Supplemental Information

Lactate Dehydrogenase A Governs Cardiac Hypertrophic Growth in Response to Hemodynamic Stress

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**Table S1. Primers used in this study. Related to STAR Methods.**

| Gene  | Species | Primers                                                                 | Test       |
|-------|---------|-------------------------------------------------------------------------|------------|
| 18s   | Mouse   | AGGTTTCGATTCCGGAGAGG CAACTTTTAATATACGCTATTGG                             | qPCR       |
| 18s   | Rat     | AAACGGCTACCACATCCAAG CCTCCAATGGGATCCTCGTTTA                            | qPCR       |
| LDHA  | Mouse   | CTCGCTTGCCCTTATGGGTTC TGCCAGTCAAGTCTCACAAGAAG                              | genotyping |
| LDHA  | Mouse   | GCATGGCAGCCTCTTCCCTTA ATCAGCAGCTTGCACTGTGG                                | qPCR       |
| LDHA  | Rat     | GACCTACGGCTTGGAAGAAG GACGTTCAACACCACAATCCACA                               | qPCR       |
| Rcan1.4 | Rat/Mouse | CCCGTGAAAAAGCAGAATGC TTCCTATATATTCTTCAAGAGGG                             | qPCR       |
| Anf   | Rat     | CTTCTTCTTCCTTGCTGGCCT TTCATCGGTCTGCTCGCTCA                                | qPCR       |
| βMHC  | Rat     | CTGAGGAGACACAGCTCTTCTGAGAGATAAGAAG                                       | qPCR       |
| NDRG3 | Rat     | AACACCCCATGGATATGTCC GTGTGAGAAACGGGAGGCA                                  | qPCR       |
| Anf   | Mouse   | CTTCTTCTCGCTTGGGCT CTGCTTCTCAGTCTCTGCA                                  | qPCR       |
| Bnp   | Mouse   | CATGGATCTCTCTGAGGCT GCCTCAAGAGCTGTCTCGTG                                  | qPCR       |
| Cre   |         | GATTTCGACCAGGTCTCTGCTC GCTAACAGCGTCTTCTGCTC                                | genotyping |
| Rcan1.1 | Mouse | TGACCCGGCGCTGTTC TGTATGTTCTGAAGAGGAATC                                  | qPCR       |
**A** 907 genes upregulated (IGF1 vs. veh)
1,227 genes upregulated (PE vs. veh)
213 genes upregulated shared (IGF1 and PE)
594 genes downregulated (PE vs. veh)
406 genes, downregulated (IGF vs. veh)
134 genes downregulated shared (IGF1 and PE)

**B**

**C**

**D**

**E**

**F**

**G**

| Compounds | MRM | MRM Transition (M/Z) | MS parameters |
|-----------|-----|----------------------|---------------|
| Lactate   | (MRM-) | 89.00>45.00 | 19 | 13 | 17 |
| F6P       | (MRM-) | 259.10>97.00 | 12 | 16 | 16 |
| GAP       | (MRM-) | 169.00>97.00 | 12 | 9  | 16 |
| PEP       | (MRM-) | 167.10>79.00 | 17 | 13 | 30 |
| PYR       | (MRM-) | 87.00>43.00  | 19 | 12 | 15 |
| ADP       | (MRM-) | 426.20>79.00 | 14 | 46 | 29 |
| FBP       | (MRM-) | 389.10>97.00 | 16 | 21 | 15 |
| ATP       | (MRM-) | 506.20>79.00 | 24 | 55 | 30 |

**H**

**FPK**
Figure S1. Upregulation of LDHA in cardiomyocytes during hypertrophic growth. Related to Figure 1.

(A) Pie chart showing differentially expressed genes. Neonatal rat ventricular myocytes (NRVMs) were isolated from 1-2 days old Sprague-Dawley rats. After plating for 24 hours, phenylephrine (50 µM) or insulin-like growth factor 1 (IGF1, 10 nM) was used to treat cells in serum-free media for 24 hours. Total RNA was then extracted for RNA-seq analysis.

(B) Glycolytic genes showed significant changes by hypertrophic growth in NRVMs, analyzed from the RNA-seq data. FKPM, fragments per kilobase of transcript per million mapped reads. N=3. Comparisons were done between PE or IGF1 and vehicle treatments.

(C) PE treatment in NRVMs led to an increase in maximal glycolytic capacity as shown by elevation of ECAR (extracellular acidification rate). N=3.

(D) LDHA protein expression was increased by endothelin-1 (ET-1) in NRVMs after 24 hours of treatment. N=5-7.

(E) LDHA mRNA level was augmented by ET-1 treatment. N=4.

(F) Representative mass spectra for glycolytic metabolites. F6P, fructose 6-phosphate; GAP, glyceraldehyde 3-phosphate; PYR, pyruvate; FBP, fructose 1,6-bisphosphate; PEP, phosphoenolpyruvate; ADP, adenosine diphosphate; ATP, adenosine triphosphate.

(G) MS parameters for various glycolytic metabolites.

(H) Cardiac levels of glycolytic metabolites as determined by MS. Wild type C57BL/6 mice were subjected to sham or transverse aortic constriction (TAC) for a week. N=5-7. Data are represented as mean ± SEM. Student’s t test was conducted. *, p<0.05; **, p<0.01; ***, p<0.001.
Figure S2. Cardiac specific knockout of LDHA leads to deterioration of heart function in response to pressure overload. Related to Figure 2.

(A) LDHA protein expression in control (F/F) and conditional knockout (cKO) mice was determined by Western blotting (left), and quantified (right). N=6-7.

(B) LDHA mRNA expression was decreased in cKO mouse hearts. N=3.

(C) Schematic presentation showing the experimental design. TAC, transverse aortic constriction; echo, echocardiography.

(D) Heart rate was not affected. N=5-15.

(E) LVID (left ventricular internal diameter) at diastole was elevated in the cKO mice after TAC, indicating impaired systolic performance. N=5-15.

(F) LDHA deficiency in the heart caused a significant increase in LVID at systole. N=5-15.

(G) LDHA knockout led to a significant decrease in cardiac growth as revealed by a lower heart weight/body weight (HW/BW) ratio. N=8-9.

(H) TAC caused an increase of LDH isoenzymes with more LDHA, and this trend was diminished in the cKO heart. Data are represented as mean ± SEM. Student’s t test was conducted for (A-B). Two-way ANOVA was conducted, followed by Tukey’s test for (D-G). *, p<0.05; **, p<0.01; ***, p<0.001.
Figure S3. Flux analysis of LDHA deficient mice in response to pressure overload. Related to Figure 2.

(A) LDHA deficiency caused an increase in cell death-related proteins in the heart after TAC for 8 weeks. N=6.

(B) LDHA knockout led to more severe fibrosis 8 weeks after TAC. Scale bar, 100 μm. N=4-10.

(C) Schematic for metabolism of [1,6-13C]glucose, [2-13C]lactate/pyruvate, and [U-13C]long chain fatty acids (LCFA) at 1 week after TAC. Note that 13C labeling for glucose, lactate-pyruvate, and long-chain fatty acids is indicated as black, blue, and red filled circles, respectively.

(D) Representative spectra for glutamate C-4 and glutamate C-5 are shown. These multiplets were taken from the region around 34.2 ppm and 180 ppm from a proton-decoupled 13C NMR spectrum. The splittings arise from 13C - 13C coupling and under these conditions are assigned exclusively to oxidation of fatty acids (red), lactate or pyruvate (blue) or glucose (black).

(E) Representative spectra for lactate from effluents (WT, sham).

(F) Glycolytic flux rate was calculated from lactate production in effluents.

(G) Fractional oxidation of various substrates. Lac, lactate; pyr, pyruvate; endo, endogenous. N=4.

(H) Oxidation ratio of glucose/LCFA and lac-pyr/LCFA. N=4. Data are represented as mean ± SEM. One-way ANOVA was conducted, followed by Tukey’s test. *, p<0.05; **, p<0.01; ***, p<0.001.
Figure S4. LDHA is required for hypertrophic growth in cardiomyocytes. Related to Figure 3.

(A) Silencing of LDHA in NRVMs by siRNA transfection. Note that LDHA si-1 was used for data in Figure 3, while LDHA si-2 was used as an independent siRNA oligo here.

(B) Knockdown of LDHA protein expression in NRVMs as quantified by immunoblotting. N=3-6.

(C) Knockdown of LDHA mRNA level as determined by real-time RT-PCR. N=3-6.

(D) PE treatment increased LDH isoenzymes containing LDHA, and siRNA against LDHA reverted this effect in NRVMs.

(E) Mitochondrial respiration was examined by measuring oxygen consumption rate (OCR) in NRVMs after LDHA silencing (si-1). N=3.

(F) Basal and maximal OCRs were decreased by LDHA knockdown (si-1). N=3.

(G) Cardiomyocyte size was decreased by LDHA silencing, as evaluated by an independent siRNA (si-2) against LDHA. Scale bar, 50 µm. N=91-115 cardiomyocytes were quantified for individual groups.

(H) LDHA silencing by si-2 suppressed PE-induced protein synthesis, as assayed by radioactive leucine incorporation. N=5.

(I) Hypertrophic molecular markers were reduced by LDHA silencing via si-2 at protein level. N=4-6.

(J) LDHA inhibition by FX11 reduced cardiomyocyte growth from PE treatment. Scale bar, 50 µm. N=84-101 cardiomyocytes were evaluated for each group.

(K) FX11 treatment led to a decrease in protein synthesis. N=5.

(L) LDHA inhibition by FX11 reduced gene expression of hypertrophic markers in NRVMs. N=4. Data are represented as mean ± SEM. Student’s t test was conducted for (B-C). Two-way ANOVA was conducted, followed by Tukey’s test, to determine statistical differences for (E-L). *, p<0.05; **, p<0.01; ***, p<0.001.
Figure S5. NMN treatment does not rescue the growth defect of cardiomyocyte from LDHA silencing. Related to Figure 4 and Figure 5.

(A) Lactate treatment in NRVMs increased cellular lactate level as determined by LC/MS/MS. N=3.

(B) Lactate treatment alone stimulated gene expression of hypertrophic markers in NRVMs, as examined by realtime RT-PCR. N=6.

(C) LDHA silencing led to a decrease in cardiomyocyte size; NMN supplementation did not show a rescue effect. Scale bar, 50 µm.

(D) Quantification of (C) showed NMN treatment did not revert cell growth defect from LDHA silencing. N=50-54 cardiomyocytes were examined for each group.

(E) NMN treatment significantly increased intracellular NAD⁺ content in NRVMs after LDHA knockdown. N=3.

(F) PE treatment in NRVMs led to an increase in NDRG3 expression as assessed by immunofluorescent staining. Scale bar, 50 µm.

(G) NDRG3 protein expression was increased by ET-1 treatment in NRVMs. N=4.

(H) LDHA enzymatic inhibition by FX11 decreased protein expression of NDRG3. N=4.

(I) LDHA silencing by siRNA in NRVMs caused a decrease in NDRG3 protein expression. N=3.

(J) NDRG3 knockdown by 2 independent siRNA oligos. N=3. Note that NDRG si-1 was used for Figure 5; NDRG3 si-2 was used as an independent siRNA oligo here.

(K) NDRG3 silencing by si-2 decreased cardiomyocyte size after PE treatment. N=50-51 cardiomyocytes were measured for each group. Scale bar, 50 µm.

(L) NDRG3 knockdown by si-2 reduced protein synthesis in NRVMs, evaluated by leucine incorporation. N=3.

(M) Knockdown of NDRG3 by si-2 led to a significant decrease in protein expression of Rcan1.4. N=4. Data are represented as mean ± SEM. Student’s t test was conducted for (B, G, H, and J). Two-way ANOVA was conducted, followed by Tukey’s test, to determine statistical differences for (A, D, E, I, K, L, and M). *, p<0.05; **, p<0.01; ***, p<0.001.
Figure S6. Overexpression of NDRG3 in NRVMs increases cardiomyocyte growth. Related to Figure 5 and Figure 6.

(A) Overexpression of NDRG3 in NRVMs by adenovirus led to an increase in cardiomyocyte size. Scale bar, 50 µm. Note that PE treatment did not further increase cell size. N=57-82 cardiomyocytes were quantified for each group.

(B) NDRG3 overexpression increased protein synthesis in NRVMs as measured by radioactive leucine incorporation. N=6. Note that no significant difference was identified after PE treatment.
(C) Overexpression of NDRG3 increased protein expression of hypertrophic markers. N=4. Data are represented as mean ± SEM.

(D) NRVMs were transfected by LDHA siRNA and PE treatment was conducted. Hypertrophic markers were examined by immunoblotting. LDHA silencing suppressed the induction of hypertrophic marker expression from PE. Supplementation of lactate (lac) significantly rescued gene expression. These results suggest that the LDHA effect in cell growth may be attributed to lactate. Co-silencing of NDRG3 strongly inhibited the rescue action from lactate. N=4. Data are represented as mean ± SEM. Two-way ANOVA was conducted, followed by Tukey’s test, to determine statistical differences. *, p<0.05; ***, p<0.001; NS, not significant.
Relative protein levels

A
B
C
D
E
F
G
H
I
J
K
Figure S7. LDHA is essential for ERK activation in cardiomyocyte hypertrophic growth. Related to Figure 7.

(A) PE treatment in NRVMs led to an increase in ERK phosphorylation. N=4.
(B) ET-1 treatment increased ERK phosphorylation in NRVMs. N=4.
(C) Pressure overload by TAC in mice activated ERK in the heart. N=5-7.
(D) LDHA knockdown in NRVMs decreased ERK phosphorylation. N=4.
(E) LDHA inhibition by FX11 suppressed ERK phosphorylation in NRVMs. N=4.
(F) Lactate treatment in NRVMs led to a further increase in ERK phosphorylation compared with PE alone. N=3.
(G) Conditional knockout of LDHA in the heart decreased ERK phosphorylation after TAC. N=6.
(H) PE treatment in NRVMs led to an increase in c-Raf phosphorylation. NDRG3 overexpression potentiated this effect. N=4.
(I) PE treatment in NRVMs stimulated the co-localization of p-c-Raf and NDRG3. Silencing of LDHA suppressed PE-induced activation of c-Raf in NRVMs. Scale bar, 50 µm.
(J) Pressure overload in the heart stimulated c-Raf phosphorylation, which was suppressed by LDHA conditional knockout. N=6.
(K) LDHA deficiency inhibited TAC-induced phosphorylation of c-Raf in the heart. Scale bar, 50 µm. Data are represented as mean ± SEM. Student’s t test was conducted for (A-F). Two-way ANOVA was conducted, followed by Tukey’s test, to determine statistical differences for (G, H, and J). *, p<0.05; **, p<0.01; ***, p<0.001.