RESEARCH ARTICLE

A simple method reveals minimum time required to quantify steady-rate metabolism and net cost of transport for human walking

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ABSTRACT

The U-shaped net cost of transport (COT) curve of walking has helped scientists understand the biomechanical basis that underlies energy minimization during walking. However, to produce an individual's net COT curve, data must be analyzed during periods of steady-rate metabolism. Traditionally, studies analyze the last few minutes of a 6–10 min trial, assuming that steady-rate metabolism has been achieved. Yet, it is possible that an individual achieves steady rates of metabolism much earlier. However, there is no consensus on how to objectively quantify steady-rate metabolism across a range of walking speeds. Therefore, we developed a simple slope method to determine the minimum time needed for humans to achieve steady rates of metabolism across slow to fast walking speeds. We hypothesized that a shorter time window could be used to produce a net COT curve that is comparable to the net COT curve created using traditional methods. We analyzed metabolic data from 21 subjects who completed several 7 min walking trials ranging from 0.50 to 2.00 m s^{-1}. We partitioned the metabolic data for each trial into moving 1, 2 and 3 min intervals and calculated their slopes. We statistically compared these slope values with values derived from the last 3 min of the 7 min trial, our 'gold' standard comparison. We found that a minimum of 2 min is required to achieve steady-rate metabolism and that data from 2–4 min yields a net COT curve that is not statistically different from the one derived from experimental protocols that are generally accepted in the field.

KEY WORDS: Metabolic power, Metabolic rate, Steady-state metabolism, Energetics

INTRODUCTION

The U-shaped net cost of transport (COT) curve of walking is a highly conserved feature in humans (Ralston, 1958), which has helped scientists understand the mechanical determinants that underlie metabolic energy minimization during walking (Alexander, 1989; Kuo and Donelan, 2010; Ralston, 1958). Understanding the mechanical determinants that allow humans to minimize their net COT during walking can act as a key measure for diagnosing and treating individuals with gait pathologies (Kuo and Donelan, 2010; Ralston, 1958; Schwartz, 2007; Waters and Mulroy, 1999). However, producing a net COT curve is a time-consuming

process that requires each subject to walk across a range of slow to fast walking speeds and reach a steady rate of metabolism at each speed. In past and recent experiments, it has been common for walking trials to last 6–10 min with the average metabolic data of the last 2–3 min of the trial being used for analysis (Arellano et al., 2020; Donelan et al., 2001; Ralston, 1958). This is done under the assumption that a subject has achieved steady-rate metabolism during this period. However, it is possible that steady-rate metabolism is reached earlier than expected and, thus, data collection times could be substantially reduced. Reducing the protocol time for generating a net COT curve can help lessen the overall burden on both experimenter and subject. Yet, there has been no consensus on how to define steady-rate metabolism to date. Therefore, as a field, we lack a simple, objective method for defining steady-rate metabolism.

For human walking, Duffy et al. (1996) stated that steady-rate metabolism usually occurs after 2 min, but it is unclear how they reached this conclusion. Since then, there have been several attempts to develop criteria for defining steady-rate metabolism. For example, Schwartz (2007) used Kendall's tau, a non-parametric rank correlation coefficient, as a statistical means to determine steady rates of oxygen consumption. For reasons unknown, this approach has not been widely adopted in the field of locomotion energetics. In contrast, other scientists have used a slope method, which quantifies the rate of change in oxygen consumption as a function of time. Plasschaert et al. (2009), for instance, used a slope threshold of 0.00025 ml O$_2$ kg^{-1} s^2 to define steady-rate metabolism. Along similar lines, others (Dennis et al., 2006; Kramer et al., 2018) have defined steady-rate metabolism as a time period when a subject exhibits low variability in their oxygen consumption values across time (a change of <10% or ≤2.0 ml O$_2$ kg^{-1} min^{-1}). While these variability criteria appear reasonable, there still lacks a methodological explanation as to why these thresholds were selected. This may be the reason why these approaches have not been widely adopted by others. And lastly, others have defined periods of steady-rate metabolism by visual identification of a plateau in the rate of O$_2$ consumption (e.g. Sims et al., 2018), which fits the classical definition seen in many exercise physiology textbooks (Brooks et al., 2004).

While there is a lack of consensus as to how to objectively identify periods of steady-rate metabolism, there have been significant advancements in decreasing the time required to estimate the metabolic demand of human walking using more advanced computational methods. For instance, Selinger and Donelan (2014) developed a mathematical approach that models the dynamics of breath-by-breath data during walking. In their study, they evoked a rapid change in the demand for metabolic energy under various conditions during treadmill walking. Subjects were tasked with initially walking at a fixed speed with their preferred step frequency

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enforced with a metronome. Then an unexpected change in both treadmill speed (faster or slower) and metronome-enforced step frequency (faster or slower) demanded a spontaneous adjustment in their walking pattern, requiring subjects to reach a new steady-rate of metabolic energy consumption. The change in metabolic energy consumption, which was captured from the initial to final steady state, followed an exponential rise or decay that approached the final value. They discovered that the underlying dynamics of the breath-by-breath data could be accurately modeled by a first-order linear differential equation. Based on the model's time constant, they found that human subjects reached 95% of their steady-state metabolic energy consumption by the 2 min mark. This methodological approach has re-shaped experimental designs and proven extremely useful in studies of 'human-in-the-loop' optimization of walking, allowing engineers and scientists to optimize the use of powered prostheses in real time (Zhang et al., 2017). While Selinger and Donelan (2014) used model fits to breath-by-breath metabolic data, other approaches have used different types of movement data (e.g. ground reaction forces, electromyography and body-worn sensors; see Slade et al., 2019, for a concise review) to predict the metabolic cost of human walking. A noteworthy example has been laid out by Slade et al. (2019), who propose a data-driven modeling approach that uses linear regression and neural network models to predict the metabolic cost of walking. They used left and right vertical ground reaction forces and lower limb electromyographic data to estimate metabolic energy from previous experiments studying conditions of walking with a powered ankle exoskeleton (Jackson and Collins, 2015) and conditions of walking with different loads and inclines (Silder et al., 2012). While it was noted that this approach lacks the accuracy of indirect calorimetry, an estimate of metabolic energy consumption could be achieved from an individual gait cycle or from a 4 s interval of data.

Although these approaches provide significant advancements in estimating metabolic cost during walking with the minimum amount of time possible, some of their drawbacks may limit their generalizability. In particular, the method of Slade et al. (2019) can estimate metabolic cost with average errors in the range 4.4−11.7%. The relatively larger errors in predicting metabolic energy cost may pose problems in settings where: (i) accuracy is critical for understanding how metabolic cost changes across speed (Thomas et al., 2021); (ii) various populations walk under different biomechanical constraints (Antonellis et al., 2022); and (iii) time-dependent adaptation processes are involved (Finley et al., 2013; Huang et al., 2012; Selinger and Donelan, 2014). For example, it has been shown that by making small adjustments to their walking step frequency, humans can converge to a new metabolic optimum when driven by a cost saving that ranges between ∼4% and 8% (Selinger et al., 2015). While predicting metabolic cost using some form of gait information may be helpful in cases where metabolic measurements cannot be acquired using standard equipment, we favor an approach like that of Selinger and Donelan (2014), where estimates of metabolic power are based on the captured rates of oxygen consumption and carbon dioxide production ($\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$, respectively). Capturing $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ not only informs an experimenter of the demand for metabolic power but also provides critical information as to whether this demand is met within the expected physiological range, where respiratory exchange ratios (RER) should reside at values less than 1.0, indicating that energy is predominantly derived by aerobic pathways. Given the complexity and limited generalizability of the current approaches, we set out to understand whether a simpler approach, one that would not require some form of advanced computational modeling, could offer a means to identify steady rates of metabolism based on the data,

without the need to make any assumptions about the underlying dynamics that govern the demand for metabolic energy.

In the spirit of simplicity and generalizability, we developed a systematic approach that would allow us to both define steady-rate metabolism and identify the minimum time required to generate the net COT curve for human walking, which may provide evidence in support of decreasing protocol times. We explored the slope method carried out by Plasschaert et al. (2009) because it is relatively simple, and could be easily understood and applied by novice and experienced scientists undertaking measurements of metabolic energy consumption during walking. As our approach relies on a simple slope method, it avoids the need for advanced, complex computational modeling of first-order dynamical processes (Selinger and Donelan, 2014) or neural networks (Slade et al., 2019). Based on the prediction of Duffy et al. (1996), we hypothesized that our slope method would identify an earlier time interval that would yield a net COT curve that is not statistically different from the net COT curve produced by the last 3 min of a 7 min walking trial. For practical purposes, we considered a net COT curve produced by the last 3 min of a 7 min walking trial as our 'gold' standard, as this reflects a generally accepted methodology in the field of locomotion biomechanics and energetics.

MATERIALS AND METHODS
Participants and experimental protocol
Twenty-one young, healthy adults (13 females, 8 males) participated in this study (mean±s.d. age 25.38±2.92 years, mass 68.37±12.41 kg, height 1.70±0.09 m) (Thomas et al., 2021). They were non-smokers and were physically active according to ACSM guidelines (American College of Sports Medicine Guidelines, 2018), with a body mass index <30.0. All participants gave written informed consent as per the University of Houston Institutional Review Board rules.

The experiment began with measurement of the subjects' standing metabolic energy consumption for 7 min using a metabolic cart (Parvo Medics TrueMax2400, Salt Lake City, UT, USA), with a setting that reported average $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ approximately every 15 s. In brief, average data were calculated from the accumulated V_{O_2}, starting with the beginning of the first detectable breath and ending with a complete breath detected close to or after time exceeds 15 s. Therefore, the average data were quantified by dividing the accumulated V_{O_2} by the accumulated time window of roughly 15 s (personal communication, Pat Yeh, Parvo Medics). The same procedure was followed for average $\dot{V}_{CO_2}$. We followed the guidelines for metabolic testing described by the Parvo Medics manual, starting with a 3 l syringe flowmeter calibration procedure. The flowmeter calibration comprised defining room temperature, barometric pressure and relative humidity. Then, the 3 l syringe was used to achieve slow to fast peak stroke rates, in the range 50–100, 100–199, 200–299, 300–399 and 400–499 l min^{-1}. We continued the flowmeter calibration until reaching a ±0.5% difference between the average volume measured during the calibration and the known volume of the 3 l syringe. This was usually achieved within two attempts. After successful flowmeter calibration, gas analyzers were calibrated with a gas tank with known percentages of CO_2 and O_2. Again, this process was continued until the gas concentration readings from the metabolic cart matched the known gas tank concentrations. This was also typically achieved within two attempts.

The subjects walked on a dual-belt treadmill (Bertec Corp., Columbus, OH, USA), completing the 7 min trials in a randomized order for all seven speeds, which ranged from 0.50 to 2.00 m s^{-1}
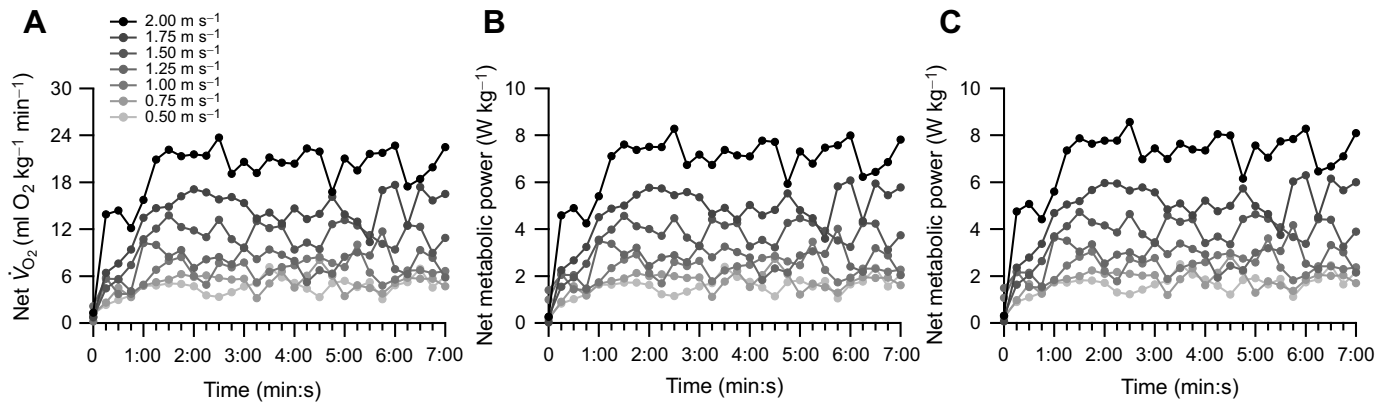
Fig. 1. Calculation of net $\dot{V}_{O_2}$ and net metabolic power. Representative time-series data from a single subject illustrating the demand for net oxygen consumption (A) and net metabolic power, according to the equations of Brockway (1987) (B) and Péronnet and Massicotte (1991) (C) while walking across slow to fast speeds. The first data point at zero marks the value for the 15 s before the start of the 7 min trial. Note the sharp rise in the demand for metabolic energy when the subject started to walk at moderate and fast speeds ($\geq$1.25 m s^{-1}). Even though the energy demand increased steadily, the subject reached a steady rate of metabolism at 2 min.

in 0.25 m s^{-1} intervals (Fig. 1). To minimize the effects of fatigue, participants rested for 5 min between the trials. The RER was monitored for each subject to ensure values remained <1.00, indicating that metabolic energy was provided primarily by aerobic pathways. If any subject was unable to maintain a walking gait at a given speed, or produced a RER above 1.00, the trial was stopped and excluded from data analysis. We excluded 2 subjects at 1.75 m s^{-1} (because their RER was >1.0) and 9 subjects at 2.00 m s^{-1} (7 could not maintain walking gait and 2 had a RER >1.0). Additionally, we excluded 1 subject at 1.00 m s^{-1} who did not reach a steady rate of metabolism during the trial.

Data analysis

We used the average $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ values to calculate metabolic power for each standing and walking trial using the Brockway equation (Brockway, 1987) and the Péronnet and Massicotte equation (Péronnet and Massicotte, 1991; Kipp et al., 2018). For each speed, we calculated net metabolic power by subtracting each participant's standing metabolic power from their gross metabolic power values in each walking trial (Fig. 1). To mirror typical data collection methods, we divided the 7 min trials into overlapping 3, 2 and 1 min intervals. For the 3 min analyses, for example, the first window started at 15 s and ended at 180 s. Note that the 15 s mark represents the average metabolic data sampled between the start of the walking trial and the subsequent 15 s. For simplicity, we refer to the first window as 0–3:00 min:s. Then, this window moved to the next time point, starting at 30 s and ending at 195 s. The 30 s mark represents the average metabolic data sampled between the end of the first 15 s of the trial and the following 15 s. We refer to this second window as 0:15–3:15 min:s. This process continued until reaching the end of the entire 7 min time-series. This analysis was replicated for the 2 min and 1 min intervals. This resulted in 17 intervals lasting 3 min each, 21 intervals lasting 2 min each, and 25 intervals lasting 1 min each. For each subject and speed, we used the time-series intervals to quantify the magnitude of the slope (ml O$_2$ kg^{-1} min^{-1} s^{-1} or W kg^{-1} s^{-1}) using linear regression and refer to this variable as the slope time window (slope$_{TW}$) throughout the rest of the text. By quantifying the slope$_{TW}$ along each 7 min trial, we expected its magnitude to reach a value of ~0, reflecting a flat line and, thus, a period of time when subjects have reached steady-rate metabolism. For subsequent analyses, we also quantified the net cost of transport

by dividing net $\dot{V}_{O_2}$ (ml O$_2$ kg^{-1} min^{-1}×1 min/60 s) by walking speed (m s^{-1}) and net metabolic power (W kg^{-1}) by walking speed (m s^{-1}). We used MATLAB (Mathworks, Inc., Natick, MA, USA) to perform all computational calculations and descriptive analyses.

Statistical analysis

For each 1, 2 and 3 min interval, we aggregated the individual data points at each speed for all subjects and then calculated the regression line that estimated the change in slope$_{TW}$ as a function of speed (i.e. 0.5 m s^{-1} to 2.0 m s^{-1}; see Fig. 2). For instance, a regression line was derived for each 1 min interval, starting from 0–1:00 min:s, then from 0:15 to 1:15 min:s, then from 0:30 to 1:30 min:s and so on, until reaching the final 6:00–7:00 min:s. The same approach was carried out for the 2 min and 3 min intervals. We then used the Dunnett method of multiple comparison (MC) to determine whether each regression line was statistically different from the regression line that was based on the last 3 min of the 7 min trial, predesignated here as the control. The criteria for significance were based on a directional one-tailed test, an alpha value equal to 0.05, k number of comparisons, and v degrees of freedom based on the residual sum of squares. To calculate the critical t-value for the Dunnett multiple comparison method, we used the 'nCDunnett' package provided in R software, which yielded the following results: t_{1min}=2.721 (k=25, v=266), t_{2min}=2.674 (k=21, v=266) and t_{3min}=2.598 (k=16, v=266).

Based on the outcomes of the Dunnett method, we moved forward with a non-linear regression analysis using R software to compare the net rate of oxygen consumption, net metabolic power and net COT curves derived from a 2–4 min and 2–5 min window with that derived from the last 4–7 min window (see Discussion for details). We then followed up with pre-planned comparisons between the average net COT values at each level of speed using paired t-tests with α=0.05 (SPSS Inc., Chicago, IL, USA). Note that for each level of speed, where values were compared using a paired t-test, sample sizes were always equal. If the data did not meet the assumptions of normality, we used the non-parametric Wilcoxon signed-rank test. For clarity, the mean and s.d. for net COT at each speed and time window are provided in Tables 1–3 while the regression equations signifying the slope, intercept, adjusted coefficient of determination (r^2_{adj}), along with the F and P values are provided in Table 4.
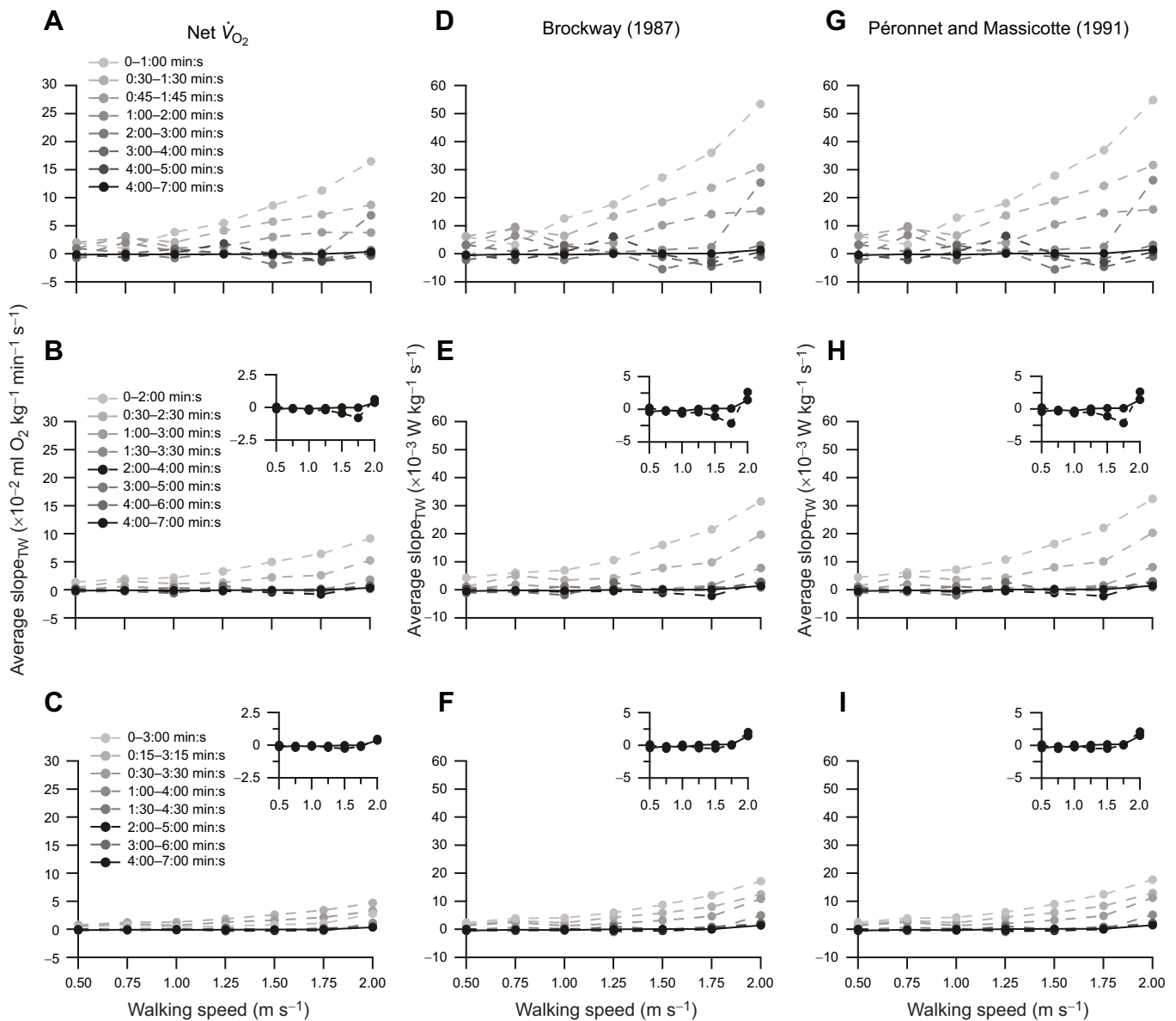
Fig. 2. Change in slope time window (slope$_{TW}$) as a function of speed. The trends illustrate the change in the average slope$_{TW}$ quantified for a 1 min (A,D,G), 2 min (B,E,H) and 3 min (C,F,I) time window. The slope$_{TW}$ at each walking speed represents the change in net $\dot{V}_{O_2}$ (left) or net metabolic power according to the equations of Brockway (1987) (middle) and Péronnet and Massicotte (1991) (right) over the specific time window across the time-series data. By plotting slope$_{TW}$ as a function of speed (using the individual data points), a regression line was fitted across all the 1 min intervals, starting from 0–1:00 min:s, then 0:15–1:15 min:s, then 0:30–1:30 min:s and so on, until reaching the final 6:00–7:00 min:s. The same procedure was followed for the 2 min and 3 min intervals. The trends show that as the time window moved along, toward the end of the original metabolic time-series data, the unit increase in slope$_{TW}$ decreased, eventually reaching similar values to those observed from the 4–7 min time window, our 'gold' standard (solid black line). To allow for closer inspection, the insets for the 2 and 3 min graphs highlight the earliest time window that is not statistically different from the gold standard. No inset is included for the 1 min intervals as they were not considered for curve comparisons. For clarity, several time intervals were omitted; however, those omitted exhibited the same downward trend.

RESULTS
Influence of time window across speed
The regression lines based on the initial time window of 1, 2 and 3 min intervals revealed that the magnitude of slope$_{TW}$ significantly increased as a function of speed (Fig. 2). For example, the slope$_{TW}$ derived for a 1 min interval was closer to zero for the slowest speed (0.5 m s^{-1}) and increased to its highest value at 2.0 m s^{-1}. As the time window moved along, toward the end of the metabolic time-series data, the unit increase in the magnitude of slope$_{TW}$ decreased and, therefore, the linear relationship between slope$_{TW}$ and speed became less steep. In general, the linear relationship between the magnitude

of slope$_{TW}$ and speed for the last 2 min and 1 min time windows overlapped closely with the last 3 min time window, i.e. the control.

In the sections that follow, we summarize the results of our linear regression analyses, which quantified the change in slope$_{TW}$ as a function of speed. The linear regression analyses are reflected in general form as $Y=B_{YX}X+B_o$, after the notation adopted by Cohen et al. (2002). In this case, Y is the estimate of slope$_{TW}$, X is speed, B_o is the Y intercept and B_{YX} is the regression coefficient for estimating slope$_{TW}$ from speed and represents the rate of change in slope$_{TW}$ per unit change in speed. For ease of interpretation, the regression lines are only reported for

Table 1. Net cost of transport (COT) derived from net $\dot{V}_{O_2}$ data extracted from the selected time windows at each speed

Walking speed (m s⁻¹)	Net COT (ml O₂ kg⁻¹ m⁻¹)		
	2 min interval (2.00–4.00 min)	3 min interval (2.00–5.00 min)	'Gold' standard (4.00–7.00 min)
0.50	0.132±0.026 (n=21) d=0.306 P=0.322 (WS)	0.132±0.027 (n=21) d=0.479 P=0.040	0.129±0.026 (n=21)
0.75	0.113±0.022 (n=21) d=0.406 P=0.078	0.112±0.022 (n=21) d=0.414 P=0.050 (WS)	0.109±0.020 (n=21)
1.00	0.103±0.017 (n=20) d=0.108 P=0.634	0.104±0.016 (n=20) d=0.274 P=0.236	0.102±0.016 (n=20)
1.25	0.105±0.017 (n=21) d=0.327 P=0.150	0.104±0.016 (n=21) d=0.259 P=0.250	0.103±0.014 (n=21)
1.50	0.115±0.016 (n=21) d=0.423 P=0.062 (WS)	0.114±0.016 (n=21) d=0.353 P=0.085 (WS)	0.113±0.015 (n=21)
1.75	0.131±0.020 (n=19) d=−0.204 P=0.385	0.132±0.195 (n=19) d=−0.201 P=0.494 (WS)	0.132±0.020 (n=19)
2.00	0.155±0.018 (n=12) d=−0.942 **P=0.008**	0.156±0.018 (n=12) d=−1.242 **P=0.001**	0.161±0.017 (n=12)

Values are expressed as means±s.d. Effect size was calculated based on Cohen's $d=t/\sqrt{n}$. All comparisons at each speed were made between an earlier interval and the 'gold' standard interval with Bonferroni-adjusted significance set at $P<0.025$. WS indicates P-values from Wilcoxon signed rank test and bold denotes significant differences when compared with the gold standard.

Table 2. Net COT values derived from net metabolic power data extracted from the selected time windows at each speed using the Brockway equation

Walking speed (m s⁻¹)	Net COT (J kg⁻¹ m⁻¹)		
	2 min interval (2.00–4.00 min)	3 min interval (2.00–5.00 min)	'Gold' standard (4.00–7.00 min)
0.50	2.663±0.531 (n=21) d=0.233 P=0.298	2.666±0.543 (n=21) d=0.395 P=0.085	2.617±0.537 (n=21)
0.75	2.271±0.437 (n=21) d=0.359 P=0.115	2.251±0.429 (n=21) d=0.380 P=0.097	2.210±0.393 (n=21)
1.00	2.087±0.338 (n=20) d=0.038 P=0.865	2.102±0.318 (n=20) d=0.190 P=0.406	2.081±0.317 (n=20)
1.25	2.125±0.327 (n=21) d=0.149 P=0.502	2.106±0.307 (n=21) d=−0.026 P=0.906	2.108±0.290 (n=21)
1.50	2.325±0.328 (n=21) d=0.069 P=0.614 (WS)	2.322±0.332 (n=21) d=0.028 P=0.821 (WS)	2.320±0.30 (n=21)
1.75	2.692±0.417 (n=19) d=−0.342 P=0.153	2.711±0.406 (n=19) d=−0.380 P=0.115	2.743±0.418 (n=19)
2.00	3.226±0.397 (n=12) d=−1.116 **P=0.003**	3.253±0.395 (n=12) d=−1.388 **P=0.001**	3.359±0.372 (n=12)

Values are expressed as means±s.d. Effect size was calculated based on Cohen's $d=t/\sqrt{n}$. Net metabolic power was estimated from the equation published by Brockway (1987). All comparisons at each speed were made between an earlier interval and the gold standard interval with Bonferroni-adjusted significance set at $P<0.025$. WS indicates P-values from Wilcoxon signed rank test and bold denotes significant differences when compared with the gold standard.

Table 3. Net COT values derived from net metabolic power data extracted from the selected time windows at each speed using the Péronnet and Massicotte equation

Walking speed (m s⁻¹)	Net COT (J kg⁻¹ m⁻¹)		
	2 min interval (2.00–4.00 min)	3 min interval (2.00–5.00 min)	'Gold' standard (4.00–7.00 min)
0.50	2.809±0.546 (n=21) d=0.229 P=0.306	2.81±0.56 (n=21) d=0.389 P=0.090	2.763±0.553 (n=21)
0.75	2.382±0.451 (n=21) d=0.357 P=0.117	2.362±0.442 (n=21) d=0.377 P=0.099	2.320±0.405 (n=21)
1.00	2.182±0.348 (n=20) d=0.035 P=0.877	2.197±0.328 (n=20) d=0.186 P=0.416	2.176±0.327 (n=20)
1.25	2.213±0.336 (n=21) d=0.141 P=0.524	2.194±0.315 (n=21) d=−0.038 P=0.862	2.197±0.298 (n=21)
1.50	2.415±0.338 (n=21) d=0.053 P=0.689 (WS)	2.412±0.342 (n=21) d=0.013 P=0.876 (WS)	2.411±0.311 (n=21)
1.75	2.791±0.430 (n=19) d=−0.348 P=0.147	2.812±0.418 (n=19) d=−0.388 P=0.108	2.844±0.431 (n=19)
2.00	3.339±0.410 (n=12) d=−1.123 **P=0.003**	3.367±0.409 (n=12) d=−1.392 **P=0.001**	3.477±0.385 (n=12)

Values are expressed as means±s.d. Effect size was calculated based on Cohen's $d=t/\sqrt{n}$. Net metabolic power was estimated from the equation published by Péronnet and Massicotte (1991). All comparisons at each speed were made between an earlier interval and the gold standard interval with Bonferroni-adjusted significance set at $P<0.025$. WS indicates P-values from Wilcoxon signed rank test and bold denotes significant differences when compared with the gold standard.

the initial and last time window for the 1, 2 and 3 min intervals. As we will see, the regression coefficient (B_{YX}) was high when the best fit lines were based on using initial time windows and lowest (approximating a value of zero) when the best fit lines were based on using the last time windows of the metabolic time-series data.

Change in slope_{TW} based on net $\dot{V}_{O_2}$

The regression lines for the initial and last time window for the 1 min intervals were slope_{TW}=0.094(speed)−0.049, r^2_{adj}=0.413 and slope_{TW}=0.010(speed)−0.010, r^2_{adj}=0.012, respectively. The regression lines for the initial and last time window for the 2 min intervals were slope_{TW}=0.047(speed)−0.018, r^2_{adj}=0.412 and slope_{TW}=0.002(speed)−0.003, r^2_{adj}=−0.002, respectively. And finally, the regression lines for the initial and last time window for the 3 min intervals were slope_{TW}=0.024(speed)−0.007, r^2_{adj}=0.369 and slope_{TW}=0.002(speed)−0.003, r^2_{adj}=0.017, respectively.

Change in slope_{TW} based on net metabolic power via the Brockway equation

The regression lines for the initial and last time window for the 1 min intervals were slope_{TW}=0.030(speed)− 0.016, r^2_{adj}=0.416 and slope_{TW}=0.003(speed)−0.003, r^2_{adj}=0.013, respectively. The regression lines for the initial and last time window for the 2 min intervals were slope_{TW}=0.016(speed)−0.007, r^2_{adj}=0.442 and slope_{TW}=0.001(speed)−0.001, r^2_{adj}=0.001, respectively. And finally, the regression lines for the initial and last time window for the 3 min intervals were slope_{TW}=0.009(speed)−0.003, r^2_{adj}=0.429 and slope_{TW}=0.001(speed)−0.001, r^2_{adj}=0.027, respectively.

Table 4. Best fit curve equations and mean square error of the regression fits for the time windows displayed in Figs 4–6

	Net $\dot{V}_{O_2}$	Brockway (1987)	Péronnet and Massicotte (1991)
Equations reflect per unit time			
2–4 min	Net $\dot{V}_{O_2}$ (ml O_2 kg^{-1} min^{-1})= $(5.879\pm0.597)speed^2 - (5.426\pm1.470)speed +(5.507\pm0.813)$, $R^2_{adj}=0.904$, $F_{2132}=632.46$, $P<0.001$ $MSE_{2-4min}=2.050$	Net metabolic power (W kg^{-1})= $(2.137\pm0.205)speed^2 - (2.117\pm0.505)speed +(1.967\pm0.279)$, $R^2_{adj}=0.906$, $F_{2132}=650.20$, $P<0.001$ $MSE_{2-4min}=0.242$	Net metabolic power (W kg^{-1})= $(2.205\pm0.212)speed^2 - (2.190\pm0.521)speed +(2.063\pm0.288)$, $R^2_{adj}=0.906$, $F_{2132}=649.07$, $P<0.001$ $MSE_{2-4min}=0.257$
2–5 min	Net $\dot{V}_{O_2}$ (ml O_2 kg^{-1} min^{-1})= $(6.058\pm0.586)speed^2 - (5.798\pm1.442)speed +(5.653\pm0.797)$, $R^2_{adj}=0.909$, $F_{2132}=669.067$, $P<0.001$ $MSE_{2-5min}=1.973$	Net metabolic power (W kg^{-1})= $(2.203\pm0.202)speed^2 - (2.249\pm0.498)speed +(2.020\pm0.275)$, $R^2_{adj}=0.911$, $F_{2132}=685.33$, $P<0.001$ $MSE_{2-5min}=0.235$	Net metabolic power (W kg^{-1})= $(2.273\pm0.208)speed^2 - (2.324\pm0.513)speed+(2.117\pm0.284)$, $R^2_{adj}=0.911$, $F_{2132}=683.91$, $P<0.001$ $MSE_{2-5min}=0.250$
4–7 min	Net $\dot{V}_{O_2}$ (ml O_2 kg^{-1} min^{-1})= $(6.584\pm0.571)speed^2 - (6.812\pm1.408)speed +(5.983\pm0.778)$, $R^2_{adj}=0.918$, $F_{2132}=751.320$, $P<0.001$ $MSE_{4-7min}=1.879$	Net metabolic power (W kg^{-1})= $(2.378\pm0.198)speed^2 - (2.563\pm0.488)speed +(2.121\pm0.270)$, $R^2_{adj}=0.920$, $F_{2132}=768.82$, $P<0.001$ $MSE_{4-7min}=0.226$	Net metabolic power (W kg^{-1})= $(2.452\pm0.204)speed^2 - (2.646\pm0.504)speed+(2.221\pm0.278)$, $R^2_{adj}=0.920$, $F_{2132}=767.35$, $P<0.001$ $MSE_{4-7min}=0.240$
Equations reflect per unit distance			
2–4 min	Net COT (ml O_2 kg^{-1} m^{-1})= $(0.069\pm0.008)speed^2 - (0.157\pm0.020)speed +(0.192\pm0.011)$, $R^2_{adj}=0.374$, $F_{2132}=41.007$, $P<0.001$ $MSE_{2-4min}=0.000388$	Net COT (J kg^{-1} m^{-1})= $(1.477\pm0.166)speed^2 - (3.292\pm0.408)speed +(3.925\pm0.225)$, $R^2_{adj}=0.412$, $F_{2132}=47.88$, $P<0.001$ $MSE_{2-4min}=0.158$	Net COT (J kg^{-1} m^{-1})= $(1.551\pm0.171)speed^2 - (3.496\pm0.420)speed+ (4.151\pm0.232)$, $R^2_{adj}=0.411$, $F_{2132}=47.81$, $P<0.001$ $MSE_{2-4min}=0.167$
2–5 min	Net COT (ml O_2 kg^{-1} m^{-1})= $(0.071\pm0.008)speed^2 - (0.160\pm0.020)speed +(0.193\pm0.011)$, $R^2_{adj}=0.396$, $F_{2132}=44.935$, $P<0.001$ $MSE_{2-5min}=0.000376$	Net COT (J kg^{-1} m^{-1})= $(1.514\pm0.164)speed^2 - (3.366\pm0.403)speed +(3.952\pm0.223)$, $R^2_{adj}=0.433$, $F_{2132}=52.12$, $P<0.001$ $MSE_{2-5min}=0.154$	Net COT (J kg^{-1} m^{-1})= $(1.591\pm0.169)speed^2 - (3.574\pm0.415)speed+ (4.180\pm0.229)$, $R^2_{adj}=0.432$, $F_{2132}=52.06$, $P<0.001$ $MSE_{2-5min}=0.163$
4–7 min	Net COT (ml O_2 kg^{-1} m^{-1})= $(0.074\pm0.008)speed^2 - (0.164\pm0.019)speed +(0.192\pm0.011)$, $R^2_{adj}=0.459$, $F_{2132}=57.905$, $P<0.001$ $MSE_{4-7min}=0.000342$	Net COT (J kg^{-1} m^{-1})= $(1.573\pm0.158)speed^2 - (3.427\pm0.389)speed +(3.924\pm0.215)$, $R^2_{adj}=0.491$, $F_{2132}=65.57$, $P<0.001$ $MSE_{4-7min}=0.144$	Net COT (J kg^{-1} m^{-1})= $(1.650\pm0.163)speed^2 - (3.635\pm0.401)speed +(4.151\pm0.222)$, $R^2_{adj}=0.488$, $F_{2132}=64.93$, $P<0.001$ $MSE_{4-7min}=0.153$

Change in slope$_{TW}$ based on net metabolic power via the Péronnet and Massicotte equation

The regression lines for the initial and last time window for the 1 min intervals were slope$_{TW}$=0.031(speed)−0.016, r^2_{adj}=0.416 and slope$_{TW}$= 0.004(speed)−0.003, r^2_{adj}=0.013, respectively. The regression lines for the initial and last time window for the 2 min intervals were slope$_{TW}$=0.017(speed)−0.007, r^2_{adj}=0.444 and slope$_{TW}$= 0.001(speed)−0.001, r^2_{adj}=0.001, respectively. And finally, the regression lines for the initial and last time window for the 3 min intervals were slope$_{TW}$=0.009(speed)−0.003, r^2_{adj}=0.431 and slope$_{TW}$=0.001(speed)−0.001, r^2_{adj}=0.028, respectively.

Dunnett's MC method to determine earliest time window

As shown in Fig. 3, the comparisons between the regression lines revealed that for 1 min window comparisons, the t-statistic fell below the critical t-value at an interval between 1:15 and 2:15 min:s; however, when contrasted with the 2 min and 3 min windows, the t-statistic exhibited greater fluctuations for the subsequent comparisons against the control. For the 2 min window, comparisons of regression lines revealed that a window between 1:30 and 3:30 min:s was not statistically different from the last 3 min window. And finally, for the 3 min window, comparison of regression lines revelated that a window between 2:00 and 5:00 min was not significantly different from the last 3 min window. Given these observations, we chose to compare the 3 min window defined from 2:00 to 5:00 min against the control. And to keep consistent with our 3 min window comparison, we also chose to compare the 2 min window defined from 2:00 to 4:00 min against the control. Although the t-statistic fell below the Dunnett's critical t-value for the 1 min window, we avoided any regression fit comparisons using this window because of higher fluctuations in the pattern.

Net $\dot{V}_{O_2}$, net metabolic power and net COT curves

The non-linear regression line comparisons characterizing net $\dot{V}_{O_2}$ as a function of speed did not significantly differ between the last 3 min interval and the 3 min interval between 2:00 and 5:00 min or between the last 3 min interval and the 2 min interval between 2:00 and 4:00 min (Fig. 4; P-values provided in the caption). In addition, the non-linear regression line comparisons characterizing the net COT as a function of speed did not significantly differ between the last 3 min interval and the 3 min interval between 2:00 and 5:00 min or between the last 3 min interval and the 2 min interval between 2:00 and 4:00 min. The same results were observed when carrying out the non-linear comparisons for net metabolic power and net COT, regardless of whether these data were
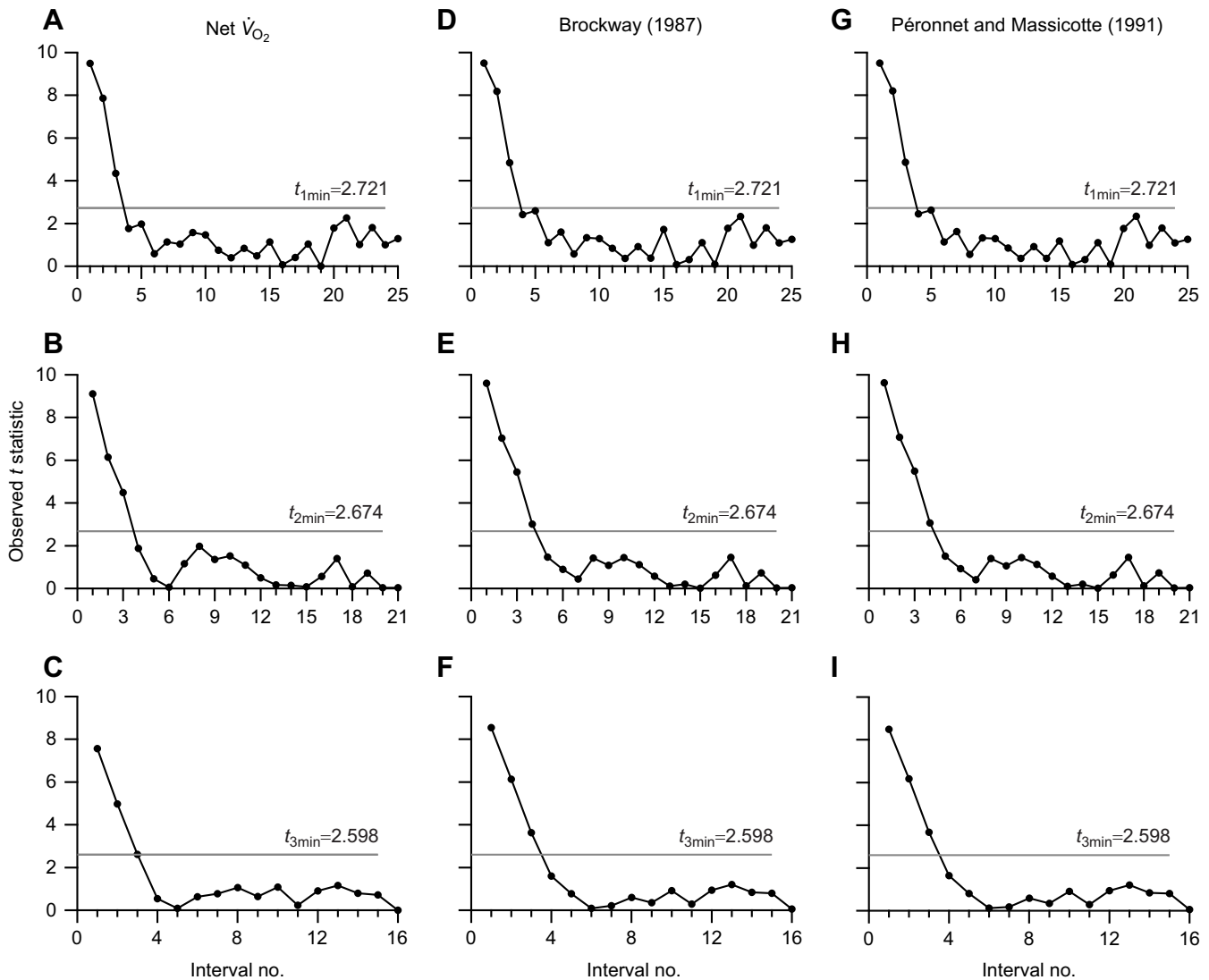
Fig. 3. Determining the earliest time window for steady-rate metabolism. The trends illustrate changes in the observed Dunnett's t-statistic derived from systematic comparisons between regression lines based on net $\dot{V}_{O_2}$ (left) or net metabolic power according to the equations of Brockway (1987) (middle) and Péronnet and Massicotte (1991) (right). Each interval, from start to end, was compared against the control, defined as the last 3 min of a 7 min walking trial. In general, the observed t-statistic was highest for the first interval, then showed a downward trend until reaching a value below Dunnett's critical t-statistic (t_{1min}, t_{2min}, t_{3min}), indicating that subjects had achieved a steady rate of metabolism that was not statistically different from that of the last 3 min of the 7 min trial. Interval number 1 represents the time window 0:00–1:00 min:s and interval number 2 represents the 0:15–1:15 min:s, and so on. Regression line comparisons revealed that the 1 min window between 0:45 and 1:45 min:s (interval number 4) reached a value below threshold (A,D,G), but the observed t-statistic had higher fluctuations in the pattern. In contrast, regression line comparisons revealed that a 2 min window between 1:00 and 3:00 min:s (interval number 5) (B,E,H) and a 3 min window between 0:45 and 3:45 min:s (interval number 4) (C,F,I) reached a value below Dunnett's threshold, and remained consistent for subsequent comparisons. Overall, the observed t-statistic trends for 2 min and 3 min windows demonstrate that subjects reached a steady rate of metabolism much earlier that the last 3 min of a 7 min walking trial.

derived using the Brockway (1987) equation or the Péronnet and Massicotte (1991) equation (Figs 5 and 6; P-values provided in the captions). Follow-up pairwise comparisons between the last 3 min interval and the earlier time intervals showed that the net COT values were not statistically different at speeds of 0.5–1.75 m s^{-1} (all $P>0.05$). However, when compared with the last 3 min interval, the net COT values for the fastest speed of 2.00 m s^{-1} were consistently lower for the earlier time intervals (Tables 1–3).

DISCUSSION

In this paper, we used a slope method as a simple approach to identify when humans achieve steady rates of metabolism while

walking across a range of slow to fast speeds (0.5–2.0 m s^{-1}). We applied this method using a window that quantified the slopes across a 1, 2 and 3 min time period that moved along the time series from beginning to end. In support of our hypothesis, we discovered that at minimum, 4 min of walking at each speed is needed to produce a net COT curve that is not statistically different from a net COT curve obtained from traditional, longer data collection times.

When do subjects reach a steady rate of metabolism?

Our slope method revealed that across the walking speeds tested here, subjects reached a steady rate of metabolism by 2 min, confirming the prediction of Duffy et al. (1996). We came to this conclusion based on our systematic analysis, which provided a clear
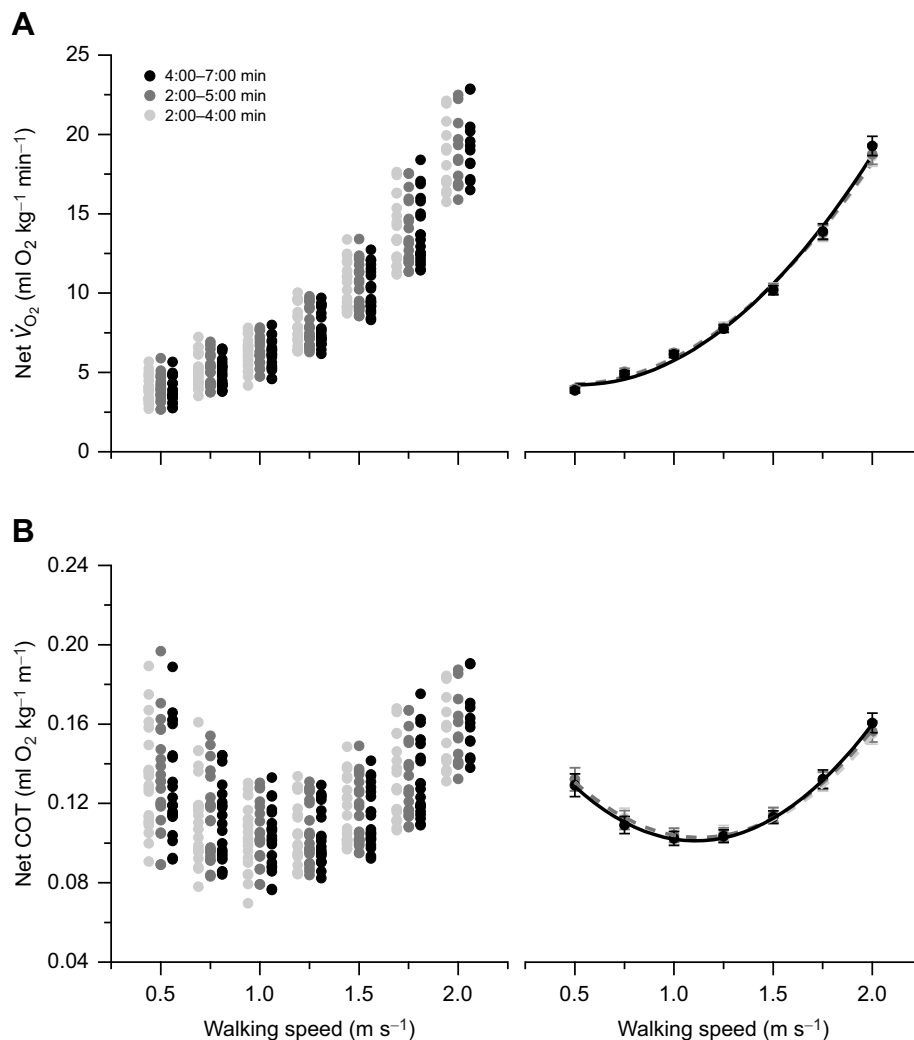
Fig. 4. Net $\dot{V}_{O_2}$ and cost of transport as a function of speed. (A) Net $\dot{V}_{O_2}$ and (B) net cost of transport (COT) changed non-linearly with walking speed, with estimates based on the average $\dot{V}_{O_2}$ measured (see Materials and Methods). The left column represents the individual data points and the right column represents the regression curve fits to these individual data points. For ease of comparing the regression curve fits, the data in the right column represent the mean±s.e.m. and are superimposed to illustrate the high degree of similarity. As expected, net $\dot{V}_{O_2}$ increased curvilinearly with speed. In contrast, net COT exhibited a U-shaped curve, highlighting the observation that walking at a speed between 1 and 1.3 m s^{-1} minimizes the net COT, i.e. the net oxygen cost required to move 1 kg of body mass 1 m. For ease of visual inspection, a small offset was applied to the original data points along the abscissa in the left column. Statistical comparisons made from the regression equations characterizing the non-linear relationship of net $\dot{V}_{O_2}$ versus speed and net COT versus speed were not statistically different for the time windows taken from 2–4 min, 2–5 min and 4–7 min (net oxygen consumption comparisons: 2–4 min versus 4–7 min P=0.741 and 2–5 min versus 4–7 min P=0.796; net COT comparisons: 2–4 min versus 4–7 min P=0.974 and 2–5 min versus 4–7 min P=0.972). Note that the regression curves for the 2–4 min window overlap with those for the 2–5 min window; therefore, they are indistinguishable from one another. The equations characterizing the polynomial non-linear regression fits in the right column are listed in Table 4.

picture as to how the slope of the net $\dot{V}_{O_2}$ versus time and the slope of net metabolic power versus time changed as each 1, 2 or 3 min window moved along the time-series data. From Dunnett's multiple comparison method, one can see that for the 2 and 3 min intervals (Fig. 3), the *t*-statistic values were highest for the first interval, then decreased steadily until reaching the first minimum value at a time window between 1:30–3:30 min:s and 1:30–4:30 min:s. Overall, a longer time window of 2 and 3 min fared much better than a 1 min time window.

Comparing net COT curves for walking

To keep our comparisons consistent, we calculated the net $\dot{V}_{O_2}$, net metabolic power and net COT curves derived from a 2–4 min and 2–5 min window. We chose these windows because the regression lines characterizing the change in average slope as a function of speed (Fig. 2) did not differ between a 2–4 min and 4–7 min window and a 2–5 min and 4–7 min window. As illustrated in Figs 4–6, the net $\dot{V}_{O_2}$, net metabolic power and net COT curves were not statistically different from the curves produced from the last 3 min of the 7 min trial, our gold standard. Follow up comparisons at each speed revealed that pairwise differences between the mean values for net COT (e.g. mean at 2–4 min versus 4–7 min at 0.5 m s^{-1}; mean at 2–5 min versus 4–7 min at 0.5 m s^{-1}, and so on) were not statistically different, except for the fastest walking speed of 2.0 m s^{-1} (Tables 1–3). At this speed, the net COT derived from a

2–4 min and 2–5 min window was slightly higher than our gold standard. We suspect that these differences at 2.0 m s^{-1} can be explained by the relatively small sample size of 12 subjects. Nonetheless, including a speed of 2.0 m s^{-1} in the net COT curve should be interpreted with caution because this particular speed is where humans prefer to transition from a walk to a run (Farris and Sawicki, 2012; Minetti et al., 1994). This might explain why many of our subjects could not keep up with the treadmill when attempting to walk at 2.0 m s^{-1}. If we restrict our walking speeds to a typical range of 0.5–1.75 m s^{-1}, our analyses suggest that either a 2–4 min or a 2–5 min window will yield average net COT values and curves that are not statistically different from our gold standard.

For cases where the entire net COT curve is not of primary scientific interest, our simple method suggests that it is robust and reliable for estimating net COT values at speeds below 2.0 m s^{-1}. In many walking experiments, it is quite common to measure metabolic energy consumption across varying conditions, while speed is held fixed at magnitudes between 1.0 and 1.3 m s^{-1}. When comparing the net COT values derived from net $\dot{V}_{O_2}$ data, the 2–4 min and 2–5 min windows were not significantly different from the 4–7 min window (Table 1). In addition, the effect sizes for these speeds ranged between 0.306 and 0.479, reflecting differences that are <0.5 s.d. away from the gold standard values. The same results hold when comparing the net COT values derived from either Brockway (1987) or Péronnet and Massicotte (1991), which are
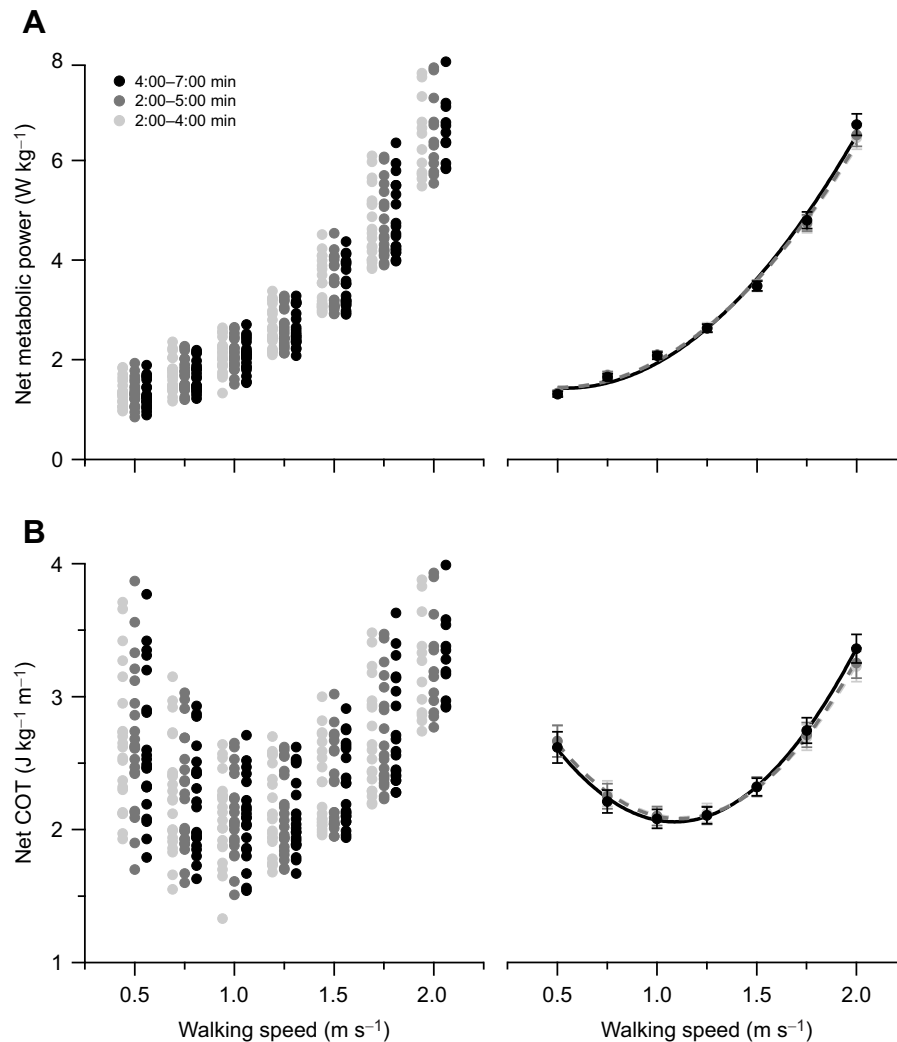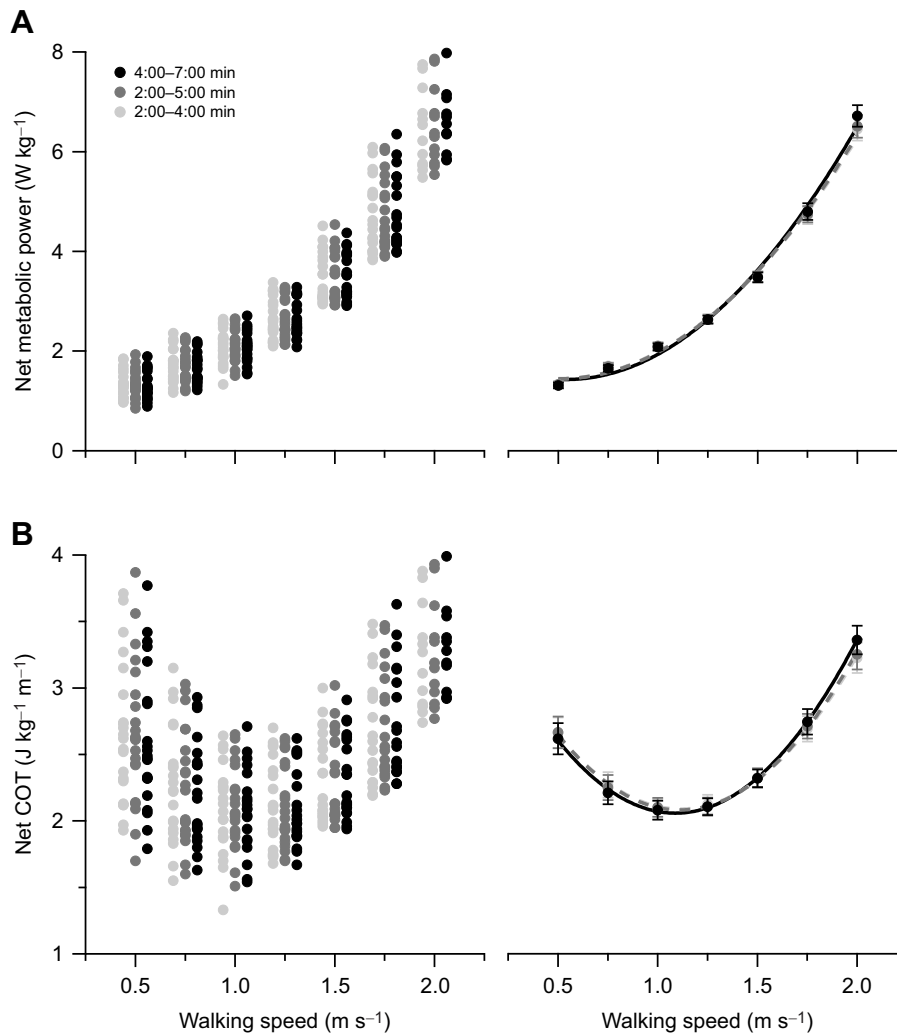
Fig. 5. Net metabolic power and net COT derived using the Brockway equation, and expressed as a function of speed. (A) Net metabolic power demand and (B) net COT changed non-linearly with walking speed. The left column represents the individual data points estimated from the Brockway (1987) equation and the right column represents the regression curves fitted to these individual data points. For ease of comparing the regression curve fits, the data in the right column represent the mean ±s.e.m. and are superimposed to illustrate the high degree of similarity. Similar to the trends and interpretation in Fig. 4, the demand for net metabolic power increased curvilinearly with speed while the net COT exhibited a U-shaped curve, highlighting the observation that walking at a speed between 1 and 1.3 m s^{-1} minimizes the net COT. Statistical comparisons made from the regression equations characterizing the non-linear relationship of net metabolic power versus speed and net COT versus speed were not statistically different for the time windows taken from 2–4 min, 2–5 min and 4–7 min (net metabolic power comparisons: 2–4 min versus 4–7 min P=0.431 and 2–5 min versus 4–7 min P=0.543; net COT comparisons: 2–4 min versus 4–7 min P=0.710 and 2–5 min versus 4–7 min P=0.781). Note that the regression curves for the 2–4 min window overlap with those for the 2–5 min window; therefore, they are indistinguishable from one another. The equations characterizing the polynomial non-linear regression fits in the right column are listed in Table 4.

depicted in Tables 2 and 3. This was the case for all speeds, with the exception arising at the fastest speed of 2.0 m s^{-1}. One can avoid this discrepancy by either not including 2.0 m s^{-1} in the derivation of the net COT curve or ensuring that at 2.0 m s^{-1}, the net COT value is derived from a 4–7 min window.

Limitations and future work

While our slope method was successful for identifying steady rates of metabolism across a range of slow to fast speeds, there are some limitations that warrant further analysis. First, we were unable to determine the effect of sampling rate on identifying periods of steady rates of metabolism. Our data were based on a previous study (Thomas et al., 2021) where our metabolic system was configured to sample average rates of O_2 consumption and CO_2 production approximately every 15 s. It is possible that applying our slope method on metabolic data that were sampled breath by breath, as done by Schwartz (2007), could have identified shorter time windows; however, breath-by-breath data are inherently noisier. While we show that our results are independent of the typical equations used in the field of locomotion energetics and biomechanics (Brockway, 1987; Péronnet and Massicotte, 1991), further analysis is needed to address the potential effect of different sampling rates and filtering techniques. Overall, we recommend that any post-processing of these types of data should be kept simple.

In line with the analyses carried out by Schwartz (2007), we also compared our quadratic regression fits and found that the mean square error for the net $\dot{V}_{O_2}$, net metabolic power and net COT curves remains small, differing between 6.8% and 12.6% from the gold standard (Table 4). This provides evidence that the total time required for data collection can be substantially reduced without sacrificing the accuracy of estimating an individual's net metabolic power and net COT curve. We can appreciate this finding by comparing the values derived from the 2–4 min window with that of the gold standard. For speeds ranging from 0.5 to 1.75 m s^{-1}, an example calculation using the Péronnet and Massicotte (1991) equation shows that a 2–4 min and 4–7 min window yield similar values for net COT (2.791 versus 2.746 J kg^{-1} m^{-1} at 0.50 m s^{-1}; 2.401 versus 2.353 J kg^{-1} m^{-1} at 0.75 m s^{-1}; 2.206 versus 2.166 J kg^{-1} m^{-1} at 1.00 m s^{-1}; 2.204 versus 2.185 J kg^{-1} m^{-1} at 1.25 m s^{-1}; 2.397 versus 2.411 J kg^{-1} m^{-1} at 1.50 m s^{-1}; 2.783 versus 2.843 J kg^{-1} m^{-1} at 1.75 m s^{-1}; and 3.363 versus 3.481 J kg^{-1} m^{-1} at 2.00 m s^{-1}). The 2–4 min window predicts net COT values that differ between −2.064% and 3.39% across the entire speed range. Similar conclusions are reached for the 2–5 min window and when using $\dot{V}_{O_2}$ values or values derived from the Brockway (1987) equation to estimate the net COT for walking. While these estimates are robust, we recognize that our data are based on a sample of healthy young adults; however, this slope method could be easily applied to any population (e.g. clinical,

Fig. 6. Net metabolic power and net COT derived using the Péronnet and Massicotte equation, as a function of speed. (A) Net metabolic power demand and (B) net COT changed non-linearly with walking speed. The left column represents the individual data points estimated from the Péronnet and Massicotte (1991) equation and the right column represents the regression curves fitted to these individual data points. For ease of comparing the regression curve fits, the data in the right column represent the mean±s.e.m. and are superimposed to illustrate the high degree of similarity. Similar to the trends and interpretation in Figs 4 and 5, the demand for net metabolic power increased curvilinearly with speed while the net COT exhibited a U-shaped curve, highlighting the observation that walking at a speed between 1 and 1.3 m s^{-1} minimizes the net COT. Statistical comparisons made from the regression equations characterizing the non-linear relationship of net metabolic power versus speed and net COT versus speed were not statistically different for the time windows taken from 2–4 min, 2–5 min and 4–7 min (net metabolic power comparisons: 2–4 min versus 4–7 min P=0.421 and 2–5 min versus 4–7 min P=0.532; net COT comparisons: 2–4 min versus 4–7 min P=0.699 and 2–5 min versus 4–7 min P=0.774). Note that the regression curves for the 2–4 min window overlap with those for the 2–5 min window; therefore, they are indistinguishable from one another. The equations characterizing the polynomial non-linear regression fits in the right column are listed in Table 4.

young children, older adults, etc.) where metabolic measurements are experimentally feasible. And because there are no assumptions underlying the transient dynamics of metabolic energy consumption, this slope method could be applied to different tasks such as cycling, hopping and running, and potentially any task where energy is provided primarily by aerobic pathways. One point to consider is that although our approach provides the advantage of simplicity, it leaves unanswered the question of how the transient dynamics unfold during the period of non-steady-rate metabolism. Modeling these transient dynamics has important implications for understanding the physiological control processes that govern pulmonary gas exchange during exercise (Whipp and Ward, 1990). If a key objective is to model and understand these transient dynamics during walking, then the methodology of Selinger and Donelan (2014) should be adopted. If not, then one could easily adopt our simple slope method to confirm periods of steady-rate metabolism during exercise. Nonetheless, we encourage the application of our simple slope method in future experiments that study different tasks at varying exercise intensities, which will help determine the generalizability of this approach and justify its use as an analytical tool.

Conclusion

In summary, we used a slope method as a viable approach for identifying steady rates of metabolism during human walking. Our analyses explored a wide range of slow to fast walking speeds (0.5–2.0 m s^{-1}), revealing that at minimum, 4 min of metabolic data for each walking trail is required to estimate a net COT curve that is not statistically different from a net COT curve derived from traditional data collection times. A key takeaway from our analyses is that once steady-rate metabolism has been achieved after 2 min, one only needs to average over 2 min of metabolic data, reflecting a time window between 2 and 4 min. Our findings suggest that if desirable, shorter trials can be performed, which will help decrease total data collection time. Based on the traditional trial durations that typically range from 6 to 10 min, we estimate a decrease in experimental time of 14–42 min across all speeds per subject. For a sample size of 21 subjects, this equates to a decrease of ~5–15 h in total data collection time. While it is common for steady rates of metabolism to be confirmed through visual inspection, our slope method provides an objective and simple way to accomplish this experimental goal, which may prove useful for scientists studying problems in or at the interface of exercise physiology and locomotion biomechanics.

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