NOVEL CONCEPTS IN CARDIAC ENERGY METABOLISM: FROM BIOLOGY TO DISEASE

EDITED BY: Thomas Pulinilkunnil, Petra Kienesberger and Jeevan Nagendran

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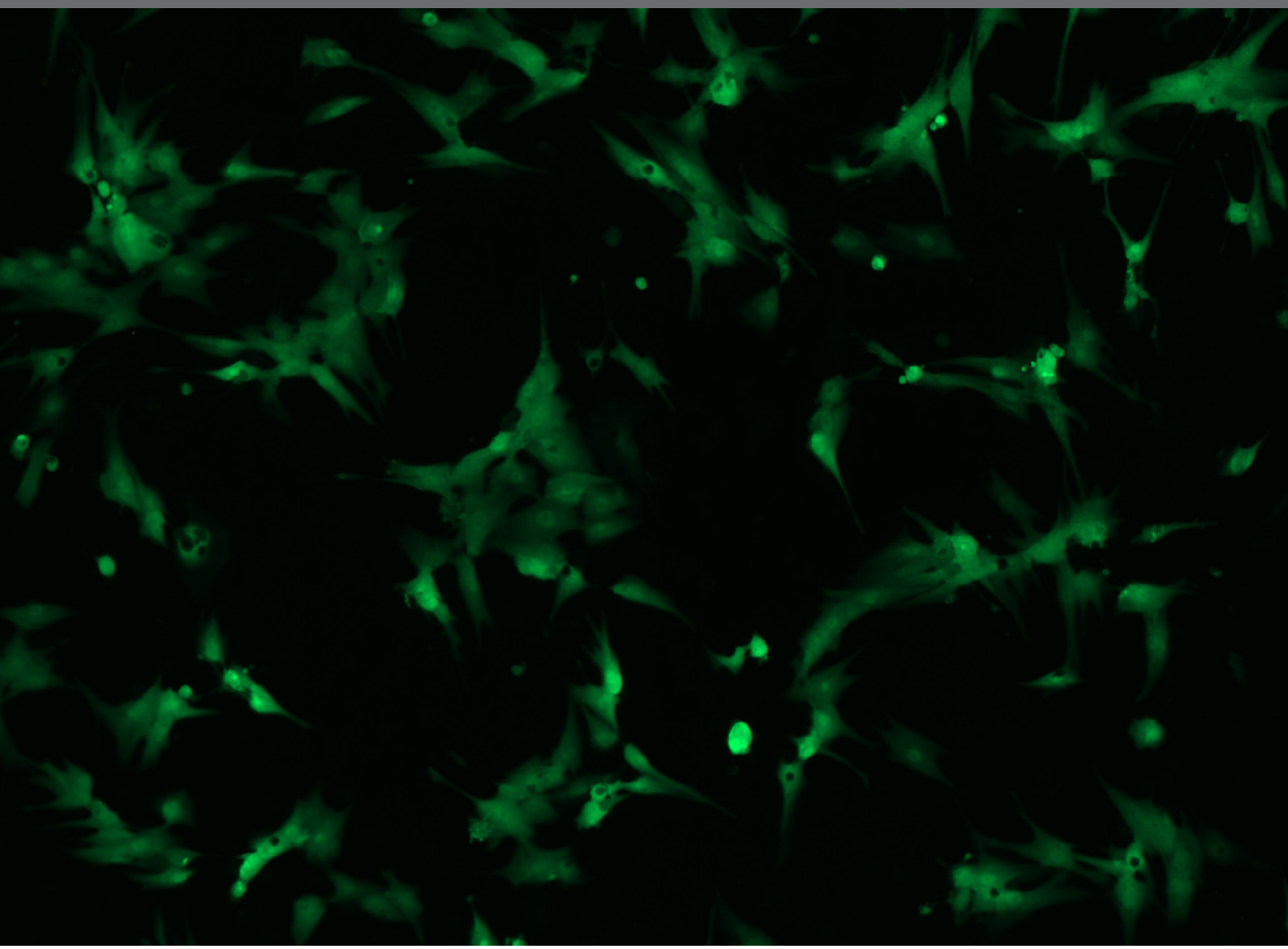

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NOVEL CONCEPTS IN CARDIAC ENERGY METABOLISM: FROM BIOLOGY TO DISEASE

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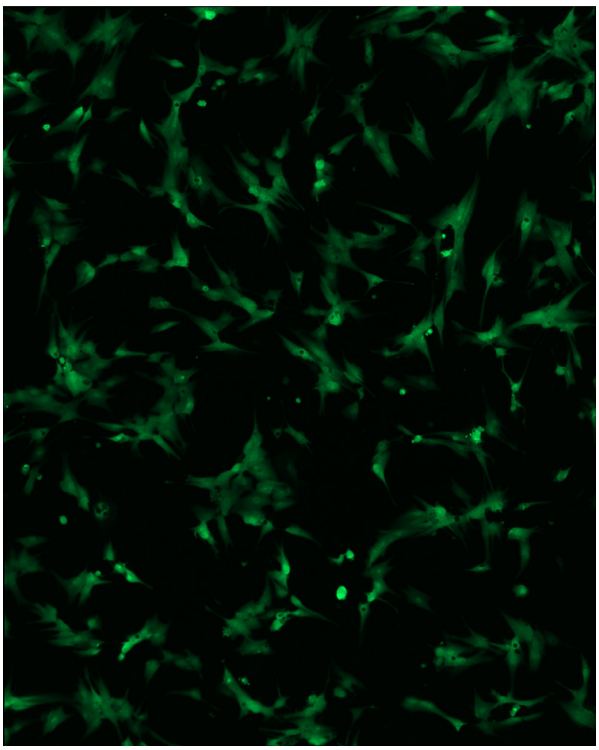

Image: Neonatal rat cardiomyocytes transduced with adenovirus encoding green fluorescent protein.
Courtesy of Pulinilkunnil and Kienesberger laboratory, Department of Biochemistry and Molecular Biology, Dalhousie University, Dalhousie Medicine New Brunswick.

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EDITORIAL
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Editorial: Novel Concepts in Cardiac Energy Metabolism: From Biology to Disease

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Keywords: energy metabolism, ATP, cardiomyocyte, heart failure, fatty acid

Editorial on the Research Topic

Novel Concepts in Cardiac Energy Metabolism: From Biology to Disease

Energy metabolism is a process that is central to cardiac health and disease. High ATP turnover in the myocardium is required to maintain contractile function. ATP generation within the mitochondria involves oxidative decarboxylation of fatty acids, pyruvate, and the Krebs cycle to generate reducing equivalents for the oxidative phosphorylation of ADP in the electron transport chain. In the healthy adult heart, the majority of ATP is generated through the oxidation of fatty acids, and a tight balance between the utilization of fatty acids and other energy substrates is maintained (1). In metabolic heart disease myocardial ATP synthesis rate and free energy of ATP hydrolysis are decreased while ATP concentration is preserved (2). Metabolic remodeling and the decline of cardiac ATP production precede structural remodeling of the stressed heart and result from progressive maladaptation in substrate use and mitochondrial biogenesis and function (3, 4). Disrupted energy flux within the myocyte is recognized as a hallmark of cardiac failure (5). Metabolic remodeling not only disrupts cardiac energetics but also induces changes in cellular processes such as growth, redox homeostasis, and autophagy (6). Maladaptive changes in nutrient uptake, oxidation, and storage can lead to reduced energetic efficiency, ATP starvation, and ultimately cardiac dysfunction. This Research Topic is dedicated to articles (1) highlighting novel mechanisms that influence myocardial energy metabolism, (2) illustrating the role of cardiac metabolic pathways in health and disease, and (3) exploring translational avenues to target cardiac metabolism for the treatment of cardio-metabolic disorders.

In this Research Topic, Gopal et al. used a mouse model with cardiomyocyte-specific deficiency of pyruvate dehydrogenase to show that impaired myocardial glucose oxidation is sufficient to hinder diastolic, but not systolic heart function. Since obesity and diabetes-induced cardiomyopathy is often associated with reduced glucose oxidation, this study suggests that impairment of glucose oxidation *per se* can drive the development of cardiomyopathy and diastolic dysfunction and that this change in cardiac energy metabolism contributes to diabetic cardiomyopathy. Novel mechanisms underlying metabolic inflexibility and impaired glucose metabolism in diabetic cardiomyopathy are also highlighted in the mini-review article by Renguet et al. The authors summarized and interpreted our current understanding of the role of protein acetylation induced by the metabolism of non-glucosidic substrates in the impairment of insulin-stimulated glucose uptake in the heart during cardio-metabolic disease. While reduced myocardial glucose oxidation is implicated in the development of cardiomyopathy related to diabetes and obesity, excessive glucose utilization has also been associated with cardiac hypertrophy. In this Research Topic, Papur et al. discuss the role

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of protein kinase D in the regulation of cardiac glucose uptake and hypertrophy and delineate how protein kinase D isoforms influence cardiac energy metabolism, morphology, function, and hypertrophy. The relationship between loss of metabolic flexibility, mitochondrial ATP production, and heart failure is described in a review article by Karwi et al. examining preclinical and clinical studies. This review focuses on the alterations in fatty acid oxidation, carbohydrate metabolism, and ketone body metabolism in the heart in the setting of heart failure and illustrates the interplay among transcriptional regulation, post-translational modifications, and cytosolic/mitochondrial signaling kinases in regulating cardiac energy metabolism. The potential of modulating cardiac metabolism to enhance the efficiency of substrate utilization and mitigate cardiac dysfunction is discussed. While maladaptation of cardiac energy metabolism occurs in disease, including obesity, diabetes, and hypertrophy, physiological changes in cardiac substrate utilization are observed during exercise. Kolwicz provides a detailed overview of changes in glucose, fatty acid, ketone body, and amino acid metabolism in the heart during chronic exercise and discusses the exercise-induced adaptation of cardiac metabolism as a potential therapy for cardiac diseases such as hypertrophy.

Aberrant cardiac lipid metabolism is a hallmark of cardiometabolic diseases including diabetic cardiomyopathy and atherosclerosis. Yang et al. outline how myocardial autophagy of lipids, i.e., lipophagy, impacts lipid homeostasis in the heart muscle and atherosclerotic plaque and offer insight into the therapeutic potential of targeting autophagy for cardiometabolic diseases. A review article by Lal et al. shines a new light on vascular endothelial growth factor B and highlights its potential role in protecting against diabetic cardiomyopathy and heart failure by modulating cardiac metabolism and promoting cell survival. Saleme and Sutendra focus in their opinion article on heart failure triggered by chemotherapy-induced cardiotoxicity. They compare the metabolic signatures of the tumor and failing heart and offer directions to protect the heart during chemotherapy. The role of chronic low-grade inflammation in cardiovascular disease is examined by Ye and Ghosh. The authors provide their opinion on the usefulness of omega-3-polyunsaturated fatty acids compared to non-steroidal anti-inflammatory drugs in the prevention of myocardial inflammation. Because cardiac energy substrate metabolism is implicated in cardiac health and disease advancing our understanding of the complexities in the cardiac metabolic network will rationalize the utility of metabolic therapies targeting cardiovascular disease.

AUTHOR'S NOTE

PK is a Heart and Stroke Foundation of Canada New Investigator and TP is a Diabetes Canada Scholar.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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ORIGINAL RESEARCH
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Cardiac-Specific Deletion of Pyruvate Dehydrogenase Impairs Glucose Oxidation Rates and Induces Diastolic Dysfunction

*Keshav Gopal [1,2,3], Malak Almutairi [1,2,3], Rami Al Batran [1,2,3], Farah Eaton [1,2,3], Manoj Gandhi [1,2,3] and John Reyes Ussher [1,2,3]**

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Obesity and type 2 diabetes (T2D) increase the risk for cardiomyopathy, which is the presence of ventricular dysfunction in the absence of underlying coronary artery disease and/or hypertension. As myocardial energy metabolism is altered during obesity/T2D (increased fatty acid oxidation and decreased glucose oxidation), we hypothesized that restricting myocardial glucose oxidation in lean mice devoid of the perturbed metabolic milieu observed in obesity/T2D would produce a cardiomyopathy phenotype, characterized via diastolic dysfunction. We tested our hypothesis via producing mice with a cardiac-specific gene knockout for pyruvate dehydrogenase (PDH, gene name *Pdha1*), the rate-limiting enzyme for glucose oxidation. Cardiac-specific *Pdha1* deficient (*Pdha1*$^{\text{Cardiac}-/-}$) mice were generated via crossing a tamoxifen-inducible Cre expressing mouse under the control of the alpha-myosin heavy chain (αMHC-MerCreMer) promoter with a floxed *Pdha1* mouse. Energy metabolism and cardiac function were assessed via isolated working heart perfusions and ultrasound echocardiography, respectively. Tamoxifen administration produced an ~85% reduction in PDH protein expression in *Pdha1*$^{\text{Cardiac}-/-}$ mice versus their control littermates, which resulted in a marked reduction in myocardial glucose oxidation and a corresponding increase in palmitate oxidation. This myocardial metabolism profile did not impair systolic function in *Pdha1*$^{\text{Cardiac}-/-}$ mice, which had comparable left ventricular ejection fractions and fractional shortenings as their αMHC-MerCreMer control littermates, but did produce diastolic dysfunction as seen via the reduced mitral E/A ratio. Therefore, it does appear that forced restriction of glucose oxidation in the hearts of *Pdha1*$^{\text{Cardiac}-/-}$ mice is sufficient to produce a cardiomyopathy-like phenotype, independent of the perturbed metabolic milieu observed in obesity and/or T2D.

Keywords: pyruvate dehydrogenase, glucose oxidation, diabetic cardiomyopathy, cardiac function, diastolic dysfunction

INTRODUCTION

The healthy heart is a metabolic omnivore that is dynamically flexible, constantly switching between carbohydrates (i.e., glucose) and fatty acids as its primary fuel source between repeated periods of refeeding and fasting, respectively (1, 2). In the setting of obesity and/or type 2 diabetes (T2D), the heart's metabolic flexibility dissipates, as the heart increases its reliance on fatty acids as the primary fuel source to meet its oxidative energy requirements (2, 3). For example, studies from Abel and colleagues have demonstrated that both leptin deficient *ob*/*ob* and leptin receptor deficient *db*/*db* mice exhibit robust increases in myocardial fatty acid oxidation rates, which is associated with a marked reduction in glucose oxidation rates (4). Moreover, Larsen and colleagues have observed identical findings in hearts from mice subjected to a sucrose-enriched diet to induce experimental obesity (5). Similarly, short-term high fat feeding of C57BL/6J mice for 2 weeks also produces significant increases in myocardial fatty acid oxidation rates and a corresponding decline in myocardial glucose oxidation rates (6). Such observations have been recapitulated in humans, as PET imaging studies by Peterson et al. have demonstrated a marked increase in fatty acid oxidation rates in the hearts of obese women, which positively correlated with overall insulin resistance (7).

These metabolic observations can be partly explained by the marked increase in circulating free fatty acid and triacylglycerol levels characteristic of obesity/T2D, which increases myocardial fatty acid delivery and subsequent fatty acid oxidation. An increase in myocardial fatty acid oxidation rates leads to a corresponding decrease in glucose oxidation rates through the "Randle Cycle" mechanism, by which fatty acids and glucose compete for oxidative acetyl CoA production (1, 8). In addition, it has also been demonstrated that insulin's ability to stimulate glucose oxidation is severely diminished in the hearts of animals or humans with obesity and/or T2D (3, 9). However, despite well documented observations regarding the myocardial metabolic phenotype in obesity/T2D, it remains enigmatic as to whether these metabolic perturbations are driving forces behind the pathology of the cardiomyopathy and diastolic dysfunction that characterizes patients with T2D.

Our objective was to determine whether a specific defect in myocardial glucose oxidation was sufficient to reproduce the cardiomyopathy phenotype observed in obesity/T2D, independent of the perturbed metabolic milieu associated with obesity/T2D. Such observations would support the notion that the myocardial metabolic perturbations observed in obesity/T2D are mechanistically involved in the pathology of diabetic cardiomyopathy. As pyruvate dehydrogenase (PDH, gene name *Pdha1*) is the rate-limiting enzyme of glucose oxidation (10), to address our aim we generated and characterized cardiac specific *Pdha1* (*Pdha1*$^{\text{Cardiac}-/-}$) mice, investigating potential changes in cardiac function and cardiac glucose/fatty acid oxidation.

METHODS

Animal Care

All animals received care according to the Canadian Council on Animal Care and all procedures were approved by the University of Alberta Health Sciences Animal Welfare Committee. C57BL/6J wild-type (WT), alpha-myosin heavy chain (αMHC)-MerCreMer (stock no. 005657) and *Pdha1*$^{\text{flox}}$ (stock no. 017443) mice were purchased from the Jackson Laboratory, USA. To generate *Pdha1*$^{\text{Cardiac}-/-}$ mice, αMHC-MerCreMer transgenic mice expressing tamoxifen-inducible Cre in cardiac myocytes were bred with *Pdha1*$^{\text{flox}}$ mice. Cre-induced inactivation of the *Pdha1* gene was carried out via 6 intraperitoneal (i.p.) injections of tamoxifen (50 mg/kg) spread over 8 days in male mice starting at 6–7 weeks of age (11, 12). All mice were allowed five weeks washout post-tamoxifen administration prior to experimentation.

Western Blotting

Frozen ventricular tissue (20 mg) was homogenized in buffer containing 50 mM Tris HCl (pH 8 at 4°C), 1 mM EDTA, 10% glycerol (wt/vol), 0.02% Brij-35 (wt/vol), 1 mM dithiothreitol, protease and phosphatase inhibitors (Sigma) to prepare myocardial protein extracts as previously described (12, 13). Protein concentration of homogenates was determined via Bradford protein assay kit (Bio-Rad). Samples were resolved via 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a 0.45 μm nitrocellulose membrane. Membranes were blocked with 10% fat free milk for 1 h and probed with either anti-PDH (Cell Signaling Technologies) or anti-hsp90 (BD Biosciences) antibodies in 5% fatty acid free bovine serum albumin overnight at 4°C. Immunoblots were visualized with the enhanced chemiluminescence western blot detection kit (Perkin Elmer), visualized with a geldoc imager (Bio-Rad) and quantified with ImageJ software.

Determination of Plasma Triacylglycerol (TAG), Insulin, and Free Fatty Acid (FFA) Levels

All animals had their food removed for a 2 h period, following which whole-blood was collected from mice via tail bleed. Tail whole-blood was centrifuged at 2,000 × *g* at 4°C for 10 min and the supernatant (plasma) was collected. Plasma TAG (Wako Pure Chemical Industries), insulin levels (ALPCO Diagnostics) and FFA levels (Roche) were quantified using commercially available enzymatic assay kits as per manufacturer instructions.

Ultrasound Echocardiography

Transthoracic echocardiography ultrasound was performed in 3% isoflurane-anesthetized mice (30–40 MHz; Vevo3100, VisualSonics,Toronto, Canada) to assess left ventricular systolic and diastolic function as previously described (14).

Isolated Working Heart Perfusions and Assessment of Energy Metabolism

Two weeks post-ultrasound echocardiography analysis, all mice were anaesthetized with i.p. sodium pentobarbital (60 mg/kg), following which the hearts were subsequently excised and immersed in ice-cold Krebs-Henseleit bicarbonate solution. The aorta was then cannulated and equilibrated in the Langendorff mode, following which the left atria was

subsequently cannulated and hearts were switched to and perfused in the working mode as previously described (13, 15). Oxygenated Krebs-Henseleit solution consisting of 1.2 mM [9,10-^{3}H]palmitate bound to 3% fatty acid free bovine serum albumin and 11 mM [U-^{14}C]glucose was delivered to the left atrium at a preload pressure of 15 mmHg, while perfusate was ejected from hearts into the aortic outflow line against a hydrostatic afterload pressure of 50 mmHg. Hearts were perfused aerobically for 40 min in the absence of insulin, and glucose and palmitate oxidation were assessed via measuring $^{3}H_2O$ and $^{14}CO_2$ production as previously described (13, 15). At the end of perfusion, hearts were immediately frozen in liquid N_2 with

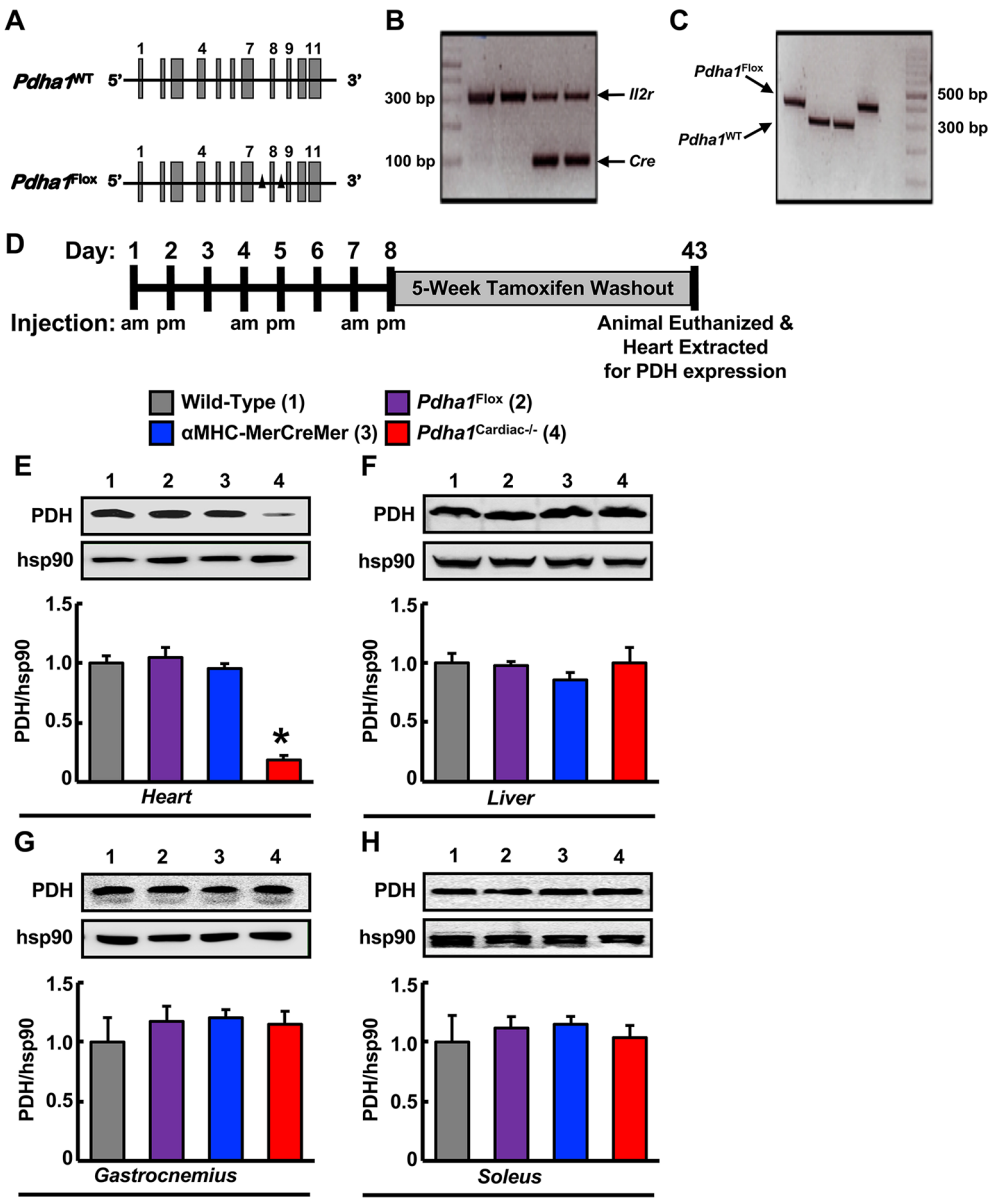

FIGURE 1 | *Generation of cardiac-specific Pdha1-deficient (Pdha1*$^{Cardiac-/-}$*) mice*. **(A)** Depiction of the *Pdha1* gene (11 exons) in *Pdha1*^{WT} mice and *Pdha1*^{Flox} mice. Black triangles depict the loxP sites flanking exon 8 of the *Pdha1* gene in the *Pdha1*^{Flox} mice. **(B/C)** Cardiac-specific *Pdha1*-deficient mouse was generated by breeding αMHC-MerCreMer transgenic mice expressing a tamoxifen-inducible Cre in cardiac myocytes with *Pdha1*^{flox} mice. PCR genotyping of mouse offspring showing presence of *Cre* **(B)** as amplification of a 100 base pair fragment, or presence of a floxed *Pdha1* or wild-type *Pdha1* **(C)** gene as amplification of a 380 base pair or 303 base pair fragment, respectively. Amplification of the *Il2r* was utilized as a positive control for PCR amplification. **(D)** Schematic model for induction of cardiac-specific *Pdha1* knockout by 6-injections of tamoxifen indicating whether mice were injected in the morning (am) or late afternoon (pm) of the day. Mice were allowed a 5 week washout period following the last tamoxifen injection prior to experimentation. **(E-H)** Western blot analysis of protein lysates of heart **(E)**, liver **(F)**, gastrocnemius muscle **(G)**, and soleus muscle **(H)** comparing PDH expression in *Pdha1*$^{Cardiac-/-}$ mice (4) versus their various control littermates [Wild-type mice (1), *Pdha1*^{Flox} mice (2), and αMHC-MerCreMer mice (3)] (n = 4–7). Values represent mean ± SEM. Differences were determined using 1-way ANOVA followed by a Bonferroni post-hoc analysis. *$P < 0.05$, significantly different versus all other genotypes.

Wollenberger tongs pre-cooled to the temperature of liquid N_2, and stored at −80°C.

Statistical Analysis

All values are presented as means ± standard error of the mean (SEM). Significant differences were determined by the use of an unpaired, two-tailed Student's *t*-test, or a one-way ANOVA followed by a Bonferroni post-hoc analysis. Differences were considered significant when $P < 0.05$.

RESULTS

Generation of Cardiac-Specific *Pdha1* Deficient Mice

In order to generate *Pdha1*$^{Cardiac-/-}$ mice, floxed *Pdha1* (*Pdha1*^{Flox}) mice possessing loxP sites flanking exon 8 of the E1 subunit of *Pdha1* (**Figure 1A**) were crossed to mice expressing Cre recombinase driven by the cardiac-specific αMHC (αMHC-MerCreMer) promoter (**Figure 1B/C**). Cre recombinase expression in cardiac myocytes from αMHC-MerCreMer mice is flanked by mutated estrogen receptor ligand-binding domains bound to heat shock protein 90 (hsp90), which can be activated via the selective estrogen receptor antagonist, tamoxifen, but remain insensitive to endogenous estrogen. Thus, we treated WT, *Pdha1*^{Flox}, αMHC-MerCreMer, and *Pdha1*$^{Cardiac-/-}$male mice with tamoxifen (50 mg/kg, 6 i.p. injections spread over 8 days) to induce Cre recombinase-mediated excision of loxP flanked DNA regions (**Figure 1D**). 5 weeks post-tamoxifen treatment, hearts were extracted from all mice with free access to food in the middle of the light cycle for assessment of myocardial PDH expression. As anticipated, we observed an ~85% gene knockdown of *Pdha1* only in myocardial protein extracts from *Pdha1*$^{Cardiac-/-}$ mice, but not from WT, *Pdha1*^{Flox}, or αMHC-MerCreMer mice (**Figure 1E**), similar to what we've seen in our previous studies using this approach (11, 12, 16). Importantly, PDH protein expression was similar in the skeletal muscle (gastrocnemius and soleus) and livers of *Pdha1*$^{Cardiac-/-}$ mice and their various control littermates (**Figure 1F–H**), indicating we had successfully generated a cardiac-specific PDH deficient mouse model. Because cardiac-specific Cre recombinase expression controlled via the αMHC promoter produces a transient cardiomyopathy (17), all subsequent studies were performed using the αMHC-MerCreMer mouse as the control littermate. Assessment of body weight and baseline plasma parameters demonstrated no overt phenotype in *Pdha1*$^{Cardiac-/-}$ mice, as their body weight and circulating glucose, insulin, triacylglycerol, and FFA levels were comparable to their αMHC-MerCreMer control littermates (**Figure 2A–E**).

Pdha1$^{Cardiac-/-}$ Mice Exhibit Normal Systolic Function with Signs of Diastolic Dysfunction

We next performed ultrasound echocardiography studies in αMHC-MerCreMer and *Pdha1*$^{Cardiac-/-}$ mice to assess *in vivo* cardiac function. Systolic function appeared normal in *Pdha1*$^{Cardiac-/-}$ mice, since left ventricular (LV) ejection fraction (LVEF), fractional shortening, and cardiac output were similar in *Pdha1*$^{Cardiac-/-}$ mice and their αMHC-MerCreMer control littermates (**Figure 3A–C**), indicating that impaired myocardial glucose oxidation rates do not adversely affect LV systolic function. Conversely, the mitral E/A ratio was decreased by ~28% in *Pdha1*$^{Cardiac-/-}$ mice (**Figure 3D**), suggestive of diastolic dysfunction. Regarding cardiac wall dimensions, *Pdha1*$^{Cardiac-/-}$ mice demonstrated no differences in LV posterior and anterior wall thickness in comparison to their αMHC-MerCreMer control littermates (**Figure 3E–H**).

Pdha1$^{Cardiac-/-}$ Mice Exhibit a Marked Reduction in Glucose Oxidation Rates and a Subsequent Increase in Fatty Acid Oxidation Rates

Two weeks following the assessment of *in vivo* cardiac function via ultrasound echocardiography, all mice were subjected to isolated working heart perfusion studies for the assessment of cardiac glucose and fatty acid oxidation rates during hrs 4 through 8 of their light cycle. Consistent with a cardiac-specific

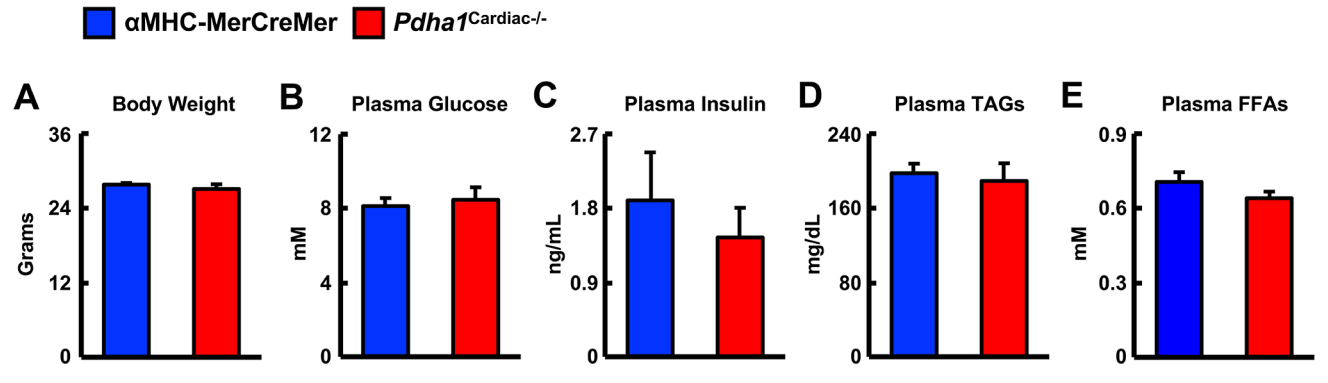

FIGURE 2 | *Baseline parameters in Pdha1*$^{Cardiac-/-}$ *mice.* **(A)** Body weights, **(B)** plasma glucose levels, **(C)** plasma insulin levels, **(D)** plasma TAG levels, and **(E)** plasma FFA levels in *Pdha1*$^{Cardiac-/-}$ mice and their αMHC-MerCreMer control littermates at 5 weeks post-tamoxifen administration ($n = 4$). Values represent mean ± SEM.

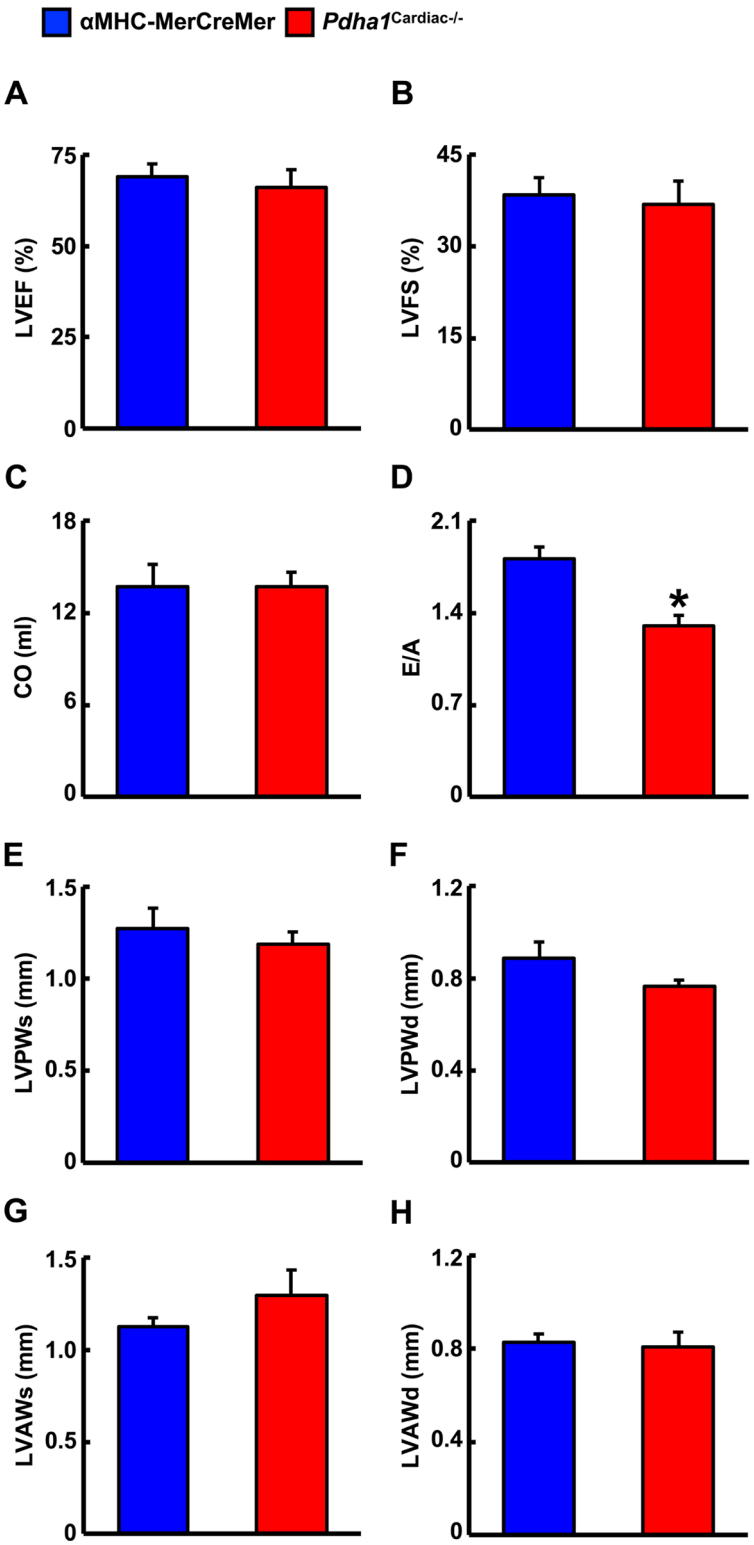

FIGURE 3 | *Pdha1*$^{Cardiac-/-}$ *mice exhibit normal systolic function with evidence of diastolic dysfunction*. Ultrasound echocardiography was performed in *Pdha1*$^{Cardiac-/-}$ mice and their αMHC-MerCreMer control littermates to assess indices of systolic function; **(A)** left ventricular ejection fraction (LVEF), **(B)** left ventricular fractional shortening (LVFS), and **(C)** cardiac output (CO). **(D)** Diastolic function was assessed via measurement of the mitral E/A ratio. Left ventricular wall dimensions were assessed via **(E)** measurement of posterior wall thickness during systole (LVPWs), **(F)**, posterior wall thickness during diastole (LVPWd), **(G)**, anterior wall thickness during systole (LVAWs), and **(H)** anterior wall thickness during diastole (LVAWd). Values represent means ± SEM (n = 6–7). Differences were determined using an unpaired, two-tailed Student's t-test. *$P < 0.05$.

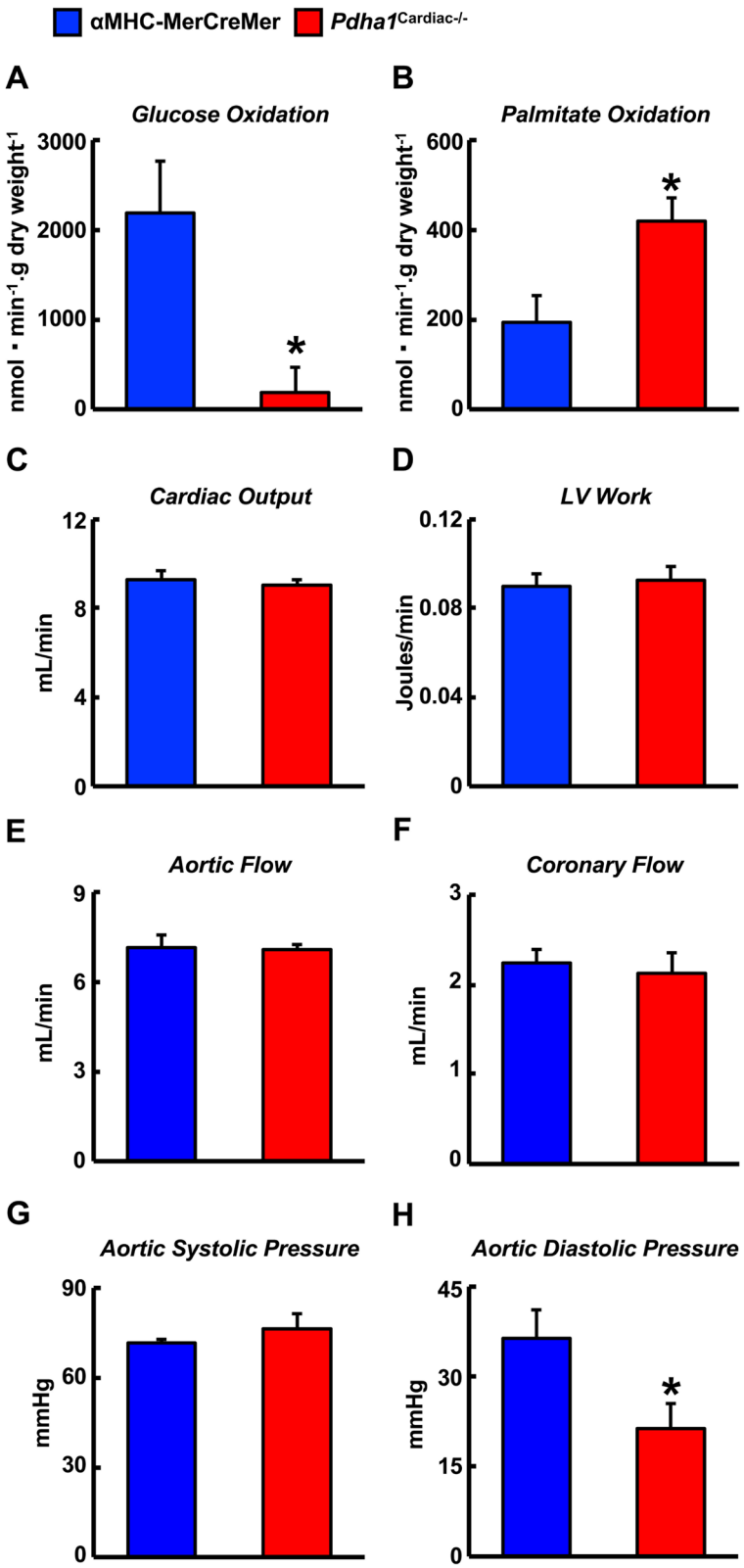

FIGURE 4 | *Altered myocardial energy metabolism and normal ex vivo cardiac function in Pdha1*$^{Cardiac-/-}$ *mice*. **(A)** Glucose oxidation rates, **(B)** palmitate oxidation rates, **(C)** cardiac output, **(D)** left ventricular (LV) work, **(E)** aortic flow, **(F)** coronary flow, **(G)** aortic systolic pressure, and **(H)** aortic diastolic pressure during isolated aerobic working heart perfusions from *Pdha1*$^{Cardiac-/-}$ and their αMHC-MerCreMer control littermates (n = 4, 5). Values represent means ± SEM. Differences were determined using an unpaired, two-tailed Student's t-test. *$P < 0.05$.

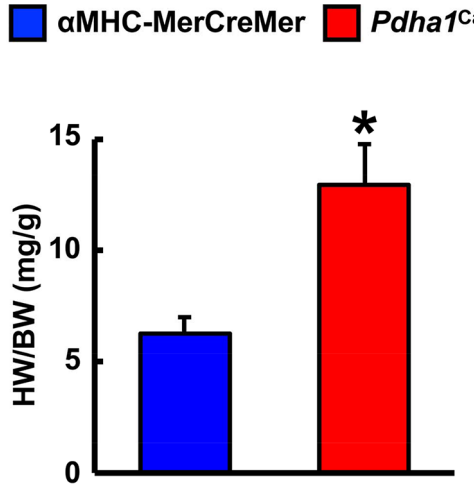

FIGURE 5 | *Pdha1*$^{Cardiac-/-}$ *mice exhibit cardiac hypertrophy*. Heart weight was normalized to body weight (HW/BW) in *Pdha1*$^{Cardiac-/-}$ mice and their αMHC-MerCreMer control littermates following completion of 40 min aerobic perfusion. Values represent means ± SEM (n = 5). Differences were determined using an unpaired, two-tailed Student's t-test. *P < 0.05.

deficiency of PDH, *Pdha1*$^{Cardiac-/-}$ mice exhibited a marked reduction in glucose oxidation rates following aerobic perfusion in the isolated working mode (**Figure 4A**). In contrast, fatty acid oxidation rates were increased in working hearts from *Pdha1*$^{Cardiac-/-}$ mice (**Figure 4B**), likely due to a "Randle Cycle" effect (1, 8). Importantly, the altered metabolic profile in hearts from *Pdha1*$^{Cardiac-/-}$ mice did not adversely affect their *ex vivo* cardiac function, as both cardiac output and left ventricular work were similar in *Pdha1*$^{Cardiac-/-}$ mice and their αMHC-MerCreMer control littermates (**Figure 4C/D**). Moreover, there were no differences in aortic and coronary flows, or aortic systolic pressure during isolated working heart perfusions from *Pdha1*$^{Cardiac-/-}$ mice and their αMHC-MerCreMer control littermates (**Figure 4E–G**), though aortic diastolic pressure was significantly decreased in *Pdha1*$^{Cardiac-/-}$ mice (**Figure 4H**). Interestingly, measurement of heart weight/body weight ratios upon completion of the 40 min aerobic perfusion protocol, revealed substantial cardiac hypertrophy in *Pdha1*$^{Cardiac-/-}$ mice (**Figure 5**).

DISCUSSION

Despite being a major fuel source for the heart, our observations demonstrate that a marked reduction in glucose oxidation in the heart secondary to cardiac-specific deletion of PDH does not adversely affect LV systolic function. This is likely due to the corresponding increase in fatty acid oxidation, ensuring that the oxidative energy needs of the heart are being met. On the contrary, our study provides support for the concept that a specific defect in myocardial glucose oxidation is sufficient to produce a diabetic cardiomyopathy-like phenotype, as *Pdha1*$^{Cardiac-/-}$ mice exhibited signs of diastolic dysfunction, reflected by their decreased mitral E/A ratio in comparison to their αMHC-MerCreMer control littermates.

Numerous preclinical studies have demonstrated that obesity and/or diabetes severely alters myocardial energy metabolism, such that the myocardium is heavily dependent on fatty acid oxidation to meet its energy requirements, often at the expense of declining glucose oxidation rates (1, 3). Indeed, myocardial metabolism in obese leptin receptor-deficient *db*/*db* mice is strikingly similar to *Pdha1*$^{Cardiac-/-}$ mice, as isolated working heart perfusion studies have demonstrated marked increases in fatty acid oxidation rates and decreases in glucose oxidation rates (4, 18). Moreover, *db*/*db* mice exhibit a decline in the mitral E/A ratio that is comparable to what we observed in *Pdha1*$^{Cardiac-/-}$ mice (19). Similarly, experimental obesity due to chronic supplementation with a high-fat diet also results in marked increases and decreases in fatty acid oxidation and glucose oxidation, respectively, during isolated working heart perfusion experiments, whereas insulin-stimulated glucose oxidation is heavily diminished (6, 9). These preclinical findings appear to translate to the clinical scenario, as PET imaging studies in either obese women or men with T2D demonstrate marked increases in myocardial fatty acid oxidation rates and a decline in glucose utilization (7, 20). Intriguingly, therapeutic interventions that restore glucose oxidation rates in the hearts of mice subjected to experimental models of obesity and/or T2D, either directly or secondary to an inhibition of fatty acid oxidation, result in a mitigation of the ensuing cardiomyopathy (9, 14, 21). Likewise, this has also been seen in experimental models of type 1 diabetes, as pharmacological activation of PDH via treatment with dichloroacetate, augmented contractile function in isolated working hearts from rats treated with streptozotocin (22).

Recent studies also suggest that glucose oxidation is perturbed during diastolic dysfunction due to chronic infusion with angiotensin II (23), whereas treatment with an angiotensin II type 1 receptor antagonist (irbesartan) reverses experimental diastolic dysfunction, which is associated with a restoration of myocardial glucose oxidation rates (23). Of interest, mice harboring a whole-body deficiency of PDH kinase 4 (*Pdk4*$^{-/-}$), a key enzyme that inactivates PDH/glucose oxidation, were protected against angiotensin II-induced diastolic dysfunction (24). Furthermore, isolated working heart perfusion studies demonstrated that glucose oxidation rates were increased in angiotensin II-infused *Pdk4*$^{-/-}$ mice versus their wild-type littermates (24).

Our findings share some similarities but also some differences from a previous study that generated a *Pdha1*$^{Cardiac-/-}$ mouse model (25). While Sun and colleagues also observed a marked reduction in glucose oxidation rates as expected for a mouse heart deficient in PDH, they did not observe a corresponding increase in fatty acid oxidation rates. This may explain why their *Pdha1*$^{Cardiac-/-}$ mice exhibited premature mortality, as evidenced by no *Pdha1*$^{Cardiac-/-}$ mice surviving beyond 16 weeks of age in this particular study, which could be due to energetically compromised hearts and death via heart failure. The *Pdha1*$^{Cardiac-/-}$ mice studied by Sun and colleagues did not demonstrate significant reductions in systolic function at 1 month post-tamoxifen administration, similar to what we observed in our *Pdha1*$^{Cardiac-/-}$ mice at 5 weeks post-tamoxifen administration. Nevertheless, at 2 months post-

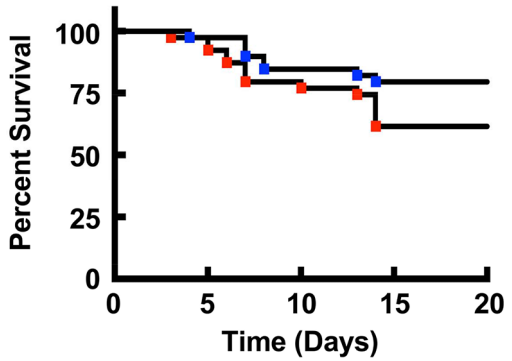

FIGURE 6 | *Pdha1*$^{Cardiac-/-}$ *mice exhibit increased sensitivity to Cre recombinase-related mortality due to fatal cardiomyopathy*. Survival rates in *Pdha1*$^{Cardiac-/-}$ mice and their αMHC-MerCreMer control littermates starting from the day of first tamoxifen injection (n = 39). Differences were determined using a Kaplan-Meier survival curve analysis. *$P < 0.05$.

tamoxifen administration, their *Pdha1*$^{Cardiac-/-}$ mice showed signs of heart failure with LVEFs <30%. Thus, it is possible that if we allowed our mice to age longer that perhaps we would have also observed systolic dysfunction in our *Pdha1*$^{Cardiac-/-}$ mice. Moreover, another aspect worth considering is that the method of tamoxifen-mediated gene deletion via Cre recombinase was not identical between our study and that of Sun et al. We administered tamoxifen to our mice 6 times at a dose of 50 mg/kg body weight spread over 8 days (**Figure 1D**), whereas Sun and colleagues administered tamoxifen for 5 consecutive days to their mice at a dose of 80 mg/kg body weight. Since Cre-recombinase activity in cardiac myocytes can produce a potentially fatal cardiomyopathy in αMHC-MerCreMer mice (17), it is possible that the severity of the Cre-recombinase-induced cardiomyopathy was exacerbated by cardiac-specific deletion of *Pdha1*, explaining the accelerated mortality in *Pdha1*$^{Cardiac-/-}$ mice from Sun et al. (25). In support of this notion, our *Pdha1*$^{Cardiac-/-}$ mice were more sensitive to the Cre recombinase mediated cardiomyopathy/mortality during tamoxifen treatment when compared to their αMHC-MerCreMer control littermates (**Figure 6**). However, all our *Pdha1*$^{Cardiac-/-}$ mice that survived the tamoxifen treatment protocol were able to survive until study completion. Interestingly, while ultrasound echocardiography revealed no gross LV structural abnormalities in hearts from *Pdha1*$^{Cardiac-/-}$ mice, when we perfused our mice for assessment of cardiac energy metabolism at 2 weeks post-ultrasound, *Pdha1*$^{Cardiac-/-}$ mice now demonstrated a marked cardiac hypertrophy, similar to that reported by Sun and colleagues (25). Likewise, Sun et al. also observed indices of impaired diastolic function in their *Pdha1*$^{Cardiac-/-}$ mice, as reflected by elevations in left ventricular interior diameter during diastole.

Despite the plethora of evidence showing a strong association between diabetic cardiomyopathy and impaired myocardial glucose oxidation, our study demonstrates that extinguishing glucose oxidation via cardiac-specific deletion of PDH is sufficient to produce a cardiomyopathy-like phenotype characterized via diastolic dysfunction and cardiac hypertrophy. Importantly, our studies were done in lean animals in the absence of the perturbed metabolic milieu associated with obesity and/or T2D, indicating that a reduction in myocardial glucose oxidation rates *per se* is likely a contributing factor to the pathology of obesity- and/or diabetes-related cardiomyopathy. Taken together, it would suggest that pharmacological development of PDH agonists to stimulate glucose oxidation (e.g., dichloroacetate) may be a novel therapeutic approach to attenuate diabetic cardiomyopathy, and the generation of *Pdha1*$^{Cardiac-/-}$ mice will prove to be a valuable tool in confirming the validity of such a strategy.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Canadian Council on Animal Care. All animal protocols were approved by the University of Alberta Health Sciences Animal Welfare Committee.

AUTHOR CONTRIBUTIONS

KG, MA, RB, FE and MG performed the research. KG and JU designed the research study. KG and MA analyzed the data. KG and JU wrote the paper. KG, MA, RB, and JU edited and revised the discussion. All authors approved the final version of the manuscript.

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frontiers in Cardiovascular Medicine

MINI REVIEW
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The Regulation of Insulin-Stimulated Cardiac Glucose Transport via Protein Acetylation

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Cellular catabolism is the cell capacity to generate energy from various substrates to sustain its function. To optimize this energy production, cells are able to switch between various metabolic pathways in accordance to substrate availability via a modulation of several regulatory enzymes. This metabolic flexibility is essential for the healthy heart, an organ requiring large quantities of ATP to sustain its contractile function. In type 2 diabetes, excess of non-glucidic nutrients such as fatty acids, branched-chain amino-acids, or ketones bodies, induces cardiac metabolic inflexibility. It is characterized by a preferential use of these alternative substrates to the detriment of glucose, this participating in cardiomyocytes dysfunction and development of diabetic cardiomyopathy. Identification of the molecular mechanisms leading to this metabolic inflexibility have been scrutinized during last decades. In 1963, Randle demonstrated that accumulation of some metabolites from fatty acid metabolism are able to allosterically inhibit regulatory steps of glucose metabolism leading to a preferential use of fatty acids by the heart. Nevertheless, this model does not fully recapitulate observations made in diabetic patients, calling for a more complex model. A new piece of the puzzle emerges from recent evidences gathered from different laboratories showing that metabolism of the non-glucidic substrates induces an increase in acetylation levels of proteins which is concomitant to the perturbation of glucose transport. The purpose of the present review is to gather, in a synthetic model, the different evidences that demonstrate the role of acetylation in the inhibition of the insulin-stimulated glucose uptake in cardiac muscle.

Keywords: glucose uptake, protein acetylation, cardiac metabolism, diabetic cardiomyopathies, insulin resistance

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INTRODUCTION

The heart is a muscular pump requiring an enormous quantity of energy to sustain its function. The left ventricle of a human heart ejects 80 ml of blood per heartbeat, the equivalent of a small cup of coffee. However, it beats 75 times per minute. At the end of the day, the human heart will have beaten 100,000 times, ejecting more than 8 tons of blood. This daily work needs 6 kg of ATP, the main cellular energy-providing molecule. This corresponds to 20-times the weight of the heart, which makes it the most energy-consuming organ (per unit weight) of our body (1). The intracellular energy stores (mainly ATP and phosphocreatine) of the heart are negligible. In absence of extracellular substrates, this energy store would be depleted in a few seconds if

rescue mechanisms (such as halt of contraction during myocardial ischemia) do not occur. In consequence, the heart has to uninterruptedly produce energy from available nutrients, working on a "just-in-time" basis. This energy production depends on body needs (resting vs. exercise), nutrient availability and hormonal status. In other words, the heart equilibrates energy production to energetic demand and this fine-tuned energetic homeostasis is a prerequisite for optimal cardiac function. Any defect in this equilibrium will have, *de facto*, negative consequences. It is currently recognized that energetic disequilibrium is not just a consequence of various cardiac pathologies but is a trigger in the onset of such pathologies including heart failure (2, 3).

THE METABOLIC FLEXIBILITY OF THE HEALTHY HEART

Most of the energy produced by cardiac muscle comes from substrate oxidation in mitochondria. The main substrates used are glucose and fatty acids (1). Fatty acids are preferentially oxidized under fasting and glucose becomes the main ATP-generating metabolite during postprandial state. This metabolic switch has been firstly described by Randle and colleagues in 1963 (4, 5). They have demonstrated that fatty acid oxidation intrinsically inhibits glucose use. The molecular mechanisms involved in this inhibition are partially identified and are a combination of allosteric regulations, phosphorylation events and transcriptional regulations [reviewed in (6, 7)]. First, pyruvate dehydrogenase (PDH), responsible for glucose oxidation, is inhibited by acetyl-coenzyme A (acetyl-CoA) and nicotinamide adenine dinucleotide (NADH), the final products of fatty acid oxidation. They act allosterically on PDH but also activate PDH kinases that phosphorylate and inactivate PDH. Second, acetyl-CoA can be metabolized into citrate. Citrate leaves mitochondria to inhibit 6-phosphophofructo-1-kinase, the enzyme that controls glycolytic flux. Third, fatty acid oxidation has been shown to directly prevent glucose uptake into myocytes (8, 9). Mechanisms proposed to be involved in glucose uptake inhibition included the impairment of insulin signaling (9) but also acetylation events (summarized in the present review). Next to these short-term mechanisms, fatty acids promote the transcriptional activity of peroxisome-proliferator-activated receptor (PPAR) family. PPARs are transcription factors that increase the expression of genes involved in fatty acid oxidation. On the other hand, the expression of several genes involved in glucose catabolism are decreased by PPARs (2, 10).

Although fatty acids are preferentially used under fasting, glucose becomes the major ATP-providing substrate in postprandial situation, which is characterized by elevated plasma glucose level and insulin secretion. Insulin stimulates glucose uptake, glycolysis, and glucose oxidation whereas it blocks fatty acid oxidation (6). Consequently, the heart constantly shifts from fatty acids to glucose and vice-versa along the day, depending on its nutritional status.

Besides glucose and fatty acids, the heart also uses alternative substrates including ketone bodies, lactate and branched chain amino acids such as leucine (1). Ketone bodies raise in plasma under prolonged fasting. Exercise increases lactate concentration whereas plasma leucine level is elevated under particular diet or during persistent fasting. When elevated, these metabolites counteract the use of other substrates to become the main ATP contributors (11). We can cite the strong and acute inhibition of cardiac glucose uptake by ketone bodies and leucine (12). Similarly, the group of Kieran Clarke has nicely showed that ketone body ester infusion inhibits glycolysis in humans (13).

The multi-faceted metabolic flexibility of the healthy heart allows optimizing energy production, providing the precise amount of ATP required for adequate contractile function. By contrast, cardiac pathologies are generally characterized by metabolic inflexibility, with only one main substrate catabolized to produce energy (2, 3, 14). Hearts of diabetic patients uses quasi exclusively fatty acids, whatever the substrate or hormonal status. In parallel, glucose uptake and catabolism are significantly inhibited. The high level of circulating fatty acids and insulin resistance are two main factors of metabolic inflexibility of the diabetic heart (2).

MOLECULAR MECHANISMS REGULATING GLUCOSE UPTAKE

Uptake of glucose, which is the first step of glucose utilization, is inhibited acutely by alternative substrates under physiological conditions but is also blocked under pathological situations such as diabetes. Cardiac glucose transport is principally under the control of glucose transporter 4 (GLUT4) (**Figure 2**). Under basal conditions, GLUT4 is mainly located in intracellular vesicles. Glucose uptake depends on their translocation into sarcolemmal membrane by stimuli such as insulin (6). Insulin promotes the activation of protein kinase B (PKB)/Akt, which can phosphorylate Akt substrate of 160 kDa (AS160). AS160 is the GTPase-activating protein of the G protein Rab that is located on GLUT4 vesicles and responsible for GLUT4 translocation into plasma membrane. The insulin-mediated AS160 phosphorylation inactivates its GAP function, promoting Rab activation. Recent studies strongly suggest that protein acetylation events are, at least partially, involved in the inhibition of glucose transport by ATP-producing substrates such as fatty acids, ketone bodies, and leucine under acute and/or chronic conditions. These studies are summarized in the next chapter.

PROTEIN ACETYLATION, A POST-TRANSLATIONAL MECHANISM SENSING CELLULAR ENERGETIC STATUS

The first observation of an acetylated peptide was made by Narita in 1958 (15, 16). This was followed by others, highlighting amino-terminal acetylation of non-histone proteins. Few years later, the discovery by Phillips (17) of the acetylation of histones on lysine residues by enzymes that were firstly called histone acetyltransferases (HATs) opened a new field of investigation.

Several decades later, tubulin was described as the first non-histone protein acetylated on a lysine residue (18).

Despite having a long history, protein acetylation remains a hot topic today with the frequent description of new acetylated proteins. A panel of recent reviews nicely describes the molecular mechanisms involved in this post-translational modification (19–21). To summarize, acetyl group provided by acetyl-CoA is transferred on epsilon-amino group of lysine residues of proteins (histones and non-histones) by spontaneous transfer from acetyl-CoA to proteins (22) or by reactions catalyzed by HAT enzymes that have been renamed lysine acetyltransferases (KATs) (**Figure 1**). Over 20 different KATs have been identified and can be classified into three major families that share similar structure and function: The general control non-derepressible 5 (GCN5)-related (GNAT) family, the CREB-binding protein (CBP)/p300 family and the MYST family. KATs are mainly localized in the cytoplasm and the nucleus (23). The lack of identified KATs in mitochondria, where more than 60% of the proteins are potentially acetylated, suggests that non-enzymatic acetylation is the main mechanism involved in this organelle (24). Acetylation is a reversible modification with lysine deacetylases (KDACs) catalyzing the deacetylation reaction. Two main families of KDACs were identified and are characterized by different catalytic mechanisms (**Figure 1**). The first family is composed of Zn^{2+}-dependent DACs and are called histone deacetylases (HDAC1 to HDAC11) even if they have multiple substrates including non-histone proteins. The second family is the NAD+-dependent KDACs called sirtuins (SIRT1 to SIRT7). HDAC1, HDAC11, SIRT6, and SIRT7 are localized into the nucleus. SIRT2 is mainly cytoplasmic whereas SIRT3 and SIRT5 are found in the mitochondria. The remaining KDACs shuttle between the nucleus and the cytoplasm (23).

Besides its action in the regulation of gene expression via the regulation of histones, protein acetylation is closely linked to cellular energy status and metabolism via its dependence on acetyl-CoA and NAD+ levels. Indeed, protein acetylation levels are tightly regulated by the availability of acetyl-CoA (20, 25). When available, ATP-generating substrates such as fatty acids, glucose, ketone bodies, and amino acids converge to the mitochondrial production of acetyl-CoA. This acetyl-CoA

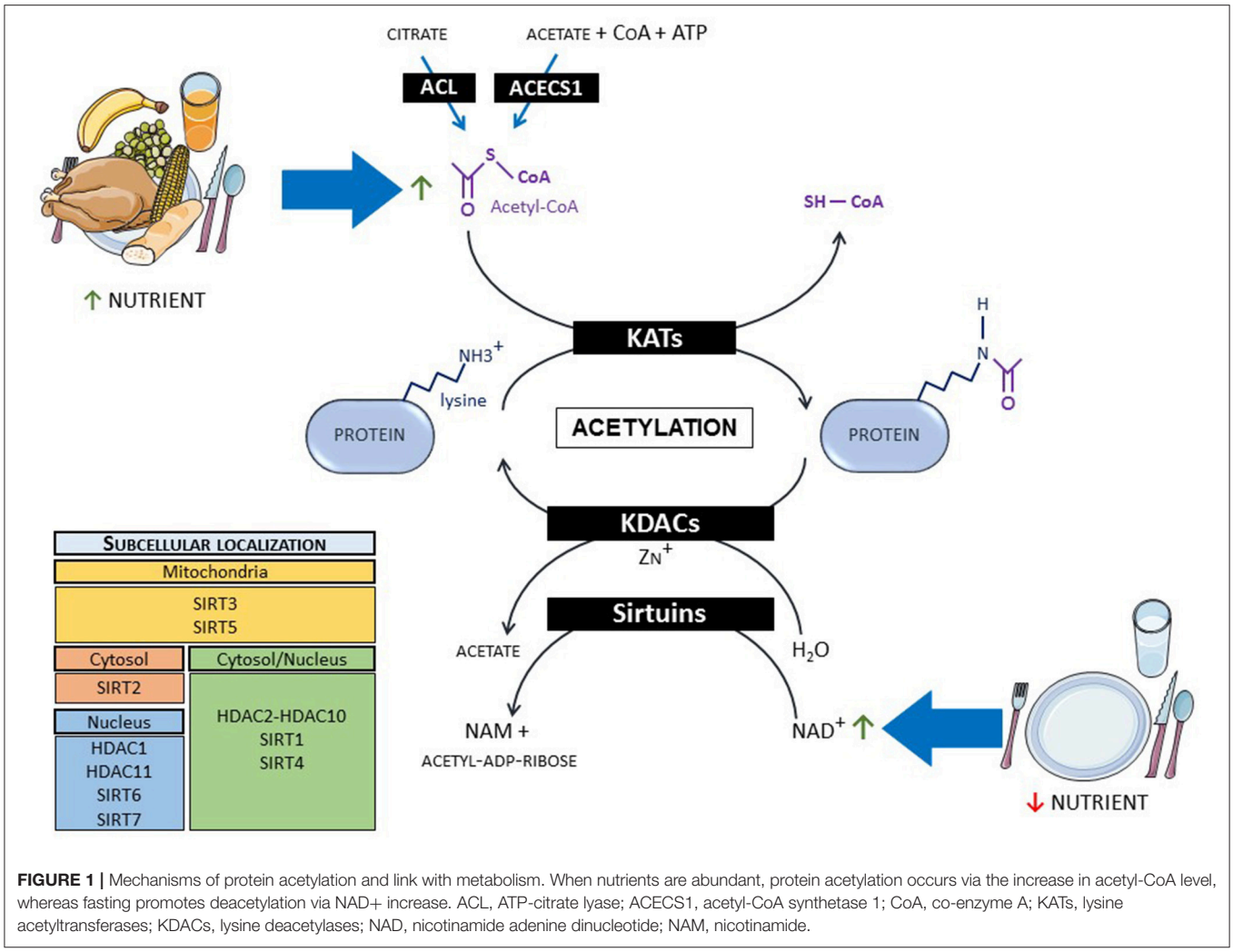

FIGURE 1 | Mechanisms of protein acetylation and link with metabolism. When nutrients are abundant, protein acetylation occurs via the increase in acetyl-CoA level, whereas fasting promotes deacetylation via NAD+ increase. ACL, ATP-citrate lyase; ACECS1, acetyl-CoA synthetase 1; CoA, co-enzyme A; KATs, lysine acetyltransferases; KDACs, lysine deacetylases; NAD, nicotinamide adenine dinucleotide; NAM, nicotinamide.

production enters into the tricarboxylic acid (TCA) cycle but also provokes mitochondrial protein acetylation. The generation of acetyl-CoA in cytoplasm and nucleus mainly depends on the activity of two enzymes, the ATP-citrate lyase (ACL) and the acetyl-CoA synthetase 1 (ACECS1). ACL uses citrate, which increases proportionally to mitochondrial acetyl-CoA, to produce cytosolic and nuclear acetyl-CoA, promoting protein acetylation under fed state. On the other hand, ACECS1 produces acetyl-CoA from acetate, CoA, and ATP, typically in nutrient-restricted conditions. More recently, carnitine acetyltransferase (CrAT) has been proposed to play an important role in cardiac metabolism producing cytoplasmic acetyl-CoA from carnitine and CoA (26). Sirtuins are controlled by cellular NAD+ level. Inasmuch as NADH oxidation gives rise to NAD+, its level fluctuates with respect to the redox state of the cell (21, 27). NAD+ synthesis also depends on its precursor availability, namely nicotinamide, nicotinamide riboside, and nicotinamide mononucleotide.

PROTEIN ACETYLATION ALTERS GLUCOSE UPTAKE

Keeping in mind the tight interconnection between acetylation and energy status, numerous studies evaluated the control of metabolism by acetylation processes, including in the heart. Fukushima and Lopaschuk recently summarized the impact of protein acetylation on cardiac fatty acid metabolism, particularly in diabetes where mitochondrial acetylation of numerous metabolic enzymes (including long chain acyl-CoA dehydrogenase, β-hydroxyacyl CoA dehydrogenase, carnitine palmitoyl transferase-2, and CrAT) participates in the chronic increase in fatty acid oxidation (21, 27). This increase in fatty acid oxidation will decrease glucose uptake and metabolism via Randle mechanisms explained above (**Figure 2**). Besides fatty acid metabolism, protein acetylation takes a growing importance in the direct regulation of glucose metabolism. In 2005, Rogers and colleagues demonstrated that fasting enhanced SIRT1 expression and activity, inducing deacetylation of the transcriptional factor peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1-α), responsible for the induction of gluconeogenic genes and the repression of glycolytic genes in liver (28). Shortly afterwards, several studies have shown that SIRT1 activation with small molecules such as SRT1720 and resveratrol improved glycaemia of diabetic rodent models (29, 30). This was associated with a better glucose disposable rate, reflecting an improvement of glucose uptake in muscle (29, 30). The beneficial effects observed in diabetic models by activation of KDACs were largely described to be mediated via a regulation of key transcriptional factors (including PGC1-α and forkhead box O) involved in regulation of mitochondrial biogenesis and expression of key metabolic enzymes (31). Interestingly, several high-throughput mass-spectrometry studies revealed that, besides histones and transcriptional regulators, numerous enzymes involved in metabolism were acetylated (32–35). Impact of the majority of these acetylation events on metabolism remains currently unknown. Current research starts to identify which ones are meaningful and how they will influence cellular metabolism.

In this mini-review, we particularly focus our interest on the impact of acetylation on insulin-stimulated glucose uptake in the heart, but also depict related studies performed in muscle and other tissues. To summarize, protein acetylation can potentially affect glucose uptake by directly targeting glucose transport but also by regulating the insulin signaling pathway or by acting on glucose oxidation (**Figure 2**). Concerning the insulin pathway, both PKB/Akt and its upstream regulator phosphoinositide-dependent kinase 1 (PDK1) were shown to be acetylated on lysine residues located in their pleckstrin homology (PH) domain. Acetylation of PH domains interfered with their binding to phosphatidylinositol (3,4,5)-trisphosphate (PIP3), a crucial step for PKB/Akt activation (36) (**Figure 2**). SIRT1 can deacetylate PKB/Akt and PDK1, enhancing their binding to PIP3 and therefore, increasing insulin signaling (36). Accordingly, SIRT1 deficient mice present a higher PKB/Akt acetylation level in the heart, affecting its activation in response to hypertrophic stimulus (36). PKB/Akt acetylation is also increased in heart of mice undergoing a high fat diet (HFD) (37). This increase correlates with a lower expression of SIRT3 and a lower activation of PKB/Akt. In adipose tissues, SIRT2 forms a complex with PKB/Akt under nutrient deprivation. Once complexed, SIRT2 can reduce PKB/Akt acetylation promoting its phosphorylation/activation after insulin stimulation (38). Upstream of PKB/Akt, SIRT1 can deacetylate the insulin receptor substrate-2 (IRS-2), which links the activated insulin receptor to its downstream effectors (39). Deacetylation of IRS2 promotes its phosphorylation on tyrosine residues and its activation.

Several studies showed that increase in acetylation correlates with a reduction in glucose oxidation and/or glycolysis, leading to an accumulation of metabolic intermediates, resulting *in fine* in glucose uptake inhibition (**Figure 2**). Strong evidences are described for a control of PDH by acetylation. Mori et al. have revealed the presence of an increased acetylation level of PDH in hypertrophic hearts (40). This PDH acetylation, which is due to a reduction in SIRT3 expression, correlates with a decrease in its activity and in glucose oxidation. Similar observations were made in muscle cells (41). Horton and collaborators have shown that succinate dehydrogenase (SDHA), a protein involved in TCA cycle and member of the respiratory complex II, was acetylated on Lys-179 in the failing heart (42). This acetylation in the FAD+ binding-region decreased SDHA activity, resulting in succinate accumulation and a reduced complex II-driven respiratory rate. SIRT3 also deacetylates various members of the mitochondrial respiratory complex I and SIRT3-deficient cells presented a lower complex 1-driven respiratory rate (43). Taken together, these results show that a global increase in mitochondrial protein acetylation will decrease TCA cycle and electron transport chain activities in heart resulting in a lower capacity to oxidize glucose, accumulating intermediates of glucose catabolism that could finally decrease glucose uptake. However, the relative implication of all these acetylation processes in the acute regulation of cardiac glucose metabolism under physiological state (Randle effect) and/or in its chronic inhibition in diabetes (metabolic inflexibility) remain largely unknown.

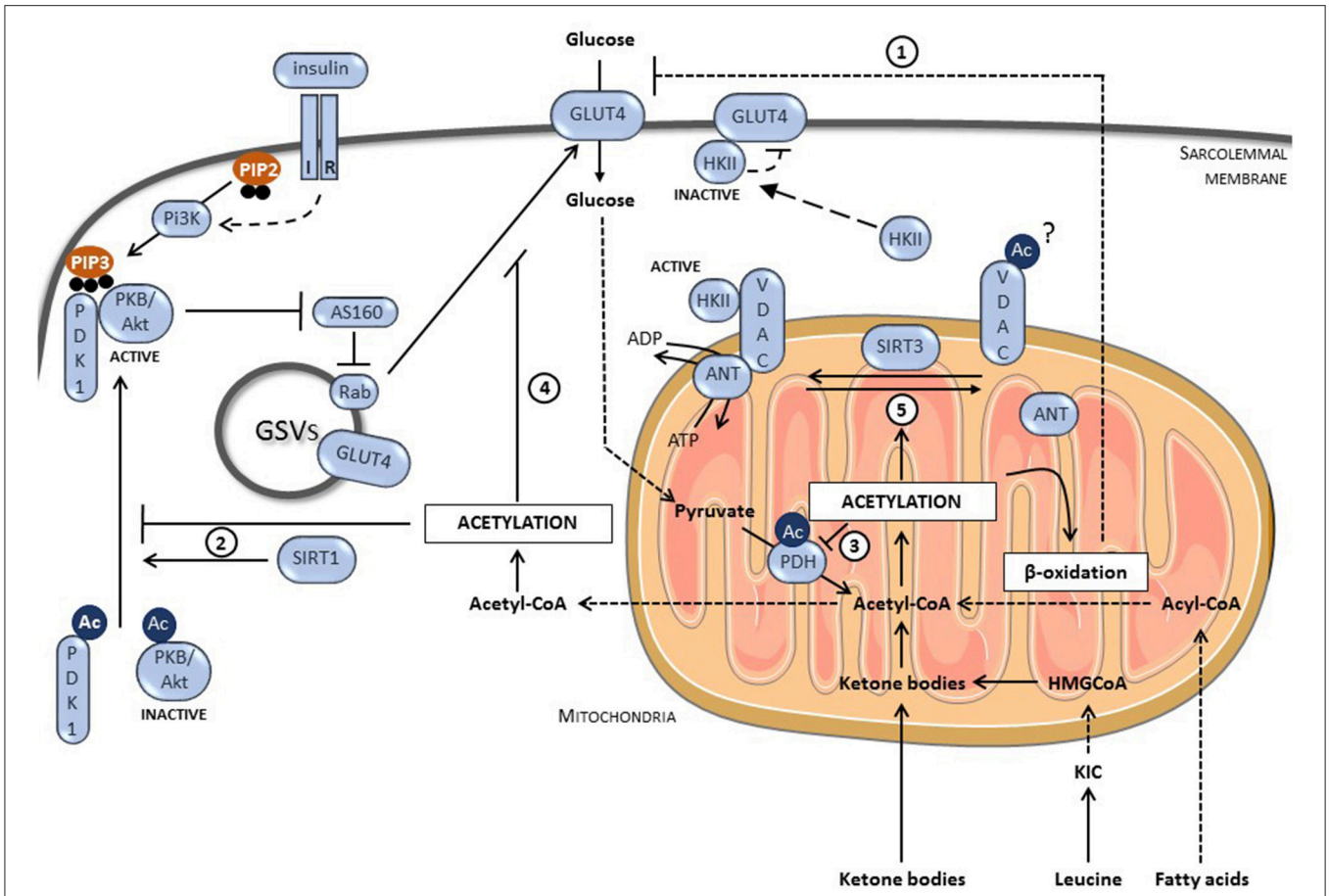

FIGURE 2 | Role of protein acetylation on glucose transport in GLUT4-dependent muscle cells**.** (1) Protein Acetylation increases fatty acid oxidation, which negatively regulates glucose transport via the mechanisms described by Randle. (2) The deacetylation of PKB/Akt and PDK1 by SIRT1 allows their interaction with PIP3 and subsequent activation. In consequence, protein acetylation limits insulin signaling and reduces glucose uptake. (3) Protein acetylation inhibits glucose oxidation, inducing accumulation of glycolytic intermediates that could block glucose uptake. (4) Protein acetylation inhibits GLUT4 translocation by a still unknown mechanism. (5) Protein acetylation prevents the formation of the VDAC/ANT complex required for HKII binding and activation. Inactive HKII promotes the accumulation of intracellular glucose that inhibits GLUT4. IR, insulin receptor; PI3K, phosphatidyl-inositol-3 kinase; PIP2, phosphatidylinositol (4,5)-trisphosphate; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; GLUT4, glucose transporter 4; PKB/Akt, protein kinase B; PDK1, phosphoinositide-dependent kinase 1; AS160, Akt substrate of 160 kDa; GSVs, GLUT4 storage vesicles; HKII, hexokinase II; VDAC, voltage-dependent anion channel; ANT, anion nucleotide transporter; SIRT3, sirtuin 3; SIRT1, sirtuin 1; KIC, α-ketoisocaproate; HMGCoA, β-Hydroxy β-methylglutaryl-CoA; Ac, acetylated-lysine.

Finally, protein acetylation directly affects glucose uptake via its action on GLUT4 (**Figure 2**). Our laboratory has recently proposed that a global increase in protein acetylation by leucine or ketone bodies (two acetyl-CoA-providing metabolites), in primary cultured cardiomyocytes and *ex vivo* perfused hearts, decreases the insulin-stimulated glucose transport via a blockage of GLUT4 translocation to the cell membrane (12). The exact mechanisms responsible for the defective GLUT4 translocation remain unknown but give another clue in the loss of glucose utilization by the diabetic heart, inasmuch as the plasmatic level of both leucine and ketone bodies is increased in diabetic patients. Lantier and colleagues have also linked the acetylation-dependent decrease of muscle glucose transport to an inhibition of hexokinase II (HKII), the enzyme responsible for glucose phosphorylation after its uptake (44). Indeed, in order to be fully activated, HKII binds the mitochondrial permeability transition pore (mPTP) composed of the voltage-dependent anion channel (VDAC) located in the outer membrane and the anion nucleotide transporter (ANT) (45). In muscle from SIRT3-deficient mice, especially under HFD, the interaction between mPTP and HKII as well as between VDAC and ANT were weaker, resulting in lower HK activity, in intracellular glucose accumulation and, subsequently, to a reduction in glucose uptake. This supports the model showing that HKII interaction with its partners is important for its activity (46).

In contrast to all these reports, it must be mentioned that another study proposed a model where the acetylation of a protein called TUG releases GLUT4 storage vesicles (GSVs) from the Golgi apparatus. This promotes GSV translocation to the plasma membrane and glucose uptake (47). An explanation of such discrepancy could be linked to the fact that this study has been performed in adipocytes whereas most of the studies

presented in this review have been performed in muscle. It will be highly interesting to study the role of TUG acetylation in GLUT4 translocation in muscle.

CONCLUSIONS

Except the last finding, there is a large consensus indicating that an increase in protein acetylation diminishes cardiac glucose uptake after insulin stimulation. Such event could occur under physiological condition when non-glucidic substrate levels increase in plasma, but could also participate in the establishment of cardiac metabolic inflexibility in pathologies. Fatty acids and leucine, which are both early markers of diabetes (48), induce acetyl-CoA accumulation and protein acetylation (12, 37), suggesting that acetylation could be an early event in insulin resistance development. This makes protein acetylation a valuable target for new treatment of diabetes. Acetylation level could be controlled by affecting the activity or the expression of KDACs and KATs. Therefore, a tight control of the activity of these enzymes could prevent, reverse or, at least, delay the establishment of the metabolic inflexibility in the diabetic heart. This could be achieved by a modulation, via dietary or pharmaceutical intervention, of NAD+ and/or acetyl-CoA levels (20, 27, 49). Even if we can conclude that protein acetylation is definitely a crucial actor in the apparition of metabolic inflexibility in the heart during diabetes, numerous questions remain unanswered. Future research will allow us to fully understand the mechanisms involved in the deregulation of acetylation events and to assess its validity as a target for diabetic treatment.

AUTHOR CONTRIBUTIONS

ER, LaB, CB, SH, and LuB wrote and edited the present review.

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REVIEW
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Acute and Chronic Effects of Protein Kinase-D Signaling on Cardiac Energy Metabolism

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Protein kinase-D (PKD) is increasingly recognized as a key regulatory signaling hub in cardiac glucose uptake and also a major player in the development of hypertrophy. Glucose is one of the predominant energy substrates for the heart to support contraction. However, a cardiac substrate switch toward glucose over-usage is associated with the development of cardiac hypertrophy. Hence, regulation of PKD activity must be strictly coordinated. This review provides mechanistic insights into the acute and chronic regulatory functions of PKD signaling in the healthy and hypertrophied heart. First an overview of the activation pathways of PKD1, the most abundant isoform in the heart, is provided. Then the various regulatory roles of the PKD isoforms in the heart in relation to cardiac glucose and fatty acid metabolism, contraction, morphology, function, and the development of cardiac hypertrophy are described. Finally, these findings are integrated and the possibility of targeting this kinase as a novel strategy to combat cardiac diseases is discussed.

Keywords: protein kinase D, PKD isoforms, glucose uptake, fatty acid uptake, cardiac hypertrophy, membrane substrate transporter

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INTRODUCTION

The heart has a high demand for energy substrates in order to sustain perpetual cycles of contraction and relaxation. The predominant substrates used under physiological conditions are (long chain) fatty acids and glucose (1). As a result, there is an almost continuous delivery of these substrates to the heart. Substrate uptake by cardiac myocytes occurs by facilitated diffusion and involves specific membrane transporters, i.e., CD36 (SR-B2) for fatty acids (2) and members of the GLUT (SLC2) family of glucose transporters for glucose (3). Importantly, for both substrates, the transporter-mediated uptake presents the rate-controlling site in their cellular utilization (4, 5). The heart possesses mainly two types of glucose transporters, i.e., GLUT1 and GLUT4. GLUT1 is known as the constitutive glucose transporter that permanently resides at the sarcolemma. In contrast, GLUT4 is present mainly in intracellular compartments from where it can be induced to translocate to the cell surface. Hence, GLUT1 mainly contributes to basal (non-stimulated) glucose uptake and GLUT4 to glucose uptake induced by a variety of physiological and pharmacological stimuli (3, 6). Because GLUT4 is considerably more abundant in the heart than GLUT1 (7), the inducible component of glucose uptake is critically important in cardiac energy metabolism. Increased cardiac contractile activity and increased circulating insulin levels are the main physiological

stimuli for inducing GLUT4 translocation to the sarcolemma and increasing cellular glucose uptake. In both cases the cellular signaling pathways involved have been largely disclosed. For regulation of contraction-induced GLUT4 translocation, the key signaling kinase was long thought to be AMP-activated kinase (AMPK) (8). However, more recently, work in our laboratory has revealed that cardiac contraction-induced GLUT4 translocation requires the additional activation of another kinase, namely protein kinase D1 (PKD1) (9, 10). Conversely, PKD1 appears not involved in insulin-stimulated glucose uptake (9, 10).

The signaling kinase PKD1 is involved in several receptor-mediated signal transduction cascades that function in multiple fundamental cellular processes, such as cell proliferation and differentiation, membrane trafficking, immune response, inflammation, angiogenesis, and cancer [for review see (11, 12)]. With respect to the cardiovascular system, PKD1 has been studied mainly in the context of cardiac hypertrophy and remodeling (13). The notion that PKD1 also functions in the contraction-induced regulation of cardiac glucose uptake indicates that PKD1 signaling is involved in both acute and chronic adaptations of the heart to external stimuli. Interestingly, PKD1's closely related relatives PKD2 and PKD3 have also been implicated in the regulation of cardiac glucose uptake and cardiac hypertrophy (12), together resulting in a complex signaling network.

The aim of this review is to provide mechanistic insight into the role of PKD—with focus on PKD1—in the healthy heart as well as in the development of cardiac disease. For this, we will first provide an overview of the PKD family, mostly focused on PKD1 activation, then we will discuss the various roles of the PKDs in the heart related to cardiac glucose and fatty acid metabolism, contraction, morphology and function. Finally, we will integrate these findings and discuss the application of PKD1 as target for therapeutic intervention.

PKD1 AND DIFFERENT ROUTES TO ACTIVATION

The protein kinase-D family makes up a family of three closely related Ser/Thr kinases: PKD1, PKD2, and PKD3. They were formerly categorized within the protein kinase C family based on the presence of two regulatory C1 domains, which are homologous to the diacylglycerol (DAG) binding domain of the PKC members. Hence, PKD1 is also referred to as PKCμ, and PKD3 as PKCν. Yet, the PKDs display unique structural properties that set them apart from the PKCs. Namely, they lack an auto-inhibitory pseudo-substrate region, a characteristic feature of the PKCs, but additionally possess a pleckstrin homology (PH) domain, which is absent in the PKCs. Moreover, the catalytic domain is different from the PKCs and shows more homology with that of the calcium/calmodulin-dependent kinases (CaMKs).

PKD1 is the most intensively studied member, and is not only activated by DAG, similarly to the classical and novel PKCs, but also directly by the novel PKCs themselves, i.e., via phosphorylation at two serines (human Ser738/Ser742, rodent Ser744/Ser748) within the activation loop, which is situated in the catalytic domain (**Figure 1**). This PKC-mediated PKD1 phosphorylation is initiated by binding of growth factors, secretory peptides (like endothelin) or α-adrenergic agonists (like phenylephrine) to G protein-coupled receptors (GPCRs), leading to activation of phospholipase-C and formation of DAG and subsequent PKC activation (**Figure 2**). The latter triggers a conformational change within PKD1 leading to release of auto-inhibition and increased auto-phosphorylation at the extreme C-terminal end (human Ser910, rodent Ser916). This mechanism of PKD activation is now regarded as the canonical PKD signaling pathway. Furthermore, auto-phosphorylation of PKD1 likely reflects activation, although this might not hold for every circumstance (14).

Interestingly, there are subtle differences between PKD1 activation by endothelin and by the α-agonist phenylephrine, which stimuli operate through closely related yet slightly different GPCRs (15). Both stimuli induce PKD1 translocation to the sarcolemma, which brings it in close contact to its upstream kinases to undergo subsequent release of its auto-inhibition. However, endothelin-induced PKD1 association with the sarcolemma is more long-lasting. Additionally, PKD1 translocation to the nucleus is more pronounced in response to phenylephrine compared to endothelin (16).

Another mechanism of PKD1 activation occurs in response to oxidative stress. Increased levels of mitochondrially produced reactive oxygen species (mROS) cause a series of tyrosine phosphorylation of PKD1 extra to the activation loop Ser-phosphorylations of the canonical activation pathway. These tyrosine phosphorylations are mediated by proto-oncogene tyrosine kinases Abl and/or Src, and precede the Ser phosphorylations (for details, see **Figure 1**). Especially, phosphorylation at human Tyr463 (rodent Tyr469) within the PH domain is needed for full PKD1 activation (17), and initiates docking of PKD1 at the mitochondria (18). Additionally, mROS-induced phospholipase D1 activation and generation of DAG is needed to support binding of PKD1 to mitochondrial membranes. ROS-induced PKD1 activation results in a markedly different pattern of activation of downstream kinases compared to the canonical pathway, and also sarcolemmal PKD1 translocation is not involved (19). mROS-induced PKD1 activation plays an important role in the development of defense mechanisms in tumor cells upon increased oxidative stress (19).

Besides the cytoplasmic pool of PKD1, which translocates to the plasma membrane or nucleus in response to GPCR agonists and oxidative stress there is also a PKD1 population localized to the trans-Golgi—endosomal continuum, as reported for several non-muscle cell lines under non-stimulated conditions (20, 21). Trans-Golgi PKD1 is involved in vesicle transport between endosomes and the plasma membrane. Also in rodent cardiomyocytes there is an endosomal population of PKD1, contributing to ⅓ of total cellular PKD1 under non-stimulated conditions (22). Using an *in vitro* kinase assay, we found this pool to be activated upon contraction stimulation (22). This activation was accompanied by Ser916 auto-phosphorylation without any trace of Ser744/748 trans-phosphorylation. Hence, contraction-induced PKD1 activation in the heart has a unique signature,

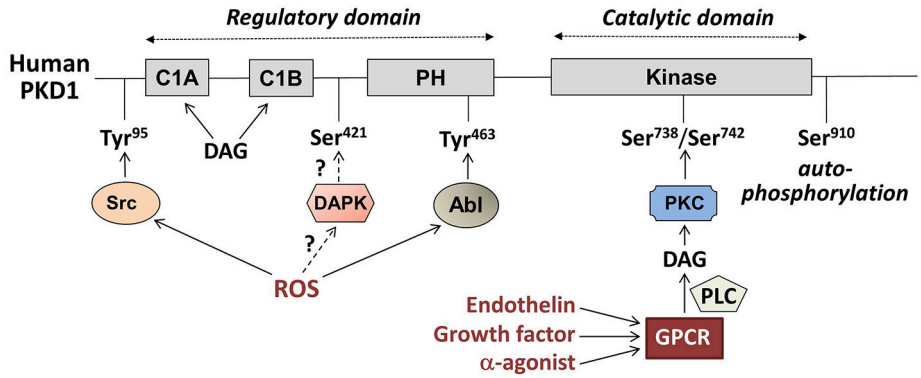

FIGURE 1 | Main regulatory phosphorylation sites of human Protein Kinase D1 (PKD1) and its upstream pathways of activation. PKD1 consist of two main domains: a regulatory domain **(Left)** and a catalytic domain **(Right)**. The locations of the regulatory tyrosine (Tyr) and serine (Ser) phosphorylation sites are indicated. DAG, diacylglycerol; PH, pleckstrin homology domain; DAPK, death-associated protein kinase; ROS, reactive oxygen species; PKC, protein kinase C; PLC, phospholipase C; GPCR, G protein-coupled receptor.

most likely via DAPK (as explained in the next section), which is different from GPCR agonist-induced or Abl/Src-mediated PKD1 activation (14).

In greater perspective, PKD activation is cell-type and stimulus-dependent. Moreover, PKD1 resides at different subcellular locations (i.e., cytosol and trans-Golgi/endosomes), and these different PKD1 pools are subject to activation by distinct stimuli (**Figure 2**).

PKD1 AND REGULATION OF GLUCOSE UPTAKE

Contraction is a main physiological stimulus impacting on cardiac metabolism. An increase in contractile activity markedly increases the metabolic demands of the heart for ATP generation which, therefore, results in the increased oxidation of glucose and (long-chain) fatty acids. In non-contracting primary cardiomyocytes *in vitro*, both respective membrane transporters GLUT4 and CD36 are largely present in intracellular membrane compartments (recycling endosomes). Electrically-induced contractions stimulate the translocation of transporters from these intracellular storage compartments (endosomes) to the cell surface within minutes, thereby increasing the uptake of both glucose and fatty acids (4). PKD1, identified as a contraction-activated kinase is involved in contraction-stimulated regulation of cardiac substrate uptake. Studies with PKD1-silenced HL-1 cardiomyocytes and with cardiomyocytes from cardio-specific PKD1 knockout mice have shown that acute contraction-induced GLUT4 translocation and glucose uptake are dependent on PKD1 activation, while contraction-induced CD36 translocation and fatty acid uptake do not need PKD1 (9). Hence, PKD1 activation has the potential to shift the cardiac substrate preference away from fatty acids toward increased glucose utilization (23). This role of PKD1 in GLUT4 translocation resembles the role of PKD1 in Golgi transport and secretion of proteins in non-muscular cells. In both cases, the same subcellular pool of PKD1 may be activated, being the PKD1 pool resident at the Golgi/endosomes. Furthermore, in both cases PKD1 activation initiates budding of vesicles for translocation to the cell surface. In case of Golgi-mediated transport of secretory proteins, PKD1 phosphorylates and activates a lipid kinase, phosphatidylinositol-4 kinase (21), which initiates curvature into the bilayers and also serves as platform for scission proteins involved in vesicle excision (24). Similarly, in the endosomes there may be similar lipid kinases present to support vesicle budding in response to PKD1 activation. Indeed, several other Golgi/endosomally present proteins involved in vesicle budding possess a PKD consensus motif, such as CtBP3/BARS or diacylglycerol kinase (24).

The underlying mechanism by which contraction stimulates PKD1-mediated GLUT4 translocation was pinpointed to ROS levels. This is based on the observation that ROS levels are increased in contracting cardiomyocytes, and that both contraction-induced PKD1 activation and GLUT4 translocation are sensitive to inhibition by ROS scavengers (9). As mentioned in the previous section, contraction-induced PKD1 activation does not require PKD1 Ser744/748 trans-phosphorylation, thereby proposing that contraction induces a novel unique activation mechanism of PKD1. This novel activation mechanism was found to include death-activated protein kinase (DAPK), a kinase mainly known from the cancer field. DAPK induces cellular apoptosis upon several death-inducing signals [for review see (25)]. In line with this, DAPK is proposed to act as a tumor suppressor, as its expression is lost in several human malignancies (26). Just like PKD1, DAPK is activated by ROS, as observed in HEK293T cells in which DAPK undergoes an activating Ser308 de-phosphorylation upon H_2O_2 stimulation (27). Additionally, DAPK and PKD1 physically interact with each other upon contraction stimulation of cardiomyocytes, which binding is necessary for PKD1 activation (9). This mechanism closely resembles oxidative stress-mediated PKD1 activation in HEK293T cells, which is

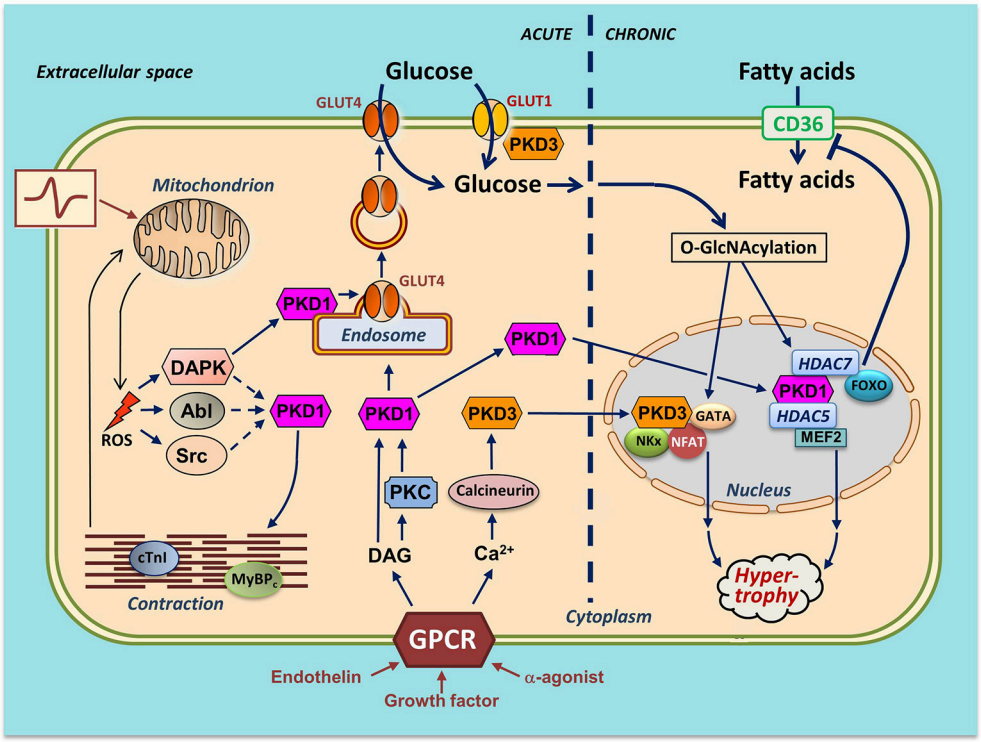

FIGURE 2 | Integration of actions of PKD1 and PKD3 in cardiac energy metabolism—a hypothetical scheme. Several stimuli induce activation of PKDs in cardiomyocytes. Endothelin, growth factors and α-adrenergic agonists activate distinct members of the family of G protein-coupled receptors (GPCR), leading to sarcolemmal phospholipase C activation and subsequent increases in diacylglycerol (DAG) and Ca^{2+}. DAG, as part of the canonical PKD1 activation pathway, binds to PKD1 thereby bringing it to the sarcolemma for further activation by PKCs. From there, the activated PKD1 migrates either to the nucleus or to endosomes. Please note that the sarcolemmal activation step of the cytoplasmic PKD1 pool is not displayed. In the nucleus, PKD1 phosphorylates the histone deacetylases (HDAC)5 and HDAC7. HDAC5 phosphorylation results in release and activation of the hypertrophic transcription factor MEF2. HDAC7 phosphorylation leads to binding to and inhibition of FOXO1, thereby depressing CD36 expression and cellular fatty acid uptake. In the cytosol, DAG stimulates PKD1 to migrate to the endosomes to acutely stimulate GLUT4 translocation and glucose uptake. Increased Ca^{2+} stimulates calcineurin-induced upregulation of PKD3 and activation of three additional hypertrophic transcription factors (GATA, NFAT, and NKx). PKD3 also regulates sarcolemmal GLUT1 localization. An increase in physical work (contractile activity) increases mitochondrial formation of ROS as by-product of elevated oxidative phosphorylation flux. DAPK migrates to the endosomes to activate the endosomally resident PKD1 pool to initiate vesicle-mediated GLUT4 translocation. DAPK, as well as Abl/Src may stimulate phosphorylation of myofilament proteins thereby further stimulating contractile activity. mROS production activates the Ser/Thr-kinase DAPK and the Tyr-kinases Abl and Src. For clarity, the effects of the PKD are divided into acute (signaling, metabolism and contraction; *left part of figure*) and chronic (nuclear; *right part of figure*). It is of importance to note that PKD1 and PKD3 integrate cardiac metabolism with contraction and also with remodeling. In case of integration of metabolism with contraction (as occurs upon an acute increase in workload), PKD1 activation ensures that the increase in contractility occurs hand in hand with increased glucose uptake. If the workload transcends from acute into chronic (as occurs upon chronic pressure overload), the PKDs are also needed for integration of metabolism with remodeling. Specifically, chronically elevated GLUT4 translocation and elevated GLUT1 expression together lead to increased glucose uptake and subsequent O-GlcNAcylation of myocellular proteins including the hypertrophic transcription factors. Together, the PKD1/PKD3-mediated direct activation of the transcription factors and the PKD1/PKD3 mediated O-GlcNAcylation and further activation of the transcription factors may lead to full-blown hypertrophic cardiac remodeling.

dependent on DAPK-Ser308 de-phosphorylation and subsequent DAPK–PKD1 interaction independent of PKCs and PKD1-Ser744/748 trans-phosphorylation (27). The role of DAPK in glucose uptake was assessed in HL-1 cells, in which DAPK was silenced via siRNA transfection. Not only was contraction-induced GLUT4 translocation abolished, also CD36 translocation was unimpaired (9), thereby providing additional support for the existence of a DAPK–PKD axis in selective glucose uptake into the heart. In conclusion, ROS activates DAPK via a yet unidentified phosphatase, which then travels to the endosomes to bind to and activate PKD1, which on its turn activates an endosomal budding kinase to stimulate formation of GLUT4-containing vesicles that translocate to the cell surface (**Figure 2**).

There is also some evidence that PKD1 activation in the heart upon α-adrenergic stimulation leads to acute increases in glucose uptake. It is known for more than two decades that α-agonists, such as epinephrine (28) and phenylephrine (29), induce both glucose uptake and GLUT4 translocation in cardiomyocytes in an insulin-independent manner. It is presumed that the α-agonists use the canonical activation pathway, including activation of novel PKCs and transphosphorylation of cytoplasmic PKD1 pools at Ser744/748, which then migrate to endosomes to induce GLUT4-vesicle budding.

PKD1 AND REGULATION OF LIPID UPTAKE

As stated in the introduction, CD36 is the main cardiac fatty acid transporter, and therefore a major site of regulation of lipid utilization in the heart. Interestingly, contraction-stimulated PKD1 activation does not induce CD36 translocation to the cell surface. This is remarkable because CD36 and GLUT4 are stored at least partly within the same intracellular compartments, i.e., the endosomes (4). At present it is unknown why only GLUT4 (not CD36) is sorted into transport vesicles during PKD1 activation.

At the level of regulation of CD36 expression, PKD1 activation downregulates transcription of the CD36 gene (30). This was shown in endothelial cells exposed to angiogenic growth factors, thereby activating PKD1 via the canonical pathway including PKCs. Subsequently, PKD1 increases phosphorylation and nuclear accumulation of HDAC7, which then associates with the transcription factor FoxO1 and depresses CD36 transcription (30). Whether PKD1 activation also directly decreases expression of CD36 in the heart via this pathway is not known, but seems likely. Specifically, in hypertrophic hearts from rodents that have undergone a transverse aortic constriction, there is an upregulation of PKD1-Ser916 phosphorylation (31) and also a downregulation of CD36 expression (32). Hence, it appears that PKD1 activation (acutely) upregulates cardiac glucose utilization and (chronically) downregulates cardiac fatty acid uptake and utilization.

In contrast, another report has suggested that PKD1 activation leads to increased cardiac lipid utilization. This study reported that the upregulation of lipoprotein lipase (LPL) activity upon experimental induction of type-1 diabetes in rats is PKD1 dependent (33). LPL mediates the hydrolysis of triacylglycerol-rich lipoproteins to fatty acids, thereby contributing to increased fatty acid delivery to the heart. PKD1 activation in these diabetic animals was due to phosphorylation of heat shock protein-25 (HSP25) by unknown mechanisms, thereby dissociating from and de-inhibiting the novel PKC isoform PKCδ, subsequently resulting in PKD1-Ser744/748 transphosphorylation. The latter was associated with secretion of LPL, in line with the role of PKD1 in Golgi transport and vesicle budding (21). However, it has not been assessed whether PKD1-mediated LPL secretion leads to increased cardiac fatty acid utilization. Such PKD1-mediated LPL upregulation would contrast the PKD1 mediated downregulation of CD36. The apparent paradox may be solved by the possibility that these opposing actions of PKD1 may not operate under the same circumstances. For instance, LPL upregulation may be confined to specific animal models of type-1 diabetes, while CD36 downregulation may be a characteristic feature of the pressure-overloaded heart.

PKD1 AND REGULATION OF MYOFILAMENT CONTRACTILITY

In a yeast-two hybrid screen of a human cardiac library using a catalytically inactive mutant of the catalytic domain of PKD1 as interaction partner, several myofilament proteins were identified as potential downstream targets, including troponin-I (TnI), telethonin, and cardiac myosin-binding protein-C (cMyBP-C) (34). This implemented PKD1 as potential regulator of myocyte contractility. Some of these phosphorylation sites overlap with protein kinase-A (PKA) phosphorylation sites, suggesting that PKD activation, just like β-adrenergic stimulation, leads to increased cross-bridge cycle kinetics and increased force development [see review (13)]. The phosphorylation of TnI by PKD1 at Ser22/23 has been shown to alter myofilament Ca^{2+} sensitivity (35). This phosphorylation appears to be a monophosphorylation, and not a bisphosphorylation as mediated by PKA (36). The phosphorylation of cMyBP-C by PKD1 at Ser315 does not alter myofilament Ca^{2+} sensitivity but increases maximal tension of contraction. Thus, the combined actions of PKD1 on distinct myofilament proteins fine-tune the increase in force development during times of increased cardiac contractile activity.

It is not yet known which of the PKD activation routes are involved in contraction-induced phosphorylation of myofilament proteins. Most likely it is a ROS-induced signaling pathway. It could be the ROS-activated Abl/Src pathway leading to PKD1-Tyr95/463 phosphorylation, although activation of PKD1 in an Abl/Src dependent manner has not yet been investigated in the heart. Alternatively, DAPK could be involved in these contractile protein phosphorylations. In relation to GLUT4 translocation, DAPK would selectively activate the endosomal PKD1 population to induce endosomal vesicle budding. For contractile protein phosphorylation, DAPK would additionally need to activate cytoplasmic pools of PKD1 for subsequent migration to the contractile apparatus. This topic requires further studies.

PKD1 AND CARDIAC HYPERTROPHY

The canonical PKD1 activation pathway has been implicated in the pathological remodeling events in the heart that occur in response to hypertension. During hypertension, a number of GPCR agonists is elevated such as endothelin and several catecholamines, which activate PKD1 in a PKC-dependent manner and via phosphorylation at Ser744/748, i.e., the canonical PKD1 pathway. A key substrate of PKD1 in hypertension-induced cardiac remodeling is histone deacetylase-5 (HDAC5), a member of the HDAC family negatively regulating the acetylation status of nucleosomal histones, and thereby repressing transcription. Moreover, HDAC5 is a negative regulator of cardiac remodeling via binding to the myocyte enhancer factor-2 (MEF2). MEF2 transcription factors contain a MEF2-specific domain and an adjacent dimerization domain (named MADS box) (37), allowing these transcription factors to bind as dimer to specific A/T-rich sequences in enhancer regions of a number of fetal genes. Increased transcription of these genes in the adult heart results in a hypertrophic phenotype. PKD1 phosphorylation of HDAC5 induces HDAC5 binding to the adaptor protein

14-3-3 and subsequent disassociation from MEF2. This de-repression of MEF2 then switches on the fetal gene program. The precise mechanisms controlling nuclear import of Ser744/748-transphosphorylated PKD are incompletely understood, but do include another adaptor protein, AKAP13, from the A-kinase-anchoring proteins (AKAPs) acting as scaffolds for signaling proteins. AKAP13 is predominantly expressed in the heart and mediates PKD1 phosphorylation by bringing PKD1 and PKC in close proximity (38). AKAP13 is also involved in subsequent transport of PKD1 into the nucleus. Ablation of the PKD1 binding domain of AKAP13 leads to a decreased hypertrophic response upon hypertrophic stimulation. Interestingly AKAP13 expression is increased upon the onset of cardiac hypertrophy (38).

Recently, another mechanism via which PKD1 may contribute to cardiac hypertrophy, has been revealed by using the transverse aortic constriction (TAC)-induced cardiac hypertrophy mouse model (39). This novel mechanism includes an inhibitory action of PKD1 on autophagy via upregulation of the Akt/mTOR pathway.

As expected, PKD1 activation is indeed observed in the hypertrophic heart, as in mice that have undergone TAC surgery, there is a marked increase in PKD1 phosphorylation (31, 39). Powerful genetic evidence for a crucial role of PKD1 in cardiac hypertrophy comes from studies with mice with cardio-specific overexpression of a constitutively active PKD1 mutant. These mice develop left ventricular chamber dilatation, myocyte hypertrophy, and wall thinning already early in life together with a severe impairment of fractional shortening (40).

PKD2 AND PKD3 AND THEIR ROLES IN CARDIAC ENERGY METABOLISM AND PATHOPHYSIOLOGY

PKD1 is by far the most abundant isoform in the heart, but the other two isoforms are also present and appear to fulfill several non-redundant roles in cardiac metabolism and pathophysiology (41). PKD2 most closely resembles PKD1 as it also contains the C-terminal auto-phosphorylation site, i.e., at Ser876, while also the flanking amino acid sequences are conserved. In contrast, the N-terminal sequences are highly variable between PKD1 and PKD2 (and also PKD3), which might explain the agonist-specific regulation of each isoform. For instance, PKD2 is not activated by the α-agonist norepinephrine (41). Moreover, PKD2, but not PKD1, auto-phosphorylation is upregulated in hearts of *db/db* mice, a mouse model of diabetic cardiomyopathy (42). Remarkably, increased PKD2 phosphorylation was not accompanied by changes in phosphorylation of HDAC5, the classical PKD1 substrate so that the observed hypertrophy is not due to activation of the MEF2-hypertrophic program. Treatment of *db/db* mice with the pan-PKD inhibitor CID755673 decreased the cardiac hypertrophy and restored cardiac function, but strangely did not affect HDAC5 phosphorylation (42). Unfortunately, this study did not describe cardiac function in *db/db* mice in which the PKD2 gene was ablated, so that off-target actions of this drug cannot be excluded. In contrast to *db/db* mice, increased PKD1 and PKD2 auto-phosphorylation was not detected in mice fed a Western (45 en% fat) diet, another model of diabetic cardiomyopathy (23). Hence, the role of PKD2 in the development of cardiac hypertrophy in *db/db* mice awaits further investigations.

PKD3 is more different from PKD1 than PKD2, as it is missing the C-terminal autophosphorylation domain, and therefore displays a slightly smaller molecular mass (90 vs. 115 kDa for PKD1 and 105 kDa for PKD2). Remarkably just like PKD1, PKD3 is also involved in regulation of glucose uptake, as established in skeletal muscle cell lines (42), but not yet confirmed in the heart. However, PKD3's involvement is different: in contrast to PKD1, PKD3 is not regulating stimulus-induced GLUT4 translocation and glucose uptake, but regulates the subcellular localization of GLUT1 and basal glucose uptake (43). Also remarkably, PKD3, just like PKD1, is involved in mediating the morphological and functional changes as seen during the development of cardiac hypertrophy. The transcription factor MEF2, which is upregulated upon PKD1 activation, is not the only transcription factor mediating the full pleiotropy of changes displayed by the hypertrophic heart. Also other transcription factors contribute to these changes, among which nuclear factor of activated T-cells (NFATc4), NK family of transcription factor 2.5 (Nkx2.5) and GATA4 [for review see (44)]. In isoproterenol-induced hypertrophy, PKD3 is substantially upregulated, and this upregulation is required for increased expression of the latter three transcription factors, whereas PKD3 does not regulate MEF2 (45). The upregulation of PKD3 expression in isoproterenol-treated neonatal cardiomyocytes is due to activation of the Ca^{2+}-sensitive phosphatase calcineurin upon increased Ca^{2+} levels. Calcineurin then stimulates the nuclear import of the transcription factors NFATc1 and NFATc3, followed by upregulation of PKD3 expression (45). It remains to be investigated if this PKD3 involvement, as seen in neonatal cardiomyocytes, can be reproduced in the adult heart.

In conclusion, the role of PKD2 in cardiac metabolism and remodeling is still unclear, whereas PKD3, in conjunction with PKD1, appears to regulate both glucose uptake and cardiac growth in a non-redundant manner.

INTEGRATION OF ROLES OF PKD1 AND FAMILY MEMBERS IN CARDIAC ENERGY METABOLISM AND PATHOPHYSIOLOGY

From the evidence collected over the last two decades there now is consensus that the PKD family—especially PKD1—plays a key role in cardiac metabolism, morphology and function. First of all, upon an acute increase in cardiac workload as occurs at the onset of physical work (e.g., exercise), PKD1 becomes rapidly activated, leading to the combined phosphorylation of contractile proteins and of a protein component of the vesicle budding machinery in the recycling endosomes. This then leads to combined increases in contractile force and in GLUT4-mediated glucose uptake. Hence, PKD1 synchronizes the increased mechanical performance of the heart with the increased energetic demands

in order to appropriately react to an acute increase in physical work.

In situations that PKD1 is chronically activated, such as during chronic stress or increased blood pressure, or a combination thereof, it activates in combination with PKD3 a set of transcription factors with established involvement in hypertrophic growth of the heart. The activation of MEF2 is not the only action of PKD1 involved in cardiac hypertrophy. As mentioned in sections "PKD1 and Regulation of Glucose Uptake" and "PKD2 and PKD3 and Their Roles in cardiac Energy Metabolism and Pathophysiology," PKD1 and PKD3 activation leads to increased glucose uptake. Hence, upon chronical stimulation of GLUT4 translocation and GLUT1 expression by PKD1 and PKD3, respectively, glucose uptake markedly increases and feeds forward to increased glycolytic rates, increased accumulation of glucose metabolites and of glucose-mediated post-translational modifications of proteins (glycosylation and O-GlcNAcylation). Especially protein O-GlcNAcylation is currently regarded as a sensitive indicator of glucose fluxes (46), and is observed to be increased in the hypertrophic heart (47, 48). Among the O-GlcNAcylated proteins are transcription factors, as observed during increased glucose fluxes in cancer cells (49). Possibly, the PKD1/PKD3-activated hypertrophic transcription factors may also undergo increased O-GlcNAcylation, thereby installing a vicious cycle of PKD-mediated actions in the heart on its way to hypertrophy. Specifically, the transcription factor O-GlcNAcylations following increased PKD1/PKD3-mediated myocellular glucose uptake may reinforce the direct activation of these transcription factors by PKD1/PKD3 resulting from PKD1/PKD3 translocation to the nucleus (**Figure 2**).

On top of this, PKD1 activation decreases CD36 expression in a FOXO1-dependent manner (see section "PKD1 and Regulation of Lipid Uptake"), which further may contribute to the development of cardiac hypertrophy. The consequently decreased fatty acid uptake rates will lead to de-activation of lipid-induced transcription factors such as members of the family of peroxisome proliferator-activated receptors (PPARs) (50). Because, especially PPARα activation has been shown to be cardio-protective in the hypertrophic heart (51), de-activation of this transcription factor may further worsen the metabolic and energetic state of the hypertrophic heart.

Considering these various PKD actions together in a chronic setting, it becomes increasingly evident that the PKD family is a major novel player in the development of cardiac hypertrophy. This notion is boosted by the observation that PKD1 overexpression in the heart is sufficient by itself for the development of a severe form of cardiac hypertrophy. For future research, it may be of interest to investigate which of these PKD1-stimulated pathways is ultimately responsible for this hypertrophy. It is also conceivable that either pathway by itself is not sufficient, and it is the combination of all these PKD1/PKD3-stimulated pathways that drives maladaptive hypertrophic growth.

Based on the foregoing, the hypertrophic heart displaying a substrate switch toward increased glucose utilization might benefit from drugs that specifically inhibit PKD1, especially in advanced stages of cardiac hypertrophy (pathological remodeling). This would counteract the hypertrophic programming and the chronic GLUT4 translocation, thereby giving the heart the ability to switch back to lowered glucose and increased fatty acid utilization. On the other hand, pharmacological drugs that would specifically induce PKD1 activation might be beneficial for the treatment of the condition of diabetic cardiomyopathy. This is especially relevant since heart failure is the leading cause of death in type-2 diabetic patients. The type-2 diabetic heart is characterized by massive lipid accumulation which over time induces insulin resistance, decreased glucose utilization and ultimately loss of contractile force [for review see (52)]. PKD1 activation may reverse the state of decreased glucose utilization, by forcing GLUT4 translocation independently of the defective insulin signaling pathway. This would be expected to also normalize fatty acid uptake via Randle cycle-mediated inhibition (53). Examples in rodent studies that this strategy might be successful include the observation that cardio-specific PKD1 overexpressor mice are resistant to develop insulin resistance upon a Western (45 en% fat) diet (23). Furthermore, PKD1 activation would also explain the observation that TAC surgery restored cardiac substrate uptake in *db/db* mice, a mouse model of type-2 diabetes displaying cardiac lipid accumulation and decreased glucose uptake (31). In this model the chronic PKD1 activation following pressure overload would restore glucose uptake to subsequently lead to normalization of the elevated fatty acid fluxes.

In conclusion, PKD is expected to be an attractive target to treat the failing heart whereby it would be dependent on the specific metabolic state of the heart whether to use PKD inhibiting drugs vs. PKD activating drugs. As a result, PKD offers a potential target for personalized medicine.

AUTHOR CONTRIBUTIONS

OS and JL wrote the first draft of the manuscript. AS, JG, and MN reviewed and edited the manuscript. Final editing was done by MN. All authors read and approved the submitted version.

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REVIEW
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Loss of Metabolic Flexibility in the Failing Heart

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To maintain its high energy demand the heart is equipped with a highly complex and efficient enzymatic machinery that orchestrates ATP production using multiple energy substrates, namely fatty acids, carbohydrates (glucose and lactate), ketones and amino acids. The contribution of these individual substrates to ATP production can dramatically change, depending on such variables as substrate availability, hormonal status and energy demand. This "metabolic flexibility" is a remarkable virtue of the heart, which allows utilization of different energy substrates at different rates to maintain contractile function. In heart failure, cardiac function is reduced, which is accompanied by discernible energy metabolism perturbations and impaired metabolic flexibility. While it is generally agreed that overall mitochondrial ATP production is impaired in the failing heart, there is less consensus as to what actual switches in energy substrate preference occur. The failing heart shift toward a greater reliance on glycolysis and ketone body oxidation as a source of energy, with a decrease in the contribution of glucose oxidation to mitochondrial oxidative metabolism. The heart also becomes insulin resistant. However, there is less consensus as to what happens to fatty acid oxidation in heart failure. While it is generally believed that fatty acid oxidation decreases, a number of clinical and experimental studies suggest that fatty acid oxidation is either not changed or is increased in heart failure. Of importance, is that any metabolic shift that does occur has the potential to aggravate cardiac dysfunction and the progression of the heart failure. An increasing body of evidence shows that increasing cardiac ATP production and/or modulating cardiac energy substrate preference positively correlates with heart function and can lead to better outcomes. This includes increasing glucose and ketone oxidation and decreasing fatty acid oxidation. In this review we present the physiology of the energy metabolism pathways in the heart and the changes that occur in these pathways in heart failure. We also look at the interventions which are aimed at manipulating the myocardial metabolic pathways toward more efficient substrate utilization which will eventually improve cardiac performance.

Keywords: fatty acid oxidation, glucose oxidation, ketone oxidation, cardiac metabolism, heart failure, insulin resistant

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INTRODUCTION

Heart failure is a major cause of death and disability, with more than 26 million people diagnosed with heart failure worldwide (1). The mortality rate of heart failure is approximately 30% within 5 years following diagnosis (2). Thus, heart failure presents a tremendous burden on society, the health care system and the economy (3, 4). While pharmacological management, primary

prevention and earlier diagnosis have dramatically improved in the last 20 years, there is still a high morbidity and mortality associated with heart failure.

Different energy substrates, namely fatty acids, carbohydrates (glucose and lactate), ketones and amino acid, contribute differently to meet the high energy demand of the heart. Fatty acid oxidation is the biggest contributor to ATP production (∼40–60%) while carbohydrates metabolism generates the remainder (∼20–40%). The heart has a "metabolic flexibility," a virtue which allows it to switch between these energy substrates according to the workload, availability of the substrates, and the hormonal status. There is a general consensus that this metabolic flexibility is impaired in heart failure which affects ATP production and, consequently, cardiac contractility. Another important dimension of the metabolic flexibility is the effect of inotropic agents on energy substrates mobilization and use which occurs in response to an increase in heart work. It has been demonstrated that adrenergic stimulation, using epinephrine, enhances glycogenolysis and increases glucose oxidation in the normal rat heart (5, 6). Nevertheless, whether this form of metabolic flexibility is also impaired in the failing heart has yet to be characterized. As a disease, the complexity of heart failure is represented by differences in etiology, severity and the presence of comorbidities, such as hypertension, diabetes and obesity. Despite its diverse nature, the failing heart is characterized by its energy deficient state, as evidenced by a 30–40% reduction in ATP production and reduced phosphocreatine/ATP (PCr/ATP) ratio (7–10). This energy-starved state may be due to mitochondrial dysfunction as a result of reactive oxygen species generation, compromised bioenergetics and impaired mitochondrial fission and fusion [see De Jong et al. (11) and Neubauer (10) for reviews]. Furthermore, mitochondrial dysfunction also directly affects myocardial energy substrate metabolism further aggravating the energy crisis. Alterations in myocardial energy substrate metabolism are discernible in the failing heart as a result of compromised mitochondrial oxidative phosphorylation [see Lopaschuk et al. (12) for review]. This results in an increased reliance on glycolysis and decreased glucose oxidation to produce the required energy (13, 14). While ketone oxidation increases in the failing heart (15–17), fatty acid oxidation has been suggested to decrease (10, 18–20), or not change (13, 21). Regardless, these metabolic shifts in heart failure can negatively affect cardiac contractility and myocardial energy management, leading to decreased metabolic flexibility at a time when the heart needs it most. While the pathological consequences of changes to fatty acid and glucose metabolism have been extensively studied, the alterations and implications of altered ketone body metabolism during heart failure remain unclear even though the heart uses the most ketone bodies per unit mass (22, 23). Lastly, there is an increasing amount of evidence suggesting that restoring metabolic flexibility by enhancing glucose oxidation directly (24) or indirectly through inhibition of fatty acid oxidation (25) could improve myocardial ATP production, improve cardiac function and limit cardiac remodeling (12, 13, 26, 27).

This review will focus on the alterations in fatty acid oxidation, carbohydrate metabolism and ketone metabolism that occur in the heart in the setting of ischemic heart failure. Amino acid metabolism is out of the scope of this review and the reader is referred to other reviews about the subject (28, 29). In addition, we will illustrate how energy metabolism in ischemic heart failure is controlled by the interplay of transcriptional regulation, post-translational modifications, and cytosolic/mitochondrial signaling kinases. We will also critically appraise some of the published data and discuss the controversies surrounding energy substrate preference in the failing heart. Lastly, we will briefly discuss the advances in pharmacological interventions which target myocardial energy metabolism as an approach to treat heart failure.

CARDIAC METABOLISM IN THE HEALTHY HEART

Cardiomyocytes have a virtue of metabolic adaptability as they can omnivorously utilize different energy substrates, including fatty acids, carbohydrates (glucose and lactate), ketones and amino acids, to meet the high energy demand of the heart and to sustain contractile function. Due to this metabolic flexibility, the heart's substrate preference can rapidly change based on the availability of energy substrates supplied to the heart, hormonal status and the changing workload of the heart. Mitochondrial oxidative phosphorylation produces the majority of the high-energy phosphates needed to sustain contractile function (**Figure 1**). Of this, fatty acid oxidation is the biggest contributor to mitochondrial oxidative metabolism, providing approximately 40–60% of the total energy produced, with the oxidation of glucose, lactate, ketone and amino acids providing the remaining 20–40%. To better understand the use of each substrate, first, we will discuss glucose, fatty acid, and ketone metabolism in the normal healthy heart.

GLUCOSE METABOLISM

Locke and Rosenheim in 1907 were the first to study myocardial glucose uptake in an isolated rabbit heart model (30). Thirty years later, Albert Szent-Gyorgyi, Hans A. Krebs and William A. Johnson reported the conversion of pyruvate to succinate, unearthing the Krebs cycle (31). The uptake of glucose into the cardiomyocyte occurs though insulin-independent (GLUT1) and insulin-dependent (GLUT4) transporters. Transported glucose is first phosphorylated by hexokinase to glucose-6-phosphate (G-6-P), which can then undergo different metabolic pathways. G-6-P can be used as a substrate for glycolysis to produce pyruvate, NADH and 2 ATP (**Figure 1**) or it can enter the hexosamine biosynthesis pathway. In addition, G-6-P can be used for glycogen synthesis or shuttled to the pentose phosphate pathway (32). Glycolysis-derived pyruvate can either be converted to lactate by lactate dehydrogenase (LDH) or alternatively transferred to the mitochondria matrix by the mitochondrial pyruvate carrier (MPC) (33) (**Figure 1**). In the mitochondrial matrix, pyruvate dehydrogenase (PDH), the rate limiting enzyme of glucose oxidation, catalyzes the conversion of pyruvate to acetyl CoA which feeds into the tricarboxylic acid (TCA) cycle. Mitochondrial pyruvate can also supply the

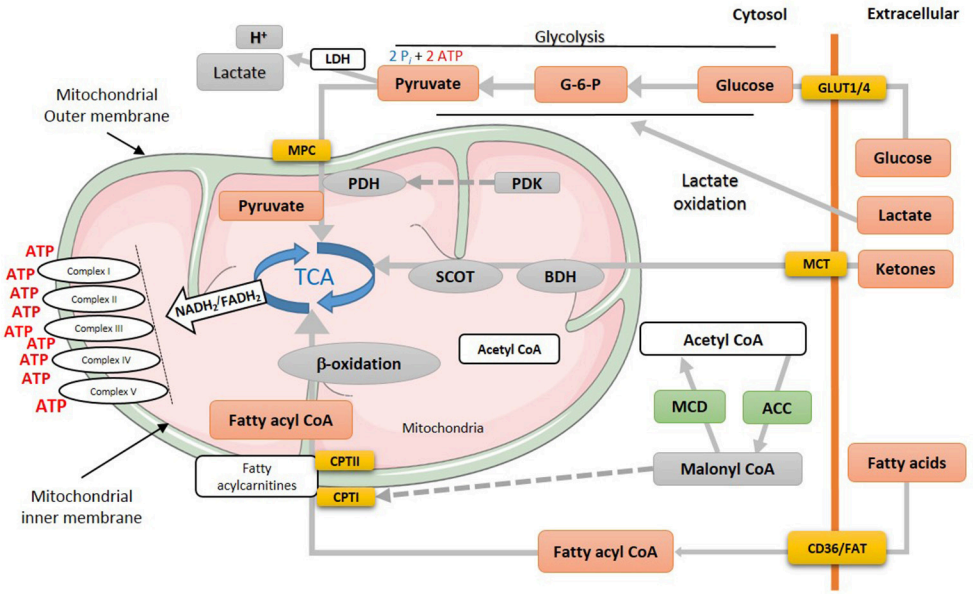

FIGURE 1 | Energy metabolism in normal heart. Various metabolic pathways contribute to mitochondrial ATP production in the heart. Mitochondrial ATP production uses mostly fatty acids, glucose and ketones as a fuel source. The production of acetyl CoA by fatty acid ß-oxidation first requires the mitochondrial uptake of fatty acids via a carnitine carrier system. Oxidation of glucose involves the production of pyruvate via glycolysis, which produces acetyl CoA for the TCA cycle via PDH. Acetyl CoA production by ketone body oxidation is facilitated by BDH and SCOT. MPC, mitochondrial pyruvate career; PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; TCA, tricarboxylic acid cycle; SCOT, succinyl-CoA-3-oxaloacid CoA transferase; BDH, β-hydroxybutyrate dehydrogenase; CPT, Carnitine palmitoyltransferase; MCD, malonyl CoA dehydrogenase; ACC, acetyl CoA carboxylase; MCT, monocarboxylate transporter; CD36, cluster of differentiation; FAT, fatty acid translocase; GLUT, glucose transporter type (1 or 4).

TCA cycle with essential intermediates, namely oxaloacetate and malate, via carboxylation. The enzymatic activity of PDH can be inhibited through phosphorylation by PDH kinase (PDK) and be reactivated by PDH phosphatase (34).

In terms of energy producing efficiency, oxidation of each glucose molecule requires 6 O_2 molecules and produces 31 high-energy phosphate (ATP) molecules. Accordingly, glucose's phosphate/oxygen (P/O) ratio is 2.58, making glucose the most efficient energy substrate. Glucose metabolism also produces two high energy phosphate (P_i) molecules by converting glucose to pyruvate through glycolysis (**Figure 1**).

FATTY ACID OXIDATION

Beta-oxidation of fatty acids was first proposed by a work of Knoop (35) which was later verified by a following study by Dakin (36). After a half century, Richard Bing and colleagues discovered that the human heart prefers fatty acids for respiration (37). Later in 1963, Philip Randle and his group (38) proposed that glucose and fatty acids are working in a concert to meeting the high energy demand of the heart, which is later known as the Randle cycle. The source of fatty acids for mitochondrial oxidative metabolism originates from circulating free fatty acids (FFAs) bound to albumin and/or released from triacylglycerols (TAG) contained in chylomicrons or very-low-density lipoproteins (VLDL) (39–41). Some fatty acids also originate from intracellular TAG stores (42). Extracellular fatty acids are transported to the cardiomyocytes through passive diffusion or via the fatty acid transport protein (FATP) or fatty acid translocase (FAT, CD36) (39, 43, 44). Once FFAs are in the cytosol, they are esterified to long-chain fatty acyl CoA via an ATP-dependent pathway initiated by a family of fatty acyl-CoA synthase enzymes (45). The majority (90%) of long-chain fatty acyl CoAs are shuttled directly to the mitochondria, facilitated by carnitine palmitoyltransferase isomers (CPTI and CPTII), with the remainder stored in the myocardial TAG reservoir. CPTI converts long chain fatty acyl CoAs to long chain acylcarnitine in the outer mitochondrial membrane which can then be converted back to long chain fatty acyl CoA by CPTII in the inner mitochondrial membrane (27, 46–48). Flux of fatty acids through CPT1 is regulated by malonyl CoA, a potent inhibitor of CPT1 (49–51). Malonyl CoA levels in turn are primarily dependent on a balance between acetyl CoA synthesis by acetyl CoA carboxylase (ACC) (52–54) and degradation by malonyl CoA decarboxylase (MCD) (27, 55, 56) (**Figure 1**). It is worth mentioning that via a carnitine shuttle system, mitochondrial acetyl CoA can be shuttled back to the cytosol to further affect malonyl CoA levels (57). Additionally, citrates produced from the TCA cycle can also translocate to the cytosolic compartment where it can activate ACC1, produce more malonyl CoA, and send a feedback response to decrease fatty acid uptake into the mitochondria (27).

In terms of ATP production efficiency, the oxidation of a representative fatty acid molecule, namely palmitate, consumes 23 molecules of O_2 and produces 105 high-energy phosphate (ATP) molecules. This means that fatty acids are the least efficient energy substrate with a P/O ratio of 2.33 compared to glucose

oxidation (2.58). Moreover, esterification of fatty acid to fatty acyl CoA requires 2 ATP-derived P_i. Similarly, cytosolic processing of fatty acid to fatty acyl CoA or TAG as well as reconverting TAG to fatty acid also consumes 2 P_i. This further reduces the total energy profit from fatty acid oxidation.

KETONE BODY OXIDATION

Ketone bodies are produced during times of fasting and starvation via hepatic ketogenesis, producing three types of ketone bodies: acetone, β-hydroxybutyrate (βOHB) and acetoacetate. Acetone, present in low abundance, is excreted by exhalation while βOHB and acetoacetate are the main ketone bodies circulating in our blood (58, 59). The predominant amount of ketogenesis occurs in the liver and the heart itself cannot produce ketones. Therefore, once circulating ketones reach the heart, ketones can enter the cardiomyocytes and be transported to the mitochondrial matrix where βOHB is oxidized into acetoacetate by βOHB dehydrogenase (BDH1), after which acetoacetate is activated by succinyl-CoA to acetoacetyl-CoA by the rate limiting enzyme, succinyl-CoA:3-oxoacid-CoA-transferase (SCOT) which is encoded by the gene *Oxct1*. Lastly, acetyl-CoA acetyltransferase converts acetoacetyl-CoA into acetyl-CoA (60, 61). Acetyl-CoA can then enter the tricarboxylic acid cycle and electron transport chain to generate ATP. Regulation of ketone oxidation enzymes in the heart remains poorly understood although hepatic BDH1 has been shown to be post-translationally modified by SIRT5 (62) and cardiac SCOT may be regulated by residue-specific nitration in the settings of diabetes (63, 64) and aging (65).

Ketone body as a fuel for the heart has long been recognized (37, 66). Barnes and Waters along with their associates (67, 68) first demonstrated that the heart can use ßOHB in 1938. In 1965, Rudolph et al. (69) showed that ßOHB and acetoacetate accounted for 2.6% ± 0.3% and 6.0% ± 1.0%, respectively, of the heart's total oxidative metabolism. Despite minor contribution to the total ATP production compared to fatty acids or glucose (12), myocardial oxidation of ketones can increase significantly in response to changes in the arterial concentration (69). Therefore, blood ketone levels are a key factor in determining myocardial ketone body oxidation rates and influencing the heart's metabolic flexibility. Lastly, it is important to consider, since the P/O ratio of ßOHB, is 2.50, this would make ketones less efficient than glucose but more efficient than palmitate.

CARDIAC METABOLISM IN THE FAILING HEART

In heart failure, cardiac energy metabolism is compromised due to impaired cardiac ATP production, metabolic flexibility, mitochondrial TCA cycle activity and overall oxidative metabolism (9, 70, 71). More specifically, impaired mitochondrial function and oxidative capacity (10, 19, 72) results in reduced ATP production by up to 40% compared to the normal heart. In humans, the phosphocreatine/ATP ratio has been shown to decrease in heart failure patients (73–75), alongside impaired mitochondrial electron transport chain activity (19, 76). However, the exact energy metabolic profile of heart failure remains controversial as there is still discrepancy regarding substrate preferences. For example, both myocardial fatty acid, glucose, and ketone body oxidation rates have been shown to vary depending on the heart failure model used and the duration of heart failure. Other molecular changes may also contribute to the diverse nature of heart failure energy metabolic pathology. This includes changes to transcriptional control, post-translational modifications, and mitochondrial biogenesis, all of which occur in response to heart failure's metabolic inflexibility.

GLUCOSE METABOLISM IN THE FAILING HEART

As discussed, the ischemic failing heart has a considerable energy deficit (30–40%) due to impaired mitochondrial oxidative metabolism, as well as due to the fact that there is a shortage in oxygen transport and an increased reliance on less efficient energy substrates which have a low O_2/ATP ratio (9, 26). Importantly, other forms of heart failure, including non-ischemic failing heart, might not have the same oxygen deficit as was shown in animals (77) and human (78). This could potentially have implications on substrates metabolism, but it is out of the scope of this review. While there is a general consensus that the heart switches from fatty acid oxidation to glucose metabolism to produce energy in heart failure, we believe it is more plausible to state that the failing heart switches from mitochondrial oxidative phosphorylation to glycolysis as a main source of energy.

Glycolysis

Glycolysis is anaerobic metabolic process which its rates increase in the failing heart (18) (**Figure 2**). Direct measurements of myocardial glycolytic rates showed a marked increase in both abdominal aorta constriction (ACC)- and transverse aortic-constriction (TAC)-induced heart failure animal models (21, 79–81). In patients with heart failure with reduced ejection fraction (HFrEF), there is a marked increase in glucose uptake and glycolysis that is associated with an increased lactate and pyruvate accumulation (82). This increase in glycolysis and the accumulation of glycolysis by-products, namely lactate, and proton, is also seen in heart failure in Dahl salt-sensitive rats fed a high salt diet (83), and in patients with heart failure (82). While glycolysis provides an anaerobic source of ATP, the accumulation of glycolysis-derived protons in the cytosol can contribute to acidosis which can reduce cardiac contractility, as it desensitizes contractile proteins to Ca^{2+} and inhibits the slow Ca^{2+} current (27, 84). It also leads to Na^{+} and Ca^{2+} overload as cardiomyocytes attempt to extrude excess proton to the extracellular space. It can also lead to an ionic imbalance which can aggravate the energy deficit seen in heart failure, as the already limited cardiac ATP supply is shifted toward re-establishing ionic homeostasis.

An important point to consider when measuring myocardial glycolytic rates is the animal model used. Employing murine hearts to address myocardial energy metabolism has increased

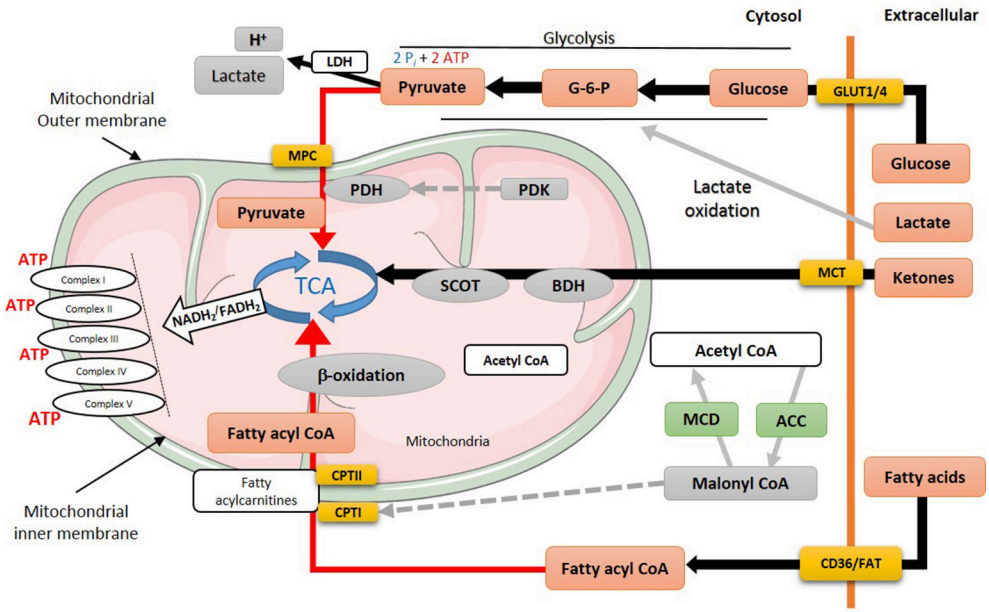

FIGURE 2 | Energy metabolism in heart failure. During heart failure, overall mitochondrial oxidative metabolism and electron transport chain activity is compromised. Increased flux is indicated by black lines, while red lines indicate impaired utilization of different substrates. MPC, mitochondrial pyruvate career; PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; TCA, tricarboxylic acid cycle; SCOT, succinyl-CoA-3-oxaloacid CoA transferase; BDH, β-hydroxybutyrate dehydrogenase; CPT, carnitine palmitoyltransferase; MCD, malonyl CoA dehydrogenase; ACC, acetyl CoA carboxylase; MCT, monocarboxylate transporter; CD36, cluster of differentiation; FAT, fatty acid translocase; GLUT, glucose transporter type (1 or 4).

dramatically due to the flexibility mice provide for knockdown or overexpression of target genes. The murine model, however, may not be ideal in cases where myocardial glycolytic measurements are required, especially if glycolytic rates are differentially affected due to a pathological disease. For example, heart failure-induced up-regulation in glycolysis rate is not detectable if the mouse heart is used as the model to study heart failure (13, 14, 83). This is probably due to the fact that the mouse heart is highly glycolytic compared to other animals models (85) and this high glycolysis is already saturating its contribution to overall ATP production (∼20%). Therefore, differences in energy substrates utilization between different animal models need to be considered (86, 87). Nevertheless, other studies have also reported a marked increase in glycolysis rate in other animal models of heart failure (80, 88–90).

One of the characteristic changes in heart failure is the development of insulin resistance which has been shown *in vivo* (13, 14, 91–95) and in human (96–98). Insulin regulates glucose uptake by enhancing GLUT4 translocation (99, 100) and increases glycolysis (101–103). In insulin resistance in heart failure, the heart switches to GLUT1 to take up glucose. Despite this impaired insulin signaling, glycolysis is increased in the failing heart.

Glucose Oxidation

We and others have reported that impairment of glucose oxidation is a metabolic marker that precedes the development of cardiac dysfunction in different animal models of heart failure (14, 94). Although glycolysis rates increase in heart failure, this does not necessarily translate into an increase in glucose oxidation since glycolysis and glucose oxidation are differentially regulated in the heart (104). The majority of studies directly examining the failing heart's glucose oxidation rates in humans and animals show a marked decrease in glucose oxidation in the failing heart, and a reduced contribution of glucose oxidation to overall ATP production (13, 14, 91, 92, 94, 96, 98, 105). A study by Diakos et al. (82) also demonstrated that the increase in cardiac glycolysis seen in severe heart failure patients was not accompanied by an increase in lactate and pyruvate accumulation, suggesting that the increase in glycolysis is not matched by an increase in glucose oxidation. In support of this, Paolisso et al. (96) reported an abrogated rate of glucose oxidation in patients with congestive heart failure. Furthermore, impairment of pyruvate oxidation in transgenic mice is associated with the development of left ventricular hypertrophy (89), emphasizing the relationship between maintained glucose oxidation and normal cardiac function. In support of this, Kato et al. (106) showed that in Dahl salt sensitive rats with heart failure (which have high cardiac glucose uptake and glycolysis), stimulating PDH with dichloroacetate improved heart function and decreased lactate production (presumably due to an increase in glucose oxidation). Combined, these studies suggest an important role of cardiac metabolic inflexibility, which occurs in heart failure, with regards to glucose oxidation in mediating heart failure severity.

While the majority of studies suggest a decrease in glucose oxidation in the failing heart, not all studies are consistent with this finding. The extent of reduction in cardiac glucose oxidation

in heart failure varies according to the severity of heart failure, as well as the experimental model of heart failure used and the availability of other energy substrates. In a rat model of transverse aortic constriction (TAC), for instance, Doenst et al. (107) showed that glucose oxidation rates remained unchanged in a rat model of compensated heart failure (due to mild TAC) glucose oxidation was only reduced after systolic dysfunction occurred. Whether the slow development of diastolic dysfunction over a relatively long period of time in animal models has an impact on energy metabolism changes needs further investigation. In support of this, Zhang et al. (14) also reported that glucose oxidation rate was only decreased as an early sign of cardiac dysfunction in another mild heart failure model induced by AAC. As the severity of heart failure increases, this will have a direct impact on glucose oxidation rate. This was shown in a number of studies where a mouse model of pressure-overload-heart failure with severe cardiac dysfunction showed a marked impaired glucose oxidation rates (93–95). However, Osorio et al. (108) reported that glucose oxidation rate was increased in a canine cardiac pacing model of heart failure which, however, contrasts the decrease in glucose oxidation seen in pacing-induced heart failure seen in pigs (88). Unfortunately, cardiac glycolytic rates were not measured in the study by Osorio et al. (108). In a TAC model of murine heart failure, an increase in the proportion of glucose oxidized by the hearts was also seen (109). The reason for these discrepant findings is unclear. However, it should be acknowledged that high pyruvate supply from increased glycolysis has the potential to augment glucose oxidation (110). Indeed, the study by Kolwicz et al. (109), which suggests that glucose oxidation was increased in TAC hearts from mice, also observed a marked increase in lactate and alanine production, indicative of increased glycolysis rates. Furthermore, while pacing-induced heart failure in dogs showed an increase in glucose oxidation (111), cardiac function was markedly low in these dogs [left ventricular work (LVW) was 148 $\pm$ 28 Hg*mm while normal LVW is 800–900 Hg*mm]. In severe heart failure, it is possible that mitochondrial calcium control is compromised, potentially leading to mitochondrial calcium accumulation, and activation of the PDH complex due to calcium activation of PDH phosphatase (112).

The reduction in glucose oxidation that occurs in the failing heart is due, in part, to an overall deterioration in mitochondrial oxidative capacity, as well as due to an impaired activity of PDH, the rate-limiting enzyme for glucose oxidation (13, 14, 92, 98). There is also some evidence that the machinery of glucose oxidation could be impaired in the failing heart (104). Gupte et al. (113) demonstrated that the mRNA expression of PDH, MCT1, and pyruvate/alanine aminotransferase, were decreased in heart failure, suggesting impairment in pyruvate metabolism. In line, Dodd et al. (89) demonstrated, using hyperpolarised ^{13}C-magnetic resonance spectroscopy, that PDH complex activity is impaired in a rat model of myocardial infarction-induced heart failure. Of importance, is that PDH complex impairment was progressive and proportional to the degree of cardiac dysfunction.

As it was discussed earlier (see Glycolysis section), it is generally accepted that insulin-induced stimulation of glucose oxidation is markedly attenuated in obesity and diabetes (114, 115), contributing to myocardial metabolic inflexibility (116). Impairment in insulin signaling and development of insulin resistant myocardium precedes cardiac dysfunction in heart failure and it is a major determine of its progression (14, 94, 117). Rutter et al. (118) showed that increased insulin resistance, which is also associated with obesity, was accompanied with worsening cardiac remodeling. In consistence, Peterson et al. (119) also, in consistence, demonstrated that insulin resistance in obese women was associated with deterioration in cardiac efficiency. Taken together, it seems plausible to suggest that insulin resistance is, at least in part, responsible for the reduction in glucose oxidation in heart failure. In a rat model of streptozotocin-induced diabetic cardiomyopathy, insulin resistance is associated with a marked decrease in PDH flux and diastolic function (120). Of interest, is that dichloroacetate (DCA), a PDK inhibitor that enhances PDH activity, treatment reversed insulin resistance, increase PDH flux and improving cardiac function (120). This further emphasizes the potential therapeutic applications of improving glucose oxidation to mitigate cardiac dysfunction in heart failure. In the same context, cardiac-specific PDHA1($^{-/-}$) knockout mice showed impaired insulin signaling which was associated with diastolic dysfunction (121). Consistent with this, Sankaralingam et al. (115) demonstrated that cardiac dysfunction and insulin resistance were both worsened by high fat diet-induced obesity in abdominal aortic constriction-induced heart failure mice model. Collectively, this further emphasizes the crucial role of insulin resistance and high circulating FFAs in the pathogenesis of heart failure.

Another mechanism by which glucose oxidation could be attenuated in heart failure is through heart failure-induced mitochondrial hyperacetylation. Hyperacetylation occurs in the failing heart (122) and hyperacetylation of PDH has previously been shown to have an inhibitory effect on its activity (123, 124). Thus, heart-failure induced hyperacetylation could inhibit PDH activity and decrease glucose oxidation rates. It has also been reported that hyperacetylation can increase the activity of fatty acid oxidation enzymes, such as LCAD and β-HAD (125). As such, enhancing the activity of these ß-oxidation enzymes could negatively feedback to inhibit glucose oxidation through the Randle cycle (38, 126).

As discussed, the failing heart has a considerable energy deficit (30–40%) due to impaired mitochondrial oxidative metabolism, as well as due to the fact that there is a shortage in oxygen transport and an increased reliance on less efficient energy substrates which have a low O_2/ATP ratio (9, 26). While there is a general consensus that the heart switches from fatty acid oxidation to glucose metabolism to produce energy in heart failure, we believe it is more plausible to state that the failing heart switches from mitochondrial oxidative phosphorylation to glycolysis as a main source of energy.

FATTY ACID OXIDATION IN THE FAILING HEART

While it is generally agreed that the failing heart has reduced cardiac energetics, mitochondrial TCA cycle activity and overall

oxidative metabolism (9, 70, 71), it is less clear whether myocardial fatty acid oxidation rates are also decreased. It is generally assumed that cardiac fatty acid oxidation is decreased in heart failure (10, 127–129), which is supported by decreased transcription of a number of enzymes involved in fatty acid oxidation (10, 128–132). However, direct measurements of fatty acid oxidation rates in both human and experimental models of heart failure do not always support this assumption.

Heart failure can be associated with an increase in circulating FFA levels due to high lipolysis rates (96, 133, 134). Of importance, is that increased level of circulating FFAs in failing heart is an important determinant of fatty acid oxidation rates in the heart. For example, decompensated heart failure patients show an increase in circulating FFAs levels, which is accompanied by enhanced myocardial fatty acid uptake and fatty acid oxidation (135, 136). In support of this, Taylor et al. (137) also demonstrated that fatty acid uptake rates are up-regulated in patients with severe heart failure (ejection fraction ∼24%), using a positron emission tomography (PET) imaging technique. In contrast, Dávila-Román et al. (73), who also using (PET) imaging, showed a decrease in circulating FFAs level in patients with non-ischaemic heart failure. Neglia et al. (75) also showed a decreased fatty acid oxidation in patients with idiopathic dilated cardiomyopathy.

Animal studies also show differing results as to what happens to fatty acid oxidation in the failing heart. Studies in mice in which heart failure was produced secondary to pressure overload or a myocardial infarction have shown that cardiac fatty acid oxidation rates are unchanged (94, 95, 110). Mori et al. (91) also showed that fatty acid oxidation rate was not changed in Ang II-induced heart failure. However, it should be recognized that these maintained rates were seen despite a decrease in cardiac function, suggesting that fatty acid oxidation per unit work may actually increase in heart failure. In addition, these studies showed that the contribution of fatty acid oxidation to total ATP production increased, due primarily to a decreased contribution of glucose oxidation to ATP production. Others have also shown that with compensatory heart failure, fatty acid oxidation enzymes are preserved (21, 79). In contrast, Byrne et al. (94) and Sung et al. (93) have shown that cardiac fatty acid oxidation rates are impaired in TAC-induced severe heart failure in mice. In rats subjected to TAC, Doenst et al. (107) also demonstrated that fatty acid oxidation rates decreased in parallel with an overall decrease in mitochondrial oxidative phosphorylation (107). Moreover, a reduction in fatty acid oxidation rates was also seen in canine models of severe heart failure (138, 139).

The issue of what happens to fatty acid oxidation in the failing heart becomes more complex in the presence of obesity and/or diabetes is present. Even in the absence of heart failure, fatty acid oxidation rates are elevated under these conditions (119, 140–142). These high cardiac fatty acid oxidation rates persist if evidence of heart failure is seen in obesity and diabetes (115, 141, 143). Furthermore a strong link between reduced cardiac efficiency and excessive reliance on fatty acid oxidation has been shown in *ob/ob* mice (141) and obese humans (119).

The reasons for the confusion as to what is happening to fatty acid oxidation in heart failure, may be related to alterations in the control of fatty acid oxidation at multiple levels, including changes in fatty acid supply to the heart, alterations in allosteric control of fatty acid oxidation, alterations in transcriptional control of fatty acid oxidation, and alterations in post-translational control of fatty acid oxidation. Increased fatty acid supply to the heart will increase fatty acid oxidation, as will the presence of insulin resistance (12). Indeed, Tuunanen et al. (144) showed that while cardiac fatty acid oxidation rates were decreased in patients with idiopathic dilated cardiomyopathy, as cardiac function deteriorated insulin resistance occurred with a subsequent increase in fatty acid oxidation rates. As discussed, in heart failure a marked cardiac insulin resistance occurs (13, 14, 92). This includes a decreased ability of insulin to inhibit fatty acid oxidation (14, 92). In the presence of obesity and/or diabetes, this cardiac insulin resistance is even more dramatic (115). As result, an increased cardiac insulin resistance in heart failure may contribute to maintaining fatty acid oxidation rates. Heart failure is often associated with impairment in insulin signaling which could have a marked impact on energy metabolism in the heart. Insulin has an inhibitory effect on fatty acid oxidation through enhancing the activity of acetyl CoA carboxylase which increases the tissue level of malonyl CoA thereby decreasing mitochondrial fatty acid uptake. Insulin-induced inhibition of fatty acid oxidation is impaired in the failing heart leading to an increase in fatty acid contribution to the total ATP production, despite being inefficient substrate during heart failure. Furthermore, inactivation of the carnitine shuttle system increases cytosolic fatty acyl CoA levels (or long chain acyl CoA) and, in addition to the accumulation of TAG and diacylglycerol (DAG), can have a negative impact on insulin signaling (14, 145, 146). For instance, excess lipid metabolites can phosphorylate serine residues on IRS-1 by activating IKK-NF-κB, JNK-AP-1, and the PKC pathway all of which can reduce glucose uptake by decreasing Akt and PI3K activity (14, 147).

Changes at the transcriptional level of genes involved in fatty acid oxidation are often cited as a key reason why cardiac fatty acid oxidation rates may be decreased in heart failure (148, 149). A down regulated gene expression of fatty acid oxidative enzyme (LCAD, MCAD) has been observed in heart failure patients, as well as during the progression of heart failure in animal models (130). Three distinct isoforms of peroxisome proliferator activated receptor [PPARα (cardiac abundant), PPARβ/δ, and PPARγ] are responsible for the transcriptional changes of fatty acid metabolic genes (150, 151). PPAR and the retinoid X receptor (RXR) complex are transferred to the nucleus to bind with a specific PPAR response element (PPRE), which is located in the target gene's promoter. An inducible PPARγ coactivator-1α (PGC-1α) is also correlated with the transcriptional activity of PPAR superfamily (149, 152). Fatty acids are the endogenous ligand of the PPARα and may activate the PPARα/PGC1α pathway for the transcriptional regulations. PPARα/PGC1α transcriptional activity has also been shown to regulate pyruvate dehydrogenease kinase 4 (PDK4), which can reduce glucose oxidation by inactivation of PDH activity (153, 154), but not glycolysis. Again in heart failure patients, cardiac PPARa expression was shown

to down regulated (113, 155). The expression of PGC-1α, important for mitochondrial biogenesis, is also down regulated in heart failure [(20, 21, 48, 156)]. In pressure-overload-induced heart failure, abnormal mitochondrial morphology and reduced mitochondrial density is seen, which is associated with altered electron transport chain proteins expression (20). Patients with heart failure also show a reduction in mitochondrial DNA contents which was accompanied by down regulation of PGC-1α-associated proteins (157). Furthermore, based on DNA microarray analysis, it has been shown that a subset of downstream gene targets of PGC-1a are also down-regulated in the failing heart, which is correlated with the reduced left ventricular ejection fraction (158). However, it is still not clear whether this attenuation in the role of PGC-1α in heart failure is enough to manipulate mitochondrial biogenesis. However, a reduction in the number of mitochondria could contribute to the changes in fatty acid oxidation observed in heart failure.

Post-translational modifications may also alter fatty acid oxidation in the failing heart This includes mitochondrial lysine acetylation, in which an acetyl group is transferred to a lysine residues or mitochondrial proteins. Acetylation can be mediated through histone and non-histone acetyl-transferase (159). Furthermore, mitochondrial acetyltransferase, namely GCN5L, has also been shown to promote acetylation (160). On the reverse reaction, sirtuins (SIRTs) act as deacetylases to reverse the effect of acetylation (161, 162). Acetylation controls the activity of number of metabolic enzymes (163). Hyperacetylation of LCAD and ßHAD results in an increase in fatty acid oxidation rates (125). In obese mice with heart failure, an elevated GCN5L expression in abdominal aortic constriction-induced heart failure is associated with increased in LCAD acetylation and an increase in fatty acid oxidation (115). Furthermore, switching to a low fat diet in obese mice showed the opposite effect on the post-translational modification associated with reduced fatty acid oxidation (115). Moreover, we also showed glucose oxidation could be inhibited through hyperacetylation in heart failure (92). Taken together, post-translational modifications may be another factor to be considered which might to explain the metabolic inflexibility during heart failure.

INSULIN RESISTANCE AND HEART FAILURE

Glucose and fatty acid metabolism are tightly controlled by insulin signaling in the heart. Evidence from clinical studies have shown a strong association between insulin resistance and cardiac dysfunction (118, 164). Moreover, patients with insulin resistance have high rates of lipolysis in adipose tissue with increases in TAG hydrolysis (164–166). Of importance, insulin resistance-induced shifts in favor of fatty acid oxidation and is associated with attenuation of glucose uptake by the heart [see review by (116)]. This change in metabolic preference was also observed in an experimental setting (13, 14, 91, 167) and clinical studies of heart failure (82, 88). Of interest, insulin resistance precedes any changes in cardiac energy metabolism in mice subjected to abdominal aortic constriction (14). Impairment in insulin signaling primarily has a direct inhibitory effect on fatty acid oxidation by increasing the malonyl CoA levels and secondarily inhibiting glucose oxidation through a negative feedback effect of the Randle cycle (38). Moreover, increasing fatty acid oxidation rates could also indirectly cause attenuation in glucose oxidation by triggering the activity of PDK and limiting the activity of the PDH complex. In a model of angiotensin II-induced heart failure with preserved ejection fraction (HFpEF), glucose oxidation rates were reduced by 45% as PDK activity was enhanced and PDH complex activity was attenuated (91). These results are further supported as PDK deletion improved the HFpEF-induced reduction in glucose oxidation (92).

KETONE OXIDATION IN THE FAILING HEART

Circulating Ketone Body Concentrations in Heart Failure

A major determinant of ketone oxidation rates in the heart are the levels of circulating ketones. Earlier studies have suggested that blood ketone levels are elevated in congestive heart failure (with reduced ejection fraction) patients proportional to the severity of cardiac dysfunction (168, 169). These results were recently challenged by Melenovsky et al. (170) who found that the plasma level of ßOHB in heart failure patients were similar to healthy subjects. However, in support of the earlier observations by Lommi et al. (168) and Lommi et al. (169), recent metabolomics studies have found increased blood ketone levels in HFrEF patients (171) and HFpEF patients (172). It is interesting to note however that Zordoky and colleagues also found that HFpEF patients had significantly higher blood ketone levels than HFrEF patients while HFrEF patients had lower ketone levels than healthy controls (172). The discrepancy in reported levels of circulating ketones in heart failure patients may be due to multiple reasons including differences in severity, duration and type of heart failure. This is especially the case in severe heart failure where insulin resistance-induced increases in hepatic ketogenesis could be inevitably contributing to increases in circulating ketones (173). In addition, it is important to note that cardiac ketone levels are dynamic. In general, the circulating levels and cellular uptake of ketones is proportional to its contribution to ATP production (174). In that regard, it is difficult to pin point cardiac ketone levels without concurrently considering pathological circulating serum levels, uptake, oxidative rates and secretion rates of ketone (175). For example, it is still not clear whether HFrEF patients, with lower blood ketone levels than HFpEF patients (172), have a greater reliance on ketone bodies and increased myocardial ketone body oxidation. Alternatively, it is possible that HFpEF patients with high circulating ketone body levels could be associated with increased muscle uptake and oxidation of ketone bodies. Therefore, changes in circulating ketone

body levels are temporal, indefinite, and direct measurements of flux through myocardial ketone body oxidation rates are required.

Ketone Body Oxidation in Heart Failure

Arterio-venous measurements for ßOHB in HFrEF patients, a surrogate for myocardial ketone body utilization, reported no differences compared to healthy controls (176), and a slight increase, however not significant, in HFrEF patients (98). However, recent studies have suggested that myocardial ketone body oxidation is increased in heart failure. In a mouse model of compensated and decompensated pressure overload cardiac hypertrophy, proteomics data demonstrated that a key enzyme involved in ketone body oxidation, namely ß-hydroxybutyrate dehydrogenase (BDH1), was up-regulated 2 to 3-fold (15). Moreover, the myocardial metabolite profile of mice with heart failure was comparable to mice fed a 4-week ketogenic diet. In parallel, Bedi et al. (16) also observed an increased ratio of serum to myocardial ketone bodies with up-regulated expression of BDH1, BDH2, and succinyl-CoA:3-ketoacid CoA transferase (SCOT) in human heart failure patients. We have also seen an increase in myocardial ketone body oxidation rates in the *ex vivo* isolated failing murine heart (unpublished data) (17). Taken together, these studies suggest that the failing heart has an increased reliance on ketone body oxidation. Nevertheless, Nagao et al. (177) found that βOHB levels were elevated in mice with ascending aortic banding-induced heart failure. *In vitro*, subjecting cardiomyocytes to oxidative stress also resulted in elevated levels of βOHB, increased levels of anti-oxidative factors, and concurrent down-regulation of the rate limiting enzyme in ketone body oxidation, SCOT (177). These findings would suggest that ketone body oxidation decreases during heart failure to maintain elevated levels of βOHB as a compensatory response to protect the heart against oxidative stress. The reason for the contradictory results could be due to the severity and duration of heart failure in these studies (15, 177). Since heart failure is a chronic condition, it seems plausible to suggest that in the early stage of heart failure, βOHB may have an antioxidant role and only become an adaptive fuel source in the end-stage heart failure (177). It is worth mentioning that βOHB has previously been shown to be an HDAC inhibitor and protects against oxidative stress (178). However, with this uncertainty comes the question of whether ketone body oxidation in heart failure is adaptive or maladaptive (23, 173)?

To address whether ketones are adaptive or maladaptive in failing hearts, several recent studies have investigated this. Schugar et al. (179) reported that mice with a cardiac-specific knockout of *Oxct1* (or SCOT, the rate limiting enzyme in ketone body oxidation) subjected to TAC-induced heart failure was associated with increased rates of anaplerosis, mitochondrial ultrastructure abnormality and accelerated pathological cardiac remodeling. Similarly, overexpression of cardiac BDH1, the first enzyme in the ketone body oxidation pathway, mitigated oxidative stress and attenuated cardiac remodeling following pressure overload-induced hypertrophy (180). Together, these studies suggest that heart failure-induced increases in ketone body oxidation are adaptive for a failing heart. However, there are still several aspects to consider in light of the preliminary suggestion that ketone body oxidation is adaptive in the setting of heart failure. One factor to consider is the change in cardiac ketone levels, a dynamic concentration that would decrease if heart failure is characterized by elevated myocardial ketone body oxidation rates. Keeping this in mind, the up-regulation of cardiokine/myokine follistatin-like protein 1 (FSTL1), having been shown to be cardioprotective in heart failure, is accompanied by a reduction in cardiac ketone body uptake in a canine model of tachypacing-induced heart failure (181). In such a scenario, high cardiac ketone levels would be suggested to be undesirable and accelerating myocardial ketone body oxidation would be adaptive in heart failure. Alternatively, since ßOHB has been shown to be an HDAC inhibitor (178) and recent work has demonstrated that HDAC inhibitors can enhance myofibril relaxation kinetics and improve diastolic function (182), maintaining ketone levels may indeed be beneficial in the setting of HFpEF as opposed to HFrEF. Second, another factor to consider are post-translational modifications which may be responsible for myocardial energy metabolic derangements that contribute to the progression of heart failure (122). In this case, increasing myocardial ketone body oxidation and increasing the myocardial acetyl CoA pool has been suggested to provide more substrate for lysine acetylation, ultimately contributing to the failing heart's hyperacetylated state (122). This may or may not be desirable since hyperacetylation of glucose and fatty acid oxidation enzymes (183), and its effects on enzyme activity, require further investigation in the setting of heart failure.

Ketones' Effects on Glucose and Fatty Acid Oxidation

Ketones have the potential to suppress glucose oxidation and *vice versa* (37, 69, 184) as they both compete for available oxygen and as a source of TCA cycle acetyl CoA. Williamson and Krebs (184) observed that in the presence of insulin, acetoacetate decreased glucose oxidation by half in the perfused rat heart. This may be explained by the ability of ketones to increase the mitochondrial acetyl-CoA to CoA ratio and consequently inhibit the activity and flux through PDH, the rate limiting enzyme of glucose oxidation (185–187). Of importance is whether ketones add a new dimension of complexity to the Randle cycle (38). Furthermore, the influence of ketone levels and its inhibitory effect on glucose oxidation also needs to be characterized to understand whether it is beneficial to enhance either of these pathways. Recently, ketone bodies were found to decrease myocardial glucose uptake and increase myocardial blood flow in a PET study in healthy humans (188). In connection with this displaced glucose uptake, leucine metabolism into ketone bodies has also been shown to inhibit GLUT4 translocation in cardiomyocytes due to an increase in lysine acetylation, ultimately hampering cardiac glucose uptake (189). Since heart failure is characterized by an increase in acetylation (122), the failing heart's hyperacetylated state could be potentiating leucine to ketone-mediated GLUT 4 inhibition and partially

conferring insulin resistance. However, this is in contrast to a study that showed that administration of ßOHB and acetoacetate were able to recapitulate insulin-induced improvements in an isolated perfused rat heart's *ex vivo* cardiac efficiency (190). Since the failing heart is insulin resistant, ketones may be a viable substrate to improve cardiac efficiency in the failing heart (190). However, more studies measuring ketone body oxidation flux in the presence and absence of insulin are required.

It has also been reported that ketones can inhibit myocardial fatty acid oxidation (191, 192). For example, intravenous infusion of ßOHB suppressed myocardial fatty acid oxidation independent of changes in malonyl-CoA levels or the ratio of acetyl-CoA to CoA in pigs (193). Since fatty acid oxidation rates in heart failure remains controversial, it is unclear whether the inhibitory effects of ketones on myocardial fatty acid oxidation are beneficial or detrimental for the failing heart. This, however, further underlines the crucial role of ketone bodies in myocardial energy metabolism and implicates ketones as an important role-player that is currently neglected from the Randle cycle.

THERAPEUTIC APPROACHES TO ADDRESS THE FAILING HEART's METABOLIC PROFILE

Stimulating Glucose Oxidation

Targeting glucose metabolism has been shown to be an effective approach to mitigate cardiac remodeling and improve heart function. Ikegami et al. (117) and Liao et al. (194) both reported that enhancing glucose uptake by GLUT1 and GLUT4 improved cardiac function and attenuated pressure-overload-induced hypertrophy in mice. Dichloroacetate (DCA) is a direct PDK inhibitor which increases glucose oxidation via enhancing PDH complex activity in the setting of heart failure. In the isolated working rat heart DCA enhances post-ischemic cardiac function and efficiency which is associated with improved coupling between glycolysis and glucose oxidation (195). DCA-induced improvement in coupling between glycolysis and glucose oxidation was also later demonstrated in suprarenal abdominal aortic constriction in rat (196). Similarly, Kato et al. (106) demonstrated that DCA administration to Dahl-salt sensitive rats increased cardiac energy reserve, reduced oxidative stress and slowed the transition from compensated heart failure to failing heart. In line with experimental studies, small clinical studies, although few, have shown promising improvement in cardiac contractility with DCA treatment in patients with coronary artery disease (197) and heart failure (198). Clinical data were not all consistent as DCA infusion in patients with congestive heart failure did not show significant beneficial effects (199).

Inhibiting Fatty Acid Oxidation

There are number of pharmacological approaches which are shown to successfully reduce fatty acid oxidation. Two molecules, namely etomoxir and perhexiline, is shown to inhibit CPT1 (**Figure 3**) and limit fatty acid oxidation with parallel increase in glucose oxidation in mouse and rat models of heart failure (200, 201). In humans, etomoxir showed improvement in ejection fraction and cardiac output (202, 203). Perhexiline also improves cardiac function and symptoms of heart failure (204) However, clinical trials to validate the preliminary encouraging finding with these two molecules were terminated due to the hepatotoxicity (204).

Furthermore, sulfo-N-succinimidyl-oleate (SSO) is an inhibitor of CD36 and has been shown to decrease fatty acid oxidation followed by an indirect increase in glucose oxidation (205). While these approaches showed beneficial effects in experimental studies involving heart failure, clinical studies with these agents have yet to be performed.

Another approach to inhibiting fatty acid oxidation is to inhibit the last enzyme of fatty acid ß-oxidation, 3-keotacyl CoA thiolase, with trimetazidine (206). Clinically trimetazidine has shown beneficial effects by increasing cardiac efficiency, where there is a shift in myocardial substrate utilization from fatty acid oxidation to glucose oxidation. It has been using as an antianginal agent in more than 100 countries (207). A combination of chronic trimetazidine treatment along with the other conventional therapy in heart failure patients improves cardiac function in humans (208). Treatment with trimetazidine in heart failure patients with idiopathic dilated cardiomyopathy shows a decrease in myocardial fatty acid oxidation rates, as well as improved left ventricular function and insulin sensitivity (208). A meta-analysis of clinical trials with trimetazidine in heart failure, showed a beneficial effect of trimetazidine on left ventricular systolic function, clinical symptoms for patients with chronic heart failure and importantly may result in decreasing all-cause mortality (209). We have also shown that trimetazidine prevents obesity-related reductions in cardiac function in obese mice with heart failure (210, 211).

Another potential intervention to decrease fatty acid oxidation in heart failure is with ranolazine. Ranolazine has been clinically used as anti-anginal agent since 2006 (212, 213). While considered to be an inhibitor of the late Na^+ current, ranolazine is also a fatty acid oxidation inhibitor, which is capable of activating PDH a rate limiting enzyme for glucose oxidation (214). However, ranolazines efficacy in treating heart failure has not been extensively studied.

Enhancing Ketone Body Oxidation

In diabetic cardiomyopathy, ketones have been popularized as a thrifty fuel substrate for the heart (215, 216). The role of myocardial ketone metabolism has attracted huge attention since empagliflozin, a sodium glucose co-transporter-2 inhibitor used to treat type 2 diabetes, has shown cardioprotective effects in type 2 diabetic patients. This cardioprotection was associated with increased plasma ketone levels which led to the proposed cardioprotective role of increased ketone body oxidation in the diabetic failing heart (215–217). Furthermore, a recent study found that non-diabetic mice with experimental TAC-induced heart failure was protected against heart failure-induced decreases in *in vivo* and *ex vivo* function following a

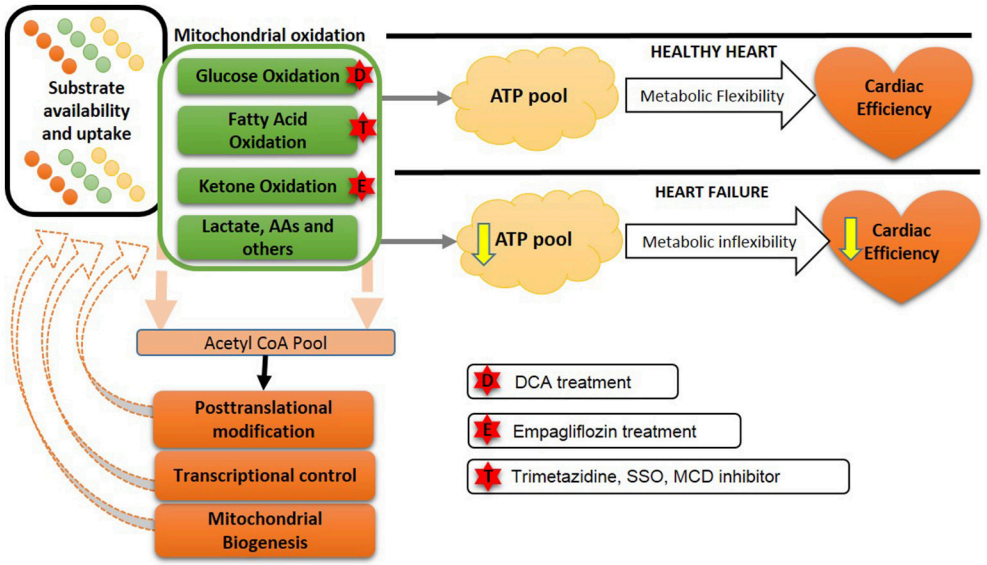

FIGURE 3 | Future approaches to overcome the metabolic balance or inflexibility: In the healthy heart, a variety of energy substrates produce ATP to maintain metabolic flexibility and cardiac efficiency. However, in heart failure a reduced ATP production occurs due to a decreased metabolic inflexibility and a less efficient heart. Transcriptional changes and altered mitochondrial biogenesis also contribute to this metabolic inflexibility in heart failure. Possible approaches to improve metabolic flexibility are shown by stars. DCA, dichloroacetate; SSO, sulfo-N-succinimidyl-oleate; MCD, malonyl CoA dehydrogenase; AA, amino acids.

2-week treatment with empagliflozin (218). However, despite the promising and exciting findings, it is still not clear whether empagliflozin increases ketone oxidation in the heart, or whether empagliflozin-mediated cardioprotection is through a ketone-independent mechanism (219). Therefore, future studies are required to elucidate whether empagliflozin's cardiovascular benefits are mediated by changes in myocardial ketone oxidation.

FUTURE DIRECTIONS

There is a growing recognition and understanding of the importance of metabolic flexibility of the heart and how metabolic inflexibility in heart failure could contribute or even cause deterioration of cardiac contractility and affect the disease progression. Of importance, is that metabolic inflexibility could also be influenced by other comorbidities such as diabetes, obesity, hyperlipidemia and hypertension. Taken together, aiming to re-establish metabolic flexibility in the failing heart is shown to be an effective approach to improve cardiac function and therapeutic outcome. In addition, it is important to recognize the need for a "tailored therapy" for different categories of patients with heart failure based on the type and severity of heart failure as well as the comorbidities which co-exist.

Here, we will discuss some of the recent pharmacological interventions which could have clinical value for failing heart patients.

Glucose Oxidation

The shift toward utilizing a more oxygen-efficient substrate, namely glucose, could potentially have favorable effects in terms of the energy production and cardiac function of the ischemic failing heart which is oxygen deficient. This approach would also improve the coupling between glycolysis and glucose oxidation and produce more ATP per mole of glucose oxidized. It would also limit glycolysis-induced acidosis and its consequent inhibitory effect on cardiac contractility. As discussed earlier, DCA is shown to increase the contribution of glucose to the total ATP production through increasing the glucose oxidation rate with a secondary reduction in fatty acid oxidation in different experimental models and in pilot human studies. However, it is important to note that DCA has a poor pharmacokinetic profile (short half-life) and a low potency. Therefore, future investigations using DCA treatment should potentially consider continuous infusion to administer DCA to maintain effective concentration.

Fatty Acid Oxidation

In the same context of utilizing oxygen efficient energy substrate in a failing heart which is under oxygen deficit, enhancing cardiac function could be achieved by reducing the reliance of the heart on fatty acid oxidation. While a considerable number of approaches exist that can directly or indirectly inhibit fatty acid oxidation, clinical trials targeting a reduction of fatty acid uptake or enzymatic activity are either limited with the confounding factors or underpowered. Therefore, framing animal models along with potential drug targeting fatty acid oxidation to optimize its effective dose, length and other possible side effects, under different diseases states (i.e. obesity, diabetes), different age, and sex should be a prime consideration prior to design future heart failure clinical studies. Unlike etomoxir and perhexiline (CPT1 inhibitors), it is not known if SSO cause

hepatotoxicity. Another possible therapeutic approach is using malonyl CoA decarboxylase (MCD) inhibitors. It has been shown that by increasing malonyl CoA levels, fatty acid oxidation is reduced with a compensatory increase in glucose oxidation (25, 220). Inhibition of MCD, using the novel compound CBM-301106, increases cardiac malonyl CoA levels and decreases fatty acid oxidation (221). However, MCD inhibitors have yet to be tested in the clinical

Furthermore, inhibition of beta-oxidation enzymes, such as 3-keotacyl CoA thiolase, also has potential in reducing fatty acids oxidation l. Based on the promising outcomes in animal and human studies (described in Therapeutic Approaches section), modulation of fatty acid oxidation using trimetazidine is a potential approach to treating heart failure. Again, large randomized clinical trials are still needed to confirm this.

Of importance, is that modulating a particular energy substrate use byu the mitochondria to enhance the overall oxidative phosphorylation could be hindered by a decrease in the number and quality of the mitochondria in the myocardium. Targeting PGC1α to enhance mitochondrial biogenesis and improve the transcriptional changes in the failing heart could potentially have a therapeutic application in heart failure. Furthermore, a combination of the potential therapeutic components (trimetazidine, CD36 inhibitors, MCD inhibitors, PDK inhibitors), targeting both fatty acid and glucose oxidation during heart failure could potentially restore metabolic inflexibility and improve cardiac function in heart failure.

Ketone Oxidation

Stimulating ketone oxidation has been proposed as a potential approach for improving cardiac function in the failing heart (180). Increasing myocardial ketone oxidation has also been indirectly implies to be beneficial in the context of diabetic cardiomyopathy through empagliflozin's "thrifty fuel hypothesis." However, there are presently no cardiac-specific drugs that can specifically modulate myocardial ketone body metabolism. While ketogenic diets are available, the extraneous systemic effects and cardiovascular risk factors associated with these high-fat diets need to be assessed as it may not be appropriate for heart failure patients. Furthermore, in light of recent work suggesting that increased ketone body oxidation is adaptive in the setting of heart failure (179, 180), increasing myocardial ketone body oxidation may be desirable, assuming it is not doing so at the cost of displacing glucose uptake, glucose oxidation or fatty acid oxidation. This would be undesirable in the context of an already depressed glucose oxidation (see section "Glucose Metabolism in Heart Failure"). Therefore, future studies characterized by normalizing ketone body oxidation in the setting of heart failure need to be conducted with measurements of the effects on other substrates and its overall cardiac energy metabolic consequences.

Targeting the Mitochondria

Recognising the central role of the mitochondria in energy metabolism and how impaired oxidative phosphorylation influences the progression of heart failure, targeting the mitochondria is another approach to regain metabolic flexibility and to improve cardiac function. Preclinical studies using mitochondrial-targeted antioxidants, such as AP39 and elamipretide, can preserve mitochondrial integrity through a marked reduction in mitochondrial ROS generation, which is associated with an improved post-infarction cardiac function *in vivo* (222, 223). Consistent with this, chronic treatment with elamipretide mitigates cardiac dysfunction in an advance heart failure model in dog, induced by a serial intracoronary microembolizations, which is accompanied with enhanced mitochondrial respiration and ATP production (224). Very recently, a double-blind, placebo-controlled clinical trial using elamipretifde in patients with HPrEF (ejection fraction $\leq$ 35%) showed a good tolerability and safety profile of the employed dosing range (225), encouraging future studies to characterize long-term safety and efficacy.

Targeting Cardiac Contractility

As contraction and metabolism are so inextricably linked, another approach to improve cardiac metabolism is through "rationalization of ATP usage." This approach could involve more reliance on less ATP-consuming processes to maintain ionic homeostasis and efficient utilization of the limited ATP to provide more ATP for cardiac contraction. For example, cytoplasmic Ca^{2+} handling which is mainly governed by the sarcoplasmic reticulum (SR) ATPase (SERCA2a) activity, which requires ATP to transfer Ca^{2+} into the SR. Therefore, enhancing the Ca^{2+} sensitivity of the myofibrils, which is impaired due to glycolysis-induced acidosis, could not only improve cardiac contractility but also reduce energy cost of contractility through reducing the amount of ATP used for Ca^{2+} homeostasis. Levosimendan is a Ca^{2+} sensitizer and it is shown to improve cardiac contractility in animal (226) and human (227), through enhancing Ca^{2+} sensitivity of troponin C without affecting Ca^{2+}-influx. Following small clinical trials have shown that levosimendan infusion improved left ventricle contractility in different types of heart failure including congestive (228), decompensated (229) advanced/end-stage (230–232) heart failure. Future large clinical trials are warranted to validate the promising effect of Levosimendan in failing heart patients.

CONCLUSIONS

Due to the heart's constant high energy demand, a fine balance between energy substrate utilization is crucial in maintaining metabolic flexibility. The metabolic profile of the failing heart is not simply a shift from "fatty acids to glucose." Rather, the failing heart can be considered to have increased rates of glycolysis, depressed glucose oxidation rates and increased ketone body oxidation rates. With regards to the controversial nature of fatty acid oxidation, while the genes involved in fatty acid oxidation are down-regulated, direct measurements of rates have presented conflicting results. Thus, future studies that consider the transcriptional regulation, post-translational modifications (acetylation), absolute metabolic rates, and mitochondrial biogenesis are all required to fully understand the way in which fatty acid oxidation is perturbed in heart failure. Finally,

definitively characterizing the metabolic profile of the failing heart will help direct future pharmacological therapies that can combine approaches to harmonize and normalize the metabolic flexibility of the failing heart.

AUTHOR CONTRIBUTIONS

QK, GU, KH, and GL designed the literature search strategies and contributed to the critical analysis and interpretation of the published data. QK, GU, and KH carried out the literature search, collected the data and wrote the manuscript which was edited by GL and approved my all authors.

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REVIEW
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An "Exercise" in Cardiac Metabolism

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Research has demonstrated that the high capacity requirements of the heart are satisfied by a preference for oxidation of fatty acids. However, it is well known that a stressed heart, as in pathological hypertrophy, deviates from its inherent profile and relies heavily on glucose metabolism, primarily achieved by an acceleration in glycolysis. Moreover, it has been suggested that the chronically lipid overloaded heart augments fatty acid oxidation and triglyceride synthesis to an even greater degree and, thus, develops a lipotoxic phenotype. In comparison, classic studies in exercise physiology have provided a basis for the acute metabolic changes that occur during physical activity. During an acute bout of exercise, whole body glucose metabolism increases proportionately to intensity while fatty acid metabolism gradually increases throughout the duration of activity, particularly during moderate intensity. However, the studies in chronic exercise training are primarily limited to metabolic adaptations in skeletal muscle or to the mechanisms that govern physiological signaling pathways in the heart. Therefore, the purpose of this review is to discuss the precise changes that chronic exercise training elicits on cardiac metabolism, particularly on substrate utilization. Although conflicting data exists, a pattern of enhanced fatty oxidation and normalization of glycolysis emerges, which may be a therapeutic strategy to prevent or regress the metabolic phenotype of the hypertrophied heart. This review also expands on the metabolic adaptations that chronic exercise training elicits in amino acid and ketone body metabolism, which have become of increased interest recently. Lastly, challenges with exercise training studies, which could relate to several variables including model, training modality, and metabolic parameter assessed, are examined.

Keywords: exercise training, lipid metabolism, metabolic remodeling, fatty acid oxidation, exercise adaptation, heart failure, cardiac hypertrophy

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INTRODUCTION

The physiological benefits of exercise training have long been appreciated. Research has demonstrated enormous cardiovascular benefits including decreased blood pressure in hypertensive individuals (1), improved glycemic control in diabetics (2), improved blood lipid profiles (3), and improved quality of life in heart failure patients (4). Exercise has also been shown to have beneficial effects on the vasculature including improvements in endothelial function (5) and atherosclerosis and plaque stability (6, 7). Recent evidence has indicated that exercise may increase cardiac myocyte proliferation (8, 9), even after myocardial infarction (9). Therefore, exercise prescription remains an essential component of cardiac rehabilitation in patients after myocardial infarction, coronary artery by-pass grafting (CABG) surgery, and heart failure with reduced ejection fraction (HFrEF) (10–12). Surprisingly, although exercise intolerance is a primary manifestation of

heart failure with preserved ejection fraction (HFpEF), a disproportional amount of research has been performed on this population (13). Despite the well-accepted benefits of exercise training in diseased population, the precise molecular adaptations that exercise elicits on the system are still not understood. Because of this, the National Institutes of Health (NIH) recently established a common fund aimed at identification of the molecular benefits that occur due to chronic exercise (14). Several excellent reviews on the cardiovascular adaptations that result from chronic exercise training have been published recently (15–17). However, these articles are limited in the discussion of the adaptations that occur in the cardio-metabolic pathways. Therefore, the purpose of this review is to summarize the existing literature that report adaptations in cardiac metabolism that result from chronic exercise training. For this review, the focus will be primarily limited to the adaptations that govern myocardial substrate utilization. Systemic adaptations, particularly contributing to oxygen delivery via enhanced coronary blood flow, are reviewed elsewhere (18, 19).

METABOLIC REMODELING IN THE PATHOLOGICAL HEART

A myriad of studies elucidated the major substrates that supply the substantial energy requirements of the incessantly contracting heart in both health and disease. In the healthy myocardium, the literature demonstrates that fatty acids supply approximately 50–70% of the necessary substrates to fuel continual ATP resynthesis (20). Moreover, it is well accepted that during ischemic conditions as well as the development of pathological hypertrophy, the metabolic profile of the heart converts to a glucose-dependent phenotype, where glycolysis is markedly upregulated (21). The increased reliance on glucose as a fuel is matched by a decline in fatty acid oxidation present in both compensated and decompensated hypertrophy (22). Conversely, conditions of lipid overload such as diabetes and obesity, subjects the heart to a condition where the supply of fatty acids exceeds oxidation, leading to the development of cardio-lipotoxicity (23–25). In a diseased state, the chronic deviation from the inherent cardio-metabolic profile may result in the loss of metabolic flexibility that contributes to the development of cardiac dysfunction (26). Therefore, novel strategies that target metabolic therapies for the treatment of cardiac pathologies is a focus of several research initiatives.

EXERCISE TRAINING AND CARDIAC DISEASE

Exercise training has long been known to elicit positive adaptations in both healthy and diseased populations. Up until the early 1950s, 4–6 weeks of complete bed rest was the traditional treatment for myocardial infarction (27). However, the controversial ideas of Herman Hellerstein, followed by seminal publications in the 1960s from Naughton (28) and Saltin (29) as well as Hellerstein (30), provided the foundation for the development of modern cardiac rehabilitation programs. Since then, numerous studies investigating the consequences of exercise-training, within the context of cardiac rehabilitation, on mortality, risk factors, and psychosocial factors have been conducted and are reviewed elsewhere (27).

The American Heart Association (AHA) declared physical activity as a major modifiable risk factor for cardiovascular disease (31). Moreover, low cardiorespiratory fitness levels are associated with an elevated mortality risk from cardiovascular disease (32). To this end, the AHA, the American College of Cardiology, and the American College of Sports Medicine put forth specific recommendations for the inclusion of cardiorespiratory exercise at a moderate-intensity for 30–40 min at 3–5 times per week (33, 34). Exercise training results in a condition of chronic volume overload, which induces myocardial remodeling and increased end-diastolic volume. In addition, myocardial contractility is also enhanced, reducing the end-systolic volume. As a result, the major physiological adaptations of exercise training is an increased stroke volume at rest (35). Because cardiac output is unchanged at rest, an additional side effect of chronic exercise training is a reduction in resting heart rate. Since heart failure is defined as an inability of the heart to maintain cardiac output to match systemic metabolic demands, exercise training, due to its ability to modify both stroke volume and heart rate, may be a promising therapeutic intervention.

Numerous studies tested the ability of chronic exercise training to elicit positive benefits in both animal models of heart failure as well as in patients with HFpEF or HFrEF. Additional efforts have been undertaken to determine the effectiveness of pre-operative exercise training for improving outcomes from cardiac surgery (36–38). In smaller studies of patients with dilated cardiomyopathy, 5–8 months of exercise training at a moderate intensity was sufficient to improve exercise performance and left ventricular function (39, 40). In addition, positive changes in metabolism were also noted with improved oxidative metabolism (40) or a tendency to augment myocardial phosphocreatine levels (39). Recently, a meta-analysis of 7 studies in patients with HFpEF determined that exercise capacity, diastolic function, and quality of life measures were all significantly increased with exercise training (41). In addition, the Exercise Training in Diastolic Heart Failure (Ex-DHF) reported improvements in exercise capacity and diastolic function (42). The elevation in exercise performance measures with exercise training are also echoed in studies of HFrEF patients (43–45). Despite positive changes in cardiac function in small population studies, larger studies including the Exercise Rehabilitation Trial (EXERT), the Heart Failure: A Controlled Trial Investigating Outcomes of Exercise Training (HF-ACTION), and the Exercise Training Meta-Analysis of Trials in Patients with Chronic Heart failure (ExTraMATCH) do not consistently find improvements in cardiac function or mortality (46–48). In accordance, inconsistent findings in changes in cardiac function are also observed in animal models as ejection fraction or diastolic function may increase (49–51), decrease (52), or remain unchanged (53). Several factors may contribute to the discrepant findings. In human studies, the presence of additional co-morbidities, varied disease

etiologies, and unknown side effects of medications could hamper improvements in exercise performance. In animal studies, sex differences and training protocols clearly contribute to conflicting reports as discussed later in this review. This certainly highlights the need for additional studies that account for these numerous confounding factors.

THE EXERCISE PHYSIOLOGIST'S TAKE ON METABOLISM

The typical undergraduate exercise physiology textbook discusses major concepts regarding bioenergetics pathways during exercise (54). One primary focus is the time and intensity dependent contributions of the three major energy pathways. First, the phosphagen system, the PCr to ATP reaction regulated by creatine kinase, resynthesizes ATP during immediate, high intensity work. Second, the short-term lactic acid system, relies on glycogen-dependent glycolysis to fuel intermediate, moderate to high intensity activity (55). Third, the long-term aerobic system requires efficient oxidative metabolism to support moderately intense, long-duration exercise. In parallel to the energy systems, careful consideration of the oxygen demands and time course of oxygen uptake are also necessary. In the early course of exercise, an increase in the cellular energetic demand occurs, requiring increased oxygen uptake. However, despite constant intensity, there is a slight delay (up to several minutes) in oxygen uptake to match the steady-state metabolic demands. This phenomenon, deemed the "oxygen deficit," represents the mismatch between total oxygen uptake and the steady-state oxygen requirement (56). During this time, ATP resynthesis is supported by both the immediate and short-term energy systems (i.e., PCr and glycolysis). In time, the oxygen uptake matches the oxygen demand and the steady-state metabolic needs are met primarily by long-term aerobic metabolism. Research has shown that exercise-trained individuals have a reduced "oxygen deficit" and reach steady-state, aerobic metabolism at a faster rate compared to sedentary counterparts (57). In other words, trained individuals have an increased capacity to utilize oxidative pathways to fuel exercise. The enhanced metabolic capacity of the system is likely furnished by a combination of augmented oxygen delivery and improved biochemical processes.

One long-standing dogma is that the failing heart is a metabolically comprised organ the contributes dysfunctional status, representing an "engine out of fuel" (58). Coinciding with this concept, a heart subjected to pressure-overload hypertrophy could be paralleled to the initial phase of intense exercise where a new steady state aerobic metabolism has not yet been achieved. In this case, the "oxygen deficit" is initially compensated by phosphocreatine and glycolysis. Indeed, accelerated glycolysis is a hallmark of the pathological myocardium (59) and alterations in the PCr/ATP ratio have been reported (60, 61). In this scenario, the hypertrophied heart would require a strategy to achieve steady state aerobic metabolism and return to its preferred fatty acid oxidation. Based on the ability to reduce the "oxygen deficit" and promote aerobic metabolism, perhaps exercise training could serve as a suitable intervention.

CARDIAC METABOLISM IN RESPONSE TO EXERCISE

When interpreting findings of exercise-based research, it is important to elucidate the acute vs. chronic responses of the physiological stress of exercise. Acute exercise, a single bout typically lasting from several minutes to hours, results in a host of cardiovascular and biochemical changes that return to baseline in a short time after cessation of the activity. Conversely, chronic exercise, or exercise training, refers to repeated bouts of acute exercise that occur over an extended period time (i.e., weeks, months, years) that result in distinct cardiovascular and biochemical adaptations that can be present for a prolonged duration. In some instances, the changes between acute and chronic exercise may be in opposition. For example, heart rate increases with acute exercise but tends to decrease with chronic exercise training. Therefore, it is critical to make these distinctions.

Past research has clearly delineated the changes that occur in the systemic usage of glucose and fatty acids in response to both acute and chronic exercise Likewise, research performed in the field of cardiac metabolism largely uncovered the relationship between the utilization of glucose and fatty acids, particularly during acute and chronic pathological stress. Although the exercise literature explored skeletal muscle metabolism of both amino acids and ketone bodies, these substrates are just starting to gain prominence in cardiac metabolism. In the ensuing section, an attempt to merge the two fields of exercise and cardiac metabolism is taken in order to understand adaptations that occur in metabolic pathways of the heart in response to chronic exercise training.

CHANGES IN GLUCOSE AND FATTY ACID METABOLISM

The systemic usage of glucose and fatty acids in the response to acute exercise has been well established by the scientific literature primarily by measuring the respiratory exchange ratio (RER) or respiratory quotient (RQ). RER or RQ is a ratio of the output of carbon dioxide divided by the intake of oxygen. RER values of 1.0 represent carbohydrate (i.e., glucose) while 0.7 represent fatty acids. It is suggested that the typical human has a resting RER of 0.85 representing a mixture of fuel usage (62, 63). During the early course of an exercise bout, the RER value rapidly approaches 1.0, proportionate to intensity, indicating a rapid utilization of glucose, presumably by the contracting skeletal muscle (64). This abrupt increase in glucose uptake and oxidation during exercise has been observed in the human heart as well in perfused hearts during acute workload (65, 66). Moreover, a significant portion of the myocardial glucose utilization is supplied by endogenous glycogen stores (67, 68), which is similar to observations made in skeletal muscle (69). If exercise intensity is moderate enough and continues for an extended duration, the RER value will return to values closer to 0.70, indicating a greater percentage of fatty acid usage (64). This coincides with elevated plasma fatty acid concentrations due to enhanced adipose tissue lipolysis (70). In

summary, the relative usage of glucose vs. fatty acids during acute exercise is based on the intensity and duration of the activity.

Past research in exercise physiology has determined the systemic adaptations that result from engagement in long-term exercise training programs. These findings generally show that chronic endurance exercise training results in an increased capacity to oxidize fatty acids at rest and during sub-maximal exercise, partly due to skeletal muscle adaptations (64, 71, 72). However, the metabolic adaptations that occur in the heart in response to chronic exercise are still not completely elucidated. Despite numerous studies investigating various aspects of metabolic responses to exercise training, a disproportionate number of studies over the last 20 years directly assessed changes in myocardial substrate utilization. Due to the logistical and technological challenges with performing these analyses in humans, most of these studies were performed in rodent models and relied on data obtained from gene expression analysis or enzymatic activity assays. However, several of the studies did utilize more traditional methods of analyzing cardiac metabolism including, isotopic tracing techniques in isolated perfused hearts and positron emission tomography (PET). **Table 1** summarizes the major findings of these chronic exercise-training studies in non-diseased mice.

Based on the data presented in **Table 1**, it is difficult to determine the exact changes that occur in myocardial substrate utilization due to chronic exercise training. Using small animal PET scanning, glucose uptake was found to be decreased (73–75) or unchanged (74) while fatty acid uptake was likewise unaltered (74, 75) or increased (76). Using isolated rodent heart perfusions, exercise training resulted in an elevation (77–80) or no change (78, 81) in glucose oxidation while glycolysis was increased (79, 80), decreased (77) or unchanged (81). With likewise inconsistencies, fatty acid oxidation was found to be increased (77, 80), decreased (78), or unaffected (78, 79) by exercise training. The duration of training in the above studies largely ranged from 4 to 10 weeks using both mice and rodents. However, these differences do not appear to account for the lack of agreement in the data. Interestingly, 5 weeks of swim training resulted in significant increases in glucose oxidation, fatty acid oxidation, and glycolysis suggesting that this mode of exercise might be preferable for eliciting metabolic adaptations (80). However, 15 weeks of swim training in rats did not result in similar changes (83). Divergent results were also reported in females (77, 84). One notable finding is the overall decrease or no change in metabolic parameters in mice subjected to 15 months of wheel running (75), which may suggest a potential aging effect or a specific requirement to monitor the intensity of exercise.

Unfortunately, potential mechanistic targets for modulation of cardiac metabolism are also lacking from the current literature primarily due to limited exploration of the associated pathways. The findings are summarized in **Figure 1**. Detailed transcriptomic and metabolomics analyses of exercise-trained mouse hearts yielded minimal changes except for a significant upregulation of phosphofructokinase 2 (PFK2), accounting for glycolytic remodeling (79). Swim training in mice enhanced citrate synthase (CS) and hydroxyacyl-coenzyme A dehydrogenase (HADH) activity and led to increased

TABLE 1 | Cardio-metabolic effects of chronic exercise training in healthy animals.

Species	Sex	Age	Mode	Intensity	Time	Length	GLO	FAO	Glycolysis	Data	References
Mice	M/F	5 weeks	TM	15 m/min, 7°	90 min	4 weeks	M: ↔ F: ↓	M: ↔ F: ↔	ND	PET mRNA	(74)
Mice	F	5 weeks	TM	15 m/min, 7°	90 min	4 weeks	↓	↑	ND	PET	(73)
Mice	M	Adult	TM	20.4 m/min, 10°	60 min	4 weeks	↑	↔	↑	Isolated heart Transcriptomics Metabolomics	(79)
Mice	M	6–7 weeks	Swim	N/A	90 min (2x/day)	5 weeks	↑	↑	↑	Working heart	(80)
Mice	M	7–9 weeks	TM	MIT: 13 m/min, 25° HIT: 26 m/min, 25°	MIT: 120 min HIT: 40 min	10 weeks	MIT: ↔ HIT: ↑	MIT: ↔ HIT: ↓	ND	Working heart mRNA	(78)
Mice	M	12 weeks	WHL	N/A	N/A	15 months	↓	↔	↓	PET Plasma Western	(75)
Rat	M	11 weeks	TM	16–28 m/min, 0°	60 min	6 weeks	ND	↑	ND	mRNA Western	(82)
Rat	M	ND	TM	18–32 m/min, 8°	80–100 min	7 weeks	↑	↑	ND	Affymetrix	(85)
Rat	M	ND	TM	22–32 m/min, 8°	60 min	10 weeks	↔	ND	↔	Working heart	(81)
Rat	ND	4 weeks	Swim	N/A	75 min	15 weeks	↔	↑	↔	mRNA	(83)
Rat	F	ND	TM	25 m/min, 16°	90 min	10 weeks	↑	↑	↓	Working heart	(77)
Rat	F	ND	TM	30 m/min, 15°	120 min	6 weeks	↔	↓	↔	Enzyme activity	(84)
Dog	M/F	ND	TM	11.3 km/h, 8–16°	75 min	9 weeks	↑	↑	↑	Enzyme activity	(93)

The metabolic changes that occur in the heart during chronic exercise training in animal models are presented and organized according to species, sex, mode, intensity, time, and duration of study. The changes that occur in glucose oxidation (GLO); fatty acid oxidation (FAO), and glycolysis are indicated as increased (↑), decreased (↓), or no change (↔). The type of data collected to determine the change in metabolism is listed along with the associated reference. M, Male; F, Female; ND, no data presented; TM, treadmill; WHL, voluntary wheel running; MIT, moderate-intensity training; HIT, high-intensity training; PET, positron emission tomography, Swim, swim training.

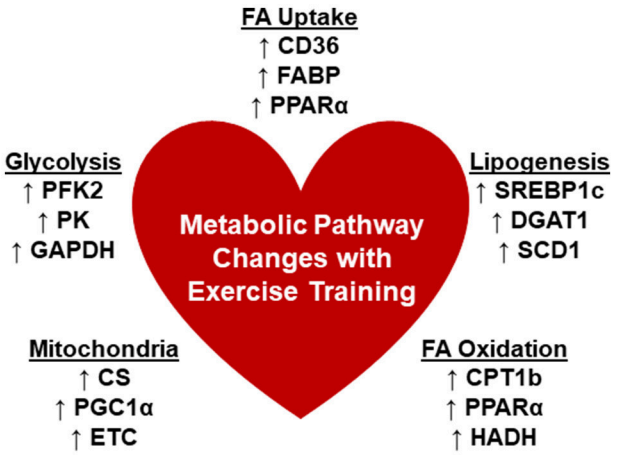

FIGURE 1 | Changes in cardiometabolic pathways with chronic exercise trainingx. There are limited reports that detailed the changes that occur in the metabolic pathways associated with myocardial substrate utilization. Summarized are the reported changes in enzymes or genes that are involved in the processes: Fatty acid (FA) uptake, lipogenesis, FA oxidation, mitochondrial function, and glycolysis. CD36, cluster of differentiation 36; CS, citrate synthase; CPT1b, carnitine palmitoyltransferase 1b; DGAT1, diacylglycerol acyltransferase 1; ETC, electron transport chain; FABP, fatty acid binding protein; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HADH, hydroxyacyl-coenzyme A dehydrogenase; PPARα, peroxisome proliferator-activated receptor, alpha; PGC1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PFK2, phosphofructokinase 2; PK, pyruvate kinase; PFK; SCD1, stearoyl CoA desaturase 1; SREBP1c, sterol regulatory element-binding protein 1c.

expression of the carnitine palmitoyltransferase I (CPT1), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), and subunits of the electron transport chain (80). Treadmill training in mice also increased CPT1b expression as well as regulators of lipid metabolism, peroxisome proliferator-activated receptor (PPARα) and sterol regulatory element-binding protein 1c (SREBP1c) (82). In addition, gene expression of CD36 was shown to be upregulated (85). However, other studies found no change in PPARα (75), CD36 (83), or CPT1b (83). Overall, these studies might suggest the need to standardize training protocols including the mode, time and duration of training, and other requirements, such as intensity, in order for more solid conclusions to be drawn.

High intensity interval training (HIIT) has traditionally been utilized to increase exercise performance in athletes but has gained mainstream and clinical attention recently. HIIT incorporates exercises that require near-maximal efforts for seconds to minutes interspersed with frequent longer duration rest periods (86). Although the physiological benefits of HIIT are apparent, there is debate whether HIIT is superior to the commonly recommended moderate-intensity continuous training (MICT) (76, 87, 88). Moreover, HIIT has been considered for patients with heart disease [for review see (89)] and is the focus of a current randomized controlled study in the United Kingdom (90). However, a recent evaluation of 261 patients with heart failure found no benefit of HIIT over MICT on left ventricular dimensions or aerobic capacity (91). In regards to cardiac substrate utilization, HIIT training in mice led to a reduction in myocardial fatty acid oxidation and increased reliance of glucose oxidation, which was associated with a decrease in the expression of PPARα and increase in glycolytic genes (78), a metabolic profile more similar to the pathological heart. Furthermore, 4 weeks of HIIT was not as effective as MICT in reducing fibrosis or enhancing angiogenesis in hypertensive rats (92). Certainly, additional research in both animal and human models is needed before any conclusions can be reached.

Despite the overall variability in the reported metabolic adaptations, the most common reported change was elevated fatty acid oxidation by isolated perfused heart experiments (77, 80), gene expression (79, 82, 83, 85), or enzymatic activities (93). These potential findings are significant based on the known down-regulation of fatty acid oxidation that occurs in the pathologically hypertrophied heart (60, 94). Our recent work demonstrated that increasing myocardial fatty acid oxidation, via cardiac-specific deletion of acetyl CoA carboxylase 2 (ACC2), prevented the impairments in fatty acid oxidation that occurred during pressure-overload hypertrophy by transverse aortic constriction (TAC) or chronic angiotensin II treatment (60, 95). In addition to prevention of the metabolic remodeling process, systolic (60) or diastolic (95) function was maintained. Overall, these data suggested that targeting myocardial fatty acid oxidation was a promising therapeutic intervention. Since the above exercise training studies appear to indicate a potential to elicit positive adaptations in myocardial fatty acid oxidation, perhaps, exercise training either as a primary or secondary intervention might demonstrate likewise results as in the ACC2 mouse studies.

In addition to the decrements observed in oxidation of exogenous fatty acids, a decreased ability of hypertrophied hearts to oxidize endogenous fatty acids, from triacylglycerol (TAG) stores, also exists (96). Interestingly, recent work demonstrated that provision of unsaturated fatty acids improved endogenous fatty acid oxidation and cardiac function parameters in isolated perfused hypertrophied hearts (97). In conjunction, enhancing myocardial triacylglycerol turnover via diacylglycerol acyltransferase 1 (DGAT1) overexpression was sufficient to prevent impaired functional recovery from ischemia (98) and prevent cardiac dysfunction due to lipotoxicity (99, 100). Previous research showed that several genes in the TAG pathway are enhanced in exercise-trained skeletal muscle (101, 102) which also appears to hold true in trained cardiac muscle (82, 99). In this regard, exercise training might be beneficial in upregulating both exogenous and endogenous fatty acid metabolism and aid in the treatment of cardiac dysfunction, although additional research is certainly required in support of this hypothesis.

There are limited reports supporting the hypothesis that exercise training may prevent the appearance of the fetal metabolic profile in pathological cardiac hypertrophy. There is also a paucity of data investigating the effects of exercise training on the modulation of cardiac metabolism in the

diabetic heart, which has been reviewed recently (103). Studies performed in rats revealed that treadmill running led to a distinct cardio-metabolic gene profile compared to aortic banding (82) or myocardial infarction (85). Specifically, genes involved in endogenous lipid metabolism (82) or beta-oxidation (85) were upregulated in hearts from trained rats. However, these studies did not test the interventional effects of training in the pathological models. However, endurance exercise training was effective in normalization of genes associated with glucose or fatty acid metabolism in spontaneously hypertensive rats (83). Likewise, exercise training was sufficient to partially normalize glycolytic, beta-oxidation, or mitochondrial enzymatic activities in volume overloaded rat hearts due to aortic regurgitation (104). Despite these findings, future research in this area is certainly warranted.

THE IMPORTANCE OF LACTATE METABOLISM IN EXERCISE

As discussed previously, there is a significant upregulation of glycolysis in skeletal muscle during the early course of an exercise bout, which is proportional to intensity. As a result, plasma lactate concentrations can increase 3- to 5-fold (105). Because of its omnivorous capacity, the heart can readily utilize the excess lactate to satisfy energetic demands. Previous studies demonstrated myocardial oxidation of lactate is significant and may be proportional to exogenous concentration within a physiological range (106, 107) or during elevated workloads (108). Interestingly, this elevated concentration of lactate can supplant fatty acid oxidation in the heart (107), providing a mechanism to preferentially oxidize the surplus lactate generated during intense activity.

AMINO ACID METABOLISM AND EXERCISE

In the exercise literature, amino acids are generally considered a necessary nutrient for the post-exercise recovery period, providing necessary substrate for skeletal muscle repair. Despite numerous studies, the promotion of exercise capacity with amino acid supplementation, particularly with branched-chain amino acids (BCAAs), is still debated (109). Recent evidence has correlated cardiovascular disease with elevated plasma BCAAs levels (110). Moreover, disruption of BCAA catabolism via genetic deletion of the mitochondrial localized 2C-type serine-threonine protein phosphatase (PP2cm) has been linked to heart failure (111), cardiac dysfunction after myocardial infarction (112), and impaired functional recovery from ischemia (113). However, the contribution of amino acid to overall cardiac metabolism has generally been considered minimal, equating to less than 5% of the total, although studies directly testing this assertion are limited (114). Likewise, the metabolic adaptations in the cardiac amino acid pathway after exercise training require additional exploration.

KETONE BODY METABOLISM AND EXERCISE

The contribution of ketone bodies to both cardiac and systemic metabolism has become of increased interest in the last several years. Recent work observed an increase in the enzyme, mitochondrial β-hydroxybutyrate dehydrogenase (BDH1), which coincided with elevated plasma levels of β-hydroxybutyrate (BHB) in both rodent and human models of heart failure (115, 116). In addition, increased measures of BHB oxidation in isolated perfused hearts was also noted (115). These studies suggested that an increased reliance on ketone body metabolism could be an additional hallmark of metabolic remodeling in the failing heart; however, whether this is an adaptive or maladaptive response remains to be seen (117). Of note, plasma ketone body concentrations and myocardial uptake are also increased in Type II diabetic patients without cardiac dysfunction (118), suggesting that the pathological consequence is due to increased availability. Indeed, it is known that ketone body uptake and oxidation in brain, heart, and skeletal muscle is proportional to the delivery (119).

In contrast to the pathological conditions of heart failure and diabetes, nutritional provision of ketone bodies in the form of ketone esters appears to improve exercise performance in both humans and rodents (120, 121), and is likely to gain increased scrutiny in the athletic performance field. In humans, ketone body ester supplementation decreased the reliance of skeletal muscle metabolism on glucose, evidenced by decreased glycolytic intermediates and blood lactate accumulation (120). The ketone body supplement also appeared to promote oxidation of intramuscular triacylglycerol during exercise (120). Interestingly, rodents fed a ketone body ester diet had improved cardiac energetics when exposed to acute isoproterenol stimulation (121). From these limited studies, the metabolic effect of ketone bodies has the potential to reduce reliance on glycolysis, promote endogenous lipid metabolism, and preserve energetics in actively working muscle. However, more research is needed to support these observations.

So, does exercise training result in any metabolic adaptations of the ketone body pathway in the heart? In essence, the answer remains unknown. There are limited reports of ketone body metabolism in exercise with one report demonstrating that 14-weeks of training in rats did not significantly change activities of enzymes associated with ketone body utilization (122). Interestingly, the cardiac activities of various ketone body enzymes, including BDH1, were 2- to 5-fold higher than that of slow-red oxidative (i.e., Type I) skeletal muscle (122), suggesting a relatively high robustness of myocardial ketone body metabolism. Overall, cardiac oxidation of ketone bodies has been suggested to be relatively minor (10–20%) in healthy hearts under physiological concentrations (114, 123). In skeletal muscle, activities of enzymes involved in ketone body hydrolysis have been reported to be up-regulated with exercise training which corresponds to both increased uptake and oxidation of ketone bodies in trained vs. untrained skeletal muscle, for review see Evans

et al. (124). Whether exercise training also confers increased capacity of cardiac ketone body metabolism remains relatively unexplored.

CHALLENGES WITH EXERCISE TRAINING RESEARCH

Research statistics reveal that ∼43% of adults in the United States (∼31% worldwide) are physical inactive, defined as performing less than 30 min of moderately intense activity on 5 days per week or 20 min of highly intense activity on 3 days per week (125). Therefore, the potential population for exercise related studies might be biased toward active individuals. Because of this, most of the existing literature focused on populations that were easily recruited, i.e., athletes in various academic institutions. In addition, performing molecular based inquiries requires invasive data collection techniques, such as blood draws and muscle biopsies, which tend to make participation in the study less attractive.

Because of the above challenges, exercise-training studies in animals, particularly rodents, are preferable. Beyond the translational difficulties, numerous other factors need to be considered. Several different modalities of exercise are often employed: swim training, treadmill running, and voluntary wheel running. All of these have their advantages and disadvantages. For example, with swim training, appropriate temperature control of the "pool" is critical. In addition, constant monitoring to avoid mortality due to drowning is necessary. Rodents tend to have unpredictable behaviors during swimming, (i.e., "floating") which can make monitoring intensity difficult (126). In treadmill running, many researchers employ an electric shock grid to "motivate" the animals. This presents potential ethical issues and may confound results particularly since the sedentary animals do not receive this same stimulus. However, less aversive motivational techniques exist, which can eliminate this concern (127). Voluntary wheel running eliminates the "forced" aspect of exercise but results in the inability to monitor intensity closely and requires single housing of the animals (128). However, voluntary wheel running may be preferred to treadmill particularly considering the reproducibility in evaluation of endurance exercise (127). Despite these issues, all of these modalities are frequently used in the exercise literature for training protocols and endurance capacity tests.

Regardless of the specific modality used, researchers must also consider general parameters of exercise prescription, namely intensity, frequency, and duration. Treadmill running presents an advantage by allowing the researcher to set a constant running speed that is equivalent to intensity. Although the animals need to be monitored closely to ensure adherence to the exercise period, some mouse strains have varied inherent running capabilities, termed critical running speed, which needs consideration (129). In general, the majority of exercise training studies employ a frequency of 5 days per week. Duration for treadmill running typically last for 60 min per session whereas swim training may encompass two 90-min sessions, for a total of 3 h per day (80). Furthermore, the length of the exercise training treatment period is traditionally from 4 weeks (130) to 12 weeks (131). Although frequency and duration are usually similar to humans, the determination of exercise intensity is difficult. Therefore, a biochemical marker documenting that a training effect has been achieved is necessary. Citrate synthase activity, a surrogate marker of mitochondrial density, in skeletal muscle is often used (80, 132). It should be noted that acute effects of exercise might persist for up to 24 h (64), so it is advisable to adjust the harvesting of animal tissues accordingly. A final challenge with conducting exercise-training research is critical in studies that use bioengineered mice. It is more frequently noted that the mouse strain can greatly influence the treatment response, including high fat feeding (133) and pressure-overload hypertrophy (134). This is also true for exercise as recent studies demonstrate a profound difference in exercise performance in a variety of mouse strains. Of note, the FVB/NJ, commonly used in transgenic colonies, significantly outperform the frequently used strain for knockout models, the C57BL/6J (130, 135, 136). Further complicating matters, there also appears to be a sexual dimorphism as female mice exhibit greater exercise performance and capacity (137–139) and more pronounced physiological hypertrophy (74, 137, 138). Therefore, careful planning of exercise training studies is definitely required.

CONCLUSIONS AND PERSPECTIVES

One potential critique with exercise training research is the inability of dissecting a specific mechanism due to the intricate systemic interactions that are caused by the exercise treatment. However, any pathological model used in research ultimately affects the entire system, so focusing on the outcomes of any particular organ is viable in the research setting. Several studies discussed above reported various positive outcomes in response to exercise training. However, additional research is required to conclude whether exercise training prevents or reverses cardiac function in models of pathological hypertrophy. Furthermore, although the precise metabolic adaptations that occur in the heart from chronic exercise training are not definitive, some evidence suggests that fatty acid oxidation may be enhanced, although it is not clear whether this represents a change in substrate preference or an increase in the metabolic pathway. However, promoting myocardial fatty acid oxidation, particularly in diseased models, is still debated (140, 141). Therefore, more in-depth research focusing on the cardio-metabolic adaptations that result from exercise training may uncover a novel therapeutic intervention to combat the metabolic derangements that occur in the pathological heart.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

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MINI REVIEW
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Autophagic Regulation of Lipid Homeostasis in Cardiometabolic Syndrome

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As an important protein quality control process, autophagy is essential for the degradation and removal of long-lived or injured cellular components and organelles. Autophagy is known to participate in a number of pathophysiological processes including cardiometabolic syndrome. Recent findings have shown compelling evidence for the intricate interplay between autophagy and lipid metabolism. Autophagy serves as a major regulator of lipid homeostasis while lipid can also influence autophagosome formation and autophagic signaling. Lipophagy is a unique form of selective autophagy and functions as a fundamental mechanism for clearance of lipid excess in atherosclerotic plaques. Ample of evidence has denoted a novel therapeutic potential for autophagy in deranged lipid metabolism and management of cardiometabolic diseases such as atherosclerosis and diabetic cardiomyopathy. Here we will review the interplays between cardiac autophagy and lipid metabolism in an effort to seek new therapeutic options for cardiometabolic diseases.

Keywords: heart, autophagy, lipid metabolism, lipophagy, cardiometabolic diseases

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CARDIOMETABOLIC SYNDROME

Cardiometabolic syndrome, also termed metabolic syndrome (MetS), is a clinical group of inter-related risk factors associated with atherosclerotic cardiovascular disease (ASCVD) and other metabolic diseases such as type 2 diabetes mellitus and stroke. The major risk factors for MetS include abdominal obesity, dyslipidemia, hypertension, insulin resistance and glucose intolerance (1). According to the National Health and Nutrition Examination Survey (NHANES), the age-adjusted prevalence of cardiometabolic syndrome was 22.9% (95% CI: 20.3 to 25.5%) between 2009 and 2010 in adults (≥20 years old) in the United States, with a prevalence of hypertriglyceridemia, elevated blood pressure, hyperglycemia and elevated waist circumference at 24.3, 24.0, 19.9 and 56.1%, respectively (2). Likewise, data from the International Diabetes Federation (IDF) supported that approximately 25% of adults worldwide suffer from metabolic syndrome, especially in those with obesity (with a rate of 60%) (3).

Despite of the improved understanding of the risk factors, the underlying mechanism(s) of cardiometabolic syndrome remains elusive at this point. A number of possible theories have been postulated including genetic and epigenetic factors, oxidative stress, apoptosis, insulin resistance, endothelial dysfunction and dysregulated lipid metabolism (4, 5). Recent findings have also suggested a key role of dysregulated autophagy in the pathophysiological change seen in cardiometabolic syndrome. Nonetheless, it remains unknown whether defective autophagy is a cause or result of

cardiometabolic syndrome (6). As dyslipidemia acts as a key component of cardiometabolic syndrome, we will briefly discuss the complex interplay between autophagy and lipid metabolism, with an emphasis on cardiovascular diseases among various complications of cardiometabolic syndrome.

AUTOPHAGY AND ITS DYSREGULATION

Autophagy is classified into three types, including macroautophagy, microautophagy and chaperone-mediated autophagy. In macroautophagy (or autophagy thereafter), autophagosomes transfer aged or damaged cellular cargo components and organelles to lysosomes for degradation, which serves as an important recycling process to maintain cellular homeostasis. As depicted in **Figure 1**, mammalian target of rapamycin (mTOR) is the key regulator in the autophagy pathway. With sufficient nutrient supply, mTOR binds with UNC-51-like kinase 1 (ULK1) and inhibits the initiation of autophagy. However, AMP-activated protein kinase (AMPK) is activated under starvation, which promotes autophagy through phosphorylation of ULK1. The ULK1 complex (ULK1-Atg13-Atg17) turns on the Beclin1 complex [Beclin1-Atg14-Vps34/Class III phosphoinositide 3-kinase (PI3K)-Vps15], fostering autophagosome nucleation. Atg12, Atg5 and Atg16L1 bind together with the help of Atg7 and Atg10, which promotes the elongation of autophagosome. LC3II (microtubule-associated protein 1 light chain 3) is also recruited into the growing membranes during this process. Finally, mature autophagosomes fuse with lysosomes and form autophagolysosomes, where damaged proteins and organelles are degraded and the breakdown products released into cytoplasm (7).

Apart from removal of unnecessary protein aggregates and organelles, degradation products such as amino acids and lipids also serve as materials for new synthesis in nutrient deprivation (8). Besides, autophagy also plays an essential regulatory role in lipid metabolism (or lipophagy), lipoprotein assembly and metabolic homeostasis (9). Abundant experiments and clinical evidence has shown that impaired autophagy disturbs cellular homeostasis and therefore contributes to the onset and development of many metabolic diseases including obesity, diabetes, atherosclerosis, and steatohepatitis (10, 11).

AUTOPHAGY REGULATES LIPID METABOLISM

Lipid overload is a pivotal element of cardiometabolic syndrome and recent evidence has suggested a role for defective autophagy in the dysregulation of lipid metabolism (12). Various genetically

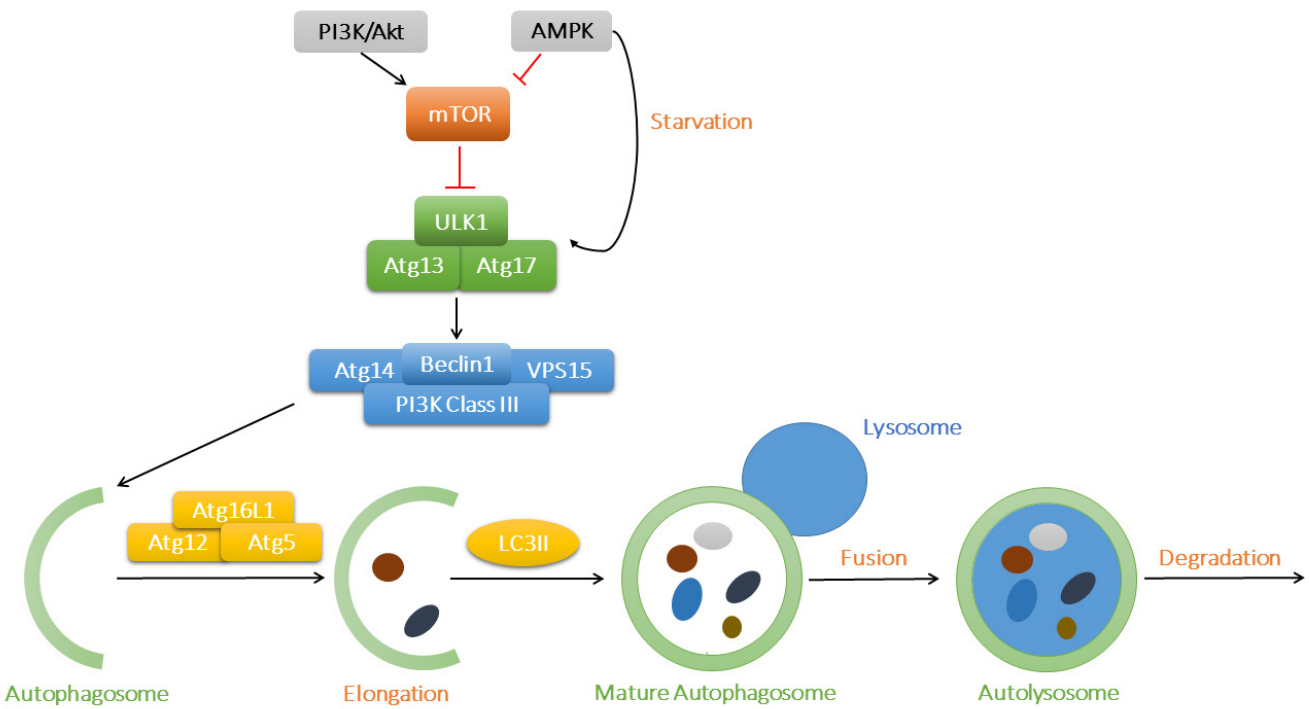

FIGURE 1 | The process of macroautophagy and its main signaling regulatory mechanisms. The key regulator mTOR binds to ULK1 to suppress its activation, while AMPK and PI3K/Akt act as the primary upstream signaling regulatory molecules for mTOR. AMPK might be activated in starvation to promote the initiation of autophagy. ULK1 complex then activates Beclin1 complex and contributes to autophagosome nucleation. Atg12, Atg5 and Atg16L1 complex is involved in the elongation of autophagosome membrane, with LC3II being recruited into expanding cargos. Finally, mature autophagosomes fuse with lysosomes and generate autolysosomes, where waste protein aggregates and organelles were broken down into raw materials such as amino acids and lipids.

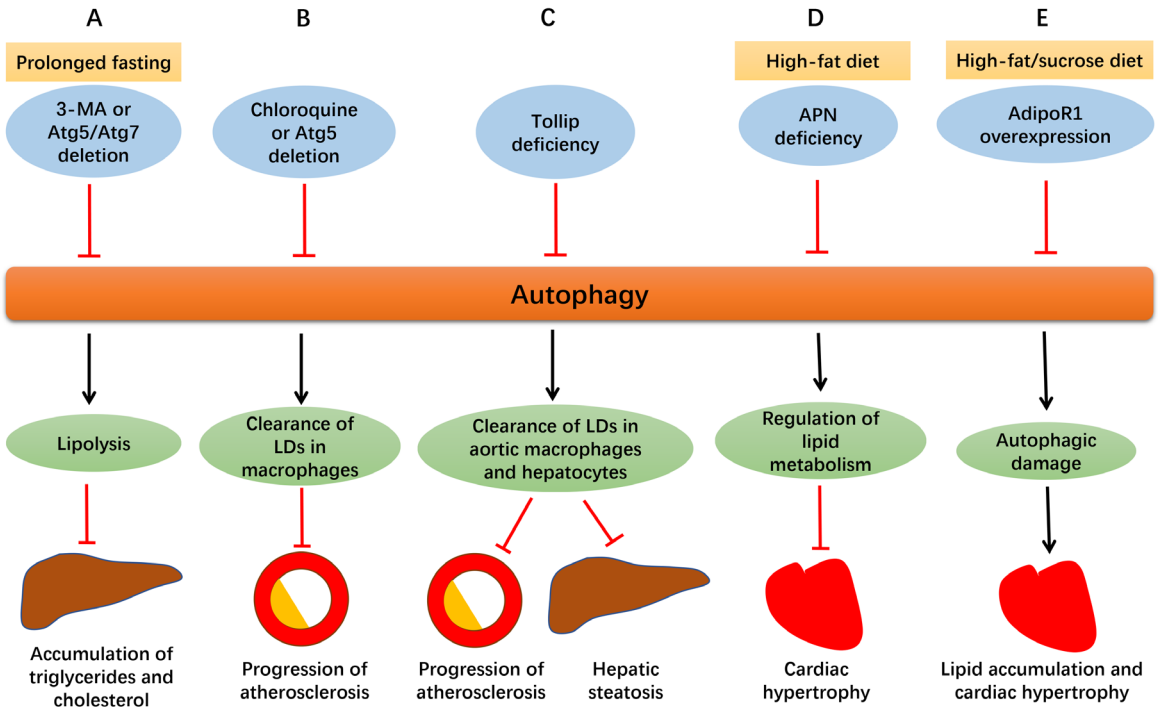

FIGURE 2 | Defective autophagy alters lipid homeostasis. **(A)** In prolonged fasting, suppressed autophagy by 3-MA or genetically ablation of Atg5 or Atg7 promotes delivery of fatty acids to the liver with suppressed hepatic lipolysis at the same time, resulting in the accumulation of triglycerides and cholesterol in the liver. **(B)** Suppressed autophagy by chloroquine or Atg5 deletion in macrophages disrupts cholesterol efflux, leading to excessive lipid droplets (LDs) deposition and ultimately, enlargement of atherosclerotic plaques. **(C)** Tollip deficiency impairs lipophagy and contributes to lipid accumulation in aortic macrophages and hepatocytes, which aggravates atherosclerosis and hepatic steatosis. **(D)** Adiponectin (APN) deficiency disturbs autophagy and leads to accentuation of obesity and cardiac hypertrophy in high-fat diet intake. **(E)** In the face of prolonged high-fat/sucrose diet intake, AdipoR1 overexpression disrupts excessive autophagy, alleviates lipid accumulation and lipotoxicity, and retards cardiac hypertrophy.

engineered murine models of autophagy deficiency have been employed in an effort to unveil the precise role of autophagy in metabolic diseases. For example, deletion of p62 led to the increase of body fat and insulin resistance (13, 14). Fibronectin type III domain containing protein 5 (FNDC5) deficiency impaired autophagy and fatty acid oxidation, as well as enhanced lipogenesis through AMPK-mTOR pathway, the effect of which was rescued by rapamycin to restore autophagy (15).

Autophagy influences lipid metabolism in a number of ways, from lipogenesis to lipolysis (16). Autophagy and lipolysis are both decreased with enough food supply, while both are increased in nutrient deprivation (17). As depicted in **Figure 2**, in prolonged fasting, using 3-methyladenine (3-MA) or genetically ablation of Atg5 or Atg7 increased fatty acid transfer to the liver, along with suppressed hepatic lipolysis, thus leading to the accumulation of triglycerides and cholesterol in the liver (18, 19). Besides, inhibition of Notch signaling using DAPT triggered early autophagy via phosphatase and tensin homolog (PTEN)-PI3K/AKT/mTOR pathway and led to adipogenic differentiation from human bone marrow mesenchymal stromal cells (MSCs), the effect of which was abolished by chloroquine or 3-MA (20). It was also reported that chronic stress prevented MSCs from differentiating into adipocytes due to the inhibited autophagy and elevated CD99 (21).

Apart from lipid metabolism, autophagy also plays an essential role in the regulation of lipoprotein metabolism. Lipoprotein contains hydrophobic lipids and amphipathic proteins in order to transfer lipids in circulation, among which low-density lipoprotein (LDL) is commonly considered an independent risk factor of cardiovascular diseases while high-density lipoprotein (HDL) is deemed cardioprotective (17). It was found that phospholipid sphingosine-1-phosphate (S1P) might be a novel mechanism of HDL cardioprotection. HDL-S1P binds with S1P receptors in heart and activates PI3K/AKT signaling pathway, which would suppress autophagic damage and improve heart function in pressure-overload heart failure (22, 23). What's more, mechanical stretch induced cardiac hypertrophy and elevated autophagy as manifested by LC3II and Beclin1, through upregulating angiotensin II receptor 1 (AT1) receptor. HDL inhibited mechanical stress-induced autophagy and mitigated cardiac hypertrophy via the AT1-PI3K/AKT pathway (24). Besides, apoB100 is a protein involved in VLDL (low-density lipoprotein) and LDL formation. Disruption of apoB100 degradative pathway results in the increase of plasma triglycerides and LDL. Omega-3 poly-saturated fatty acids promote autophagic degradation of apoB100 and suppress VLDL accumulation, denoting a potential therapeutic target for lipid disorders (25). In conclusion, autophagy may play an indispensable

role in regulating lipid metabolism, including lipogenesis, lipolysis and adipogenic differentiation from multipotential stem cells. Besides, autophagy is also involved in governing lipoprotein metabolism. As a good example, lowered autophagic damage may contribute to the cardioprotective role of HDL.

AUTOPHAGY INDUCED BY LIPIDS

Autophagy may be stimulated by saturated or unsaturated fatty acids such as palmitate and oleate. Levels of Beclin1 and Atg7 were elevated in adipose tissues following a prolonged high-monounsaturated fatty acid diet intake (26). Palmitate induced autophagy in various cell types and EIF2AK2/PKR (eukaryotic translation initiation factor 2α kinase 2/protein kinase R) and STAT3 (signal transducer and activator of transcription 3) may be involved in the facilitated autophagy process. Genetic or pharmacological inhibition of STAT3 stimulated autophagy both *in vitro* and *in vivo*, while overexpression of STAT3 inhibited starvation-induced autophagy, possibly through its interaction with the dsRNA-activated protein kinase (PKR). STAT3 acted as a competitive inhibitor of PKR to inhibit PKR phosphorylation. Palmitate is capable of disrupting the inhibitory STAT3-PKR interactions and led to phosphorylation of PKR-dependent EIF2α, which promoted autophagic induction (27, 28).

Diabetic cardiomyopathy is a common complication in type 2 diabetes mellitus patients, featured by cardiac hypertrophy and heart failure. Russo and colleagues used milk fat-based diet consisting of abundant saturated fatty acids (SFA) to induce diabetic cardiomyopathy-like hypertrophy and left ventricular dysfunction in mice. SFA diet promoted autophagy indicated by increased LC3II and Beclin1, and sphingolipids are required in the pathogenesis (29). Moreover, dietary lipids are packaged and stored mainly in triglyceride-containing droplets in enterocytes, which triggers autophagy instantly for lysosomal degradation (30). Likewise, autophagy can be stimulated by oxidized lipids such as 4-hydroxynonenal (4-HNE) and oxidized (ox) LDL in advanced atherosclerotic plaques (31). These findings suggest that autophagy can be induced by fatty acids and lipids through various mechanisms.

LIPOPHAGY AND DRUG TARGETS FOR ATHEROSCLEROSIS

Intracellular lipids including triglycerides and cholesterol are stored in the form of lipid droplets (LDs). Lipids may induce autophagy and undergo autophagic-lysosomal degradation in order to avoid lipotoxicity caused by excessive lipid accumulation, which is often termed as lipophagy, a special form of selective macroautophagy. LDs are transferred by autophagosomes to lysosomes and degraded into free fatty acids and cholesterol, and defective lipophagy results in excessive lipid accumulation. Besides, the amount of lipids targeted for lipophagy varies according to different nutritional status. Studies showed increased association of LC3 with LDs under starvation, and the percentage of autophagosomes containing lipids increased markedly with increased time of energy deprivation. Lipophagy is selectively upregulated when facing extra energy needs, while the breakdown products free fatty acids undergo β-oxidation to supply ATP. Increased lipophagy enables the cell to generate energy timely for cell survival under starvation (32). Therefore, lipophagy regulates not only intracellular lipid stores, but also energy homeostasis especially in face of nutrient deprivation.

As commonly known, accumulation of lipids and lipoproteins are important early pathophysiological changes in atherosclerosis, and macrophage is closely involved in the disease. Atherosclerosis usually begins with the retention of the lipoproteins into the subendothelial space, where they are oxidized and accumulate, leading to the formation of plaques gradually. For example, apoliprotein B enters subendothelial space of the artery wall and triggers secretion of inflammatory cytokines, and then monocytes are attracted here and differentiate into macrophages. Accumulated lipids and lipoproteins are engulfed by macrophages to form foam cells, which contributes to progression of atherosclerotic plaques (33). Autophagy serves as a significant mechanism for clearance of lipid excess in these plaques. Lipids are carried to lysosomes by autophagosomes where they are degraded into free cholesterol and released out of macrophages (34). As depicted in Figure2, impaired autophagy either by chloroquine or Atg5 deletion in macrophages disrupted cholesterol efflux to apolipoprotein A-I (ApoA-I) and led to accumulation of intracellular lipid droplets and macrophage dysfunction, which resulted in progression of atherosclerosis eventually. Macrophage lipophagy and cholesterol efflux was upregulated both *in vitro* and *in vivo* in response to lipid excess, which may become a novel therapeutic target for atherosclerosis (35).

As mentioned above, impaired lipophagy leads to intracellular lipid accumulation, thus contributing to atherosclerosis and hepatic steatosis (36). As shown in Figure2, toll-interacting protein (Tollip), a molecule associated with autolysosome fusion, is believed to play an essential role in this pathological process. Deletion of both ApoE and Tollip disturbed the fusion of lipid droplets with lysosomes in aortic macrophages and hepatocytes, and aggravated atherosclerosis and hepatic steatosis, compared to deletion of ApoE alone. It may be concluded that Tollip deficiency may impair lipophagy, and contribute to lipid accumulation and enlargement of atherosclerotic plaques (36). Another report suggested that inhibition of mTOR offers anti-atherosclerotic property through activation of autophagy and cholesterol efflux and depletion of macrophages in plaques. However, lipid stores are reduced with increased LDL levels at the same time, which may become a side effect if utilized as an anti-atherosclerotic therapy (37).

Moreover, ORMDL sphingolipid biosynthesis regulator 3 (ORMDL3), as an essential regulator of lipid metabolism, inflammation and ER stress, is involved in the pathogenesis of atherosclerosis. Expression levels of ORMDL3 were elevated in the Chinese Han population carrying alleles of the rs7216389 and rs9303277, exhibiting overtly elevated atherosclerotic risk. Experimentally, oxidized low-density lipoprotein (ox-LDL) stimulated ORMDL3 expression in endothelial cells. ORMDL3

silencing reduced basal and ox-LDL-induced autophagy, and suppressed BECN1 expression, which is a protein vital to autophagic initiation. Therefore, ORMDL3 mediates ox-LDL-induced autophagy in endothelial cells in atherosclerosis (38).

Based on these findings, it is concluded that lipophagy serves as a significant mechanism for the clearance of excessive lipids, maintenance of cellular homeostasis, and prevention against the progression of atherosclerotic plaques. Impaired lipophagy causes accumulation of lipids and lipoproteins, thus autophagy may be considered a possible therapeutic target for atherosclerosis.

MITOPHAGY AND DYSLIPIDEMIA

Mitochondria are organelles responsible for energy supply and are pivotal to cell survival, in particular in organs with a great need for energy such as the heart. Mitochondrial dysfunction causes profound damage to cellular homeostasis, necessitating the need for mitochondrial quality control. Mitophagy serves as an indispensable mechanism to transfer damaged mitochondria for lysosomal degradation by autophagosomes in order to clear aberrant mitochondria in metabolic diseases. Regulatory machineries of mitophagy are involved with PTEN-induced putative kinase 1 (PINK1), Parkin (the E3 ligase Parkinson protein-2), Mfn2 (mitochondrial fusion 2 protein mitofusin), the Nix/Bnip3L-Atg8-LC3II complexes and Fun14 domain containing 1 (FundC1) (39). PINK1 phosphorylates Mfn2, accumulates on damaged mitochondria and recruits Parkin to mitochondrial outer membrane, which initiates Mfn2 degradation and mediates the clearance of defective mitochondria ultimately (40).

As mitophagy plays a vital role in the clearance of unwanted mitochondria, impaired mitophagy was closely associated with mitochondrial injury and dyslipidemia in cardiometabolic diseases such as atherosclerosis (41, 42). Inflammation is widely accepted to be a key factor in the formation of atherosclerotic plaques and defective mitophagy activated inflammation, which led to secretion of inflammatory cytokines such as IL-1β. One possible mechanism may be due to the inability of defective mitophagy to clear damaged mitochondria, resulting in production of superoxide/ROS (reactive oxygen species), inflammation and disrupted lipid metabolism, leading to the ultimate plaque expansion. Though limited reports are available at this time, it is plausible to credit the essential roles for mitophagy in the regulation of lipid metabolism and thus outcome of cardiometabolic diseases.

CONCLUSION AND DISCUSSION

Autophagic-lysosomal degradation pathway is an indispensable mechanism for clearing and recycling waste cellular components to maintain homeostasis and to provide materials for new synthesis. Recent evidence has emphasized a vital role for autophagy in lipid metabolism in cardiometabolic diseases such as atherosclerosis. Lipophagy, as a selective form of autophagy, takes charge of translocating lipids for lysosomal degradation, and prevents excess lipid deposit in macrophages and expansion of atherosclerotic plaques, which should be a promising drug target for the management of cardiometabolic diseases.

It is noteworthy that the role of autophagy in cardiometabolic diseases can be complex. Different reports indicate both protective and detrimental roles in atherosclerosis. Most studies do favor a protective role for autophagy in the prevention of atherosclerosis (31, 43). Autophagy of SMCs (smooth muscle cells) in fibrous caps of advanced atherosclerotic lesions helps to degrade damaged components caused by oxidative stress or other injury, thus maintaining plaque stability (44). Still, cellular damage will accumulate if too much or persistent oxidative stress exists. The damaged lysosomal membranes are unable to fuse with autophagosomes and autophagy no longer works. However, autophagic death of SMCs and endothelial cells will result in plaque destabilization and thrombosis and deteriorate the disease, which means excessive autophagy can also be detrimental during this pathophysiological change (45).

Apart from atherosclerosis, autophagy also plays both beneficial and detrimental roles in other cardiometabolic diseases. As depicted in **Figure 2**, adipocyte-derived cytokine adiponectin (APN) and its receptor 1 (AdipoR1) are essential for the regulation of lipid metabolism in cardiometabolic diseases through altering autophagic process. APN is cardioprotective in high-fat diet induced obesity and APN deficiency impaired autophagy, which caused accentuation of obesity, metabolic intolerance, cardiac hypertrophy and dysfunction (46). In another study with 6 month high-fat/sucrose diet (HFSD) treatment, autophagic genes Beclin1 and Lamp2A were upregulated in cardiomyocytes, which was believed to be detrimental to heart since prolonged HFSD feeding led to lipotoxicity and cardiomyopathy. AdipoR1 overexpression disrupted excessive autophagy, reduced lipid accumulation and cardiac hypertrophy, and ameliorated cardiac function, which may suggest that its cardioprotective role is attributed to decreased autophagic damage (47).

In summary, basal level of autophagy is of great significance to clear damaged cellular components and maintain lipid homeostasis, while excessive autophagy may be detrimental and leads to cell death. Due to its regulatory effects on lipid metabolism, autophagy and lipophagy is considered a novel therapeutic target for cardiometabolic syndrome and atherosclerosis, which deserves to be explored further more.

AUTHOR CONTRIBUTIONS

MY drafted and revised the manuscript. YZ revised the manuscript. JR revised the manuscript and provided financial support.

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REVIEW
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Vascular Endothelial Growth Factor B and Its Signaling

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In diabetes, compromised glucose utilization leads the heart to use FA almost exclusively for ATP generation. Chronically, this adaptation unfortunately leads to the conversion of FA to potentially toxic FA metabolites. Paired with increased formation of reactive oxygen species related to excessive mitochondrial oxidation of FA, can provoke cardiac cell death. To protect against this cell demise, intrinsic mechanisms must be available to the heart. Vascular endothelial growth factor B (VEGFB) may be one growth factor that plays an important role in protecting against heart failure. As a member of the VEGF family, initial studies with VEGFB focused on its role in angiogenesis. Surprisingly, VEGFB does not appear to play a direct role in angiogenesis under normal conditions or even when overexpressed, but has been implicated in influencing vascular growth indirectly by affecting VEGFA action. Intriguingly, VEGFB has also been shown to alter gene expression of proteins involved in cardiac metabolism and promote cell survival. Conversely, multiple models of heart failure, including diabetic cardiomyopathy, have indicated a significant drop in VEGFB. In this review, we will discuss the biology of VEGFB, and its relationship to diabetic cardiomyopathy.

Keywords: STZ diabetes, VEGFB, angiogenesis, cell death, cardiomyopathy

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INTRODUCTION

The incidence of diabetes has reached epidemic proportions, with approximately 366 million people affected globally. Cardiovascular disease is the leading cause of diabetes-related death and this heart failure could be an outcome of atherosclerotic coronary artery disease or a consequence of an intrinsic malfunction of the heart muscle (labeled diabetic cardiomyopathy) (1–3). Diabetic cardiomyopathy is a complicated disorder and several factors have been associated with its development. These include an accumulation of connective tissue and insoluble collagen, impaired sensitivity to various ligands (e.g., β-agonists), mitochondrial dysfunction, ER stress, RAAS activation and abnormalities in proteins that regulate intracellular calcium (4). Additionally, changes in cardiac metabolism have also been reported in diabetic cardiomyopathy and are considered a principal culprit in its initiation. Metabolic changes embrace reduced glucose consumption, with a switch to predominant fatty acid (FA) utilization (5). Unlike glucose, the oxidation of FAs requires proportionally greater oxygen to produce a similar amount of ATP (6). Regrettably, augmented FA oxidation increases the generation of reactive oxygen species (ROS) which have been implicated in apoptotic cell death. Therefore, in the diabetic heart it would be useful to have arrangements to (a) promote angiogenesis (to ensure a steady supply of oxygen to metabolize this excess of FA), and (b) prevent cell demise (associated with increased FA oxidation). Members belonging to the vascular endothelial growth factor (VEGF)

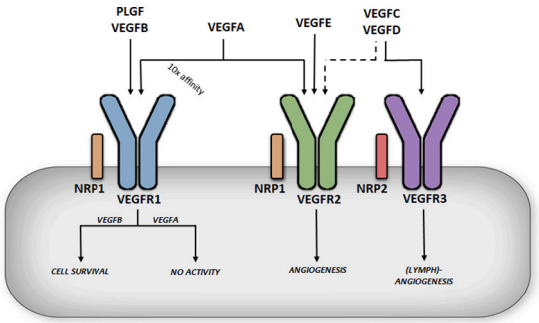

FIGURE 1 | Differential functions of vascular endothelial growth factor receptors. VEGFA binding to VEGFR1 does not produce significant receptor activation (in this case the receptor acts a decoy), whereas VEGFB binding to VEGFR1 has been described to promote cell survival. VEGFA can also bind to VEGFR2, albeit with a lower affinity, but this is considered a key regulator of angiogenesis, promoting endothelial cell migration and proliferation.

family of proteins are unique in their ability to modulate both oxygen delivery and inhibit programmed cell death (7).

VEGF Family of Proteins

The VEGF family consists of 6 growth factors; VEGFA, VEGFB, VEGFC, VEGFD, VEGFE and placental growth factor (PLGF) (8). These growth factors are able to bind and activate tyrosine kinase receptors called vascular endothelial growth factor receptors, of which there are three major types (VEGFR1-3) (**Figure 1**). VEGFA is able to bind VEGFR1 and VEGFR2, VEGFB and PLGF can only bind VEGFR1, VEGFC and VEGFD bind to both VEGFR2 and VEGFR3 while VEGFE only binds to VEGFR2. In addition to VEGFRs, there are two co-receptors, Neuropilin-1 (NRP1) and Neuropilin-2 (NRP2) (**Figure 1**). These co-receptors can bind to VEGFRs to potentiate the latter's action; some VEGFs can also bind independently to NRPs (9).

VEGFA

The most extensively studied member of the VEGF family, VEGFA, was first isolated and cloned in 1989 (10). The VEGFA gene is located on chromosome 6p21.1 in humans, contains 8 exons, and alternative splicing through exons 6 and 7 leads to a number of isoforms named after the number of amino acid left after cleavage of the signal peptide (11). $VEGFA_{121}$ lacks both exons 6 and 7, which encode highly basic heparin binding domains, making this isoform acidic and freely soluble once secreted. $VEGFA_{186}$, which lacks exon 6B, and the full length $VEGFA_{206}$ contain multiple heparin binding domains allowing for almost complete sequestration once secreted, due to their high heparin affinity. The most abundant and biologically relevant isoform, $VEGFA_{165}$ (henceforth referred to as VEGFA), lacks exon 6 but contains the heparin binding domain encoded in exon 7, allowing it to be sequestered onto the cell surface or extra cellular matrix once secreted. VEGFA is considered a key regulator of angiogenesis, promoting endothelial cell (EC) migration and proliferation (12) (**Figure 1**). This important role was illustrated in genetic studies where manipulation of this growth factor revealed severe defects in angiogenesis, with *VEGFA* $^{+/-}$ mice dying *in utero* (13). VEGFA also exhibits a number of additional functions; through the regulation of endothelial nitric oxide synthase (eNOS), it can increase vascular permeability and vasodilation (14), whereas increased Akt signaling gives VEGFA a role in cell survival (15). It has also been implicated in monocyte chemotaxis (16) and colony formation (17). The functions of VEGFA are mediated primarily through binding and activation of VEGFR2. Interestingly, VEFGA also binds to VEGFR1 and that too, with a 10-fold higher affinity (18). Paradoxically, binding of VEGFA to VEGFR1 has limited receptor activation, suggestive of VEGFR1 being a negative regulator of VEGFA action (19) (**Figure 1**). In this regard, VEGFR1 knockout is embryonically lethal as in the absence of this receptor, VEGFA only binds to VEGFR2 leading to hyper vascularization (20).

Other VEGFS

VEGFC and VEGFD play a prominent role in lymphoangiogenesis (21, 22). Both of these growth factors are secreted as pro-proteins with long C and N terminals that require cleaving for them to become fully active and bind to VEGFR2 and VEGFR3 (**Figure 1**). Deletion of VEGFD does not produce any obvious phenotype (23), while experiments with recombinant VEGFD promote lymphatic EC angiogenesis. Deletion of VEGFC is embryonically lethal due to lack of lymphatic vessel development (24) while overexpression of VEGFC results in selective induction of lymphatic EC proliferation (25). VEGFE was discovered in the genome of the Orf virus which can occasionally infect humans through contact with goats and sheep, and leads to highly vascularized skin lesions (26). VEGFE acts in a similar fashion as $VEGFA_{165}$ but only binds VEGFR2 (**Figure 1**) and is structurally similar to $VEGFA_{121}$ with no heparin binding capabilities. PLGF is structurally similar to VEGFA, containing four isoforms (PLGF1-4) (27). PLGF1 and PLGF3 are freely diffusible lacking a heparin binding domain, while PLGF2 and PLGF4 contain an additional 21 basic amino acids enabling these isoforms to be sequestered. PLGF can only bind VEGFR1 but unlike VEGFA, PLGF is pro-angiogenic on binding to this receptor (**Figure 1**). PLGF null mice are healthy indicating PLGF as being redundant for vascular development. However, knockout of PLGF shows impaired angiogenesis in pathological conditions such as ischemia (28). For additional information regarding the above VEGF family members, the reader is directed towards these excellent reviews (8, 12, 29, 30). The current review will focus on VEGFB.

VEGFB

Overview

The VEGFB gene is located on chromosome 11q13.1 and alternative splicing leads to two isoforms. $VEGFB_{167}$ encompasses over 80% of transcripts (31) and contains a highly basic C-terminal heparin binding domain allowing it to be sequestered onto the cell surface, much like $VEGFA_{165}$. The other isoform, $VEGFB_{186}$ has a hydrophobic C-terminal making it freely soluble (32) (**Figure 2**). Tissue expression analysis of VEGFB observed this growth factor to be highly expressed in the heart and skeletal muscle,

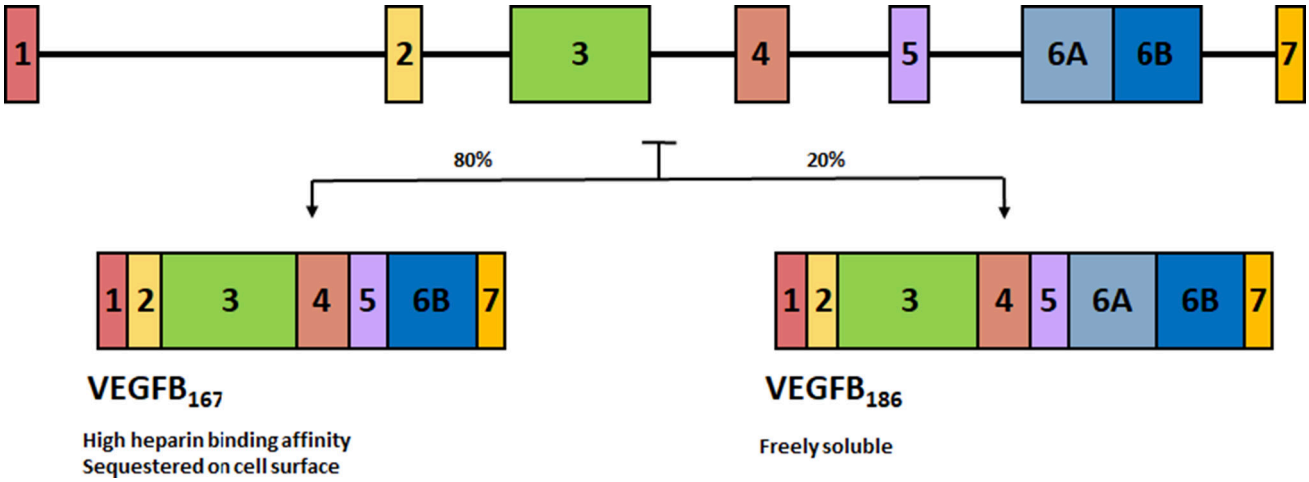

FIGURE 2 | Alternative splicing of VEGFB. Alternative splicing leads to two isoforms. VEGFB167 encompasses over 80% of transcripts and contains a highly basic C-terminal heparin binding domain allowing it to be sequestered onto the cell surface. The other isoform, VEGFB186 has a hydrophobic C-terminal making it freely soluble.

with limited expression in most other tissues (33). The genetic expression of VEGFB is fairly stable and is not regulated by growth factors, hypoxia or hormones (34). Genetic knockout of VEGFB demonstrated that this growth factor is not relevant for normal health (35), as VEGFB null mice developed only a mild phenotype with no effect on mortality.

Function

VEGFB shares 47% of its amino acid sequence with VEGFA and contains the hallmark PXCVXXX-RCXGCC VEGF family motif (33), which lead to the initial studies focused on a role for VEGFB in angiogenesis. Unlike the other members of the VEGF family, VEGFB uncharacteristically does not promote angiogenesis (36–38). For example, although it was suggested that VEGFB is able to induce EC growth, this was ultimately attributed to VEGFA/VEGFB heterodimer formation, and not a direct function of VEGFB (39). Through the use of adenoviral vectors to promote overexpression in muscle or peri-adventital tissue, VEGFB was unable to stimulate vessel growth (40). In mice transgenically overexpressing VEGFB in the skin, there was limited effect on blood vessel density although an increase in capillary diameter was observed (41). Finally, ischemic limb studies provided additional evidence against VEGFB being an angiogenic growth factor as it was ineffective in aiding vascular growth (42). Due to an absence of a role in angiogenesis, the novelty of this growth factor diminished after its discovery. More recently, the importance of VEGFB has been described in cell survival (38) (**Figure 3**), a function that is especially relevant under pathological conditions (43), and will be discussed later.

Receptor Binding

The action of VEGFB is coordinated by its binding to VEGFR1. The VEGFR1 gene is located on chromosome 13q12.3 and encodes a tyrosine kinase receptor comprised of an extracellular ligand binding domain, a transmembrane domain, intracellular tyrosine kinase domain and a carboxy-terminal region (18). Alternative splicing also generates a soluble form of VEGFR1 (sVEGFR1) that only contains the extra cellular ligand binding domain. Three members of the VEGF family, VEGFA, VEGFB and PLGF can bind VEGFR1, each with distinct functions. VEGFB binding leads to activation of a number of downstream activators similar to most tyrosine kinase receptors, including p38 MAPK, ERK/MAPK, PKB/AKT and PI3K (44). VEGFA binding to VEGFR1 does not produce significant receptor activation (44) (**Figure 1**), indicating that VEGFR1 could act as a negative regulator of VEGFA action. In this regard, deletion of the VEGFR1 gene is embryonically lethal (45), likely due to enhanced VEGFA action on VEGFR2, promoting uncontrolled angiogenesis. Interestingly, deletion of only the tyrosine kinase domain results in normal healthy mice (46), suggesting that the ligand binding domain is necessary for VEGFR1 regulation of VEGFA. Further evidence for a role of this receptor in regulating proper angiogenesis is the expression of this receptor primarily in the blood vessels of numerous organs

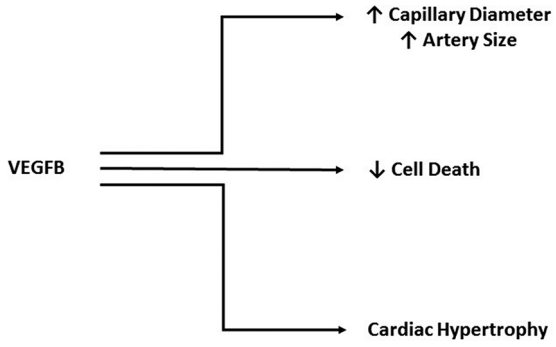

FIGURE 3 | Cardio protective actions of VEGFB. Although it does not play a direct role in angiogenesis, VEGFB has been implicated in increasing capillary diameter and artery size. Additionally, VEGFB has a significant role in protecting against cell death and promoting adaptive hypertrophy.

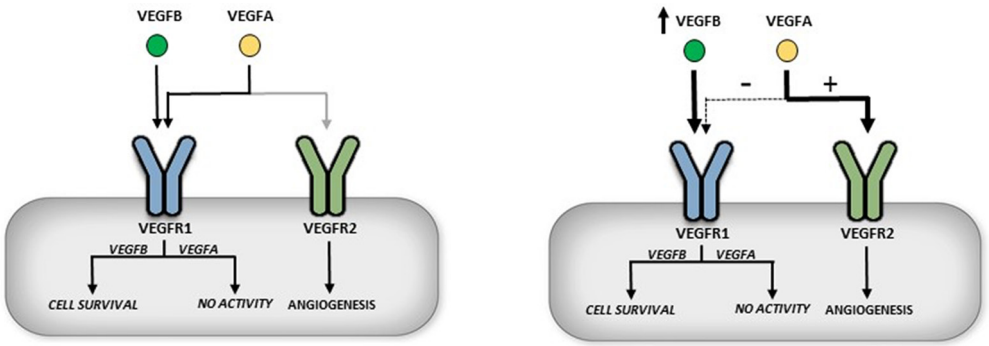

FIGURE 4 | Indirect role of VEGFB in angiogenesis. VEGFR1 has a 10× fold greater affinity for binding VEGFA than VEGFR2 (left panel). Overexpression of VEGFB is suggested to occupy and thus displace VEGFA from VEGFR1. This allows more VEGFA to bind to VEGFR2 and can initiate angiogenesis (right panel).

such as the heart, kidney, liver and brain (47). Soluble VEGFR1 is also a potent anti-angiogenic factor that can bind VEGFA in the plasma. PLGF will bind VEGFR1, but unlike VEGFB, can induce angiogenesis (48) (**Figure 1**). The effect of binding of PLGF to VEGFR1 to induce angiogenesis has been suggested to be a consequence of its interaction with the immunoglobulin domains two and three of the receptor, an action that is not seen with VEGFB that only binds to Ig domain two (49).

The Action of VEGFB to Influence Angiogenesis

Unlike the futile studies related to examining the function of VEGFB in angiogenesis, recent studies have recognized a prominent role of this growth factor in sensitizing cells to VEGFA induced angiogenesis (43, 50). This phenomenon can be explained through the specificity of VEGFA and VEGFB binding to VEGFR1 and VEGFR2. VEGFA can bind to both VEGFR1 and VEGFR2, but only by binding VEGFR2 does VEGFA activate downstream signaling. It should be noted that VEGFR1 has an order of magnitude greater affinity for VEGFA (18). Conversely, binding of VEGFB to VEGFR1 initiates downstream signals (**Figure 1**). Transgenic (TG) overexpression of VEGFB revealed an interesting mechanism in which excess VEGFB can occupy VEGFR1, allowing more VEGFA activation of VEGFR2 (**Figure 4**). In a cardiomyocyte specific overexpression of VEGFB, TG rat hearts displayed enhanced activation of multiple downstream signaling VEGFA targets (43). Moreover, VEGFA administration to VEGFB TG animals presented greater downstream signaling after 10 min compared to wildtype. Accordingly, VEGFB knockout animals demonstrated blunted VEGFA action compared to wildtype, likely a result of increased available VEGFR1 negatively regulating this growth factor. In a more recent study, adeno-associated virus (AAV) VEGFB transduction in mice showed unanticipated vascular effects in adipose tissue (50). As quickly as two weeks post AAV administration, increased capillary density and vessel size was observed. This enhanced capillary network displayed a normal pattern compared to AAV administration of VEGFA which revealed abnormal vasculature and increased infiltration of inflammatory cells. This study reasoned that the increased vasculature seen in VEGFB AAV animals was not due to a direct effect of VEGFB but a result of increased VEGFA action of VEGFR2 due to less available VEGFR1 (**Figure 4**).

Genetic Manipulation

In addition to these indirect effects of VEGFB in enhancing VEGFA action, recent studies have recognized a prominent role of this growth factor in cell survival, and a number of exciting areas of research have emerged. While knockout of the VEGFB gene has limited consequences, employing this mouse model in various disease conditions has uncovered a protective role for VEGFB. In a cerebral ischemic injury model, $VEGFB^{-/-}$ mice displayed a 40% greater increase in infarct size as well as severity of brain dysfunction compared to wildtype animals (51). This study also examined cultured neurons exposed to hypoxia to induce cell death and determined that cells cultured with 100 ng/mL VEGFB had less demise, further reinforcing a neuroprotective role for VEGFB. Through the use of a mouse cornea pocket assay which causes degradation of corneal blood vessels, VEGFB null mice displayed accelerated degeneration and after three weeks had fewer blood vessels (38). Furthermore, in an oxygen induced blood vessel regression model, VEGFB deficient mice had increased regression and treatment with a VEGFB neutralizing antibody further intensified this observation. Conversely, intravitreal VEGFB treatment inhibited blood vessel regression. This survival effect of VEGFB was also observed in cells other than vascular EC. Hence, when primary chordial EC, retinal EC, $CD133^{+}CD34^{+}$ stem cells and aortic smooth muscle cells (SMC) isolated from VEGFB null mice were cultured in serum free medium or under H_2O_2 induced stress, they exhibited increased apoptosis and VEGFB treatment of these cells reduced this effect. Culture of retinal EC, chordial EC, pericytes, and SMC immortalized cell lines also show decrease serum starved cell death when treated with VEGFB (**Figure 3**). Finally, in a model of acute myocardial infarction, $VEGFB^{-/-}$ mice demonstrated reduced revascularization of the ischemic border 7 days post MI as a consequence of fewer thrombomodulin positive capillaries and smooth muscle α-actin positive covered vessels in the infarct area compared to wildtype animals (42). As was seen in other studies and models, administration of VEGFB to these $VEGFB^{-/-}$ mice induced revascularization.

Unlike VEGFB knockout animals, TG overexpression of VEGFB (particularly in the heart) produced significant alterations that were cardio protective. Rats with the VEGFB gene attached to the αMHC promoter generates a cardiomyocyte specific overexpression of VEGFB. These animals displayed a robust increase in arteries of all sizes, especially vessels >150 μm, in which there was a five-fold increase. Additionally, these hearts had capillaries with larger diameters (43) (**Figure 3**). A unique feature of these TG hearts was that they exhibited cardiac hypertrophy, but this was not pathological as even at 22 months, there was no difference in ejection fraction, fractional shortening or maximal exercise capability. Moreover, gene expression analysis identified no change in pathological hypertrophy genes. Exposing these animals to experimental MI revealed marked differences between the groups. Echocardiography showed a less severe decrease in ejection fraction, fractional shortening and an increase in left ventricular systolic and diastolic diameters in TG rats, when measured at both 1 and 4 weeks post MI. The TG hearts also demonstrated better perfusion in both the non-infarcted area as well as the infarcted and border areas. Analysis of the infarct size post-mortem confirmed a substantial decrease in infarct size in TG hearts from both male and female rats. Additionally, this study utilized a 2 week angiotensin II treatment to model pathological hypertrophy and found decreased VEGFB. Along similar lines, in human heart samples from patients that underwent heart transplant, there was decreased VEGFB mRNA in hearts from patients with ischemic heart disease or dilated cardiomyopathy compared to donor hearts that were not used for transplant (43). In another human study, patients that had suffered an acute myocardial infarction (AMI) displayed increased plasma VEGFB compared to healthy volunteers (52). However, within the AMI group, those patients that were on the lower scale of the plasma VEGFB spectrum prior to discharge, exhibited increased left ventricular remodeling six months post MI, a marker for potential left ventricular dysfunction and heart failure. These studies have led to investigation of VEGFB gene therapy in a number of heart disease models. In one study, mice that underwent transversal aortic constriction displayed decreased ejection fraction and fractional shortening, along with left ventricular hypertrophy and decreased VEGFB mRNA four weeks after surgery compared to sham treated animals (53). Treatment via VEGFB viral vectors 2 weeks post-surgery abolished the decreases in ejection fraction and fractional shortening; animals also displayed less severe hypertrophy. These effects were suggested to be due to an increase in cardiomyocyte proliferation (detected by Ki-67 immunostaining) and decreased apoptosis (seen via cleaved caspase 3 immunostaining). In another study which used $VEGFB_{186}$, dogs were exposed to 28 days of left ventricular pacing via an external pacemaker to induce a model of dilated cardiomyopathy (54). Intracoronary $VEGFB_{186}$ was delivered either 2 days prior to pacing or 2 weeks after initiation of the pacing protocol (these animals were labeled delayed AAV-CMV-VEGFB). Paced animals that did not receive $VEGFB_{186}$ transgene displayed typical signs of decompensated heart failure with increased left ventricular end-diastolic pressure, decreased left ventricular systolic pressure and decreased mean arterial pressure. Animals given $VEGFB_{186}$ viral vectors prior to pacing showed no significant changes while the delayed $VEGFB_{186}$ treated animals had significant changes after 2 weeks of pacing but no further changes once VEGFB treatment was initiated. Finally, the cardio-protective ability of VEGFB was examined with respect to mitigating the cardio toxic effects of drugs like doxorubicin (55). Doxorubicin is a commonly used anti-cancer drug that is effective against a variety of cancers by inhibiting cell cycle progression and stopping proliferation of malignant cells (56). However, multiple doses of doxorubicin have been found to be cardio toxic, leading to left ventricular dysfunction and heart failure. Mice injected with AAV9-VEGFB seven days before initiation of a multiple doxorubicin dose protocol, saw no decrease in heart weight and cardiomyocyte size as seen in control mice (55). Furthermore, VEGFB pretreatment prevented cardiac microvasculature damage. Additionally, a single high dose injection of doxorubicin induced DNA double-strand breaks which was reduced in VEGFB pretreated animals.

In contrast to the genetic studies with VEGFB suggesting a role for this growth factor in cell survival, genetic manipulation of VEGFR1, particularly sVEGFR1, is seen as an anti-angiogenic cancer therapy (57). Anti-VEGF treatments continue to be heavily investigated in cancer therapy due to the hypoxic conditions found in most tumors leading to enhanced VEGF action. As outlined previously, VEGFR1 has an interesting interaction with VEGFA; this receptor has a 10 times greater affinity for VEGFA compared to VEGFR2 but produces minimal downstream signals (18). Soluble VEGFR1 also has this unique property for VEGFA binding, as well as being freely diffusible in the circulation to take up any excess VEGFA, limiting tumor angiogenesis. When tumor cells were transfected with sVEGFR1 cDNA and injected into nude mice to observe tumor growth, cells that had higher sVEGFR1 expression grew much slower than unaffected tumor cells (58). In a different experiment, animals injected with tumor cells expressing more sVEGFR1 lived nearly twice as long as control.

Protective Cell Survival Role of Recombinant VEGFB

In addition to the genetic studies examining the effects of VEGFB on cell survival, the use of recombinant VEGFB protein has also been employed to investigate the mechanisms of this growth factor in preventing cell death (**Figure 3**). Treatment of primary aortic SMC with human recombinant VEGFB downregulated many genes involved in apoptosis such as *Bmf, TrP53inp1*, and *DCN (59)*. In cell lines treated with VEGFB, there was a substantial downregulation of many BH3-only protein genes such as *Bad* and *Bid,* and other genes related to cell death like *Casp9, Bax* and *TNF-α*.

A rat ganglion cell line treated with VEGFB and exposed to hydrogen peroxide (H_2O_2) or serum starvation to induce cell death revealed that VEGFB was protective in both instances and promoted cell survival (59). This serum starved cell survival effect was not seen with other VEGFR1 ligands like PLGF, whereas VEGFA had a weaker effect. Additionally, in an optic nerve crush injury model, injection of VEGFB into the eye following injury resulted in an increase in the number of viable ganglion cells when measured 2 weeks post procedure. Similarly, inhibition of VEGFB with a neutralizing antibody decreased the number of viable cells in this model. VEGFB treatment was also able to reduce neuronal apoptosis following NMDA or ischemia induced apoptosis. In all

of these models, real-time PCR revealed that VEGFB treatment reduced the expression of apoptotic genes and VEGFR1 blockade using a neutralizing antibody eliminated the protective effects of VEGFB, implying that the benefits of this growth factor are found by binding VEGFR1 (59). In another study, cardiomyocytes were exposed to 48 h of hypoxia and then 24 h of reoxygenation with or without VEGFB. The presence of this growth factor decreased the percentage of apoptotic cells and a similar finding was seen with cells treated with the cardiotoxic drug epirubicin (60). VEGFB treatment of cardiomyocytes for 24 h increased the expression of many genes involved in contractility (*αMHC*), calcium handling (*SERCA2a*) and mitochondrial function (PGC1α) in a manner similar to that seen with compensatory hypertrophy induced by the thyroid hormone T3 (**Figure 3**).

VEGFB in Cancer

Many pro survival factors that have beneficial effects in numerous disease and injury conditions have unfortunately also been implicated in other pathologies, and VEGFB is no exception. VEGFB expression can be found in many different tumor types and its expression has been found to be increased in multiple cancers such as renal carcinomas and hepatocellular carcinomas (61). In examining patients with hepatocellular carcinomas, researchers found that higher VEGFB expression correlated with advanced stage, multiple tumors, positive vascular invasion and lack of capsule formation (62). Interestingly, a study found that gain of VEGFB function in a cancer cell line with low VEGFB resulted in an increase in circulating tumor cells and metastases (63). Enhanced VEGFB function led to reduced perivascular cells and increased vascular leakiness, inflammation, hypoxia and M2-like macrophages. These effects of VEGFB were independent of VEGFA as a cancer cell line that doesn't express VEGFA as well as cells treated with a VEGFA neutralizing antibody still displayed increased circulating tumor cells with increased VEGFB expression. Implanting high VEGFB expressing tumors into mice lacking the tyrosine kinase domain of VEGFR1 did not entirely stop VEGFB induced metastasis, suggesting a possible VEGFR1 independent mechanism. VEGFR1 also has a prominent role in cancer and interestingly, like VEGFB, VEGFA can also signal through this receptor (64). Hence, treatment of the colorectal carcinoma cell line HT-29, (which does not express VEGFR2), with either VEGFA or VEGFB produced signal activation with increased phosphorylation of extracellular signal-regulated kinases 1/2 (ERK 1/2) and c-Jun N-terminal kinases (JNK). These treatments also lead to increased cell migration and invasion which was inhibited with the VEGFR1 neutralizing antibody 18F1. A similar study was done using a number of pancreatic carcinoma cell lines that expressed VEGFR1 but no VEGFR2 (65). VEGFA or VEGFB treatment lead to phosphorylation of ERK 1/2 but not JNK. Increased cell proliferation and invasion was seen with VEGFA or VEGFB treatment and inhibited by 18F1.

VEGFB in Diabetes

As outlined in this review, VEGFB has been indicated to play a role in cell survival and indirectly promote VEGFA induced angiogenesis (11, 38, 43) (**Figure 4**). Both of these functions are highly desirable, particularly during diabetes, with increased cardiomyocyte demise and poor angiogenesis in the heart being hallmark conditions associated with this disease (66, 67). Currently it is unclear whether this increased cell death or decreased angiogenesis in the heart is an outcome of changes in VEGFB. We injected rats with streptozotocin (STZ), a β-cell-specific toxin to induce diabetes. A single dose of 55 mg/kg (D55) STZ was used to induce moderate diabetes. Our contention is that this D55 model of diabetes imitates the clinical phenomenon of insufficient glycemic management in T1D where multiple finger pricks and daily insulin injections (3–4/day) mean poor patient compliance and repeated exposure to bouts of acute hyperglycemia. These animals were kept for 6 weeks, a well-established model of diabetic cardiomyopathy. Analysis of cardiomyocyte VEGFB protein and mRNA expression revealed a significant decrease in the production of this growth factor (68). Furthermore, there was reduced cell survival signaling as well as a corresponding increase in cell death markers such as cleaved caspase 3 and cleaved PARP. Interestingly, there was a robust increase in VEGFR1 expression in the diabetic animals. However, treatment with recombinant VEGFB did not elicit downstream signaling as seen with control cardiomyocytes, suggesting a defect in VEGFR1 in the diabetic heart. These results (low VEGFB, increased VEGFR1 and blunted signaling) were also duplicated in animals made severely diabetic with 100 mg/kg STZ (D100) and monitored for 4 days. Interestingly, although insulin treatment of these D100 animals to produce euglycemia restored VEGFB protein expression, there was no change in VEGFR1 expression or its downstream signaling. These data for the first time suggested that the loss of VEGFB and its downstream signaling events is an early event after hyperglycemia, is sustained with disease progression, and could be added to the list of potential instigators that lead to cardiomyopathy in these T1D animals. In this regard, a reduction of VEGFB will be unable to withstand the forces like oxidative stress that are propelling the diabetic cardiomyocyte towards cell death. As cardiomyopathy is also a feature of Type 2 diabetes (66), the role of VEGFB in progression of heart dysfunction in animal models of T2D is also of interest and should be examined. In addition to cardiomyopathy, diabetic retinopathy is also a major consequence of diabetes and human retinal endothelial cells cultured in high glucose displayed decreased VEGFB gene expression (69). In rats made diabetic with 50 mg/kg STZ and injected with multiple intravitreal VEGFB injections 10 weeks later, TUNEL staining of the rat ganglion cell layer revealed a decrease in the number of apoptotic cells in the STZ animals treated with VEGFB (70).

Therapeutic Options

During diabetes, the heart can no longer utilize glucose as an energy source and must adapt to use FA to generate ATP (5). While this switch ensures the heart is able to manage its constant energy demands, the increased reliance upon FA leads to a number of consequences. Generating ATP through FA oxidation requires more oxygen than using glucose and in diabetes there is blunted VEGFA mediated angiogenesis leading to a reduced supply of oxygen (6, 71). This lack of oxygen bottlenecks FAO resulting in the diabetic heart having to store the excess FA as triglycerides (72). Furthermore, the accumulation of triglycerides leads to the

formation of ceramides and diacylglycerols which can lead to cardiac cell death (3). It is within this paradigm that the efficacy of VEGFB, as a therapeutic opportunity for the diabetic heart, is compelling. VEGFB has the capability of enhancing VEGFA induced angiogenesis (50) which may aid in providing the heart the necessary oxygen to metabolize the increased supply of FA. In addition, through its actions promoting cell survival, VEGFB could limit cardiac cell death and help delay heart failure. In conclusion, we suggest that using VEGFB as a cardio protective therapy in diabetes is an intriguing concept and should be explored.

Limitations

Although the role of VEGFB in angiogenesis and cell death is an emerging topic, its connection to diabetes is in its infancy. Some studies have suggested that knockout of VEGFB promotes insulin sensitivity and decreased fatty acid accumulation (73, 74). However, these studies were all conducted in mice, and were not always repeatable (75). The beneficial effects of VEGFB have largely been obtained in transgenic rats and models of cell death in dogs and mice. Our own studies show that in an STZ model of Type 1 diabetes in rats, there is a robust decrease in VEGFB. Hence, when investigating the actions of VEGFB, it is critical that consideration should be given to the animal model being used. It should also be noted that one third of patients with diabetes show signs of diabetic retinopathy (76) and higher levels of VEGFB have been reported in the vitreous of patients with diabetic ocular disease (77). Hence, the therapeutic value of VEGFB in treating diabetic cardiomyopathy needs to be considered in relation to the possible accompaniment of retinopathy in these patients.

AUTHOR CONTRIBUTIONS

All authors listed have made substantial, direct, and intellectual contribution to the work and approved it for publication.

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OPINION
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A Similar Metabolic Profile Between the Failing Myocardium and Tumor Could Provide Alternative Therapeutic Targets in Chemotherapy-Induced Cardiotoxicity

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Chemotherapy-induced cardiotoxicity (CIC) is an emerging clinical problem with significant healthcare costs and no preventative therapies (1, 2). Identifying selective therapeutic targets in CIC is difficult, in part, because the mechanisms of drug toxicity vary between chemotherapeutics. For example, cardiotoxicity can be acute or chronic, transient or permanent, and can affect myocardial contractility, cardiomyocyte conduction or the myocardial vascular system (3). Thus, candidate CIC therapies would need to target many features involved in cardiac dysfunction, and additionally should not prevent chemotherapy-mediated tumor regression. Although most would agree that investing in new therapies that specifically target the tumor, while not affecting other normal tissues, including the heart would be ideal, this approach is currently impractical, as even the most selective cancer therapies have been associated with cardiotoxicity (1). For example, Bcr-Abl kinase is a specific gene fusion that causes chronic myeologenous leukemia (CML) (4), and although Bcr-Abl kinase inhibitors, including imatinib mesylate are effective in treating CML (5), they are also associated with cardiotoxicity in pre-clinical animal studies and patients (6), suggesting that alternative adjuvant therapies that can prevent, limit or improve CIC need to be developed. The most commonly used preventative therapy for CIC is dexrazoxane (7), and although dexrazoxane has shown some benefit in preventing CIC (7), it has also been associated with prevention of chemotherapy-induced tumor regression (8), and increased incidence in the development of certain types of cancer in pediatric patients (9, 10). In addition, current treatment guidelines for patients diagnosed with CIC often result in discontinuation of the chemotherapy (regardless of the tumor responsiveness) and initiation into standard heart failure treatment regimes (which include β-blockers and angiotensin inhibitors) (11). In both options, for either prevention or treatment of CIC, the myocardium appears to have precedence over the tumor, with patients receiving suboptimal care for their cancer. Rather than separating our treatment regime to focus either on heart failure or cancer, an ideal approach would look for common pathways identified in both tissues, with the aim to limit or improve chemotherapy-induced heart failure, but not prevent (or even enhance) chemotherapy-induced tumor regression. In this opinion article,

we will discuss metabolic pathways that appear to be induced in both the failing heart and tumor, suggesting that metabolic therapies could provide an alternative approach for treating CIC, without hindering or potentially even improving chemotherapy-induced tumor regression.

In recent years several metabolic pathways have been identified in the failing myocardium, resulting in the emergence of metabolic therapies that appear to be beneficial against several forms of heart failure in both animals and patients (12, 13). The myocardium is the most energetically demanding organ of our body, and predominantly utilizes long-chain fatty acids and glucose as the primary substrates to generate adenosine triphosphate (ATP), which is required for myocardial contractility (14). In normal conditions, glucose is metabolized to pyruvate in the cytoplasm by glycolysis (GLY), generating ∼2 ATP (14). Pyruvate can be further metabolized in the mitochondria to acetyl-CoA, the substrate for the Krebs' cycle, in a process termed glucose oxidation (GO), and this requires the pyruvate dehydrogenase complex (PDC) (14). Alternatively, long-chain fatty acids can also be metabolized in the mitochondria to generate acetyl-CoA *via* fatty acid β-oxidation (FAO) (14). The reducing equivalents NADH and $FADH_2$ produced from the Krebs' cycle can enter the electron transport chain (ETC) to produce ∼32 ATP (14). The normal myocardium generates the majority of its ATP (∼60–90%) from mitochondrial fatty acid β-oxidation (FAO) and glucose oxidation (GO), with cytoplasmic GLY providing a minimal alternative energy-producing pathway (14, 15). Several studies have reported that a transition from a normal to failing myocardium is associated with a switch in energy metabolism from mitochondrial GO to cytoplasmic GLY (13–15). Furthermore, GLY appears to be uncoupled from GO, in part, because PDC is actively inhibited by pyruvate dehydrogenase kinase (PDK) (14). The increase in GLY (and uncoupling to GO) results in an increase in the production of lactate and protons (H^+) in the cytoplasm. This buildup of H^+ eventually results in a decrease in cardiac efficiency, since the cardiomyocytes utilize a large amount of ATP to restore ion homeostasis, at the expense of ATP-dependent contractility (14). Thus, this shift in energy metabolism impairs cardiac contractility and conductance.

A prominent metabolic transcription factor that has been shown to be important in the switch in energy metabolism from GO to GLY is hypoxia-inducible factor 1α (HIF1α) (16). HIF1α is a transcription factor that is induced in the failing myocardium, and is associated with increased expression of glucose transporters, glycolytic enzymes, and PDK (17–19). Thus, HIF1α can directly increase GLY (*via* increasing glucose uptake into the cell and increasing the levels of glycolytic enzymes) and inhibit GO (*via* the induction of PDK), resulting in decreased cardiac efficiency. Several studies have shown that coupling GLY with GO can improve cardiac function in several heart failure models. For example, inhibition of PDK with the small molecule compound dichloroacetate (DCA) improves cardiac function in both ischemic and afterload-induced heart failure models (20–24). Furthermore, inhibition of FAO with Ranolazine or Trimetazidine, which subsequently increase GO [*via* the Randle cycle; (25)], improves cardiac function in multiple preclinical heart failure models and in patients (26–29). Therefore, increasing GO (either directly with PDK inhibitors or indirectly with FAO inhibitors) appears to reverse the metabolic remodeling observed in the failing heart and improve cardiac efficiency and function. A recent study has implicated a similar metabolic remodeling in sunitinib-induced heart failure [i.e., increased GLY (30)], suggesting that therapeutically increasing GO in CIC would be beneficial in this form of heart failure as well. In addition, several chemotherapeutics, including anthracyclines or tyrosine kinase inhibitors are associated with cardiac metabolic dysfunction (30–38), providing further evidence that metabolic therapies could be beneficial against a variety of cardiotoxic chemotherapy agents.

Intriguingly, a similar metabolic remodeling has also been identified in cancer progression (39, 40). In 1927 Otto Warburg observed that most cancer cells utilized aerobic GLY, and this was associated with decreased mitochondrial respiration (41). It is now well described that cancer cells have a similar uncoupling of GLY with GO to the failing myocardium, however, unlike the failing myocardium, this metabolic profile provides cancer cells with a survival advantage (39, 42). For example, the increase in GLY in cancer results in an increase in other glycolytic branching pathways, including the pentose phosphate pathway or serine biosynthetic pathway, which generates nucleotides or amino acids, respectively, both required for cell proliferation (40, 43). Alternatively, the decrease in mitochondrial GO provides cancer cells with apoptosis resistance (19, 39, 44). The inhibition of PDC (and GO) in cancer cells is associated with an increase in the mitochondrial membrane potential, which subsequently increases the threshold for activation of the mitochondrial permeability transition pore and thus, mitochondrial dependent apoptosis (19, 39, 44). Similar to the failing myocardium, HIF1α is also induced in cancer cells and is associated with an increase in the expression of glucose transporters, glycolytic enzymes and PDK (resulting in suppressed mitochondrial GO). Inhibition of PDK (and increasing GO), with DCA in cancer cells results in decreased proliferation and enhanced mitochondrial-dependent apoptosis, resulting in decreased tumor growth in several pre-clinical animal models (19, 44–47), and in a small clinical trial in glioblastoma patients (48). Alternatively, other compounds that also increase GO, including the pyruvate kinase activator TEPP-46, has shown benefit against tumor progression (49). Taken together, these studies provide strong evidence that therapeutically increasing GO is a valid approach for decreasing tumor progression. In addition, our group had shown that increasing GO with DCA was sufficient to decrease HIF1α activity (44), providing a strong positive feedback loop that would potentiate the increase in the GO/GLY ratio, in cancer.

Recent evidence has also implicated HIF1α with the reductive glutamine pathway in cancer (50–52). The reductive glutamine pathway is associated with decreased GO, and provides cancer

cells with sufficient mitochondrial substrates (i.e., citrate) to sustain lipogenesis, a critical requirement for proliferating cancer cells (50). Similarly the reductive glutamine pathway has also been implicated in right-sided heart failure, as well (53), and inhibition of this pathway has been shown to be beneficial against both heart failure and cancer progression (51–53). Intriguingly, enhancing GO has been shown to inhibit the reductive glutamine pathway (51, 53), suggesting that metabolic therapies which

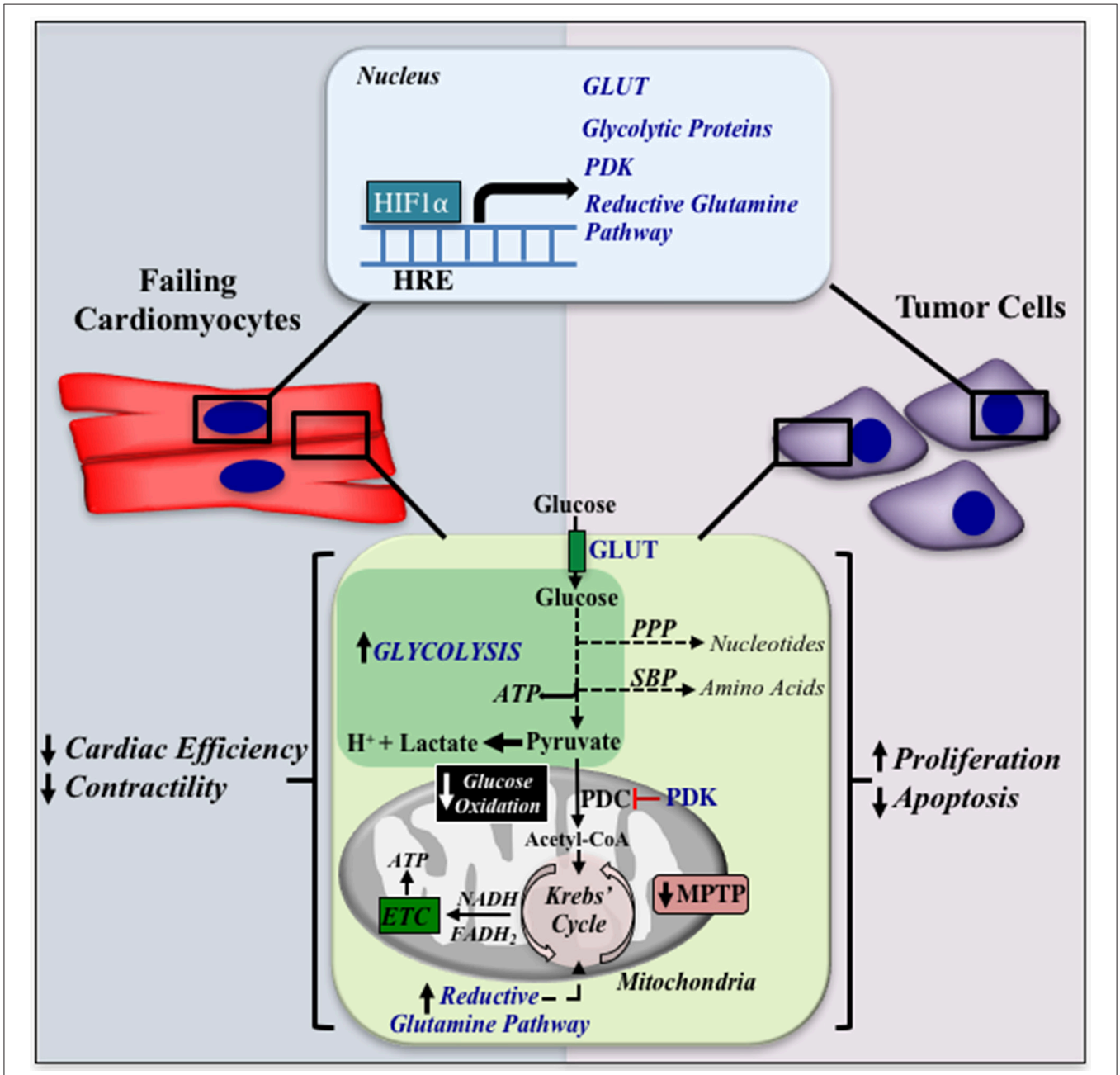

FIGURE 1 | A similar metabolic remodeling in heart failure and cancer. HIF1α is induced in both the failing cardiomyocyte and cancer cells, and can increase the expression of glucose transporters, glycolytic enzymes, pyruvate dehydrogenase kinase (PDK), and the reductive glutamine pathway (shown in blue font). The increase in glycolysis is accompanied with the shuttling of glycolytic intermediates into the PPP and SBP, increasing nucleotide and amino acid synthesis, respectively. Furthermore, uncoupling of glycolysis with glucose oxidation results in an increase in lactate and H^+ production in the cytoplasm. Inhibition of glucose oxidation is associated with closure of the MPTP, leading to apoptosis resistance. These metabolic alterations result in decreased cardiac efficiency and contractility in the heart and increased proliferation and apoptosis resistance in the tumor. HRE, Hypoxia Response Element; GLUT, Glucose Transporter; PPP, Pentose Phosphate Pathway; SBP, Serine Biosynthetic Pathway; PDK, Pyruvate Dehydrogenase Kinase; PDC, Pyruvate Dehydrogenase Complex; ETC: Electron Transport Chain; MPTP, Mitochondrial Permeability Transition Pore.

increase GO could have alternative benefits against heart failure and cancer progression, in addition to altering energy metabolism.

In conclusion, a similar metabolic profile (i.e., uncoupling of GLY with GO) appears to be prominent in both heart failure and cancer (see **Figure 1**). Therapeutically increasing GO in either the failing myocardium or tumor results in improved cardiac function or tumor regression, respectively, suggesting that a similar metabolic therapy could be beneficial in CIC. Although intriguing, much work is required to address if metabolic therapies could be advantageous against this emerging and prominent clinical condition.

AUTHOR CONTRIBUTIONS

All authors listed, have made substantial, direct and intellectual contribution to the work, and approved it for publication.

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Omega-3 PUFA vs. NSAIDs for Preventing Cardiac Inflammation

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INTRODUCTION

Inflammatory cell accumulation occurs in the cardiac muscle during cardiac injury and repair (1). From an evolutionary perspective, inflammation is required for immunosurveillance and host defense. However, such cardinal signs of acute inflammation, such as redness, pain, swelling etc., due to injury or infection. Might be typically absent in chronic low-grade inflammation (LGI). Current literature suggests that chronic low-grade inflammation (LGI) is a primary causative factor behind chronic diseases like cardiovascular diseases (CVD), non-alcoholic fatty liver disease (NAFLD) and obesity (2).

COMMON MECHANISMS FOR NSAIDs

NSAIDs are anti-inflammatory drugs which as a class block the generation of prostaglandins (PGs), leukotrienes (LT) or epoxides (3), which are upregulated during inflammation Therefore, NSAIDs are commonly used for prevention of multiple chronic inflammatory conditions including CVD. The major enzyme participating in PG biosynthesis is cyclooxygenase (COX), which is subdivided as constitutive COX-1 and inducible COX-2 forms. These isoforms of COX show differential activity in inflamed tissues. COX-2 is expressed 10- to 80-fold, whereas COX-1 expressed 2- to 4-fold (4). Both COX isoforms are responsible for converting arachidonic acid (ARA) to intermediate PGs, the PGG_2, and the PGH_2. ARA is the precursor of eicosanoids which is cleaved by phospholipase A_2 (PLA_2) from membrane phospholipids. Then thromboxane synthase and various isomerases are activated which generates thromboxane A_2 (TxA2) and PGs (PGE_2, $PGF_{2\alpha}$, PGD_2, PGI_2) (5). These four PGs have the common function of vasodilation as well as increasing permeability of membranes (thus promoting "redness" due to increased blood flow). PGE_2 and $PGF_{2\alpha}$ are mainly produced by monocytes and macrophages, mast cells produce PGD_2 and endothelial cells produce PGI_2 (6). Long-term treatment with NSAIDs lower beneficial PGs as well (7). PGE_2 and $PGF_{2\alpha}$ control water and electrolyte absorption and maintain secretion in gastric mucosa. Thus, NSAIDs can decrease the secretion of mucous-bicarbonate barrier between the gastric lumen and epithelial cells. Subsequently, in contact with low pH of the stomach, epithelial cells are killed and the integrity of the mucosa is lost, causing ulceration (7).

ASPIRIN

Aspirin is a widely used anti-inflammatory drug. Aspirin inhibits the COX activity by acetylating the hydroxyl group on COX, which specific acts on serine residues. This leads to the irreversible inhibition of COX, as well as causes the ARA binding restriction (8).

Aspirin is easily and quickly absorbed in the GI tract and hydrolyzes to salicylic acid (SA) in the stomach and intestine. However, SA and aspirin can strongly bind to albumin. This avoids the hydrolysis of aspirin too fast (9), as albumin concentration often decreases dramatically under acute inflammation due to the formation of complex albumin-hyaluronic acid or due

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to decreased albumin synthesis (9, 10). Thus, SA and aspirin hydrolyze faster under acute inflammation. In this case, regulating the range of SA (or aspirin dose) concentration is critical in order to avoid further adverse effects. The half-life of aspirin is relatively short, for 15–20 min in adults. Aspirin inhibits the PGs production mainly due to the blockage of COX-2. However, aspirin also inhibits the cytoprotective PGs in gastric mucosa, which impairs the integrity of epithelial cells and also destabilizes the lysosomal membrane (11).

INDOMETHACIN

Indomethacin is a NSAID, and is effective against fever and pain. Like aspirin, indomethacin is a non-selective NSAIDs, which block both COX-1 and COX-2 (12). Thus, PGE_1 and PGE_2 in gastric mucosa can be reduced, resulting in gastric and intestinal ulceration (11). Similarly, with the inhibitory effect of TxA_2, the platelet aggregation ability decreases dramatically to induce bleeding (13). Studies also show that indomethacin can potentially increase the blood pressure in patients (14). It is easily absorbed by the GI tract and bind with the protein in plasma and injured tissues, specifically albumin as aspirin.

IBUPROFEN

Ibuprofen is an effective analgesic and an antipyretic and especially recommended for children because of its better safety profile. Ibuprofen needs longer time than other NSAIDs as an antipyretic and it has a more intense effect on pain relief than aspirin (15). The advantage of this drug is the lighter side effects on the GI tract. High dose for long-term use is mainly for the chronic inflammatory diseases, including arthritis (15).

RECENT PROBLEMS WITH USING NSAIDs IN CARDIAC DISEASES

NSAIDs like aspirin has been used for decades to protect against low grade inflammation in cardiovascular disorders. It was initially thought to be safe and effective against systemic inflammation affecting the cardiovascular system (16, 17). However, evidence of benefit as not been consistent (18) and is plagued by major side effects like GI bleeding, which can make this therapeutic approach questionable in vulnerable populations (19). In essence, a core impact of NSAIDs is to inhibit COX activity. However, COX activity, especially COX-2, is responsible for also maintaining aorta function. COX2 disruption can harden aorta leading to aortic fibrosis (20) and atherosclerosis (21). In addition, inhibition of COX2 reduces the benefits of statin on cardioprotection (22). This might be the reason why all NSAIDs like ibuprofen and naproxen in addition to aspirin in recent years have demonstrated cardiovascular effects including heart attacks (23, 24). More importantly, according to a recent study, long-term users of aspirin have >30% increased chance of a cardiovascular event upon withdrawal of the drug (25). Therefore, preventative therapies that avoid long term NSAID use is warranted in chronic inflammatory diseases including CVDs.

OMEGA-3 PUFA

Omega-3 PUFA are polyunsaturated fatty acids with the first double bond on the third carbon from the terminal methyl end. Fish and flaxseed oils are rich in omega-3 PUFA with protective functions for the heart (26), liver (27), and brain (28). The major fatty acids contained in the fish oil supplement are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the long chain members of the omega-3 family. In contrast, flaxseed oil is mainly composed of alpha-linolenic acid (ALA), the parent omega-3 PUFA.

MECHANISM OF ANTI-INFLAMMATORY RESPONSE

Similar to NSAIDs, omega-3 PUFA, especially EPA and DHA inhibit the production of pro-inflammatory eicosanoids. However, instead of blocking COX activity, they use the same COX to increase the production of anti-inflammatory eicosanoids by providing a different substrate. Twenty-carbon omega-3 PUFA and ARA compete with each other for the use of the COX enzyme. This increases the production of the anti-inflammatory mediators like LTB_5 and PGE_3 from EPA and at the same time limits the inflammatory LTB_4 and PGE_2 production from ARA (29). In this case, the mucosa protective PGs (PGE_2) are still available, albeit reduced. As a result, the side effects caused by anti-inflammatory drugs are drastically reduced. Given the basic differences in the mechanism of NSAIDs and omega-3 PUFAs in blocking COX vs. providing an alternate substrate to COX enzyme, the timeline for actions are vastly different. NSAIDs are more acute in action due to direct enzymatic blockade, whereeas omega-3 PUFAs act slower due to its gradual replacement of membrane phospholipid ARA, which might take weeks if not months to have a biologically plausible effect. Clearly, NSAIDs are preferred for acute inflammatory challenges resulting from physical injury or trauma whereas omega-3 PUFAs are at best a long term, mild anti-inflammatory solution. Thus, consuming omega-3 supplement can be considered as a preventative therapy, alternate to NSAIDs on resolving long-term chronic inflammatory stage, with some major differences as listed in **Table 1** (65).

In addition to modulating PGs, EPA and DHA produce lipid mediators responsible for anti-inflammation and resolution: resolvins and protectins (66). Due to the different substrates of resolvins' production, they are divided into E-series from EPA and D-series from DHA (66). Although they are from different sources, they show very similar effects on preventing inflammation. Both of them increase with the presence of aspirin or with higher EPA/DHA consumption, which are stimulated by the aspirin acetylated COX-2. As mentioned before, COX-2 is the rate-limiting enzyme promoting the synthesis of pro and anti-inflammatory eicosanoids, depending on the different substrates. COX-2 dependent resolvins attenuate inflammation and block

TABLE 1 | Comparison of anti-inflammatory role between omega-3 PUFA and NSAIDs.

	Omega-3 PUFA	NSAIDs
Main effect	Reducing inflammatory eicosanoids production (30)	Blocking inflammatory eicosanoids production (31)
Mechanism	Compete with ARA for COX binding sites (30)	Broadly block COXs activity (31)
Lipid profile	↓TC, ↓TG, ↓LDL, ↑HDL (32)	↑LDL, ↑ TG, ↑ TC, ↓HDL (33, 34)
Blood pressure	↓(35)	↑(36, 37)
Cytokine	↓IL-1 (38), ↓TNFα (38), ↓IL-6 (39)	↓IL-1, ↓TNFα, ↓IL-6 (40)
ROS	↓(41)	↑(42)
Low-dose effect*	Slowing renal dysfunction (43) Reduce seizure and improve epilepsy (44) Improvement on mild to moderate depression (45) Decreased oxidative stress in type 2 diabetes (46)	Primary Cardiovascular Diseases Prevention (47) cerebrovascular disease prevention (48) analgesic effect on dental pain(49) and osteoarthritis pain (50) inhibitory effect on tumor cell growth and metastasis (51) Intraventricular Hemorrhage prevention (52)
High-dose effect*	Substitution of NSAIDs in rheumatoid arthritis (53) Secondary prevention of cardiac disease (54) Slow renal dysfunction (43)	First-line desmoid tumor treatment (55) Long-term treatment attenuates cystic fibrosis (56)
Side-effect	↑ LDL, ↑ HDL, ↑ insulin resistance (57) Higher risk of sudden cardiac death (58) ↑ lipid peroxidation (59, 60)	Gastrointestinal bleeding (61) Increased risk of spinal fusion after sugery (62) Increased risk of coronary heart disease (63) Worsened kidney function (64)

**Omega-3 low dose <1.2 g/d, high dose >2 g/d. NSAIDs dose is various depending on the drug.*

the human neutrophil transendothelial migration by competing for the leukotriene B4 receptors (BLT1) with LTB4 (67). Protectins are the other group of new pro-resolving and anti-inflammatory lipid mediators, which are derived from DHA only (66). They block the immigration of T-cells, promote the T cell apoptosis, and reduce the potent inflammatory factor TNFα (68).

Other than directly affecting the eicosanoid pathway, ALA, EPA, and DHA also helps to reduce pro-inflammatory cytokines, including TNFα, IL-1, and IL-6 (30). These potent cytokines initiate the cascade of pro-inflammatory mediators, including cytokines, chemokines and adhesion molecules following injury. This leads to the high recruitment of immune cells, such as neutrophils, monocytes, B cells and T cells. In most acute and chronic inflammatory diseases, it has been shown that omega-3 PUFA attenuates the inflammatory response by reducing the inflammatory hallmarks (30). In cardiac diseases, omega-3 PUFA can inhibit the secretion of lipopolysacchrides (LPS). This limits the initiation of LPS-induced inflammatory pathway, including NF-κB and toll-like receptor 4 (TLR4) (69). Along with such inhibition of pro-inflammatory signaling, nitric oxide (NO) production increases (45). This leads to improved endothelial function (70).

Considering of the close relation between inflammation and oxidative stress, omega-3 PUFA can also lower oxidative stress through increased cellular antioxidant capacity. However, this result can only be reached with over 3.4 g/day EPA/DHA consumption (71). Having a high dose of omega-3 PUFA on the other hand can cause excess fatty acid accumulation, which potentially can also increase oxidative stress, given that omega-3 PUFAs have multiple double bonds amenable to oxidation.

POTENTIAL RISKS OF OMEGA-3 PUFA SUPPLEMENTATION

Inflammation exists to fight off infection or injury. Thus, while LGI may be perceived as detrimental, acute inflammation especially in the context of infection is a protective response that needs to be sustained at least for some time. In a chronic inflammatory state, such as rheumatoid arthritis (RA), EPA/DHA reduce RA inflammation and benefits the patient. However, the similar effects during infection or tumor surveillance can result in a negative health outcome (72–74). In 2005, IOM summarized that intake of 0.9–9.4 g/day of EPA and 0.6–6 g/day of DHA was linked to an impairment of immune responses. It is now known that DHA and EPA can both improve and impair host resistance to a number of pathogens (75, 76). These adverse outcomes of omega-3 intake were observed with bacterial, fungal and viral pathogen models (77). Given the potent anti-inflammatory effects of DHA and EPA, it is thus conceivable omega-3 PUFA can be both helpful or detrimental specific to the disease context. Moreover, like most nutrients or anti-inflammatory drugs, there is a potential for negative health effects under excess intakes.

NSAIDs AND OMEGA-3 PUFA IN COMBINATION?

An intriguing idea would be to use both low dose NSAID and long chain omega-3s like DHA/EPA in combination for prevention of cardiac and other LGI states. In theory, as both these classes of drugs act on the same COX/LOX pathway, the requirements/dosing of each might be lower due to their synergistic effects. The problem with such an approach is that long term safety of omega-3 supplementation in the pill form still remains unestablished in patients with various LGI states including CVD. With recent reports of long term ill effects of NSAIDs at the current dosing levels in cardiac patients, there is evidence that it might be risky to carry on such a trial for potential negative effects on coagulation (78).

In conclusion, the effectiveness of NSAIDs for acute inflammation has not translated to a safe strategy for long term prevention of CVD. Controversy also surrounds the long term impact of omega-3 PUFA as a preventative measure against chronic low grade inflammation (77). However, in patients unable to take NSAIDs in the long term due to GI or bleeding problems, due to the similarity in their mechanism of action,

low dose omega-3 PUFA could be a substitute to prevent LGI associated with cardiovascular diseases.

AUTHOR CONTRIBUTIONS

JY wrote the paper. SG edited and revised the paper. Both authors read and approved the final manuscript.

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