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SYMPOSIUM

Fuel Use in Mammals: Conserved Patterns and Evolved Strategies for Aerobic Locomotion and Thermogenesis

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Synopsis Effective aerobic locomotion depends on adequate delivery of oxygen and an appropriate allocation of metabolic substrates. The use of metabolic substrates during exercise follows a predictive pattern of lipid and carbohydrate oxidation that is similar in lowland native cursorial mammals. We have found that in two highland lineages of mice (*Phyllotis* and *Peromyscus*) the fuel use pattern is shifted to a greater reliance on carbohydrates compared to their lowland conspecifics and congenerics. However, there is variation between lineages in the importance of phenotypic plasticity in the expression of this metabolic phenotype. Moreover, this metabolic phenotype is independent of running aerobic capacity and can also be independent of thermogenic capacity. For example, wild-caught mice from a highland population of deer mice (*Peromyscus maniculatus*) housed in warm normoxic laboratory conditions maintain higher maximum cold-induced oxygen consumption in acute hypoxia than lowland congenerics, but shivering and non-shivering thermogenesis is supported by high rates of lipid oxidation. This is reflected in the consistently higher activities of oxidative and fatty acid oxidation enzymes in the gastrocnemius of highland deer mice compared to lowlanders, which are resistant to hypoxia acclimation. While a fixed trait in muscle aerobic capacity may reflect the pervasive and unremitting low PO_2 at high altitudes, muscle capacities for substrate oxidation may be more flexible to match appropriate substrate use with changing energetic demands. How shivering thermogenesis and locomotion potentially interact in the matching of muscle metabolic capacities to appropriate substrate use is unclear. Perhaps it is possible that shivering serves as "training" to ensure muscles have the capacity to support locomotion or visa-versa.

Introduction

The ability to effectively engage in exercise is a performance trait that is a potential target of natural selection (Dalziel et al. 2009). Locomotion takes many forms, including bouts of burst or sprint exercise powered by rapid rates of ATP production independent of O_2 supply (i.e., anaerobic). On the other hand, for long-term aerobic locomotion the coordinated supply of sufficient O_2 and the appropriate allocation of metabolic substrates are necessary for effective and homeostatic ATP production. Currently, there are many unknowns concerning how substrate use has evolved for aerobic locomotion in mammals. We have very little information on how variation in substrate allocation during exercise may influence performance across species, populations, or between environments. It is also unclear how patterns of fuel use with locomotion compare to fuel use during another task of equivalent metabolic rate, such as thermogenesis. Subordinate traits such as selective recruitment of metabolic pathways in muscle fibers may directly relate to performance, be it locomotion or shivering thermogenesis. There may also be interactions between the capacity for effective exercise and capacity to thermoregulate that influence skeletal muscle phenotype. Finally, we do not yet understand what trade-offs or benefits may have shaped the evolution of these metabolic strategies. This review will discuss current research and the many unanswered questions regarding the variation in aerobic ATP production across species, populations, and between environments.

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Variation in fuel allocation in mammals and across environments

The variation in capacity for oxygen delivery from the atmosphere to working muscle mitochondria has been thoroughly studied (e.g., Taylor et al. 1981). These studies have been inspired by early observations of the allometric scaling of metabolic rate and body mass (Klieber 1932), and later by work examining the adaptive variation in aerobic exercise capacity between species of similar body mass (Taylor et al. 1987). This natural variation in aerobic capacity across mammals allowed researchers to determine structural and functional capacities for flux at the various steps in O_2 transport, and correlate these with maximal flux through the entire pathway (Weibel et al. 1992). One of the reasons this was possible for the O_2 cascade is that the pathway for O_2 from source to sink is somewhat linear, flux at each step is equivalent, and tissue stores of O_2 are relatively small for most terrestrial mammals (Taylor et al. 1996).

Implicit to aerobic ATP production is a commensurate flow of electrons along the electron transport chain that combine with O_2 at complex IV and produce H_2O. Thus, there must be a coordinated upregulation of both O_2 and metabolic substrate flux to support the large-scale changes in ATP turnover rates that occur in muscle and other tissues. Surprisingly, compared to O_2 flux there is considerably less known regarding the evolution of substrate flux pathways across exercising mammals. One possible explanation for this dearth of information is that pathways for metabolic substrate delivery are arranged as a network consisting of four major storage depots and four substrate pathways that converge to contribute to mitochondrial ATP production in the working tissue (Roberts et al. 1996; Taylor et al. 1996; McClelland 2004; Weber 2010). In the post-absorptive state, working muscles can draw upon the ample lipid stores in adipose tissue or lipids stored in myocytes as intramuscular triglycerides (IMTG). Carbohydrate stores are more limited, located mainly as liver and muscle glycogen (Weber 1992). This makes the investigation of the capacities for flux at specific steps of the substrate supply cascade difficult to assess and to compare with maximal rate of their oxidation.

Both the intensity and duration of exercise interact to influence the allocation of substrates, both in terms of absolute flux and in their proportional use (Bock et al. 1928; Felig and Wahren 1975; Brooks and Mercier 1994; Roberts et al. 1996; Weber 2010; Schippers et al. 2014). Longer running bouts at low intensity are accompanied by a progressive increase in lipid oxidation and a decrease in carbohydrate use, as more free fatty acids (FFA) become available (Wolfe et al. 1990), and likely reflect a refractory period as exercise begins for the mobilization and transport of FFA to mitochondria to reach sufficient rates to meet energetic demand. As work rate increases, lipid oxidation alone cannot sustain sufficiently high rates of ATP supply, and carbohydrates become the predominate fuel (Romijn et al. 1993; Brooks and Mercier 1994; Weber 2010; Schippers et al. 2014).

This phenomenon has been extensively studied in humans but more recent work in other mammals show that the absolute flux of substrates vary considerably across exercising mammals. Indeed, rates of carbohydrate and lipid oxidation can vary by an order of magnitude between species running at equivalent exercise intensities (Schippers et al. 2014). Due to the relationship between intensity and duration with substrate oxidation, it is essential that comparisons be made using standardized work rates (e.g., as % VO_2max or % aerobic scope) and durations. To understand if relative allocations of different fuel types vary between species one must ask if each fuel type supports equivalent proportions of total metabolism. This has been termed the relative mix of fuels supporting aerobic ATP production (Weber 2010). When expressed in this way it becomes clear that variation in aerobic capacity is matched with equivalent variation in absolute rates of carbohydrate and lipid oxidation during submaximal exercise. Comparisons made at the same relative exercise intensity (as % aerobic scope = ((VO_2exercise − VO_2rest)/VO_2max − VO_2rest)) × 100; Schippers et al. 2014), show that absolute rates of carbohydrate oxidation scale with species' aerobic capacity (Fig. 1). When carbohydrate use is expressed as a percentage total VO_2 to correct for differences in metabolic rate, proportional use is independent of aerobic capacity (e.g., all species used the same % carbohydrates to power moderate exercise). Thus, variation in fuel use can be explained by differences in VO_2max rather than by distinct metabolic strategies not linked to aerobic capacity (Fig. 1), at least across the subset of mammalian species tested so far (Schippers et al. 2014). This remarkable conservation of relative fuel use suggests the basis for a predictive model for exercising mammals.

It is unclear why exercise fuel use is so predictable in some mammals. One explanation involves the energetic properties of the available stored substrate supplies used to support locomotion. There exist energetic trade-offs between these fuels in supporting

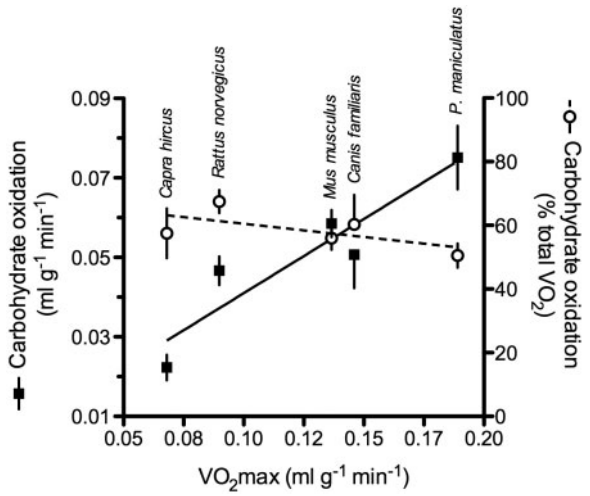

Fig. 1 Maximum oxygen consumption (VO_2max) during forced treadmill running compared to the absolute carbohydrate oxidation (in mL/g/min) and the percentage of total oxygen consumption used for carbohydrate oxidation (% total VO_2) during submaximal aerobic exercise at equivalent intensities relative to VO_2max in dogs (*Canis familiaris*) and goats (*Capra hircus*) (Roberts et al. 1996), rats (*Rattus norvegicus*) (McClelland et al. 1998), mice (*Mus musculus*) (Schippers et al. 2014), and deer mice (*Peromyscus maniculatus*) (Lau et al. 2017). Linear regression of aerobic capacity (VO_2max) with absolute carbohydrate oxidation showed a strong correlation ($r^2 = 0.86$) and a slope significantly different from zero ($P = 0.02$). When aerobic capacity was regressed against relative carbohydrate oxidation as % total VO_2, there was a weak correlation ($r^2 = 0.41$), and the slope of this relationship was not significantly different from zero ($P = 0.244$).

degrees of work and for different durations. Lipids are highly reduced (e.g., 34–38 kJ/g versus 4.2 kJ/g wet mass, or 17.5 kJ/g dry mass, for glycogen) and are a major depot of stored energy (Roberts et al. 1996; Weber 2010). However, for mammals, lipids can only sustain relatively low ATP synthesis rates, which limits their ability to maintain ATP homeostasis at high work rates. On the other hand, mammals are able to support work rates with very high ATP turnover rates using carbohydrates. However, carbohydrates stores are relatively small in most mammals (~7500 kJ compared to 600,000 kJ for lipids in humans, representing ~1% and 80% total energy reserves; Newsholme 1982), and are unable on their own to support exercise for very long durations (Fournier and Weber 1994; Weber 2010).

Since the use of abundant lipids stores is restricted to low and moderate exercise intensities, it has been suggested that an advantage of a high VO_2max is that faster running speeds represent low percentages of VO_2max, intensities that rely on a significant involvement of lipids (Roberts et al. 1996). In contrast, species with lower VO_2max running at the same speed would be exercising at relatively high percentage of VO_2max supported, to a large extent, by the valuable and limited carbohydrate stores. This may provide a selective advantage for highly aerobic species that rely on long duration low-intensity locomotion for capturing prey and for foraging.

Is the pattern of fuel use with exercise truly invariant across all mammalian species? Available data span a limited number of species and draw principally upon studies of laboratory animals (Fig. 1). However, the predictive pattern of fuel use may not hold for species or populations living in extreme environments. In other words, selective pressures for an alternative allocation pattern that disentangles fuel use from aerobic capacity may occur to improve performance and provide a selective advantage in these environments. For example, high altitude offers an extreme environment where selection for efficient use of oxygen might shift fuel use to a more carbohydrate-based strategy. Both theoretical (Brand 2005) and empirical evidence (Welch et al. 2007) support the O_2-saving advantage of glucose oxidation for aerobic ATP production relative to lipids.

To test this hypothesis we undertook a comparative study using two low-altitude and two high-altitude mouse species from the genus *Phyllotis* native to Peru. We reduced the potential influence of adult phenotypic plasticity by housing the wild-caught mice at sea level in common conditions and diets for several weeks. We also controlled for the effect of exercise intensity on fuel use by standardizing work rates to each individual's running VO_2max. We found using a phylogenetically-based analysis that when exercising at the same moderate running intensity in hypoxia, high-altitude native mice used carbohydrates at a higher absolute rate, resulting in greater relative use compared to their low altitude congenerics (Schippers et al. 2012). These results suggest a putative metabolic adaptation to high altitude as a result of genetic differences between species. However, we cannot rule out the influences of developmental and phenotypic plasticity in our experiments. For example, it is possible that *Phyllotis* species vary in their acclimation response to the common conditions in captivity that do not reflect a reversal of any acclimatization experienced in their native environments. We also studied exercise metabolism in another mouse lineage, the North American deer mouse (*P. maniculatus*). We found in first generation mice born and raised in common warm normoxic laboratory conditions that lowland and highland ancestry mice had similar relative use of lipids and carbohydrates when exercising at a common submaximal running intensity. However, when these mice were acclimated for several weeks

to hypobaric hypoxia, the lowland native mice showed no change in fuel use—similar to laboratory rats (McClelland et al. 1998), but in the highland deer mice there was a shift to a more carbohydrate-based exercise metabolism (Lau et al. 2017). This is tantalizing evidence suggesting that convergence to a similar exercise fuel use strategy is the product of either genetically fixed or phenotypically plastic traits, depending on the high altitude lineage. Future studies should fully test this hypothesis by examining both of these lineages of wild mice *in situ* in their native environments. These data are some of the very few examples showing fuel selection that is independent of aerobic capacity (Fig. 2).

What about more sustained activities other than exercise that elevate metabolic rate and necessitate increased fuel use above resting levels, such as thermogenesis? Given the limited availability of stored carbohydrates, long-term heat production is likely not sustainable using high rates of glucose oxidation. Indeed, moderate rates of shivering thermogenesis are supported by a mix of lipid and carbohydrate oxidation in both rats and humans (Weber and Haman 2005; Vaillancourt et al. 2009). Small mammals have a particularly difficult task of maintaining body temperature due to their high surface area to volume ratios and reduced capacity for insulation compared to larger mammals. These small endotherms thermoregulate using both shivering thermogenesis by skeletal muscle and non-shivering thermogenesis occurring principally in brown adipose tissue (BAT) but also in other tissues (i.e., beige or brite fat; see Cohen and Spiegelman 2015). BAT has a large store of triglycerides (TG) that are mobilized to support heat production by this tissue (Cannon and Nedergaard 2010). The importance of the different thermo-effector organs to total heat production varies depending on many factors, including body size and thermal history, but shivering can contribute up to a remarkable 70% of total thermogenesis in wild *Phyllotis xanthopygus* from 2529 m, housed briefly in warm and normoxia, and then cold acclimated for 1 month to 15 °C (Nespolo et al. 1999). Shivering and non-shivering thermogenesis are highly aerobic and challenging for small mammals living at high altitude where there is a decreased oxygen availability. High altitude environments are also very cold compared to lowland environments of similar latitudes. For example, the average annual minimum (−6.4 °C) and average winter (−13.8 °C) temperatures experienced by deer mice at the summit of Mt Evans (Western Regional Climate Centre) are much colder and those experienced by lowland deer mice from the plains of

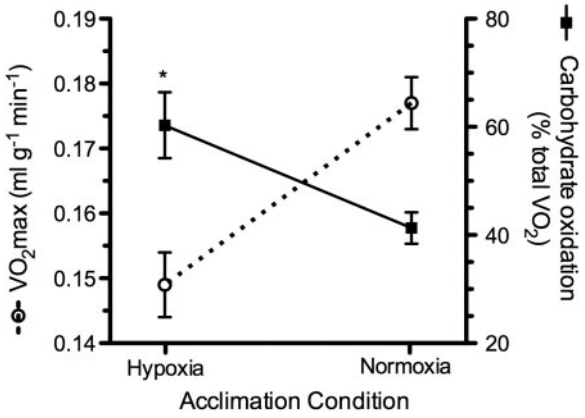

Fig. 2 Maximum oxygen consumption (VO_2max) in normoxia and hypoxia during forced treadmill running and the percentage of oxygen consumption for carbohydrate oxidation (% total VO_2) during exercise at 75% VO_2max in F1 laboratory-born and raised high-altitude native deer mice with reaction norms depicting changes with acclimation to normoxia and to hypoxia (Lau et al. 2017). *indicates a significant difference from normoxic controls.

Nebraska (Velotta et al. 2016). A high selective pressure for elevated thermogenesis in hypoxia may drive organismal aerobic capacity at altitude. Indeed, mark and recapture studies at high altitude have shown higher winter survivability in wild deer mice with greater cold-induced maximal oxygen consumption (VO_2summit) in hypoxia (Hayes and O'Connor 1999). Moreover, wild, high altitude *P. maniculatus* acclimated to warm normoxic laboratory conditions for 6 weeks have higher VO_2summit in acute hypoxia than low altitude native mice from the same or different species (*Peromyscus leucopus*) held under the same conditions (Cheviron et al. 2012). This was also true when wild highland deer mice were compared to their wild lowland congenerics in their respective environments, and in the first generation (F1) descendants born and raised in captivity in common-garden conditions of warm and normoxia (Cheviron et al. 2014). Similar results have been seen in deer mouse strains developed to carry distinct α-haemoglobin haplotypes generally fixed at either low or high altitude and acclimated to 340 m and 3800 m (Chappell and Snyder 1984).

It is unclear what substrates support this higher thermogenic capacity in highland deer mice, but respiratory exchange ratios (RER = VCO_2/VO_2) approach 0.70 at VO_2summit in both wild-caught lowland and highland deer mice acclimated to warm normoxic conditions and tested in acute hypoxia (Cheviron et al. 2012) suggesting a high reliance on lipids. This high reliance on lipids to support heat production is independent of thermogenic capacity with consistent RER values across very different

VO_2summit (Cheviron et al. 2012; Fig. 3). Thus, the greater aerobic capacity in high altitude mice would be accompanied by higher rates of lipid oxidation to support these high rates of heat production. This is consistent with observed population differences in muscle transcriptional profiles and biochemical phenotypes showing distinct capacities to support lipid-fueled shivering thermogenesis (Cheviron et al. 2012, 2014; Lau et al. 2017; see below).

Variation in muscle phenotype and regulation of fuel use

Skeletal muscles are the primary consumers of oxygen and substrates during exercise (Armstrong et al. 1992; Weibel and Hoppeler 2005) and can contribute between 38% and 70% of total thermogenesis as shivering thermogenesis in high altitude native mice (Nespolo et al. 1999; Van Sant and Hammond 2008). Thus, whole animal fuel use patterns may reflect variation in features of skeletal muscle involved in substrate oxidation. The order in which muscles are recruited to support rising work rates may explain patterns of fuel use across exercise intensity (Roberts et al. 1996; McClelland 2004). Thus, if recruitment patterns of homologous muscles are conserved across species, populations, and environments, examining variation in phenotypic traits of these muscles may be sufficient to understand variation in exercise metabolism. Muscle substrate use may be regulated at many different levels: mobilization from external or intramuscular stores, convective transport in the circulation, membrane transport into cells and into the mitochondria. However, the best understood mechanisms involve the selective recruitment of intracellular pathways. Data on muscle metabolic phenotypes in species where fuel use has also been measured are rare. When species and populations of mice from low and high altitude were compared they showed distinct correspondence of muscle phenotype to whole animal exercise fuel use. These comparisons are informative to understand if variation in capacity for fuel use at the muscle fiber level can be explained by either a change in biochemical capacity (hierarchical regulation) or by altered regulation of existing metabolic machinery (metabolic regulation) (Suarez and Moyes 2012). In other words, one might expect the increased reliance on carbohydrate use with exercise in high altitude native mice to be accompanied with increases in muscle capacity for glucose oxidation. Surprisingly, we saw no differences in the apparent maximal activity (Vmax) for four enzymes in glycolysis between low and high altitude species of the genus *Phyllotis*. Nor

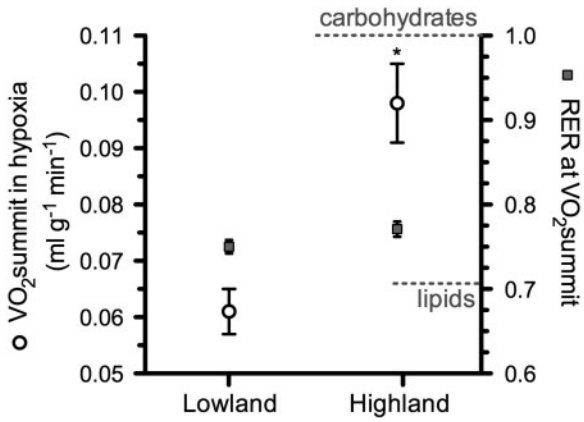

Fig. 3 Cold-induced rates of maximum oxygen consumption (VO_2summit) in acute hypoxia for wild-caught deer mice from lowland and highland populations acclimated for 6 weeks to normoxic laboratory conditions (Cheviron et al. 2012). Respiratory exchange ratios (RER = VCO_2/VO_2) were determined at VO_2summit. Dotted lines denote RER values representing 100% carbohydrate-based or 100% lipid-based aerobic metabolism. *indicates a significant difference from normoxic controls.

were there any differences in the aerobic capacity of the gastrocnemius muscle as determined using citrate synthase and cytochrome oxidase activities (Schippers et al. 2012). Therefore, for wild-caught Andean mice acclimated to normoxic laboratory conditions, muscle phenotype does not correlate with whole-animal exercise fuel use. These data also suggest that species-specific metabolic regulation of muscle carbohydrate use contributes to their distinct exercise fuel use strategies. It would be informative to examine these species in their native environments, as well as after rearing in common conditions to understand the relative roles of genetic specialization and phenotypic plasticity of muscle traits in relation to organismal performance. In contrast to *Phyllotis* species, when we examined the same muscle in deer mice, we found that tissue aerobic capacity appears to be genetically fixed at a higher level in high-altitude native mice as a result of higher type I and type IIa fiber area, and that this phenotype was unaffected by hypoxia acclimation (Lui et al. 2015; Lau et al. 2017). High-altitude deer mice born and raised in captivity at sea level also exhibit higher mitochondrial volume densities in aerobic fibers of the gastrocnemius, when compared to sea-level mice, and hypoxia acclimation did not alter this trait in either population (Mahalingam et al. 2017). However, other metabolic properties of the muscle appear to be more labile. The increase in carbohydrate use seen during exercise after hypoxia acclimation in highland mice was accompanied by

increased capacity for muscle glucose uptake and phosphorylation (Lau et al. 2017). Interestingly, in highland mice the more aerobic muscle phenotype is accompanied by higher capacity for β-oxidation of FFA (Cheviron et al. 2012; Lui et al. 2015; Lau et al. 2017). This phenotype seems to be a hallmark of high altitude deer mice and is reflected in the transcriptional plasticity differences from low altitude mice observed in wild mice *in situ*, after 6 weeks in common laboratory conditions, and in F1 descendants of these wild parents born and raised at sea level (Cheviron et al. 2012; Cheviron et al. 2014; Scott et al. 2015). The higher thermogenic capacities in hypoxia seen in highland mice were found to be attributable, in part, to up-regulation of transcription for muscle genes involved in lipid metabolism and mitochondrial respiratory capacities (Cheviron et al. 2012, 2014).

Effects of multiple natural stressors *in situ* on fuel use and muscle phenotype

It is unclear how hypoxia and cold may interact at high altitude to affect muscle metabolic capacities for substrate use or in shaping how mammals power exercise and thermogenesis. Moreover, information is lacking on the intensity that wild mammals may exercise when behaving naturally *in situ*. For example, do highland mammals move around at moderate intensities and use carbohydrates at the relatively high rates compared to lowlanders as has been measured during forced treadmill exercise? Field metabolic rates (FMR) have shown that highland and lowland deer mice have similar hourly metabolic costs in their native environments at similar environmental temperatures (Hayes 1989). However, FMR cannot distinguish between locomotion and thermogenesis. There are obvious trade-offs for exercising mammals at high altitude, since an increased reliance on carbohydrates risks the depletion of this valuable and limited energy store. Indeed we found that after only 15 min of moderate treadmill exercise at an intensity of 75% VO_2max, high altitude deer mice showed a significant reduction in muscle glycogen (Lau et al. 2017). In fact, when total carbohydrate stores are estimated from resting concentrations in liver and muscle (extrapolating from gastrocnemius assuming muscle mass is 60% of body mass), the 260 μmoles total glucose could sustain a carbohydrate oxidation rate of ~9.5–12 μmoles of glucose per min at 75% VO_2max for approximately 20–25 min (Lau et al. 2017). However, this estimate assumes that all the carbon is lost upon glucose catabolism, which is unlikely, as some will appear as lactate to be recycled back into glycogen. Indeed, livers of high altitude deer mice have a higher aerobic capacity and a trend for higher phosphoenolpyruvate carboxykinase activities compared to low altitude mice (Lui et al. 2017), suggesting that gluconeogenesis may be enhanced in this population. This would increase the ability to supply circulating glucose, an important energy source for running mice. In support of this notion, studies have found that genetically modified laboratory mice lacking the muscle isoform of glycogen synthase do not have impaired endurance (Pederson et al. 2005) suggesting that the ability to use circulating glucose independent of muscle glycogen breakdown is very beneficial.

Regardless of these estimates of exercise endurance, it is unknown if mice in the wild perform at this level of exercise or for how long. Clues into the locomotory habits of wild mice *in situ* come from studies of voluntary locomotion using running wheels in captive mice. When voluntary wheel running was assessed in captive deer mice, 2–3 generations removed from a wild highland population (3500–3900 m), mice were observed to run at submaximal intensities equivalent to 62–73% VO_2max (Chappell et al. 2004). The predictive model for mammal fuel use suggests that these mice would power wheel running with a roughly equal mix of lipids and carbohydrates, at least if in a post-absorptive state (Schippers et al. 2014). These mice were also observed to display an activity pattern of several exercise bouts of very short durations that resulted in many hours of running per night. They were also feeding and these data suggest that the availability of carbohydrates for exercise does not limit their locomotory behavior.

What about the high capacity for lipid oxidation observed in the muscles of high altitude deer mice that support shivering thermogenesis (e.g., Cheviron et al. 2012)? How might this metabolic phenotype impact locomotion that relies more heavily on carbohydrate metabolism? The answer may lie in how the animals recover from exercise. Studies have shown that reductions in IMTG can be modest over the course of an exercise bout. However, in recovery there is a significant decline in IMTG that mirrors the repletion of muscle glycogen stores (Kiens and Richter 1998; Van Loon 2004). These data suggest that lipid oxidation provides ATP necessary for the energy consuming reactions of glycogen formation from plasma-borne glucose (Bangsbo et al. 1991). An increased capacity for rapid ATP production by catabolizing lipids in recovery would be beneficial for the activity patterns observed in deer mice during voluntary wheel running. This leads to the enticing

idea that cold exposure may enhance not only heat generation, but also that chronic shivering may "train" skeletal muscle for enhanced locomotor capacity. Cold acclimation does increase muscle capillarity (guinea pigs, Sillau et al. 1980) and aerobic capacity in some laboratory models (rats, Harri and Valtola 1975) but not others (CD-1 mice, Beaudry and McClelland 2010). It remains to be seen if altitude ancestry impacts the cold acclimation response and how this may influence exercise metabolism in different populations of deer mice.

Exercising in the cold and hypoxia

How might environmental variables affect exercise and influence substrate use or locomotory endurance? Specifically, if adaptation or acclimation to chronic cold increases aerobic capacity, does this help animals operate at a low %VO_2max during routine locomotory activities? Conversely, does metabolic heat generated during exercise substitute for thermogenesis and minimize the energetic cost of these combined tasks? When directly compared, VO_2summit elicited by cold and exercise-induced VO_2max are equivalent in mice or rats kept in normoxic and warm laboratory conditions (Chappell 1984; Conley et al. 1985; Chappell and Hammond 2004; but see Hayes and Chappell 1986, where VO_2max < VO_2summit and Seeherman et al. 1981, where VO_2max > VO_2summit). VO_2summit has also been found to be ~15% higher than VO_2max in captive-bred *P. leucopus* housed in warm and normoxic conditions (Segrem and Hart 1967). Acclimation to chronic cold can have distinct influences on VO_2summit and running VO_2max. It has been repeatedly shown that cold acclimation increases VO_2summit in small mammals such as mice. For example, cold acclimation in deer mice can increase VO_2summit by a dramatic 65% (Chappell and Hammond 2004). Other studies also find high plasticity in thermogenic capacity to chronic cold with increases ranging from 30–70% in *Peromyscus* species in laboratory and field studies (Wickler 1981; Hayes and Chappell 1986) and by 94% in *P. xanthopygus* with laboratory acclimation to cold (Nespolo et al. 1999). These increases in aerobic capacity appear to be driven by changes in both shivering and nonshivering thermogenesis (Nespolo et al. 1999). Few studies have examined how cold acclimation affects running VO_2max or compared it to VO_2summit. In general, cold acclimation has been found to increase VO_2max in some studies (Turner et al. 1995) but not others (Chappell and Hammond 2004), and to a lesser extent than cold acclimation affects VO_2summit (Hayes and Chappell 1986). On the other hand, endurance training, while effective in increasing running VO_2max, can have little impact on thermogenic capacity, as seen in laboratory rats (Conley et al. 1985). These data suggest that in cold acclimated mice, central O_2 and substrate supply systems are sufficient to support much higher aerobic metabolic rates than during exercise VO_2max, and that maximal aerobic locomotion is possibly limited by peripheral factors such as muscle respiratory capacity.

A study looking at voluntary wheel running in both warm and cold shows that exercise is partially substitutive for thermogenesis in deer mice (Chappell et al. 2004). When running in the cold, deer mice had higher VO_2 at low exercise intensities but this increase in energy expenditure was lower than expected if thermoregulatory costs were additive to locomotory costs. Interestingly the net cost of transport (COT = slope of VO_2 versus speed) declined with running at lower temperatures, so that it was less costly for mice running in the cold to increase speed than those running in warm conditions. Although seemingly advantageous, the absolute cost of locomotion overall remains higher in the cold (Chappell et al. 2004). At high altitude, mice are running in both cold and hypoxia. Work looking at the energetics of voluntary wheel running in wild least chipmunks (*Tamias minimus*) at both moderate and high elevations shows a lower net COT at higher altitudes (Chappell and Dlugosz 2009). Interestingly, this study also looked at another larger species, the golden-mantled ground squirrel (*Spermophilus lateralis*), which did not show the same effect of altitude on running energetics. It is possible that COT reflects a change in the substrate use since an increase in carbohydrate oxidation would decrease the VO_2 at any work rate (Welch et al. 2007). However, we have recently reported that F1 laboratory-reared high altitude deer mice have a lower COT than low altitude mice that is independent of fuel use (Lau et al. 2017). This suggests a biomechanical explanation, but does not rule out a role for other metabolic explanations for increased locomotory efficiency.

Conclusions

The characteristics of lipids and carbohydrates make them appropriate fuel sources for different activities, work intensities, and environments in mammals. This disparity provides opportunity for selection to act on metabolic processes under extreme environmental conditions such as those found at high altitude. We have shown that mice native to high altitude diverge from the predicted pattern of fuel

use based on aerobic capacity. The preference for carbohydrates during submaximal running in lineages of high altitude mice is a potential example of convergent evolution, driven by both fixed genetic traits and phenotypic plasticity of the substrate flux pathways. However, until studied comparing different highland lineages both *in situ* and after 1–2 generations bred in common garden conditions, it is premature to suggest this trait as an adaptation common to highland mammals. The dual roles of skeletal muscle in both locomotion and shivering thermogenesis add another layer of complexity to this story. We suggest that the enhanced capacity for lipid oxidation in high altitude deer mice that supports shivering may also prepare skeletal muscle for carbohydrate-fueled locomotion by enhancing recovery from intense exercise.

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