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Review

Post-translational modifications of pyruvate dehydrogenase complex in cardiovascular disease

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SUMMARY

Pyruvate dehydrogenase complex (PDC) is a crucial enzyme that connects glycolysis and the tricarboxylic acid (TCA) cycle pathway. It plays an essential role in regulating glucose metabolism for energy production by catalyzing the oxidative decarboxylation of pyruvate to acetyl coenzyme A. Importantly, the activity of PDC is regulated through post-translational modifications (PTMs), phosphorylation, acetylation, and O-GlcNAcylation. These PTMs have significant effects on PDC activity under both physiological and pathophysiological conditions, making them potential targets for metabolism-related diseases. This review specifically focuses on the PTMs of PDC in cardiovascular diseases (CVDs) such as myocardial ischemia/reperfusion injury, diabetic cardiomyopathy, obesity-related cardiomyopathy, heart failure (HF), and vascular diseases. The findings from this review offer theoretical references for the diagnosis, treatment, and prognosis of CVD.

INTRODUCTION

Cardiovascular disease (CVD) is a prevalent global health concern.[1] The pyruvate dehydrogenase complex (PDC) plays a crucial role in maintaining energy metabolism in the myocardium by connecting glycolysis and the tricarboxylic acid (TCA) cycle pathway. Post-translational modifications (PTMs), which link changes in cellular metabolism to protein function, regulate cellular processes involved in cardiovascular homeostasis.[2,3] Notably, numerous studies have demonstrated that PDC subunits undergo various PTMs, including phosphorylation, dephosphorylation, acetylation, and glycosylation. These PTMs have significant effects on PDC activity under both physiological and pathophysiological conditions, making them potential targets for CVD. Summarizing the changes in PTMs of PDC that occur in CVD can provide further insights into CVD and mitochondrial metabolic disorders.

COMPOSITION AND FUNCTION OF THE PDC

The mammalian PDC is a 9.5 million-Dalton protein machine organized about a 60-meric core, consisting of pyruvate dehydrogenase (E1), dihydrolipoamide acetyltransferase (E2), dihydrolipoamide dehydrogenase (E3), and E3-binding protein (E3BP), as well as isoforms of pyruvate dehydrogenase kinase (PDKs 1–4) and pyruvate dehydrogenase phosphatase (PDPs 1–2) are attached through ionic interactions.[4] E1 component is a heterotetramer consisting of two alpha and two beta subunits.[5]

PDC plays a crucial role in maintaining the balance between glucose oxidation and the TCA cycle, thereby regulating glucose metabolism and ATP production. Insufficient PDC activity results in the failure of pyruvate metabolism and a shortage of TCA cycle substrates, which is a common cause of abnormal mitochondrial energy metabolism and cellular dysfunction. Moreover, PDC activity has significant implications in various diseases including cardiomyopathy, diabetes, obesity, neurological disorders, and cancer. By effectively regulating PDC activity providing prevention and treatment options for CVD.

The activity and stability of PDC are influenced by multiple factors. Patients with PDC deficiency experience reduced cellular PDC activity, which leads to symptoms like lactic acidosis, elevated pyruvate levels, and ataxia.[6] In addition to the increased substrate utilization resulting from the expression of PDC through transcriptional regulation, notably, PTMs also significantly impact the activity of PDC. Therefore, uncovering the modulation of PDC activity through various PTMs and identifying potential modification sites in CVD hold promise as potential therapeutic approaches for these diseases.

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PTMs OF PDC IN CVD

Ischemic/reperfusion injury

Ischemic cardiomyopathy is characterized by the reduced blood and oxygen supply to the myocardium caused by the narrowing and blockage of coronary arteries, as well as chronic myocardial ischemia. This ultimately leads to impaired myocardial function. Timely reperfusion is crucial for saving the ischemic heart, but it also causes severe myocardial ischemia/reperfusion (I/R) injury. Unfortunately, there is currently a lack of clinically effective interventions to address this issue.[7,8] I/R injury increases the risk of myocardial infarction, arrhythmias, cardiac hypertrophy and heart failure (HF).[9] Numerous studies have demonstrated a strong association between I/R injury and PTMs of proteins.[10,11] For example, the novel modification of croton acylation is a key response of cardiomyocytes to I/R injury, inhibiting the occurrence of fibrosis and apoptosis.[12] I/R reduces glucose oxidation and decoups glucose oxidation from glycolysis.[13]

Phosphorylation

I/R causes changes in the phosphorylation levels of specific proteins in cardiomyocytes. These proteins, including kinases and phosphatases, reduce the severity of I/R damage and protect the heart by reducing cardiac cell death. For example, Protein kinase B, which activates Akt through phosphorylation, is an important component of cardiac protection.[14] During myocardial I/R, phosphorylation changes can trigger a series of biological effects. On the one hand, phosphorylation can promote the adaptation and recovery of cardiomyocytes, for example, by activating certain protective signaling pathways to reduce ischemic injury, inhibit autophagy, inhibit the opening of mitochondrial permeability transition pores, and prevent reperfusion arrhythmia. On the other hand, excessive phosphorylation may also cause damage to cardiomyocytes, exacerbating I/R injury by activating apoptotic pathways or interfering with ion balance. Hyperphosphorylation of Connexin 43 at serine 282 triggers apoptosis of rat cardiomyocytes by activating the mitochondrial apoptotic pathway.[15] I/R is a frequently observed pathological process that disrupts cellular energy metabolism. In particular, I/R has been shown to cause an increase in the phosphorylation of PDCE1α. A study by Churchill et al. in 2005 showed that cardiac ischemia for 30 min resulted in a decrease in PDC activity, which was only partially restored after reperfusion for 120 min[16] δ-isoform of protein kinase C (PKC-δ) is usually inactivated in the cytoplasm. However, during reperfusion, PKC-δ is translocated and associated with the phosphorylation of PDCE1α. Specifically, PKC-δ interacts with PDK and phosphorylates PDCE1α.[17] This translocation of PKC-δ to the mitochondria leads to an increase in superoxide anion production. As a result, there is a loss of mitochondrial respiratory activity and apoptosis during myocardial reperfusion. Blocking the translocation of PKC-δ effectively prevents these processes. Clifford Dl Folmes et al. showed that PKC may also directly phosphorylate PDCE1β, exerting its effect on PDC activity.[18] This may account for a 35% reduction in glucose oxidation during reperfusion and lead to PDC inactivation. Phosphorylation-related PKC-δ translocations lead to apoptosis. PKC is a kind of protein kinase widely existing in cells. It regulates the activity of various intracellular proteins through phosphorylation, and thus participates in many biological processes such as cell signal transduction, cell proliferation and apoptosis. These findings highlight the significance of protein PTMs as a crucial molecular mechanism underlying I/R injury, which ultimately affects cellular energy metabolism.

Decreased PDC activity in ischemic myocardial tissue has been observed in most studies, but this is not universally observed.[19,20] The reperfusion stage also showed inconsistencies.[21] The current literature is limited, and further studies are necessary to reveal the role of PDC and its phosphorylation in cardiometabolism and function.

Acetylation

Mitochondrial hyperacetylation is widely recognized to impair oxidative metabolism.[22] It has been suggested that acetylation, along with other acyl PTMs, may lead to reduced cardiac energetics and impaired function in pathophysiologic conditions. Protein acetylation occurs irreversibly on the α-amino group at the N-terminal amino acid or reversibly on the ε-amino group on the side chain of the lysine residue.[23] Acetylases are enzymes that transfer acetyl groups from acetyl coenzyme A to lysine, while deacetylases remove the acetyl group from lysine. Deacetylases can reverse the effects of acetylases by deacetylating histones and non-histone proteins.[24] Targeting acetylation shows promise in the treatment of I/R damage,[25] while deacetylation holds potential for reducing acute I/R injury. For instance, p53 acetylation at the K118 site plays a regulatory role in cardiac I/R injury. In the ischemic heart, elevated levels of p53 acetylation result in p53 binding to the promoter of the pro-apoptotic gene bax, leading to cardiomyocyte death.[26] Further research is needed to elucidate the molecular mechanism of PDC acetylation and its interaction with other protein acetylation, which could provide a stronger foundation and more insights for the prevention and treatment of ischemic cardiomyopathy.

O-GlcNAcylation

The process of protein O-GlcNAcylation plays a role in redox regulation and various cellular activities. Studies have demonstrated that increasing the activity of the hexosamine biosynthesis pathway and promoting O-GlcNAcylation can provide protection against acute I/R injury.[27,28] Li et al. revealed that branched-chain amino acids(BCAAs) hindered the entry of pyruvate into the TCA cycle by inhibiting PDC activity. Moreover, BCAAs further suppressed PDC by downregulating hexosamine biosynthesis pathway, leading to a decrease in protein O-GlcNAcylation.[29] This modification was observed in the PDCE2 and PDCE3/E3bp subunits, but not in the PDCE1α subunit.

These findings highlight the widespread impact of I/R injury on PTMs involving the three subunits of PDCE1α, PDCE2, and PDCE3/E3bp. It has been reported that Dichloroacetate (DCA) activates the PDC and stimulates glucose aerobic metabolism in the heart, reducing of oxygen consumption and lactate production, and this may improve cardiac efficiency during I/R,[30–33] resulting in a reduction of myocardial infarction size.[34] Previous studies have shown that insulin stimulates the interaction between the O-GlcNAc transferase(OGT) and PDK1, resulting in

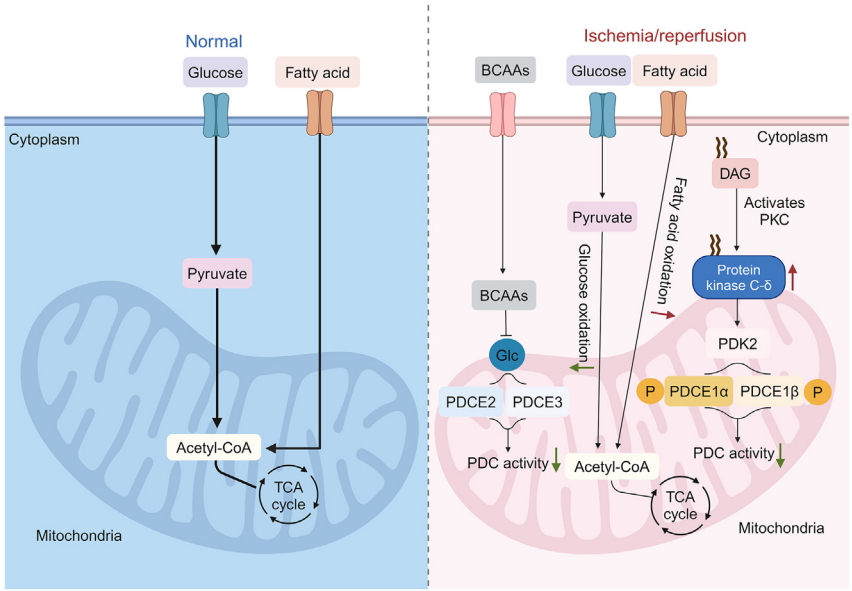

Figure 1. Post-translational modifications of pyruvate dehydrogenase complex in ischemia/reperfusion injury

Translocation of δPKC to mitochondria occurs during reperfusion. Myocardial ischemia-reperfusion leads to activation of δPKC, which translocates to mitochondria after myocardial reperfusion. δPKC specifically interacts with PDK2 and phosphorylates it, resulting in decreased PDC activity. BCAAs reduced the *o*-glcn acylation of PDCE2/E3 and also resulted in decreased PDC activity. δPKC, The delta-isoform of protein kinase C; BCAAs, Branched-Chain Amino Acids; Glc, O-GlcNAcylation; P, Phosphorylation.

O-GlcNAc modification in adipocytes, while DCA inhibits the activity of PDK1, and DCA indirectly activates PDC by inhibiting the activity of PDK1, confirming the correlation between PDC and O-GlcNAc modification.[35] Although there is relatively limited research on the direct link between PDC and O-GlcNAcylation. Similarly, baicalin, a flavonoid extracted from the root of *Scutellaria baicalensis*, has been found to have antihypertensive, hypolipidemic, and inhibitory effects on the development of atherosclerosis. It also has an ameliorative effect on CVD. Baicalin can induce metabolic reprogramming by targeting PDC activity, and pre-treatment with baicalin reduces PDC phosphorylation at S293 at 30 min after reperfusion, thereby upregulating PDC activity and increasing ATP production during reperfusion.[36] Baicalin has also shown a significant anti-apoptotic protective effect against I/R damage in cardiomyocytes.[37] Therefore, potential PDC agonists, whether derived through chemical synthesis or natural products, have the potential to act as novel targets for enhancing energy metabolism in CVD by regulating the activity of PDC and its PTMs (Figure 1).

Diabetic cardiomyopathy

Phosphorylation

Diabetic cardiomyopathy is a condition caused by impaired insulin signaling, increased fatty acids uptake and reduced glucose utilization.[38] When the insulin signal of the heart is impaired, the sensitivity of the heart cells to insulin is reduced, resulting in the weakened role of insulin in lowering blood sugar and regulating fat metabolism in the heart. This leads to an increase in blood sugar levels and an increase in the rate of fatty acid oxidation, which in turn increases the burden on the heart and promotes the occurrence and development of diabetic cardiomyopathy. The development of diabetic cardiomyopathy is strongly associated with the phosphorylation level of PDC, which has been extensively studied. In the heart, many studies have reported rapid activation of PDC by insulin, allegedly by activating PDP due to reduced insulin levels during starvation, and recent evidence suggests that PKC-δ plays a role in insulin-mediated PDP activation.[39] Streptozotocin induces a significant decrease in PDP2 protein level and PDP activity in the heart, leading to hyperphosphorylation of PDC in diabetic patients.[40] In myocardial tissues of diabetic cardiomyopathy, the phosphorylation level of PDC is increased as a result of impaired insulin signaling, leading to decreased activity of the enzyme.[41] This prevents cardiomyocytes from effectively using glucose as an energy source, causing reliance on fatty acid oxidation instead.[42] In the diabetic heart, the reduced activity of PDC is primarily caused by phosphorylation. Mitochondria from OVE26, a genetic model of chronic type 1 diabetes, exhibit hyperacetylation of protein lysines and elevated levels of phosphorylation at the PDCE1α S293.[43] Hyperacetylation may contribute to the increased oxidative stress associated with the progression of diabetes and diabetic cardiomyopathy. Cardiac mitochondria from OVE26 primarily demonstrate metabolic inflexibility, which can be further enhanced by hyperacetylation of lysines, significantly impacting mitochondrial function. In case of insulin-dependent diabetes mellitus (IDDM), the expression of PDK is increased, whereas the expression of PDP is decreased, leading to enhanced PDC phosphorylation.[44] Other factors influencing PDC phosphorylation in IDDM hearts include the activation of kinase activator protein, which phosphorylates PDC and deactivates the enzyme, and the increased levels of taurine in the diabetic heart may be inhibiting this phosphorylation.[44]

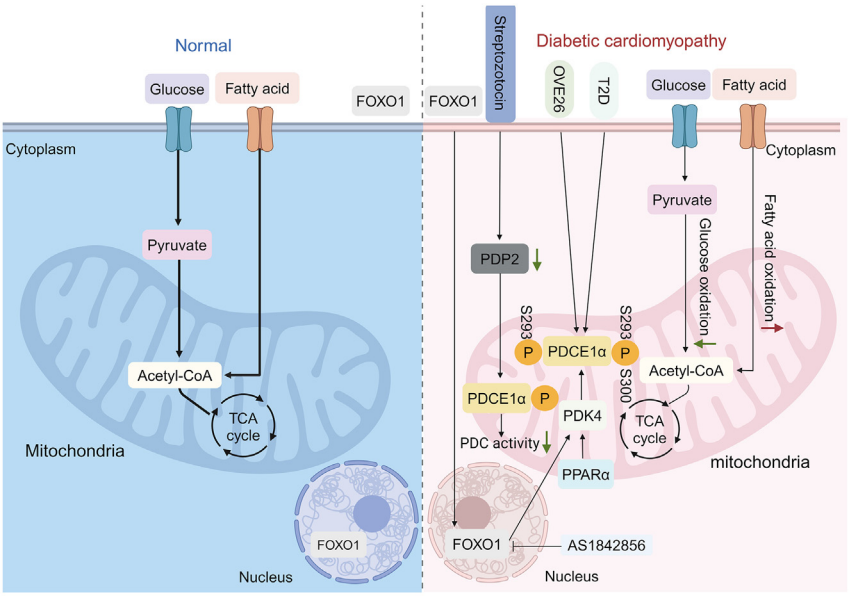

Figure 2. Post-translational modifications of pyruvate dehydrogenase complex in diabetic cardiomyopathy

PDCE1α is phosphorylated in both disease T2D and OVE26, leading to dysregulation of glucose and fatty acid metabolism. Mitochondria from OVE26, a genetic model of chronic type 1 diabetes, exhibit elevated levels of phosphorylation at the PDCE1α S293.T2D exhibit elevated levels of phosphorylation at the PDCE1α S293 and S300.FOXO1 indirectly affects PDC phosphorylation by regulating the expression of PDK4. FOXO1 nuclear translocation leads to the up-regulation of PDK4 expression and an increase in the phosphorylation of PDCE1α. Streptozotocin induces a significant decrease in PDP2 protein level and PDP activity in the heart, leading to hyperphosphorylation of PDC in diabetic patients. Treatment with the FOXO1 antagonist AS1842856 has been shown to reduce myocardial PDK4 expression. T2D, Type 2 diabetes; OVE26, A genetic model of chronic type 1 diabetes; STZ, Streptozotocin; PPARα, Peroxisome proliferator-activated receptor alpha.

A study in type 2 diabetes mellitus demonstrated that after 28 days of DCA treatment, blood glucose levels, PDC flux, and diastolic dysfunction were all normalized.[45] However, further basic and clinical studies are required to confirm and optimize these targets. The novel PDK inhibitors PS8 and PS10 have shown potential in improving glucose tolerance, reducing hepatic steatosis, and promoting myocardial glucose metabolism, thereby improving diabetic cardiac hypertrophy in obese mice.[46] Taurine, another inhibitor mentioned earlier, also holds promise for the treatment of diabetic cardiomyopathy. However, it requires further exploration and development, including larger and longer-term studies for validation.

Studies have demonstrated that reduced FOXO1 protein expression in cardiomyocytes is associated with decreased PDK4 protein expression and PDC phosphorylation.[47,48] By inhibiting FOXO1, PDC activity can be increased, thereby attenuating the development of diabetic cardiomyopathy. Treatment with the FOXO1 antagonist AS1842856 has been shown to reduce myocardial PDK4 expression, leading to decreased PDC phosphorylation at S293 and S300, it can increase the rate of glucose oxidation in the isolated heart of diabetic C57BL/6J mice, while improving diastolic function.[49] This corresponds to another finding, after pharmacological inhibition of FoxO1, Gopal K et al. observed reduced PDC phosphorylation at site 1 S293 and site 2 S300, which was consistent with PDK4 not phosphorylating PDC site 3 S232.[50]

In addition, PPARα (Peroxisome proliferator-activated receptor alpha) regulates the expression of multiple genes involved in fatty acid oxidation and metabolism, is a ligand-activated transcription factor belonging. Activation of PPARα induces the expression of PDK4, which phosphorylates PDC, resulting in reduced PDC activity and impaired glucose oxidation. PDK1-4 negatively regulates the activity of PDC through phosphorylation,[51] and activation of PDK1-4 will directly affect the phosphorylation level and activity of PDC. PDK4 is the target gene of PPAR. PPAR agonist WY-14,643 and dexamethasone can increase PDK4 levels.[52] Insulin down-regulates the abundance of PDK2 and PDK4 mRNA transcripts.[53] Amp-activated protein kinase and fatty acid synergistically induce PDK4.[54] Further research could be carried out in the heart.

O-GlcNAcylation

Hu et al. found that streptozotocin-induced dysfunction of diabetic mouse cardiomyocytes was accompanied by increased OGT expression and O-GlcNAcylation, the proportion of PDC dephosphorylation decreased.[55] However, the changes in O-GlcNAcylation of PDC in diabetic cardiomyopathy mice still require further exploration. Investigating the alterations in O-GlcNAcylation of PDC is crucial for understanding the pathogenesis and identifying potential therapeutic targets of diabetic cardiomyopathy.

The other PTMs of PDC have been investigated to a lesser degree, and the specific subunits and sites of action are not as well characterized, either. Therapeutic targets for diabetic cardiomyopathy involve several aspects, including FOXO1, PDK, and PDC, which can affect myocardial metabolism, structure, and function through different pathways to protect the heart (Figure 2).

Table 1. Obesity leads to phosphorylation of PDC

Induction mode	Tissue/Cell	Species	Regulator	Manifestation	Reference
High-fat diet	Liver	Mice	ATP	Phosphorylation of PDC	Zhang et al.[61]
Gold thioglucose	Heart	Mice	Unknown	Dephosphorylation of PDC↓	Kerbey et al.[60]
Gold thioglucose	Heart	Mice	Lipid oxidation	Dephosphorylation of PDC↓	Caterson et al.[59]
Circulating lymphocytes	Lymphocytes	Human	PDP1 is not sensitive to Mg/Ca	PDP1 activity↓	Piccinini et al.[118]

Obesity cardiomyopathy

Obesity cardiomyopathy is a disease characterized by abnormal structure and function of the myocardium due to obesity.[56] The incidence of HF is higher in individuals who are overweight or obese.[57] The enzyme PDC plays a crucial role in fatty acid metabolism, regulating their oxidation and synthesis within cardiomyocytes. In obesity, there is an excessive accumulation of fatty acid in cardiomyocytes, which inhibits PDC activity. As a result, cardiomyocytes are unable to efficiently utilize glucose for energy metabolism, leading to impaired cardiac function.[58]

Phosphorylation

The accumulation of fatty acid interferes with the PTMs of PDC in obese individuals. Specifically, there is an increase in phosphorylation levels of PDC, which competes with lipid oxidation. When lipid oxidation is enhanced, PDC phosphorylation is also increased (Table 1).[58–61] Moreover, consumption of a high-calorie unsaturated fatty acid diet (UNSAT) reduces the content of PDCE1α protein and increases PDCE1α S300 phosphorylation.[62] Therefore, phosphorylation of PDC may have implications for the development of obesity cardiomyopathy. Sugden MC's study also confirmed this point, and PDC deactivation caused by phosphorylation was catalyzed by PDK1-4, and fatty acid oxidation inhibited PDC activity.[63]

Acetylation

In addition to this, PDC acetylation also affects the heart. Previous studies have shown that decreased PDC activity is a contributing factor in the development of diastolic dysfunction.[64] In obese mice, cardiac diastolic dysfunction is associated with hyperacetylation of PDCE1α, which negatively impacts PDC activity in cardiac cells. Using a diet-induced obesity model in aged mice, it has been suggested that inhibitory lysine acetylation of PDC may contribute to the development of diastolic dysfunction in the mouse heart.[65] This hyperacetylated state may affect the overall activity of PDC by altering the structure and function of PDCE1α. Since PDC plays a crucial role in cardiomyocyte energy metabolism, a decrease in its activity may result in insufficient energy supply to cardiomyocytes, thus affecting the diastolic function of the heart. High acetylation of PDCE1α inhibits pyruvate oxidation.[66] Long-term exposure to a high-fat diet has previously been shown to promote diastolic dysfunction, which has been of concern in terms of PDC specific loss, reduced pyruvate oxidation, and decreased PDC activity.[67] Therefore, therapies targeting PDC could potentially benefit individuals with obesity cardiomyopathy by improving the metabolic balance of cardiomyocytes, reducing myocardial injury, and enhancing cardiac function. However, the successful application of these therapies in obesity cardiomyopathy treatment requires a deeper understanding of cardiac metabolism in obese populations (Figure 3).

Heart failure

HF is a result of various CVD,[68] which can be caused by different factors but share common risk factors. It poses a significant threat to human health due to its high morbidity and mortality rates, ultimately leading to cardiac structural and functional remodeling.[69]

Phosphorylation

Changes in heart metabolism in HF lead to impaired energy production, which in turn affects heart function. In HF, overloading the heart causes dramatic metabolic changes that impair the heart's energy production and lead to worsening systolic function and reduced PDC flux.[70] Myocardial glucose and fatty acid oxidation imbalances and altered cardiac energy metabolism in failing hearts are co-important factors in cardiac pathology associated with obesity, diabetes, and HF. In the early stages of HF, there is an increase in glucose uptake and utilization, while fatty acid oxidation decreases. However, in the later stages, glucose metabolism decreases due to insulin resistance.[71] In the end-stage failing heart, there is an increase in PDC protein expression and a decrease in PDK4 expression. The relative expression levels of the E1α, E1β, E2, and E3bp subunits of PDC were found to be higher in the failing LV compared to the nonfailing LV.[72]

Acetylation

Lysine acetylation of non-histones, which controls multiple families of mitochondrial metabolic pathways, contributes to cardiac energy disturbances that occur in obesity, diabetes, and HF. Alterations in the post-translational control of energy metabolic processes have

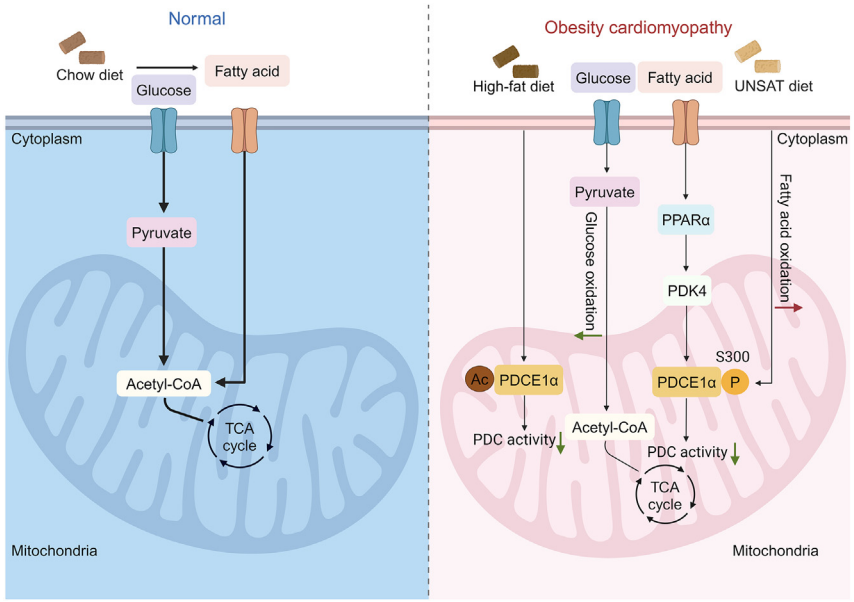

Figure 3. Post-translational modifications of pyruvate dehydrogenase complex in obesity cardiomyopathy

Fatty acid synthesis is increased in obesity cardiomyopathy, and fatty acid and PPARα target and regulate PDK4, which promotes phosphorylation of PDCE1α at S300. Acetylation of PDCE1α occurs in high-fat diet-induced obesity. Similarly, consumption of UNSAT reduces the content of PDCE1α protein and increases phosphorylation of PDCE1α at S300. PPARα, Peroxisome proliferator-activated receptor alpha; UNSAT, A high-calorie unsaturated fatty acid diet.

recently been identified as important contributors to these metabolic changes. In particular, lysine acetylation of non-histones, which controls multiple families of mitochondrial metabolic pathways, contributes to cardiac energy disturbances that occur in obesity, diabetes, and HF. One of the important targets for mitochondrial acetylation is fatty acid beta-oxidase, which helps to alter cardiac substrate preference and promote fatty acid beta oxidation rates during obesity, diabetes, and HF.[73] And eventually lead to cardiac dysfunction in these disease states.

Lysine residue acetylation targets a majority of metabolic pathway proteins in mitochondria. Many microRNA are involved in pathophysiological events associated with disease. Such as miR-21, miR-1, miR-23a, miR-142-5p, miR-126, miR-29, miR-195, and miR-499, as they are most commonly mentioned as important specific indicators of cardiac hypertrophy and fibrosis leading to HF. miR-195 plays a role in metabolic regulation in HF.[74] Previous studies have identified a novel miR-195-regulated pathway in the failing heart, which results in hyperacetylation of key metabolic proteins, namely PDC and ATP synthase, and these studies have also demonstrated that miR-195 directly targets SIRT3, a member of sirtuins family that play an essential role in deacetylation.[75] Further investigations have shown that the acetylation level of PDCE2 and PDCE3 is increased in the myocardium of miR-195 overexpressing mice, leading to reduced PDC activity and impaired mitochondrial respiration in patients with HF. In addition, highly acetylated proteins were found in the hearts of mice with HF whose ejection fraction was preserved, and these highly acetylated proteins were enriched in mitochondria. High acetylation in HF with ejection fraction preservation suggests that mitochondrial function is associated with impaired TCA circulation and the abundance of TCA intermediates is down-regulated.[76] In the context of other diseases, such as tumors, SIRT3 is involved in PDCE1α deacetylation, affecting PDC activity and cellular metabolism.[77] Activation of SIRT3 has shown a protective effect in diseases like CVD and tumors, improving myocardial function and inhibiting tumor growth and metastasis. Another member, SIRT6, also correlates with PDC. Pamela Becherini et al. showed that SIRT6 promotes PDC activity in breast cancer cells by at least two mechanisms, namely, increasing PDC levels and enhancing intracellular Ca^{2+} concentration, which promotes mammary tumorigenesis in mice.[78] The deacetylated molecules represented by SIRT3 and other members of sirtuins family may improve myocardial damage and protect heart function by regulating the acetylation level in the heart. Deacetylation sites of PDC are unknown, and a study using a mass spectrometry-based proteomics approach analyzed three potential acetylation sites for PDCE1α in thymic epithelial cells, which inspires us to further explore specific sites with the help of techniques such as mass spectrometry.[79]

O-GlcNAcylation

One example of how heart disease may lead to an increase in O-GlcNAcylation is that an acute increase of the protein O-GlcNAcylation prevents TNF-α-induced vascular dysfunction by inhibiting iNOS expression.[80] O-GlcNAcylation is an autoprotective response in acute stress models (hypoxia, ischemia, oxidative stress).[81] The increase of O-GlcNAcylation is currently considered to be a self-protective mechanism of the heart in response to various stressful conditions. The continuous abnormal increase of O-GlcNAc glycosylation is harmful to the human

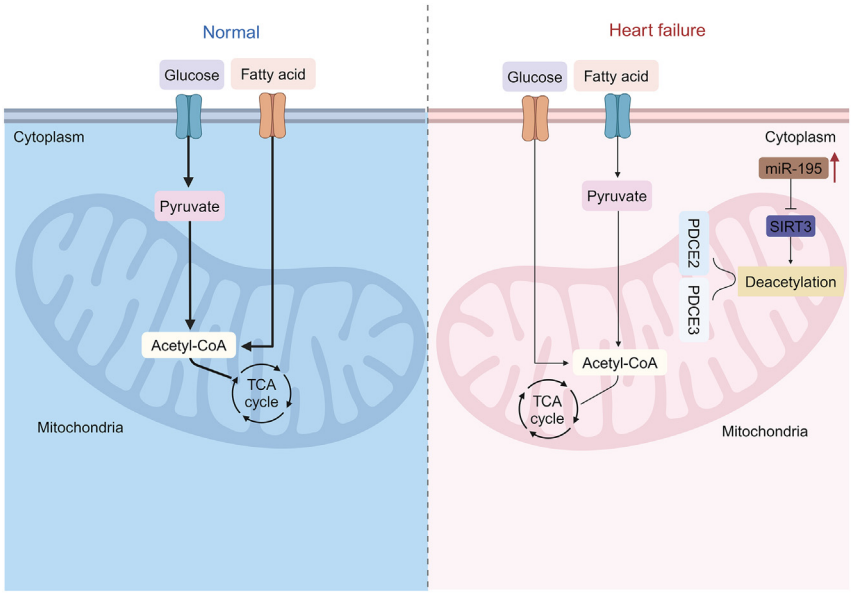

Figure 4. Post-translational modifications of pyruvate dehydrogenase complex in heart failure

miR-195 levels were elevated in failing myocardium. miR-195 targets SIRT3 in the failing heart, and the deacetylation of PDCE2 and PDCE3 are inhibited, further reducing PDC activity.

body. In recent years, a growing number of studies have demonstrated chronic abnormal increases in protein O-GlcNAc glycosylation in patients with a variety of CVDs, including HF, myocardial hypertrophy, hypertension, and vascular dysfunction.[81]

Ida G Lunde showed that global O-GlcNAcylation was increased by 60% in failing hearts.[82] Although they did not specifically investigate the changes in O-GlcNAcylation of individual proteins in their study, immunoblots revealed the presence of certain O-GlcNAcylation bands with specific molecular weights.[83]Notably, a significant increase in cardiac O-GlcNAcylation at around 50 kDa was observed in all four cardiac insufficiencies: aortic stenosis and hypertension, myocardial infarction, and aortic fasciculations. It is worth noting that the molecular weight of PDC falls within this range, but further scientific verification is necessary (Figure 4).

Vascular diseases

Vascular calcification is a common occurrence in advanced vascular disease and is often associated with aging, diabetes, and chronic kidney disease. Notably, diabetic patients tend to have more severe atherosclerosis and a higher prevalence of vascular calcification, which ultimately leads to increased CVD mortality.[84,85] The results of this study are summarized in the following table. A study by Lee SJ et al. found that smooth muscle cells (VSMCs) in the vascular wall and calcified blood vessels of patients with atherosclerosis exhibited increased phosphorylation of S293 and S300 in PDCE1α and SMAD 1/5/8, furthermore, the researchers observed that the use of DCA for PDK4 inhibition led to the alleviation of vascular calcification.[86] Another study by Maria J Forteza et al. demonstrated that inhibition of PDK restored PDC activity and reduced pro-inflammatory signaling in the vessel wall, and this inhibition also suppressed the pro-atherosclerotic and plaque destabilizing pathways associated with NLRP3 inflammasome activation and IL-1β secretion.[87] The finding has implications for the treatment of vascular diseases. These findings have significant implications for the treatment of vascular diseases, offering new ideas and targets. In particular, inhibiting PDC phosphorylation may serve as a potential strategy for the prevention and treatment of vascular diseases.

Pulmonary hypertension is widely recognized for its notable increase in right ventricular pressure overload, which can eventually result in myocardial hypertrophy and remodeling of the right heart. Interestingly, elevated levels of PDK4 have been observed in patients with pulmonary hypertension. In a study, the use of LCZ696, which targeted PDK4 and inhibits its expression, inhibited the phosphorylation of GSK3-β, showed promising results in attenuating pulmonary hypertension-induced right ventricular remodeling.[88] These findings suggest that the PDC-related signaling pathways play a role in the development of pulmonary arterial hypertension and warrant further investigation (Figure 5).

CROSSTALK BETWEEN PTMs

Crosstalk between PTMs is often utilized as a fine-tuning mechanism to adapt to changes in the external environment.[89] Common PTMs Crosstalk includes phosphorylation and acetylation, as well as phosphorylation and glycosylation. These PTMs can occur on the same residues in the substrate protein sequence, on closely located S/T residues, or even on distantly positioned S/T residues.[90,91] Additionally,

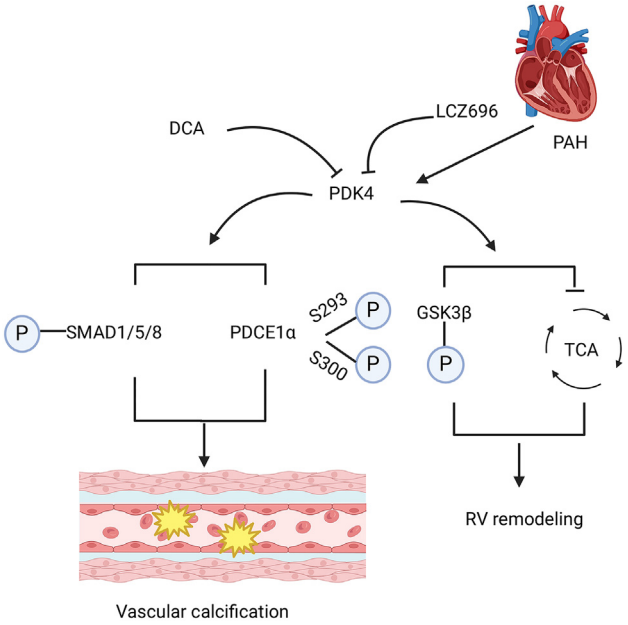

Figure 5. Post-translational modifications of pyruvate dehydrogenase complex in vascular diseases

In the vessel wall of patients with atherosclerosis and in VSMCs of calcified vessels, S293 and S300 of PDCE1α and SMAD 1/5/8 were phosphorylated. DCA decreased the phosphorylation levels of PDCE1α S293 and S300.LCZ696 attenuates RV remodeling and inhibits the phosphorylation of GSK3-β by downregulating PDK4. Symptoms of vascular calcification and pulmonary hypertension were alleviated by inhibiting PDK4. DCA, Dichloroacetate; PAH, Pulmonary arterial hypertension.

PTMs can occur both on closely located S/T residues and at distant but spatially close positions.[92] An example of this is p53. Phosphorylation of p53 takes place at the N-terminal end, while acetylation occurs at the C-terminal end.[93] Phosphorylation enhances the interaction of p53 with CBP/p300 and promotes C-terminal acetylation. Likewise, there was a study on the crosstalk between SUMOylation and acetylation in PDC. The study confirmed that SUMO 2/3 modification of PDCE1α was unchanged but SUMO 2/3 modification of PDCE1β was significantly increased and acetylation was significantly decreased in A431 cells after H_2O_2 treatment. This study suggests for the first time that there may be a crosstalk between acetylation and SUMOylation in PDCE1β.[94] This led us to realize that PTMs are highly dynamic and that crosstalk has a more complex and enriched regulatory system, as well as the need to fill the current gap in crosstalk in CVD. Although the functional regulation of PTMs in CVD has been extensively studied, there has been relatively little research on the specific role of PTM of PDC crosstalk in CVD. This may be due to the difficulty of detecting and analyzing crosstalk techniques, as well as the complexity of PTM crosstalk itself. In response to the emerging research shortfall, detection techniques can be improved so that PTMs communication can be accurately identified and quantified in complex biological samples. Modeling system and data sharing methods promote the development of PDC crosstalk (Table 2).

PERSPECTIVES ON CLINICAL PRACTICE

For phosphorylation, PDK regulates PDC activity and inhibits it by phosphorylating the E1 subunit of PDC. Treatment with PDK inhibitors has been shown to ameliorate myocardial I/R and diabetic cardiomyopathy as well as HF in a wide range of animal and cellular models. For I/R injury, DCA treatment of C57BL/6J mice after ligation of the left anterior descending coronary artery to obtain an *in vivo* model of ischemia significantly reduced the infarct size induced by myocardial I/R, in a cellular model DCA treatment ameliorated cardiomyocyte obstruction under H/R conditions.[95] For diabetic cardiomyopathy, it was shown that after 28 days of DCA treatment, PDC fluxes returned to normal levels, blood glucose levels normalized, and diabetes restored homeostasis of myocardial substrate selection and reversed diastolic dysfunction.[96] However, it is important to note that DCA is a nonspecific and ineffective PDK inhibitor, requiring high doses to achieve therapeutic effects.[97] Therefore, there is a need to develop and synthesize efficient and low-toxicity PDK inhibitors that can offer highly potent therapeutic effects. PS10 is another novel PDK inhibitor for the treatment of diabetic cardiomyopathy.[98] PS10 significantly augmented PDC activity and reduced phosphorylation in different tissues. PS10 treatment improved glucose tolerance and significantly attenuated hepatic steatosis in a mouse model.[99] AZD7545 and Nov3r are also a novel inhibitor of the steatosis and necrosis that may occur in the myocardium with AZD7545.[100] For HF, because of the high PDK4 inhibitory activity of vitamin K3 due to its highly similar ATP-binding site to PDK4, researchers synthesized novel PDK4 inhibitor K3 derivatives8 that are more active than the existing PDK4 inhibitor, DCA, and tested their cardioprotective effects in a mouse model of HF.[101] PDK inhibitors provide drug targets for the treatment of CVD by modulating PDC. There are few clinical studies on

Table 2. Post-translational modifications of PDC subunits and sites in cardiovascular diseases

Subunit	Site	PTM	Disease	Mechanism	PDC activity	Regulator	Reference
E1α	S232	Phosphorylation	Unknown	Unknown	Decrease	PDK4	Unknown
E1α	S293	Phosphorylation	Diabetic cardiomyopathy, vascular calcification	Impaired diastolic function, Translocation of PKCδ	Decrease	PDK4	Gopal et al.[49] and Lee et al.[86]
E1α	S300	Phosphorylation	Diabetic/Obesity cardiomyopathy, vascular calcification	Impaired diastolic function, Translocation of PKCδ	Decrease	PDK4	Gopal et al.[49] and Lee et al.[86]
E1α	Unknown	Acetylation	Obesity cardiomyopathy	Restrict pyruvate oxidation	Increase	Unknown	Thapa et al.[65]
E1β	Unknown	Phosphorylation	I/R injury	Decreased glucose oxidation rate, Translocation of PKCδ	Decrease	Unknown	Folmes et al.[18]
E2	Unknown	Acetylation	Heart failure	Lower mitochondrial respiration	Decrease	SIRT3	Zhang et al.[75]
E2	Unknown	O-GlcNAcylation	I/R injury	Vulnerable to I/R injury	Decrease	Unknown	Li et al.[29]
E3	Unknown	Acetylation	Heart failure	Lower mitochondrial respiration	Decrease	SIRT3	Zhang et al.[75]
E3bp	Unknown	O-GlcNAcylation	I/R injury	Vulnerable to I/R injury	Decrease	Unknown	Li et al.[29]

PDK inhibitors, but their pharmacological effects vary depending on the type of disease and the type of drug, so future studies may focus more on the modulation aspect of the pharmacological effects of PDK inhibitors.

Regarding glycosylation, in recent years more and more studies have shown that glycosylation levels are abnormal in patients with a variety of CVD, including HF and vascular dysfunction. The role of glycosylation of PDC in CVD has now received some attention, but research in this area is still limited, with some studies focusing on exploring the molecular mechanisms of PDC glycosylation and its specific manifestations in disease. Research on specific mechanisms has been limited to the aspect of I/R injury. As mentioned previously, BCAAs impede pyruvate entry into the TCA cycle by inhibiting PDC activity during I/R, and in diabetic cardiomyopathy, this is accompanied by decreased dephosphorylation of PDC and elevated OGT expression and O-GlcNAcylation. Clinically, based on glycosylation, relevant glycosylation inhibitors have been applied in the treatment of some diseases, such as as anti-inflammatory drugs, blocking inflammatory response and antitumor and anticoagulant drugs, etc. For glycosylation, cellular O-GlcNAcylation levels can be regulated by the abundance of UDP-GlcNAc, as well as by regulating OGT, the O-GlcNAcase (OGA) and its GFAT. Various categories of OGT inhibitors have been identified, including substrate analogs, dual substrate analogs, and small molecule inhibitors. The first reported OGT inhibitor is Alloxan, but it has potential cytotoxic effects.[102] Several OGA inhibitors have also been identified, which can have therapeutic effects on diseases by inhibiting OGA activity and increasing the level of O-GlcNAc modification. PUGNAc is one of the earliest and most widely used potent OGA inhibitors, but it lacks specificity.[103,104] Another inhibitor, MK87199,[105] has shown the ability to increase the level of intracellular O-GlcNAc modifications and has entered clinical trials for the treatment of Alzheimer's disease, but it has not been applied to CVD. However, there are currently no approved drugs targeting OGT activity, and the development of OGT inhibitors still faces significant challenges, including selectivity, stability, permeability, and toxicity. Hence, it is imperative to conduct further optimization and evaluation of the current OGT inhibitors, alongside the development of novel inhibitors. The current understanding of the mechanism of action of glycosylation modifications in CVD is still in its infancy for two main reasons: one is the technical difficulty in identifying how individual glycoform changes affect protein properties and cellular functions, and the other is that little is known about the regulatory mechanisms associated with the control of glycosylation modifications in CVD. The role of glycosylation of PDC in CVD is an important but understudied area. Future studies should simultaneously reveal more about both the molecular mechanisms of PDC glycosylation in CVD and potential therapeutic strategies.

Regarding acetylation and deacetylation, PDC-related studies in the context of CVD have only been involved in obesity cardiomyopathy and HF, and only in a fundamental way. One of the molecules involved in the mechanistic pathway is SIRT3. given the importance of SIRT3, it is crucial to develop drugs that target it. One example of this is Honokiol, a small molecular mass compound that activates SIRT3 and has various pharmacological properties. Studies have shown that Honokiol can partially reverse the adverse effects of diabetes and cardiac hypertrophy on the heart.[106,107] However, there is limited information on the role of SIRT3 deacetylation in PDC studies. Further studies are needed to elucidate the mechanisms involved and contribute to the understanding of CVD as well as to the development of relevant drugs to study for clinical efficacy and safety in CVD.

The onset and progression of CVD involve various mechanisms. Consequently, numerous drugs have been developed and investigated to target different pathways and achieve diverse selectivity. However, there is a lack of research on PTMs of PDC, despite their crucial regulatory role in myocardial energy metabolism and protection. Therefore, it is imperative to explore the mechanism of action of PTMs of PDC in CVD, as well as the development and study of drugs that can modulate PDC activity through PTMs, are pressing issues in the field of cardiovascular pharmacology.

Conclusions and outlook

PDC catalyzes the conversion of pyruvate to acetyl-CoA, which is a bridge between glycolysis and TCA. The activity of PDC is regulated by a variety of PTMs, including phosphorylation, dephosphorylation, acetylation, and glycosylation. PTMs are post-translational chemical modifications of proteins that alter protein structure and function. PTMs of PDC do play an important role in CVD, they affect the energy metabolism of myocardium by influencing the activity of PDC. These changes may lead to myocardial diastolic dysfunction, cardiomyocyte apoptosis, myocardial fibrosis and mitochondrial respiratory function injury. By combing PTMs of PDC in various CVD, it was found that kinases, phosphatases, transcription factors and small non-coding RNA were all involved in the regulation of PTMs related to PDC. These regulatory mechanisms work together to affect energy metabolism of cardiomyocytes, which is of great significance for the development and treatment of CVD. A classic modification, ubiquitination, occurs in PDCE1β, where high EGFR-PTK activity leads to phosphorylation of tyrosine residues of PDCE1β, which in turn bind to ubiquitin ligase, resulting in ubiquitination of PDCE1β protein. PDC deficiency occurs when it is recognized and degraded by the proteasome.[108] Recent advancements in proteomics and bioinformatics have identified novel PTMs such as crotonylation, lactylation, and β-hydroxybutyrylation.[109,110] These PTMs of novel acylation have been reported to be involved in a variety of important biological processes, Arata Fukushima et al. found that succinylation of PDC in the neonatal heart plays a crucial role in energy metabolism as the heart matures.[111] Li Fei Wu et al. found that lysine alaninization is associated with the development of cardiac hypertrophy and may be a new therapeutic target for cardiac hypertrophy.[112] For lactylation, Naijin Zhang et al. found that p300 and SIRT1 act as acyltransferases and delactases of α-MHC, respectively, and regulate the level of lactic acidification of α-MHC.[113] By decreasing lactate production, lactonization of α-MHC can be reduced, leading to further deterioration of myocardial function. lactonization of α-MHC can alleviate HF. In another research, p300, as an acyltransferase, can be inhibited by Rg3 to inhibit its acyltransferase activity, which reduces the level of 2-hydroxyisobutylation of PDCE3 to restore PDC activity reverses cardiac hypertrophy.[114] It seems that the potential of these novel modifications is great and these findings provide new perspectives for better understanding and interpreting medical treatments for CVD. Among them, p300 has a non-negligible value in PTMs and can be used as a starting point for research. A Study has shown that lactate first stimulates electron transport chain activity, after which PDC activation occurs.[115] There is room for extensive research on whether lactate modification is involved in the mechanism of lactate as an ETC messenger and whether it causes PDC-related changes. The exact regulatory mechanism of the interaction between SUMO molecules and the key mitochondrial protein PDC remains unclear. Surprisingly, however, SUMOylation of mitochondria-associated proteins modulates mitochondrial autophagy, which has been implicated in the onset and progression of CVD.[116] Whether SUMOylation of PDC affects cardiac function by influencing mitochondrial biofunction remains to be investigated. Regarding SUMOylation of PDC, a study demonstrated for the first time the possible crosstalk between acetylation and SUMOylation in PDCE1β, which further suggests the value of the study of SUMOylation of PDC.[117] Further analysis is required to understand the involvement of these novel PTMs in PDC regulation, particularly in relation to substrate binding, cofactor binding, active state transitions, and subcellular localization. As CVD is closely associated with metabolic disorders, modulation the activity of PDC through various PTMs to improve energy supply is of great significance. Exploring the modification types, sites, and mechanism of action of novel PDC in CVD is of great significance for uncovering the molecular basis of metabolic regulation in the cardiovascular system and identifying new therapeutic targets and strategies for CVD.

The clarification of PTMs of PDC offers multiple advantages in CVD research. Firstly, investigating PTMs of PDC can help in understanding the regulation of energy metabolism in cardiomyocytes, providing valuable insights into the complex mechanisms involved. Additionally, exploring PTMs of PDC may lead to the identification of new molecular markers for CVD, which could improve the accuracy of diagnosis and prognosis. Moreover, studying the mechanisms associated with PTMs related to CVD can aid in the identification of new therapeutic targets. By specifically targeting these PTMs, novel drugs or intervention strategies can be developed for the treatment of CVD.

Further studies in proteomics and molecular biology studies are necessary to identify any unknown types and locations of PTMs of PDC and their impact on both PDC and CVD. In the future, the investigation of PTMs in PDC faces the following challenges, such as elucidating the interactions between different PTMs and assessing the role of PTMs of PDC in various types and stages of CVD. The patterns and mechanisms of PTMs of PDC in different types and stages of CVD remain unclear, and additional fundamental and clinical studies are required. Additionally, it is crucial to overcome the limitations of current detection and analysis methods for PTMs of PDC and develop more efficient and specific techniques.

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AUTHOR CONTRIBUTIONS

B.G., F.J.Z., and Y.Y. are responsible for writing the article and have the equal contribution. X.M.N., Z.Z.H., Q.L.M., Z.Q.Y., W.H.J., M.L.L., Y.S.W., and S.L.J. made the drawings and adjustments to the references. L.Y. and N.M. supervised the project.

DECLARATION OF INTERESTS

All the authors confirm that there are no conflicts of interest.

REFERENCES

1. Roth, G.A., Mensah, G.A., Johnson, C.O., Addolorato, G., Ammirati, E., Baddour, L.M., Barengo, N.C., Beaton, A.Z., Benjamin, E.J., Benziger, C.P., et al. (2020). Global burden of cardiovascular diseases and risk factors, 1990-2019: update from the gbd 2019 study. J. Am. Coll. Cardiol. *76*, 2982–3021. https://doi.org/10.1016/j.jacc.2020.11.010.
2. Yan, K., Wang, K., and Li, P. (2019). The role of post-translational modifications in cardiac hypertrophy. J. Cell Mol. Med. *23*, 3795–3807. https://doi.org/10.1111/jcmm.14330.
3. Hou, J., Wen, X., Long, P., Xiong, S., Liu, H., Cai, L., Deng, H., and Zhang, Z. (2022). The role of post-translational modifications in driving abnormal cardiovascular complications at high altitude. Front. Cardiovasc. Med. *9*, 886300. https://doi.org/10.3389/fcvm.2022.886300.
4. Tso, S.C., Qi, X., Gui, W.J., Wu, C.Y., Chuang, J.L., Wernstedt-Asterholm, I., Morlock, L.K., Owens, K.R., Scherer, P.E., Williams, N.S., et al. (2014). Structure-guided development of specific pyruvate dehydrogenase kinase inhibitors targeting the atp-binding pocket. J. Biol. Chem. *289*, 4432–4443. https://doi.org/10.1074/jbc.M113.533885.
5. Ho, L., Hu, C.W., Packman, S., and Patel, M.S. (1986). Deficiency of the pyruvate dehydrogenase component in pyruvate dehydrogenase complex-deficient human fibroblasts. Immunological identification. J. Clin. Invest. *78*, 844–847. https://doi.org/10.1172/JCI112651.
6. Patel, K.P., O'Brien, T.W., Subramony, S.H., Shuster, J., and Stacpoole, P.W. (2012). The spectrum of pyruvate dehydrogenase complex deficiency: clinical, biochemical and genetic features in 371 patients. Mol. Genet. Metab. *105*, 34–43. https://doi.org/10.1016/j.ymgme.2011.09.032.
7. Lv, B., Zhou, J., He, S., Zheng, Y., Yang, W., Liu, S., Liu, C., Wang, B., Li, D., and Lin, J. (2022). Induction of myocardial infarction and myocardial ischemia-reperfusion injury in mice. J. Vis. Exp. *10*, 63257.
8. Gabaldon-Perez, A., Marcos-Garces, V., Gavara, J., Rios-Navarro, C., Miñana, G., Bayes-Genis, A., Husser, O., Sanchis, J., Nunez, J., Chorro, F.J., and Bodi, V. (2021). Coronary revascularization and long-term survivorship in chronic coronary syndrome. J. Clin. Med. *10*, 610. https://doi.org/10.3390/jcm10040610.
9. Elgendy, I.Y., Mahtta, D., and Pepine, C.J. (2019). Medical therapy for heart failure caused by ischemic heart disease. Circ. Res. *124*, 1520–1535. https://doi.org/10.1161/CIRCRESAHA.118.313568.
10. Ou, W., Liang, Y., Qin, Y., Wu, W., Xie, M., Zhang, Y., Zhang, Y., Ji, L., Yu, H., and Li, T. (2021). Hypoxic acclimation improves cardiac redox homeostasis and protects heart against ischemia-reperfusion injury through upregulation of o-glcnacylation. Redox Biol. *43*, 101994. https://doi.org/10.1016/j.redox.2021.101994.
11. Yao, L., He, F., Zhao, Q., Li, D., Fu, S., Zhang, M., Zhang, X., Zhou, B., and Wang, L. (2023). Spatial multiplexed protein profiling of cardiac ischemia-reperfusion injury. Circ. Res. *133*, 86–103. https://doi.org/10.1161/CIRCRESAHA.123.322620.
12. Cai, W., Xu, D., Zeng, C., Liao, F., Li, R., Lin, Y., Zhao, Y., Dong, W., Wang, Q., Yang, H., et al. (2022). Modulating lysine crotonylation in cardiomyocytes improves myocardial outcomes. Circ. Res. *131*, 456–472. https://doi.org/10.1161/CIRCRESAHA.122.321054.
13. Li, T., Xu, J., Qin, X., Hou, Z., Guo, Y., Liu, Z., Wu, J., Zheng, H., Zhang, X., and Gao, F. (2017). Glucose oxidation positively regulates glucose uptake and improves cardiac function recovery after myocardial reperfusion. Am. J. Physiol. Endocrinol. Metab. *313*, E577–E585. https://doi.org/10.1152/ajpendo.00014.2017.
14. Casin, K.M., and Calvert, J.W. (2021). Dynamic regulation of cysteine oxidation and phosphorylation in myocardial ischemia-reperfusion injury. Cells *10*, 2388. https://doi.org/10.3390/cells10092388.
15. Fu, Z.P., Wu, L.L., Xue, J.Y., Zhang, L.E., Li, C., You, H.J., and Luo, D.L. (2022). Connexin 43 hyper-phosphorylation at serine 282 triggers apoptosis in rat cardiomyocytes via activation of mitochondrial apoptotic pathway. Acta Pharmacol. Sin. *43*, 1970–1978. https://doi.org/10.1038/s41401-021-00824-z.
16. Churchill, E.N., Murriel, C.L., Chen, C.H., Mochly-Rosen, D., and Szweda, L.I. (2005). Reperfusion-induced translocation of deltapkc to cardiac mitochondria prevents pyruvate dehydrogenase reactivation. Circ. Res. *97*, 78–85. https://doi.org/10.1161/01.RES.0000173896.32522.6e.
17. Churchill, E.N., and Szweda, L.I. (2005). Translocation of deltapkc to mitochondria during cardiac reperfusion enhances superoxide anion production and induces loss in mitochondrial function. Arch. Biochem. Biophys. *439*, 194–199. https://doi.org/10.1016/j.abb.2005.05.007.
18. Folmes, C.D., Sawicki, G., Cadete, V.J., Masson, G., Barr, A.J., and Lopaschuk, G.D. (2010). Novel o-palmitolylated beta-e1 subunit of pyruvate dehydrogenase is phosphorylated during ischemia/reperfusion injury. Proteome Sci. *8*, 38. https://doi.org/10.1186/1477-5956-8-38.
19. Kobayashi, K., and Neely, J.R. (1983). Effects of ischemia and reperfusion on pyruvate dehydrogenase activity in isolated rat hearts. J. Mol. Cell. Cardiol. *15*, 359–367. https://doi.org/10.1016/0022-2828(83)90320-6.
20. Terrand, J., Papageorgiou, I., Rosenblatt-Velin, N., and Lerch, R. (2001). Calcium-mediated activation of pyruvate dehydrogenase in severely injured postischemic myocardium. Am. J. Physiol. Heart Circ. Physiol. *281*, H722–H730. https://doi.org/10.1152/ajpheart.2001.281.2.H722.
21. Han, Y., Sun, W., Ren, D., Zhang, J., He, Z., Fedorova, J., Sun, X., Han, F., and Li, J. (2020). Sirt1 agonism modulates cardiac nlrp3 inflammasome through pyruvate dehydrogenase during ischemia and reperfusion. Redox Biol. *34*, 101538. https://doi.org/10.1016/j.redox.2020.101538.
22. Davidson, M.T., Grimsrud, P.A., Lai, L., Draper, J.A., Fisher-Wellman, K.H., Narowski, T.M., Abraham, D.M., Koves, T.R., Kelly, D.P., and Muoio, D.M. (2020). Extreme acetylation of the cardiac mitochondrial proteome does not promote heart failure. Circ. Res. *127*, 1094–1108. https://doi.org/10.1161/CIRCRESAHA.120.317293.
23. Kosanam, H., Thai, K., Zhang, Y., Advani, A., Connelly, K.A., Diamandis, E.P., and Gilbert, R.E. (2014). Diabetes induces lysine acetylation of intermediary metabolism enzymes in the kidney. Diabetes *63*, 2432–2439. https://doi.org/10.2337/db12-1770.
24. Wang, Z.A., Markert, J.W., Whedon, S.D., Yapa Abeywardana, M., Lee, K., Jiang, H., Suarez, C., Lin, H., Farnung, L., and Cole, P.A. (2023). Structural basis of sirtuin 6-catalyzed nucleosome deacetylation. J. Am. Chem. Soc. *145*, 6811–6822. https://doi.org/10.1021/jacs.2c13512.
25. Zuurbier, C.J., Bertrand, L., Beauloye, C.R., Andreadou, I., Ruiz-Meana, M., Jespersen, N.R., Kula-Alwar, D., Prag, H.A., Eric Botker, H., Dambrova, M., et al. (2020). Cardiac metabolism as a driver and therapeutic target of myocardial infarction. J. Cell Mol. Med. *24*, 5937–5954. https://doi.org/10.1111/jcmm.15180.
26. Gogna, R., Madan, E., Khan, M., Pati, U., and Kuppusamy, P. (2013). P53's choice of myocardial death or survival: oxygen protects infarct myocardium by recruiting p53 on nos3 promoter through regulation of p53-lys(118) acetylation. EMBO Mol. Med. *5*, 1662–1683. https://doi.org/10.1002/emmm.201202055.
27. Jensen, R.V., Andreadou, I., Hausenloy, D.J., and Bøtker, H.E. (2019). The role of o-glcnacylation for protection against ischemia-reperfusion injury. Int. J. Mol. Sci. *20*, 404. https://doi.org/10.3390/ijms20020404.
28. Wright, J.N., Collins, H.E., Wende, A.R., and Chatham, J.C. (2017). O-glcnacylation and cardiovascular disease. Biochem. Soc. Trans. *45*, 545–553. https://doi.org/10.1042/BST20160164.
29. Li, T., Zhang, Z., Kolwicz, S.C., Jr., Abell, L., Roe, N.D., Kim, M., Zhou, B., Cao, Y., Ritterhoff, J., Gu, H., et al. (2017). Defective branched-chain amino acid catabolism disrupts glucose metabolism and sensitizes the heart to ischemia-reperfusion injury. Cell Metab. *25*, 374–385. https://doi.org/10.1016/j.cmet.2016.11.005.
30. Jaimes, R., 3rd, Kuzmiak-Glancy, S., Brooks, D.M., Swift, L.M., Posnack, N.G., and Kay, M.W. (2016). Functional response of the isolated, perfused normoxic heart to pyruvate dehydrogenase activation by dichloroacetate and pyruvate. Pflugers Arch. *468*, 131–142. https://doi.org/10.1007/s00424-015-1717-1.
31. Kato, T., Niizuma, S., Inuzuka, Y., Kawashima, T., Okuda, J., Tamaki, Y., Iwanaga, Y., Narazaki, M., Matsuda, T., Soga, T., et al. (2010). Analysis of metabolic remodeling in compensated left ventricular hypertrophy and heart failure. Circ. Heart Fail. *3*, 420–430. https://doi.org/10.1161/CIRCHEARTFAILURE.109.888479.
32. Niewisch, M.R., Kuçi, Z., Wolburg, H., Sautter, M., Krampen, L., Deubzer, B., Handgretinger, R., and Bruchelt, G. (2012). Influence of dichloroacetate (dca) on lactate production and oxygen consumption in neuroblastoma cells: is dca a suitable drug for neuroblastoma therapy? Cell. Physiol. Biochem. *29*, 373–380. https://doi.org/10.1159/000338492.
33. Taniguchi, M., Wilson, C., Hunter, C.A., Pehowich, D.J., Clanachan, A.S., and Lopaschuk, G.D. (2001). Dichloroacetate improves cardiac efficiency after ischemia

independent of changes in mitochondrial proton leak. Am. J. Physiol. Heart Circ. Physiol. *280*, H1762–H1769. https://doi.org/10.1152/ajpheart.2001.280.4.H1762.

34. Ussher, J.R., Wang, W., Gandhi, M., Keung, W., Samokhvalov, V., Oka, T., Wagg, C.S., Jaswal, J.S., Harris, R.A., Clanachan, A.S., et al. (2012). Stimulation of glucose oxidation protects against acute myocardial infarction and reperfusion injury. Cardiovasc. Res. *94*, 359–369. https://doi.org/10.1093/cvr/cvs129.
35. Whelan, S.A., Dias, W.B., Thiruneelakantapillai, L., Lane, M.D., and Hart, G.W. (2010). Regulation of insulin receptor substrate 1 (irs-1)/akt kinase-mediated insulin signaling by o-linked beta-n-acetylglucosamine in 3t3-l1 adipocytes. J. Biol. Chem. *285*, 5204–5211. https://doi.org/10.1074/jbc.M109.077818.
36. Li, J., Chang, W.T., Qin, G., Wojcik, K.R., Li, C.Q., Hsu, C.W., Han, M., Zhu, X., Vanden Hoek, T.L., and Shao, Z.H. (2022). Baicalein preconditioning cardioprotection involves pro-oxidant signaling and activation of pyruvate dehydrogenase. Am. J. Chin. Med. *50*, 1255–1267. https://doi.org/10.1142/S0192415X22500513.
37. Chang, W.T., Li, J., Vanden Hoek, M.S., Zhu, X., Li, C.Q., Huang, H.H., Hsu, C.W., Zhong, Q., Li, J., Chen, S.J., et al. (2013). Baicalein preconditioning protects cardiomyocytes from ischemia-reperfusion injury via mitochondrial oxidant signaling. Am. J. Chin. Med. *41*, 315–331. https://doi.org/10.1142/S0192415X13500237.
38. Cannon, M.V., Silljé, H.H.W., Sijbesma, J.W.A., Khan, M.A.F., Steffensen, K.R., van Gilst, W.H., and de Boer, R.A. (2016). Lxralpha improves myocardial glucose tolerance and reduces cardiac hypertrophy in a mouse model of obesity-induced type 2 diabetes. Diabetologia *59*, 634–643. https://doi.org/10.1007/s00125-015-3827-x.
39. Hopf, A.E., Andresen, C., Kötter, S., Isić, M., Ulrich, K., Sahin, S., Bongardt, S., Röll, W., Drove, F., Scheerer, N., et al. (2018). Diabetes-induced cardiomyocyte passive stiffening is caused by impaired insulin-dependent titin modification and can be modulated by neuregulin-1. Circ. Res. *123*, 342–355. https://doi.org/10.1161/CIRCRESAHA.117.312166.
40. Huang, B., Wu, P., Popov, K.M., and Harris, R.A. (2003). Starvation and diabetes reduce the amount of pyruvate dehydrogenase phosphatase in rat heart and kidney. Diabetes *52*, 1371–1376. https://doi.org/10.2337/diabetes.52.6.1371.
41. Kuo, T.H., Giacomelli, F., Wiener, J., and Lapanowski-Netzel, K. (1985). Pyruvate dehydrogenase activity in cardiac mitochondria from genetically diabetic mice. Diabetes *34*, 1075–1081. https://doi.org/10.2337/diab.34.11.1075.
42. Belke, D.D., Larsen, T.S., Gibbs, E.M., and Severson, D.L. (2000). Altered metabolism causes cardiac dysfunction in perfused hearts from diabetic (db/db) mice. Am. J. Physiol. Endocrinol. Metab. *279*, E1104–E1113. https://doi.org/10.1152/ajpendo.2000.279.5.E1104.
43. Vadvalkar, S.S., Baily, C.N., Matsuzaki, S., West, M., Tesiram, Y.A., and Humphries, K.M. (2013). Metabolic inflexibility and protein lysine acetylation in heart mitochondria of a chronic model of type 1 diabetes. Biochem. J. *449*, 253–261. https://doi.org/10.1042/BJ20121038.
44. Militante, J.D., Lombardini, J.B., and Schaffer, S.W. (2000). The role of taurine in the pathogenesis of the cardiomyopathy of insulin-dependent diabetes mellitus. Cardiovasc. Res. *46*, 393–402. https://doi.org/10.1016/s0008-6363(00)00025-0.
45. Le Page, L.M., Rider, O.J., Lewis, A.J., Ball, V., Clarke, K., Johansson, E., Carr, C.A., Heather, L.C., and Tyler, D.J. (2015). Increasing pyruvate dehydrogenase flux as a treatment for diabetic cardiomyopathy: a combined 13c hyperpolarized magnetic resonance and echocardiography study. Diabetes *64*, 2735–2743. https://doi.org/10.2337/db14-1560.
46. Wu, C.Y., Satapati, S., Gui, W., Wynn, R.M., Sharma, G., Lou, M., Qi, X., Burgess, S.C., Malloy, C., Khemtong, C., et al. (2018). A novel inhibitor of pyruvate dehydrogenase kinase stimulates myocardial carbohydrate oxidation in diet-induced obesity. J. Biol. Chem. *293*, 9604–9613. https://doi.org/10.1074/jbc.RA118.002838.
47. Gopal, K., Al Batran, R., Altamimi, T.R., Greenwell, A.A., Saed, C.T., Tabatabaei Dakhili, S.A., Dimaano, M.T.E., Zhang, Y., Eaton, F., Sutendra, G., and Ussher, J.R. (2021). Foxo1 inhibition alleviates type 2 diabetes-related diastolic dysfunction by increasing myocardial pyruvate dehydrogenase activity. Cell Rep. *35*, 108935. https://doi.org/10.1016/j.celrep.2021.108935.
48. Gopal, K., Saleme, B., Al Batran, R., Aburasayn, H., Eshreif, A., Ho, K.L., Ma, W.K., Almutairi, M., Eaton, F., Gandhi, M., et al. (2017). Foxo1 regulates myocardial glucose oxidation rates via transcriptional control of pyruvate dehydrogenase kinase 4 expression. Am. J. Physiol. Heart Circ. Physiol. *313*, H479–H490. https://doi.org/10.1152/ajpheart.00191.2017.
49. Heather, L.C., Gopal, K., Srnic, N., and Ussher, J.R. (2024). Redefining Diabetic Cardiomyopathy: Perturbations in Substrate Metabolism at the Heart of Its Pathology. Diabetes *73*, 659–670. https://doi.org/10.2337/dbi23-0019.
50. Guo, H., Wu, H., and Li, Z. (2023). The Pathogenesis of Diabetes. Int. J. Mol. Sci. *24*, 6978. https://doi.org/10.3390/ijms24086978.
51. Wu, C.Y., Tso, S.C., Chuang, J.L., Gui, W.J., Lou, M., Sharma, G., Khemtong, C., Qi, X., Wynn, R.M., and Chuang, D.T. (2018). Targeting hepatic pyruvate dehydrogenase kinases restores insulin signaling and mitigates chrebp-mediated lipogenesis in diet-induced obese mice. Mol. Metab. *12*, 12–24. https://doi.org/10.1016/j.molmet.2018.03.014.
52. Huang, B., Wu, P., Bowker-Kinley, M.M., and Harris, R.A. (2002). Regulation of pyruvate dehydrogenase kinase expression by peroxisome proliferator-activated receptor-alpha ligands, glucocorticoids, and insulin. Diabetes *51*, 276–283. https://doi.org/10.2337/diabetes.51.2.276.
53. Abbot, E.L., Mccormack, J.G., Reynet, C., Hassall, D.G., Buchan, K.W., and Yeaman, S.J. (2005). Diverging regulation of pyruvate dehydrogenase kinase isoform gene expression in cultured human muscle cells. FEBS J. *272*, 3004–3014. https://doi.org/10.1111/j.1742-4658.2005.04713.x.
54. Houten, S.M., Chegary, M., Te Brinke, H., Wijnen, W.J., Glatz, J.F.C., Luiken, J.J.F.P., Wijburg, F.A., and Wanders, R.J.A. (2009). Pyruvate dehydrogenase kinase 4 expression is synergistically induced by amp-activated protein kinase and fatty acids. Cell. Mol. Life Sci. *66*, 1283–1294. https://doi.org/10.1007/s00018-009-9066-x.
55. Hu, Y., Belke, D., Suarez, J., Swanson, E., Clark, R., Hoshijima, M., and Dillmann, W.H. (2005). Adenovirus-mediated overexpression of o-glcnacase improves contractile function in the diabetic heart. Circ. Res. *96*, 1006–1013. https://doi.org/10.1161/01.RES.0000165478.06813.58.
56. Hubert, H.B., Feinleib, M., Mcnamara, P.M., and Castelli, W.P. (1983). Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the framingham heart study. Circulation *67*, 968–977. https://doi.org/10.1161/01.cir.67.5.968.
57. Ren, J., Wu, N.N., Wang, S., Sowers, J.R., and Zhang, Y. (2021). Obesity cardiomyopathy: evidence, mechanisms, and therapeutic implications. Physiol. Rev. *101*, 1745–1807. https://doi.org/10.1152/physrev.00030.2020.
58. Lopaschuk, G.D., Ussher, J.R., Folmes, C.D.L., Jaswal, J.S., and Stanley, W.C. (2010). Myocardial fatty acid metabolism in health and disease. Physiol. Rev. *90*, 207–258. https://doi.org/10.1152/physrev.00015.2009.
59. Caterson, I.D., Kerbey, A.L., Cooney, G.J., Frankland, R., Denyer, G.S., Nicks, J., and Williams, P.F. (1988). Inactivation of pyruvate dehydrogenase complex in heart muscle mitochondria of gold-thioglucose-induced obese mice is not due to a stable increase in activity of pyruvate dehydrogenase kinase. Biochem. J. *253*, 291–294. https://doi.org/10.1042/bj2530291.
60. Kerbey, A.L., Caterson, I.D., Williams, P.F., and Turtle, J.R. (1984). Proportion of active dephosphorylated pyruvate dehydrogenase complex in heart and isolated heart mitochondria is decreased in obese hyperinsulinaemic mice. Biochem. J. *217*, 117–121. https://doi.org/10.1042/bj2170117.
61. Zhang, Y., Zhao, Z., Ke, B., Wan, L., Wang, H., and Ye, J. (2016). Induction of posttranslational modifications of mitochondrial proteins by atp contributes to negative regulation of mitochondrial function. PLoS One *11*, e0150454. https://doi.org/10.1371/journal.pone.0150454.
62. Lundsgaard, A.M., Sjøberg, K.A., Høeg, L.D., Jeppesen, J., Jordy, A.B., Serup, A.K., Fritzen, A.M., Pilegaard, H., Myrmel, L.S., Madsen, L., et al. (2017). Opposite regulation of insulin sensitivity by dietary lipid versus carbohydrate excess. Diabetes *66*, 2583–2595. https://doi.org/10.2337/db17-0046.
63. Holness, M.J., and Sugden, M.C. (2003). Regulation of pyruvate dehydrogenase complex activity by reversible phosphorylation. Biochem. Soc. Trans. *31*, 1143–1151. https://doi.org/10.1042/bst0311143.
64. Elnwasany, A., Ewida, H.A., Szweda, P.A., and Szweda, L.I. (2023). Inhibition of Pyruvate Dehydrogenase in the Heart as an Initiating Event in the Development of Diabetic Cardiomyopathy. Antioxidants (Basel) *12*, 756. https://doi.org/10.3390/antiox12030756.
65. Thapa, D., Bugga, P., Mushala, B.A.S., Manning, J.R., Stoner, M.W., Mcmahon, B., Zeng, X., Cantrell, P.S., Yates, N., Xie, B., et al. (2022). Gcn5l1 impairs diastolic

function in mice exposed to a high fat diet by restricting cardiac pyruvate oxidation. Physiol. Rep. *10*, e15415. https://doi.org/10.14814/phy2.15415.

66. Tong, M., Saito, T., Zhai, P., Oka, S.I., Mizushima, W., Nakamura, M., Ikeda, S., Shirakabe, A., and Sadoshima, J. (2021). Alternative mitophagy protects the heart against obesity-associated cardiomyopathy. Circ. Res. *129*, 1105–1121. https://doi.org/10.1161/CIRCRESAHA.121.319377.
67. Gopal, K., Almutairi, M., Al Batran, R., Eaton, F., Gandhi, M., and Ussher, J.R. (2018). Cardiac-specific deletion of pyruvate dehydrogenase impairs glucose oxidation rates and induces diastolic dysfunction. Front. Cardiovasc. Med. *5*, 17. https://doi.org/10.3389/fcvm.2018.00017.
68. McMurray, J.J.V., and Pfeffer, M.A. (2005). Heart failure. Lancet *365*, 1877–1889. https://doi.org/10.1016/S0140-6736(05)66621-4.
69. Tanai, E., and Frantz, S. (2015). Pathophysiology of heart failure. Compr. Physiol. *6*, 187–214. https://doi.org/10.1002/cphy.c140055.
70. Bøgh, N., Hansen, E., Omann, C., Lindhardt, J., Nielsen, P.M., Stephenson, R.S., Laustsen, C., Hjortdal, V.E., and Agger, P. (2020). Increasing carbohydrate oxidation improves contractile reserves and prevents hypertrophy in porcine right heart failure. Sci. Rep. *10*, 8158. https://doi.org/10.1038/s41598-020-65098-7.
71. Schroeder, M.A., Lau, A.Z., Chen, A.P., Gu, Y., Nagendran, J., Barry, J., Hu, X., Dyck, J.R.B., Tyler, D.J., Clarke, K., et al. (2013). Hyperpolarized (13)c magnetic resonance reveals early- and late-onset changes to in vivo pyruvate metabolism in the failing heart. Eur. J. Heart Fail. *15*, 130–140. https://doi.org/10.1093/eurjhf/hfs192.
72. Sheeran, F.L., Angerosa, J., Liaw, N.Y., Cheung, M.M., and Pepe, S. (2019). Adaptations in protein expression and regulated activity of pyruvate dehydrogenase multienzyme complex in human systolic heart failure. Oxid. Med. Cell. Longev. *2019*, 4532592. https://doi.org/10.1155/2019/4532592.
73. Fukushima, A., and Lopaschuk, G.D. (2016). Acetylation control of cardiac fatty acid beta-oxidation and energy metabolism in obesity, diabetes, and heart failure. Biochim. Biophys. Acta *1862*, 2211–2220. https://doi.org/10.1016/j.bbadis.2016.07.020.
74. Sigutova, R., Evin, L., Stejskal, D., Ploticova, V., and Svagera, Z. (2022). Specific micrornas and heart failure: time for the next step toward application? Biomed. Pap-Olomouc *166*, 359–368. https://doi.org/10.5507/bp.2022.028.
75. Zhang, X., Ji, R., Liao, X., Castillero, E., Kennel, P.J., Brunjes, D.L., Franz, M., Möbius-Winkler, S., Drosatos, K., George, I., et al. (2018). Microrna-195 regulates metabolism in failing myocardium via alterations in sirtuin 3 expression and mitochondrial protein acetylation. Circulation *137*, 2052–2067. https://doi.org/10.1161/CIRCULATIONAHA.117.030486.
76. Liu, X., Zhang, Y., Deng, Y., Yang, L., Ou, W., Xie, M., Ding, L., Jiang, C., Yu, H., Li, Q., and Li, T. (2022). Mitochondrial protein hyperacetylation underpins heart failure with preserved ejection fraction in mice. J. Mol. Cell. Cardiol. *165*, 76–85. https://doi.org/10.1016/j.yjmcc.2021.12.015.
77. Ozden, O., Park, S.H., Wagner, B.A., Song, H.Y., Zhu, Y., Vassilopoulos, A., Jung, B., Buettner, G.R., and Gius, D. (2014). Sirt3 deacetylates and increases pyruvate dehydrogenase activity in cancer cells. Free Radic. Biol. Med. *76*, 163–172. https://doi.org/10.1016/j.freeradbiomed.2014.08.001.
78. Becherini, P., Caffa, I., Piacente, F., Damonte, P., Vellone, V.G., Passalacqua, M., Benzi, A., Bonfiglio, T., Reverberi, D., Khalifa, A., et al. (2021). Sirt6 enhances oxidative phosphorylation in breast cancer and promotes mammary tumorigenesis in mice. Cancer Metab. *9*, 6. https://doi.org/10.1186/s40170-021-00240-1.
79. Zhang, Y., Wen, P., Luo, J., Ding, H., Cao, H., He, W., Zen, K., Zhou, Y., Yang, J., and Jiang, L. (2021). Sirtuin 3 regulates mitochondrial protein acetylation and metabolism in tubular epithelial cells during renal fibrosis. Cell Death Dis. *12*, 847. https://doi.org/10.1038/s41419-021-04134-4.
80. Hilgers, R.H.P., Xing, D., Gong, K., Chen, Y.F., Chatham, J.C., and Oparil, S. (2012). Acute o-glcnacylation prevents inflammation-induced vascular dysfunction. Am. J. Physiol. Heart Circ. Physiol. *303*, H513–H522. https://doi.org/10.1152/ajpheart.01175.2011.
81. Dassanayaka, S., and Jones, S.P. (2014). O-glcnac and the cardiovascular system. Pharmacol. Ther. *142*, 62–71. https://doi.org/10.1016/j.pharmthera.2013.11.005.
82. Watson, L.J., Facundo, H.T., Ngoh, G.A., Ameen, M., Brainard, R.E., Lemma, K.M., Long, B.W., Prabhu, S.D., Xuan, Y.T., and Jones, S.P. (2010). O-linked beta-n-acetylglucosamine transferase is indispensable in the failing heart. Proc. Natl. Acad. Sci. USA *107*, 17797–17802. https://doi.org/10.1073/pnas.1001907107.
83. Lunde, I.G., Aronsen, J.M., Kvaløy, H., Qvigstad, E., Sjaastad, I., Tønnessen, T., Christensen, G., Grønning-Wang, L.M., and Carlson, C.R. (2012). Cardiac o-glcnac signaling is increased in hypertrophy and heart failure. Physiol. Genomics *44*, 162–172. https://doi.org/10.1152/physiolgenomics.00016.2011.
84. Jono, S., Shioi, A., Ikari, Y., and Nishizawa, Y. (2006). Vascular calcification in chronic kidney disease. J. Bone Miner. Metab. *24*, 176–181. https://doi.org/10.1007/s00774-005-0668-6.
85. Giachelli, C.M. (2009). The emerging role of phosphate in vascular calcification. Kidney Int. *75*, 890–897. https://doi.org/10.1038/ki.2008.644.
86. Lee, S.J., Jeong, J.Y., Oh, C.J., Park, S., Kim, J.Y., Kim, H.J., Doo Kim, N., Choi, Y.K., Do, J.Y., Go, Y., et al. (2015). Pyruvate dehydrogenase kinase 4 promotes vascular calcification via smad1/5/8 phosphorylation. Sci. Rep. *5*, 16577. https://doi.org/10.1038/srep16577.
87. Forteza, M.J., Berg, M., Edsfeldt, A., Sun, J., Baumgartner, R., Kareinen, I., Casagrande, F.B., Hedin, U., Zhang, S., Vuckovic, I., et al. (2023). Pyruvate dehydrogenase kinase regulates vascular inflammation in atherosclerosis and increases cardiovascular risk. Cardiovasc. Res. *119*, 1524–1536. https://doi.org/10.1093/cvr/cvad038.
88. Shen, M., Zheng, C., Chen, L., Li, M., Huang, X., He, M., Liu, C., Lin, H., Liao, W., Bin, J., et al. (2023). Lcz696 (sacubitril/valsartan) inhibits pulmonary hypertension induced right ventricular remodeling by targeting pyruvate dehydrogenase kinase 4. Biomed. Pharmacother. *162*, 114569. https://doi.org/10.1016/j.biopha.2023.114569.
89. Pillai, V.B., Samant, S., Sundaresan, N.R., Raghuraman, H., Kim, G., Bonner, M.Y., Arbiser, J.L., Walker, D.I., Jones, D.P., Gius, D., and Gupta, M.P. (2015). Honokiol blocks and reverses cardiac hypertrophy in mice by activating mitochondrial sirt3. Nat. Commun. *6*, 6656. https://doi.org/10.1038/ncomms7656.
90. Torres, C.R., and Hart, G.W. (1984). Topography and polypeptide distribution of terminal n-acetylglucosamine residues on the surfaces of intact lymphocytes. J. Biol. Chem. *259*, 3308–3317.
91. Dong, D.L., Xu, Z.S., Chevrier, M.R., Cotter, R.J., Cleveland, D.W., and Hart, G.W. (1993). Glycosylation of mammalian neurofilaments. Localization of multiple o-linked n-acetylglucosamine moieties on neurofilament polypeptides l and m. J. Biol. Chem. *268*, 16679–16687.
92. Schjoldager, K.T., Narimatsu, Y., Joshi, H.J., and Clausen, H. (2020). Global view of human protein glycosylation pathways and functions. Nat. Rev. Mol. Cell Biol. *21*, 729–749. https://doi.org/10.1038/s41580-020-00294-x.
93. Lambert, P.F., Kashanchi, F., Radonovich, M.F., Shiekhattar, R., and Brady, J.N. (1998). Phosphorylation of p53 serine 15 increases interaction with cbp. J. Biol. Chem. *273*, 33048–33053. https://doi.org/10.1074/jbc.273.49.33048.
94. Horita, H., Law, A., and Middleton, K. (2018). Utilizing optimized tools to investigate ptm crosstalk: identifying potential ptm crosstalk of acetylated mitochondrial proteins. Proteomes *6*, 24. https://doi.org/10.3390/proteomes6020024.
95. Li, X., Liu, J., Hu, H., Lu, S., Lu, Q., Quan, N., Rousselle, T., Patel, M.S., and Li, J. (2019). Dichloroacetate ameliorates cardiac dysfunction caused by ischemic insults through ampk signal pathway-not only shifts metabolism. Toxicol. Sci. *167*, 604–617. https://doi.org/10.1093/toxsci/kfy272.
96. Heather, L.C., Gopal, K., Srnic, N., and Ussher, J.R. (2024). Redefining Diabetic Cardiomyopathy: Perturbations in Substrate Metabolism at the Heart of Its Pathology. Diabetes *73*, 659–670. https://doi.org/10.2337/dbi23-0019.
97. Mjos, O.D., Miller, N.E., Riemersma, R.A., and Oliver, M.F. (1976). Effects of dichloroacetate on myocardial substrate extraction, epicardial st-segment elevation, and ventricular blood flow following coronary occlusion in dogs. Cardiovasc. Res. *10*, 427–436. https://doi.org/10.1093/cvr/10.4.427.
98. Haythorne, E., Lloyd, M., Walsby-Tickle, J., Tarasov, A.I., Sandbrink, J., Portillo, I., Exposito, R.T., Sachse, G., Cyranka, M., Rohm, M., et al. (2022). Altered glycolysis triggers impaired mitochondrial metabolism and mTORC1 activation in diabetic β-cells. Nat. Commun. *13*, 6754. https://doi.org/10.1038/s41467-022-34095-x.
99. Jeon, J.H., Thoudam, T., Choi, E.J., Kim, M.J., Harris, R.A., and Lee, I.K. (2021). Loss of metabolic flexibility as a result of overexpression of pyruvate dehydrogenase kinases in muscle, liver and the immune system: Therapeutic targets in metabolic

diseases. J. Diabetes Investig. *12*, 21–31. https://doi.org/10.1111/jdi.13345.

100. Jones, H.B., Reens, J., Johnson, E., Brocklehurst, S., and Slater, I. (2014). Myocardial steatosis and necrosis in atria and ventricles of rats given pyruvate dehydrogenase kinase inhibitors. Toxicol. Pathol. *42*, 1250–1266. https://doi.org/10.1177/0192623314530195.
101. Aizawa, K., Ikeda, A., Tomida, S., Hino, K., Sugita, Y., Hirose, T., Sunazuka, T., Kido, H., Yokoyama, S., and Nagai, R. (2023). A potent pdk4 inhibitor for treatment of heart failure with reduced ejection fraction. Cells *13*, 87. https://doi.org/10.3390/cells13010087.
102. Tiedge, M., Richter, T., and Lenzen, S. (2000). Importance of cysteine residues for the stability and catalytic activity of human pancreatic beta cell glucokinase. Arch. Biochem. Biophys. *375*, 251–260. https://doi.org/10.1006/abbi.1999.1666.
103. Macauley, M.S., Bubb, A.K., Martinez-Fleites, C., Davies, G.J., and Vocadlo, D.J. (2008). Elevation of global o-glcnac levels in 3t3-l1 adipocytes by selective inhibition of o-glcnacase does not induce insulin resistance. J. Biol. Chem. *283*, 34687–34695. https://doi.org/10.1074/jbc.M804525200.
104. Macauley, M.S., Whitworth, G.E., Debowski, A.W., Chin, D., and Vocadlo, D.J. (2005). O-glcnacase uses substrate-assisted catalysis: kinetic analysis and development of highly selective mechanism-inspired inhibitors. J. Biol. Chem. *280*, 25313–25322. https://doi.org/10.1074/jbc.M413819200.
105. Selnick, H.G., Hess, J.F., Tang, C., Liu, K., Schachter, J.B., Ballard, J.E., Marcus, J., Klein, D.J., Wang, X., Pearson, M., et al. (2019). Discovery of mk-8719, a potent o-glcnacase inhibitor as a potential treatment for tauopathies. J. Med. Chem. *62*, 10062–10097. https://doi.org/10.1021/acs.jmedchem.9b01090.
106. Shen, J., Lan, Y., Ji, Z., and Liu, H. (2024). Sirtuins in intervertebral disc degeneration: current understanding. Mol. Med. *30*, 44. https://doi.org/10.1186/s10020-024-00811-0.
107. Huang, X., Shu, H., Ren, C., and Zhu, J. (2022). Sirt3 improves bone regeneration and rescues diabetic fracture healing by regulating oxidative stress. Biochem. Biophys. Res. Commun. *604*, 109–115. https://doi.org/10.1016/j.bbrc.2022.03.001.
108. Han, Z., Zhong, L., Srivastava, A., and Stacpoole, P.W. (2008). Pyruvate dehydrogenase complex deficiency caused by ubiquitination and proteasome-mediated degradation of the e1 subunit. J. Biol. Chem. *283*, 237–243. https://doi.org/10.1074/jbc.M704748200.
109. Wang, H., Yang, L., Liu, M., and Luo, J. (2023). Protein post-translational modifications in the regulation of cancer hallmarks. Cancer Gene Ther. *30*, 529–547. https://doi.org/10.1038/s41417-022-00464-3.
110. Zhou, T., Cheng, X., He, Y., Xie, Y., Xu, F., Xu, Y., and Huang, W. (2022). Function and mechanism of histone beta-hydroxybutyrylation in health and disease. Front. Immunol. *13*, 981285. https://doi.org/10.3389/fimmu.2022.981285.
111. Fukushima, A., Alrob, O.A., Zhang, L., Wagg, C.S., Altamimi, T., Rawat, S., Rebeyka, I.M., Kantor, P.F., and Lopaschuk, G.D. (2016). Acetylation and succinylation contribute to maturational alterations in energy metabolism in the newborn heart. Am. J. Physiol. Heart Circ. Physiol. *311*, H347–H363. https://doi.org/10.1152/ajpheart.00900.2015.
112. Wu, L.F., Wang, D.P., Shen, J., Gao, L.J., Zhou, Y., Liu, Q.H., and Cao, J.M. (2022). Global profiling of protein lysine malonylation in mouse cardiac hypertrophy. J. Proteomics *266*, 104667. https://doi.org/10.1016/j.jprot.2022.104667.
113. Zhang, N., Zhang, Y., Xu, J., Wang, P., Wu, B., Lu, S., Lu, X., You, S., Huang, X., Li, M., et al. (2023). Alpha-myosin heavy chain lactylation maintains sarcomeric structure and function and alleviates the development of heart failure. Cell Res. *33*, 679–698. https://doi.org/10.1038/s41422-023-00844-w.
114. Ni, J., Zhang, H., Wang, X., Liu, Z., Nie, T., Li, L., Su, J., Zhu, Y., Ma, C., Huang, Y., et al. (2022). Rg3 regulates myocardial pyruvate metabolism via p300-mediated dihydrolipoamide dehydrogenase 2-hydroxyisobutyrylation in tac-induced cardiac hypertrophy. Cell Death Dis. *13*, 1073. https://doi.org/10.1038/s41419-022-05516-y.
115. Cai, X., Ng, C.P., Jones, O., Fung, T.S., Ryu, K.W., Li, D., and Thompson, C.B. (2023). Lactate activates the mitochondrial electron transport chain independently of its metabolism. Mol. Cell *83*, 3904–3920.e7. https://doi.org/10.1016/j.molcel.2023.09.034.
116. Xiao, H., Zhou, H., Zeng, G., Mao, Z., Zeng, J., and Gao, A. (2022). Sumoylation targeting mitophagy in cardiovascular diseases. J. Mol. Med. *100*, 1511–1538. https://doi.org/10.1007/s00109-022-02258-4.
117. Pieroni, S., Castelli, M., Piobbico, D., Ferracchiato, S., Scopetti, D., Di-Iacovo, N., Della-Fazia, M.A., and Servillo, G. (2022). The Four Homeostasis Knights: In Balance upon Post-Translational Modifications. Int. J. Mol. Sci. *23*, 14480. https://doi.org/10.3390/ijms232214480.
118. Piccinini, M., Mostert, M., Alberto, G., Ramondetti, C., Novi, R.F., Dalmasso, P., and Rinaudo, M.T. (2005). Down-regulation of pyruvate dehydrogenase phosphatase in obese subjects is a defect that signals insulin resistance. Obes. Res. *13*, 678–686. https://doi.org/10.1038/oby.2005.76.