluctuations [ $F_{4,9} = 1.17$ ,  $P = 0.342$ ,  $\eta_p^2 = 0.115$  (Fig. 1)].

#### Net MCOT and step-to-step variability

Mixed model ANOVA revealed that speed ( $P < 0.001$ ), the square of speed ( $P < 0.001$ ), and step length variations ( $P < 0.001$ ) were significant predictors of net MCOT (Fig. 2). Step length fluctuations had a non-significant effect on net MCOT and were therefore removed from the model. The resulting model equation was:

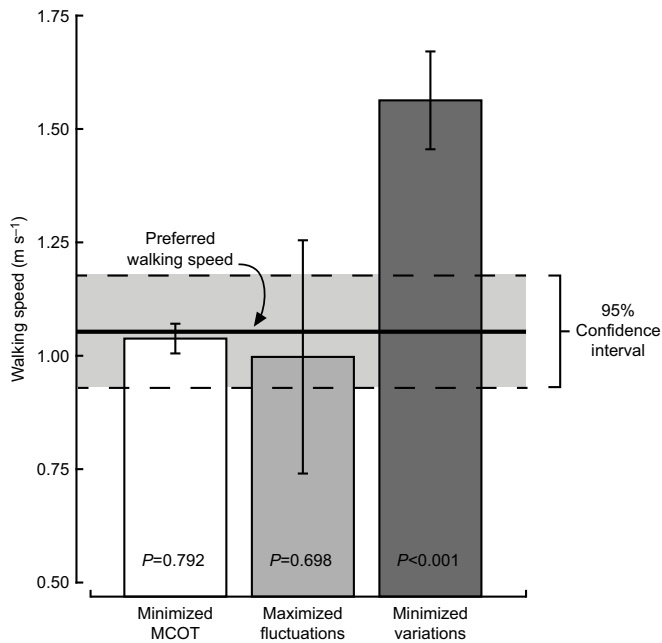
$$\text{Net MCOT} = 2.8 - (2.9 \times \text{Speed}) + (1.5 \times \text{Speed}^2) + (11 \times \text{Variations}), \quad (2)$$

where net MCOT is measured in J kg $^{-1}$  m $^{-1}$ , Speed is measured in m s $^{-1}$ , and Variations is the coefficient of variation of step length (unitless ratio). The model's estimated net MCOT values related to the measured values with an adjusted  $R^2 = 0.917$ . It should be noted that, if this analysis is run without the variations term (i.e. speed terms only), the adjusted  $R^2 = 0.906$ , indicating that the addition of variations provides a modest improvement to the model.

#### Preferred walking speed and speeds predicted by net MCOT, variations and fluctuations

Mauchly's test,  $\chi^2_5 = 15.597$ , indicated a violation of the assumption of sphericity ( $P = 0.009$ ); therefore, degrees of freedom were corrected using Greenhouse–Geisser estimates of sphericity ( $\epsilon = 0.466$ ). A main effect was found on predicted speed [ $F_{1.40,9} = 12.6$ ,  $P = 0.002$ ,  $\eta_p^2 = 0.583$  (Fig. 3)].

*Post hoc* comparisons using least significant difference revealed that the measured PWS ( $1.05 \pm 0.20$  m s $^{-1}$ ) differed from the speed at which step length variations were minimized ( $1.56 \pm 0.17$  m s $^{-1}$ ;  $P < 0.001$ ). No significant difference was observed between the measured PWS and the speed of minimum MCOT ( $1.04 \pm 0.05$  m s $^{-1}$ ;  $P = 0.792$ ), as well as the maximum of step length fluctuations ( $1.00 \pm 0.41$  m s $^{-1}$ ;  $P = 0.698$ ).



**Fig. 3. Speeds at which net MCOT and variations (step length CV) were estimated to be minimized, and at which fluctuations (step length DFA- $\alpha$ ) were estimated to be maximized.** The minimum of net MCOT was not significantly different ( $P=0.792$ ) from the mean PWS (solid horizontal line; dashed lines=95% confidence interval), nor was the maximum of fluctuations ( $P=0.698$ ). However, the minimum of variations was significantly different from the PWS ( $P<0.001$ ). Bars represent mean values with error bars indicating 95% confidence intervals. Least significant difference was used for *post hoc* comparisons. CV, coefficient of variation.

## DISCUSSION

### Interaction between net MCOT and variations

We had hypothesized that increased variations would be associated with increased MCOT. This hypothesis was based on the assumption that there is a metabolically ‘optimal’ step length for each speed (Minetti et al., 1995), and that increases in step length variations represent an increased occurrence of suboptimal steps. In support of our hypothesis, an interaction was observed between step length variations and MCOT (Fig. 2). Similar to previous research relating step variability to metabolic rate (O’Connor et al., 2012), the model from the current study shows that increases in step length variations may be linearly associated with increases in MCOT. For example, when walking at  $1.25 \text{ m s}^{-1}$ , each increase in step length variations of 0.01 (or 1% of the mean step length) will see a corresponding increase in MCOT of 5.9% ( $0.11 \text{ J kg}^{-1} \text{ m}^{-1}$ ). To put this value in context, an increase in speed from  $1.25$  to  $1.35 \text{ m s}^{-1}$  would achieve a similar increase in MCOT ( $0.10 \text{ J kg}^{-1} \text{ m}^{-1}$ ), suggesting that the effect of variations on MCOT may be substantial. Despite this substantial effect of variations, walking speed appeared to be a better predictor of MCOT in the current study. Further research could determine whether the interaction between variations and MCOT is more pronounced in different tasks or populations.

Step length variations and net MCOT were both dependent on walking speed (Fig. 1), which agrees with previous research in metabolic expenditure (Alexander, 2002; Browning and Kram, 2005; Ralston, 1958) and step variability (Jordan et al., 2007). Also, the net MCOT calculated in this study resembled the values observed in previous studies (Bastien et al., 2005; Peterson and Martin, 2010). The range of step length variations observed in this

study was similar to that of previous research (Jordan et al., 2007; Roemmich et al., 2013). However, where previous research has shown a decline in step length coefficient of variation with increasing speed (Jordan et al., 2007), the results of the current study may indicate that step length coefficient of variation reaches a minimum and stabilizes, or begins increasing again with very fast walking speeds.

### No interaction between net MCOT and fluctuations

We also hypothesized that increased step length fluctuations would be associated with decreased MCOT. Although step length fluctuations have been studied previously (Jordan et al., 2007) at a range of walking speeds (75–125% PWS), the current study employed a wider range of walking speeds in order to further test how speed may affect fluctuations. The employed speeds ( $0.75$ – $1.75 \text{ m s}^{-1}$ ) ranged from  $74.5 \pm 20.2$  to  $173.9 \pm 47.3\%$  PWS. Despite this range, a main effect of speed was not observed on step length fluctuations, and no interaction was observed between step length fluctuations and MCOT.

Within the range of speeds tested in the current study, it is likely that MCOT is not affected by step length fluctuations, or *vice versa*. As such, it may be that changes in fluctuations can occur independently of MCOT. Differences in fluctuations have previously been observed between young and older adults (Chien et al., 2015; Hausdorff et al., 1997), as well as between healthy and pathological populations, such as those with Huntington’s or Parkinson’s disease (Hausdorff, 2009; Hausdorff et al., 1997). Similarly, metabolic differences are observed with aging and pathology (Mian et al., 2006; Waters and Mulroy, 1999), indicating that a potential function of fluctuations is to decrease energy expenditure. However, the results of the current study do not support the hypothesis that fluctuations minimize energy expenditure. As such, it may be that the function of fluctuations is to facilitate other gait requirements. For example, fluctuations have been speculated to be reflective of resistance to perturbations, or adaptability (Hausdorff et al., 1995; Stergiou et al., 2006). If this is the case, then it would seem that adaptability in the gait pattern, via fluctuations, may be adjusted without compromising MCOT. Also, these results may indicate that fluctuations, and thereby adaptability, may be robust to changes in energy expenditure. Further experimentation is needed to determine whether different levels of fluctuations relate to adaptability, perhaps via changing terrain surfaces or task demands.

### Interactions with preferred walking speed

It has been shown that the human PWS closely coincides with the minimization of MCOT (Alexander, 2002; Browning and Kram, 2005; Ralston, 1958). The PWS measured in the current study ( $1.05 \pm 0.20 \text{ m s}^{-1}$ ) was slightly lower than other reported treadmill values for healthy young participants; for example,  $1.15 \text{ m s}^{-1}$  (Roerdink et al., 2015) and  $1.18 \text{ m s}^{-1}$  (Dal et al., 2010). The reason for this difference is unclear, although one subject in the current study did have a very low PWS ( $0.58 \text{ m s}^{-1}$ ), which lowered the group average by about  $0.05 \text{ m s}^{-1}$ . The speed at which net MCOT was minimized ( $1.04 \pm 0.05 \text{ m s}^{-1}$ ) was in line with other studies, which found minima of net MCOT at approximately  $1.00 \text{ m s}^{-1}$  (Bastien et al., 2005; Peterson and Martin, 2010). The similarity between the PWS and the predicted minimum of MCOT shows the strong effect of minimizing energy expenditure on preferred movement strategies.

We hypothesized that more persistent fluctuations may facilitate a reduction of MCOT, which led us to hypothesize that the speed at

which fluctuations were maximized would coincide with the PWS. Previous studies have shown interactions between PWS and the statistical persistence of other measures such as stride time and stride length (Chien et al., 2015; Jordan et al., 2007), but it would appear that this may not hold true for step length fluctuations. Although we did not find a significant difference between the speed at which fluctuations were maximized and the PWS, it should be noted that the majority of the subject-specific maxima occurred at either the slowest or fastest speed. As such, it is possible that the lack of a statistical difference is due to the high subject-to-subject variability in the fluctuations maximum (Fig. 3 and Fig. S1), and thus does not provide strong support for an interaction between PWS and fluctuations. This inter-subject variability may be attributable to subject-specific gait strategies across walking speeds. It should also be noted that these results were obtained during treadmill walking at a constant speed and therefore may not directly transfer to overground walking. Future studies may investigate whether different types of people (e.g. young/old, athlete/non-athlete) display consistent trends of fluctuations in different conditions.

Due to the previously shown interaction between step length variations and MCOT (O'Connor et al., 2012), we hypothesized that the minimization of variations would coincide with the PWS. However, the minimization of variations occurred at a higher speed ( $1.56 \pm 0.17 \text{ m s}^{-1}$ ) than the PWS ( $1.05 \pm 0.20 \text{ m s}^{-1}$ ) and was also higher than the speed predicted to minimize net MCOT ( $1.04 \pm 0.05 \text{ m s}^{-1}$ ). Compared to walking speed, variations have a relatively small impact on MCOT (Fig. 2). Therefore, it may be that the ability of variations to reduce MCOT is only employed when MCOT is high (i.e. fast walking speeds) in an attempt to mitigate the metabolic increase due to speed. However, this does not explain the potential increase in step length variations observed in the fastest walking speed (Fig. 1). While the current study is unable to fully explain the potential increase in step length variations at the fastest speed, one might speculate that it is due to the participants approaching the walk-to-run transition speed ( $1.9\text{--}2.1 \text{ m s}^{-1}$ ; Bartlett and Kram, 2008; Malcolm et al., 2009; Prilutsky and Gregor, 2001). It may be that variations increase as the person comes close to transitioning from unstable walking to stable running, as has been seen when a person transitions between stable attractor states (Diedrich and Warren, 1995). However, such an explanation is beyond the scope of this study and requires further experimentation.

Furthermore, because variations were not minimized at the PWS, it may be possible that MCOT could further be reduced at PWS by reducing variations. For example, if the step length coefficient of variation was reduced from its predicted value at PWS ( $\sim 0.026$ ; Fig. 1, dashed line) to the predicted minimum ( $\sim 0.020$ ; Fig. 1), then the model would predict a 3.8% decrease in MCOT. Reducing MCOT has been a common goal of various interventions, and recent advancements have been made using wearable devices. For example, powered ankle exoskeletons have thus far been capable of reducing metabolic expenditure by about 8–24% (Malcolm et al., 2013; Mooney et al., 2014; Zhang et al., 2017), and unpowered devices by up to 7.2% (Collins et al., 2015). The results of the current study indicate that there may be room for metabolic energy reduction without an external device. This reduction may even be possible at the PWS, which is often considered the metabolically optimal speed. Future studies could implement training protocols that reduce variations in order to investigate whether this is a feasible approach to reducing metabolic energy expenditure.

## Conclusion

The current study aimed to characterize the interaction between net MCOT and step-to-step variability while walking at different speeds. The amount of step length variations was linearly associated with MCOT, likely because increases in variations are indicative of a higher occurrence of metabolically suboptimal steps. The step length fluctuations were not predictive of changes in net MCOT. This indicates that the persistence of fluctuations likely does not function to reduce MCOT. Despite its potential to reduce MCOT, step length variations were not minimized at the PWS but were at fast walking speeds, where they may be reduced in order to mitigate the increased MCOT due to increased walking speed.

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## Competing interests

The authors declare no competing or financial interests.

## Author contributions

Conceptualization: C.G.R., V.M., J.M.Y., K.Z.T.; Methodology: C.G.R., V.M., J.M.Y., K.-C.S., K.Z.T.; Software: V.M., K.Z.T.; Validation: V.M., J.M.Y., K.-C.S., K.Z.T.; Formal analysis: C.G.R.; Investigation: C.G.R.; Resources: K.Z.T.; Data curation: C.G.R.; Writing - original draft: C.G.R.; Writing - review & editing: C.G.R., V.M., J.M.Y., K.-C.S., K.Z.T.; Visualization: C.G.R., K.Z.T.; Supervision: V.M., J.M.Y., K.Z.T.; Project administration: K.Z.T.; Funding acquisition: C.G.R.

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## Supplementary information

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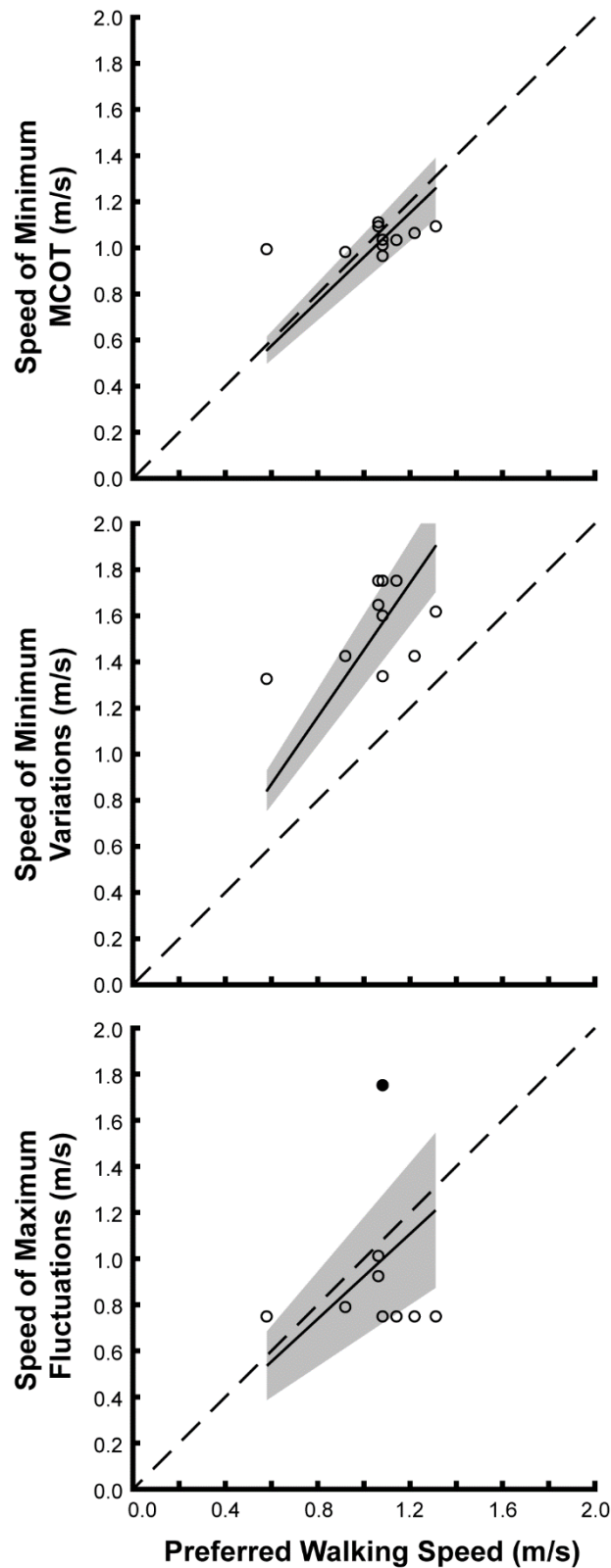
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## Supplementary Information

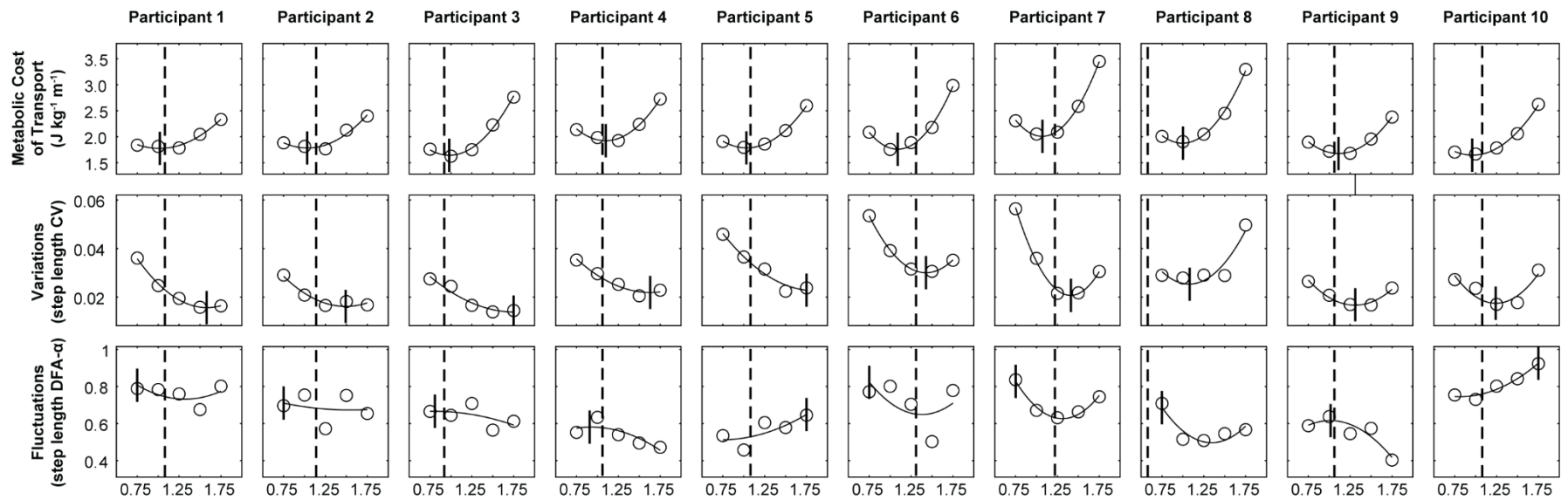


**Figure S1. No-intercept regressions showing the relationships between the preferred walking speed and minimum of net metabolic cost of transport (MCOT) and Variations (step length CV) and the maximum of Fluctuations (DFA- $\alpha$ ).** A slope of 1 (dashed line for reference) would indicate that the variable is perfectly capable of predicting preferred walking speed. The slopes from MCT ( $p = 0.447$ ) and Fluctuations ( $p = 0.564$ ) were not significantly different from one, but the slope from Variations was significantly different than one ( $p < 0.001$ ). Shaded areas indicate 95% confidence intervals around each regression line. Each circle represents a single subject. Filled circle denotes two data points at same position. CV = Coefficient of Variation; DFA = Detrended Fluctuation Analysis



Table S1. **Step parameters for each condition, reported as mean (s.d.).**

Speed (m s <sup>-1</sup> )	0.75	1.00	1.25	1.50	1.75
Step Width (cm)	9.0(3.6)	9.2(3.2)	9.5(3.1)	10.4(2.8)	11.1(3.1)
Step Length (m)	0.50(0.04)	0.59(0.05)	0.67(0.05)	0.74(0.05)	0.80(0.06)
Right only (m)	0.51(0.04)	0.59(0.05)	0.68(0.05)	0.75(0.05)	0.81(0.06)
Left only (m)	0.50(0.03)	0.59(0.05)	0.67(0.05)	0.74(0.05)	0.79(0.05)
Step time (s)	0.68(0.05)	0.60(0.05)	0.55(0.04)	0.51(0.04)	0.47(0.03)
Stance time (s)	0.92(0.07)	0.79(0.07)	0.71(0.06)	0.65(0.05)	0.60(0.04)



**Figure S2. Subject-specific data across speeds.** Row 1: Net metabolic cost of transport; solid vertical line denotes estimated minimum. Row 2: Variations (step length CV). Solid vertical line denotes estimated minimum Row 3: Fluctuations (step length DFA- $\alpha$ ). Solid vertical line denotes estimated maximum. Dashed vertical line denotes preferred walking speed. CV = Coefficient of Variation; DFA = Detrended Fluctuation Analysis