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Metabolic recovery from submaximal exercise in hypoxia acclimated high altitude deer mice (*Peromyscus maniculatus*)

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ABSTRACT

Animals living at high-altitude are faced with unremitting low oxygen availability. This can make it difficult to perform daily tasks that require increases in aerobic metabolism. An activity important for survival is aerobic locomotion, and the rapid recovery of muscle metabolism post exercise. Past work shows that hypoxia acclimated high-altitude mice (*Peromyscus maniculatus*) have a greater reliance on carbohydrates to power exercise than low altitude mice. However, it is unclear how quickly after aerobic exercise these mice can recovery and replenish muscle glycogen stores. The gastrocnemius muscle of high-altitude deer mice has a more aerobic phenotype and a greater capacity to oxidize lipids than low altitude deer mice. This suggests that high altitude mice may recover more rapidly from exercise than their lowland counterparts due to a greater capacity to support glycogen replenishment using intramuscular triglycerides (IMTG). To explore this possibility, we used low- and high-altitude native deer mice born and raised in common lab conditions and acclimated to chronic hypoxia. We determined changes in oxygen consumption following 15 min of aerobic exercise in 12% O_2 and sampled skeletal muscles and liver at various time points during recovery to examine changes in key metabolites, including glycogen and IMTG. We found depletion in glycogen stores during exercise only in lowlanders, which returned to resting levels following 90 min of recovery. In contrast, IMTG did not change significantly with exercise or during recovery in either population. These data suggest that exercise recovery is influenced by altitude ancestry in deer mice.

1. Introduction

The capacity for aerobic exercise is important for fitness and survival in wild animals (Storz et al., 2019). This performance measure is often used to define species and population differences resulting from local selection pressures. Differences in the capacity for aerobic exercise involves variation in traits that ensure adequate delivery of oxygen and the appropriate metabolic substates to support the metabolism of working muscles. Exercise also leads to accumulation and/or depletion of circulating and tissue metabolites, and re-establishing resting levels of these metabolites in recovery is important to allow for subsequent bouts of exercise. The capacity for recovery from aerobic exercise likely has fitness consequences as it could impact an individual's ability to evade predators or in their success at capturing prey. Interestingly, metabolic recovery from exercise has been little studied in mammals, outside of work on humans and laboratory rodents. Past studies on humans show that recovery from high intensity exercise is associated with aerobic capacity (Tomlin and Wenger, 2001). This suggests that metabolic recovery should vary across species with both aerobic capacity and environmental O_2 availability. The low environmental PO_2 at high altitude is known to constrain aerobic exercise in lowland native mammals (Lui et al., 2015). Thus, exercise recovery is likely also constrained in hypoxic environments. However, little is known about how species native to high altitude compensate for reduced oxygen availability to effectively recover from aerobic exercise.

Endurance or sustained exercise requires a sufficient supply of oxygen and appropriate substrates to the mitochondria to support rates of ATP production that match ATP demand (Lau et al., 2017). High altitude poses many challenges to aerobic exercise capacity as oxygen availability decreases with increases in elevation. To compensate for the low PO_2, some species native to the high alpine have evolved a greater maximal oxygen consumption ($\dot{V}O_2max$) in hypoxia. For example,

Abbreviations: EPOC, Excess post-exercise oxygen consumption; GLUT, Glucose transporter; G6PDH, glucose-6-phosphate dehydrogenase; IMTG, intramuscular triglycerides; $\dot{V}O_2$, oxygen consumption; $\dot{V}O_2max$, maximal oxygen consumption..

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highland populations of the North American deer mouse (*Peromyscus maniculatus*) have evolved a greater exercise-induced $\dot{V}O_2$max compared with a lowland conspecific population, even when both are born and raised in a common garden condition (Lui et al., 2015). Overlaid on this fixed genetic difference is an environmentally induced increase in exercise-induced $\dot{V}O_2$max with hypoxia acclimation (Lui et al., 2015). During submaximal aerobic exercise individuals living in oxygen limiting environments should preferentially oxidize carbohydrates due to an increased efficiency of ATP production per mole of substrate and per mole O_2, compared with anaerobic ATP production and lipid oxidation, respectively (Brand, 2005; Hochachka, 1985). Indeed, highland deer mice running for 15 min at ~75% of their $\dot{V}O_2$max in hypoxia (12% O_2), show an increased reliance on carbohydrates after hypoxia acclimation, associated with decreases in their muscle glycogen at the end of exercise (Lau et al., 2017).

Although exercise is important for survival, recovery from a bout of aerobic exercise may be just as important, but has been much less studied. The time needed to restore homeostasis disrupted during the sustained activity may vary across species and between environments (Tomlin and Wenger, 2001). Patterns of recovery from exercise can be determined at both the whole animal and tissue levels. At the whole animal level, the period of excess post-exercise oxygen consumption (EPOC) is a common indicatory of exercise recovery (Baker and Gleeson, 1998). Since whole animal measurements reflect recovery of tissue metabolism, re-establishment of resting levels of tissue metabolites has been extensively studied, principally in humans (Brooks et al., 1973; Fournier et al., 2002). Replenishment of glycogen stores following exhaustive exercise has been used as a key metric of exercise recovery in humans but also in lab rodents (Brooks et al., 1973; Fell et al., 1980; Fournier et al., 2002). It is important to examine muscle metabolite recovery since re-establishing metabolite baseline levels are necessary for the proper functioning of metabolic pathways. Without repletion of key substrates, these individuals may be unable to effectively exercise (Muoio et al., 2002), possibly having consequences for survival in the wild.

Exercise recovery in the high-altitude environment may be prolonged due to the reduced oxygen availability for aerobic ATP production for the recovery process. It has been suggested that lipids may provide the ATP necessary for post-exercise glycogen resynthesis, where lipolysis in muscle is stimulated during recovery and the resulting free fatty acids catabolized. Studies on humans exercised for exhaustive periods found that the levels of intramuscular triglycerides (IMTG) in the vastus lateralis muscle remained unchanged during exercise but were significantly depleted during the first three hours post exercise and continued to decline for up to 18 h of recovery (Kiens and Richter, 1998). A similar finding was observed in rats following a two-hour swimming exercise trial where IMTG levels were significantly decreased in the soleus and vastus lateralis muscles during the recovery period (Pearsall and Palmer, 1990). In highland deer mice, the gastrocnemius muscle has greater capillary densities, capillary abundance, and generally a greater oxidative phenotype compared to lowlander conspecifics (Lui et al., 2015) that may aid in exercise recovery. As well, this muscle has a greater mitochondrial volume density and these mitochondria have a greater respiratory capacity than seen in lowland deer mice (Mahalingam et al., 2017). These evolved features of skeletal muscle allow highland mice to have increases in their overall aerobic metabolism in hypoxia (Lui et al., 2015), suggesting a greater capacity to support glycogen resynthesis when recovering from exercise (Lau et al., 2017). The increase in oxidative traits of the gastrocnemius of highland deer mice is associated with higher activity of β-hydroxyacyl-CoA dehydrogenase (HOAD), an enzyme that plays a major role in fatty acid oxidation (Cheviron et al., 2014; Lau et al., 2017). Although in mammals lipids are only used during moderate submaximal exercise (McClelland et al., 2017), this fuel source may be essential in effective exercise recovery in muscles of highlanders. The evolved increase in both aerobic capacity and lipid oxidation suggest that highland mice have an enhanced capacity for exercise recovery in hypoxia.

To understand if altitude ancestry impacts whole-animal and muscle recovery from submaximal aerobic exercise, we used lab born and raised lowland and highland populations of deer mice acclimated to hypoxia and exercised at a submaximal intensity in hypoxic conditions. We used submaximal aerobic exercise normalized to $\dot{V}O_2$max because intensity is known to affect fuel use (Schippers et al., 2014) and during voluntary wheel running highland deer mice select intensities that are within a similar range (Chappell et al., 2004). We examined changes in key muscle metabolites immediately following exercise and during 90 min of recovery. We test the hypothesis that, due to their greater aerobic capacity, highland deer mice recover more quickly from submaximal aerobic exercise than lowland conspecifics. We predict that this will include replenishing their muscle glycogen stores in their gastrocnemius muscle more rapidly than lowlanders due to their more aerobic muscle phenotype. We also predict that depletion of IMTG will occur to a greater extent during exercise recovery in highlanders as compared to lowlanders.

2. Materials and methods

2.1. Experimental animals

All animal procedures used in this study were approved by the McMaster University Animal Research Ethics Board following guidelines from the Canadian Council on Animal Care. Wild deer mice (*Peromyscus maniculatus*) were trapped at high altitude (4350 m a.s.l at the summit of Mount Blue Sky in Clear Creek County, CO, USA) and low altitude (600 m a.s.l., Kearney, NE). Wild mice were caught using Sherman live traps (model LFATG) containing nesting material and baited with peanut butter and oatmeal, as described previously (Cheviron et al., 2013) and in accordance with the recommendations of the American Society of Mammalogists Animal Care and Use Committee (Sikes et al., 2011). Mice were transported to McMaster University (90 m a.s.l.) and bred within their respective populations to the second or third generation (G2 or G3) in common garden lab conditions at 23 °C, 12:12 light: dark, and provided standard mouse chow (Tekland Global Rodent Diets; Envigo, Mississauga, ON) and water *ad libitum*.

2.2. Treadmill familiarization period

Before experiments began, mice undergoing exercise testing, lowland (sample size, $N = 25$) and highland ($N = 27$) deer mice, were familiarized to running on a rodent treadmill on three separate days separated by 24–48 h. On the first day of familiarization mice ran for 10 min at 12 m min^{-1}. On the second day, mice ran for 13 min starting at 12 m min^{-1} and speed was increased by 2 m min^{-1} every two minutes to 20 m min^{-1}. Finally on the third day, mice ran for 15 min, starting at 16 m min^{-1} and speed was increased by 2 m min^{-1} every minute until they reached 20 m min^{-1} for the remaining 15 min. Mice were scored on their running performance on a scale from 1 to 10, with a score of 10 indicating they ran consistently without stopping, 5 being they stopped for about 50% of the time, and 0 being that they did not run at all. Only mice who scored above a 6 were considered good runners and used for further experiments.

2.3. Hypoxia acclimation

In a previous study, hypoxia acclimation was shown to lead to an increased reliance on carbohydrates in high altitude highlander (HA) mice during submaximal exercise (Lau et al., 2017). Thus, following the treadmill familiarization period, all lowland and highland mice were acclimated to hypobaric hypoxia conditions for 6 weeks as previously described (Lau et al., 2017; Lui et al., 2015). Briefly, deer mice were

placed in specially designed hypobaric chambers (McClelland et al., 1998) held at a barometric pressure of ~60 kPa using a vacuum pump, mimicking conditions at high altitude (~12 kPa O_2). This hypobaric exposure is known to induce phenotypic plasticity of cardiorespiratory and metabolic traits in low and high altitude deer mice (*e.g.*, Lau et al., 2017; Tate et al., 2017). The mice were returned to normobaria 1–2 times per week for animal husbandry. At the end of the acclimation period there was no significant difference in body mass between lowland (21.5 ± 0.8 g, $N = 25$) and highland (19.8 ± 0.7 g, $N = 27$) mice ($P > 0.05$).

2.4. Respirometry

The rate of oxygen consumption ($\dot{V}O_2$) was determined during treadmill exercise and in recovery from exercise using an open-flow respirometry system (Sable Systems), as previously described for deer mice (Lau et al., 2017; Lui et al., 2015; Schippers et al., 2012). Briefly, incurrent air (~12% O_2 balance N_2) was pushed into a plexiglass enclosed motorized treadmill (chamber volume ~ 800 ml) at a rate of 1–2 L min^{-1} using a mass flow controller. A subsample of the excurrent air was dried using prebaked Drierite (W.A. Hammond Company, OH; (White et al., 2006)) and passed through an oxygen and carbon-dioxide analyzer using a subsampler pump (Sable Systems, NV). Mice were run in the postabsorptive state by fasting them for 3–4 h prior to being placed in the treadmill chamber and allowed to adjust for 5 min before the trial began. Running occurred for 15 min in hypoxia (12% O_2) and at speeds corresponding to a target intensity of ~80% $\dot{V}O_2$max (21.3 m min^{-1} and 24.4 m min^{-1} for lowland and highland mice, respectively) as previously determined (Lau et al., 2017) for hypoxia acclimated lowlander (LA) and HA deer mice. Recovery occurred in hypoxia (12% O_2) for 90 min with the enclosed treadmill covered with a cloth to minimize any external stressors. $\dot{V}O_2$ during exercise and recovery was calculated according to eq. 3b from Withers (1977). $\dot{V}O_2$ was averaged for the last 5 min of exercise, then at 5-min time periods for the first 30 min of recovery, followed by every 10 min for the remaining 60 min of recovery. Following the 90-min recovery, mice were returned their cages and returned to the hypobaric chambers to fully recover for at least 72 h before they ran on the treadmill a second time for tissue sampling (see below).

2.5. Exercise recovery and tissue sampling

Mice were run a second time under the same conditions as above, except at the end of the 15 min of exercise each individual mouse was randomly assigned to one of 4 recovery groups to be sampled at 0, 10, 60, or 90 min during post exercise recovery. During the recovery period, the treadmill was covered with an opaque cloth to minimize disturbance of the animals. Once mice reached the end of their assigned recovery period, they were removed from the treadmill chamber and placed into a container containing isoflurane-soaked cotton balls. Alternatively, isoflurane was flowed directly into the treadmill chamber until the mice were unconscious, and then mice were decapitated. Blood was collected and blood glucose was measured using a whole blood glucose meter (Acu-Chek, Roche). The remaining blood sample was centrifuged and the plasma fraction frozen. Following blood collection, tissues were always collected in the same order and freeze clamped between liquid N_2 cooled aluminum plates. The right gastrocnemius muscle was removed and separated into white and red muscle, followed by the liver, the right vastus medialis, and finally the whole left gastrocnemius. Samples were placed immediately in liquid N_2 and then stored at −80 °C for later analysis. Samples collected at rest were taken from a separate group of mice (lowland, $N = 7$; highland, $N = 6$) who had undergone 6 weeks of hypoxia acclimation but without the treadmill familiarization period. All mice were fasted for 3 h, and to ensure mice were undisturbed, were then euthanized by carefully covering their cage with a lid affixed with isoflurane-soaked cotton ball. Blood and muscles were sampled in the same order as above.

2.6. Glucose, glycogen, and glucose-6-phosphate assays

Muscle and liver samples were powdered using a liquid N_2 cooled mortar and pestle. Glycogen was determined in frozen muscle samples as previously described (Bergmeyer et al., 1974; Lau et al., 2017). Briefly, powdered muscle was added to cold 6% PCA at a 10× dilution (mg/ml). Samples were then homogenized for 20 s on ice using a PowerGen 125 homogenizer (Fisher Scientific) or a cooled glass-on-glass homogenizer. An aliquot (400 μl) was removed from the final homogenate and frozen in liquid N_2 for storage at −80 °C to be used for lactate determination (see below). The remainder was aliquoted (100 μl) for glycogen analysis. Following thawing of the 100 μl aliquot, 20 μl of 1 M $KHCO_3$ and 40 μl of 400 mM acetate buffer (pH 4.8) were added. Half of this mixture was set aside on ice to determine free glucose. To the remaining homogenate amyloglucosidase (10 U/sample, Roche Diagnostics) was added and the samples were incubated for 2 h at 40 °C in a shaking water bath. Samples were then neutralized using 1 M K_2CO_3. Total glucose content was measured in duplicate at 37 °C in a 96-well plate format using a SpectraMax Plus 384 Plate Reader (Molecular Devices, CA). Assay conditions were (in mM) 20 imidazole, pH 7.4, 1 ATP, 0.5 NAD^+, and 5 $MgCl_2$. Absorbance was recorded at 340 nm before and after the addition of glycogen-6-phosphate dehydrogenase (G6PDH, 10 U/well, suspended in 300 mM TEA buffer and 4.05 mM $MgSO_4$ at pH 7.5 (Sigma Aldrich)) to determine glycogen-6-phosphate content. Hexokinase was then added (10 U/well, Roche) to determine total glucose content. Glycogen concentrations are expressed as glycosyl units and the concentration of free glucose was subtracted from total glucose to report final amount of glycogen in each tissue sample.

2.7. Lactate assay

To assess lactate tissue accumulation the homogenates from above were thawed on ice and centrifuged (Eppendorf Centrifuge 5810 R) at 10,000 ×*g* for 10 min at 4 °C. The supernatant was transferred to a new tube and neutralized with 3 M K_2CO_3 for appropriate assay conditions. The samples were once again centrifuged for 10,000 ×*g* for 10 min at 4 °C. The neutralized supernatant was then stored in −80 °C before analysis. Muscle or plasma samples were loaded in triplicate in a 96-well plate and read in a SpectraMax Plus 384 Plate Reader (Molecular Devices, CA) as follows. The homogenate was loaded into wells with reaction buffer (0.2 M Glycine buffer (600 mM glycine and 500 mM hydrazine), 2 mM NAD^+, and H_2O). Absorbances were measured at 340 nm before and after a 30-min incubation at 37 °C with the addition of lactate dehydrogenase (LDH, 8 U/well, Sigma L1378). Lactate concentrations were determined using the standard curve.

2.8. Intramuscular triglycerides

Intramuscular triglyceride (IMTG) concentrations were determined in muscle at rest and in recovery from exercise, as described previously (Lyons and McClelland, 2022; McClelland et al., 1999). Briefly, powdered tissue (~15 mg) was placed in 30 μl/mg Folch solution (1:1 chloroform/methanol *v*/v, Folch et al., 1957) in a glass tube and homogenized using a PowerGen 125 homogenizer (Fisher Scientific). The homogenizer was then rinsed with an additional 1 ml of Folch and this was combined with the original homogenate. Samples were manually shaken for 20 min and then centrifuged (Eppendorf Centrifuge 5810 R) for 10 min at 3000 rpm. The supernatant was transferred to a new tube and the pellet was rinsed with 1 ml of Folch and centrifuged once again for 10 min at 3000 rpm. Following centrifugation, the supernatants were combined and an additional 0.5 × volume of chloroform was added resulting in a 2:1 chloroform:methanol ratio. A total of 0.25 × of 0.2% KCl was then added, and the sample was centrifuged at 2500 rpm for 10

min. After centrifuging, the aqueous layer was discarded, and 1–2 ml of 99% ethanol was added to aid evaporation. The sample was then evaporated under a steady stream of N_2 at 40 °C. Dried samples were stored in −20 °C until analysis. Prior to assay, samples were resuspended in 250 μl of isopropanol. IMTG were measured using Sigma-Aldrich total triglyceride kit in a 96 well format. Samples were read at 540 nm and IMTG concentrations within the muscles were calculated using the standard curve.

2.9. Statistical analysis

Statistical analyses were performed using RStudio version 2022.07.2 (RStudio, 2022) and GraphPad Prism for Mac (Version 9.5.1, 2023). Respirometry data were analyzed using a repeated measures analysis of variance (ANOVA). Metabolite data were analyzed using linear mixed models using the lme4 package in RStudio with body mass included as a covariate and sex as a random effect (Bates et al., 2015). Two-way ANOVAs were used to determine the effects of population and recovery time and their interactions on the metabolites. *Post hoc* testing was done with lsmeans using Kenward-Rodger test in R. Analysis of the breakpoint between the slopes of rapid and slow recovery was analyzed using threshold regression model estimation with the package chngpt in R (Fong et al., 2017). Breakpoint and absolute change in $\dot{V}O_2$max were compared using a Student's *t*-test (Graphpad Prism). Data that did not pass the normality test were transformed. To compared relative changes in tissue metabolites, we also expressed data relative to rest (where rest was set to a value of 1). All data are presented as means SEM.

3. Results

3.1. Oxygen consumption

To examine if recovery from exercise at the whole animal level differed between populations, $\dot{V}O_2$ was measured during exercise and for 90 min in the recovery after 15 min of submaximal exercise. We found that $\dot{V}O_2$ significantly declined by 3 min into recovery, compared with rates during exercise ($F_{3.5,\ 137.5} = 55.28$, $p < 0.0001$). However, after this time point $\dot{V}O_2$ did not significantly change for the duration of recovery (Fig. 1A). There were no significant differences in $\dot{V}O_2$ between lowland and highland deer mice ($p > 0.05$) during either exercise or recovery. Since previous studies on exercise recovery have identified two main phases of $\dot{V}O_2$, the rapid and slow curve components (Gaesser and Brooks, 1984), we determined if the transition between the two recovery phases were different between lowland and highland deer mice. We examined the "breakpoint", where the initial rapid recovery phase ended, and the slow phase began (Fig. 1B). There were no significant differences between populations in the time where the breakpoint occurred and slopes of $\dot{V}O_2$ *versus* time significantly changed in recovery. Breakpoints occurred at 2.53 ± 0.24 min and 2.54 ± 0.41 min of recovery in lowland and highland deer mice, respectively ($F_{12,\ 15} = 2.51$, $p = 0.631$). We also determined the change in $\dot{V}O_2$ from the end of exercise to this breakpoint (delta$\dot{V}O_2$). Similar to the results for breakpoint, we found no significant difference in delta $\dot{V}O_2$ between populations ($F_{16,\ 12} = 1.31$, $p = 0.785$, Fig. 1C).

3.2. Blood glucose and lactate

To help understand how circulating metabolites in deer mice may be affected by exercise and change in recovery from exercise, we measured levels of circulating lactate and glucose (Fig. 2; Tables 2 and 3). Exercise led to a significant increase in plasma lactate with a main effect of time, but not population or a significant interaction (Table 2, Table S2). In the lowland mice plasma lactate remained significantly elevated at 0 and 10 min into recovery (*post hoc* analysis compared to rest, at 0 min, $p =$ 0.001; at 10 min, $p = 0.004$). After 60 min of recovery plasma lactate was no longer significantly different than resting values. Highlanders show an elevated plasma lactate concentration immediately after exercise that approach statistical significance ($p = 0.056$, Table 2). Similarly, when plasma lactate was expressed relative to resting values for each population there was a significant effect of time ($F_{4,\ 52.1} = 5.03$, $p =$ 0.002) but not of population ($F_{1,\ 52.4} = 0.40$, $p = 0.531$) nor a significant interaction ($F_{1,\ 52.4} = 0.69$, $p = 0.602$; Fig. 2A). Relative to levels at rest, plasma lactate was significantly higher in lowlanders immediately after exercise and 10 min into recovery. Highland mice also showed elevated plasma lactate immediately following exercise (*post hoc* analysis, $p =$ 0.046) but returned to resting levels after 10 min of recovery. Plasma lactate in the lowlanders increased twice as much as the highlanders immediately following exercise and remained 50% higher than highlanders after 10 min of recovery.

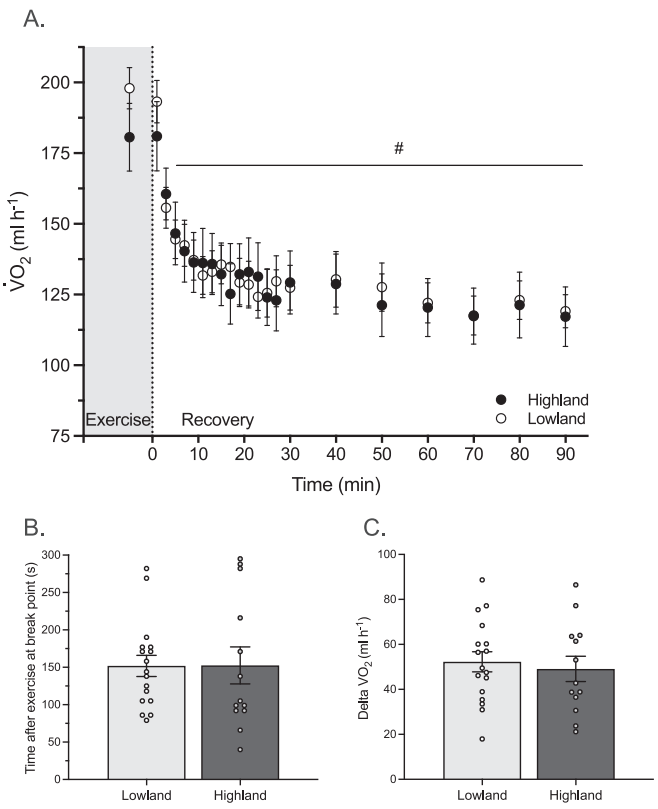

Fig. 1. Oxygen consumption ($\dot{V}O_2$) (A) in lowland and highland deer mice acclimated to chronic hypoxia during 15 min of submaximal exercise (~80% $\dot{V}O_2$max) and 90 min of post-exercise recovery in hypoxia. Each data point represents the mean $\dot{V}O_2$ (ml h^{-1}) ± SEM. Lowland deer mice are shown in open circles and highland deer mice are shown in filled circles. $n = 20$ per population. Threshold break point (B) between rapid and slow $\dot{V}O_2$ recovery phases, and absolute change in $\dot{V}O_2$ (C) from the end of exercise to the threshold breakpoint (delta$\dot{V}O_2$). Sample sizes in B and C are $n = 16$ for lowland and $n = 13$ for highland deer mice. #, significantly different from exercise and immediately post exercise in both populations.

Circulating glucose did not change significantly with either exercise or recovery but there was a main effect of population, but not of time nor was there a significant interaction (Table 3, Table S3). When the data were expressed relative to the resting values there was a significant main effect of population ($F_{1,\ 52} = 4.56$, $p = 0.037$; Fig. 2), likely driven by a 30% increase in blood glucose in highland mice immediately after exercise had ended. There was no significant effect of time ($F_{4,\ 52} = 1.11$, $p =$ 0.361), nor was there a significant interaction ($F_{4,\ 52} = 0.45$, $p =$ 0.775).

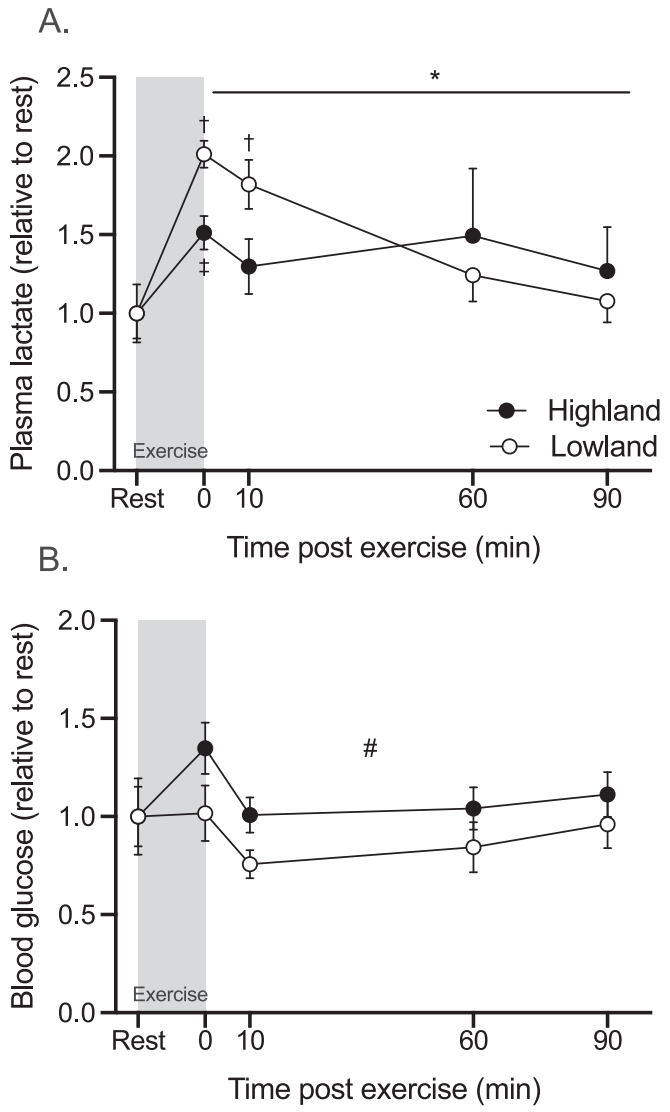

Fig. 2. Plasma lactate and blood glucose concentrations relative to resting values in lowland and highland deer mice acclimated to chronic hypoxia during submaximal exercise and recovery from exercise in hypoxia. Relative change (mean ± SEM) from resting values for A) plasma lactate and B) blood glucose levels at rest and during recovery following 15 min of submaximal exercise (~80% $\dot{V}O_2$max) in hypoxia. Lowlanders are shown in open circles and highlanders are shown in filled circles. Samples sizes are $n = 6$–7 per time point (see Tables 3 and 4). *, significant main effect of time ($F_{4, 52.1} = 5.03$, $p = 0.002$). #, significant main effect of population ($F_{1, 52} = 4.56$, $p = 0.037$). †, significantly different than rest in lowlanders ($p < 0.05$). ‡, significantly different than rest in highlanders ($p < 0.05$).

3.3. Tissue glycogen

We determine changes in glycogen concentration in the red and white gastrocnemius and of the vastus medialis with exercise and over the recovery period. Glycogen was significantly depleted in the red gastrocnemius and showed a significant main effect of time, but not of population, nor was there a significant interaction (Table 1; Table S1). In the lowland deer mice, glycogen concentration in the red gastrocnemius was significantly decreased from resting values until 90 min of recovery (*post hoc* analysis compared to rest, at 0 min, $p < 0.0001$; at 10 min, $p = 0.002$; at 60 min, $p = 0.025$). In highlanders, red gastrocnemius glycogen tended to be lower immediately after exercise when compared to rest, but did not reach a level of statistical significance ($p = 0.085$). When data were expressed relative to resting values within population, there was a significant main effect of time ($F_{4, 53.1} = 4.75$, $p = 0.002$) and population ($F_{1, 53.6} = 11.17$, $p = 0.002$; Fig. 3A), but there was no significant interaction between time and population ($F_{4, 53.17} = 1.81$, $p = 0.140$). Glycogen concentrations in lowland mice decreased by 55% with exercise and were significantly different than resting levels immediately following exercise until 90 min into recovery (*post hoc* analysis compared to rest, at 0 min, $p < 0.0001$; at 10 min, $p = 0002$; at 60 min, $p = 0.025$). Highland mice showed a decrease in red gastrocnemius glycogen concentration by 35% compared to rest following exercise, but this decline failed to reach statistically significant ($p = 0.085$). Relative levels of red gastrocnemius glycogen were significantly different between populations during recovery with lowlanders having relative levels that were 27–46% lower than in highlanders at 0 min ($p = 0.029$) 10 min ($p = 0.007$), and at 60 min ($p = 0.027$) post exercise.

Muscle glycogen in the vastus medialis did not show a significant change with either time or population (Table 1; Table S1). When the data were expressed relative to resting levels there were also no differences across time ($F_{4, 41} = 0.37$, $p = 0.832$) or between populations ($F_{1, 41} = 3.78$, $p = 0.059$), nor was there an interaction ($F_{4, 41} = 0.25$, $p = 0.909$; Fig. 3B). Glycogen concentrations in the white gastrocnemius muscle were significantly depleted with exercise and showed a significant main effect of time, but not of population (Table 1; Table S1). In lowland deer mice, glycogen in the white gastrocnemius did not return to resting levels until after 60 min of recovery (*post hoc* analysis compared to rest, at 0 min, $p = 0.032$; at 10 min, $p = 0.01$; at 60 min, $p = 0.048$). Highland mice also showed a decline in white gastrocnemius glycogen immediately post exercise that approached statistical significance ($p = 0.059$). When data were expressed relative to rest within population there was a significant main effect of time ($F_{4, 54} = 2.97$, $p = 0.027$) and glycogen concentrations in the lowlanders declined by 50% compared to rest following 10 min of recovery (*post hoc* analysis, 0 min, $p = 0.015$; 10 min, $p = 0.006$; 60 min, $p = 0,041$; Fig. 3C). In highlanders, relative glycogen levels tended to be lower immediately after exercise ($p = 0.064$). For this muscle, there was also a significant main effect of population ($F_{1, 54} = 5.24$, $p = 0.026$) where glycogen concentrations were 45% lower than in highlanders at 10 min of recovery ($p = 0.034$), but there was no significant interaction ($F_{4, 54} = 0.71$, $p = 0.588$).

Since liver glycogen is a valuable source of glucose for exercising muscles, we examined changes in this fuel source with exercise and into recovery. We found that during exercise and recovery liver glycogen showed a significant main effect of time but not for population, nor was there a significant interaction (Table 1; Table S1). Liver glycogen concentrations in lowland deer mice did not change significantly from resting values to immediately following exercise (*post hoc* analysis compared to rest, at 0 min, $p = 0.104$) but decreased significantly during exercise recovery and did not return to resting levels even after 90 min of recovery (at 10 min, $p = 0.032$; at 60 min, $p = 0.024$; at 90 min, $p = 0.046$). At 60 min post exercise, highland mice tended to have liver glycogen levels that were lower than at rest ($p = 0.061$). When data were expressed relative to rest for each population, there was a main effect of time ($F_{4, 52.2} = 2.63$, $p = 0.045$) but not population ($F_{1, 53.69} = 0.67$, $p = 0.417$) nor a significant interaction ($F_{4, 52.18} = 0.44$, $p = 0.778$; Fig. 3D). Highland deer mice showed a decrease in their liver glycogen by 60% following 60 min of recovery ($p = 0.036$). Lowland mice also tended to have liver glycogen that was reduced at 10 min ($p = 0.054$), and at 60 min ($p = 0.51$) of recovery from exercise.

3.4. Free glucose and glucose-6-phosphate

We investigated changes in concentrations of free glucose in the gastrocnemius, vastus lateralis, and the liver to gain a better understanding of regulation of cellular glucose metabolism during exercise. We found that free glucose did change significantly with exercise and recovery from exercise in the red gastrocnemius with highlanders having significantly elevated concentrations from rest following 90 min of

Table 1
Glycogen concentrations with exercise and in post-exercise recovery in lowland and highland deer mice acclimated to chronic hypoxia. Glycogen (in μmol g^{-1} w.w.) was determined for red gastrocnemius, white gastrocnemius, vastus medialis, and liver at rest, immediately after 15 min of submaximal exercise (~80% $\dot{V}O_2$max) and in recovery in hypoxia. Data are presented as mean ± SEM, with sample sizes in parentheses. There was a significant effect of time for both red ($F_{4,\ 53.1} = 4.75$, p = 0.002), white ($F_{4,54} = 2.95$, $p = 0.028$), and liver ($F_{4,\ 52.2} = 2.65$, $p = 0.044$) tissues.

Glycogen (μmol g^{-1} w.w.)		Rest	0 min	10 min	60 min	90 min
Red gastrocnemius	lowland	16.35 ± 0.70 (7)	7.21 ± 0.33 (7)[a]	8.54 ± 0.69 (6)[a]	8.40 ± 0.40 (6)[a]	10.43 ± 0.59 (6)
	highland	13.29 ± 1.54 (6)	8.81 ± 0.71 (6)	11.50 ± 0.53 (7)	12.54 ± 0.85 (7)	9.55 ± 0.66 (7)
White gastrocnemius	lowland	15.10 ± 0.82 (7)	8.56 ± 0.40 (7)[a]	7.12 ± 0.38 (6)[a]	8.51 ± 0.79 (6)[a]	9.03 ± 0.68 (6)
	highland	13.08 ± 1.09 (6)	8.64 ± 0.93 (6)	11.25 ± 0.96 (7)	8.89 ± 0.47 (7)	11.37 ± 0.68 (7)
Vastus medialis	lowland	24.83 ± 1.76 (7)	25.59 ± 3.30 (4)	25.83 ± 3.17 (6)	26.45 ± 2.92 (5)	18.84 ± 1.80 (4)
	highland	15.91 ± 1.16 (5)	19.83 ± 1.42 (3)	21.51 ± 1.53 (6)	22.34 ± 1.57 (7)	20.97 ± 2.20 (5)
Liver	lowland	101.48 ± 21.75 (7)	68.20 ± 14.17 (7)	50.11 ± 20.68 (6)[a]	41.59 ± 17.72 (6)[a]	52.52 ± 15.05 (6)[a]
	highland	81.77 ± 22.20 (5)	54.28 ± 6.06 (6)	69.28 ± 17.19 (7)	34.07 ± 8.21 (7)	39.91 ± 15.99 (7)

[a] Significantly different from resting values for lowland mice.

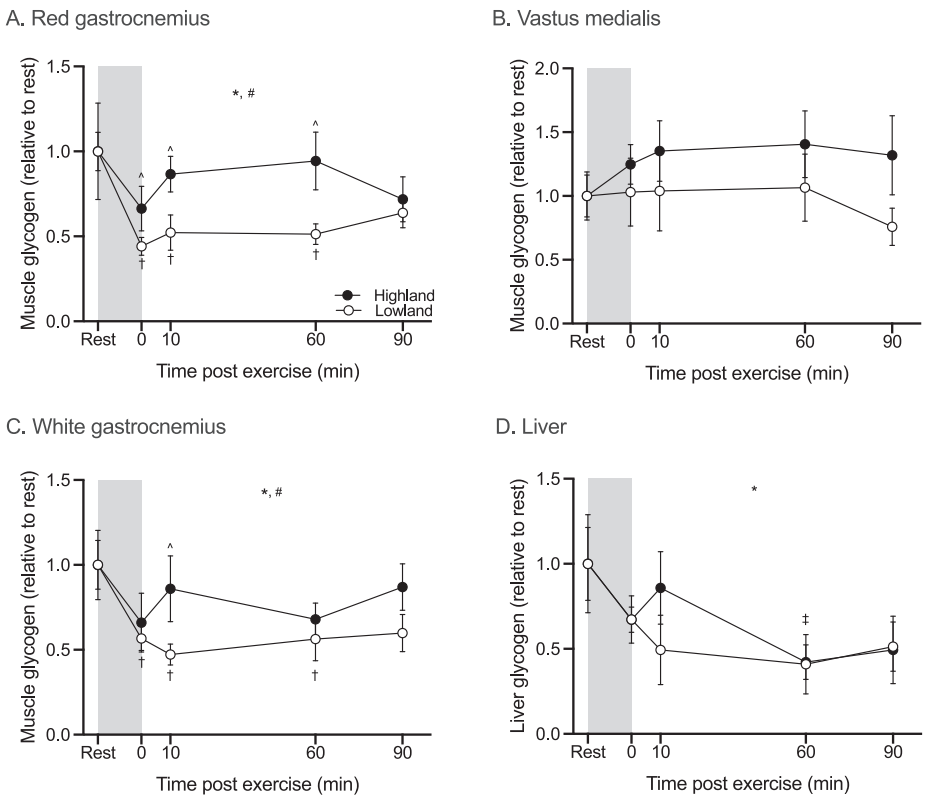

Fig. 3. Glycogen concentration relative to resting values with exercise and in post-exercise recovery in lowland and highland deer mice acclimated to chronic hypoxia. Glycogen was determined for, A) Red gastrocnemius muscle, B) Vastus medialis muscle, C) White gastrocnemius muscle, and D) Liver at rest, immediately after 15 min of submaximal exercise (~80% $\dot{V}O_2$max) and in recovery in hypoxia. Data are standardized to rest and presented as mean ± SEM. Lowlanders are shown in open circles and highlanders are shown in filled circles. Sample sizes are $n = 3$–7 for each time point (see Table 2). *, significant main effect of time in red ($F_{4,\ 53.1} = 4.75$, p = 0.002), white ($F_{4,\ 54} = 2.97$, $p = 0.027$), and liver ($F_{4,52.2} = 2.63$, $p = 0.045$). #, significant main effect of population in red ($F_{1,\ 53.6} = 11.17$ p = 0.002) and white ($F_{1,\ 54} = 5.24$, $p = 0.026$) gastrocnemius. †, significantly different than rest in lowlanders ($p < 0.05$). ‡, significantly different than rest in highlanders ($p < 0.05$). ˆ, significant difference between populations at a given time point ($p < 0.05$).

recovery ($p = 0.008$, Table 3). There were no significant differences between populations nor a significant interaction (Table S3). When data were expressed relative to resting values within population, there was a significant main effect of time ($F_{4,52.1} = 2.60$ p = 0.046) with highlanders having significantly elevated free glucose concentrations following 90 min of recovery ($p = 0.006$; Fig. 4A). There was also a main effect of population ($F_{1,\ 52.8} = 3.80$ $p = 0.033$) with lowlanders and highlanders differing at 90 min of recovery ($p = 0.023$).

In the white gastrocnemius there was no significant effect of time and there were no significant differences between populations nor a significant interaction (Table 3, Table S3). When data were expressed relative to resting values, there were no differences in time ($F_{4,\ 54} = 1.72$, $p = 0.158$), population ($F_{1,\ 54} = 0.07$, $p = 0.796$), or interaction ($F_{1,\ 54} = 1.07$, $p = 0.380$; Fig. 4C).

Free glucose was not significantly affected by exercise or recovery in the vastus medialis and there were no significant differences between populations nor a significant interaction (Table 3; Table S3). Even when data were expressed relative to rest there were no differences with time ($F_{4,\ 43} = 0.15$, $p = 0.964$), population ($F_{1,\ 43} = 0.36$, $p = 0.551$) or interaction ($F_{4,\ 43} = 0.30$, $p = 0.875$; Fig. 4B). The liver also showed no significant changes in free glucose during exercise or in recovery and there were no differences between populations nor a significant interaction (Table 3; Table S3). When data were expressed relative to resting values within population, there was no significant effect of time ($F_{4,\ 54} = 0.47$, $p = 0.754$) but there was a significant main effect of population ($F_{1,\ 54} = 7.27$, $p = 0.009$) but no significant interaction (Fig. 4D). Free

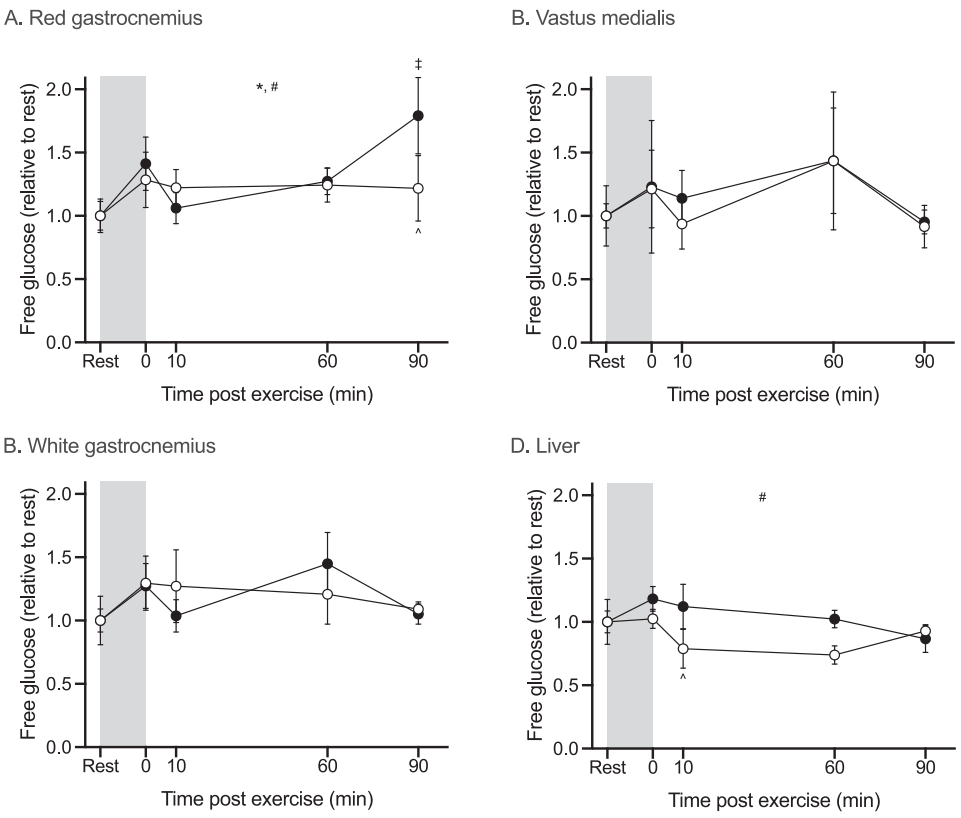

Fig. 4. Free glucose concentrations relative to resting values with exercise and in recovery in hypoxia for lowland and highland deer mice acclimated to chronic hypoxia. Free glucose was determined for, A) Red gastrocnemius muscle, B) Vastus medialis muscle, C) White gastrocnemius muscle, and D) Liver at rest, immediately after 15 min of submaximal exercise (~80% $\dot{V}O_2$max) and during recovery in hypoxia. Data are standardized to resting values within population and presented as mean ± SEM. Lowlanders are shown in open circles and highlanders are shown in filled circles. Sample sizes are n = 4–7 for each time point (see Table 4). *, significant main effect of time in red gastrocnemius ($F_{4,\ 521} = 2.60$, $p = 0.046$). #, significant main effect of population in red gastrocnemius ($F_{1,\ 52.8} = 3.80$ $p = 0.033$) and liver ($F_{1,\ 54} = 7.27$, $p = 0.009$). ‡, significantly different than rest in highlanders ($p < 0.05$). ^, significant difference between populations at a given time point ($p < 0.05$).

glucose concentrations in the liver were significantly different between populations at 10 min post exercise ($p = 0.016$), with lowland mice having 34% lower relative free glucose than highlanders.

Glucose-6-phosphate is the product of an initial step in glycolysis where glucose is phosphorylated to glucose-6-phosphate by the enzyme hexokinase or glucokinase. We found that glucose-6-phosphate concentration changed significantly with time in the red gastrocnemius following exercise, but there was no significant population effect nor was there a significant interaction (Table 4, Table S4). In the lowlanders glucose-6-phosphate was significantly different from resting levels immediately after exercise ($p = 0.0005$) but returned to levels not significantly different than resting as recovery progressed. When the data were expressed relative to resting levels within population, there was a significant main effect of population ($F_{1,\ 53} = 9.69$, $p = 0.003$) and time ($F_{4,\ 53} = 3.11$, $p = 0.023$) but no significant interaction ($F_{4,\ 53} = 1.42$, $p = 0.239$; Fig. 5A). Glucose-6-phosphate levels declined by 70% in lowlanders following exercise, which was 50% lower than in highlanders ($p = 0.005$). Relative concentrations of glucose-6-phosphate significantly differed between populations and was 64% lower at 0 and 52% lower at 90 min post exercise during recovery than in highlanders (0 min, $p = 0.019$; 90 min, $p = 0.007$).

In contrast to the gastrocnemius, the vastus medialis showed no change in glucose-6-phosphate following exercise or recovery or between populations nor a significant interaction (Table 4; Table S4). When the data were expressed relative to rest there was a significant main effect of population ($F_{1,\ 37} = 12.02$, $p = 0.001$) but not of time ($F_{4,\ 37} = 0.46$, $p = 0.761$) or an interaction ($F_{4,\ 37} = 1.85$, $p = 0.141$; Fig. 5B). Concentrations of glucose-6-phosphate significantly differed between populations immediately post exercise (0 min, $p = 0.003$).

The white gastrocnemius also showed no significant change in glucose-6-phosphate levels following exercise or in recovery from exercise and there was no significant effect of population nor a significant interaction (Table 4; Table S4). When the data were expressed relative to rest there was no significant effect of time ($F_{4,\ 52.1} = 0.89$, $p = 0.479$) but there was a significant main effect of population ($F_{1,\ 53} = 17.44$, $p = 0.0001$) but no significant interaction ($F_{4,\ 52.4} = 0.60$, $p = 0.67$; Fig. 5C). Concentrations of glucose-6-phosphate were significantly lower (42–51%) in lowland deer mice at 0, 10, and 90 min post exercise in recovery (0 min, $p = 0.015$; 10 min, $p = 0.024$; 90 min, $p = 0.033$).

Similarly, the liver showed no change in glucose-6-phosphate following exercise or recovery and the effect of population approached statistical significance (Table 4; Table S4). When the data were expressed relative to rest, there was no significant main effect of time ($F_{4,\ 46} = 0.68$, $p = 0.661$), population ($F_{1,46} = 2.87$, $p = 0.097$) nor any significant interaction ($F_{4,46} = 0.59$, $p = 0.670$; Fig. 5D).

3.5. Intramuscular triglycerides

To assess if changes in muscle glycogen were associated with reciprocal changes in muscle triglyceride stores, we measured IMTG in the red gastrocnemius. We found that there were no significant changes in IMTG concentration with either exercise or in recovery from exercise (Table 5). There was also no significant effect of population, nor a significant interaction (Table S5). When the data were expressed relative to rest there was a significant main effect of population ($F_{1,\ 44} = 4.65$, $p = 0.036$), but not of time ($F_{4,\ 44} = 0.06$, $p = 0.993$) or a significant

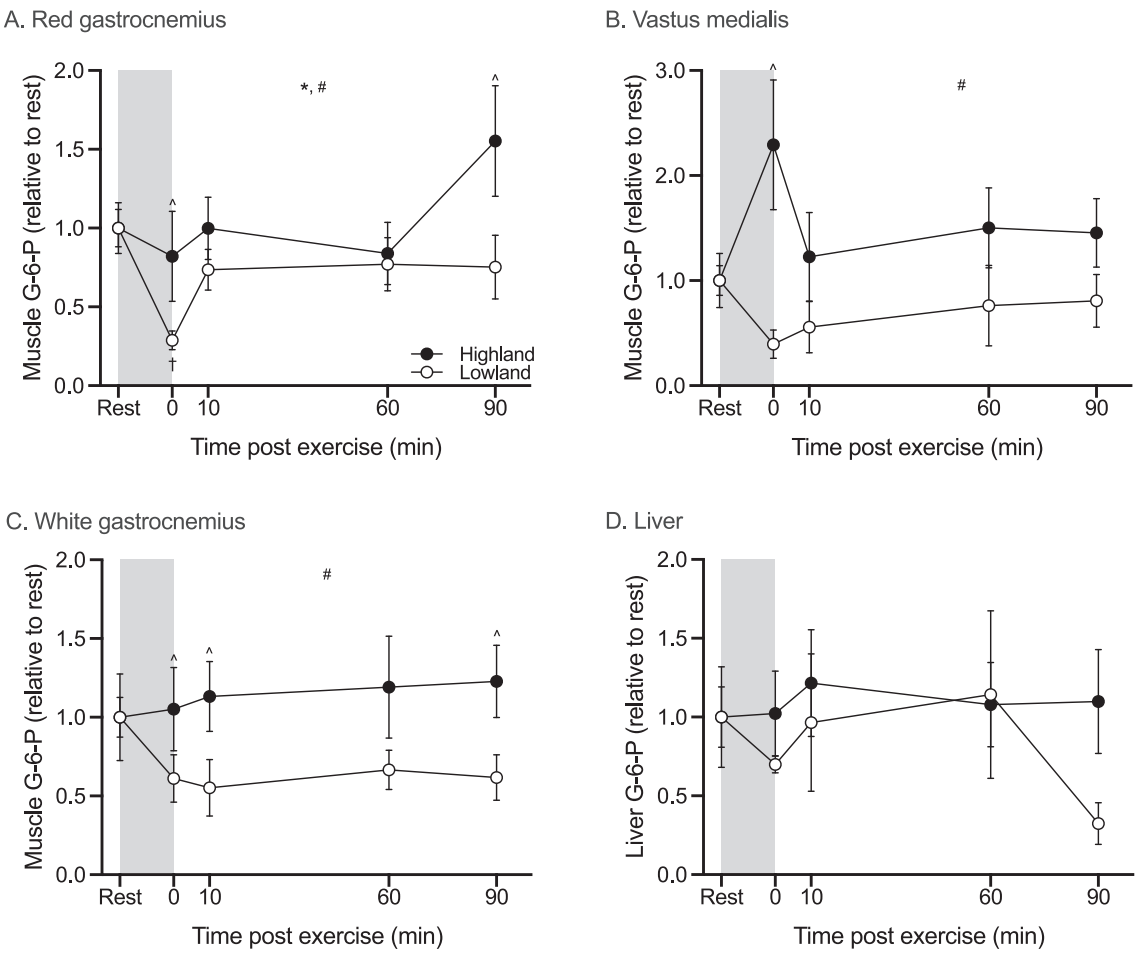

Fig. 5. Glucose-6-phosphate (G-6-P) concentration relative to resting values during exercise and recovery in hypoxia for lowland and highland deer mice acclimated to chronic hypoxia. Glucose-6-phosphate was determined for, A) Red gastrocnemius muscle, B) Vastus medialis muscle, C) White gastrocnemius muscle, and D) Liver at rest, immediately after 15 min of submaximal exercise (~80% $\dot{V}O_2$max) and in recovery in hypoxia. Data are standardized to resting values within population and presented as mean ± SEM. Lowlanders are shown in open circles and highlanders are shown in filled circles. Sample sizes are n = 3–7 for each time point (see Table 5). *, significant main effect of time ($F_{4, 53} = 3.11$, $p = 0.023$). #, significant main effect of population in red gastrocnemius ($F_{1, 53} = 9.69$, $p = 0.003$), vastus medialis ($F_{1, 37} = 12.02$, $p = 0.001$), and white gastrocnemius ($F_{1, 53} = 17.44$, $p = 0.0001$). †, significantly different than rest in lowlanders ($p < 0.05$). ^, significant difference between populations at a given time point ($p < 0.05$).

interaction ($F_{4, 44} = 0.01$, $p = 0.924$; Fig. 6). Relative concentrations of IMTG were significantly different between populations and was 85% lower in lowlanders at 60 min in of post exercise recovery ($p = 0.039$).

3.6. Muscle lactate

To understand if metabolic changes during exercise and recovery were associated with variation in lactate production, or if plasma lactate reflect tissue levels, we measured lactate concentrations in muscle and liver (Table 2; Fig. 7). Lactate concentrations in both the red and white gastrocnemius did not change with exercise or recovery and there were no differences with population (Table 2; Table S2). The liver also showed no changes in lactate concentration with exercise or recovery and there were no differences with population (Table S2). When the data were expressed relative to rest there was a significant main effect of population in the red gastrocnemius ($F_{1, 54} = 7.02$, $p = 0.011$; Fig. 7A) and white gastrocnemius ($F_{1, 54} = 12.69$, $p = 0.001$; Fig. 7B), but not for the liver ($F_{1, 54} = 0.01$, $p = 0.968$; Fig. 7C). Concentrations of lactate were significantly different between populations with lowlanders having 16–28% lower muscle lactate at 0 min and 60 min during post exercise recovery in the red and white gastrocnemius (red gastrocnemius, 0 min, $p = 0.038$; 60 min, $p = 0.017$; white gastrocnemius, 0 min, $p = 0.046$; 60 min, $p = 0.035$). However, there were no significant effects of time for any tissue measured (red gastrocnemius, $F_{4, 54} = 1.68$, $p = 0.168$; white gastrocnemius, $F_{4, 54} = 0.42$, $p = 0.791$; liver, $F_{4, 54} = 2.00$, $p = 0.107$).

4. Discussion

The main objective of this study was to determine if altitude ancestry in deer mice impacts recovery from aerobic exercise in hypoxia. We tested the hypothesis that highland deer mice recover more rapidly from exercise, due to their higher aerobic capacity in hypoxia. This recovery would be associated with more rapid recovery of muscle metabolites, compared with lowlanders. However, we found that highland deer mice showed little change in muscle metabolites with exercise and EPOC did not differ from lowlanders. In contrast, lowland deer mice showed significant changes in muscle metabolites post-exercise. Muscle glycogen in both the red and white gastrocnemius were significantly depleted in lowlanders following exercise, and only returned to resting levels by 90 min of recovery. Changes in red gastrocnemius glycogen did not correspond with levels of IMTG, which did not vary with either exercise or in recovery. In contrast with muscle, liver glycogen was depleted in both populations with exercise and into recovery. These results suggest that altitude ancestry affects changes in muscle metabolites during submaximal exercise in hypoxia, with changes occurring in muscles of

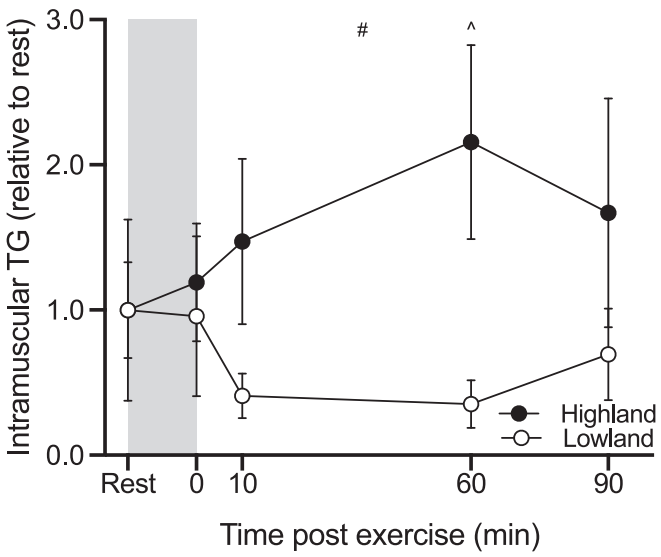

Fig. 6. Intramuscular triglyceride (TG) concentration of the red gastrocnemius relative to resting values during exercise and recovery in hypoxia for lowland and highland deer mice acclimated to chronic hypoxia. Concentration of IMTG in red gastrocnemius muscle was determined at rest and recovery following 15 min of submaximal exercise (~80% $\dot{V}O_2$max) in hypoxia. Data are standardized to resting values within population and presented as mean ± SEM. Lowlanders are shown in open circles and highlanders are shown in filled circles. Sample sizes are n = 4–7 for each timepoint (see Table 5). #, significant main effect of population ($F_{1, 44} = 4.65$, $p = 0.036$). ^, significant difference between populations at a given time point ($p < 0.05$).

lowland deer mice that returned to resting levels during recovery. The maintenance of muscle and blood metabolite levels, including lactate, in highlanders suggests a reduced reliance on anaerobic ATP production at a similar submaximal exercise compared with lowlanders. This would improve the efficiency of exercise metabolism in hypoxia and reduce costs in recovery from an exercise bout at high altitude.

4.1. Muscle glycogen replenishment following exercise

We found that glycogen was significantly depleted in the red and white portions of the gastrocnemius muscle following submaximal exercise, but only in lowland deer mice and to levels significantly lower than in highlanders (Fig. 3). This is consistent with previous studies on deer mice, where glycogen in the whole gastrocnemius muscle (red and white combined) was significantly depleted immediately after 15 min of exercise in hypoxia at a similar submaximal intensity used in the current study (Lau et al., 2017). Lab born and raised highland deer mice increase their reliance on carbohydrates to power submaximal exercise after hypoxia acclimation, a response not seen in lowland deer mice (Lau et al., 2017). Interestingly, although hypoxia acclimated highlanders tended to deplete muscle stores immediately after exercise (Fig. 3) as we observed previously (Lau et al., 2017), it was not to levels that were statistically different than rest, nor were they as low as in lowlanders. Studies on humans have shown that glycogen availability can affect muscle glycogen use during exercise (Areta and Hopkins, 2018). We did not find that resting muscle glycogen differed between lowland and highland mice, suggesting glycogen availability cannot explain differences in exercise fuel use. Previous studies on these populations found hypoxia acclimation increased maximal activities of hexokinase only in highland deer mice, suggesting a greater capacity to take up circulating glucose into the working muscles during exercise (Lau et al., 2017; Fueger et al., 2005). Lowlanders on the other hand, may rely to a greater extent on intramuscular glycogen stores to power exercise at the submaximal intensity used in this study. The sparing of muscle glycogen may be advantageous to highlanders to avoid depleting this valuable fuel source. The gastrocnemius of highland deer mice is also highly aerobic, with a greater mitochondrial density and respiratory capacity, than in lowlanders (Mahalingam et al., 2017). Taken together, these results suggest that the highland phenotype leads to reduced exercise muscle glycogen use and a reduced reliance on anaerobic ATP production (Lui et al., 2015).

Fuel use, including muscle glycogen, is a function of exercise intensity and duration (Felig and Wahren, 1975; Weber et al., 1996). The exercise intensity we used in this study was within the range observed for voluntary wheel running in captive highland deer mice bred for 2 or 3 generations in captivity (Chappell et al., 2004). Running at this intensity occurred in many short bouts of exercise but resulted in significant running over a night (Chappell et al., 2004). This suggests our experimental protocol was ecologically relevant and likely reflects recovery from aerobic exercise in naturally behaving mice. However, studies on other rodents, such as lab rats, show greater reductions in muscle glycogen than we observed but after a two-hour exhaustive swimming exercise. After the two hours of swimming, glycogen concentrations declined by 65% in the soleus muscle, and full glycogen repletion only occurred after four hours of recovery (Pearsall and Palmer, 1990). This demonstrates that exhaustive exercise can lead to large changes in muscle glycogen and differs from recovery from aerobic locomotion. Indeed, this difference in experimental protocols may help explain the modest to no glycogen depletion seen here. There are also differences in the type of muscle sampled. The soleus muscle is highly oxidative, containing mostly type I fibers (Armstrong and Phelps, 1984), and therefore has a higher capacity for substrate oxidation than the gastrocnemius, which is less oxidative due to a mixed fiber-type composition. Neither lowland nor highland deer mice showed significant changes in glycogen during exercise and recovery in the vastus medialis (Fig. 3; Table 1). This muscle may not be recruited at this running intensity or possibly relies primarily on exogenous fuel sources.

Table 2
Lactate concentrations with exercise and in post-exercise recovery in lowland and highland deer mice acclimated to chronic hypoxia. Lactate (in μmol g^{-1} w.w.) was determined for red gastrocnemius, white gastrocnemius, liver, and plasma during rest, immediately after 15 min of submaximal exercise (~80% $\dot{V}O_2$max) and in recovery in hypoxia. Data are presented as mean ± SEM, with sample sizes in parentheses. There was a significant effect of time for plasma lactate ($F_{4, 52.1} = 4.94$, $p = 0.002$).

Lactate (μmol g^{-1} w.w.)		Rest	0 min	10 min	60 min	90 min
Red gastrocnemius	lowland	6.29 ± 0.16 (7)	5.01 ± 0.16 (7)	5.88 ± 0.21 (6)	5.39 ± 0.11 (6)	5.43 ± 0.14 (6)
	highland	5.35 ± 0.11 (6)	5.10 ± 0.10 (6)	4.91 ± 0.14 (7)	5.89 ± 0.18 (7)	5.08 ± 0.16 (7)
White gastrocnemius	lowland	7.43 ± 0.17 (7)	6.09 ± 0.23 (7)	6.04 ± 0.39 (6)	6.27 ± 0.28 (6)	6.53 ± 0.33 (6)
	highland	5.28 ± 0.32 (6)	5.61 ± 0.18 (6)	5.61 ± 0.21 (7)	6.16 ± 0.22 (7)	6.20 ± 0.18 (7)
Liver	lowland	17.45 ± 0.47 (7)	16.70 ± 0.74 (7)	15.65 ± 3.19 (6)	12.19 ± 1.03 (6)	14.34 ± 0.53 (6)
	highland	17.75 ± 0.86 (6)	15.05 ± 1.36 (6)	13.76 ± 1.46 (7)	13.82 ± 0.73 (7)	14.43 ± 1.95 (7)
Plasma	lowland	1.98 ± 0.13 (6)	3.98 ± 0.06 (7)[a]	3.60 ± 0.13 (6)[a]	2.46 ± 0.13 (6)	2.14 ± 0.11 (6)
	highland	1.90 ± 0.14 (6)	2.87 ± 0.08 (6)	2.46 ± 0.13 (7)	2.83 ± 0.31 (7)	2.41 ± 0.20 (7)

[a] Significantly different from resting values for lowland mice.

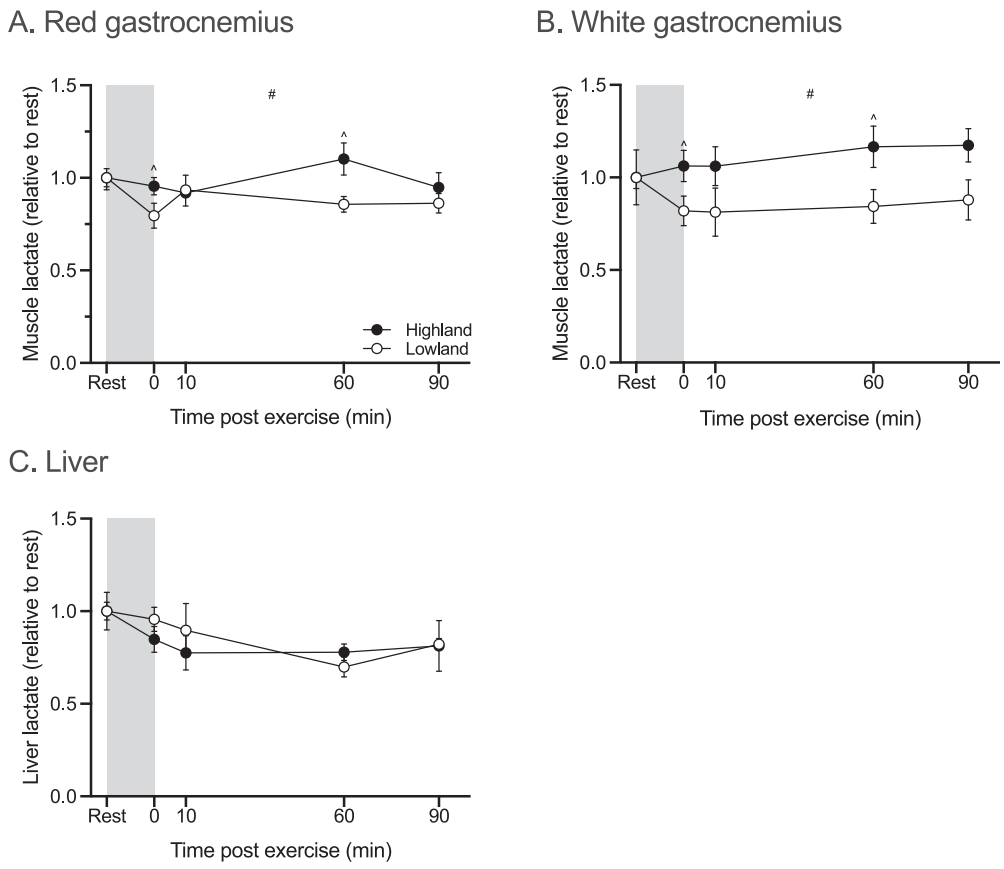

Fig. 7. Lactate concentrations relative to resting values during exercise and recovery in hypoxia for lowland and highland deer mice acclimated to chronic hypoxia. Lactate was determined in A) Red gastrocnemius muscle, B) White gastrocnemius muscle, and C) Liver. Concentrations of lactate were determined at rest and recovery following 15 min of submaximal exercise (~80% $\dot{V}O_2$max) in hypoxia. Data are standardized to resting values within population and presented as mean ± SEM. Lowlanders are shown in open circles and highlanders are shown in filled circles. Sample sizes are n = 6–7 per time point (see Table 2). #, significant main effect of population in red ($F_{1, 54} = 7.02$, $p = 0.011$) and white ($F_{1, 54} = 12.69$, p = 0.001) gastrocnemius. ^, significant difference between populations at a given time point ($p < 0.05$).

Table 3
Free glucose concentrations with exercise and in post-exercise recovery in lowland and highland deer mice acclimated to chronic hypoxia. Free glucose (in μmol g^{-1} w.w.) was determined for red gastrocnemius, white gastrocnemius, vastus lateralis, and liver during rest, immediately after 15 min of submaximal exercise (~80% $\dot{V}O_2$max) and in recovery in hypoxia. Data are presented as mean ± SEM, with sample sizes in parentheses. There was a significant effect of time for the red gastrocnemius ($F_{4, 52.1} = 2.65$, p = 0.044).

Free glucose (μmol g^{-1} w.w.)		Rest	0 min	10 min	60 min	90 min
Red gastrocnemius	lowland	1.72 ± 0.08 (6)	2.21 ± 0.14 (7)	2.11 ± 0.10 (6)	2.14 ± 0.10 (6)	2.10 ± 0.18 (6)
	highland	1.60 ± 0.09 (6)	2.26 ± 0.14 (6)	1.70 ± 0.08 (7)	2.04 ± 0.06 (7)	2.86 ± 0.18 (7)[a]
White gastrocnemius	lowland	2.12 ± 0.07 (7)	2.75 ± 0.17 (7)	2.70 ± 0.25 (6)	2.57 ± 0.21 (6)	2.31 ± 0.05 (6)
	highland	2.13 ± 0.17 (6)	2.71 ± 0.16 (6)	2.21 ± 0.10 (7)	3.09 ± 0.20 (7)	3.09 ± 0.20 (7)
Vastus medialis	lowland	3.82 ± 0.14 (7)	4.63 ± 0.52 (5)	3.58 ± 0.31 (6)	5.48 ± 0.93 (5)	3.50 ± 0.29 (5)
	highland	3.58 ± 0.38 (5)	4.40 ± 1.08 (3)	4.07 ± 0.32 (6)	5.14 ± 0.56 (7)	3.40 ± 0.15 (5)
Liver	lowland	29.84 ± 2.59 (7)	30.56 ± 2.26 (7)	23.52 ± 4.59 (6)	23.45 ± 2.64 (6)	27.70 ± 1.48 (6)
	highland	24.24 ± 4.31 (6)	28.66 ± 2.35 (6)	27.21 ± 4.27 (7)	24.80 ± 1.66 (7)	21.00 ± 2.60 (7)
Blood (mmol L^{-1})	lowland	4.69 ± 0.91 (7)	4.77 ± 0.67 (6)	3.55 ± 0.34 (6)	3.95 ± 0.60 (6)	4.50 ± 0.57 (6)
	highland	4.37 ± 0.66 (6)	5.88 ± 0.57 (6)	4.40 ± 0.39 (6)	4.54 ± 0.48 (7)	4.86 ± 0.49 (7)

[a] Significantly different from resting values for highland mice.

4.2. Free glucose, glucose-6-phosphate, and lactate

Changes in muscle metabolites such as glucose and glucose-6-phosphate can provide insight into flux through specific pathway steps during exercise and recovery. We observed no differences in the free glucose with exercise or into recovery for any of the tissues (Fig. 4). In contrast, glucose-6-phosphate levels were significantly depleted in the gastrocnemius of the lowland deer mice immediately following exercise (Fig. 5). When compared to highlanders, lowlanders had glucose-6-phosphate levels that were 50% lower in the red gastrocnemius and 40% lower in the white gastrocnemius following exercise. This decline in lowlanders suggests an increased flux through glycolysis that exceeds the rate of glucose-6-phosphate production. Lowland deer mice also showed increased accumulation of plasma lactate following exercise, which was significantly elevated above resting values and that of highlanders early in recovery (Fig. 2; Table 2). This suggests a mismatch between pyruvate production in working tissues and its oxidation by the mitochondria. These results are consistent with the lower respiratory capacity of the gastrocnemius muscle in lowlander compared with highlander deer mice (Mahalingam et al., 2017). Lactate accumulation

Table 4
Glucose-6-Phosphate concentrations with exercise and in post-exercise recovery in lowland and highland deer mice acclimated to chronic hypoxia. Glucose-6-Phosphate (in μmol g^{-1} w.w.) was determined for red gastrocnemius, white gastrocnemius, vastus lateralis, and liver during rest, immediately after 15 min of submaximal exercise (~80% $\dot{V}O_2$max) and in recovery in hypoxia. Data are presented as mean ± SEM, with sample sizes in parentheses. There was a significant effect of time for red gastrocnemius ($F_{4, 53} = 3.56$, $p = 0.012$).

Glucose-6-Phosphate (μmol g^{-1} w.w.)		Rest	0 min	10 min	60 min	90 min
Red gastrocnemius	lowland	2.68 ± 0.16 (7)	0.77 ± 0.06 (7)[a]	1.97 ± 0.16 (5)	2.06 ± 0.18 (6)	2.01 ± 0.22 (6)
	highland	1.50 ± 0.09 (6)	1.44 ± 0.20 (6)	1.75 ± 0.13 (7)	1.47 ± 0.13 (7)	2.72 ± 0.23 (7)
White gastrocnemius	lowland	2.97 ± 0.14 (7)	1.81 ± 0.17 (7)	1.63 ± 0.24 (5)	1.98 ± 0.15 (6)	1.83 ± 0.18 (6)
	highland	1.90 ± 0.21 (6)	1.99 ± 0.20 (6)	2.15 ± 0.16 (7)	2.26 ± 0.23 (7)	2.33 ± 0.17 (7)
Vastus medialis	lowland	3.44 ± 0.18 (7)	1.36 ± 0.23 (4)	1.91 ± 0.42 (4)	2.62 ± 0.59 (5)	2.78 ± 0.43 (4)
	highland	1.48 ± 0.17 (5)	2.29 ± 0.53 (3)	1.81 ± 0.26 (6)	2.22 ± 0.25 (5)	2.15 ± 0.22 (5)
Liver	lowland	0.66 ± 0.13 (6)	0.46 ± 0.03 (6)	0.64 ± 0.29 (5)	0.76 ± 0.35 (5)	0.22 ± 0.09 (5)
	highland	0.66 ± 0.21 (6)	0.68 ± 0.18 (5)	0.81 ± 0.23 (7)	0.72 ± 0.18 (6)	0.73 ± 0.22 (6)

[a] Significantly different from resting values for lowland mice.

Table 5
Intramuscular triglyceride (IMTG) concentrations with exercise and in post-exercise recovery in lowland and highland deer mice acclimated to chronic hypoxia. Intramuscular triglyceride (in μmol g^{-1} w.w.) was determined for red gastrocnemius during rest, immediately after 15 min of submaximal exercise (~80% $\dot{V}O_2$max) and in recovery in hypoxia. Data are presented as mean ± SEM, with sample sizes in parentheses.

IMTG (μmol g^{-1} w.w.)		Rest	0 min	10 min	60 min	90 min
Red gastrocnemius	lowland	9.10 ± 1.23 (6)	8.70 ± 2.05 (6)	3.71 ± 0.70 (4)	3.20 ± 0.87 (3)	6.32 ± 1.28 (5)
	highland	3.90 ± 1.09 (5)	4.64 ± 0.65 (6)	5.74 ± 0.91 (6)	8.40 ± 0.98 (7)	6.50 ± 1.16 (7)

in the muscles can occur with increasing exercise intensity as reliance on anaerobic glycolysis increases (Rabinowitz and Enerbäck, 2020). Although not well defined for mice, the threshold where lactate accumulation sharply increases (the lactate threshold) or more appropriately for aerobic exercise, the maximal lactate steady state (the maximal intensity of exercise where blood lactate remains constant), generally occurs with exercise intensities above 70% of maximal aerobic capacity (Ferreira et al., 2007; Lønbro et al., 2019). In CD-1 strain lab mice, acute hypoxia leads to this threshold occurring at a lower running intensity (Le Moine et al., 2011). However, hypoxia acclimation resulted in a lactate threshold that was not different than mice in normoxia (Le Moine et al., 2011). It is unclear how hypoxia acclimation impacts the lactate threshold in lowland and highland deer mice. However, differences in blood lactate accumulation with submaximal exercise (Table 2) suggests that hypoxia acclimated lowlanders were at a running intensity above lactate threshold, while highlanders were not. However, the reliability of using blood lactate as an indicator of exercise intensity in mice has recently been questioned (Lønbro et al., 2019). Nevertheless, methods for standardizing exercise and tissue sampling protocols would facilitate comparisons of exercise recovery between different studies.

4.3. Liver glycogen use with exercise and in recovery

During exercise the liver provides a valuable source of circulatory glucose for working muscles (López-Soldado et al., 2021; Wasserman and Cherrington, 1991). We found that liver glycogen declined significantly with exercise and in recovery. By 10 min of recovery, liver glycogen had declined by almost 50% in lowland mice. By 60 min of exercise recovery highlanders also showed a significant decline in liver glycogen. In contrast to skeletal muscle, liver glycogen remained significantly depleted following 90 min of recovery in lowlanders (Fig. 3, Table 1). Previous studies have demonstrated the importance of hepatic glycogen stores as a fuel source for exercise in humans and rodents, as it supplies circulatory glucose for uptake into the working muscles (Brooks et al., 1973; Lau et al., 2017; Wasserman, 1995). Rats and mice fasted before exercise showed significantly depleted liver glycogen following exercise, and it remained depleted for 24 h (Brooks et al., 1973; Fell et al., 1980). Since exercise recovery took place in this study in the absence of food, hepatic stores likely provide the glucose necessary for muscle glycogen replenishment (Fell et al., 1980). Moreover, glycogen replenishment in muscle may be prioritized, at least in the absence of feeding (Fell et al., 1980). Gluconeogenesis in the liver can also provide glucose necessary for muscle glycogen replenishment. Using precursors such as lactate or glycerol, gluconeogenesis can increase the flux of glucose to muscles without changes in liver glycogen (Wasserman and Cherrington, 1991). In highland deer mice, liver has a greater aerobic capacity and possibly a greater gluconeogenic ability, suggesting a supply of circulatory glucose during exercise and recovery independent of liver glycogen depletion (Lau et al., 2017).

4.4. IMTG in exercise recovery

In lowland mammals, lipid oxidation is maximal at moderate rates (~60% $\dot{V}O_2$max) of aerobic exercise (Schippers et al., 2014). However, data supporting the use of IMTG to power exercise remain equivocal (Stokie et al., 2023). There is compelling evidence that IMTG may provide the energy necessary for glycogen replenishment in exercise recovery (Kiens and Richter, 1998; Pearsall and Palmer, 1990). Although highlanders had higher relative amounts of IMTG in the red gastrocnemius during periods of recovery, compared with lowlanders, IMTG concentrations did not change significantly with exercise or recovery in either population (Fig. 6, Table 5). Highlander deer mice have an additional source of lipid fuel that could support exercise recovery with higher concentrations of circulating triglycerides than lowland mice (Lyons and McClelland, 2022). As well, highlanders have rates of circulatory delivery of triglycerides during peak thermogenesis that are 2–3 times higher than in lowlanders (Lyons and McClelland, 2022). Together these findings suggest highlanders may use circulatory triglycerides, rather than IMTG, during muscle metabolite recovery from submaximal exercise (Pearsall and Palmer, 1990).

4.5. Oxygen consumption during recovery

Oxygen consumption after exercise can be used as a marker of whole animal recovery. During the initial drop in oxygen consumption immediately after exercise, the 'rapid recovery phase', 90% of EPOC can occur over this first minute of exercise recovery (Baker and Gleeson, 1998). This rapid phase of EPOC was similar between deer mouse populations and occurred within approximately 3 min of exercise ending (Fig. 1). In general, EPOC was similar between lowlanders and highlanders, despite suggestion that having a greater aerobic capacity can lead to improved recovery (Tomlin and Wenger, 2001). Although the

highlanders are known to have an increased aerobic capacity, the mice in our study were run at the same relative % $\dot{V}O_2$max, leading to similar EPOC. Lowland deer mice did not incur a greater EPOC, despite disruptions in plasma and tissue metabolites immediately after exercise and early into recovery. It is unclear if this is the result of population differences in efficiency of oxygen use post-exercise or our exercise protocol. Indeed, potential population differences in EPOC may only occur with other exercise interventions, such as running to exhaustion.

5. Conclusions

This study is the first to investigate the influence of altitude ancestry in whole animal and muscle metabolite recovery during submaximal exercise in hypoxia. Previous reports have shown that high and low altitude deer mice differ in their aerobic exercise capacity (Lui et al., 2015; Lau et al., 2017). The current study demonstrates that muscle metabolite recovery from aerobic exercise is also influenced by altitude ancestry. The mice used in this study were all acclimated to hypobaric hypoxia, so it is unclear if differences in exercise recovery are the result of fixed genetic differences or environmentally induced plasticity. Nevertheless, our results showed that muscle glycogen concentrations were depleted significantly in both the red and white gastrocnemius in lowlanders but not in highlanders. Liver glycogen concentrations had a main effect of time during exercise and recovery. Previous work on humans and rodents suggested a use of intramuscular fats as an energy source to power muscle glycogen replenishment during recovery (Kiens and Richter, 1998; Pearsall and Palmer, 1990). The current study did not support these findings as IMTG levels of the red gastrocnemius did not change throughout exercise or recovery. Future studies should investigate the role of other fuel sources for muscle recovery, such as circulating lipids, which are elevated in highland mice in response to acute cold (Lyons and McClelland, 2022). Highland deer mice may also have a unique response to different combinations of acclimation time and exercise training that could enhance overall performance (Scariot et al., 2023).

CRediT authorship contribution statement

Lauren M. Dessureault: Writing – original draft, Methodology, Formal analysis, Data curation. **Reegan A. Tod:** Methodology, Data curation. **Grant B. McClelland:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The author is an Editorial Board Member for *CBP* and was not involved in the editorial review or the decision to publish this article.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

No financial interests or personal relationships to declare.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpb.2024.111004.

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