

SHORT COMMUNICATION

Patterns of fuel use during locomotion in mammals revisited: the importance of aerobic scope

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ABSTRACT

Fuel selection patterns during exercise are thought to be conserved among sea-level native mammals when intensity is expressed relative to maximum aerobic capacity ($\dot{V}_{O_{2,max}}$). However, this claim is based on data from only a few species larger than rats, and has never been tested statistically. Thus, we investigated fuel use in a small mammal (*Mus musculus*, CD-1 strain), and combined these data with published data on rats, dogs, goats and humans to evaluate the robustness of the mammalian fuel selection model. We found that mice rely less on carbohydrates to power moderate intensity exercise at the same % $\dot{V}_{O_{2,max}}$ than larger mammals. We suggest that this difference is due to a decline in aerobic scope (O_2 available for exercise above resting metabolism) as body size decreases. We propose a redefined fuel use model that reflects changes in fractional aerobic scope with body size. Our results indicate that exercise defined as percent aerobic scope is a better predictor of fuel use across a wide range of quadruped species from mice to dogs and running humans.

KEY WORDS: Aerobic scope, Exercise, Carbohydrates, Respirometry, Aerobic capacity, Fuel use, Mammals

INTRODUCTION

Animals power aerobic exercise by using the appropriate mix of available fuels based on size of stores, ease of delivery to muscle, and capacity for membrane transport and oxidation. Early work in humans has shown that the mix of fuels used varies with exercise intensity (Edwards et al., 1934), and it is now well recognized that with increasing work rate the proportion of carbohydrates oxidized increases while lipids oxidation decreases (Brooks and Mercier, 1994). Changes in protein oxidation are generally not discussed because they contribute less than 5% of the total ATP production during exercise in the post-absorptive state (Carraro et al., 1994; Rennie et al., 1981). Surprisingly, this pattern of fuel use appears to be conserved across many mammalian species. Over the last 15 years, comparative physiologists examining fuel use in various mammals have found that the data converge on a general model where intensity of exercise, when expressed as a fraction of an individual's aerobic capacity (% $\dot{V}_{O_{2,max}}$), determines proportional fuel use (McClelland, 2004; Roberts et al., 1996; Weber and Haman, 2004). That is, an amalgamation of published means from rats, dogs, goats and humans has been used to propose that all mammals oxidize the same mixture of carbohydrates and lipids at any given intensity of exercise relative to $\dot{V}_{O_{2,max}}$. Even though it is quite remarkable that such a global pattern emerges in spite of the great

phylogenetic distances between species studied and wide variations in body mass and aerobic capacities, a statistical examination of the conserved nature of this model has not been performed. Moreover, current available data do not cover an extensive range of mammal species, nor do they extend the range to small body size.

Small mammals, such as mice, are routinely used as model species to study energetics and muscle metabolism. Yet, prior to our recent study on wild Andean mice (genus *Phyllotis*) (Schippers et al., 2012), fuel selection patterns had not been described in a mammal smaller than a rat. Because mass-specific resting metabolic rate increases as body size decreases (Kleiber, 1932), mice and other small mammals have a smaller aerobic scope (increase in oxidative metabolism from resting rates to $\dot{V}_{O_{2,max}}$) compared with larger mammals of comparable aerobic capacities (Bishop, 1999). Reduction in aerobic scope could have profound effects on fuel use patterns when expressed as a function of aerobic capacity because resting metabolic rates constitutes a much higher proportion of $\dot{V}_{O_{2,max}}$ in these small animals.

Extending the mammalian fuel use model to small mammals would provide a valuable hypothesis-testing tool for many popular experimental models including laboratory and wild rodents. For example, species of wild mice, especially those native to different altitudes (genera *Peromyscus* and *Phyllotis*), represent unique systems to investigate appropriate allocation of metabolic fuels and other metabolic adaptations to extreme environments of low pressure and temperature (Cheviron et al., 2014; Hayes and O'Connor, 1999; Nespolo et al., 2003; Russell et al., 2008; Schippers et al., 2012; Storz, 2007). In addition, a more comprehensive fuel use model could be used to test predictions regarding fuel use without the confounding influence of exercise intensity. For example, properly accounting for hypoxia-induced changes in resting \dot{V}_{O_2} and $\dot{V}_{O_{2,max}}$ is necessary to test the prediction that high-altitude mammals increase the reliance on carbohydrate oxidation as an oxygen-saving adaptation (15% ATP per mol of oxygen) for exercise in hypoxia (Brand, 2005; Daut and Elzinga, 1989; Welch et al., 2007).

To extend the current fuel use model to small mammals, we investigated fuel oxidation rates during exercise in laboratory mice (*Mus musculus*, CD-1 strain). We combined these data with those previously published on other mammalian quadrupeds [Wistar rats (McClelland et al., 1998), dogs and goats (Roberts et al., 1996)] and humans during treadmill exercise (Achten et al., 2003). We statistically evaluated the potentially conserved nature of mammalian fuel selection patterns. We tested the null hypotheses that small mammals show the same pattern of fuel use as a function of % $\dot{V}_{O_{2,max}}$ as other larger mammalian quadrupeds.

RESULTS AND DISCUSSION

In this study we found that, similar to other mammals, mice increase the use of carbohydrates as exercise intensity increases towards an aerobic maximum (Fig. 1A,B). However, the overall pattern of fuel use during exercise is different in mice compared to larger

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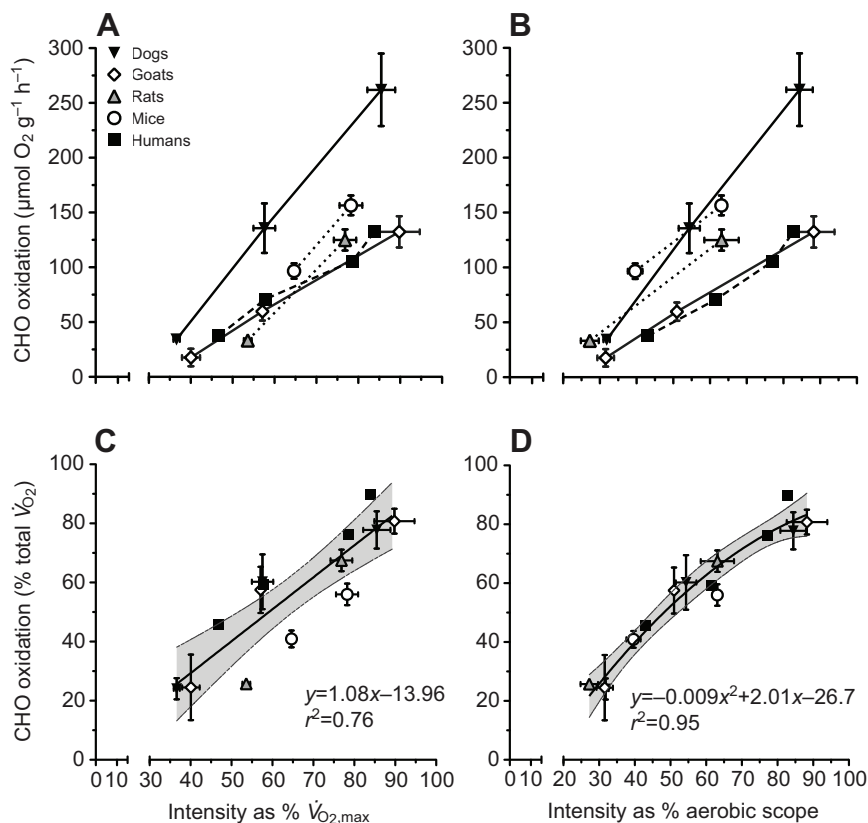


Fig. 1. Absolute rate of carbohydrate oxidation (CHO) and proportional CHO use relative to exercise intensities expressed as % aerobic capacity (% $\dot{V}_{O_2,max}$) and % aerobic scope. Absolute rates of carbohydrate oxidation (A,B) and percent of oxygen used for CHO oxidation (% \dot{V}_{O_2} ; C,D) with exercise intensities expressed as (A,C) % $\dot{V}_{O_2,max}$ [$(\dot{V}_{O_2,exercise}/\dot{V}_{O_2,max}) \times 100$] and (B,D) % aerobic scope [$(\dot{V}_{O_2,exercise} - \dot{V}_{O_2,resting}) / (\dot{V}_{O_2,max} - \dot{V}_{O_2,resting}) \times 100$], in CD-1 mice (present study), Wistar rats (McClelland et al., 1998), dogs and goats (Roberts et al., 1996), and humans during treadmill exercise (Achten et al., 2003). Data are shown as means \pm s.e.m. Bold lines in C and D represent the regression for all means, with 95% confidence intervals as the grey shaded areas between the dashed lines.

quadrupeds and to humans, as CD-1 mice were found to rely on carbohydrates to a lesser extent at submaximal exercise intensities (% $\dot{V}_{O_2,max}$; Fig. 1C). Mean % carbohydrate oxidation used by mice at both exercise intensities studied fell outside the 95% confidence intervals of the linear regression for data means from dogs, goats, rats, mice and humans. Moreover, the 95% confidence intervals of the linear regression generated from mice alone do not overlap with those from the larger mammals (dogs and goats). Interestingly, at low exercise intensities, published data from rats (McClelland et al., 1998) also deviate from the existing mammalian fuel use model, falling outside the 95% confidence interval of the linear regression for all species (Fig. 1C). The current model for mammal fuel use (McClelland, 2004; Weber, 2011) is not sufficient to describe exercise metabolism across a wide range of body size. This is likely due to reductions in aerobic scope as body size decreases, resulting in very low work efforts representing high % $\dot{V}_{O_2,max}$ in small mammals (see Table 1).

When considering the intensity of aerobic exercise, two definitions are important to understanding relative levels of effort.

Aerobic scope expresses the range of oxidative metabolism from rest to maximal aerobic exercise, whereas $\dot{V}_{O_2,max}$ is defined as the maximal aerobic capacity of an animal with no direct consideration given to basal metabolism. When exercise is expressed as a percentage of aerobic scope rather than % $\dot{V}_{O_2,max}$, an inclusive overall model of mammalian fuel use emerges (Fig. 1D). In fact, when % aerobic scope is used to define work intensity, the relative contribution of carbohydrate oxidation to total oxygen consumption (% total \dot{V}_{O_2}) for mice and rats falls within the 95% confidence intervals of the linear regression from all species (Fig. 1D). Regression analyses show that both normalisers for intensity (% aerobic scope and % $\dot{V}_{O_2,max}$) explain much of the variation in relative carbohydrate use, and with slopes significantly greater than 0 ($P < 0.001$). However, fractional carbohydrate use was more strongly correlated ($P < 0.001$) to exercise intensity relative to aerobic scope ($F = 165.6$, d.f. = 11, $r^2 = 0.952$, $P < 0.001$) than relative to $\dot{V}_{O_2,max}$ ($F = 38.5$, d.f. = 12, $r^2 = 0.762$, $P < 0.001$). This new and refined model is a better predictor of the fuel use during exercise because it takes into account differences in aerobic scope and extends across a wider

Table 1. Body mass, resting oxygen consumption (\dot{V}_{O_2}) and maximum aerobic capacity ($\dot{V}_{O_2,max}$) used to calculate aerobic scope for mice, rats, goats, dogs and humans

	<i>N</i>	Body mass (kg)	Resting \dot{V}_{O_2} (ml O_2 g $^{-1}$ h $^{-1}$)	$\dot{V}_{O_2,max}$ (ml O_2 g $^{-1}$ h $^{-1}$)	Aerobic scope (ml O_2 g $^{-1}$ h $^{-1}$)	Resting \dot{V}_{O_2} as % $\dot{V}_{O_2,max}$	Study
Mice	12	0.0385 \pm 0.0011	3.3 \pm 0.1	8.1 \pm 0.2	4.8 \pm 0.2	41	Present study
Rats	12	0.307 \pm 0.005	1.9 \pm 0.1	5.4 \pm 0.1	3.5 \pm 0.1	35	McClelland et al., 1998
Goats	4	30 \pm 3	0.504 ^a	4.1 \pm 0.1	3.6 \pm 0.1	12	Roberts et al., 1996
Dogs	3	25 \pm 2	0.612 ^a	8.8 \pm 0.2	8.1 \pm 0.2	7	Roberts et al., 1996
Humans	12	74 \pm 1	0.258 ^b	4.0 \pm 0.1	3.7	7	Achten et al., 2003

^aMean resting \dot{V}_{O_2} from Taylor et al. (Taylor et al., 1987).

^bResting \dot{V}_{O_2} for humans from Mazzeo et al. (Mazzeo et al., 1995).

range of body size from 0.039 kg mice to 74 kg humans (Table 1, Fig. 1D). The equation that describes this relationship can be used as a predictive tool of fuel use at any given exercise intensity for a variety of mammals spanning three orders of magnitude in body weight.

Aerobic scope is generally reduced in small compared with large mammals because mass-specific resting (Kleiber, 1932) and maximal metabolic rates (Taylor et al., 1987) increase as body mass declines, but with different slopes. Resting \dot{V}_{O_2} therefore represents a higher fraction of $\dot{V}_{O_{2,max}}$ in small mammals compared with larger mammals. For example, the resting metabolism of humans, dogs and goats represents only ~10% of their $\dot{V}_{O_{2,max}}$. In contrast, rats and mice at rest are at 35 and 41% of their $\dot{V}_{O_{2,max}}$, respectively (see Table 1). This difference in aerobic scope is particularly apparent when comparing large and small mammals at low exercise intensities, where 50% $\dot{V}_{O_{2,max}}$ may correspond to running in a dog but slow walking in a mouse. It is not surprising that smaller mammals use a lower proportion of carbohydrates compared with larger mammals when intensity of exercise is expressed relative to $\dot{V}_{O_{2,max}}$. Most studies on exercise metabolism fail to measure resting metabolic rates. In addition, true resting metabolism can be difficult to obtain, leading to underestimated aerobic scopes. Future studies should aim to carefully determine either resting or basal metabolic rates to accurately evaluate individual aerobic scopes.

Conclusions

This study proposes a new model of fuel use for exercising mammals, where exercise intensity relative to aerobic scope (in lieu of $\dot{V}_{O_{2,max}}$) determines the proportional contribution of carbohydrate and lipid oxidation to total energy expenditure. Unlike the previous cross-species model first proposed by Roberts and colleagues (Roberts et al., 1996), and more recently endorsed by others (McClelland, 2004; Weber and Haman, 2004; Weber, 2011), this pattern promises to hold across a wider range of body size, including mice and potentially even smaller mammals. We predict that this new model will serve as a useful hypothesis-testing and predictive tool for comparative and evolutionary physiologists, but also as a translational tool to compare data across mammals.

MATERIALS AND METHODS

Study design

All procedures were approved by the McMaster University Animal Research Ethics Board. Outbred male CD-1 mice were obtained at 6 weeks of age (Charles-River, Wilmington, MA, USA). Mice ($N=12$) were kept under a 12 h:12 h light:dark cycle with the lights coming on at 07:00 h. All mice were subjected to the following *in vivo* experimental measurements using a respirometry system and metabolic chamber as previously described (Schippers et al., 2012): (1) $\dot{V}_{O_{2,max}}$, and after at least 48 h recovery, (2) rates of oxygen consumption (\dot{V}_{O_2}) and carbon dioxide production (\dot{V}_{CO_2}) at rest in a 600 ml metabolic chamber or during exercise at 65 and 80% of individual $\dot{V}_{O_{2,max}}$ in a rodent treadmill (~800 ml) using a flow-through respirometry system (Sables Systems, Las Vegas, NV, USA) as previously described (Schippers et al., 2012). These submaximal exercise measurements were performed in a random order between 12:00 and 19:00 h following a 6 h fast. Mice were not fasted before the $\dot{V}_{O_{2,max}}$ trials. Each mouse was never subjected to more than one measurement in a single day.

Exercise and resting protocols for mice

$\dot{V}_{O_{2,max}}$ was determined using criteria previously described (Schippers et al., 2012) by increasing the speed of the treadmill by 3 m min⁻¹ every 2 min from an initial speed of 7 m min⁻¹ at a constant angle of 10 deg. Submaximal exercise intensities were determined from the relationship between \dot{V}_{O_2} and speed but also by adjusting treadmill speed in real time to maintain the target \dot{V}_{O_2} . We used data from at least 5 min of recordings between the fifth and

20th minute of exercise from each mouse to calculate group means. Data from five of the 12 mice were not used at 65% $\dot{V}_{O_{2,max}}$ because behaviours in addition to running at this low intensity caused high and non-steady-state \dot{V}_{O_2} .

Calculations and statistical analyses

\dot{V}_{O_2} , \dot{V}_{CO_2} and rates of carbohydrate and lipid oxidation were calculated as previously described (Withers et al., 1977; Schippers et al., 2012) using the indirect calorimetry equations of Frayn (Frayn, 1983). We used raw data obtained from previously published studies on rats (McClelland et al., 1998), dogs and goats (Roberts et al., 1996) and for comparison, means obtained for moderately trained humans (men) during treadmill running (Table 1) because fuel use differs between bicycle and treadmill exercise in humans (Achten et al., 2003). We used individual $\dot{V}_{O_{2,max}}$ and resting \dot{V}_{O_2} values for each rat and mouse (Table 1). We used previously published mean resting \dot{V}_{O_2} values for same-sized dogs and goats (Taylor et al., 1987) in the calculation of aerobic scope of each individual. For humans, we used representative resting \dot{V}_{O_2} for men similar in age, weight and training status to the exercise data (Mazzeo et al., 1995). Exercise intensity as a fraction of aerobic scope was determined according to the equation:

$$\% \text{ Aerobic scope} = \frac{(\dot{V}_{O_2, \text{exercise}} - \dot{V}_{O_2, \text{resting}})}{\text{Aerobic scope}} \times 100, \quad (1)$$

where aerobic scope = $\dot{V}_{O_{2,max}} - \dot{V}_{O_{2,resting}}$. We performed regression analysis of carbohydrate oxidation with relative exercise intensity expressed as % $\dot{V}_{O_{2,max}}$ and as % aerobic scope. Both linear and quadratic polynomial regressions were fitted to group means to obtain predictive equations for these five species. Changes in coefficients of determination (r^2) were found to be statistically significant between linear and polynomial regressions for % carbohydrate (CHO) use by % aerobic scope ($P=0.03$) but not for % $\dot{V}_{O_{2,max}}$ (F -test). Multiple regression and an F -test were used to determine significant differences in r^2 between the regressions between % CHO use and % $\dot{V}_{O_{2,max}}$ and % aerobic scope. Values are presented as means \pm s.e.m.

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Competing interests

The authors declare no competing financial interests.

Author contributions

M.-P.S. and G.B.M. designed the experiment; M.-P.S. and C.L.M. collected the data; M.-P.S. analyzed the data and wrote the manuscript; G.B.M. and C.L.M. edited the manuscript.

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