



# Mitochondria-associated lactate dehydrogenase is not a biologically significant contributor to bioenergetic function in murine striated muscle

Kyle L. Fulghum<sup>a,b</sup>, Benjamin R. Rood<sup>a</sup>, Velma O. Shang<sup>a,c</sup>, Lindsey A. McNally<sup>a</sup>, Daniel W. Riggs<sup>a,d</sup>, Yu-Ting Zheng<sup>a</sup>, Bradford G. Hill<sup>a,b,c,\*</sup>

<sup>a</sup> Diabetes and Obesity Center, Envirome Institute, Department of Medicine, University of Louisville, Louisville, KY, USA

<sup>b</sup> Department of Physiology and Biophysics, University of Louisville, Louisville, KY, USA

<sup>c</sup> Department of Biochemistry and Molecular Genetics, University of Louisville, Louisville, KY, USA

<sup>d</sup> Department of Bioinformatics and Biostatistics, University of Louisville, Louisville, KY, USA

## ARTICLE INFO

### Keywords:

Cardiac hypertrophy  
Exercise  
Mitochondria  
Respiration  
Bioenergetics  
Glycolysis

## ABSTRACT

Previous studies indicate that mitochondria-localized lactate dehydrogenase (mLDH) might be a significant contributor to metabolism. In the heart, the presence of mLDH could provide cardiac mitochondria with a higher capacity to generate reducing equivalents directly available for respiration, especially during exercise when circulating lactate levels are high. The purpose of this study was to test the hypothesis that mLDH contributes to striated muscle bioenergetic function. Mitochondria isolated from murine cardiac and skeletal muscle lacked an appreciable ability to respire on lactate in the absence or presence of exogenous NAD<sup>+</sup>. Although three weeks of treadmill running promoted physiologic cardiac growth, mitochondria isolated from the hearts of acutely exercised or exercise-adapted mice showed no further increase in lactate oxidation capacity. In all conditions tested, cardiac mitochondria respired at > 20-fold higher levels with provision of pyruvate compared with lactate. Similarly, skeletal muscle mitochondria showed little capacity to respire on lactate. Protease protection assays of isolated cardiac mitochondria confirmed that LDH is not localized within the mitochondrion. We conclude that mLDH does not contribute to cardiac bioenergetics in mice.

## 1. Introduction

The heart requires large amounts of energy for contraction. Periods of heightened physical activity further increase the energy demands of the heart. During exercise, higher circulating levels of lactate provide the heart with increased levels of oxidizable substrate [1,2]. Although high blood lactate levels increase lactate oxidation and diminish myocardial glucose catabolism [2–4], the mechanisms by which lactate regulates cardiac energetics and function remain incompletely understood.

Lactate oxidation occurs via cytosolic lactate dehydrogenase (LDH), which converts lactate to pyruvate in an NAD<sup>+</sup>-dependent manner; the pyruvate can then be oxidized in mitochondria. Interestingly, several studies suggest that LDH also exists within mitochondria [5–8], yet other studies indicate that LDH is not within mitochondria and that the contribution of mitochondrial LDH (mLDH) to energetics is negligible [9–11]. Intramitochondrial LDH could be advantageous to the heart, especially during exercise, because the additional NADH generated in

the LDH reaction would be directly available for respiration, without the need for the malate-aspartate shuttle system to transport reducing equivalents across the inner mitochondrial membrane.

The goal of this study was to determine whether mLDH exists in the heart and to delineate whether exercise influences LDH localization or intramitochondrial lactate oxidation. We find that cardiac mitochondria do not contain LDH and that exercise does not influence mLDH abundance or its contribution to respiration. These results indicate that cytosolic, and not mitochondrial, LDH promotes cardiac lactate oxidation.

## 2. Methods

### 2.1. Experimental animals

All procedures were approved by the Institutional Animal Care and Use Committee at the University of Louisville. Adult, male FVB/NJ mice (15–20 weeks of age) on a 12 h:12 h light: dark cycle were used in all

\* Corresponding author. Department of Medicine, Christina Lee Brown Envirome Institute, Diabetes and Obesity Center, University of Louisville, 580 S. Preston St., Rm 321E, Louisville, KY, 40202, USA.

E-mail address: [bradford.hill@louisville.edu](mailto:bradford.hill@louisville.edu) (B.G. Hill).

<https://doi.org/10.1016/j.redox.2019.101177>

Received 22 February 2019; Received in revised form 8 March 2019; Accepted 21 March 2019

Available online 22 March 2019

2213-2317/ © 2019 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

experiments. Chow and water were provided *ad libitum*. Upon completion of each experiment, mice were anesthetized with sodium pentobarbital (150 mg/kg, i.p.) prior to tissue harvest.

## 2.2. Exercise capacity testing and treadmill training

Exercise familiarization, capacity testing, and training were performed as described previously [4,12] for up to three weeks. We acclimated the mice to treadmill running for two days before the first exercise capacity test (ECT). Mice were exercised at 75% of their initial exercise capacity, at a 10° incline for 40 min (Week 1, 5 d/wk), 50 min (Week 2, 5 d/wk), and 60 min (Week 3, 5 d/wk). We performed ECTs at the end of each week and continued the training regimen until work determined during the ECT increased by at least an average of 1.5-fold above pretraining values. Then, we trained the mice one additional week prior to euthanasia. Work was calculated as the product of bodyweight (kg) and vertical distance (m), where vertical distance = distance run (m)  $\times$   $\sin \theta$  ( $\theta$  = inclination angle) [12,13].

To examine the acute effects of exercise, mice ran at a 10° incline at 10 m/min for the first 10 min, then the speed was adjusted to 19.5 m/min for the next 40 min. In this protocol, we confirmed compliance if > 90% of the 19.5 m/min portion of the exercise bout was completed (i.e. > 36 min at 19.5 m/min). We euthanized mice immediately following the exercise protocol and excised cardiac and skeletal muscle tissue for mitochondrial isolation and biochemical analyses.

## 2.3. Circulating lactate measurements

Blood samples for circulating glucose and lactate were obtained via tail clip and measured using the Accu-Check Aviva meter (Roche) and the Lactate Plus meter (Nova Biomedical), respectively.

## 2.4. Mitochondrial isolation

Hearts or gastrocnemius muscles from sedentary and exercised mice were isolated and homogenized in 1 ml of isolation buffer (Buffer A: 220 mM mannitol, 70 mM sucrose, 5 mM MOPS, 1 mM EGTA, 0.2% fatty acid-free BSA, pH 7.2) using a Potter Elvehjem tube and a Teflon pestle. The homogenate was centrifuged at 800g for 10 min at 4 °C. The supernatant was then centrifuged at 10,000g for 15 min at 4 °C to obtain the mitochondrial fraction. The mitochondrial pellets were washed twice in 500  $\mu$ L of isolation buffer and then resuspended in 400  $\mu$ L of respiration buffer (120 mM KCl, 25 mM sucrose, 10 mM HEPES, 1 mM MgCl<sub>2</sub>, 5 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.2) for extracellular flux analysis and biochemical assays. Protein concentration was assessed using the Lowry DC Protein Assay kit (Biorad).

## 2.5. Extracellular flux analysis

Mitochondrial respiration was assessed using a Seahorse XF24 analyzer (Agilent), as described previously [4,14,15]. For each group, 10  $\mu$ g of mitochondrial protein was suspended in 50  $\mu$ L of respiration buffer and loaded into 24-well XF culture microplates. The microplates were centrifuged at 500g for 3 min at 4 °C, followed by addition of 625  $\mu$ L of warm (37 °C) respiration buffer. We used the following substrates to stimulate state 3 respiration: for cardiac mitochondria, we provided 5 mM pyruvate, 2.5 mM malate, and 1 mM ADP; for skeletal muscle mitochondria, we provided 5 mM glutamate, 2.5 mM malate, and 1 mM ADP; and for both cardiac and skeletal muscle mitochondria, we provided 5 mM lactate, 2.5 mM malate, and 1 mM ADP. We next provided 1 mM NAD<sup>+</sup> (final concentration), which enabled understanding of how LDH localized to the intermembrane space or outer mitochondrial membrane might contribute to mitochondrial energetics [16]. At the end of the respiration assays, 10  $\mu$ M antimycin A was added to ensure that all oxygen consumption was due to mitochondrial respiration.

## 2.6. Protease protection assay

To examine mitochondrial LDH localization, freshly isolated mitochondria (100  $\mu$ g cardiac mitochondrial protein) were suspended in Buffer A in the presence or absence of 0.25 mg/ml trypsin (Becton, Dickinson and Company, product number 215240). Mitochondria solubilized with 1% (v/v) Triton X-100 in the presence of 0.25 mg/ml trypsin served as an additional control. Reaction volumes (100  $\mu$ L) were incubated at room temperature for 15 min, and the reaction was stopped by addition of 2  $\mu$ L of 100 $\times$  protease inhibitor cocktail (Sigma P8340). We then collected mitochondria by centrifugation at 10,000g for 10 min at 4 °C. The supernatants were discarded from non-solubilized treatment groups. The mitochondrial pellets were then solubilized in Buffer A containing 1% Triton X-100 and 2% SDS (v/v). Protein amount was determined via Lowry DC Protein Assay Kit (BioRad), and samples were prepared for protein separation via SDS-PAGE.

## 2.7. Immunoblotting

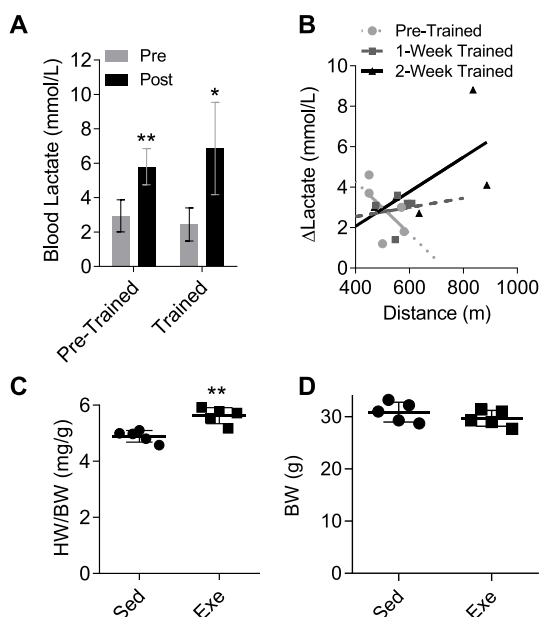
Mitochondrial proteins were separated by SDS-PAGE (12% resolving gel) and transferred to PVDF membranes. After blocking in 5% milk, the membranes were incubated with antibodies against LDHB (1:2000, Abcam), GAPDH with HRP-conjugated (1:4000, Cell Signaling), ALDH2 (1:4000, Invitrogen), or HSP60 (1:2000, Cell Signaling) overnight at 4 °C. After incubation with HRP-linked secondary antibodies (anti-mouse, 1:2500, Cell Signaling; anti-rabbit, 1:2500, Cell Signaling; and anti-goat, 1:2500; Invitrogen), the membranes were developed using Pierce™ ECL Western Blotting Substrate (ThermoFisher). Immunoreactive proteins were imaged using a BioRad™ ChemiDoc™ MP Imager.

## 2.8. Statistical analyses

Data are mean  $\pm$  S.D. Statistical analyses were performed using two-tailed, paired or unpaired Student's t-tests, or two-way ANOVA with Tukey's post-tests, where appropriate. Linear mixed-effects models were conducted to estimate associations between lactate and distance. These models used the difference between pre-ECT and post-ECT lactate levels ( $\Delta$  lactate) as the outcome. An interaction term was included to test whether this association was modified by weeks of training. The null hypothesis was rejected if  $p < 0.05$ . Statistical analyses were performed using SAS, version 9.4 (SAS Institute, Inc., Cary, North Carolina) or GraphPad Prism, version 7 (GraphPad Software, La Jolla, California).

## 3. Results

*Exercise training elicits cardiac growth and shifts associations between lactate and running distance:* Following two weeks of exercise training, the mice met our *a priori* criteria for exercise adaptation, i.e., at least 50% increase in work during exercise capacity testing. Mice were then exercised one additional week before euthanasia. Blood lactate levels significantly increased after each exercise capacity test, indicating that the mice ran to exhaustion (Fig. 1A). Initial exercise capacity tests in untrained mice suggested a negative relationship ( $\beta = -0.013$ ) between running distance and circulating blood lactate levels at fatigue. One week of training led to a minimally positive relationship ( $\beta = +0.002$ ) and two weeks of training led to a positive relationship between lactate and distance ( $\beta = +0.009$ ) (Fig. 1B). The distance  $\times$  week interaction term showed that the relationship between lactate and distance was modified by the duration of training ( $p = 0.015$ ). Similar to our previous findings [4,12], exercise-adapted mice showed a 15% increase in heart weight to body weight ratio (Fig. 1C). There were no differences in body weight after completion of the training regimen (Fig. 1D). These findings could suggest adaptations that improve the ability of organs to use lactate as an energy source.



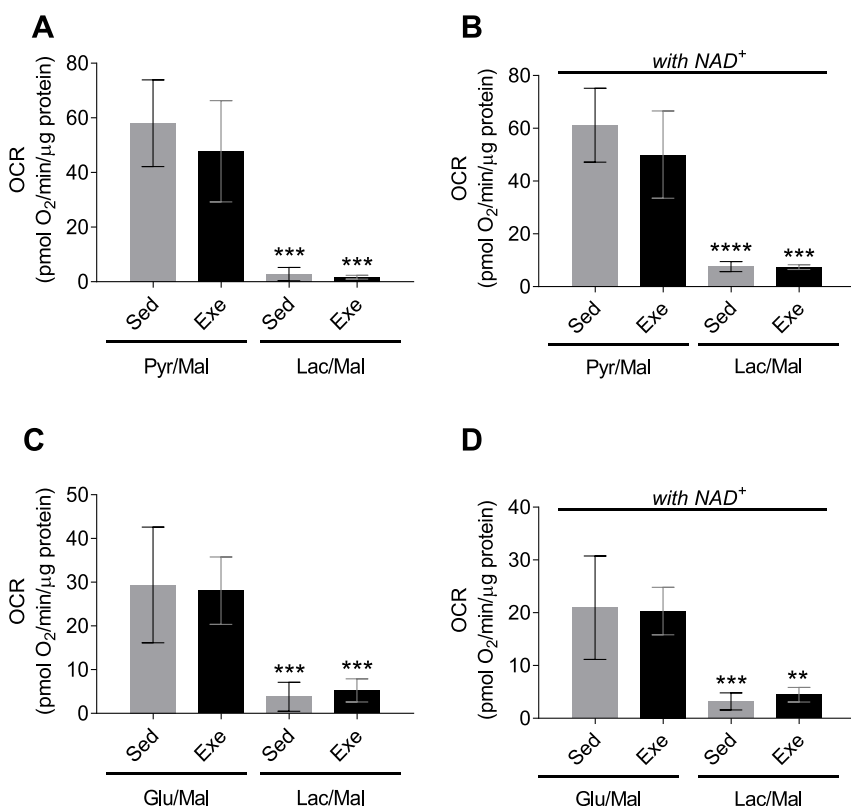
**Fig. 1. Treadmill training elicits physiological adaptation in mice.** (A) Change in blood lactate level before (Pre) and after (Post) exercise capacity tests in pre-trained mice and in mice subjected to treadmill training for two weeks; (B) Linear regression models comparing the distance run and pre-vs. post-lactate levels ( $\Delta$ Lactate) measured during each exercise capacity test; (C) Myocardial growth as indicated by heart weight (HW) normalized to body weight (BW) in sedentary (Sed) and exercise-trained (Exe) mice; and (D) BW in sedentary and exercise-trained mice. Statistical tests included: paired *t*-test (panel A), linear mixed effects models (panel B) and unpaired *t*-tests (panels C and D). *n* = 5 per group, \**p* < 0.05, \*\**p* < 0.01.

**Lactate does not drive substantial respiration in isolated mitochondria:** Because previous studies suggest that mitochondria may harbor LDH [5–8], which could provide an energetic advantage to the heart when

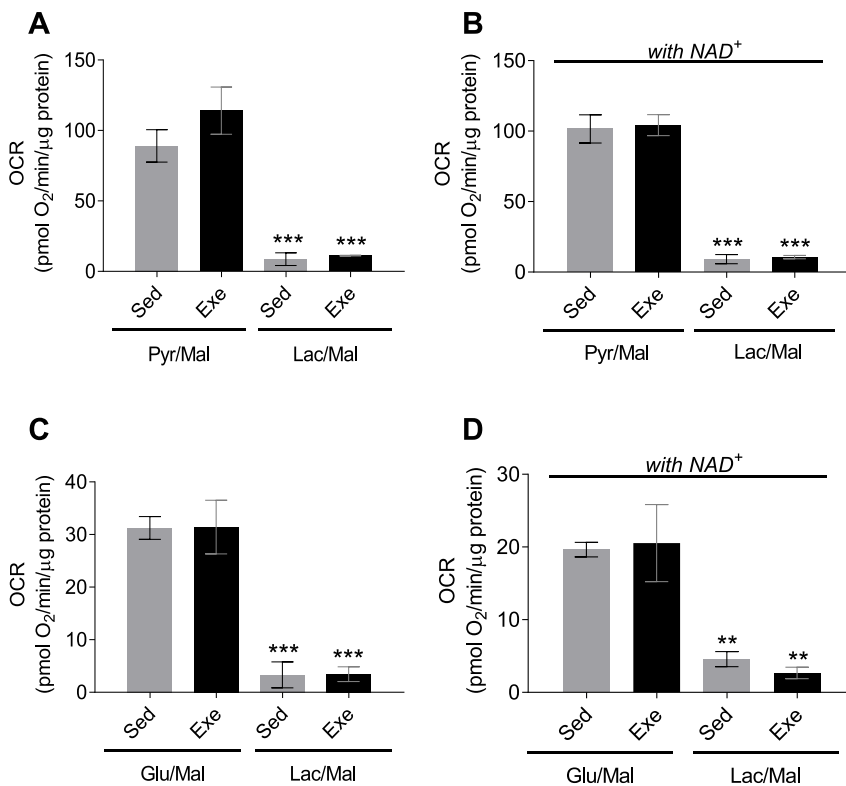
circulating lactate levels are high, we next assessed whether isolated cardiac mitochondria from sedentary or exercise-adapted mice respire on lactate. As shown in Fig. 2A, mitochondria isolated from hearts of sedentary and exercise-adapted mice showed > 20-fold higher state 3 respiration when provided with pyruvate and malate as a substrate compared with lactate and malate. Because studies suggest that mLDH is in the intermembrane space of mitochondria and thus requires extramitochondrial  $\text{NAD}^+$  for activity [16], we also tested whether  $\text{NAD}^+$  would stimulate lactate oxidation to appreciable levels. As shown in Fig. 2B,  $\text{NAD}^+$  did not provide mitochondria with a significant ability to respire on lactate. Similar results were obtained for skeletal muscle mitochondria, which lacked the ability to respire on lactate compared with typical substrates that support Complex I-driven respiration (i.e., glutamate + malate) in the absence (Fig. 2C) or presence (Fig. 2D) of exogenous  $\text{NAD}^+$ . Exercise did not significantly affect cardiac or skeletal muscle mitochondrial respiration.

**Acute exercise does not influence mitochondrial lactate utilization:** Because recent studies indicate that exercise acutely augments respiratory capacity of cardiac mitochondria [17], we next tested whether an acute bout of exercise affects lactate oxidation in isolated mitochondria. For this, we subjected mice to an intense bout of treadmill running and immediately isolated mitochondria for respirometry. Mitochondria supplied with pyruvate-driven respiration had higher state 3 respiration in acutely exercised mice compared with sedentary mice; however, this did not reach statistical significance (*p* = 0.136). Lactate did not drive appreciable levels of respiration in either the sedentary or acutely exercised groups (Fig. 3A), and exogenous  $\text{NAD}^+$  did not bolster respiration (Fig. 3B). Skeletal muscle mitochondrial respiration was not affected by acute bouts of exercise, in the absence (Fig. 3C) or presence (Fig. 3D) of  $\text{NAD}^+$ .

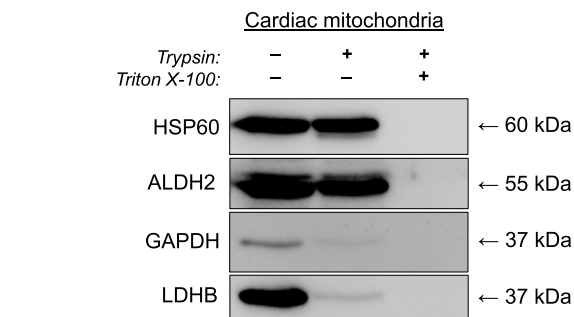
**LDH is not located in the matrix of cardiac mitochondria:** The respirometry results suggest that intramitochondrial LDH is not a significant source of lactate oxidation in striated muscle, especially in cardiac muscle, which consumes lactate for energy. To confirm that LDH is not an intramitochondrial protein in the heart, we performed



**Fig. 2. Lactate contributes minimally to respiration in isolated mitochondria from striated muscle.** State 3 respiration of mitochondria isolated from sedentary (Sed) and exercise-adapted (Exe) mice: (A) Oxygen consumption rate (OCR) of cardiac mitochondria provided with 5 mM pyruvate, 2.5 mM malate, and 1 mM ADP (Pyr/Mal) or 5 mM lactate, 2.5 mM malate, and 1 mM ADP (Lac/Mal); (B) OCR of cardiac mitochondria provided with Pyr/Mal or Lac/Mal in the presence of 1 mM  $\text{NAD}^+$ ; (C) OCR of skeletal muscle mitochondria provided with Pyr/Mal or Lac/Mal; and (D) OCR of skeletal muscle mitochondria provided with Pyr/Mal or Lac/Mal in the presence of 1 mM  $\text{NAD}^+$ . The statistical test used was two-way ANOVA with Tukey's test for multiple comparisons. *n* = 5 per group, \*\**p* < 0.01, \*\*\**p* < 0.001, \*\*\*\**p* < 0.0001 vs. corresponding Sed or Exe group.



**Fig. 3. Acute exercise does not promote mitochondrial lactate utilization.** State 3 respiration in mitochondria isolated immediately after one bout of exercise (Exe) or from sedentary (Sed) mice: (A) Oxygen consumption rate (OCR) of cardiac mitochondria provided with 5 mM pyruvate, 2.5 mM malate, and 1 mM ADP (Pyr/Mal) or 5 mM lactate, 2.5 mM malate, and 1 mM ADP (Lac/Mal); (B) OCR of cardiac mitochondria provided with Pyr/Mal or Lac/Mal in the presence of 1 mM NAD<sup>+</sup>; (C) OCR of skeletal muscle mitochondria provided with Pyr/Mal or Lac/Mal; and (D) OCR of skeletal muscle mitochondria provided with Pyr/Mal or Lac/Mal in the presence of 1 mM NAD<sup>+</sup>. The statistical test used was two-way ANOVA with Tukey's test for multiple comparisons. n = 3 per group, \*\*p < 0.01, \*\*\*p < 0.001 vs. corresponding Sed or Exe group.



**Fig. 4. LDHB is not localized within cardiac mitochondria of mice.** Protease protection assay: Isolated cardiac mitochondria were treated with or without trypsin (0.25 mg/ml) for 15 min in the absence or presence of Triton X-100 (1% v/v). Mitochondrial lysates were examined by Western blotting for the presence of: lactate dehydrogenase B (LDHB); the known intramitochondrial proteins, heat shock protein 60 (HSP60) and aldehyde dehydrogenase 2 (ALDH2); and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), which served as a control for cytosolic contaminants. Data are representative of two independent experiments.

protease protection assays of isolated mitochondria. For this, isolated mitochondria were incubated with trypsin, which results in proteolysis of extramitochondrial proteins that persist as either contaminants or outer mitochondrial membrane-associated proteins. As shown in Fig. 4, treatment of isolated mitochondria with trypsin resulted in loss of GAPDH and LDHB immunoreactivity; however, the matrix-residing proteins ALDH2 and HSP60 were maintained in mitochondrial fractions in the absence or presence of trypsin. Solubilization of mitochondria with non-ionic detergent (Triton X-100) enabled trypsin-mediated degradation of ALDH2 and HSP60 (Fig. 4). These findings show that LDH is not an intramitochondrial protein in mouse heart.

#### 4. Discussion

Several studies indicate that LDH may be localized to mammalian

mitochondria [5–8]. The presence of LDH within mitochondria could be particularly important to the heart, which has high energetic requirements that further increase with exercise. The rationale for examining mLDH is strengthened by the fact that circulating lactate concentration correlates positively with myocardial lactate uptake and oxidation [3,18,19] and can contribute remarkably to cardiac ATP production in mammals [2]. Importantly, any NADH generated within mitochondria during the LDH reaction would be directly available to the respiratory chain, bypassing the need to transport reducing equivalents across the inner mitochondrial membrane via the malate-aspartate shuttle. Therefore, we examined whether striated muscle mitochondria have the capacity to oxidize lactate. The salient findings of this study are: 1) intramitochondrial lactate oxidation does not occur to significant levels in cardiac or skeletal muscle mitochondria; 2) the presence of exogenous NAD<sup>+</sup> does not augment lactate oxidation in isolated mitochondria from striated muscle; and 3) LDH does not exist within cardiac mitochondria. These findings indicate that in murine heart lactate oxidation occurs after extramitochondrial LDH-mediated conversion of lactate to pyruvate.

Results of our exercise capacity tests indicated that lactate abundance negatively correlated with running distance only in untrained mice, which suggests potential adaptations to circulating lactate. Because the heart strongly adapts to exercise [2], is a net lactate consumer [2], and is a primary contributor to exercise capacity [20], we tested whether exercise influences lactate-supported respiration in isolated heart mitochondria. In both cardiac and skeletal muscle mitochondria, lactate failed to support significant amounts of respiration. Furthermore, provision of extramitochondrial NAD<sup>+</sup> did not significantly affect lactate oxidation by isolated mitochondria, which excludes the possibility that extramitochondrial LDH in the mitochondrial isolates contributes significantly to respiration. These findings appear to support the idea that LDH catalysis occurs primarily in the cytosol [9–11] and to contradict previous findings that suggest LDH is within mitochondria [5–8].

Results from protease protection assays further support this conclusion. The protease protection assay is based on the concept that



intramitochondrial proteins are protected from protease-mediated degradation: the defined exclusion limit of the outer mitochondrial membrane is 3–5 kDa [21,22] and trypsin is approximately 23 kDa. Therefore, only proteins that contaminate the mitochondrial preparation or that are associated with the outer mitochondrial membrane can be degraded by trypsin. In our mitochondrial preparations, the intramitochondrial proteins HSP60 and ALDH2 remained unaffected by trypsin; however, trypsin proteolyzed LDHB and GAPDH, the latter of which served as a control for cytosolic protein contaminants. LDHB is the predominant LDH isozyme expressed in mammalian heart, and, unlike LDHA, it has a higher affinity for lactate than pyruvate [23]. Thus, although LDHB is likely to contribute most to lactate oxidation by the heart, its catalytic activity appears confined to extramitochondrial locales.

It remains possible that the discrepancy between our results and previous studies could be due to model-specific factors. Nevertheless, even in similar models, there appear to be converse findings. For example, previous studies suggest intramitochondrial LDH in rat heart or skeletal muscle tissue [5,8] and in human cell lines (e.g., HeLa and H460 cells [6], CCF-STTG1 astrocytoma cell line [7]); however, other studies demonstrate negligible intramitochondrial lactate oxidation and LDH localization in human [9] and rat muscle tissue [9–11]. These contrasting reports leave the issue of mLDH unresolved, at least for striated muscle. We chose the FVB/NJ mouse strain to address this issue. This strain demonstrates exceptional treadmill exercise capacity and an ability to adapt to chronic exercise regimens [12,13,24]. Furthermore, cardiac mitochondria from acutely exercised FVB/NJ mice show evidence of rapid mitochondrial adaptations (i.e., mitochondrial fission) [17]. Our findings show negligible levels of lactate oxidation in isolated mitochondria from heart and skeletal muscle in sedentary, acutely exercised, and exercise-adapted conditions. While it remains unclear whether mitochondria from female mice have the capacity for intramitochondrial lactate oxidation, our findings suggest that LDH catalysis is not an intramitochondrial phenomenon in the hearts of male mice.

## Acknowledgements

The authors declare no conflicts of interest. This study was supported in part by the National Institutes of Health, USA (HL130174, HL78825, ES28268) and the American Diabetes Association Pathway to Stop Diabetes Grant (1-16-JDF-041).

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.redox.2019.101177>.

## References

[1] A.A. Gibb, B.G. Hill, Metabolic coordination of physiological and pathological

- cardiac remodeling, *Circ. Res.* 123 (2018) 107–128.
- [2] K. Fulghum, B.G. Hill, Metabolic mechanisms of exercise-induced cardiac remodeling, *Front. Cardiovasc. Med.* 5 (2018) 127.
- [3] B.O. Schonekess, Competition between lactate and fatty acids as sources of ATP in the isolated working rat heart, *J. Mol. Cell. Cardiol.* 29 (1997) 2725–2733.
- [4] A.A. Gibb, P.N. Epstein, S. Uchida, Y. Zheng, L.A. McNally, D. Obal, K. Katragadda, P. Trainor, D.J. Conklin, K.R. Brittain, M.T. Tseng, J. Wang, S.P. Jones, A. Bhatnagar, B.G. Hill, Exercise-induced changes in glucose metabolism promote physiological cardiac growth, *Circulation* 136 (2017) 2144–2157.
- [5] G.A. Brooks, H. Dubouchaud, M. Brown, J.P. Sicurello, C.E. Butz, Role of mitochondrial lactate dehydrogenase and lactate oxidation in the intracellular lactate shuttle, *Proc. Natl. Acad. Sci. U. S. A.* 96 (1999) 1129–1134.
- [6] Y.J. Chen, N.G. Mahieu, X. Huang, M. Singh, P.A. Crawford, S.L. Johnson, R.W. Gross, J. Schaefer, G.J. Patti, Lactate metabolism is associated with mammalian mitochondria, *Nat. Chem. Biol.* 12 (2016) 937–943.
- [7] J. Lemire, R.J. Mailloux, V.D. Appanna, Mitochondrial lactate dehydrogenase is involved in oxidative-energy metabolism in human astrocytoma cells (CCF-STTG1), *PLoS One* 3 (2008) e1550.
- [8] R.B. Brandt, J.E. Laux, S.E. Spainhour, E.S. Kline, Lactate dehydrogenase in rat mitochondria, *Arch. Biochem. Biophys.* 259 (1987) 412–422.
- [9] H.N. Rasmussen, G. van Hall, U.F. Rasmussen, Lactate dehydrogenase is not a mitochondrial enzyme in human and mouse vastus lateralis muscle, *J. Physiol.* 541 (2002) 575–580.
- [10] K. Sahlin, M. Fernstrom, M. Svensson, M. Tonkonogi, No evidence of an intracellular lactate shuttle in rat skeletal muscle, *J. Physiol.* 541 (2002) 569–574.
- [11] Y. Yoshida, G.P. Holloway, V. Ljubcic, H. Hatta, L.L. Spriet, D.A. Hood, A. Bonen, Negligible direct lactate oxidation in subsarcolemmal and intermyofibrillar mitochondria obtained from red and white rat skeletal muscle, *J. Physiol.* 582 (2007) 1317–1335.
- [12] A.A. Gibb, L.A. McNally, D.W. Riggs, D.J. Conklin, A. Bhatnagar, B.G. Hill, FVB/NJ mice are a useful model for examining cardiac adaptations to treadmill exercise, *Front. Physiol.* 7 (2016) 636.
- [13] M.P. Massett, B.C. Berk, Strain-dependent differences in responses to exercise training in inbred and hybrid mice, *Am. J. Physiol. Regulat. Integr. Comp. Physiol.* 288 (2005) R1006–1013.
- [14] S.M. Hindi, S. Sato, G. Xiong, K.R. Bohnert, A.A. Gibb, Y.S. Gallot, J.D. McMillan, B.G. Hill, S. Uchida, A. Kumar, TAK1 regulates skeletal muscle mass and mitochondrial function, *JCI Insight* 3 (2018).
- [15] S. Sato, Y. Ogura, V. Mishra, J. Shin, S. Bhatnagar, B.G. Hill, A. Kumar, TWEAK promotes exercise intolerance by decreasing skeletal muscle oxidative phosphorylation capacity, *Skeletal Muscle* 3 (2013) 18.
- [16] R.A. Jacobs, A.K. Meinild, N.B. Nordsborg, C. Lundby, Lactate oxidation in human skeletal muscle mitochondria, *Am. J. Physiol. Endocrinol. Metab.* 304 (2013) E686–E694.
- [17] M. Coronado, G. Fajardo, K. Nguyen, M. Zhao, K. Kooiker, G. Jung, D.Q. Hu, S. Reddy, E. Sandoval, A. Stotland, R.A. Gottlieb, D. Bernstein, Physiological mitochondrial fragmentation is a normal cardiac adaptation to increased energy demand, *Circ. Res.* 122 (2018) 282–295.
- [18] E.W. Gertz, J.A. Wisneski, W.C. Stanley, R.A. Neese, Myocardial substrate utilization during exercise in humans. Dual carbon-labeled carbohydrate isotope experiments, *J. Clin. Investig.* 82 (1988) 2017–2025.
- [19] L. Kaijser, B. Berglund, Myocardial lactate extraction and release at rest and during heavy exercise in healthy men, *Acta Physiol. Scand.* 144 (1992) 39–45.
- [20] D.R. Bassett Jr., E.T. Howley, Limiting factors for maximum oxygen uptake and determinants of endurance performance, *Med. Sci. Sports Exerc.* 32 (2000) 70–84.
- [21] R. Benz, Biophysical properties of porin pores from mitochondrial outer membrane of eukaryotic cells, *Experientia* 46 (1990) 131–137.
- [22] C.A. Mannella, The 'ins' and 'outs' of mitochondrial membrane channels, *Trends Biochem. Sci.* 17 (1992) 315–320.
- [23] J.A. Read, V.J. Winter, C.M. Eszes, R.B. Sessions, R.L. Brady, Structural basis for altered activity of M- and H-isozyme forms of human lactate dehydrogenase, *Proteins* 43 (2001) 175–185.
- [24] I. Lerman, B.C. Harrison, K. Freeman, T.E. Hewett, D.L. Allen, J. Robbins, L.A. Leinwand, Genetic variability in forced and voluntary endurance exercise performance in seven inbred mouse strains, *J. Appl. Physiol.* 92 (2002) 2245–2255 1985.