Inhibitory Acetylation of Pyruvate Dehydrogenase Promotes Diastolic Dysfunction in Mice

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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, ketones, and glucose in failing hearts [2,3]. The HFpEF phenotype in these two distinct mouse models is also characterized by an increase in 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 and utilization) [3], resulted in normalized cardiac bioenergetics and amelioration of the HFpEF phenotype [2,3].

These studies suggest that tight regulation of mitochondrial protein acetylation is required to prevent the development of HFpEF, and approaches that reduce maladaptive hyperacetylation are potentially cardioprotective. Using a mouse model of constitutively-reduced mitochondrial protein acetylation, we examined the metabolic mechanisms underlying protection from diet-induced 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 30 weeks, and measured heart structure and function by echocardiography. No changes were observed on either diet between WT and KO mice in either morphological

62 parameters or systolic function (**Figure A**). In contrast, WT mice displayed a significant
63 increase in E/e' ratio (a marker of HFpEF-related diastolic dysfunction) [2] on a HFD,
64 which was absent in acetylation-deficient KO mice under the same conditions (**Figure**
65 **A**).
66
67 To understand which fuel metabolism pathways are disrupted in mice with diastolic
68 dysfunction, we performed Oroboros respirometry in mitochondrial-enriched cardiac
69 tissue preparations from HFD-fed WT and KO mice. Previous reports have shown that
70 fatty acid oxidation is significantly modified in hyperacetylated mitochondria from HFpEF
71 mice [2,3]. Unexpectedly, ADP-stimulated state 3 respiration was not different between
72 WT (hyperacetylated) and KO (hypoacetylated) samples provided with palmitate as a
73 fuel substrate (**Figure B**). Instead, we observed a significant increase in maximal state
74 3 respiration in KO samples provided with pyruvate relative to their WT counterparts
75 (**Figure B**), indicating that decreased glucose oxidation may underpin diastolic
76 dysfunction in this animal model.
77
78 The pyruvate dehydrogenase (PDH) complex regulates mitochondrial glucose
79 utilization, and previous work has demonstrated that increased flux through PDH can
80 reverse diastolic dysfunction in diabetic rats [5]. Consistent with this, we found that PDH
81 activity was significantly increased in mitochondria from KO mice on a HFD, relative to
82 both WT diet groups (**Figure C**). Elevated PDH activity is commonly associated with a
83 decrease in inhibitory phosphorylation at Ser-293 [2], however we detected no difference
84 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 across 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-derived 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 inhibitory PDH 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 hyperacetylation 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 mimic 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 overexpressing 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**). Combined, 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 altered fuel metabolism underpins the development of HFpEF, and shed new light on the regulatory role played by mitochondrial protein acetylation on glucose utilization in the failing heart.

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139 **FIGURE**

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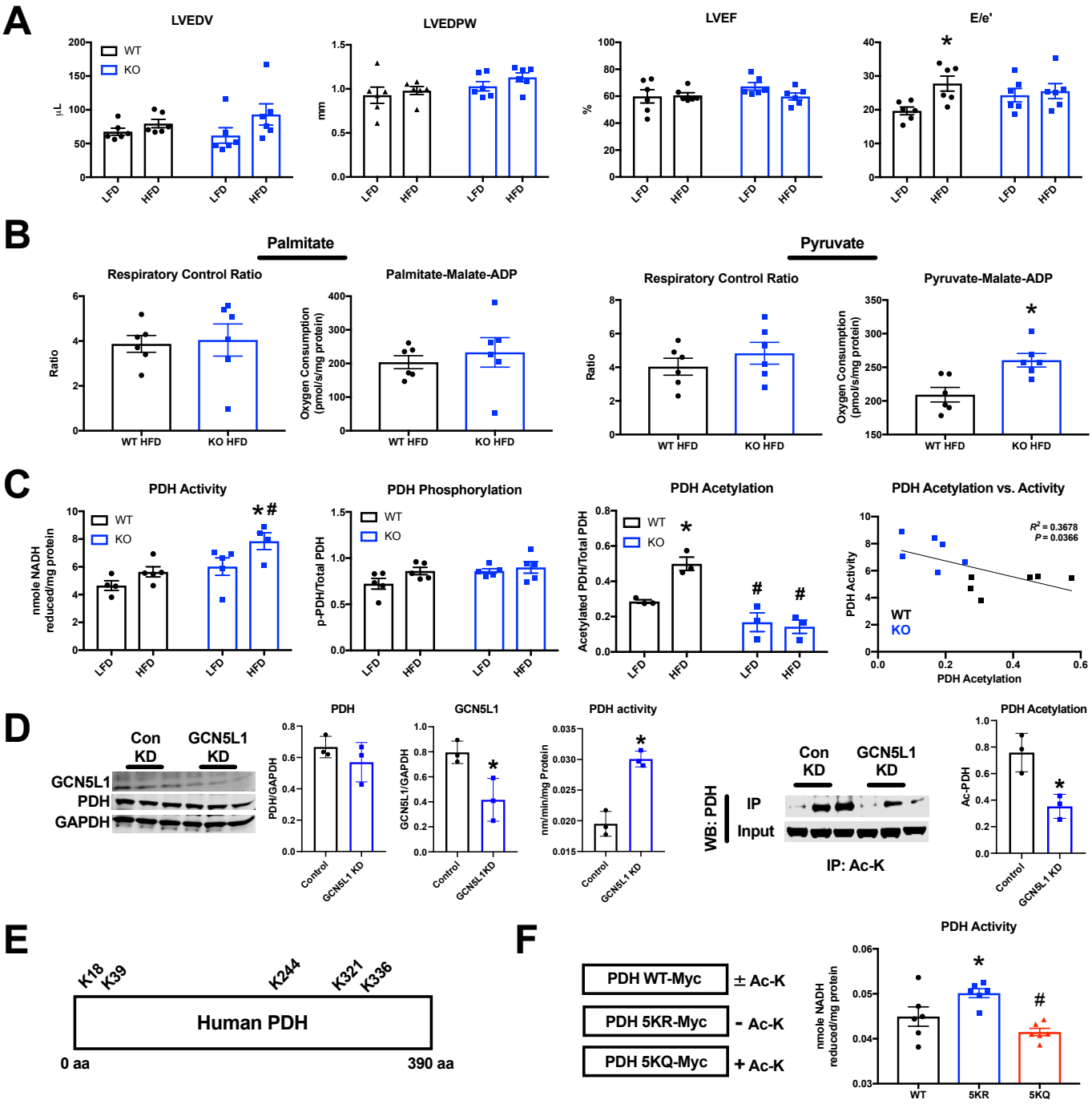

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142 **Mice deficient in PDH acetylation are protected from diastolic dysfunction. A.**

143 Wildtype (WT) and cardiac-specific GCN5L1 knockout (KO) mice were placed on low fat

(LFD; 10% fat), or high fat diets (HFD; 60% fat) for 30 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 palmitate or pyruvate 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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DISCLOSURES

None