Inhibitory Acetylation of Pyruvate Dehydrogenase Promotes Diastolic Dysfunction in Mice

Dharendra Thapa[1,2,3 # $], Paramesha Bugga[1,2,3 #], Bellina A.S. Mushala[1,2,3], Janet R. Manning[1,2,3], Michael W. Stoner[1,2,3], Brenda McMahon[2], Bingxian Xie[3,4], Lia R. Edmunds[3,4], Michael J. Jurczak[3,4], and Iain Scott[1,2,3 *]

[1] Division of Cardiology, Department of Medicine, University of Pittsburgh, Pittsburgh, PA
[2] Vascular Medicine Institute, Department of Medicine, University of Pittsburgh, Pittsburgh, PA
[3] Center for Metabolism and Mitochondrial Medicine, Department of Medicine, University of Pittsburgh, Pittsburgh, PA
[4] Division of Endocrinology and Metabolism, Department of Medicine, University of Pittsburgh, Pittsburgh, PA
[$] Present Address: Department of Exercise Physiology, West Virginia University School of Medicine, Morgantown, WV

[#] These authors contributed equally to this work

* To whom correspondence should be addressed:

Iain Scott, PhD, FAHA, FCVS
Division of Cardiology
Department of Medicine
University of Pittsburgh
BST E1259
200 Lothrop Street
Pittsburgh PA
15261
Email: iain.scott@pitt.edu
Tel: (412) 648-7691

ABSTRACT

Alterations in cardiac fuel metabolism underpin the development of heart failure with preserved ejection fraction (HFpEF). Mouse models of HFpEF, in addition to changes in fuel choice, display elevated levels of mitochondrial protein acetylation. Reversal of mitochondrial hyperacetylation restores cardiac bioenergetics and function. In this study, we examined the metabolic mechanisms of diastolic dysfunction in diet-induced failing hearts, using a model of constitutively-reduced mitochondrial protein acetylation.

LETTER

Deleterious changes in energy metabolism underpin the development of heart failure with preserved ejection fraction (HFpEF) [1]. Studies on two recently developed murine models of HFpEF have reported altered cardiac fuel substrate utilization, culminating in changes to the relative oxidation rates of fatty acids, ketone bodies, and glucose in failing hearts [2,3]. These two distinct murine HFpEF models are also characterized by increased mitochondrial protein acetylation, a reversible posttranslational modification that is commonly linked to reduced metabolic enzyme activity [2,3]. Restoring normal mitochondrial protein acetylation through supplementation with either nicotinamide riboside (to stimulate Sirtuin 3-mediated protein deacetylation [2]), or ketone bodies (which inhibit fatty acid uptake [3]), resulted in normalized cardiac bioenergetics and amelioration of the HFpEF phenotype [2,3].

These findings suggest that tight regulation of mitochondrial protein acetylation is required to prevent the development of HFpEF, and approaches that reduce maladaptive hyperacetylation may therefore be cardioprotective. Using a mouse model of constitutively-reduced mitochondrial protein acetylation, we examined the mechanisms underlying metabolic protection from HFpEF. We placed wildtype (WT) and cardiac-specific GCN5L1 knockout (KO) mice (which display global reductions in mitochondrial protein acetylation [4]) on low fat (LFD; 10% fat) or high fat (HFD; 60% fat) diets for 24 weeks, and measured heart structure and function by echocardiography. No changes were observed between WT and KO mice in either morphological parameters (LVEDV and LVEDPW) or systolic function (LVEF) on either diet (**Figure A**). In contrast, WT mice displayed a significant increase in E/e' ratio (a marker of HFpEF-related diastolic dysfunction [2]) on a HFD, which was absent in acetylation-deficient GCN5L1 KO mice (**Figure A**).

To understand which fuel metabolism pathways may be disrupted in mice with diastolic dysfunction, we performed Oroboros respirometry in mitochondrial-enriched cardiac tissue preparations from HFD-fed WT and KO mice. Previous reports have shown that fatty acid oxidation is significantly modified in hyperacetylated mitochondria from HFpEF mice [2,3]. Surprisingly, ADP-stimulated state 3 respiration was not different between WT (hyperacetylated) and KO (hypoacetylated) samples provided with palmitate as a fuel substrate (**Figure B**). Instead, we observed a significant increase in

maximal state 3 respiration in KO samples provided with pyruvate relative to their WT counterparts (**Figure B**), indicating that changes in glucose oxidation may underpin the diastolic dysfunction phenotype in this model.

The pyruvate dehydrogenase (PDH) complex regulates mitochondrial glucose utilization, and previous work has demonstrated that increased flux through PDH can reverse diastolic dysfunction in diabetic rats [5]. We found that PDH activity was significantly increased in mitochondria from KO mice on a HFD relative to both WT diet groups (**Figure C**). Elevated PDH enzyme activity is typically associated with decreased inhibitory phosphorylation at Ser-293 [2], however we detected no difference in PDH phosphorylation between WT and KO mice on either diet (**Figure C**). Instead, we found a significant increase in PDH hyperacetylation in HFD-fed WT mice relative to LFD controls, and a significant decrease in PDH acetylation in both KO diet groups (**Figure C**). PDH acetylation status in all animal groups was negatively correlated with PDH activity (R^2 = 0.3678; P = 0.037; **Figure C**). To verify these findings independently, we examined PDH activity and acetylation status in human AC16 cardiomyocyte cells following shRNA-mediated GCN5L1 depletion. Knockdown of GCN5L1 in AC16 cells resulted in a ~40% increase in PDH activity and a ~50% decrease in PDH acetylation, without observed changes in total PDH abundance (**Figure D**). Combined, these data suggest that GCN5L1-mediated hyperacetylation of PDH, rather than PDH inhibitory phosphorylation, is a key driver of cardiac fuel metabolism defects in the context of diastolic dysfunction.

Finally, we sought to determine the specific effect of acetylation on PDH enzymatic activity. Published studies in the PhosphoSitePlus proteomics database (www.phosphosite.org) have identified five acetylation sites on human PDH at K18, K39, K244, K321, and K336 (**Figure E**). We generated novel PDH expression constructs where each of these five lysines was replaced with either arginine (5KR; to model a non-acetylated state) or glutamine (5KQ; to mimic a hyperacetylated state), and expressed them in GCN5L1 knockdown AC16 cells (which represent a basal, non-acetylated state for endogenous PDH; **Figure C**). PDH activity was significantly increased in non-acetylated PDH 5KR expressing cells relative to those over-expressing wildtype PDH (**Figure F**). Conversely, PDH activity was significantly decreased in hyperacetylated PDH 5KQ expressing cells relative to those deficient in PDH acetylation (**Figure F**). Overall, these data indicate that lysine hyperacetylation has a specific negative effect on PDH activity in cardiomyocytes.

In summary, we report that inhibitory acetylation of PDH drives deleterious changes in fuel metabolism in a model of cardiac diastolic dysfunction. These studies add to the developing consensus that changes in fuel metabolism underpin the development of HFpEF, and shed new light on the regulatory role played by mitochondrial protein acetylation on glucose metabolism in the failing heart.

BIBLIOGRAPHY

[1] Lopaschuk GD, Karwi QG, Tian R, Wende AR, Abel ED. Cardiac Energy Metabolism in Heart Failure. Circ Res. 2021 May 14;128(10):1487-1513. doi: 10.1161/CIRCRESAHA.121.318241. Epub 2021 May 13. PMID: 33983836; PMCID: PMC8136750.

[2] Tong D, Schiattarella GG, Jiang N, Altamirano F, Szweda PA, Elnwasany A, Lee DI, Yoo H, Kass DA, Szweda LI, Lavandero S, Verdin E, Gillette TG, Hill JA. NAD+ Repletion Reverses Heart Failure With Preserved Ejection Fraction. Circ Res. 2021 May 28;128(11):1629-1641. doi: 10.1161/CIRCRESAHA.120.317046. Epub 2021 Apr 22. PMID: 33882692; PMCID: PMC8159891.

[3] Deng Y, Xie M, Li Q, Xu X, Ou W, Zhang Y, Xiao H, Yu H, Zheng Y, Liang Y, Jiang C, Chen G, Du D, Zheng W, Wang S, Gong M, Chen Y, Tian R, Li T. Targeting Mitochondria-Inflammation Circuit by β-Hydroxybutyrate Mitigates HFpEF. Circ Res. 2021 Jan 22;128(2):232-245. doi: 10.1161/CIRCRESAHA.120.317933. Epub 2020 Nov 12. PMID: 33176578.

[4] Thapa D, Manning JR, Stoner MW, Zhang M, Xie B, Scott I. Cardiomyocyte-specific deletion of GCN5L1 in mice restricts mitochondrial protein hyperacetylation in response to a high fat diet. Sci Rep. 2020 Jun 30;10(1):10665. doi: 10.1038/s41598-020-67812-x. PMID: 32606301; PMCID: PMC7326908.

[5] Le Page LM, Rider OJ, Lewis AJ, Ball V, Clarke K, Johansson E, Carr CA, Heather LC, Tyler DJ. Increasing Pyruvate Dehydrogenase Flux as a Treatment for Diabetic Cardiomyopathy: A Combined 13C Hyperpolarized Magnetic Resonance and Echocardiography Study. Diabetes. 2015 Aug;64(8):2735-43. doi: 10.2337/db14-1560. Epub 2015 Mar 20. PMID: 25795215; PMCID: PMC4516266.

31 **FIGURE**

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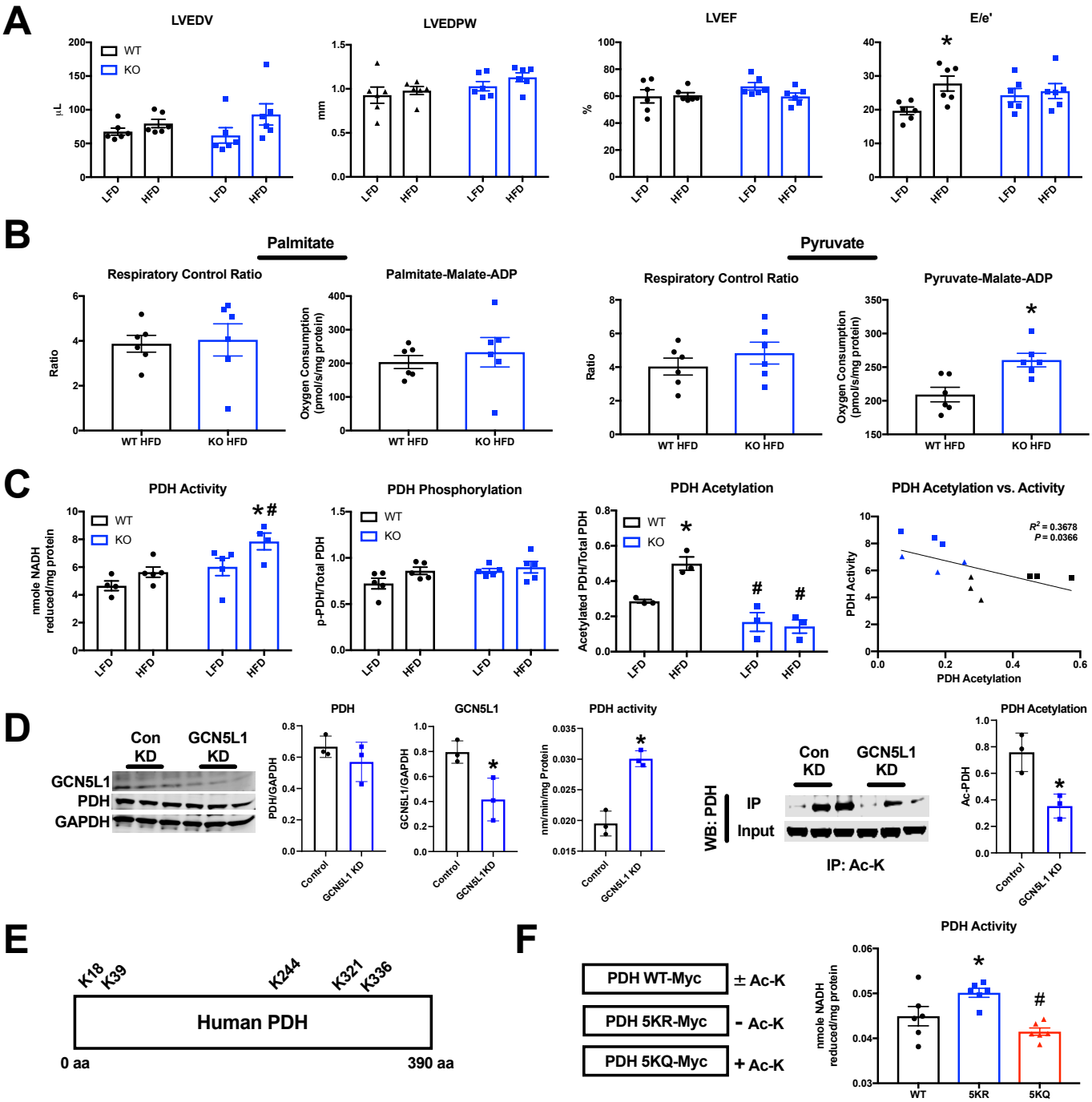

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35 **Mice deficient in PDH acetylation are protected from diastolic dysfunction. A.** Wildtype (WT)

36 and cardiac-specific GCN5L1 knockout (KO) mice were placed on low fat (LFD; 10% fat), or high fat

diets (HFD; 60% fat) for 24 weeks, followed by echocardiography to measure LV end diastolic volume (LVEDV), LV end diastolic posterior wall thickness (LVEDPW), LV ejection fraction (LVEF), and E/e' ratio. N = 6; * = $P < 0.05$ relative to WT LFD. **B.** Oxygen consumption in cardiac tissues from HFD-fed WT and KO mice was measured in response to either pyruvate or palmitate using Oroboros respirometry. N = 6; * = $P < 0.05$ relative to WT. **C.** PDH activity, phosphorylation (S293), and acetylation were measured in WT and KO mice following LFD or HFD. N = 3-5 * = $P < 0.05$ relative to WT LFD, # = $P < 0.05$ to WT HFD **D.** Total PDH acetylation and enzymatic activity was measured in stable Control shRNA or GCN5L1 shRNA knockdown (KD) human cardiac AC16 cells. N = 3; * = $P < 0.05$ relative to WT. **E.** Schematic of human PDH showing published lysine acetylation sites listed on PhosphoSitePlus (www.phosphosite.org). **F.** GCN5L1 KD AC16 cells were transfected with Myc-tagged wildtype PDH (WT-Myc), a non-acetylated PDH mimic in which lysines K18/K39/K244/K321/K336 were replaced with arginine (5KR-Myc), and an acetylated PDH mimic where lysines K18/K39/K244/K321/K336 were replaced with glutamine (5KQ-Myc). N = 6; * = $P < 0.05$ relative to WT, # = $P < 0.05$ to 5KR.

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