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ORIGINAL PAPER

Function of left ventricle mitochondria in highland deer mice and lowland mice

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Abstract

To gain insight into the mitochondrial mechanisms of hypoxia tolerance in high-altitude natives, we examined left ventricle mitochondrial function of highland deer mice compared with lowland native deer mice and white-footed mice. Highland and lowland native deer mice (*Peromyscus maniculatus*) and lowland white-footed mice (*P. leucopus*) were first-generation born and raised in common lab conditions. Adult mice were acclimated to either normoxia or hypoxia (60 kPa) equivalent to ~ 4300 m for at least 6 weeks. Left ventricle mitochondrial physiology was assessed by determining respiration in permeabilized muscle fibers with carbohydrates, lipids, and lactate as substrates. We also measured the activities of several left ventricle metabolic enzymes. Permeabilized left ventricle muscle fibers of highland deer mice showed greater rates of respiration with lactate than either lowland deer mice or white-footed mice. This was associated with higher activities of lactate dehydrogenase in tissue and isolated mitochondria in highlanders. Normoxia-acclimated highlanders also showed higher respiratory rates with palmitoyl-carnitine than lowland mice. Maximal respiratory capacity through complexes I and II was also greater in highland deer mice but only compared with lowland deer mice. Acclimation to hypoxia had little effect on respiration rates with these substrates. In contrast, left ventricle activities of hexokinase increased in both lowland and highland deer mice after hypoxia acclimation. These data suggest that highland deer mice support an elevated cardiac function in hypoxia, in part, with high ventricle cardiomyocyte respiratory capacities supported by carbohydrates, fatty acids, and lactate.

Keywords High altitude · Heart · Hypoxia · Lactate · Mitochondria · Phenotypic plasticity

Introduction

Cardiac muscle is a highly aerobic, continuously working tissue that relies primarily on oxidative phosphorylation (OXPHOS) to support resting to maximal cardiac output (reviewed in Glancy and Balaban 2021). Indeed, the demand for O_2 and substrates can increase several-fold to support an elevated cardiac output during aerobic exercise or increased demand for thermogenesis (Tate et al. 2017). The highly aerobic nature of cardiomyocyte metabolism makes it sensitive to decreased O_2 delivery, and hypoxia can negatively affect cardiac physiology in lowland native animals, including laboratory rodents (Dong et al. 2021; Pirzada et al. 1975; Rumsey et al. 1999; Templeman et al. 2010; Turek et al. 1972a, b; Tyberg et al. 1970). However, the effects of hypoxia on cardiomyocyte metabolism in animals adapted to hypoxic environments remain unclear. Proper cardiac function is critical for survival, so those species living in environments with low O_2 availability, such as at high altitude, have likely evolved mechanisms to ensure sustained cardiac function in the face of environmental hypoxia.

Chronic hypoxia impacts cardiac form and function in mammals, but the magnitude of effects varies with altitudinal ancestry. Many lowland rodents, including white-footed mice (WFM, *Peromyscus leucopus,* Rafinesque, 1818) and lowland populations of deer mice (DM, *P. maniculatus,* Wagner 1845), increase total heart mass in

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response to chronic hypoxia (La Padula and Costa 2005; Turek et al. 1972a, b). This response is driven primarily by a right ventricle hypertrophy (with no change in the combined mass of the left ventricle and septum), and in lowland DM is associated with an increase in right ventricular pressure and thickness of pulmonary artery smooth muscle (Velotta et al. 2018; West et al. 2021) resulting from a hypoxia-induced pulmonary hypertension. In contrast, mammals native to hypoxic environments may not express a similar maladaptive right ventricle plasticity due to a blunting of hypoxia-induced pulmonary hypertension (reviewed in Storz et al. 2010b). Indeed, first-generation laboratory born and raised highland DM do not develop a right ventricle hypertrophy with exposure to chronic hypoxia (Velotta et al. 2018; West et al. 2021). Instead, highland DM respond to chronic hypoxia with an elevation in maximal heart rate and cardiac output to support an enhanced thermogenic capacity, compared with lowland mice in the same conditions (Tate et al. 2017, 2020). Surprisingly, this increase in cardiac performance capacity is not associated with a left ventricle hypertrophy commonly observed with beneficial changes in heart function (e.g., with exercise training; Radovits et al. 2013). This suggests that mechanisms independent of hypertrophy, such as metabolic remodeling of cardiomyocytes, may underlie the capacity for higher cardiac output in highland mice.

To meet chronic increases in energy demand, tissues may undergo a metabolic remodeling with elevations in total mitochondrial volume and/or the respiratory capacity per unit of mitochondrial volume. Whether this is also true of the myocardium for highland species to compensate for reduced environmental O_2 availability is unclear. The left ventricle of laboratory born and raised highland DM does show an increase in capillarization in response to chronic hypoxia, demonstrating phenotypic plasticity of this tissue to improve O_2 supply (Tate et al. 2017). Increased capacity for mitochondrial respiration could further enhance tissue O_2 utilization in hypoxia by multiple mechanisms. First, by increasing the capacity for mitochondrial respiration relative to the capacity for O_2 transport from the circulation (i.e., excess mitochondrial capacity), mitochondria operate at a lower submaximal activation of the electron transport system, such that mitochondrial respiration can be maintained at lower O_2 tensions and the O_2 gradient driving diffusion from the blood can be enhanced (Boushel et al. 2011; Cardinale et al. 2019; Larsen et al. 2020). Second, by increasing reliance on the subsarcolemmal fraction of mitochondria (those nearest the cell membrane), overall diffusion distances from the sarcolemma are decreased. Each of these mechanisms have been previously observed in skeletal muscle of highland DM, which have higher volume and respiratory capacity of sarcolemmal mitochondria when compared with lowland DM and/or WFM (Dawson and Scott 2022; Mahalingam et al. 2017), but these mechanisms have not been investigated in the myocardium.

Although cardiomyocytes readily oxidize a wide variety of metabolic substrates, in lowland native mammals, cardiac mitochondria preferentially oxidize fatty acids (reviewed in Stanley et al. 2005). A reduction in the capacity of cardiomyocytes for fatty acid oxidation, and a shift toward carbohydrate use, occurs with chronic hypoxia in laboratory rodents, generally with no change in mitochondrial abundance (Daneshrad et al. 2000; Dong et al. 2021; Razeghi et al. 2001; Templeman et al. 2010). These metabolic responses in lowlanders are associated with a deterioration in cardiac function when accompanied by a pressure-overload cardiac hypertrophy (Rumsey et al. 1999; Storz et al. 2010b). However, an increased capacity for carbohydrate respiration may result in a greater reliance on this substrate and provide an O_2-saving advantage for those species native to high-altitude regions due to a greater ATP yield per O_2 consumed (Brand 2005; Korvald et al. 2000). Indeed, cardiac glucose uptake was higher in Andean Quechua and Sherpas when compared to lowland humans (Holden et al. 1995). Similarly, skeletal muscles in highland leaf-eared mice showed an increased reliance on carbohydrates during exercise (Schippers et al. 2012, 2021). The same was true for laboratory born and raised highland DM after hypoxia acclimation (Lau et al. 2017). Cardiomyocytes are also known to be net lactate consumers (Dong et al. 2021; Kaijser and Berglund 1992; Massie et al. 1994), and will readily oxidize lactate when plasma concentrations are high (Gertz et al. 1988; Glancy and Balaban 2021). Cardiomyocytes express high activities of lactate dehydrogenase (LDH), primarily the H-isoform, which favors conversion of lactate to pyruvate (Schultheiss et al. 1981) for aerobic ATP production. An increased capacity for lactate oxidation at high altitude could reduce plasma and cytosolic lactate accumulation in hypoxia and avoid carbon loss from glycogenolysis occurring in other tissues (Glancy and Balaban 2021). Currently it is unclear if cardiac metabolism has evolved in highland mammals to alter capacities for using lactate or other substrates, and how hypoxia acclimation contributes to these changes.

To address these issues, we compared mitochondrial physiology and enzyme activities of the left ventricle between DM native to high altitude and of lowland DM and WFM. To distinguish between genetically fixed traits and environmentally induced changes, all mice were born and raised in common laboratory conditions and acclimated to either normoxia or chronic hypoxia conditions. We hypothesized that high-altitude native mice have evolved an increased respiratory capacity of left ventricle cardiomyocytes, which will further increase with hypoxia acclimation. We predicted that the capacity of cardiomyocytes for respiration with carbohydrates or lactate as substrates will be higher in highland deer mice than in lowlanders.

Materials and methods

Lowland and highland mice

This study used captive breeding populations of highland and lowland DM and the strictly lowland WFM, established as previously described (Cheviron et al. 2012; Coulson et al. 2021; Lau et al. 2017; Lui et al. 2015). Wild highland DM (*P.m. rufinus*) were trapped at ~ 4300 m (Clear Creek County, CO; 39°35′18″N, 105°38′38″W). Lowland DM (*P.m. nebracensis*) and WFM were trapped in Nine-Mile Prairie (Lancaster County, NE; 40°52′12″N, 96°48′20.3″W) at ~ 400 m above sea level. Mice were transferred to McMaster University (90 m, Hamilton, ON) and bred within population to the first generation. Progeny were raised to adulthood in common laboratory conditions with a constant temperature (23 °C) and light cycle (12L:12D) and provided chow (Teklad Global Rodent Diets, Envigo) and water ad libitum. Adult mice from each group were randomly assigned to one of two acclimation conditions for a period of at least 6 weeks; 1) normoxia in standard holding conditions, or 2) hypobaric hypoxia (60 kPa simulating ~ 4300 m) in specially constructed chambers, as described previously (Lui et al. 2015; McClelland et al. 1998). Hypobaric chambers were returned to normobaric conditions for a brief period (< 30 min) every 3–4 days for cage cleaning. Mice used were from several unique breeding families (11 for highland DM, 5 for lowland DM, and 9 for WFM) and a mix of males and females. All procedures were approved by the McMaster University Animal Research Ethics Board in accordance with guidelines established by the Canadian Council on Animal Care.

Permeabilized muscle fiber respirometry

After the acclimation period was complete, mice were euthanized via isoflurane overdose followed by cervical dislocation. Permeabilized fibers were prepared using methods described previously (Kuznetsov et al. 2008; Mahalingam et al. 2017). Briefly, the heart was removed, and the left ventricle was cut into small ~ 5 mg pieces and placed in ice-cold relaxing and preservation buffer (mM: 2.77 CaK_2EGTA, 7.23 K_2EGTA, 5.77 Na_2ATP, 6.56 $MgCl_2 \cdot 6H_2O$, 20 taurine, 15 Na_2Phosphocreatine, 20 imidazole, 0.5 dithiothreitol, 50 methanesulfonate; pH 7.1). The muscle fibers were then manually teased apart using dissecting probes under a stereomicroscope and then permeabilized for 30 min in the same buffer but with saponin (50 µg ml^{-1}). These fibers underwent three 10 min rinses in ice-cold respiration solution (in mM: 0.5 EGTA, 3 $MgCl_2$, 60 K-lactobionate, 20 taurine, 10 KH_2PO_4, 20 Hepes, 110 sucrose, 1 g L^{-1} fatty acid-free bovine serum albumin (BSA); pH 7.1). Fibers were patted dry and weighed immediately before respirometry measurements. Remaining portions of the left ventricle from lowland and highland DM were frozen in liquid nitrogen immediately after sampling and were stored at −80℃ for enzyme measurements (see below).

Respiration rates were measured using high-resolution respirometry (Oxygraph-2k, Oroboros Instruments, Innsbruck, Austria) in 2 mL of respiration buffer at 37 °C. Separate protocols were used to assess respiration rates with different combinations of substrates. For all protocols, the respiration buffer was initially oxygenated to 450 µM O_2 by bubbling O_2 into the chamber. First, leak respiration was stimulated without the addition of adenylates by the addition of 5 mM pyruvate and 2 mM malate ($L_{N,PM}$). Phosphorylating respiration was stimulated with progressive additions of 2 mM ADP (P_{PM}, using substrates of complex I), 20 mM of glutamate (P_{PMG}, complex I), and 20 mM of succinate (P_{PMGS}, complexes I + II). In a separate set of fiber preparations from the same individuals as above, the capacity for respiration was stimulated with the addition of fatty acid. Leak respiration was stimulated without adenylates by the addition of 2 mM malate and 40 µM palmitoyl-carnitine ($L_{N,\,Pc}$), and phosphorylating respiration was then stimulated with the addition of 2 mM ADP (P_{Pc}). We also determined fiber respiration with lactate in a third set of fiber preparations, as described previously (Elustondo et al. 2013). We stimulated phosphorylating respiration with progressive additions of 1.25 mM ADP, 0.2 mM malate, and 2 mM NAD^+ (P_{M+NAD^+}), followed by 30 mM L-lactate (P_{Lac}). We then added a low concentration (5 µM) of α-cyano-4-hydroxycinnamate (CINN) to specifically inhibit pyruvate transport across the inner mitochondrial membrane ($P_{Lac+Cinn}$) (Elustondo et al. 2013). One preparation (a hypoxia-acclimated lowland DM) did not respond to CINN and was removed from the dataset. We determined respiration that was directly attributable to lactate entering the mitochondria as P_{Lac}–$P_{Lac+Cinn}$, the difference between total respiration with lactate and respiration after inhibition of inner membrane pyruvate transport.

Enzyme activities

Previously frozen tissue from lowland and highland DM was powdered under liquid N_2 using a mortar and pestle. A portion of this powder was weighed and homogenized on ice using a glass-on-glass homogenizer in 20 volumes of homogenization buffer (in mM: 100 KH_2PO_4, 5 EDTA, and 0.1% (v/v) Triton-X-100; pH 7.2). The apparent Vmax of citrate synthase (CS), hexokinase (HK), ß-hydroxyacyl-CoA dehydrogenase (HOAD), and lactate dehydrogenase (LDH)

were measured in the left ventricle tissue as previously described for DM (Cheviron et al. 2012; Lau et al. 2017; Lui et al. 2015) using a Spectromax Plus 384 microplate reader (Molecular Devices; San Jose, CA, USA). Assays were conducted at 37 °C under the following conditions in mM unless stated otherwise: HK, 50 HEPES, 8 $MgCl_2$, 0.5 NADP, 8 ATP, 4 U glucose-6-PDH, 5 glucose (omitted for background) (pH 7.6); HOAD, 100 TEA-HCl, 5 EDTA, 0.28 NADH, 0.1 acetyl-CoA (omitted for background) (pH 7.0); LDH, 40 Tris–HCl, 0.28 NADH, 2.4 pyruvate-Na (omitted for background) (pH 7.4); CS, 40 Tris–HCl, 0.22 acetyl-CoA, 0.1 dithiobisnitrobenzoic acid (DTNB), 0.5 oxaloacetate (omitted for background) (pH 8.0).

LDH activities were assayed in freshly isolated and intact left ventricle mitochondria using a separate group of mice kept in the same acclimation conditions as described above. Mice were euthanized (as described above) and the left ventricle was immediately transferred to 10 mL of ice-cold isolation buffer (in mM: 100 sucrose, 50 Tris base, 5 $MgCl_2$, 5 EGTA, 100 KCl, 1 ATP). The ventricular muscle was minced and then digested for 5 min in the same buffer containing protease (1 mg g^{-1} muscle tissue). The digested muscle was then gently homogenized with six passes of a Potter–Elvehjem Teflon on glass homogenizer (100 rpm). Homogenates were centrifuged at 700 × g for 10 min. The resulting supernatant was filtered through cheesecloth and then centrifuged at 1000 × g for 10 min. The supernatant was centrifuged at 8700 × g for 10 min and the pellet was resuspended in 10 mL of isolation buffer with 1% w/v BSA (fatty acid-free) and centrifuged again at 8700 × g. The pellet was resuspended in 10 mL of storage buffer (in mM: 0.5 EGTA, 3 $MgCl_2$, 60 K-methanesulfonate, 20 taurine, 10 KH_2PO_4, 20 Hepes, 110 sucrose, 0.02 vitamin E succinate, 2 malate, pH 7.1) and centrifuged a final time at 8700 × g. The resulting pellet was resuspended in 250–400 µL of storage buffer. Apparent Vmax of LDH in isolated mitochondria were measured at 37 °C using a Spectromax Plus 384 microplate reader (Molecular Devices). To determine if LDH activity was intra-mitochondrial, activity was determined in both intact and disrupted mitochondria. Intact mitochondria were assayed in isotonic storage buffer, with 0.28 mM NADH, 2.4 mM pyruvate-Na (omitted for background). LDH activity was negligible when mitochondria were kept intact. We then disrupted the isolated mitochondria by freeze thawing in liquid nitrogen and repeated the LDH activity measurement as described previously (Passarella et al. 2014). Due to low mouse availability, mitochondrial LDH activity from the lowland species was pooled (5 WFM and 1 DM for normoxia, and 2 WFM and 6 DM for hypoxia acclimation) to compare with that of highland DM.

Statistical analyses

Data are presented as means ± SEM. Two-factor ANOVA and Tukey's or Holm-Sidak's multiple-comparisons post-tests were used as appropriate to determine the main effects of and interactions between population altitude and acclimation environment using GraphPad Prism version 9.1.0 for Mac, and in R (https://www.r-project.org/). A significance level of $P < 0.05$ was used throughout. One significant outlier for the P_{Lac}- $P_{Lac+CINN}$ measurement (a hypoxia-acclimated lowland DM) was identified using a Grubbs' test and removed.

Results

Permeabilized fiber respiratory capacities

We used permeabilized left ventricle muscle fibers to determine respiratory capacities of this tissue in highland DM compared with that of lowland DM and WFM, and in response to hypoxia acclimation. Leak respiration was determined in the absence of ADP and showed a significant effect

Table 1 Respiration rates (JO_2 in nmol min^{-1} mg^{-1} ventricle weight) of permeabilized muscle fibers of left ventricles from highland and lowland deer mice and white-footed mice acclimated to normoxic control conditions or to hypobaric hypoxia conditions for 6–8 weeks. Values are presented as means ± S.E.M

	Highland deer mice		Lowland deer mice		White-footed mice	
	Normoxia	Hypoxia	Normoxia	Hypoxia	Normoxia	Hypoxia
$L_{N,PM}$	2.13 ± 0.13	2.51 ± 0.12	1.61 ± 0.18	1.35 ± 0.20*	2.20 ± 0.24	2.18 ± 0.16
$L_{N,Pc}$	2.09 ± 0.21	1.65 ± 0.13	0.97 ± 0.18*	0.53 ± 0.15*	1.42 ± 0.21	1.55 ± 0.11
P_{PM}	9.24 ± 0.54	9.16 ± 0.82	5.55 ± 0.66*	5.06 ± 0.93*	8.08 ± 0.52	8.43 ± 0.28
P_{PMG}	9.85 ± 0.59	9.86 ± 0.87	5.87 ± 0.74*	5.91 ± 1.00*	8.80 ± 0.67	9.48 ± 0.34
P_{PMGS}	11.29 ± 0.61	11.43 ± 1.17	7.81 ± 1.08	7.78 ± 1.13*	9.80 ± 1.06	11.12 ± 0.59
RCR	5.38 ± 0.29	4.60 ± 0.58	4.97 ± 0.09	6.03 ± 0.84	4.60 ± 0.53	5.27 ± 0.47
$1-(L_N / P_{PMGS})$	0.81 ± 0.01	0.77 ± 0.03	0.76 ± 0.05	0.81 ± 0.02	0.77 ± 0.03	0.80 ± 0.02

Leak respiration in the absence of ADP (L_N) and oxidative phosphorylation (P) with substrates P pyruvate, Pc palmitoyl-carnitine, M malate, G glutamate, S succinate, respiratory acceptor control ratio (RCR = $P_{PM}/L_{N,PM}$); OXPHOS coupling efficiency ($1-(L_N/P_{PMGS})$). *Significantly different from highland deer mice within an acclimation

Table 2 Statistical summary for data that appear in Table 1

	Population		Acclimation		Pop × Accl	
	F	*P*	*F*	*P*	*F*	*P*
$L_{N,PM}$	13.74	< 0.001	0.004	0.950	1.473	0.244
$L_{N,Pc}$	24.03	< 0.001	3.766	0.061	1.866	0.171
P_{PM}	19.51	< 0.001	0.097	0.757	0.186	0.831
P_{PMG}	17.34	< 0.001	0.071	0.792	0.161	0.852
P_{PMGS}	6.829	0.003	0.032	0.858	0.443	0.646
RCR	0.467	0.630	0.521	0.475	1.247	0.300
$1-(L_N/P_{PMGS})$	0.005	0.665	0.347	0.560	1.487	0.240

of population when either pyruvate and malate ($L_{N,PM}$), or palmitoyl-carnitine ($L_{N,Pc}$) were used as substrates. Leak was unaffected by acclimation (Tables 1 and 2). Highland DM had higher $L_{N,PM}$ compared with lowland DM (by 1.9-fold) when both were acclimated to hypoxia. $L_{N,Pc}$ was also greater in highland DM compared with lowland DM and in both normoxia (2.2-fold) and hypoxia (3.1-fold) acclimations (Table 1). Similarly, there was a significant effect of population but not of acclimation for OXPHOS stimulated by the addition of ADP with pyruvate and malate as substrates (P_{PM}), after the addition of glutamate (P_{PMG}, Table 1, Table 2), and for maximal respiratory capacity through complexes I and II with further addition of succinate (P_{PMGS}, $F_{2,34}=6.548$, $P=0.004$; Table 1, Fig. 1A). *Post hoc* analysis revealed that P_{PMGS} was equivalent between hypoxia-acclimated highland DM and WFM, but both groups had significantly higher rates of respiration than lowland DM ($P=0.025$ for highland DM, and $P=0.025$ for WFM). There were no significant differences between groups or with acclimation in either respiratory acceptor control ratio (RCR) or OXPHOS coupling efficiency (Tables 1 and 2). OXPHOS respiration with palmitoyl-carnitine (P_{Pc}) as a substrate also showed a significant effect of population ($F_{2,33}=14.74$, $P<0.0001$), but not of hypoxia acclimation (Fig. 1B). In normoxia acclimation, P_{Pc} was greater in highland DM compared with both lowland DM and WFM (4.32 ± 0.35 versus 1.95 ± 0.36 and 2.68 ± 0.49 nmol min^{-1} mg^{-1}; $P<0.02$). After hypoxia acclimation, acyl-carnitine fueled respiration was twofold higher in highland DM and WFM compared with lowland DM (3.51 ± 0.36 and 3.51 ± 0.36 versus 1.73 ± 0.40 nmol min^{-1} mg^{-1}; $P=0.0071$).

Lactate respiration of permeabilized fibers

Cardiac muscle in mammals is known to be a net consumer of lactate during rest and exercise (Bergman et al. 2009). Therefore, we assessed permeabilized left ventricle fiber respiration with lactate as the primary substrate. We first initiated respiration using malate and NAD^+ ($P_{M+NAD}{}^+$), resulting in respiration rates that were similar across DM populations and WFM (Tables 3 and 4). Acclimation to hypoxia led to an increase in $P_{M+NAD}{}^+$ but only in lowland DM (significant population × acclimation effect). The subsequent addition of lactate increased respiration rates, and there was a significant effect of population, with respiration in normoxia-acclimated highland DM that was higher than WFM (Tables 3 and 4). The inhibition of pyruvate transport across the inner mitochondrial membrane by the addition of 5 µM CINN decreased respiration ($P_{Lac+Cinn}$) to similar rates as those without added lactate (Tables 3 and 4). We determined respiration due to lactate entering mitochondria as the difference between P_{Lac} and $P_{Lac+Cinn}$ and found it varied significantly with population ($F_{2,37}=5.47$, $P=0.008$), but there was no effect of hypoxia acclimation (Fig. 2). *Post hoc* analysis revealed that within the normoxia acclimation treatment, lactate-specific respiration was significantly higher in highland DM than WFM ($P<0.05$) and approached statistical significance compared with lowland DM ($P=0.07$). However, lowland DM and WFM had equivalent respiration rates ($P>0.05$).

Enzyme activities

We used the apparent Vmax for enzymes at key steps in metabolic pathways to predict how the capacity for pathway flux varies between lowland DM and highland DM and with hypoxia acclimation. We found few significant differences in enzyme activity of left ventricle between populations or with hypoxia acclimation (Table 5). One exception was the Krebs cycle enzyme IDH, which was 1.2-fold higher in the left ventricle of highland DM than in lowland DM. We also found that HK, an important regulator of circulatory glucose uptake, showed a significant effect of acclimation, with increased activity in both lowland and highland DM with chronic hypoxia (Table 5). To help understand the underlying mechanisms responsible for differences in left ventricle respiration with lactate as a substrate, we determined LDH activity in whole tissue homogenates and in isolated left ventricle mitochondria (Fig. 3). At the tissue level, LDH showed a significant effect of population ($F_{1,35}=10.61$, $P=0.025$) but not of acclimation ($F_{1,35}=0.305$, $P=0.584$). We found that LDH activity was greater in normoxia-acclimated

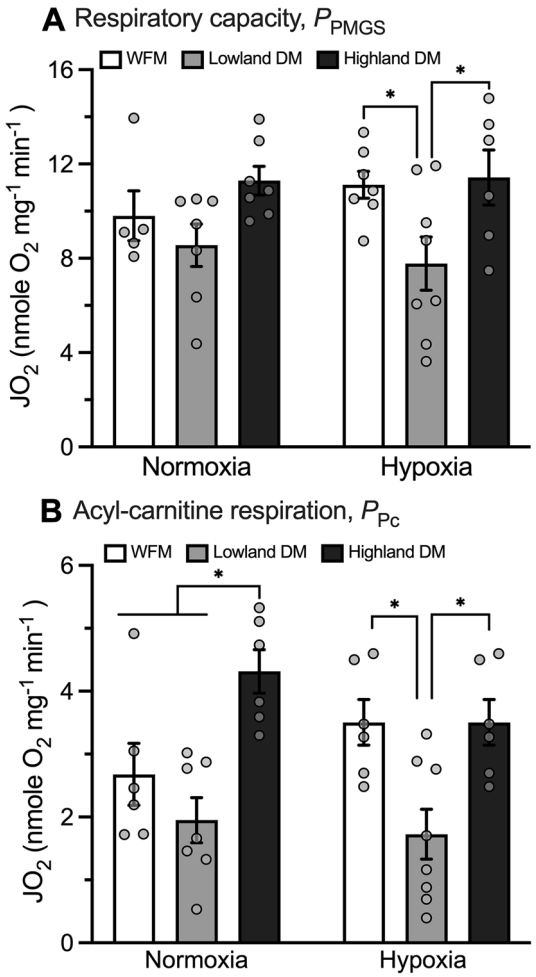

Fig. 1 Respiratory capacity of oxidative phosphorylation using **A**. *P* pyruvate, *M* malate, *G* glutamate, and *S* succinate, as substrates (P_{PMGS}) for permeabilized fibers from left ventricle of highland and lowland deer mice (DM) and white-footed mice (WFM) acclimated to normoxia control conditions [$N=5$ (2 males, 3 females) for WFM, 7 (3 M, 4F) for lowland DM, and 7 (1 M, 6F) for highland DM] or hypobaric hypoxia [$N=7$ (3 M, 4F) for WFM, 8 (6 M, 2F) for lowland DM, and 7 (1 M, 6F) for highland DM] for 6–8 weeks. P_{PMGS} showed a significant effect of population ($F_{2,33}=6.829$, $P=0.003$) but not of acclimation ($F_{1,33}=0.032$, $P=0.858$). Respiration using **B** palmitoyl-carnitine (P_{Pc}) also showed a significant effect of population ($F_{2,33}=13.89$, $P<0.001$) but not of acclimation ($F_{1,33}=0.114$, $P=0.737$). Sample sizes for P_{Pc} in mice acclimated to normoxia were $N=6$ (3 M, 3F) for WFM, 7 (4 M, 3F) for lowland DM, and 6 (0 M, 6F) for highland DM, and hypoxia were $N=6$ (3 M, 3F) for WFM, 8 (6 M, 2F) for lowland DM, and 6 (3 M, 3F) for highland DM. Respiration rates are standardized to fiber wet weight. Values are presented as means ± S.E.M. *Significantly different within an acclimation

highland DM compared with lowlanders (Fig. 3A). In the hypoxia-acclimated mice, LDH tended to be higher in the highland DM and this difference approached statistical significance ($P=0.079$). The activity of LDH was also assessed in isolated mitochondria, which showed a significant effect of population ($F_{1,26}=6.353$, $P=0.018$; Fig. 3B), driven by higher overall enzyme activities in highlanders.

Discussion

The goal of this study was to determine if highland native DM have a greater left ventricle cardiomyocyte respiratory capacity to support an enhanced cardiac performance in hypoxia. We also wished to determine if environmentally induced plasticity contributes to variation in respiratory capacities in highland DM compared with a lowland conspecific DM population and a lowland congeneric species (WFM). We found similar CS activities in the left ventricle of all groups and activity did not change with hypoxia acclimation, suggesting equivalent mitochondrial volume densities. However, respiratory capacities of left ventricle permeabilized fibers were often greater in highland DM compared to lowland DM. Similarly, fatty acid-supported OXPHOS capacity with palmitoyl-carnitine was ~ twofold greater in highland compared with lowland DM regardless of acclimation conditions. In contrast, permeabilized fibers from left ventricles of lowland native WFM tended to have equivalent respiratory capacities to highland DM, suggesting that some of the observed differences between DM populations could be explained by evolved reductions in respiratory capacity in lowland DM rather than evolved increases in highland DM. One exception was OXPHOS capacity supported by lactate, which was highest in highland DM, associated with higher LDH activities in highlanders in both left ventricle tissue and within mitochondria. Hypoxia acclimation had little influence on most of the traits we measured, except for a hypoxia-induced increase in HK activity in both populations. These data suggest that highland DM support an elevated cardiac function in hypoxia, in part, with high ventricle cardiomyocyte respiratory capacities supported by variation in mitochondrial carbohydrate, fatty acid, and lactate oxidation.

Respiration with lactate was greater in highland DM compared with lowland DM and WFM (by ~ 1.7-fold, Fig. 2), which appears to be an evolved change in highlanders that could represent an adaptation to high altitude. We saw no effect of acclimation on respiration supported with lactate, in contrast to data on lab rats showing that chronic hypoxia impacted the machinery for left ventricle lactate metabolism (McClelland and Brooks 2002). The greater lactate-stimulated respiration in highland DM is likely facilitated by higher LDH activities in isolated mitochondria, and increased lactate availability in vivo by greater cytosolic LDH activities (Fig. 3). The association between cytosolic LDH activity and capacity for pyruvate production is well established, but the location of mitochondrial LDH and its role in lactate oxidation is unresolved (Glancy et al. 2021). Current evidence suggests that mitochondrial LDH is not

Table 3 Respiration rates (JO_2 in nmol min^{-1} mg^{-1} ventricle weight) of permeabilized muscle fibers of left ventricles from highland and lowland deer mice and white-footed mice acclimated to normoxic control conditions or to hypobaric hypoxia conditions for 6–8 weeks. Values are presented as means ± S.E.M

	Highland deer mice		Lowland deer mice		White-footed mice	
	Normoxia	Hypoxia	Normoxia	Hypoxia	Normoxia	Hypoxia
$P_{M+NAD}{}^{+}$	10.61 ± 0.84	8.95 ± 0.77	6.60 ± 0.80	11.92 ± 2.3†	7.17 ± 0.87	9.24 ± 0.95
P_{Lac}	16.70 ± 1.51	14.47 ± 1.1	11.68 ± 1.84	16.22 ± 2.72	10.60 ± 1.0*	13.03 ± 1.01
$P_{Lac+Cinn}$	11.10 ± 1.28	9.20 ± 0.61	7.97 ± 1.10	12.45 ± 1.68	7.18 ± 0.87	8.89 ± 0.92

Oxidative phosphorylation (P) in the presence of ADP with substrates M, malate; NAD^{+}; *Lac* lactate, and 5 µM transport inhibitor CINN, α-cyano-4 hydroxycinnamate

†Significantly different than normoxia acclimation within a population. *Significantly different from highland deer mice within an acclimation

Table 4 Statistical summary for data that appear in Table 3

	Population		Acclimation		Pop × Accl	
	F	*P*	*F*	*P*	*F*	*P*
$P_{M+NAD}{}^{+}$	1.289	0.287	2.715	0.107	4.855	0.013
P_{Lac}	3.663	0.035	0.896	0.350	2.611	0.086
$P_{Lac+Cinn}$	2.714	0.079	1.488	0.230	4.122	0.024

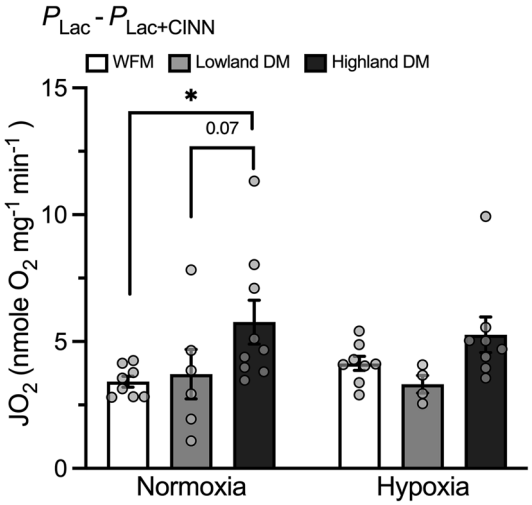

Fig. 2 Respiration during oxidative phosphorylation supported by malate and lactate (Lac) as substrates in the presence of NAD^{+} (P_{Lac}) that was sensitive to inhibition of pyruvate transport by α-cyano-4 hydroxycinnamate (i.e., P_{Lac}–$P_{Lac+CINN}$) for permeabilized fibers from left ventricle of highland and lowland deer mice (DM) and white-footed mice (WFM) acclimated to normoxia control conditions [$N = 8$ (4 males, 4 females) for WFM, 6 (4 M, 2F) for lowland DM, and 9 (9 M, 0F) for highland DM)] or hypobaric hypoxia [($N = 8$ (5 M, 3F) for WFM, 4 (3 M, 1F) for lowland DM), and 8 (3 M, 5F) for highland DM] for 6–8 weeks. Respiration showed a significant effect of population ($F_{2,37} = 5.47$, $P = 0.008$) but not acclimation ($F_{1,37} = 0.011$, $P = 0.917$). Respiration rates are standardized to fiber wet weight. Values are presented as means ± S.E.M. *Significantly different within an acclimation

localized to the matrix, but likely exists in the intermembrane space (Elustondo et al. 2013; Glancy et al. 2021; Hashimoto et al. 2006). Excess lactate crossing the outer mitochondrial membrane would be converted to pyruvate in the intermembrane space and the resulting pyruvate oxidized in the matrix. Indeed, lactate-stimulated respiration returned to baseline levels when we blocked pyruvate transport into the matrix (Table 3). Nevertheless, an increase in the capacity for lactate oxidation in the left ventricle could be beneficial in hypoxic environments, in which other tissues may produce lactate via anaerobic glycolysis, because it would decrease plasma and cytosolic lactate levels and minimize carbon loss. The higher ability to consume lactate may enhance the capacity for aerobic ATP production and help highland DM support heart rates and cardiac output that are 1.5 to 1.7-fold greater than seen in lowland mice at maximal hypoxic thermogenic capacities (Tate et al. 2017, 2020).

We also found that left ventricles from highland DM had a high capacity to support mitochondrial respiration using palmitoyl-carnitine. Permeabilized left ventricle fibers of normoxia-acclimated highland DM showed fatty-acid-stimulated respiration that was approximately double the rates determined for both lowland DM and WFM (Fig. 1B). The greater P_{Pc} was not associated with increased capacity for ß-oxidation, as HOAD activities were not different between lowland and highland DM (Table 5). Mitochondrial membrane transport of fatty acids by carnitine

Table 5 Apparent enzyme Vmax (in µmoles min^{-1} g^{-1} w.w.) from left ventricles of highland and lowland deer mice acclimated to normoxic control conditions or to hypobaric hypoxia conditions for 6–8 weeks. Values are presented as means ± S.E.M

	Highland deer mice		Lowland deer mice		Population		Acclimation		Pop × Accl	
	Normoxia	Hypoxia	Normoxia	Hypoxia	*F*	*P*	*F*	*P*	*F*	*P*
HK	5.00 ± 0.54	6.59 ± 0.35	5.02 ± 0.55	6.08 ± 0.67	0.158	0.693	6.018	0.019	0.238	0.629
PK	44.7 ± 3.3	46.6 ± 5.7	40.1 ± 2.1	39.8 ± 1.5	2.509	0.122	0.054	0.818	0.103	0.750
HOAD	151.2 ± 15.4	132.3 ± 5.6	127.8 ± 10.7	144.3 ± 13.3	0.197	0.660	0.020	0.889	2.203	0.147
CS	165.3 ± 13.8	171.7 ± 8.3	149.3 ± 15.1	165.9 ± 12.3	0.241	0.627	0.743	0.395	0.720	0.402
IDH	71.0 ± 5.4	72.6 ± 3.4	63.5 ± 5.8	58.5 ± 5.2	5.026	0.031	0.102	0.752	0.453	0.506
COX	216.7 ± 24.2	203.7 ± 12.1	197.0 ± 27.4	181.5 ± 17.0	1.066	0.309	0.475	0.495	0.003	0.954

HK hexokinase, *PK* pyruvate kinase, *HOAD* ß-hydroxyacyl-CoA dehydrogenase, *CS* citrate synthase, *IDH* isocitrate dehydrogenase, *COX* cytochrome-c oxidase

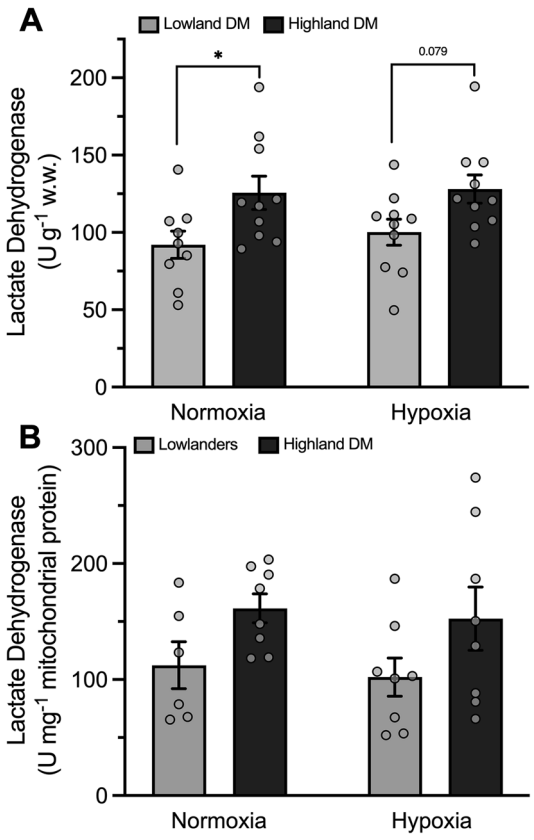

Fig. 3 The apparent Vmax for lactate dehydrogenase (LDH) per gram tissue wet weight (w.w.) **A** in left ventricle from lowland deer mice (DM) and highland DM acclimated to normoxia control conditions ($N=9$ and 10) or hypobaric hypoxia ($N=10$) for 6–8 weeks. Vmax showed a significant effect of population ($F_{1,35}=10.606$, $P=0.025$), but not of acclimation ($F_{1,35}=0.305$, $P=0.584$). The apparent Vmax for LDH was also measured in isolated left ventricle mitochondria **B** for highland DM ($N=8$) and lowlanders ($N=6$ and 8, lowland DM and white-footed mice combined) and showed a significant effect of population ($F_{1,26}=6.353$, $P=0.018$) but not acclimation ($F_{1,26}=0.224$, $P=0.640$). Values are presented as means ± S.E.M. U, units of activity in µmoles min^{-1}. *Significantly different within an acclimation

palmitoyl-transferase is an important bottleneck for oxidation (McClelland 2004), and an increase in activity of this enzyme in highlanders could explain population differences in respiration. Highland DM also show a greater capacity for respiration with carbohydrates, through complex I, and complex I and II combined, compared with lowland DM (Table 1, Fig. 1A). Perhaps highland DM would show a shift in ventricle fuel use in vivo, to a greater reliance on carbohydrates, at least relative to lowland DM. However, equivalent P_{Pc}/P_{PMGS} ratios in both DM populations (data not shown) suggest relative capacities for lipid and carbohydrate substrates to support OXPHOS are not altered at high altitude. That is, highlanders have a similarly enhanced capacity to supported respiration with both substrates, compared to lowland DM. It is worth noting that substrate use in vivo is complex and depends on many factors, including substrate availability and regulation of metabolic pathways. Indeed, the Vmax of the enzyme HK increased in the left ventricle with hypoxia acclimation for both lowland and highland DM (Table 5). This enzyme plays an essential role in circulatory glucose uptake in skeletal muscle (Wasserman et al. 2011) suggesting an increased cytosolic availability after acclimation. Increased glucose availability and a greater capacity for respiration using pyruvate may result in a greater reliance on carbohydrates to support cardiomyocyte metabolism in hypoxia-acclimated highlanders. In contrast to HK activities, respiratory capacities did not vary in highland DM as a result of environmentally induced plasticity. These data suggest that highlanders maintain cardiac muscle respiratory capacities that are fixed at a higher level compared with lowland DM. We also found that left ventricle CS activities were similar across all mice, suggesting variation in respiratory capacities was not the result of differences in mitochondrial volume density, but to a remodeling of mitochondrial metabolism. The basis of this remodeling is unclear but could involve changes in the activity of individual electron transport chain complexes (Mahalingam et al. 2017).

The differences in cardiomyocyte respiratory capacities between highland and lowland DM were not generally seen between highlanders and the strictly lowland WFM, thought to represent the ancestral lowland condition (Velotta et al. 2018). With some exceptions (e.g., lactate), respiratory capacities of left ventricle fibers were similar between WFM and highland DM. This would suggest that the enhanced left ventricle aerobic metabolism seen in highlanders may not have evolved to increase survival in the cold alpine environment. However, WFM have lower hypoxic maximal aerobic capacities, associated with lower maximal heart rates and cardiac outputs than seen in highland DM (Tate et al. 2017, 2020). These data suggest that a limitation in O_2 supply, or some other trait, may limit cardiac performance of WFM in hypoxia. Whereas lowland DM have similar heart tissue capillarization (Tate et al. 2017) but lower respiratory capacities than highlanders, WFM have similar respiratory capacities but perhaps lower capillarization. The result would be similar: an inability to support high cardiac function in hypoxia.

Conclusion

Here we show that highland DM maintain higher respiratory capacities of their left ventricle cardiomyocytes compared with lowland DM. The capacity for higher respiration with pyruvate, acyl-carnitines, and lactate likely aid highland DM in maintaining high levels of cardiovascular performance in hypoxia. Highland DM rely on an elevated cardiac output in hypoxia for appropriate O_2 and substrate delivery to support the high thermogenic demands they experience in the cold high alpine habitat (Tate et al. 2017, 2020; Lyons and McClelland 2022). In fact, cardiac output in highland DM can reach rates that are almost double that of lowland mice, when both are at their maximal rate of aerobic heat production (Tate et al. 2020). Cardiac performance in highlanders likely profits from the combined increases in circulatory O_2 transport (Storz et al. 2010a), capillary surface area for tissue uptake (Tate et al. 2017), and respiratory capacity (this study). The absence of one or more of these traits may limit the ability for aerobic ATP production and lead to a loss of performance. This seems to be the case for WFM, who had similar cardiomyocyte respiratory capacities to highland DM but have much lower maximal cardiac output in hypoxia (Tate et al. 2020), suggesting a limitation in tissue O_2 delivery in this exclusively low-altitude species.

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Data availability Data are available from Figshare: https://doi.org/10.6084/m9.figshare.20449983.v2.

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