# Blood as fuel: the metabolic cost of pedestrian locomotion in Rhodnius prolixus 

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#### Abstract

Active searching for vertebrate blood is a necessary activity for haematophagous insects, and it can be assumed that this search should also be costly in terms of energetic expenditure. Whether by swimming, walking, running or flying, active movement requires energy, increasing metabolic rate relative to resting situations. We analysed the respiratory pattern and energetic cost of pedestrian locomotion in the blood-sucking bug Rhodnius prolixus using flowthrough respirometry, by measuring carbon dioxide emission and water loss before, during and after walking. We observed an increase in the metabolic rate during walking as compared with resting of up to 1.7 -fold in male $R$. prolixus and 1.5 -fold in females, as well as a change in their respiratory pattern, which switched from cyclic during rest to continuous when the insects started to walk, remaining in this condition during locomotion and for several minutes after stopping. Walking induced a significant loss of mass in both males and females. This can be explained by an increase in both metabolic rate and water loss during walking. These data constitute the first metabolic measures of active haematophagous insects and provide the first insights into the energetic expenditure associated with the active search for blood in this group.


KEY WORDS: Respiration, Activity, Metabolic rate, Disease vectors, Chagas

## INTRODUCTION

Resource procurement requires active searching for most animals. For limbed animals, locomotion is the mechanism that facilitates this task. Whether by swimming, walking, running or flying, active movement involves energy expenditure, which induces a measurable increase in metabolic rate, respect to resting periods.

The amount of energy necessary for self-transport depends on several factors. For terrestrial locomotion, the weight of the animal, the distance displaced and the speed of movement should be considered as suitable bases for measurement and comparison (Herreid, 1981; Schmidt-Nielsen, 1972; Tucker, 1970). Full (1989) found a remarkable similarity between data on the metabolic cost of terrestrial locomotion for different arthropods and larger animals such as lizards, birds and mammals. As the energetic cost of transport is directly proportional to the metabolic rate, which in turn depends directly on the rate of oxygen consumption, assessing the exchange of respiratory gases in an animal appears to be an adequate way to estimate the energy costs of locomotion.

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Respiratory metabolism and energetics during terrestrial locomotion in invertebrates such as insects have been relatively less studied over the last 30 years in comparison to vertebrates (Lipp et al., 2005). Most studies on insects have focused on the energetics of horizontal running (Bartholomew et al., 1985, 1988; Duncan and Lighton, 1997; Herreid et al., 1981; Jensen and Holm-Jensen, 1980; Lehmann and Schützner, 2010; Rogowitz and Chappell, 2000) and climbing slopes (Full and Tullis, 1990; Lipp et al., 2005), as well as load carriage (Bartholomew et al., 1988; Schilman and Roces, 2005). Few studies, however, have assessed metabolic rate and energetic expenditure during voluntary walking (Berrigan and Lighton, 1994; Lipp et al., 2005; Rothe and Nachtigall, 1989).

Metabolic rate during pedestrian locomotion is probably much lower than during flight. However, metabolic costs of pedestrian locomotion could reach an important portion of the total energy budget associated with flight, particularly in species that search for food or explore potential substrates for oviposition by walking (Berrigan and Lighton, 1994; Full, 2010).

For haematophagous insects, starvation appears to be a main cause of locomotion initiation (Lehane, 2005; Lehane and Schofield, 1982; McEwen and Lehane, 1993). In the case of disease vectors, this association between locomotion and host search for feeding acquires particular relevance for the spread of transmitted pathogens. Yet, there is at present no information available relating to the energetic cost of locomotion in bloodfeeding insects.

One way of evaluating the cost associated with self-transport is by comparing the metabolism of an organism at rest and during locomotion. Metabolic activity has been successfully measured in different species of blood-sucking arthropods, such as fleas, bedbugs, ticks and mosquitoes (Lighton et al., 1993; Gray and Bradley, 2003, 2006; DeVries et al., 2013). However, the species that has been best characterized in terms of respiration dynamics and energetic cost in different situations is the kissing bug Rhodnius prolixus (Bradley et al., 2003; Contreras and Bradley, 2009, 2010; Rolandi et al., 2014; Heinrich and Bradley, 2014; Leis et al., 2016; Paim et al., 2016). As obligated blood-feeding hemimetabolous insects, they approach their hosts mostly by walking. Hence, kissing bugs constitute a suitable model system to evaluate the energetics of pedestrian locomotion in blood-sucking insects. Rhodnius prolixus is, in addition, a classical model in insect physiology and an important vector of the causative agent of a major health problem in Central and South America, Chagas disease (Lazzari, 2017).

The aim of this work was to characterize the energetic costs associated with walking in R. prolixus. Our knowledge about the evolutionary ecology of triatomines is still limited (Menu et al., 2010). In order to dig deeper on this aspect of their biology, quantitative estimates of the costs and benefits associated with different activities (e.g. feeding, locomotion, egg laying, etc.) and life history traits (tolerance to starvation, reproductive strategy, dispersal, etc.) are required. Our aim here was to provide
information in line with this need and shed additional light on the dispersal capacity of Chagas disease vectors.

## MATERIALS AND METHODS

## Experimental animals and set-up

Fifth-instar nymphs of Rhodnius prolixus Stål 1859 from our laboratory colony were fed on heparinized sheep blood offered in an artificial feeder, and used for experiments $10-15$ days after their imaginal ecdysis. Groups of insects were maintained in a climatic chamber in 0.51 plastic jars at $25 \pm 1^{\circ} \mathrm{C}, 60 \pm 5 \%$ relative humidity and subjected to a $12 \mathrm{~h}: 12 \mathrm{~h}$ light:dark cycle.

After the imaginal moult, 15 females and males were separately weighed to the nearest 0.1 mg , individually placed into 5 ml disposable syringes (Terumo) and kept unfed until experiments began. Each insect was glued to a 1 cm long piece of toothpick by the pronotum and then placed into a 30 ml respirometric chamber, made by modifying 50 ml disposable syringes, for measuring resting, walking and recovery metabolic rate. Measurements of resting metabolic rate started 1 min after insects stopped moving their legs. Together with the insect, a polystyrene ball was introduced into the chamber in order to provide a walking substrate that the insect could grasp with its legs (Rothe and Nachtigall, 1989) (Fig. 1). Of several ball sizes tested, the most adequate in terms of facility for the bugs to grasp, maintain natural leg position and perform longer walking periods were balls of 3.1 cm diameter ( 341 mg ). Each assay was video recorded for posterior calculation of walking speed and dynamics.

## Walking and recovery metabolism

In our study, walking was defined as the turning of a polystyrene ball by continuous movement of the insect legs as proposed by Rothe and Nachtigall (1989). Walking was stimulated by gently turning the respirometric chamber upside down until the polystyrene ball contacted the insect tarsi (Fig. 1B). To trigger walking, a researcher gently touched the external wall of the chamber in front of the starved insects with a finger to provide a stimulus (heat). After 30 min trial, the respirometric chamber was gently tapped to stimulate the release of the polystyrene ball by the insect, and so allow measurement of the recovery metabolism. Afterwards, individuals were removed from the chamber, detached from the support and weighed again.

## Respiratory measurements

Metabolic measurements were carried out in a room kept at a constant temperature of $25 \pm 1^{\circ} \mathrm{C}$. Flow-through respirometry, following methods already validated (Bradley et al., 2003; Contreras and Bradley, 2010; Lighton, 2008; Leis et al., 2016), was used to measure $\mathrm{CO}_{2}$ production and water loss. Measurements were performed during rest (basal metabolism) and during and after walking using a Sable Systems (Henderson, NV, USA) respirometric chain. Expedata software controlled an 8-channel multiplexer (only two channels used, one for experiments and the another as baseline) switching a water vapour (Drierite) and $\mathrm{CO}_{2}$ (Ascarite) scrubbed airflow of $200 \mathrm{ml} \mathrm{min}^{-1}$ (Sierra mass flow controller). The multiplexer made the airflow pass through the respirometric chambers and then conducted it to a water vapour analyser (RH-300, Sable Systems), after which the water vapour was scrubbed using magnesium perchlorate. Finally, the airflow reached an infrared $\mathrm{CO}_{2}$ analyser (CA-10, Sable Systems). An electronic interphase (UI-2, Sable Systems) gathered the data from the instruments and fed them to a computer through Expedata for analysis.

Baseline measurements of 15 min were obtained using an empty chamber, both before and after each recording event, in order to determine zero $\mathrm{CO}_{2}$ level and to correct for instrument noise.

## Metabolic calculations, data analysis and statistics

The initial 5 min of recording from the experimental and empty chambers were excluded from the analysis to eliminate effects of the accumulated $\mathrm{CO}_{2}$ derived from insect respiration and/or valve action. The respiratory pattern $\left(\dot{V}_{\mathrm{CO}_{2}}, \mu \mathrm{~min} \mathrm{~min}^{-1}\right)$ and water loss ( $\dot{V}_{\mathrm{H}_{2} \mathrm{O}}, \mu 1 \mathrm{~min}^{-1}$ ) were determined during rest, walking and recovery. We calculated the mean rate and the mass-dependent mean rate of $\mathrm{CO}_{2}$ emission ( $\dot{V}_{\mathrm{CO}_{2}}$ ) and water loss ( $\dot{V}_{\mathrm{H}_{2} \mathrm{O}}$ ) using the methods and equations established by Lighton (2008). To determine an energy equivalent of metabolic rate measured as the rate of $\mathrm{CO}_{2}$ produced, we also followed the methods proposed by Lighton (2008), using the respiratory quotient (RQ) of 0.83 obtained by Leis et al. (2016) for resting $R$. prolixus. Walking speed was calculated from the recorded videos using the software Tracker (Open Source Physics). We calculated the energy cost of walking as the gross cost of transport (GCOT) and the net cost of transport (NCOT) after Herreid (1981), Bartholomew et al. (1988) and Schilman and Roces (2005). The


Fig. 1. Respirometric chamber for measuring $\mathrm{CO}_{2}$ release and water loss during rest, walking and recovery. (A) Set-up for measurement of resting and recovery metabolic rate. (B) Set-up for measurement of walking metabolic rate. 1, plastic syringe; 2 , incurrent air; 3 , excurrent air; 4, toothpick; 5 , polystyrene ball; 6 , Rhodnius prolixus adult.



Fig. 2. Body mass of bugs before and after respirometric measurements. (A) Body mass during rest and after recovery. (B) Walking-dependent mass loss. Data are means+s.e.m. ( $n=10$ for both females and males) before (black bars) and after (grey bars) respirometric measurements. * $P<0.05$, ${ }^{* *} P<0.001$.
movement of the polystyrene ball was fitted to a sinusoidal model by the software. A two-way ANOVA with a repeated measures factor was used to compare data for rest, walking and recovery and sex. When required, the variables were transformed to meet the assumptions of the model. Finally, factors were compared by conducting a post hoc Holm-Šidák pairwise multiple comparison method.

## RESULTS

## Body mass

In order to assess the cost of walking, first we had to characterize the dynamics associated with tethered insects walking voluntarily over a polystyrene ball.

Fig. 2 depicts the body mass change after walking, as mass decreases and the rate of mass loss increases as a result of walking. The insects decreased their mass significantly after walking, regardless of sex, i.e. female or male ( $P<0.001$; Fig. 2A). Females were significantly heavier than males regardless of the experimental conditions, i.e. before or after walking ( $P<0.05$; Fig. 2B). Detailed descriptive statistics of the data are presented in Table S1.

## Water loss

A typical respirometric run shows an augmentation of $\dot{V}_{\mathrm{H}_{2} \mathrm{O}}$ release during walking (Fig. 3).


Fig. 3. Example of $\mathrm{H}_{2} \mathrm{O}$ release for a single insect during rest, walking and recovery. WLR, water loss rate. Grey shading indicates periods of stress due to induced polystyrene ball grabbing or release.

The analysis of $\ln$-transformed water loss rate (WLR) revealed that actual as well as $\ln$-transformed mass-specific WLR increase during walking and then decrease during recovery to a similar rate to that during rest, regardless of sex ( $P<0.005$; Fig. 4), and no differences between rest and recovery were observed. Descriptive statistics are presented in Table S1. This increase reached 2.4 -fold for females and 2.9-fold for males.

## $\dot{\mathbf{V}}_{\mathbf{C O}_{2}}$ emission and metabolic rate

A typical run of gas exchange is shown in Fig. 5. Prior to walking, resting bugs exhibited a cyclic pattern of $\mathrm{CO}_{2}$ emission, showing clear and regular peaks of $\mathrm{CO}_{2}$ release. Once an insect began to walk, the gas exchange pattern became irregular and remained that way during recovery.

The computation of respirometric and energetic variables revealed that actual as well as mass-specific metabolic rate, in terms of the rate of $\mathrm{CO}_{2}$ release $\left(\dot{V}_{\mathrm{CO}_{2}}\right)$ and energetic cost, significantly increased during walking as compared with rest and recovery $\left(\dot{V}_{\mathrm{CO}_{2}}, P<0.005\right.$, Figs 3 and 6 A ; mass-specific $\dot{V}_{\mathrm{CO}_{2}}$, $P<0.005$, Fig. 6B; metabolic rate, $P<0.001$, Fig. 6C; mass-specific metabolic rate, $P<0.001$, Fig. 6D). Descriptive statistics are presented in Table S1.

## Cost of walking

Full results of the statistical analyses of metabolic variables measured in this study are presented in Table 1.

Under our conditions, walking bugs displayed an increase in energy expenditure during walking of around 1.5 -fold for females and 1.7 -fold for males. The GCOT was about $421.05 \mathrm{~J} \mathrm{~kg}^{-1} \mathrm{~m}^{-1}$ for females and $457 \mathrm{~J} \mathrm{~kg}^{-1} \mathrm{~m}^{-1}$ for males. NCOT was near to $139 \mathrm{~J} \mathrm{~kg}^{-1} \mathrm{~m}^{-1}$ for females and $180 \mathrm{~J} \mathrm{~kg}^{-1} \mathrm{~m}^{-1}$ for males. Despite the higher energy cost of walking shown by males, no significant difference was observed associated with the sex of the individuals.

## DISCUSSION

Rhodnius prolixus showed a notable mass loss after a few minutes of walking. Almost $20 \%$ mass loss after walking was observed for both males and females, at a rate of $1.47 \pm 0.14 \mathrm{mg} \mathrm{h}^{-1}$ for females and $0.992 \pm 0.165 \mathrm{mg} \mathrm{h}^{-1}$ for males. This variation may appear relatively high, but it should be noted that it does only refer to a short period at the beginning of the experiments, during which the insects could eliminate excrement. A similar initial mass loss has been noted by us to occur when insects were forced to walk on an arena (M.A.L. and C.R.L., unpublished observations).


Fig. 4. Water loss associated with walking. (A) WLR during rest, walking and recovery. (B) Mass-specific WLR during rest, walking and recovery. Data are means+s.e.m. $(n=10)$ for adult $R$. prolixus of both sexes. ${ }^{*} P<0.05$.

Our experimental bugs exhibited a cyclic respiratory pattern during rest. This pattern changed during walking, switching to an irregular gas exchange (Fig. 5), which suggests a sustained opening of spiracles. As this occurred, the rate of water loss significantly increased during walking (Figs 3 and 4). This could explain some of the mass loss of the insects during locomotion activity. Rolandi et al. (2014) showed a positive relationship between WLR and metabolic rate in R. prolixus. As metabolic rate and activity are also positively related, we can link the physical activity with WLR and some of the mass loss. We suppose that both water loss and mass loss stabilize during sustained walking because, as we discuss below, adults of $R$. prolixus are potentially capable of walking considerable distances with the energy provided by a full blood meal.

## Varying respiratory patterns according to behaviour

As previously described by others (Contreras and Bradley, 2009; Leis et al., 2016; Paim et al., 2016), R. prolixus displays different respiratory patterns according to their physiological condition and activity. Three different dynamics of gas exchange have been described in this species: continuous, discontinuous cyclic (DGC) and cyclic. Two of them, DGC and cyclic, have been proposed in mosquitoes to be variants of the same dynamic, with the cyclic pattern considered to be a manifestation of the DGC (Gray and Bradley, 2006). In the present study, our time constant was 9 s and we used the same airflow ( $200 \mathrm{ml} \mathrm{min}^{-1}$ ), but a different chamber size $(30 \mathrm{ml})$, as that employed by Contreras and Bradley (2009). Under these conditions, two different patterns clearly appeared as manifestations of gas exchange in $R$. prolixus, i.e. cyclic and
continuous. But, in a previous article, we reported the observation of the three patterns described by others for this species (Leis et al., 2016).

Unfed resting bugs usually exhibit DGC (Bradley et al., 2003; Contreras and Bradley, 2009) but in our experimental conditions, insects showed a cyclic respiratory pattern before walking, maybe as a consequence of stress produced by previous manipulations or as a consequence of our time constant. Gray and Bradley (2006) suggested that insects showing a cyclic gas exchange pattern could be respiring with a discontinuous pattern. As our time constant allowed $99 \%$ of the air in the respirometric chamber to be cleared in 45 s , we cannot completely discard the possibility of our insects showing a DCG respiratory pattern during rest. Then, during walking, a switch to a continuous pattern was observed. This change was revealed in our experiments as a marked increase and dynamic change in $\mathrm{CO}_{2}$ emission. Water loss was predominantly continuous during both phases of our experiments, i.e. previous to and during walking, and a high increase in the rate of water loss was only observed during walking, but without a change in the dynamics.

## Energetic cost of walking

As we have shown previously, the metabolic rate of $R$. prolixus increased during walking 1.5 times for females and 1.7 times for males as compared with that during rest. The energy needed to deal with this energetically demanding activity is provided by the nutrients found in blood: 100 ml of human blood, for example, contains 0.652 g of lipids, 0.088 g of carbohydrates and 20.5 g of proteins (Lehane, 2005). If all these nutriments are metabolized, this means that the energetic value of 1 mg of human blood is


Fig. 5. Example of $\mathrm{CO}_{2}$ release for a single insect during rest, walking and recovery. A change of pattern when insects started to walk was observed. Grey shading indicates periods of stress due to induced polystyrene ball grabbing or release.


Fig. 6. Metabolic rate associated with walking. (A) Metabolic rate in terms of $\mathrm{CO}_{2}$ release during rest, walking and recovery. (B) Mass-specific metabolic rate in terms of $\mathrm{CO}_{2}$ release during rest, walking and recovery. (C) Metabolic rate in terms of energy cost using a respiratory quotient ( RQ ) $=0.83$ during rest, walking and during recovery. (D) Mass-specific metabolic rate in terms of energy cost using $\mathrm{RQ}=0.83$ during rest, walking and recovery. Data are means+s.e.m. ( $n=10$ for both females and males). ${ }^{*} P<0.05$.
approximatively 3.91 J . Thus, a full blood meal ( 150 mg ) can provide 586 J of energy and allow adults of $R$. prolixus to rest for up to 43 days in females and 57 days in males (Fig. 7). These times correspond to our experimental conditions, where previous
manipulation and hanging in the chamber could induce some stress; so, longer times could be expected for completely undisturbed bugs in thigmotactic akinesis in a natural refuge. Assuming that an individual uses its available energy only for

Table 1. Results of statistical analyses



Fig. 7. Energetic value of blood. In R. prolixus, 1 mg of human blood can provide the energy required during rest for close to 7 h in females and 9 h in males. It can also provide the energy required for walking at $0.6 \mathrm{~cm} \mathrm{~s}^{-1}$ for almost 5 h or 100 m in females and for almost 6 h or 123 m in males.
walking, a full blood meal can provide the energy required to walk up to 15 km in females and 18 km in males, at an average speed of $0.6 \mathrm{~cm} \mathrm{~h}^{-1}$ (Fig. 7).

Comparing the energetic cost of locomotion is not always an easy task, even when metabolic data for a single species during walking are available. The cost of locomotion can be assessed by obtaining the GCOT, the NCOT and/or the minimum cost of transport (MCOT). GCOT is calculated by dividing the mass-dependent metabolic rate during walking by the walking speed, NCOT is determined by subtracting the mass-dependent metabolic rate during rest from the mass-dependent metabolic rate during walking and then dividing it by the walking speed. Finally, MCOT is defined as the slope of the relationship between the massdependent metabolic rate during walking and the walking speed of each individual (Herreid et al., 1981; Schilman and Roces, 2005). GCOT is the most direct estimation of the cost of transport and may be the most relevant measure in an ecological approach. This measure has two main disadvantages: it is dependent on the resting metabolic rate of the animals and it drops toward minimum levels as the speed increases. NCOT accounts for the resting metabolic rate issue by simply subtracting it from the walking metabolic rate, but NCOT still drops as the speed increases. MCOT, in contrast, is not dependent on either the speed or the resting metabolic rate, allowing comparison of the locomotion of organisms walking or running at different velocities (Herreid et al., 1981; Lighton et al., 1993). In this study, resting metabolic rate and walking speed did not differ among the different individuals of $R$. prolixus analysed, making the calculation of GCOT and NCOT very robust (Schilman and Roces, 2005), but not MCOT, rendering comparisons with other species unpractical. However, it is possible to compare the increment of the metabolic cost during locomotion relative to their respective resting metabolic cost. From this perspective, the increase of metabolic cost of R. prolixus is not as important as that for some species of ants. Individuals of the ant Camponotus sp. showed an increment of metabolic rate of 4.3 times during level walking at $0-5 \mathrm{~mm} \mathrm{~s}^{-1}$ over resting rates (Lipp et al., 2005). That represents an augmentation of almost 2 times and 1.5 times the equivalent metabolic rate increase of females and males of $R$. prolixus, respectively. The increase associated with walking is even lower than the metabolic increase of flying over resting in terms of mass-specific rate of $\mathrm{O}_{2}$ consumption
for Drosophila melanogaster (Hocking, 1953; Lehmann et al., 2000; Niven and Scharlemann, 2005).

Knowledge of the cost of locomotion of blood-sucking bugs can provide valuable information about their dispersive capacity. There is little published information on the dispersive capacity of R. prolixus either by walking or flight. There is some information on field dispersive flight of another blood-sucking bug, Triatoma infestans, but no data related to metabolic cost. Triatoma infestans are capable of long flights and walking excursions (Lehane and Schofield, 1981; Abrahan et al., 2011). Assuming that flight in triatomines, as shown by other flying insects (Niven and Scharlemann, 2005), is far more expensive in terms of metabolic cost, we can argue that the dispersive capacity by walking is much higher than by flying. Our results reflect that assumption and show the first experimental data that allows estimation of how far an adult individual of $R$. prolixus can go by walking with the energy provided by a full meal on human blood, revealing an impressive dispersive capacity of almost 15 km in females and 18 km in males.

It is worth mentioning that water loss was measured under respirometric conditions, i.e. using a continuous current of dry air. Despite this fact, the loss of water during walking was only $0.5-$ $0.7 \%$ of the insect mass per hour, which seems relatively low for an active insect under these conditions. We can therefore expect that water loss would not be a limiting factor for the dispersion capacity of kissing bugs.

Summarizing, the present work presents the first analysis of the energetic cost of walking in a haematophagous insect. We provide measured quantitative data for adults of both sexes and calculated the energetic value of blood as energy source. This information is essential for the elaboration of realistic models of the dispersive capacity of Chagas disease vectors, as well for a comprehensive analysis of the ecophysiology and evolutionary ecology of triatomines, and the epidemiology of American trypanosomiasis. Yet, a note of caution should be given to the reader, concerning the consideration of these findings in a natural situation. The biology of kissing bugs makes it particularly difficult to perform metabolic measurements under conditions closely resembling those of an insect spontaneously walking in the wild. In the laboratory, triatomines either tend to remain immobile when undisturbed or they run when manipulated. When they are put in an arena, instead
of exploring it, they usually run to reach a wall and remain in thigmotactic akinesis. In contrast, their resting metabolic rate (Leis et al., 2016) is relatively low, as compared with that of more active insects, such as bees or ants. To ensure a reliable quantification of respiratory variables, the use of small chambers is required, as well as precise control of the experimental conditions. In order to cope with these constraints, we chose to adapt to bugs the method employed by Rothe and Nachtigall (1989) for evaluating the cost of walking in honeybees. Given that in this situation bugs are not carrying their own mass in a natural way, but are keeping a load with their legs, our method probably overestimates or underestimates real costs. It is worth mentioning, however, that our experimental results and estimations are in line with the dispersive potential of bugs observed in the field (Abrahan et al., 2011). Despite the fact that the costs could not be exact, having unravelled that water and energy reserves obtained in a single meal allow bugs to walk distances of several kilometres is relevant from an epidemiological point of view, even assuming them to be approximate figures of such capacity.

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## Competing interests

The authors declare no competing or financial interests.

## Author contributions

Conceptualization: C.R.L.; Methodology: M.A.L., C.R.L.; Software: M.A.L.;
Validation: M.A.L.; Formal analysis: M.A.L., C.R.L.; Investigation: M.A.L.; Writing original draft: M.A.L., C.R.L.; Writing - review \& editing: M.A.L., C.R.L.; Supervision: C.R.L.; Funding acquisition: C.R.L.

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## Data availability

Data are available from Mendeley Data: http://dx.doi.org/10.17632/x2dmzft9jc.1.

## Supplementary information

Supplementary information available online at
https://jeb.biologists.org/lookup/doi/10.1242/jeb.227264.supplemental

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Table S1. Descriptive statistics (means $\pm$ s.e.m) of weight, speed, metabolic values and water loss in both sexes. Results of statistical analyses can be found in the Table 1.

|  | Female | Male |
| :---: | :---: | :---: |
| Temperature ( ${ }^{\circ} \mathrm{C}$ ) | 25 | 25 |
| n | 10 | 10 |
| Weight before walking (mg) | $99.68 \pm 5.17$ | $72.88 \pm 5.6$ |
| Weight loss (mg) | $19.78 \pm 1.49$ | $13.19 \pm 1.94$ |
| Weight loss rate ( $\mathrm{mg} / \mathrm{min}$ ) | $1.47 \pm 0.14$ | $0.992 \pm 0.165$ |
| RMR ( $\mu$ l $\mathrm{CO}_{2} / \mathrm{h}$ ) | $23.88 \pm 1.54$ | $17.83 \pm 1.82$ |
| Mass-dependent $\mathrm{RMR}(\mu \mathrm{l} \mathrm{CO} 2 / \mathrm{h} / \mathrm{mg})$ | $0.242 \pm 0.009$ | $0.237 \pm 0.007$ |
| RMR (J/h) | $0.574 \pm 0.037$ | $0.429 \pm 0.043$ |
| Mass-dependent RMR (J/h/mg) | $0.00583 \pm 0.0002$ | $0.0057 \pm 0.0002$ |
| Speed (cm/s) | $0.596 \pm 0.041$ | $0.594 \pm 0.013$ |
| Walking metabolic rate ( $\mu \mathrm{lCO} \mathrm{CO}_{2} / \mathrm{h}$ ) | $35.42 \pm 1.53$ | $28.40 \pm 1.87$ |
| Mass-dependent Walking metabolic rate ( $\mu \mathrm{CO}_{2} / \mathrm{h} / \mathrm{mg}$ ) | $0.364 \pm 0.013$ | $0.401 \pm 0.03$ |
| Walking metabolic rate ( $\mathrm{J} / \mathrm{h}$ ) | $0.852 \pm 0.037$ | $0.683 \pm 0.045$ |
| Mass-dependent Walking metabolic rate (J/h/mg) | $0.00877 \pm 0.0003$ | $0.00965 \pm 0.0007$ |
| GCOT (J/Kg/m) | $421.05 \pm 34.31$ | $457.48 \pm 28.28$ |
| NCOT (J/Kg/m) | $138.7 \pm 19.75$ | $179.52 \pm 27.7$ |
| Recovery metabolic rate ( $\mu \mathrm{lCO} \mathrm{CO}_{2} / \mathrm{h}$ ) | $21.6 \pm 1.37$ | $18.91 \pm 1.68$ |
| Mass-dependent Recovery metabolic rate ( $\mu \mathrm{l}$ $\mathrm{CO}_{2} / \mathrm{h} / \mathrm{mg}$ ) | $0.228 \pm 0.01$ | $0.266 \pm 0.028$ |
| Recovery metabolic rate (J/h) | $0.519 \pm 0.033$ | $0.782 \pm 0.18$ |
| Mass-dependent Recovery metabolic rate ( $\mathrm{J} / \mathrm{h} / \mathrm{mg}$ ) | $0.00549 \pm 0.0002$ | $0.0117 \pm 0.003$ |
| Resting WLR (mg/h) | $2.003 \pm 0.211$ | $1.65 \pm 0.14$ |
| Resting mass-dependent WLR (mg/h/mg) | $0.0215 \pm 0.003$ | $0.0239 \pm 0.025$ |
| Walking WLR (mg/h) | $4.8 \pm 1.1$ | $4.5 \pm 0.82$ |
| Walking Mass-dependent WLR (mg/h/mg) | $0.0538 \pm 0.015$ | $0.065 \pm 0.011$ |
| Recovery WLR (mg/h) | $2.426 \pm 0.32$ | $1.55 \pm 0.11$ |
| Recovery Mass-dependent WLR (mg/h/mg) | $0.0245 \pm 0.003$ | $0.0234 \pm 0.004$ |


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