UNDERSTANDING MOLECULAR MECHANISMS IN DIABETIC CARDIOMYOPATHY (DCM)

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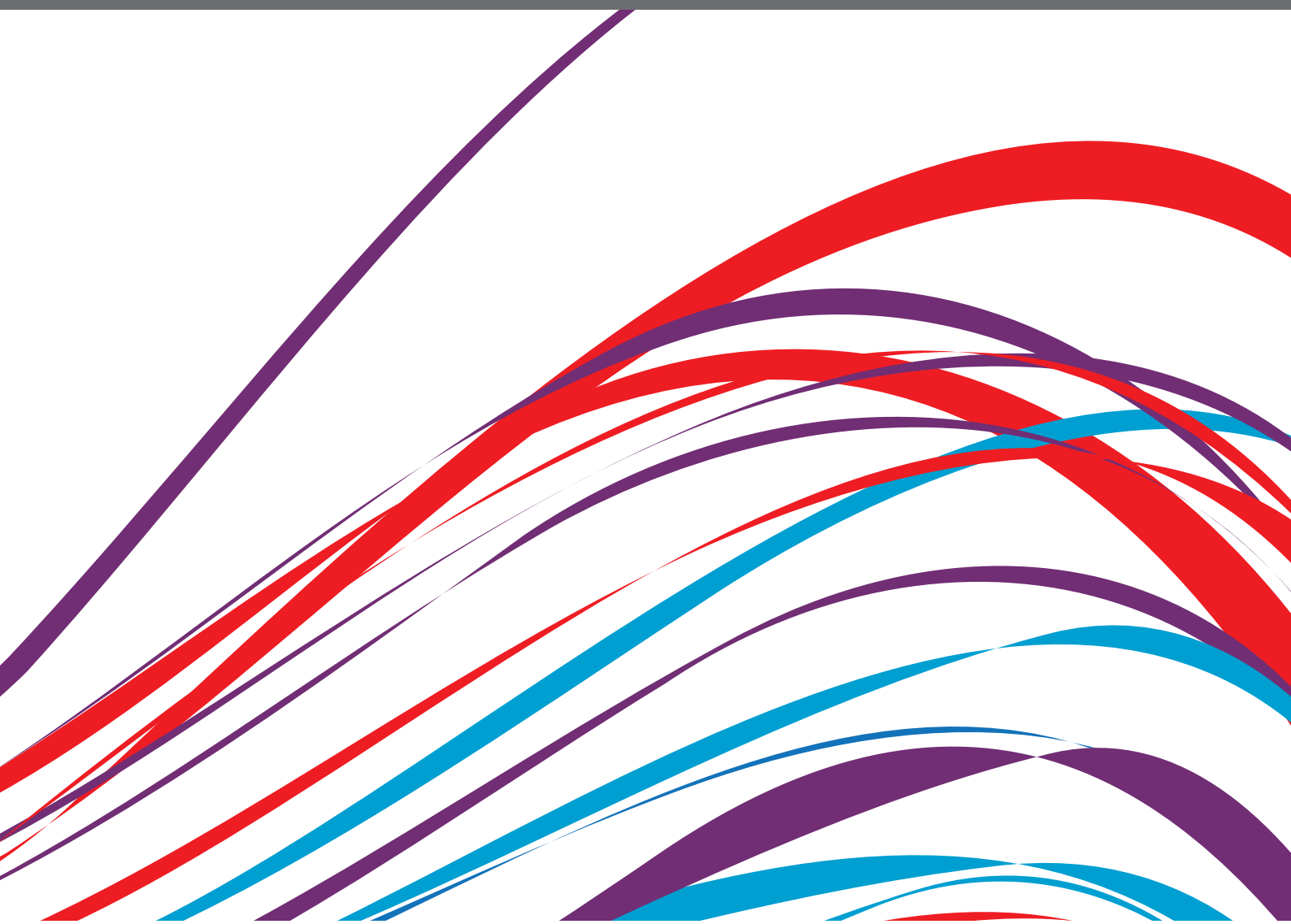

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UNDERSTANDING MOLECULAR MECHANISMS IN DIABETIC CARDIOMYOPATHY (DCM)

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EDITORIAL
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Editorial: Understanding Molecular Mechanisms in Diabetic Cardiomyopathy (DCM)

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Editorial on the Research Topic

Understanding Molecular Mechanisms in Diabetic Cardiomyopathy (DCM)

Cardiovascular disease is the leading cause of death worldwide, and diabetes, the most common metabolic syndrome, causes a 2–5 times higher risk for heart disease (1). Specifically, cardiovascular complications in people with diabetes mellitus (DM) are 2-3-fold more elevated than in non-diabetic counterparts, leading to a higher chance of causing diabetic cardiomyopathy (DCM). DCM is a prominent disease in people with DM, and molecular mechanisms that drive DCM are not fully understood as the disease itself is multifactorial and challenging potential treatment options (2). Therefore, this Research Topic is focused on understanding the molecular mechanism(s) contributing to DCM. The Research Topic comprised review and research articles from different experts working on DCM. The Research Topic of original research and review topics highlights the multifactorial molecular mechanisms involved in the DCM and some recent therapeutic advances in preclinical approaches at various levels in the pipeline.

One of the major issues during cardiac pathology is impaired protein quality of the myocardium, which is more prone to damage under diabetic conditions (3). The review article by Kaur et al. comprehensively focused on the protein quality control (PQC) system that appears to play a vital role in maintaining cardiomyocyte viability under physiological and pathological conditions. Protein homeostasis is preserved by the molecular process of protein translation, degradation of misfolded or unfolded proteins, recycling of amino acids, and disposing of toxic substances by various quality control pathways. These pathways include unfolded protein response (UPR), ubiquitin-protease system (UPS), autophagy and mitophagy. In addition, the role of unfolded protein response (UPR) in Endoplasmic reticulum (UPR^{ER}) and mitochondria (UPR^{Mt}), and the molecules involved in these pathways and their contribution to DCM are discussed. Compromised PQC leads to impaired cellular homeostasis that results in the aggregation of misfolded proteins and toxic substances, which provokes the onset of heart failure in diabetes. Therefore, the finely tuned manipulation of PQC in the myocardium is essential to maintain cellular equilibrium in response to the diabetic condition and may be a promising therapeutic strategy for the betterment of cardiac complications in diabetes patients.

Sudden cardiac death (SCD) in patients with heart failure preserved with ejection fraction (HFpEF) is another major problem in people with DM, as these patients are more prone to morbidity and cardiac mortality (4). Patel et al. found that the upregulation of Osteopontin (OPN)

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shows adverse cardiac remodeling, whereas OPN knockdown reduced HFpEF, improved glucose tolerance, and reduced insulin resistance in mice. Therefore, the expression level of OPN, along with its correlated proteins such as low-density lipoprotein receptor (LDLR), dynamin2 (DNM2), fibronectin-1(FN1), and 2-oxoglutarate dehydrogenase-like (OGDHL) are predicted to serve as a potential risk marker for SCD in DCM. Authors also find that dysregulated expression of these proteins in patients with DM and HFpEF who experienced SCD may serve as a predictive plasma biomarker that determines whether its expression level alters SCD risk in patients with DM and HFpEF. As they are secreted into plasma, OPN and LDLR levels could serve as potential biomarkers in DCM that require further investigation.

A common feature of the diabetic heart is the excess production of ROS due to metabolic inflexibility that accelerates the progression of DCM, which ultimately triggers mitochondrial dysfunction. Parker et al. established a model to study DCM, based on the mitochondrial structure, replicating diabetic myocardium that could be useful to research mitochondrial targets in DCM. In this work, DCM was induced by low-dose streptozotocin (STZ) and a high-fat diet that resulted in a spectrum of mitochondrial changes observed in diabetes. They found that this model replicated several features of DCM, such as decreased mitochondrial area, reduced complex-II dependent oxygen consumption, and increased levels of Complex-III and V proteins.

Cardiovascular disease is the major obstacle to diabetes-associated complications, besides creating mechanical abnormalities of the myocardium. Another prominent feature of diabetic myocardium is the altered electrical remodeling causing cardiac arrhythmia. In this regard, Gallego et al. presented a comprehensive review on the importance of considering the electrical features of the myocardium when treated for diabetes. They provided details of the underlying cellular level mechanisms that alter cardiac ion channels, regulatory proteins, and a subsequent change in sodium, calcium, and potassium currents. Altogether, this collectively lengthens the QT interval duration, increasing the risk of developing life-threatening ventricular arrhythmias and sudden death. As QT duration strongly correlates with the risk of developing ventricular tachycardia followed by ventricular fibrillation, the thorough QT/QTc analysis evaluates QT interval prolongation that may act as a qualitative marker for proarrhythmic risk. Diabetic patients treated with the glucose-lowering drug showed prolonged QTc intervals, indicating that strict glycemic control is insufficient to normalize the electrophysiological disturbances. Careful studies are required to elucidate if cardio protection includes electrical remodeling and prolonged repolarization, as authors predict that the mechanism of cardioprotection might involve a reduction of arrhythmia.

A review article by Kassab et al. focused on the role of mitochondrial outer membrane protein, Miro1, which mediates the movement of mitochondria along the microtubules. Authors emphasized the importance of mitochondrial dynamic, motility, shape, and structure in the diabetic myocardium as reports show smaller mitochondria associated with diabetic myocardium. They also pointed out in this review that HF and diabetes are recognized with enhanced activation of the NLRP3 inflammasome that links microtubule organization, inflammation, and the association to mitochondrial motility, which may lead to novel therapeutic approaches toward the mitochondrial-microtubule-inflammatory axis. Furthermore, the expression of Miro1 and whether it influences the processes of fission and fusion for mitophagy in the heart remains unclear, which led to this review. Based on the existing literature, the authors suggest a possible unifying mechanism linking impaired mitophagy, the MT network, and the inflammatory response to arrested mitochondrial movement. As the causative agents and mechanisms of mitochondrial dysfunction and impaired motility are discovered, new promising treatment therapies may emerge to promote better cardiac outcomes in DCM/HF.

In another interesting study, Huang et al. targeted a specific protein, P2X7R, whose role in diabetic myocardium is not studied but predicted to play an essential role. When they treated mice with a P2X7R inhibitor (A438079), they reduced myocardial hypertrophy, fibrosis, and apoptosis and improved cardiac function. Mechanistically they showed that P2X7R plays an essential role in the pathogenesis of STZ-induced diabetic cardiac damage and remodeling through the PKCβ/ERK axis and suggest that P2X7R may be a potential target in the treatment of DCM. Although one of the critical features of DCM is cardiac fibrosis (CF), which is still not successfully targeted in treating heart failure, Lin et al. showed that inhibition of sodium-dependent glucose transporter 1 (SGLT1) attenuates cardiomyocyte apoptosis and delays the development of DCM. Further, they evaluated the changes in the expression of SGLT1 in the progression of diabetic cardiac fibrosis. They identified a significant increase in SGLT1 expression in the diabetic heart, concluding that SGLT1 is involved in cardiac fibrosis *via* the p38 and ERK1/2 signaling pathways.

Shraim et al. reviewed the Epidermal Growth Factor Receptor (EGFR/ErbB/HER) family of tyrosine kinases in diabetes myocardium as chronic dysregulation of EGFR may act as mediating diabetes-induced cardiovascular pathology. Authors examined their potential interplay with the Renin-Angiotensin-Aldosterone System heptapeptide, Angiotensin-(1-7), as well the arachidonic acid metabolite, 20-HETE (20-hydroxy-5, 8, 11, 14-eicosatetraenoic acid). Its greater understanding of other critical modulators of cardiovascular function could facilitate the development of novel therapeutic strategies for treating diabetes-induced cardiovascular complications.

Muñoz-Córdova et al. critically discussed the mechanism of diabetic mediated cardiac dysfunction by highlighting three critical areas: inflammatory signaling, mitochondrial alterations, and autophagic flux, their contribution to the pathogenesis, and their potential as pharmacological targets. Here, they critically discussed the mechanisms by highlighting the latest evidence, their contribution to the pathogenesis, and their potential as pharmacological targets. Though plenty of antidiabetic agents benefit HF, there is scarce information available for DCM. Authors cited the combined effect of drugs such as liraglutide and dapagliflozin that showed improved diastolic function and regression of left ventricular mass in patients with T2DM. On

the other hand, Sitagliptin prevents the exacerbation of DCM in T2DM when it is used together with other antidiabetic drugs. Therefore, the authors highlight the importance of considering already approved therapies in searching for new DCM as a study of the mechanism can lead to discovering new molecules with better therapeutical properties.

Association of epigenetics and various factors and how those contributes to the development of DCM is comprehensively reviewed by Mittal et al. They discussed different epigenetic mechanisms such as histone modifications (acetylation and methylation), DNA methylation and non-coding RNA in modulating molecular pathways regulating the expression of important molecules. Authors cleverly explored the disease with complex etiology and cumulative effects of crosstalk between genetic and epigenetic factors, which has several inducers such as ROS-mediated oxidative stress, hyperglycemic conditions, cytokines-mediated inflammation, cell death, and epigenetic regulation of dysregulated molecular pathways induced by these mediators. They also discussed the possibility of inhibiting HDACs as promising therapeutic targets for DCM. In another review article, Mohan et al. discussed and evaluated critical clinical studies that studied the effect of drugs such as allopurinol, SGLT2 inhibitor, and metformin in reverting left ventricular hypertrophy patients with and without T2DM.

Interestingly, Liu et al. demonstrated that a combinational exposure of bisphenol A (BPA) and a high-fat diet (HFD) in female mice during the perinatal period can cause susceptibility to insulin resistance, obesity, impaired glucose tolerance, and increased blood pressure, cardiac hypertrophy, and impaired endothelial function in their F2 offspring. In addition, these inherited transgenerational abnormalities showed a sex-specific pattern. They strongly recommend adjusting lifestyle and alleviating exposure to environmental EDSs during pregnancy to reduce the risk of metabolic and cardiovascular diseases such as DCM in the offspring.

In conclusion, this Research Topic emphasizes various molecular mechanisms involved in DCM development and explores potential therapeutic targets against DCM. Although DCM is a multifactorial complex metabolic disease, this Research Topic will be an essential key to conceptual advancement in the field of DCM.

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

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REFERENCES

1. Martin-Timon I, Sevillano-Collantes C, Segura-Galindo A, Del Canizo-Gomez FJ. Type 2 diabetes and cardiovascular disease: have all risk factors the same strength? *World J Diabetes.* (2014) 5:444–70. doi: 10.4239/wjd.v5.i4.444
2. Low Wang CC, Hess CN, Hiatt WR, Goldfine AB. Clinical update: cardiovascular disease in diabetes mellitus: atherosclerotic cardiovascular disease and heart failure in type 2 diabetes mellitus - mechanisms, management, and clinical considerations. *Circulation.* (2016) 133:2459–502. doi: 10.1161/CIRCULATIONAHA.116.022194
3. Baseler WA, Dabkowski ER, Williamson CL, Croston TL, Thapa D, Powell MJ, et al. Proteomic alterations of distinct mitochondrial subpopulations in the type 1 diabetic heart: contribution of protein import dysfunction. *Am J Physiol Regul Integr Comp Physiol.* (2011) 300:R186–200. doi: 10.1152/ajpregu.00423.2010
4. Walker AM, Cubbon RM. Sudden cardiac death in patients with diabetes mellitus and chronic heart failure. *Diab Vasc Dis Res.* (2015) 12:228–33. doi: 10.1177/1479164115573225

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REVIEW
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Cellular Protein Quality Control in Diabetic Cardiomyopathy: From Bench to Bedside

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Heart failure is a serious comorbidity and the most common cause of mortality in diabetes patients. Diabetic cardiomyopathy (DCM) features impaired cellular structure and function, culminating in heart failure; however, there is a dearth of specific clinical therapy for treating DCM. Protein homeostasis is pivotal for the maintenance of cellular viability under physiological and pathological conditions, particularly in the irreplaceable cardiomyocytes; therefore, it is tightly regulated by a protein quality control (PQC) system. Three evolutionarily conserved molecular processes, the unfolded protein response (UPR), the ubiquitin-proteasome system (UPS), and autophagy, enhance protein turnover and preserve protein homeostasis by suppressing protein translation, degrading misfolded or unfolded proteins in cytosol or organelles, disposing of damaged and toxic proteins, recycling essential amino acids, and eliminating insoluble protein aggregates. In response to increased cellular protein demand under pathological insults, including the diabetic condition, a coordinated PQC system retains cardiac protein homeostasis and heart performance, on the contrary, inappropriate PQC function exaggerates cardiac proteotoxicity with subsequent heart dysfunction. Further investigation of the PQC mechanisms in diabetes propels a more comprehensive understanding of the molecular pathogenesis of DCM and opens new prospective treatment strategies for heart disease and heart failure in diabetes patients. In this review, the function and regulation of cardiac PQC machinery in diabetes mellitus, and the therapeutic potential for the diabetic heart are discussed.

Keywords: diabetic cardiomyopathy (DCM), cardiovascular disease, protein quality control (PQC), autophagy, proteostasis, unfolded protein response, proteasome

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INTRODUCTION

Diabetes mellitus is one of the fastest-growing health issues worldwide, and it is a major threat to cardiovascular health. In 2019, it was estimated that 463 million people had diabetes, a number predicted to reach 700 million by 2045 (1), and diabetes patients have a 2–5-fold increased risk of developing heart failure (2, 3). Diabetic cardiomyopathy (DCM) refers to the cardiac dysfunction and structural abnormalities subsequent to diabetes, and independent of coronary artery disease, hypertension, and valve malfunctions (4, 5). The systemic metabolic alterations caused by reduced insulin secretion, in type 1 diabetes mellitus (T1DM), or progressive insulin resistance, in type 2 diabetes mellitus (T2DM), constitute continuous cardiac stress that leads to the activation of numerous cellular responses. DCM is characterized by impaired cellular homeostasis,

the progressive accumulation of reactive oxygen species (ROS), reactive nitrogen species (RNS) and advanced-end glycation products, organelle dysfunction, and chronic inflammation. Eventually, DCM promotes pathological myocardial remodeling, resulting in cardiac dysfunction. Clinically, diastolic dysfunction is the first manifestation of DCM, followed by systolic dysfunction in later stages, and, ultimately, heart failure (6, 7). However, a single anti-diabetes agent (e.g., metformin or fibrate) is unable to ameliorate multiple comorbid conditions. The combination of individual therapies is indispensable for T2DM patients with other complications, including DCM.

Proteins are the primary managers of cellular homeostasis; therefore, regulation of their synthesis, maturation, and degradation in cardiomyocytes is essential for cardiac performance. To cope with the imbalance in the cardiac protein cycle in response to pathological stress, crucial protein quality control (PQC) systems participate in maintaining cellular protein homeostasis (8, 9) (**Figure 1**). Endoplasmic reticulum (ER) stress activates the unfolded protein response (UPR^{ER}) to reduce protein synthesis, increase the expression of folding chaperones, and degrade non-functional proteins through the ER-associated protein degradation (ERAD) pathway. ERAD recognizes and translocates non-functional proteins into the cytosol for degradation. The ubiquitin-proteasome system (UPS) breaks down most proteins secreted by ERAD and those that have reached the end of their lifespan. Proteins that cannot be processed by the proteasome or protein aggregates are broken down via the autophagy-lysosome system. Similar to ER, mitochondria have a specific UPR (UPR^{mt}) signaling to manage their unfolded protein load and can be selectively marked for autophagic degradation when the damage surpasses their coping capabilities (11). Coordination of PQC systems is adaptive and protective, while impaired PQC contributes to cardiac aging and diseases (12), including DCM (9). Therefore, it is crucial to comprehensively understand the function and regulation of PQC pathways to identify potential therapeutic targets and strategies for DCM.

UPR

The ER is a central organelle for cellular PQC, operating as the keeper of the multistep maturation process of nascent polypeptides into functional proteins. The ER serves several cellular functions, comprising protein folding, posttranslational modifications, trafficking, calcium homeostasis, and lipid biosynthesis (13–16). Any intracellular and extracellular perturbations to its protein folding capacity result in ER stress and trigger the multi-faceted UPR^{ER} necessary for cellular PQC (17–20). Recently, mitochondria have been found to possess their own stress response to manage the unfolded proteins contained within them, also contributing to protein homeostasis (21).

The UPR^{ER} Process

The primary intent of the UPR^{ER} is to adapt to any cellular changes by restoring protein homeostasis. The chaperones

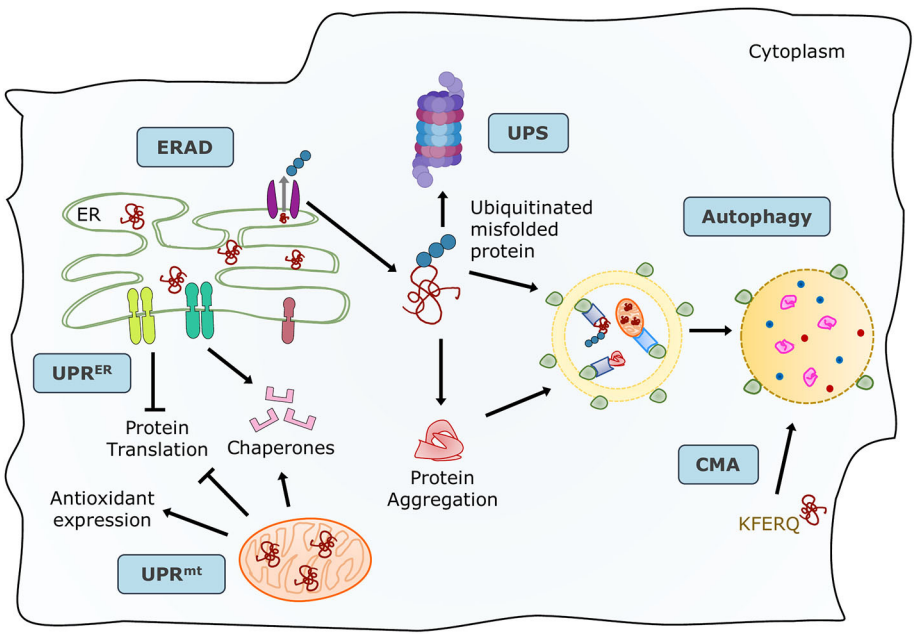

FIGURE 1 | Maintenance of protein homeostasis by the principal PQC systems. Accumulation of misfolded proteins in the ER trigger the UPR to increase its folding capacity by upregulating chaperone expression, and to decrease protein load by inhibiting translation. Misfolded proteins are exported by ERAD complexes that label them and direct them to degradation through the UPS and autophagy. Misfolded or damaged proteins are also sequestered into protein aggregates to reduce their toxicity, these aggregates are processed by the autophagy-lysosome system. Proteins with the KFERQ motif are translocated into the lysosomes via CMA. Additionally, unfolded proteins in mitochondria induce the mtUPR to increase mitocondrial proteases and chaperones. Adapted from Ciechanover et al. (10) and used under CC BY 3.0.

in the ER-lumen assist protein folding by binding to the hydrophobic regions of the nascent proteins (GRP78, GRP94), promoting glycoprotein interactions (Calreticulin, Calnexin), and facilitating the formation of disulfide bridges (ERP57, ERP78) (22). The master chaperone glucose regulatory protein 78 (GRP78) also binds to calcium, assists in ER permeability during protein translocation, and guides the misfolded proteins for degradation. The initial step of UPR^{ER} is the release of the transmembrane sensors, primarily bound to the master chaperone under non-stressed conditions (23). These UPR^{ER} sensors, including protein kinase RNA-like endoplasmic reticulum kinase (PERK), inositol-requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6), are distinctively activated by stress stimuli and elicit varied adaptive downstream responses. Upon ER stress, PERK is majorly involved in attenuation of translation for lessening ER protein load via eukaryotic translation initiation factor 2α (eIF2α). PERK-eIF2α increases the expression of key genes facilitating UPR^{ER} via activating transcription factor (ATF4). On the other hand, the endoribonuclease activity of IRE1 causes splicing of the transcription factor, X-box binding protein 1 (XBP1). The spliced XBP1 (sXBP1) upregulates the expression of genes involved in UPR^{ER} signaling (9) and the ERAD pathway (24). IRE1-dependent decay (RIDD) is known to regulate essential ER-localized messenger RNAs (mRNAs) to reduce the inflow of newly synthesized proteins into the ER (25). IRE1 also enhances the degradation of terminally misfolded proteins via ERAD (UPS section) (18). Finally, upon activation, ATF6 translocates from the ER luminal domain to the Golgi apparatus, where site-1 and site-2 proteases cleave it to form an active segment, p50ATF6. The activated ATF6 transcriptionally regulates essential genes responsible for UPR^{ER} (26). All three UPR^{ER} branches are required to upregulate chaperones expression for assisting protein proper folding (27).

The ER stress response (ERSR) initially induces an adaptive UPR^{ER} to a certain threshold. In the face of chronic pathological stresses, oversaturated ER ensues apoptotic ERSR (28). Overexpression of ATF4 upregulates C/EBP homologous protein (CHOP), growth arrest, and DNA damage-inducible 34 (GADD34) and other pro-apoptotic genes (25). CHOP induces cell death by dysregulating the balance between pro- and anti-apoptotic genes from B-cell lymphoma 2 (BCL2) family. Also, oligomerization of the pro-apoptotic proteins BAX and BAK on the ER membrane causes calcium release into the cytosol, eventually promoting mitochondria-dependent apoptotic pathways (29). IRE1 induces ER-mediated apoptotic mechanisms via recruitment of TNF receptor-associated factor (TRAF) 2 and apoptosis signal-regulating kinase 1 (ASK1), leading to activation of c-Jun N-terminal kinase (JNK), and caspase-12 signaling pathways. In addition to IRE1-regulated caspase 12 cleavage, m-calpain, a cysteine protease, directly cleaves caspase-12 upon stimulation, resulting in its activation (30). The three UPR^{ER} branches exist to facilitate both cytoprotective and apoptotic responses depending on the nature of the stimulus (25, 27); therefore, it is not surprising that temporal dynamics of the UPR^{ER} has an important role in determining cellular fate.

Physiopathological Role of UPR^{ER} in the Heart

ER-resident genes have been deemed essential in the heart. GRP78 or XBP1 deficiency is implicated in impaired cardiac development (31, 32) and cardiac dysfunction in response to pathological stresses (33, 34). The increase in protein disulfide isomerase (PDI) (35), an ER chaperone, and sXBP1 expression (36) in ischemic human hearts suggest UPR^{ER} is an adaptive component of the cardiac stress response. However, a maladaptive stress response is evident in dilated and failing human hearts marked by an increase in CHOP expression and cell death (37, 38). In light of the clinical evidence, it is apparent that the ERSR has both adaptive and maladaptive roles in cardiac pathology.

ER chaperones promote cell survival under pathological stress in the heart; nevertheless, overexpression is damaging. Cardiac GRP78 knockout in adult mice induced increased cell death, reduced cardiac performance, and caused early mortality (31). Moreover, the pre-induction of GRP78 and GRP94 had a cardioprotective role under oxidative damage in ischemia/reperfusion (39). On the contrary, increased protein synthesis under cardiac hypertrophy upregulated GRP78 expression, simultaneously, under pressure overload, GRP78 overexpression further potentiated hypertrophy by stimulating expression of hypertrophic factors resulting in cardiac dysfunction (40). Additionally, the overexpression of calreticulin, an ER chaperone, resulted in cardiac remodeling, dysfunction, and heart failure due to prolonged UPR^{ER} activation. This damaging effect of calreticulin overexpression *in vivo* was abated by inhibition of IRE1 (41, 42), overall suggesting the importance of balanced UPR^{ER} to tackle pathological stress in the heart.

Several animal studies targeting the individual UPR^{ER} branches emphasized the importance of UPR^{ER} in the pathological hearts of different etiologies. Cardiac PERK deficiency aggravated heart function in response to pressure overload in mice (43), indicating the cytoprotective role of the PERK branch. Moreover, transient IRE1-XBP1 response following pressure overload in mice (44) limited myocardial injury by reducing ER-associated cell death and inflammation (45) and promoting adaptive hypertrophy, in turn preserving contractility in hypertrophic failing hearts (25, 46). Similarly, cardiac XBP1 deficiency enhanced pathological remodeling and dysfunction (47). Lastly, ATF6 deletion in mouse hearts resulted in increased oxidative stress and decreased function after ischemia/reperfusion. The equivalent *in vitro* ATF6 knockdown model in cardiomyocytes showed similar results, which were obliterated by ATF6 overexpression (48). As noted, transient activation of all three UPR^{ER} branches has an adaptive function succeeding acute cardiac ER stress, while sustained activation of UPR^{ER} results in irreversible damage to the myocardium. This persistent stress signaling induces cardiomyocyte death via activation of ER-mediated apoptosis following myocardial infarction, ischemia/reperfusion, and pressure overload (23, 49). Also, Miyazaki et al. (50) demonstrated that cardiac CHOP deficiency inhibits ER-mediated myocardial apoptosis and

inflammation following reperfusion injury, highlighting the role of maladaptive ERSR.

The UPR^{ER} in DCM

Role of the UPR^{ER} and Apoptotic ERSR in DCM Development

The role of ER stress in the development of DCM was first observed in failing diabetic human hearts with swollen ERs (51), indicating protein imbalance. They also presented ER-mediated apoptosis, evidenced by increased CHOP and cell death (52). These clinical findings imply that the impaired UPR^{ER} predisposes the diabetic heart to failure; however, the precise nexus is elusive. The cardiac fate following ERSR has since been ascertained in several animal models of DCM. In the diabetic models, the elevation of cardiac ER stress-related markers (53) and UPR^{ER} genes (54–57) is associated with cardiac abnormalities (58) and apoptosis (59). Although the canonical UPR^{ER} signaling is an adaptive response, chronic ER stress is deleterious in the diabetic heart. In T1DM, prolonged ATF6 activation-induced cell death (60), extracellular matrix gene expression, cardiac fibrosis (61), and reduced cardiac compliance in rat models. Moreover, oxidative stress resulted in cardiac dysfunction in type 1 diabetic hearts via persistent PERK signaling (62). The role of over-activated PERK-CHOP and ATF6 ensuing apoptotic signaling via BCL2 associated agonist of cell death (BAD) and contributing to ER-mediated cardiac dysfunction was also recapitulated in T2DM rodent hearts (63). Apoptotic ERSR is associated with pathogenesis of DCM due to irreplaceable cardiomyocyte loss associated with the upregulation of cleaved-caspase 12, CHOP, and JNK in type 1 and type 2 diabetic hearts (51, 54). Altogether, the maladaptive ERSR in DCM prompts organelle dysfunction, cell death, and subsequent myocardial remodeling (64), suggesting that hyperactivated PERK and ATF6 are detrimental in DCM.

Metabolic Triggers of UPR^{ER} in DCM

Pathological remodeling and cardiac dysfunction in DCM are accompanied by alterations in cellular protein synthesis, which can facilitate ER stress and UPR^{ER}. ER stress is an early event in DCM, and the major triggers include hyperglycemia, hyperlipidemia, insulin deficiency/resistance, and inflammation (65, 66) (**Figure 2**). High glucose and lipid overload induce oxidative stress interceding dysregulated protein homeostasis, prolonged UPR^{ER}, and cardiomyocyte death (67). Glucose and lipids upregulated adaptive IRE1-XBP1 signaling (51), and prolonged stress triggered apoptotic CHOP (63), IRE1-JNK (68), and caspase 12 activation in human cardiac cells (69), type 1 (55), and type 2 (30) diabetic rodent hearts. Inflammation and hyperinsulinemia are other factors that instigate ER stress and can be further potentiated by ER stress in a detrimental loop. As a coping mechanism, hyperinsulinemia-induced ER stress has emerged as a new player in the onset of insulin resistance (70, 71), possibly via IRE1/JNK signaling (58), contributing to reduced cardiac function in T2DM (72). On the other hand, pro-inflammatory cytokine interleukin-1β via interleukin 1 receptor-associated kinase 2 (IRAK2) promoted CHOP expression and cell death in T1DM, thereby impairing cardiac function (73). IRAK2 is known to be elevated in the condition of ER stress (74), suggesting a feedback loop mechanism accountable for unalleviated ER stress. However, the direct mechanism of ER-mediated inflammation and cardiac dysfunction in DCM is yet to be determined. Further mechanistic study of the intrinsic details of preferential UPR^{ER} under the numerous drivers of ER stress in DCM is essential.

Intrinsic Regulation of UPR^{ER} in DCM

The involvement of ERSR in DCM progression is well-accepted; nonetheless, only a few regulatory mechanisms of UPR^{ER} in diabetes are documented, where the ER machinery coordinates with several cellular molecules and signaling pathways (**Figure 3**). For instance, downregulated NAD-dependent protein deacetylase sirtuin 1 (SIRT1) promoted stress signaling pathways such as IRE1-JNK in T1DM (75), and PERK-CHOP and IRE1-caspase 12 signaling in T2DM (76), resulting in ER-mediated apoptosis and cardiac dysfunction. A protein kinase, general control nonderepressible (GCN2), triggered cell death, and cardiac dysfunction directly via the eIF2α-ATF4-CHOP pathway in T1DM and T2DM (77). In addition, increased EGFR tyrosine kinase receptor activation instigated ER stress in T1DM (78) and in T2DM following myocardial infarction (79) by increasing CHOP associated cell death. The ERSR is also regulated via transcription factors. Forkhead box O1 (FOXO1) activation leads to direct and indirect induction of ER stress in DCM via PERK signaling (80, 81), and peroxisome-proliferator activator receptor (PPAR) β/γ activity promotes XBP1 splicing restoring ER balance and providing cryoprotection under diabetic stress in human cardiac cells (69). Additionally, microRNAs (miRNAs) have been observed to regulate UPR^{ER} in the diabetic heart. *mir455* and *mir22* are cardioprotective in T1DM (61) and T2DM (82), respectively. *Mir455* reduces cardiac fibrosis via calreticulin suppression, and *mir22* alleviates ER-mediated apoptosis via SIRT1 upregulation.

Role of Non-canonical UPR^{ER} in DCM

Apart from the regulated framework of UPR^{ER}, diabetic condition also impairs UPR^{ER} capacity by directly regulating ER chaperones. Elevated PDI, despite the cardioprotective action under ischemic cardiomyopathy (35), was associated with increased cell death in hearts from diabetes patients (56). The lack of protective effect was attributable to the altered redox state of PDI under type 1 diabetes. Moreover, dual-color fluorescence imaging indicated an abnormal ER oxidative state and altered polarity in diabetic myocardial tissue (83), suggesting ER oxidative state may contribute to impaired UPR^{ER} under diabetes. Therefore, the maladaptive ERSR can be promoted by factors independent of canonical UPR^{ER} pathways in the diabetic heart. Also, post-translational modifications such as O-GlcNACylation are essential for protein stability and function. The protective effect of XBP1 on O-GlcNACylation (8, 46, 84) is absent in T2DM, leading to cardiac dysfunction (85), which is

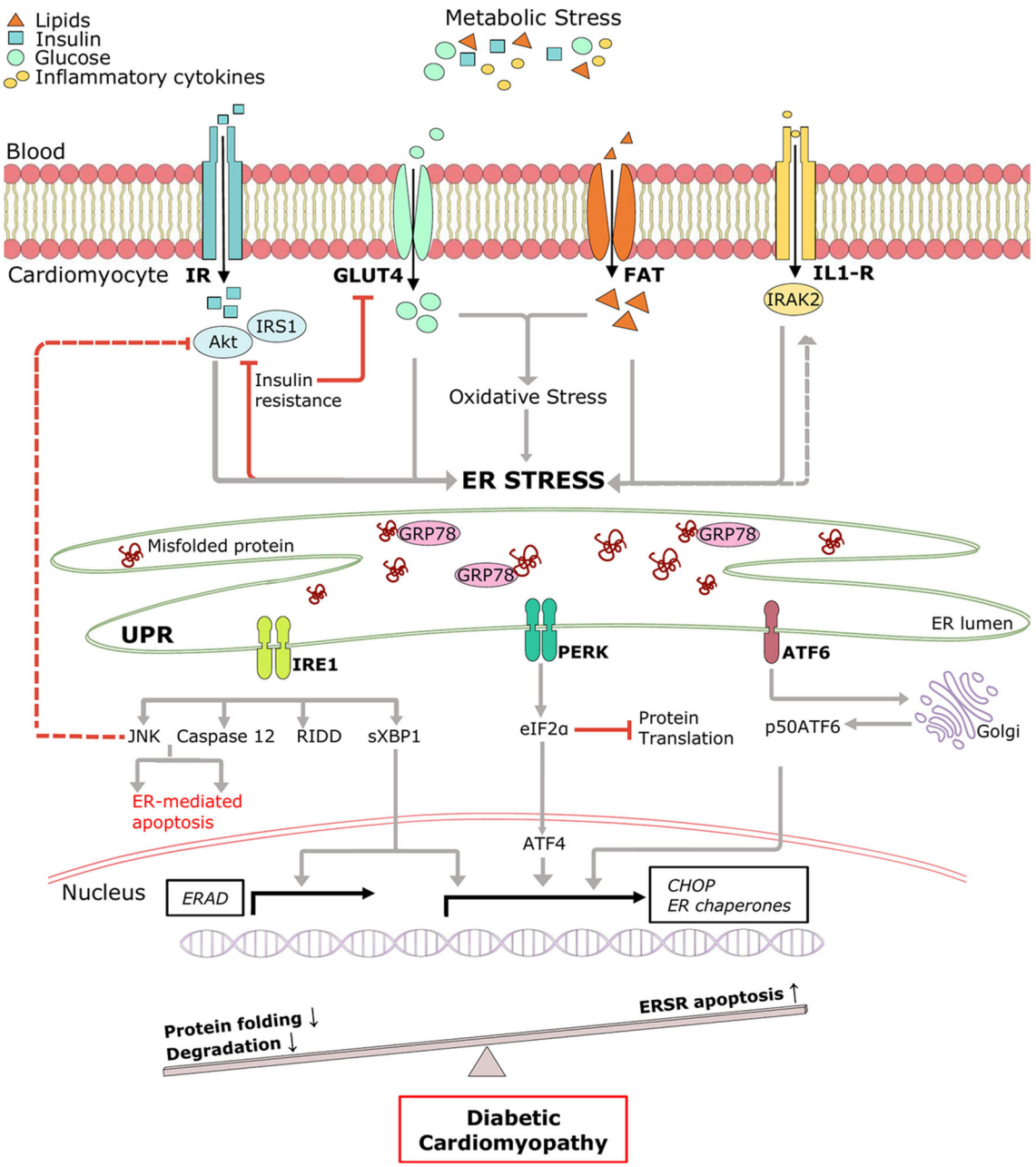

FIGURE 2 | Pictorial representation of triggers of ER stress and role of ERSR activated UPR in the progression of DCM. Under systemic metabolic stress, lipid accumulation, and high glucose directly triggers ER stress and indirectly via oxidative stress. Hyperinsulinemia and inflammatory cytokines also induce ER stress, and in a feedback loop mechanism, ER stress triggers insulin resistance and inflammation (dotted lines). Upon accumulation of misfolded proteins, UPR signaling is activated following dissociation from GRP78. UPR sensors, IRE1, PERK, and ATF6 and its downstream network drives multiple signal outputs such as inhibition of protein translation and increased gene upregulation. Balanced UPR restores protein homeostasis by increasing ER chaperones and ERAD genes. Prolonged stress induces apoptotic ERSR via upregulation of CHOP, contributing to the pathogenesis of DCM. IR, Insulin receptor; IRS1, insulin receptor substrate 1; Akt, protein kinase B; GLUT4, glucose transporter type 4; FAT, fatty acid transporter; IL-R, Interleukin 1β receptor.

likely due to delayed UPR^{ER} action, as the timely UPR^{ER} lacks in the diabetic heart. Despite the growing knowledge about ERSR following various stresses faced by the diabetic heart, it is still unclear about how or when the switch between adaptive UPR^{ER} and apoptotic ERSR supervenes.

Mitochondrial UPR (UPR^{mt}) in the Heart

The mitochondrial proteome contains more than 1,300 proteins and the majority of the nuclear-encoded proteins are imported into the organelle in an unfolded state (86). Mitochondrial PQC entails protein import and folding via chaperones (HSP60,

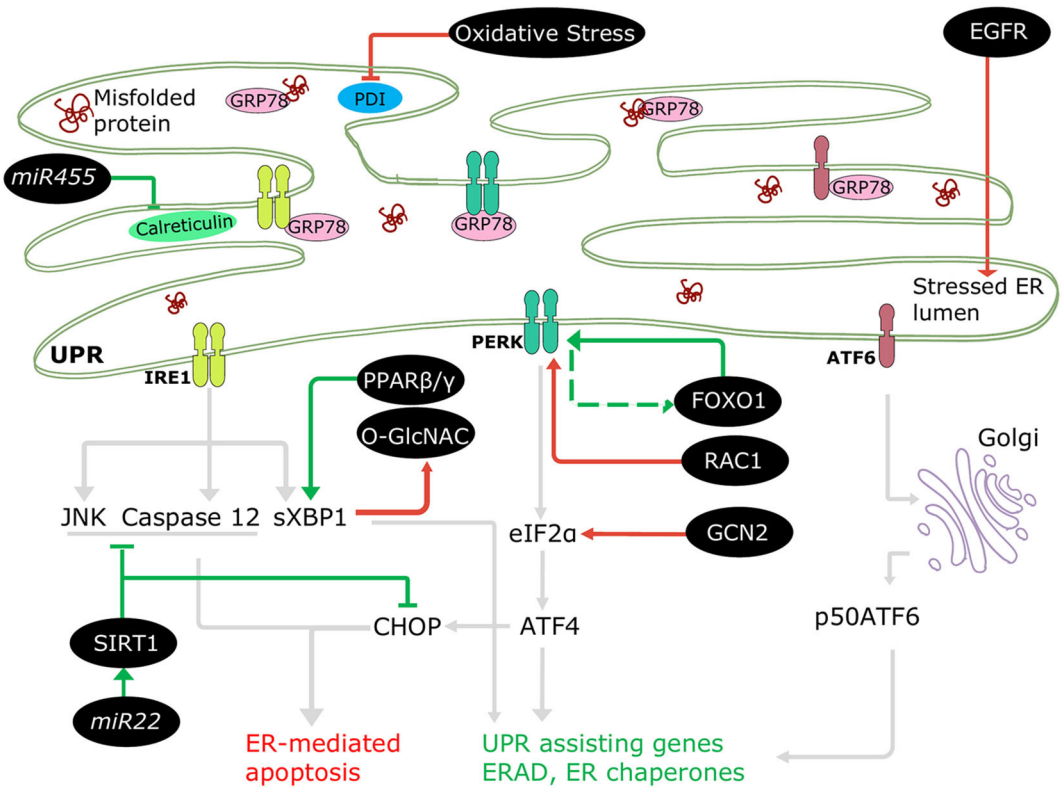

FIGURE 3 | Regulation of ERSR in the diabetic heart. Some cellular molecules (FOXO1, PPARβ/γ, SIRT1, *mir22*, *mir455*) participate in regulation of adaptive ERSR by activating UPR and inhibiting ER-mediated apoptosis (green lines). On the other hand, some molecules and stresses (oxidative stress, O-GlcNAC, EGFR, RAC1, GCN2) stimulate maladaptive ERSR (red lines).

HSP70, and TRAP1) and degradation of misfolded proteins by proteases (ClpP, YME1L1, LonP1, HTRA2/Omi, and Oma1) (87). In response to stresses, the UPR^{mt} initiates a retrograde response to the nucleus to ensure proteome integrity via induction of the UPR^{mt}-related chaperones (88). Akin to UPR^{ER}, UPR^{mt} transiently inhibits protein translation and aims to mitigate proteotoxic stress inside the mitochondrion (89, 90). Under physiological conditions, activating transcription factor 5 (ATF5) is imported into the mitochondrion and degraded by LonP1; however, stress targets ATF5 to the nucleus as the transcription factor for the induction of UPR^{mt} (91). Owing to the cross-over among certain stress response proteins (PERK, ATF4, and CHOP) and the physical linkage between ER and mitochondria, both UPR^{ER} and UPR^{mt} participate in an integrated stress response to maintain cellular proteostasis (21). For instance, consequent to eIF2α activation, translation of ATF4, CHOP and ATF5 regulate the UPR^{mt}. UPR^{mt-}associated expression of CHOP is identified by binding of c-Jun to the AP-1 promoter region in the CHOP gene (92). CHOP binding, along with MURE1/2 elements in the promoter region, increases transcription of HSP60, ClpP, ATF5, and LonP1. Additionally, misfolded proteins are ubiquitinated in the inner mitochondrial space and degraded by the UPS in the cytosol in a process called mitochondrial associated degradation (93).

Mitochondrial PQC is essential for cardiac structure and function (94). In clinic, patients with ventricular pressure overload due to aortic stenosis had elevated ATF5 and reduced apoptosis (95), suggesting its protective role under cardiac stress. Analogous to clinical observation, silencing ATF5 in cardiomyocytes abated UPR^{mt} and its protection against pressure overload (96). Similarly, pharmacological stimulation of UPR^{mt} ameliorated cardiac dysfunction following ischemic injury via ATF5 induction (97). Moreover, HSP70 overexpression increased the import of antioxidant proteins, reduced cell death, and improved cardiac function against ischemic stress (98). As such, UPRmt is protective under cardiac stress; nonetheless, the role of mitochondrial proteases is still unclear. For instance, under hypoxia, mitochondrial protease LonP1 contributed to ROS accumulation and cell death in cultured cardiomyocytes (99). On the other hand, LonP1 overexpression was found to be protective following ischemic/reperfusion injury in mouse hearts (100) while reduced LonP1 activity in mitochondria contributed to contractile dysfunction after pressure overload (101). Interestingly, the same study demonstrated that LonP1 activation induces UPR^{ER}; however, UPR^{ER} is activated before UPR^{mt}, suggesting a fine-tuning role of LonP1 in the integrated stress response. More importantly, LonP1 deficiency was compensated via ATF4-dependent fibroblast growth factor

(FGF21) activation (92, 101), a marker for mitochondrial stress signaling involved in mediating metabolic changes and ameliorating cardiac dysfunction under several cardiac etiologies, including diabetes (21, 102). Also, the mitochondrial protease, Oma1, is upregulated under cardiac ischemic stress; however, its ablation is protective against heart failure in mice (103).

UPR^{mt} chaperones are likely protective under diabetic stress in the heart. There is reduced expression of the mitochondrial chaperone, HSP70, in T2DM human hearts (104), indicating decreased protein import and UPR^{mt} induction. In the hearts of pre-diabetic rats with hyperinsulinemia, there was an increase in HSP60 expression; however, after prolonged diabetic stress HSP60-mediated myocardial protection decreased due to abated expression (105). Moreover, hyperglycemia reduced TRAP1 expression and activity, ultimately reducing cardiomyocyte viability (106). Furthermore, in T2DM rodent hearts, UPR^{mt} is responsible for the dysregulation of the mitochondrial permeability transition pore, associated with elevated cell death and ischemic injury (107). Overall, adaptive UPR^{mt} is critical for cardiac structure and function under diabetic stress; however, the detailed mechanistic role of the mitochondrial PQC and its therapeutic applications is yet to be cemented in DCM.

UPS

The UPS is a major quality control pathway in eukaryotic cells, which plays a fundamental role in maintaining cellular proteostasis and, as such, ensures cell viability and function. The UPS is the primary proteolytic path for ∼80% of cellular proteins, most of which are short-lived, misfolded, or damaged (108, 109). Mechanistically, the ubiquitin proteolytic pathway involves two distinct steps: ubiquitylation of protein substrates and degradation of the ubiquitylated proteins by the proteasome (110).

UPS Process

Ubiquitylation

Ubiquitin is a 76-amino acid globular protein that is highly conserved in eukaryotes, and its transfer to target proteins is mediated by a carefully choreographed enzymatic cascade. Initially, ubiquitin is activated to a high-energy thiol ester state by the ubiquitin-activating enzyme E1 in an ATP-dependent manner. Following activation, the ubiquitin moiety is transferred to ubiquitin-conjugating proteins E2 by transesterification. Finally, an E3 ubiquitin ligase catalyzes ubiquitin transfer from the E2-ubiquitin thioester intermediate to a lysine residue on the substrate protein (111–113) (**Figure 4A**). The human genome encodes ∼1,000 E3 ubiquitin ligases, which are subdivided into three major groups, depending on which of the following three domains they possess, namely, really interesting new gene (RING), RING-in-between-RING (RBR), and homologous to the E6-AP carboxyl terminus (HECT). It has been widely reported that the E3 ubiquitin ligases confer specificity to the ubiquitylation process (116). The proteins are targeted by either a single ubiquitin molecule (monoubiquitylation) or ubiquitin chains (polyubiquitylation). To date, eight structurally and functionally distinct ubiquitin linkages (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48, Lys63, and Met1) have been identified, among of them, Lys48 and Lys63 are the most prominent linkage types (117–119). Lys48-ubiquitylated proteins are typically subjected to proteasomal degradation, while Lys63-linked ubiquitin chains mediate autophagic protein quality control (120, 121) (**Figure 4B**).

Akin to other posttranslational modifications, the ubiquitylation process is reversible; removal of ubiquitin molecules from substrate proteins is mediated by deubiquitylating enzymes (DUBs) (122). DUBs perform critical roles in the ubiquitylation pathway (123). First, *de novo* ubiquitin is translated as either linear polyubiquitylated chains, or ubiquitin fused to small ribosomal proteins, and DUBs are required to free mono ubiquitin from these precursors. Second, in consort with the E3 ligases, DUBs mediate ubiquitin chain editing, which can alter the ubiquitin signal or protein stability. Finally, DUBs maintain ubiquitin homeostasis by recycling ubiquitin molecules (122, 123).

Proteasomal Degradation

The degradation of polyubiquitylated proteins is catalyzed by the 26S proteasome, a large ATP-dependent multicatalytic complex composed of a barrel-shaped 20S core protease (CP) capped at one or both ends by the 19S regulatory particle (RP) (124) (**Figure 4C**). The 20S CP is composed of 28 subunits that are arranged as a cylindrical stack containing four hetroheptameric rings, two peripheral α-rings (α_{1-7}), and two inner β-rings (β_{1-7}). The two β-rings form the central proteolytic chamber, whereas the α subunits guard substrate entry into the chamber, impeding access when the proteasome is in an inactivated state (125, 126). The proteolytic activity of the 20S CP is activated by binding to the 19S RP to establish the proteasome holoenzyme (127). Protein components of the 19S RP recognize ubiquitylated substrates and transport them to the proteolytic core in an ATP-dependent manner (128–131). The peptidase activity of the 20 CP is also activated by other regulatory particles such as the 11S RP, which mediates protein degradation via a ubiquitin- and ATP-independent manner (132). The output of proteasomal degradation is small peptides, which, upon proteasomal exit, are further degraded by a plethora of cytosolic peptidases to generate amino acids to be recycled.

Chaperone-Assisted Proteasomal Degradation (CAP)

Molecular chaperones are essential for the folding fidelity and conformational integrity of proteins (133, 134), by participating in nascent polypeptide folding, protein transport, assembly of oligomeric complexes, and repair of misfolded proteins (133). In addition, chaperones can also facilitate the degradation of folding-incompetent proteins, thereby preventing their aggregation (133, 135). The C-terminus of Hsc70-interacting protein (CHIP) is a central player in chaperone-mediated degradation (136, 137). CHIP binds with the constitutively expressed HSP70/HSC70 chaperones and members of the ubiquitin conjugating enzyme, such as the Ubc4/5 family, to initiate chaperone substrate sorting to the proteasome or lysosome (135, 138). BAG family molecular chaperone regulator

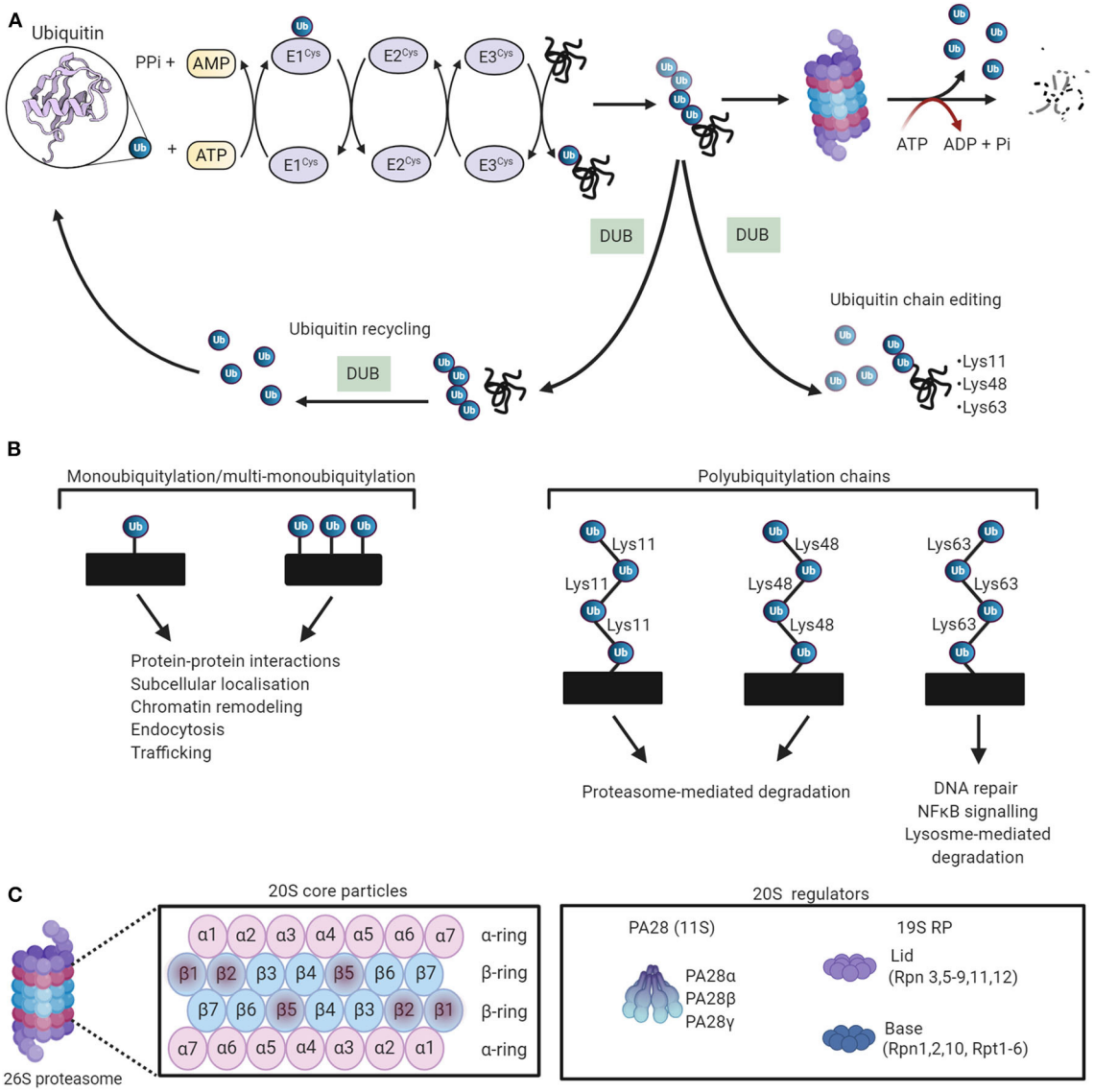

FIGURE 4 | The ubiquitin-proteasome system (UPS) working theory. **(A)** The UPS marks substrate proteins for degradation via a ubiquitylation system where internal lysine residues of substrate proteins are tagged with ubiquitin (PDB ID: 1UBQ) (114). These ubiquitylated proteins are then degraded by the proteasome. Deubiquitylating enzymes (DUBs) edit ubiquitin chains and mediate ubiquitin recycling. Adapted from Zheng et al. (115) and used under CC BY. **(B)** The different ubiquitin linkages and their unique biological functions. **(C)** The proteosomal 20S core particle (CP) consists of four stacked rings, two outer rings composed of seven α subunits, and two inner rings composed of seven β subunits. The proteolytically active sites are localized in the β1, β2, and β5 subunits. The majority of 20S proteasomes are capped with 19S regulatory particles. The 20S can also be activated by PA28. Created with Biorender.com.

1 (BAG1) is a co-chaperone which functions as a nucleotide exchange factor triggering ADP dissociation from HSP70/HSC70 proteins and thereafter promoting chaperone substrate release (139). Interestingly, BAG1 can also simultaneously bind to the proteasome via its Ub-like (UBL) domain thereby providing a functional link between chaperones and the proteolytic machinery (140). Conversely, the co-chaperone HSPBP1 attenuates CHIP ubiquitin ligase activity when it is complexed with HSP70/HSC70 and thus inhibits CHIP-mediated degradation (141). Notably, both CHIP and BAG1 exert cytoprotective effects in the heart following ischemia-reperfusion injury (142, 143).

In addition to CHIP and HSP70, chaperones such as HSP20, HSP90, and αB-crystallin (CryAB) are also induced in cardiomyocytes in an effort to buffer misfolded proteins during cardiac stress (137). Numerous studies have highlighted the protective role of these proteins in the heart (137). For instance, HSP90 appears to be cardioprotective in both doxorubicin-induced heart failure and high-glucose induced cell injury (144, 145). Moreover, cardiac specific over-expression of HSP20

attenuates apoptosis, reduces infarct size, and improves cardiac function in mice following ischemia-reperfusion injury (146). Mutations which impair the function of chaperones have been implicated in numerous diseases including cardiomyopathies (147). Pre-clinical studies have demonstrated that transgenic mice expressing an R120G-missense mutation in CRYAB develop restrictive cardiomyopathies and manifest pathological characteristics similar to those observed in clinical desmin-related myopathy (DRC); aberrant protein aggregation in cardiomyocytes and cardiac dysfunction (148, 149).

ERAD

ERAD is an integral facet of the UPS pathway (150). It is an evolutionarily conserved PQC mechanism in mammalian cells that orchestrates the function of numerous proteins to maintain ER homeostasis (151, 152). Through ERAD, aberrant ER luminal and transmembrane proteins are recognized and retrotranslocated to the cytosolic face where they are modified by the ubiquitylation machinery. The E3 ligases implicated in ERAD include soluble proteins, such as PRKN, ubiquitin conjugation factor E4 A (UBE4A), and CHIP, and ER transmembrane proteins, such as synoviolin (also known as HRD1), TEB4, GP78, and RMA1 (150). ERAD substrates are commonly conjugated to Lys48- and Lys11-linked polyubiquitin chains (153). Once ERAD substrates are adequately ubiquitylated, they are extracted from the ER membrane into the cytosol by the p97-UFD1-NPL4 complex to facilitate their proteasomal degradation (154). As such, if this adaptive ERAD function is defective or insufficient, the UPR^{ER} activates destructive cell pathways by transforming into an alternative signaling platform known as the terminal UPR^{ER} (155–158).

The UPS in Cardiac Physiopathology

UPS activity is imperative in the heart as cardiomyocytes are highly susceptible to protein damage due to their constant exposure to metabolic and mechanical stress (159). Additionally, as terminally differentiated cells, cardiomyocytes possess minimal replicative potential; thus, failure to eliminate damaged proteins triggers excessive apoptosis, which is detrimental to the heart. Over the past decade, numerous clinical and experimental studies have documented impaired proteasome function, accumulation of ubiquitylated proteins, and alterations in the expression of UPS components in diseased hearts (159–164). Highlighting the importance of proteasomal integrity, cardiac proteasome inhibition induces heart dysfunction, and pathological hypertrophy in a preclinical mouse model (165). The pharmacological impediment of proteasome activity also leads to maladaptive structural and functional changes in pig hearts, which are consistent with a hypertrophic cardiomyopathy phenotype (166). Similarly, genetic inhibition of cardiac 20S proteasome promotes cardiac dysfunction in mice during systolic overload (138). Moreover, use of proteasome inhibitors (bortezomib, carfilzomib, and ixazomib), as targeted chemotherapeutics, is related to cardiovascular adverse events, including congestive heart failure (167). Of note, perturbations in UPS function have also been documented in doxorubicin-induced cardiotoxicity (168–170).

Inhibition of UBE2V1, a member of the E2 protein family, reduces protein aggregation in a $CryAB^{R120G}$-desmin related myopathy mouse model, improves cardiac function, and enhances survival *in vivo* (171). Likewise, it has been firmly established that E3 ligases play a significant role in the pathogenesis of heart diseases (**Table 1**). In a preclinical model of pressure-overload, MURF1 knockout mice displayed exacerbated cardiac hypertrophy in response to mechanical stress (176). Similarly, transgenic mice expressing mutations in *Trim63*, the gene encoding MURF1, develop cardiac hypertrophy (204). Moreover, *Chip*–/– mice challenged with ischemia-reperfusion injury were more prone to arrhythmias and had decreased survival rates (143). However, research on the pathological implications of DUBs in the heart is limited (**Table 1**). A recent study revealed that the expression of ubiquitin carboxyl-terminal hydrolase isozyme L1 (UCHL1) was increased in the cardiomyocytes of hypertrophic and failing hearts (199). Overexpression of UCHL1 exacerbates pressure-overload induced cardiac hypertrophy and dysfunction, which can be reversed by systemic administration of the UCHL1 inhibitor LDN-57444 in mice (199). These studies demonstrate the detrimental effects of UPS malfunction in the myocardium.

Furthermore, Doroudgar et al. demonstrated that HRD1 plays an essential role in the adaptive ERSR in cardiomyocytes and that cardiac-specific overexpression of HRD1 preserves cardiac structure and function in a mouse model of pathological cardiac hypertrophy (197). Moreover, overexpression of Derlin3, a component of the ERAD retrotranslocation channel, enhances ERAD-dependent disposal of misfolded proteins, attenuates exorbitant ERSR, and reduces caspase activity in response to ischemia/reperfusion injury (205). Conversely, knockdown of Derlin3 impairs the clearance of misfolded ER proteins and augments ischemia-mediated cell death in cardiomyocytes (205). Collectively, these findings suggest that ERAD-associated UPS plays a crucial role in myocardial viability and underscore the importance of PQC mechanisms in the setting of cardiac injury.

The UPS in DCM

The E3 Ubiquitin Ligases in DCM

The E3 ubiquitin ligases participate in cardiac metabolic regulation, by regulating numerous transcription factors involved in DCM (206) (**Figure 5**). FOXO1 has emerged as an influential player in the pathogenesis of DCM, which is overactivated in the hearts of murine models of T2DM. This aberrant activation is associated with the development of cardiomyopathy, evidenced by the cardiac-specific deletion of FOXO1 ameliorating high fat diet-induced cardiac dysfunction and preserved insulin responsiveness (80). At the molecular level, several E3 ubiquitin ligases, including CHIP, MDM, and COP1, regulate FOXO (190, 207, 208), as a consequence, the functions of FOXOs are subdued by virtue of their ubiquitin-mediated proteasomal degradation.

In addition, GATA4, a member of the GATA zinc-finger transcription factor family, is abundantly expressed in the heart. GATA4 regulates the transcription of numerous cardiac genes,

TABLE 1 | The role of the ubiquitylation pathway in cardiovascular disease.

		Cardiac hypertrophy	Ischemia-reperfusion injury	Diabetic cardiomyopathy	Heart failure
Cardiac-specific E3 ligases	**MURF1**	Potentially cardioprotective (172)	Cardioprotective (173, 174)		Deleterious (175)
	MURF2	Dispensable (176)		Cardioprotective (177)	
	MURF3		Potentially Cardioprotective (178)	Cardioprotective (179)	
	Atrogin1/MAFbox	Cardioprotective (180)	Deleterious (181)		Potentially deleterious (182)
Non-cardiac specific E3 ligases	**TTRIM72 (MG53)**	Cardioprotective (183)	Cardioprotective (184, 185)	Deleterious (186)	Cardioprotective (187)
	TRIM21	Deleterious (188)			
	MDM2	Cardioprotective (189)	Cardioprotective (189)	Potentially cardioprotective (80, 190)	
	c-CB1		Deleterious (191)		Potentially deleterious (191)
ERAD-associated E3 ligases	**CHIP**	Cardioprotective (192)	Cardioprotective (143)		
	Parkin		Cardioprotective (193, 194)	Potentially Cardioprotective (195, 196)	
	HRD1	Cardioprotective (197)			
	GP78			Potentially deleterious (198)	
Deubiquitylating enzymes	**UCHL1**	Deleterious (199)			
	CYLD	Deleterious (200)			
	A20	Cardioprotective (201)	Cardioprotective (202)		
	USP4	Cardioprotective (203)			

including those involved in myocyte growth and survival (209). In both STZ-induced type 1 diabetic mice and *db/db* type 2 diabetic mice, GATA4 protein levels are significantly diminished in the heart (210), which is likely associated with the E3 ubiquitin ligase, CHIP (210).

Furthermore, PPARα is a member of the PPAR subfamily of nuclear receptors and acts as a prominent regulator of myocardial fatty acid utilization (211). Transgenic mice with cardiac-specific PPARα overexpression showed cardiac insulin resistance, reduced glucose utilization, lipid accumulation, and cardiomyopathy (212). MG53, an E3 ubiquitin ligase, and MURF1 are both regulators of PPARα (186, 213). Increased protein levels of MG53 results in a DCM-like phenotype (186). Mechanistically, not only does MG53 deteriorate insulin sensitivity, it also positively regulates PPARα, thereby inducing an energy source shift of glucose to fatty acid oxidation (186). Of interest, both MG53 and PPARα were also elevated in the hearts of *db/db* mice, HFD-induced obese mice, and rhesus monkeys with a spontaneous metabolic syndrome characterized by obesity, hyperlipidemia, and hyperglycemia (186). Moreover, MURF2 and MURF3 attenuate cardiac PPAR isoform activities and protect against DCM in HFD-challenged mice (177, 179).

Finally, the nuclear factor erythroid 2-related factor 2 (NRF2) is the master regulator of the cellular antioxidant response. NRF2 exerts transcriptional action on antioxidant genes through binding to the antioxidant response element (ARE), such as quinone oxidoreductase 1 (NQO1) (214), heme oxygenase-1 (HO1) (215), and superoxide dismutase 1 (SOD1). In addition to its antioxidant capabilities, NRF2 also enhances the clearance of toxic ubiquitylated proteins in the heart (216, 217). KEAP1, as an adaptor of the CUL3-RBX1 E3 ubiquitin ligase, binds NRF2, leading to its ubiquitylation and subsequent proteasomal degradation (218, 219). Human diabetic hearts show a significant reduction in NRF2 protein expression (220), associated with early-onset maladaptive cardiac remodeling and heart failure (220, 221). Both oxidative stress and misfolded proteins synergistically contribute to DCM; therefore, KEAP1 and the CUL3-RBX1 E3 ubiquitin ligase complex represent promising therapeutic targets for diabetic heart disease.

The Cardiac Proteasome in DCM

Diabetes induces both structural and functional alterations in the proteasome (**Figure 5**). In a recent study, Li et al. reported that STZ-induced diabetic mice exhibit a severe and

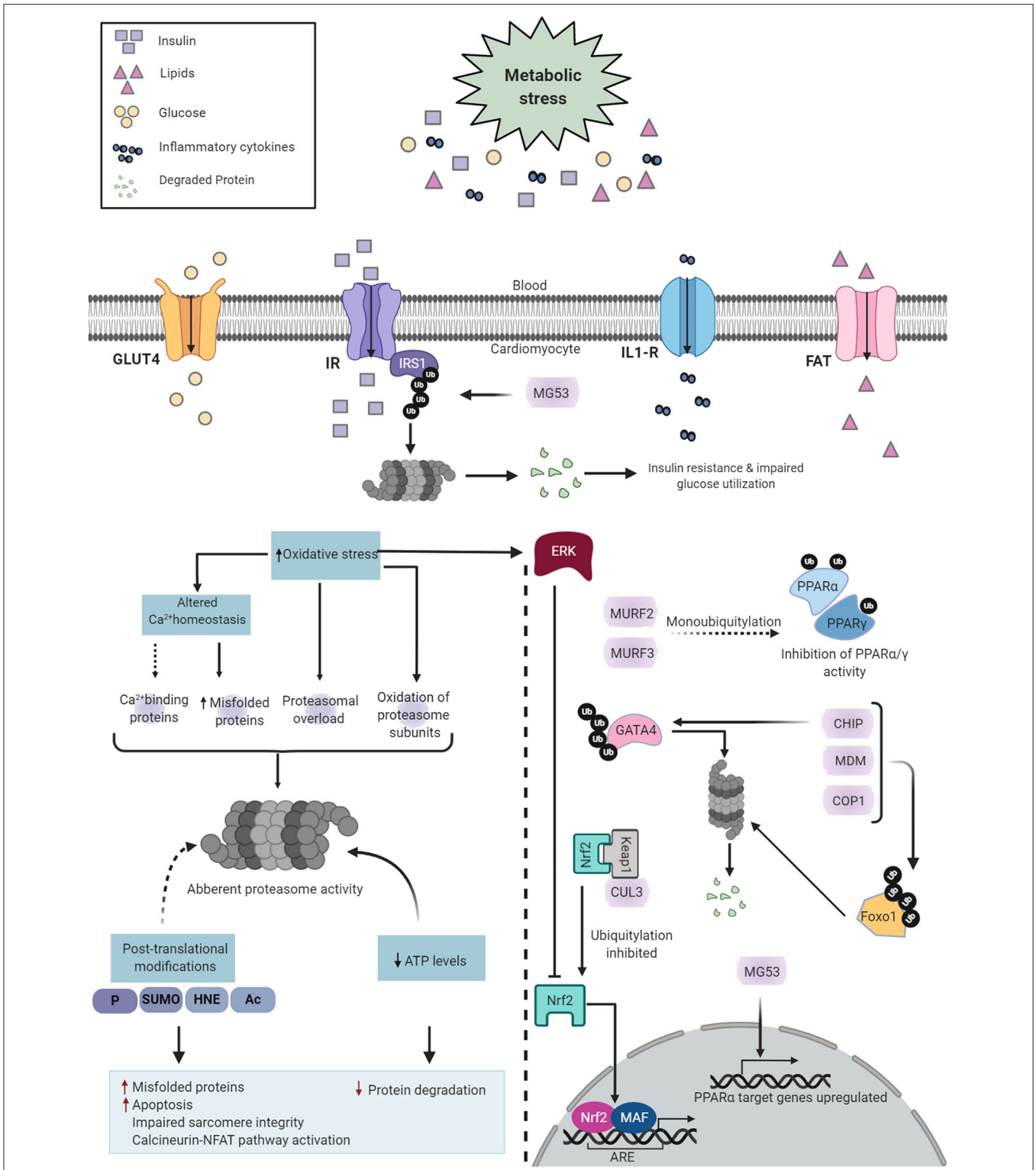

FIGURE 5 | Diabetes-induced cardiac UPS dysfunction. Diabetes induces metabolic alterations in the heart that cause proteasome dysfunction in cardiomyocytes. Proteasome impairment may induce cardiomyopathy through multiple distinct mechanisms such as accumulation of misfolded proteins, enhanced apoptotic activity, contractile dysfunction and activation of calcineurin-NFAT pathway (Left). The E3 ligases regulate key transcription factors involved in DCM (Right). Created with Biorender.com.

progressive decline in cardiac proteasome activity, evidenced by a cumulative increase in GFPdgn (UPS function reporter) and Lys48-linked ubiquitylated protein levels in the heart (222). These alterations in proteasome activity precede the onset of cardiac dysfunction and thus could potentially play a pathogenic role in diabetic heart disease. In line with this, proteasome functional insufficiency was also reported in the myocardium of Sprague–Dawley rats subjected to T1DM, accompanied by higher levels of ubiquitylated and oxidized proteins (223). Taken together, these studies suggest that diabetes induces dissonance in proteasome activity and thereby distorts myocardial proteostasis.

Although the regulatory events underpinning these observations remain largely elusive, there are multiple mechanisms by which diabetes could lead to proteasome dysfunction, such as ATP depletion, oxidative stress, calcium imbalance, and diabetes-induced posttranslational modifications. ATP is required for both ubiquitin conjugation and the activation of the proteasome (224). Cardiomyocytes subjected to severe ATP depletion manifested profound proteotoxicity and stress-induced cell death (225). As such, reduced ATP levels, as observed in diabetic hearts (226–228), likely contribute to proteasome dysfunction.

Likewise, oxidative damage to proteasome subunits affects proteasome activity (229). Oxidation of the 19S regulatory particles Rpt3 and Rpt5 impairs the degradative capacity of the 26S proteasome (230). Bulteua et al. demonstrated that oxidation of the 20S proteasome also blocks proteasomal peptidase activity (231). Consistently, treatment with the NSAID meclofenamate sodium resulted in increased oxidative stress and concomitant oxidation of proteasome subunits, reducing proteasome activity (159, 232). Moreover, mitochondrial dysfunction-associated accumulation of 4-HNE, a secondary product of lipid peroxidation, directly inhibits the proteasome activity in failing rat heart (233). On the other hand, hyperglycemia-induced oxidative stress promotes aberrant cellular Ca^{2+} homeostasis (234, 235), subsequently leading to the accumulation of misfolded proteins and proteasomal overload. Altered cellular Ca^{2+} concentrations may influence the activity of the proteasome more directly by modulating the activity of Ca^{2+}-binding proteins that interact with the proteasome. For instance, calmodulin binds to several non-ATPase subunits of the 26S proteasome and could alter proteasome activity (236).

Cardiac proteasome activity is influenced by posttranslational modifications, such as SUMOylation, glycosylation nitrosylation, and phosphorylation (237), which could be modified by the diabetic myocardial environment (222). Accordingly, protein kinase CβII (PKCβII), a classical PKC isoform, phosphorylates, and inhibits the proteasome activity in failing rat hearts (238). Treatment with a PKCβII inhibitor improves cardiac PQC, function, and survival (238). Abnormal proteasome activity compromises cardiac function through numerous mechanisms (239). Primarily, proteasomal derailment induces cardiac contractile inefficiency by impairing sarcomeres (239). Two parallel processes, assembly, and degradation, are necessary to maintain sarcomere integrity (175). The degradation of sarcomeric proteins is regulated almost exclusively by the UPS (240). Also, proteasome inhibition activates the calcineurin-NFAT pathway in the heart (241), which induces pathological hypertrophic growth (242). Finally, proteasome inhibition has been shown to induce apoptosis in cultured cardiac myocytes (243, 244).

AUTOPHAGY

Autophagy is the homeostatic process through which cellular components are delivered to the lysosomes for degradation into their basic units. The cargo managed by the autophagic process includes insoluble and large misfolded proteins that cannot be degraded by the UPS (245), protein aggregates (246), and the proteasome itself (247). Autophagy can be triggered in the heart by various stress signals, such as nutrient deprivation, the absence of growth factors, and UPS malfunction (248).

Autophagy Process

Autophagy comprises three types of processes (249). Macroautophagy (hereafter referred to as autophagy) requires the formation of double-membrane vesicles, named autophagosomes, to sequester cytoplasmic components and organelles. Fully developed autophagosomes are fused with the lysosomes, where lysosomal hydrolases break down all the elements contained, including the inner membrane. The second type, microautophagy, is when cytoplasmic components are engulfed and degraded through the invagination of the lysosomal membrane. The third type, chaperone-mediated autophagy (CMA), is the process through which proteins exposing a KFERQ motif are translocated into the lysosomes. Approximately 75% of the human proteome has potential KFERQ motifs (250). Even though autophagy and microautophagy are bulk processes engulfing everything in a section of the cytoplasm, they also function selectively. Organelle (251) and protein aggregate (252) labeling consists of ubiquitination, a process shared with the UPS (253). This label is recognized by autophagy receptors such as p62/sequestosome 1 (SQSTM1), BCL2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3), and BNIP3-like (NIX) (254). Selective autophagy is vital to prevent proteotoxicity and promote cellular survival.

The autophagic molecular machinery consists of numerous autophagy-related proteins (ATG), directing all the stages of autophagy: initiation, elongation, maturation, and degradation (**Figure 6A**). Initiation begins with ATG1, also known as ULK1, forming the serine/threonine-kinase ULK complex. This complex phosphorylates the class III phosphatidylinositol 3-kinase complex I (PI3KC3-C1) containing beclin 1 (BECN1). The latter complex produces phosphatidylinositol 3,4,5-trisphosphate (PIP3), initiating the formation of the phagophore. Elongation is driven by the lipidation of microtubule-associated proteins 1A/1B light chain 3 (LC3) by ATG7 and the ATG5/ATG12 complex, during which LC3-I is conjugated with phosphatidylethanolamine to form LC3-II. This process allows the incorporation of LC3-II into the membrane and stabilization of the phagophore. At this stage, the autophagy receptors recognize labeled components and bind to LC3-II. When the autophagosome is closed, small GTPases of the Ras-related protein in brain (RAB) family recruit tethering proteins

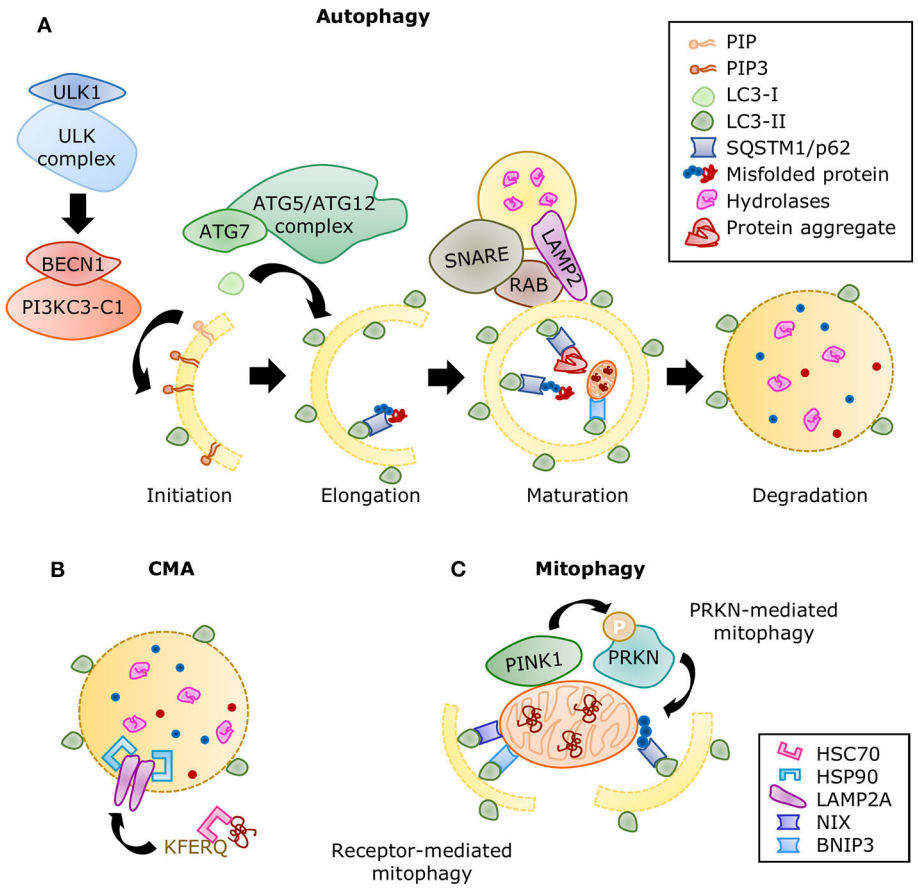

FIGURE 6 | Summary of the autophagic processes. **(A)** A series of protein complexes drive autophagosome formation and fusion with the lysosome. The autolysosome breaks down misfolded proteins, protein aggregates, and organelles using a variety of hydrolases. Misfolded proteins are directed to selective autophagy through ubiquitination and detection by SQSTM1/p62. Adapted from Ciechanover et al. (10) and used under CC BY 3.0. **(B)** During CMA, proteins containing the KFERQ motif are recognized by the chaperone HSC70 and translocated directly into the lysosome by a complex of LAMP2A proteins stabilized with HSP90 chaperones. **(C)** Mitophagy is the selective degradation of nonfunctional mitochondria that have been labeled by the PINK1/PRKN system. Adaptor proteins such as SQSTM1 recognize the ubiquitinated proteins and linking it to the growing autophagosome. The receptor-mediated mitophagy is independent of PINK1/PRKN labeling, but directed by receptors, such as BNIP3 and NIX, which also contain LC3-binding domains.

to anchor the autophagosome and the lysosome together, while snap receptor (SNARE) proteins and lysosome-associated membrane glycoprotein 2 (LAMP2) regulate their fusion (255), this is the maturation to autolysosome. A variety of enzymes in the autolysosome carry out the degradation process, after which the macromolecules are released into the cytosol for anabolic reactions.

Molecular chaperones also have a significant role in all three types of autophagy mediating selectivity and stability of the processes. If protein refolding fails, they can also direct cargo for degradation. The function of chaperone heat shock cognate 71kDa protein (HSC70) was first described in CMA. HSC70 recognizes the KFERQ motif in proteins and facilitates their translocation into the lysosome through LAMP2A (249). A second chaperone, heat shock protein (HSP90) enhances binding of the substrates and LAMP2A stability (256) (**Figure 6B**). Furthermore, HSC70 was later associated with the targeting of cytosolic proteins toward endosomal microautophagy (257) and chaperone-assisted selective autophagy (CASA), both of which can manage the degradation of protein aggregates (258). The substrate and process specificity of chaperones participating in different types of degradation is believed to come from the formation of complexes with co-chaperones, whose availability depends on cell type and stress or stimulus conditions. For example, BAG3 is a co-chaperone that interacts with HSP70 and HSPB8 to trigger selective autophagy of aggregated proteins (259). Its counterpart, BAG1, guides proteins toward proteasomal degradation. Several other co-chaperones have been described (260); however, their specific part in cardiac function is still being explored.

Physiopathological Role of Autophagy in the Heart

Basal autophagic activity in the myocardium is required to prevent the accumulation of misfolded proteins and the recycling of essential components from defective organelles to sustain

cardiac function. Mutations in autophagy-related genes are the cause of dilated cardiomyopathies. The most common is Danon disease (261), a mutation of the *LAMP2* gene, characterized by the weakening of the heart, protein aggregation, accumulation of autophagic vesicles in the muscle, and cardiac hypertrophy. The multisystemic disorders rising from loss-of-function mutations in the *EPG5*(262), *PLEKHM2* (263), and *BAG3* (264–266) genes have significant cardiovascular manifestations and are the result of defective autophagy. On the contrary, inducing autophagy ameliorates desmin-related cardiomyopathy by clearing the protein aggregates that originate from the mutation of the *CRYAB* gene (267). Additionally, cardiomyocyte-specific ATG5 deletion in mice, since birth (268) and in the adult stage (269), caused systolic dysfunction and sarcomeric structure disarray without any further stress. Conversely, augmenting basal autophagy by ATG5 overexpression (270) or BECN1 mutation to prevent its association with BCL2 (271) increased longevity in mice. A certain capacity for autophagic processing is elementary for normal cardiac function, and its sustenance counteracts proteotoxicity.

Cardiac autophagic flux is strongly induced by fasting (272), caloric restriction (273), exercise (274), and in neonatal mouse hearts after the placental supply is interrupted at birth (275), which is pivotal for cardiac contractility and survival (276, 277). Fasting and caloric restriction stimulate autophagy in the heart through 5′-AMP-activated protein kinase (AMPK) phosphorylation (272, 273) and increased SIRT1-mediated FOXO1 activity (278). Upon nutrient restoration after birth, insulin and amino acids moderate autophagy. Insulin signaling inhibits autophagy through AKT serine/threonine kinase 1 (AKT)-mediated activation of the mechanistic target of rapamycin kinase complex 1 (MTORC1), an autophagy inhibitor. When postnatal autophagy inhibition was disrupted by genetic deletion of the insulin receptors, cardiomyocyte death, and heart failure occurred due to excessive autophagy (279). Unrestrained autophagy was diminished by the supplementation of amino acids, which suppressed autophagy (279) through RAG protein family regulation of MTORC1 (280). On the other hand, in exercised mouse hearts, AMPK activation promoted the expression of autophagic genes (274) and dissociated the BCL2/BECN1 complex to increase autophagy level (281). However, in the long-term, exercise increased the autophagic capacity by augmenting LC3 expression in cardiac muscle without boosting autophagic flux (282). Nevertheless, this increased capacity limited cardiac injury and improved function after myocardial infarction (283, 284). These observations suggest that the regulation of autophagy by exercise is multifaceted and adaptive.

The role of autophagy in cardiac disease has been more challenging to determine, since it can be adaptive or maladaptive depending on the specific pathology and pathogenesis stage. The presence of abnormal protein aggregation in the myocardium of dilated cardiomyopathy patients was associated with impaired cardiac autophagy (12), while the detection of autophagic vacuoles was associated with improved heart failure prognosis (285). In preclinical studies, myocardial ischemia-induced autophagy in mouse (286) and swine models (287), moderating apoptosis, and autophagy induction, in turn, limited myocardial injury (272, 288, 289). Autophagy was reduced after prolonged pressure overload in mouse hearts, and ATG5 deletion aggravated cardiac remodeling and performance (269). These results indicate that autophagy is required to preserve cardiac function in response to pathological stresses. However, excessive BECN1 expression and autophagosome formation were found to be detrimental during reperfusion (290) and pressure overload (291), also in mouse studies. The seemingly confusing outcomes could be explained by the discovery that BECN1 association to the Rubicon protein was able to inhibit autophagic flux by interfering with autophagosome maturation (292), which has been recently termed autosis (293), a form of cell death. In addition, substantial evidence indicate that maladaptive autophagy was observed in atrial fibrillation by the degradation of cardiac troponin I/T (294) and calcium channel CAV1.2 (295), resulting in contractility and electrical alterations. Collectively, adaptive autophagy is essential to cardiac health, whereas either insufficient or excessive autophagy is detrimental.

Autophagy in DCM

The elucidation of the role of autophagy in DCM has been intricate due to the complexities of the disease and the duality of the nature of autophagy. In T2DM, autophagic flux is increased in the early stages of disease (296) and later reduced, with cardiac function improvement mostly being associated with therapeutic restoration of the autophagic flux (297–305). Increased expression of autophagy proteins was observed in human atrial samples and obese mouse myocardium, while fractional shortening was maintained (306). However, impaired autophagy by long-term chloroquine administration (300) or cardiac ULK1 deficiency was detrimental for cardiac function in obesity, resulting from fibrosis and apoptosis (297). On the contrary, boosting autophagic flux in a later stage by rapamycin administration improved systolic performance in high-fat diet (HFD)-induced diabetes (302). It has been suggested that autophagy contributes to high fructose-induced cardiomyopathy (307); nonetheless, these samples also displayed signs indicating that autophagic flux might have been blunted. In myocardial samples of obese and T2DM patients, amylin aggregates were detected and found to induce cardiac dysfunction (308). These aggregates, also known as islet amyloid polypeptide (IAPP) oligomers, disrupted autophagy-associated disposal, increasing their toxicity (309, 310). All together, autophagy is stimulated in T2DM stages with preserved cardiac function, while its abnormalities likely cause the onset and development of DCM and heart failure.

In T1DM models, the regulation and function of autophagy in the heart are elusive. Cardiac autophagy could be enhanced at an early time point (311); nonetheless, most evidence indicates it is suppressed. More importantly, preclinical experiments suggest that autophagy inhibition could be therapeutic in this case. Cardiac BECN1 overexpression in streptozotocin (STZ)-induced diabetes aggravated cardiac function (312). Conversely, autophagy reduction by BECN1 insufficiency and hypomorphic ATG16 improved echocardiographic measurements and hemodynamic analysis in the same model and in OVE26

mice, which develop severe early-onset T1DM due to deficient insulin production. Of note, even though autophagic flux was diminished, the functional improvements were accompanied by increased expression of RAB9 (312), which directed a non-canonical form of autophagy (313); therefore, it is speculated that non-canonical autophagy fulfills beneficial effects in T1DM. Akin to observations in T2DM patients (308), STZ-injected mice displayed toxic cardiac protein aggregation that can be improved by boosting autophagy (314). Interestingly, in T1DM and T2DM, CMA was found to be promoted even after autophagy was suppressed; however, evidence showed it could be contributing to metabolic inflexibility (7). As such, the molecular mechanisms underlying cardiac autophagy in DCM require further investigation.

Despite the distinct etiologies and biochemical stresses present in T1DM and T2DM, a few regulatory pathways have been demonstrated to be involved in autophagy suppression and the development of cardiac dysfunction (**Figure 7**). AMPK phosphorylation is reduced in the hearts of a number of DCM mouse models, including OVE26 (315), STZ-induced (316), diet-induced (302), and genetically obese mice. In OVE26 mice, the stimulation of AMPK phosphorylation by metformin restored autophagy and cardiac dysfunction (315). AMPK-mediated autophagy regulation is attained through numerous molecular mechanisms (316, 317), but mainly through repression of MTORC1 activity (302, 304, 318). MTORC1 suppresses autophagy by phosphorylating ULK1 (319, 320) and transcription factor EB (TFEB) (321), a master regulator of autophagy gene expression. Cardiac TFEB is suppressed in both T1DM and T2DM (322). AKT, mitogen-activated protein kinases 1 and 3 (ERK1/2), and the SIRT family are additional nodal points for autophagy regulation in DCM. AKT (57) and ERK1/2 (323) inhibited autophagic flux in the hearts of obese mice through MTORC1 activation. Strikingly, cardiac *Akt2* knockout preserved cardiac function in high-fat diet-induced obesity by rescuing cardiac autophagosome maturation (301). In contrast, SIRT3 and SIRT1 were downregulated in both STZ-induced (324) and HFD-induced diabetic hearts (325). Consistently, SIRT3 (326) and SIRT1 (327) mediated the cardioprotective effects of resveratrol observed in T1DM by enhancing autophagic flux via activation of FOXO3A (324) and FOXO1 (327). As such, MTORC1, AKT, and ERK1/2 act as negative regulators of autophagy during the development of DCM, while AMPK and the SIRT family are considered as the enhancers.

More recently, non-coding RNAs have been found to be involved in numerous mechanisms underlying the development of DCM, with some of them regulating autophagy. Dysregulation of miRNAs was analyzed in the left ventricle of diabetic mice after STZ-injection, revealing that *mir30a*, *mir133a*, *mir212*, and *mir221* are particularly associated with autophagy regulation. Among them, *mir212* and *mir221* were significantly upregulated in diabetic ventricles and even remained increased after insulin treatment (328), suggesting that they are likely involved in cardiac deterioration even with proper glycemic control. *Mir212* targets *Foxo3a* (329), while *mir221* targets *p27* mRNA, modulating MTORC1 activity (330), both of which inhibit autophagy. Additionally, m*ir30d* also targets *Foxo3a* to suppress autophagy in DCM (331). On the other hand, *mir30c* targets *Becn1*, and cardiac overexpression of this miRNA improved cardiac function in genetically obese mice (332), possibly by decreasing BECN1-Rubicon association (292) and improving autophagosome clearance. Lastly, the long non-coding RNA *H19* is downregulated in the heart of STZ-induced diabetic rats, allowing for increased MTORC1 signaling and suppressed autophagy (333). Therefore, emerging evidence demonstrates that non-coding RNA regulation of autophagy also acts as potential therapeutic targets for treating DCM.

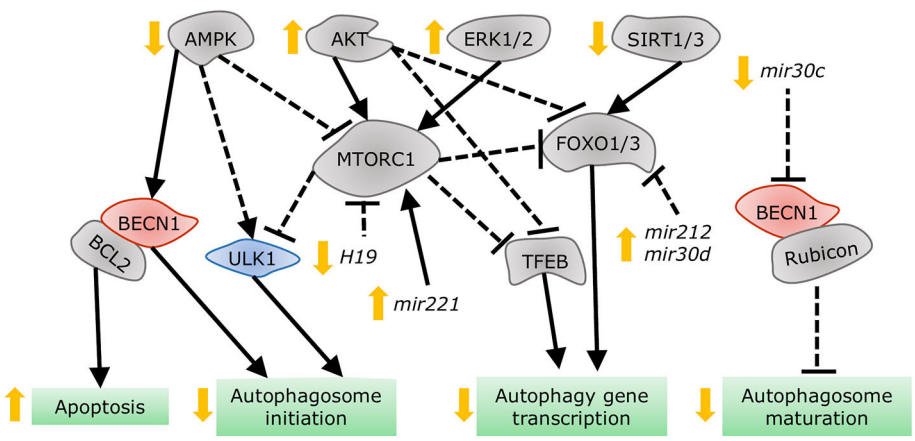

FIGURE 7 | Dysregulation of autophagy in the diabetic heart. AMPK and SIRT1/3 inhibition, in addition to AKT and ERK1/2 stimulation, impair autophagy gene transcription, and autophagosome initiation via MTORC1, TFEB, and FOXO1/3 regulation. Decreased dissociation of the BECN1/BCL2 complex augments apoptosis, while increased association of BECN1 with Rubicon blunts autophagosome maturation. MiRNAs altered in diabetes interfere with autophagy by acting on players such as MTORC1, FOXO1/3, and BECN1.

Mitophagy in DCM

Even though mitochondria possess their own UPR, proteotoxic stress or damage can surpass their capacity, requiring a more robust response. Mitochondria can be selectively targeted for autophagy. Without adequate mitochondrial clearance, remaining damaged mitochondria are unable to meet ATP demand, produce excessive ROS, and promote cell death and inflammation (195). Mitochondrial autophagy, termed mitophagy, is directed by the serine/threonine-protein kinase PINK1 (PINK1) phosphorylating the E3 ubiquitin-protein ligase PRKN (PRKN) and fostering mitochondrial protein ubiquitination. The adaptor proteins SQSTM1, neighbor of BRCA1 (NBR1), nuclear dot protein 52 (NDP52), optineurin (OPTN), TAX1BP1 (TBK1), BNIP3, and NIX link damaged mitochondria to autophagosomes for their degradation (334) and can sometimes induce PINK1-independent mitophagy (**Figure 6C**). PINK1 deletion results in mitochondrial dysfunction, oxidative stress, and cardiomyopathy (335). Similarly, knocking out cardiac PRKN in mice accelerated the development of systolic dysfunction after HFD-feeding, accompanied by the accumulation of dysfunctional mitochondria and lipids (296). ULK1 (336) and RAB9-dependent (312) mitophagy has also been observed in the hearts of diabetic mice, and its impairment aggravated cardiac function. However, the levels of these proteins continued to increase when diastolic (336) and systolic dysfunction (312) were detected; therefore, there is doubt on whether alternative mitophagy could be sufficient to sustain cardiac function during metabolic stress.

Impaired mitophagy is a major contributor to the pathogenesis of DCM. In a compensated stage of HDF feeding, mitophagy is upregulated by the general autophagy ATG7-dependent mechanism, and disrupting mitophagy by deleting cardiac PRKN expression accelerates the appearance of cardiac dysfunction (296). Consistently, PINK1 and PRKN levels were found to be downregulated in the myocardium of STZ-induced and OVE26 diabetic mice with systolic dysfunction (312). Different mechanisms have been proposed to contribute to the loss of mitophagy after metabolic stress. MST1 was found to inhibit cardiac mitophagy in the hearts of diabetic mice via SIRT3- mediated PRKN suppression (196). SIRT3 and PRKN activities were ameliorated by melatonin (337) and icariin administration (338), resulting in improved mitochondrial function. In pancreatic islets and hepatocytes from obese mice, an increase in P53 protein suppressed mitophagy by direct interaction of P53 and PRKN, blunting mitochondrial uptake by autophagosomes (339, 340). Lipid metabolism was associated to HFD-induced PRKN reduction, given that stimulation of lipid catabolism by overexpressing acetyl-coA 2 (ACC2) restored mitophagy and cardiac function in mice (341). Adequate mitochondrial morphology and dynamics are also vital to facilitate mitophagy during DCM. HFD feeding induced dynamin-related protein 1 (DRP1) activity in the hearts of mice and monkeys. DRP1 is the primary regulator of mitochondrial fission, and its increased activity suppressed mitophagy and resulted in cardiac inflammation and heart failure (342, 343). On the contrary, myocardial samples of *ob/ob* mice showed reduced levels of mitofusin 2 (MFN2), the lead regulator of mitochondrial fusion. Restoration of MFN2 expression in cardiomyocytes exposed to high-glucose and high-fat treatment recovered mitochondrial membrane potential and function (344). MFN2 also promotes PRKN translocation and mitophagy in cardiomyocytes (345). Albeit the need to continue exploring the mechanisms governing mitophagy during DCM, its role in maintaining mitochondrial quality has been acknowledged.

CROSSTALK BETWEEN PQC SYSTEMS

The different PQC systems act as an integrated stress response. They are interconnected and regulate each other at the transcriptional and protein level, and this interdependence is relevant for health and disease. For instance, the UPS downregulates autophagy by processing transcription factors, such as P53, NFkB, HIF, and FOXO (346), and autophagy components, such as BECN1 (347, 348), LC3, p62, and ULK1(346). In cancer cells, chemotherapy resistance to bortezomib, a proteasomal inhibitor, arises from the induction of autophagy as a compensatory mechanism (349). The combination of bortezomib with hydroxychloroquine improved the treatment outcome (350). In turn, proteasomes can be degraded by autophagy. Amino acid starvation-induced autophagic activity also enhances polyubiquitination of 19S regulatory particle, targeting it for autophagic uptake and decreasing proteasomal activity level (351, 352).

Similarly, the UPR^{ER} components, ATF6, CHOP and IRE1 are degraded by the UPS, while two UPR^{ER} branches, PERK-ATF4 and IRE1-XBP1 regulate the expression of UPS components (346). IRE1 is handled through direct interaction with the ERAD complex SEL1-HRD1 and during ER stress, this interaction is broken for UPR^{ER} initiation (353). At the same time, IRE1-XBP1 pathways stimulate the transcription of SEL1L and HRD1 for further UPS function of misfolded proteins (354). This self-modulating feedback loop prevents overactivation and ER-mediated cell death. In *db/db* mice, cardiac expression of HRD1 is reduced, suggesting blunted ERAD activity contributes to prolonged ERSR (355). On the contrary, in doxorubicin-induced cardiomyopathy, UPS activity was observed to be increased (168, 169), perhaps furthering the impairment of UPR^{ER} function (356). Even though the goal of all PQC system is the restoration of protein homeostasis, the impact of each one in cellular function under stress conditions differs.

Clear links between the UPR^{ER}, UPS, and autophagy have been acknowledged; however, few of them have been explored in the diabetic heart. Both IRE1 and PERK branches of the UPR^{ER} induce autophagy by promoting BECN1/BCL2 dissociation and upregulating autophagy genes, such as ATG12, BECN1, and LC3. In addition, ER calcium release can stimulate the Ca^{2+}/calmodulin-dependent kinase kinase β (CaCMKKβ) that phosphorylates and activates AMPK, resulting in autophagy stimulation (357). UPS malfunction has also been found to provoke autophagy through NRF2-mediated SQSTM1 upregulation (358) and calcineurin-TFEB activation (165), suggesting that autophagy acts as a compensatory mechanism upon proteasomal insufficiency. Proteasomal insufficiency was

detected in diabetic hearts previous to cardiac dysfunction (UPS section); therefore, it is possible that these mechanisms contribute to autophagy induction in early stage of DCM. On the other hand, autophagy suppression inhibits UPS function due to the accumulation of SQSTM1 that drives the excessive sequestration of ubiquitinated proteins in protein aggregates, preventing proteasomal degradation (359). Autophagy inhibition in late DCM could be aggravating UPS malfunction and further damaging cardiac function. The exploration of these crosstalks in the heart of diabetes patients and animal models could clarify the status of PQC components and regulatory mechanisms.

THERAPEUTICS

Proper regulation of the PQC system in the myocardium is vital to maintain cardiac physiology and preserve heart performance in response to pathological stresses. Studies on the alterations and regulation of PQC in the heart in the fact of diabetes mellitus have provided new insights into the molecular pathogenesis of DCM, as well as delivered proof-of-concept evidence that the fine-tuned modulation of the UPR^{ER}, UPS, and autophagic event is a potential therapeutic strategy to treat DCM and prevent heart failure in the diabetic populations.

Targeting the UPR^{ER}

As a metabolic disorder, DCM embodies subsequent UPR^{ER} events, where protective ER response is dominant in early stages, followed by decreased UPR^{ER} signaling, and ultimately, irreversible ERSR associated with structural abnormalities in the myocardium (360). Therefore, potentiating initial UPR^{ER} activation to restore protein homeostasis and impeding ER apoptotic response to prevent cell death qualifies as a therapeutic strategy for managing ER stress in DCM (17, 51). Interestingly, exercise, a known physiological UPR^{ER} inducer, mitigated apoptotic ERSR by reducing CHOP and caspase 12 expression, and augmented cardiac function in STZ induced type 1 diabetes (361). This suggests that exercise is beneficial by restoring protein homeostasis.

Modulation of the UPR^{ER} sensors and GRP78 restores cellular homeostasis and improve heart function in multiple cardiovascular disorders. Although GRP78 overexpression is cardioprotective in hypoxia-induced injury (362), normalizing GRP78 is shown to be beneficial for disorders with overactivated UPR^{ER} such as DCM. Moreover, chemically enhancing IRE1-XBP1 (363) and ATF6 activity (364) reduced ER-associated apoptosis following myocardial infarction. Contrastingly, IRE1 and PERK inhibition alleviated atherosclerosis development and decreased cell death in cardiac arrhythmias, respectively. As stated above, PERK and ATF6 hyperactivation are deleterious in the diabetic heart, indicating that our UPR^{ER} knowledge is incomplete in the context of different cardiac etiologies. Nonetheless, the pharmacological modulation of UPR^{ER} signaling following numerous diabetic stresses has dramatically increased in the past few years (**Table 2**).

Poor glycemic control is associated with increased ER stress and decreased function in the diabetic heart. Sodium-glucose cotransporter 2 (SGLT2) inhibitors as an effective-glucose lowering therapy showed robust cardioprotective outcomes in clinical trials (371) and pre-clinical studies (372) by reduced ER-mediated apoptosis following oxidative stress. However, glucagon-like peptide 1 (GLP-1) agonists are shown to exacerbate heart failure or have no significant cardiovascular outcome in T2DM patients (371). GLP-1 agonists are cardioprotective in DCM rodent models by inhibiting UPR^{ER} signaling and ER-mediated apoptosis (368–370, 381). Therefore, these drugs fall short of mitigating heart failure in diabetes patients, possibly due to inhibition of adaptive ERSR. Also, metformin (382) and thiazolidinediones (TZD) (367) lower cardiovascular events in T2DM patients by its antihyperglycemic effects. Meanwhile, in pre-clinical studies, metformin also induced the protective UPR^{ER} function (365, 366), and TZDs improved insulin sensitivity by upstream mediated attenuation of inflammation and ER-associated apoptosis (69), thereby ameliorating cardiac function in diabetes. Taken together, clinically antihyperglycemic drugs fulfil cardioprotective role in DCM, although their function on UPR^{ER} needs to be further confirmed.

Multiple approaches are being employed to improve cardiac function by the administration of anti-ER stress chemicals, which may facilitate UPR^{ER} action (60). Chemical chaperones restored the UPR^{ER}, which attenuates maladaptive ERSR under pathological stresses (383), including diabetes (71, 384). 4-phenyl butyric acid (4-PBA) and tauroursdoeoxycholic (TUDCA) improved heart function in doxorubicin-induced cardiomyopathy (356), emphasizing their potential as cardioprotective drugs. Moreover, these chaperones can reduce ER protein load in cardiomyocytes by reducing fatty acid uptake (380) and normalizing GRP78 expression (64) in T2DM rat models. Additionally, TUDCA is currently employed in three clinical trials in amyloid cardiomyopathy associated with the onset of type 1 diabetes (385). Of note, given the ubiquitous nature of UPR^{ER} signaling, these multi-organ targeting drugs may have off-target effects. Therefore, further clarification of specific drug targets is of considerable significance to improve the efficacy of these drugs as DCM therapy.

Other strategies to target ER stress as DCM therapy include antihypertensives, antioxidants, and antiinflammatory compounds. Besides the metabolic alterations, increased angiotensin II signaling in diabetes also induces ER stress in the heart (60). The antihypertensive drugs, such as valsartan, are shown to downregulate CHOP expression and reduce cardiac remodeling in DCM (373, 374). On the other hand, phytochemicals, such as matrine, have attracted attention in attenuating maladaptive ERSR (376) and preserving UPR^{ER} signaling (61, 377), subsequently improving cardiac function in STZ-induced DCM. Moreover, vanadium derivative (55) and endogenous hormones, such as melatonin (379) and FGF21 (386, 387), have been investigated for their cardioprotective role by suppressing oxidative stress-mediated ERSR and cell death in T2DM. Furthermore, targeting upstream regulators of UPR^{ER} signaling, such as SIRT1 (76, 375), might be beneficial as DCM therapy. Therefore, these molecules may be further developed as novel therapeutic agents with clinical efficacy to target UPR^{ER} signaling in DCM. In conclusion, since targeting UPR^{ER} signaling is a two-edged sword, proper UPR^{ER} regulation

TABLE 2 | UPR-targeting drugs.

	Therapeutic application	Mechanism/Target	Effect on cardiac ER and UPR	Type of DM	References
Metformin	Antihyperglycemic	AMPK activation	↑GRP78 ↑PERK/eIF2α ↓ CHOP	T2DM	(365, 366)
Thiazolidinediones	Antihyperglycemic	PPAR activation	↑IRE/XBP1 ↓ IRE/JNK ↓ CHOP	T2DM	(69, 366, 367)
DPP4 inhibitors	Antihyperglycemic	DPP4 inhibition	↓ IRE/JNK ↓ CHOP	T2DM	(366)
GLP1-agonists	Antihyperglycemic	GLP1 receptor activation	↓ CHOP ↓ JNK	T2DM High glucose cardiomyocytes	(368–370)
SGLT2 inhibitors	Antihyperglycemic	SGLT2 inhibition	↓ CHOP ↓ Caspase 12	T2DM	(371, 372)
Adiponectin	Appetite and metabolic regulators	Adiponectin receptor activation	↓ IRE/JNK	T2DM	(58)
Angiotensin-II receptor type 1 blockers	Antihypertensive	Angiotensin receptor inhibition	↓ CHOP ↓ GRP78	T1DM T2DM	(373, 374)
Resvertrol	Antioxidant	SIRT activation	↓ PERK/CHOP ↓ ATF6/CHOP ↓ IRE1/JNK	T2DM	(76, 375)
Rapamycin	Antihyperglycemic	UPR inhibition	↓ IRE1/JNK	T2DM	(366)
Tanshinone II	Antioxidant	Superoxide mutase activation	↓ CHOP ↓ GRP78	T1DM	(376)
Matrine	Antioxidant Antiinflammatory	N/A	↓ ATF6 ↓ calreticulin ↓ PERK ↓ GRP78	T1DM T1DM	(61, 377)
Anthocyanins	Antioxidant	Inhibition of oxidation promoting enzymes and ROS scavenging	↓ CHOP ↓ GRP78 ↓ XBP1	T2DM	(378)
Apocyanin	Antioxidant	NAPDH oxidase inhibitor	↓ PERK ↓ GRP78 ↓ ATF6	T1DM	(66)
Melatonin	Antioxidant	Melatonin receptor activator	↓ PERK/CHOP	T2DM	(379)
IL-1 receptor anagonist	Antiinflammatory	IL-1	↓ CHOP	T1DM	(73)
Vanadium deravative	Antihyperglycemic	PPARγ activation	↓ CHOP ↓ GRP78 ↓ XBP1	T2DM	(55)
EGFR inhibitor	Antioxidant	Tyrosine kinase receptor inhibition	↓ CHOP	T1DM/T2DM	(78, 79)
TUDCA	Chemical ER chaperone	GRP78	Binds to misfolded proteins	T2DM/T1DM	(41, 64)
4-PBA	Chemical ER chaperone	N/A	Binds to misfolded proteins	T2DM	(356, 380)

List of drugs proven to alter cardiac UPR during DCM treatment.

is essential to restore protein homeostasis in the cardiomyocytes, while inappropriate suppression of ERSR may have unpredictable effects on cardiac function in DM populations.

Targeting the UPS

Targeting the Proteasome

Given its indispensable role in maintaining cellular proteostasis, the proteasome is a potent therapeutic target to treat proteotoxic stress in the heart. Benign enhancement of proteasomal function by overexpression of the 11S proteasomal subunit PA28α markedly reduced aberrant protein aggregation and cardiac hypertrophy in a mouse model of $CryAB^{R120G}$ proteinopathy (388). Likewise, cardiac-specific proteasome enhancement partially improved right ventricular dysfunction and survival in mice subjected to pressure overload (389). More recently, Li et al. reported that restoration of proteasome function facilitated by PA28α overexpression preserves cardiac hemodynamics and ameliorates diabetes-induced pathological cardiac remodeling in STZ-induced diabetic mice (222). These salient findings suggest that genetic proteasome enhancement restores PQC and improves cardiac function in response to various pathological conditions, including metabolic stress.

Pharmacological stimulation of cAMP-PKA and cGMP-PKG signaling by phosphodiesterase (PDE) inhibitors can also activate cardiac proteasome activity. The synthesis of cAMP and cGMP is mediated by adenylyl cyclases or guanylyl cyclases, respectively, whereas their degradation is mediated by PDEs (149). Thus, inhibiting PDEs increases cellular levels of cAMP and cGMP. Eleven PDE families have been identified; among them, PDE1, PDE2, PDE3, PDE4, PDE5, and PDE8 are expressed in the

heart (390). Ranek et al. (391) demonstrated that sildenafil, an FDA approved PDE5 inhibitor functioning on activation of PKG, stimulates proteasome peptidase activity, enhances the clearance of misfolded proteins, and decreases aberrant protein aggregation, thereby improving cardiac proteostasis. More recently, it has been reported that pharmacological inhibition of PDE1 (IC86430) increases cardiac proteasome function and accelerates proteasomal degradation of aberrant myocardial proteins in a PKA- and PKG-mediated manner (392). Strikingly, the administration of IC86430 at an overt disease stage markedly improved diastolic function and delayed premature death in $CryAB^{R120G}$ mice (392). Conclusively, pharmacological enhancement of proteasome activity by stimulating PKA or PKG is likely a novel strategy to treat DCM by eliminating aggregation of damaged proteins and alleviating cellular proteotoxicity.

Targeting the E3 Ubiquitin Ligases

Due to their ability to regulate UPS, the E3 ubiquitin ligases represent promising drug targets for patients with diabetic heart disease. Hydrogen sulfide (H_2S) is a gastotransmitter to maintain cardiovascular homeostasis, which is blunted in various cardiovascular diseases, including DCM (355). H_2S primarily signals through a specific protein modification termed sulfhydration, whereby the thiol group of a reactive cysteine is converted to an hydropersulfide (–SSH) group (393, 394). Recent work by Yu et al. (355) has shown that exogenous H_2S reduces translocation of the free fatty acid (FFA) transporter CD36 from intracellular stores to the plasma membrane by promoting sulfhydration of the ER-resident ubiquitin ligase, HRD1; thereby attenuating myocardial fatty acid uptake and lipotoxicity in *db/db* mice. More specifically, HRD1 S-sulfhydration regulates the ubiquitylation of VAMP3 (involved in CD36 trafficking) and promotes its degradation. Interestingly, H_2S-generating compounds have been tested in various preclinical models of heart disease (395). For instance, SG-1002, an orally active H_2S prodrug, attenuates cardiac dysfunction in HFD-induced type II diabetic mice (394). Of note, SG-1002 has been clinically investigated in patients with cardiovascular disease (396). Thus, H_2S may hold therapeutic potential for the treatment of DCM.

Additionally, AMPK has been shown to regulate the transcription of two ubiquitin ligases in the heart; Atrogin-1 and MURF1 (397). AMPK activation leads to increased rates of UPS-mediated protein degradation, thereafter increasing amino acid availability for protein synthesis or ATP production as the heart adapts to a deteriorating metabolic milieu (397). In the diabetic heart, AMPK-mediated protein lysis is cardioprotective due to preserved PQC (22, 397). Numerous studies have reported that metformin improves clinical outcomes in patients with diabetic heart failure by activating AMPK (398–400); however, whether and how metformin regulates cardiac UPS in the progression of DCM requires further investigation.

Targeting Autophagy

Autophagy has been targeted for the treatment of cardiovascular disease; however, in DCM, it is not yet determined whether the induction or inhibition of autophagy has potential treatment effects. Preventive and therapeutic advice in diabetes includes lifestyle modifications, such as exercise and caloric restriction, which have been shown to induce cardiac autophagy and diminish the risk of cardiac events (401). Caloric restriction is sometimes supported by the prescription of appetite suppressors, among which adiponectin (302, 402), leptin (403), and GLP1 receptor agonists (404, 405) have also been proved to induce cardiac autophagy in diabetic models.

Pharmacological treatment for DCM relies mostly on the attenuation of the systemic derangements that lead to cardiac stress; nevertheless, several of these treatments are also able to activate autophagy in cardiac cells (**Table 3**). As mentioned above, one of the most widely prescribed insulin sensitizers is metformin. This AMPK activator improved cardiac function in animal DCM (315) and human heart failure (400). Similarly, improving glycemic control by other insulin sensitizers (302, 408–412), stimulating insulin secretion (413, 414, 416–418), or reducing glucose uptake in the renal tube (419–421) has cardioprotective effects, also restoring myocardial autophagy in DCM models. Lipid-lowering treatments (422, 423) and antihypertensives (424, 428, 452) enhanced myocardial autophagy in preclinical models of T1DM and T2DM through different molecular mechanims, such as calcium-mediated autophagosome-lysosome fusion and receptors modulation. Despite some conflicting results (453), the antioxidant supplements, resveratrol (327, 432, 433, 454, 455), spermidine (437–440), and epigallocatechin gallate (EGCG) (441–444) protected the heart in clinical and preclinical studies in both types of diabetes, also by regulating myocardial autophagy. While the systemic effects of these treatments are essential, the cardiac-specific regulation of autophagy is necessary for the maintenance of cardiac function in diabetes. Further research is required to delineate which drugs in each class are the most beneficial.

Some nondiabetic treatments also display benefits in cardiovascular health in diabetes through autophagy regulation. Suberoylanilide hydroxamic acid (SAHA), a histone deacetylase inhibitor, restored cardiomyocyte contractility in STZ-injected rats (445) and can promote cardiac autophagy (446). On the other hand, the granulocyte-colony stimulating factor (GCSF) stimulates bone marrow function and ameliorated diastolic dysfunction in rodent T2DM models (447, 456, 457) by downregulating autophagy (448). The antimalarial drug and lupus treatment hydroxychloroquine is mostly associated with high risks of cardiotoxicity and heart failure (458); nevertheless, it improved β-cell function in obese nondiabetic patients (449) and reduced glycemic levels in T2DM patients (450). Hydroxychloroquine represses autophagy by accumulating in lysosomes and inhibiting autophagosome fusion (459). Considering this, it remains to be explored whether reduced autophagy might be a therapeutic strategy in early stages of disease and whether targeting cardiac autophagy is an adjuvant strategy for the current metabolic treatments to prevent DCM and heart failure in the diabetic populations.

TABLE 3 | Autophagy-targeting drugs.

Drug	Therapeutic application	Mechanism/Target	Effect on cardiac autophagy	Type of DM	References
Adiponectin	Appetite and metabolic regulators	Adiponectin receptor activation	Induction	T2DM	(302, 402)
Leptin	Appetite and metabolic regulators	Leptin receptor activation	Induction	T2DM	(403, 406)
Metformin	Antihyperglycemic	AMPK activation	Induction	T1DM/T2DM	(315, 316, 400, 407)
Rapamycin and analogs	Antihyperglycemic	MTORC1 inhibition	Induction	T2DM	(302, 408, 409)
Thiazolidinediones	Antihyperglycemic	PPAR activation	Induction	T2DM/T1DM	(410–412)
DPP4 inhibitors	Antihyperglycemic	DPP4 inhibition	Induction	T2DM	(413–415)
GLP1-agonists	Antihyperglycemic	GLP1 receptor activation	Induction	T2DM	(404, 416–418)
SGLT2 inhibitors	Antihyperglycemic	SGLT2 inhibition	Induction	T2DM	(419–421)
Fenofibrate	Lipid-lowering	PPARα activation	Induction	T1DM	(422)
Statins	Lipid-lowering	HMG-CoA reductase inhibition	Induction	T2DM	(423)
Verapamil	Antihypertensive	Calcium channel inhibition	Induction	T1DM/T2DM	(424, 425)
Nifedipine	Antihypertensive	Calcium channel inhibition	Induction	T1DM	(426, 427)
Valsartan	Antihypertensive	Angiotensin receptor inhibition	Induction	T1DM/T2DM	(428–431)
Resveratrol	Antioxidants	SIRT activation	Induction	T1DM/T2DM	(327, 432–436)
Spermidine	Dietary supplement	Acetylase inhibitor	Induction	T1DM	(437–440)
EGCG	Antioxidant and antiinflammatory	SIRT1 activation	Inhibition	T1DM/T2DM	(441–444)
Suberoylanilide hydroxamic acid (SAHA)	Antineoplastic	HDAC inhibition	Induction	T1DM	(445)
Granulocyte-colony stimulating factor (GCSF)	Hematopoietic cytokine	GCSF receptor activation	Inhibition	T2DM	(446, 447)
Hydroxychloroquine	Antimalarial and immunosuppressive	Lysosomal inhibition	Inhibition	T2DM	(448–451)

List of drugs proven to affect cardiac autophagy during DCM treatment.

CONCLUSIONS

Accumulating evidence on the molecular pathogenesis of DCM has revealed the essential roles of proper cellular protein quality control in diabetes-associated heart disease. Upon diabetic stress, the PQC machinery senses, detects, and disposes of the damaged proteins by multiple processes, including the UPR, the UPS, and autophagy. Concerted action of the three cellular systems can tackle proteotoxicity, subsequently improving the cardiac outcome in diabetes; accordingly, compromised PQC mechanisms appear to contribute to heart disease as a result of impaired cellular homeostasis. Dysregulation of the UPR results in the accumulation of misfolded proteins, while malfunction of the UPS and autophagy lead to aggregation of toxic proteins in the cytosol, all of which triggers cell death and provokes the onset and development of heart failure in diabetes. Therefore, it is suggested that the maintenance of protein homeostasis may be a valuable and promising therapeutic strategy to treat cardiac complications in diabetes patients. Of note, either insufficient or exaggerated action of the principal mechanisms exacerbates cytotoxicity in the face of pathological stresses, including diabetic stress (**Figure 8**). Given this dual role in the heart, finely tuned manipulation of the UPR, the UPS, and autophagy in the myocardium is mandatory to maintain cellular equilibrium in response to the diabetic condition with increased demand of protein turnover. As such, despite the growing knowledge of the mechanisms underlying cardiac proteostasis networks, further research is indispensable to investigate the therapeutic potential for heart disease by targeting PQC molecules in diabetes mellitus. As the understanding of molecular regulation of PQC function develops, so will our capability to exploit pharmacological

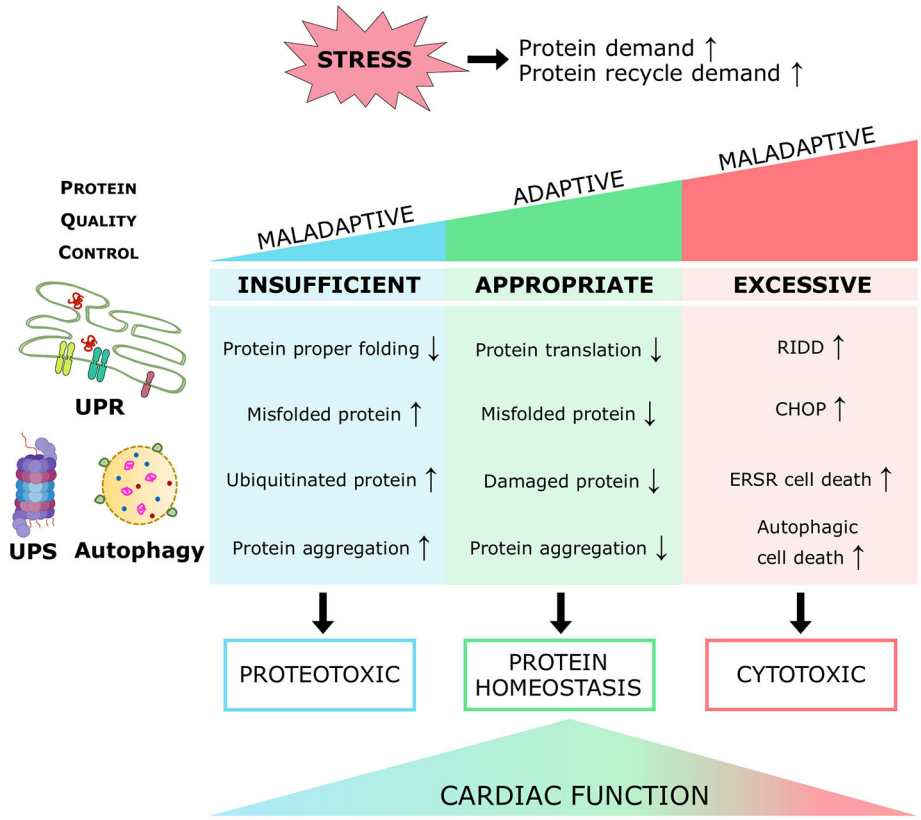

FIGURE 8 | Role of PQC systems in cardiac function under pathological stress. Either insufficient or excessive activity of the main PQC systems is maladaptive. By inducing protein or cellular toxicity, they contribute to cardiac dysfunction. Appropriate level of PQC maintains cardiac protein homeostasis and sustains cardiac function.

interventions to prevent proteotoxicity and cardiac dysfunction in diabetic populations.

AUTHOR CONTRIBUTIONS

NK, RR, and AR-V collected references, generated tables and figures, and drafted the manuscript. AR-V and WL designed the work, wrote, and proofread the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. IDF Diabetes Atlas, 9th edn. Federation ID, editor. Brussels: International Diabetes Federation (2019).
2. Kannel WB, Hjortland M, Castelli WP. Role of diabetes in congestive heart failure: the Framingham study. *Am J Cardiol.* (1974) 34:29–34. doi: 10.1016/0002-9149(74)90089-7
3. Preis SR, Pencina MJ, Hwang S-J, D'Agostino RB, Savage PJ, Levy D, et al. Trends in cardiovascular disease risk factors in individuals with and without diabetes mellitus in the Framingham Heart Study. *Circulation.* (2009) 120:212–20. doi: 10.1161/CIRCULATIONAHA.108.846519
4. Rubler S, Dlugash J, Yuceoglu YZ, Kumral T, Branwood AW, Grishman A. New type of cardiomyopathy associated with diabetic glomerulosclerosis. *Am J Cardiol.* (1972) 30:595–602. doi: 10.1016/0002-9149(72)90595-4
5. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE, Drazner MH, et al. 2013 ACCF/AHA guideline for the management of heart failure. *J Am Coll Cardiol.* (2013) 62:e147–e239. doi: 10.1016/j.jacc.2013.05.019
6. Jia G, Hill MA, Sowers JR. Diabetic cardiomyopathy. *Circ Res.* (2018) 122:624–38. doi: 10.1161/CIRCRESAHA.117.311586
7. Tan Y, Zhang Z, Zheng C, Wintergerst KA, Keller BB, Cai L. Mechanisms of diabetic cardiomyopathy and potential therapeutic strategies: preclinical and clinical evidence. *Nat Rev Cardiol.* (2020) 17:585–607. doi: 10.1038/s41569-020-0339-2
8. Wang ZV, Hill JA. Protein quality control and metabolism: bidirectional control in the heart. *Cell Metab.* (2015) 21:215–26. doi: 10.1016/j.cmet.2015.01.016
9. Yang L, Zhao D, Ren J, Yang J. Endoplasmic reticulum stress and protein quality control in diabetic cardiomyopathy. *Biochim Biophys Acta.* (2015) 1852:209–18. doi: 10.1016/j.bbadis.2014.05.006

10. Ciechanover A, Kwon YT. Degradation of misfolded proteins in neurodegenerative diseases: therapeutic targets and strategies. *Exp Mol Med.* (2015) 47:e147. doi: 10.1038/emm.2014.117
11. Melber A, Haynes CM. UPRmt regulation and output: a stress response mediated by mitochondrial-nuclear communication. *Cell Res.* (2018) 28:281–95. doi: 10.1038/cr.2018.16
12. Henning RH, Brundel BJJM. Proteostasis in cardiac health and disease. *Nat Rev Cardiol.* (2017) 14:637–53. doi: 10.1038/nrcardio.2017.89
13. Chen B, Retzlaff M, Roos T, Frydman J. Cellular strategies of protein quality control. *Cold Spring Harb Perspect Biol.* (2011) 3:a004374. doi: 10.1101/cshperspect.a004374
14. Hwang J, Qi L. Quality control in the endoplasmic reticulum: crosstalk between ERAD and UPR pathways. *Trends Biochem Sci.* (2018) 43:593–605. doi: 10.1016/j.tibs.2018.06.005
15. Moon HW, Han HG, Jeon YJ. Protein quality control in the endoplasmic reticulum and cancer. *Int J Mol Sci.* (2018) 19:3020. doi: 10.3390/ijms19103020
16. McLendon PM, Robbins J. Proteotoxicity and cardiac dysfunction. *Circ Res.* (2015) 116:1863–82. doi: 10.1161/CIRCRESAHA.116.305372
17. Dubnikov T, Ben-Gedalya T, Cohen E. Protein quality control in health and disease. *Cold Spring Harb Perspect Biol.* (2017) 9. doi: 10.1101/cshperspect.a023523
18. Brodsky JL. Cleaning up: ER-associated degradation to the rescue. *Cell.* (2012) 151:1163–7. doi: 10.1016/j.cell.2012.11.012
19. Kozutsumi Y, Segal M, Normington K, Gething MJ, Sambrook J. The presence of malfolded proteins in the endoplasmic reticulum signals the induction of glucose-regulated proteins. *Nature.* (1988) 332:462–4. doi: 10.1038/332462a0
20. Groenendyk J, Agellon LB, Michalak M. Coping with endoplasmic reticulum stress in the cardiovascular system. *Annu Rev Physiol.* (2013) 75:49–67. doi: 10.1146/annurev-physiol-030212-183707
21. Arrieta A, Blackwood EA, Stauffer WT, Glembotski CC. Integrating ER and mitochondrial proteostasis in the healthy and diseased heart. *Front Cardiovasc Med.* (2020) 6:193. doi: 10.3389/fcvm.2019.00193
22. Wang X, Robbins J. Heart failure and protein quality control. *Circ Res.* (2006) 99:1315–28. doi: 10.1161/01.RES.0000252342.61447.a2
23. Tarone G, Brancaccio M. Keep your heart in shape: molecular chaperone networks for treating heart disease. *Cardiovasc Res.* (2014) 102:346–61. doi: 10.1093/cvr/cvu049
24. Hetz C, Zhang K, Kaufman RJ. Mechanisms, regulation and functions of the unfolded protein response. *Nat Rev Mol Cell Biol.* (2020) 21:421–38. doi: 10.1038/s41580-020-0250-z
25. Lindholm D, Korhonen L, Eriksson O, Koks S. Recent insights into the role of unfolded protein response in ER stress in health and disease. *Front Cell Dev Biol.* (2017) 5:48. doi: 10.3389/fcell.2017.00048
26. Ghemrawi R, Battaglia-Hsu SF, Arnold C. Endoplasmic reticulum stress in metabolic disorders. *Cells.* (2018) 7:63. doi: 10.3390/cells7060063
27. Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. *Science.* (2011) 334:1081–6. doi: 10.1126/science.1209038
28. Maamoun H, Abdelsalam SS, Zeidan A, Korashy HM, Agouni A. Endoplasmic reticulum stress: a critical molecular driver of endothelial dysfunction and cardiovascular disturbances associated with diabetes. *Int J Mol Sci.* (2019) 20:1658. doi: 10.3390/ijms20071658
29. Scorrano L, Oakes SA, Opferman JT, Cheng EH, Sorcinelli MD, Pozzan T, et al. BAX and BAK regulation of endoplasmic reticulum $Ca2^{+}$: a control point for apoptosis. *Science.* (2003) 300:135–9. doi: 10.1126/science.1081208
30. Nakagawa T, Yuan J. Cross-talk between two cysteine protease families. Activation of caspase-12 by calpain in apoptosis. *J Cell Biol.* (2000) 150:887–94. doi: 10.1083/jcb.150.4.887
31. Wang X, Bi X, Zhang G, Deng Y, Luo X, Xu L, et al. Glucose-regulated protein 78 is essential for cardiac myocyte survival. *Cell Death Differ.* (2018) 25:2181–94. doi: 10.1038/s41418-018-0109-4
32. Masaki T, Yoshida M, Noguchi S. Targeted disruption of CRE-binding factor TREB5 gene leads to cellular necrosis in cardiac myocytes at the embryonic stage. *Biochem Biophys Res Commun.* (1999) 261:350–6. doi: 10.1006/bbrc.1999.0972
33. Shintani-Ishida K, Nakajima M, Uemura K, Yoshida K. Ischemic preconditioning protects cardiomyocytes against ischemic injury by inducing GRP78. *Biochem Biophys Res Commun.* (2006) 345:1600–5. doi: 10.1016/j.bbrc.2006.05.077
34. Sun Y, Liu G, Song T, Liu F, Kang W, Zhang Y, et al. Upregulation of GRP78 and caspase-12 in diastolic failing heart. *Acta Biochim Pol.* (2008) 55:511–6. doi: 10.18388/abp.2008_3057
35. Severino A, Campioni M, Straino S, Salloum FN, Schmidt N, Herbrand U, et al. Identification of protein disulfide isomerase as a cardiomyocyte survival factor in ischemic cardiomyopathy. *J Am Coll Cardiol.* (2007) 50:1029–37. doi: 10.1016/j.jacc.2007.06.006
36. Sawada T, Minamino T, Fu HY, Asai M, Okuda K, Isomura T, et al. X-box binding protein 1 regulates brain natriuretic peptide through a novel AP1/CRE-like element in cardiomyocytes. *J Mol Cell Cardiol.* (2010) 48:1280–9. doi: 10.1016/j.yjmcc.2010.02.004
37. Castillero E, Akashi H, Pendrak K, Yerebakan H, Najjar M, Wang C, et al. Attenuation of the unfolded protein response and endoplasmic reticulum stress after mechanical unloading in dilated cardiomyopathy. *Am J Physiol Heart Circ Physiol.* (2015) 309:H459–70. doi: 10.1152/ajpheart.00056.2015
38. Yao Y, Lu Q, Hu Z, Yu Y, Chen Q, Wang QK. A non-canonical pathway regulates ER stress signaling and blocks ER stress-induced apoptosis and heart failure. *Nat Commun.* (2017) 8:133. doi: 10.1038/s41467-017-00171-w
39. Martindale JJ, Fernandez R, Thuerauf D, Whittaker R, Gude N, Sussman MA, et al. Endoplasmic reticulum stress gene induction and protection from ischemia/reperfusion injury in the hearts of transgenic mice with a tamoxifen-regulated form of ATF6. *Circ Res.* (2006) 98:1186–93. doi: 10.1161/01.RES.0000220643.65941.8d
40. Zhang G, Wang X, Bi X, Li C, Deng Y, Al-Hashimi AA, et al. GRP78 (Glucose-Regulated Protein of 78 kDa) promotes cardiomyocyte growth through activation of GATA4 (GATA-Binding Protein 4). *Hypertension.* (2019) 73:390–8. doi: 10.1161/HYPERTENSIONAHA.118.12084
41. Groenendyk J, Lee D, Jung J, Dyck JR, Lopaschuk GD, Agellon LB, et al. Inhibition of the unfolded protein response mechanism prevents cardiac fibrosis. *PLoS One.* (2016) 11:e0159682. doi: 10.1371/journal.pone.0159682
42. Okada K, Minamino T, Tsukamoto Y, Liao Y, Tsukamoto O, Takashima S, et al. Prolonged endoplasmic reticulum stress in hypertrophic and failing heart after aortic constriction: possible contribution of endoplasmic reticulum stress to cardiac myocyte apoptosis. *Circulation.* (2004) 110:705–12. doi: 10.1161/01.CIR.0000137836.95625.D4
43. Liu X, Kwak D, Lu Z, Xu X, Fassett J, Wang H, et al. Endoplasmic reticulum stress sensor protein kinase R-like endoplasmic reticulum kinase (PERK) protects against pressure overload-induced heart failure and lung remodeling. *Hypertension.* (2014) 64:738–44. doi: 10.1161/HYPERTENSIONAHA.114.03811
44. Binder P, Wang S, Radu M, Zin M, Collins L, Khan S, et al. Pak2 as a novel therapeutic target for cardioprotective endoplasmic reticulum stress response. *Circ Res.* (2019) 124:696–711. doi: 10.1161/CIRCRESAHA.118.312829
45. Steiger D, Yokota T, Li J, Ren S, Minamisawa S, Wang Y. The serine/threonine-protein kinase/endoribonuclease IRE1alpha protects the heart against pressure overload-induced heart failure. *J Biol Chem.* (2018) 293:9652–61. doi: 10.1074/jbc.RA118.003448
46. Wang ZV, Deng Y, Gao N, Pedrozo Z, Li DL, Morales CR, et al. Spliced X-box binding protein 1 couples the unfolded protein response to hexosamine biosynthetic pathway. *Cell.* (2014) 156:1179–92. doi: 10.1016/j.cell.2014.01.014
47. Duan Q, Ni L, Wang P, Chen C, Yang L, Ma B, et al. Deregulation of XBP1 expression contributes to myocardial vascular endothelial growth factor-A expression and angiogenesis during cardiac hypertrophy *in vivo*. *Aging Cell.* (2016) 15:625–33. doi: 10.1111/acel.12460
48. Jin JK, Blackwood EA, Azizi K, Thuerauf DJ, Fahem AG, Hofmann C, et al. ATF6 decreases myocardial ischemia/reperfusion damage and links ER stress and oxidative stress signaling pathways in the heart. *Circ Res.* (2017) 120:862–75. doi: 10.1161/CIRCRESAHA.116.310266
49. Minamino T, Kitakaze M. ER stress in cardiovascular disease. *J Mol Cell Cardiol.* (2010) 48:1105–10. doi: 10.1016/j.yjmcc.2009.10.026
50. Miyazaki Y, Kaikita K, Endo M, Horio E, Miura M, Tsujita K, et al. C/EBP homologous protein deficiency attenuates myocardial reperfusion injury by

inhibiting myocardial apoptosis and inflammation. *Arterioscler Thromb Vasc Biol.* (2011) 31:1124–32. doi: 10.1161/ATVBAHA.111.224519
51. Xu J, Zhou Q, Xu W, Cai L. Endoplasmic reticulum stress and diabetic cardiomyopathy. *Exp Diabetes Res.* (2012) 2012:827971. doi: 10.1155/2012/827971
52. Ljubkovic M, Gressette M, Bulat C, Cavar M, Bakovic D, Fabijanic D, et al. Disturbed fatty acid oxidation, endoplasmic reticulum stress, and apoptosis in left ventricle of patients with type 2 diabetes. *Diabetes.* (2019) 68:1924–33. doi: 10.2337/db19-0423
53. Yan R, Hu S, Ming Z, Lin L, Le H, Jin W. GW26-e2443 changes in gene expression of calreticulin in the myocardium of streptozotocin-induced diabetic cardiomyopathy. *J Am Coll Cardiol.*. (2015) 66(16 Suppl):C88–C9. doi: 10.1016/j.jacc.2015.06.350
54. Li Z, Zhang T, Dai H, Liu G, Wang H, Sun Y, et al. Involvement of endoplasmic reticulum stress in myocardial apoptosis of streptozocin-induced diabetic rats. *J Clin Biochem Nutr.* (2007) 41:58–67. doi: 10.3164/jcbn.2007008
55. Cong XQ, Piao MH, Li Y, Xie L, Liu Y. Bis(maltolato)oxovanadium(IV) (BMOV) attenuates apoptosis in high glucose-treated cardiac cells and diabetic rat hearts by regulating the unfolded protein responses (UPRs). *Biol Trace Elem Res.* (2016) 173:390–8. doi: 10.1007/s12011-016-0668-5
56. Toldo S, Boccellino M, Rinaldi B, Seropian IM, Mezzaroma E, Severino A, et al. Altered oxido-reductive state in the diabetic heart: loss of cardioprotection due to protein disulfide isomerase. *Mol Med.* (2011) 17:1012–21. doi: 10.2119/molmed.2011.00100
57. Pei Z, Deng Q, Babcock SA, He EY, Ren J, Zhang Y. Inhibition of advanced glycation endproduct (AGE) rescues against streptozotocin-induced diabetic cardiomyopathy: role of autophagy and ER stress. *Toxicol Lett.* (2018) 284:10–20. doi: 10.1016/j.toxlet.2017.11.018
58. Dong F, Ren J. Adiponectin improves cardiomyocyte contractile function in db/db diabetic obese mice. *Obesity (Silver Spring).* (2009) 17:262–8. doi: 10.1038/oby.2008.545
59. Sun X, Zhao D, Lu F, Peng S, Yu M, Liu N, et al. Hydrogen sulfide regulates muscle RING finger-1 protein S-sulfhydration at Cys(44) to prevent cardiac structural damage in diabetic cardiomyopathy. *Br J Pharmacol.* (2020) 177:836–56. doi: 10.1111/bph.14601
60. Xu J, Wang G, Wang Y, Liu Q, Xu W, Tan Y, et al. Diabetes- and angiotensin II-induced cardiac endoplasmic reticulum stress and cell death: metallothionein protection. *J Cell Mol Med.* (2009) 13:1499–512. doi: 10.1111/j.1582-4934.2009.00833.x
61. Liu Z, Zhang Y, Tang Z, Xu J, Ma M, Pan S, et al. Matrine attenuates cardiac fibrosis by affecting ATF6 signaling pathway in diabetic cardiomyopathy. *Eur J Pharmacol.* (2017) 804:21–30. doi: 10.1016/j.ejphar.2017.03.061
62. Liu ZW, Zhu HT, Chen KL, Dong X, Wei J, Qiu C, et al. Protein kinase RNA-like endoplasmic reticulum kinase (PERK) signaling pathway plays a major role in reactive oxygen species (ROS)-mediated endoplasmic reticulum stress-induced apoptosis in diabetic cardiomyopathy. *Cardiovasc Diabetol.* (2013) 12:158. doi: 10.1186/1475-2840-12-158
63. Lakshmanan AP, Harima M, Suzuki K, Soetikno V, Nagata M, Nakamura T, et al. The hyperglycemia stimulated myocardial endoplasmic reticulum (ER) stress contributes to diabetic cardiomyopathy in the transgenic non-obese type 2 diabetic rats: a differential role of unfolded protein response (UPR) signaling proteins. *Int J Biochem Cell Biol.* (2013) 45:438–47. doi: 10.1016/j.biocel.2012.09.017
64. Miki T, Miura T, Hotta H, Tanno M, Yano T, Sato T, et al. Endoplasmic reticulum stress in diabetic hearts abolishes erythropoietin-induced myocardial protection by impairment of phospho-glycogen synthase kinase-3beta-mediated suppression of mitochondrial permeability transition. *Diabetes.* (2009) 58:2863–72. doi: 10.2337/db09-0158
65. Rutkowski DT, Hegde RS. Regulation of basal cellular physiology by the homeostatic unfolded protein response. *J Cell Biol.* (2010) 189:783–94. doi: 10.1083/jcb.201003138
66. Li J, Zhu H, Shen E, Wan L, Arnold JM, Peng T. Deficiency of rac1 blocks NADPH oxidase activation, inhibits endoplasmic reticulum stress, and reduces myocardial remodeling in a mouse model of type 1 diabetes. *Diabetes.* (2010) 59:2033–42. doi: 10.2337/db09-1800
67. He Y, Zhou L, Fan Z, Liu S, Fang W. Palmitic acid, but not high-glucose, induced myocardial apoptosis is alleviated by Nacetylcysteine due to attenuated mitochondrial-derived ROS accumulation-induced endoplasmic reticulum stress. *Cell Death Dis.* (2018) 9:568. doi: 10.1038/s41419-018-0593-y
68. Hotamisligil GS. Role of endoplasmic reticulum stress and c-Jun NH2-terminal kinase pathways in inflammation and origin of obesity and diabetes. *Diabetes.* (2005) 54 (Suppl 2):S73–8. doi: 10.2337/diabetes.54.suppl_2.S73
69. Palomer X, Capdevila-Busquets E, Botteri G, Salvado L, Barroso E, Davidson MM, et al. PPARbeta/delta attenuates palmitate-induced endoplasmic reticulum stress and induces autophagic markers in human cardiac cells. *Int J Cardiol.* (2014) 174:110–8. doi: 10.1016/j.ijcard.2014.03.176
70. Boden G, Cheung P, Kresge K, Homko C, Powers B, Ferrer L. Insulin resistance is associated with diminished endoplasmic reticulum stress responses in adipose tissue of healthy and diabetic subjects. *Diabetes.* (2014) 63:2977–83. doi: 10.2337/db14-0055
71. Gray S, Kim JK. New insights into insulin resistance in the diabetic heart. *Trends Endocrinol Metab.* (2011) 22:394–403. doi: 10.1016/j.tem.2011.05.001
72. Park SY, Cho YR, Kim HJ, Higashimori T, Danton C, Lee MK, et al. Unraveling the temporal pattern of diet-induced insulin resistance in individual organs and cardiac dysfunction in C57BL/6 mice. *Diabetes.* (2005) 54:3530–40. doi: 10.2337/diabetes.54.12.3530
73. Liu Z, Zhao N, Zhu H, Zhu S, Pan S, Xu J, et al. Circulating interleukin-1beta promotes endoplasmic reticulum stress-induced myocytes apoptosis in diabetic cardiomyopathy via interleukin-1 receptor-associated kinase-2. *Cardiovasc Diabetol.* (2015) 14:125. doi: 10.1186/s12933-015-0288-y
74. Cao SS, Luo KL, Shi L. Endoplasmic reticulum stress interacts with inflammation in human diseases. *J Cell Physiol.* (2016) 231:288–94. doi: 10.1002/jcp.25098
75. Luo W, Jin Y, Wu G, Zhu W, Qian Y, Zhang Y, et al. Blockage of ROS and MAPKs-mediated inflammation via restoring SIRT1 by a new compound LF10 prevents type 1 diabetic cardiomyopathy. *Toxicol Appl Pharmacol.* (2019) 370:24–35. doi: 10.1016/j.taap.2019.03.005
76. Guo R, Liu W, Liu B, Zhang B, Li W, Xu Y. SIRT1 suppresses cardiomyocyte apoptosis in diabetic cardiomyopathy: an insight into endoplasmic reticulum stress response mechanism. *Int J Cardiol.* (2015) 191:36–45. doi: 10.1016/j.ijcard.2015.04.245
77. Feng W, Lei T, Wang Y, Feng R, Yuan J, Shen X, et al. GCN2 deficiency ameliorates cardiac dysfunction in diabetic mice by reducing lipotoxicity and oxidative stress. *Free Radic Biol Med.* (2019) 130:128–39. doi: 10.1016/j.freeradbiomed.2018.10.445
78. Galan M, Kassan M, Choi SK, Partyka M, Trebak M, Henrion D, et al. A novel role for epidermal growth factor receptor tyrosine kinase and its downstream endoplasmic reticulum stress in cardiac damage and microvascular dysfunction in type 1 diabetes mellitus. *Hypertension.* (2012) 60:71–80. doi: 10.1161/HYPERTENSIONAHA.112.192500
79. Mali V, Haddox S, Hornersmith C, Matrougui K, Belmadani S. Essential role for EGFR tyrosine kinase and ER stress in myocardial infarction in type 2 diabetes. *Pflugers Arch.* (2018) 470:471–80. doi: 10.1007/s00424-017-2097-5
80. Battiprolu PK, Hojayev B, Jiang N, Wang ZV, Luo X, Iglewski M, et al. Metabolic stress-induced activation of FoxO1 triggers diabetic cardiomyopathy in mice. *J Clin Invest.* (2012) 122:1109–18. doi: 10.1172/JCI60329
81. Xin Z, Ma Z, Jiang S, Wang D, Fan C, Di S, et al. FOXOs in the impaired heart: new therapeutic targets for cardiac diseases. *Biochim Biophys Acta Mol Basis Dis.* (2017) 1863:486–98. doi: 10.1016/j.bbadis.2016.11.023
82. Tang Q, Len Q, Liu Z, Wang W. Overexpression of miR-22 attenuates oxidative stress injury in diabetic cardiomyopathy via Sirt 1. *Cardiovasc Ther.* (2018) 36. doi: 10.1111/1755-5922.12318
83. Xiao H, Wu C, Li P, Tang B. Simultaneous fluorescence visualization of endoplasmic reticulum superoxide anion and polarity in myocardial cells and tissue. *Anal Chem.* (2018) 90:6081–8. doi: 10.1021/acs.analchem.7b05440
84. Du Toit A. Post-translational modification: sweetening protein quality control. *Nat Rev Mol Cell Biol.* (2014) 15:295. doi: 10.1038/nrm3787
85. Ducheix S, Magre J, Cariou B, Prieur X. Chronic O-GlcNAcylation and diabetic cardiomyopathy: the bitterness of glucose. *Front Endocrinol.* (2018) 9:642. doi: 10.3389/fendo.2018.00642

86. Shpilka T, Haynes CM. The mitochondrial UPR: mechanisms, physiological functions and implications in ageing. *Nat Rev Mol Cell Biol.* (2018) 19:109–20. doi: 10.1038/nrm.2017.110
87. Jovaisaite V, Mouchiroud L, Auwerx J. The mitochondrial unfolded protein response, a conserved stress response pathway with implications in health and disease. *J Exp Biol.* (2014) 217(Pt 1):137–43. doi: 10.1242/jeb.090738
88. Zhao Q, Wang J, Levichkin IV, Stasinopoulos S, Ryan MT, Hoogenraad NJ. A mitochondrial specific stress response in mammalian cells. *EMBO J.* (2002) 21:4411–9. doi: 10.1093/emboj/cdf445
89. Martinus RD, Garth GP, Webster TL, Cartwright P, Naylor DJ, Høj PB, et al. Selective induction of mitochondrial chaperones in response to loss of the mitochondrial genome. *Eur J Biochem.* (1996) 240:98–103. doi: 10.1111/j.1432-1033.1996.0098h.x
90. Haynes CM, Ron D. The mitochondrial UPR - protecting organelle protein homeostasis. *J Cell Sci.* (2010) 123(Pt 22):3849–55. doi: 10.1242/jcs.075119
91. Li J, Zhang D, Brundel B, Wiersma M. Imbalance of ER and mitochondria interactions: prelude to cardiac ageing and disease? *Cells.* (2019) 8:1617. doi: 10.3390/cells8121617
92. Yi HS, Chang JY, Shong M. The mitochondrial unfolded protein response and mitohormesis: a perspective on metabolic diseases. *J Mol Endocrinol.* (2018) 61:R91–R105. doi: 10.1530/JME-18-0005
93. Ravanelli S, den Brave F, Hoppe T. Mitochondrial quality control governed by ubiquitin. *Front Cell Dev Biol.* (2020) 8:270. doi: 10.3389/fcell.2020.00270
94. Oh CM, Ryu D, Cho S, Jang Y. Mitochondrial quality control in the heart: new drug targets for cardiovascular disease. *Korean Circ J.* (2020) 50:395–405. doi: 10.4070/kcj.2019.0416
95. Glembotski CC, Arrieta A, Blackwood EA. Unfolding the roles of mitochondria as therapeutic targets for heart disease. *J Am Coll Cardiol.* (2019) 73:1807–10. doi: 10.1016/j.jacc.2018.12.089
96. Smyrnias I, Gray SP, Okonko DO, Sawyer G, Zoccarato A, Catibog N, et al. Cardioprotective effect of the mitochondrial unfolded protein response during chronic pressure overload. *J Am Coll Cardiol.* (2019) 73:1795–806. doi: 10.1016/j.jacc.2018.12.087
97. Wang YT, Lim Y, McCall MN, Huang KT, Haynes CM, Nehrke K, et al. Cardioprotection by the mitochondrial unfolded protein response requires ATF5. *Am J Physiol Heart Circ Physiol.* (2019) 317:H472–H8. doi: 10.1152/ajpheart.00244.2019
98. Williamson CL, Dabkowski ER, Dillmann WH, Hollander JM. Mitochondria protection from hypoxia/reoxygenation injury with mitochondria heat shock protein 70 overexpression. *Am J Physiol Heart Circ Physiol.* (2008) 294:H249–56. doi: 10.1152/ajpheart.00775.2007
99. Kuo CY, Chiu YC, Lee AY, Hwang TL. Mitochondrial Lon protease controls ROS-dependent apoptosis in cardiomyocyte under hypoxia. *Mitochondrion.* (2015) 23:7–16. doi: 10.1016/j.mito.2015.04.004
100. Venkatesh S, Li M, Saito T, Tong M, Rashed E, Mareedu S, et al. Mitochondrial LonP1 protects cardiomyocytes from ischemia/reperfusion injury in vivo. *J Mol Cell Cardiol.* (2019) 128:38–50. doi: 10.1016/j.yjmcc.2018.12.017
101. Lu B, Shangguan F, Huang D, Gong S, Shi Y, Song Z, et al. LonP1 orchestrates UPRmt and UPRER and mitochondrial dynamics to regulate heart function. *bioRxiv [Preprint].* (2019). doi: 10.1101/564492
102. Dogan SA, Pujol C, Maiti P, Kukat A, Wang S, Hermans S, et al. Tissue-specific loss of DARS2 activates stress responses independently of respiratory chain deficiency in the heart. *Cell Metab.* (2014) 19:458–69. doi: 10.1016/j.cmet.2014.02.004
103. Acin-Perez R, Lechuga-Vieco AV, Del Mar Munoz M, Nieto-Arellano R, Torroja C, Sanchez-Cabo F, et al. Ablation of the stress protease OMA1 protects against heart failure in mice. *Sci Transl Med.* (2018) 10:eaan4935. doi: 10.1126/scitranslmed.aan4935
104. Shepherd DL, Hathaway Q, Nichols CE, Durr AJ, Pinti MV, Hughes KM, et al. Mitochondrial proteome disruption in the diabetic heart through targeted epigenetic regulation at the mitochondrial heat shock protein 70 (mtHsp70) nuclear locus. *J Mol Cell Cardiol.* (2018) 119:104–15. doi: 10.1016/j.yjmcc.2018.04.016
105. Chen HS, Wu TE, Juan CC, Lin HD. Myocardial heat shock protein 60 expression in insulin-resistant and diabetic rats. *J Endocrinol.* (2009) 200:151–7. doi: 10.1677/JOE-08-0387
106. Zhang X, Zhong Z, Li W. Downregulation of TRAP1 aggravates injury of H9c2 cardiomyocytes in a hyperglycemic state. *Exp Ther Med.* (2019) 18:2681–6. doi: 10.3892/etm.2019.7847
107. Itoh T, Kouzu H, Miki T, Tanno M, Kuno A, Sato T, et al. UPR in endoplasmic reticulum vs. UPR in mitochondria in diabetic myocardium: which is responsible for deficient regulation of mitochondrial permeability transition pores? *Circulation.* (2018) 124:A15050.
108. Dikic I. Proteasomal and autophagic degradation systems. *Annu Rev Biochem.* (2017) 86:193–224. doi: 10.1146/annurev-biochem-061516-044908
109. Rock KL, Gramm C, Rothstein L, Clark K, Stein R, Dick L, et al. Inhibitors of the proteasome block the degradation of most cell proteins and the generation of peptides presented on MHC class I molecules. *Cell.* (1994) 78:761–71. doi: 10.1016/S0092-8674(94)90462-6
110. Schwartz AL, Ciechanover A. The ubiquitin-proteasome pathway and pathogenesis of human diseases. *Annu Rev Med.* (1999) 50:57–74. doi: 10.1146/annurev.med.50.1.57
111. Deshaies RJ. Make it or break it: the role of ubiquitin-dependent proteolysis in cellular regulation. *Trends Cell Biol.* (1995) 5:428–34. doi: 10.1016/S0962-8924(00)89102-3
112. Hochstrasser M. Ubiquitin-dependent protein degradation. *Annu Rev Genet.* (1996) 30:405–39. doi: 10.1146/annurev.genet.30.1.405
113. Jentsch S, Schlenker S. Selective protein degradation: a journey's end within the proteasome. *Cell.* (1995) 82:881–4. doi: 10.1016/0092-8674(95)90021-7
114. Vijay-Kumar S, Bugg CE, Cook WJ. Structure of ubiquitin refined at 1.8 A resolution. *J Mol Biol.* (1987) 194:531–44. doi: 10.1016/0022-2836(87)90679-6
115. Zheng Q, Huang T, Zhang L, Zhou Y, Luo H, Xu H, et al. Dysregulation of ubiquitin-proteasome system in neurodegenerative diseases. *Front Aging Neurosci.* (2016) 8:303. doi: 10.3389/fnagi.2016.00303
116. Finley D. Recognition and processing of ubiquitin-protein conjugates by the proteasome. *Annu Rev Biochem.* (2009) 78:477–513. doi: 10.1146/annurev.biochem.78.081507.101607
117. Kulathu Y, Komander D. Atypical ubiquitylation - the unexplored world of polyubiquitin beyond Lys48 and Lys63 linkages. *Nat Rev Mol Cell Biol.* (2012) 13:508–23. doi: 10.1038/nrm3394
118. Lopez-Mosqueda J, Dikic I. Deciphering functions of branched ubiquitin chains. *Cell.* (2014) 157:767–9. doi: 10.1016/j.cell.2014.04.026
119. Yau R, Rape M. The increasing complexity of the ubiquitin code. *Nat Cell Biol.* (2016) 18:579–86. doi: 10.1038/ncb3358
120. Thrower JS, Hoffman L, Rechsteiner M, Pickart CM. Recognition of the polyubiquitin proteolytic signal. *EMBO J.* (2000) 19:94–102. doi: 10.1093/emboj/19.1.94
121. Kwon YT, Ciechanover A. The ubiquitin code in the ubiquitin-proteasome system and autophagy. *Trends Biochem Sci.* (2017) 42:873–86. doi: 10.1016/j.tibs.2017.09.002
122. Komander D. The emerging complexity of protein ubiquitination. *Biochem Soc Trans.* (2009) 37(Pt 5):937–53. doi: 10.1042/BST0370937
123. Reyes-Turcu FE, Ventii KH, Wilkinson KD. Regulation and cellular roles of ubiquitin-specific deubiquitinating enzymes. *Annu Rev Biochem.* (2009) 78:363–97. doi: 10.1146/annurev.biochem.78.082307.091526
124. Voges D, Zwickl P, Baumeister W. The 26S proteasome: a molecular machine designed for controlled proteolysis. *Annu Rev Biochem.* (1999) 68:1015–68. doi: 10.1146/annurev.biochem.68.1.1015
125. Groll M, Bajorek M, Kohler A, Moroder L, Rubin DM, Huber R, et al. A gated channel into the proteasome core particle. *Nat Struct Biol.* (2000) 7:1062–7. doi: 10.1038/80992
126. Groll M, Ditzel L, Lowe J, Stock D, Bochtler M, Bartunik HD, et al. Structure of 20S proteasome from yeast at 2.4 A resolution. *Nature.* (1997) 386:463–71. doi: 10.1038/386463a0
127. Glickman MH, Ciechanover A. The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. *Physiol Rev.* (2002) 82:373–428. doi: 10.1152/physrev.00027.2001
128. Elsasser S, Chandler-Militello D, Muller B, Hanna J, Finley D. Rad23 and Rpn10 serve as alternative ubiquitin receptors for the proteasome. *J Biol Chem.* (2004) 279:26817–22. doi: 10.1074/jbc.M404020200
129. Husnjak K, Elsasser S, Zhang N, Chen X, Randles L, Shi Y, et al. Proteasome subunit Rpn13 is a novel ubiquitin receptor. *Nature.* (2008) 453:481–8. doi: 10.1038/nature06926

130. Navon A, Goldberg AL. Proteins are unfolded on the surface of the ATPase ring before transport into the proteasome. *Mol Cell.* (2001) 8:1339–49. doi: 10.1016/S1097-2765(01)00407-5
131. Rabl J, Smith DM, Yu Y, Chang SC, Goldberg AL, Cheng Y. Mechanism of gate opening in the 20S proteasome by the proteasomal ATPases. *Mol Cell.* (2008) 30:360–8. doi: 10.1016/j.molcel.2008.03.004
132. Baumeister W, Walz J, Zuhl F, Seemuller E. The proteasome: paradigm of a self-compartmentalizing protease. *Cell.* (1998) 92:367–80. doi: 10.1016/S0092-8674(00)80929-0
133. Hartl FU, Bracher A, Hayer-Hartl M. Molecular chaperones in protein folding and proteostasis. *Nature.* (2011) 475:324–32. doi: 10.1038/nature10317
134. Hipp MS, Kasturi P, Hartl FU. The proteostasis network and its decline in ageing. *Nat Rev Mol Cell Biol.* (2019) 20:421–35. doi: 10.1038/s41580-019-0101-y
135. Kettern N, Dreiseidler M, Tawo R, Hohfeld J. Chaperone-assisted degradation: multiple paths to destruction. *Biol Chem.* (2010) 391:481–9. doi: 10.1515/bc.2010.058
136. Arndt V, Rogon C, Hohfeld J. To be, or not to be–molecular chaperones in protein degradation. *Cell Mol Life Sci.* (2007) 64:2525–41. doi: 10.1007/s00018-007-7188-6
137. Wang X, Su H, Ranek MJ. Protein quality control and degradation in cardiomyocytes. *J Mol Cell Cardiol.* (2008) 45:11–27. doi: 10.1016/j.yjmcc.2008.03.025
138. Ranek MJ, Zheng H, Huang W, Kumarapeli AR, Li J, Liu J, et al. Genetically induced moderate inhibition of 20S proteasomes in cardiomyocytes facilitates heart failure in mice during systolic overload. *J Mol Cell Cardiol.* (2015) 85:273–81. doi: 10.1016/j.yjmcc.2015.06.014
139. Takayama S, Bimston DN, Matsuzawa S, Freeman BC, Aime-Sempe C, Xie Z, et al. BAG-1 modulates the chaperone activity of Hsp70/Hsc70. *EMBO J.* (1997) 16:4887–96. doi: 10.1093/emboj/16.16.4887
140. Alberti S, Demand J, Esser C, Emmerich N, Schild H, Hohfeld J. Ubiquitylation of BAG-1 suggests a novel regulatory mechanism during the sorting of chaperone substrates to the proteasome. *J Biol Chem.* (2002) 277:45920–7. doi: 10.1074/jbc.M204196200
141. Alberti S, Bohse K, Arndt V, Schmitz A, Hohfeld J. The cochaperone HspBP1 inhibits the CHIP ubiquitin ligase and stimulates the maturation of the cystic fibrosis transmembrane conductance regulator. *Mol Biol Cell.* (2004) 15:4003–10. doi: 10.1091/mbc.e04-04-0293
142. Townsend PA, Cutress RI, Carroll CJ, Lawrence KM, Scarabelli TM, Packham G, et al. BAG-1 proteins protect cardiac myocytes from simulated ischemia/reperfusion-induced apoptosis via an alternate mechanism of cell survival independent of the proteasome. *J Biol Chem.* (2004) 279:20723–8. doi: 10.1074/jbc.M400399200
143. Zhang C, Xu Z, He XR, Michael LH, Patterson C. CHIP, a cochaperone/ubiquitin ligase that regulates protein quality control, is required for maximal cardioprotection after myocardial infarction in mice. *Am J Physiol Heart Circ Physiol.* (2005) 288:H2836–42. doi: 10.1152/ajpheart.01122.2004
144. Bartha E, Solti I, Szabo A, Olah G, Magyar K, Szabados E, et al. Regulation of kinase cascade activation and heat shock protein expression by poly(ADP-ribose) polymerase inhibition in doxorubicin-induced heart failure. *J Cardiovasc Pharmacol.* (2011) 58:380–91. doi: 10.1097/FJC.0b013e318225c21e
145. Ke X, Chen J, Peng L, Zhang W, Yang Y, Liao X, et al. Heat shock protein 90/Akt pathway participates in the cardioprotective effect of exogenous hydrogen sulfide against high glucose-induced injury to H9c2 cells. *Int J Mol Med.* (2017) 39:1001–10. doi: 10.3892/ijmm.2017.2891
146. Fan GC, Ren X, Qian J, Yuan Q, Nicolaou P, Wang Y, et al. Novel cardioprotective role of a small heat-shock protein, Hsp20, against ischemia/reperfusion injury. *Circulation.* (2005) 111:1792–9. doi: 10.1161/01.CIR.0000160851.41872.C6
147. Macario AJ, Grippo TM, Conway de Macario E. Genetic disorders involving molecular-chaperone genes: a perspective. *Genet Med.* (2005) 7:3–12. doi: 10.1097/01.GIM.0000151351.11876.C3
148. Wang X, Osinska H, Klevitsky R, Gerdes AM, Nieman M, Lorenz J, et al. Expression of R120G-alphaB-crystallin causes aberrant desmin and alphaB-crystallin aggregation and cardiomyopathy in mice. *Circ Res.* (2001) 89:84–91. doi: 10.1161/hh1301.092688
149. Wang X, Wang H. Priming the proteasome to protect against proteotoxicity. *Trends Mol Med.* (2020) 26:639–48. doi: 10.1016/j.molmed.2020.02.007
150. Christianson JC, Ye Y. Cleaning up in the endoplasmic reticulum: ubiquitin in charge. *Nat Struct Mol Biol.* (2014) 21:325–35. doi: 10.1038/nsmb.2793
151. Qi L, Tsai B, Arvan P. New insights into the physiological role of endoplasmic reticulum-associated degradation. *Trends Cell Biol.* (2017) 27:430–40. doi: 10.1016/j.tcb.2016.12.002
152. Olzmann JA, Kopito RR, Christianson JC. The mammalian endoplasmic reticulum-associated degradation system. *Cold Spring Harb Perspect Biol.* (2013) 5:a013185. doi: 10.1101/cshperspect.a013185
153. Locke M, Toth JI, Petroski MD. Lys11- and Lys48-linked ubiquitin chains interact with p97 during endoplasmic-reticulum-associated degradation. *Biochem J.* (2014) 459:205–16. doi: 10.1042/BJ20120662
154. Ye Y, Meyer HH, Rapoport TA. Function of the p97-Ufd1-Npl4 complex in retrotranslocation from the ER to the cytosol: dual recognition of nonubiquitinated polypeptide segments and polyubiquitin chains. *J Cell Biol.* (2003) 162:71–84. doi: 10.1083/jcb.200302169
155. Hetz C. The unfolded protein response: controlling cell fate decisions under ER stress and beyond. *Nat Rev Mol Cell Biol.* (2012) 13:89–102. doi: 10.1038/nrm3270
156. Hetz C, Chevet E, Harding HP. Targeting the unfolded protein response in disease. *Nat Rev Drug Discov.* (2013) 12:703–19. doi: 10.1038/nrd3976
157. Hetz C, Papa FR. The unfolded protein response and cell fate control. *Mol Cell.* (2018) 69:169–81. doi: 10.1016/j.molcel.2017.06.017
158. Kleiger G, Mayor T. Perilous journey: a tour of the ubiquitin-proteasome system. *Trends Cell Biol.* (2014) 24:352–9. doi: 10.1016/j.tcb.2013.12.003
159. Gilda JE, Gomes AV. Proteasome dysfunction in cardiomyopathies. *J Physiol.* (2017) 595:4051–71. doi: 10.1113/JP273607
160. Day SM. The ubiquitin proteasome system in human cardiomyopathies and heart failure. *Am J Physiol Heart Circ Physiol.* (2013) 304:H1283–H93. doi: 10.1152/ajpheart.00249.2012
161. Weekes J, Morrison K, Mullen A, Wait R, Barton P, Dunn MJ. Hyperubiquitination of proteins in dilated cardiomyopathy. *Proteomics.* (2003) 3:208–16. doi: 10.1002/pmic.200390029
162. Willis MS, Bevilacqua A, Pulinilkunnil T, Kienesberger P, Tannu M, Patterson C. The role of ubiquitin ligases in cardiac disease. *J Mol Cell Cardiol.* (2014) 71:43–53. doi: 10.1016/j.yjmcc.2013.11.008
163. Predmore MJ, Wang VP, Davis BF, Bartolone RS, Westfall MM, Dyke MD, et al. Ubiquitin proteasome dysfunction in human hypertrophic and dilated cardiomyopathies. *Circulation.* (2010) 121:997–1004. doi: 10.1161/CIRCULATIONAHA.109.904557
164. Liu J, Chen Q, Huang W, Horak KM, Zheng H, Mestril R, et al. Impairment of the ubiquitin-proteasome system in desminopathy mouse hearts. *FASEB J.* (2006) 20:362–4. doi: 10.1096/fj.05-4869fje
165. Pan B, Li J, Parajuli N, Tian Z, Wu P, Lewno MT, et al. The calcineurin-TFEB-p62 pathway mediates the activation of cardiac macroautophagy by proteasomal malfunction. *Circ Res.* (2020) 127:502–18. doi: 10.1101/2019.12.27.889519
166. Herrmann J, Wohlert C, Saguner AM, Flores A, Nesbitt LL, Chade A, et al. Primary proteasome inhibition results in cardiac dysfunction. *Eur J Heart Failure.* (2013) 15:614–23. doi: 10.1093/eurjhf/hft034
167. Cole DC, Frishman WH. Cardiovascular complications of proteasome inhibitors used in multiple myeloma. *Cardiol Rev.* (2018) 26:122–9. doi: 10.1097/CRD.0000000000000183
168. Kumarapeli AR, Horak KM, Glasford JW, Li J, Chen Q, Liu J, et al. A novel transgenic mouse model reveals deregulation of the ubiquitin-proteasome system in the heart by doxorubicin. *FASEB J.* (2005) 19:2051–3. doi: 10.1096/fj.05-3973fje
169. Liu J, Zheng H, Tang M, Ryu YC, Wang X. A therapeutic dose of doxorubicin activates ubiquitin-proteasome system-mediated proteolysis by acting on both the ubiquitination apparatus and proteasome. *Am J Physiol Heart Circ Physiol.* (2008) 295:H2541–50. doi: 10.1152/ajpheart.01052.2008
170. Lindsten K, Menendez-Benito V, Masucci MG, Dantuma NP, Kumarapeli AR, Horak KM, et al. GFP reporter mouse models of UPS proteolytic function. *FASEB J.* (2006) 20:1027; author reply 8. doi: 10.1096/fj.06-0504ufm

171. Xu N, Gulick J, Osinska H, Yu Y, McLendon PM, Shay-Winkler K, et al. Ube2v1 positively regulates protein aggregation by modulating ubiquitin proteasome system performance partially through K63 ubiquitination. *Circ Res.* (2020) 126:907–22. doi: 10.1161/CIRCRESAHA.119.316444
172. Maejima Y, Usui S, Zhai P, Takamura M, Kaneko S, Zablocki D, et al. Muscle-specific RING finger 1 negatively regulates pathological cardiac hypertrophy through downregulation of calcineurin A. *Circ Heart Failure.* (2014) 7:479–90. doi: 10.1161/CIRCHEARTFAILURE.113.000713
173. Li H-H, Du J, Fan Y-N, Zhang M-L, Liu D-P, Li L, et al. The ubiquitin ligase MuRF1 protects against cardiac ischemia/reperfusion injury by its proteasome-dependent degradation of phospho-c-Jun. *Am J Pathol.* (2011) 178:1043–58. doi: 10.1016/j.ajpath.2010.11.049
174. Mattox TA, Young ME, Rubel CE, Spaniel C, Rodriguez JE, Grevengoed TJ, et al. MuRF1 activity is present in cardiac mitochondria and regulates reactive oxygen species production *in vivo*. *J Bioenerg Biomembr.* (2014) 46:173–87. doi: 10.1007/s10863-014-9549-9
175. Willis MS, Schisler JC, Portbury AL, Patterson C. Build it up-tear it down: protein quality control in the cardiac sarcomere. *Cardiovasc Res.* (2009) 81:439–48. doi: 10.1093/cvr/cvn289
176. Willis MS, Ike C, Li L, Wang DZ, Glass DJ, Patterson C. Muscle ring finger 1, but not muscle ring finger 2, regulates cardiac hypertrophy in vivo. *Circ Res.* (2007) 100:456–9. doi: 10.1161/01.RES.0000259559.48597.32
177. He J, Quintana MT, Sullivan J, T LP, T JG, Schisler JC, et al. MuRF2 regulates PPARgamma1 activity to protect against diabetic cardiomyopathy and enhance weight gain induced by a high fat diet. *Cardiovasc Diabetol.* (2015) 14:97. doi: 10.1186/s12933-015-0252-x
178. Fielitz J, van Rooij E, Spencer JA, Shelton JM, Latif S, van der Nagel R, et al. Loss of muscle-specific RING-finger 3 predisposes the heart to cardiac rupture after myocardial infarction. *Proc Natl Acad Sci U S A.* (2007) 104:4377–82. doi: 10.1073/pnas.0611726104
179. Quintana MT, He J, Sullivan J, Grevengoed T, Schisler J, Han Y, et al. Muscle ring finger-3 protects against diabetic cardiomyopathy induced by a high fat diet. *BMC Endocr Disord.* (2015) 15:36. doi: 10.1186/s12902-015-0028-z
180. Li HH, Kedar V, Zhang C, McDonough H, Arya R, Wang DZ, et al. Atrogin-1/muscle atrophy F-box inhibits calcineurin-dependent cardiac hypertrophy by participating in an SCF ubiquitin ligase complex. *J Clin Invest.* (2004) 114:1058–71. doi: 10.1172/JCI200422220
181. Xie P, Guo SB, Fan Y, Zhang H, Gu D, Li H. Atrogin-1/MAFbx enhances simulated ischemia/reperfusion-induced apoptosis in cardiomyocytes through degradation of MAPK phosphatase-1 and sustained JNK activation. *J Biol Chem.* (2009) 284:5488–96. doi: 10.1074/jbc.M806487200
182. Galasso G, De Rosa R, Piscione F, Iaccarino G, Vosa C, Sorriento D, et al. Myocardial expression of FOXO3a-Atrogin-1 pathway in human heart failure. *Eur J Heart Fail.* (2010) 12:1290–6. doi: 10.1093/eurjhf/hfq102
183. Liu W, Wang G, Zhang C, Ding W, Cheng W, Luo Y, et al. MG53, a novel regulator of KChIP2 and Ito, plays a critical role in electrophysiological remodeling in cardiac hypertrophy. *Circulation.* (2019) 139:2142–56. doi: 10.1161/CIRCULATIONAHA.118.029413
184. Cao CM, Zhang Y, Weisleder N, Ferrante C, Wang X, Lv F, et al. MG53 constitutes a primary determinant of cardiac ischemic preconditioning. *Circulation.* (2010) 121:2565–74. doi: 10.1161/CIRCULATIONAHA.110.954628
185. Zhang Y, Lv F, Jin L, Peng W, Song R, Ma J, et al. MG53 participates in ischaemic postconditioning through the RISK signalling pathway. *Cardiovasc Res.* (2011) 91:108–15. doi: 10.1093/cvr/cvr029
186. Liu F, Song R, Feng Y, Guo J, Chen Y, Zhang Y, et al. Upregulation of MG53 induces diabetic cardiomyopathy through transcriptional activation of peroxisome proliferation-activated receptor alpha. *Circulation.* (2015) 131:795–804. doi: 10.1161/CIRCULATIONAHA.114.012285
187. He B, Tang RH, Weisleder N, Xiao B, Yuan Z, Cai C, et al. Enhancing muscle membrane repair by gene delivery of MG53 ameliorates muscular dystrophy and heart failure in delta-Sarcoglycan-deficient hamsters. *Mol Ther.* (2012) 20:727–35. doi: 10.1038/mt.2012.5
188. Pan JA, Sun Y, Jiang YP, Bott AJ, Jaber N, Dou Z, et al. TRIM21 ubiquitylates SQSTM1/p62 and suppresses protein sequestration to regulate redox homeostasis. *Mol Cell.* (2016) 62:149–51. doi: 10.1016/j.molcel.2016.03.015
189. Hauck L, Stanley-Hasnain S, Fung A, Grothe D, Rao V, Mak TW, et al. Cardiac-specific ablation of the E3 ubiquitin ligase Mdm2 leads to oxidative stress, broad mitochondrial deficiency and early death. *PLoS One.* (2017) 12:e0189861. doi: 10.1371/journal.pone.0189861
190. Fu W, Ma Q, Chen L, Li P, Zhang M, Ramamoorthy S, et al. MDM2 acts downstream of p53 as an E3 ligase to promote FOXO ubiquitination and degradation. *J Biol Chem.* (2009) 284:13987–4000. doi: 10.1074/jbc.M901758200
191. Rafiq K, Kolpakov MA, Seqqat R, Guo J, Guo X, Qi Z, et al. c-Cbl inhibition improves cardiac function and survival in response to myocardial ischemia. *Circulation.* (2014) 129:2031–43. doi: 10.1161/CIRCULATIONAHA.113.007004
192. Schisler JC, Rubel CE, Zhang C, Lockyer P, Cyr DM, Patterson C. CHIP protects against cardiac pressure overload through regulation of AMPK. *J Clin Invest.* (2013) 123:3588–99. doi: 10.1172/JCI69080
193. Kubli DA, Zhang X, Lee Y, Hanna RA, Quinsay MN, Nguyen CK, et al. Parkin protein deficiency exacerbates cardiac injury and reduces survival following myocardial infarction. *J Biol Chem.* (2013) 288:915–26. doi: 10.1074/jbc.M112.411363
194. Sun T, Ding W, Xu T, Ao X, Yu T, Li M, et al. Parkin regulates programmed necrosis and myocardial ischemia/reperfusion injury by targeting cyclophilin-D. *Antioxid Redox Signal.* (2019) 31:1177–93. doi: 10.1089/ars.2019.7734
195. Tang Y, Liu J, Long J. Phosphatase and tensin homolog-induced putative kinase 1 and Parkin in diabetic heart: role of mitophagy. *J Diabetes Investig.* (2015) 6:250–5. doi: 10.1111/jdi.12302
196. Wang S, Zhao Z, Fan Y, Zhang M, Feng X, Lin J, et al. Mst1 inhibits Sirt3 expression and contributes to diabetic cardiomyopathy through inhibiting Parkin-dependent mitophagy. *Biochim Biophys Acta.* (2019) 1865:1905–14. doi: 10.1016/j.bbadis.2018.04.009
197. Doroudgar JS, Völkers LM, Thuerauf JD, Khan HTM, Mohsin AS, Respress CJ, et al. Hrd1 and ER-associated protein degradation, ERAD, are critical elements of the adaptive ER stress response in cardiac myocytes. *Circ Res.* (2015) 117:536–46. doi: 10.1161/CIRCRESAHA.115.306993
198. Wang Y, Gao P, Wei C, Li H, Zhang L, Zhao Y, et al. Calcium sensing receptor protects high glucose-induced energy metabolism disorder via blocking gp78-ubiquitin proteasome pathway. *Cell Death Dis.* (2017) 8:e2799. doi: 10.1038/cddis.2017.193
199. Bi HL, Zhang XL, Zhang YL, Xie X, Xia YL, Du J, et al. The deubiquitinase UCHL1 regulates cardiac hypertrophy by stabilizing epidermal growth factor receptor. *Sci Adv.* (2020) 6:eaax4826. doi: 10.1126/sciadv.aax4826
200. Wang H, Lai Y, Mathis BJ, Wang W, Li S, Qu C, et al. Deubiquitinating enzyme CYLD mediates pressure overload-induced cardiac maladaptive remodeling and dysfunction via downregulating Nrf2. *J Mol Cell Cardiol.* (2015) 84:143–53. doi: 10.1016/j.yjmcc.2015.04.012
201. Huang H, Tang QZ, Wang AB, Chen M, Yan L, Liu C, et al. Tumor suppressor A20 protects against cardiac hypertrophy and fibrosis by blocking transforming growth factor-beta-activated kinase 1-dependent signaling. *Hypertension.* (2010) 56:232–9. doi: 10.1161/HYPERTENSIONAHA.110.149963
202. Li HL, Zhuo ML, Wang D, Wang AB, Cai H, Sun LH, et al. Targeted cardiac overexpression of A20 improves left ventricular performance and reduces compensatory hypertrophy after myocardial infarction. *Circulation.* (2007) 115:1885–94. doi: 10.1161/CIRCULATIONAHA.106.656835
203. He B, Zhao YC, Gao LC, Ying XY, Xu LW, Su YY, et al. Ubiquitin-specific protease 4 is an endogenous negative regulator of pathological cardiac hypertrophy. *Hypertension.* (2016) 67:1237–48. doi: 10.1161/HYPERTENSIONAHA.116.07392
204. Chen S, Czernuszewicz G, Lombardi R, Jin JP, Willerson J, Marian A. Human molecular genetic and functional studies identify TRIM63, encoding muscle RING ringer protein 1, as a novel gene for human hypertrophic cardiomyopathy. *Circulation.* (2012) 126:907–16. doi: 10.1161/CIRCRESAHA.112.270207
205. Belmont JP, Chen JW, San Pedro NM, Thuerauf JD, Lowe GN, Gude AN, et al. Roles for endoplasmic reticulum-associated degradation and the novel endoplasmic reticulum stress response gene Derlin-3 in the ischemic heart. *Circ Res.* (2010) 106:307–16. doi: 10.1161/CIRCRESAHA.109.203901
206. Bai T, Wang F, Mellen N, Zheng Y, Cai L. Diabetic cardiomyopathy: role of the E3 ubiquitin ligase. *Am J Physiol Endocrinol Metab.* (2016) 310:E473–83. doi: 10.1152/ajpendo.00467.2015

207. Kato S, Ding J, Pisck E, Jhala US, Du K. COP1 functions as a FoxO1 ubiquitin E3 ligase to regulate FoxO1-mediated gene expression. *J Biol Chem.* (2008) 283:35464–73. doi: 10.1074/jbc.M801011200
208. Li F, Xie P, Fan Y, Zhang H, Zheng L, Gu D, et al. C terminus of Hsc70-interacting protein promotes smooth muscle cell proliferation and survival through ubiquitin-mediated degradation of FoxO1. *J Biol Chem.* (2009) 284:20090–8. doi: 10.1074/jbc.M109.017046
209. Oka T, Maillet M, Watt AJ, Schwartz RJ, Aronow BJ, Duncan SA, et al. Cardiac-specific deletion of Gata4 reveals its requirement for hypertrophy, compensation, and myocyte viability. *Circ Res.* (2006) 98:837–45. doi: 10.1161/01.RES.0000215985.18538.c4
210. Kobayashi S, Mao K, Zheng H, Wang X, Patterson C, O'Connell TD, et al. Diminished GATA4 protein levels contribute to hyperglycemia-induced cardiomyocyte injury. *J Biol Chem.* (2007) 282:21945–52. doi: 10.1074/jbc.M703048200
211. Madrazo JA, Kelly DP. The PPAR trio: regulators of myocardial energy metabolism in health and disease. *J Mol Cell Cardiol.* (2008) 44:968–75. doi: 10.1016/j.yjmcc.2008.03.021
212. Park SY, Cho YR, Finck BN, Kim HJ, Higashimori T, Hong EG, et al. Cardiac-specific overexpression of peroxisome proliferator-activated receptor-alpha causes insulin resistance in heart and liver. *Diabetes.* (2005) 54:2514–24. doi: 10.2337/diabetes.54.9.2514
213. Rodriguez JE, Liao JY, He J, Schisler JC, Newgard CB, Drujan D, et al. The ubiquitin ligase MuRF1 regulates PPARα activity in the heart by enhancing nuclear export via monoubiquitination. *Mol Cell Endocrinol.* (2015) 413:36–48. doi: 10.1016/j.mce.2015.06.008
214. Kim HJ, Zheng M, Kim SK, Cho JJ, Shin CH, Joe Y, et al. CO/HO-1 induces NQO-1 expression via Nrf2 activation. *Immune Netw.* (2011) 11:376–82. doi: 10.4110/in.2011.11.6.376
215. Gong P, Stewart D, Hu B, Li N, Cook J, Nel A, et al. Activation of the mouse heme oxygenase-1 gene by 15-deoxy-Delta(12,14)-prostaglandin J(2) is mediated by the stress response elements and transcription factor Nrf2. *Antioxid Redox Signal.* (2002) 4:249–57. doi: 10.1089/152308602753666307
216. Li J, Ichikawa T, Villacorta L, Janicki JS, Brower GL, Yamamoto M, et al. Nrf2 protects against maladaptive cardiac responses to hemodynamic stress. *Arterioscler Thromb Vasc Biol.* (2009) 29:1843–50. doi: 10.1161/ATVBAHA.109.189480
217. Wang W, Li S, Wang H, Li B, Shao L, Lai Y, et al. Nrf2 enhances myocardial clearance of toxic ubiquitinated proteins. *J Mol Cell Cardiol.* (2014) 72:305–15. doi: 10.1016/j.yjmcc.2014.04.006
218. Yamamoto M, Kensler TW, Motohashi H. The KEAP1-NRF2 system: a thiol-based sensor-effector apparatus for maintaining redox homeostasis. *Physiol Rev.* (2018) 98:1169–203. doi: 10.1152/physrev.00023.2017
219. Kobayashi A, Kang MI, Okawa H, Ohtsuji M, Zenke Y, Chiba T, et al. Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. *Mol Cell Biol.* (2004) 24:7130–9. doi: 10.1128/MCB.24.16.7130-7139.2004
220. Tan Y, Ichikawa T, Li J, Si Q, Yang H, Chen X, et al. Diabetic downregulation of Nrf2 activity via ERK contributes to oxidative stress-induced insulin resistance in cardiac cells *in vitro* and *in vivo*. *Diabetes.* (2011) 60:625–33. doi: 10.2337/db10-1164
221. Xing Y, Niu T, Wang W, Li J, Li S, Janicki JS, et al. Triterpenoid dihydro-CDDO-trifluoroethyl amide protects against maladaptive cardiac remodeling and dysfunction in mice: a critical role of Nrf2. *PLoS One.* (2012) 7:e44899. doi: 10.1371/journal.pone.0044899
222. Li J, Ma W, Yue G, Tang Y, Kim I-M, Weintraub NL, et al. Cardiac proteasome functional insufficiency plays a pathogenic role in diabetic cardiomyopathy. *J Mol Cell Cardiol.* (2017) 102:53–60. doi: 10.1016/j.yjmcc.2016.11.013
223. Powell SR, Samuel SM, Wang P, Divald A, Thirunavukkarasu M, Koneru S, et al. Upregulation of myocardial 11S-activated proteasome in experimental hyperglycemia. *J Mol Cell Cardiol.* (2008) 44:618–21. doi: 10.1016/j.yjmcc.2007.12.009
224. Smith DM, Fraga H, Reis C, Kafri G, Goldberg AL. ATP binds to proteasomal ATPases in pairs with distinct functional effects, implying an ordered reaction cycle. *Cell.* (2011) 144:526–38. doi: 10.1016/j.cell.2011.02.005
225. Kabakov AE, Budagova KR, Latchman DS, Kampinga HH. Stressful preconditioning and HSP70 overexpression attenuate proteotoxicity of cellular ATP depletion. *Am J Physiol Cell Physiol.* (2002) 283:C521–34. doi: 10.1152/ajpcell.00503.2001
226. Boudina S, Abel E. Mitochondrial uncoupling: a key contributor to reduced cardiac efficiency in diabetes. *Physiology.* (2006) 21:250–8. doi: 10.1152/physiol.00008.2006
227. Boudina S, Sena S, Theobald H, Sheng X, Wright JJ, Hu XX, et al. Mitochondrial energetics in the heart in obesity-related diabetes: direct evidence for increased uncoupled respiration and activation of uncoupling proteins. *Diabetes.* (2007) 56:2457–66. doi: 10.2337/db07-0481
228. Boudina TS, Sena ES, O'neill DB, Tathireddy DP, Young DM, Abel DE. Reduced mitochondrial oxidative capacity and increased mitochondrial uncoupling impair myocardial energetics in obesity. *Circulation.* (2005) 112:2686–95. doi: 10.1161/CIRCULATIONAHA.105.554360
229. Aiken CT, Kaake RM, Wang X, Huang L. Oxidative stress-mediated regulation of proteasome complexes. *Mol Cell Proteomics.* (2011) 10:R110.006924. doi: 10.1074/mcp.M110.006924
230. Divald A, Kivity S, Wang P, Hochhauser E, Roberts B, Teichberg S, et al. Myocardial ischemic preconditioning preserves postischemic function of the 26S proteasome through diminished oxidative damage to 19S regulatory particle subunits. *Circ Res.* (2010) 106:1829–38. doi: 10.1161/CIRCRESAHA.110.219485
231. Bulteau AL, Szweda LI, Friguet B. Age-dependent declines in proteasome activity in the heart. *Arch Biochem Biophys.* (2002) 397:298–304. doi: 10.1006/abbi.2001.2663
232. Ghosh R, Hwang SM, Cui Z, Gilda JE, Gomes AV. Different effects of the nonsteroidal anti-inflammatory drugs meclofenamate sodium and naproxen sodium on proteasome activity in cardiac cells. *J Mol Cell Cardiol.* (2016) 94:131–44. doi: 10.1016/j.yjmcc.2016.03.016
233. Campos JC, Queliconi BB, Dourado PM, Cunha TF, Zambelli VO, Bechara LR, et al. Exercise training restores cardiac protein quality control in heart failure. *PLoS One.* (2012) 7:e52764. doi: 10.1371/journal.pone.0052764
234. Bouchard RA, Bose D. Influence of experimental diabetes on sarcoplasmic reticulum function in rat ventricular muscle. *Am J Physiol.* (1991) 260(2 Pt 2):H341–54. doi: 10.1152/ajpheart.1991.260.2.H341
235. Lagadic-Gossmann D, Buckler KJ, Le Prigent K, Feuvray D. Altered $Ca2^+$ handling in ventricular myocytes isolated from diabetic rats. *Am J Physiol.* (1996) 270(5 Pt 2):H1529–H37. doi: 10.1152/ajpheart.1996.270.5.H1529
236. Shen X, Valencia CA, Szostak JW, Dong B, Liu R. Scanning the human proteome for calmodulin-binding proteins. *Proc Natl Acad Sci U S A.* (2005) 102:5969–74. doi: 10.1073/pnas.0407928102
237. Cui Z, Scruggs SB, Gilda JE, Ping P, Gomes AV. Regulation of cardiac proteasomes by ubiquitination, SUMOylation, and beyond. *J Mol Cell Cardiol.* (2014) 71:32–42. doi: 10.1016/j.yjmcc.2013.10.008
238. Ferreira JC, Boer BN, Grinberg M, Brum PC, Mochly-Rosen D. Protein quality control disruption by PKCbetaII in heart failure; rescue by the selective PKCbetaII inhibitor, betaIIV5-3. *PLoS One.* (2012) 7:e33175. doi: 10.1371/journal.pone.0033175
239. Wang X, Li J, Zheng H, Su H, Powell SR. Proteasome functional insufficiency in cardiac pathogenesis. *Am J Physiol Heart Circ Physiol.* (2011) 301:H2207–19. doi: 10.1152/ajpheart.00714.2011
240. Hnia K, Clausen T, Moog-Lutz C. Shaping striated muscles with ubiquitin proteasome system in health and disease. *Trends Mol Med.* (2019) 25:760–74. doi: 10.1016/j.molmed.2019.05.008
241. Tang M, Li J, Huang W, Su H, Liang Q, Tian Z, et al. Proteasome functional insufficiency activates the calcineurin-NFAT pathway in cardiomyocytes and promotes maladaptive remodelling of stressed mouse hearts. *Cardiovasc Res.* (2010) 88:424–33. doi: 10.1093/cvr/cvq217
242. Wilkins BJ, Molkentin JD. Calcium-calcineurin signaling in the regulation of cardiac hypertrophy. *Biochem Biophys Res Commun.* (2004) 322:1178–91. doi: 10.1016/j.bbrc.2004.07.121
243. Dong X, Liu J, Zheng H, Glasford JW, Huang W, Chen QH, et al. In situ dynamically monitoring the proteolytic function of the ubiquitin-proteasome system in cultured cardiac myocytes. *Am J Physiol Heart Circ Physiol.* (2004) 287:H1417–25. doi: 10.1152/ajpheart.01233.2003
244. Tsukamoto O, Minamino T, Okada K, Shintani Y, Takashima S, Kato H, et al. Depression of proteasome activities during the progression of cardiac dysfunction in pressure-overloaded heart of mice. *Biochem Biophys Res Commun.* (2006) 340:1125–33. doi: 10.1016/j.bbrc.2005.12.120

245. Ding W-X, Yin X-M. Sorting, recognition and activation of the misfolded protein degradation pathways through macroautophagy and the proteasome. *Autophagy.* (2008) 4:141–50. doi: 10.4161/auto.5190
246. Tannous P, Zhu H, Nemchenko A, Berry JM, Johnstone JL, Shelton JM, et al. Intracellular protein aggregation is a proximal trigger of cardiomyocyte autophagy. *Circulation.* (2008) 117:3070–8. doi: 10.1161/CIRCULATIONAHA.107.763870
247. Zhang T, Shen S, Qu J, Ghaemmaghami S. Global analysis of cellular protein flux quantifies the selectivity of basal autophagy. *Cell Rep.* (2016) 14:2426–39. doi: 10.1016/j.celrep.2016.02.040
248. Zheng Q, Su H, Tian Z, Wang X. Proteasome malfunction activates macroautophagy in the heart. *Am J Cardiovasc Dis.* (2011) 1:214–26.
249. Orogo AM, Gustafsson ÅB. Therapeutic targeting of autophagy. *Circ Res.* (2015) 116:489–503. doi: 10.1161/CIRCRESAHA.116.303791
250. Kirchner P, Bourdenx M, Madrigal-Matute J, Tiano S, Diaz A, Bartholdy BA, et al. Proteome-wide analysis of chaperone-mediated autophagy targeting motifs. *PLOS Biol.* (2019) 17:e3000301. doi: 10.1371/journal.pbio.3000301
251. Anding AL, Baehrecke EH. Cleaning house: selective autophagy of organelles. *Dev Cell.* (2017) 41:10–22. doi: 10.1016/j.devcel.2017.02.016
252. Kraft C, Peter M, Hofmann K. Selective autophagy: ubiquitin-mediated recognition and beyond. *Nat Cell Biol.* (2010) 12:836–41. doi: 10.1038/ncb0910-836
253. Shaid S, Brandts CH, Serve H, Dikic I. Ubiquitination and selective autophagy. *Cell Death Differ.* (2013) 20:21–30. doi: 10.1038/cdd.2012.72
254. Zientara-Rytter K, Subramani S. The roles of ubiquitin-binding protein shuttles in the degradative fate of ubiquitinated proteins in the ubiquitin-proteasome system and autophagy. *Cells.* (2019) 8:40. doi: 10.3390/cells8010040
255. Dikic I, Elazar Z. Mechanism and medical implications of mammalian autophagy. *Nat Rev Mol Cell Biol.* (2018) 19:349–64. doi: 10.1038/s41580-018-0003-4
256. Bandyopadhyay U, Kaushik S, Varticovski L, Cuervo AM. The chaperone-mediated autophagy receptor organizes in dynamic protein complexes at the lysosomal membrane. *Mol Cell Biol.* (2008) 28:5747–63. doi: 10.1128/MCB.02070-07
257. Sahu R, Kaushik S, Clement CC, Cannizzo ES, Scharf B, Follenzi A, et al. Microautophagy of cytosolic proteins by late endosomes. *Dev Cell.* (2011) 20:131–9. doi: 10.1016/j.devcel.2010.12.003
258. Arndt V, Dick N, Tawo R, Dreiseidler M, Wenzel D, Hesse M, et al. Chaperone-assisted selective autophagy is essential for muscle maintenance. *Curr Biol.* (2010) 20:143–8. doi: 10.1016/j.cub.2009.11.022
259. Stürner E, Behl C. The role of the multifunctional BAG3 protein in cellular protein quality control and in disease. *Front Mol Neurosci.* (2017) 10:177. doi: 10.3389/fnmol.2017.00177
260. Kaushik S, Cuervo AM. Chaperones in autophagy. *Pharmacol Res.* (2012) 66:484–93. doi: 10.1016/j.phrs.2012.10.002
261. D'Souza RS, Levandowski C, Slavov D, Graw SL, Allen LA, Adler E, et al. Danon disease. *Circulation.* (2014) 7:843–9. doi: 10.1161/CIRCHEARTFAILURE.114.001105
262. Cullup T, Kho AL, Dionisi-Vici C, Brandmeier B, Smith F, Urry Z, et al. Recessive mutations in EPG5 cause Vici syndrome, a multisystem disorder with defective autophagy. *Nat Genet.* (2013) 45:83–7. doi: 10.1038/ng.2497
263. Muhammad E, Levitas A, Singh SR, Braiman A, Ofir R, Etzion S, et al. PLEKHM2 mutation leads to abnormal localization of lysosomes, impaired autophagy flux and associates with recessive dilated cardiomyopathy and left ventricular noncompaction. *Hum Mol Genet.* (2015) 24:7227–40. doi: 10.1093/hmg/ddv423
264. Norton N, Li D, Mark, Jill, Rampersaud E, Züchner S, et al. Genome-wide studies of copy number variation and exome sequencing identify rare variants in BAG3 as a cause of dilated cardiomyopathy. *Am J Hum Genet.* (2011) 88:273–82. doi: 10.1016/j.ajhg.2011.01.016
265. Villard E, Perret C, Gary F, Proust C, Dilanian G, Hengstenberg C, et al. A genome-wide association study identifies two loci associated with heart failure due to dilated cardiomyopathy. *Eur Heart J.* (2011) 32:1065–76. doi: 10.1093/eurheartj/ehr105
266. Inomata Y, Nagasaka S, Miyate K, Goto Y, Hino C, Toukairin C, et al. Bcl-2-associated athanogene 3 (BAG3) is an enhancer of small heat shock protein turnover via activation of autophagy in the heart. *Biochem Biophys Res Commun.* (2018) 496:1141–7. doi: 10.1016/j.bbrc.2018.01.158
267. Bhuiyan MS, Pattison JS, Osinska H, James J, Gulick J, McLendon PM, et al. Enhanced autophagy ameliorates cardiac proteinopathy. *J Clin Invest.* (2013) 123:5284–97. doi: 10.1172/JCI70877
268. Taneike M, Yamaguchi O, Nakai A, Hikoso S, Takeda T, Mizote I, et al. Inhibition of autophagy in the heart induces age-related cardiomyopathy. *Autophagy.* (2010) 6:600–6. doi: 10.4161/auto.6.5.11947
269. Nakai A, Yamaguchi O, Takeda T, Higuchi Y, Hikoso S, Taniike M, et al. The role of autophagy in cardiomyocytes in the basal state and in response to hemodynamic stress. *Nat Med.* (2007) 13:619–24. doi: 10.1038/nm1574
270. Pyo J-O, Yoo S-M, Ahn H-H, Nah J, Hong S-H, Kam T-I, et al. Overexpression of Atg5 in mice activates autophagy and extends lifespan. *Nat Commun.* (2013) 4:2300. doi: 10.1038/ncomms3300
271. Fernández ÁF, Sebti S, Wei Y, Zou Z, Shi M, McMillan KL, et al. Disruption of the beclin 1-BCL2 autophagy regulatory complex promotes longevity in mice. *Nature.* (2018) 558:136–40. doi: 10.1038/s41586-018-0162-7
272. Godar RJ, Ma X, Liu H, Murphy JT, Weinheimer CJ, Kovacs A, et al. Repetitive stimulation of autophagy-lysosome machinery by intermittent fasting preconditions the myocardium to ischemia-reperfusion injury. *Autophagy.* (2015) 11:1537–60. doi: 10.1080/15548627.2015.1063768
273. Wohlgemuth SE, Julian D, Akin DE, Fried J, Toscano K, Leeuwenburgh C, et al. Autophagy in the heart and liver during normal aging and calorie restriction. *Rejuvenation Res.* (2004) 10:281–92. doi: 10.1089/rej.2006.0535
274. Li H, Miao W, Ma J, Xv Z, Bo H, Li J, et al. Acute exercise-induced mitochondrial stress triggers an inflammatory response in the myocardium via NLRP3 inflammasome activation with mitophagy. *Oxid Med Cell Longev.* (2016) 2016:1–11. doi: 10.1155/2016/1987149
275. Kuma A, Hatano M, Matsui M, Yamamoto A, Nakaya H, Yoshimori T, et al. The role of autophagy during the early neonatal starvation period. *Nature.* (2004) 432:1032–6. doi: 10.1038/nature03029
276. Komatsu M, Waguri S, Ueno T, Iwata J, Murata S, Tanida I, et al. Impairment of starvation-induced and constitutive autophagy in Atg7-deficient mice. *J Cell Biol.* (2005) 169:425–34. doi: 10.1083/jcb.200412022
277. Yan Z, Kronemberger A, Blomme J, Call JA, Caster HM, Pereira RO, et al. Exercise leads to unfavourable cardiac remodelling and enhanced metabolic homeostasis in obese mice with cardiac and skeletal muscle autophagy deficiency. *Sci Rep.* (2017) 7:7894. doi: 10.1038/s41598-017-08480-2
278. Hariharan N, Maejima Y, Nakae J, Paik J, Depinho RA, Sadoshima J. Deacetylation of FOXO by SIRT1 plays an essential role in mediating starvation-induced autophagy in cardiac myocytes. *Circ Res.* (2010) 107:1470–82. doi: 10.1161/CIRCRESAHA.110.227371
279. Riehle C, Wende AR, Sena S, Pires KM, Pereira RO, Zhu Y, et al. Insulin receptor substrate signaling suppresses neonatal autophagy in the heart. *J Clin Invest.* (2013) 123:5319–33. doi: 10.1172/JCI71171
280. Russell RC, Yuan H-X, Guan K-L. Autophagy regulation by nutrient signaling. *Cell Research.* (2014) 24:42–57. doi: 10.1038/cr.2013.166
281. He C, Bassik MC, Moresi V, Sun K, Wei Y, Zou Z, et al. Exercise-induced BCL2-regulated autophagy is required for muscle glucose homeostasis. *Nature.* (2012) 481:511–5. doi: 10.1038/nature10758
282. Tam BT, Pei XM, Yung BY, Yip SP, Chan LW, Wong CS, et al. Autophagic adaptations to long-term habitual exercise in cardiac muscle. *Int J Sports Med.* (2015) 36:526–34. doi: 10.1055/s-0034-1398494
283. Campos JC, Queliconi BB, Bozi LHM, Bechara LRG, Dourado PMM, Andres AM, et al. Exercise reestablishes autophagic flux and mitochondrial quality control in heart failure. *Autophagy.* (2017) 13:1304–17. doi: 10.1080/15548627.2017.1325062
284. Chen CY, Hsu HC, Lee BC, Lin HJ, Chen YH, Huang HC, et al. Exercise training improves cardiac function in infarcted rabbits: involvement of autophagic function and fatty acid utilization. *Eur J Heart Fail.* (2010) 12:323–30. doi: 10.1093/eurjhf/hfq028
285. Saito T, Asai K, Sato S, Hayashi M, Adachi A, Sasaki Y, et al. Autophagic vacuoles in cardiomyocytes of dilated cardiomyopathy with initially decompensated heart failure predict improved prognosis. *Autophagy.* (2016) 12:579–87. doi: 10.1080/15548627.2016.1145326
286. Matsui Y, Takagi H, Qu X, Abdellatif M, Sakoda H, Asano T, et al. Distinct roles of autophagy in the heart during ischemia and reperfusion. *Circ Res.* (2007) 100:914–22. doi: 10.1161/01.RES.0000261924.76669.36

287. Yan L, Vatner DE, Kim SJ, Ge H, Masurekar M, Massover WH, et al. Autophagy in chronically ischemic myocardium. *Proc Natl Acad Sci U S A.* (2005) 102:13807–12. doi: 10.1073/pnas.0506843102
288. Kanamori H, Takemura G, Goto K, Maruyama R, Ono K, Nagao K, et al. Autophagy limits acute myocardial infarction induced by permanent coronary artery occlusion. *Am J Physiol Heart Circ Physiol.* (2011) 300:H2261–H71. doi: 10.1152/ajpheart.01056.2010
289. Sciarretta S, Yee D, Nagarajan N, Bianchi F, Saito T, Valenti V, et al. Trehalose-induced activation of autophagy improves cardiac remodeling after myocardial infarction. *J Am Coll Cardiol.* (2018) 71:1999–2010. doi: 10.1016/j.jacc.2018.02.066
290. Ma X, Liu H, Foyil SR, Godar RJ, Weinheimer CJ, Hill JA, et al. Impaired autophagosome clearance contributes to cardiomyocyte death in ischemia/reperfusion injury. *Circulation.* (2012) 125:3170–81. doi: 10.1161/CIRCULATIONAHA.111.041814
291. Zhu H, Tannous P, Johnstone JL, Kong Y, Shelton JM, Richardson JA, et al. Cardiac autophagy is a maladaptive response to hemodynamic stress. *J Clin Invest.* (2007) 117:1782–93. doi: 10.1172/JCI27523
292. Zhong Y, Wang QJ, Li X, Yan Y, Backer JM, Chait BT, et al. Distinct regulation of autophagic activity by Atg14L and Rubicon associated with Beclin 1-phosphatidylinositol-3-kinase complex. *Nat Cell Biol.* (2009) 11:468–76. doi: 10.1038/ncb1854
293. Nah J, Zhai P, Huang C-Y, Fernández ÁF, Mareedu S, Levine B, et al. Upregulation of Rubicon promotes autosis during myocardial ischemia/reperfusion injury. *J Clin Invest.* (2020) 130:2978–991. doi: 10.1172/JCI132366
294. Wiersma M, Meijering RAM, Qi XY, Zhang D, Liu T, Hoogstra-Berends F, et al. Endoplasmic reticulum stress is associated with autophagy and cardiomyocyte remodeling in experimental and human atrial fibrillation. *J Am Heart Assoc.* (2017) 6:e006458. doi: 10.1161/JAHA.117.006458
295. Yuan Y, Zhao J, Gong Y, Wang D, Wang X, Yun F, et al. Autophagy exacerbates electrical remodeling in atrial fibrillation by ubiquitin-dependent degradation of L-type calcium channel. *Cell Death Dis.* (2018) 9:873. doi: 10.1038/s41419-018-0860-y
296. Tong M, Saito T, Zhai P, Oka S-I, Mizushima W, Nakamura M, et al. Mitophagy is essential for maintaining cardiac function during high fat diet-induced diabetic cardiomyopathy. *Circ Res.* (2019) 124:1360–71. doi: 10.1161/CIRCRESAHA.118.314607
297. An M, Ryu D-R, Won Park J, Ha Choi J, Park E-M, Eun Lee K, et al. ULK1 prevents cardiac dysfunction in obesity through autophagy-meditated regulation of lipid metabolism. *Cardiovasc Res.* (2017) 113:1137–47. doi: 10.1093/cvr/cvx064
298. Cao L, Qin X, Peterson MR, Haller SE, Wilson KA, Hu N, et al. CARD9 knockout ameliorates myocardial dysfunction associated with high fat diet-induced obesity. *J Mol Cell Cardiol.* (2016) 92:185–95. doi: 10.1016/j.yjmcc.2016.02.014
299. Sciarretta S, Zhai P, Shao D, Maejima Y, Robbins J, Volpe M, et al. Rheb is a critical regulator of autophagy during myocardial ischemia. *Circulation.* (2012) 125:1134–46. doi: 10.1161/CIRCULATIONAHA.111.078212
300. Kanamori H, Takemura G, Goto K, Tsujimoto A, Mikami A, Ogino A, et al. Autophagic adaptations in diabetic cardiomyopathy differ between type 1 and type 2 diabetes. *Autophagy.* (2015) 11:1146–60. doi: 10.1080/15548627.2015.1051295
301. Xu X, Hua Y, Sreejayan N, Zhang Y, Ren J. Akt2 knockout preserves cardiac function in high-fat diet-induced obesity by rescuing cardiac autophagosome maturation. *J Mol Cell Biol.* (2013) 5:61–3. doi: 10.1093/jmcb/mjs055
302. Guo R, Zhang Y, Turdi S, Ren J. Adiponectin knockout accentuates high fat diet-induced obesity and cardiac dysfunction: Role of autophagy. *Biochim Biophys Acta.* (2013) 1832:1136–48. doi: 10.1016/j.bbadis.2013.03.013
303. Hu N, Zhang Y. TLR4 knockout attenuated high fat diet-induced cardiac dysfunction via NF-κB/JNK-dependent activation of autophagy. *Biochim Biophys Acta.* (2017) 1863:2001–11. doi: 10.1016/j.bbadis.2017.01.010
304. Kandadi MR, Panzhinskiy E, Roe ND, Nair S, Hu D, Sun A. Deletion of protein tyrosine phosphatase 1B rescues against myocardial anomalies in high fat diet-induced obesity: role of AMPK-dependent autophagy. *Biochim Biophys Acta.* (2015) 1852:299–309. doi: 10.1016/j.bbadis.2014.07.004
305. Xu X, Ren J. Macrophage migration inhibitory factor (MIF) knockout preserves cardiac homeostasis through alleviating Akt-mediated myocardial autophagy suppression in high-fat diet-induced obesity. *Int J Obesity.* (2015) 39:387–96. doi: 10.1038/ijo.2014.174
306. Munasinghe PE, Riu F, Dixit P, Edamatsu M, Saxena P, Hamer NSJ, et al. Type-2 diabetes increases autophagy in the human heart through promotion of Beclin-1 mediated pathway. *Int J Cardiol.* (2016) 202:13–20. doi: 10.1016/j.ijcard.2015.08.111
307. Mellor KM, Bell JR, Young MJ, Ritchie RH, Delbridge LMD. Myocardial autophagy activation and suppressed survival signaling is associated with insulin resistance in fructose-fed mice. *J Mol Cell Cardiol.* (2011) 50:1035–43. doi: 10.1016/j.yjmcc.2011.03.002
308. Despa S, Margulies KB, Chen L, Knowlton AA, Havel PJ, Taegtmeyer H, et al. Hyperamylinemia contributes to cardiac dysfunction in obesity and diabetes: a study in humans and rats. *Circ Res.* (2012) 110:598–608. doi: 10.1161/CIRCRESAHA.111.258285
309. Rivera JF, Gurlo T, Daval M, Huang CJ, Matveyenko AV, Butler PC, et al. Human-IAPP disrupts the autophagy/lysosomal pathway in pancreatic β-cells: protective role of p62-positive cytoplasmic inclusions. *Cell Death Differ.* (2011) 18:415–26. doi: 10.1038/cdd.2010.111
310. Shigihara N, Fukunaka A, Hara A, Komiya K, Honda A, Uchida T, et al. Human IAPP-induced pancreatic β cell toxicity and its regulation by autophagy. *J Clin Invest.* (2014) 124:3634–44. doi: 10.1172/JCI69866
311. Eguchi M, Kim YH, Kang KW, Shim CY, Jang Y, Dorval T, et al. Ischemia-reperfusion injury leads to distinct temporal cardiac remodeling in normal versus diabetic mice. *PLoS One.* (2012) 7:e30450. doi: 10.1371/journal.pone.0030450
312. Xu X, Kobayashi S, Chen K, Timm D, Volden P, Huang Y, et al. Diminished autophagy limits cardiac injury in mouse models of type 1 diabetes. *J Biol Chem.* (2013) 288:18077–92. doi: 10.1074/jbc.M113.474650
313. Nishida Y, Arakawa S, Fujitani K, Yamaguchi H, Mizuta T, Kanaseki T, et al. Discovery of Atg5/Atg7-independent alternative macroautophagy. *Nature.* (2009) 461:654–8. doi: 10.1038/nature08455
314. Zhang M, Zhang L, Hu J, Lin J, Wang T, Duan Y, et al. MST1 coordinately regulates autophagy and apoptosis in diabetic cardiomyopathy in mice. *Diabetologia.* (2016) 59:2435–47. doi: 10.1007/s00125-016-4070-9
315. Xie Z, Lau K, Eby B, Lozano P, He C, Pennington B, et al. Improvement of cardiac functions by chronic metformin treatment is associated with enhanced cardiac autophagy in diabetic OVE26 mice. *Diabetes.* (2011) 60:1770–8. doi: 10.2337/db10-0351
316. He C, Zhu H, Li H, Zou MH, Xie Z. Dissociation of Bcl-2-Beclin1 complex by activated AMPK enhances cardiac autophagy and protects against cardiomyocyte apoptosis in diabetes. *Diabetes.* (2013) 62:1270–81. doi: 10.2337/db12-0533
317. Guo Y, Yu W, Sun D, Wang J, Li C, Zhang R, et al. A novel protective mechanism for mitochondrial aldehyde dehydrogenase (ALDH2) in type i diabetes-induced cardiac dysfunction: role of AMPK-regulated autophagy. (2015) 1852:319–31. doi: 10.1016/j.bbadis.2014.05.017
318. Liang L, Shou X-L, Zhao H-K, Ren G-Q, Wang J-B, Wang X-H, et al. Antioxidant catalase rescues against high fat diet-induced cardiac dysfunction via an IKKβ-AMPK-dependent regulation of autophagy. *Biochim Biophys Acta.* (2015) 1852:343–52. doi: 10.1016/j.bbadis.2014.06.027
319. Kim J, Kundu M, Viollet B, Guan K-L. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol.* (2011) 13:132–41. doi: 10.1038/ncb2152
320. Kobayashi S, Xu X, Chen K, Liang Q. Suppression of autophagy is protective in high glucose-induced cardiomyocyte injury. *Autophagy.* (2012) 8:577–92. doi: 10.4161/auto.18980
321. Yang M, Liu E, Tang L, Lei Y, Sun X, Hu J, et al. Emerging roles and regulation of MiT/TFE transcriptional factors. *Cell Commun Signal.* (2018) 16:31. doi: 10.1186/s12964-018-0242-1
322. Trivedi PC, Bartlett JJ, Perez LJ, Brunt KR, Legare JF, Hassan A, et al. Glucolipotoxicity diminishes cardiomyocyte TFEB and inhibits lysosomal autophagy during obesity and diabetes. *Biochim Biophys Acta.* (2016) 1861:1893–910. doi: 10.1016/j.bbalip.2016.09.004
323. Pires KM, Buffolo M, Schaaf C, David Symons J, Cox J, Abel ED, et al. Activation of IGF-1 receptors and Akt signaling by systemic hyperinsulinemia contributes to cardiac hypertrophy but does not regulate cardiac autophagy in obese diabetic mice. *J Mol Cell Cardiol.* (2017) 113:39–50. doi: 10.1016/j.yjmcc.2017.10.001

324. Yu W, Gao B, Li N, Wang J, Qiu C, Zhang G, et al. Sirt3 deficiency exacerbates diabetic cardiac dysfunction: role of Foxo3A-Parkin-mediated mitophagy. *Biochim Biophys Acta.* (2017) 1863:1973–83. doi: 10.1016/j.bbadis.2016.10.021
325. Wang S, Wang C, Turdi S, Richmond KL, Zhang Y, Ren J. ALDH2 protects against high fat diet-induced obesity cardiomyopathy and defective autophagy: role of CaM kinase II, histone H3K9 methyltransferase SUV39H, Sirt1, and PGC-1α deacetylation. *Int J Obes.* (2018) 42:1073–87. doi: 10.1038/s41366-018-0030-4
326. Zhang M, Wang S, Cheng Z, Xiong Z, Lv J, Yang Z, et al. Polydatin ameliorates diabetic cardiomyopathy via Sirt3 activation. *Biochem Biophys Res Commun.* (2017) 493:1280–7. doi: 10.1016/j.bbrc.2017.09.151
327. Wang B, Yang Q, Sun Y-Y, Xing Y-F, Wang Y-B, Lu X-T, et al. Resveratrol-enhanced autophagic flux ameliorates myocardial oxidative stress injury in diabetic mice. *J Cell Mol Med.* (2014) 18:1599–611. doi: 10.1111/jcmm.12312
328. Costantino S, Paneni F, Lüscher TF, Cosentino F. MicroRNA profiling unveils hyperglycaemic memory in the diabetic heart. *Eur Heart J.* (2016) 37:572–6. doi: 10.1093/eurheartj/ehv599
329. Ucar A, Gupta SK, Fiedler J, Erikci E, Kardasinski M, Batkai S, et al. The miRNA-212/132 family regulates both cardiac hypertrophy and cardiomyocyte autophagy. *Nat Commun.* (2012) 3:1078. doi: 10.1038/ncomms2090
330. Su M, Wang J, Wang C, Wang X, Dong W, Qiu W, et al. MicroRNA-221 inhibits autophagy and promotes heart failure by modulating the p27/CDK2/mTOR axis. *Cell Death Differ.* (2015) 22:986–99. doi: 10.1038/cdd.2014.187
331. Li X, Du N, Zhang Q, Li J, Chen X, Liu X, et al. MicroRNA-30d regulates cardiomyocyte pyroptosis by directly targeting foxo3a in diabetic cardiomyopathy. *Cell Death Dis.* (2014) 5:e1479. doi: 10.1038/cddis.2014.430
332. Chen C, Yang S, Li H, Yin Z, Fan J, Zhao Y, et al. Mir30c is involved in diabetic cardiomyopathy through regulation of cardiac autophagy via BECN1. *Mol Ther Nucleic Acids.* (2017) 7:127–39. doi: 10.1016/j.omtn.2017.03.005
333. Zhuo C, Jiang R, Lin X, Shao M. LncRNA H19 inhibits autophagy by epigenetically silencing of DIRAS3 in diabetic cardiomyopathy. *Oncotarget.* (2017) 8:1429–37. doi: 10.18632/oncotarget.13637
334. Morciano G, Patergnani S, Bonora M, Pedriali G, Tarocco A, Bouhamida E, et al. Mitophagy in cardiovascular diseases. *J Clin Med.* (2020) 9:892. doi: 10.3390/jcm9030892
335. Billia F, Hauck L, Konecny F, Rao V, Shen J, Mak TW. PTEN-inducible kinase 1 (PINK1)/Park6 is indispensable for normal heart function. *Proc Natl Acad Sci U S A.* (2011) 108:9572–7. doi: 10.1073/pnas.1106291108
336. Tong M, Saito T, Zhai P, Oka S, Sadoshima J. ULK1-dependent mitophagy is essential for maintaining cardiac function during high fat diet-induced diabetic cardiomyopathy. *FASEB J.* (2019) 33:660.13. doi: 10.1096/fasebj.2019.33.1_supplement.660.13
337. Wang S, Zhao Z, Feng X, Cheng Z, Xiong Z, Wang T, et al. Melatonin activates Parkin translocation and rescues the impaired mitophagy activity of diabetic cardiomyopathy through Mst1 inhibition. *J Cell Mol Med.* (2018) 22:5132–44. doi: 10.1111/jcmm.13802
338. Ni T, Lin N, Huang X, Lu W, Sun Z, Zhang J, et al. Icariin ameliorates diabetic cardiomyopathy through Apelin/Sirt3 signalling to improve mitochondrial dysfunction. *Front Pharmacol.* (2020) 11:256. doi: 10.3389/fphar.2020.00256
339. Song Y, Lee W, Lee Y-H, Kang E, Cha B-S, Lee B-W. Metformin restores Parkin-mediated mitophagy, suppressed by cytosolic p53. *Int J Mol Sci.* (2016) 17:122. doi: 10.3390/ijms17010122
340. Hoshino A, Ariyoshi M, Okawa Y, Kaimoto S, Uchihashi M, Fukai K, et al. Inhibition of p53 preserves Parkin-mediated mitophagy and pancreatic β-cell function in diabetes. *Proc Natl Acad Sci U S A.* (2014) 111:3116–21. doi: 10.1073/pnas.1318951111
341. Shao D, Kolwicz Stephen C, Wang P, Roe Nathan D, Villet O, Nishi K, et al. Increasing fatty acid oxidation prevents high fat diet induced cardiomyopathy through regulating parkin mediated mitophagy. *Circulation.* (2020) 42:983–97. doi: 10.1016/j.yjmcc.2019.11.128
342. Cahill TJ, Leo V, Kelly M, Stockenhuber A, Kennedy NW, Bao L, et al. Resistance of dynamin-related protein 1 oligomers to disassembly impairs mitophagy, resulting in myocardial inflammation and heart failure. *J Biol Chem.* (2015) 290:25907–19. doi: 10.1074/jbc.M115.665695
343. Hu Q, Zhang H, Gutiérrez Cortés N, Wu D, Wang P, Zhang J, et al. Increased Drp1 acetylation by lipid overload induces cardiomyocyte death and heart dysfunction. *Circ Res.* (2020) 126:456–70. doi: 10.1161/CIRCRESAHA.119.315252
344. Hu L, Ding M, Tang D, Gao E, Li C, Wang K, et al. Targeting mitochondrial dynamics by regulating Mfn2 for therapeutic intervention in diabetic cardiomyopathy. *Theranostics.* (2019) 9:3687–706. doi: 10.7150/thno.33684
345. Xiong W, Ma Z, An D, Liu Z, Cai W, Bai Y, et al. Mitofusin 2 participates in mitophagy and mitochondrial fusion against angiotensin II-induced cardiomyocyte injury. *Front Physiol.* (2019) 10:411. doi: 10.3389/fphys.2019.00411
346. Kocaturk NM, Gozuacik D. Crosstalk between mammalian autophagy and the ubiquitin-proteasome system. *Front Cell Dev Biol.* (2018) 6:128. doi: 10.3389/fcell.2018.00128
347. Xu C, Feng K, Zhao X, Huang S, Cheng Y, Qian L, et al. Regulation of autophagy by E3 ubiquitin ligase RNF216 through BECN1 ubiquitination. *Autophagy.* (2014) 10:2239–50. doi: 10.4161/15548627.2014.981792
348. Jin S, Tian S, Chen Y, Zhang C, Xie W, Xia X, et al. USP19 modulates autophagy and antiviral immune responses by deubiquitinating Beclin-1. *EMBO J.* (2016) 35:866–80. doi: 10.15252/embj.201593596
349. Obeng EA, Carlson LM, Gutman DM, Harrington WJ, Jr., Lee KP, et al. Proteasome inhibitors induce a terminal unfolded protein response in multiple myeloma cells. *Blood.* (2006) 107:4907–16. doi: 10.1182/blood-2005-08-3531
350. Vogl DT, Stadtmauer EA, Tan K-S, Heitjan DF, Davis LE, Pontiggia L, et al. Combined autophagy and proteasome inhibition: a phase 1 trial of hydroxychloroquine and bortezomib in patients with relapsed/refractory myeloma. *Autophagy.* (2014) 10:1380–90. doi: 10.4161/auto.29264
351. Cuervo AM, Palmer A, Rivett AJ, Knecht E. Degradation of proteasomes by lysosomes in rat liver. *Eur J Biochem.* (1995) 227:792–800. doi: 10.1111/j.1432-1033.1995.tb20203.x
352. Cohen-Kaplan V, Livneh I, Avni N, Fabre B, Ziv T, Kwon YT, et al. p62- and ubiquitin-dependent stress-induced autophagy of the mammalian 26S proteasome. *Proc Natl Acad Sci U S A.* (2016) 113:E7490–E9. doi: 10.1073/pnas.1615455113
353. Sun S, Shi G, Sha H, Ji Y, Han X, Shu X, et al. IRE1α is an endogenous substrate of endoplasmic-reticulum-associated degradation. *Nat Cell Biol.* (2015) 17:1546–55. doi: 10.1038/ncb3266
354. Travers KJ, Patil CK, Wodicka L, Lockhart DJ, Weissman JS, Walter P. Functional and genomic analyses reveal an essential coordination between the unfolded protein response and ER-associated degradation. *Cell.* (2000) 101:249–58. doi: 10.1016/S0092-8674(00)80835-1
355. Yu M, Du H, Wang B, Chen J, Lu F, Peng S, et al. Exogenous H(2)S induces Hrd1 S-sulfhydration and prevents CD36 translocation via VAMP3 ubiquitylation in diabetic hearts. *Aging Dis.* (2020) 11:286–300. doi: 10.14336/AD.2019.0530
356. Fu HY, Sanada S, Matsuzaki T, Liao Y, Okuda K, Yamato M, et al. Chemical endoplasmic reticulum chaperone alleviates doxorubicin-induced cardiac dysfunction. *Circ Res.* (2016) 118:798–809. doi: 10.1161/CIRCRESAHA.115.307604
357. Zhang C, Syed TW, Liu R, Yu J. Role of endoplasmic reticulum stress, autophagy, and inflammation in cardiovascular disease. *Front Cardiovasc Med.* (2017) 4:29. doi: 10.3389/fcvm.2017.00029
358. Su H, Wang X. p62 stages an interplay between the ubiquitin-proteasome system and autophagy in the heart of defense against proteotoxic stress. *Trends Cardiovasc Med.* (2011) 21:224–8. doi: 10.1016/j.tcm.2012.05.015
359. Tian Z, Wang C, Hu C, Tian Y, Liu J, Wang X. Autophagic-lysosomal inhibition compromises ubiquitin-proteasome system performance in a p62 dependent manner in cardiomyocytes. *PLoS ONE.* (2014) 9:e100715. doi: 10.1371/journal.pone.0100715
360. Yang Q, Gao H, Dong R, Wu YQ. Sequential changes of endoplasmic reticulum stress and apoptosis in myocardial fibrosis of diabetes mellitus-induced rats. *Mol Med Rep.* (2016) 13:5037–44. doi: 10.3892/mmr.2016.5180
361. Chengji W, Xianjin F. Exercise protects against diabetic cardiomyopathy by the inhibition of the endoplasmic reticulum stress pathway in rats. *J Cell Physiol.* (2019) 234:1682–8. doi: 10.1002/jcp.27038
362. Bi X, Zhang G, Wang X, Nguyen C, May HI, Li X, et al. Endoplasmic reticulum chaperone GRP78 protects heart from

ischemia/reperfusion injury through Akt activation. *Circ Res.* (2018) 122:1545–54. doi: 10.1161/CIRCRESAHA.117.312641
363. Wang S, Wang Z, Fan Q, Guo J, Galli G, Du G, et al. Ginkgolide K protects the heart against endoplasmic reticulum stress injury by activating the inositol-requiring enzyme 1alpha/X box-binding protein-1 pathway. *Br J Pharmacol.* (2016) 173:2402–18. doi: 10.1111/bph.13516
364. Zhang G, Wang X, Gillette TG, Deng Y, Wang ZV. Unfolded protein response as a therapeutic target in cardiovascular disease. *Curr Top Med Chem.* (2019) 19:1902–17. doi: 10.2174/1568026619666190521093049
365. Quentin T, Steinmetz M, Poppe A, Thoms S. Metformin differentially activates ER stress signaling pathways without inducing apoptosis. *Dis Model Mech.* (2012) 5:259–69. doi: 10.1242/dmm.008110
366. Jung TW, Choi KM. Pharmacological modulators of endoplasmic reticulum stress in metabolic diseases. *Int J Mol Sci.* (2016) 17:192. doi: 10.3390/ijms17020192
367. DeFronzo RA, Inzucchi S, Abdul-Ghani M, Nissen SE. Pioglitazone: the forgotten, cost-effective cardioprotective drug for type 2 diabetes. *Diab Vasc Dis Res.* (2019) 16:133–43. doi: 10.1177/1479164118825376
368. Liu J, Liu Y, Chen L, Wang Y, Li J. Glucagon-Like Peptide-1 analog Liraglutide protects against diabetic cardiomyopathy by the inhibition of the endoplasmic reticulum stress pathway. *J Diabetes Res.* (2013) 2013:630537. doi: 10.1155/2013/630537
369. Dong Y, Yan S, Li GY, Wang MN, Leng L, Li Q. Identification of key candidate genes and pathways revealing the protective effect of liraglutide on diabetic cardiac muscle by integrated bioinformatics analysis. *Ann Transl Med.* (2020) 8:181. doi: 10.21037/atm.2020.01.94
370. Younce CW, Burmeister MA, Ayala JE. Exendin-4 attenuates high glucose-induced cardiomyocyte apoptosis via inhibition of endoplasmic reticulum stress and activation of SERCA2a. *Am J Physiol Cell Physiol.* (2013) 304:C508–18. doi: 10.1152/ajpcell.00248.2012
371. Ritchie RH, Abel ED. Basic mechanisms of diabetic heart disease. *Circ Res.* (2020) 126:1501–25. doi: 10.1161/CIRCRESAHA.120.315913
372. Zhou Y, Wu W. The sodium-glucose co-transporter 2 inhibitor, Empagliflozin, protects against diabetic cardiomyopathy by inhibition of the endoplasmic reticulum stress pathway. *Cell Physiol Biochem.* (2017) 41:2503–12. doi: 10.1159/000475942
373. Wu T, Dong Z, Geng J, Sun Y, Liu G, Kang W, et al. Valsartan protects against ER stress-induced myocardial apoptosis via CHOP/Puma signaling pathway in streptozotocin-induced diabetic rats. *Eur J Pharm Sci.* (2011) 42:496–502. doi: 10.1016/j.ejps.2011.02.005
374. Liu X, Xu Q, Wang X, Zhao Z, Zhang L, Zhong L, et al. Irbesartan ameliorates diabetic cardiomyopathy by regulating protein kinase D and ER stress activation in a type 2 diabetes rat model. *Pharmacol Res.* (2015) 93:43–51. doi: 10.1016/j.phrs.2015.01.001
375. Wu H, Li GN, Xie J, Li R, Chen QH, Chen JZ, et al. Resveratrol ameliorates myocardial fibrosis by inhibiting ROS/ERK/TGF-beta/periostin pathway in STZ-induced diabetic mice. *BMC Cardiovasc Disord.* (2016) 16:5. doi: 10.1186/s12872-015-0169-z
376. Tao S, Chen L, Song J, Zhu N, Song X, Shi R, et al. Tanshinone IIA ameliorates diabetic cardiomyopathy by inhibiting Grp78 and CHOP expression in STZ-induced diabetes rats. *Exp Ther Med.* (2019) 18:729–34. doi: 10.3892/etm.2019.7580
377. Hou H, Zhang Q, Dong H, Ge Z. Matrine improves diabetic cardiomyopathy through TGF-beta-induced protein kinase RNA-like endoplasmic reticulum kinase signaling pathway. *J Cell Biochem.* (2019) 120:13573–82. doi: 10.1002/jcb.28632
378. Zhao R, Xie X, Le K, Li W, Moghadasian MH, Beta T, et al. Endoplasmic reticulum stress in diabetic mouse or glycated LDL-treated endothelial cells: protective effect of Saskatoon berry powder and cyanidin glycans. *J Nutr Biochem.* (2015) 26:1248–53. doi: 10.1016/j.jnutbio.2015.05.015
379. Xiong FY, Tang ST, Su H, Tang HQ, Jiang P, Zhou Q, et al. Melatonin ameliorates myocardial apoptosis by suppressing endoplasmic reticulum stress in rats with longterm diabetic cardiomyopathy. *Mol Med Rep.* (2018) 17:374–81. doi: 10.3892/mmr.2017.7841
380. Ge CX, Xu MX, Qin YT, Gu TT, Lou DS, Li Q, et al. Endoplasmic reticulum stress-induced iRhom2 up-regulation promotes macrophage-regulated cardiac inflammation and lipid deposition in high fat diet (HFD)-challenged mice: intervention of fisetin and metformin. *Free Radic Biol Med.* (2019) 141:67–83. doi: 10.1016/j.freeradbiomed.2019.05.031
381. Ravassa S, Zudaire A, Carr RD, Diez J. Antiapoptotic effects of GLP-1 in murine HL-1 cardiomyocytes. *Am J Physiol Heart Circ Physiol.* (2011) 300:H1361–72. doi: 10.1152/ajpheart.00885.2010
382. Griffin SJ, Leaver JK, Irving GJ. Impact of metformin on cardiovascular disease: a meta-analysis of randomised trials among people with type 2 diabetes. *Diabetologia.* (2017) 60:1620–9. doi: 10.1007/s00125-017-4337-9
383. Park CS, Cha H, Kwon EJ, Sreenivasaiah PK, Kim DH. The chemical chaperone 4-phenylbutyric acid attenuates pressure-overload cardiac hypertrophy by alleviating endoplasmic reticulum stress. *Biochem Biophys Res Commun.* (2012) 421:578–84. doi: 10.1016/j.bbrc.2012.04.048
384. Ozcan U, Yilmaz E, Ozcan L, Furuhashi M, Vaillancourt E, Smith RO, et al. Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. *Science.* (2006) 313:1137–40. doi: 10.1126/science.1128294
385. Malick R. Endoplasmic reticulum stress and heart complication in diabetes. *J Diabetes Metab.* (2015) 6:12. doi: 10.4172/2155-6156.1000630
386. Zhang X, Yang L, Xu X, Tang F, Yi P, Qiu B, et al. A review of fibroblast growth factor 21 in diabetic cardiomyopathy. *Heart Fail Rev.* (2019) 24:1005–17. doi: 10.1007/s10741-019-09809-x
387. Yang H, Feng A, Lin S, Yu L, Lin X, Yan X, et al. Fibroblast growth factor-21 prevents diabetic cardiomyopathy via AMPK-mediated antioxidation and lipid-lowering effects in the heart. *Cell Death Dis.* (2018) 9:227. doi: 10.1038/s41419-018-0307-5
388. Li J, Horak KM, Su H, Sanbe A, Robbins J, Wang X. Enhancement of proteasomal function protects against cardiac proteinopathy and ischemia/reperfusion injury in mice. *J Clin Invest.* (2011) 121:3689–700. doi: 10.1172/JCI45709
389. Rajagopalan V, Zhao M, Reddy S, Fajardo G, Wang X, Dewey S, et al. Altered ubiquitin-proteasome signaling in right ventricular hypertrophy and failure. *Am J Physiol Heart Circ Physiol.* (2013) 305:H551–H62. doi: 10.1152/ajpheart.00771.2012
390. Miller CL, Yan C. Targeting cyclic nucleotide phosphodiesterase in the heart: therapeutic implications. *J Cardiovasc Transl Res.* (2010) 3:507–15. doi: 10.1007/s12265-010-9203-9
391. Ranek MJ, Terpstra EJ, Li J, Kass DA, Wang X. Protein kinase g positively regulates proteasome-mediated degradation of misfolded proteins. *Circulation.* (2013) 128:365–76. doi: 10.1161/CIRCULATIONAHA.113.001971
392. Zhang H, Pan B, Wu P, Parajuli N, Rekhter MD, Goldberg AL, et al. PDE1 inhibition facilitates proteasomal degradation of misfolded proteins and protects against cardiac proteinopathy. *Sci Adv.* (2019) 5:eaaw5870. doi: 10.1126/sciadv.aaw5870
393. Paul BD, Snyder SH. H(2)S signalling through protein sulfhydration and beyond. *Nat Rev Mol Cell Biol.* (2012) 13:499–507. doi: 10.1038/nrm3391
394. Barr LA, Shimizu Y, Lambert JP, Nicholson CK, Calvert JW. Hydrogen sulfide attenuates high fat diet-induced cardiac dysfunction via the suppression of endoplasmic reticulum stress. *Nitric Oxide.* (2015) 46:145–56. doi: 10.1016/j.niox.2014.12.013
395. Li Z, Polhemus DJ, Lefer DJ. Evolution of hydrogen sulfide therapeutics to treat cardiovascular disease. *Circ Res.* (2018) 123:590–600. doi: 10.1161/CIRCRESAHA.118.311134
396. Polhemus DJ, Li Z, Pattillo CB, Gojon G, Sr., Gojon G, et al., Giordano T, et al. A novel hydrogen sulfide prodrug, SG1002, promotes hydrogen sulfide and nitric oxide bioavailability in heart failure patients. *Cardiovasc Ther.* (2015) 33:216–26. doi: 10.1111/1755-5922.12128
397. Baskin KK, Taegtmeyer H. AMP-activated protein kinase regulates E3 ligases in rodent heart. *Circ Res.* (2011) 109:1153–61. doi: 10.1161/CIRCRESAHA.111.252742
398. Masoudi FA, Inzucchi SE, Wang Y, Havranek EP, Foody JM, Krumholz HM. Thiazolidinediones, metformin, and outcomes in older patients with diabetes and heart failure: an observational study. *Circulation.* (2005) 111:583–90. doi: 10.1161/01.CIR.0000154542.13412.B1
399. Shah DD, Fonarow GC, Horwich TB. Metformin therapy and outcomes in patients with advanced systolic heart failure and diabetes. *J Card Fail.* (2010) 16:200–6. doi: 10.1016/j.cardfail.2009.10.022

400. Wong AK, Symon R, AlZadjali MA, Ang DS, Ogston S, Choy A, et al. The effect of metformin on insulin resistance and exercise parameters in patients with heart failure. *Eur J Heart Fail.* (2012) 14:1303–10. doi: 10.1093/eurjhf/hfs106
401. Gulsin GS, Athithan L, McCann GP. Diabetic cardiomyopathy: prevalence, determinants and potential treatments. *Ther Adv Endocrinol Metab.* (2019) 10:2042018819834869. doi: 10.1177/2042018819834869
402. Liu Y, Palanivel R, Rai E, Park M, Gabor TV, Scheid MP, et al. Adiponectin stimulates autophagy and reduces oxidative stress to enhance insulin sensitivity during high-fat diet feeding in mice. *Diabetes.* (2015) 64:36–48. doi: 10.2337/db14-0267
403. Malik SA, Mariño G, Benyounès A, Shen S, Harper F, Maiuri MC, et al. Neuroendocrine regulation of autophagy by leptin. *Cell Cycle.* (2011) 10:2917–23. doi: 10.4161/cc.10.17.17067
404. Yu W, Zha W, Ren J. Exendin-4 and liraglutide attenuate glucose toxicity-induced cardiac injury through mTOR/ULK1-dependent autophagy. *Oxid Med Cell Longev.* (2018) 2018:5396806. doi: 10.1155/2018/5396806
405. Margulies KB, Anstrom KJ, Hernandez AF, Redfield MM, Shah MR, Braunwald E, et al. GLP-1 agonist therapy for advanced heart failure with reduced ejection fraction. *Circ Heart Failure.* (2014) 7:673–9. doi: 10.1161/CIRCHEARTFAILURE.114.000346
406. McGaffin KR, Sun C-K, Rager JJ, Romano LC, Zou B, Mathier MA, et al. Leptin signalling reduces the severity of cardiac dysfunction and remodelling after chronic ischaemic injury. *Cardiovasc Res.* (2008) 77:54–63. doi: 10.1093/cvr/cvm023
407. Johnson JA, Majumdar SR, Simpson SH, Toth EL. Decreased mortality associated with the use of metformin compared with dulfonylurea monotherapy in type 2 diabetes. *Diabetes Care.* (2002) 25:2244–8. doi: 10.2337/diacare.25.12.2244
408. Rovira J, Marcelo Arellano E, Burke JT, Brault Y, Moya-Rull D, Bañón-Maneus E, et al. Effect of mTOR inhibitor on body weight: from an experimental rat model to human transplant patients. *Transpl Int.* (2008) 21:992–8. doi: 10.1111/j.1432-2277.2008.00710.x
409. Das A, Durrant D, Koka S, Salloum FN, Xi L, Kukreja RC. Mammalian target of rapamycin (mTOR) inhibition with rapamycin improves cardiac function in type 2 diabetic mice. *J Biol Chem.* (2014) 289:4145–60. doi: 10.1074/jbc.M113.521062
410. Hsiao P-J, Chiou H-YC, Jiang H-J, Lee M-Y, Hsieh T-J, Kuo K-K. Pioglitazone enhances cytosolic lipolysis, β-oxidation and autophagy to ameliorate hepatic steatosis. *Sci Rep.* (2017) 7:9030. doi: 10.1038/s41598-017-09702-3
411. Elrashidy RA, Asker ME, Mohamed HE. Beneficial effects of pioglitazone against cardiovascular injury are enhanced by combination with aliskiren in a rat model of diabetic nephropathy. *J Pharm Pharmacol.* (2012) 64:862–71. doi: 10.1111/j.2042-7158.2012.01508.x
412. Bhandari U, Kumar V, Kumar P, Tripathi CD, Khanna G. Protective effect of pioglitazone on cardiomyocyte apoptosis in low-dose streptozotocin & high-fat diet-induced type-2 diabetes in rats. *Indian J Med Res.* (2015) 142:598–605. doi: 10.4103/0971-5916.171290
413. Murase H, Kuno A, Miki T, Tanno M, Yano T, Kouzu H, et al. Inhibition of DPP-4 reduces acute mortality after myocardial infarction with restoration of autophagic response in type 2 diabetic rats. *Cardiovasc Diabetol.* (2015) 14:103. doi: 10.1186/s12933-015-0264-6
414. Bostick B, Habibi J, Ma L, Aroor A, Rehmer N, Hayden MR, et al. Dipeptidyl peptidase inhibition prevents diastolic dysfunction and reduces myocardial fibrosis in a Mouse model of Western diet induced obesity. *Metabolism.* (2014) 63:1000–11. doi: 10.1016/j.metabol.2014.04.002
415. Packer M. Worsening heart failure during the use of DPP-4 inhibitors. *JACC Heart Failure.* (2018) 6:445–51. doi: 10.1016/j.jchf.2017.12.016
416. Marso SP, Bain SC, Consoli A, Eliaschewitz FG, Jódar E, Leiter LA, et al. Semaglutide and cardiovascular outcomes in patients with type 2 diabetes. *N Engl J Med.* (2016) 375:1834–44. doi: 10.1056/NEJMoa1607141
417. Marso SP, Daniels GH, Brown-Frandsen K, Kristensen P, Mann JFE, Nauck MA, et al. Liraglutide and cardiovascular outcomes in type 2 diabetes. *N Engl J Med.* (2016) 375:311–22. doi: 10.1056/NEJMoa1603827
418. Noyan-Ashraf MH, Shikatani EA, Schuiki I, Mukovozov I, Wu J, Li RK, et al. A glucagon-like peptide-1 analog reverses the molecular pathology and cardiac dysfunction of a mouse model of obesity. *Circulation.* (2013) 127:74–85. doi: 10.1161/CIRCULATIONAHA.112.091215
419. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med.* (2015) 373:2117–28. doi: 10.1056/NEJMoa1504720
420. Hammoudi N, Jeong D, Singh R, Farhat A, Komajda M, Mayoux E, et al. Empagliflozin improves left ventricular diastolic dysfunction in a genetic model of type 2 diabetes. *Cardiovasc Drugs Therapy.* (2017) 31:233–46. doi: 10.1007/s10557-017-6734-1
421. Aragón-Herrera A, Feijóo-Bandín S, Otero Santiago M, Barral L, Campos-Toimil M, Gil-Longo J, et al. Empagliflozin reduces the levels of CD36 and cardiotoxic lipids while improving autophagy in the hearts of Zucker diabetic fatty rats. *Biochem Pharmacol.* (2019) 170:113677. doi: 10.1016/j.bcp.2019.113677
422. Zhang J, Cheng Y, Gu J, Wang S, Zhou S, Wang Y, et al. Fenofibrate increases cardiac autophagy via FGF21/SIRT1 and prevents fibrosis and inflammation in the hearts of Type 1 diabetic mice. *Clin Sci.* (2016) 130:625–41. doi: 10.1042/CS20150623
423. Sabe AA, Elmadhun NY, Sadek AA, Chu LM, Bianchi C, Sellke FW. Differential effects of atorvastatin on autophagy in ischemic and nonischemic myocardium in Ossabaw swine with metabolic syndrome. *J Thorac Cardiovasc Surg.* (2014) 148:3172–8. doi: 10.1016/j.jtcvs.2014.07.104
424. Park H-W, Park H, Semple IA, Jang I, Ro S-H, Kim M, et al. Pharmacological correction of obesity-induced autophagy arrest using calcium channel blockers. *Nat Commun.* (2014) 5:4834. doi: 10.1038/ncomms5834
425. Afzal N, Ganguly PK, Dhalla KS, Pierce GN, Singal PK, Dhalla NS. Beneficial effects of verapamil in diabetic cardiomyopathy. *Diabetes.* (1988) 37:936–42. doi: 10.2337/diabetes.37.7.936
426. Brown RA, Lee MM, Sundareson AM, Woodbury DJ, Savage AO. Influence of calcium channel blocker treatment on the mechanical properties of diabetic rat myocardium. *Acta Diabetol.* (1996) 33:7–14. doi: 10.1007/BF00571933
427. Pushparaj C, Das A, Purroy R, Nàger M, Herreros J, Pamplona R, et al. Voltage-gated calcium channel blockers deregulate macroautophagy in cardiomyocytes. *Int J Biochem Cell Biol.* (2015) 68:166–75. doi: 10.1016/j.biocel.2015.09.010
428. Zhang X, Li Z-L, Crane JA, Jordan KL, Pawar AS, Textor SC, et al. Valsartan regulates myocardial autophagy and mitochondrial turnover in experimental hypertension. *Hypertension.* (2014) 64:87–93. doi: 10.1161/HYPERTENSIONAHA.113.02151
429. Guan J, Liu W-Q, Xing M-Q, Shi Y, Tan X-Y, Jiang C-Q, et al. Elevated expression of periostin in diabetic cardiomyopathy and the effect of valsartan. *BMC Cardiovasc Disord.* (2015) 15:90. doi: 10.1186/s12872-015-0084-3
430. Yang Z-h, Peng X-d. Effects of valsartan on diabetic cardiomyopathy in rats with type 2 diabetes mellitus. *Chin Med J.* (2010) 123:3640–3.
431. Brandenburg VM, Rocca H-PB-L, Marx N. Herzinsuffizienztherapie bei Patienten mit Diabetes. *Dtsch Med Wochenschr.* (2016) 141:1647–9. doi: 10.1055/s-0042-000001
432. Zhu X, Wu C, Qiu S, Yuan X, Li L. Effects of resveratrol on glucose control and insulin sensitivity in subjects with type 2 diabetes: systematic review and meta-analysis. *Nutr Metab.* (2017) 14:60. doi: 10.1186/s12986-017-0217-z
433. Sai M, Dong H, Jiangwei C, Xiujuan L, Tao G, Feng C. GW25-e3514 resveratrol alleviates diabetic cardiomyopathy through improvement of mitochondrial biogenesis and function in a SIRT1-dependent manner. *J Am Coll Cardiol.* (2014) 64:C24–C5. doi: 10.1016/j.jacc.2014.06.120
434. Timmers S, Konings E, Bilet L, Riekelt, Tineke, Gijs, et al. Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. *Cell Metab.* (2011) 14:612–22. doi: 10.1016/j.cmet.2011.10.002
435. Zordoky BNM, Robertson IM, Dyck JRB. Preclinical and clinical evidence for the role of resveratrol in the treatment of cardiovascular diseases. *Biochim Biophys Acta Mol Basis Dis.* (2015) 1852:1155–77. doi: 10.1016/j.bbadis.2014.10.016
436. Lv W, Zhang J, Jiao A, Wang B, Chen B, Lin J. Resveratrol attenuates hIAPP amyloid formation and restores the insulin secretion ability in hIAPP-INS1 cell line via enhancing autophagy. *Can J Physiol Pharmacol.* (2018) 97:82–9. doi: 10.1139/cjpp-2016-0686
437. Yan J, Yan JY, Wang YX, Ling YN, Song XD, Wang SY, et al. Spermidine-enhanced autophagic flux improves cardiac dysfunction following

myocardial infarction by targeting the AMPK/mTOR signalling pathway. *Br J Pharmacol.* (2019) 176:3126–42. doi: 10.1111/bph.14706
438. Hu J, Lu X, Zhang X, Shao X, Wang Y, Chen J, et al. Exogenous spermine attenuates myocardial fibrosis in diabetic cardiomyopathy by inhibiting endoplasmic reticulum stress and the canonical Wnt signaling pathway. *Cell Biol Int*. (2020) 44:1660–70. doi: 10.1002/cbin.11360
439. Wang Y, Chen J, Li S, Zhang X, Guo Z, Hu J, et al. Exogenous spermine attenuates rat diabetic cardiomyopathy via suppressing ROS-p53 mediated downregulation of calcium-sensitive receptor. *Redox Biol.* (2020) 32:101514. doi: 10.1016/j.redox.2020.101514
440. Eisenberg T, Abdellatif M, Schroeder S, Primessnig U, Stekovic S, Pendl T, et al. Cardioprotection and lifespan extension by the natural polyamine spermidine. *Nat Med.* (2016) 22:1428–38. doi: 10.1038/nm.4222
441. Zhou H, Chen Y, Huang S-W, Hu P-F, Tang L-J. Regulation of autophagy by tea polyphenols in diabetic cardiomyopathy. *J Zhejiang Univ Sci B.* (2018) 19:333–41. doi: 10.1631/jzus.B1700415
442. Othman AI, El-Sawi MR, El-Missiry MA, Abukhalil MH. Epigallocatechin-3-gallate protects against diabetic cardiomyopathy through modulating the cardiometabolic risk factors, oxidative stress, inflammation, cell death and fibrosis in streptozotocin-nicotinamide-induced diabetic rats. *Biomed Pharmacother.* (2017) 94:362–73. doi: 10.1016/j.biopha.2017.07.129
443. Meng J-M, Cao S-Y, Wei X-L, Gan R-Y, Wang Y-F, Cai S-X, et al. Effects and mechanisms of tea for the prevention and management of diabetes mellitus and diabetic complications: an updated review. *Antioxidants.* (2019) 8:170. doi: 10.3390/antiox8060170
444. Wu Y, Xia Z-Y, Zhao B, Leng Y, Dou J, Meng Q-T, et al. (−)-Epigallocatechin-3-gallate attenuates myocardial injury induced by ischemia/reperfusion in diabetic rats and in H9c2 cells under hyperglycemic conditions. *Int J Mol Med.* (2017) 40:389–99. doi: 10.3892/ijmm.2017.3014
445. Bocchi L, Motta BM, Savi M, Vilella R, Meraviglia V, Rizzi F, et al. The histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) restores cardiomyocyte contractility in a rat model of early diabetes. *Int J Mol Sci.* (2019) 20:1873. doi: 10.3390/ijms20081873
446. Xie M, Kong Y, Tan W, May H, Battiprolu PK, Pedrozo Z, et al. Histone deacetylase inhibition blunts ischemia/reperfusion injury by inducing cardiomyocyte autophagy. *Circulation.* (2014) 129:1139–51. doi: 10.1161/CIRCULATIONAHA.113.002416
447. Shin JH, Lim Y-H, Song Y-S, So B-I, Park J-Y, Fang C-H, et al. Granulocyte-colony stimulating factor reduces cardiomyocyte apoptosis and ameliorates diastolic dysfunction in Otsuka long-evans tokushima fatty rats. *Cardiovasc Drugs Therapy.* (2014) 28:211–20. doi: 10.1007/s10557-014-6519-8
448. Miyata S, Takemura G, Kawase Y, Li Y, Okada H, Maruyama R, et al. Autophagic cardiomyocyte death in cardiomyopathic hamsters and its prevention by granulocyte colony-stimulating factor. *Am J Pathol.* (2006) 168:386–97. doi: 10.2353/ajpath.2006.050137
449. Wasko MCM, McClure CK, Kelsey SF, Huber K, Orchard T, Toledo FGS. Antidiabetogenic effects of hydroxychloroquine on insulin sensitivity and beta cell function: a randomised trial. *Diabetologia.* (2015) 58:2336–43. doi: 10.1007/s00125-015-3689-2
450. Gerstein HC, Thorpe KE, Wayne Taylor D, Brian Haynes R. The effectiveness of hydroxychloroquine in patients with type 2 diabetes mellitus who are refractory to sulfonylureas-a randomized trial. *Diabetes Res Clin Pract.* (2002) 55:209–19. doi: 10.1016/S0168-8227(01)00325-4
451. Bourke L, McCormick J, Taylor V, Pericleous C, Blanchet B, Costedoat-Chalumeau N, et al. Hydroxychloroquine protects against cardiac ischaemia/reperfusion injury in vivo via enhancement of ERK1/2 phosphorylation. *PLoS One.* (2015) 10:e0143771. doi: 10.1371/journal.pone.0143771
452. Qian L-B, Jiang S-Z, Tang X-Q, Zhang J, Liang Y-Q, Yu H-T, et al. Exacerbation of diabetic cardiac hypertrophy in OVE26 mice by angiotensin II is associated with JNK/c-Jun/miR-221-mediated autophagy inhibition. *Oncotarget.* (2017) 8:106661–71. doi: 10.18632/oncotarget.21302
453. Yoshino J, Conte C, Fontana L, Mittendorfer B, Imai S-I, Kenneth, et al. Resveratrol supplementation does not improve metabolic function in nonobese women with normal glucose tolerance. *Cell Metab.* (2012) 16:658–64. doi: 10.1016/j.cmet.2012.09.015
454. Szkudelski T, Szkudelska K. Resveratrol and diabetes: from animal to human studies. *Biochim Biophys Acta.* (2015) 1852:1145–54. doi: 10.1016/j.bbadis.2014.10.013
455. Song Y-J, Zhong C-B, Wu W. Resveratrol and diabetic cardiomyopathy: focusing on the protective signaling mechanisms. *Oxid Med Cell Longev.* (2020) 2020:1–19. doi: 10.1155/2020/7051845
456. Lim Y-H, Joe J-H, Jang K-S, Song Y-S, So B-I, Fang C-H, et al. Effects of granulocyte-colony stimulating factor (G-CSF) on diabetic cardiomyopathy in Otsuka Long-Evans Tokushima Fatty rats. *Cardiovasc Diabetol.* (2011) 10:92. doi: 10.1186/1475-2840-10-92
457. Daltro PS, Alves PS, Castro MF, Azevedo CM, Vasconcelos JF, Allahdadi KJ, et al. Administration of granulocyte-colony stimulating factor accompanied with a balanced diet improves cardiac function alterations induced by high fat diet in mice. *BMC Cardiovasc Disord.* (2015) 15:162. doi: 10.1186/s12872-015-0154-6
458. Tönnesmann E, Stroehmann I, Kandolf R, Wolburg H, Strach K, Musshoff F, et al. Cardiomyopathy caused by longterm treatment with chloroquine: a rare disease, or a rare diagnosis? *J Rheumatol.* (2012) 39:1099–103. doi: 10.3899/jrheum.110959
459. Mauthe M, Orhon I, Rocchi C, Zhou X, Luhr M, Hijlkema K-J, et al. Chloroquine inhibits autophagic flux by decreasing autophagosome-lysosome fusion. *Autophagy.* (2018) 14:1435–55. doi: 10.1080/15548627.2018.1474314

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Osteopontin and LDLR Are Upregulated in Hearts of Sudden Cardiac Death Victims With Heart Failure With Preserved Ejection Fraction and Diabetes Mellitus

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Background: Diabetes mellitus (DM) is associated with increased risk of sudden cardiac death (SCD), particularly in patients with heart failure with preserved ejection fraction (HFpEF). However, there are no known biomarkers in the population with DM and HFpEF to predict SCD risk.

Objectives: This study was designed to test the hypothesis that osteopontin (OPN) and some proteins previously correlated with OPN, low-density lipoprotein receptor (LDLR), dynamin 2 (DNM2), fibronectin-1 (FN1), and 2-oxoglutarate dehydrogenase-like (OGDHL), are potential risk markers for SCD, and may reflect modifiable molecular pathways in patients with DM and HFpEF.

Methods: Heart tissues were obtained at autopsy from 9 SCD victims with DM and HFpEF and 10 age and gender-matched accidental death control subjects from a Finnish SCD registry and analyzed for the expression of OPN and correlated proteins, including LDLR, DNM2, FN1, and OGDHL by immunohistochemistry.

Results: We observed a significant upregulation in the expression of OPN, LDLR, and FN1, and a marked downregulation of DNM2 in heart tissues of SCD victims with DM and HFpEF as compared to control subjects ($p < 0.01$).

Conclusions: The dysregulated protein expression of OPN, LDLR, FN1, and DNM2 in patients with DM and HFpEF who experienced SCD provides novel potential modifiable molecular pathways that may be implicated in the pathogenesis of SCD in these patients. Since secreted OPN and soluble LDLR can be measured in plasma, these results support

the value of further prospective studies to assess the predictive value of these plasma biomarkers and to determine whether tuning expression levels of OPN and LDLR alters SCD risk in patients with DM and HFpEF.

Keywords: sudden cardiac death (SCD), diabetes mellilus, osteopontin (OPN), LDLR, HFpEF—heart failure with preserved ejection fraction

INTRODUCTION

Heart failure with preserved ejection fraction (HFpEF) is the most common form of heart failure, affecting more than 3 million adults in the United States, and is represented by multiple subgroups or phenotypes (1). Type 2 diabetes mellitus (DM) is present in up to 45% of patients with HFpEF, and is associated with higher rates of morbidity and long-term mortality (2). Pathophysiology of HFpEF with DM include endothelial dysfunction, increased interstitial and perivascular fibrosis, cardiomyocyte stiffness, and left ventricular hypertrophy (3). DM is also associated with an increased risk of sudden cardiac death (SCD), in part linked with the presence of microvascular disease and autonomic neuropathy.

A common cause of non-ischemic SCD in younger subjects is primary myocardial fibrosis (4, 5), which is defined as myocardial fibrosis in the absence of other associated causes of fibrosis, and also has been demonstrated to be a common phenotype in SCD associated with HFpEF and DM (6). Risk stratification within the patient population diagnosed with DM and HFpEF remains a challenge.

Osteopontin (OPN) is a matricellular pro-fibrotic phosphorylated glycoprotein whose upregulation contributes to various pathological diseases such as cancers, chronic kidney disease, atherosclerosis, and adverse cardiac remodeling (7). In experimental models, lower OPN was associated with reduced adipose tissue inflammation, improved glucose tolerance and reduced insulin resistance. We recently showed that genetic knock down of OPN reduced HFpEF pathology in a cardio-renal model of HFpEF in mice (8, 9). Of great significance was the finding that pharmacological blockade of OPN by an OPN aptamer reversed pressure overload-induced heart failure (10).

In HF patients at high risk for SCD, plasma OPN and Galectin-3 levels were associated with sustained ventricular tachycardia and ventricular fibrillation (11). Plasma levels of OPN are increased in patients with heart failure with preserved ejection fraction (HFpEF) and predict mortality (12, 13).

In this study, we investigated the expression levels of OPN and correlated proteins, dynamin2 (DNM2), low density lipoprotein (LDLR), 2-oxoglutarate dehydrogenase-like (OGDHL), and fibronectin-1 (FN1), in the hearts of SCD victims who had both DM and HFpEF. The correlation of the selected proteins with OPN is based on our previous findings that these proteins were positively or negatively correlated with OPN expression in the heart and/or kidney in a mouse model of HFpeF (8, 14). Our hypothesis is that OPN and FN1 may contribute to the extracellular matrix remodeling in HFpEF that can be exacerbated through advanced glycation end products associated with diabetes (15). and may be a target for drug therapy. We reasoned that potential differential expression in the heart, specifically in proteins detected in the plasma such as OPN and LDLR, may identify biomarkers for SCD in patients with DM and HFpEF and generate novel future targets for prevention of SCD.

METHODS

Sample Collection

A death was classified as sudden if it was either a witnessed event within 6 h of the onset of symptoms or an unwitnessed death within 24 h when the subject was last seen alive in a normal state of health. The current criteria for SCD were chosen to cover as many subjects with SCD as possible, considering that many subjects with SCD would be found dead and would have been missed with the 1 h definition for SCD originally proposed by Hinkle and Thaler (16). All of the subjects are from a defined geographical area in Northern Finland.

Autopsied human heart samples from the left ventricular free wall from 9 SCD victims with DM and HFpEF and 10 previously healthy individuals who died as a result of an accident were obtained from a Finnish SCD registry as paraffin-embedded sections and analyzed for the expression of seven proteins by immunohistochemistry (see **Table 1**).

The study complies with the Declaration of Helsinki and was approved by the Ethics Committee of Northern Ostrobothnia Hospital District and the National Authority for Medicolegal Affairs (Valvira). Consent from next of kin was waived by the Ethics Committee since according to the Finnish law, medicolegal autopsy does not require consent.

Immunohistochemistry

The slides with paraffin-embedded heart sections were incubated for 45 min at 70 degrees, then dewaxed with two 5 min xylene washes and hydrated by 3 min graded ethanol washes of 100% (twice), 95, 80, and 70%, followed by two 4 min water immersions. Heat-mediated antigen retrieval was then done using 1X Citrate Antigen Retrieval Buffer (Ab93678) in a steamer for 75 min followed by permeabilization using 0.2% Triton X-100 (Sigma-Aldrich) in PBS for 30 min, and then blocking with 10% donkey serum in 1% TBST for 45 min. The slides were then incubated with the following antibodies for 2 h at room temperature: osteopontin (hOPN, R&D AF1433; dilution 1:50), low density lipoprotein receptor (LDLR, Abcam Ab52818; dilution 1:200), dynamin 2 (DNM2, Abcam Ab3475; dilution 1:200), fibronectin 1 (FN1, Sigma F3648, dilution 1:200), 2-oxoglutarate dehydrogenase-like (OGDHL, Proteintech 17110-1-AP; dilution 1:200), very low density lipoprotein receptor (VLDLR, Abcam Ab203271; dilution 1:200), and desmoplakin (DSP, Santa Cruz Biotechnology sc-390975, dilution 1:100).

TABLE 1 | Study population information.

No.	Gender	Age (Years)	Hypertension	Hypercholesterolemia	BMI (kg/m^2)	DM status
SCD victim 1	Male	48	Yes	No	37.6	Type 2
SCD victim 2	Male	70	Yes	No	40.3	Type 2
SCD victim 3	Male	75	No	No	30.8	Type 2
SCD victim 4	Female	52	No	No	76.8	Type 2
SCD victim 5	Male	69	No	Yes	28.4	Type 2
SCD victim 6	Male	67	No	No	31.7	Type 2
SCD victim 7	Male	64	Yes	No	31.2	Type 2
SCD victim 8	Male	63	Yes	No	27.4	Type 2
SCD victim 9	Male	73	Yes	No	42.8	Type 2
Accidental death control 1	Male	70	No	No	22.8	No
Accidental death control 2	Male	46	No	No	26.1	No
Accidental death control 3	Male	49	No	No	32	No
Accidental death control 4	Male	71	Yes	No	41.8	No
Accidental death control 5	Male	76	No	No	28.3	No
Accidental death control 6	Male	64	No	No	21.6	No
Accidental death control 7	Male	67	No	No	34.2	No
Accidental death control 8	Male	69	Yes	Yes	33.3	No
Accidental death control 9	Male	73	No	No	28.9	No
Accidental death control 10	Male	62	No	No	43.7	Type 1

BMI, Body Mass Index; SCD, Sudden Cardiac Death; DM, Diabetes Mellitus.

Slides were then washed with PBS and staining was detected by incubation with various species-specific biotinylated secondary antibodies [biotinylated goat IgG (Vector Laboratories BA-9500; dilution 1:200), biotinylated rabbit IgG (Vector Laboratories BA-1000, dilution 1:200), biotinylated mouse IgG BA-9200 (Vector Laboratories, dilution 1:200)] at room temperature for 30 min, followed by DAB peroxidase (HRP) amplification (SK-4100, Vector Laboratories). Primary and secondary antibody incubations were done in 10% donkey serum in 1% Tris-Buffered Saline, 0.1% Tween® 20 Detergent (TBST). Each single-antibody staining was repeated 3 times (on 3 different slides from the same paraffin block). No-primary control staining was performed with each of the secondary antibodies used.

Imaging and Protein Expression Quantification

The stained slides were scanned at 20-X magnification using the Olympus VS120–L100 Virtual Slide Microscope (Tokyo, Japan). Images were quantified using ImageJ (NIH) by 2 independent examiners blinded to the groups. For each sample, 5 images (of 5 fields) were used for quantification and the average positive area normalized to the total stained area (as percentage) was used for statistical analysis.

Statistics

For all experiments, N refers to the number of individual subjects. All data are expressed as mean ± SEM. *P*-values were calculated using unpaired Student's *t*-tests and $p < 0.05$ was considered significant. Repeated symbols represent *p*-values of different orders of magnitude, i.e., $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. Similar significant differences were confirmed by two independent examiners.

RESULTS

Study Population

Table 1 summarizes demographic and clinical information of the SCD victims and accidental death controls. All SCD victims had cardiac cause of death (either ischemic or non-ischemic) with significant coronary artery disease or left ventricular hypertrophy with fibrosis at autopsy.

Osteopontin and LDLR Are Upregulated in Cardiac Autopsies of SCD Victims With DM+HFpEF

We have previously shown that OPN and LDLR are highly expressed in the renal tubules of a mouse model of HFpEF, and that OPN knockout reduced the pathologically high levels of LDLR and improved renal and cardiac function (8, 14). We found that cardiac OPN expression in hearts of SCD victims with DM+HFpEF was significantly increased by 1.52-fold (28.4 ± 2.4 vs. 18.7 ± 3.2% in the control group, $p < 0.05$, **Figure 1A**). Moreover, our results showed a 3.58-fold increase in cardiac LDLR protein levels in SCD victims with DM+HFpEF (57.6 ± 5.0 vs. 16.1 ± 0.9% in control group, $p < 0.0001$, **Figure 1B**).

DNM2 Is Downregulated in Cardiac Autopsies of SCD Victims DM+HFpEF

We have previously shown that OPN and DNM2 are highly expressed in the renal tubules of a mouse model of HFpEF, and that OPN knockout reduced the pathologically high levels

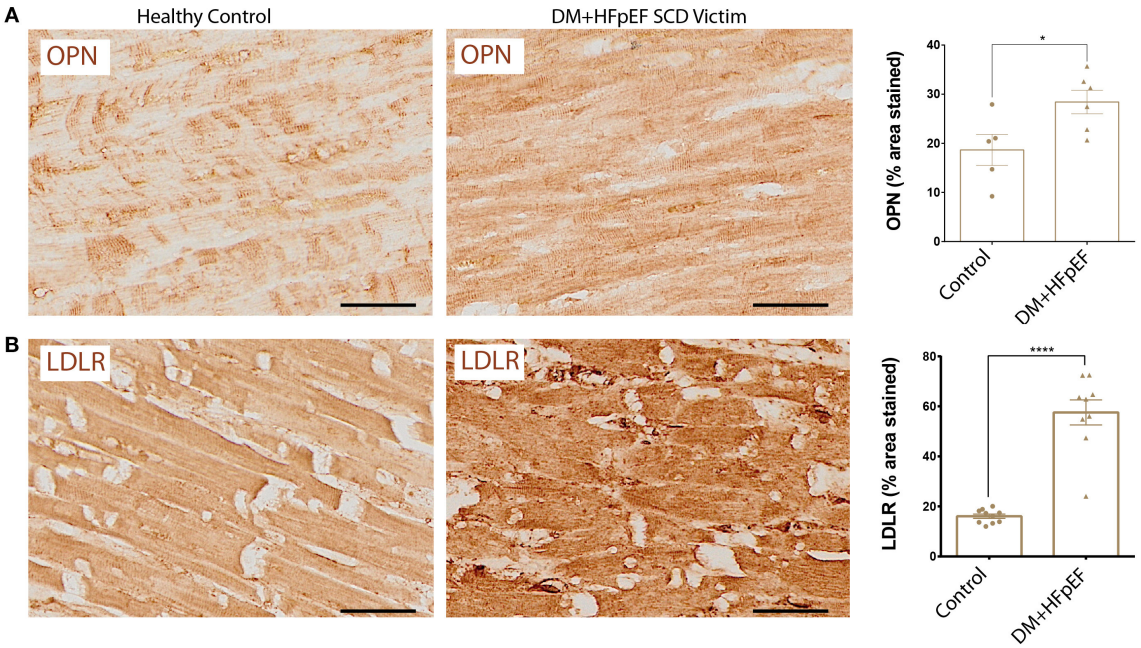

FIGURE 1 | Upregulation of OPN and LDLR in SCD victims with DM+HFpEF. Immunostaining of cardiac biopsies of SCD victims with both HFpEF and DM shows upregulated OPN **(A)** and LDLR **(B)** protein expression compared to control hearts. Data are mean ± SEM. $N = 5$–10 subjects/group. Scale bar = 50 μm. HFpEF, Heart Failure with Preserved Ejection Fraction; DM, Diabetes Mellitus; LDLR, Low Density Lipoprotein Receptor; OPN, Osteopontin. *$P < 0.05$, ****$P < 0.0001$ using Student's *t*-test.

of DNM2 and improved renal and cardiac function (8, 14). When compared with control hearts, cardiac DNM2 expression was significantly lower by 0.69-fold in cardiac tissue from SCD victims with DM+HFpEF (32.3 ± 5.1 vs. 46.5 ± 3.6% in control group, $p < 0.05$, **Figure 2**).

FN1 Is Upregulated in Cardiac Autopsies of SCD Victims With DM+HFpEF

We have previously shown that in a mouse model of pressure overload, treatment with an OPN aptamer prevented cardiomyocyte hypertrophy and cardiac fibrosis, blocked OPN downstream signaling, and reduced expression of extracellular matrix proteins including FN1 (10). Cardiac FN1 expression in SCD victims with DM+HFpEF was significantly increased by 1.6-fold (51.2 ± 7.1 vs. 32.3 ± 3.3% in control group, $p < 0.05$, **Figure 3**).

OGDHL, DSP, and VLDLR Remain Unchanged in Cardiac Autopsies of SCD Victims With DM+HFpEF

We recently reported that OPN negatively regulates the expression of mitochondrial enzyme, OGDHL in the heart of a mouse model of HFpEF, and that OGDHL expression is downregulated in cardiac biopsies from patients with HFpEF and DM (8). We investigated the expression levels of OGDHL, as well as 2 other proteins not previously correlated (DSP and VLDLR) to serve as negative controls, in cardiac tissue from autopsies of SCD victims with HFpEF and DM by immunohistochemistry. Our findings showed that there were no significant changes in the expression patterns of these three proteins (**Figure 4**). Negative control staining (without primary antibodies) showed no signal as shown in **Figure 5**.

DISCUSSION AND CONCLUSIONS

Diabetes Mellitus (DM) is a major public health problem affecting ∼29.1 million Americans. DM is present in about 45% of patients with heart failure with preserved ejection fraction (HFpEF). Furthermore, SCD occurs in ∼20% of HFpEF patients (17). Notably, the I-Preserve trial identified DM as a multivariable predictor of SCD in HFpEF patients (18, 19). In HFpEF, type 2 DM comorbidity is associated with smaller left ventricular volumes, worse diastolic dysfunction, poorer quality of life, and overall worse outcomes (20). Among the factors that contribute to increased SCD risk in patients with DM are silent myocardial ischemia, autonomic nervous system dysfunction, and abnormal cardiac repolarization (21). Two important factors that have contributed to current risk stratification in SCD victims who had HFpEF are male gender and insulin-treated DM. In a 2020 study by Adabag and Langsetmo (22), DM was identified as one of the main risk factors associated with significantly increased SCD incidence. In a cohort of 615 HFpEF patients used to validate the survival prediction model, DM was a comorbidity in 52% of SCD victims, while only 29% of the survivors and 36% of those who died due to non-SCD reasons had DM. These results validated the same group's 2014 study (19). which reported that DM was

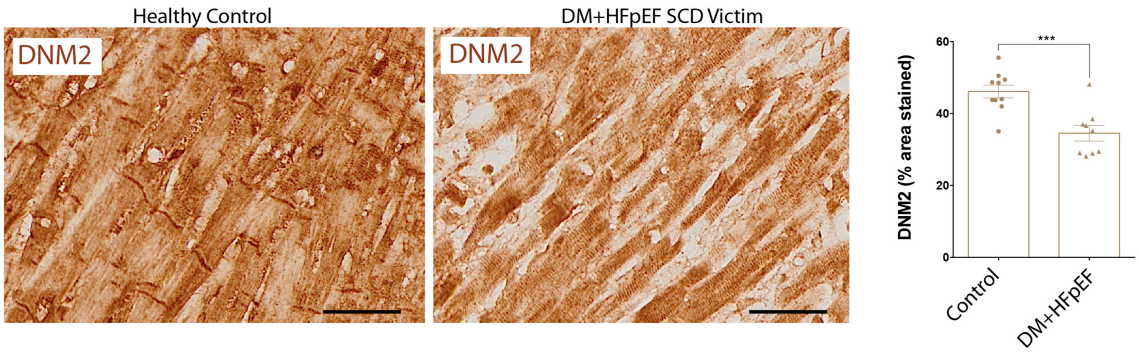

FIGURE 2 | Downregulation of DNM2 in SCD victims with DM+HFpEF. Immunostaining of cardiac biopsies of SCD victims with both HFpEF and DM shows lower DNM2 protein expression compared to control hearts. Data are mean ± SEM. $N = 9$–10 subjects/group. Scale bar = 50 μm. HFpEF, Heart Failure with Preserved Ejection Fraction; DM, Diabetes Mellitus; DNM2, Dynamin-2. ***$p < 0.001$ using Student's *t*-test.

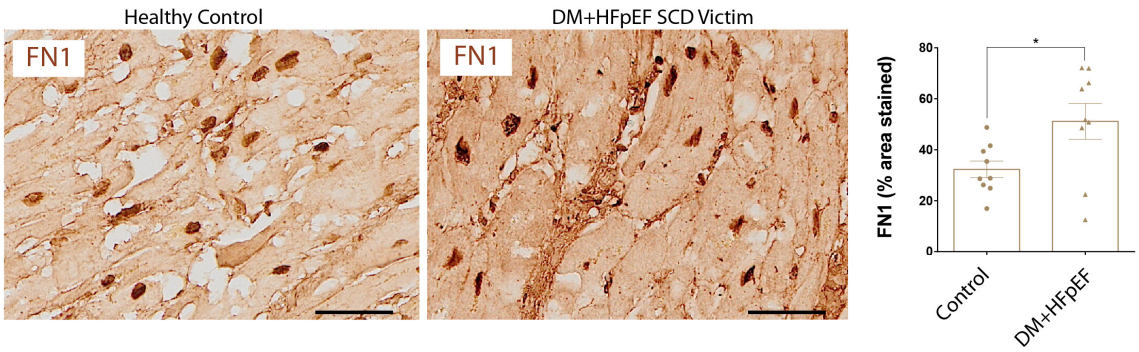

FIGURE 3 | Upregulation of FN1 protein in SCD victims with DM+HFpEF. Immunostaining of cardiac biopsies of SCD victims with both HFpEF and DM shows higher FN1 protein expression compared to control hearts. Data are mean ± SEM. $N = 9$ subjects/group. Scale bar = 50 μm. HFpEF, Heart Failure with Preserved Ejection Fraction; DM, Diabetes Mellitus; FN1, Fibronectin 1. *$P < 0.05$ using Student's *t*-test.

present in 38% of HFpEF patients who died of SCD compared to 35% prevalence in non-SCD deaths and 25% in survivors over a 5 year period. These reports consistently corroborate the elevated risk of SCD in HFpEF patients with DM. The currently known factors that contribute to risk stratification are not useful in lowering mortality attributable to SCD in patients with both DM and HFpEF (17). Thus, the focus of this study was to analyze the expression levels of specific proteins expressed in patients with the phenotype of interest—DM+HFpEF with SCD.

OPN is a matricellular protein that has been implicated in many inflammatory and profibrotic events, especially in the heart. In patients with severe aortic stenosis, elevated OPN levels correlated with increased rates of atrial arrhythmia, and an increased risk of death (23). In our previous work (8, 14), we showed that OPN is highly expressed in kidneys and circulation in a cardio-renal mouse model of HFpEF, and that genetic knock down of OPN reduced HFpEF pathology. The work also showed that OPN regulates LDLR expression *in vivo*. Therefore, we analyzed the protein expression levels of both OPN and its related molecules (previously shown to be correlated to OPN expression), as well as other proteins not previously correlated to OPN, to serve as negative controls.

The results of this study showed that the expression levels of OPN and correlated proteins LDLR, FN1, and DNM2 are highly dysregulated in SCD victims with DM+HFpEF, while those proteins not previously correlated to OPN including VLDLR and DSP were not changed. LDLR, FN1, and OPN showed a significantly increased expression in the diseased hearts, while DNM2 was reduced. Osteopontin had increased expression in the diseased hearts, supporting its role in the pathological process in patients with DM+HFpEF. Increased plasma OPN levels have been directly associated with cardiac dysfunction in HFpEF patients (13). On the other hand, we recently showed that OPN dysregulates the signaling of the β_2-adrenergic receptor in cardiac cells (7), a receptor known to be associated with SCD in humans (24). Further investigation is required to elucidate the role of elevated OPN levels in SCD victims hearts in regards to SCD pathophysiology.

We had previously demonstrated that OPN regulates LDLR expression and subsequent lipid accumulation and metabolic abnormalities in renal tubules of a mouse model of renal-induced HFpEF (14). Therefore, the elevated LDLR levels in SCD victims with HFpEF and DM could be

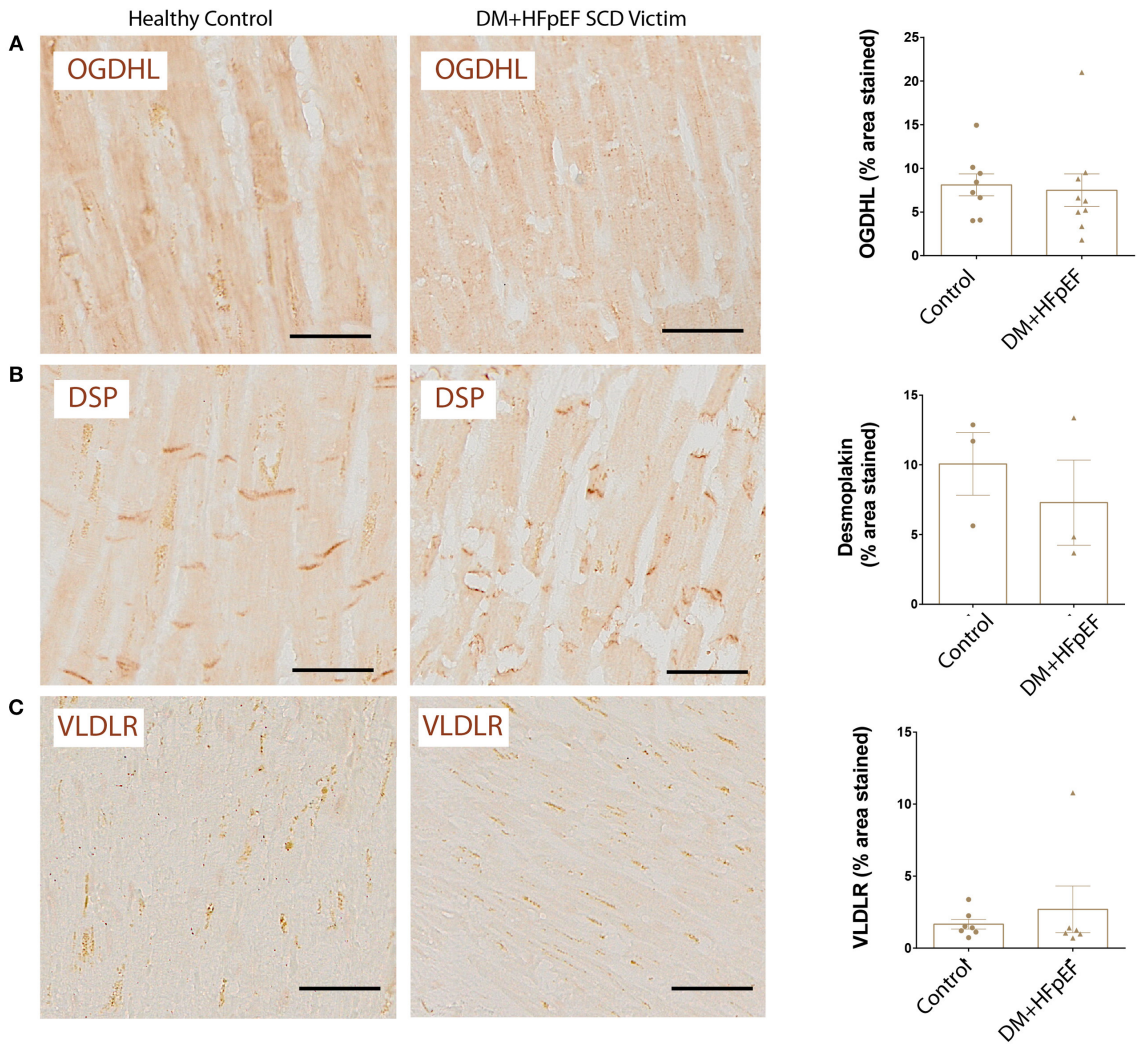

FIGURE 4 | Unchanged expression of OGDHL, DSP, and VLDLR proteins in SCD victims with DM+HFpEF. Immunostaining of cardiac biopsies of SCD victims with both HFpEF and DM shows no significant changes in the expression pattern of OGDHL **(A)**, DSP **(B)** and VLDLR **(C)** compared to control hearts. Data are mean ± SEM. *N* = 3–10 subjects/group. Scale bar = 50 μm. HFpEF, Heart Failure with Preserved Ejection Fraction; DM, Diabetes Mellitus; DSP, Desmoplakin; OGDHL, 2-Oxoglutarate Dehydrogenase Like; VLDLR, Very Low Density Lipoprotein Receptor. No significance was detected by Student's *t*-test.

FIGURE 5 | Negative control staining shows no signal. Immunostaining of cardiac biopsies of SCD victims without primary antibodies shows no signal with anti-goat **(A)**, anti-rabbit **(B)** or anti-mouse **(C)** IgG secondary antibodies. Scale bar = 50 μm. These images serve as negative controls for images in **Figures 1–4**.

attributed to the increased OPN levels and may indicate impaired lipid homeostasis in the population studied. FN1 is a matricellular protein that is elevated following myocardial injury or fibrosis (25). Increased expression of FN1 in the diseased hearts alludes to its role in fibrosis that is a major pathology of both DM and HFpEF. DNM2 is involved in

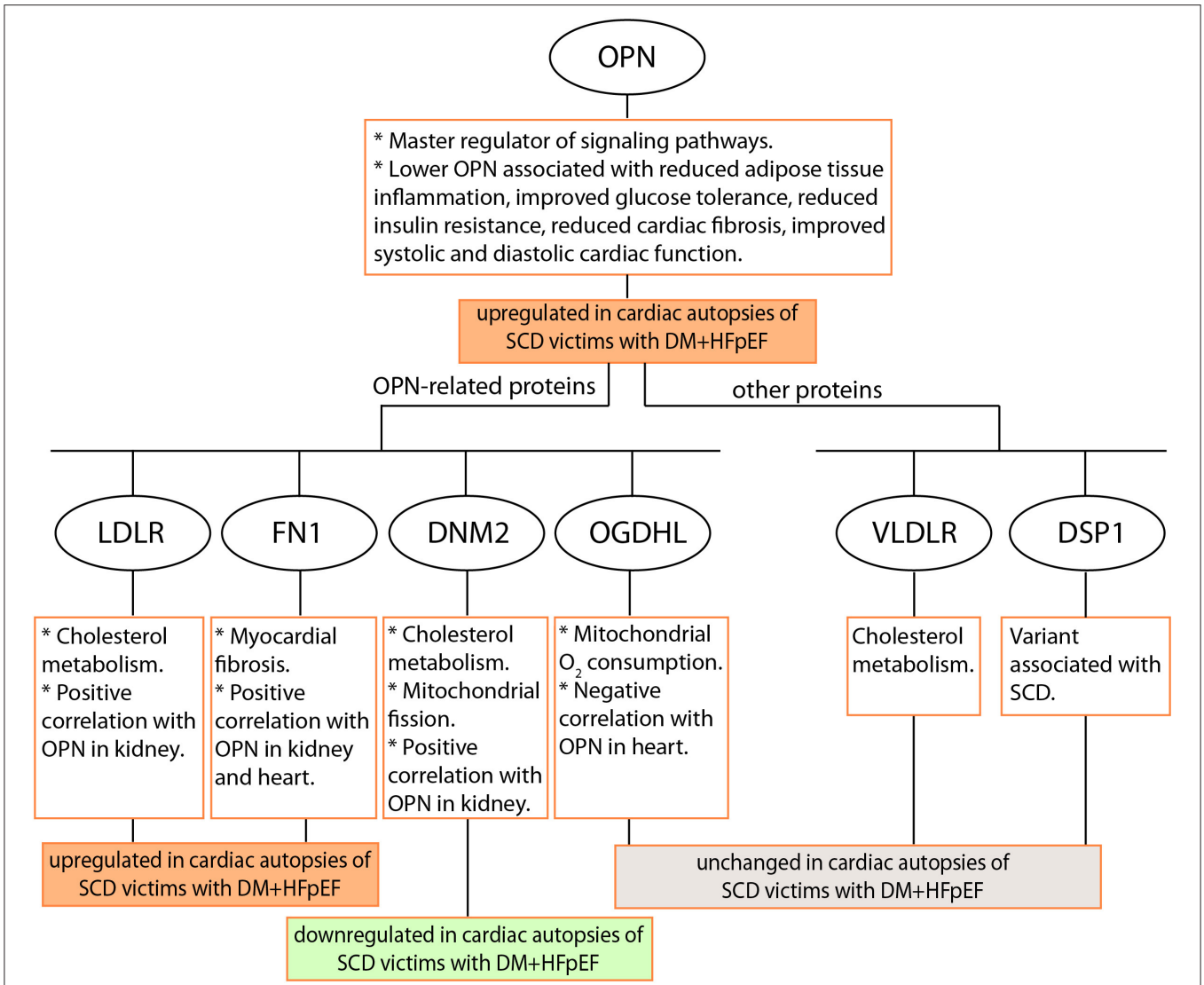

FIGURE 6 | Schematic of the study. Seven proteins were investigated by immunostaining in cardiac autopsies from sudden cardiac death (SCD) victims with Heart Failure with preserved Ejection Fraction (HFpEF) and Type 2 Diabetes Mellitus (DM). OPN, Osteopontin; LDLR, Low Density Lipoprotein Receptor; DNM2, Dynamin-2; FN1, Fibronectin 1; OGDHL, 2-Oxoglutarate Dehydrogenase Like; VLDLR, Very Low Density Lipoprotein Receptor; DSP, Desmoplakin.

endocytosis including LDLR-mediated LDL uptake via clathrin-coated vesicles (26). Therefore, the reduced DNM2 expression in SCD victims could potentially serve a compensatory mechanism to prevent excessive lipid accumulation as a result of LDLR overexpression.

OGDHL is a mitochondrial protein which catalyzes the conversion of 2-oxoglutarate to succinyl-CoA during the TCA cycle and its decreased expression could be associated with mitochondrial dysfunction; impaired energy metabolism could affect efficient glucose utilization (e.g., increased glycolysis and reduced oxidative phosphorylation) (8, 27). In diabetes, OGDHL may have greater consequences for cardiac cells/tissues since there is already reduced TCA cycle flux (28). In our previous study, we detected significant (though modest) changes in OGDHL RNA and protein expression in biopsies obtained from the right ventricular septum from patients with DM and HFpEF (8). However, in this study, we did not detect changes in OGDHL expression by staining in this population.

Our team previously reported that the DSP gene, which codes for the structural protein Desmoplakin, harbors 2 variants associated with sudden cardiac death in subjects with primary myocardial fibrosis (6). Finding no changes in protein expression in another population is not surprising or even expected. In addition, the LDLR and VLDLR are structurally related members of the LDL receptor family with different tissue distribution and functional roles. While the LDLR is predominantly involved in lipid metabolism, the VLDLR has a more limited role in uptake of TG-rich lipoproteins in peripheral tissues [e.g., muscle,

heart, adipose tissues (29)]. Compared to the LDLR, the VLDLR binds a variety of non-lipoprotein receptors (thrombospondin-1, LPL, urokinase plasminogen activator /plasminogen activator inhibitor-1 complex, Reelin and fibrin as wells as common ligands with LDLR [LDL, apoE, Lp(a), PCSK9]. These receptors are also differentially regulated; for example, the VLDLR is upregulated by fenofibrate through PPAR-α (30), while the LDLR is not (31). Conversely, the LDLR is downregulated by sterol negative feedback, but the VLDLR is not affected by sterols (32). VLDLR was chosen as a protein that was never correlated with Osteopontin. We found low expression levels of cardiac VLDLR that was not changed between the groups.

The dysregulation of OPN, LDLR, DNM2, and FN1 protein levels in heart tissues (as shown in study schematic in **Figure 6**) provides supportive evidence and calls for further investigation to elucidate the role(s) and contribution(s) of these potentially detrimental alterations in the pathogenesis of SCD in patients with HFpEF and DM. The fact that secreted OPN and soluble LDLR can be detected in the plasma calls for future investigation of plasma OPN and LDLR levels as risk markers for SCD in patients with DM+HFpEF. Specifically the roles of OPN and LDLR in increased cholesterol accumulation and subsequent pathological effects in the hearts of patients with HFpEF and DM are yet to be elucidated.

LIMITATIONS

This study is limited by the lack of control groups of patients with DM alone, HFpEF alone, and DM+HFpEF without SCD, and by its small numbers. We acknowledge that the levels of LDL-cholesterol, medications history, and HbA1c% would have been informative in our study. Unfortunately, since our subjects are autopsied sudden cardiac death victims, we do not have the detailed blood chemistry and medication history data for all of them. In addition, since the controls are age- and gender-matched accident victims chosen from the Finland registry, we also lack any blood chemistry and medication history data for them.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of Northern Ostrobothnia Hospital District and the National Authority for Medicolegal Affairs (Valvira). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

KJ-W performed the experiments. MP, DR, KY, and LS analyzed the data and prepared the figures. MP, DR, KY, AL, AM, RG, LT, JG, RM, JJ, and LS interpreted the results of experiments. MP, DR, and KY drafted the manuscript. MP, DR, KY, KJ-W, AL, AM, RG, LT, JG, RM, JJ, and LS edited and revised the manuscript and approved the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Shah SJ, Katz DH, Selvaraj S, Burke MA, Yancy CW, Gheorghiade M, et al. Phenomapping for novel classification of heart failure with preserved ejection fraction. *Circulation*. (2015) 131:269–79. doi: 10.1161/CIRCULATIONAHA.114.010637
2. McHugh K, DeVore AD, Wu J, Matsouaka RA, Fonarow GC, Heidenreich PA, et al. Heart failure with preserved ejection fraction and diabetes: Jacc state-of-the-art review. *J Am Coll Cardiol*. (2019) 73:602–11. doi: 10.1016/j.jacc.2018.11.033
3. Meagher P, Adam M, Civitarese R, Bugyei-Twum A, Connelly KA. Heart failure with preserved ejection fraction in diabetes: mechanisms and management. *Can J Cardiol*. (2018) 34:632–43. doi: 10.1016/j.cjca.2018.02.026
4. Hookana E, Junttila MJ, Puurunen VP, Tikkanen JT, Kaikkonen KS, Kortelainen ML, et al. Causes of nonischemic sudden cardiac death in the current era. *Heart Rhythm*. (2011) 8:1570–75. doi: 10.1016/j.hrthm.2011.06.031
5. di Gioia CR, Giordano C, Cerbelli B, Pisano A, Perli E, De Dominicis E, et al. Nonischemic left ventricular scar and cardiac sudden death in the young. *Hum Pathol*. (2016) 58:78–89. doi: 10.1016/j.humpath.2016.08.004
6. Junttila MJ, Holmstrom L, Pylkas K, Mantere T, Kaikkonen K, Porvari K, et al. Primary myocardial fibrosis as an alternative phenotype pathway of inherited cardiac structural disorders. *Circulation*. (2018) 137:2716–26. doi: 10.1161/CIRCULATIONAHA.117.032175
7. Pollard CM, Desimine VL, Wertz SL, Perez A, Parker BM, Maning J, et al. Deletion of osteopontin enhances beta(2)-adrenergic receptor-dependent anti-fibrotic signaling in cardiomyocytes. *Int J Mol Sci*. (2019) 20:1396. doi: 10.20944/preprints201902.0266.v1
8. Yousefi K, Irion CI, Takeuchi LM, Ding W, Lambert G, Eisenberg T, et al. Osteopontin promotes left ventricular diastolic dysfunction through a mitochondrial pathway. *J Am Coll Cardiol*. (2019) 73:2705–18. doi: 10.1016/j.jacc.2019.02.074
9. Yousefi K, Dunkley JC, Shehadeh LA. A preclinical model for phenogroup 3 hfpef. *Aging*. (2019) 11:4305–7. doi: 10.18632/aging.102102

10. Li J, Yousefi K, Ding W, Singh J, Shehadeh LA. Osteopontin rna aptamer can prevent and reverse pressure overload-induced heart failure. *Cardiovasc Res*. (2017) 113:633–43. doi: 10.1093/cvr/cvx016
11. Francia P, Adduci C, Semprini L, Borro M, Ricotta A, Sensini I, et al. Osteopontin and galectin-3 predict the risk of ventricular tachycardia and fibrillation in heart failure patients with implantable defibrillators. *J Cardiovasc Electrophysiol*. (2014) 25:609–16. doi: 10.1111/jce.12364
12. Tromp J, Khan MA, Klip IT, Meyer S, de Boer RA, Jaarsma T, et al. Biomarker profiles in heart failure patients with preserved and reduced ejection fraction. *J Am Heart Assoc*. (2017) 6:e003989. doi: 10.1161/JAHA.116.003989
13. Krum H, Elsik M, Schneider HG, Ptaszynska A, Black M, Carson PE, et al. Relation of peripheral collagen markers to death and hospitalisation in patients with heart failure and preserved ejection fraction: results of the i-preserve collagen sub-study. *Circ Heart Fail*. (2011) 4:561–8. doi: 10.1161/CIRCHEARTFAILURE.110.960716
14. Ding W, Yousefi K, Goncalves S, Goldstein BJ, Sabater AL, Kloosterboer A, et al. Osteopontin deficiency ameliorates alport pathology by preventing tubular metabolic deficits. *JCI Insight*. (2018) 3:e94818. doi: 10.1172/jci.insight.94818
15. Jia G, Hill MA, Sowers JR. Diabetic cardiomyopathy: an update of mechanisms contributing to this clinical entity. *Circ Res*. (2018) 122:624–38. doi: 10.1161/CIRCRESAHA.117.311586
16. Hinkle LE Jr., Thaler HT. Clinical classification of cardiac deaths. *Circulation*. (1982) 65:457–64. doi: 10.1161/01.CIR.65.3.457
17. Vaduganathan M, Claggett BL, Chatterjee NA, Anand IS, Sweitzer NK, Fang JC, et al. Sudden death in heart failure with preserved ejection fraction: a competing risks analysis from the topcat trial. *JACC Heart Fail*. (2018) 6:653–61. doi: 10.1016/j.jchf.2018.02.014
18. Zile MR, Gaasch WH, Anand IS, Haass M, Little WC, Miller AB, et al. Mode of death in patients with heart failure and a preserved ejection fraction: results from the irbesartan in heart failure with preserved ejection fraction study (i-preserve) trial. *Circulation*. (2010) 121:1393–405. doi: 10.1161/CIRCULATIONAHA.109.909614
19. Adabag S, Rector TS, Anand IS, McMurray JJ, Zile M, Komajda M, et al. A prediction model for sudden cardiac death in patients with heart failure and preserved ejection fraction. *Eur J Heart Fail*. (2014) 16:1175–82. doi: 10.1002/ejhf.172
20. Yap J, Tay WT, Teng TK, Anand I, Richards AM, Ling LH, et al. Association of diabetes mellitus on cardiac remodeling, quality of life, and clinical outcomes in heart failure with reduced and preserved ejection fraction. *J Am Heart Assoc*. (2019) 8:e013114. doi: 10.1161/JAHA.119.013114
21. Bergner DW, Goldberger JJ. Diabetes mellitus and sudden cardiac death: what are the data? *Cardiol J*. (2010) 17:117–29.
22. Adabag S, Langsetmo L. Sudden cardiac death risk prediction in heart failure with preserved ejection fraction. *Heart Rhythm*. (2020) 17:358–64. doi: 10.1016/j.hrthm.2019.12.009
23. Lutz M, von Ingersleben N, Lambers M, Rosenberg M, Freitag-Wolf S, Dempfle A, et al. Osteopontin predicts clinical outcome in patients after treatment of severe aortic stenosis with transcatheter aortic valve implantation (tavi). *Open Heart*. (2017) 4:e000633. doi: 10.1136/openhrt-2017-000633
24. Gavin MC, Newton-Cheh C, Gaziano JM, Cook NR, VanDenburgh M, Albert CM. A common variant in the β2-adrenergic receptor and risk of sudden cardiac death. *Heart Rhythm*. (2011) 8:704–10. doi: 10.1016/j.hrthm.2011.01.003
25. Valiente-Alandi I, Potter SJ, Salvador AM, Schafer AE, Schips T, Carrillo-Salinas F, et al. Inhibiting fibronectin attenuates fibrosis and improves cardiac function in a model of heart failure. *Circulation*. (2018) 138:1236–52. doi: 10.1161/CIRCULATIONAHA.118.034609
26. Bitoun M, Durieux AC, Prudhon B, Bevilacqua JA, Herledan A, Sakanyan V, et al. Dynamin 2 mutations associated with human diseases impair clathrin-mediated receptor endocytosis. *Hum Mutat*. (2009) 30:1419–27. doi: 10.1002/humu.21086
27. Stobbe MD, Houten SM, van Kampen AH, Wanders RJ, Moerland PD. Improving the description of metabolic networks: the tca cycle as example. *FASEB J*. (2012) 26:3625–36. doi: 10.1096/fj.11-203091
28. Schrauwen P, Hesselink MK. Reduced tricarboxylic acid cycle flux in type 2 diabetes mellitus? *Diabetologia*. (2008) 51:1694–7. doi: 10.1007/s00125-008-1069-x
29. Takahashi S. Triglyceride rich lipoprotein -lpl-vldl receptor and lp(a)-vldl receptor pathways for macrophage foam cell formation. *J Atheroscler Thromb*. (2017) 24:552–9. doi: 10.5551/jat.RV17004
30. Gao Y, Shen W, Lu B, Zhang Q, Hu Y, Chen Y. Upregulation of hepatic vldlr via pparα is required for the triglyceride-lowering effect of fenofibrate. *J Lipid Res*. (2014) 55:1622–33. doi: 10.1194/jlr.M041988
31. Staels B, Dallongeville J, Auwerx J, Schoonjans K, Leitersdorf E, Fruchart J-C. Mechanism of action of fibrates on lipid and lipoprotein metabolism. *Circulation*. (1998) 98:2088–93. doi: 10.1161/01.CIR.98.19.2088
32. Wittmaack FM, Gåfvels ME, Bronner M, Matsuo H, McCrae KR, Tomaszewski JE, et al. Localization and regulation of the human very low density lipoprotein/apolipoprotein-e receptor: trophoblast expression predicts a role for the receptor in placental lipid transport. *Endocrinology*. (1995) 136:340–8. doi: 10.1210/endo.136.1.7828550

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frontiers in Pharmacology

ORIGINAL RESEARCH
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SGLT1 Knockdown Attenuates Cardiac Fibroblast Activation in Diabetic Cardiac Fibrosis

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Background: Cardiac fibroblast (CF) activation is a hallmark feature of cardiac fibrosis in diabetic cardiomyopathy (DCM). Inhibition of the sodium-dependent glucose transporter 1 (SGLT1) attenuates cardiomyocyte apoptosis and delays the development of DCM. However, the role of SGLT1 in CF activation remains unclear.

Methods: A rat model of DCM was established and treated with si-SGLT1 to examine cardiac fibrosis. In addition, *in vitro* experiments were conducted to verify the regulatory role of SGLT1 in proliferation and collagen secretion in high-glucose– (HG–) treated CFs.

Results: SGLT1 was found to be upregulated in diabetic cardiac tissues and HG-induced CFs. HG stimulation resulted in increased proliferation and migration, increased the expression of transforming growth factor-β1 and collagen I and collagen III, and increased phosphorylation of p38 mitogen-activated protein kinase and extracellular signal-regulated kinase (ERK) 1/2. These trends in HG-treated CFs were significantly reversed by si-SGLT1. Moreover, the overexpression of SGLT1 promoted CF proliferation and collagen synthesis and increased phosphorylation of p38 mitogen-activated protein kinase and ERK1/2. SGLT1 silencing significantly alleviated cardiac fibrosis, but had no effect on cardiac hypertrophy in diabetic hearts.

Conclusion: These findings provide new information on the role of SGLT1 in CF activation, suggesting a novel therapeutic strategy for the treatment of DCM fibrosis.

Keywords: cardiac fibroblasts, sodium–glucose cotransporter, high glucose, diabetic cardiomyopathy, mitogen-activated protein kinase

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INTRODUCTION

Diabetic cardiomyopathy (DCM) is a myocardial disease that is specific to patients with diabetes and is independent of various types of heart diseases, including hypertension, coronary, and valvular (Bugger and Abel, 2014; Seferovic and Paulus, 2015). Cardiac fibrosis caused by abnormal glucose metabolism and microangiopathy are the main pathological features of DCM, leading to impairment

Abbreviations: DCM, diabetic cardiomyopathy; CFs, cardiac fibroblasts; SGLT1, sodium–glucose cotransporter; FBG, fasting blood glucose; IPGTTs, intraperitoneal glucose tolerance tests; HG, high glucose; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; TGF-β1, transforming growth factor-β1.

of cardiac function and eventual progression to heart failure (Wang et al., 2021). The activation of cardiac fibroblasts (CFs) and degeneration of cardiomyocytes provide the biological basis for cardiac remodeling and the pathophysiological basis of DCM formation (Zhang et al., 2020). CFs switched from a resting type to an activated type increasing their proliferation and migration capacity and began to secrete large amounts of extracellular matrix, causing fibrosis of the heart (Frangogiannis, 2021). The development of fibrosis-targeting therapies for patients with DCM will help to further understand the functional pluralism of CFs and dissect the molecular basis for fibrotic remodeling.

Sodium–glucose cotransporter (SGLT) belongs to the solute carrier five gene family, which transports glucose against a concentration gradient in an energy-consuming manner and plays an important role in the active transport of glucose (Wood and Trayhurn, 2003; Sano et al., 2020). Sodium–glucose cotransporter 1 (SGLT1) is expressed in various human tissues and organs, including the intestine, lung, heart, skeletal muscle, and kidney (Gyimesi et al., 2020). SGLT1 is essential for the quick absorption of glucose and galactose in the intestine, and increases in SGLT1 protein expression cause interstitial fibrosis and cardiac remodeling in mice (Ramratnam et al., 2014). SGLT1 expression is also elevated in hypertrophic cardiomyopathy, ischemic cardiomyopathy, and DCM in humans (Song et al., 2016). Selective inhibition of SGLT1 expression has a protective effect against myocardial-infarction-induced ischemic cardiomyopathy (Sawa et al., 2020). In addition, Hirose et al. (2018) demonstrated that SGLT1 knockout effectively alleviated pressure-overload–induced cardiomyopathy, suggesting that SGLT1 inhibitors have an active effect on hypertrophic cardiomyopathy. More importantly, our previous study found that SGLT1 inhibition could attenuate apoptosis and relieve myocardial fibrosis, thus suppressing DCM development by regulating the JNK/p38 signaling pathway (Lin et al., 2021). However, in the abovementioned study, we only investigated the role of SGLT1 in cardiomyocytes and rat H9C2 cells. It would be more appropriate to study the role of SGLT1 in the activation of CFs during the development of DCM.

Our previous study found that high-glucose (HG) levels promote SGLT1 and matrix metalloproteinase 2 expression in CFs (Meng et al., 2018), but whether HG levels promote cardiac fibrosis by inducing CF activation and whether SGLT1 is involved in HG-induced CF activation have not been reported. Thus, arrays of experiments were performed in this study to determine the role of SGLT1 in CF activation during DCM. Moreover, we tried to characterize the role of the p38 and ERK1/2 signaling pathways in the regulatory mechanism of SGLT1 expression for CF activation.

MATERIALS AND METHODS

Ethics Statement

All animal procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Medicine Animal Welfare Committee of Shaoxing People's Hospital.

Culture of Rat Primary CFs

Primary rat CFs were isolated from the ventricles of neonatal male Sprague Dawley rats (2–3 days old) using enzyme digestion. The tissue was cut into 1 mm cube pieces and digested with trypsin/EDTA (Gibco, NY, United States) and collagenase II (Sigma, United States) at 37°C. The mixture (collagenase and trypsin, 100: 1) was placed in a shaker at 37°C for 20 min, and the supernatant was collected and combined with DMEM containing 10% FBS, and this process was repeated until the tissue was fully digested. Cardiomyocytes were separated from CFs using centrifugation at a low speed (300 g), and the supernatant containing CFs was collected. The isolated CFs were cultured in DMEM supplemented with 10% FBS and 1% penicillin–streptomycin at 37°C in a humidified incubator with 5% CO_2. Cells were divided into four groups: 1) a control group, in which CFs were incubated with DMEM containing 5.5 mmol/L normal glucose for 48 h; 2) an HG group, in which CFs were incubated with DMEM containing 33 mmol/L glucose (HG) for 48 h; 3) an HG + si-NC group, in which CFs were transfected with si-NC and cultured under HG conditions for 48 h; and 4) an HG + si-SGLT1 group, in which CFs were transfected with si-SGLT1 and cultured under HG conditions for 48 h.

Small Interference RNA Transfection

To knockdown the expression of SGLT1 in CFs, small interfering RNAs against the SGLT1 gene (si-SGLT1) and the siRNA negative control (si-NC) were synthesized at Guangzhou RiboBio Co., Ltd. Briefly, CFs grown to 70–80% confluence were incubated with Lipofectamine 3,000 transfection reagent (Invitrogen, Waltham, MA, United States) loaded with siRNAs for 48 h. Transfection efficiency was evaluated using RT-qPCR analysis.

Cell Counting Kit-8 Assay

CFs were cultured in 96-well plates at a density of 1×10^4 cells/well. A 10 μl aliquot of the Cell Counting Kit-8 (MCE, Shanghai, China) solution was added to each well, and the plates were incubated at 37°C for 1 h. Absorbance was measured using a microplate reader (Molecular Devices, CA) at 450 nm.

Wound Scratch Assay

Cells were grown to 90% confluence in 6-well plates in DMEM supplemented with 10% FBS, and the medium was replaced with serum-free DMEM to starve cells for 24 h. Wounds were made with a sterile 200 μl pipette tip by drawing a line through the plated cells perpendicular to the abovementioned line. CFs were transfected with si-NC or si-SGLT1 and exposed to HG conditions for 24 h. Images were acquired using a Leica microscope (DM 2000, Leica, Wetzlar, Germany).

Western Blotting

Protein samples from CFs and cardiac tissues were extracted using the RIPA buffer, and equal amounts of proteins from each group were separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Then, the separated proteins were transferred to polyvinylidene fluoride membranes and blocked using 5% nonfat milk for 1 h at room temperature. Subsequently, the membranes were incubated with primary antibodies at 4°C overnight, followed by incubation with horseradish peroxidase– (HRP–) conjugated goat anti-rabbit secondary antibody (1:5,000, Santa Cruz) for 1 h at room temperature. The following primary antibodies were purchased from Abcam and used at a 1:1,000 dilution: β-actin (ab5694), SGLT1 (ab14686), collagen I (ab34710), and collagen III (ab6310). The following antibodies were purchased from Cell Signaling Technology and used at a 1:1,000 dilution: ERK1/2 (cat. 4695), phospho-ERK1/2 (cat. 4376), p38 (cat. 8690), and phospho-p38 (cat. 4511).

RNA Analysis

TRIzol reagent (Invitrogen, Carlsbad, CA, United States) was used to extract RNA from cardiac tissues, and cDNA was synthesized using a PrimeScript Reverse Transcription Reagent Kit (Takara, Otsu, Japan). RT-PCR was performed using a SYBR Premix Ex Taq Kit (Takara). The following primer sequences were used: SGLT1 forward, 5′-GGACAGTAGCACCTTGAGC-3′; reverse, 5′-CCAACAGTCCCACGATTAG-3′; β-actin forward, 5′-CCAGATCATGTTTGAGACCT-3′; and reverse, 5′-TCTCTTGCTCGAAGTCTAGG-3′. All reactions were performed in triplicate.

Animal Experiments

Sprague Dawley rats were obtained from the Nanjing Biomedical Research Institute of Nanjing University (China). A total of 24 6-week-old male SD rats were assigned to four groups (control, STZ, si-NC, and si-SGLT1 groups) using the random number method, with six rats in each group. A 12-h light–dark cycle was used, and the rats were provided *ad libitum* access to food and water. After acclimation for 1 week, rats in the diabetes groups were fed a high-fat diet (fat provided 60% of total calories, Research Diet D12492) for 4 weeks and then intraperitoneally injected with 60 mg/kg STZ (Sigma) dissolved in a citrate buffer (pH 4.5), whereas the control group received normal chow. We performed intraperitoneal glucose tolerance tests (IPGTTs) to identify the insulin-resistant rats, and fasting blood glucose (FBG) levels were measured seven days after injection. Body weights were recorded. Successful induction of diabetes was defined by an FBG value higher than 16.7 mmol (Feng et al., 2019). After successful establishment of the rat model with DCM, the rats in the si-NC and si-SGLT1 groups were injected with 5 μl of siRNA or 5 μl of si-SGLT1 (200 nmol/500 g) in PBS, once a week. All rats were sacrificed after 16 weeks of feeding. The left ventricular tissues were removed and cut into pieces for histomorphological analysis.

ELISA

After the rats were fasted overnight, blood samples were obtained from the postcaval vein and processed for plasma extraction within 1 h (centrifuged at 3,000 x g for 10 min at 4°C), and the plasma was stored at −80°C in polypropylene tubes for further analysis. The expression levels of collagen I, collagen III, and transforming growth factor-β1 (TGF-β1) in CFs and rat serum were detected using the rat collagen I Type I ELISA Kit (abx052369, Abbexa, United Kingdom), rat collagen type III ELISA Kit (abx573727, Abbexa), and TGF-β1 ELISA Kit (PT878, Beyotime, Jiangsu, China), respectively, following the instructions of the manufacturer.

Histology

Tissues from rats were fixed using 10% buffered formalin, dehydrated, embedded in paraffin, and sectioned into 5 μm-thick sections. Hematoxylin and eosin (HE) staining was used to assess cardiac injury, whereas Masson's trichrome staining was used to detect collagen fibers, and the slides were observed under an optical microscope. For immunohistochemistry, sections were stained with a primary antibody against SGLT1 (1:200, Abcam) and then stained with a secondary antibody. After washing with PBS, the slides were incubated with 3,3′-diaminobenzidine. The detailed procedure has been described previously (Lin et al., 2019). Semiquantitative analysis was performed using image analysis software (Image-Pro Plus, Media Cybernetics).

Wheat Germ Agglutinin Staining

Slides were stained with Alexa Fluor 488–conjugated wheat germ agglutinin WGA (Sigma). In brief, slides were dewaxed, rehydrated, and blocked with 3% BSA for 20 min. The slides were then incubated in WGA solubilized in PBS for 30 min at room temperature in the dark. After washing with PBS, the sections were stained with DAPI (Invitrogen) for 5 min and images were acquired using a Nikon Eclipse Ti-U fluorescence microscope (Minato-ku, Tokyo, Japan). The cardiomyocyte size was determined by dividing the total area by the number of cardiomyocytes using Image J software (NIH, Bethesda, MD, Unites States).

Statistical Analysis

The experimental data are expressed as the mean ± standard deviation. All statistical analyses were performed using GraphPad Prism 8.0 software (GraphPad Software, San Diego, CA, United States). Data are presented as the mean ± standard deviation. The *t*-test was used to perform comparisons between the two different groups. One-way analysis of variance was used to compare multiple groups. Statistical significance was set at $p < 0.05$.

RESULTS

SGLT1 Is Upregulated in Diabetic Cardiac Tissues and High-Glucose–Induced Cardiac Fibroblasts

The results of IPGTT revealed that blood glucose levels peaked half an hour after intraperitoneal injection, slowly decreased thereafter, and remained at 6–8 mmol/L in the control group

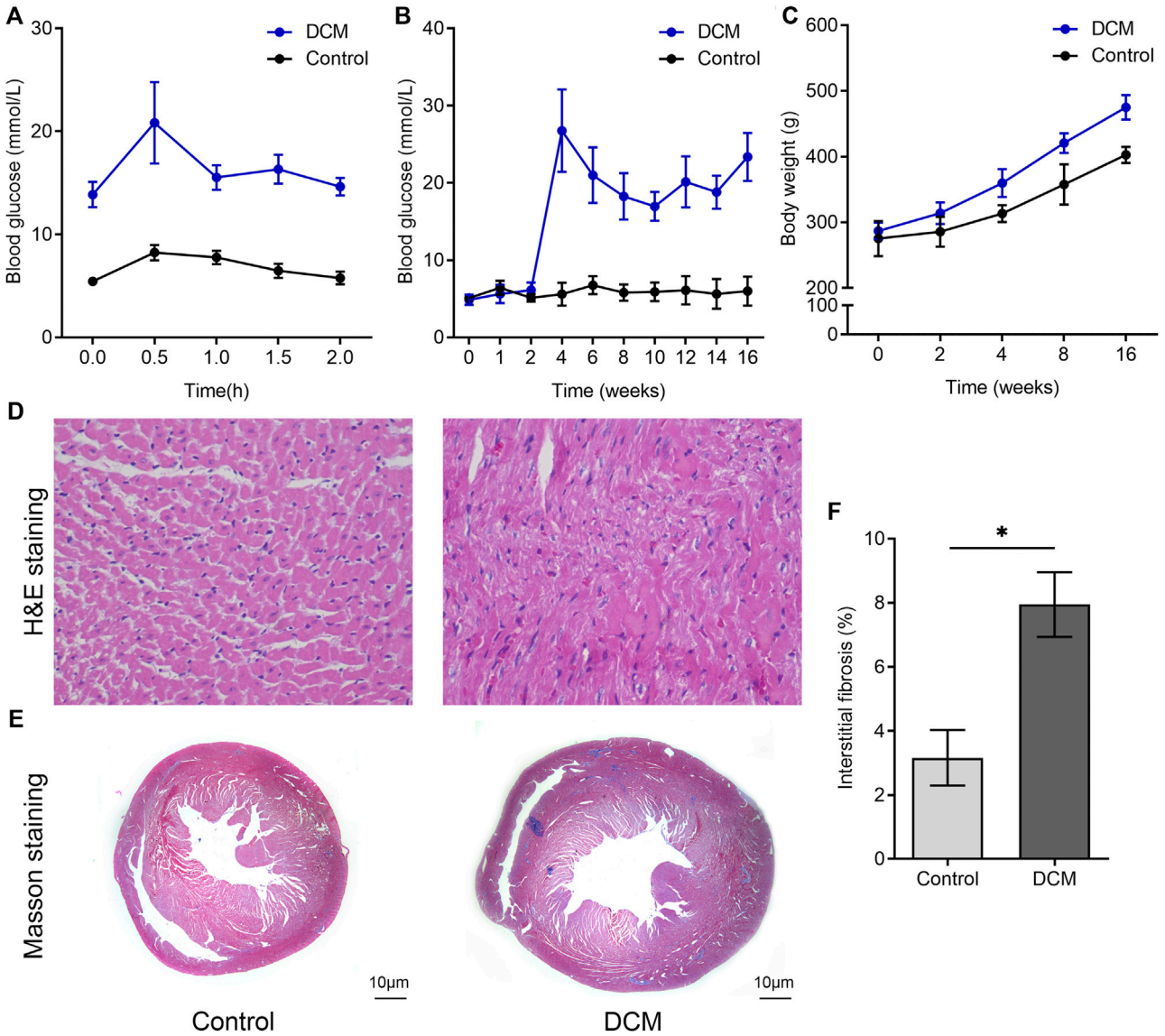

FIGURE 1 | Changes of blood glucose level and histopathology in DCM rats. SD rats were fed a high-fat diet (HFD) for 4 weeks before streptozotocin (STZ) was injected intraperitoneally at a dose of 60 mg/kg, and rats were fed for another 12 weeks. **(A)** Intraperitoneal glucose tolerance tests (IPGTTs) were used to identify insulin resistance in the two groups. **(B)** The FBG levels of the two groups measured at indicated times. **(C)** The body weight of the two groups was monitored at the indicated time. The paraffin sections of rat left ventricular tissues after **(D)** hematoxylin and eosin (H&E) staining and **(E)** Masson's trichrome staining. **(F)** Quantitative analysis of interstitial Masson's trichrome staining (n = 6 per group).

throughout the entire process. In contrast, the blood glucose levels of diabetic rats demonstrated evident hyperglycemia throughout the entire process (**Figure 1A**). **Figure 1B** shows that rats in the control group had a relatively stable FBG level, whereas the FBG level of the DCM group increased significantly after injection with STZ in the fourth week ($p < 0.05$). The body weight of the DCM group was higher than that of the control group (**Figure 1C**). HE and Masson staining were performed to examine the changes in cardiac pathology (**Figures 1D,E**). Increased interstitial fibrotic areas were observed in the DCM group compared to the NC group (**Figure 1F**).

We then validated that SGLT1 mRNA and protein levels were both upregulated in the DCM group compared to those in the control group (**Figures 2A,B**). Moreover, using immunohistochemistry, we found that CFs in diabetic rats expressed higher SGLT1 levels than those in normal rats (**Figures 2C,D**). In contrast to the control group, diabetic rats showed a significant increase in fibrosis-related proteins, including collagen I and collagen III expression (**Figures 2C,E**). *In vitro*, CFs changed from a long and thin shape to fusiform under HG conditions (**Figure 2F**), and the results of immunofluorescence revealed that the fluorescence intensity of SGLT1 was markedly increased in the HG medium (**Figure 2G**).

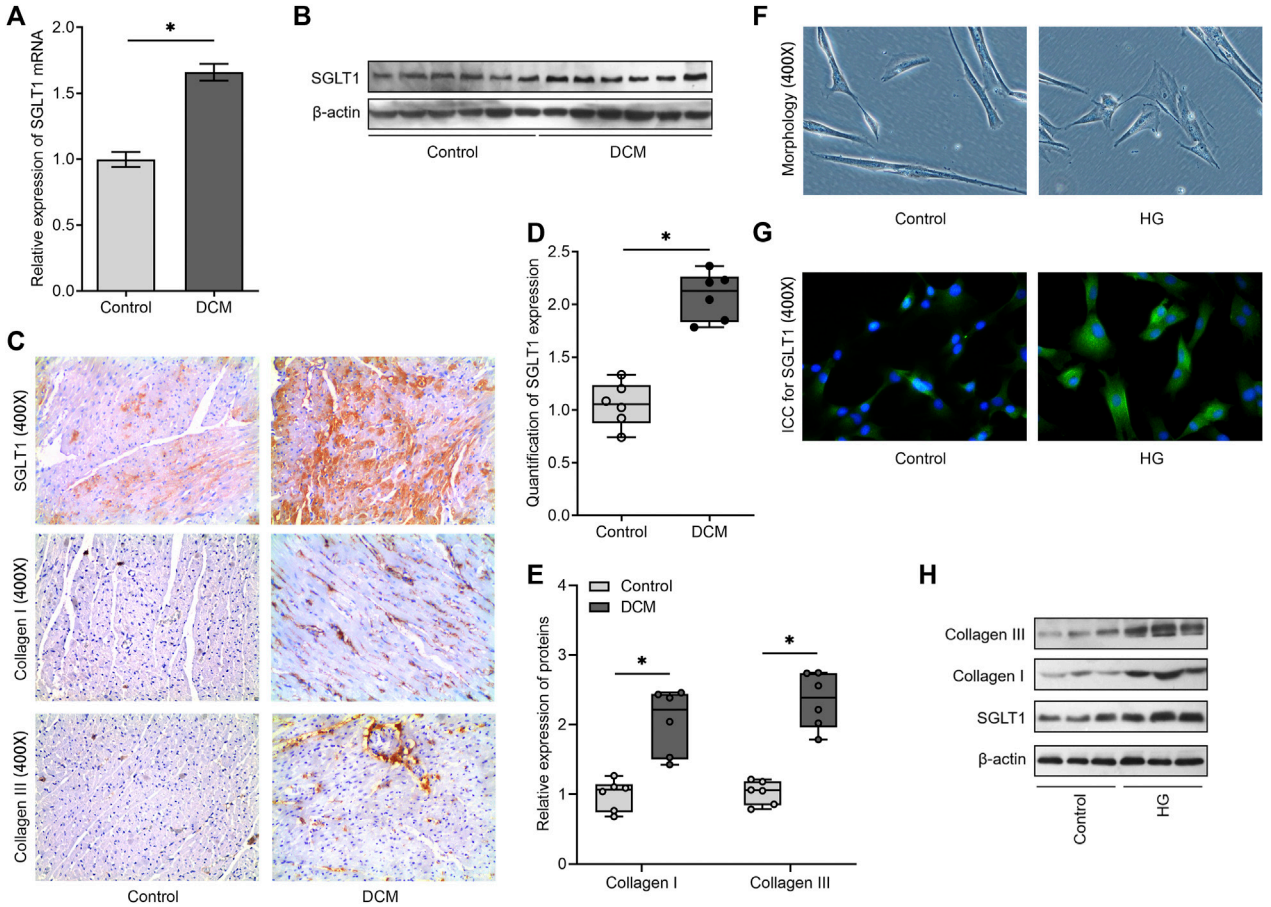

FIGURE 2 | SGLT1 is downregulated in the experimental diabetic cardiac fibrosis model and high-glucose–induced CFs. **(A)** The mRNA level of SGLT1 was detected using RT-qPCR ($n = 6$). **(B)** The protein level of SGLT1 was analyzed using western blot analysis ($n = 6$). **(C)** The protein levels of SGLT1, collagen I, and collagen III were evaluated using immunohistochemical staining. Relative expression of SGLT1 **(D)** and collagen I and collagen III **(E)** was found using semiquantitative analysis based on immunohistochemistry. **(F)** The morphology of CFs with or without high-glucose stimulation was imaged under an optical microscope. **(G)** The protein level of SGLT1 was analyzed by immunofluorescent staining in high-glucose–induced CFs. **(H)** The protein levels of SGLT1, collagen I, and collagen III were analyzed using western blot analysis in high-glucose–induced CFs ($n = 3$).

Accordingly, western blotting analysis revealed that compared to the control group, the levels of SGLT1, collagen I, and collagen III proteins were significantly upregulated in the HG group (**Figure 2H**).

Knockdown of SGLT1 Inhibits High-Glucose–Induced Cardiac Fibroblast Activation

To investigate the role of SGLT1 in CF activation, we knocked down SGLT1 by transfecting CFs with specific siRNAs against SGLT1. The results of RT-qPCR (**Figure 3A**) and western blotting (**Figure 3B**) analysis validated that SGLT1 si-RNA2 exerted the highest knockdown efficiency and was, therefore, chosen to perform the subsequent assays. Subsequent characterization of the CFs showed that HG stimulation significantly increased cell viability (**Figure 3C**) and migration (**Figures 3D,E**). We found that CFs with SGLT1 inhibition had markedly reduced cell viability and migration compared with those in the HG + si-NC group (**Figures 3C–E**). Furthermore, ELISA revealed that HG stimulation caused high expression of TGF-β1, collagen I, and collagen III, indicating the synthesis of collagen in CFs, whereas the inhibition of SGLT1 effectively reversed this increase (**Figures 3F–H**). We further analyzed the potential involvement of the p38 mitogen-activated protein kinase (MAPK) and ERK1/2 signaling pathways in the regulatory role of SGLT1 in HG-mediated CF activation. As expected, HG significantly activated the phosphorylation of p38 MAPK and ERK1/2, whereas SGLT1 silencing reduced the effects of HG (**Figure 3I**). Therefore, these data indicate that SGLT1 regulates the function of CFs.

Sodium–Glucose Cotransporter 1 Regulates p38 MAPK and ERK1/2 Signaling and Collagen Synthesis in Cardiac Fibroblasts

We further investigated the potential mechanism underlying the involvement of SGLT1 in the activation of CFs. SGLT1 overexpression was achieved by transfecting CFs with a

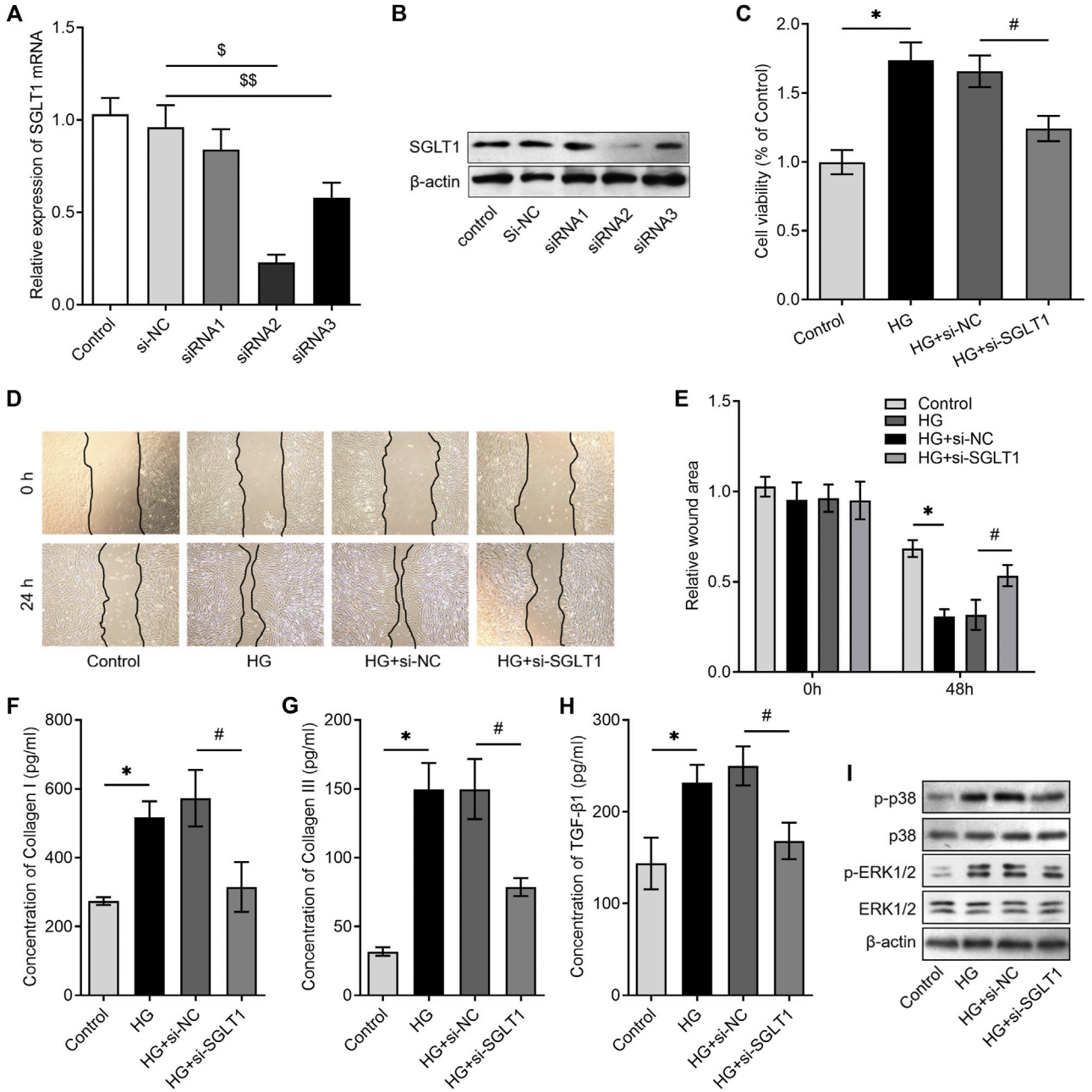

FIGURE 3 | Knockdown of SGLT1 inhibited high-glucose–induced CF activation. CFs were transfected with SGLT1 siRNAs, and SGLT1 mRNA and protein levels were detected using RT-qPCR **(A)** and western blotting, respectively **(B)**. CCK-8 assay was used to detect the proliferation of CFs under high-glucose condition with or without SGLT1 inhibition. **(D, E)** The representative images of the wound-healing assay were obtained at 0 and 24 h after knockdown of SGLT1, and the migrative ability of CFs was compared. **(F–H)** ELISA was used to detect the levels of collagen-synthesis–related markers, including TGF-β1, collagen I, and collagen III in the cell supernatant (n = 6). **(I)** Western blotting analysis was performed to investigate the phosphorylation levels of p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK)1/2 in CFs under high-glucose condition with or without SGLT1 inhibition.

plasmid containing the SGLT1 gene, and RT-qPCR and western blotting analysis revealed significantly higher SGLT1 levels in the SGLT1-transfected group when compared with the control group (**Figures 4A,B**). Interestingly, we found that the overexpression of SGLT1 significantly increased the proliferation of CFs under both normal and HG conditions (**Figure 4C**). In addition, the overexpression of SGLT1 effectively promoted the secretion of TGF-β1, collagen I, and collagen III in the cell supernatant, under

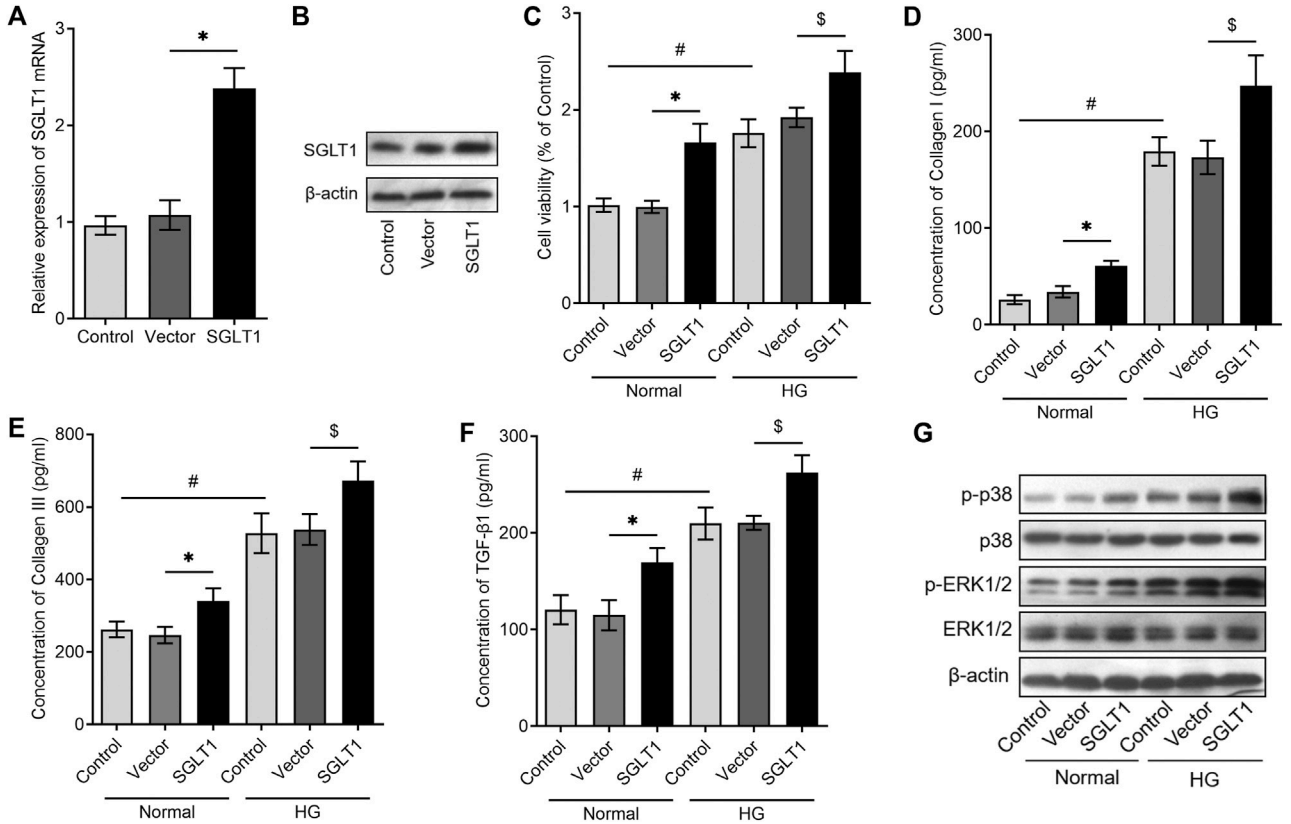

FIGURE 4 | SGLT1 overexpression promotes collagen release *via* the p38 and ERK1/2 signaling pathway in CFs. CFs were transfected with an SGLT1 plasmid or vector, and SGLT1 expression levels were detected using RT-qPCR **(A)** or western blotting analysis **(B)**. **(C)** After overexpression of SGLT1 in CFs, CCK-8 assay was used to detect the proliferation of CFs under normal or high-glucose condition ($n = 6$). **(D–F)** After overexpressing SGLT1 in CFs, the collagen-synthesis–related markers, including TGF-β1, collagen I, and collagen III, in the cell supernatant were measured using ELISA ($n = 6$). **(G)** After overexpressing SGLT1 in CFs, the protein levels of phosphorylated p38 and p-ERK1/2 were analyzed using western blotting in CFs both under normal or high-glucose condition ($n = 3$).

both normal and HG conditions (**Figures 4D–F**). Furthermore, overexpression of SGLT1 significantly increased p38 and p-ERK1/2 levels under both normal and HG conditions (**Figure 4G**). These data suggest that SGLT1 might activate CFs by activating the p38 and p-ERK1/2 pathways.

Sodium–Glucose Cotransporter 1 Inhibition Alleviates Fibrosis in the Diabetic Heart

Since cardiac fibrosis and hypertrophy are important pathological structural features of DCM, we investigated whether SGLT1 regulates fibrosis and hypertrophy in diabetic hearts. We knocked down SGLT1 in rats with DCM by continuously administering si-SGLT1 *via* intravenous injection in the tail vein. As shown in **Figures 5A,B**, the expression of SGLT1 mRNA and protein in the heart was reduced in the DCM + si-SGLT1 group when compared with that in the DCM + si-NC group. Accordingly, the immunohistochemistry results indicated that CFs expressed low SGLT1 levels after SGLT1 inhibition (**Figure 5C**). Interestingly, compared with the DCM + si-NC group, a significant reduction in interstitial fibrosis was observed in the DCM + si-SGLT1 group (**Figure 5D**). The hallmarks of fibrosis and protein levels of collagen I and collagen III were reduced by SGLT1 knockdown (**Figures 5E,F**). However, SGLT1 knockdown had no significant effect on cardiac hypertrophy, as examined by WGA staining (**Figure 5G**). Collectively, our results indicated that the knockdown of SGLT1 reduced cardiac fibrosis but had no effect on cardiac hypertrophy in DCM.

DISCUSSION

Increasing attention has been placed on the utility of SGLT2 inhibitors because, in addition to controlling blood glucose levels, they have been shown to provide significant cardiovascular benefits in T2DM patients (Packer et al., 2021). Rather than SGLT2, recent findings have also emphasized the potential role of SGLT1 in the development of cardiovascular diseases. The myocardial expression of SGLT1 in humans is altered in various cardiovascular disease states. Compared with controls, left ventricular SGLT1 mRNA and protein expression was significantly upregulated in heart failure patients with DCM (Sayour et al., 2020). Individuals carrying loss-of-function mutations in the SGLT1 gene are estimated to have a lower

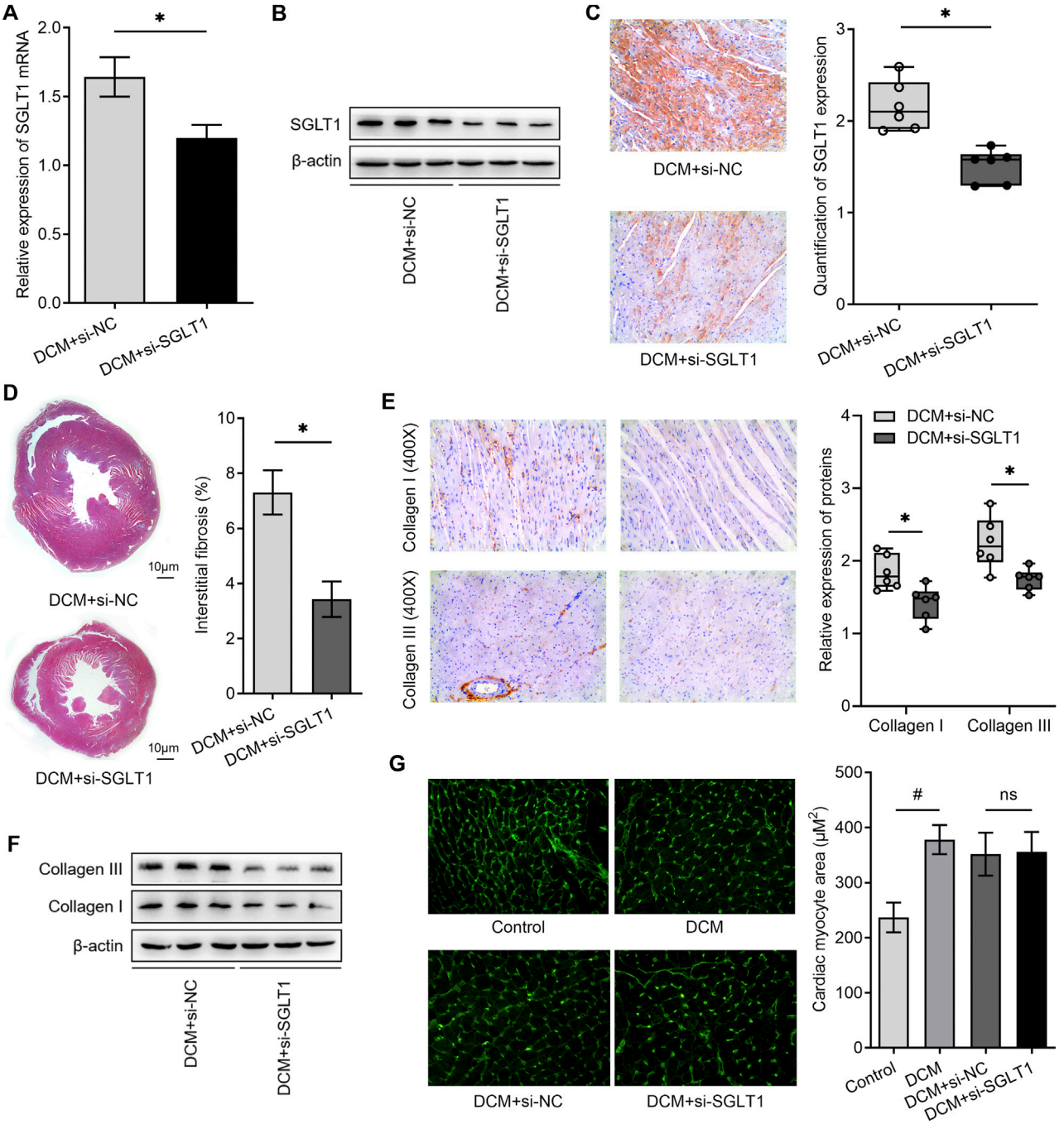

FIGURE 5 | SGLT1 inhibition alleviated myocardial fibrosis and apoptosis in diabetic rat hearts (n = 6). **(A, B)** Knockdown of SGLT1 was achieved by continuous intravenous injection into the tail vein with specific SGLT1 siRNA in DCM rats, and the knockdown efficiency was identified by detecting SGLT1 expression in myocardial tissues using RT-qPCR and western blotting analysis. **(C)** SGLT1 protein levels in myocardial tissue were measured by immunohistochemistry staining. **(D)** Masson's trichrome staining of myocardial tissue shows interstitial fibrosis in DCM rats treated with si-NC or si-SGLT1. **(E, F)** Relative protein levels of collagen I and collagen III in myocardial tissue were measured using immunohistochemical staining and western blotting analysis (n = 6). **(G)** Wheat germ agglutinin staining and quantitation of myocardial cell size in DCM rats treated with si-NC or si-SGLT1 (n = 6).

risk of developing heart failure, driven by mitigation of postprandial hyperglycemic episodes (Seidelmann et al., 2018). In endothelial cells, angiotensin II upregulates SGLT1 expression to promote sustained oxidative stress, and inhibition of SGLT1 appears to be an attractive strategy to enhance protective endothelial function (Park et al., 2021). Apart from cardiomyocytes and endothelial cells in the heart, we first identified that SGLT1 was expressed in human CFs (Meng

et al., 2018), and in the present study, the significant finding was that an increase in SGLT1 expression in rat hearts triggered the development of cardiac fibrosis through activation of CFs by upregulating the p38 MAPK and ERK1/2 signaling pathways.

Targeting SGLT1 has also been found to have cardioprotective effects in DCM. RNA-mediated inhibition of SGLT1 gene glycemic variability and cardiac damage were seen in type 2 diabetes mellitus mice *in vivo* (Sun et al., 2021). In cultured cardiomyocytes, SGLT1 knockdown restored cell proliferation, suppressed reactive oxygen species, and induced cytotoxicity (Chai et al., 2021). These data supported the notion that SGLT1 might serve as a target for myocardial injury in the diabetic heart. It is well known that CF activation plays an essential role during the development of cardiac fibrosis. However, the role of SGLT1 in CF activation remains unclear.

Several lines of experimental evidence suggest that SGLT1 silencing may attenuate cardiac fibrosis. Ramratnam et al. (2014) demonstrated that cardiac overexpression of SGLT1 increases collagen I gene expression and interstitial fibrosis in mouse hearts. Another study found that SGLT1 knockout downregulated CTGF and collagen I gene expression and interstitial fibrosis in pressure-overload–increased mouse hearts (Matsushita et al., 2018). Similar to these studies, we also found that knockdown of SGLT in diabetic hearts suppressed the synthesis of TGF-β1, collagen I, and collagen III. Furthermore, in cultured CFs, we found that SGLT1 regulates cell proliferation and collagen synthesis, suggesting the role of SGLT1 in regulating CF activation. To the best of our knowledge, this study is the first to demonstrate that SGLT1 regulates the activation of CFs in DCM.

Our *in vitro* experiments showed that HG upregulated SGLT1 expression in CFs, which was accompanied by an increase in the abundance of p-p38 and p-ERK1/2. SGLT1 overexpression significantly induced the abundance of these proteins in CFs under both normal and HG conditions. TGF-β1 stimulation in CFs resulted in increased proliferation, increased collagen I and collagen III expression, and increased p38 and ERK1/2 phosphorylation (Xu et al., 2017), whereas inhibition of the activation of p38 kinase and ERK1/2 could effectively attenuate cardiac fibrosis (Tao et al., 2016). Activation of MAPKs participates in the upregulation of cerebral SGLT-1 expression (Yamazaki et al., 2018). Moreover, the relationship between the SGLT1 and MAPK signaling pathways in the heart has also been reported in our previous study (Lin et al., 2021). Based on the abovementioned results, we deduced that the increase in SGLT1 expression in the diabetic heart is involved in triggering CF proliferation and subsequent cardiac fibrosis.

Furthermore, we noticed that the study performed by Matsushita et al. suggested that SGLT1 knockout could prevent chronic pressure-overload–induced hypertrophic cardiomyopathy (Matsushita et al., 2018). However, in our study, we found that knockdown of SGLT1 had no effect on hyperglycemia-related hypertrophy in diabetic hearts. This discrepancy may be because of the differences in experimental animal models. We used SD rats to establish a DCM model, and Matsushita et al. (2018) used mice that underwent transverse aortic constriction surgery. The other significant difference is that we only knocked down SGLT1 in rats using specific siRNA, rather than using gene knockout technology. A previous study suggested that SGLT1-deficient mice need to consume a glucose–galactose-free diet because they show symptoms of glucose–galactose malabsorption syndrome (Gorboulev et al., 2012). Therefore, SGLT1 knockout may not be appropriate in DCM. More studies are needed to investigate the exact role of SGLT1 in cardiac hypertrophy.

In summary, our study evaluated the changes in the expression of SGLT1 in the progression of diabetic cardiac fibrosis and identified a significant increase in SGLT1 expression in the diabetic heart. SGLT1 is involved in cardiac fibrosis via the p38 and ERK1/2 signaling pathways. Our findings suggest that SGLT1 is a potential therapeutic target for the prevention of diabetic cardiac fibrosis.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by the Medicine Animal Welfare Committee of Shaoxing People's Hospital.

AUTHOR CONTRIBUTIONS

LM and HU designed the project; HL and LG performed animal experiments and analyzed the data; LM and HL performed *in vitro* experiments and wrote the draft manuscript; HG supervised and funded the project; and HU and HG made the modification.

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REFERENCES

Bugger, H., and Abel, E. D. (2014). Molecular Mechanisms of Diabetic Cardiomyopathy. *Diabetologia* 57 (4), 660–671. doi:10.1007/s00125-014-3171-6

Chai, Q., Miao, J., Liu, M., Zhang, Z., Meng, Z., and Wu, W. (2021). Knockdown of SGLT1 Prevents the Apoptosis of Cardiomyocytes Induced by Glucose Fluctuation Via Relieving Oxidative Stress and Mitochondrial Dysfunction. *Biochem. Cell Biol.* 99 (3), 356–363. doi:10.1139/bcb-2020-0491

Feng, Y., Xu, W., Zhang, W., Wang, W., Liu, T., and Zhou, X. (2019). LncRNA DCRF Regulates Cardiomyocyte Autophagy by Targeting miR-551b-5p in Diabetic Cardiomyopathy. *Theranostics* 9 (15), 4558–4566. doi:10.7150/thno.31052

Frangogiannis, N. G. (2021). Cardiac Fibrosis. *Cardiovasc Res.* 117 (6), 1450–1488. doi:10.1093/cvr/cvaa324

Gorboulev, V., Schürmann, A., Vallon, V., Kipp, H., Jaschke, A., Klessen, D., et al. (2012). Na+-D-glucose Cotransporter SGLT1 Is Pivotal for Intestinal Glucose Absorption and Glucose-dependent Incretin Secretion. *Diabetes* 61 (1), 187–196. doi:10.2337/db11-1029

Gyimesi, G., Pujol-Giménez, J., Kanai, Y., and Hediger, M. A. (2020). Sodium-coupled Glucose Transport, the SLC5 Family, and Therapeutically Relevant Inhibitors: from Molecular Discovery to Clinical Application. *Pflugers Arch. - Eur. J. Physiol.* 472 (9), 1177–1206. doi:10.1007/s00424-020-02433-x

Hirose, M., Matsushita, N., Ishida, N., Ibi, M., and Saito, M. (2018). Roles of Sodium-Glucose Cotransporter 1 (SGLT1) in the Induction of Cardiac Remodeling. *Yakugaku Zasshi* 138 (7), 939–943. doi:10.1248/yakushi.17-00223-2

Lin, H., Zhang, J., Ni, T., Lin, N., Meng, L., Gao, F., et al. (2019). Yellow Wine Polyphenolic Compounds Prevents Doxorubicin-induced Cardiotoxicity through Activation of the Nrf2 Signalling Pathway. *J. Cel Mol Med* 23 (9), 6034–6047. doi:10.1111/jcmm.14466

Lin, N., Lin, H., Yang, Q., Lu, W., Sun, Z., Sun, S., et al. (2021). SGLT1 Inhibition Attenuates Apoptosis in Diabetic Cardiomyopathy via the JNK and P38 Pathway. *Front. Pharmacol.* 11, 598353. doi:10.3389/fphar.2020.598353

Matsushita, N., Ishida, N., Ibi, M., Saito, M., Sanbe, A., Shimojo, H., et al. (2018). Chronic Pressure Overload Induces Cardiac Hypertrophy and Fibrosis via Increases in SGLT1 and IL-18 Gene Expression in Mice. *Int. Heart J.* 59 (5), 1123–1133. doi:10.1536/ihj.17-565

Meng, L., Uzui, H., Guo, H., and Tada, H. (2018). Role of SGLT1 in High Glucose Level-Induced MMP-2 Expression in Human Cardiac Fibroblasts. *Mol. Med. Rep.* 17 (5), 6887–6892. doi:10.3892/mmr.2018.8688

Packer, M., Anker, S. D., Butler, J., Filippatos, G., Ferreira, J. P., Pocock, S. J., et al. (2021). Empagliflozin in Patients with Heart Failure, Reduced Ejection Fraction, and Volume Overload. *J. Am. Coll. Cardiol.* 77 (11), 1381–1392. doi:10.1016/j.jacc.2021.01.033

Park, S. H., Belcastro, E., Hasan, H., Matsushita, K., Marchandot, B., Abbas, M., et al. (2021). Angiotensin II-Induced Upregulation of SGLT1 and 2 Contributes to Human Microparticle-Stimulated Endothelial Senescence and Dysfunction: Protective Effect of Gliflozins. *Cardiovasc. Diabetol.* 20 (1), 65. doi:10.1186/s12933-021-01252-3

Ramratnam, M., Sharma, R. K., D'Auria, S., Lee, S. J., Wang, D., Huang, X. Y., et al. (2014). Transgenic Knockdown of Cardiac Sodium/glucose Cotransporter 1 (SGLT1) Attenuates PRKAG2 Cardiomyopathy, whereas Transgenic Overexpression of Cardiac SGLT1 Causes Pathologic Hypertrophy and Dysfunction in Mice. *J. Am. Heart Assoc.* 3 (4), e000899. doi:10.1161/jaha.114.000899

Sano, R., Shinozaki, Y., and Ohta, T.. Sodium-glucose Co-transporters: Functional Properties and Pharmaceutical Potential. *J. Diabetes Investig.*, 2020.11(4): 770–782. doi: doi:10.1111/jdi.13255

Sawa, Y., Saito, M., Ishida, N., Ibi, M., Matsushita, N., Morino, Y., et al. (2020). Pretreatment with KGA-2727, a Selective SGLT1 Inhibitor, Is Protective against Myocardial Infarction-Induced Ventricular Remodeling and Heart Failure in Mice. *J. Pharmacol. Sci.* 142 (1), 16–25. doi:10.1016/j.jphs.2019.11.001

Sayour, A. A., Oláh, A., Ruppert, M., Barta, B. A., Horváth, E. M., Benke, K., et al. (2020). Characterization of Left Ventricular Myocardial Sodium-Glucose Cotransporter 1 Expression in Patients with End-Stage Heart Failure. *Cardiovasc. Diabetol.* 19 (1), 159. doi:10.1186/s12933-020-01141-1

Seferovic, P. M., and Paulus, W. J. (2015). Clinical Diabetic Cardiomyopathy: a Two-Faced Disease with Restrictive and Dilated Phenotypes. *Eur. Heart J.* 36 (27), 1718–1727. 1727a-1727c. doi:10.1093/eurheartj/ehv134

Seidelmann, S. B., Feofanova, E., Yu, B., Franceschini, N., Claggett, B., Kuokkanen, M., et al. (2018). Genetic Variants in SGLT1, Glucose Tolerance, and Cardiometabolic Risk. *J. Am. Coll. Cardiol.* 72 (15), 1763–1773. doi:10.1016/j.jacc.2018.07.061

Song, P., Onishi, A., Koepsell, H., and Vallon, V. (2016). Sodium Glucose Cotransporter SGLT1 as a Therapeutic Target in Diabetes Mellitus. *Expert Opin. Ther. Targets* 20 (9), 1109–1125. doi:10.1517/14728222.2016.1168808

Sun, Z., Chai, Q., Zhang, Z., Lu, D., Meng, Z., and Wu, W. (2021). Inhibition of SGLT1 Protects against Glycemic Variability-Induced Cardiac Damage and Pyroptosis of Cardiomyocytes in Diabetic Mice. *Life Sci.* 271, 119116. doi:10.1016/j.lfs.2021.119116

Tao, L., Bei, Y., Chen, P., Lei, Z., Fu, S., Zhang, H., et al. (2016). Crucial Role of miR-433 in Regulating Cardiac Fibrosis. *Theranostics* 6 (12), 2068–2083. doi:10.7150/thno.15007

Wang, L., Cai, Y., Jian, L., Cheung, C. W., Zhang, L., and Xia, Z. (2021). Impact of Peroxisome Proliferator-Activated Receptor-α on Diabetic Cardiomyopathy. *Cardiovasc. Diabetol.* 20 (1), 2. doi:10.1186/s12933-020-01188-0

Wood, I. S., and Trayhurn, P. (2003). Glucose Transporters (GLUT and SGLT): Expanded Families of Sugar Transport Proteins. *Br. J. Nutr.* 89 (1), 3–9. doi:10.1079/bjn2002763

Xu, Y., Xiao, H., Luo, H., Chen, Y., Zhang, Y., Tao, L., et al. (2017). Inhibitory Effects of Oxymatrine on TGF-B1-Induced Proliferation and Abnormal Differentiation in Rat Cardiac Fibroblasts via the p38MAPK and ERK1/2 Signaling Pathways. *Mol. Med. Rep.* 16 (4), 5354–5362. doi:10.3892/mmr.2017.7277

Yamazaki, Y., Arita, K., Harada, S., and Tokuyama, S. (2018). Activation of C-Jun N-Terminal Kinase and P38 after Cerebral Ischemia Upregulates Cerebral Sodium-Glucose Transporter Type 1. *J. Pharmacol. Sci.* 138 (4), 240–246. doi:10.1016/j.jphs.2017.02.016

Zhang, J., Zhang, M., Yang, Z., Huang, S., Wu, X., Cao, L., et al. (2020). PDCD4 Deficiency Ameliorates Left Ventricular Remodeling and Insulin Resistance in a Rat Model of Type 2 Diabetic Cardiomyopathy. *BMJ Open Diabetes Res. Care* 8 (1), e001081. doi:10.1136/bmjdrc-2019-001081

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Mitochondrial Arrest on the Microtubule Highway—A Feature of Heart Failure and Diabetic Cardiomyopathy?

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A pathophysiological consequence of both type 1 and 2 diabetes is remodelling of the myocardium leading to the loss of left ventricular pump function and ultimately heart failure (HF). Abnormal cardiac bioenergetics associated with mitochondrial dysfunction occurs in the early stages of HF. Key factors influencing mitochondrial function are the shape, size and organisation of mitochondria within cardiomyocytes, with reports identifying small, fragmented mitochondria in the myocardium of diabetic patients. Cardiac mitochondria are now known to be dynamic organelles (with various functions beyond energy production); however, the mechanisms that underpin their dynamism are complex and links to motility are yet to be fully understood, particularly within the context of HF. This review will consider how the outer mitochondrial membrane protein Miro1 (Rhot1) mediates mitochondrial movement along microtubules *via* crosstalk with kinesin motors and explore the evidence for molecular level changes in the setting of diabetic cardiomyopathy. As HF and diabetes are recognised inflammatory conditions, with reports of enhanced activation of the NLRP3 inflammasome, we will also consider evidence linking microtubule organisation, inflammation and the association to mitochondrial motility. Diabetes is a global pandemic but with limited treatment options for diabetic cardiomyopathy, therefore we also discuss potential therapeutic approaches to target the mitochondrial-microtubule-inflammatory axis.

Keywords: diabetic cardiomyopathy, heart failure, Miro1, microtubules, HDAC6, NLRP3, mitochondrial dysfunction, mitochondrial movement

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INTRODUCTION

Diabetic Mellitus (DM) remains a global epidemic, with an estimated 463 million cases worldwide in 2019, and is associated with marked morbidity and mortality rates (1). Diabetes is a major risk factor for heart failure (HF), with a three-fold higher prevalence for developing coronary artery disease, CAD (2, 3). Although, approximately 50 years ago, it was revealed that myocardial remodelling and dysfunction can also occur in diabetic patients in the absence of CAD, a condition termed diabetic cardiomyopathy (DCM) which commonly advances to HF (4, 5). Recently it has emerged that in addition to HF with reduced ejection fraction (HFrEF) roughly half of all HF cases can be classified as HF with preserved ejection fraction (HFpEF), which is less well-understood in comparison. Further, treatments developed to manage HFrEF have limited efficacy in HFpEF

patients; highlighting the need for a more detailed understanding of the disease mechanisms. Significantly, over a third of patients with HFpEF have type 2 diabetes (6, 7) indicating that the diabetic insult leads, at least initially, to the development of a distinct myocardial phenotype.

Mitochondrial dysfunction is a hallmark of HF (8) and more recently identified as a feature of HFpEF (9) and DCM (10). While mitochondrial dysfunction is a broad term a recurring feature of HF both in the presence and absence of diabetes, is morphological remodelling of mitochondria, with reports of both swelling and fragmentation [for a review see (11)]. Mitochondrial size, shape and distribution (factors dictating function) are regulated by mechanisms grouped under the umbrella term "mitochondrial dynamics," which also encompasses mitochondrial turnover, mitophagy and biogenesis, as reviewed by (12). Mitochondrial movement, although less well-studied, particularly in the heart, is driven by the outer mitochondrial membrane protein Miro1 (also termed RHOT1) and is also believed to be important for regulating and maintaining a healthy mitochondrial network (13).

DCM is a chronic low grade inflammatory condition, with inflammation associated with the pathogenesis of HF and linked to the development of mitochondrial dysfunction [as reviewed recently (14)]. Kaludercic and Di Lisa (15) provided an overview of a number of studies indicating that excessive ROS production due to mitochondrial dysfunction is a causative agent of increased expression and activity of the inflammasome, NLRP3. NLRP3 is a multimeric complex formed from NOD-like receptor 3, the apoptosis-associated speck-like protein containing (ASC) adaptor protein, and caspase-1, (16, 17). Although, details are somewhat sparse (and not within the setting of the heart or DCM) an association between proteins mediating mitochondrial dynamics and NLRP3 inflammasome assembly is also emerging (18, 19).

This review will summarise evidence for the emerging role of dysregulated mitochondrial movement in HF/DCM, the intersection with aberrant mitochondrial dynamics and NLRP3 activity focusing on the involvement of Miro1; as well as considering the putative mechanisms involved.

MITOCHONDRIAL DYNAMICS IN CARDIOVASCULAR HEALTH AND DISEASE

Previously perceived as "static" organelles within cardiomyocytes, mitochondria are now known to be highly dynamic undergoing restructuring *via* an equilibrium of fission and fusion (12). In brief, fission, mediated by the GTPase dynamin-related protein 1 (Drp1) and receptors Mitochondrial fission 1 protein (Fis1), Mitochondrial fission factor (MFF), and MiD49/51, leads to the division of a single mitochondrion into two, allowing removal of damaged mitochondria from the network. Whereas fusion, is the amalgamation of two mitochondria into one larger mitochondrion orchestrated by the outer mitochondrial membrane (OMM) proteins Mitofusin 1 (Mfn1) and Mitofusin 2 (Mfn2). Fusion of the inner mitochondrial membrane (IMM) next occurs and is regulated by Optic atrophy 1 (Opa1).

The dynamic nature of mitochondria underpins mitochondrial quality control that is, mitochondrial biogenesis (replacement with healthy mitochondria), maintenance and degradation (mitophagy). Over time mitochondria can accumulate damage as a result of multiple mutations in nuclear-encoded mitochondrial genes or oxidative damage *via* ROS formation as a by-product of OXPHOS. In brief, mitophagy, the removal of damaged mitochondria, is regulated by PTEN-induced kinase protein 1 (PINK1) which upon activation (*via* phosphorylation) recruits cytosolic Parkin (20, 21). Parkin selectively phospho-ubiquitinates the OMM proteins (including Mfn1/2) and facilitates the selective binding and extension of autophagosomes around damaged mitochondria, although details of the exact mechanisms involved remain incomplete (22).

Recently another method of "fusion" has been identified in cardiomyocytes *via* the formation of tubular protrusions known as nanotunnels, visualised by live-cell confocal imaging and electron microscopy (23, 24). Nanotunnels serve as a form of direct intercommunication between cardiac mitochondria over micron distances, which allows the exchange of matrix contents between non-adjacent mitochondria. Whether the frequency of nanotunnels is correlated to cell stress is not yet clear, although links to an imbalance in Ca^{2+} cycling has been implicated (25).

There is a plethora of studies, both clinical and preclinical, identifying decreased expression of fusion proteins and increased levels of fission proteins in the context of cardiovascular disease (26). For example, depressed levels of Mfn1/2 are a feature of human and rodent hearts with impaired contractility and mitochondrial dysfunction (27, 28). Ablation of Mfn1 and Mfn2 results in dilated cardiomyopathy, impaired mitochondrial respiration and mitochondrial fragmentation (29). Opa1 is also depressed in HF patients (30). Whereas, increased fission, fragmented mitochondria and cell death associated with Drp1 expression has been reported as a feature of HF (31). Consequently, research has focused upon developing pharmacological inhibitors of Drp1, for example, treatment of HL-1 cardiomyocytes with mitochondrial division inhibitor-1 is shown to be cardioprotective against ischemia/reperfusion injury (32). However, the Janus nature of Drp1-mediated pathways should not be ignored as in some conditions promotion of these pathways can be cardioprotective (33). Similarly, reduced levels of PINK1 and Parkin in the heart are also associated with ventricular hypertrophy, mitochondrial swelling (34) and disorganised mitochondria (35). Andres et al. (36) also reported how simvastatin provides cardioprotection by triggering Parkin-dependent mitophagy. A balance between mitochondrial fusion, fission and mitophagy is crucial for maintaining a healthy population of mitochondria.

MIRO1 PLAYS A CENTRAL ROLE GOVERNING MITOCHONDRIAL MOVEMENT

While Mfn1, Mfn2, Opa1 and Drp1 (and receptors) are essential for regulating mitochondrial size and shape, important for mitochondrial "quality control," the movement of mitochondria within the cell is also a crucial factor with distribution

tightly linked to cellular energy requirements. An elegant study from Bers and colleagues not only captured, using live imaging, fusion and fission events within cardiomyocytes but also tracked mitochondrial movement (37). Interestingly, the study showed that over a 1 h period the net movement of mitochondria between the sarcomeres (interfibrillar, IFM) was $<0.3\,\mu m$ compared to those adjacent to the nucleus (perinuclear, PNM) which traversed $2.8\,\mu m$; this difference in motility may be due to the IFM being more spatially restricted by the sarcomeric organisation.

Miro1 localised to the OMM, has been firmly established in neurons as central for regulating mitochondrial movement in response to temporal and spatial metabolic demands (13), with impaired mitochondrial trafficking associated with several neurodegenerative diseases (38). Miro1 is also highly expressed in the heart (39) and although less well-studied, knock-down of Miro1 in H9c2 cardiomyoblasts revealed a similar effect upon mitochondrial movement in a Ca^{2+} dependent manner (40). Interestingly, studies exploring mitochondrial transfer *via* transplantation of human induced pluripotent mesenchymal stem cells (iPSC-MSCs) for tissue regeneration in models of anthracycline-induced cardiomyopathy identify the intrinsically high Miro1 content of iPSC-MSCs as essential for facilitating mitochondrial relocation and improved cardiac bioenergetics (41). In contrast, a recent study suggested that knockdown of Miro1 in cultured neonatal cardiomyocytes (NRCMs) could be protective against phenylephrine-induced hypertrophy through attenuating mitochondrial fission (42). Whilst different cardiac pathologies likely require different approaches in terms of therapeutic targeting, NRCMs as a model system may not always be directly translatable to the mature cardiomyocyte in which mitochondria show a substrate preference for free fatty acids rather than pyruvate (product of glycolysis); Dorn et al. have proposed that after birth there is cell-wide replacement with "adult" mitochondria (43). It is noteworthy that Miro1 is also decreased in pancreatic cells of patients with type 2 diabetes, with a mouse model of islet Miro1 ablation developing insulin resistance, increased production of ROS, inflammation and dysregulated mitophagy (44). Evidence, mainly from studies of neuronal tissue (38), indicates that loss of Miro1 is a decisive factor leading to "arrested" motility and linked to the accumulation of damaged mitochondria.

A MIRO1-MACROMOLECULAR COMPLEX MOBILISES MITOCHONDRIA ALONG MICROTUBULES

Miro proteins bind to kinesin-1/KIF5 and Milton (also known as trafficking kinesin-binding protein 1, TRAK1 or OIP106) (39); interactions proposed to link mitochondria to the microtubule trafficking apparatus (45, 46). Miro1 also directly interacts with Mfn2 in neuronal cells, an association that is proposed as an essential step mediating mitochondrial movement (47) as shown in **Figure 1**; although, the molecular basis of this interaction remains unknown as is whether this association occurs in cardiomyocytes. Significantly, as discussed above, cardiac Mfn2 levels are reported to be down-regulated in models of DCM (48). How the loss of Mfn2 (which presumably leads to reduced Miro1-Mfn2 interactions) impacts mitochondrial movement and DCM linked phenotypic changes has yet to be clarified.

Ca^{2+} binding to Miro1, *via* two EF-hands within the primary sequence, elicits a conformational change, mediating the association and dissociation of the Miro1-complex assembly from the microtubules, MTs, (with detachment halting mitochondrial motility). This process underpins mitochondrial moment (**Figure 1**) (49). Aberrant Ca^{2+} homeostasis is a hallmark of HF in patients (50) and associated with DCM (51). Mitochondria play a central role in regulating cytosolic $[Ca^{2+}]$ and maintaining the cellular redox status (52). Organisation of mitochondria straddling either side of the dyad (formed by t-tubules, specialised regions of the sarcolemma, and junctional sarcoplasmic reticulum) is essential for Ca^{2+} uptake into the mitochondria for driving bioenergetics (52, 53). While HF is associated with displacement of mitochondria (54), how impaired Ca^{2+} cycling influences mitochondrial movement along microtubules has yet to be examined in detail. Importantly, the Bers group have shown in isolated cardiomyocytes that under stress conditions, causing mitochondrial damage, there is migration of IFM (fission products) *via* MTs to the perinuclear region where they undergo mitophagy (37).

MICROTUBULE ORGANISATION AND CARDIOVASCULAR FUNCTION AND DISEASE

The presence of MTs in cardiomyocytes has been known for many years (55). MTs are formed by the polymerisation of α/β-tubulin dimers assembling into rods roughly $\sim$ 25 nm in diameter which can extend up to tens of microns in length (56). Similar to mitochondria, MTs are organised into spatially distinct populations; (i) interfibrillar (55), (ii) those surrounding the nuclear envelope (57), and (iii) cortical MTs perpendicular to myofibrils (58). These differing populations of MTs are commonly believed to explain, in part, the diverse physiological roles of MTs in cardiomyocytes, for example, ion channel trafficking, mechanical signalling pathways fundamental for cardiac contractility and inter-organelle communication, as reviewed by Caporizzo et al. (59).

As also discussed in (59) there are numerous studies linking changes to MT properties to the development and progression of cardiovascular diseases. MT remodelling has been identified as the source of increased mechanical stiffness occurring in the early stages of diastolic dysfunction. Accordingly, there has been interest in the use of reagents that depolymerise MTs. Specifically, post-translational modification (PTM) (detyrosination) of MTs is associated with increased viscoelastic resistance in human failing hearts (60). Stiffening of the myocardium due to MT remodelling in the failing heart is also reported to displace mitochondria adjacent to the sarcolemma (subsarcolemmal mitochondria, SSM) triggering the propagation of abnormal Ca^{2+} transients, leading to arrhythmogenesis (8). However, since mitochondria have been demonstrated to provide a bridge

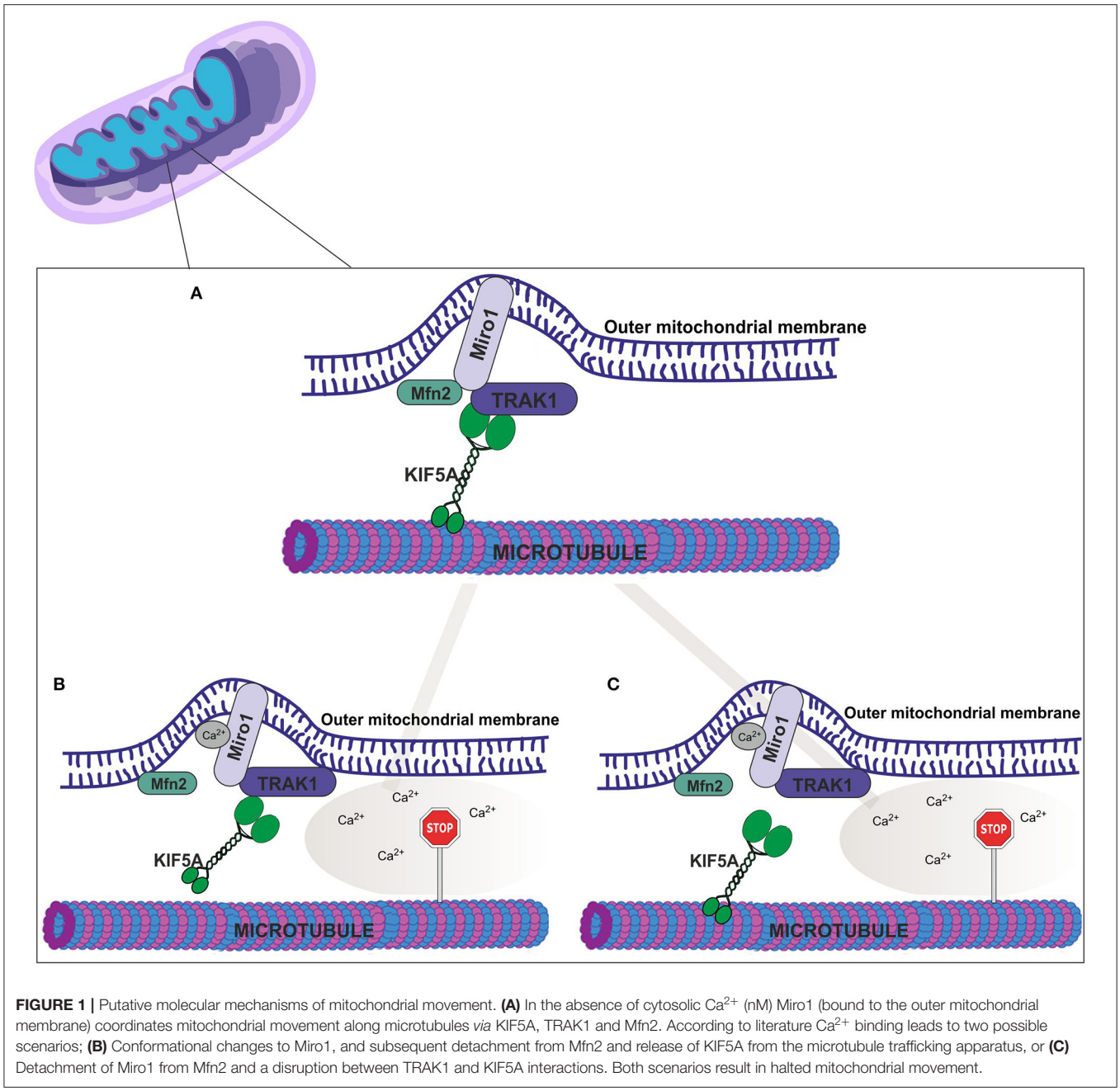

FIGURE 1 | Putative molecular mechanisms of mitochondrial movement. **(A)** In the absence of cytosolic Ca^{2+} (nM) Miro1 (bound to the outer mitochondrial membrane) coordinates mitochondrial movement along microtubules *via* KIF5A, TRAK1 and Mfn2. According to literature Ca^{2+} binding leads to two possible scenarios; **(B)** Conformational changes to Miro1, and subsequent detachment from Mfn2 and release of KIF5A from the microtubule trafficking apparatus, or **(C)** Detachment of Miro1 from Mfn2 and a disruption between TRAK1 and KIF5A interactions. Both scenarios result in halted mitochondrial movement.

between calcium cycling (and contractility) and mechano-electric and chemical MT-mediated inter-organelle tethering (61) the impact of disrupting the MT network upon mitochondrial motility and function needs further investigation.

PROTEIN POST-TRANSLATIONAL MODIFICATIONS (PTMS) AND ROLE IN MITOCHONDRIAL MOTILITY

In addition to Ca^{2+} homeostasis, regulatory mechanisms mediating mitochondrial motility include the cellular redox balance (62), as well PTMs of mitochondrial (63) and MT proteins (64). Miro1 is also regulated by PTMs being ubiquitinated after phosphorylation *via* PINK1, triggering Parkin and proteasomal pathways leading to Miro1 degradation (65). Given that the PINK1-Parkin pathway is activated in response to depolarisation of the mitochondrial membrane it is generally considered, in this context, that prevention of mitochondrial movement through Miro1 phosphorylation-degradation is important for preserving mitochondrial quality by segregating those damaged mitochondria for removal from the cell (66). How removal of damaged mitochondria is linked to the transport of mitochondria *via* Miro1 is not clear, particularly

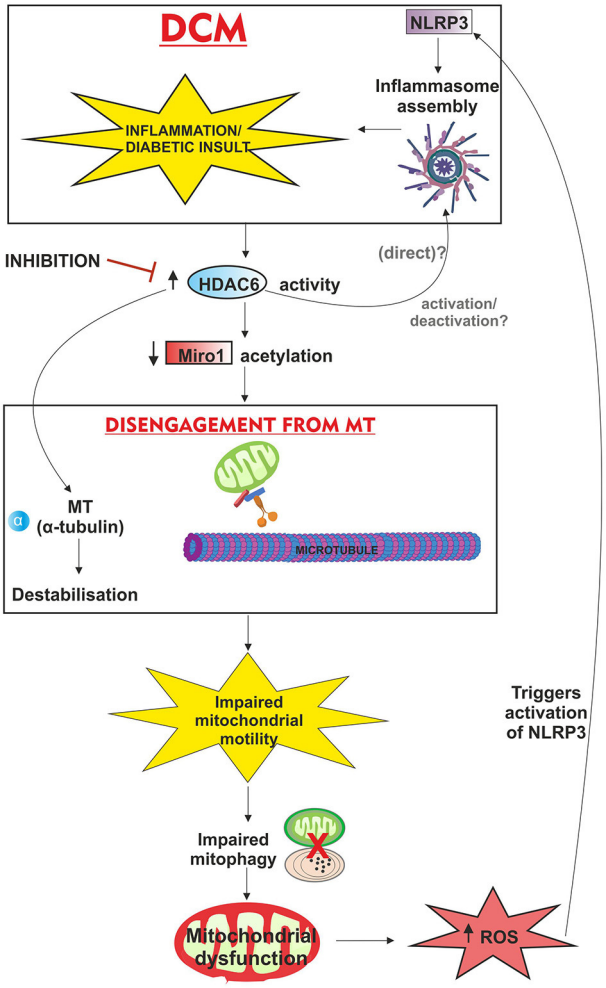

FIGURE 2 | Schematic overview of proposed mechanism of action of HDAC6 and associated deacetylation; linking mitochondrial motility, inflammation and the putative involvement of post-translational modifications in DCM. Inflammation/diabetic insult triggers HDAC6 activity which directly interacts with NLRP3 *via* ubiquitin binding domains (although it is unknown whether this activates/inactivates NLRP3 and inflammasome assembly in the heart). The combination of microtubule (alpha-tubulin) destabilisation and reduced acetylation (important for microtubule stability), contributes to disengagement of the mitochondria from the microtubule apparatus and halts mitochondrial movement. With mitochondrial motility linked to mitophagy, the removal of damaged mitochondria (potentially ROS producing) is impaired, leading to subsequent mitochondrial dysfunction, and oxidative stress further exacerbating inflammation *via* NLRP3. Inhibition of HDAC6 activity is also reported as cardioprotective.

within cardiomyocytes; although the Bers study (37) would suggest that mitochondrial transport of IFM precedes mitophagy. The relationship between mitophagy and Miro1 also appears to be cell-type dependent with Miro1 loss-of-function mutations preventing the induction of mitophagy in neurons (67) but activating mitophagy in fibroblasts (68). Miro1 is also a substrate of the class II histone deacetylase, HDAC6, with acetylation of K105 (murine and rat) corresponding to K92 in human Miro1, involving different lysine residues than those targeted for ubiquitination, indicative of two different processes (69). HDAC6 co-immunopreciptates with Miro1 supporting a direct interaction between the two proteins. Importantly, Kalinski et al. (69) also demonstrated that deactetylation of Miro1 leads to stalled mitochondrial movement with detrimental effects upon axon growth, with pharmacological inhibition or deletion of HDAC6 protecting mitochondria against damage and abnormal mitochondrial clustering.

Notably, one of the main components of MTs, α-tubulin, is also an HDAC6 substrate. Acetylation is important for MT stability, as well-inducing conformationally directed MT organisation for the recruitment of kinesin motor proteins (70). Inhibition of HDAC6 in hippocampal neurons is shown to lead to higher levels of α-tubulin and enhanced mitochondrial motility (71). While the role of MT acetylation remains to be fully understood it is intriguing that HDAC6 separately regulates both Miro1 and MT engagement and disengagement and consequently mitochondrial motility.

Significantly, inhibition of HDAC6 activity is also reported as cardioprotective (72) preventing the development of hypertrophy and fibrosis (73). Further, in the context of DCM, HDAC6 inhibition using tubastatin A (TBA) is shown to be beneficial in a rat model of type 1 diabetes for improving outcomes from ischaemia/reperfusion (I/R) injury (74). Moreover, recently, pan-inhibitors of HDACs have been proposed as novel treatments for treating HFpEF, preserving cardiac function in a small animal model (rat) of hypertension induced LV-dysfunction (75) and a larger animal (feline) model of pressure overload (76).

Increased HDAC6 activity in the failing heart has been known for the past decade (77) but a key question that remains to be fully answered is how is HDAC6 activated? Chen and colleagues demonstrated, using an atrial cell line (HL-1), that mitochondrial dysfunction (impaired OXPHOS) induced by treatment with TNF-α, could be rescued using an inhibitor of HDAC6 (78) indicating a mechanistic link between inflammation, mitochondrial function and HDAC6 activity. Further, HDAC6 inhibition in SH-SY5Y cells (a model for Parkinson's disease) is reported to lead to reduced activation of the inflammasome, NLRP3, inflammatory response concomitant with attenuation of dopaminergic neuronal degeneration (79); although the pathways involved were not described.

MICROTUBULES PLAY A CRITICAL ROLE FOR NLRP3 ACTIVATION

Activation of NLRP3 modulates the release of inflammatory cytokines, IL-1β and IL-18, cell death and fibrosis associated with the pathogenesis of DCM (80). The acetylation of α-tubulin, whilst linked to mitochondrial transport, is also shown to play a role in the movement of NLRP3 inflammasome components along microtubules leading to the subsequent apposition of NLRP3 to mitochondrion-associated ASC (81). Multiple studies have established the link between microtubule dynamics and NLRP3 inflammasome activity, for

example (82). Significantly NLRP3 activation is a feature of several cardiac pathologies as reviewed in (83). For example, colchicine (a microtubule polymerisation inhibitor) disrupts microtubule/tubulin dynamics suppressing the activation of the NLRP3 inflammasome. Animal models of myocardial infarction treated with colchicine have improved cardiac performance, improved survival rates and attenuated HF development and inflammatory response (84).

Since the acetylation of α-tubulin/NLRP3 is under the control of HDAC6 it is perhaps not surprising that beneficial effects of using HDAC6 inhibitors in preventing IL-1β generation have been demonstrated (85). Although, one macrophage study concluded that HDAC6 is a negative regulator of NLRP3, due to a direct interaction mediated by the ubiquitin binding domains (86); with this mechanism suggested to mediate NLRP3 transport into aggresomes *via* the microtubule network. More recently, Magupalli et al. also demonstrated in macrophages that specific regions of MTs, the microtubule-organising centre (the centrosome), are the sites for NLRP3 assembly and HDAC6 knockout (and loss of ubiquitin-binding) leads to impaired inflammasome assembly and activation (87). Clearly, there is a complex association between NLRP3 assembly, activation and MTs, which may also be tissue/cell type specific.

An indirect link between Miro1 and NLRP3 has also been identified in a rat pancreatic cells using high-fat and high glucose stressors to mimic T2DM conditions (88). Specifically, cells exhibited dysregulated Ca^{2+} homeostasis, which was suggested to lead to the dissociation of Miro1 from mitochondria and subsequent impaired mitochondrial movement, stalled mitophagy and accumulation of damaged ROS producing mitochondria that in turn triggered activation of NLRP3.

CONCLUDING REMARKS

Here we have highlighted evidence for the physio-pathological role of Miro1-mediated movement of mitochondria along MTs and while the majority of data is from the study of neurons evidence is emerging to support a similar role for Miro1 in the heart (40). Therefore, in addition to studies focussing upon strategies to prevent fission (89, 90) an emerging area for future studies is the delineation of the mechanisms surrounding mitophagy, mitochondrial movement, and role of the Miro1-macromolecular complex. For example, it remains unclear as to whether Miro1 expression and activity influences the processes of fission and fusion and is essential for mitophagy in the heart.

Additionally, we have highlighted a potential link between mitochondrial motility and inflammation and the involvement of deacetylation and HDAC6 (**Figure 2**). Although technically there remain significant challenges around studying PTMs (91) a better understanding of the functional effects of PTMs on the proteins underpinning mitochondrial motility will provide new avenues for future research. In conclusion, this review article summarises some of the current evidence, and areas where knowledge is lacking, for mitochondrial motility in the heart, and suggests a possible unifying mechanism linking impaired mitophagy, the MT network and the inflammatory response to arrested mitochondrial movement. As the causative agents and mechanisms of mitochondrial dysfunction and impaired motility are discovered, then new promising treatment therapies may emerge for promoting better cardiac outcomes in DCM/HF.

AUTHOR CONTRIBUTIONS

AK and SK wrote and planned the manuscript. ZA and HD contributed to the writing process. SK generated the figures in consultation with AK. All authors contributed and approved the submitted article.

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REFERENCES

1. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9(th) edition. *Diabetes Res Clin Pract.* (2019) 157:107843. doi: 10.1016/j.diabres.2019.107843
2. Aneja A, Tang WHW, Bansilal S, Garcia MJ, Farkouh ME. Diabetic cardiomyopathy: Insights into pathogenesis, diagnostic challenges, and therapeutic options. *Am J Med.* (2008) 121:748–57. doi: 10.1016/j.amjmed.2008.03.046
3. Avogaro A, de Kreutzenberg SV, Negut C, Tiengo A, Scognamiglio R. Diabetic cardiomyopathy: a metabolic perspective. *Am J Cardiol.* (2004) 93:13–6. doi: 10.1016/j.amjcard.2003.11.003
4. Rubler S, Dlugash J, Yuceoglu YZ, Kumral T, Branwood AW, Grishman A. New type of cardiomyopathy associated with diabetic glomerulosclerosis. *The Am J Cardiol.* (1972) 30:595–602. doi: 10.1016/0002-9149(72)90595-4
5. Dillmann WH. Diabetic cardiomyopathy. *Circ Res.* (2019) 124:1160–2. doi: 10.1161/CIRCRESAHA.118.314665
6. Paulus WJ, Dal Canto E. Distinct myocardial targets for diabetes therapy in heart failure with preserved or reduced ejection fraction. *JACC Heart Fail.* (2018) 6:1–7. doi: 10.1016/j.jchf.2017.07.012
7. Meagher P, Adam M, Civitarese R, Bugyei-Twum A, Connelly KA. Heart failure with preserved ejection fraction in diabetes: mechanisms and management. *Can J Cardiol.* (2018) 34:632–43. doi: 10.1016/j.cjca.2018.02.026
8. Rosca MG, Tandler B, Hoppel CL. Mitochondria in cardiac hypertrophy and heart failure. *J Mol Cell Cardiol.* (2013) 55:31–41. doi: 10.1016/j.yjmcc.2012.09.002
9. Kumar AA, Kelly DP, Chirinos JA. Mitochondrial dysfunction in heart failure with preserved ejection fraction. *Circulation.* (2019) 139:1435–50. doi: 10.1161/CIRCULATIONAHA.118.036259
10. Schilling JD. The mitochondria in diabetic heart failure: from pathogenesis to therapeutic promise. *Antioxid Redox Signal.* (2015) 22:1515–26. doi: 10.1089/ars.2015.6294
11. Daghistani HM, Rajab BS, Kitmitto A. Three-dimensional electron microscopy techniques for unravelling mitochondrial dysfunction in heart

failure and identification of new pharmacological targets. *Br J Pharmacol.* (2019) 176:4340–59. doi: 10.1111/bph.14499
12. Dorn GW 2nd. Evolving concepts of mitochondrial dynamics. *Annu Rev Physiol.* (2019) 81:1–17. doi: 10.1146/annurev-physiol-020518-114358
13. Burte F, Carelli V, Chinnery PF, Yu-Wai-Man P. Disturbed mitochondrial dynamics and neurodegenerative disorders. *Nat Rev Neurol.* (2015) 11:11–24. doi: 10.1038/nrneurol.2014.228
14. Filard T, Ghinassi B, Di Baldassarre A, Tanzilli G, Morano S, Lenzi A, et al. Cardiomyopathy associated with diabetes: the central role of the cardiomyocyte. *Int J Mol Sci.* (2019) 20:3299. doi: 10.3390/ijms20133299
15. Kaludercic N, Di Lisa F. Mitochondrial ROS formation in the pathogenesis of diabetic cardiomyopathy. *Front Cardiovasc Med.* (2020) 7:12. doi: 10.3389/fcvm.2020.00012
16. MacDonald JA, Wijekoon CP, Liao KC, Muruve DA. Biochemical and structural aspects of the ATP-binding domain in inflammasome-forming human NLRP proteins. *IUBMB Life.* (2013) 65:851–62. doi: 10.1002/iub.1210
17. Li PL. Cardiovascular pathobiology of inflammasomes: inflammatory machinery and beyond. *Antioxid Redox Signal.* (2015) 22:1079–83. doi: 10.1089/ars.2015.6319
18. Ichinohe T, Yamazaki T, Koshiba T, Yanagi Y. Mitochondrial protein mitofusin 2 is required for NLRP3 inflammasome activation after RNA virus infection. *Proc Natl Acad Sci U S A.* (2013) 110:17963–8. doi: 10.1073/pnas.1312571110
19. Park S, Won JH, Hwang I, Hong S, Lee HK, Yu JW. Defective mitochondrial fission augments NLRP3 inflammasome activation. *Sci Rep.* (2015) 5:15489. doi: 10.1038/srep15489
20. Youle RJ, Narendra DP. Mechanisms of mitophagy. *Nat Rev Mol Cell Biol.* (2011) 12:9–14. doi: 10.1038/nrm3028
21. Okatsu K, Oka T, Iguchi M, Imamura K, Kosako H, Tani N, et al. PINK1 autophosphorylation upon membrane potential dissipation is essential for Parkin recruitment to damaged mitochondria. *Nature Comm.* (2012) 3:1016. doi: 10.1038/ncomms2016
22. Dorn GW 2nd. Parkin-dependent mitophagy in the heart. *J Mol Cell Cardiol.* (2016) 95:42–9. doi: 10.1016/j.yjmcc.2015.11.023
23. Huang X, Sun L, Ji S, Zhao T, Zhang W, Xu J, et al. Kissing and nanotunneling mediate intermitochondrial communication in the heart. *Proc Natl Acad Sci U S A.* (2013) 110:2846–51. doi: 10.1073/pnas.1300741110
24. Vincent AE, Ng YS, White K, Davey T, Mannella C, Falkous G, et al. The spectrum of mitochondrial ultrastructural defects in mitochondrial myopathy. *Sci Rep.* (2016) 6:30610. doi: 10.1038/srep30610
25. Lavorato M, Iyer VR, Dewight W, Cupo RR, Debattisti V, Gomez L, et al. Increased mitochondrial nanotunneling activity, induced by calcium imbalance, affects intermitochondrial matrix exchanges. *Proc Natl Acad Sci U S A.* (2017) 114:E849–58. doi: 10.1073/pnas.1617788113
26. Vasquez-Trincado C, Garcia-Carvajal I, Pennanen C, Parra V, Hill JA, Rothermel BA, et al. Mitochondrial dynamics, mitophagy and cardiovascular disease. *J Physiol.* (2016) 594:509–25. doi: 10.1113/JP271301
27. Montaigne D, Marechal X, Coisne A, Debry N, Modine T, Fayad G, et al. Myocardial contractile dysfunction is associated with impaired mitochondrial function and dynamics in type 2 diabetic but not in obese patients. *Circulation.* (2014) 130:554. doi: 10.1161/CIRCULATIONAHA.113.008476
28. Gao Q, Wang XM, Ye HW, Yu Y, Kang PF, Wang HJ, et al. Changes in the expression of cardiac mitofusin-2 in different stages of diabetes in rats. *Mol Med Rep.* (2012) 6:811–4. doi: 10.3892/mmr.2012.1002
29. Chen Y, Liu YQ, Dorn GW. Mitochondrial fusion is essential for organelle function and cardiac homeostasis. *Circ Res.* (2011) 109:1327–36. doi: 10.1161/CIRCRESAHA.111.258723
30. Chen L, Gong Q, Stice JP, Knowlton AA. Mitochondrial OPA1, apoptosis, and heart failure. *Cardiovas Res.* (2009) 84:91–9. doi: 10.1093/cvr/cvp181
31. Givvimani S, Pushpakumar S, Veeranki S, Tyagi SC. Dysregulation of Mfn2 and Drp-1 proteins in heart failure. *Can J Physiol Pharmacol.* (2014) 92:583–91. doi: 10.1139/cjpp-2014-0060
32. Ong SB, Subrayan S, Lim SY, Yellon DM, Davidson SM, Hausenloy DJ. Inhibiting mitochondrial fission protects the heart against ischemia/reperfusion injury. *Circulation.* (2010) 121:2012–22. doi: 10.1161/CIRCULATIONAHA.109.906610
33. Shirakabe A, Zhai P, Ikeda Y, Saito T, Maejima Y, Hsu CP, et al. Drp1-dependent mitochondrial autophagy plays a protective role against pressure overload-induced mitochondrial dysfunction and heart failure. *Circulation.* (2016) 133:1249–63. doi: 10.1161/CIRCULATIONAHA.115.020502
34. Billia F, Hauck L, Konecny F, Rao V, Shen J, Mak TW. PTEN-inducible kinase 1 (PINK1)/Park6 is indispensable for normal heart function. *Proc Natl Acad Sci U S A.* (2011) 108:9572–7. doi: 10.1073/pnas.1106291108
35. Kubli DA, Quinsay MN, Gustafsson AB. Parkin deficiency results in accumulation of abnormal mitochondria in aging myocytes. *Commun Integr Biol.* (2013) 6:e24511. doi: 10.4161/cib.24511
36. Andres AM, Hernandez G, Lee P, Huang CQ, Ratliff EP, Sin J, et al. Mitophagy is required for acute cardioprotection by simvastatin. *Antioxid Redox Signal.* (2014) 21:1960–73. doi: 10.1089/ars.2013.5416
37. Lu X, Thai PN, Lu S, Pu J, Bers DM. Intrafibrillar and perinuclear mitochondrial heterogeneity in adult cardiac myocytes. *J Mol Cell Cardiol.* (2019) 136:72–84. doi: 10.1016/j.yjmcc.2019.08.013
38. Hsieh CH, Shaltouki A, Gonzalez AE, Bettencourt da Cruz A, Burbulla LF, St Lawrence E, et al. Functional impairment in miro degradation and mitophagy is a shared feature in familial and sporadic parkinson's disease. *Cell Stem Cell.* (2016) 19:709–24. doi: 10.1016/j.stem.2016.08.002
39. Fransson A, Ruusala A, Aspenstrom P. Atypical Rho GTPases have roles in mitochondrial homeostasis and apoptosis. *J Biol Chem.* (2003) 278:6495–502. doi: 10.1074/jbc.M208609200
40. Saotome M, Safiulina D, Szabadkai G, Das S, Fransson A, Aspenstrom P, et al. Bidirectional Ca^{2+}-dependent control of mitochondrial dynamics by the Miro GTPase. *Proc Natl Acad Sci U S A.* (2008) 105:20728–33. doi: 10.1073/pnas.0808953105
41. Zhang Y, Yu Z, Jiang D, Liang X, Liao S, Zhang Z, et al. iPSC-MSCs with high intrinsic MIRO1 and sensitivity to TNF-alpha yield efficacious mitochondrial transfer to rescue anthracycline-induced cardiomyopathy. *Stem Cell Rep.* (2016) 7:749–63. doi: 10.1016/j.stemcr.2016.08.009
42. Conejeros C, Parra V, Sanchez G, Pedrozo Z, Olmedo I. Miro1 as a novel regulator of hypertrophy in neonatal rat cardiomyocytes. *J Mol Cell Cardiol.* (2020) 141:65–9. doi: 10.1016/j.yjmcc.2020.03.014
43. Gong G, Song M, Csordas G, Kelly DP, Matkovich SJ, Dorn GW, et al. Parkin-mediated mitophagy directs perinatal cardiac metabolic maturation in mice. *Science.* (2015) 350:aad2459. doi: 10.1126/science.aad2459
44. Basu K, Lajoie D, Aumentado-Armstrong T, Chen J, Koning RI, Bossy B, et al. Molecular mechanism of DRP1 assembly studied in vitro by cryo-electron microscopy. *PLoS ONE.* (2017) 12:e0179397. doi: 10.1371/journal.pone.0179397
45. Brickley K, Smith MJ, Beck M, Stephenson FA. GRIF-1 and OIP106, members of a novel gene family of coiled-coil domain proteins: association in vivo and in vitro with kinesin. *J Biol Chem.* (2005) 280:14723–32. doi: 10.1074/jbc.M409095200
46. Lopez-Domenech G, Covill-Cooke C, Ivankovic D, Halff EF, Sheehan DF, Norkett R, et al. Miro proteins coordinate microtubule- and actin-dependent mitochondrial transport and distribution. *EMBO J.* (2018) 37:321–36. doi: 10.15252/embj.201696380
47. Misko A, Jiang S, Wegorzewska I, Milbrandt J, Baloh RH. Mitofusin 2 is necessary for transport of axonal mitochondria and interacts with the Miro/Milton complex. *J Neurosci.* (2010) 30:4232–40. doi: 10.1523/JNEUROSCI.6248-09.2010
48. Hu L, Ding M, Tang D, Gao E, Li C, Wang K, et al. Targeting mitochondrial dynamics by regulating Mfn2 for therapeutic intervention in diabetic cardiomyopathy. *Theranostics.* (2019) 9:3687–706. doi: 10.7150/thno.33684
49. Macaskill AF, Rinholm JE, Twelvetrees AE, Arancibia-Carcamo IL, Muir J, Fransson A, et al. Miro1 is a calcium sensor for glutamate receptor-dependent localization of mitochondria at synapses. *Neuron.* (2009) 61:541–55. doi: 10.1016/j.neuron.2009.01.030
50. Bers DM. Altered cardiac myocyte Ca regulation in heart failure Physiology. *Bethesda*. (2006) 21:380–7. doi: 10.1152/physiol.00019.2006
51. Pereira L, Matthes J, Schuster I, Valdivia HH, Herzig S, Richard S, et al. Mechanisms of [Ca2+]i transient decrease in cardiomyopathy of db/db type 2 diabetic mice. *Diabetes.* (2006) 55:608–15. doi: 10.2337/diabetes.55.03.06.db05-1284
52. Bertero E, Maack C. Calcium signaling and reactive oxygen species in mitochondria. *Circ Res.* (2018) 122:1460–78. doi: 10.1161/CIRCRESAHA.118.310082

53. Kohlhaas M, Maack C. Calcium release microdomains and mitochondria. *Cardiovasc Res.* (2013) 98:259–68. doi: 10.1093/cvr/cvt032
54. Belmonte S, Morad M. Shear fluid-induced Ca2+ release and the role of mitochondria in rat cardiac myocytes. *Ann N Y Acad Sci.* (2008) 1123:58–63. doi: 10.1196/annals.1420.007
55. Goldstein MA, Entman ML. Microtubules in mammalian heart muscle. *J Cell Biol.* (1979) 80:183–95. doi: 10.1083/jcb.80.1.183
56. Gittes F, Mickey B, Nettleton J, Howard J. Flexural rigidity of microtubules and actin filaments measured from thermal fluctuations in shape. *J Cell Biol.* (1993) 120:923–34. doi: 10.1083/jcb.120.4.923
57. Stroud MJ. Linker of nucleoskeleton and cytoskeleton complex proteins in cardiomyopathy. *Biophys Rev.* (2018) 10:1033–51. doi: 10.1007/s12551-018-0431-6
58. Steele DF, Fedida D. Cytoskeletal roles in cardiac ion channel expression. *Biochim Biophys Acta.* (2014) 1838:665–73. doi: 10.1016/j.bbamem.2013.05.001
59. Caporizzo MA, Chen CY, Prosser BL. Cardiac microtubules in health and heart disease. *Exp Biol Med.* (2019) 244:1255–72. doi: 10.1177/1535370219868960
60. Caporizzo MA, Chen CY, Bedi K, Margulies KB, Prosser BL. Microtubules increase diastolic stiffness in failing human cardiomyocytes and myocardium. *Circulation.* (2020) 141:902–15. doi: 10.1161/CIRCULATIONAHA.119.043930
61. Caffarra Malvezzi C, Cabassi A, Miragoli M. Mitochondrial mechanosensor in cardiovascular diseases. *Vasc Biol.* (2020) 2:R85–92. doi: 10.1530/VB-20-0002
62. Debattisti V, Gerencser AA, Saotome M, Das S, Hajnoczky G. ROS Control mitochondrial motility through p38 and the motor adaptor miro/trak. *Cell Rep.* (2017) 21:1667–80. doi: 10.1016/j.celrep.2017.10.060
63. Stram AR, Payne RM. Post-translational modifications in mitochondria: protein signaling in the powerhouse. *Cell Mol Life Sci.* (2016) 73:4063–73. doi: 10.1007/s00018-016-2280-4
64. Magiera MM, Singh P, Gadadhar S, Janke C. Tubulin posttranslational modifications and emerging links to human disease. *Cell.* (2018) 173:1323–7. doi: 10.1016/j.cell.2018.05.018
65. Birsa N, Norkett R, Wauer T, Mevissen TE, Wu HC, Foltynie T, et al. Lysine 27 ubiquitination of the mitochondrial transport protein Miro is dependent on serine 65 of the Parkin ubiquitin ligase. *J Biol Chem.* (2014) 289:14569–82. doi: 10.1074/jbc.M114.563031
66. Wang X, Winter D, Ashrafi G, Schlehe J, Wong YL, Selkoe D, et al. PINK1 and Parkin target Miro for phosphorylation and degradation to arrest mitochondrial motility. *Cell.* (2011) 147:893–906. doi: 10.1016/j.cell.2011.10.018
67. Berenguer-Escuder C, Grossmann D, Antony P, Arena G, Wasner K, Massart F, et al. Impaired mitochondrial-endoplasmic reticulum interaction and mitophagy in Miro1-mutant neurons in Parkinson's disease. *Hum Mol Genet.* (2020) 29:1353–64. doi: 10.1093/hmg/ddaa066
68. Grossmann D, Berenguer-Escuder C, Bellet ME, Scheibner D, Bohler J, Massart F, et al. Mutations in RHOT1 disrupt endoplasmic reticulum-mitochondria contact sites interfering with calcium homeostasis and mitochondrial dynamics in parkinson's disease. *Antioxid Redox Signal.* (2019) 31:1213–34. doi: 10.1089/ars.2018.7718
69. Kalinski AL, Kar AN, Craver J, Tosolini AP, Sleigh JN, Lee SJ, et al. Deacetylation of Miro1 by HDAC6 blocks mitochondrial transport and mediates axon growth inhibition. *J Cell Biol.* (2019) 218:1871–90. doi: 10.1083/jcb.201702187
70. Balabanian L, Berger CL, Hendricks AG. Acetylated microtubules are preferentially bundled leading to enhanced kinesin-1 motility. *Biophys J.* (2017) 113:1551–60. doi: 10.1016/j.bpj.2017.08.009
71. Chen S, Owens GC, Makarenkova H, Edelman DB. HDAC6 regulates mitochondrial transport in hippocampal neurons. *PLoS ONE.* (2010) 5:e10848. doi: 10.1371/journal.pone.0010848
72. Demos-Davies KM, Ferguson BS, Cavasin MA, Mahaffey JH, Williams SM, Spiltoir JI, et al. HDAC6 contributes to pathological responses of heart and skeletal muscle to chronic angiotensin-II signaling. *Am J Physiol Heart Circ Physiol.* (2014) 307:H252–8. doi: 10.1152/ajpheart.00149.2014
73. Kee HJ, Bae EH, Park S, Lee KE, Suh SH, Kim SW, et al. HDAC inhibition suppresses cardiac hypertrophy and fibrosis in DOCA-salt hypertensive rats via regulation of HDAC6/HDAC8 enzyme activity. *Kidney Blood Press Res.* (2013) 37:229–39. doi: 10.1159/000350148
74. Leng Y, Wu Y, Lei S, Zhou B, Qiu Z, Wang K, et al. Inhibition of HDAC6 activity alleviates myocardial ischemia/reperfusion injury in diabetic rats: potential role of peroxiredoxin 1 acetylation and redox regulation. *Oxid Med Cell Longev.* (2018) 2018:9494052. doi: 10.1155/2018/9494052
75. Jeong MY, Lin YH, Wennersten SA, Demos-Davies KM, Cavasin MA, Mahaffey JH, et al. Histone deacetylase activity governs diastolic dysfunction through a nongenomic mechanism. *Sci Transl Med.* (2018) 10:eaao0144. doi: 10.1126/scitranslmed.aao0144
76. Wallner M, Eaton DM, Berretta RM, Liesinger L, Schittmayer M, Gindlhuber J, et al. HDAC inhibition improves cardiopulmonary function in a feline model of diastolic dysfunction. *Sci Transl Med.* (2020) 12:eaay7205. doi: 10.1126/scitranslmed.aay7205
77. Lemon DD, Horn TR, Cavasin MA, Jeong MY, Haubold KW, Long CS, et al. Cardiac HDAC6 catalytic activity is induced in response to chronic hypertension. *J Mol Cell Cardiol.* (2011) 51:41–50. doi: 10.1016/j.yjmcc.2011.04.005
78. Lkhagva B, Kao YH, Lee TI, Lee TW, Cheng WL, Chen YJ. Activation of Class I histone deacetylases contributes to mitochondrial dysfunction in cardiomyocytes with altered complex activities. *Epigenetics.* (2018) 13:376–85. doi: 10.1080/15592294.2018.1460032
79. Yan S, Wei X, Jian W, Qin Y, Liu J, Zhu S, et al. Pharmacological Inhibition of HDAC6 attenuates NLRP3 inflammatory response and protects dopaminergic neurons in experimental models of parkinson's disease. *Front Aging Neurosci.* (2020) 12:78. doi: 10.3389/fnagi.2020.00078
80. Luo B, Huang F, Liu Y, Liang Y, Wei Z, Ke H, et al. NLRP3 Inflammasome as a molecular marker in diabetic cardiomyopathy. *Front Physiol.* (2017) 8:519. doi: 10.3389/fphys.2017.00519
81. Misawa T, Takahama M, Kozaki T, Lee H, Zou J, Saitoh T, et al. Microtubule-driven spatial arrangement of mitochondria promotes activation of the NLRP3 inflammasome. *Nat Immunol.* (2013) 14:454–60. doi: 10.1038/ni.2550
82. Li X, Thome S, Ma X, Amrute-Nayak M, Finigan A, Kitt L, et al. MARK4 regulates NLRP3 positioning and inflammasome activation through a microtubule-dependent mechanism. *Nat Commun.* (2017) 8:15986. doi: 10.1038/ncomms15986
83. An N, Gao Y, Si Z, Zhang H, Wang L, Tian C, et al. Regulatory mechanisms of the NLRP3 inflammasome, a novel immune-inflammatory marker in cardiovascular diseases. *Front Immunol.* (2019) 10:1592. doi: 10.3389/fimmu.2019.01592
84. Fujisue K, Sugamura K, Kurokawa H, Matsubara J, Ishii M, Izumiya Y, et al. Colchicine improves survival, left ventricular remodeling, and chronic cardiac function after acute myocardial infarction. *Circ J.* (2017) 81:1174–82. doi: 10.1253/circj.CJ-16-0949
85. Carta S, Tassi S, Semino C, Fossati G, Mascagni P, Dinarello CA, et al. Histone deacetylase inhibitors prevent exocytosis of interleukin-1beta-containing secretory lysosomes: role of microtubules. *Blood.* (2006) 108:1618–26. doi: 10.1182/blood-2006-03-014126
86. Hwang I, Lee E, Jeon SA, Yu JW. Histone deacetylase 6 negatively regulates NLRP3 inflammasome activation. *Biochem Biophys Res Commun.* (2015) 467:973–8. doi: 10.1016/j.bbrc.2015.10.033
87. Magupalli VG, Negro R, Tian Y, Hauenstein AV, Di Caprio G, Skillern W, et al. HDAC6 mediates an aggresome-like mechanism for NLRP3 and pyrin inflammasome activation. *Science.* (2020) 369:eaas8995. doi: 10.1126/science.aas8995
88. Gao J, Liu RT, Cao S, Cui JZ, Wang A, To E, et al. NLRP3 inflammasome: activation and regulation in age-related macular degeneration. *Mediators Inflamm.* (2015) 2015:690243. doi: 10.1155/2015/690243

89. Qi X, Qvit N, Su YC, Mochly-Rosen D. A novel Drp1 inhibitor diminishes aberrant mitochondrial fission and neurotoxicity. *J Cell Sci.* (2013) 126:789–802. doi: 10.1242/jcs.114439
90. Cassidy-Stone A, Chipuk JE, Ingerman E, Song C, Yoo C, Kuwana T, et al. Chemical inhibition of the mitochondrial division dynamin reveals its role in Bax/Bak-dependent mitochondrial outer membrane permeabilization. *Develop Cell.* (2008) 14:193–204. doi: 10.1016/j.devcel.2007.11.019
91. Smith LE, Rogowska-Wrzesinska A. The challenge of detecting modifications on proteins. *Essays Biochem.* (2020) 64:135–53. doi: 10.1042/EBC20190055

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REVIEW
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Electrical Features of the Diabetic Myocardium. Arrhythmic and Cardiovascular Safety Considerations in Diabetes

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Diabetes is a chronic metabolic disease characterized by hyperglycemia in the absence of treatment. Among the diabetes-associated complications, cardiovascular disease is the major cause of mortality and morbidity in diabetic patients. Diabetes causes a complex myocardial dysfunction, referred as diabetic cardiomyopathy, which even in the absence of other cardiac risk factors results in abnormal diastolic and systolic function. Besides mechanical abnormalities, altered electrical function is another major feature of the diabetic myocardium. Both type 1 and type 2 diabetic patients often show cardiac electrical remodeling, mainly a prolonged ventricular repolarization visible in the electrocardiogram as a lengthening of the QT interval duration. The underlying mechanisms at the cellular level involve alterations on the expression and activity of several cardiac ion channels and their associated regulatory proteins. Consequent changes in sodium, calcium and potassium currents collectively lead to a delay in repolarization that can increase the risk of developing life-threatening ventricular arrhythmias and sudden death. QT duration correlates strongly with the risk of developing *torsade de pointes*, a form of ventricular tachycardia that can degenerate into ventricular fibrillation. Therefore, QT prolongation is a qualitative marker of proarrhythmic risk, and analysis of ventricular repolarization is therefore required for the approval of new drugs. To that end, the Thorough QT/QTc analysis evaluates QT interval prolongation to assess potential proarrhythmic effects. In addition, since diabetic patients have a higher risk to die from cardiovascular causes than individuals without diabetes, cardiovascular safety of the new antidiabetic drugs must be carefully evaluated in type 2 diabetic patients. These cardiovascular outcome trials reveal that some glucose-lowering drugs actually reduce cardiovascular risk. The mechanism of cardioprotection might involve a reduction of the risk of developing arrhythmia.

Keywords: ion channels, currents, cardiac, arrhythmia, antidiabetics, CVOT, TQT

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INTRODUCTION

Diabetes affected 422 million adults in 2014 (WHO, 2021), but the incidence and the associated socio-sanitary cost are steadily rising. Diabetes is a chronic metabolic disease characterized by hyperglycemia in the absence of treatment. Type 1 diabetes (T1D), caused by insufficient or no insulin production, is the less common form. Type 2 diabetes (T2D) accounts for more than 90% of

the cases and starts with insulin resistance, but later progresses towards various degrees of β-cell dysfunction. As a result, many type 2 diabetic patients may need exogenous insulin.

In the last decades, available pharmacologic approaches have significantly improved the life span and the quality of life of diabetic patients. However, over time, diabetes-associated complications have emerged. Among them, cardiovascular disease stands out because is the major cause of mortality and morbidity in diabetic patients. In fact, the Framingham study reported that patients with type 2 diabetes were twice as likely as healthy people to die from cardiovascular diseases (Kannel et al., 1974). Currently, due to the advances in diabetes management, many diabetic patients achieve good glycemic control. However, cardiovascular complications remain (Food and Drug Administration, 2008; Duckworth et al., 2009) and are still the leading cause of death (Preis et al., 2009; Xu and Rajaratnam, 2017).

Besides inducing cardiovascular disease, diabetes changes the myocardial structure leading to a cardiac dysfunction known as diabetic cardiomyopathy. Diabetic cardiomyopathy was first described by Rubler et al., in 1972 as heart failure in diabetics without hypertension, myocardial ischemia, congenital or valvular disease (Rubler et al., 1972). It is currently defined as a ventricular dysfunction in the absence of coronary artery disease or hypertension (Bugger and Abel, 2014). In fact, the Framingham study described the association between diabetes and cardiac hypertrophy independently from blood pressure (Kannel et al., 1974). Moreover, the Cardiovascular Health study (Lee et al., 1997) and the Strong Heart study (Devereux et al., 2000) confirmed the association between diabetes and increased left ventricular mass and wall thickness with compromised diastolic and systolic function. Different mechanisms have been proposed to promote the development of diabetic cardiomyopathy and damage the heart. These include cardiac insulin resistance; metabolic remodeling with abnormal free fatty acids metabolism and lipotoxicity; mitochondrial dysfunction with increased ROS production; accumulation of advanced glycation end products and collagen; abnormalities in calcium handling; pro-inflammatory responses; activation of the renin-angiotensin-aldosterone system and autonomic neuropathy with increased sympathetic activity (reviewed in Casis and Echevarria, 2004; Battiprolu et al., 2013; Bugger and Abel, 2014; Onay-Besikci, 2014; Grisanti, 2018; Jia et al., 2018; Palomer et al., 2018; Quagliariello et al., 2020).

Torsade de pointes (TdP) is a ventricular arrhythmia characterized by a change in the amplitude and a twisting of the QRS complexes around the isoelectric line in the electrocardiogram. TdP usually ends spontaneously, but in some cases may degenerate into lethal ventricular fibrillation. Long QT syndrome is an inherited or drug-induced arrhythmia syndrome characterized by a prolongation of the QT interval (Sanguinetti and Tristani-Firouzi, 2006; Schwartz et al., 2012) that causes *torsade de pointes*, ventricular fibrillation and sudden death. Since TdP occurs in the setting of prolonged QT intervals, QT duration and heart rate-corrected QT duration (QTc) have become qualitative markers of proarrhythmic risk. The electrocardiogram of both type 1 and type 2 diabetic patients often shows prolonged QT (Bellavere et al., 1988; Chambers et al., 1990; Jermendy et al., 1990; Ewing et al., 1991; Veglio et al., 2000; Brown et al., 2001; Ninkovic et al., 2016), which increases the risk of ventricular arrhythmia (Tse et al., 2016; Hegyi et al., 2019). At the cellular level, diabetes lengthens the cardiac action potential duration due to changes in the expression and electrophysiological properties of various ion channels (Lengyel et al., 2007; Torres-Jacome et al., 2013; Gallego and Casis, 2014).

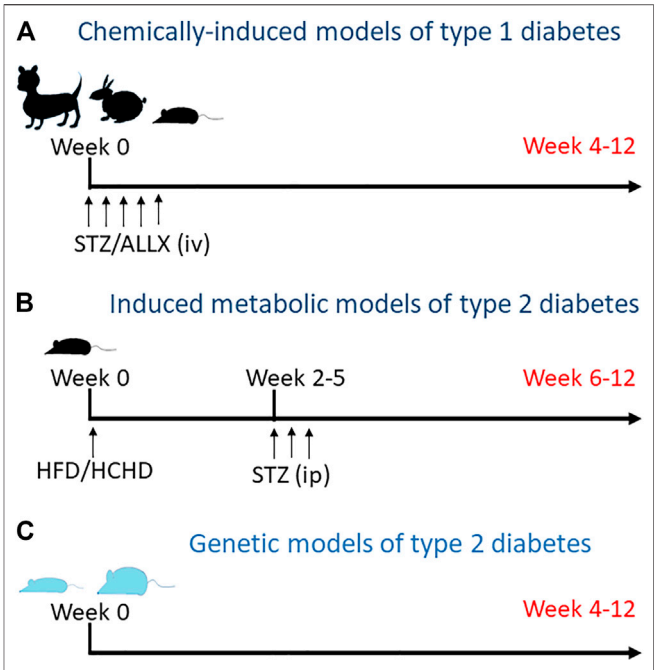


FIGURE 1 | Animal models for diabetes. **(A)** Type 1 diabetes is induced by intravenous injection of streptozotocin or alloxane to kill pancreatic beta cells. Depending on the species, one or more consecutive injections of the toxic are required. **(B)** Metabolic models for type 2 diabetes include diet-induced insulin resistance followed by mild to medium pancreatic toxicity. Animals are fed with high fat and/or high carbohidrate diet (HFD and HCHD, respectively) for some weeks to generate insulin resistance and then receive one or more intraperitoneal injections of low dose streptozotocin to reduce beta cell mass. **(C)** Genetically hyperinsulinemic and/or hyperglycemic animals, lean and obese, spontaneously develop diabetes over time.

In addition, arrhythmogenesis in diabetes might be amplified by other factors like autonomic dysregulation (Jermendy, 2003; Spallone et al., 2011; Chen et al., 2019), inflammation (Karam et al., 2017) and the presence of comorbidities such as hypertension (Yiu and Tse, 2008). This review focuses on the electrical characteristics of the diabetic myocardium, such as reduced conduction velocity and prolonged ventricular repolarization. Since these alterations may increase the risk of arrhythmia, evaluating the proarrhythmic risk of new drugs is very relevant for the case of glucose-lowering drugs.

Since 2005, potential proarrhythmic risk must be evaluated for the approval of any new drug, including new anti-hyperglycemic drugs. Currently, the Thorough QT/QTc (TQT) studies are performed with premarketing drugs in order to exclude those that prolong the QT interval (ICH E14). At the same time,

concerns about cardiovascular risk in diabetic patients has guided the policy of the regulatory agencies to establish the safety of new glucose-lowering drugs. Therefore, cardiovascular outcome trials (CVOTs) that evaluate specific cardiovascular endpoints are being conducted on type 2 diabetic patients (Goldfine, 2008). Furthermore, the information is being incorporated into the standards of medical care (Scheen, 2020).

In the preclinical setting, animal models, mainly rodents, but also guinea pigs, rabbits and dogs, are commonly used for the study of the diabetic heart. Currently, there are many different animal models for T1D and T2D (**Figure 1**). These have been extensively reviewed, and include spontaneous, transgenic as well as surgically, chemically and diet-induced models (Masiello, 2006; King, 2012; Al-awar et al., 2016; King and Bowe, 2016). Most of our knowledge regarding cardiac electrical remodeling in diabetes derives from studies with animal models of T1D (Magyar et al., 1992; Shimoni et al., 1994; Casis et al., 2000; Lengyel et al., 2007; Lengyel et al., 2008). Streptozotocin (STZ) and alloxane are glucose analogues that destroy pancreatic β-cells (Lenzen, 2008) and have been, for decades, the choice for inducing experimental diabetes because the procedure is simple and reliable. T2D has a different origin and pathophysiology. In humans, it starts with insulin resistance and eventually there is a loss of functional β-cells and hyperglycemia. In order to recapitulate this situation, a number of animal models have been generated using different strategies. Among them are the leptin-receptor deficient obese mice ($Lepr^{db/db}$), the non-obese Goto-Kakizaki (GK) rat with defective β-cells, the obese Otsuka Long Evans Tokushima Fat rat (OLETF) or the Zucker diabetic fatty rats (ZDF). In addition, metabolic models that combine high fat and/or high-carbohydrate diet with an intraperitoneal low-dose of STZ (Ionut et al., 2010; Podell et al., 2017) are becoming popular. Progressive feeding on high caloric food leads to glucose intolerance and insulin resistance, whereas STZ provides the loss of functional beta cell mass required establishing diabetes.

In the following section, we will discuss the electrical characteristics of the diabetic myocardium that may eventually increase the risk of arrhythmia: from changes in the electrocardiogram and the action potential, to altered behavior of sodium, calcium and potassium channels. Then, we will examine the proarrhythmic safety of antidiabetic drugs and the recommended treatments for type 2 diabetic patients at cardiovascular risk.

CARDIAC ELECTRICAL REMODELING

Alterations in the Cardiac Conduction System in Diabetes

Although diabetic patients frequently have increased heart rate (Ziegler et al., 2008), diagnosis of bradyarrhythmia and the need for pacemaker treatment are also more frequent in type 2 diabetic patients than in control subjects (Rautio et al., 2020). Among the diabetes-induced electrical disturbances, there is a nodal dysfunction that may be related to autonomic dysregulation. In line with this, leptin-receptor deficient db/db mice showed reduced sinus node recovery time and relative autonomic denervation that increased the risk of developing arrhythmia (Soltysinska et al., 2014). On the other hand, there are intrinsic abnormalities in the sinoatrial node that affect the generation and conduction of electricity. For example, the non-obese type 2 diabetic Goto-Kakizaki (GK) rats had a reduced expression of pacemaker channels and connexins. Among others, genes encoding connexins Cx40, Cx43, and Cx45, as well as the hyperpolarization-activated cyclic nucleotide-gated channel 4 (HCN4) that drives the funny current I_f, were downregulated (Howarth et al., 2018).

Some diabetic patients display slow ventricular depolarization that is visible in the electrocardiogram as an increase in QRS duration (Singleton et al., 2020). Reduced expression of HCN4, connexins and ion channels has also been confirmed in different regions of the cardiac conduction system in the STZ models of T1D (Howarth et al., 2007; Watanabe et al., 2012; Zhang et al., 2019).

Fibroblasts are the most abundant non-cardiomyocyte cells in atria and ventricles. Cardiac fibroblasts can undergo an activation process and differentiate to myofibroblasts that express connexins Cx43 and Cx45. Through these gap junction proteins, myofibroblasts directly interact with cardiomyocytes modulating their electrophysiological behavior and reducing cardiac impulse conduction (Miragoli et al., 2006). Diabetes favors the phenotype switch to myofibroblasts in the heart. Thus, differentiation to myofibroblasts is increased in hearts from STZ-treated and Zucker diabetic rats, which are animal models of T1D and T2D, respectively (Li et al., 2012a; Fowlkes et al., 2013).

Prior to the development of fibrosis, diabetes can induce a lateralization of connexin43 proteins that may impair electrical coupling. Although normal baseline conduction velocity was observed in diabetic rats one to 2 weeks after STZ injection, this normal functioning might be sustained by a robust ventricular conduction reserve. However, under challenging conditions like elevated potassium or ischemia, diabetic ventricles showed larger conduction times compared to controls (Nygren et al., 2007; Rahnema et al., 2011). Furthermore, overt impaired conduction velocity may develop as diabetes progresses. In this sense, long-term type 1 diabetic rabbits without cardiac fibrosis had decreased conduction already in basal conditions. Again, stressing the heart with hypo or hyperkalemia further slowed the velocity. A mechanism responsible might be the diabetes-induced reduction in the cardiac sodium current, as reported by electrophysiological techniques and mathematical modeling (Stables et al., 2014).

Atrial Fibrillation

Diabetes is an independent risk factor for atrial fibrillation (AF) and diabetic patients have one-third greater risk of incidence of AF compared with unaffected individuals (Huxley et al., 2012). Although the underlying pathophysiological mechanisms are not completely understood, diabetes-induced atrial remodeling shares some mechanisms with diabetic cardiomyopathy. Atrial autonomic dysregulation, oxidative stress, fluctuations of glucose levels and structural and electrical remodeling contribute to the development of arrhythmia (Goudis et al., 2015; Wang et al.,

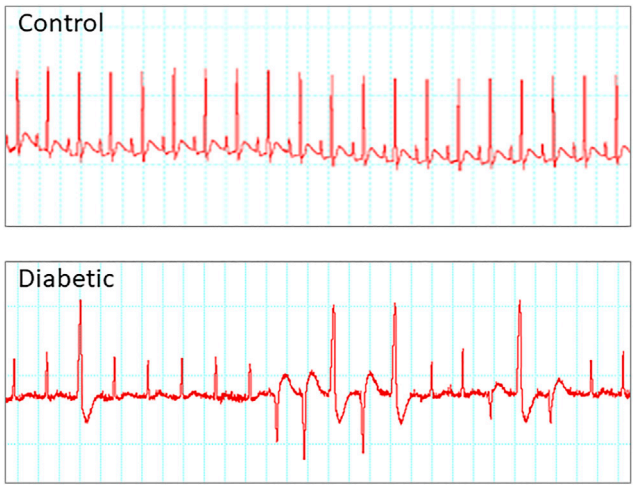


FIGURE 2 | Diabetes is a substrate for developing arrhythmia. Example of electrocardiograms recorded from control and type 2 diabetic rats upon stimulation with caffeine plus dobutamine. Only diabetic animals develop arrhythmia under cardiac challenge.

2019). For instance, the above mentioned diabetes-induced differential expression of conexins, that affects action potential conduction, might contribute to the development of atrial fibrillation (Watanabe et al., 2012). Regarding the electrical remodeling, diabetic Zucker obese rats with higher susceptibility to AF showed prolonged atrial action potential duration due to a reduction of the ultrarapid delayed rectifier and transient outward repolarizing currents (I_{kur} and I_{to}) along with a reduction of the corresponding channel forming proteins (Fu et al., 2019).

Prolonged QT Interval Duration

The most-studied electrical alteration of the diabetic myocardium is prolonged ventricular repolarization. Diabetes-induced lengthening of QT and QTc intervals associates with higher risk of developing ventricular arrhythmias and sudden cardiac death (**Figure 2**). Prolonged QTc was first reported in individuals with diabetic neuropathy (Bellavere et al., 1988; Chambers et al., 1990; Jermendy et al., 1990; Ewing et al., 1991) and later, it was also observed in newly diagnosed type 2 diabetic patients with no apparent complications (Naas et al., 1998). It is difficult to estimate the prevalence of longer QTc among diabetics. The different characteristics of the cohorts in each study and the formula used to adjust the QT for heart rate (Bazett, Fridericia, Hodges, others) lead to heterogeneous results, with prevalence estimates ranging from 30 to 66% (Veglio et al., 2002; Kumar et al., 2004; Li et al., 2012b; Cox et al., 2014; Lu et al., 2017). In these studies, recruited participants usually exhibited diabetes with several years of duration and therefore received antidiabetic medication. Metformin is the first choice to start glycemic control and many patients require additional drugs over time (Ferrannini and DeFronzo, 2015). Most prevalence studies do not differentiate subjects on monotherapy from those on combined therapy, but the fact is that antidiabetic medication does not seem to be efficient to restore normal QTc values.

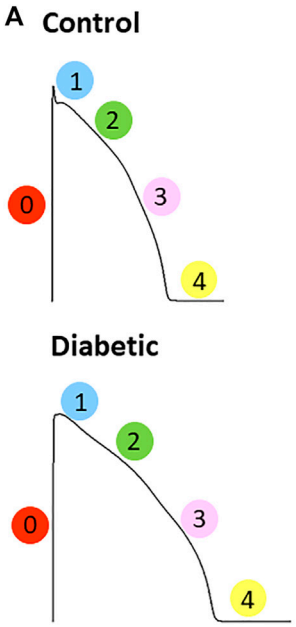
Another parameter that analyzes the repolarization is the QT dispersion or QTd, which reflects the difference between the longer and the shorter QT interval duration in the 12 leads electrocardiogram. An increase in the QTd is typically associated to an increased predisposition to ventricular arrhythmias. Diabetic patients show increased QTd and heart-rate corrected QT dispersion (QTdc) compared to non-diabetic subjects in the absence of other cardiovascular pathologies. Although the prevalence of severe prolonged QTc (>500 ms) and QTd (>80 ms) is low (Ninkovic et al., 2016), the presence of concomitant cardiovascular abnormalities such as left ventricular hypertrophy or hypertension increased the QT dispersion in diabetes mellitus (Cardoso et al., 2001).

ION CHANNELS AND CURRENTS IN THE DIABETIC MYOCARDIUM

The basic electrical activity of the cardiac cells is an action potential consisting of a rapid depolarization phase followed by a repolarization phase with a plateau. In 1983, Fein et al. described for the first time a lengthening of the cardiac action potential duration (APD) in a rat model of T1D (Fein et al., 1983), an effect consistently confirmed in rodent and non-rodent models (Magyar et al., 1992; Casis et al., 2000; Lengyel et al., 2007; Torres-Jacome et al., 2013). Thus, the APD observed in animal models of diabetes correlates with the prolonged QTc duration and increased QTd found in diabetic patients. In addition, in ventricular myocytes isolated from diabetic rats APD prolongation was not homogeneous throughout the heart, and the effect was more pronounced in the endocardium than in the epicardium (Casis et al., 2000). Prolonged APD results from diabetes-induced alterations in the expression and behavior of several ion channels that conduct depolarizing (sodium and calcium) and repolarizing (potassium) currents (summarized in **Figure 3**).

Alterations in the Sodium Current

The protein Nav1.5, encoded by the SCN5A gene, is the cardiac isoform of the voltage-gated sodium channel, which is responsible for carrying the sodium current, or I_{Na}. Diabetic rabbits displayed a reduction in the density of the cardiac I_{Na} although the Nav1.5 protein levels were not significantly reduced (Stables et al., 2014). However, in type 1 diabetic rats, as well as in culture cells exposed to hyperglycemic conditions, Yu et al. reported a reduced amount of Nav1.5 channels in the membrane. The excessive O-linked GlcNAcylation led to abnormal Nav1.5 aggregation in the cytoplasm and defective trafficking of the channel to the membrane (Yu et al., 2018). Nav1.5 channels open during the depolarization phase and then rapidly inactivate. However, some channels might reopen creating a persistent or late sodium current or $I_{Na,L}$ that interferes with the repolarization (Zaza and Rocchetti, 2013). If the contribution of the late component of the I_{Na} increases, AP duration can prolong excessively increasing the risk of arrhythmia. This might happen in diabetes; in fact, increased $I_{Na,L}$ has been found in type 1 and type 2 (db/db) mice with



B

AP Phase	Current	Protein	Type of Diabetes
0	↓ I_{Na} ↑ I_{Na-L}	↓ Nav1.5	T1D & T2D
1	↓ I_{to} ↓ I_{Kur}	↓ Kv4.3, Kv4.2 ↓ Kv1.5	T1D & T2D T1D & T2D
2	↓ I_{CaL}	↓ Cav1.2	T1D & T2D
3	↓ I_{Ks} ↓ I_{Kr}	=Kv7.1, ↓minK ↓ hERG	T1D T1D & T2D
4	= I_{K1} ? I_{K1} ? I_{NCX}	=Kir2.1 ↓ Kir2.1 ↑ NCX	T1D T2D T2D

FIGURE 3 | Diabetes affects the depolarizing and repolarizing currents responsible for the cardiac action potential. **(A)** Action potential depictions of a control and a diabetic heart (numbers indicate the phase of the AP) showing that diabetes changes action potential shape and prolongs its duration. **(B)** Summary of the alterations in the cardiac ion currents and the respective channel-forming proteins obtained in animal models of TD1 and T2D. See text for references.

prolonged QTc (Lu et al., 2013). Moreover, diabetic mice treated with the $I_{Na,L}$ inhibitor GS967 were more resistant to develop atrial fibrillation under an arrhythmia-inducing protocol than untreated diabetic animals (Jin et al., 2019). In addition, cultured cells incubated in high glucose conditions showed an increase in the late sodium current (Fouda et al., 2020). Different signaling pathways, metabolites and mechanisms regulate the late sodium current (Horváth et al., 2020). In diabetic mice, attenuated insulin/PI3K/Akt signaling increases $I_{Na,L}$, contributing to the subsequent APD and QTc prolongations (Lu et al., 2013).

Calcium Current and Calcium Handling

The L-type calcium current or I_{Ca-L} is a depolarizing current active during the phase of repolarization of the action potential and is the current responsible for the maintenance of the plateau phase. Regarding the I_{Ca-L}, experiments with animal models have yielded confusing results. Some groups found no effects upon the current amplitude and the biophysical behavior of the calcium channel (Jourdon and Feuvray, 1993; Tsuchida et al., 1994; Lengyel et al., 2007; Lengyel et al., 2008) in type 1 diabetic models. On the contrary, other groups described a reduction in the current amplitude and a slowing in the current inactivation kinetics (Horackova and Murphy, 1988; Wang et al., 1995; Chattou et al., 1999). Finally, some groups reported a reduction of I_{Ca-L} but no effect on the channel behavior. In this sense, the reduction in the cardiac I_{Ca-L} density correlated with a decreased expression of its channel-forming protein Cav1.2 in Akita mice, a genetic model of T1D with defective insulin production, as well as in models of T2D like the db/db obese mice and Zucker obese rats (Pereira et al., 2006; Lu et al., 2007; Fu et al., 2019). Electrophysiological recordings of single-channel activity showed that the biophysical properties of the calcium channel were similar in control and diabetic animals (Pereira et al., 2006).

The reason behind these discrepant results is not clear. It might be related with methodological aspects in current recordings, such as the patch-clamp configuration or the charge carrier used in the experiment (Ca^{2+} or Ba^{2+}). There could also be intrinsic differences in the diabetic models, for instance, induced *vs.* genetic. The experimental conditions for current recordings are also crucial, since the amplitude of the L-type calcium current is very sensitive to the intracellular calcium content. In physiological conditions, L-type calcium channels inactivate *via* Ca^{2+}-dependent inactivation, mainly by the calcium released from the sarcoplasmic reticulum (Pelzer et al., 1990; Sham, 1997; Kubalová, 2003). Thus, if intracellular Ca^{2+} is buffered during the current recording, differences in current behavior between control and diabetic cardiomyocytes might not be observed. However, experimental conditions that preserve the intracellular Ca^{2+} can differentially affect the behavior of the I_{Ca-L} in control and diabetic cells. In fact, intracellular calcium handling is altered in the diabetic myocardium (Allo et al., 1991), showing a characteristic reduced systolic Ca^{2+} and an augmented diastolic Ca^{2+}.

Diabetes induces changes in several proteins involved in calcium handling, what leads to an increase in the cytosolic (Ca^{2+}). Diabetes prolongs the duration of both the contraction and the relaxation phases of the cardiac cycle (Regan et al., 1974; Feuvray et al., 1979; Fein et al., 1980). In the diabetic heart, Ca^{2+} release from the sarcoplasmic reticulum during systole is

depressed, mainly due to a downregulation of Ca^{2+}-releasing proteins at the SR membrane. In this sense, mRNA and protein levels of ryanodine receptors (RYRs) are reduced in diabetes and become fully restored after insulin supplementation (Teshima et al., 2000; Netticadan et al., 2001). However, more characteristic of the diabetic myocardium is the diastolic calcium overload, which is increased about three fold (Allo et al., 1991). Diabetes increases the opening probability of RYRs and leads to diastolic Ca^{2+} leak to the cytoplasm (Singh et al., 2018). In addition, calcium reuptake to the SR is also compromised because diabetic cardiomyopathy reduces the expression of the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2) as well as its apparent affinity for Ca^{2+} (Teshima et al., 2000; Netticadan et al., 2001), (Zhong et al., 2001; Abe et al., 2002; Razeghi et al., 2002). As a result, diabetic cardiomyocytes have higher diastolic calcium levels caused by a markedly impaired calcium uptake (Penpargkul et al., 1981; Horackova and Murphy, 1988; Fein et al., 1990; Golfman et al., 1996). Thus, diabetic cardiomyocytes have less calcium available for the contraction during systole together with an excess of diastolic calcium that impairs relaxation during diastole.

The Repolarizing Potassium Currents

Several potassium currents determine the action potential repolarization phase. In the human heart these are the transient outward or I_{to}, the ultrarapid delayed rectifier or I_{Kur}, the rapid delayed rectifier or I_{Kr}, the slow delayed rectifier or I_{Ks}, and the inward rectifier or I_{K1} (Chiamvimonvat et al., 2017). Diabetes does not change the inward rectifier, the current that contributes to the final phase of the repolarization (Magyar et al., 1992; Casis et al., 2000).

On the contrary, I_{to}, which is the dominant ventricular repolarizing current in rodents, is highly affected by T1D. A reduction in the expression of the channel-forming proteins Kv4.3 and Kv4.2 and the accessory subunit KChIP2 (Qin et al., 2001; Lengyel et al., 2007; Torres-Jacome et al., 2013) caused the reduction in the amplitude of I_{to} (Magyar et al., 1992; Shimoni et al., 1994; Xu et al., 1996; Casis et al., 2000). In addition, diabetes accelerated I_{to} current inactivation (Magyar et al., 1992; Casis et al., 2000) due to a reduction in the Kv4.3 channel phosphorylation by CaMKII (Gallego et al., 2008). This contributed to the reduction of the total current and the lengthening of the action potential duration. Regarding I_{Kur}, diabetes reduced the expression of the pore forming protein Kv1.5, thus inhibiting the current (Casis et al., 2000; Torres-Jacome et al., 2013).

The delayed rectifiers are virtually absent in rodents; therefore, they have been analyzed mostly in type 1 diabetic rabbits and dogs. The amplitude of the slow delayed rectifier I_{Ks} was reduced (Lengyel et al., 2007; Lengyel et al., 2008) which correlated with the reduced expression levels of the accessory subunit mink, whereas the pore-forming subunit Kv7.1 was not or little affected (Lengyel et al., 2007; Zhang et al., 2007). Regarding the rapid delayed rectifier, most of the studies reported no effect of diabetes on I_{Kr} amplitude and properties (Lengyel et al., 2007; Lengyel et al., 2008; Torres-Jacome et al., 2013). However, experiments made in rabbits with long diabetes duration showed significant reduction of both the I_{Kr} amplitude and the expression of its pore-forming protein ERG (Zhang et al., 2006; Zhang et al., 2007).

A combination of factors can lead to the reduced electrical activity and expression of cardiac potassium channels. In type 1 diabetic animals, insulin treatment restored some but not all the altered currents (Zhang et al., 2006; Lengyel et al., 2007). The impaired metabolic status of the diabetic cells might also affect protein synthesis. For instance, *in vitro* activation of the AMP-dependent protein kinase reduced several K^+ repolarizing currents in a similar fashion than diabetes (Torres-Jacome et al., 2013). On the other hand, diabetes induced a sterile inflammation that increased the IL-1b release from cardiac macrophages and led to the reduction of the I_{to} (Monnerat et al., 2016).

The literature regarding cardiac electrical remodeling in T2D, however, is very limited. A few studies reported a prolongation of APD and a reduction of the amplitude of the ventricular I_{to} current in the genetic models WBN/Kob rats (Tsuchida et al., 1994; Tsuchida and Watajima, 1997), leptin-receptor deficient homozygous db/db mice (Shimoni et al., 2004) or the Otsuka-Long-Evans-Tokushima Fatty rats (Sato et al., 2014). Similarly, I_{to}, I_{Kur} and $I_{Ca\text{-}L}$ currents, as well as their corresponding channel-forming proteins, were reduced in the atria of Zucker diabetic fatty rats (Fu et al., 2019). Regarding humans, a study in elderly type 2 diabetic patients (Ashrafi et al., 2017) showed a reduction of the mRNAs encoding for hERG and Kir channels responsible for I_{Kr} and I_{K1}, and an increase of the Na^+/Ca^{2+} exchanger (NCX) expression. Although channel protein levels and current recordings were not assessed and the study was performed in few, aged and poly-medicated patients, this is the first work that directly compares the expression of cardiac channels between diabetic and non-diabetic humans (Hancox, 2017).

SAFETY ASSESSMENT OF ANTIDIABETIC DRUGS

Proarrhythmic Safety of New Drugs

Like diabetes, other disorders cause metabolic, endocrine, immune or autonomic disturbances, providing an environment that impairs cardiac ion channel function. Moreover, some drugs can directly inhibit and in some cases activate ion channels, affecting the overall electric response. Therefore, the proarrhythmic propensity of drugs is a matter of concern. Between 1989 and 2003 several drugs were associated with ventricular arrhythmia, mainly *torsade de pointes*, and were withdrawn from the market [reviewed in Turner et al., 2018]. As a result, in 2005 the International Council for Harmonization (ICH) of Technical Requirements for Pharmaceuticals for Human Use released two guidelines for industry, the non-clinical S7B and the clinical E14, to evaluate potential proarrhythmic effects of new drugs.

Non-clinical testing strategy focused mainly on evaluating the effects of a drug on the hERG channel *in vitro*, and in electrocardiographic recordings to analyze ventricular repolarization (ICH S7B Guideline, 2005). The hERG channel

encoded by the human ether-a-go-go-related gene conducts the I_{Kr}, the main repolarizing current in humans. The blockade of hERG channel is responsible for most of the drug-induced QT prolongations and TdPs because the protein has a peculiar site that effectively accommodates the binding of drugs and makes it particularly susceptible to blockade (Sanguinetti and Tristani-Firouzi, 2006). However, not all the hERG-channel blockers prolong the QT interval or induce TdP. Some drugs might have additional effects on other channels that counteract the lengthening of the repolarization, resulting in normal action potential and QT interval duration. Furthermore, QTc prolongation does not necessarily trigger TdP yet may discontinue compounds from development. Since 2013, the Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative works in a new paradigm for assessment of TdP proarrhythmic risk of new drugs that is not focused exclusively in hERG blockage and QT prolongation (Sager et al., 2014). Instead, the CiPA initiative includes: the *in vitro* assessment of drug effects on multiple ion channels; the prediction of proarrhythmic risk using *in silico* models; the *in vitro* confirmation of proarrhythmicity in human stem cell derived ventricular cardiomyocytes; and electrocardiograms in phase 1 clinical trials (Vicente et al., 2018).

On the other hand, the clinical evaluation of potential proarrhythmic effects consists on the "Thorough QT/QTc" or TQT Study, usually performed before phase 3 clinical development to detect whether the drug has a threshold pharmacologic effect on prolonging repolarization. In the TQT studies, healthy voluntaries receive a negative control (placebo); a positive control (a drug that prolongs the QTc); the drug under development at the maximum recommended therapeutic dose; and the drug at supratherapeutic dose to explore the "worst-case scenario", for instance, in an impaired clearance of the drug. The TQT study is negative if the drug does not prolong the mean QT/QTc interval more than 5 ms, which is considered the threshold of regulatory concern (ICH E14 Guideline, 2015). However, the TQT study is resource intensive and scientists and regulatory agencies are discussing alternatives that are more effective, such as the intensive assessment of ECG parameters in the first-in-human study (Darpo et al., 2014; ICH E14 Guideline, 2015).

Proarrhythmic Safety of Antidiabetic Drugs

Since metformin, insulin, sulfonylureas or thiazolidinedione were marketed years before the regulations about arrhythmic safety, TQT studies did not evaluate these classic antidiabetics. On the contrary, newer drugs approved after the ICH E14 guideline, such as semaglutide, have been examined in TQT studies (Demmel et al., 2018). However, evaluation of glucose lowering drugs is very challenging since, for instance, changes in blood glucose concentrations *per se* may correlate with prolonged QTc (Suys et al., 2006). The Cardiac Safety Research Consortium discussed these major confounding factors (Heller et al., 2015). Variations in glucose, insulin and potassium levels, fasted vs. fed state and the activation of the autonomous nervous system may all affect ventricular repolarization, as well as other ECG parameters like RR interval duration and the T-wave morphology. This complicates the design and interpretation of TQT studies for assessing antidiabetic compounds.

Glucose-Lowering Treatment for Patients at Cardiovascular Risk

On the other hand, although the incidence of cardiovascular disease (CVD) has declined in both adults with and without diabetes over the last few decades, diabetic patients still have 2-fold greater risk of CVD compared with the non-diabetic population (Fox et al., 2004; Preis et al., 2009). In addition, studies of the agonist of the peroxisome proliferator-activated receptor families alpha and gamma muraglitazar, an agonist of the peroxisome proliferator-activated receptor (PPAR), and the thiazolidinedione rosiglitazone, concluded an increase in the risk of death and in the incidence of major adverse cardiovascular events compared to standard therapy (Nissen et al., 2005; Nissen and Wolski, 2007). These concerning results precipitated that, in 2008, the FDA issued guidance for industry aimed to ensure that new antidiabetic therapies for T2D do not increase the CVD risk. As a result, during pharmacological development of new antidiabetic drugs, Cardiovascular Outcome Trials (CVOTs) must be performed on selected type 2 diabetic patients at higher risk of cardiovascular events (Food and Drug Administration, 2008). Primary outcome in CVOTs is typically a composite of death of cardiovascular cause, non-fatal myocardial infarction and non-fatal stroke. Secondary outcomes may include hospitalization for heart failure, acute coronary syndrome and revascularization (Schnell et al., 2016). Interestingly, diabetes is the only pathology where routine CVOTs in the absence of safety signals are mandatory.

As with the TQTs, CVOTs were not performed for the old antidiabetic drugs. Three groups of new drugs: dipeptidyl peptidase 4 (DPP-4) inhibitors, sodium-glucose cotransporter 2 (SGLT2) inhibitors and glucagon-like peptide 1 (GLP-1) receptor agonists have undertaken CVOTs. Detailed summaries of completed and ongoing studies have been recently published (Cefalu et al., 2018; Schnell et al., 2019; Schnell et al., 2020). Although some differences between drugs exist, CVOTs confirm that the tested drugs fulfill the FDA requirements because they are not associated with an unacceptable increase in cardiovascular risk; therefore, all of them are safe. The GLP1R agonist liraglutide and the SGLT2 inhibitors empagliflozin and, to a lower extent, canagliflozin, have yielded very positive results. Consequently, the American Diabetes Association recommended incorporating these drugs to the standard therapy in T2D patients with established atherosclerotic cardiovascular disease (Davies et al., 2018).

Furthermore, very recent studies have focused on the potential protective effect of SGLT2 inhibitors against developing arrhythmias. In newly diagnosed diabetic patients, SGTL2 inhibitors associates with a reduced risk of new onset arrhythmia (Chen et al., 2020). Similarly, recent meta-analysis examining arrhythmia outcomes, like atrial fibrillation and ventricular tachycardia, found that SGLT2 inhibitors treatment reduces the risk of cardiac arrhythmias in diabetic patients (Li et al., 2021). However, in most trials, information regarding pre-existing arrhythmia and anti-arrhythmic therapy were not available. More research will be required to determine the best treatment for those arrhythmic patients who develop diabetes.

Currently, the ADA recommends the use of either an SGLT2 inhibitor or a GLP-1 receptor agonist to reduce cardiovascular risk because this treatment is appropriate for many patients (ADA, 2021). Although both SGLT2i and GLP-1R agonist could be used in patients with atherosclerotic cardiovascular disease, SGLT2 inhibitors are recommended in patients with heart failure or kidney disease (Dardano et al., 2020).

Among the proposed cardioprotective mechanisms are lowering blood pressure, which may lower cardiac afterload; improving cardiac metabolism increasing energy production; reducing inflammation and cytokine release; preventing adverse cardiac remodeling; reducing sympathetic nerve activity; improving renal function; improving mitochondrial dysfunction and improving ionic dyshomeostasis. Thus, the underlying cardioprotective mechanisms of SGLT2 inhibitors and GLP-1 receptor agonists remain unclear and might involve complementary systemic and direct cardiac effects (recently reviewed in Lahnwong et al., 2018; Dardano et al., 2020; Lopaschuk and Verma, 2020).

Regarding ionic dyshomeostasis and arrhythmic risk, (Na^+) was elevated in the diabetic myocardium, directly increasing the risk of sudden arrhythmic death (Lambert et al., 2015). Empagliflocin inhibits the Na^+/H^+ exchanger and reduces the sodium and calcium concentration (Baartscheer et al., 2017). Another SGLT2 inhibitor, dapagliflozin, reduced the membrane expression of the Na^+/H^+ exchanger, the Na^+/Ca^{2+} exchanger and the L-type calcium channel in db/db mice (Arow et al., 2020). The resulting reduction of the cytoplasmic (Na^+) and (Ca^{2+}) might reduce the risk of developing potentially lethal arrhythmias. Very interestingly, in insulin resistant rats dapagliflozin-treatment improved cardiac repolarization. Daplagliflozin supressed QT interval and AP prolongation, mainly by restoring the depressed potassium currents I_{to} and I_{K1}, but also by reducing I_{Na} (Durak et al., 2018).

CONCLUSION AND FUTURE PRESPECTIVES

Both T1D and T2D induce a cardiac remodeling that leads to mechanical dysfunction and/or cardiac arrhythmias. Experiments with animal models have consistently shown that diabetes alters the expression and regulation of cardiac ion channels and transporters, which impairs impulse generation, conduction, excitation-contraction coupling and myocyte contractility.

Prolonged QTc interval duration persists in a number of treated diabetic patients, indicating that strict glycemic control is not sufficient to normalize the electrophysiological disturbances. Available glucose-lowering drugs that improve cardiovascular prognosis are crucial in the management of type 2 diabetic patients with established cardiovascular disease or at high cardiovascular risk. Further studies are needed to elucidate if cardioprotection includes electrical remodeling and prolonged repolarization. This could be of particular interest for patients with diabetes-associated complications that may increase the risk of arrhythmia.

AUTHOR CONTRIBUTIONS

MG and OC conceptualization of the manuscript. MG, OC, JZ, BA and AA writing the original draft. MG, OC, and BA: writing review and editing. All authors contributed to the article and approved the submitted version.

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REFERENCES

Abe, T., Ohga, Y., Tabayashi, N., Kobayashi, S., Sakata, S., Misawa, H., et al. (2002). Left Ventricular Diastolic Dysfunction in Type 2 Diabetes Mellitus Model Rats. *Am. J. Physiology-Heart Circulatory Physiol.* 282 (1), H138–H148. doi:10.1152/ajpheart.2002.282.1.h138

ADA (2021). Cardiovascular Disease and Risk Management: Standards of Medical Care in Diabetes-2021. *Diabetes Care* 44, S125–S150.

Al-awar, A., Kupai, K., Veszelka, M., Szűcs, G., Attieh, Z., Murlasits, Z., et al. (2016). Experimental Diabetes Mellitus in Different Animal Models. *J. Diabetes Res.* 2016, 9051426. doi:10.1155/2016/9051426

Allo, S. N., Lincoln, T. M., Wilson, G. L., Green, F. J., Watanabe, A. M., and Schaffer, S. W. (1991). Non-Insulin-Dependent Diabetes-Induced Defects in Cardiac Cellular Calcium Regulation. *Am. J. Physiology-Cell Physiol.* 260 (6), C1165–C1171. doi:10.1152/ajpcell.1991.260.6.c1165

Arow, M., Waldman, M., Yadin, D., Nudelman, V., Shainberg, A., Abraham, N. G., et al. (2020). Sodium–glucose Cotransporter 2 Inhibitor Dapagliflozin Attenuates Diabetic Cardiomyopathy. *Cardiovasc. Diabetology* 19 (1), 7. doi:10.1186/s12933-019-0980-4

Ashrafi, R., Modi, P., Oo, A. Y., Pullan, D. M., Jian, K., Zhang, H., et al. (2017). Arrhythmogenic Gene Remodelling in Elderly Patients with Type 2 Diabetes with Aortic Stenosis and normal Left Ventricular Ejection Fraction. *Exp. Physiol.* 102 (11), 1424–1434. doi:10.1113/ep086412

Baartscheer, A., Schumacher, C. A., Wüst, R. C. I., Fiolet, J. W. T., Stienen, G. J. M., Coronel, R., et al. (2017). Empagliflozin Decreases Myocardial Cytoplasmic Na+ through Inhibition of the Cardiac Na+/H+ Exchanger in Rats and Rabbits. *Diabetologia* 60 (3), 568–573. doi:10.1007/s00125-016-4134-x

Battiprolu, P. K., Lopez-Crisosto, C., Wang, Z. V., Nemchenko, A., Lavandero, S., and Hill, J. A. (2013). Diabetic Cardiomyopathy and Metabolic Remodeling of the Heart. *Life Sci.* 92 (11), 609–615. doi:10.1016/j.lfs.2012.10.011

Bellavere, F., Ferri, M., Guarini, L., Bax, G., Piccoli, A., Cardone, C., et al. (1988). Prolonged QT Period in Diabetic Autonomic Neuropathy: a Possible Role in Sudden Cardiac Death? *Heart* 59 (3), 379–383. doi:10.1136/hrt.59.3.379

Brown, D. W., Giles, W. H., Greenlund, K. J., Valdez, R., and Croft, J. B. (2001). Impaired Fasting Glucose, Diabetes Mellitus, and Cardiovascular Disease Risk Factors Are Associated with Prolonged QTc Duration. Results from the Third National Health and Nutrition Examination Survey. *Eur. J. Cardiovasc. Prev. Rehabil.* 8 (4), 227–233. doi:10.1177/174182670100800407

Bugger, H., and Abel, E. D. (2014). Molecular Mechanisms of Diabetic Cardiomyopathy. *Diabetologia* 57 (4), 660–671. doi:10.1007/s00125-014-3171-6

Cardoso, C., Salles, G., Bloch, K., Deccache, W., and Siqueira, A. G. (2001). Clinical Determinants of Increased QT Dispersion in Patients with Diabetes Mellitus. *Int. J. Cardiol.* 79 (2-3), 253–262. doi:10.1016/s0167-5273(01)00443-0

Casis, O., and Echevarria, E. (2004). Diabetic Cardiomyopathy: Electromechanical Cellular Alterations. *Cvp* 2 (3), 237–248. doi:10.2174/1570161043385655

Casis, O., Gallego, M., Iriarte, M., and Sánchez-Chapula, J. A. (2000). Effects of Diabetic Cardiomyopathy on Regional Electrophysiologic Characteristics of Rat Ventricle. *Diabetologia* 43 (1), 101–109. doi:10.1007/s001250050013

Cefalu, W. T., Kaul, S., Gerstein, H. C., Holman, R. R., Zinman, B., Skyler, J. S., et al. (2018). Cardiovascular Outcomes Trials in Type 2 Diabetes: Where Do We Go from Here? Reflections from aDiabetes CareEditors' Expert Forum. *Dia Care* 41 (1), 14–31. doi:10.2337/dci17-0057

Chambers, J. B., Sampson, M. J., Sprigings, D. C., and Jackson, G. (1990). Qt Prolongation on the Electrocardiogram in Diabetic Autonomic Neuropathy. *Diabetic Med.* 7 (2), 105–110. doi:10.1111/j.1464-5491.1990.tb01342.x

Chattou, S., Diacono, J., and Feuvray, D. (1999). Decrease in Sodium-Calcium Exchange and Calcium Currents in Diabetic Rat Ventricular Myocytes. *Acta Physiol. Scand.* 166 (2), 137–144. doi:10.1046/j.1365-201x.1999.00547.x

Chen, C., Wang, W., Zhou, W., Jin, J., Chen, W., Zhu, D., et al. (2019). Nocturnal Ventricular Arrhythmias Are Associated with the Severity of Cardiovascular Autonomic Neuropathy in Type 2 Diabetes. *J. Diabetes* 11 (10), 794–801. doi:10.1111/1753-0407.12908

Chen, H.-Y., Huang, J.-Y., Siao, W.-Z., and Jong, G.-P. (2020). The Association between SGLT2 Inhibitors and New-Onset Arrhythmias: a Nationwide Population-Based Longitudinal Cohort Study. *Cardiovasc. Diabetology* 19 (1), 73. doi:10.1186/s12933-020-01048-x

Chiamvimonvat, N., Chen-Izu, Y., Clancy, C. E., Deschenes, I., Dobrev, D., Heijman, J., et al. (2017). Potassium Currents in the Heart: Functional Roles in Repolarization, Arrhythmia and Therapeutics. *J. Physiol.* 595 (7), 2229–2252. doi:10.1113/jp272883

Cox, A. J., Azeem, A., Yeboah, J., Soliman, E. Z., Aggarwal, S. R., Bertoni, A. G., et al. (2014). Heart Rate-Corrected QT Interval Is an Independent Predictor of All-Cause and Cardiovascular Mortality in Individuals with Type 2 Diabetes: The Diabetes Heart Study. *Dia Care* 37 (5), 1454–1461. doi:10.2337/dc13-1257

Dardano, A., Miccoli, R., Bianchi, C., Daniele, G., and Del Prato, S. (2020). Invited Review. Series: Implications of the Recent CVOTs in Type 2 Diabetes. *Diabetes Res. Clin. Pract.* 162, 108112. doi:10.1016/j.diabres.2020.108112

Darpo, B., Garnett, C., Benson, C. T., Keirns, J., Leishman, D., Malik, M., et al. (2014). Cardiac Safety Research Consortium: Can the Thorough QT/QTc Study Be Replaced by Early QT Assessment in Routine Clinical Pharmacology Studies? Scientific Update and a Research Proposal for a Path Forward. *Am. Heart J.* 168 (3), 262–272. doi:10.1016/j.ahj.2014.06.003

Davies, M. J., D'Alessio, D. A., Fradkin, J., Kernan, W. N., Mathieu, C., Mingrone, G., et al. (2018). Management of Hyperglycemia in Type 2 Diabetes, 2018. A Consensus Report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD)A Consensus Report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Dia Care* 41 (12), 2669–2701. doi:10.2337/dci18-0033**2018**

Demmel, V., Sandberg-Schaal, A., Jacobsen, J. B., Golor, G., Pettersson, J., and Flint, A. (2018). No QTc Prolongation with Semaglutide: A Thorough QT Study in Healthy Subjects. *Diabetes Ther.* 9 (4), 1441–1456. doi:10.1007/s13300-018-0442-0

Devereux, R. B., Roman, M. J., Paranicas, M., O'Grady, M. J., Lee, E. T., Welty, T. K., et al. (2000). Impact of Diabetes on Cardiac Structure and Function. *Circulation* 101 (19), 2271–2276. doi:10.1161/01.cir.101.19.2271

Duckworth, W., Abraira, C., Moritz, T., Reda, D., Emanuele, N., Reaven, P. D., et al. (2009). Glucose Control and Vascular Complications in Veterans with Type 2 Diabetes. *N. Engl. J. Med.* 360 (2), 129–139. doi:10.1056/nejmoa0808431

Durak, A., Olgar, Y., Degirmenci, S., Akkus, E., Tuncay, E., and Turan, B. (2018). A SGLT2 Inhibitor Dapagliflozin Suppresses Prolonged Ventricular-Repolarization through Augmentation of Mitochondrial Function in Insulin-Resistant Metabolic Syndrome Rats. *Cardiovasc. Diabetology* 17, 144. doi:10.1186/s12933-018-0790-0

Ewing, D. J., Boland, O., Neilson, J. M. M., Cho, C. G., and Clarke, B. F. (1991). Autonomic Neuropathy, QT Interval Lengthening, and Unexpected Deaths in Male Diabetic Patients. *Diabetologia* 34 (3), 182–185. doi:10.1007/bf00418273

Fein, F., Aronson, R. S., Nordin, C., Miller-Green, B., and Sonnenblick, E. H. (1983). Altered Myocardial Response to Ouabain in Diabetic Rats: Mechanics and Electrophysiology. *J. Mol. Cell Cardiol.* 15 (11), 769–784. doi:10.1016/0022-2828(83)90336-x

Fein, F. S., Kornstein, L. B., Strobeck, J. E., Capasso, J. M., and Sonnenblick, E. H. (1980). Altered Myocardial Mechanics in Diabetic Rats. *Circ. Res.* 47 (6), 922–933. doi:10.1161/01.res.47.6.922

Fein, F. S., Zola, B. E., Malhotra, A., Cho, S., Factor, S. M., Scheuer, J., et al. (1990). Hypertensive-Diabetic Cardiomyopathy in Rats. *Am. J. Physiology-Heart Circulatory Physiol.* 258 (3), H793–H805. doi:10.1152/ajpheart.1990.258.3.h793

Ferrannini, E., and DeFronzo, R. A. (2015). Impact of Glucose-Lowering Drugs on Cardiovascular Disease in Type 2 Diabetes. *Eur. Heart J.* 36 (34), 2288–2296. doi:10.1093/eurheartj/ehv239

Feuvray, D., Idell-Wenger, J. A., and Neely, J. R. (1979). Effects of Ischemia on Rat Myocardial Function and Metabolism in Diabetes. *Circ. Res.* 44 (3), 322–329. doi:10.1161/01.res.44.3.322

Food and Drug Administration, (2008). *Guidance for Industry Diabetes Mellitus — Evaluating Cardiovascular Risk in New Antidiabetic Therapies to Treat Type 2 Diabetes.* Food and Drug Administration.

Fouda, M. A., Ghovanloo, M. R., and Ruben, P. C. (2020). Cannabidiol Protects against High Glucose-Induced Oxidative Stress and Cytotoxicity in Cardiac Voltage-Gated Sodium Channels. *Br. J. Pharmacol.*

Fowlkes, V., Clark, J., Fix, C., Law, B. A., Morales, M. O., Qiao, X., et al. (2013). Type II Diabetes Promotes a Myofibroblast Phenotype in Cardiac Fibroblasts. *Life Sci.* 92 (11), 669–676. doi:10.1016/j.lfs.2013.01.003

Fox, C. S., Coady, S., Sorlie, P. D., Levy, D., Meigs, J. B., D'Agostino, R. B., et al. (2004). Trends in Cardiovascular Complications of Diabetes. *Jama* 292 (20), 2495–2499. doi:10.1001/jama.292.20.2495

Fu, L., Rao, F., Lian, F., Yang, H., Kuang, S., Wu, S., et al. (2019). Mechanism of Electrical Remodeling of Atrial Myocytes and its Influence on Susceptibility to Atrial Fibrillation in Diabetic Rats. *Life Sci.* 239, 116903. doi:10.1016/j.lfs.2019.116903

Gallego, M., and Casis, O. (2014). *Cellular Mechanism Underlying the Misfunction of Cardiac Ionic Channels in Diabetes*, 233. NEW YORK: Springer, 199.

Gallego, M., Fernandez, D., Ahyayauch, H., Casis, E., and Casis, O. (2008). Reduced Calmodulin Expression Accelerates Transient Outward Potassium Current Inactivation in Diabetic Rat Heart. *Cell Physiol. Biochem.* 22 (5-6), 625–634. doi:10.1159/000185546

Goldfine, A. B. (2008). Assessing the Cardiovascular Safety of Diabetes Therapies. *N. Engl. J. Med.* 359 (11), 1092–1095. doi:10.1056/nejmp0805758

Golfman, L. S., Takeda, N., and Dhalla, N. S. (1996). Cardiac Membrane Ca(2+)-Transport in Alloxan-Induced Diabetes in Rats. *Diabetes Res. Clin. Pract.* 31 (Suppl. l), 73. doi:10.1016/0168-8227(96)01233-8

Goudis, C. A., Korantzopoulos, P., Ntalas, I. V., Kallergis, E. M., Liu, T., and Ketikoglou, D. G. (2015). Diabetes Mellitus and Atrial Fibrillation: Pathophysiological Mechanisms and Potential Upstream Therapies. *Int. J. Cardiol.* 184, 617–622. doi:10.1016/j.ijcard.2015.03.052

Grisanti, L. A. (2018). Diabetes and Arrhythmias: Pathophysiology, Mechanisms and Therapeutic Outcomes. *Front. Physiol.* 9, 1669. doi:10.3389/fphys.2018.01669

Group, T. A. C. (2008). Intensive Blood Glucose Control and Vascular Outcomes in Patients with Type 2 Diabetes. *New Engl. J. Med.* 358 (24), 2560–2572.

Hancox, J. C. (2017). A Basis for Human QT Interval Prolongation and Arrhythmia Risk in Type 2 Diabetes? *Exp. Physiol.* 102 (11), 1395–1396. doi:10.1113/ep086618

Hegyi, B., Bers, D. M., and Bossuyt, J. (2019). CaMKII Signaling in Heart Diseases: Emerging Role in Diabetic Cardiomyopathy. *J. Mol. Cell Cardiol.* 127, 246–259. doi:10.1016/j.yjmcc.2019.01.001

Heller, S., Darpö, B., Mitchell, M. I., Linnebjerg, H., Leishman, D. J., Mehrotra, N., et al. (2015). Considerations for Assessing the Potential Effects of Antidiabetes Drugs on Cardiac Ventricular Repolarization: A Report from the Cardiac Safety Research Consortium. *Am. Heart J.* 170 (1), 23–35. doi:10.1016/j.ahj.2015.03.007

Horackova, M., and Murphy, M. G. (1988). Effects of Chronic Diabetes Mellitus on the Electrical and Contractile activities,45Ca2+ Transport, Fatty Acid Profiles and Ultrastructure of Isolated Rat Ventricular Myocytes. *Pflugers Arch.* 411 (5), 564–572. doi:10.1007/bf00582379

Horváth, B., Hézső, T., Kiss, D., Kistamás, K., Magyar, J., Nánási, P. P., et al. (2020). Late Sodium Current Inhibitors as Potential Antiarrhythmic Agents. *Front. Pharmacol.* 11 (413). doi:10.3389/fphar.2020.00413

Howarth, F. C., Qureshi, M. A., Jayaprakash, P., Parekh, K., Oz, M., Dobrzynski, H., et al. (2018). The Pattern of mRNA Expression Is Changed in Sinoatrial Node from Goto-Kakizaki Type 2 Diabetic Rat Heart. *J. Diabetes Res.* 2018, 8454078. doi:10.1155/2018/8454078

Howarth, F. C., Nowotny, N., Zilahi, E., El Haj, M. A., and Lei, M. (2007). Altered Expression of gap junction Connexin Proteins May Partly Underlie Heart

Rhythm Disturbances in the Streptozotocin-Induced Diabetic Rat Heart. *Mol. Cell. Biochem.* 305 (1-2), 145–151. doi:10.1007/s11010-007-9537-z

Huxley, R. R., Alonso, A., Lopez, F. L., Filion, K. B., Agarwal, S. K., Loehr, L. R., et al. (2012). Type 2 Diabetes, Glucose Homeostasis and Incident Atrial Fibrillation: the Atherosclerosis Risk in Communities Study. *Heart* 98 (2), 133–138. doi:10.1136/heartjnl-2011-300503

ICH E14 Guideline, (2015). *The clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs*. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use.

ICH S7B Guideline, (2005). *The non-clinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals*. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use.

Ionut, V., Liu, H., Mooradian, V., Castro, A. V. B., Kabir, M., Stefanovski, D., et al. (2010). Novel Canine Models of Obese Prediabetes and Mild Type 2 Diabetes. *Am. J. Physiology-Endocrinology Metab.* 298 (1), E38–E48. doi:10.1152/ajpendo.00466.2009

Jermendy, G. (2003). Clinical Consequences of Cardiovascular Autonomic Neuropathy in Diabetic Patients. *Acta Diabetol.* 40, S370–S374. doi:10.1007/s00592-003-0122-y

Jermendy, G., Koltai, M. Z., and Pogátsa, G. (1990). QT Interval Prolongation in Type 2 (Non-insulin-dependent) Diabetic Patients with Cardiac Autonomic Neuropathy. *Acta Diabet. Lat* 27 (4), 295–301. doi:10.1007/bf02580933

Jia, G., Hill, M. A., and Sowers, J. R. (2018). Diabetic Cardiomyopathy. *Circ. Res.* 122 (4), 624–638. doi:10.1161/circresaha.117.311586

Jin, X., Jiang, Y., Xue, G., Yuan, Y., Zhu, H., Zhan, L., et al. (2019). Increase of Late Sodium Current Contributes to Enhanced Susceptibility to Atrial Fibrillation in Diabetic Mice. *Eur. J. Pharmacol.* 857. doi:10.1016/j.ejphar.2019.172444

Jourdon, P., and Feuvray, D. (1993). Calcium and Potassium Currents in Ventricular Myocytes Isolated from Diabetic Rats. *J. Physiology-London* 470, 411–429. doi:10.1113/jphysiol.1993.sp019866

Kannel, W. B., Hjortland, M., and Castelli, W. P. (1974). Role of Diabetes in Congestive Heart Failure: The Framingham Study. *Am. J. Cardiol.* 34 (1), 29–34. doi:10.1016/0002-9149(74)90089-7

Karam, B. S., Chavez-Moreno, A., Koh, W., Akar, J. G., and Akar, F. G. (2017). Oxidative Stress and Inflammation as central Mediators of Atrial Fibrillation in Obesity and Diabetes. *Cardiovasc. Diabetology* 16, 120. doi:10.1186/s12933-017-0604-9

King, A., and Bowe, J. (2016). Animal Models for Diabetes: Understanding the Pathogenesis and Finding New Treatments. *Biochem. Pharmacol.* 99, 1–10. doi:10.1016/j.bcp.2015.08.108

King, A. J. (2012). The Use of Animal Models in Diabetes Research. *Br. J. Pharmacol.* 166 (3), 877–894. doi:10.1111/j.1476-5381.2012.01911.x

Kubalová, Z. (2003). Inactivation of L-type Calcium Channels in Cardiomyocytes. Experimental and Theoretical Approaches. *Gen. Physiol. Biophys.* 22 (4), 441–454.

Kumar, R., Fisher, M., Whitaker, R., and MacFarlane, P. W. (2004). Effect of Controlling Hyperglycemia with Diet on QT Abnormalities in Newly Diagnosed Patients with Type 2 Diabetes. *Diabetes care* 27 (11), 2767–2768. doi:10.2337/diacare.27.11.2767-a

Lahnwong, S., Chattipakorn, S. C., and Chattipakorn, N. (2018). Potential Mechanisms Responsible for Cardioprotective Effects of Sodium–Glucose Co-transporter 2 Inhibitors. *Cardiovasc. Diabetology* 17 (1), 101. doi:10.1186/s12933-018-0745-5

Lambert, R., Srodulski, S., Peng, X., Margulies, K. B., Despa, F., and Despa, S. (2015). Intracellular Na+ Concentration ([Na+](i)) Is Elevated in Diabetic Hearts Due to Enhanced Na+-Glucose Cotransport. *J. Am. Heart Assoc.* 4 (9), e002183. doi:10.1161/jaha.115.002183

Lee, M., Gardin, J. M., Lynch, J. C., Smith, V.-E., Tracy, R. P., Savage, P. J., et al. (1997). Diabetes Mellitus and Echocardiographic Left Ventricular Function in Free-Living Elderly Men and Women: The Cardiovascular Health Study. *Am. Heart J.* 133 (1), 36–43. doi:10.1016/s0002-8703(97)70245-x

Lengyel, C., Virag, L., Biro, T., Jost, N., Magyar, J., Biliczki, P., et al. (2007). Diabetes Mellitus Attenuates the Repolarization reserve in Mammalian Heart. *Cardiovasc. Res.* 73 (3), 512–520. doi:10.1016/j.cardiores.2006.11.010

Lengyel, C., Virág, L., Kovács, P. P., Kristóf, A., Pacher, P., Kocsis, E., et al. (2008). Role of Slow Delayed Rectifier K+-current in QT Prolongation in the Alloxan-Induced Diabetic Rabbit Heart. *Acta Physiol.* 192 (3), 359–368. doi:10.1111/j.1748-1716.2007.01753.x

Lenzen, S. (2008). The Mechanisms of Alloxan- and Streptozotocin-Induced Diabetes. *Diabetologia* 51 (2), 216–226. doi:10.1007/s00125-007-0886-7

Li, C.-j., Lv, L., Li, H., and Yu, D.-m. (2012). Cardiac Fibrosis and Dysfunction in Experimental Diabetic Cardiomyopathy Are Ameliorated by Alpha-Lipoic Acid. *Cardiovasc. Diabetol.* 11, 73. doi:10.1186/1475-2840-11-73

Li, H.-L., Lip, G.-Y. H., Feng, Q., Fei, Y., Tse, Y.-K., Wu, M.-z., et al. (2021). Sodium-glucose Cotransporter 2 Inhibitors (SGLT2i) and Cardiac Arrhythmias: a Systematic Review and Meta-Analysis. *Cardiovasc. Diabetology* 20 (1), 100. doi:10.1186/s12933-021-01293-8

Li, X., Ren, H., Xu, Z. R., Liu, Y. J., Yang, X. P., and Liu, J. Q. (2012). Prevalence and Risk Factors of Prolonged QTc Interval Among Chinese Patients with Type 2 Diabetes. *Exp. Diabetes Res.* 2012, 234084. doi:10.1155/2012/234084

Lopaschuk, G. D., and Verma, S. (2020). Mechanisms of Cardiovascular Benefits of Sodium Glucose Co-transporter 2 (SGLT2) Inhibitors. *JACC: Basic Translational Sci.* 5 (6), 632–644. doi:10.1016/j.jacbts.2020.02.004

Lu, Z., Jiang, Y.-P., Wu, C.-Y. C., Ballou, L. M., Liu, S., Carpenter, E. S., et al. (2013). Increased Persistent Sodium Current Due to Decreased PI3K Signaling Contributes to QT Prolongation in the Diabetic Heart. *Diabetes* 62 (12), 4257–4265. doi:10.2337/db13-0420

Lu, Z., Jiang, Y.-P., Xu, X.-H., Ballou, L. M., Cohen, I. S., and Lin, R. Z. (2007). Decreased L-type Ca2+ Current in Cardiac Myocytes of Type 1 Diabetic Akita Mice Due to Reduced Phosphatidylinositol 3-kinase Signaling. *Diabetes* 56 (11), 2780–2789. doi:10.2337/db06-1629

Lu, Z., Lense, L., Sharma, M., Shah, A., Luu, Y., Cardinal, L., et al. (2017). Prevalence of QT Prolongation and Associated LVEF Changes in Diabetic Patients over a Four-Year Retrospective Time Period. *J. Community Hosp. Intern. Med. Perspect.* 7 (2), 87–94. doi:10.1080/20009666.2017.1320203

Magyar, J., Rusznak, Z., Szentesi, P., Szucs, G., and Kovacs, L. (1992). Action Potentials and Potassium Currents in Rat Ventricular Muscle during Experimental Diabetes. *J. Mol. Cell Cardiol.* 24 (8), 841–853. doi:10.1016/0022-2828(92)91098-p

Masiello, P. (2006). Animal Models of Type 2 Diabetes with Reduced Pancreatic Beta-Cell Mass. *Int. J. Biochem. Cel Biol.* 38 (5-6), 873–893. doi:10.1016/j.biocel.2005.09.007

Miragoli, M., Gaudesius, G., and Rohr, S. (2006). Electrotonic Modulation of Cardiac Impulse Conduction by Myofibroblasts. *Circ. Res.* 98 (6), 801–810. doi:10.1161/01.res.0000214537.44195.a3

Monnerat, G., Alarcon, M. L., Vasconcellos, L. R., Hochman-Mendez, C., Brasil, G., Bassani, R. A., et al. (2016). Macrophage-dependent IL-1 Beta Production Induces Cardiac Arrhythmias in Diabetic Mice. *Nat. Commun.* 7, 13344. doi:10.1038/ncomms13344

Naas, A. A. O., Davidson, N. C., Thompson, C., Cummings, F., Ogston, S. A., Jung, R. T., et al. (1998). QT and QTc Dispersion Are Accurate Predictors of Cardiac Death in Newly Diagnosed Non-insulin Dependent Diabetes: Cohort Study. *Bmj* 316 (7133), 745–746. doi:10.1136/bmj.316.7133.745

Netticadan, T., Temsah, R. M., Kent, A., Elimban, V., and Dhalla, N. S. (2001). Depressed Levels of Ca2+-Cycling Proteins May Underlie Sarcoplasmic Reticulum Dysfunction in the Diabetic Heart. *Diabetes* 50 (9), 2133–2138. doi:10.2337/diabetes.50.9.2133

Ninkovic, V. M., Ninkovic, S. M., Miloradovic, V., Stanojevic, D., Babic, M., Giga, V., et al. (2016). Prevalence and Risk Factors for Prolonged QT Interval and QT Dispersion in Patients with Type 2 Diabetes. *Acta Diabetol.* 53 (5), 737–744. doi:10.1007/s00592-016-0864-y

Nissen, S. E., and Wolski, K. (2007). Effect of Rosiglitazone on the Risk of Myocardial Infarction and Death from Cardiovascular Causes. *N. Engl. J. Med.* 356 (24), 2457–2471. doi:10.1056/nejmoa072761

Nissen, S. E., Wolski, K., and Topol, E. J. (2005). Effect of Muraglitazar on Death and Major Adverse Cardiovascular Events in Patients with Type 2 Diabetes Mellitus. *Jama* 294 (20), 2581–2586. doi:10.1001/jama.294.20.joc50147

Nygren, A., Olson, M. L., Chen, K. Y., Emmett, T., Kargacin, G., and Shimoni, Y. (2007). Propagation of the Cardiac Impulse in the Diabetic Rat Heart: Reduced Conduction reserve. *J. Physiology-London* 580 (2), 543–560. doi:10.1113/jphysiol.2006.123729

Onay-Besikci, A. (2014). *Substrate Metabolism in the Diabetic Heart*, 233. NEW YORK: Springer, 76.

Palomer, X., Pizarro-Delgado, J., and Vázquez-Carrera, M. (2018). Emerging Actors in Diabetic Cardiomyopathy: Heartbreaker Biomarkers or Therapeutic Targets? *Trends Pharmacological Sciences* 39 (5), 452–467. doi:10.1016/j.tips.2018.02.010

Pelzer, D., Pelzer, S., and McDonald, T. F. (1990). Properties and Regulation of Calcium Channels in Muscle Cells. *Rev. Physiol. Biochem. Pharmacol.* 114, 107–207. doi:10.1007/bfb0031019

Penpargkul, S., Fein, F., Sonnenblick, E. H., and Scheuer, J. (1981). Depressed Cardiac Sarcoplasmic Reticular Function from Diabetic Rats. *J. Mol. Cell Cardiol.* 13 (3), 303–309. doi:10.1016/0022-2828(81)90318-7

Pereira, L., Matthes, J., Schuster, I., Valdivia, H. H., Herzig, S., Richard, S., et al. (2006). Mechanisms of [Ca2+]i Transient Decrease in Cardiomyopathy of Db/db Type 2 Diabetic Mice. *Diabetes* 55 (3), 608–615. doi:10.2337/diabetes.55.03.06.db05-1284

Podell, B. K., Ackart, D. F., Richardson, M. A., DiLisio, J. E., Pulford, B., and Basaraba, R. J. (2017). A Model of Type 2 Diabetes in the guinea Pig Using Sequential Diet-Induced Glucose Intolerance and Streptozotocin Treatment. *Dis. Models Mech.* 10 (2), 151–162.

Preis, S. R., Pencina, M. J., Hwang, S.-J., D'Agostino, R. B., Savage., Levy, D., et al. (2009). Trends in Cardiovascular Disease Risk Factors in Individuals with and without Diabetes Mellitus in the Framingham Heart Study. *Circulation* 120 (3), 212–220. doi:10.1161/circulationaha.108.846519

Qin, D., Huang, B., Deng, L., El-Adawi, H., Ganguly, K., Sowers, J. R., et al. (2001). Downregulation of K+ Channel Genes Expression in Type I Diabetic Cardiomyopathy. *Biochem. biophysical Res. Commun.* 283 (3), 549–553. doi:10.1006/bbrc.2001.4825

Quagliariello, V., De Laurentiis, M., Cocco, S., Rea, G., Bonelli, A., Caronna, A., et al. (2020). NLRP3 as Putative Marker of Ipilimumab-Induced Cardiotoxicity in the Presence of Hyperglycemia in Estrogen-Responsive and Triple-Negative Breast Cancer Cells. *Int. J. Mol. Sci.* 21 (20).

Rahnema, P., Shimoni, Y., and Nygren, A. (2011). Reduced Conduction reserve in the Diabetic Rat Heart: Role of iPLA2 Activation in the Response to Ischemia. *Am. J. Physiology-Heart Circulatory Physiol.* 300 (1), H326–H334. doi:10.1152/ajpheart.00743.2010

Rautio, E., Gadler, F., Gudbjörnsdottir, S., Franzén, S., Rydén, L., Svensson, A.-M., et al. (2020). Patients with Type 2 Diabetes Have an Increased Demand for Pacemaker Treatment: A Comparison with Age- and Sex-Matched Control Subjects from the General Population. *Dia Care* 43 (11), 2853–2858. doi:10.2337/dc20-0084

Razeghi, P., Young, M. E., Cockrill, T. C., Frazier, O. H., and Taegtmeyer, H. (2002). Downregulation of Myocardial Myocyte Enhancer Factor 2C and Myocyte Enhancer Factor 2C-Regulated Gene Expression in Diabetic Patients with Nonischemic Heart Failure. *Circulation* 106 (4), 407–411. doi:10.1161/01.cir.0000026392.80723.dc

Regan, T. J., Ettinger, P. O., Khan, M. I., Jesrani, M. U., Lyons, M. M., Oldewurtel, H. A., et al. (1974). Altered Myocardial Function and Metabolism in Chronic Diabetes Mellitus without Ischemia in Dogs. *Circ. Res.* 35 (2), 222–237. doi:10.1161/01.res.35.2.222

Rubler, S., Dlugash, J., Yuceoglu, Y. Z., Kumral, T., Branwood, A. W., and Grishman, A. (1972). New Type of Cardiomyopathy Associated with Diabetic Glomerulosclerosis. *Am. J. Cardiol.* 30 (6), 595–602. doi:10.1016/0002-9149(72)90595-4

Sager, P. T., Gintant, G., Turner, J. R., Pettit, S., and Stockbridge, N. (2014). Rechanneling the Cardiac Proarrhythmia Safety Paradigm: A Meeting Report from the Cardiac Safety Research Consortium. *Am. Heart J.* 167 (3), 292–300. doi:10.1016/j.ahj.2013.11.004

Sanguinetti, M. C., and Tristani-Firouzi, M. (2006). hERG Potassium Channels and Cardiac Arrhythmia. *Nature* 440 (7083), 463–469. doi:10.1038/nature04710

Sato, T., Kobayashi, T., Kuno, A., Miki, T., Tanno, M., Kouzu, H., et al. (2014). Type 2 Diabetes Induces Subendocardium-Predominant Reduction in Transient Outward K+ Current with Downregulation of Kv4.2 and KChIP2. *Am. J. Physiology-Heart Circulatory Physiol.* 306 (7), H1054–H1065. doi:10.1152/ajpheart.00414.2013

Scheen, A. J. (2020). Series: Implications of the Recent CVOTs in Type 2 Diabetes. *Diabetes Res. Clin. Pract.* 159, 107726. doi:10.1016/j.diabres.2019.05.005

Schnell, O., Standl, E., Catrinoiu, D., Genovese, S., Lalic, N., Skra, J., et al. (2016). Report from the 1st Cardiovascular Outcome Trial (CVOT) Summit of the Diabetes & Cardiovascular Disease (D&CVD) EASD Study Group. *Cardiovasc. Diabetology* 15, 33. doi:10.1186/s12933-016-0357-x

Schnell, O., Standl, E., Catrinoiu, D., Itzhak, B., Lalic, N., Rahelic, D., et al. (2019). Report from the 4th Cardiovascular Outcome Trial (CVOT) Summit of the Diabetes & Cardiovascular Disease (D&CVD) EASD Study Group. *Cardiovasc. Diabetology* 18 (1), 30. doi:10.1186/s12933-019-0822-4

Schnell, O., Standl, E., Cos, X., Heerspink, H. J. L., Itzhak, B., Lalic, N., et al. (2020). Report from the 5th Cardiovascular Outcome Trial (CVOT) summit. *Cardiovasc. Diabetology* 19 (1), 47. doi:10.1186/s12933-020-01022-7

Schwartz, P. J., Crotti, L., and Insolia, R. (2012). Long-QT Syndrome. *Circ. Arrhythm Electrophysiol.* 5 (4), 868–877. doi:10.1161/circep.111.962019

Sham, J. S. (1997). Ca2+ Release-Induced Inactivation of Ca2+ Current in Rat Ventricular Myocytes: Evidence for Local Ca2+ Signalling. *J. Physiology-London* 500 (2), 285–295. doi:10.1113/jphysiol.1997.sp022020

Shimoni, Y., Chuang, M., Abel, E. D., and Severson, D. L. (2004). Gender-dependent Attenuation of Cardiac Potassium Currents in Type 2 Diabeticdb/dbmice. *J. Physiology-London* 555 (2), 345–354. doi:10.1113/jphysiol.2003.055590

Shimoni, Y., Firek, L., Severson, D., and Giles, W. (1994). Short-Term Diabetes Alters K+ Currents in Rat Ventricular Myocytes. *Circ. Res.* 74 (4), 620–628. doi:10.1161/01.res.74.4.620

Singh, R. M., Waqar, T., Howarth, F. C., Adeghate, E., Bidasee, K., and Singh, J. (2018). Hyperglycemia-induced Cardiac Contractile Dysfunction in the Diabetic Heart. *Heart Fail. Rev.* 23 (1), 37–54. doi:10.1007/s10741-017-9663-y

Singleton, M. J., German, C., Hari, K. J., Saylor, G., Herrington, D. M., Soliman, E. Z., et al. (2020). QRS Duration Is Associated with All-Cause Mortality in Type 2 Diabetes: The Diabetes Heart Study. *J. Electrocardiol.* 58, 150–154. doi:10.1016/j.jelectrocard.2019.11.053

Soltysinska, E., Speerschneider, T., Winther, S. V., and Thomsen, M. B. (2014). Sinoatrial Node Dysfunction Induces Cardiac Arrhythmias in Diabetic Mice. *Cardiovasc. Diabetology* 13, 122. doi:10.1186/s12933-014-0122-y

Spallone, V., Ziegler, D., Freeman, R., Bernardi, L., Frontoni, S., Pop-Busui, R., et al. (2011). Cardiovascular Autonomic Neuropathy in Diabetes: Clinical Impact, Assessment, Diagnosis, and Management. *Diabetes Metab. Res. Rev.* 27 (7), 639–653. doi:10.1002/dmrr.1239

Stables, C. L., Musa, H., Mitra, A., Bhushal, S., Deo, M., Guerrero-Serna, G., et al. (2014). Reduced Na+ Current Density Underlies Impaired Propagation in the Diabetic Rabbit Ventricle. *J. Mol. Cell Cardiol.* 69, 24–31. doi:10.1016/j.yjmcc.2013.12.031

Suys, B., Heuten, S., De Wolf, D., Verherstraeten, M., de Beeck, L. O., Matthys, D., et al. (2006). Glycemia and Corrected QT Interval Prolongation in Young Type 1 Diabetic Patients: What Is the Relation? *Diabetes care* 29 (2), 427–429. doi:10.2337/diacare.29.02.06.dc05-1450

Teshima, Y., Takahashi, N., Saikawa, T., Hara, M., Yasunaga, S., Hidaka, S., et al. (2000). Diminished Expression of Sarcoplasmic Reticulum Ca2+-ATPase and Ryanodine Sensitive Ca2+Channel mRNA in Streptozotocin-Induced Diabetic Rat Heart. *J. Mol. Cell Cardiol.* 32 (4), 655–664. doi:10.1006/jmcc.2000.1107

Torres-Jacome, J., Gallego, M., Rodríguez-Robledo, J. M., Sanchez-Chapula, J. A., and Casis, O. (2013). Improvement of the Metabolic Status Recovers Cardiac Potassium Channel Synthesis in Experimental Diabetes. *Acta Physiol.* 207 (3), 447–459. doi:10.1111/apha.12043

Tse, G., Lai, E. T., Tse, V., and Yeo, J. M. (2016). Molecular and Electrophysiological Mechanisms Underlying Cardiac Arrhythmogenesis in Diabetes Mellitus. *J. Diabetes Res.* 2016, 2848759. doi:10.1155/2016/2848759

Tsuchida, K., Watajima, H., and Otomo, S. (1994). Calcium Current in Rat Diabetic Ventricular Myocytes. *Am. J. Physiology-Heart Circulatory Physiol.* 267 (6), H2280–H2289. doi:10.1152/ajpheart.1994.267.6.h2280

Tsuchida, K., and Watajima, H. (1997). Potassium Currents in Ventricular Myocytes from Genetically Diabetic Rats. *Am. J. Physiology-Endocrinology Metab.* 273 (4), E695–E700. doi:10.1152/ajpendo.1997.273.4.e695

Turner, J. R., Rodriguez, I., Mantovani, E., Gintant, G., Kowey, P. R., Klotzbaugh, R. J., et al. (2018). Drug-induced Proarrhythmia and Torsade de Pointes: A Primer for Students and Practitioners of Medicine and Pharmacy. *J. Clin. Pharmacol.* 58 (8), 997–1012. doi:10.1002/jcph.1129

Veglio, M., Bruno, G., Borra, M., Macchia, G., Bargero, G., D'Errico, N., et al. (2002). Prevalence of Increased QT Interval Duration and Dispersion in Type 2 Diabetic Patients and its Relationship with Coronary Heart Disease: a Population-Based Cohort. *J. Intern. Med.* 251 (4), 317–324. doi:10.1046/j.1365-2796.2002.00955.x

Veglio, M., Chinaglia, A., and Cavallo Perin, P. (2000). The Clinical Utility of QT Interval Assessment in Diabetes. *Diabetes Nutr. Metab.* 13 (6), 356–365.

Vicente, J., Zusterzeel, R., Johannesen, L., Mason, J., Sager, P., Patel, V., et al. (2018). Mechanistic Model-Informed Proarrhythmic Risk Assessment of Drugs: Review of the "CiPA" Initiative and Design of a Prospective Clinical Validation Study. *Clin. Pharmacol. Ther.* 103 (1), 54–66. doi:10.1002/cpt.896

Wang, A., Green, J. B., Halperin, J. L., and Piccini, J. P. (2019). Atrial Fibrillation and Diabetes Mellitus. *J. Am. Coll. Cardiol.* 74 (8), 1107–1115. doi:10.1016/j.jacc.2019.07.020

Wang, D. W., Kiyosue, T., Shigematsu, S., and Arita, M. (1995). Abnormalities of K+ and Ca2+ Currents in Ventricular Myocytes from Rats with Chronic Diabetes. *Am. J. Physiology-Heart Circulatory Physiol.* 269 (4), H1288–H1296. doi:10.1152/ajpheart.1995.269.4.h1288

Watanabe, M., Yokoshiki, H., Mitsuyama, H., Mizukami, K., Ono, T., and Tsutsui, H. (2012). Conduction and Refractory Disorders in the Diabetic Atrium. *Am. J. Physiology-Heart Circulatory Physiol.* 303 (1), H86–H95. doi:10.1152/ajpheart.00010.2012

WHO (2021). *Global Report on Diabetes*. http://www.who.int.

Xu, J., and Rajaratnam, R. (2017). Cardiovascular Safety of Non-insulin Pharmacotherapy for Type 2 Diabetes. *Cardiovasc. Diabetology* 16, 18. doi:10.1186/s12933-017-0499-5

Xu, Z., Patel, K. P., and Rozanski, G. J. (1996). Metabolic Basis of Decreased Transient Outward K+ Current in Ventricular Myocytes from Diabetic Rats. *Am. J. Physiology-Heart Circulatory Physiol.* 271 (5), H2190–H2196. doi:10.1152/ajpheart.1996.271.5.h2190

Yiu, K.-H., and Tse, H.-F. (2008). Hypertension and Cardiac Arrhythmias: a Review of the Epidemiology, Pathophysiology and Clinical Implications. *J. Hum. Hypertens.* 22 (6), 380–388. doi:10.1038/jhh.2008.10

Yu, P., Hu, L., Xie, J., Chen, S., Huang, L., Xu, Z., et al. (2018). O-GlcNAcylation of Cardiac Nav1.5 Contributes to the Development of Arrhythmias in Diabetic Hearts. *Int. J. Cardiol.* 260, 74–81. doi:10.1016/j.ijcard.2018.02.099

Zaza, A., and Rocchetti, M. (2013). The Late Na+ Current - Origin and Pathophysiological Relevance. *Cardiovasc. Drugs Ther.* 27 (1), 61–68. doi:10.1007/s10557-012-6430-0

Zhang, Y., Wang, Y., Yanni, J., Qureshi, M. A., Logantha, S. J. R. J., Kassab, S., et al. (2019). Electrical Conduction System Remodeling in Streptozotocin-Induced Diabetes Mellitus Rat Heart. *Front. Physiol.* 10 (826). doi:10.3389/fphys.2019.00826

Zhang, Y., Xiao, J., Lin, H., Luo, X., Wang, H., Bai, Y., et al. (2007). Ionic Mechanisms Underlying Abnormal QT Prolongation and the Associated Arrhythmias in Diabetic Rabbits: A Role of Rapid Delayed Rectifier K+ Current. *Cell Physiol. Biochem.* 19 (5-6), 225–238. doi:10.1159/000100642

Zhang, Y., Xiao, J., Wang, H., Luo, X., Wang, J., Villeneuve, L. R., et al. (2006). Restoring Depressed HERG K+ Channel Function as a Mechanism for Insulin Treatment of Abnormal QT Prolongation and Associated Arrhythmias in Diabetic Rabbits. *Am. J. Physiology-Heart Circulatory Physiol.* 291 (3), H1446–H1455. doi:10.1152/ajpheart.01356.2005

Zhong, Y., Ahmed, S., Grupp, I. L., and Matlib, M. A. (2001). Altered SR Protein Expression Associated with Contractile Dysfunction in Diabetic Rat Hearts. *Am. J. Physiology-Heart Circulatory Physiol.* 281 (3), H1137–H1147. doi:10.1152/ajpheart.2001.281.3.h1137

Ziegler, D., Zentai, C. P., Perz, S., Rathmann, W., Haastert, B., Döring, A., et al. (2008). Prediction of Mortality Using Measures of Cardiac Autonomic Dysfunction in the Diabetic and Nondiabetic Population: The MONICA/KORA Augsburg Cohort Study. *Diabetes Care* 31 (3), 556–561. doi:10.2337/dc07-1615

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P2X7 Receptor Deficiency Ameliorates STZ-induced Cardiac Damage and Remodeling Through PKCβ and ERK

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Diabetic cardiomyopathy (DCM) is a complication of diabetes mellitus which result in cardiac remodeling and subsequent heart failure. However, the role of P2X7 receptor (P2X7R) in DCM has yet to be elucidated. The principal objective of this study was to investigate whether P2X7R participates in the pathogenesis of DCM. In this study, the C57BL/6 diabetic mouse model was treated with a P2X7R inhibitor (A438079). Cardiac dysfunction and remodeling were attenuated by the intraperitoneal injection of A438079 or P2X7R deficiency. *In vitro*, A438079 reduced high glucose (HG) induced cell damage in H9c2 cells and primary rat cardiomyocytes. Furthermore, HG/streptozotocin (STZ)-induced P2X7R activation mediated downstream protein kinase C-β (PKCβ) and extracellular regulated protein kinases (ERK) activation. This study provided evidence that P2X7R plays an important role in the pathogenesis of STZ-induced diabetic cardiac damage and remodeling through the PKCβ/ERK axis and suggested that P2X7R might be a potential target in the treatment of diabetic cardiomyopathy.

Keywords: diabetic cardiomyopathy, P2X7 receptor, cardiac remodeling, PKCβ, ERK

INTRODUCTION

Many patients are diagnosed with diabetes worldwide, and the complications caused by diabetes are also diverse, such as diabetic nephropathy, diabetic retinopathy, and diabetic heart diseases. Among these complications, diabetic cardiomyopathy (DCM) is a serious complication that results in a poor prognosis for patients with diabetes (Zhao et al., 2012; Forbes and Cooper, 2013; Ofstad, 2016; Ogurtsova et al., 2017). In individuals with diabetes mellitus, a high blood glucose level is an independent causal factor for cardiomyopathy, one of the leading causes of hospitalization and death worldwide (Wang et al., 2006). DCM is characterized by structural and functional disorders, including ventricular dysfunction, interstitial fibrosis, cardiomyocyte hypertrophy, myocardial apoptosis, and metabolic deregulation (Wang et al., 2006). These pathophysiological changes eventually lead to cardiac remodeling and decreased cardiac output, preventing the heart from adequately pumping blood. Thus, an effective drug or target to treat DCM must be identified.

The P2X7 receptor (P2X7R), which is composed of 595 amino acids, is a non-selective cationic gated channel that assembles into a trimeric complex and belongs to the P2X family (Surprenant et al., 1996). After binding to extracellular ATP, the ion channel opens and allows K^+, Na^+, and

Ca^{2+} plasma permeation (predominantly Ca^{2+}) (Surprenant et al., 1996). The P2X7R is widely expressed *in vivo*, including nerve cells, liver cells, cardiomyocytes, monocytes, and macrophages (Burnstock and Knight, 2004). According to recent studies, P2X7R inhibition attenuates liver and lung fibrosis (Riteau et al., 2010; Huang et al., 2014). Simultaneously, P2X7R inhibition (using a siRNA or a pharmacological inhibitor) prevents the progression of atherosclerosis and myocardial infarction (Mezzaroma et al., 2011; Peng et al., 2015; Stachon et al., 2017). In diabetic animal models, the increase in P2X7R activity is involved in the pathogenesis of diabetic nephropathy (Kreft et al., 2016). P2X7Ri has been suggested to exert specific anti-inflammatory and anti-macrophage effects on diabetic nephropathy, ameliorating glomerular damage and fibrosis (Menzies et al., 2017). However, the role of the P2X7R in DCM has not been systematically determined.

The aim of the present study was to elucidate the essential role of the P2X7R in regulating DCM and its underlying mechanisms. The P2X7R-selective inhibitor A438079 was used in a streptozotocin (STZ)-induced type I diabetes mouse model, cultured H9c2 cells, and primary rat cardiomyocytes. The expression of P2X7R was significantly upregulated in the STZ-induced diabetic mouse model and high glucose (HG)-treated cell model. Moreover, P2X7R inhibition dramatically relieved cardiac damage by ameliorating fibrosis, apoptosis, and cardiomyocyte hypertrophy in STZ- or HG-induced models *in vivo* and *in vitro*. In addition, we further described the crucial role of P2X7R in knockout mice with diabetes.

MATERIALS AND METHODS

Animal Experiments

Male C57BL/6 wild-type mice and male $P2X7R^{-/-}$ mice on a C57BL/6 background were provided by GemPharmatech Co., Ltd. (Nanjing, Jiangsu, China). Non-knockout male C57BL/6 mice were obtained from the Animal Center of Wenzhou Medical University. Animals were housed on a 12:12 h light–dark cycle at a constant room temperature and fed a standard rodent diet. The animals were acclimated to the laboratory for at least 2 weeks before initiating the studies. All operations were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Animal care and experimental protocols were approved by the Committee on Animal Care of Wenzhou Medical University.

Eight- to twelve-week-old mice weighing 23–25 g were used to develop the diabetes mellitus model. Diabetes mellitus was induced by a single intraperitoneal (i.p.) injection of STZ (Sigma Chemicals, St. Louis, MO, United States) at a dose of 50 mg/kg formulated in 100 mM citrate buffer (pH 4.5) for 5 consecutive days. After 1 week, blood samples were collected. Fasting blood glucose levels were measured using a glucometer after a 12 h fast. Mice with fasting blood glucose levels >12 mmol/L were considered diabetic and chosen for further experiments. C57BL/6 wild-type mice and $P2X7R^{-/-}$ mice were randomly divided into four groups: (i) non-treated C57BL/6 control mice that received buffered saline (WT group, n = 7); (ii) non-treated $P2X7R^{-/-}$control mice that received buffered saline ($P2X7R^{-/-}$ group, n = 7); (iii) STZ-induced diabetic mice (WT + STZ group, n = 7); and (iv) $P2X7R^{-/-}$ mice that received an i.p. injection of STZ (STZ + $P2X7R^{-/-}$ group, n = 7). Non-knockout male C57BL/6 mice were randomly divided into four groups: (I) non-treated C57BL/6 control mice that received buffered saline (Ctrl group, n = 8); (II) STZ-induced diabetic mice without treatment (STZ group, n = 8); (III) STZ-induced diabetic mice that received an i.p. injection of 10 mg/kg A438079 every second day for 16 weeks (STZ + A-10MG group, n = 8); and (IV) STZ-induced diabetic mice that received an i.p. injection of 20 mg/kg A438079 every second day for 16 weeks (STZ + A-20MG group, n = 9). All animals were sacrificed by administering sodium pentobarbital anesthesia. The hearts were collected and weighed. In addition, heart tissues were placed in 4% paraformaldehyde for the pathological analysis and/or snap-frozen in liquid nitrogen for gene and protein expression analyses.

Cardiac Function

Systolic and diastolic cardiac function were determined non-invasively using transthoracic echocardiography in mice 2 h before sacrifice. Diastolic function was assessed by performing pulsed-wave Doppler imaging of the transmitral filling pattern. The ejection fraction (EF) was calculated from the left ventricle end-diastolic volume (LVEDV) and end-systolic volume (LVESV) using the following equation: $(\mathrm{LVEDV} - \mathrm{LVESV})/\mathrm{LVEDV} \times 100\%$. Fractional shortening (FS) was calculated using the following equation: $\mathrm{FS} = \left[\left(\mathrm{LVIDd} - \mathrm{LVIDs}\right)/\mathrm{LVIDd}\right] \times 100\%$. The Tei index was determined based on the Doppler recordings of the left ventricular isovolumetric relaxation time (IRT), isovolumetric contraction time (ICT), and ejection time (ET):$\mathrm{Tei} = (\mathrm{IRT} + \mathrm{ICT})/\mathrm{ET}$.

Isolation of Primary Rat Cardiomyocytes

Newborn (2–3 days old) SD rats were obtained from the Animal Center of Wenzhou Medical University. The incision on the sternal left margin was disinfected with 75% alcohol. The hearts were removed from the chest, washed twice with cold PBS, and gently dissected. The heart fragments were digested with 0.08% trypsin for 8 min at 37°C with magnetic stirring. This step was repeated approximately 16 times until the tissue organization was no longer visible. The supernatant was collected, and the digestion was terminated by an incubation with DMEM containing 10% FBS. All the collected liquid was centrifuged at 1000 rpm for 10 min. The cells were incubated for 1 h at 37°C after suspension. The non-adherent cells were primary cardiomyocytes, and the adhesive cells were fibroblasts, which were all collected and cultivated for the experiment.

H9c2 Cell and Primary Cardiomyocyte Culture and Treatment

The rat myocardium-derived cell line H9c2 was obtained from the Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China). H9c2 cells or primary cardiomyocytes were maintained in DMEM (Gibco, Eggenstein, Germany) containing

5.5 mmol/L D-glucose, 10% FBS, 100 U/ml penicillin, and 100 mg/ml streptomycin at 37°C in a 5% CO_2 incubator (Thermo Fisher Scientific, Waltham, MA, United States). Experiments were performed when the cell density is about 70–80%. In the HG-treated group, H9c2 cells or primary cardiomyocytes were incubated with DMEM containing 33 mmol/L glucose. A438079, LY317615, and PD98095 were dissolved in DMSO and added to the cells for 1 h. The final concentration of the three inhibitors was 10 μM. Afterward, the cells were exposed to HG for 24 h.

Reagents

The P2X7R inhibitor (A438079), PKC inhibitor (LY317615), and ERK-specific inhibitor (PD98095) were purchased from Selleck (Shanghai, China). A RevertAid First Strand cDNA Synthesis Kit was obtained from Thermo Fisher Scientific (Waltham, MA, United States). SYBR Premix Ex Taq^{TM} (TliRnaseH Plus) was obtained from Takara (Dalian, China). The primary anti-P2X7R antibody (#APR-004) was obtained from Alomone Labs (Israel). Anti-collagen 1 (anti-COL-1, ab34710), anti-matrix metalloproteinase-9 (anti-MMP9, ab38898), and anti-heavy chain cardiac myosin (ab185967) antibodies were obtained from Abcam (Cambridge, United Kingdom). Moreover, antibodies against TGFβ (#3711), GAPDH (#5174), Caspase-3 (#9662), Bcl2 (#2870), phospho-PKCβII (Thr638/641) (#9375), Bax (#5023), and Phospho-p44/42 MAPK (ERK1/2) (Thr202/Tyr204; #4370) were obtained from Cell Signaling Technology (Danvers, MA, United States).

Western Blot Analysis

Protein isolation and western blot analyses were performed using methods described in the literature (Huang et al., 2008). Protein samples were loaded and separated on SDS-PAGE gels before being transferred to a PVDF membrane (Millipore, MA, United States). Membranes were blocked with a 5% fat-free milk solution in TBST (TBS containing 0.1% Tween-20) at room temperature for 1 h, followed by an overnight incubation at 4°C with the appropriate primary antibodies. Immunoreactive bands were incubated with a secondary antibody at room temperature for 1 h and labeled with horseradish peroxidase after three washes. Proteins were detected with the ECL reagent (Bio-Rad, United States).

Real-Time Quantitative PCR

Total RNA was extracted from H9c2 cells or heart tissues (20–50 mg) using TRIzol reagent according to the manufacturer's protocol (Invitrogen Life Technologies). One microgram of total RNA from each sample was used to generate cDNAs using the RevertAid First Strand cDNA Synthesis Kit (#K1622; Thermo Fisher Scientific). The resulting cDNA templates were amplified using SYBR in a real-time polymerase chain reaction with primers from Sangon Biotech (Shanghai, China) (**Table 1**). PCR was directly monitored using CFX 96 (Bio-Rad, United States). All results were normalized to GAPDH.

Terminal Deoxynucleotidyl Transferase-Mediated dUTP Nick End Labeling Staining

Paraffin tissue sections with a thickness of 5 μm were used for terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining of apoptotic cells using a One-Step TUNEL Apoptosis Assay Kit from Beyotime (Shanghai, China) according to the manufacturer's instructions. TUNEL-positive cells were imaged under a fluorescence microscope (400× amplification; Nikon, Japan).

Histopathological Masson's Trichrome, Sirius Red and Rhodamine Staining

Excised heart tissue specimens were fixed with 4% paraformaldehyde, processed in a graded series of alcohol and xylene solutions and then embedded in paraffin. Paraffin blocks were sliced into sections at a thickness of 5 μm. After rehydration, the sections were stained with hematoxylin and eosin (H&E). Images of each section were captured using a light microscope (400× amplification; Nikon, Japan) to evaluate the histopathological damage. Paraffin sections (5 μm) were stained with 0.1% Sirius Red and Masson's trichrome (Sigma) to evaluate the level of collagen deposition and fibrosis, respectively. H9c2 cells were pretreated with HG (33 mM) for 24 h in the presence or absence of A438079. Then, the cells were stained with rhodamine (Beyotime; Shanghai, China) according to the manufacturer's instructions. The stained sections or cells were

TABLE 1 | The primer sequences of each target gene.

Gene	Species	FW	RW
P2X7R	Rat	CGGGCCACAACTATACTACGA	CCTGAACTGCCACCTCTGTAA
TGF-β	Rat	GACTCTCCACCTGCAAGACC	GGACTGGCGAGCCTTAGTTT
ANP	Rat	GTACAGTGCGGTGTCCAACA	ATCCTGTCAATCCTACCCCC
MyHC	Rat	GAACACCAGCCTCATCAACC	CCTTCTTGGCCTTCTCCTCT
GAPDH	Rat	ACAGCAACAGGGTGGTGGAC	TTTGAGGGTGCAGAGAACTT
P2X7R	Mouse	AGAATGAGTTCCCCTGCAAA	AAGCTGTACCAGCGGAAAGA
TGF-β	Mouse	TGACGTCACTGGAGTTGTACGG	GGTTCATGTCxATGGATGGTGC
ANP	Mouse	AAGAACCTGCTAGACCACCTGGAG	TGCTTCCTCAGTCTGCTCACTCAG
MyHC	Mouse	CAAAGGCAAGGCAAAGAAAG	TCACCCCTGGAGACTTTGTC
GAPDH	Mouse	ACCCAGAAGACTGTGGATGG	TTCAGCTCAGGGATGACCTT

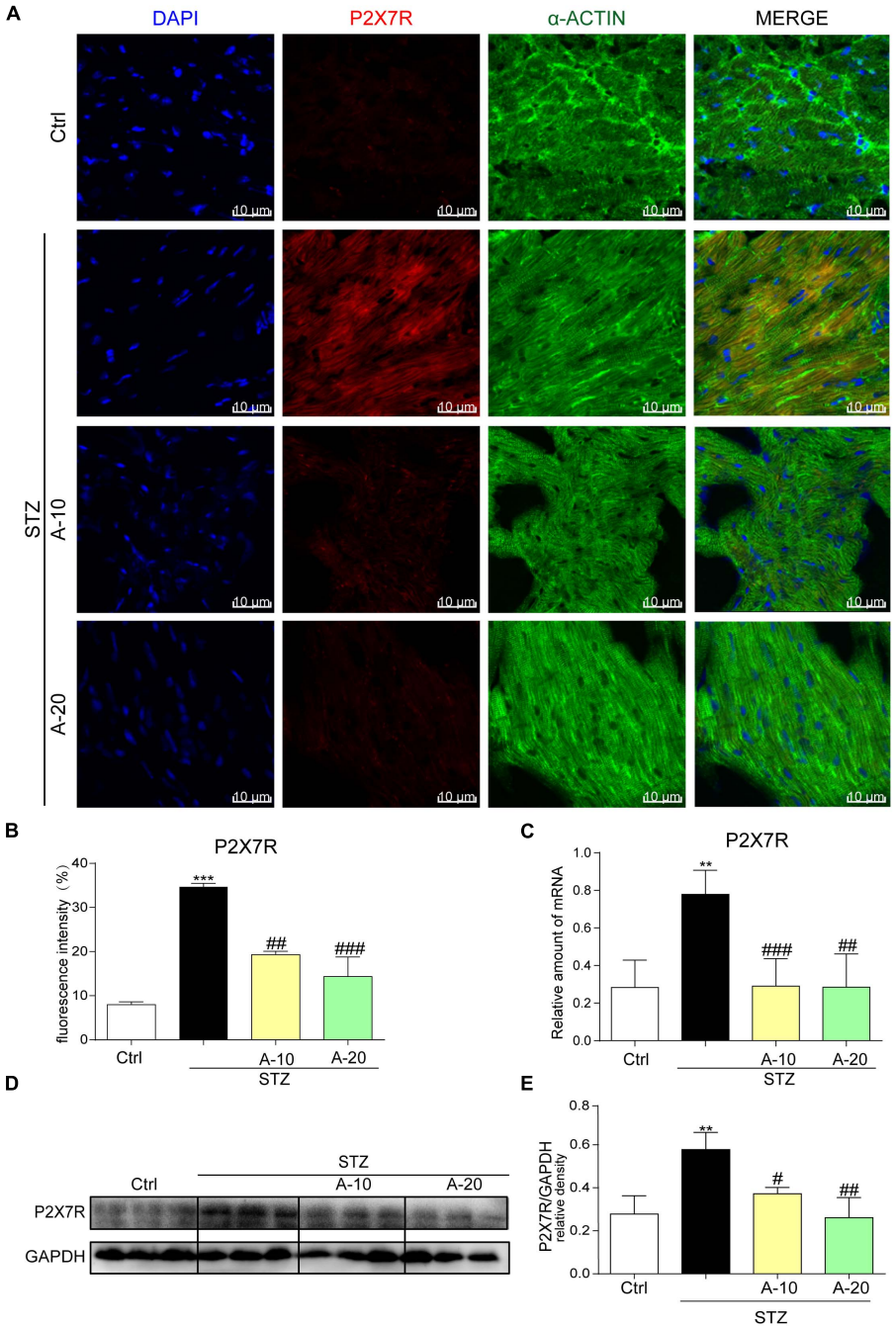

FIGURE 1 | P2X7 receptor expression was increased in the STZ-induced type 1 diabetes model and HG-treated cell model *in vitro*. Representative images **(A)** and quantification **(B)** of immunofluorescence staining for P2X7R in myocardial tissues (400× magnification). The expression of the P2X7R mRNA **(C)** and protein **(D)** with the corresponding statistics **(E)** (data from three independent experiments were analyzed: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared with the Ctrl; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ compared with STZ alone).

then viewed under a Nikon fluorescence microscope (400× amplification; Nikon, Japan).

Flow Cytometry

H9c2 cells were pretreated with HG (33 mM) for 24 h before the level of apoptosis was measures using flow cytometry. An Annexin V-EGFP/PI Cell Apoptosis Detection Kit (KeyGen Biotech, Nanjing, China) was used for this experiment. Then, 0.25% trypsin without EDTA was used to digest cells, and the cells were centrifuged at 2000 rpm for 5 min. Pellets were washed twice with PBS. Binding buffer (500 μl) was added to suspend the cell pellets. After mixing the cells with 5 μl of Annexin V-FITC and

5 μl of propidium iodide and reacting for 5–15 min lucifugally, the cells were immediately analyzed using a FACSCalibur flow cytometer (Becton Dickinson & Co., United States).

Statistical Analysis

All data were obtained from three independent experiments and are presented as the means ± SEM. All statistical analyses were performed using GraphPad Pro Prism 5.01 software (GraphPad, San Diego, CA, United States). One-way ANOVA followed by the multiple comparisons test with the Bonferroni correction were employed to analyze the differences between sets of data. A p-value < 0.05 was considered statistically significant.

RESULTS

P2X7 Receptor Expression Was Substantially Increased in the STZ-Induced Type 1 Diabetes Model and HG-Treated Cell Model *in vitro*

In the diabetic mouse model induced by STZ, immunofluorescence staining was utilized to inspect P2X7R expression in the myocardial tissue of C57BL/6 mice (**Figure 1A**). The expression of P2X7R was significantly increased after the administration of STZ. However, the administration of A438079, a selective inhibitor of the P2X7R, significantly reduced the fluorescence intensity. Moreover, compared with the low-dose group (10 mg/kg), the decreasing trend observed in the high-dose group (20 mg/kg) was more evident based on the fluorescence intensity (**Figure 1B**). As expected, the results obtained for the protein and mRNA levels were consistent with the immunofluorescence double staining (**Figures 1C–E**). Collectively, the expression of the P2X7R was upregulated in the STZ-induced diabetic model.

We used HG (33 mM) to stimulate H9c2 cells at different time points and evaluate whether the upregulation of the P2X7R *in vitro* was consistent with the changes observed *in vivo*. As shown in **Supplementary Figures 1A,B**, P2X7R levels increased and leveled off after 24 h. Thus, we chose 24 h as the time point for our subsequent experiments. In H9c2 cells and primary rat cardiomyocytes pretreated with A438079 for 1 h, P2X7R expression was significantly reduced at both the protein and mRNA levels (**Supplementary Figures 1C,D**). Interestingly, in primary fibroblasts, the expression of the P2X7R was not altered after HG stimulation (**Supplementary Figures 1E,F**). Based on this phenomenon, HG mainly altered the level of the P2X7R at the myocardial cell surface.

P2X7 Receptor Inhibition Alleviated STZ-Induced Cardiac Dysfunction

We performed additional experiments to detect the effect of P2X7R inhibition on the status and cardiac function of mice. The weight of C57BL/6 mice was decreased and fasting blood glucose levels increased significantly after the administration of STZ (**Supplementary Figures 1G,H**). However, a slight increase in body weight was detected in A438079-treated mice, and no change in fasting plasma glucose levels was observed (**Supplementary Figures 1G,H**) after treatment with A438079.

Non-invasive transthoracic echocardiography was used to examine the cardiac function of all experimental mice 2 h before sacrifice (**Table 2**). Echocardiography data revealed that the heart rate was not affected. Heart weight/body weight (HW/BW) was significantly increased in the STZ group compared with the other groups. Furthermore, STZ not only disrupted diastolic function (as observed in the IVSd, LVIDd, IRT, and Tei indices) but also reduced contraction function (EF% and FS%). As expected, these dysfunctions were substantially attenuated by the A438079 treatment.

Blockade of the P2X7 Receptor Attenuated STZ-Induced Cardiac Remodeling and Apoptosis *in vivo*

Although A438079 improved the cardiac function of diabetic mice, we were not sure whether it improved cardiac remodeling. Thus, H&E staining was used to detect the structural morphology. The hearts of STZ-challenged mice displayed structural abnormalities, such as disorganized myofibers. This disorder was improved in the other groups treated with the inhibitor (**Figure 2A**).

Fibrosis is also an important pathological variation observed in individuals with DCM (Wang et al., 2006). The connective tissue in the myocardium was examined using Masson's trichrome and Sirius Red staining for collagen (**Figure 2A**). The hearts from the STZ group showed apparent collagen and fibrous tissue accumulation, which were suppressed by treatment with A438079 (**Figures 2A,C**). At the protein level, the expression of the profibrotic markers TGF-β, COL-1, and MMP-9 was increased in the hearts of STZ-induced diabetic mice (**Figure 2B**). The results of PCR for TGF-β showed the same trend (**Figure 2F**). These molecular biological changes were remarkably reversed

TABLE 2 | Biometric and echocardiographic parameters of the C57BL/6 experimental mice.

	Ctrl (n = 8)	STZ		
		n = 8	A-10MG (n = 8)	A-20MG (n = 9)
HR, bpm	525 ± 79	489 ± 78	514 ± 54	527 ± 41
HW/BW, mg/g	5.78 ± 0.26	6.61 ± 0.36***	5.74 ± 0.34###	5.94 ± 0.23##
IVSD, mm	0.73 ± 0.04	0.75 ± 0.08	0.73 ± 0.03	0.70 ± 0.03
LVIDd, mm	2.23 ± 0.10	2.45 ± 0.06	2.17 ± 0.15#	2.16 ± 0.12##
IRT, ms	14.14 ± 2.03	24.5 ± 1.91***	16.67 ± 1.37	17.33 ± 2.18###
Tei index	0.70 ± 0.07	0.84 ± 0.14	0.73 ± 0.02	0.70 ± 0.02
EF%	82.03 ± 2.94	73.26 ± 1.45**	79.58 ± 2.11#	79.26 ± 1.86#
FS%	44.09 ± 2.37	36.85 ± 2.25**	43.36 ± 3.33#	42.99 ± 3.22#

Ctrl, control; HR, heart rate; HW/BW, heart weight/body weight; IVSD, diastole interventricular septal thickness; LVIDd, diastole left ventricle internal dimension; IRT, isovolumic relaxation time; EF%, ejection fraction %; FS%, fractional shortening %.

*Data presented as Mean ± SD, **$p < 0.01$, and ***$p < 0.001$, compared to Ctrl group; #$p < 0.05$, ##$p < 0.01$, and ###$p < 0.001$, compared to STZ group.*

by A438079 administration (**Figures 2B,F**). Moreover, higher expression of myosin heavy chain (MyHC), a biomarker of cardiac hypertrophy, was observed in the STZ-induced group than in the A438079 treatment groups (**Figures 2B,E**). Similar results were observed for the expression of the ANP mRNA (**Figure 2D**). Taken together, P2X7R inhibition significantly improved myocardial remodeling.

In addition, apoptosis plays an important role in DCM. As shown in images of TUNEL staining, A438079 played a role in preserving myocardial cell survival and reducing apoptosis caused by diabetes (**Figures 3A,C**). The levels of apoptosis-related proteins, such as Caspase-3 and Bax, were increased due to diabetes, and the level of the antiapoptotic protein Bcl-2 was decreased (**Figures 3B,E**). P2X7R inhibition led to changes in the levels of apoptotic proteins, and the Bax-to-Bcl-2 ratio was decreased (**Figure 3D**).

P2X7 Receptor Inhibition Prevented HG-Induced Phenotypic Changes in H9c2 Cells and Primary Rat Cardiomyocytes

The tests described below were performed to confirm whether P2X7R inhibition using A438079 protected the myocardium *in vitro*. H9c2 cells and primary rat cardiomyocytes were incubated with A438079 for 1 h prior to HG (33 mM) stimulation for 24 h. The protein levels of the hypertrophic marker MyHC and profibrotic proteins TGF-β, MMP-9, and COL-1 in the respective cells were remarkably decreased by the A438079 pretreatment (**Figures 4A–D**). The results of quantitative PCR showed that P2X7R inhibition alleviated HG-induced hypertrophy and fibrosis to varying degrees (**Figures 4E,F**). As intuitively observed from the rhodamine staining, the inhibition of the P2X7R improved the hypertrophic response of H9c2 cells to HG (**Figure 4G**). These phenotypic changes were consistent with the results obtained from the hearts of STZ-induced diabetic mice.

Pharmacological Inhibition of the P2X7 Receptor Attenuated HG-Induced Apoptosis in H9c2 Cells and Rat Primary Cardiomyocytes

A selective inhibitor of the P2X7R was used *in vitro* to confirm the role of the P2X7R in HG-induced apoptosis. H9c2 cells and rat primary cardiomyocytes were pretreated with A438079 for 1 h and then exposed to HG (33 mM) for 24 h. As shown in **Figures 4H,I**, the expression of Bax and Caspase-3 was significantly increased in the HG group but was obviously downregulated by the A438079 treatment in H9c2 cells and rat primary cardiomyocytes. Similar trends in the ratio of Bax to Bcl-2 and the ratio of Caspase-3 to GAPDH were also observed (**Supplementary Figures 2A–D**). The flow cytometry results for H9c2 cells revealed a higher percentage of apoptotic cells in the HG stimulation group than in the normal group. In addition, the A438079 treatment significantly suppressed apoptosis, particularly late apoptosis (**Figure 4J**).

P2X7 Receptor Knockout Improved STZ-Induced Cardiac Dysfunction

Based on previous animal experiments and *in vitro* experiments, pharmacological inhibition of the myocardial P2X7R reduced cardiac remodeling and apoptosis in the STZ-induced model or HG-treated cells. P2X7R knockout mice ($P2X7R^{-/-}$) were used as experimental models to better clarify its role. **Figure 5C** shows the expression of the P2X7R in cardiac tissue from $P2X7R^{-/-}$ mice.

All mice were subjected to echocardiography 2 h before sacrifice. A significant difference in the heart rate was not observed among the four groups. Under basal conditions, P2X7R knockout alone had no effect on cardiac function (**Figure 5A** and **Table 3**). As shown in **Table 3**, STZ caused systolic dysfunction (as evidenced by the EF% and FS% indices). As expected, these dysfunctions improved in $P2X7R^{-/-}$ mice. **Figure 5A** shows a representative echocardiogram of each group, which presents the findings more intuitively.

The P2X7 Receptor Was Involved in STZ-Induced Cardiac Remodeling and Apoptosis

P2X7R knockout mice were transformed into diabetic mouse models by administering them STZ, and then myocardial tissues were observed using histochemical staining. HE staining revealed that STZ-induced cardiomyocyte hypertrophy and histopathological alterations were alleviated by P2X7R knockout (**Figure 5B**). Masson's trichrome staining showed that $P2X7R^{-/-}$ decreased cardiac fibrosis in STZ-induced mice (**Figures 5B,D**).

Strikingly, STZ-induced changes in the expression of the profibrotic proteins collagen I, MMP 9, and TGF-β were reversed in the $P2X7R^{-/-}$ mouse hearts (**Figures 5E,F**). Myocardial tissue apoptosis is shown in **Figures 5G,H**. The STZ treatment induced Caspase 3 and Bax expression and inhibited Bcl-2 expression. As expected, these abnormal changes were ameliorated by knockout of the P2X7R (**Figures 5G,H**). Based on these results, P2X7R enhanced STZ-induced remodeling and apoptosis in the mouse myocardial tissue.

P2X7R Regulated the Activation of the PKCβ/ERK Axis in High Glucose-Induced Cardiomyocytes

The underlying mechanism by which HG causes damage must be elucidated. By reviewing previous studies, the PKC/MAPK pathway participates in the development of DCM (Soetikno et al., 2012). A PKC phosphorylation site was identified in the short N terminus of P2X7R (Boue-Grabot et al., 2000). Among the many subtypes of PKC, protein kinase C-β (PKCβ) is stimulated by HG (Hayashi et al., 2017). Furthermore, PKCβ is involved in many heart diseases (Mochly-Rosen et al., 2012; Newton et al., 2016). Here, we measured the levels of phosphorylated and total PKC and ERK in HG (33 mM)-induced myocytes treated with or without A438079. As shown in **Figures 6A,B**, the levels of phosphorylated PKCβ and ERK were increased upon HG stimulation and were remarkably reduced after the addition of

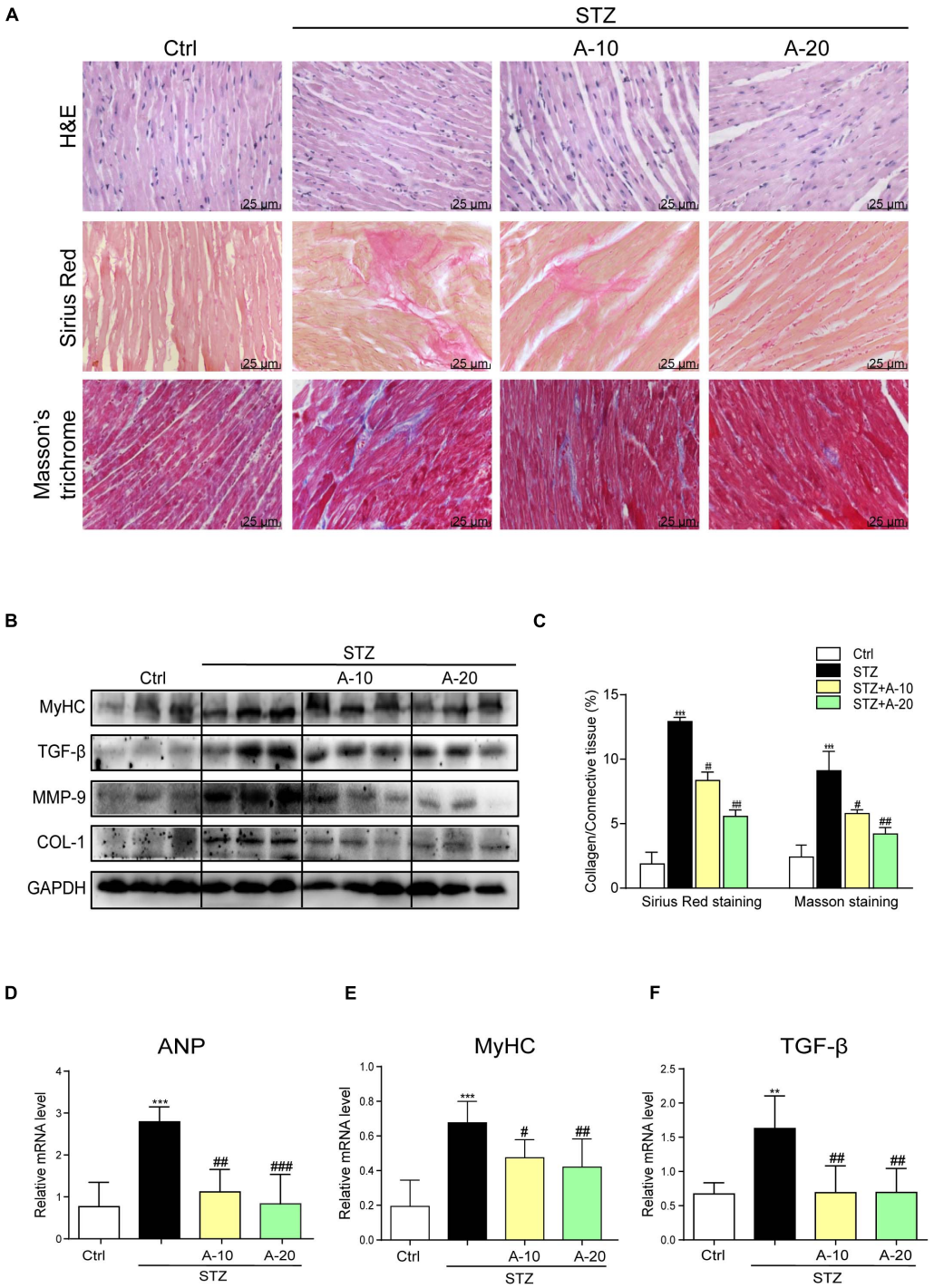

FIGURE 2 | Blockade of the P2X7 receptor attenuated STZ-induced cardiac remodeling. Representative images of hematoxylin-eosin staining (H&E) of the myocardial tissues (400× magnification), (**A**, upper panel). Myocardial fibrosis analysis was detected using Sirius Red staining (**A**, middle panel), and representative images of Masson's trichrome staining (**A**, bottom panel) are shown (400× magnification). Bar graph showing the quantified interstitial fibrotic areas (%) in images of Sirius Red staining and Masson's trichrome staining **(C)**. Levels of the MyHC, TGF-β, MMP-9, and COL-1 proteins in myocardial tissues were measured using western blotting **(B)** (n = 3 per group). The mRNA expression of the hypertrophy markers MyHC and ANP **(D,E)** and fibrosis marker TGF-β **(F)** in the myocardial tissues is shown (data from three independent experiments were analyzed: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared with the Ctrl; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ compared with STZ alone).

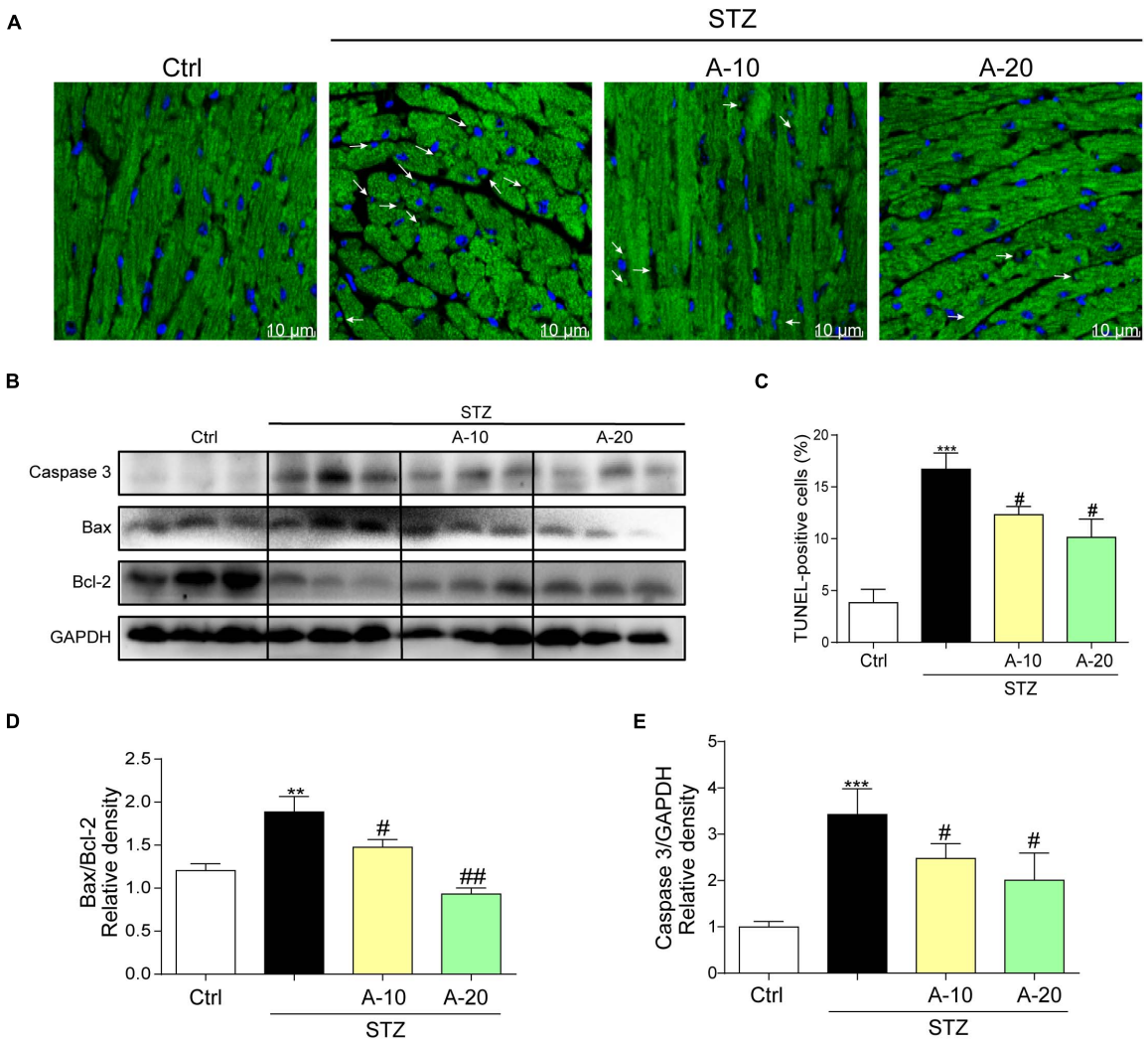

FIGURE 3 | Blockade of the P2X7 receptor improved STZ-induced cardiac apoptosis. Representative images **(A)** and quantification **(C)** of TUNEL staining in mouse myocardial tissues are shown (400× magnification). Levels of the apoptosis-related proteins Caspase-3, Bcl-2, and Bax were measured by western blot **(B)**. (n = 3 per group). The ratio of the Bcl-2 protein to the Bax protein and the ratio of Caspase3/GAPDH proteins are shown **(D,E)** (data from three independent experiments were analyzed: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared with the Ctrl; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ compared with STZ alone).

A438079, indicating that the P2X7R, an upstream regulator, may adjust PKCβ and ERK activation in HG-induced myocytes.

We pretreated cells with inhibitors of these kinases (LY317615, an inhibitor of PKCβ, or PD98059, an inhibitor of ERK) for 1 h and then incubated them with HG to further elucidate the relationship between PKCβ and ERK. After the addition of PKCβ and ERK inhibitors, the expression of the P2X7R did not change upon HG stimulation (**Figures 6C,D**). In contrast, LY317615 not only inhibited PKCβ activity but also reduced the activity of ERK (**Figures 6C,E**). However, PD98059 did not affect P2X7R and phospho-PKCβ activation (**Figures 6C,F**). Additionally, the levels of the molecular markers of cardiac hypertrophy, fibrosis, and apoptosis (such as MyHC, Col-1, MMP-9, TGF-β, Bax, Bcl-2, and Caspase-3) were decreased by PD98059 or LY317615 (**Figure 6G**). Thus, the activation of PKCβ and ERK was involved in HG-induced myocardial injury.

DISCUSSION

Cardiovascular disease remains one of the main causes of death worldwide. In the present study, we systematically revealed the novel role of the P2X7R in DCM. The P2X7R was upregulated in the diabetic hearts and in HG-treated cardiomyocytes. The inhibition or knockout of the P2X7R significantly mitigated cardiac damage, including cardiomyocyte fibrosis, apoptosis, and hypertrophy, *in vivo* and *in vitro*. We confirmed that the suppression of P2X7R inhibited PKCβ and ERK activation and then decreased the hallmarks of HG-induced myocardial remodeling. In addition, PKCβ was identified as an upstream molecule that regulates the activation of ERK in this process. **Figure 6H** summarizes the graphical abstract.

Diabetic cardiomyopathy is caused by prolonged diabetes (Aneja et al., 2008). Diabetes mainly changes the structure

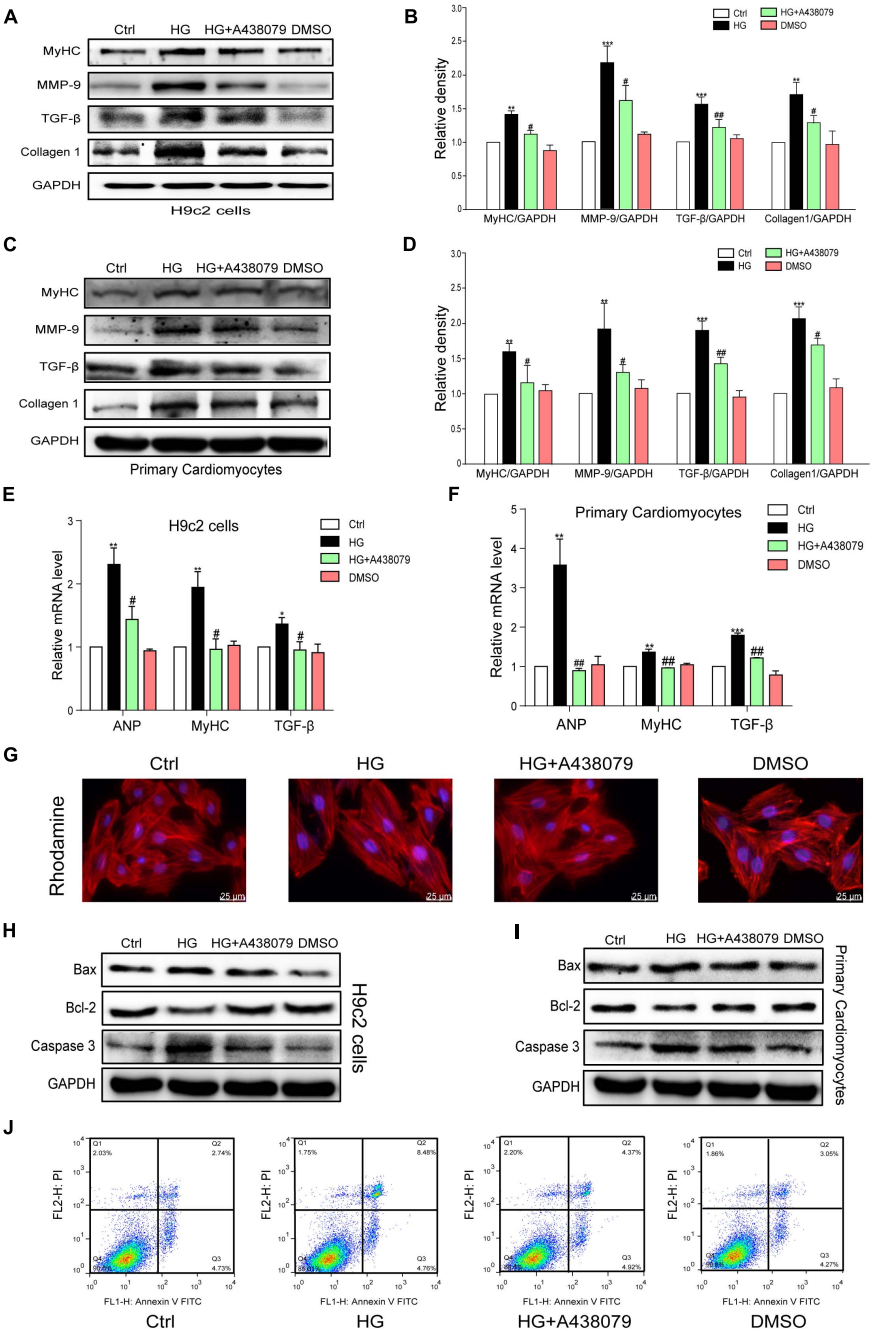

FIGURE 4 | P2X7 receptor inhibition prevented HG-induced phenotypic changes and apoptosis in H9c2 cells and rat primary cardiomyocytes. The levels of pro-fibrotic (MMP-9, TGF-β, and COL-1) and pro-hypertrophic proteins (MyHC) was examined using western blot analysis **(A,B)**. **(E)** The bar graph shows the corresponding PCR data for TGF-β, MYHC, and ANP. The same process and analysis were performed for rat primary cardiac myocytes. (WB: **C,D**, PCR: **F**). Representative images of rhodamine staining in each group of H9c2 cells **(G)**. The levels of the apoptosis-related proteins Caspase-3, Bcl-2, and Bax were measured on H9c2 cells and primary cardiac myocytes using western blotting **(H,I)**. Flow cytometry analysis showing that A438079 reduced the apoptosis of HG-treated H9c2 cells **(J)**. (Data from three independent experiments were analyzed: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared with the Ctrl; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ compared with STZ alone).

and function of the myocardium. Hyperglycemia not only causes morphological changes in the myocardium, such as cardiomyocyte hypertrophy, but also affects the myocardial structure and induces interstitial and perivascular fibrosis, eventually leading to heart failure (Kannel and McGee, 1979; Mizushige et al., 2000; Singh et al., 2008; Bugger and Abel, 2009, 2014). Reversing these detrimental processes will improve the cardiac function of patients with diabetes mellitus.

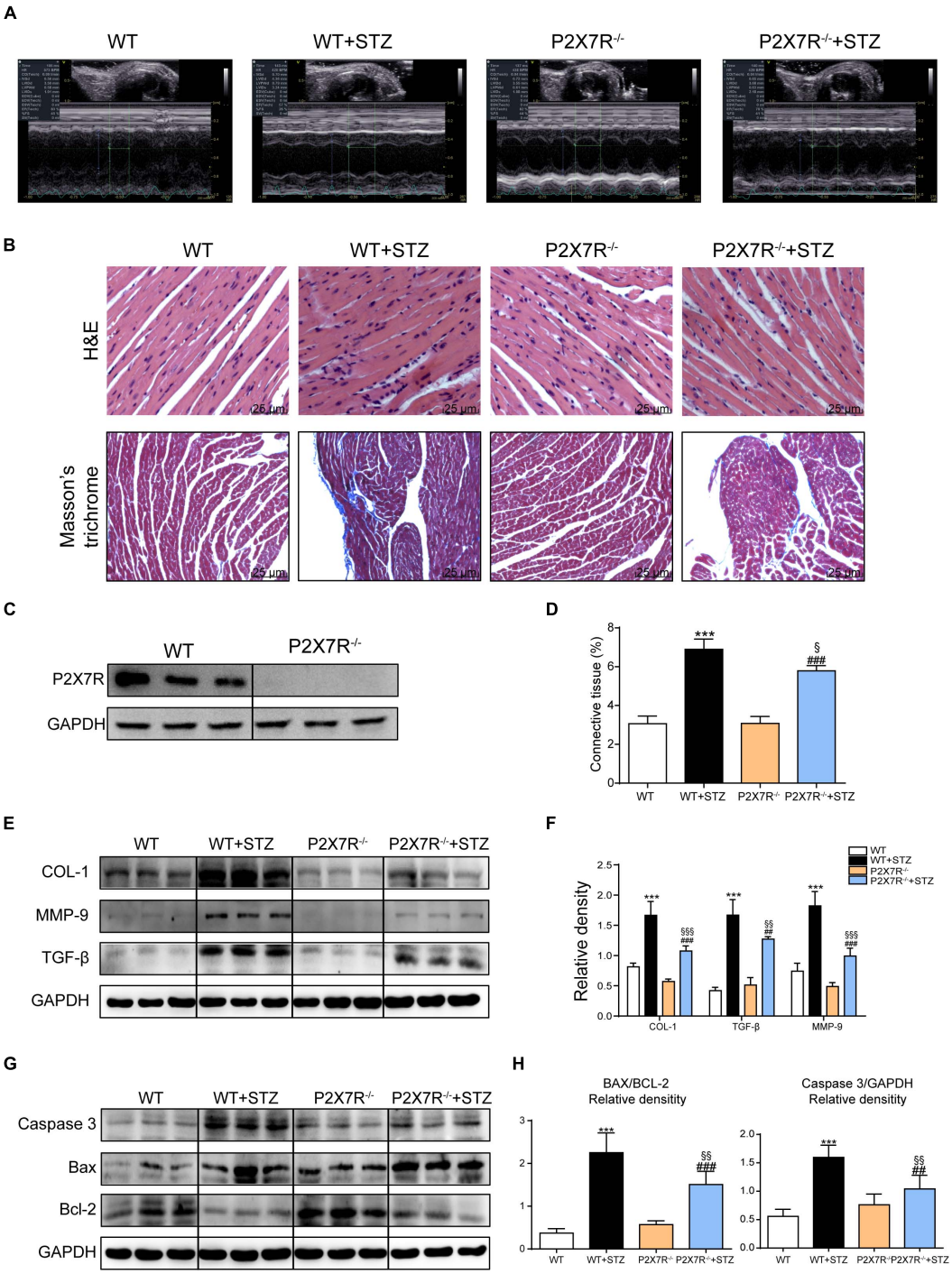

FIGURE 5 | The P2X7 receptor was involved in STZ-induced cardiac damage. Representative images **(A)** of echocardiograms from each group of knockout mice. Representative images of hematoxylin-eosin staining (H&E) of the myocardial tissues from knockout mice (400× magnification), (**B**, upper panel). Myocardial fibrosis was analyzed using Masson's trichrome staining, and representative images (**B**, bottom panel) are shown (400× magnification). Quantification of the interstitial fibrotic areas (%) in images of Masson's trichrome staining are shown in the bar graph **(D)**. The expression of the P2X7 receptor in myocardial tissues from knockout mice **(C)**. The protein levels of fibrosis markers in myocardial tissues were measured using western blotting **(E,F)** (n = 3 animals per group). The levels of the apoptosis-related proteins Caspase-3, Bcl-2, and Bax were measured using western blotting **(G)**. The ratio of the Bcl-2 protein to the Bax protein and the ratio of Caspase3/GAPDH proteins are shown **(H)**. (Data from three independent experiments were analyzed: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared with WT mice; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ compared with P2X7R–/– mice; $^{\S}p < 0.05$, $^{\S\S}p < 0.01$, and $^{\S\S\S}p < 0.001$ compared to the WT + STZ group).

TABLE 3 | Biometric and echocardiographic parameters of the C57BL/6 gene knockout experimental mice.

	WT	WT + STZ	P2X7R$^{-/-}$	P2X7R$^{-/-}$ + STZ
HR, bpm	460 ± 41	400 ± 63	460 ± 67	418 ± 54
HW/BW, mg/g	5.21 ± 0.27	6.59 ± 0.56**	5.38 ± 0.33	6.07 ± 0.58$^{\#}$
IVSD, mm	0.63 ± 0.04	0.64 ± 0.03	0.63 ± 0.08	0.63 ± 0.05
LVIDd, mm	3.55 ± 0.32	3.83 ± 0.24*	3.40 ± 0.36	3.60 ± 0.18$^{\#}$ §§
LVPWd, mm	0.62 ± 0.06	0.69 ± 0.05*	0.63 ± 0.04	0.67 ± 0.06$^{\#}$
LVIDs, mm	2.23 ± 0.27	2.50 ± 0.35*	2.15 ± 0.25	2.39 ± 0.20$^{\#\#}$
EF%	76 ± 5.94	62 ± 7.59***	77.96 ± 4.85	71.23 ± 3.91$^{\#\#\#}$ §§§
FS%	39.23 ± 5.26	28.8 ± 5.35***	40.63 ± 4.68	35.52 ± 2.66$^{\#\#\#}$ §§§

HR, heart rate; HW/BW, heart weight/body weight; IVSD, diastole interventricular septal thickness; LVIDd, diastole left ventricle internal dimension; LVPWd, left ventricular diastole posterior wall thickness; LVIDs, systole left ventricle internal dimension; EF%, ejection fraction %; FS%, fractional shortening %.
*Data presented as Mean ± SD, *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$, compared to WT; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$, compared to P2X7R$^{-/-}$; $^{\S\S}p < 0.01$, and $^{\S\S\S}p < 0.001$, compared to WT + STZ.*

The P2X7R was first cloned from a rat brain (Surprenant et al., 1996). With advances in research, the P2X7R was subsequently detected in all types of cells *in vivo* (Collo et al., 1997; Rassendren et al., 1997; Fountain and Burnstock, 2009). In addition, P2X7R expression was not obvious in the kidney under physiological conditions. However, in abnormal cases, such as diabetes and hypertension, P2X7R expression was significantly increased in rats (Vonend et al., 2004). Moreover, increased P2X7R activity may contribute to the pathogenesis of diabetic nephropathy, and P2X7R inhibition reduces renal macrophage accumulation and contributes to reducing the high prevalence of kidney disease observed in patients with diabetes (Kreft et al., 2016; Menzies et al., 2017). Thus, the P2X7R exerts detrimental effects and is associated with organ damage caused by high sugar levels. Additionally, the P2X7R also exerts important physiological and pathological effects on the cardiovascular system (Burnstock, 2017). For example, stimulation of the P2X7R regulates pro-inflammatory responses in endothelial cells (Sathanoori et al., 2015) and induces autoimmunity in individuals with dilated cardiomyopathy (Martinez et al., 2015). In the present study, the cardiac function of the STZ-induced groups was significantly worse, based on the echocardiography results (**Tables 2**, **3**). Cardiac function and corresponding parameters were significantly improved in P2X7R knockout mice or in mice administered the P2X7R inhibitor. Meanwhile, P2X7R expression was substantially upregulated, and subsequently increased the expression of β-Myhc, Collagen I, and TGF-β in both animal models and cells exposed to HG, whereas P2X7R blockade significantly reversed myocardial fibrosis and reduced hypertrophy, indicating that high P2X7R expression is involved in cardiac remodeling by modulating fibrosis and hypertrophy in subjects with DCM. Notably, cell apoptosis contributes to the development of cardiac remodeling (Wang et al., 2014), and reducing the apoptosis of cardiac tissue may be an effective therapeutic strategy. Here, Caspase 3 and Bax levels increased, whereas the level of the antiapoptotic protein Bcl-2 was decreased, similar to the results from the TUNEL assay. However, the addition of the P2X7R inhibitor (A438079) or P2X7R$^{-/-}$ reversed these aberrant changes. Collectively, the P2X7R is involved in the process of fibrosis, hypertrophy, and apoptosis in heart tissues of diabetic mice, suggesting that the inhibition of P2X7R expression to prevent cardiac remodeling will be an effective cardioprotective strategy for abolishing DCM. Interestingly, the change in P2X7R expression only occurred in cardiomyocytes, but not in cultured rat primary fibroblasts (**Supplementary Figure 1D**), which is supported by the results showing that fibroblasts in the heart mostly mediate fibrosis through P2Y receptors (Certal et al., 2017). This phenomenon indicates that cardiomyocytes are the main sources of P2X7R expression leading to cardiac remodeling in DCM.

Based on the P2X7R structure, the N terminus contains a consensus PKC phosphorylation site (Boue-Grabot et al., 2000). Furthermore, P2X7R activation promotes calcium influx, which activates the PKC protein (Surprenant et al., 1996). Thus, various phenomena indicate a link between the P2X7R and PKC protein. Naturally, the PKC protein becomes the focus because of its important function in cells (Antal et al., 2015). Given its different structures, PKC is divided into three subfamilies. PKCβ is one of the conventional PKCs detected in heart tissue. Based on ample evidence, the PKCβ isoform is overactivated in the hearts of rats with diabetes mellitus (Disatnik et al., 1994; Connelly et al., 2009; Liu et al., 2012). Mice with specific overexpression of PKCβ in the myocardium exhibit left ventricular hypertrophy, cardiac myocyte necrosis, and multifocal fibrosis (Wakasaki et al., 1997). Therefore, we investigated whether the activation of PKCβ is involved in the mechanism by which the P2X7R in cardiomyocytes regulates hyperglycemia/STZ-induced cardiac remodeling. As expected, the phosphorylation of PKCβ was increased when H9c2 cells were stimulated with HG (**Figure 6A**). On the other hand, P2X7R inhibition decreased the phosphorylation of PKCβ (**Figure 6A**). We explored the possible downstream targets of PKCβ to further mechanistically investigate the effect of PKCβ on diabetes. Extracellular signal-regulated kinases (ERK) are an important subfamily of mitogen-activated protein kinases that control a broad range of cellular activities and physiological processes (Lu and Xu, 2006; Keshet and Seger, 2010; RoskoskiJr., 2012). P2X7R stimulation in HEK-293 cells leads to the activation of ERK1 and ERK2 independent of Ca^{2+} influx (Amstrup and Novak, 2003), and ERK1/2 are activated by similar PKC-dependent signaling pathways (Bradford and Soltoff, 2002). In the present study, the P2X7R inhibitor A438079 suppressed phospho-PKCβ activity and decreased phospho-ERK activity in HG-induced H9c2 cells (**Figures 6A,B**), indicating that P2X7R is a critical upstream molecule of PKCβ and ERK. However, when the inhibitor of PKCβ was added to the cells, P2X7R activity was not affected, but ERK phosphorylation was reduced (**Figure 6C**). Interestingly, neither PKCβ nor P2X7R activity was altered by the administration of the ERK inhibitor PD98059 (**Figure 6C**). Thus, PKCβ mediates ERK activation initiated by the P2X7R in HG-induced H9c2 cells. Similarly, the inhibition of PKC and ERK abolished HG-induced cell hypertrophy, fibrosis, and apoptosis (**Figure 6G**). Taken

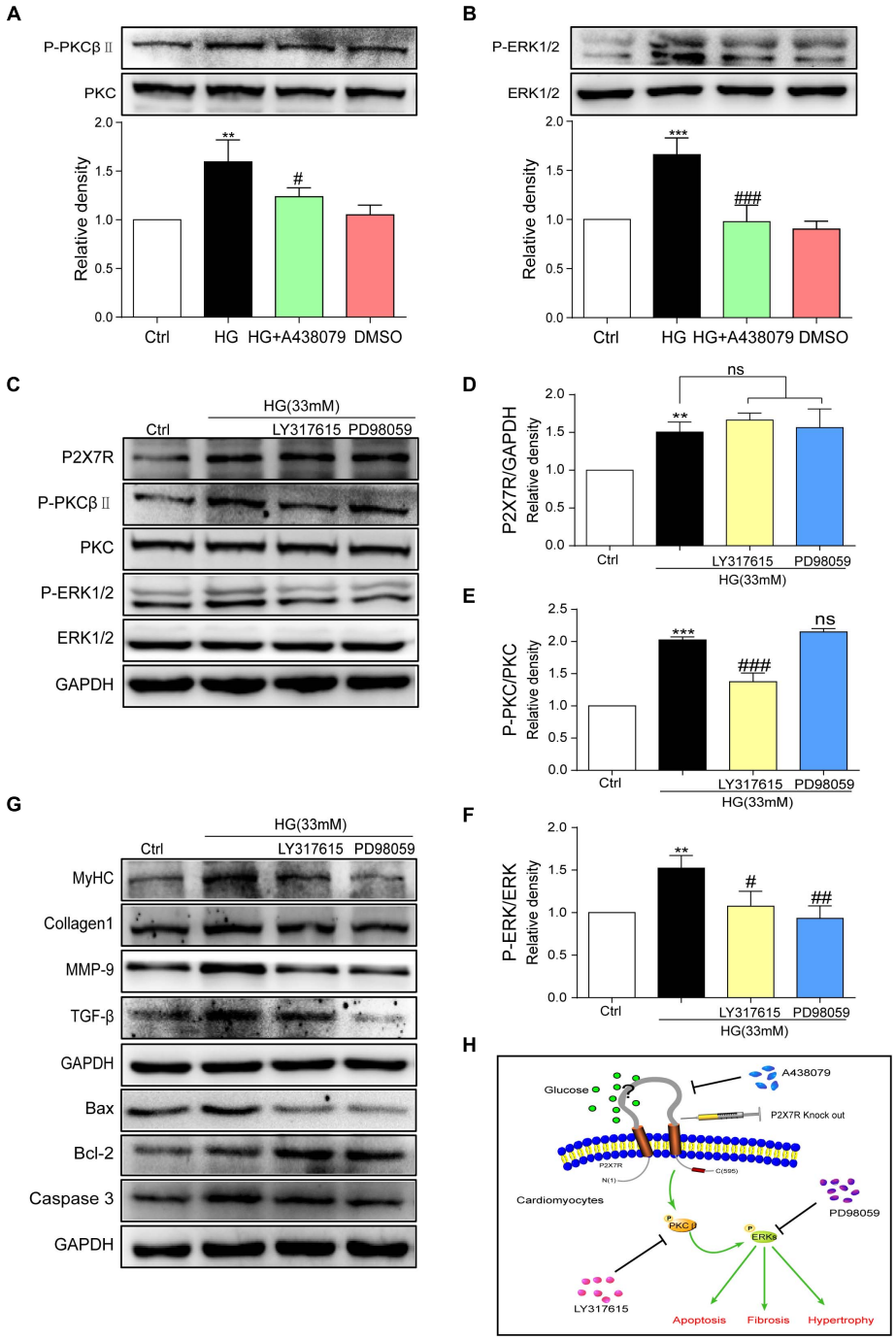

FIGURE 6 | P2X7R regulated the activation of the PKCβ/ERK pathway in high glucose-induced cardiomyocytes. Total protein was extracted and p-PKC/PKC and p-ERK/ERK levels were analyzed using western blot analyses **(A,B)**. Levels of the P2X7R, p-PKC/PKC and p-ERK/ERK proteins were analyzed using western blotting **(C)**, and the quantitative statistics are presented in graphs **(D–F)**. The levels of pro-fibrotic, pro-apoptotic, and pro-hypertrophic proteins were determined using western blot analyses **(G)**. A schematic illustrating the role of P2X7R in diabetes/HG-induced injury in cardiomyocytes, the preventative effect of A438079 and the role of P2X7R knockout. **(H)** (Data from three independent experiments were analyzed: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared with the Ctrl; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ compared with HG alone).

together, the P2X7R, which is upstream of PKC, subsequently regulates the ERK pathway to participate in the pathogenesis of DCM (**Figure 6H**).

In general, we described the role of the P2X7R in DCM using its specific inhibitors and P2X7R$^{-/-}$ mice. P2X7R inhibition or deficiency reduces myocardial hypertrophy, fibrosis, and

apoptosis to subsequently improve cardiac function. Moreover, we further clarified that the P2X7R/PKCβ/ERK pathway is involved in the process and provided a new target for the treatment of DCM.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The animal study was reviewed and approved by the Committee on Animal Care of Wenzhou Medical University.

AUTHOR CONTRIBUTIONS

SH wrote the manuscript. SH and WW conceived, designed, and analyzed the data. SH, WW, and LL researched the data and performed the animal experiments. TW, YZ, and YL performed the cell experiments. WH reviewed and edited the manuscript. YW and ZH designed and supervised the study. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcell.2021.692028/full#supplementary-material

REFERENCES

Amstrup, J., and Novak, I. (2003). P2X7 receptor activates extracellular signal-regulated kinases ERK1 and ERK2 independently of Ca2+ influx. *Biochem. J.* 374 (Pt 1), 51–61. doi: 10.1042/BJ20030585

Aneja, A., Tang, W. H., Bansilal, S., Garcia, M. J., and Farkouh, M. E. (2008). Diabetic cardiomyopathy: insights into pathogenesis, diagnostic challenges, and therapeutic options. *Am. J. Med.* 121, 748–757. doi: 10.1016/j.amjmed.2008.03.046

Antal, C. E., Hudson, A. M., Kang, E., Zanca, C., Wirth, C., Stephenson, N. L., et al. (2015). Cancer-associated protein kinase C mutations reveal kinase's role as tumor suppressor. *Cell* 160, 489–502. doi: 10.1016/j.cell.2015.01.001

Boue-Grabot, E., Archambault, V., and Seguela, P. (2000). A protein kinase C site highly conserved in P2X subunits controls the desensitization kinetics of P2X(2) ATP-gated channels. *J. Biol. Chem.* 275, 10190–10195. doi: 10.1074/jbc.275.14.10190

Bradford, M. D., and Soltoff, S. P. (2002). P2X7 receptors activate protein kinase D and p42/p44 mitogen-activated protein kinase (MAPK) downstream of protein kinase C. *Biochem. J.* 366 (Pt 3), 745–755. doi: 10.1042/BJ20020358

Bugger, H., and Abel, E. D. (2009). Rodent models of diabetic cardiomyopathy. *Dis. Model. Mech.* 2, 454–466. doi: 10.1242/dmm.001941

Bugger, H., and Abel, E. D. (2014). Molecular mechanisms of diabetic cardiomyopathy. *Diabetologia* 57, 660–671. doi: 10.1007/s00125-014-3171-6

Burnstock, G. (2017). Purinergic signaling in the cardiovascular system. *Circ. Res.* 120, 207–228. doi: 10.1161/CIRCRESAHA.116.309726

Burnstock, G., and Knight, G. E. (2004). Cellular distribution and functions of P2 receptor subtypes in different systems. *Int. Rev. Cytol.* 240, 31–304. doi: 10.1016/S0074-7696(04)40002-3

Certal, M., Vinhas, A., Barros-Barbosa, A., Ferreirinha, F., Costa, M. A., and Correia-de-Sa, P. (2017). ADP-induced Ca(2+) signaling and proliferation of rat ventricular myofibroblasts depend on phospholipase C-linked TRP channels activation within lipid rafts. *J. Cell. Physiol.* 232, 1511–1526. doi: 10.1002/jcp.25656

Collo, G., Neidhart, S., Kawashima, E., Kosco-Vilbois, M., North, R. A., and Buell, G. (1997). Tissue distribution of the P2X7 receptor. *Neuropharmacology* 36, 1277–1283. doi: 10.1016/s0028-3908(97)00140-8

Connelly, K. A., Kelly, D. J., Zhang, Y., Prior, D. L., Advani, A., Cox, A. J., et al. (2009). Inhibition of protein kinase C-beta by ruboxistaurin preserves cardiac function and reduces extracellular matrix production in diabetic cardiomyopathy. *Circ. Heart Fail.* 2, 129–137. doi: 10.1161/CIRCHEARTFAILURE.108.765750

Disatnik, M. H., Buraggi, G., and Mochly-Rosen, D. (1994). Localization of protein kinase C isozymes in cardiac myocytes. *Exp. Cell Res.* 210, 287–297. doi: 10.1006/excr.1994.1041

Forbes, J. M., and Cooper, M. E. (2013). Mechanisms of diabetic complications. *Physiol. Rev.* 93, 137–188. doi: 10.1152/physrev.00045.2011

Fountain, S. J., and Burnstock, G. (2009). An evolutionary history of P2X receptors. *Purinergic Signal.* 5, 269–272. doi: 10.1007/s11302-008-9127-x

Hayashi, T., Shibata, H., Kurihara, I., Yokota, K., Mitsuishi, Y., Ohashi, K., et al. (2017). High glucose stimulates mineralocorticoid receptor transcriptional activity through the protein kinase C beta signaling. *Int. Heart J.* 58, 794–802. doi: 10.1536/ihj.16-649

Huang, C., Yu, W., Cui, H., Wang, Y., Zhang, L., Han, F., et al. (2014). P2X7 blockade attenuates mouse liver fibrosis. *Mol. Med. Rep.* 9, 57–62. doi: 10.3892/mmr.2013.1807

Huang, Z., Wang, C., Wei, L., Wang, J., Fan, Y., Wang, L., et al. (2008). Resveratrol inhibits EMMPRIN expression via P38 and ERK1/2 pathways in PMA-induced THP-1 cells. *Biochem. Biophys. Res. Commun.* 374, 517–521. doi: 10.1016/j.bbrc.2008.07.058

Kannel, W. B., and McGee, D. L. (1979). Diabetes and cardiovascular disease. The Framingham study. *JAMA* 241, 2035–2038. doi: 10.1001/jama.1979.03290450033020

Keshet, Y., and Seger, R. (2010). The MAP kinase signaling cascades: a system of hundreds of components regulates a diverse array of physiological functions. *Methods Mol. Biol.* 661, 3–38. doi: 10.1007/978-1-60761-795-2_1

Kreft, E., Kowalski, R., Jankowski, M., and Szczepanska-Konkel, M. (2016). Renal vasculature reactivity to agonist of P2X7 receptor is increased in streptozotocin-induced diabetes. *Pharmacol. Rep.* 68, 71–74. doi: 10.1016/j.pharep.2015.06.140

Liu, Y., Lei, S., Gao, X., Mao, X., Wang, T., Wong, G. T., et al. (2012). PKCbeta inhibition with ruboxistaurin reduces oxidative stress and attenuates left ventricular hypertrophy and dysfunction in rats with streptozotocin-induced diabetes. *Clin. Sci.* 122, 161–173. doi: 10.1042/CS20110176

Lu, Z., and Xu, S. (2006). ERK1/2 MAP kinases in cell survival and apoptosis. *IUBMB Life* 58, 621–631. doi: 10.1080/15216540600957438

Martinez, C. G., Zamith-Miranda, D., da Silva, M. G., Ribeiro, K. C., Brandao, I. T., Silva, C. L., et al. (2015). P2x7 purinergic signaling in dilated cardiomyopathy induced by auto-immunity against muscarinic M2 receptors: autoantibody levels, heart functionality and cytokine expression. *Sci. Rep.* 5:16940. doi: 10.1038/srep16940

Menzies, R. I., Booth, J. W. R., Mullins, J. J., Bailey, M. A., Tam, F. W. K., Norman, J. T., et al. (2017). Hyperglycemia-induced renal P2X7 receptor activation enhances diabetes-related injury. *EBioMedicine* 19, 73–83. doi: 10.1016/j.ebiom.2017.04.011

Mezzaroma, E., Toldo, S., Farkas, D., Seropian, I. M., Van Tassell, B. W., Salloum, F. N., et al. (2011). The inflammasome promotes adverse cardiac remodeling following acute myocardial infarction in the mouse. *Proc. Natl. Acad. Sci. U.S.A.* 108, 19725–19730. doi: 10.1073/pnas.1108586108

Mizushige, K., Yao, L., Noma, T., Kiyomoto, H., Yu, Y., Hosomi, N., et al. (2000). Alteration in left ventricular diastolic filling and accumulation of myocardial collagen at insulin-resistant prediabetic stage of a type II diabetic rat model. *Circulation* 101, 899–907. doi: 10.1161/01.cir.101.8.899

Mochly-Rosen, D., Das, K., and Grimes, K. V. (2012). Protein kinase C, an elusive therapeutic target? *Nat. Rev. Drug Discov.* 11, 937–957. doi: 10.1038/nrd3871

Newton, A. C., Antal, C. E., and Steinberg, S. F. (2016). Protein kinase C mechanisms that contribute to cardiac remodelling. *Clin. Sci.* 130, 1499–1510. doi: 10.1042/CS20160036

Ofstad, A. P. (2016). Myocardial dysfunction and cardiovascular disease in type 2 diabetes. *Scand. J. Clin. Lab. Invest.* 76, 271–281. doi: 10.3109/00365513.2016.1155230

Ogurtsova, K., da Rocha Fernandes, J. D., Huang, Y., Linnenkamp, U., Guariguata, L., Cho, N. H., et al. (2017). IDF Diabetes Atlas: global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Res. Clin. Pract.* 128, 40–50. doi: 10.1016/j.diabres.2017.03.024

Peng, K., Liu, L., Wei, D., Lv, Y., Wang, G., Xiong, W., et al. (2015). P2X7R is involved in the progression of atherosclerosis by promoting NLRP3 inflammasome activation. *Int. J. Mol. Med.* 35, 1179–1188. doi: 10.3892/ijmm.2015.2129

Rassendren, F., Buell, G. N., Virginio, C., Collo, G., North, R. A., and Surprenant, A. (1997). The permeabilizing ATP receptor, P2X7. Cloning and expression of a human cDNA. *J. Biol. Chem.* 272, 5482–5486. doi: 10.1074/jbc.272.9.5482

Riteau, N., Gasse, P., Fauconnier, L., Gombault, A., Couegnat, M., Fick, L., et al. (2010). Extracellular ATP is a danger signal activating P2X7 receptor in lung inflammation and fibrosis. *Am. J. Respir. Crit. Care Med.* 182, 774–783. doi: 10.1164/rccm.201003-0359OC

Roskoski, R. Jr. (2012). ERK1/2 MAP kinases: structure, function, and regulation. *Pharmacol. Res.* 66, 105–143. doi: 10.1016/j.phrs.2012.04.005

Sathanoori, R., Sward, K., Olde, B., and Erlinge, D. (2015). The ATP receptors P2X7 and P2X4 modulate high glucose and palmitate-induced inflammatory responses in endothelial cells. *PLoS One* 10:e0125111. doi: 10.1371/journal.pone.0125111

Singh, V. P., Le, B., Khode, R., Baker, K. M., and Kumar, R. (2008). Intracellular angiotensin II production in diabetic rats is correlated with cardiomyocyte apoptosis, oxidative stress, and cardiac fibrosis. *Diabetes* 57, 3297–3306. doi: 10.2337/db08-0805

Soetikno, V., Sari, F. R., Sukumaran, V., Lakshmanan, A. P., Mito, S., Harima, M., et al. (2012). Curcumin prevents diabetic cardiomyopathy in streptozotocin-induced diabetic rats: possible involvement of PKC-MAPK signaling pathway. *Eur. J. Pharm. Sci.* 47, 604–614. doi: 10.1016/j.ejps.2012.04.018

Stachon, P., Heidenreich, A., Merz, J., Hilgendorf, I., Wolf, D., Willecke, F., et al. (2017). P2X7 deficiency blocks lesional inflammasome activity and ameliorates atherosclerosis in mice. *Circulation* 135, 2524–2533. doi: 10.1161/CIRCULATIONAHA.117.027400

Surprenant, A., Rassendren, F., Kawashima, E., North, R. A., and Buell, G. (1996). The cytolytic P2Z receptor for extracellular ATP identified as a P2X receptor (P2X7). *Science* 272, 735–738. doi: 10.1126/science.272.5262.735

Vonend, O., Turner, C. M., Chan, C. M., Loesch, A., Dell'Anna, G. C., Srai, K. S., et al. (2004). Glomerular expression of the ATP-sensitive P2X receptor in diabetic and hypertensive rat models. *Kidney Int.* 66, 157–166. doi: 10.1111/j.1523-1755.2004.00717.x

Wakasaki, H., Koya, D., Schoen, F. J., Jirousek, M. R., Ways, D. K., Hoit, B. D., et al. (1997). Targeted overexpression of protein kinase C beta2 isoform in myocardium causes cardiomyopathy. *Proc. Natl. Acad. Sci. U.S.A.* 94, 9320–9325. doi: 10.1073/pnas.94.17.9320

Wang, H. J., Wang, W., Cornish, K. G., Rozanski, G. J., and Zucker, I. H. (2014). Cardiac sympathetic afferent denervation attenuates cardiac remodeling and improves cardiovascular dysfunction in rats with heart failure. *Hypertension* 64, 745–755. doi: 10.1161/HYPERTENSIONAHA.114.03699

Wang, J., Song, Y., Wang, Q., Kralik, P. M., and Epstein, P. N. (2006). Causes and characteristics of diabetic cardiomyopathy. *Rev. Diabet. Stud.* 3, 108–117. doi: 10.1900/RDS.2006.3.108

Zhao, X., Zhu, X., Zhang, H., Zhao, W., Li, J., Shu, Y., et al. (2012). Prevalence of diabetes and predictions of its risks using anthropometric measures in southwest rural areas of China. *BMC Public Health* 12:821. doi: 10.1186/1471-2458-12-821

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REVIEW
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The Role of Epidermal Growth Factor Receptor Family of Receptor Tyrosine Kinases in Mediating Diabetes-Induced Cardiovascular Complications

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Diabetes mellitus is a major debilitating disease whose global incidence is progressively increasing with currently over 463 million adult sufferers and this figure will likely reach over 700 million by the year 2045. It is the complications of diabetes such as cardiovascular, renal, neuronal and ocular dysfunction that lead to increased patient morbidity and mortality. Of these, cardiovascular complications that can result in stroke and cardiomyopathies are 2- to 5-fold more likely in diabetes but the underlying mechanisms involved in their development are not fully understood. Emerging research suggests that members of the Epidermal Growth Factor Receptor (EGFR/ErbB/HER) family of tyrosine kinases can have a dual role in that they are beneficially required for normal development and physiological functioning of the cardiovascular system (CVS) as well as in salvage pathways following acute cardiac ischemia/reperfusion injury but their chronic dysregulation may also be intricately involved in mediating diabetes-induced cardiovascular pathologies. Here we review the evidence for EGFR/ErbB/HER receptors in mediating these dual roles in the CVS and also discuss their potential interplay with the Renin-Angiotensin-Aldosterone System heptapeptide, Angiotensin-(1-7), as well the arachidonic acid metabolite, 20-HETE (20-hydroxy-5, 8, 11, 14-eicosatetraenoic acid). A greater understanding of the multi-faceted roles of EGFR/ErbB/HER family of tyrosine kinases and their interplay with other key modulators of cardiovascular function could facilitate the development of novel therapeutic strategies for treating diabetes-induced cardiovascular complications.

Keywords: epidermal growth factor receptor, ErbB2, ErbB3, ErbB4, diabetes, heart, cardiac dysfunction, vascular dysfunction

INTRODUCTION

Diabetes mellites (DM) is a set of metabolic disorders arising from defective insulin secretion and/or action in which hyperglycemia (HG) is a common feature. Type 1 diabetes mellitus (T1DM) results from the autoimmune-mediated destruction of pancreatic β cells. The infiltration of T lymphocytes, release of cytokines, and generation of reactive oxygen species (ROS) ultimately results in the

dysfunction and apoptosis of pancreatic β cells (Morgan et al., 2014; Crevecoeur et al., 2015; Tan et al., 2019; Toi et al., 2020). In contrast, type 2 diabetes mellitus (T2DM) is characterized by target tissue resistance to the metabolic actions of insulin as well as pancreatic β cell dysfunction. Insulin resistance is strongly associated with visceral obesity and the resulting chronic low-grade inflammation as well as increased ectopic fat deposition (Saltiel, 2021; Tsatsoulis, et al., 2013).

The International Diabetes Federation declared that approximately 463 million adults are currently living with diabetes mellitus and this figure will likely reach over 700 million by the year 2045. Half of the sufferers are unaware of their condition and hence, prone to serious hyperglycemia/diabetes-related complications (IDF Diabetes Atlas, 2019). DM results in secondary pathophysiologic alterations in several organ systems that impose an immense burden on both the individual with diabetes and the public health system. Cardiovascular (CV) complications constitute the leading cause of morbidity and mortality in individuals with DM (Forbes and Cooper, 2013; Babel and Dandekar, 2021). These include microangiopathy, abnormal vascular reactivity, atherosclerosis, hypertension, cardiomyopathy, ischemic heart disease, and myocardial infarction (Akhtar and Benter, 2013; Babel and Dandekar, 2021). Moreover, the specific underlying mechanisms leading to the development of these CV complications are not fully understood and likely involve multiple intracellular signaling networks that can be activated by hyperglycemia (HG) and/or diabetes.

Epidermal growth factor receptor (EGFR/ErbB1/HER1), one of the most versatile signaling units in biology, plays a key role in regulating many cellular functions including growth, proliferation, motility, and survival (Kumagai et al., 2021; Sharifi et al., 2021). Perturbations in EGFR expression and signalling are implicated in several pathophysiological conditions including cancer and diabetes (Matroughi, 2010; Akhtar and Benter, 2013; Xu et al., 2017; Akhtar et al., 2018; Talukdar et al., 2020; Kumagai et al., 2021; Sharifi et al., 2021). Emerging research suggests that members of the Epidermal Growth Factor Receptor (EGFR/ErbB/HER) family of tyrosine kinases can have a dual role in that they are necessary (acting as "good guys") for the normal development and physiological functioning of the cardiovascular system (CVS) but their dysregulation may also be intricately involved in mediating cardiovascular pathologies (i.e., acting as "bad guys") (Matrougui, 2010; Akhtar and Benter, 2013; Schreier et al., 2014). In this article, we review the evidence for EGFR/ErbB/HER receptors in mediating these dual ("good guy" verses "bad guy") roles in the heart and then in the vascular system followed by a discussion of their potential interplay with the Renin-Angiotensin-Aldosterone System heptapeptide, Angiotensin-(1–7), as well the arachidonic acid metabolite, 20-HETE-key players in the regulation of cardiovascular function.

EPIDERMAL GROWTH FACTOR RECEPTOR FAMILY OF RECEPTOR TYROSINE KINASES: AN OVERVIEW

EGFR (a/k/a ErbB1 or HER1) is a 180 kDa transmembrane glycoprotein made up of 1,186 amino acids that belongs to the broader family of receptor tyrosine kinases (RTKs) (Rayego-Mateos et al., 2018; Kumagai et al., 2021). It is made up of four conserved domains: an extracellular ligand-binding domain, a transmembrane domain, a cytoplasmic tyrosine kinase-containing domain, and a regulatory domain (Zheng et al., 2014). Moreover, there are three other homologous members that comprise the EGFR/ErbB/HER family of RTKs: ErbB2 (EGFR2/HER2/Neu), ErbB3 (EGFR3/HER3), and ErbB4 (EGFR4/HER4). EGFR members, with the exception of ErbB2, are activated by numerous growth factors or ligands such as epidermal growth factor (EGF), betacellulin, neuregulin-1 (NRG-1) and Heparin-binding EGF-like growth factor (HB-EGF). Upon ligand interaction with its cognate receptor, either homodimerization of the receptor or heterodimerization with other members of the EGFR family occurs. ErbB2 lacks a known ligand and depends on heterodimerization with other receptors of the EGFR family for activation. ErbB3 lacks a functional kinase domain and therefore also relies on heterodimerization with other EGFR/ErbB receptors for activity. Thus, EGFR and ErbB4 receptors are the only fully functioning homodimers (Kumagai et al., 2021; Sharifi et al., 2021). Next, phosphorylation of precisely defined tyrosine residues within the cytoplasmic kinase portion of the receptor dimer occurs, eventually leading to the activation of multiple downstream signaling cascades. Such cascades include p38 Mitogen-activated protein kinase (MAPKs), Extracellular-signal regulated kinase 1/2 (ERK1/2), Phosphatidylinositol 3-kinases (PI3Ks)/Protein kinase B (AKT), phospholipase C-gamma 1 (PLCγ1), and Janus kinase (JAK) (/Signal transducers and activation of transcription (STAT) pathways (Rayego-Mateos et al., 2018). Consequently, several cellular processes, such as angiogenesis, proliferation, differentiation, motility, survival, and apoptosis can take place (Akhtar and Benter, 2013; Kumagai et al., 2021; Sharifi et al., 2021; Zheng et al., 2014).

EGFR/ErbBs can also be activated via ligand-independent pathways through a process of "transactivation" such as by peptides of the Renin-Angiotensin-Aldosterone system (RAAS) and other G-protein coupled receptors (GPCRs) (Forrester et al., 2016). For example, EGFR transactivation can occur via Angiotensin II (Ang II) Norepinephrine (NE), (leptin, thrombin, and endothelin by mechanisms involving non-ligand associated Src family kinases, and/or mediated via matrix metalloproteases (MMP), and/or a disintegrin and metalloprotease (ADAM)-dependent shedding of cell surface bound EGF-like ligands (Matrougui, 2010; Akhtar et al., 2012a; Beltowski and Jazmroz-Wisniewska, 2014; Chen et al., 2015; Forrester et al., 2016; Reichelt et al., 2017). It should be noted that all ligands of the EGFR/ErbB family of receptors exist as membrane-anchored precursors that are released by enzymatic-cleavage by sheddases such as MMPs and ADAMs (Rayego-Mateos et al., 2018). EGFR/ErbB receptors are expressed widely in epithelial, neuronal and mesenchymal tissues (Yano et al., 2003; Chen et al., 2016). Hence, EGFR/ErbBs are key regulators of many cellular homeostatic functions and their dysregulation is associated with several pathological functions including diabetes-induced cardiovascular dysfunction.

DIABETES-INDUCED CARDIAC DYSFUNCTION: THE DUAL ROLE OF EPIDERMAL GROWTH FACTOR RECEPTOR FAMILY OF RECEPTOR TYROSINE KINASES AND THEIR LIGANDS IN THE HEART

Cardiac complications including diabetic cardiomyopathy and heart failure are 2- to 5-fold more likely in patients with diabetes (for a review see Kenny and Abel, 2019). This increased risk is dependent on blood glucose levels as higher HbA1c levels (indicating worsening hyperglycemia) are associated with increased likelihood of heart failure in patients with diabetes (Erquo et al., 2013). Even short-term episodes of hyperglycemia or "glycemic variability" such as those observed postprandially are thought to increase the risk of developing cardiovascular complications associated with diabetes (Ceriello, 2005; Brownlee and Hirsch, 2006). Interestingly, in long-term studies it has been shown that subsequent therapy-induced correction of hyperglycemia does not normalise the risk of developing cardiovascular complications back to baseline (Kenny and Abel, 2019) implying that even transient excursions into hyperglycemia may induce long-term adverse molecular or signaling changes in the cardiovascular system that can lead to cardiovascular complications. This phenomenon has been described as "glycemic" or "metabolic" memory (Chalmers and Cooper, 2008; Holman et al., 2008); though recently this concept has been challenged (Miller and Orchard, 2020). Diabetes-induced cardiac complications may arise from a direct effect of hyperglycemia-induced molecular changes in the cardiac muscle as observed in cardiac myopathy (hypertrophy and fibrosis) or in the microvasculature of the heart that manifest as atherosclerosis or coronary artery disease (Falcao-Pires and Leite-Moreira, 2012; Kenny and Abel, 2019; Babel and Dandekar, 2021). Patients with diabetes who develop cardiomyopathy initially exhibit a hidden subclinical phase of myocardial remodeling that leads to compromised diastolic function, followed by systolic dysfunction that can progress to eventual heart failure (Falcao-Pires and Leite-Moreira, 2012) The exact underlying mechanisms for the development of cardiac pathologies in diabetes are not fully understood but likely involve a wide array of cell signaling networks. These include generation of reactive oxygen species (ROS)/oxidative stress, endoplasmic reticulum (ER) stress, mitochondrial dysfunction, accumulation of advanced glycated end-products (see also section on vascular dysfunction), and hyperglycemia-induced alterations in signaling by growth factors, peptides of RAAS as well as other GPCRs that are known also to transactivate EGFR (for recent reviews see Babel and Dandekar, 2021; Kenny and Abel, 2019, Forrester et al., 2016). Emerging evidence suggests that not only do EGFR/ErbB/HER family of TK receptors play a key role in the development of diabetes-induced cardiac dysfunction but they may also represent a convergent point or "hub" and a "relay" for the multiple other signaling molecules or inputs leading to cardiac dysfunction. In fact, the current understanding is that ErbBs may play a dual role, whereby they can elicit beneficial as well as detrimental signaling in the heart. In the proceeding section, we will discuss studies highlighting the dual role of EGFR/ErbB/HER receptor signaling in the normal (physiological) and the diabetic (pathological) heart.

The Beneficial Role of Epidermal Growth Factor Receptors in the Normal and Diabetic Heart

EGFR/ErbB/HER receptors are vitally important in the physiological development of the heart irrespective of whether it is normal or diabetic. Indeed, the beneficial (good guy) role of ErbB receptors starts even before birth. These receptor tyrosine kinases (RTKs) are essential for normal cardiac morphogenesis during embryogenesis, and also in maintaining proper physiology of the adult heart (Sanchez-Soria and Camenisch, 2010; Fuller et al., 2008) see also **Table 1**. Observations in engineered knockout (KO) or mutant mice of ErbB1, ErbB2, ErbB3, ErbB4 highlighted the lethal phenotypes and heart defects that can arise as a result of a deficiency in the number or signaling of these receptors (for review see also Sanchez-Soria and Camenisch, 2010). ErbB1 KO and mutant mice displayed semilunar valve defects (Sibilia and Wagner, 1995; Chen et al., 2000) whilst disrupted endocardial cushion/heart valve mesenchyme formation was noted in ErbB3 KO mice that ultimately leads to death of embryos within 2 weeks (Erickson et al., 1997; Camenisch et al., 2002). ErbB2 and ErbB4 KO mice lack ventricular trabeculation-a key process in the maturation of ventricles and necessary for physical contractility and normal blood flow (Gassmann et al., 1995; Lee et al., 1995; Chan et al., 2002; Negro et al., 2004).

ErbB2 is also important for neural development and myelination of nerve fibres (Lee et al., 1995; Garratt et al., 2000). Not only does ErbB2 signaling participate in the development of the ventricular conduction system (Negro et al., 2004) but it is also required for cardiac atrial electrical activity during development of the atrial conduction system that if disrupted leads to premature death at mid-gestation (Tenin et al., 2014). Thus, activation of ErbB signaling has been proposed as a therapeutic strategy to reduce the mortality rate of congenital heart diseases and in the prevention of cardiac damage in adults (Sanchez-Soria and Camenisch, 2010). Collectively, these studies suggest that all four EGFR/ErbB/HER receptors are necessary for the proper physiological development of the heart-the first organ to form during embryogenesis. Furthermore, all EGFR/ErbB/HER receptors and several of their ligands are now also thought to be expressed in the adult heart (either in cardiac myocytes, fibroblasts, endothelial or vascular smooth muscle cells) and are required for normal physiological functioning of the post-natal heart (Camprecios et al., 2011; Kirabo et al., 2017; Sanchez-Soria and Camenisch, 2010). Of the EGFR/ErbB receptors, ErbB2 and ErbB4 appear to be the most abundant and of the ligands, EGF, HB-EGF and NRG-1 are thought to be most important in regulating heart function (Reichelt et al., 2017).

TABLE 1 | A summary of selected studies highlighting the **beneficial** role of EGFR signaling in the heart.

Role of EGFR	Study model	Intervention	Results	Refs.
ErbB1 expression protects from myocardial I/R injury	Myocyte-specific Hif2a or ErbB1 knockout mice		• RNA-binding protein 4 suppression attenuated hypoxia-inducible factor 2A-dependent induction of ErbB1 • ErbB1 myosin Cre + mice suffered larger infarctions and could not be saved by amphiregulin	(Lee et al., 2020)
EGF protects against myocardial I/R injury	C57BL/6 (B6) mice	EGF	• EGF inhibits ROS and H_2O_2 induced cell death and by Nrf2 activation • EGF limited cardiac I/R injury and apoptosis *in vivo*	Ma and Jin, (2019)
NRG-1 receptor ErbB3 limits apoptosis and improves cell survival	Wistar rats	I/R injury and protective post-conditioning procedure	• ErbB3 expression increased after I/R injury (with and without post-conditioning) • ErbB3 expression improved cell survival and reduced mitochondrial dysfunction and apoptosis	Morano et al. (2017)
NRG-1β induces proliferation, survival and paracrine signaling	Primary human cardiac ventricular fibroblasts	NRG-1β	• NRG-1β improved proliferation and survival of human cardiac fibroblasts by inducing ErbB3-dependent activation of ErbB2	Kirabo et al. (2017)
NRG-1 inhibits ER stress and protects against myocardial I/R injury	Sprague-dawley and wistar rats	NRG-1 ligand of cardiomyocyte ErbB receptors	• NRG-1 reduced cardiomyocyte ER stress, hypoxia-reoxygenation induced apoptosis and myocardial infarct size induced by I/R injury	Fang et al. (2017)
ErbB activation alleviates doxorubicin induced cardiac toxicity	Stem cell derived human cardiac myocytes	Trastuzumab and lapatinib	• ErbB activation with NRG protected against doxorubicin-induced cardiac myocyte injury, while inhibition with trastuzumab exacerbated it	Eldridge et al. (2014)
EGFR/ErbB2 improves T1D hearts recovery from I/R injury	Wistar rat	AG825 or AG1478	• Chronic AG1478 or AG825 treatment decreased cardiac recovery in normal and diabetic rats	Akhtar et al. (2012b)
		EGF and/or losartan	• Acute EGF treatment pre or post ischemia improved cardiac recovery and opposed ischemic changes by EGFR/ErbB2 activation in T1D hearts	
EGFR protects myocytes in reperfused hearts	C57/Bl6 mice	AG1478, GM6001, or CRM197	• EGFR inhibition limited CCPA-mediated functional protection in reperfused hearts	Williams-Pritchard et al. (2011)
EGFR maintains normal cardiac function and left ventricular thickness	C57BL/6J (B6) mice	EGFR small molecule TKIs, irreversible EKB-569 and reversible AG1478	• EGFR inhibition resulted in left ventricular thickness and cardiac function changes via increasing fibrosis and altering left ventricular apoptotic gene expression	Barrick et al. (2008)
ErbB4 plays a major role in normal cardiac conduction and ventricular trabeculation	ErbB4 cardiac-knockout mice		• ErbB4 deletion resulted in severe dilated cardiomyopathy, abnormal conduction, impaired ventricular trabeculation and premature death	Garcia-Rivello et al. (2005)
EGFR/ErbB1 activation protects against stress-induced cardiac injury	Adult Swiss-CD1 male mice	EGF, AG1478	• EGF led to lower increase in total LDH, LDH-1, and creatinine kinase activity, and protected against stress-induced cardiac injury (these effects were abolished by simultaneous AG1478 administration)	Pareja et al. (2003)
ErbB2 prevents dilated cardiomyopathy	C57BL/6J mice		• Ventricular-restricted ErbB2 deletion resulted in dilated cardiomyopathy with impaired left ventricular contractility and increased susceptibility of cardiomyocytes to anthracycline toxicity	Crone et al. (2002)

I/R, ischemia/reperfusion; NRG, neuregulin; ER, endoplasmic reticulum; T1D, type-1 diabetes; CCPA, 2-chloro-N(6)-cyclopentyladenosine; CK, creatinine kinase.

The EGFR/ErbB1 receptor and some of its ligands, especially EGF and HB-EGF, are expressed in the adult heart and thus, both ligand-activated signaling and ligand-independent transactivation of EGFR likely contribute to specific protective responses in the adult heart (for review see Reichelt et al., 2017). EGFR/ErbB1 receptor appears to be important in cardiomyocyte survival and contractile function with its cardiac-specific deletion in the adult heart resulting in cardiac dysfunction (Rajagopalan et al., 2008). In a recent study, cardiomyocyte-specific ErbB1 downregulation in mice led to impaired contractility (Guo et al., 2021). Similarly,

conditional deletion of its ligand, HB-EGF, also resulted in cardiac contractile defects (Iwamoto et al., 2003) implying EGFR/ErbB1 receptors play a critical role in maintaining contractile homeostasis in the adult heart.

Endogenous or exogenously administered EGF-like ligands (e.g., EGF, HB-EGF, betacellulin and amphiregulin) and subsequent activation of EGFR/ErbB1, is thought to play a beneficial cytoprotective role in the heart against stress induced injury (Lorita et al., 2002; Pareja et al., 2003). For example, acute, high intensity stress can cause cardiac damage via elevated catecholamine release and cardiomyocyte death by apoptosis and/or necrosis (Liaudet et al., 2014). Administration of EGF can prevent the damage caused by intense and sustained β-adrenergic stimulation and catecholamine release in the heart by interfering with β -adrenergic signaling (Lorita et al., 2002; Pareja et al., 2003). Further, it was shown that activated EGFR/ErbB1 plays a critical role in the cardiac protection against the acute, high intensity stress induced in fighting mice (Pareja et al., 2003) and may also offer protection against cardiac stressors during hibernation (Childers et al., 2019). During hibernation, animals experience a reduction in blood circulation that makes the heart prone to multiple stressors. Increased EGFR phosphorylation (Y1086) was found to play a key role in activation of MAPK signaling, inhibition of downstream p-ELK1, and reduction in both p-BAD mediated pro-apoptotic signaling and caspase-9 apoptotic signaling in the heart of hibernating ground squirrels (Childers et al., 2019). Activation of cardioprotective EGFR signaling likely explains how these mammals cope with cardiac stresses during hibernation, which otherwise can lead to serious cardiac injury (Childers et al., 2019).

To examine the physiological role of ErbB2 signaling in the adult heart, rats with a ventricular-specific deletion of ErbB2 were generated (Crone et al., 2002). After physiological analysis, they discovered that these mutant mice hearts showed wall thinning, chamber dilation and decreased contractility indicative of dilated cardiomyopathy (Crone et al., 2002). Thus, increasing expression or activation of ErbB2 might prevent dilated cardiomyopathy and heart failure accordingly. Further, in a model of type 1 diabetes, the effect of acute activation or chronic inhibition of EGFR and ErbB2 signaling on heart function was also studied (Akhtar et al., 2012a). Recovery of cardiac function following I/R injury in diabetic hearts was significantly impaired, most likely as a result of reduced dimerization and signaling by cardiac ErbB2 and EGFR receptors. Chronic administration of selective pharmacological inhibitors of either ErbB2 or EGFR/ErbB1 in diabetic animals exacerbated cardiac recovery. In contrast, acute stimulation of EGFR/ErbB2 signaling with EGF improved cardiac recovery following I/R injury (Akhtar et al., 2012a). These findings confirmed the beneficial role or EGFR and ErbB2 receptors and downstream signaling via ERK1/2, p38 MAP kinase and AKT in mediating recovery of cardiac function that is normally impaired in diabetes (Akhtar et al., 2012b).

In another study using in H9c2 rat cardiomyoblasts, pretreatment with EGF attenuated H_2O_2-induced oxidative stress and inhibited ROS-induced cell death and H_2O_2-induced apoptosis through activating Nrf2 (Ma and Jin, 2019). In animal models of myocardial I/R, *in vivo* administration of EGF diminished infarct size and myocardial apoptosis (Ma and Jin, 2019). Taken together, these data demonstrate that EGF attenuates oxidative stress and cardiac I/R injury by reducing myocardial infarct size and improving cardiac function possibly via activation of Nrf2 (Akhtar et al., 2012b; Ma and Jin, 2019).

During cardiac I/R injury, the myocardium becomes depleted of zinc and the capacity to mobilise labile zinc is reduced indicative of zinc dyshomeostasis. Administration of zinc pyrithione can normalize zinc levels during I/R and also prevents apoptosis by increasing ErbB1/ErbB2 levels (Bodiga et al., 2015) further confirming the cardioprotective role of these ErbB receptors. Zinc pyrithione attenuated caspase activation, decreased the proteolytic degradation of ErbB2, enhanced activation and complexation (heterodimerization) of ErbB1/ErbB2 that resulted in increased myocyte survival after hypoxia/reoxygenation injury (Bodiga et al., 2015).

EGFR/ErbB receptors also mediate the beneficial effects of cardiac preconditioning (Benter et al., 2005c; Akhtar et al., 2012b). It is well known that ischemic preconditioning, which typically involves exposure of the heart to brief episodes of ischemia-reperfusion (I/R), protects the myocardium from the greater damaging effects of subsequent more prolonged I/R. In a rat model of type 1 diabetes, chronic *in vivo* administration of a specific pharmacological inhibitor of EGFR abrogated the cardiac benefits of preconditioning implying a critical role of EGFR/ErbB1 in cardiac preconditioning (Akhtar et al., 2012b). In models of cardiac preconditioning induced by pharmacological agents, the cardioprotective effects of GPCR agonists such as bradykinin, A1 adenosine receptors, apelin, acetylcholine and a δ-opioid peptide were found to be also mediated via transactivation of EGFR (Krieg et al., 2004; Cohen et al., 2007; Methner et al., 2009; Williams-Pritchard et al., 2011; Folino et al., 2018). For example, apelin-induced-reduction in infarct size and myocardial contracture via its GPCR (termed APJ) were prevented by the inhibition of EGFR, Src, MMP or RISK (reperfusion injury salvage kinase) pathways (Folino et al., 2018). Recently, the cardioprotective effects of myocyte-specific hypoxia-inducible factor 2A were additionally reported to be mediated via EGFR (Lee et al., 2020) further implicating EGFR/ErbB1 receptors in a protective role during myocardial I/R injury. Moreover, the fact that a pan-ErbB inhibitor, genistein, also blocked the cardioprotective effects of I/R preconditioning (Akhtar et al., 2012b), implied that other ErbBs receptors, beyond EGFR, might also be involved in mediating the beneficial effects of cardiac preconditioning.

Previously it was thought that only EGFR, ErbB2 and ErbB4 were expressed in the adult heart but subsequent studies have demonstrated that post-natal cardiomyocytes of mice express a functional ErbB3 protein as well (Camprecios et al., 2011). More recently ErbB3 gene expression has also been reported in normal human ventricular cardiac fibroblasts (Kirabo et al., 2017). Although methylation of ErbB3 gene has been reported in human dilated cardiomyopathy (Haas et al., 2013); the function of ErbB3 in the adult heart is only now being understood. For example the E3 ligase (or neuregulin receptor degradation protein-1; Nrdp1), that selectively targets ErbB3 (Diamonti et al., 2002), is upregulated following I/R injury. Mice

over-expressing Nrdp1 in the heart exhibit higher infarct size as well as increased apoptosis and inflammatory cell influx following I/R (Zhang et al., 2011) implying that Nrdp1 is a pro-apoptotic signal in the heart during I/R injury and exerts its actions via degradation of ErbB3. More recently it was reported that ErbB3 is a cardioprotective/pro-survival factor against redox stress (Morano et al., 2017). ErbB3 gene expression was transiently increased in the adult heart after I/R injury (Morano et al., 2017). However, I/R reduced ErbB3 protein levels, whereas postconditioning (that was induced by brief cycles of I/R immediately after ischemia) prevented I/R-induced reduction in ErbB3 receptor protein. Furthermore, the transient over-expression of ErbB3 gene alone was able to enhance cell survival and attenuate mitochondrial dysfunction and apoptosis in H9c2 cells exposed to redox-stress (Morano et al., 2017). This study implied that ErbB3 acts beneficially as a cytoprotective and/or pro-survival factor in the heart (Morano et al., 2017).

A cardiomyocyte-specific conditional ErbB4 KO mouse model was also used to prove that ErbB4 is required for remodeling of cardiomyocytes and essential in maintaining myocardial contractile function in the post-natal heart (Garcia-Rivello et al., 2005). Despite the normal heart morphology evident at birth, conditional ErbB4 KO mice developed severe dilated cardiomyopathy and conduction abnormalities that lead to premature death (Garcia-Rivello et al., 2005).

The HB-EGF ligand binds to and triggers signaling via several ErbB receptors including EGFR/ErbB1 and ErbB4 receptors. In experiments with HB-EGF-null mice, over half of the mice lacking this ligand died within a week. The rest showed severe heart failure, enlarged cardiac valves and ventricular chambers with a resultant impairment of cardiac function. In contrast, administration of HB-EGF in WT mice increased phosphorylation of cardiac ErbB2, ErbB4, and to a lesser degree, of EGFR (Iwamoto et al., 2003) implying that HB-EGF-induced activation of multiple ErbB receptors is crucial for normal heart function.

The cardiac effects of NRG-1, another EGF-like ligand, have been extensively studied. The stimulation of ErbB signaling by NRG-1 appears crucial for cardiomyocyte survival and furthermore, is an important compensatory mechanism in the failing adult heart (De Keulenaer et al., 2019). Neuregulin ligand-mediated ErbB receptor signaling plays a key role in cellular functions such as proliferation, differentiation, migration and protection against cell death through controlling bcl-x splicing and bcl-2 family protein expression, as well as activation of the mammalian target of rapamycin (mTOR) that induces protein synthesis and hypertrophy (for reviews see Sanchez-Soria and Camenisch, 2010; De Keulenaer et al., 2019). Thus, through multiple effectors, NRG-1 induces pro-survival gene expression and enhances proliferation and survival of human cardiac fibroblasts (Fang et al., 2017; Kirabo et al., 2017; De Keulner et al., 2019). For example, NRG-1 counters the upregulation of endoplasmic reticulum (ER) stress markers like glucose-regulated protein 78 and cleaved caspase-12 in ventricular myocytes through ErbB4 receptors. Inhibition of EGFR/ErbB1 signaling or ErbB4 knockdown reversed the beneficial effects of NRG-1 in inhibiting ER stress in cultured neonatal cardiomyocytes (Fang et al., 2017). Moreover, in an *in vivo* rat model of cardiac I/R injury, intravenous NRG-1 administration significantly decreased ER stress and myocardial infarct size (Fang et al., 2017). Thus, NRG-1 has a protective role in I/R injury by inhibiting myocardial ER stress that is likely mediated by several ErbB receptors including ErbB4. Indeed, these pro-survival effects of NRG-1 have led to its consideration as a potential therapeutic agent for treatment of heart failure in pre-clinical and human clinical studies that have collectively shown that i. v., administration of this ligand significantly improved cardiac contractility and remodelling as well as attenuated mitochondrial dysfunction and apoptosis (Liu et al., 2006; Gao et al., 2010; Jabbour et al., 2011; Guo et al., 2012; Xiao et al., 2012; Hill et al., 2013; De Keulenaer et al., 2019). Additionally, and of relevance to the diabetic heart, is the potential glucose-lowering ability of NRG-1. Acute administration of this ligand decreased the glycaemic response to an oral glucose tolerance test in rats (Caillaud et al., 2016) implying that it might have added therapeutic value as a glucose-lowering agent in diabetic patients with heart failure.

Interestingly, monoclonal antibody and small molecule-based inhibitors of EGFR/ErbB receptors are now extensively used in the treatment of cancer (Roskoski, 2019; Kumagai et al., 2021). However, their use may lead to unwanted cardiac toxicities ranging from reduced LV ejection fraction to heart failure (Kenigsberg et al., 2017; Cuomo et al., 2019). In an early study, female (but strangely, not male) mice chronically administered either of two EGFR small molecule tyrosine kinase inhibitors, lead to physiological and pathological cardiac changes such as altered LV wall thickness and increased apoptosis (Barrick et al., 2008). Using a human-induced pluripotent stem cell-derived cardiomyocyte *in vitro* model, treatment with trastuzumab, a monoclonal antibody inhibitor of ErbB2, also aggravated cardiomyocyte damage, whereas activation of ErbB signaling with NRG-1 alleviated cardiomyocyte injury (Eldridge et al., 2014). In the clinic, trastuzumab is known to exhibit cardiac toxicity particularly in patients with abnormal LV function but cardiotoxicity with other ErbB inhibitors such as gefitinib (a small molecule EGFR-specific inhibitor) or even lapatinib (a dual EGFR-ErbB2 inhibitor) appears to be rare (Kenigsberg et al., 2017). Thus, studies on the cardiotoxicity of some ErbB receptor inhibitors are supportive of a cardioprotective role of ErbBs in the adult heart. However, since not all ErbB inhibitors exhibit cardiotoxicity, the possibility that the observed adverse effects on the heart might be due to other "off-target", but drug-specific, effects remains to be elucidated.

The Detrimental Role of Epidermal Growth Factor Receptor in the Heart

The studies described in the preceding section (see also **Table 1**) clearly suggest a beneficial and necessary role of EGFR/ErbB/HER receptors in the developing heart. They further imply that acute or transient activation of EGFR/ErbB/HER receptor TKs and subsequent downstream activation of survival (or salvage/recovery) pathways such as PI3K/AKT/mTOR and RISK is

TABLE 2 | A summary of selected *in vivo* studies highlighting the **detrimental** role of EGFR/ErbB signaling in the heart.

Role of EGFR	Study model	Intervention	Results	Refs.
Amphiregulin via EGFR activation increases cardiac fibrosis after myocardial infarction	C57BL6 mice	Amphiregulin and gefitinib	• Amphiregulin, via EGFR activation, promoted cardiac fibroblast migration, proliferation, and collagen synthesis (gefitinib abrogated all these effects) • Amphiregulin deletion improved cardiac function and increased survival rate	Liu et al. (2020)
EGFR contribute to myocardial infarction in T2D	T2D mice (db−/db−)	Group 1: Untreated Group 2: AG1478 Group 3: Tudca	• EGFR and ER stress inhibitors reduced the cell apoptosis, inflammation, and myocardial infarct size in T2D mice after myocardial I/R injury induction	Mali et al. (2018)
EGFR plays a role cardiac fibrosis	C57Bl/6J mice	Generation of osteoglycin-null mice (OGN-/-) Ang II infusion	• OGN, via EGFR inhibition, negatively regulated cardiac fibrosis by attenuating myofibroblast proliferation and migration • Chronic Ang II infusion in OGN deficient mice increased cardiac fibrosis and impaired cardiac function	Zuo et al. (2018)
EGFR increases cardiac remodeling in diabetic cardiomyopathy	C57/BL6 mice	Gefitinib and ramipril	• Gefitinib, via EGFR inhibition, prevented lipid peroxidation, antioxidant enzymes damage, myocardial hypertrophy, myocardial damage and improved Ca^{2+} homeostasis in STZ-induced cardiomyopathy	Shah et al. (2018)
EGFR induces cardiac hypertrophy	C57BL/6 mice	AG1478, 542 and 54	• AG1478, 542 and 543, via EGFR inhibition, attenuated ang II- and EGF-induced cardiac hypertrophy	Peng et al. (2016)
EGFR contributes to cardiac inflammation and injury associated with high fiber diet	Apo-E knockout mice	AG1478 and 542	• EGFR inhibitors attenuated palmitic acid and hyperlipidemia-induced cardiac injury and inflammation in mice fed with high fat	(Li et al., 2016)
EGFR role in cardiac damage and remodeling through oxidative stress	STZ-induced T1D mice	AG1478 and 451	• AG1478 and 451, via EGFR inhibition, decreased diabetes-induced oxidative stress, cardiac remodeling, hypertrophy, fibrosis and apoptosis	Liang et al. (2015)
EGFR and its downstream ER stress lead to cardiac injury and microvascular dysfunction in T1D	C57BL/6J	STZ only or in combination with AG1478, tudca or insulin	• AG1478, tudca, and insulin reduced cardiac fibrosis, collagen type I, and plasminogen activator inhibitor 1 and restored the impaired epithelium dependent and independent relaxation responses in T1D mice	Galan et al. (2012)
EGFR contributes to ROS production and cardiac damage	Wistar rats	Aldosterone and EGF	• Aldosterone-induced NHE-1 stimulation, via EGFR transactivation resulted in ROS production and cardiac injury	De Giusti et al. (2011)

T2D, type 2 diabetes; ER, endoplasmic reticulum; I/R, ischemia/reperfusion; Ang II, angiotensin II; STZ, streptozocin; ApoE, apolipoprotein E; ROS, reactive oxygen species; NHE, sodium hydrogen exporter.

required for mediating beneficial cardioprotective effects in I/R injury. In contrast, there is now a growing body of evidence that supports the notion that chronic or persistent overexpression/activation of EGFR/ErbB family of receptors can play a detrimental role (see **Table 2**) in the pathological heart including in cardiac hypertrophy, fibrosis and cardiac remodeling associated with diabetes (e.g., Kobayashi and Eguchi, 2012). As EGFR/ErbB/HER receptors are known drivers of cell growth and proliferation via mitogenic signaling through Ras/Raf/MAP kinases, AKT and ERK1/2, they play key role in mediating cardiac hypertrophy (Peng et al., 2016; Bi et al., 2020). For example, Ang II-mediated cardiac hypertrophy is reported to occur via Src-dependent EGFR transactivation through AKT and ERK pathways that can be reversed with pharmacological inhibitors of EGFR (Liang et al., 2015; Peng et al., 2016). In addition to Ang II, other members of the RAAS can also transactivate EGFR/ErbB receptors to impact cardiac function. In this regard, EGFR transactivation is known to be involved in aldosterone-induced NHE-1 (Na^+/H^+ Exchanger 1) stimulation that mediates oxidative stress and subsequent cardiovascular damage (De Giusti et al., 2011).

Hyperglycemia associated with diabetes is known to lead to the generation of reactive oxygen species (ROS) such as via NADPH oxidase (NOX) activity (see also *The Link Between Epidermal Growth Factor Receptor & Nicotinamide Adenine Dinucleotide Phosphate Oxidase in Diabetes-Induced Vascular Dysfunction*) that can lead to cardiovascular complications (Brownlee, 2005; Zhang et al., 2020; Babel and Dandekar, 2021). There is emerging evidence that EGFR inhibitors can prevent ROS formation and development of oxidative stress that leads to cardiac dysfunction (Liang et al., 2015; Shah et al., 2018). EGFR inhibition with gefitinib decreased hyperglycemia-induced cardiac remodeling by preventing oxidative stress-induced changes in the diabetic heart (Shah et al., 2018). Administration of gefitinib (a selective EGFR inhibitor) in C57/BL6 mice, significantly prevented lipid peroxidation and damage of antioxidant enzymes like glutathione and thioredoxin reductase. It also prevented myocardial hypertrophy and attenuated the diabetes-induced alterations in

collagen deposition and myocardial remodeling. Administration of gefitinib also prevented depletion of SERCA2a (sarcoplasmic endoplasmic reticulum Ca^{2+} ATPase2a) and NCX1 (sodium-calcium exchanger-1) in streptozotocin-induced cardiomyopathy-indicative of improved Ca^{2+} homeostasis during myocardial contractility (Shah et al., 2018). These findings suggest that inhibition of EGFR can reduce cardiac damage in diabetic cardiomyopathy through balancing oxidant-antioxidant systems and attenuating subsequent hypertrophy and remodeling in the diabetic heart. Thus, gefitinib, and other clinically approved EGFR inhibitors that do not exhibit cardiotoxicity, may be considered for the potential treatment of diabetic cardiomyopathy.

Emerging evidence supports the view that generation of ROS and oxidative stress is strongly associated with the occurrence of endoplasmic reticulum (ER) stress (Chong et al., 2017). The ER is responsible for the correct folding, processing and trafficking of secretory and membrane-bound proteins to the Golgi apparatus, but when the capacity of this sub-cellular organelle to fold proteins becomes saturated, such as in oxidative stress, ER stress ensues that can lead to multiple pathologies (Navid and Colbert, 2017) including diabetes-induced cardiovascular complications (Xu et al., 2012; Sankrityayan et al., 2019). In a mouse model of type 1 diabetes, upregulation of EGFR tyrosine kinase led to induction of ER stress that resulted in diabetes induced cardiovascular dysfunction (Galan et al., 2012). Pharmacological inhibition of EGFR attenuated receptor phosphorylation and reduced ER stress, cardiac fibrosis, and microvascular dysfunction (Galan et al., 2012) implying a key role of EGFR in mediating ER stress and subsequent cardiac pathology. The detrimental role of EGFR in the pathogenesis of diabetes-induced heart damage was further confirmed by Liang et al. (2015). They reported that Ang II-induced cardiac hypertrophy, fibrosis and remodeling was mediated via enhanced signaling through a Src-dependent EGFR/AKT pathway. The fact that significant attenuation of these pathological changes occurred after treatment with gene silencing shRNAs or EGFR inhibitors provided strong evidence that aberrent EGFR signaling plays a crucial role in the pathogenesis of cardiac dysfunction and remodeling in diabetes (Liang et al., 2015). Additionally, EGFR signaling and ER stress are also reported to play a role in inducing myocardial infarction in type 2 diabetes (Mali et al., 2018). To investigate this role, T2D mice were treated with AG1478 (a specific EGFR inhibitor) or TUDCA (an ER stress inhibitor) for 2 weeks. After that, acute myocardial I/R injury was induced. Treatment with EGFR and ER stress inhibitors in T2D mice significantly reduced the extent of the myocardial infarct size compared to untreated T2D mice. Inhibition of EGFR and ER stress was associated with reduced myocardium p38 and ERK1/2 MAP-kinases activity, increased pro-survival signaling via AKT, and reduced inflammatory cell infiltration and apoptosis (Mali et al., 2018). By suggesting that EGFR signaling was detrimental in acute myocardial I/R injury, this study (Mali et al., 2018) was at variance with multiple other reports showing that EGFR signaling is beneficial in the recovery of cardiac function following I/R injury (Ma and Jin 2019; Akhtar et al., 2012b; Pareja et al., 2003; Lorita et al., 2002). The precise reasons for this discrepancy are not known but the use of different animal models and experimental conditions may be possible explanations. For example, Mali et al. (2018) used a T2DM (db^{-}/db^{-}) mouse model with regional ischemia compared to a T1DM (streptozotocin-induced) rat model with global ischemia used by Akhtar et al. (2012b). How these potential variables precisely impact on EGFR/ErbB signaling requires further study. Nonetheless, collectively these studies suggest that targeting of EGFR and ER stress may represent novel therapeutic strategies in the potential treatment of diabetes-induced cardiac pathologies.

Obesity is a leading risk factor for the development of type 2 diabetes and its cardiovascular complications (Leitner et al., 2017; Drucker, 2021). In a study aiming to determine if EGFR mediates the pathogenesis of hyperlipidemia/obesity-related cardiac diseases, EGFR inhibition significantly ameliorated myocardial fibrosis, apoptosis, and inflammation in two rat models of obesity (Li et al., 2016). Thus, consistent with reports in models of diabetes, this study also demonstrated a harmful effect of EGFR activation in the pathogenesis of obesity-induced cardiac pathology (Li et al., 2016) and thus, potential use of EGFR inhibitors for the treatment for obesity-related cardiovascular complications may be warranted.

EGFR signaling is additionally thought to be important in the cardiac actions of osteoglycin (OGN), a small leucine-rich proteoglycan that also regulates bone and glucose homeostasis (Lee et al., 2018; Zuo et al., 2018). OGN negatively regulates cardiac fibrosis by attenuating cardiac myofibroblast (CMF) proliferation and migration through inhibition of EGFR signaling. Thus, OGN deficiency promoted EGFR and ERK1/2 phosphorylation in CMFs cultured from adult and neonate mice. In contrast, restoration of OGN in OGN-null CMFs resulted in inhibition of cell migration and proliferation through decreased EGFR signaling (Zuo et al., 2018). Stimulation of EGFR/ErbB receptors by amphiregulin (AR), another member of EGF family of ligands, induced cardiac fibrosis after myocardial infarction (Liu et al., 2018; Liu et al., 2020) highlighting the detrimental role of EGFR/ErbBs in cardiac pathology. EGFR transactivation was also involved in mediating the non-canonical, cardiomyocyte hypertrophy-promoting, effects of the death receptor 5 that normally mediates apoptosis (Tanner et al., 2019). Interestingly a very recent study showed that increased EGFR signalling in the rat brain, namely within the hypothalamic paraventricular nucleus (PVN), may also play a key role in mediating myocardial-infarction-induced heart failure through enhancing ERK1/2-induced sympathetic overactivity (Yu et al., 2021). Selective gene silencing of EGFR in the PVN led to a correction of multiple central and peripheral markers associated with heart failure (Yu et al., 2021) implying that targeting brain EGFR might represent a novel approach in the treatment of MI-induced HF. Collectively, the above studies suggest a critical role for both peripheral and central EGFR/ErbB signaling in the development of cardiac pathologies, and further imply that these RTKs may serve as a "hub" or "relay" for other signaling molecules/inputs that induce cardiac complications in diabetes.

As a summary of their cardiac actions, **Figure 1** highlights the overall beneficial and detrimental actions of EGFR/ErbB2/HER receptors and downstream effector signaling in the heart (see also perspectives and concluding remarks section below).

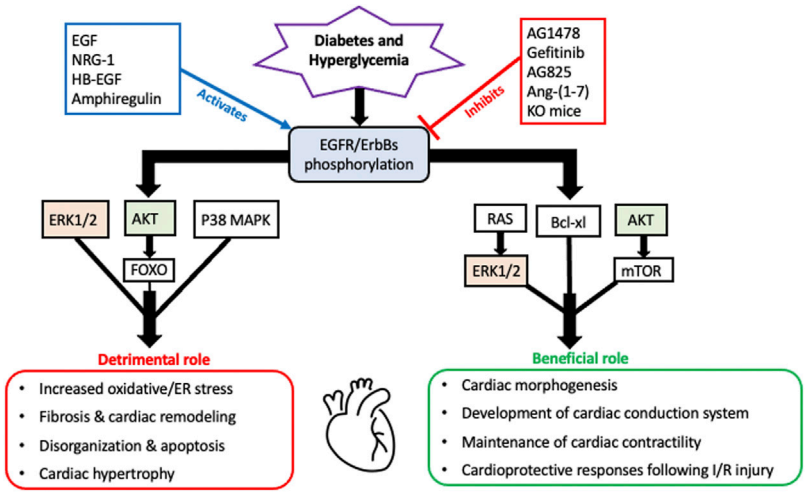

FIGURE 1 | A schematic highlighting the overall beneficial and detrimental actions of EGFR/ErbB receptors and their downstream signaling effectors in the diabetic heart. It appears that all four EGFR/ErbB receptors are necessary for the development of the heart during embryogenesis and beneficial for the proper functioning of the adult heart including a key role in maintenance of cardiac contractility. EGFR/ErbB2 signaling is also essential for normal cardiomyocyte function. Acute stimulation of EGFR/ErbB receptor signaling is also essential for activation of salvage pathways and cardiac recovery following I/R injury. In addition to these beneficial actions, EGFR/ErbB receptor signaling can also have detrimental outcomes. Persistent or chronic upregulation of EGFR/ErbB receptor signaling has been involved in the development of cardiac dysfunction and pathologies including fibrosis, remodelling and hypertrophy. Several key downstream effectors are involved in mediating both actions with some notable overlaps such as AKT and ERK1/2 (highlighted)- implying that they too can have dual actions depending on the pathophysiological context akin to the role of their upstream EGFR/ErbB receptors (see main text for further details and references).

DIABETES-INDUCED VASCULAR DYSFUNCTION

Both T1DM and T2DM can lead to potentially life-threatening microvascular and macrovascular complications such as retinopathy, neuropathy, nephropathy, as well as coronary heart disease and stroke (Kosiborod et al., 2018; Colom et al., 2021). Similar to that described in the heart (see above), diabetes-induced vascular complications generally result from chronic hyperglycemia (Van Wijngaarden et al., 2017) though repeated transient excursions into hyperglycemia and glycemic variability also increase the risk of developing vascular complications (Holman et al., 2008; Gray and Jandeleit-Dahm, 2014; Kenny and Abel, 2019; Babel and Dandekar, 2021). Correction of hyperglycemia in diabetic patients also does not completely restore the risk of developing vascular complications to baseline (Ceriello, 2005; Brownlee and Hirsch, 2006; Chalmers and Cooper, 2008; Kenny and Abel, 2019) implying that hyperglycemia-induced vascular changes, most likely at a molecular level, are long-lasting–a phenomenon termed "glycemic" or "metabolic memory". Although there appears to be some differences in the pathology of micro-and macro-vascular dysfunction (e.g., the latter is most common in T2DM which comprises around 85–90% of all diabetic patients), both involve damage to the endothelium, the innermost lining of blood vessels, that is involved in many important vascular functions including maintenance of vascular tone (Shi and Vanhoutte, 2017); indeed, endothelial dysfunction represents a significant underlying cause of organ dysfunction and failure in several pathologies including diabetes and SARS-CoV2 (COVID19) infections (Shi and Vanhoutte, 2017; Jin et al., 2020; Nagele et al., 2020). In the vasculature, key dysfunctional changes of the tunica media such as increased vascular smooth muscle responsiveness to endothelium-dependent vasoconstrictors, are early features of diabetic vasculopathy (Cooper et al., 2001; Lee et al., 2012). Since diabetes-induced cardiac dysfunction also involves endothelial dysfunction, it is not surprising that endothelial dysfunction associated with micro- or macro-vascular complications most likely arises from a similar, though not necessarily identical, concoction of underlying biological mechanisms. Of note in the diabetic vasculature are increased intracellular Ca^{2+}, diminished NO synthesis, increased polyol pathway flux, altered cellular redox state (ROS/oxidative and ER stress), prostanoid synthesis, accelerated nonenzymatic formation of advanced glycation end products (AGEs), increased formation of diacylglycerol and activation of specific protein kinase C (PKC) isoforms, inflammation and enhanced apoptosis (for review see Babel and Dandekar, 2021; Shi and Vanhoutte, 2017; Forbes and Cooper, 2013, Giacco and Brownlee, 2010; Brownlee, 2005). Since endothelial and vascular smooth muscle (VSM) cells (VSMCs) are closely linked functionally, many of the molecular pathways effecting endothelial dysfunction are also altered in VSMCs implying that a combination of endothelial and smooth muscle dysfunction is ultimately responsible for the abnormalities of vascular function in diabetes. Indeed, in patients with T2DM, VSM dysfunction was noted in both the micro- and macro-vasculature but was more pronounced in the microvasculature-i.e. blood vessels with diameter of

TABLE 3 | A summary of selected studies implicating EGFR signaling in diabetes-induced vascular dysfunction.

Role of EGFR	Study model	Intervention	Results	Refs.
Endothelial EGFR compared to vascular smooth muscle (VSM) EGFR plays a minor role in obesity/DIVD	High-fat diet T2DM mice	Endothelial cell (EC) specific EGFR knockout	• A comparison of vascular function parameters in EC verus VSM-specific EGFR KO mice implied that EC-EGFR plays a minor role in mediating obesity/T2DM-induced vascular dysfunction	Schreier et al. (2021)
Vascular smooth muscle (VSM) specific deletion of EGFR prevents obesity and DIVD	High-fat diet T2DM mice	VSM-specific EGFR-knockout (KO)	• VSM-specific EGFR via ErbB2- ROCK–MRTF–SRF signalling mediates vascular dysfunctiom	Stern et al. (2020)
DIVD and vascular remodeling can be corrected by inhibition of EGFR by novel polymeric nanoparticles	STZ-induced T1DM wistar rats	PAMAM dendrimers AG1478	• AG1478 or PAMAMs (novel inhibitors of ErbBs) corrected abnormal vascular reactivity and vascular remodeling in diabetic MVB	Akhtar et al. (2019)
Multiple downstream pathways are involved in EGFR- ErB2 mediated DIVD	STZ-induced T1DM wistar rats	Lapatinib	• lapatinib inhibits EGFR & ErbB2 activity in diabetic MVB and in VSMCs and reverses diabetes and hyperglycemia-induced changes in ERKs, ROCK, AKT, FOXO, eNOS, & NF-kB and prevents HG-induced apoptosis in VSMCs	Benter et al. (2015)
Src-dependent EGFR/ErbB2 transactivation via ang II leads to DIVD	STZ-induced T1DM wistar rats	Ang-(1–7)	• Ang-(1–7) inhibits diabetes-induced transactivation of ErbB receptors in isolated MVB and opposes downstream signaling changes in ERK1/2, p38 MAPK, ROCK, eNOS, and IkB-α	Akhtar et al. (2015)
Increased EGFR activity via NADPH-oxidase leads to DIVD	C57/BL6 and db^{-}/db^{-} T2D mice	AG1478, or exogenous EGF, or gp-91 ds-tat	• T2DM or EGF alters vascular reactivity in MRA that could be corrected by AG1478. EGF increased ROCK expression in MRA that could be corrected by NOX inhibition	Kassan et al. (2015a)
EGFR-ErbB2 heterodimers via ERK1/2-ROCKs lead to DVID	STZ-T1DM wistar rats	AG825 Anti-ErbB2 siRNA AG1478	• Chronic *in vivo* or acute *ex vivo* inhibition of ErbB2 or EGFR corrected DIVD in MVB and attenuated elevated ERK1/2 and ROCK signaling. First evidence for EGFR-ErbB2 heterodimerization presented in co-association/immunoprecipitation assays	Akhtar et al. (2013)
Elevated EGFR mRNA an early change in development of DIVD	STZ-T1DM wistar rats	AG1478	• Diabetes altered expression of over 1,300 genes in MVB. AG1478 treatment prevented 95% of these changes implying that EGFR is a key early change in the development of DIVD	Benter et al. (2009)
Elevated EGFR via reduced eNOS mediates DIVD	T2DM db/db mice	AG1478	• Altered vascular reactivity and reduced eNOS expression in MRA and CA of diabetic mice could be corrected by AG1478 treatment	Belmadani et al. (2008)
EGFR and ErbB2 mediate DIVD in carotid artery	STZ-T1DM wistar rats	AG1478 AG825	• Altered vascular reactivity in diabetic carotid artery could be corrected by AG1478 and AG825 implicating EGFR and ErbB receptors in mediating DIVD	Yousif et al. (2005)
Increased EGFR signaling leads to DIVD in renal artery	STZ-T1DM wistar rats	AG1478 Genistein diadzein	• Elevated EGFR and DIVD in renal artery could be corrected by AG1478 and genistein but not by diadzein	Benter et al. (2005b)
Elevated EGFR phosphorylation leads to DIVD in MVB	STZ-T1DM wistar rats	AG1478 Genistein	• Diabetes-induced abnormal vascular reactivity in MVB was linked to increased EGFR phosphorylation that could be corrected by AG178 treatment	Benter et al. (2005a)

STZ, streptozocin; MVB, mesenteric vascular bed; VSMC, vascular smooth muscle cell; HG, hyperglycemia; MRA, mesenteric resistance arteries, CA, coronary artery; DIVD, Diabetes induced vascular dysfunction; AG1478, A selective EGFR Inhibitor; AG825, A selective ErbB2 inhibitor; lapatanib, A dual EGFR and ErbB2 inhibitor; Genistein, A pan ErbB/tyrosine kinase inhibitor; Diadzein, an inactive analogue of genistein; Ang-(1–7), Angiotensin-(1–7); gp91 das-tat, specific inhibitor of NADPH oxidase (NOX); PAMAM, Polyamidoamine (as dendrimeric nanoparticles); ROCK, Rho kinase; MRTF, actin–myocardin-related transcription factor; SRF, serum response factor.

< 150 μm (Montero et al., 2013). In terms of mechanisms, in addition to ROS generation and oxidative stress, diabetes-induced alterations in signaling by growth factors, RTKs, peptides of RAAS as well as other GPCRs that exert significant influence on intracellular calcium levels, ROS, ER stress, inflammation and apoptosis appear to be involved in mediating VSMC dysfunction (Babel and Dandekar, 2021). For example, signalling via vascular endothelial growth factor (VEGF) appears important in mediating retinopathy in diabetes (reviewed in Wong et al., 2016), and hyperglycemia-induced up-regulation of GPCR signalling by the RAAS peptide, Ang II, has been reported in VSMCs (Montezano et al., 2014; Touyz et al., 2018). Since it is now known that GPCRs can initiate transactivation of the EGFR (e.g., Akhtar et al., 2015; Forrester et al., 2016), which is expressed in both endothelial cells and VSMCs (e.g., Akhtar et al., 2012a; Schreier et al., 2021), there is strong indications that EGFR signalling may also be a key player in mediating

vascular pathology in diabetes (Akhtar and Benter, 2013; Matrougui, 2010; Schreier et al., 2014; Schreier et al., 2018).

Role of Epidermal Growth Factor Receptors in the Diabetic Vasculature

EGFR/ErbB receptor signaling that typically regulates cell growth, differentiation and proliferation is essential to the normal physiologically development and functioning of the vasculature (Forrester et al., 2016). However, its chronic dysregulation appears to have a detrimental role in mediating diabetes-induced vascular dysfunction and remodelling (Benter et al., 2005; Akhtar and Benter, 2013; Matrougui, 2010; Akhtar et al., 2019; Schreier, et al., 2014; Schreier et al., 2018) see also **Table 3**.

Probably the early clues to the involvement of EGFR/ErbB receptors in diabetes-induced complications were the observation that excretion of EGF ligand was abnormal in many patients with diabetes (Lev-Ran et al., 1990) and that EGF gene expression was elevated in the mesenteric artery of rats bearing type 1 diabetes (Gilbert et al., 2000). Further, since vascular proliferation and remodeling (e.g., via hypertrophy, hyperplasia and fibrosis) leads to altered vascular contractility in diabetes, of note were the studies showing a pro-contractile role for EGF/EGFR signaling (Berk et al., 1985; Florian and Watts, 1999). For example, EGF via stimulation of EGFR elicited potent vasoconstriction in aortic strips from a rat model of hypertension (Florian and Watts, 1999); and EGFR activation via MMP-2 reportedly increased ROS formation and facilitated contraction in non-diabetic aortas (Prado et al., 2018). Additionally, there is also evidence in the non-diabetic vasculature that acute EGFR signaling may mediate the contractions of GPCR ligands such as Ang II and endothelin (ET-1) (Kawanabe et al., 2004; Lucchesi et al., 2004) that also play an important role in mediating diabetes-induced vascular dysfunction. A recent study also suggests that dysregulation of vascular, as opposed to endothelial, EGFR might be the dominant factor in the development of vascular pathologies associated with obesity (Schreier et al., 2021) an important risk factor for developing T2DM.

The first *in vivo* reports demonstrating a direct link between EGFR signaling and diabetes-induced vascular dysfunction appeared in 2005 which showed that enhanced EGFR/ErbB1 signaling was a key mediator of diabetes-induced vascular dysfunction as pharmacological inhibition of this RTK was corrective of this pathology in the mesenteric vascular bed as well as in renal and carotid arteries (Benter et al., 2005a; Benter et al., 2005b; Yousif et al., 2005). This initial research on vascular EGFR was conducted in a preclinical model of type 1 diabetes (streptozotocin-induced diabetes in rats) and its findings were subsequently replicated in the vasculature of animal models of type 2 diabetes (Belmadani et al., 2008; Palen and Matrougui, 2008; Choi et al., 2012; Galan et al., 2012; Kassan et al., 2015a; Stern et al., 2020), implying that dysregulated EGFR/ErbB1/HER1 signaling was a common mediator of vascular complications in both Type 1 and Type 2 diabetes. Further, through gene expression profiling studies of diabetic mesenteric vasculature, it was demonstrated that EGFR1/ErbB1/HER1 inhibition could normalize approximately 90% of the transcriptomic changes that occurred during the development of diabetes-induced vascular abnormalities (Benter et al., 2009). In addition, increased gene expression of EGFR1/ErbB1/HER1 appeared to be a critical early mRNA change resulting in diabetes-induced vascular dysfunction (Benter et al., 2009). Studies from the same group on the underlying mechanisms, identified that EGFR1/HER1 did not act alone, rather heterodimerization with ErbB2 (its preferred dimerization partner) and subsequently signaling via multiple pathways including PI3K/AKT were key mediators of diabetes-induced vascular complications (Akhtar et al., 2013; Akhtar et al., 2015). More recently, these authors showed that all ErbB receptors are activated by hyperglycemia (Akhtar et al., 2015; Akhtar et al., 2020b) and that pan-ErbB inhibitors can reverse these changes and correct vascular dysfunction in a rat model of type 1 diabetes (Akhtar et al., 2020b; Akhtar et al., 2019) implying a possible role for all EGFR/ErbB receptors in mediating diabetes-induced vascular dysfunction.

Src family of non-receptor tyrosine kinases appear to be key upstream effectors of EGFR/ErbB RTKs as hyperglycemia/diabetes is known to induce EGFR/ErbB signaling via a Src-dependent mechanism (Akhtar et al., 2012a; Akhtar et al., 2015; see also T*he Interplay of Epidermal Growth Factor Receptor and Angiotensin 1-7, a Member of the Renin-Angiotensin-Aldosterone System, in Diabetes-Induced Vascular Dysfunction* below). As to the downstream effectors of EGFR/ErbB signaling in the diabetic vasculature, several studies have now implied that multiple downstream pathways are likely involved including dysregulation of NO, Rho kinases (ROCKs), ERK1/2 and PI3K/AKT pathways (Akhtar and Benter, 2013; Benter et al., 2015; Matrougui, 2010; Schreier et al., 2014; Schreier et al., 2018). For example, diabetes enhances phosphorylation of EGFR1 and ErbB2, formation of EGFR/ErbB2 heterodimers and subsequent elevation in ERK1/2 and ROCK signaling leading to dysfunction in the mesenteric vasculature of T1DM rats (Akhtar et al., 2013; Benter et al., 2015). Administration of AG1478, a selective EGFR inhibitor, or AG825, a specific ErbB2 inhibitor, or Fasudil, a ROCK inhibitor, or PD98059, an ERK1/2 signaling inhibitor, all attenuated the observed changes associated with diabetes-induced vascular dysfunction (Akhtar et al., 2013) indicating the importance of these effectors in this pathology. Similarly, in a mouse model of T2DM, EGFR inhibition rescued abnormal ROCK activity and restored vascular function (Kassan et al., 2015b). Collectively, these results implied that EGFR/ErbB2-ERK1/2-ROCK pathway is an important mediator of diabetes-induced vascular dysfunction. The role of ROCK as a downstream effector of EGFR/ErbB2 heterodimers was also confirmed recently in a mouse model of high-fat diet induced obesity/type 2 diabetes bearing vascular smooth muscle-specific deletion of EGFR (Stern et al., 2020). This study showed that EGFR expressed in vascular smooth muscle cells mediates vascular remodeling via a ROCK-dependent activation of serum response factor - a transcription factor that regulates cell growth and proliferation (Stern et al., 2020). Interestingly, administration of the ROCK inhibitor, Fasudil, in the early stages of experimental diabetes was found to markedly suppress

vascular hyperreactivity, increase tissue perfusion, and prevent organ damage whereas late-stage Fasudil administration had little or no effect (Li et al., 2015). This observation is suggestive of a threshold of vascular damage beyond which recovery of function is limited with ROCK inhibitors (Li et al., 2015) as well as possibly with other types of pharmacological interventions. Also of interest here is that EGF-ligand induced vasoconstriction is dependent on ROCK signaling. In rat aortic rings, EGF induced Ca^{2+} sensitization of VSMCs via a Rho-kinase-dependent inactivation of myosin light chain phosphatase (MLCP) mediated by the EGFR-MEK-ERK1/2 signaling pathway (Sasahara et al., 2015). Taken together, these studies suggest an important role of hyperglycemia-induced Src-dependent transactivation of EGFR/ErbB receptors and a downstream ROCK-dependent pro-contractile action in mediating diabetes-induced vascular dysfunction.

Endothelial Nitric Oxide Synthase as an Effector of Epidermal Growth Factor Receptor or ErbB2 in Diabetes-Induced Vascular Dysfunction

Endothelial and thereby vascular dysfunction is characterised by a decreased production of nitric oxide (NO). In the vasculature, NO is mainly produced from L-arginine by endothelial nitric oxide synthase (eNOS) in a Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) dependent reaction (Forstermann and Sessa, 2012; Zhao et al., 2015; Murthy et al., 2017). NO-mediated signaling is fundamental in maintaining vascular function as it can potentially prevent vascular inflammation, thrombosis, and cell proliferation (Bian et al., 2008; Zhao et al., 2015). eNOS activity is controlled through multi-site phosphorylation. For instance, enhanced phosphorylation of eNOS at Thr495 and decreased phosphorylation at Ser1177 site in the vascular endothelium leads to inadequate NO synthesis and impaired endothelium-dependent relaxation (Long et al., 2007). Diabetes reduces eNOS Ser1177 phosphorylation, thereby decreasing NO levels in endothelial cells and VSMCs (Tousoulis et al., 2012; Benter et al., 2015). Additionally, several studies have now determined that eNOS is a downstream effector of EGFR or ErbB2 signaling in T1DM and T2DM (Belmadani et al., 2008; Galan et al., 2012; Benter et al., 2015), and that exaggerated ErbB2-EGFR heterodimerization in the diabetic vasculature leads to diminished NO activity (Benter et al., 2015). Administration of lapatinib, which is a dual inhibitor of EGFR and ErbB2 RTKs, ameliorates vascular dysfunction by normalizing the decreased NO levels in the diabetic vasculature (Benter et al., 2015). Regarding restoration of NO activity in the diabetic vasculature by selective EGFR inhibitors, studies have shown that chronic *in vivo* AG1478 treatment rescued eNOS phosphorylation and expression (Belmadani et al., 2008; Galan et al., 2012) whereas acute *in vitro* AG1478 treatment appeared not to (Kassan et al., 2015a). It is worth mentioning that ROS generation by NADPH oxidase accounts partly for the reduced NO bioavailability in the vascular bed (Kassan et al., 2015a), consequently inducing dysfunction of the vascular endothelium (Sena et al., 2008; Akar et al., 2011; Meyrelles et al., 2011). Interestingly, a CaMKII inhibitor, KN-93, was also able to prevent the development of abnormal vascular reactivity in a rat model of diabetes and hypertension (Yousif et al., 2008) implying that NO production might also involve CaMKII-independent pathways in such pathologies.

PI3K as an Effector of Epidermal Growth Factor Receptor in Diabetes-Induced Vascular Dysfunction

PI3Ks are lipid kinases that belong to a group of enzymes involved in the regulation of multiple signaling cascades that control multiple cellular processes including cell growth, proliferation, motility, and survival. It is well known that PI3K/AKT signaling is downstream of EGFR/ErbB signalling (Roskoski 2019) and also can activate eNOS and subsequent NO generation to regulate vascular function (Kobayashi et al., 2005). Impairment in PI3K signaling pathway has been demonstrated in aortas of diabetic mice (Kobayashi et al., 2004). The activated PI3K-Akt signaling pathway can also improve insulin sensitivity and protect the vascular endothelium (Feng et al., 2018).

Activation of P13K/Akt/eNos pathway, such as by components of green tea, has been reported to have beneficial effects on diabetes-induced vascular dysfunction (Bhardwaj et al., 2014; Zhang and Zhang, 2020). For example, (-)-Epigallocatechin-3-gallate (EGCG), a highly effective component in green tea that has anti-inflammatory as well as antioxidant and free radical scavenging properties, was able to inhibit eNOS uncoupling and prevent hyperglycemia-induced endothelial dysfunction and apoptosis by activating the PI3K/AKT/eNOS pathway (Zhang and Zhang, 2020). Similarly, in a rat model of T1DM, chronic administration of catechin, another key component of green tea, markedly attenuated diabetes-induced vascular dysfunction and vascular oxidative stress via activation of endothelial PI3K signaling and the subsequent downstream eNOS signaling system that generates NO (Bhardwaj et al., 2014). Although PI3K-eNOS signaling can be a downstream consequence of EGFR activation, the relationship between EGFR activation in diabetic vasculature and administration of green tea components was not investigated in these studies. Nonetheless, these studies suggest that PI3K signalling is beneficial in maintaining vascular function as its attenuation is associated with diabetes induced vascular complications.

In contrast to these studies, in a rat model of TIDM, chronic selective inhibition of PI3K with LY294002 significantly attenuated the development of diabetes-induced abnormal vascular reactivity in the carotid artery (Yousif et al., 2006) implying that that PI3K pathway might also mediate vascular dysfunction. The reasons for this discrepancy are not clear but several reports have now confirmed AKT, and by implication PI3K to which it often coupled, as a downstream player in ErbB receptor-dependent vascular dysfunction in models of diabetes (Benter et al., 2015; Kasan et al., 2015b; Amin et al., 2011). For example, in the diabetic mesenteric vascular bed, hyperglycemia-induced upregulation of Akt signaling was prevented by lapatinib, a dual inhibitor of EGFR and ErbB2 (Benter et al., 2015). With the caveat that AKT signaling does not always have to be coupled to PI3K, these studies are suggestive of a possible detrimental role of

PI3K/AKT pathway in diabetes-induced vascular dysfunction. Supportive of this notion, is the finding that ANG II, that is known to be elevated in diabetes, increases aortic contractile responses via PI3-kinase pathway in a rat model of diabetes with systemic hyperinsulinemia (Kobayashi et al., 2006). Collectively, the data on the role of PI3K in mediating vascular dysfunction appears contradictory and may be indicative of a dual role for this molecule whereby depending on the context it too may behave as a good guy or a bad guy, akin to the role of EGFR/ErbB receptors in cardiovascular complications as discussed herein.

Interplay of Epidermal Growth Factor Receptor and Forkhead Transcription Factors in Diabetes-Induced Vascular Dysfunction

FOXO3 is a pro-apoptotic protein that belongs to the forkhead family of transcriptional regulators which are critical mediators of oxidative stress (Ponugoti, et al., 2012). FOXO activity, amongst others, is regulated via phosphorylation, whereby increased phosphorylation renders it inactive via mechanisms including nuclear exclusion, polyubiquitination, and degradation (Oellerich and Potente, 2012). ROS/oxidative stress can induce FOXO3 phosphorylation which results in its release from the 14–3-3 binding protein, and subsequent translocation to the nucleus where it modulates target gene expression (Ponugoti, et al., 2012). The role of FOXO3 as a downstream effector of EGFR/ErbB2 receptors was studied in the mesenteric vascular bed of T1DM rats (Benter et al., 2015). Diabetes-induced vascular dysfunction arising from enhanced EGFR/ErbB2 signaling also led to downstream activation of FOXO3 (as evidenced by reduced phosphorylation at Ser253) through an AKT-independent manner that ultimately leads to apoptosis and vascular dysfunction (Benter et al., 2015). Hyperglycemia-mediated decrease in FOXO3A phosphorylation and increased total FOXO3A levels could be reversed upon lapatinib treatment (a dual inhibitor of EGFR and ErbB2 receptors) (Benter et al., 2015). Thus, FOXO-mediated pro-apoptotic signaling is likely an important downstream contributor to EGFR/ErbB receptor-mediated vascular dysfunction in diabetes.

The Link Between Epidermal Growth Factor Receptor and NF-kB Transcription Factor in Diabetes-Induced Vascular Dysfunction

The Nuclear Factor Kappa B (NF-kB) transcription factor is a key player in the development of diabetes-induced vascular complications through regulation of many cellular processes such as inflammation, cell growth, proliferation, and apoptosis (Suryavanshi and Kulkarni., 2017; Taniguchi and Karin, 2018). In unstimulated cells, inactive NF-kB is bound in the cytoplasm to inhibitory proteins in the inhibitor of kB (IkB) family. Activation of NF-kB occurs via signal transduction pathways that stimulate the IkB kinase (IKK) complex resulting in a series of reactions that ultimately cause degradation of IkB and releasing of active NF-kB. Aberrant NF-kB activity has been implicated in many diseases including diabetes and its complications. Chronic hyperglycemia is known to activate NF-kB that triggers expression of pro-inflammatory cytokines and pro-apoptotic genes. It can also induce calcification of endothelial cells leading to endothelial and vascular dysfunction (Suryavanshi and Kulkarni., 2017). In the vasculature of T1DM rats, diabetes leads to the enhanced phosphorylation of IkB-α (Benter et al., 2015), a component of IKK complex and a universal marker of NF-kB activation. Data from this study illustrated that the increased ErbB2-EGFR hetero-dimerization in the vasculature leads to elevated NF-kB activation, which eventually induced apoptosis and vascular dysfunction (Benter et al., 2015). This was evidenced by *in vivo* and *ex vivo* lapatinib administration which attenuated the increased phosphorylation of IkB-α in VSMCs grown under high glucose conditions (Benter et al., 2015). Furthermore, administration of BAY 11–7,082, an NF-kB antagonist, corrected the altered vascular responsiveness to norephinephrine in the diabetic mesenteric vascular bed and hyperglycemia-induced apoptosis in VSMCs (Benter et al., 2015). Thus, it was determined from these results that NF-kB is downstream of EGFR-ErbB2 signaling in T1DM (Benter et al., 2015). These findings were also confirmed in coronary arteries of mice bearing T2DM (Kassan et al., 2015b). Intriguingly, it was determined that eNOS signaling was coupled with NF-kB activation in the diabetic mesenteric vasculature, where eNOS was shown to be upstream of NF-kB in mediating vascular dysfunction associated with diabetes (Benter et al., 2015).

The Link Between Epidermal Growth Factor Receptor & Nicotinamide Adenine Dinucleotide Phosphate Oxidase in Diabetes-Induced Vascular Dysfunction

Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) enzyme complex comprises of several sub-units, namely, p22phox, NOX2; p47phox; p67phox; and p40phox; also known as NOX1, NOX2, NOX3, NOX4, and NOX5 respectively (Volpe et al., 2018). It is well established that the NOX family are major producers of reactive oxygen species that mediate cardiovascular complications associated with diabetes (Volpe et al., 2018; Iacobini et al., 2021). Several studies have now shown that EGFR inhibition leads to a reduction in NOX activity in models of diabetes-induced. cardiovascular complications (Belmadani et al., 2008; Choi et al., 2012; Galan et al., 2012; Kassan et al., 2015a)- implying that EGFR/ErbB receptor activation and subsequent NOX-mediated ROS/oxidative stress are critical in mediating vascular dysfunction associated with diabetes. In the mesenteric artery, mRNA levels of Nox2 and Nox4 and NADPH oxidase activity were upregulated in a mouse model of T1DM and reduced upon EGFR inhibition (Galan et al., 2012). Similarly, in an experimental model of TD2M, selective EGFR inhibition improved vascular function and reduced p22phox-NADPH expression (Kassan et al., 2015b). Collectively these studies provide a direct link between EGFR and NADPH oxidase in mediating oxidative stress that leads to diabetes-induced vascular dysfunction.

The Interplay of Epidermal Growth Factor Receptor and Angiotensin 1-7, a Member of the Renin-Angiotensin-Aldosterone System, in Diabetes-Induced Vascular Dysfunction

The renin-angiotensin-aldosterone system (RAAS) is a key regulator of homeostasis within the cardiovascular system (Ferrario et al., 2010; Akhtar et al., 2020a; Paz Ocaranza 2020). Its main peptide, Angiotensin II (Ang II), is known to be detrimental in the diabetic vasculature where it mediates oxidative stress, pro-inflammatory signaling and vasoconstriction. Enhanced signaling via this octapeptide in the diabetic vasculature involves transactivation and cross-talk with EGFR/ErbB/HER family of receptors (Akhtar et al., 2012a; Akhtar et al., 2015; Akhtar et al., 2016; Sur and Agrawal, 2014).

Ang II-mediated detrimental effects on the vasculature are exerted through the classical arm of the RAAS constituting angiotensin converting enzyme (ACE)/Ang II/Angiotensin II type 1 (AT1) receptors. The other arm of the RAAS that counter-regulates or opposes the actions of Ang II involves the heptapeptide, Angiotensin-(1–7) and comprises ACE2/Ang-(1–7)/Mas receptor (for recent review see Akhtar et al., 2020a). Ang-(1–7) can be derived from the catalytic metabolism of Ang II via the actions of angiotensin converting enzyme 2 (ACE2) (Ferrario et al., 2010). Ang-(1–7) induces vasodilation and attenuates Ang II-induced vasoconstriction in animal models (Benter et al., 1993). In humans, Ang-(1–7) also improves insulin-stimulated endothelium-dependent vasodilation and attenuates endothelin-1–dependent vasoconstrictor tone in obese patients (Schinzari et al., 2018). In endothelial cells Ang-(1–7) increases production of nitric oxide and prostacyclin and in vascular smooth muscle cells it inhibits pro-contractile and pro-inflammatory signalling (Benter et al., 1993; Benter et al., 1995a; Ferrario et al., 2010). It is known that Ang-(1–7) exhibits antihypertensive, antithrombotic and antiproliferative properties and can correct abnormal vascular reactivity including that observed in diabetes (Benter et al., 1995a; Benter et al., 1995b; Benter et al., 2006; Benter et al., 2007; Benter et al., 2008; Benter et al., 2011; Chappell et al., 2014; Dhaunsi et al., 2010; Patel et al., 2014; Yousif et al., 2012; Yousif et al., 2014). In animal models of diabetes, Ang-1-7 can attenuate NADPH oxidase and NF-kB activity and prevent vascular dysfunction without markedly correcting hyperglycemia (Benter et al., 2007; Benter et al., 2008; Al-Maghrebi et al., 2009; Yousif et al., 2014). Although the precise mechanistic details through which Ang-(1–7) exerts its beneficial actions are not yet fully elucidated, it appears that Ang-(1–7) prevents diabetes-induced vascular dysfunction, at least in part, via inhibiting Ang II-mediated transactivation of EGFR/ErbB family of receptor tyrosine kinases (Akhtar et al., 2012a; Akhtar et al., 2015). In addition to inhibiting EGFR phosphorylation at multiple tyrosine sites in the vasculature of diabetic animals, Ang-(1–7) inhibited ErbB2 phosphorylation at tyrosine residues Y1221/22, Y1248, Y877, with modulation of associated downstream signaling pathways involving ERK1/2, p38 MAPK, ROCK, eNOS, and lkB-α in the diabetic mesenteric vascular bed (Akhtar et al., 2015). Furthermore, high glucose- or diabetes-induced ErbB3, and Erbb4 transactivation could be ameliorated by Ang-(1–7) treatment thereby suggesting that Ang-(1–7) acts as a pan-inhibitor of the EGFR/ErbB/HER family of receptor tyrosine kinases (Akhtar et al., 2015). More recently other pan-ErbB inhibitors have been shown to be beneficial in reversing or preventing diabetes-induced vascular dysfunction (Akhtar et al., 2019; Akhtar et al., 2020b) and as such pan-ErbB inhibition might prove to be a useful strategy in the treatment of diabetes induced vascular complications.

As to the mechanism of diabetes-induced transactivation of ErbB receptors, high glucose-mediated ErbB2 transactivation occurred via a Src-dependent mechanism in VSMCs as evidenced by increased phosphorylation of Src at Y416. Ang-(1–7) acting via its MAS receptor blocked the phosphorylation of Src at this site-an upstream effector of ErbB2 transactivation (Akhtar et al., 2015). Thus, it was proposed that Ang-(1–7) inhibited diabetes or hyperglycemia induced transactivation of ErbB receptors in the diabetic vasculature by acting as an inhibitor of Src (Akhtar et al., 2012a; Akhtar et al., 2015). Subsequent studies in cardiac fibroblasts (Tao et al., 2014) and in activated macrophages (Souza and Costa-Neto, 2012) further supported the notion that Ang-(1–7) can inhibit Src phosphorylation. Recent studies in other disease models suggest that inhibition of Src-dependent EGFR transactivation by Ang-(1–7) may be a general mechanism of action of this heptapeptide (Kilarkaje et al., 2013; Yousif et al., 2014; El-Hashim et al., 2019) and may represent an important mechanism by which it may exert its beneficial effects in diabetes-induced vascular complications.

It is noteworthy that, despite its inhibitory effects on EGFR and other ErbB receptors (Akhtar et al., 2012b; Akhtar et al., 2015), Ang-(1–7) exerts considerable cardioprotective effects in models of diabetes (Dhaunsi et al., 2010; Yousif et al., 2012; Benter et al., 2014; Abwainy et al., 2016). Thus, the precise mechanisms underlying this phenomenon, especially as to how Ang-(1–7) interplays with the dual role of EGFR/ERbB signaling in the CVS, remain to be elucidated.

The Arachidonic Acid Metabolite, 20-HETE and Its Interplay With Epidermal Growth Factor Receptor in Diabetes-Induced Vascular Dysfunction

The arachidonic acid metabolite, 20-HETE (20-hydroxy-5, 8, 11, 14-eicosatetraenoic acid), plays an important role in controlling cardiovascular function (Rocic and Schwartzman, 2018; Yousif et al., 2009a; Yousif et al., 2009b). In blood vessels, it acts as a potent vasoconstrictor, regulates myogenic tone and also potentiates the vasoconstrictor response to peptides like angiotensin II and endothelin (Roman 2002; Fan et al., 2016). 20-HETE induces endothelial dysfunction and adverse vascular remodeling that leads to increased blood pressure (Fan et al., 2016). Its actions are most likely exerted through its newly discovered receptor, GPR75, an orphan G-protein ($G\alpha_{q/11}$) coupled receptor (Garcia et al., 2017).

20-HETE is produced from arachidonic acid metabolism, not via the classical cyclooxygenase (COX) or lipoxygenase (LOX) pathways, but by a third pathway involving cytochrome P450-mediated ω-hydroxylation via CYP4A and CYP4F enzyme subfamilies (Fan et al., 2016; Fan et al., 2015; Roman, 2002). Its presence has been reported in the microvasculature of the brain, lungs, kidneys, and the peripheral blood vessels (Miyata and Roman, 2005). There is accumulating evidence that CYP4A/4F and 20-HETE pathway play a major role in vascular dysfunction and changes in 20-HETE levels is implicated in many diseases like diabetes and hypertension (Fan et al., 2016; Miyata and Roman, 2005; Rocic and Schwartzman, 2018; Yousif et al., 2009a; Yousif et al., 2009b). For example, 20-HETE contributed to elevated vascular reactivity in renal and mesenteric vasculature of streptozotocin-induced diabetic mice (Yousif et al., 2009a). Moreover, 20-HETE stimulates mitogenic and angiogenic responses (*in vivo* and *in vitro*) to various growth factors such as EGF (Miyata and Roman, 2005). This was demonstrated in a study whereby inhibition of 20-HETE by HET0016 (an inhibitor of 20-HETE synthesis) almost completely abolished the angiogenic responses of EGF and other growth factors (Chen et al., 2005). Also, 20-HETE has been implicated as a key regulator of NO production and function. For instance, 20-HETE leads to eNOS uncoupling and impairs acetylcholine-induced relaxation through tyrosine kinase-, MAPK/ERK-, and IKK- dependent mechanisms that involve HSP90-eNOS dissociation and HSP90-IKKβ association (Cheng et al., 2010) leading to endothelial dysfunction. Moreover, 20-HETE stimulates NF-kB and increases levels of proinflammatory cytokines causing endothelial activation. This is partly mediated via MAPK-ERK1/2 signalling (Ishizuka et al., 2008).

Further studies on the mechanism by which 20-HETE exerts its actions in the vasculature (Chen, et al., 2012; Guo et al., 2007; Guo et al., 2009) showed that 20-HETE stimulates endothelial progenitor cell proliferation, migration and tube formation as well as enhancing release of proangiogenic factors such as VEGF. Importantly, these studies revealed the interplay with EGFR/ErbB receptors in that these vascular effects of 20-HETE were mediated through Src and EGFR with downstream activation of NADPH oxidases/reactive oxygen species (ROS) system, eNOS uncoupling and stimulation of MAPK and PI3K/Akt pathways (Chen, et al., 2012; Guo et al., 2007; Guo et al., 2009).

In VSMC, it was shown that norephinephrine (NE), Ang II, and EGF can activate the Ras/MAPK pathway through generation of 20-HETE (Muthalif et al., 1988). Since ACE/Ang II/AT1 receptor pathway is known to lead to transactivation of ErbBs in VSMC (Akhtar et al., 2015; Akhtar et al., 2016), the fact that 20-HETE is a potent activator of endothelial ACE expression and activity might represent another point of interplay between EGFR and 20-HETE signaling. Indeed, 20-HETE induces ACE expression through increased NF-κB binding to the ACE promoter (Cheng et al., 2012; Garcia et al., 2016). Endothelial ACE transcription is in turn mediated by EGFR via a MAPK/IkappaB kinase signaling cascade that ultimately results in increased ACE activity (Garcia et al., 2016). Furthermore, in parallel enhanced synthesis of 20-HETE *in vivo* leads to increased ACE expression in the vasculature (Garcia et al., 2015; Garcia et al., 2016; Garcia et al., 2017; Garcia and Schwartzman, 2017) as well as increased serum Ang II levels (Sodhi et al., 2010). Blocking 20-HETE synthesis prevented these changes, implicating 20-HETE in the regulation of ACE expression and AngII levels *in vivo* that indirectly could have an impact on ACE/AngII/AT1 receptor mediated transactivation of ErbBs. Thus, EGFR is likely involved in mediating the actions of 20-HETE in stimulating ACE expression and Ang II levels as well being involved in the downstream actions of ACE/AngII/AT1 receptor signaling possibly via Src phosphorylation. Collectively the above studies suggest that EGFR/ErbB receptor transactivation may represent an important signaling convergence point or "hub" by which many cardiovascular risk factors promote diabetes-induced vascular dysfunction and remodeling.

PERSPECTIVES AND CONCLUDING REMARKS

Signaling via EGFR/ErbB family of receptor tyrosine kinases appears to be a central hub or relay in mediating vital functions in the normal development and physiology of the cardiovascular system as well as in the development of cardiovascular complications and pathologies. All four members, ErbB1-4, appear to be expressed both in the normal and diabetic heart as well as normal and diabetic vasculature where EGFR/ErbB receptors appear to have a dual (or "good guy" v "bad guy"; "Jekyll and Hyde" or "Janus-faced") role. EGFR/ErbB receptors generally have beneficial "good-guy" actions in normal physiological function of the cardiovascular system and in mediating key "salvage" or recovery pathways following cardiac ischemia-reperfusion injury. However, several studies highlighted in this review confirm also the detrimental "bad-guy" effect of upregulated EGFR/ErbB signaling in the pathogenesis of diabetic-induced cardiac pathologies (Galan et al., 2012; Liang et al., 2015). Further highlighting the dual role of EGFR/ErbB receptors, these findings apparently contradict those showing protective effects of EGFR/ErbBs following ischemia/reperfusion injury in diabetic animals (Akhtar et al., 2012a). In an attempt to reconcile this contradiction, it has been postulated that EGFR/ErbB activation could be protective in the context of acute injury such as acute ischemia/reperfusion injury, but detrimental in chronic pathologies where persistent upregulation of EGFR/ErbB receptor signaling is observed such as diabetes-induced cardiac fibrosis and remodeling (Xu and Cai, 2015).

Similarly, upregulation of all four ErbBs has been implicated in the development of diabetes-induced vascular dysfunction through mechanisms involving multiple downstream effectors such as ERK1/2, ROCKs, p38 MAPK, FOXO, AKT, eNOS, and NF-kB [see also **Figure 2**] (Akhtar et al., 2015; Akhtar et al., 2020a). Both in the vasculature and in the heart, much of the evidence suggests that EGFR/ErbB receptors might function as key relays for the actions of the RAAS especially in mediating the pathological actions of its detrimental arm comprising the ACE/Ang II/AT1 receptor signaling cascade-that is known to lead to transactivation of EGFR/ErbBs with subsequent development of

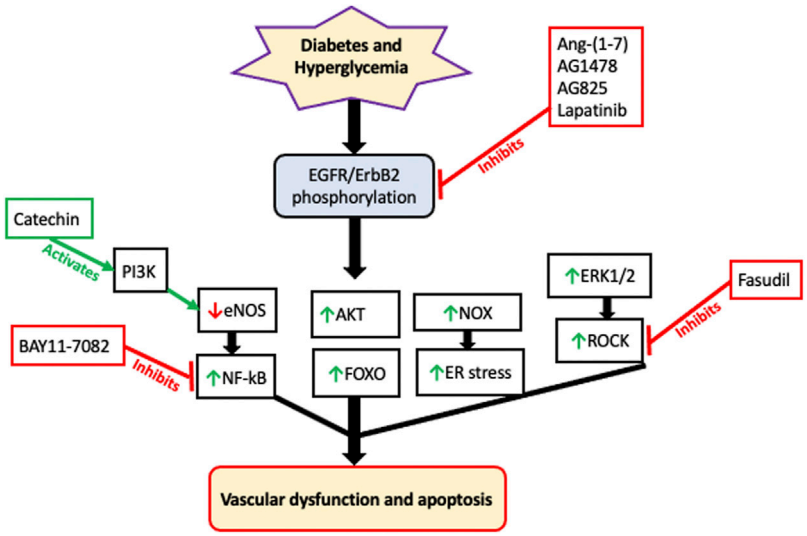

FIGURE 2 | A schematic of the likely signaling effectors of EGFR/ErbB receptors in mediating diabetes-induced vascular dysfunction. Although there is now evidence that all four ErbB receptors may be activated by hyperglycemia, the majority of the data suggests that diabetes induced vascular dysfucntion proceeds via phosphorylation and subsequent hetero-dimerization EGFR and ErbB2 receptors. This is turn begins a cascade of downstream signaling pathways including attenuation of eNOS that results in decreased NO production. Activation of P13K, such as by green tea components like catechin, can rescure eNOS and reduction in NO. eNOS appears to be upstream of NF-kB activation. Additionally, ERK1/2 activation leads to downstream ROCK signaling–a key driver of pro-contractile changes leading to vascular dysfunction. Interestingly, activation of AKT and FOXO occur independently of each other implying that uncoupling of AKT-FOXO axis is a key component in developing diabetes-induced vascular complications. There is also evidence that EGFR/ErbB2 signaling via NADPH-oxidase (NOX) leads to increased ROS/oxidative and ER stress in the diabetic cardiovascular system. The precise cross-talk and interplay between these and other pathways remains to be fully elucidated. Furthermore, since multiple gene expression changes are known to corrected by EGFR inhibition in the diabetic vasculature, it is very likely that the signaling pathways discussed here will act in concert with multiple other, as yet unconfirmed, signaling cascades that are downstream of EGFR/ErbB receptors to eventually lead to diabetes-induced vascular cell apoptosis and dysfunction. Some of the reported pharmacologic interventions used in the analyses of these pathways are also shown; red dead-end arrow indicates inhibition; green arrow indicates activation (refer to the main text for more details and references).

cardiovascular pathologies. Similarly, EGFR/ErbB receptors appear to be a key target for the actions of the beneficial counter-regulatory, ACE2/Ang-(1–7)/Mas receptor, pathway or "arm" of the RAAS. For example, the vasculo-protective effects of Ang-(1–7)/Mas receptor appear to be mediated, at least in part, via blockade of a Src-dependent EGFR/ErbB receptor phosphorylation and attenuation of downstream effectors (Akhtar et al., 2012a; Akhtar et al., 2015).

Interestingly, EGFR/ErbB receptor signaling also appears important in mediating the vascular effects of the arachidonic acid metabolite, 20-HETE-a key regulator of cardiovascular function that also exhibits significant cross-talk with RAAS. EGFR/ErbB receptors are likely involved in mediating the actions of 20-HETE especially those leading to enhancing ACE expression and serum Ang II levels. Further, EGFR/ErbB receptors are also likely be involved in the downstream actions of ACE/AngII/AT1 receptor signaling such as in the Src-dependent transactivation of EGFR/ErbB receptor family members that ultimately leads to diabetes-induced vascular complications. Therefore, EGFR/ErbBs might represent a key point of convergence for actions of the RAAS and other molecules that are involved in cross-talk with RAAS such as 20-HETE. Thus, targeting ErbB receptor might represent a novel strategy for the treatment of cardiovascular complications.

EGFR inhibition is reported to have several benefits in diabetes including normalization of cardiovascular function, reduction of blood pressure, lowering of blood glucose levels and reversing insulin resistance (Hao et al., 2004; Nagareddy et al., 2010; Akhtar et al., 2013; Kassan et al., 2015a; Akhtar et al., 2015; Benter et al., 2015; Fountas et al., 2015). Inhibitors of EGFR/ErbB receptors (e.g., lapatinib) are already clinically available for the treatment of several cancers but could also be repurposed for treatment of diabetes-induced cardiovascular complications (Akhtar et al., 2015). In contrast, inducers or activators of EGFR/ErbB receptors (e.g., NRG or EGF) might be useful in recovering hearts from ischemia-reperfusion injury especially in diabetes where cardiac function is already compromised. Interestingly, in this regard it was found that the beneficial actions of RAAS inhibitors such as the angiotensin receptor blockers (e.g., losartan), that are clinically approved for use in myocardial infarction/ischemic heart disease, might not be optimal and cardiac function recovery could be improved by co-administering ligands or activators of EGFR/ErbB signaling (Akhtar et al., 2012b). Consistent with losartan's known inhibition of Angiotensin II-mediated transactivation of EGFR (e.g., Akhtar et al., 2016), reduced EGFR phosphorylation was observed in losartan-treated diabetic hearts following ischemia-reperfusion injury compared to untreated diabetic hearts. Co-treatment of losartan with EGF, a ligand for EGFR, prevented the inhibitory effects of losartan on EGFR transactivation with a parallel improvement in cardiac function recovery greater than either agent alone. These findings may have important clinical

implications as they suggest that rescuing the EGFR inhibitory effect of AT1 receptor antagonists by activators of the EGFR/ErbB family of receptor tyrosine kinases may represent a novel clinical approach to improve protection against end-organ damage in diabetic hearts. However, future treatments targeting EGFR/ErbBs will need to balance their beneficial versus the detrimental effects in a given cardiovascular pathology as well as the differential expression of EGFR/ErbB receptors and their ligands in different tissues. A greater understanding of the multi-faceted roles of EGFR/ErbB/HER family of tyrosine kinases, their interplay with other key modulators of cardiovascular function, and the development of smart or intelligent drug delivery systems (e.g., Mitchel et al., 2021) that can spatially and temporally control delivery of EGFR/ErbB/HER receptor modulators for a specific application, could facilitate the development of novel therapeutic strategies/formulations for treating diabetes-induced cardiovascular complications. Indeed, engineered nanoparticles, that could potentially encapsulate and site-specifically deliver EGFR/ErbB receptor modulators for a specific purpose, are now in advanced stages of development (Mitchell et al., 2021). By controlling nanoparticle surface and nanomaterial properties, their size, shape and architecture, as well as through careful selection of cell or tissue-specific targeting moieties and built-in responsiveness to environmental cues such as hyperglycemia, nanoparticle delivery systems can now be produced with intelligent designs to tailor the delivery of EGFR/ErbB receptor drugs to a specific application or condition as new-age precision medicines. Such approaches may have broader application beyond diabetes-induced cardiovascular dysfunction as dysregulated EGFR/ErbB/HER signaling also leads to diabetes-induced retinal and corneal complications (Akhtar et al., 2009; Ju et al., 2019), diabetic foot ulcers (Qi et al., 2018; Yang et al., 2020) and renal pathologies (Harskamp et al., 2016; Rayego-Mateos et al., 2018; Sheng et al., 2021) implying that aberrant signalling via EGFR/ErbB/HER receptors and their ligands might represent a common underlying mechanism in the development of diabetes complications.

AUTHOR CONTRIBUTIONS

SA conceived the idea. SA, BS, MM, AH and IB searched, wrote and edited the manuscript.

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REFERENCES

Abwainy, A. a., Babiker, F., Akhtar, S., and Benter, I. F. (2016). Endogenous angiotensin-(1-7)/Mas Receptor/NO Pathway Mediates the Cardioprotective Effects of Pacing Postconditioning. *Am. J. Physiology-Heart Circulatory Physiol.* 310 (1), H104–H112. doi:10.1152/ajpheart.00121.2015

Akar, F., Pektas, M. B., Tufan, C., Soylemez, S., Sepici, A., Ulus, A. T., et al. (2011). Resveratrol Shows Vasoprotective Effect Reducing Oxidative Stress without Affecting Metabolic Disturbances in Insulin-dependent Diabetes of Rabbits. *Cardiovasc. Drugs Ther.* 25 (2), 119–131. doi:10.1007/s10557-010-6255-7

Akhtar, S., and Benter, I. F. (2013). The Role of Epidermal Growth Factor Receptor in Diabetes-Induced Cardiac Dysfunction. *Bioimpacts* 3 (1), 5–9. doi:10.5681/bi.2013.008

Akhtar, S., Chandrasekhar, B., Attur, S., Dhaunsi, G. S., Yousif, M. H. M., and Benter, I. F. (2015). Transactivation of ErbB Family of Receptor Tyrosine Kinases Is Inhibited by Angiotensin-(1-7) via its Mas Receptor. *PLoS One* 10 (11), e0141657. doi:10.1371/journal.pone.0141657

Akhtar, S., Vranic, S., Cyprian, F. S., and Al Moustafa, A. E. (20182018). Epstein-Barr Virus in Gliomas: Cause, Association, or Artifact? *Front. Oncol.* 8, 123. doi:10.3389/fonc.2018.00123

Akhtar, S., Almubrad, T., Bron, A. J., Yousif, M. H. M., Benter, I. F., and Akhtar, S. (20092009). Role of Epidermal Growth Factor Receptor (EGFR) in Corneal Remodelling in Diabetes. *Acta Ophthalmologica* 87 (8), 881–889. doi:10.1111/j.1755-3768.2008.01434.x

Akhtar, S., Benter, I. F., Danjuma, M. I., Doi, S. A. R., Hasan, S. S., and Habib, A. M. (2020a). Pharmacotherapy in COVID-19 Patients: A Review of ACE2-Raising Drugs and Their Clinical Safety. *J. Drug Target.* 28, 683–699. doi:10.1080/1061186X.2020.1797754

Akhtar, S., Chandrasekhar, B., and Benter, I. (2020b). 455-P: Pan-ErbB Inhibition Attenuates Diabetes-Induced Vascular Dysfunction in an Experimental Model of Diabetes. *Diabetes* 69 (Suppl. 1), 455–P. doi:10.2337/db20-455-P

Akhtar, S., Chandrasekhar, B., Yousif, M. H., Renno, W., Benter, I. F., and El-Hashim, A. Z. (2019). Chronic Administration of Nano-Sized PAMAM Dendrimers *In Vivo* Inhibits EGFR-ERK1/2-ROCK Signaling Pathway and Attenuates Diabetes-Induced Vascular Remodeling and Dysfunction. *Nanomedicine: Nanotechnology, Biol. Med.* 18, 78–89. doi:10.1016/j.nano.2019.02.012

Akhtar, S., El-Hashim, A. Z., Chandrasekhar, B., Attur, S., and Benter, I. F. (2016). Naked Polyamidoamine Polymers Intrinsically Inhibit Angiotensin II-Mediated EGFR and ErbB2 Transactivation in a Dendrimer Generation- and Surface Chemistry-dependent Manner. *Mol. Pharmaceutics* 13 (5), 1575–1586. doi:10.1021/acs.molpharmaceut.6b00045

Akhtar, S., Yousif, M. H., Dhaunsi, G. S., Chandrasekhar, B., Al-Farsi, O., and Benter, I. F. (2012a). Angiotensin-(1-7) Inhibits Epidermal Growth Factor Receptor Transactivation via a Mas Receptor-dependent Pathway. *Br. J. Pharmacol.* 165 (5), 1390–1400. doi:10.1111/j.1476-5381.2011.01613.x

Akhtar, S., Yousif, M. H. M., Chandrasekhar, B., and Benter, I. F. (2012b). Activation of EGFR/ERBB2 via Pathways Involving ERK1/2, P38 MAPK, AKT and FOXO Enhances Recovery of Diabetic Hearts from Ischemia-Reperfusion Injury. *PLoS One* 7 (6), e39066. doi:10.1371/journal.pone.0039066

Akhtar, S., Yousif, M. H. M., Dhaunsi, G. S., Sarkhouh, F., Chandrasekhar, B., Attur, S., et al. (2013). Activation of ErbB2 and Downstream Signalling via Rho Kinases and ERK1/2 Contributes to Diabetes-Induced Vascular Dysfunction. *PLoS One* 8 (6), e67813. doi:10.1371/journal.pone.0067813

Al-Maghrebi, M., Benter, I. F., and Diz, D. I. (2009). Endogenous Angiotensin-(1-7) Reduces Cardiac Ischemia-Induced Dysfunction in Diabetic Hypertensive Rats. *Pharmacol. Res.* 59 (4), 263–268. doi:10.1016/j.phrs.2008.12.008

Amin, A. H., Abd Elmageed, Z. Y., Partyka, M., and Matrougui, K. (2011). Mechanisms of Myogenic Tone of Coronary Arteriole: Role of Down Stream Signaling of the EGFR Tyrosine Kinase. *Microvasc Res.* 81 (1), 135–42. doi:10.1016/j.mvr.2010.11.001

Babel, R. A., and Dandekar, M. P. (2021). A Review on Cellular and Molecular Mechanisms Linked to the Development of Diabetes Complications. *Cdr* 17 (4), 457–473. doi:10.2174/1573399816666201103143818

Barrick, C. J., Yu, M., Chao, H.-H., and Threadgill, D. W. (2008). Chronic Pharmacologic Inhibition of EGFR Leads to Cardiac Dysfunction in C57BL/6J Mice. *Toxicol. Appl. Pharmacol.* 228 (3), 315–325. doi:10.1016/j.taap.2007.12.012

Belmadani, S., Palen, D. I., Gonzalez-Villalobos, R. A., Boulares, H. A., and Matrougui, K. (2008). Elevated Epidermal Growth Factor Receptor Phosphorylation Induces Resistance Artery Dysfunction in Diabetic Db/db Mice. *Diabetes* 57 (6), 1629–1637. doi:10.2337/db07-0739

Beltowski, J., and Jazmroz-Wisniewska, A. (2014). Transactivation of ErbB Receptors by Leptin in the Cardiovascular System: Mechanisms, Consequences and Target for Therapy. *Cpd* 20 (4), 616–624. doi:10.2174/13816128004140213155050

Benter, I. F., Juggi, J. S., Khan, I., Yousif, M. H., Canatan, H., and Akhtar, S. (2005c). Signal Transduction Mechanisms Involved in Cardiac Preconditioning: Role of Ras-GTPase, Ca2+/calmodulin-dependent Protein Kinase II and Epidermal Growth Factor Receptor. *Mol. Cel Biochem*. 268 (1-2), 175–183. doi:10.1007/s11010-005-3895-1

Benter, I. F., Yousif, M. H. M., Juggi, J., and Akhtar, S. (2014). "The Angiotensin-Converting Enzyme 2/Angiotensin-(1-7)/Mas Receptor Axis: A Potential Target for Treating Diabetic Cardiovascular Disease," in *Diabetic Cardiomyopathy: Biochemical and Molecular Mechanisms. Advances in Biocehmistry in Health and Disease*. Editors B. Turan and N. S. Dhalla (New York: Springer Science + Business Media).

Benter, I. F., Benboubetra, M., Hollins, A. J., Yousif, M. H. M., Canatan, H., and Akhtar, S. (2009). Early Inhibition of EGFR Signaling Prevents Diabetes-Induced Up-Regulation of Multiple Gene Pathways in the Mesenteric Vasculature. *Vasc. Pharmacol.* 51 (4), 236–245. doi:10.1016/j.vph.2009.06.008

Benter, I. F., Diz, D. I., and Ferrario, C. M. (1993). Cardiovascular Actions of Angiotensin(1-7). *Peptides* 14 (4), 679–684. doi:10.1016/0196-9781(93)90097-z

Benter, I. F., Diz, D. I., and Ferrario, C. M. (1995b). Pressor and Reflex Sensitivity Is Altered in Spontaneously Hypertensive Rats Treated with Angiotensin-(1-7). *Hypertension* 26 (6 Pt 2), 1138–1144. doi:10.1161/01.hyp.26.6.1138

Benter, I. F., Ferrario, C. M., Morris, M., and Diz, D. I. (1995a). Antihypertensive Actions of Angiotensin-(1-7) in Spontaneously Hypertensive Rats. *Am. J. Physiology-Heart Circulatory Physiol.* 269 (1 Pt 2), H313–H319. doi:10.1152/ajpheart.1995.269.1.h313

Benter, I. F., Juggi, J. S., Khan, I., Yousif, M. H. M., Canatan, H., and Akhtar, S. (2005). Signal Transduction Mechanisms Involved in Cardiac Preconditioning: Role of Ras-GTPase, Ca2 +/calmodulin-dependent Protein Kinase II and Epidermal Growth Factor Receptor. *Mol. Cel Biochem*. 268 (1-2), 175–183. doi:10.1007/s11010-005-3895-1

Benter, I. F., Sarkhou, F., Al-Khaldi, A. T., Chandrasekhar, B., Attur, S., Dhaunsi, G. S., et al. (2015). The Dual Targeting of EGFR and ErbB2 with the Inhibitor Lapatinib Corrects High Glucose-Induced Apoptosis and Vascular Dysfunction by Opposing Multiple Diabetes-Induced Signaling Changes. *J. Drug Target.* 23 (6), 506–518. doi:10.3109/1061186x.2015.1057150

Benter, I. F., Yousif, M. H. M., Al-Saleh, F. M., Raghupathy, R., Chappell, M. C., and Diz, D. I. (2011). Angiotensin-(1-7) Blockade Attenuates Captopril- or Hydralazine-Induced Cardiovascular protection in Spontaneously Hypertensive Rats Treated with NG-nitro-L-arginine Methyl Ester. *J. Cardiovasc. Pharmacol.* 57 (5), 559–567. doi:10.1097/fjc.0b013e31821324b6

Benter, I. F., Yousif, M. H. M., Anim, J. T., Cojocel, C., and Diz, D. I. (2006). Angiotensin-(1-7) Prevents Development of Severe Hypertension and End-Organ Damage in Spontaneously Hypertensive Rats Treated with L-NAME. *Am. J. Physiology-Heart Circulatory Physiol.* 290 (2), H684–H691. doi:10.1152/ajpheart.00632.2005

Benter, I. F., Yousif, M. H. M., Cojocel, C., Al-Maghrebi, M., and Diz, D. I. (2007). Angiotensin-(1-7) Prevents Diabetes-Induced Cardiovascular Dysfunction. *Am. J. Physiology-Heart Circulatory Physiol.* 292 (1), H666–H672. doi:10.1152/ajpheart.00372.2006

Benter, I. F., Yousif, M. H. M., Dhaunsi, G. S., Kaur, J., Chappell, M. C., and Diz, D. I. (2008). Angiotensin-(1-7) Prevents Activation of NADPH Oxidase and Renal Vascular Dysfunction in Diabetic Hypertensive Rats. *Am. J. Nephrol.* 28 (1), 25–33. doi:10.1159/000108758

Benter, I. F., Yousif, M. H. M., Griffiths, S. M., Benboubetra, M., and Akhtar, S. (2005a). Epidermal Growth Factor Receptor Tyrosine Kinase-Mediated Signalling Contributes to Diabetes-Induced Vascular Dysfunction in the Mesenteric Bed. *Br. J. Pharmacol.* 145 (6), 829–836. doi:10.1038/sj.bjp.0706238

Benter, I. F., Yousif, M. H. M., Hollins, A. J., Griffiths, S. M., and Akhtar, S. (2005b). Diabetes-induced Renal Vascular Dysfunction Is Normalized by Inhibition of Epidermal Growth Factor Receptor Tyrosine Kinase. *J. Vasc. Res.* 42 (4), 284–291. doi:10.1159/000085904

Berk, B. C., Brock, T. A., Webb, R. C., Taubman, M. B., Atkinson, W. J., Gimbrone, M. A., Jr, et al. (1985). Epidermal Growth Factor, a Vascular Smooth Muscle Mitogen, Induces Rat Aortic Contraction. *J. Clin. Invest.* 75, 1083–1086. doi:10.1172/jci111772

Bhardwaj, P., Khanna, D., and Balakumar, P. (2014). Catechin Averts Experimental Diabetes Mellitus-Induced Vascular Endothelial Structural and Functional Abnormalities. *Cardiovasc. Toxicol.* 14 (1), 41–51. doi:10.1007/s12012-013-9226-y

Bi, H.-L., Zhang, X.-L., Zhang, Y.-L., Xie, X., Xia, Y.-L., Du, J., et al. (2020). The Deubiquitinase UCHL1 Regulates Cardiac Hypertrophy by Stabilizing Epidermal Growth Factor Receptor. *Sci. Adv.* 6 (16), eaax4826. doi:10.1126/sciadv.aax4826

Bian, K., Doursout, M.-F., and Murad, F. (2008). Vascular System: Role of Nitric Oxide in Cardiovascular Diseases. *J. Clin. Hypertens.* 10 (4), 304–310. doi:10.1111/j.1751-7176.2008.06632.x

Blaine, S. A., Ray, K. C., Branch, K. M., Robinson, P. S., Whitehead, R. H., and Means, A. L. (2009). Epidermal Growth Factor Receptor Regulates Pancreatic Fibrosis. *Am. J. Physiology-Gastrointestinal Liver Physiol.* 297 (3), G434–G441. doi:10.1152/ajpgi.00152.2009

Bodiga, V. L., Thokala, S., Vemuri, P. K., and Bodiga, S. (2015). Zinc Pyrithione Inhibits Caspase-3 Activity, Promotes ErbB1-ErbB2 Heterodimerization and Suppresses ErbB2 Downregulation in Cardiomyocytes Subjected to Ischemia/reperfusion. *J. Inorg. Biochem.* 153, 49–59. doi:10.1016/j.jinorgbio.2015.09.010

Brownlee, M., and Hirsch, I. B. (2006). Glycemic Variability: a Hemoglobin A1c-independent Risk Factor for Diabetic Complications. *JAMA* 295, 1707–1708. doi:10.1001/jama.295.14.1707

Brownlee, M. (2005). The Pathobiology of Diabetic Complications: a Unifying Mechanism. *Diabetes* 54 (6), 1615–1625. doi:10.2337/diabetes.54.6.1615

Caillaud, K., Boisseau, N., Ennequin, G., Chavanelle, V., Etienne, M., Li, X., et al. (2016). Neuregulin 1 Improves Glucose Tolerance in Adult and Old Rats. *Diabetes Metab.* 42 (2), 96–104. doi:10.1016/j.diabet.2015.08.003

Camenisch, T. D., Schroeder, J. A., Bradley, J., Klewer, S. E., and McDonald, J. A. (2002). Heart-valve Mesenchyme Formation Is Dependent on Hyaluronan-Augmented Activation of ErbB2-ErbB3 Receptors. *Nat. Med.* 8 (8), 850–855. doi:10.1038/nm742

Camprecios, G., Lorita, J., Pardina, E., Peinado-Onsurbe, J., Soley, M., and Ramírez, I. (2011). Expression, Localization, and Regulation of the Neuregulin Receptor ErbB3 in Mouse Heart. *J. Cell. Physiol.* 226, 450–455. doi:10.1002/jcp.22354

Ceriello, A. (2005). Postprandial Hyperglycemia and Diabetes Complications: Is it Time to Treat?. *Diabetes* 54, 1–7. doi:10.2337/diabetes.54.1.1

Chalmers, J., and Cooper, M. E. (2008). UKPDS and the Legacy Effect. *N. Engl. J. Med.* 359, 1618–1620. doi:10.1056/nejme0807625

Chan, R., Hardy, W. R., Laing, M. A., Hardy, S. E., and Muller, W. J. (2002). The Catalytic Activity of the ErbB-2 Receptor Tyrosine Kinase Is Essential for Embryonic Development. *Mol. Cel Biol* 22 (4), 1073–1078. doi:10.1128/mcb.22.4.1073-1078.2002

Chappell, M. C, Marshall, A. C., Alzayadneh, E. M., Shaltout, H. A., and Diz, D. I. (2014). Update on the Angiotensin converting enzyme 2-Angiotensin (1-7)-MAS Receptor Axis: Fetal Programing, Sex Differences, and Intracellular Pathways. *Front Endocrinol (Lausanne)* 9 (4), 201. doi:10.3389/fendo.2013.00201

Chen, B., Bronson, R. T., Klaman, L. D., Hampton, T. G., Wang, J.-f., Green, P. J., et al. (2000). Mice Mutant for Egfr and Shp2 Have Defective Cardiac Semilunar Valvulogenesis. *Nat. Genet.* 24 (3), 296–299. doi:10.1038/73528

Chen, J., Chen, J.-K., and Harris, R. C. (2015). EGF Receptor Deletion in Podocytes Attenuates Diabetic Nephropathy. *Jasn* 26 (5), 1115–1125. doi:10.1681/asn.2014020192

Chen, J., Zeng, F., Forrester, S. J., Eguchi, S., Zhang, M.-Z., and Harris, R. C. (2016). Expression and Function of the Epidermal Growth Factor Receptor in Physiology and Disease. *Physiol. Rev.* 96 (3), 1025–1069. doi:10.1152/physrev.00030.2015

Chen, L., Ackerman, R., and Guo, A. M. (2012). 20-HETE in Neovascularization. *Prostaglandins & Other Lipid Mediators* 98, 63–68. doi:10.1016/j.prostaglandins.2011.12.005

Chen, P., Guo, M., Wygle, D., Edwards, P. A., Falck, J. R., Roman, R. J., et al. (2005). Inhibitors of Cytochrome P450 4A Suppress Angiogenic Responses. *Am. J. Pathol.* 166 (2), 615–624. doi:10.1016/s0002-9440(10)62282-1

Cheng, J., Garcia, V., Ding, Y., Wu, C.-C., Thakar, K., Falck, J. R., et al. (2012). Induction of Angiotensin-Converting Enzyme and Activation of the Renin-Angiotensin System Contribute to 20-Hydroxyeicosatetraenoic Acid-Mediated Endothelial Dysfunction. *Atvb* 32, 1917–1924. doi:10.1161/atvbaha.112.248344

Cheng, J., Wu, C.-C., Gotlinger, K. H., Zhang, F., Falck, J. R., Narsimhaswamy, D., et al. (2010). 20-Hydroxy-5,8,11,14-eicosatetraenoic Acid Mediates Endothelial Dysfunction via IκB Kinase-dependent Endothelial Nitric-Oxide Synthase Uncoupling. *J. Pharmacol. Exp. Ther.* 332 (1), 57–65. doi:10.1124/jpet.109.159863

Childers, C. L., Tessier, S. N., and Storey, K. B. (2019). The Heart of a Hibernator: EGFR and MAPK Signaling in Cardiac Muscle during the Hibernation of Thirteen-Lined Ground squirrels,Ictidomys Tridecemlineatus. *PeerJ* 7, e7587. doi:10.7717/peerj.7587

Choi, S.-K., Galán, M., Partyka, M., Trebak, M., Belmadani, S., and Matrougui, K. (2012). Chronic Inhibition of Epidermal Growth Factor Receptor Tyrosine Kinase and Extracellular Signal-Regulated Kinases 1 and 2 (ERK1/2) Augments Vascular Response to Limb Ischemia in Type 2 Diabetic Mice. *Am. J. Pathol.* 180 (1), 410–418. doi:10.1016/j.ajpath.2011.09.016

Chong, W., Shastri, M., and Eri, R. (2017). Endoplasmic Reticulum Stress and Oxidative Stress: A Vicious Nexus Implicated in Bowel Disease Pathophysiology. *Ijms* 18 (4), 771. doi:10.3390/ijms18040771

Cohen, M. V., Philipp, S., Krieg, T., Cui, L., Kuno, A., Solodushko, V., et al. (2007). Preconditioning-mimetics Bradykinin and DADLE Activate PI3-Kinase through Divergent Pathways. *J. Mol. Cell. Cardiol.* 42 (4), 842–851. doi:10.1016/j.yjmcc.2007.01.004

Colom, C., Rull, A., Sanchez-Quesada, J. L., and Pérez, A. (2021). Cardiovascular Disease in Type 1 Diabetes Mellitus: Epidemiology and Management of Cardiovascular Risk. *Jcm* 10 (8), 1798. doi:10.3390/jcm10081798

Cooper, M., Bonnet, F., Oldfield, M., and Jandeleit-Dahm, K. (2001). Mechanisms of Diabetic Vasculopathy: an Overview. *Am. J. Hypertens.* 14 (5 Pt 1), 475–486. doi:10.1016/s0895-7061(00)01323-6

Crèvecoeur, I., Rondas, D., Mathieu, C., and Overbergh, L. (2015). The Beta-Cell in Type 1 Diabetes: What Have We Learned from Proteomic Studies?. *Prot. Clin. Appl.* 9 (7-8), 755–766. doi:10.1002/prca.201400135

Crone, S. A., Zhao, Y.-Y., Fan, L., Gu, Y., Minamisawa, S., Liu, Y., et al. (2002). ErbB2 Is Essential in the Prevention of Dilated Cardiomyopathy. *Nat. Med.* 8 (5), 459–465. doi:10.1038/nm0502-459

Cuomo, A., Rodolico, A., Galdieri, A., Russo, M., Campi, G., Franco, R., et al. (2019). Heart Failure and Cancer: Mechanisms of Old and New Cardiotoxic Drugs in Cancer Patients. *Card. Fail. Rev.* 5 (2), 112–118. doi:10.15420/cfr.2018.32.2

De Giusti, V. C., Nolly, M. B., Yeves, A. M., Caldiz, C. I., Villa-Abrille, M. C., Chiappe de Cingolani, G. E., et al. (2011). Aldosterone Stimulates the Cardiac Na +/H + Exchanger via Transactivation of the Epidermal Growth Factor Receptor. *Hypertension* 58 (5), 912–919. doi:10.1161/hypertensionaha.111.176024

De Keulenaer, G. W., Eline, Feyen., Lindsey, Dugaucquier., Shakeri., Hadis., Anastasia, Shchendrygina., Belenkov., Yury. N., et al. (2019). Mechanisms of the Multitasking Endothelial Protein NRG-1 as a Compensatory Factor during Chronic Heart Failure Circulation. *Heart Fail.* 12, e006288. doi:10.1161/circheartfailure.119.006288

Dhaunsi, G. S., Yousif, M. H., Akhtar, S., Chappell, M. C., Diz, D. I., and Benter, I. F. (2010). Angiotensin-(1-7) Prevents Diabetes-Induced Attenuation in PPAR-Gamma and Catalase Activities. *Eur. J. Pharmacol.* 638 (1-3), 108–114. doi:10.1016/j.ejphar.2010.04.030

Diamonti, A. J., Guy, P. M., Ivanof, C., Wong, K., Sweeney, C., and Carraway, K. L. (2002). An RBCC Protein Implicated in Maintenance of Steady-State Neuregulin Receptor Levels. *Proc. Natl. Acad. Sci.* 99, 2866–2871. doi:10.1073/pnas.052709799

Drucker, D. J. (2021). Diabetes, Obesity, Metabolism, and SARS-CoV-2 Infection: the End of the Beginning. *Cel Metab.* 33 (3), 479–498. doi:10.1016/j.cmet.2021.01.016

El-Hashim, A. Z., Khajah, M. A., Babyson, R. S., Renno, W. M., Ezeamuzie, C. I., Benter, I. F., et al. (2019). Ang-(1-7)/MAS1 Receptor axis Inhibits Allergic Airway Inflammation via Blockade of Src-Mediated EGFR Transactivation in a Murine Model of Asthma. *PLoS One* 14 (11), e0224163. doi:10.1371/journal.pone.0224163

Eldridge, S., Guo, L., Mussio, J., Furniss, M., Hamre, J., 3rd, and Davis, M. (2014). Examining the Protective Role of ErbB2 Modulation in Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes. *Toxicol. Sci.* 141 (2), 547–559. doi:10.1093/toxsci/kfu150

Erickson, S. L., O'Shea, K. S., Ghaboosi, N., Loverro, L., Frantz, G., Bauer, M., et al. (1997). ErbB3 Is Required for normal Cerebellar and Cardiac Development: A Comparison with ErbB2-And Heregulin-Deficient Mice. *Development* 124 (24), 4999–5011. doi:10.1242/dev.124.24.4999

Erqou, S., Lee, C.-T. C., Suffoletto, M., Echouffo-Tcheugui, J. B., de Boer, R. A., van Melle, J. P., et al. (2013). Association between Glycated Haemoglobin and the Risk of Congestive Heart Failure in Diabetes Mellitus: Systematic Review and Meta-Analysis. *Eur. J. Heart Fail.* 15, 185–193. doi:10.1093/eurjhf/hfs156

Falcão-Pires, I., and Leite-Moreira, A. F. (2012). Diabetic Cardiomyopathy: Understanding the Molecular and Cellular Basis to Progress in Diagnosis and Treatment. *Heart Fail. Rev.* 17 (3), 325–344. doi:10.1007/s10741-011-9257-z

Fan, F., Ge, Y., Lv, W., Elliott, M. R., Muroya, Y., Hirata, T., et al. (2016). Molecular Mechanisms and Cell Signaling of 20-hydroxyeicosatetraenoic Acid in Vascular Pathophysiology. *Front. Biosci. (Landmark Ed.* 21, 1427–1463. doi:10.2741/4379

Fan, F., Geurts, A. M., Murphy, S. R., Pabbidi, M. R., Jacob, H. J., and Roman, R. J. (2015). Impaired Myogenic Response and Autoregulation of Cerebral Blood Flow Is Rescued in CYP4A1 Transgenic Dahl Salt-Sensitive Rat. *Am. J. Physiology-Regulatory, Integr. Comp. Physiol.* 308, R379–R390. doi:10.1152/ajpregu.00256.2014

Fang, S.-J., Li, P.-Y., Wang, C.-M., Xin, Y., Lu, W.-W., Zhang, X.-X., et al. (2017). Inhibition of Endoplasmic Reticulum Stress by Neuregulin-1 Protects against Myocardial Ischemia/reperfusion Injury. *Peptides* 88, 196–207. doi:10.1016/j.peptides.2016.12.009

Feng, C., Jin, Z., Chi, X., Zhang, B., Wang, X., Sun, L., et al. (2018). SHBG Expression Is Correlated with PI3K/AKT Pathway Activity in a Cellular Model of Human Insulin Resistance. *Gynecol. Endocrinol.* 34 (7), 567–573. doi:10.1080/09513590.2017.1411474

Ferrario, C. M., Ahmad, S., Joyner, J., and Varagic, J. (2010). Advances in the Renin Angiotensin System. *Adv. Pharmacol.* 59, 197–233. doi:10.1016/S1054-3589(10)59007-0

Florian, J. A., and Watts, S. W. (1999). Epidermal Growth Factor: a Potent Vasoconstrictor in Experimental Hypertension. *Am. J. Physiology-Heart Circulatory Physiol.* 276 (3), H976–H983. doi:10.1152/ajpheart.1999.276.3.H976

Folino, A., Accomasso, L., Giachino, C., Montarolo, P. G., Losano, G., Pagliaro, P., et al. (2018). Apelin-induced Cardioprotection against Ischaemia/reperfusion Injury: Roles of Epidermal Growth Factor and Src. *Acta Physiol.* 222 (2), e12924. doi:10.1111/apha.12924

Forbes, J. M., and Cooper, M. E. (2013). Mechanisms of Diabetic Complications. *Physiol. Rev.* 93 (1), 137–188. doi:10.1152/physrev.00045.2011

Forrester, S. J., Kawai, T., O'Brien, S., Thomas, W., Harris, R. C., and Eguchi, S. (2016). Epidermal Growth Factor Receptor Transactivation: Mechanisms, Pathophysiology, and Potential Therapies in the Cardiovascular System. *Annu. Rev. Pharmacol. Toxicol.* 56, 627–653. doi:10.1146/annurev-pharmtox-070115-095427

Förstermann, U., and Sessa, W. C. (2012). Nitric Oxide Synthases: Regulation and Function. *Eur. Heart J.* 33 (7), 829–837. doi:10.1093/eurheartj/ehr304

Fountas, A., Diamantopoulos, L.-N., and Tsatsoulis, A. (2015). Tyrosine Kinase Inhibitors and Diabetes: A Novel Treatment Paradigm? *Trends Endocrinol. Metab.* 26 (11), 643–656. doi:10.1016/j.tem.2015.09.003

Fuller, S. J., Sivarajah, K., and Sugden, P. H. (2008). ErbB Receptors, Their Ligands, and the Consequences of Their Activation and Inhibition in the Myocardium. *J. Mol. Cell Cardiol.* 44 (5), 831–854. doi:10.1016/j.yjmcc.2008.02.278

Galán, M., Kassan, M., Choi, S.-K., Partyka, M., Trebak, M., Henrion, D., et al. (2012). A Novel Role for Epidermal Growth Factor Receptor Tyrosine Kinase and its Downstream Endoplasmic Reticulum Stress in Cardiac Damage and Microvascular Dysfunction in Type 1 Diabetes Mellitus. *Hypertension* 60 (1), 71–80. doi:10.1161/hypertensionaha.112.192500

Gao, R., Zhang, J., Cheng, L., Wu, X., Dong, W., Yang, X., et al. (2010). A Phase II, Randomized, Double-Blind, Multicenter, Based on Standard Therapy, Placebo-Controlled Study of the Efficacy and Safety of Recombinant Human Neuregulin-1 in Patients with Chronic Heart Failure. *J. Am. Coll. Cardiol.* 55, 1907–1914. doi:10.1016/j.jacc.2009.12.044

Garcia, V., and Schwartzman, M. L. (2017). Recent Developments on the Vascular Effects of 20-hydroxyeicosatetraenoic Acid. *Curr. Opin. Nephrol. Hypertens.* 26, 74–82. doi:10.1097/MNH.0000000000000302

Garcia, V., Gilani, A., Shkolnik, B., Pandey, V., Zhang, F. F., Dakarapu, R., et al. (2017). 20-HETE Signals through G-Protein-Coupled Receptor GPR75 (G Q)

to Affect Vascular Function and Trigger Hypertension. *Circ. Res.* 120, 1776–1788. doi:10.1161/circresaha.116.310525

Garcia, V., Joseph, G., Shkolnik, B., Ding, Y., Zhang, F. F., Gotlinger, K., et al. (2015). Angiotensin II Receptor Blockade or Deletion of Vascular Endothelial ACE Does Not Prevent Vascular Dysfunction and Remodeling in 20-HETE-dependent Hypertension. *Am. J. Physiology-Regulatory, Integr. Comp. Physiol.* 309, R71–R78. doi:10.1152/ajpregu.00039.2015

Garcia, V., Shkolnik, B., Milhau, L., Falck, J. R., and Schwartzman, M. L. (2016). 20-HETE Activates the Transcription of Angiotensin-Converting Enzyme via Nuclear Factor- B Translocation and Promoter Binding. *J. Pharmacol. Exp. Ther.* 356, 525–533. doi:10.1124/jpet.115.229377

García-Rivello, H., Taranda, J., Said, M., Cabeza-Meckert, P., Vila-Petroff, M., Scaglione, J., et al. (2005). Dilated Cardiomyopathy in Erb-B4-Deficient Ventricular Muscle. *Am. J. Physiology-Heart Circulatory Physiol.* 289 (3), H1153–H1160. doi:10.1152/ajpheart.00048.2005

Garratt, A. N., Voiculescu, O., Topilko, P., Charnay, P., and Birchmeier, C. (2000). A Dual Role of erbB2 in Myelination and in Expansion of the Schwann Cell Precursor Pool. *J. Cel Biol.* 148, 1035–1046. doi:10.1083/jcb.148.5.1035

Gassmann, M., Casagranda, F., Orioli, D., Simon, H., Lai, C., Klein, R., et al. (1995). Aberrant Neural and Cardiac Development in Mice Lacking the ErbB4 Neuregulin Receptor. *Nature* 378 (6555), 390–394. doi:10.1038/378390a0

Geraldes, P., Hiraoka-Yamamoto, J., Matsumoto, M., Clermont, A., Leitges, M., Marette, A., et al. (2009). Activation of PKC-δ and SHP-1 by Hyperglycemia Causes Vascular Cell Apoptosis and Diabetic Retinopathy. *Nat. Med.* 15 (11), 1298–1306. doi:10.1038/nm.2052

Giacco, F., and Brownlee, M. (2010). Oxidative Stress and Diabetic Complications. *Circ. Res.* 107 (9), 1058–1070. doi:10.1161/CIRCRESAHA.110.223545

Gilbert, R. E., Rumble, J. R., Cao, Z., Cox, A. J., van Eeden, P., Allen, T. J., et al. (2000). Endothelin Receptor Antagonism Ameliorates Mast Cell Infiltration, Vascular Hypertrophy, and Epidermal Growth Factor Expression in Experimental Diabetes. *Circ. Res.* 86 (2), 158–165. doi:10.1161/01.res.86.2.158

Gray, S. P., and Jandeleit-Dahm, K. (2014). The Pathobiology of Diabetic Vascular Complications-Cardiovascular and Kidney Disease. *J. Mol. Med.* 92 (5), 441–452. doi:10.1007/s00109-014-1146-1

Guo, A. M., Arbab, A. S., Falck, J. R., Chen, P., Edwards, P. A., Roman, R. J., et al. (2007). Activation of Vascular Endothelial Growth Factor through Reactive Oxygen Species Mediates 20-hydroxyeicosatetraenoic Acid-Induced Endothelial Cell Proliferation. *J. Pharmacol. Exp. Ther.* 321, 18–27. doi:10.1124/jpet.106.115360

Guo, A. M., Scicli, G., Sheng, J., Falck, J. C., Edwards, P. A., and Scicli, A. G. (2009). 20-HETE Can Act as a Nonhypoxic Regulator of HIF-1α in Human Microvascular Endothelial Cells. *Am. J. Physiology-Heart Circulatory Physiol.* 297, H602–H613. doi:10.1152/ajpheart.00874.2008

Guo, S., Okyere, A. D., McEachern, E., Strong, J. L., Carter, R. L., Patwa, V. C., et al. (2021). Epidermal Growth Factor Receptor-dependent Maintenance of Cardiac Contractility. *Cardiovasc. Res.*, cvab149. doi:10.1093/cvr/cvab149

Guo, Y. F., Zhang, X. X., Liu, Y., Duan, H. Y., Jie, B. Z., and Wu, X. S. (2012). Neuregulin-1 Attenuates Mitochondrial Dysfunction in a Rat Model of Heart Failure. *Chin. Med. J. (Engl)* 125, 807–814.

Haas, J., Frese, K. S., Park, Y. J., Keller, A., Vogel, B., Lindroth, A. M., et al. (2013). Alterations in Cardiac DNA Methylation in Human Dilated Cardiomyopathy. *EMBO Mol. Med.* 5, 413–429. doi:10.1002/emmm.201201553

Hao, L., Du, M., Lopez-Campistrous, A., and Fernandez-Patron, C. (2004). Agonist-induced Activation of Matrix Metalloproteinase-7 Promotes Vasoconstriction through the Epidermal Growth Factor-Receptor Pathway. *Circ. Res.* 94 (1), 68–76. doi:10.1161/01.res.0000109413.57726.91

Harskamp, L. R., Gansevoort, R. T., van Goor, H., and Meijer, E. (2016). The Epidermal Growth Factor Receptor Pathway in Chronic Kidney Diseases. *Nat. Rev. Nephrol.* 12 (8), 496–506. doi:10.1038/nrneph.2016.91

Hill, M. F., Patel, A. V., Murphy, A., Smith, H. M., Galindo, C. L., Pentassuglia, L., et al. (2013). Intravenous Glial Growth Factor 2 (GGF2) Isoform of Neuregulin-1 Beta Improves Left Ventricular Function, Gene and Protein Expression in Rats after Myocardial Infarction. *PLoS One* 8, e55741. doi:10.1371/journal.pone.0055741

Holman, R. R., Paul, S. K., Bethel, M. A., Matthews, D. R., and Neil, H. A. W. (2008). 10-year Follow-Up of Intensive Glucose Control in Type 2 Diabetes. *N. Engl. J. Med.* 359, 1577–1589. doi:10.1056/nejmoa0806470

Iacobini, C., Vitale, M., Pesce, C., Pugliese, G., and Menini, S. (2021). Diabetic Complications and Oxidative Stress: A 20-Year Voyage Back in Time and Back to the Future. *Antioxidants* 10 (5), 727. doi:10.3390/antiox10050727

IDF Diabetes Atlas (2019). *IDF Diabetes Atlas*. 9th edn.. retrieved from: https://www.diabetesatlas.org/en/sections/individual-social-and-economic-impact.html (accessed on April 20, 2021).

Ishizuka, T., Cheng, J., Singh, H., Vitto, M. D., Manthati, V. L., Falck, J. R., et al. (2008). 20-Hydroxyeicosatetraenoic Acid Stimulates Nuclear Factor-κB Activation and the Production of Inflammatory Cytokines in Human Endothelial Cells. *J. Pharmacol. Exp. Ther.* 324 (1), 103–110. doi:10.1124/jpet.107.130336

Iwamoto, R., Yamazaki, S., Asakura, M., Takashima, S., Hasuwa, H., Miyado, K., et al. (2003). Heparin-binding EGF-like Growth Factor and ErbB Signaling Is Essential for Heart Function. *Proc. Natl. Acad. Sci.* 100 (6), 3221–3226. doi:10.1073/pnas.0537588100

Jabbour, A., Hayward, C. S., Keogh, A. M., Kotlyar, E., McCrohon, J. A., England, J. F., et al. (2011). Parenteral Administration of Recombinant Human Neuregulin-1 to Patients with Stable Chronic Heart Failure Produces Favourable Acute and Chronic Haemodynamic Responses. *Eur. J. Heart Fail.* 13, 83–92. doi:10.1093/eurjhf/hfq152

Jin, Y., Ji, W., Yang, H., Chen, S., Zhang, W., and Duan, G. (2020). Endothelial Activation and Dysfunction in COVID-19: from Basic Mechanisms to Potential Therapeutic Approaches. *Sig Transduct Target. Ther.* 5, 293. doi:10.1038/s41392-020-00454-7

Ju, X., Yang, X., Yang, T., Chen, H., Song, Z., Zhang, Z., et al. (2019). EGFRinhibitor,AG1478, Inhibits Inflammatory Infiltration and Angiogenesis in Mice with Diabetic Retinopathy. *Clin. Exp. Pharmacol. Physiol.* 46 (1), 75–85. doi:10.1111/1440-1681.13029

Kassan, M., Ait-Aissa, K., Ali, M., Trebak, M., and Matrougui, K. (2015a). Augmented EGF Receptor Tyrosine Kinase Activity Impairs Vascular Function by NADPH Oxidase-dependent Mechanism in Type 2 Diabetic Mouse. *Biochim. Biophys. Acta (Bba) - Mol. Cel Res.* 1853 (10 Pt A), 2404–2410. doi:10.1016/j.bbamcr.2015.05.032

Kassan, M., Choi, S.-K., Galán, M., Trebak, M., Belmadani, S., and Matrougui, K. (2015b). Nuclear Factor Kappa B Inhibition Improves Conductance Artery Function in Type 2 Diabetic Mice. *Diabetes Metab. Res. Rev.* 31 (1), 39–49. doi:10.1002/dmrr.2542

Kawanabe, Y., Masaki, T., and Hashimoto, N. (2004). Involvement of Epidermal Growth Factor Receptor-Protein Tyrosine Kinase Transactivation in Endothelin-1-Induced Vascular Contraction. *J. Neurosurg.* 100 (6), 1066–1071. doi:10.3171/jns.2004.100.6.1066

Kenigsberg, B., Jain, V., and Barac, A. (2017). Cardio-oncology Related to Heart Failure. *Heart Fail. Clin.* 13 (2), 297–309. doi:10.1016/j.hfc.2016.12.002

Kenny, H. C., and Abel, E. D. (2019). Heart Failure in Type 2 Diabetes Mellitus. *Circ. Res.* 124 (1), 121–141. doi:10.1161/CIRCRESAHA.118.311371

Kilarkaje, N., Yousif, M. H. M., El-Hashim, A. Z., Makki, B., Akhtar, S., and Benter, I. F. (2013). Role of Angiotensin II and Angiotensin-(1-7) in Diabetes-Induced Oxidative DNA Damage in the Corpus Cavernosum. *Fertil. Sterility* 100 (1), 226–233. doi:10.1016/j.fertnstert.2013.02.046

Kirabo, A., Ryzhov, S., Gupte, M., Sengsayadeth, S., Gumina, R. J., Sawyer, D. B., et al. (2017). Neuregulin-1β Induces Proliferation, Survival and Paracrine Signaling in normal Human Cardiac Ventricular Fibroblasts. *J. Mol. Cell Cardiol.* 105, 59–69. doi:10.1016/j.yjmcc.2017.03.001

Kobayashi, T., and Eguchi, S. (2012). The Epidermal Growth Factor Receptor. *Hypertension* 60 (1), 20–21. doi:10.1161/hypertensionaha.112.197038

Kobayashi, T., Hayashi, Y., Taguchi, K., Matsumoto, T., and Kamata, K. (2006). ANG II Enhances Contractile Responses via PI3-Kinase P110δ Pathway in Aortas from Diabetic Rats with Systemic Hyperinsulinemia. *Am. J. Physiology-Heart Circulatory Physiol.* 291 (2), H846–H853. doi:10.1152/ajpheart.01349.2005

Kobayashi, T., Matsumoto, T., and Kamata, K. (2005). The PI3-K/Akt Pathway: Roles Related to Alterations in Vasomotor Responses in Diabetic Models. *J. Smooth Muscle Res.* 41 (6), 283–302. doi:10.1540/jsmr.41.283

Kobayashi, T., Taguchi, K., Yasuhiro, T., Matsumoto, T., and Kamata, K. (2004). Impairment of PI3-K/Akt Pathway Underlies Attenuated Endothelial Function in Aorta of Type 2 Diabetic Mouse Model. *Hypertension* 44 (6), 956–962. doi:10.1161/01.HYP.0000147559.10261.a7

Kosiborod, M., Gomes, M. B., Gomes, M. B., Nicolucci, A., Pocock, S., Rathmann, W., et al. (2018). Vascular Complications in Patients with

Type 2 Diabetes: Prevalence and Associated Factors in 38 Countries (The DISCOVER Study Program). *Cardiovasc. Diabetol.* 17, 150. doi:10.1186/s12933-018-0787-8

Krieg, T., Cui, L., Qin, Q., Cohen, M. V., and Downey, J. M. (2004). Mitochondrial ROS Generation Following Acetylcholine-Induced EGF Receptor Transactivation Requires Metalloproteinase Cleavage of proHB-EGF. *J. Mol. Cell Cardiol.* 36, 435–443. doi:10.1016/j.yjmcc.2003.12.013

Kumagai, S., Koyama, S., and Nishikawa, H. (2021). Antitumour Immunity Regulated by Aberrant ERBB Family Signalling. *Nat. Rev. Cancer* 21 (3), 181–197. doi:10.1038/s41568-020-00322-0

Lee, H.-Y., Youn, S.-W., Oh, B.-H., and Kim, H.-S. (2012). Krüppel-Like Factor 2 Suppression by High Glucose as a Possible Mechanism of Diabetic Vasculopathy. *Korean Circ. J.* 42 (4), 239–245. doi:10.4070/kcj.2012.42.4.239

Lee, J. W., Koeppen, M., Seo, S.-W., Bowser, J. L., Yuan, X., Li, J., et al. (2020). Transcription-independent Induction of ERBB1 through Hypoxia-Inducible Factor 2A Provides Cardioprotection during Ischemia and Reperfusion. *Anesthesiology* 132 (4), 763–780. doi:10.1097/aln.0000000000003037

Lee, K.-F., Simon, H., Chen, H., Bates, B., Hung, M.-C., and Hauser, C. (1995). Requirement for Neuregulin Receptor erbB2 in Neural and Cardiac Development. *Nature* 378 (6555), 394–398. doi:10.1038/378394a0

Lee, N. J., Ali, N., Zhang, L., Qi, Y., Clarke, I., Enriquez, R. F., et al. (2018). Osteoglycin, a Novel Coordinator of Bone and Glucose Homeostasis. *Mol. Metab.* 13, 30–44. doi:10.1016/j.molmet.2018.05.004

Leitner, D. R., Frühbeck, G., Yumuk, V., Schindler, K., Micic, D., Woodward, E., et al. (2017). Obesity and Type 2 Diabetes: Two Diseases with a Need for Combined Treatment Strategies - EASO Can Lead the Way. *Obes. Facts* 10 (5), 483–492. doi:10.1159/000480525

Lev-Ran, A., Hwang, D. L., Miller, J. D., and Josefsberg, Z. (1990). Excretion of Epidermal Growth Factor (EGF) in Diabetes. *Clinica Chim. Acta* 192 (3), 201–206. doi:10.1016/0009-8981(90)90222-e

Li, T., Yang, G.-m., Zhu, Y., Wu, Y., Chen, X.-y., Lan, D., et al. (2015). Diabetes and Hyperlipidemia Induce Dysfunction of VSMCs: Contribution of the Metabolic Inflammation/miRNA Pathway. *Am. J. Physiology-Endocrinology Metab.* 308 (4), E257–E269. doi:10.1152/ajpendo.00348.2014

Li, W., Fang, Q., Zhong, P., Chen, L., Wang, L., Zhang, Y., et al. (2016). EGFR Inhibition Blocks Palmitic Acid-Induced Inflammation in Cardiomyocytes and Prevents Hyperlipidemia-Induced Cardiac Injury in Mice. *Sci. Rep.* 6, 24580. doi:10.1038/srep24580

Liang, D., Zhong, P., Hu, J., Lin, F., Qian, Y., Xu, Z., et al. (2015). EGFR Inhibition Protects Cardiac Damage and Remodeling through Attenuating Oxidative Stress in STZ-Induced Diabetic Mouse Model. *J. Mol. Cell Cardiol.* 82, 63–74. doi:10.1016/j.yjmcc.2015.02.029

Liaudet, L., Calderari, B., and Pacher, P. (2014). Pathophysiological Mechanisms of Catecholamine and Cocaine-Mediated Cardiotoxicity. *Heart Fail. Rev.* 19 (6), 815–824. doi:10.1007/s10741-014-9418-y

Liu, L., Jin, X., Hu, C.-F., Zhang, Y.-P., Zhou, Z. e., Li, R., et al. (2018). Amphiregulin Enhances Cardiac Fibrosis and Aggravates Cardiac Dysfunction in Mice with Experimental Myocardial Infarction Partly through Activating EGFR-dependent Pathway. *Basic Res. Cardiol.* 113 (2), 12. doi:10.1007/s00395-018-0669-y

Liu, L., Song, S., Zhang, Y. P., Wang, D., Zhou, Z. e., Chen, Y., et al. (2020). Amphiregulin Promotes Cardiac Fibrosis post Myocardial Infarction by Inducing the Endothelial-Mesenchymal Transition via the EGFR Pathway in Endothelial Cells. *Exp. Cel Res.* 390 (2), 111950. doi:10.1016/j.yexcr.2020.111950

Liu, X., Gu, X., Li, Z., Li, X., Li, H., Chang, J., et al. (2006). Neuregulin-1/erbB-activation Improves Cardiac Function and Survival in Models of Ischemic, Dilated, and Viral Cardiomyopathy. *J. Am. Coll. Cardiol.* 48, 1438–1447. doi:10.1016/j.jacc.2006.05.057

Long, C., Cook, L. G., Hamilton, S. L., Wu, G.-Y., and Mitchell, B. M. (2007). FK506 Binding Protein 12/12.6 Depletion Increases Endothelial Nitric Oxide Synthase Threonine 495 Phosphorylation and Blood Pressure. *Hypertension* 49 (3), 569–576. doi:10.1161/01.hyp.0000257914.80918.72

Lorita, J., Escalona, N., Faraudo, S., Soley, M., and Ramirez, I. (2002). Effects of Epidermal Growth Factor on Epinephrine-Stimulated Heart Function in Rodents. *Am. J. Physiology-Heart Circulatory Physiol.* 283, H1887–H1895. doi:10.1152/ajpheart.00217.2002

Lucchesi, P. A., Sabri, A., Belmadani, S., and Matrougui, K. (2004). Involvement of Metalloproteinases 2/9 in Epidermal Growth Factor Receptor Transactivation in Pressure-Induced Myogenic Tone in Mouse Mesenteric Resistance Arteries. *Circulation* 110 (23), 3587–3593. doi:10.1161/01.CIR.0000148780.36121.47

Ma, J., and Jin, G. (2019). Epidermal Growth Factor Protects against Myocardial Ischaemia Reperfusion Injury through Activating Nrf2 Signalling Pathway. *Free Radic. Res.* 53, 313–323. doi:10.1080/10715762.2019.1584399

Mali, V., Haddox, S., Hornersmith, C., Matrougui, K., and Belmadani, S. (2018). Essential Role for EGFR Tyrosine Kinase and ER Stress in Myocardial Infarction in Type 2 Diabetes. *Pflugers Arch. - Eur. J. Physiol.* 470 (3), 471–480. doi:10.1007/s00424-017-2097-5

Matrougui, K. (2010). Diabetes and Microvascular Pathophysiology: Role of Epidermal Growth Factor Receptor Tyrosine Kinase. *Diabetes Metab. Res. Rev.* 26 (1), 13–16. doi:10.1002/dmrr.1050

Methner, C., Donat, U., Felix, S. B., and Krieg, T. (2009). Cardioprotection of Bradykinin at Reperfusion Involves Transactivation of the EGF Receptor via Metalloproteinase-8. *Acta Physiol. (Oxf)* 197, 265–271.

Meyrelles, S. S., Peotta, V. A., Pereira, T. M., and Vasquez, E. C. (2011). Endothelial Dysfunction in the Apolipoprotein E-Deficient Mouse: Insights into the Influence of Diet, Gender and Aging. *Lipids Health Dis.* 10, 211. doi:10.1186/1476-511x-10-211

Miller, R. G., and Orchard, T. J. (2020). Understanding Metabolic Memory: A Tale of Two Studies. *Diabetes* 69 (3), 291–299. doi:10.2337/db19-0514

Mitchell, M. J., Billingsley, M. M., Haley, R. M., Wechsler, M. E., Peppas, N. A., and Langer, R. (2021). Engineering Precision Nanoparticles for Drug Delivery. *Nat. Rev. Drug Discov.* 20 (2), 101–124. doi:10.1038/s41573-020-0090-8

Miyata, N., and Roman, R. J. (2005). Role of 20-hydroxyeicosatetraenoic Acid (20-HETE) in Vascular System. *J. Smooth Muscle Res.* 41 (4), 175–193. doi:10.1540/jsmr.41.175

Montero, D., Walther, G., Pérez-Martin, A., Vicente-Salar, N., Roche, E., and Vinet, A. (2013). Vascular Smooth Muscle Function in Type 2 Diabetes Mellitus: a Systematic Review and Meta-Analysis. *Diabetologia* 56 (10), 2122–2133. doi:10.1007/s00125-013-2974-1

Montezano, A. C., NguyenCat, Dinh. A., Rios, F. J., and Touyz, R. M. (2014). Angiotensin II and Vascular Injury. *Curr. Hypertens. Rep.* 16, 431. doi:10.1007/s11906-014-0431-2

Morano, M., Angotti, C., Tullio, F., Gambarotta, G., Penna, C., Pagliaro, P., et al. (2017). Myocardial Ischemia/reperfusion Upregulates the Transcription of the Neuregulin1 Receptor ErbB3, but Only Postconditioning Preserves Protein Translation: Role in Oxidative Stress. *Int. J. Cardiol.* 233, 73–79. doi:10.1016/j.ijcard.2017.01.122

Morgan, N. G., Leete, P., Foulis, A. K., and Richardson, S. J. (2014). Islet Inflammation in Human Type 1 Diabetes Mellitus. *IUBMB Life* 66 (11), 723–734. doi:10.1002/iub.1330

Murthy, S., Koval, O. M., Ramiro Diaz, J. M., Kumar, S., Nuno, D., Scott, J. A., et al. (2017). Endothelial CaMKII as a Regulator of eNOS Activity and NO-Mediated Vasoreactivity. *PloS one* 12 (10), e0186311. doi:10.1371/journal.pone.0186311

Muthalif, M. M., Benter, I. F., Karzoun, N., Fatima, S., Harper, J., Uddin, M. R., et al. (1998). 20-Hydroxyeicosatetraenoic Acid Mediates Calcium/calmodulin-dependent Protein Kinase II-Induced Mitogen-Activated Protein Kinase Activation in Vascular Smooth Muscle Cells. *Proc. Natl. Acad. Sci.* 95 (21), 12701–12706. doi:10.1073/pnas.95.21.12701

Nagareddy, P. R., MacLeod, K. M., and McNeill, J. H. (2010). GPCR Agonist-Induced Transactivation of the EGFR Upregulates MLC II Expression and Promotes Hypertension in Insulin-Resistant Rats. *Cardiovasc. Res.* 87 (1), 177–186. doi:10.1093/cvr/cvq030

Nägele, M. P., Haubner, B., Tanner, F. C., Ruschitzka, F., and Flammer, A. J. (2020). Endothelial Dysfunction in COVID-19: Current Findings and Therapeutic Implications. *Atherosclerosis* 314, 58–62. doi:10.1016/j.atherosclerosis.2020.10.014

Navid, F., and Colbert, R. A. (2017). Causes and Consequences of Endoplasmic Reticulum Stress in Rheumatic Disease. *Nat. Rev. Rheumatol.* 13, 25–40. doi:10.1038/nrrheum.2016.192

Negro, A., Brar, B. K., and Lee, K. F. (2004). Essential Roles of Her2/erbB2 in Cardiac Development and Function. *Recent Prog. Horm. Res.* 59 (1), 1–12. doi:10.1210/rp.59.1.1

Oellerich, M. F., and Potente, M. (2012). FOXOs and Sirtuins in Vascular Growth, Maintenance, and Aging. *Circ. Res.* 110 (9), 1238–1251. doi:10.1161/circresaha.111.246488

Palen, D. I., and Matrougui, K. (2008). Role of Elevated EGFR Phosphorylation in the Induction of Structural Remodelling and Altered Mechanical Properties of Resistance Artery from Type 2 Diabetic Mice. *Diabetes Metab. Res. Rev.* 24 (8), 651–656. doi:10.1002/dmrr.905

Pareja, M., Sánchez, O., Lorita, J., Soley, M., and Ramírez, I. (2003). Activated Epidermal Growth Factor Receptor (ErbB1) Protects the Heart against Stress-Induced Injury in Mice. *Am. J. Physiology-Regulatory, Integr. Comp. Physiol.* 285 (2), R455–R462. doi:10.1152/ajpregu.00588.2002

Patel, V. B., Parajuli, N., and Oudit, G. Y. (2014). Role of angiotensin-converting enzyme 2 (ACE2) in diabetic cardiovascular complications. *Clin Sci (Lond)* 126 (7), 471–82. doi:10.1042/CS20130344

Paz Ocaranza, M., Riquelme, J. A., García, L., Jalil, J. E., Chiong, M., Santos, R. A. S., et al. (2020). Counter-regulatory Renin-Angiotensin System in Cardiovascular Disease. *Nat. Rev. Cardiol.* 17 (2), 116–129. doi:10.1038/s41569-019-0244-8

Peng, K., Tian, X., Qian, Y., Skibba, M., Zou, C., Liu, Z., et al. (2016). Novel EGFR Inhibitors Attenuate Cardiac Hypertrophy Induced by Angiotensin II. *J. Cell. Mol. Med.* 20 (3), 482–494. doi:10.1111/jcmm.12763

Ponugoti, B., Dong, G., and Graves, D. T. (2012). Role of Forkhead Transcription Factors in Diabetes-Induced Oxidative Stress. *Exp. Diabetes Res.* 2012, 1–7. doi:10.1155/2012/939751

Prado, A. F., Pernomian, L., Azevedo, A., Costa, R. A. P., Rizzi, E., Ramos, J., et al. (2018). Matrix Metalloproteinase-2-Induced Epidermal Growth Factor Receptor Transactivation Impairs Redox Balance in Vascular Smooth Muscle Cells and Facilitates Vascular Contraction. *Redox Biol.* 18, 181–190. doi:10.1016/j.redox.2018.07.005

Qi, M., Zhou, Q., Zeng, W., Wu, L., Zhao, S., Chen, W., et al. (2018). Growth Factors in the Pathogenesis of Diabetic Foot Ulcers. *Front. Biosci. (Landmark Ed.* 23, 310–317. doi:10.2741/4593

Rajagopalan, V., Zucker, I. H., Jones, J. A., Carlson, M., and Ma, Y. J. (2008). Cardiac ErbB-1/ErbB-2 Mutant Expression in Young Adult Mice Leads to Cardiac Dysfunction. *Am. J. Physiology-Heart Circulatory Physiol.* 295, H543–H554. doi:10.1152/ajpheart.91436.2007

Rayego-Mateos, S., Rodrigues-Diez, R., Morgado-Pascual, J. L., Valentijn, F., Valdivielso, J. M., Goldschmeding, R., et al. (2018). Role of Epidermal Growth Factor Receptor (EGFR) and its Ligands in Kidney Inflammation and Damage. *Mediators Inflamm.* 2018, 1–22. doi:10.1155/2018/8739473

Reichelt, M. E., O'Brien, S., Thomas, W. G., and Headrick, J. P. (2017). Transactivation of the Epidermal Growth Factor Receptor in Responses to Myocardial Stress and Cardioprotection. *Int. J. Biochem. Cel Biol.* 83, 97–110. doi:10.1016/j.biocel.2016.12.014

Rocic, P., and Schwartzman, M. L. (2018). 20-HETE in the Regulation of Vascular and Cardiac Function. *Pharmacol. Ther.* 192, 74–87. doi:10.1016/j.pharmthera.2018.07.004

Roman, R. J. (2002). P-450 Metabolites of Arachidonic Acid in the Control of Cardiovascular Function. *Physiol. Rev.* 82, 131–185. doi:10.1152/physrev.00021.2001

Roskoski, R., Jr. (2019). Small Molecule Inhibitors Targeting the EGFR/ErbB Family of Protein-Tyrosine Kinases in Human Cancers. *Pharmacol. Res.* 139, 395–411. doi:10.1016/j.phrs.2018.11.014

Saltiel, A. R. (2021). Insulin Signaling in Health and Disease. *J. Clin. Invest.* 131 (1), e142241. doi:10.1172/JCI142241

Sanchez-Soria, P., and Camenisch, T. D. (2010). ErbB Signaling in Cardiac Development and Disease. *Semin. Cel Develop. Biol.* 21 (9), 929–935. doi:10.1016/j.semcdb.2010.09.011

Sankrityayan, H., Oza, M. J., Kulkarni, Y. A., Mulay, S. R., and Gaikwad, A. B. (2019). ER Stress Response Mediates Diabetic Microvascular Complications. *Drug Discov. Today* 24 (12), 2247–2257. doi:10.1016/j.drudis.2019.08.003

Sasahara, T., Okamoto, H., Ohkura, N., Kobe, A., and Yayama, K. (2015). Epidermal Growth Factor Induces Ca2+ Sensitization through Rho-kinase-dependent Phosphorylation of Myosin Phosphatase Target Subunit 1 in Vascular Smooth Muscle. *Eur. J. Pharmacol.* 762, 89–95. doi:10.1016/j.ejphar.2015.05.042

Schinzari, F., Tesauro, M., Veneziani, A., Mores, N., Di Daniele, N., and Cardillo, C. (2018). Favorable Vascular Actions of Angiotensin-(1-7) in Human Obesity. *Hypertension* 71, 185–191. doi:10.1161/HYPERTENSIONAHA.117.10280

Schreier, B., Gekle, M., and Grossmann, C. (2014). Role of Epidermal Growth Factor Receptor in Vascular Structure and Function. *Curr. Opin. Nephrol. Hypertens.* 23 (2), 113–121. doi:10.1097/01.mnh.0000441152.62943.29

Schreier, B., Hünerberg, M., Mildenberger, S., Rabe, S., Bethmann, D., Wickenhauser, C., et al. (2018). Deletion of the EGF Receptor in Vascular Smooth Muscle Cells Prevents Chronic Angiotensin II-Induced Arterial wall Stiffening and media Thickening. *Acta Physiol.* 222 (3), e12996. doi:10.1111/apha.12996

Schreier, B., Stern, C., Dubourg, V., Nolze, A., Rabe, S., Mildenberger, S., et al. (2021). Endothelial Epidermal Growth Factor Receptor Is of Minor Importance for Vascular and Renal Function and Obesity-Induced Dysfunction in Mice. *Sci. Rep.* 11, 7269. doi:10.1038/s41598-021-86587-3

Sena, C. M., Nunes, E., Louro, T., Proença, T., Fernandes, R., Boarder, M. R., et al. (2008). Effects of α-lipoic Acid on Endothelial Function in Aged Diabetic and High-Fat Fed Rats. *Br. J. Pharmacol.* 153 (5), 894–906. doi:10.1038/sj.bjp.0707474

Shah, S., Akhtar, M. S., Hassan, M. Q., Akhtar, M., Paudel, Y. N., and Najmi, A. K. (2018). EGFR Tyrosine Kinase Inhibition Decreases Cardiac Remodeling and SERCA2a/NCX1 Depletion in Streptozotocin Induced Cardiomyopathy in C57/BL6 Mice. *Life Sci.* 210, 29–39. doi:10.1016/j.lfs.2018.08.018

Sharifi, J., Khirehgesh, M. R., Safari, F., and Akbari, B. (2021). EGFR and Anti-EGFR Nanobodies: Review and Update. *J. Drug Target.* 29 (4), 387–402. doi:10.1080/1061186X.2020.1853756

Sheng, L., Bayliss, G., and Zhuang, S. (2021). Epidermal Growth Factor Receptor: A Potential Therapeutic Target for Diabetic Kidney Disease. *Front. Pharmacol.* 11, 598910. doi:10.3389/fphar.2020.598910

Shi, Y., and Vanhoutte, P. M. (2017). Macro- and Microvascular Endothelial Dysfunction in Diabetes. *J. Diabetes* 9 (5), 434–449. doi:10.1111/1753-0407.12521

Sibilia, M., and Wagner, E. (1995). Strain-dependent Epithelial Defects in Mice Lacking the EGF Receptor. *Science* 269 (5221), 234–238. doi:10.1126/science.7618085

Sodhi, K., Wu, C.-C., Cheng, J., Gotlinger, K., Inoue, K., Goli, M., et al. (2010). CYP4A2-Induced Hypertension Is 20-Hydroxyeicosatetraenoic Acid- and Angiotensin II-dependent. *Hypertension* 56, 871–878. doi:10.1161/hypertensionaha.110.154559

Souza, L. L., and Costa-Neto, C. M. (2012). Angiotensin-(1-7) Decreases LPS-Induced Inflammatory Response in Macrophages. *J. Cell. Physiol.* 227 (5), 2117–2122. doi:10.1002/jcp.22940

Stern, C., Schreier, B., Nolze, A., Rabe, S., Mildenberger, S., and Gekle, M. (2020). Knockout of Vascular Smooth Muscle EGF Receptor in a Mouse Model Prevents Obesity-Induced Vascular Dysfunction and Renal Damage *In Vivo*. *Diabetologia* 63, 2218–2234. doi:10.1007/s00125-020-05187-4

Sur, S., and Agrawal, D. (2014). Transactivation of EGFR by G Protein-Coupled Receptor in the Pathophysiology of Intimal Hyperplasia. *Cvp* 12 (2), 190–201. doi:10.2174/15701611126661402261237 45

Suryavanshi, S. V., and Kulkarni, Y. A. (2017). NF-κβ: A Potential Target in the Management of Vascular Complications of Diabetes. *Front. Pharmacol.* 8, 798. doi:10.3389/fphar.2017.00798

Taguchi, K., Kobayashi, T., Takenouchi, Y., Matsumoto, T., and Kamata, K. (2011). Angiotensin II Causes Endothelial Dysfunction via the GRK2/Akt/eNOS Pathway in Aortas from a Murine Type 2 Diabetic Model. *Pharmacol. Res.* 64 (5), 535–546. doi:10.1016/j.phrs.2011.05.001

Talukdar, S., Emdad, L., Das, S. K., and Fisher, P. B. (2020). EGFR: An Essential Receptor Tyrosine Kinase-Regulator of Cancer Stem Cells. *Adv. Cancer Res.* 147, 161–188. doi:10.1016/bs.acr.2020.04.003

Tan, S. Y., Mei Wong, J. L., Sim, Y. J., Wong, S. S., Mohamed Elhassan, S. A., Tan, S. H., et al. (2019). Type 1 and 2 Diabetes Mellitus: A Review on Current Treatment Approach and Gene Therapy as Potential Intervention. *Diabetes Metab. Syndr. Clin. Res. Rev.* 13 (Issue 1), 364–372. doi:10.1016/j.dsx.2018.10.008

Taniguchi, K., and Karin, M. (2018). NF-κB, Inflammation, Immunity and Cancer: Coming of Age. *Nat. Rev. Immunol.* 18, 309–324. doi:10.1038/nri.2017.142

Tanner, M. A., Thomas, T. P., and Grisanti, L. A. (2019). Death Receptor 5 Contributes to Cardiomyocyte Hypertrophy through Epidermal Growth Factor Receptor Transactivation. *J. Mol. Cell Cardiol.* 136, 1–14. doi:10.1016/j.yjmcc.2019.08.011

Tao, X., Fan, J., Kao, G., Zhang, X., Su, L., Yin, Y., et al. (2014). Angiotensin-(1-7) Attenuates Angiotensin II-Induced Signalling Associated with Activation of a

Tyrosine Phosphatase in Sprague-Dawley Rats Cardiac Fibroblasts. *Biol. Cel* 106 (6), 182–192. doi:10.1111/boc.201400015

Tenin, G., Clowes, C., Wolton, K., Krejci, E., Wright, J. A., Lovell, S. C., et al. (2014). Erbb2 Is Required for Cardiac Atrial Electrical Activity during Development. *PLoS One* 9 (9), e107041. doi:10.1371/journal.pone.0107041

Toi, P. L., Anothaisintawee, T., Chaikledkaew, U., Briones, J. R., Reutrakul, S., and Thakkinstian, A. (2020). Preventive Role of Diet Interventions and Dietary Factors in Type 2 Diabetes Mellitus: An Umbrella Review. *Nutrients* 12 (9), 2722. doi:10.3390/nu12092722

Tousoulis, D., Kampoli, A.-M., Tentolouris Nikolaos Papageorgiou, C., Stefanadis, C., and Stefanadis, C. (2012). The Role of Nitric Oxide on Endothelial Function. *Cvp* 10 (1), 4–18. doi:10.2174/157016112798829760

Touyz, R. M., Alves-Lopes, R., Rios, F. J., Camargo, L. L., Anagnostopoulou, A., Arner, A., et al. (2018). Vascular Smooth Muscle Contraction in Hypertension. *Cardiovasc. Res.* 114 (4), 529–539. doi:10.1093/cvr/cvy023

Tsatsoulis, A., Mantzaris, M. D., Bellou, S., and Andrikoula, M. (2013). Insulin Resistance: an Adaptive Mechanism Becomes Maladaptive in the Current Environment - an Evolutionary Perspective. *Metabolism* 62 (5), 622–633. doi:10.1016/j.metabol.2012.11.004

van Wijngaarden, R. P. T., Overbeek, J. A., Heintjes, E. M., Schubert, A., Diels, J., Straatman, H., et al. (2017). Relation between Different Measures of Glycemic Exposure and Microvascular and Macrovascular Complications in Patients with Type 2 Diabetes Mellitus: an Observational Cohort Study. *Diabetes Ther.* 8, 1097–1109. doi:10.1007/s13300-017-0301-4

Volpe, C. M. O., Villar-Delfino, P. H., dos Anjos, P. M. F., and Nogueira-Machado, J. A. (2018). Cellular Death, Reactive Oxygen Species (ROS) and Diabetic Complications. *Cell Death Dis* 9, 119. doi:10.1038/s41419-017-0135-z

Williams-Pritchard, G., Knight, M., Hoe, L. S., Headrick, J. P., and Peart, J. N. (2011). Essential Role of EGFR in Cardioprotection and Signaling Responses to A1 Adenosine Receptors and Ischemic Preconditioning. *Am. J. Physiology-Heart Circulatory Physiol.* 300 (6), H2161–H2168. doi:10.1152/ajpheart.00639.2010

Wong, T. Y., Cheung, C. M., Larsen, M., Sharma, S., and Simó, R. (2016). Diabetic Retinopathy. *Nat. Rev. Dis. Primers* 2, 16012. doi:10.1038/nrdp.2016.12

Xiao, J., Li, B., Zheng, Z., Wang, M., Peng, J., Li, Y., et al. (2012). Therapeutic Effects of Neuregulin-1 Gene Transduction in Rats with Myocardial Infarction. *Coron. Artery Dis.* 23, 460–468. doi:10.1097/mca.0b013e32835877da

Xu, J., Zhou, Q., Xu, W., and Cai, L. (2012). Endoplasmic Reticulum Stress and Diabetic Cardiomyopathy. *Exp. Diabetes Res.* 2012, 1–12. doi:10.1155/2012/827971

Xu, Z., and Cai, L. (2015). Diabetic Cardiomyopathy: Role of Epidermal Growth Factor Receptor Tyrosine Kinase. *J. Mol. Cell Cardiol.* 84, 10–12. doi:10.1016/j.yjmcc.2015.04.002

Xu, Z., Zhao, Y., Zhong, P., Wang, J., Weng, Q., Qian, Y., et al. (2017). EGFR Inhibition Attenuates Diabetic Nephropathy through Decreasing ROS and Endoplasmic Reticulum Stress. *Oncotarget* 8 (20), 32655–32667. doi:10.18632/oncotarget.15948

Yang, Q., Zhang, Y., Yin, H., and Lu, Y. (2020). Topical Recombinant Human Epidermal Growth Factor for Diabetic Foot Ulcers: A Meta-Analysis of Randomized Controlled Clinical Trials. *Ann. Vasc. Surg.* 62, 442–451. doi:10.1016/j.avsg.2019.05.041

Yano, S., Kondo, K., Yamaguchi, M., Richmond, G., Hutchison, M., Wakeling, A., et al. (2003). Distribution and Function of EGFR in Human Tissue and the Effect of EGFR Tyrosine Kinase Inhibition. *Anticancer Res.* 23 (5A), 3639–3650.

Yousif, M. H., Benter, I. F., and Roman, R. J. (2009a). Cytochrome P450 Metabolites of Arachidonic Acid Play a Role in the Enhanced Cardiac Dysfunction in Diabetic Rats Following Ischaemic Reperfusion Injury. *Auton. Autacoid Pharmacol.* 29 (1-2), 33–41. doi:10.1111/j.1474-8673.2009.00429.x

Yousif, M. H. M., Makki, B., El-Hashim, A. Z., Akhtar, S., and Benter, I. F. (2014). Chronic Treatment with Ang-(1-7) Reverses Abnormal Reactivity in the Corpus Cavernosum and Normalizes Diabetes-Induced Changes in the Protein Levels of ACE, ACE2, ROCK1, ROCK2 and omega-hydroxylase in a Rat Model of Type 1 Diabetes. *J. Diabetes Res.* 2014, 142154. doi:10.1155/2014/142154

Yousif, M. H. M., Akhtar, S., Walther, T., and Benter, I. F. (2008). Role of Ca2+/calmodulin-dependent Protein Kinase II in Development of Vascular Dysfunction in Diabetic Rats with Hypertension. *Cell Biochem. Funct.* 26 (2), 256–263. doi:10.1002/cbf.1446

Yousif, M. H. M., Benter, I. F., and Akhtar, S. (2005). The Role of Tyrosine Kinase-Mediated Pathways in Diabetes-Induced Alterations in Responsiveness of Rat Carotid Artery. *Autonom Auta Pharm.* 25 (2), 69–78. doi:10.1111/j.1474-8673.2004.00333.x

Yousif, M. H. M., Benter, I. F., Dunn, K. M. J., Dahly-Vernon, A. J., Akhtar, S., and Roman, R. J. (2009b). Role of 20-hydroxyeicosatetraenoic Acid in Altering Vascular Reactivity in Diabetes. *Auton. Autacoid Pharmacol.* 29 (1-2), 1–12. doi:10.1111/j.1474-8673.2009.00426.x

Yousif, M. H. M., Benter, I. F., Hares, N., Canatan, H., and Akhtar, S. (2006). Phosphoinositide 3-kinase Mediated Signalling Contributes to Development of Diabetes-Induced Abnormal Vascular Reactivity of Rat Carotid Artery. *Cel Biochem. Funct.* 24 (1), 13–22. doi:10.1002/cbf.1278

Yousif, M. H. M., Dhaunsi, G. S., Makki, B. M., Qabazard, B. A., Akhtar, S., and Benter, I. F. (2012). Characterization of Angiotensin-(1-7) Effects on the Cardiovascular System in an Experimental Model of Type-1 Diabetes. *Pharmacol. Res.* 66 (3), 269–275. doi:10.1016/j.phrs.2012.05.001

Yu, Y., Wei, S.-G., Weiss, R. M., and Felder, R. B. (2021). Silencing Epidermal Growth Factor Receptor in Hypothalamic Paraventricular Nucleus Reduces Extracellular Signal-Regulated Kinase 1 and 2 Signaling and Sympathetic Excitation in Heart Failure Rats. *Neuroscience* 463, 227–237. doi:10.1016/j.neuroscience.2021.01.025

Zhang, C., Booz, G. W., Yu, Q., He, X., Wang, S., and Fan, F. (2018). Conflicting Roles of 20-HETE in Hypertension and Renal End Organ Damage. *Eur. J. Pharmacol.* 833, 190–200. doi:10.1016/j.ejphar.2018.06.010

Zhang, Y., Murugesan, P., Huang, K., and Cai, H. (2020). NADPH Oxidases and Oxidase Crosstalk in Cardiovascular Diseases: Novel Therapeutic Targets. *Nat. Rev. Cardiol.* 17 (3), 170–194. doi:10.1038/s41569-019-0260-8

Zhang, Y., Zeng, Y., Wang, M., Tian, C., Ma, X., Chen, H., et al. (2011). Cardiacspecific Overexpression of E3 Ligase Nrdp1 Increases Ischemia and Reperfusioninduced Cardiac Injury. *Basic Res. Cardiol.* 106, 371–383. doi:10.1007/s00395-011-0157-0

Zhang, Z., and Zhang, D. (2020). (-)-Epigallocatechin-3-Gallate Inhibits eNOS Uncoupling and Alleviates High Glucose-Induced Dysfunction and Apoptosis of Human Umbilical Vein Endothelial Cells by PI3K/AKT/eNOS Pathway. *Dmso* 13, 2495–2504. doi:10.2147/DMSO.S260901

Zhao, Y., Vanhoutte, P. M., and Leung, S. W. S. (2015). Vascular Nitric Oxide: Beyond eNOS. *J. Pharmacol. Sci.* 129 (2), 83–94. doi:10.1016/j.jphs.2015.09.002

Zheng, K., Kitazato, K., and Wang, Y. (2014). Viruses Exploit the Function of Epidermal Growth Factor Receptor. *Rev Med Virol.* 24 (4), 274–86. doi:10.1002/rmv.1796

Zhou, J., Du, T., Li, B., Rong, Y., Verkhratsky, A., and Peng, L. (2015). Crosstalk between MAPK/ERK and PI3K/AKT Signal Pathways during Brain Ischemia/Reperfusion. *ASN Neuro* 7 (5), 175909141560246. doi:10.1177/1759091415602463

Zuo, C., Li, X., Huang, J., Chen, D., Ji, K., Yang, Y., et al. (2018). Osteoglycin Attenuates Cardiac Fibrosis by Suppressing Cardiac Myofibroblast Proliferation and Migration through Antagonizing Lysophosphatidic Acid 3/matrix Metalloproteinase 2/epidermal Growth Factor Receptor Signalling. *Cardiovasc. Res.* 114 (5), 703–712. doi:10.1093/cvr/cvy035

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Characterisation of the Myocardial Mitochondria Structural and Functional Phenotype in a Murine Model of Diabetic Cardiomyopathy

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People affected by diabetes are at an increased risk of developing heart failure than their non-diabetic counterparts, attributed in part to a distinct cardiac pathology termed diabetic cardiomyopathy. Mitochondrial dysfunction and excess reactive oxygen species (ROS) have been implicated in a range of diabetic complications and are a common feature of the diabetic heart. In this study, we sought to characterise impairments in mitochondrial structure and function in a recently described experimental mouse model of diabetic cardiomyopathy. Diabetes was induced in 6-week-old male FVB/N mice by the combination of three consecutive-daily injections of low-dose streptozotocin (STZ, each 55 mg/kg i.p.) and high-fat diet (42% fat from lipids) for 26 weeks. At study end, diabetic mice exhibited elevated blood glucose levels and impaired glucose tolerance, together with increases in both body weight gain and fat mass, replicating several aspects of human type 2 diabetes. The myocardial phenotype of diabetic mice included increased myocardial fibrosis and left ventricular (LV) diastolic dysfunction. Elevated LV superoxide levels were also evident. Diabetic mice exhibited a spectrum of LV mitochondrial changes, including decreased mitochondria area, increased levels of mitochondrial complex-III and complex-V protein abundance, and reduced complex-II oxygen consumption. In conclusion, these data suggest that the low-dose STZ-high fat experimental model replicates some of the mitochondrial changes seen in diabetes, and as such, this model may be useful to study treatments that target the mitochondria in diabetes.

Keywords: diabetes, heart, experimental – animal models, mitochondria, diabetic cardiomyopathy, mitochondrial function

Abbreviations: AMREP, Alfred Medical Research and Education Precinct; ANOVA, analysis of variance; AUC, area-under-the-curve; D, diabetic; H_2O_2, hydrogen peroxide; HbA1c, glycated haemoglobin; IPGTT, intraperitoneal glucose tolerance test; LV, left ventricular; ND, non-diabetic; ROS, reactive oxygen species; SEM, standard error of the mean; STZ, streptozotocin.

INTRODUCTION

The global prevalence of diabetes mellitus in adults has increased from an estimated 108 million in 1980, to 422 million by 2014, and is projected to reach 693 million by 2045 (Cho et al., 2018). Type 2 diabetes is the predominant type, accounting for approximately 90% of cases in adults (Zhou et al., 2016). Heart failure incidence is roughly 3-times greater in patients with concomitant diabetes, compared to patients without diabetes (Nichols et al., 2004). Furthermore, diabetes patients have considerably worse clinical outcomes associated with heart failure, in contrast to those without diabetes (Jia et al., 2018). This is in part explained by a distinct form of heart failure that can manifest in diabetes patients, termed diabetic cardiomyopathy. Diabetic cardiomyopathy is typified by maladaptive changes in cardiac structure and function that occur independently of other cardiovascular risk factors, including hypertension, coronary artery disease, and atherosclerosis (Rubler et al., 1972; Ritchie and Abel, 2020). Despite our progress in understanding this disease process, there are currently no effective treatments that specifically target the underlying pathogenesis contributing to diabetic cardiomyopathy (Marwick et al., 2018).

Excess levels of reactive oxygen species (ROS) are a common feature of the diabetic heart, and are thought to accelerate the progression of diabetic cardiomyopathy (Teshima et al., 2014; Ritchie and Abel, 2020). In diabetes, as endogenous antioxidants become overwhelmed, there is typically an imbalance between ROS generation and ROS removal, leading to oxidative stress in the heart (Liu et al., 2014; Xu et al., 2017). Importantly, mitochondria are a major site of ROS generation in the heart, due to the high energy demand and oxygen consumption (Kaludercic and Di Lisa, 2020). This is further exacerbated by metabolic inflexibility in diabetes, where there is abnormal myocardial substrate utilisation (Amaral and Okonko, 2015). Ultimately, these conditions trigger mitochondrial dysfunction, leading to increased electron leakage from the mitochondrial respiratory chain, potentiating excessive levels of ROS (Fillmore et al., 2014).

Conventional, widely-used experimental models of type 2 diabetes, such as the *db/db* mouse and *ob/ob* mouse, display increased fatty acid oxidation in the cardiac mitochondria, leading to mitochondrial dysfunction and oxidative stress (Mazumder et al., 2004; Boudina et al., 2007). However, these models possess several confounding factors, including impaired leptin signalling and supra-pathological obesity, that represent important limitations (Barouch et al., 2003; Burke et al., 2017). In a previous study from our laboratory, we described an alternative model of diabetic cardiomyopathy, that combined low-dose streptozotocin (STZ) with high-fat diet (Tate et al., 2019). Importantly, this experimental model has a milder systemic phenotype than the genetic models described above, whilst replicating several of the key clinical features of diabetic cardiomyopathy, including robust diastolic dysfunction and characteristic changes in myocardial structure in the context of elevated body weight and impaired glucose tolerance (Tate et al., 2019). Whilst this earlier study reported changes in the expression of genes associated with left ventricular (LV) mitochondrial function (Tate et al., 2019), the direct impact on mitochondrial morphology was beyond its scope. Therefore, this study sought to investigate the structure and function of cardiac mitochondria in this murine model of diabetic cardiomyopathy.

MATERIALS AND METHODS

Animals

The use of mice for this study was approved by the Alfred Medical Research and Education Precinct (AMREP) Animal Ethics Committee. All research activities involving animals were in accordance with the guidelines provided by the National Health and Medical Research Council of Australia for animal experimentation. Mice were sourced, housed, and maintained in the AMREP Animal Centre under a 12-h light/dark cycle at 22°C ± 1°C with access to food and water.

Experimental Design and Assessment of Systemic Phenotype

The primary aim of this study was to characterise the structure and function of cardiac mitochondria in a model of diabetic cardiomyopathy that combines low-dose STZ and high-fat diet (and which replicates many aspects of human type 2 diabetes as a result; Tate et al., 2019). We have included flow charts for reporting animal use in all experiments (**Supplementary Figure 1**). FVB/N male mice (6-week-old) were randomly allocated to the non-diabetic or diabetes group. To induce diabetes, mice received three consecutive-daily intraperitoneal (i.p.) injections of STZ (55 mg/kg/day, in 0.1 mol/L citric acid vehicle dissolved in saline). STZ-administered mice were then placed on a high-fat diet (42% energy from lipids; SF04-001, Speciality Feeds) for the remainder of the study (to induce diabetes, impair glucose tolerance and elevate body weight). Control mice received citrate vehicle followed by a standard laboratory chow diet for the study duration. Fortnightly blood glucose measurements using a glucometer (Accu-Chek, Roche) were carried out to assess the presence of diabetes via the saphenous vein. Diabetic mice received daily monitoring and husbandry. Toward study end, mice were individually housed in Promethion metabolic cages for 24 h (Sable Systems International). Individual cages were thermally controlled and contained a ceiling-mounted food hopper and water bottle. Cages contained a lid-mounted house that records body mass. A running wheel was available for the duration of the experiment (Soto et al., 2019). Whole body composition was analysed using an Echo-MRI^{TM} 4-in-1 700 Analyzer one week before tissue collection, as previously described (Tate et al., 2019). Intraperitoneal glucose tolerance tests (IPGTT) were performed one week prior to tissue collection. Mice were fasted for 5 h before recording baseline blood glucose levels. After mice received a glucose challenge (25% i.p.), blood glucose levels were measured using a glucometer (Accu-Chek, Roche) at 15, 30, 45, 60, 90, and 120 min after collecting a drop of blood from the saphenous vein (Tate et al., 2019). Glucose clearance rate was later determined by calculating area-under-the-curve (AUC) using the baseline blood glucose concentration for each experimental group. A minimum 24-h rest period was included between experiments. At study

endpoint, glycated haemoglobin (HbA1c) analysis was performed using the Cobas-b-101 POC system (Roche). Prior to tissue collection, mice were anaesthetised using ketamine/xylazine (85/8.5 mg/kg i.p.) followed by exsanguination via rapid removal of the heart. All mice were sacrificed for tissue collection at approximately the same time of each day (always between 9am and 12pm) to minimise any influence due to circadian effect. LV was dissected for immediate superoxide measurements, histological processing, morphological and functional assessment of mitochondria or snap-frozen in liquid nitrogen and stored at −80°C for subsequent biochemical analysis.

Assessment of Cardiac Phenotype: Fibrosis, ROS Detection, and LV Function

Myocardial Histology

LV tissues were placed in 10% neutral buffered formalin for 24 h before being embedded in paraffin. Paraffin-embedded LV tissues were cut (4 μm thick) and stained by the Monash University Histology Platform. Images were collected using an Olympus BX60 microscope. Picrosirius red stain was used to assess the level of LV interstitial collagen (polarised microscopy was used to specifically identify type I and III collagen). Scanned images were blinded then quantified using ImageJ software (picrosirius red at ×200 magnification, 10–32 images per sample; Tate et al., 2019).

Myocardial ROS Detection

LV superoxide levels were detected via lucigenin-enhanced chemiluminescence. During tissue collection, fresh LV sections (4 mm^3 × 1 mm^3 per sample) were placed into individual wells of an opaque 96-well optiplate (Perkin Elmer) containing 100 μL of Krebs-HEPES buffer. β-NADPH was added to three of the four tissue-containing wells, assigning one as the non-substrate control. After a 1-h incubation at 37°C, lucigenin (5 μM) was added to every well, before being placed into an EnSpire Plate reader (Perkin Elmer) for superoxide detection via chemiluminescence (Tate et al., 2019). An Amplex Red assay kit (Invitrogen) was used to assess LV hydrogen peroxide content, as per manufacturer's instructions. Hydrogen peroxide standards and LV protein samples were added to a black 96-well plate (Sigma-Aldrich). Standards and samples were then incubated for 30 min with Amplex Red/horseradish peroxidase (0.1 mM/0.2 U/ml, respectively) in the dark, prior to hydrogen peroxide detection using a fluorescence CLARIOstar plate reader (530 nm/590 nm excitation/emission).

Myocardial Function

One week prior to tissue collection, echocardiography was carried out in anaesthetised mice (ketamine/xylazine/atropine; 80/8/0.96 mg/kg i.p.) using the Vevo 2100 ultrasound machine (VisualSonics). Echocardiography was performed (technicians were blinded to treatment group) and validated by the Baker Institute Preclinical Cardiology Platform (Donner et al., 2018). M-mode function was used to assess LV chamber dimensions and fractional shortening. The B-mode function was used to measure LV volume and area during the systolic and diastolic phase to quantify ejection fraction, and cardiac output. The transmitral Doppler flow function was used to assess LV filling (early [E] and late [A] filling), E/A ratio and deceleration time. Tissue Doppler echocardiography technique was used to evaluate tissue velocity (early [e'] and late [a'] filling) and the e'/a' ratio.

Morphological Analysis of Mitochondria

At the time of tissue collection, fresh tissues were dissected (4 pieces each approximately 1 mm^3) from the apex of LV and placed in microfuge tubes (Eppendorf) containing 1 mL of fixative (2% paraformaldehyde, 2.5 glutaraldehyde in 0.1 M sodium-cacodylate buffer), before being processed by the Monash Ramaciotti Centre for Cryo-Electron Microscopy. Imaging was carried out by the Monash Ramaciotti Centre for Cryo-Electron Microscopy. Only one of the four pieces was utilised for imaging by electron microscopy. From this we obtained 10 images per mouse heart sample and analysed them using ImageJ software with the investigator blinded to the experimental groups. Total number of mitochondria or lipid droplets per image were counted and averaged per sample. The mitochondrial width (Feret diameter), area, length and aspect ratio, and lipid droplet count, were analysed.

Abundance of Mitochondrial Respiratory Chain Proteins

The abundance of mitochondrial respiratory chain proteins was determined via Western blot analysis of key subunits in each of complexes I-V. Protein was homogenised in ice-cold RIPA buffer using the Tissue Lyser II machine (Qiagen) at 30 Hz for 1 min. 30 μg of protein was loaded into 4–15% Tris-glycine gradient gel (Cat. no. 4561026, Bio Rad; 4–15% Mini-PROTEAN® TGX Protein Gels) and separated by SDS-PAGE gel electrophoresis. To assess relative protein abundance of mitochondrial complexes I-V, the membrane was incubated overnight with the Total OXPHOS Rodent WB Antibody Cocktail (Cat. no. ab110413, Abcam; 1:1000 dilution) or Anti-Calnexin C-Terminal Rabbit Polyclonal Antibody housekeeper (Cat. no. 208880, Abcam; 1:1000) at 4°C. A 60-min incubation was then carried out using a polyclonal rabbit anti-mouse immunoglobulins/horseradish peroxidase secondary antibody (1:3000 dilution, Dako), prior to membrane imaging using the ChemiDoc imaging system (Bio-Rad), and quantification using Image Lab software.

Assessment of Myocardial Mitochondrial Respiration

At study end, freshly dissected LV tissue was permeabilised in saponin (50 μg/mL)/biopsy preservation solution (BIOPS: 10 mM Ca-EGTA buffer, 0.1 μM free calcium, 20 mM imidazole, 20 mM taurine, 50 mM K-MES, 0.5 mM DTT, 6.56 mM $MgCl_2$, 5.77 mM adenosine triphosphate (ATP), 15 mM phosphocreatine, pH 7.1) for 20 min at 4°C with gentle rocking, as previously described (Horscroft et al., 2015). Tissues were washed three times (5-min intervals) with mitochondrial respiratory medium (MiR05: 0.5 mM EGTA, 3 mM $MgCl_2.6H_2O$, 60 mM K-lactobionate, 20 mM taurine, 10 mM KH_2PO_4, 20 mM HEPES, 110 mM sucrose, 1 g/L, defatted BSA, pH 7.4; (Horscroft et al., 2015). Samples (1–2 mg) were then placed in the Oxygraph-O2k (Oroboros Instruments, Innsbruck, Austria) chambers containing 2 mL MiR05 at 37°C. A substrate-uncoupler-inhibitor

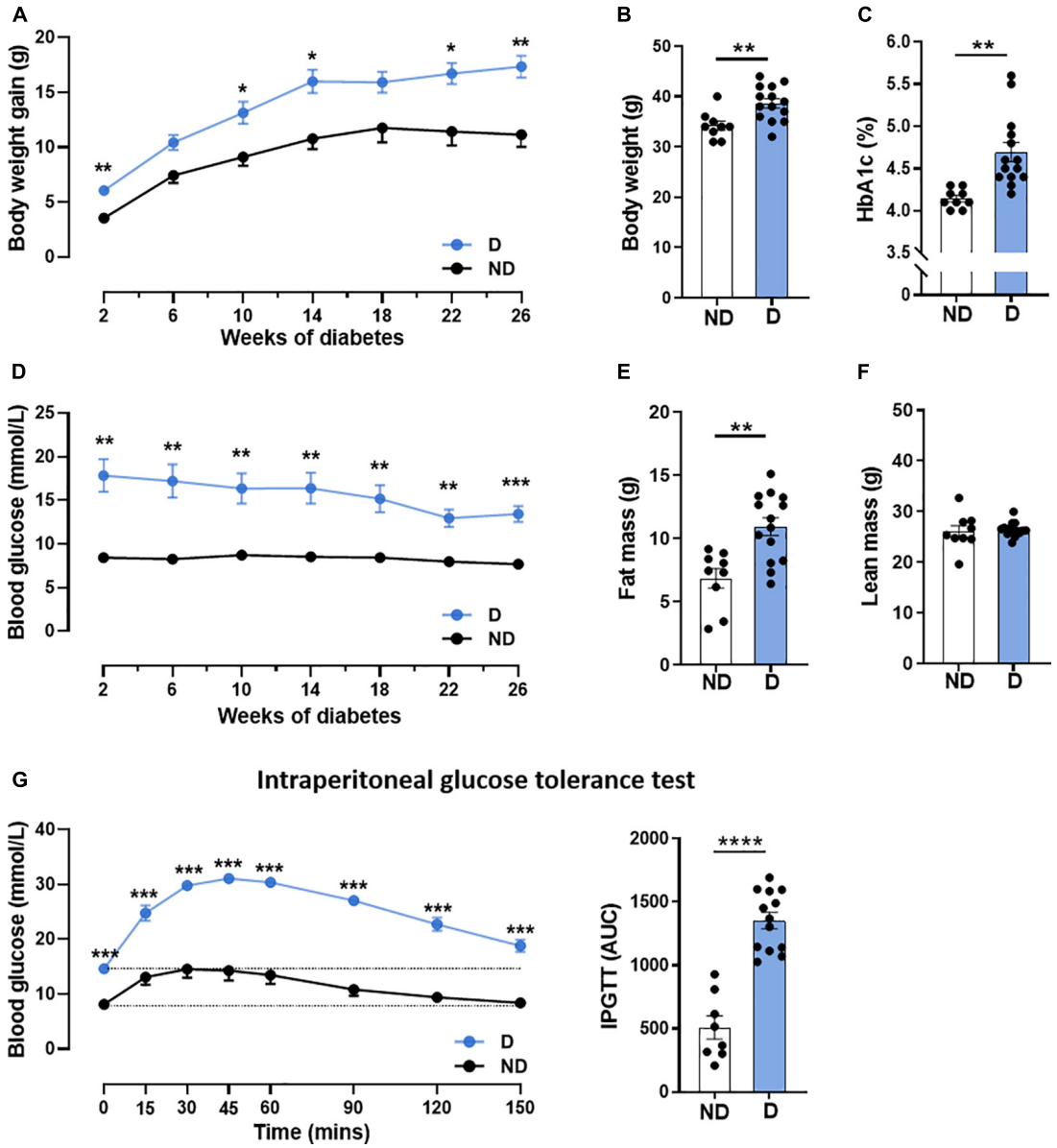

FIGURE 1 | Characterisation of systemic phenotype in diabetic mice. **(A)** Body weight gain over time, **(B)** end-point body weight, **(C)** glycated haemoglobin (HbA1c), **(D)** blood glucose over time, **(E)** fat mass, **(F)** lean mass, **(G)** intraperitoneal glucose tolerance test (IPGTT); dotted line indicates basal glucose for each group where AUC is calculated. Data represented as mean ± SEM. n = 8–14 per group (note individual data points). Data analysis used unpaired t-test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. ND, non-diabetic; D, diabetes; AUC, area under curve.

titration (SUIT) protocol (Pesta and Gnaiger, 2012) was used as follows: 5 mM Glutamate and 10 mM malate were added to assess leak respiration (L) in the absence of adenylates and limitation of flux by electron input through complex I (CI_{LEAK}); 1 mM adenosine diphosphate was added to asses OXPHOS capacity [P] with limitation of flux by electron input through CI (CI_{OXPHOS}); complex I was then inhibited by addition of 0.5 μM rotenone, and 10 mM succinate were subsequently added to asses [P] with limitation of flux by electron input through CII (CII_{OXPHOS}); 10 μM cytochrome *c* was added to test for outer mitochondrial membrane integrity (not shown in representative trace), before final addition of 5 μM antimycin A to determine the residual non-mitochondrial oxygen consumption [ROX]. Oxygen concentration in the chambers was maintained between 250 and 400 μM. Data of mitochondrial respiration were normalised by wet tissue mass and are presented as [pmol O2/s/mg wet weight].

Gene Expression Analysis

RNA extraction was performed using the commercially-available GenElute^{TM} Mammalian Total RNA Miniprep Kit

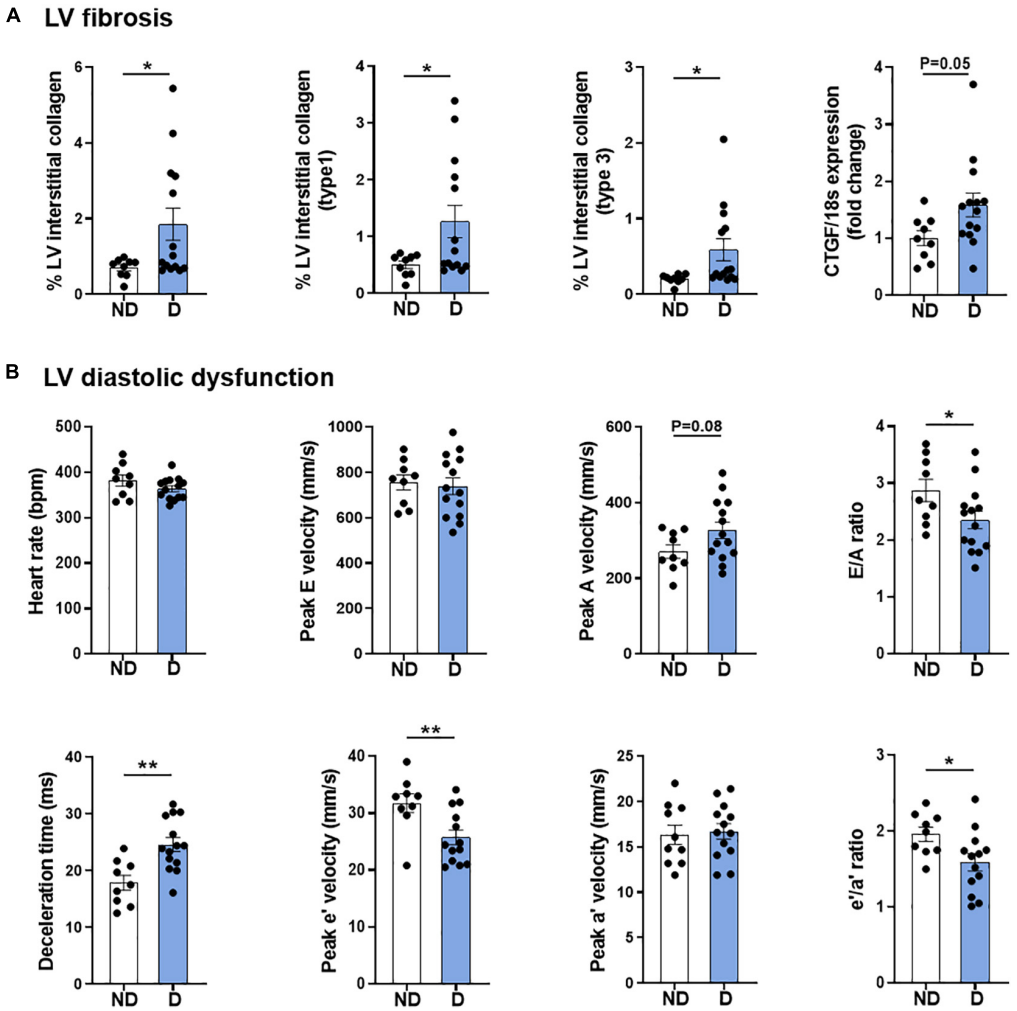

FIGURE 2 | Cardiac phenotype of diabetic mice. **(A)** LV fibrosis: Total interstitial collagen, type 1 collagen and type 3 collagen analysis using polarised light in picrosirius-red stained LV sections, and gene expression of fibrosis marker connective tissue growth factor (CTGF). **(B)** LV diastolic function: Heart rate, peak E wave velocity, peak A wave velocity, E/A ratio, deceleration time, peak e' wave velocity, peak a' wave velocity, and e'/a' ratio. Data represented as mean ± SEM. n = 9–14 per group (note individual data points). Data analysis used unpaired t-test. $^{*}P < 0.05$, $^{**}P < 0.01$. ND, non-diabetic; D, diabetes; H_2O_2, hydrogen peroxide; LV, left ventricle; NOX, NADPH oxidase; ROS, reactive oxygen species.

(Sigma-Aldrich) and reversed-transcribed using the high-capacity cDNA reverse-transcription kit (Thermo Fisher Scientific), both as per the manufacturer's instructions. LV gene expression was carried out using real-time PCR with SYBR green chemistry (ThermoFisher). Primers were generated using Primer3 Plus from mouse sequences in PubMed gene database (**Supplementary Table 1**). Relative gene expression was detected and quantified via QuantStudio7 Flex system (Applied Biosystems), using the comparative delta-delta cycle threshold ($\Delta\Delta$CT) method to determine fold-change relative to non-diabetic mice.

Statistical Analysis

Data analysis was performed using GraphPad Prism 9.0.0 statistical software. Comparisons between treatment groups were analysed using unpaired t-test. $P < 0.05$ was considered statistically significant.

RESULTS

Confirmation of Systemic Phenotype in Diabetic Mice

Patients with diabetes commonly exhibit an increase in blood glucose with altered glucose handling and weight gain (Ritchie and Abel, 2020). Hence, we first confirmed that diabetic mice receiving high-fat diet in combination with STZ in this study exhibited significantly higher weight gain throughout the 26 weeks of diabetes (**Figure 1A**), leading to an increase in body weight at study end (**Figure 1B**). No differences were seen in tibial length, a marker of animal size, between diabetic mice

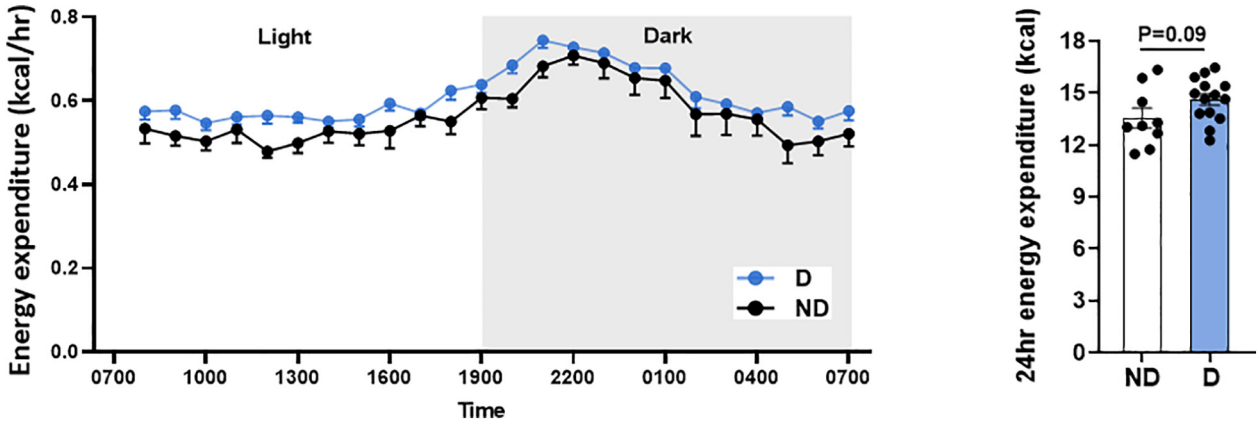

FIGURE 3 | Energy expenditure over 24 h in diabetic mice. Data represented as mean ± SEM. *n* = 9–14 per group (note individual data points). Data analysis used unpaired *t*-test. ND, non-diabetic; D, diabetes.

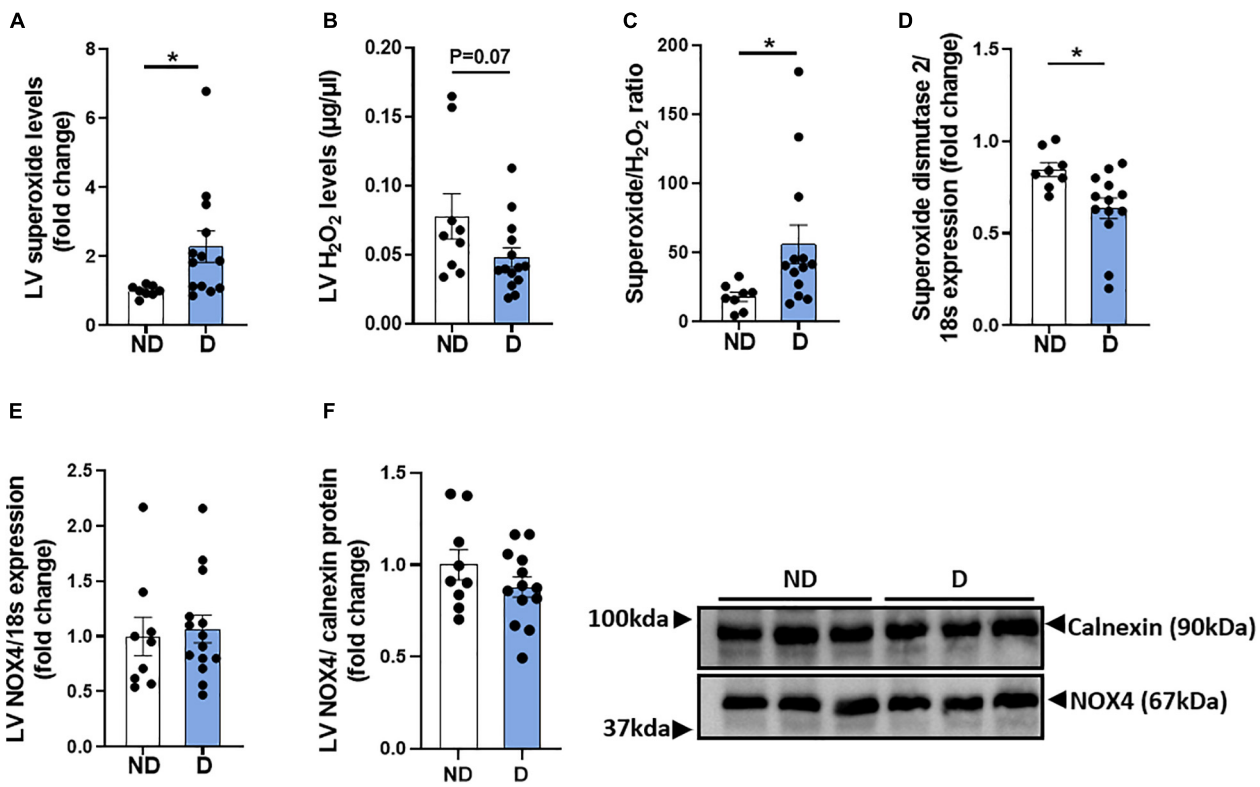

FIGURE 4 | LV ROS production in diabetic mice. **(A)** LV Superoxide generation, **(B)** LV H_2O_2 generation, **(C)** LV superoxide/H_2O_2 ratio. Markers of LV ROS generation; gene expression of **(D)** superoxide dismutase-2 and **(E)** NADPH oxidase-4 (NOX4), and **(F)** protein abundance of NOX4. Data represented as mean ± SEM. *n* = 8–14 per group (note individual data points). Data analysis used unpaired *t*-test. *$P < 0.05$. ND, non-diabetic; D, diabetes.

and non-diabetic mice (**Supplementary Table 2**). At study end, diabetic mice had elevated glycated haemoglobin (HbA1c) levels, a measure of long-term blood glucose levels (**Figure 1C**). Diabetic mice also exhibited a significant increase in blood glucose levels by the 2-week timepoint, and this remained elevated for the duration of the study (**Figure 1D**). Whole body composition analysis in conscious mice was assessed using Echo-MRI; this revealed a significantly increased fat mass in diabetic mice, compared to non-diabetic control mice (**Figure 1E**), however, there was no difference in lean mass between groups (**Figure 1F**). Glucose tolerance tests are a routine clinical method to assess glucose handling (Marwick et al., 2018). Glucose tolerance was significantly impaired in diabetic mice over the course of 150 min, as demonstrated by the larger area-under-the-curve

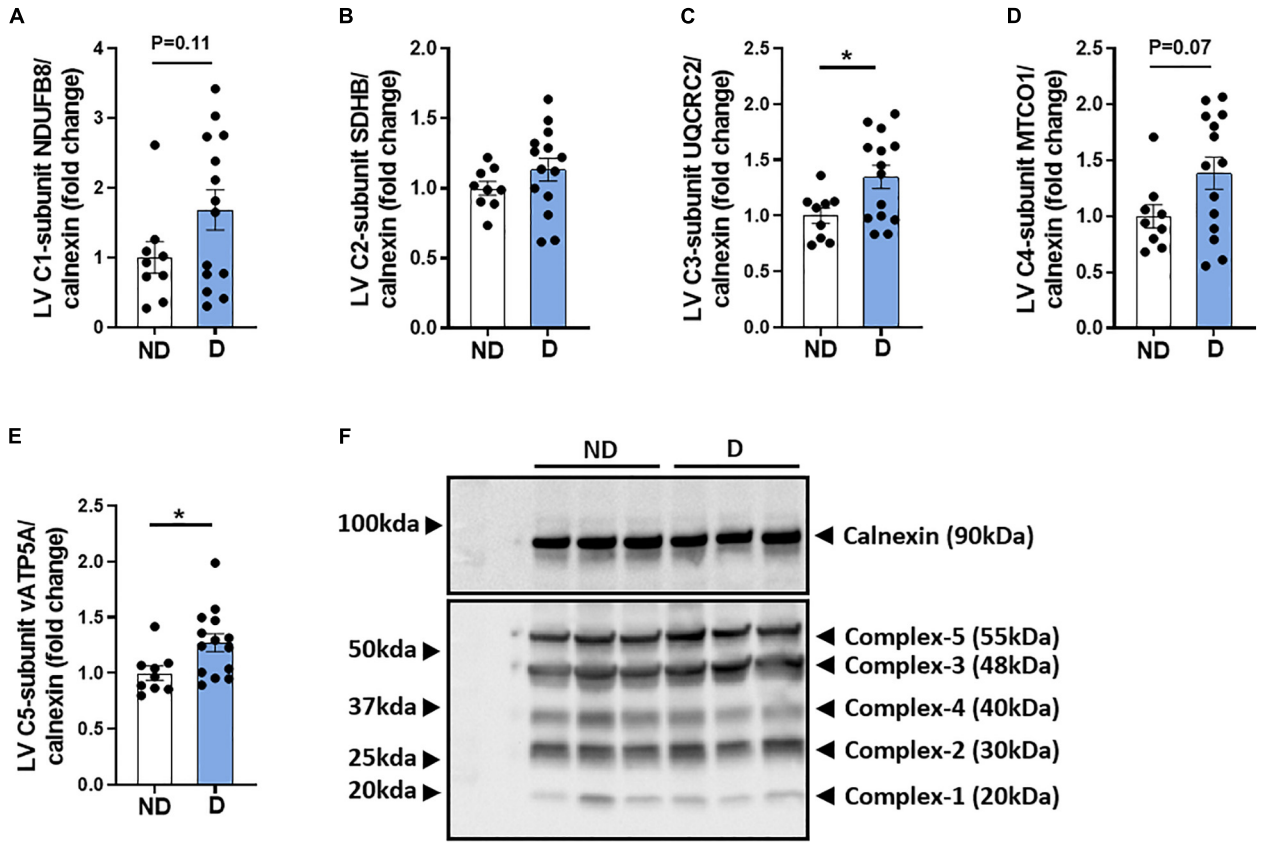

FIGURE 5 | Protein abundance of mitochondria complexes in diabetic mice. Western blot quantification of **(A)** LV complex-I-subunit NDUFB8/calnexin, **(B)** LV complex-II-subunit SDHB/calnexin, **(C)** LV complex-III-subunit UQCRC2/calnexin, **(D)** LV complex-IV -subunit MTCO1/calnexin, **(E)** LV complex-V -subunit vATP5A/calnexin. **(F)** Representative blot images of mitochondria complexes. Data represented as mean ± SEM. n = 9–14 per group (note individual data points). Data analysis used unpaired t-test. *$P < 0.05$. ND, non-diabetic; D, diabetes; LV, left ventricle.

(**Figure 1G**). Diabetic mice also exhibited larger liver and spleen weights (**Supplementary Table 2**) compared to their non-diabetic counterparts. The weights of individual fat pads, including perirenal and inguinal fat, were also significantly elevated in diabetic mice (**Supplementary Table 2**).

Confirmation of Cardiac Phenotype in Diabetic Mice

As described previously, this model combining low-dose STZ superimposed on high-fat diet mimics several features of diabetic cardiomyopathy, including LV fibrosis and ROS generation, and most importantly, robust LV diastolic dysfunction at 26 weeks (**Figure 2**; Tate et al., 2019). Corroborating observations from our previous publication using a different cohort of mice (Tate et al., 2019), whole heart, LV, right ventricle, and atria weights were unchanged with diabetes (**Supplementary Table 2**). These observations corresponded with characteristic cardiac structural changes typically observed in diabetic cardiomyopathy, including an increase in myocardial fibrosis, as highlighted by an increase in LV interstitial collagen deposition (**Figure 2A**), and an increase in the mRNA expression of pro-fibrotic growth factor, connective tissue growth factor (CTGF; **Figure 2A**). As mentioned, diastolic dysfunction was present in diabetic mice as highlighted by several markers, including E/A ratio, deceleration time and e'/a' ratio (**Figure 2B**). Importantly, heart rate was the same in both groups (**Figure 2B**).

Impact of Diabetes on Systemic Energy Expenditure

Type 2 diabetes patients commonly have higher energy expenditure (Bitz et al., 2004). In this study, there was a tendency toward higher energy expenditure over 24 h in diabetic mice, however, this was not statistically different (**Figure 3**).

Impact of Diabetes on LV ROS Production

Diabetes-induced ROS generation and subsequent oxidative stress are contributing factors in the development and progression of diabetic cardiomyopathy (Jia et al., 2018). LV Superoxide levels were elevated in diabetic mice in this experimental model of diabetic cardiomyopathy (**Figure 4A**). There was also a trend toward a reduction in hydrogen peroxide levels in diabetic mice, compared to non-diabetic mice (**Figure 4B**). Accordingly, diabetic mice exhibited a

significant increase in the LV superoxide/hydrogen peroxide ratio (**Figure 4C**). LV mRNA expression levels of superoxide dismutase-2 was significantly lower in diabetic mice, compared to non-diabetic mice (**Figure 4D**). No differences were observed in LV mRNA expression or protein abundance of the NADPH-oxidase subunit NOX4 (**Figures 4E,F**).

Abundance of Mitochondrial Respiratory Chain Proteins in Diabetic Mice

The mitochondria respiratory chain is a major contributor to excess ROS levels and the subsequent oxidative damage that is characteristic of the diabetic heart (Ding et al., 2019; Kaludercic and Di Lisa, 2020). There was a tendency toward an increase in complex-I protein abundance in diabetic mice (**Figure 5A**). No difference was observed in complex-II abundance between groups (**Figure 5B**). Diabetic mice exhibited a significant increase in complex-III protein abundance (**Figure 5C**), a tendency for elevated complex-IV protein abundance (**Figure 5D**), and a significantly elevated complex-V protein abundance (**Figure 5E**). Representative images of individual mitochondrial protein complex abundance in diabetic and non-diabetic mice are shown (**Figure 5F**).

Characterisation of LV Mitochondrial Morphology in Diabetic Mice

Changes in mitochondrial ultrastructure have previously been observed in the diabetic heart, in both the clinical and experimental setting (Dabkowski et al., 2010; Montaigne et al., 2014; Daghistani et al., 2019). In the current study, no differences were observed in the total number of mitochondria, or in mitochondrial length between experimental groups (**Figures 6A,B**). Interestingly, diabetic mice showed a significant decrease in mitochondria width and area (**Figures 6C,D**). The mitochondrial aspect ratio was not altered by diabetes (ND: 0.646 ± 0.009; D: 0.616 ± 0.017, NS). The total number of lipid droplets was elevated in diabetic mice (**Figure 6E**). Accordingly, the lipid droplet number to mitochondria ratio was significantly higher in diabetic mice (**Figure 6F**). Representative electron microscopy images of cardiac mitochondria are displayed (**Figure 6G**). Western blot analysis showed no differences in the protein expression of LV dynamin-related protein 1 (DRP1) (**Figure 6H**), however, there was a tendency for diabetic mice to exhibit lower protein expression of LV Mitofusin 1 (MFN1) (**Figure 6I**), and a significant reduction in LV Mitofusin 2 (MFN2) (**Figure 6J**). Representative western blot images are presented (**Figure 6K**).

Characterisation of Cardiac Mitochondrial Respiratory Function in Diabetic Mice

Changes in cardiac mitochondrial electron transport chain activity and mitochondrial respiration have previously been observed in patients with diabetes (Montaigne et al., 2014). In this study high resolution respirometry was carried out using the Oroboros Instruments Oxygraph-O2k to assess cardiac mitochondrial function. **Figure 7A** displays a representative trace of oxygen consumption over time in non-diabetic and diabetic mice. No differences were seen in $[CI]_{LEAK}$ between groups (**Figure 7B**), however, there was a tendency toward reduced CI-linked mitochondrial respiration $[CI]_{OXPHOS}$ in diabetic mice (**Figure 7C**). Diabetic mice also displayed a significant decrease in CII-linked mitochondrial respiration (**Figure 7D**). Gene expression analysis of LV tissue revealed a significant increase in mitochondrial uncoupled protein-3 in diabetic mice (UCP3; **Figure 7E**), compared to non-diabetic mice.

DISCUSSION

This study builds upon previous reports from our laboratory describing an experimental model of diabetic cardiomyopathy that recapitulates several of the clinical features of human disease, including robust diastolic dysfunction and myocardial structure changes. The model combines a low-dose STZ approach with high-fat diet (Tate et al., 2019). Mitochondrial dysfunction and an overproduction of ROS are common features of the diabetic heart, as well as major drivers of cardiac remodelling (Gollmer et al., 2020). In our previous study we noted changes in the expression of genes associated with LV mitochondrial respiratory function, providing a rationale to study mitochondria structure and function in more detail in this model (Tate et al., 2019). Mitochondrial dysfunction and oxidative stress are present in commonly-utilised experimental models of diabetes, including the *db/db* and *ob/ob* spontaneous genetic models (Mazumder et al., 2004; Boudina et al., 2007). However, these models have confounding factors, including impaired leptin signalling and supra-pathological obesity, that need to be taken into consideration when interpreting findings (Barouch et al., 2003; Burke et al., 2017). Therefore, introducing a new experimental model to the toolkit of biomedical scientists, with a milder phenotype, will help us to further understand the underlying mechanisms of diabetic cardiomyopathy.

In this study we followed mice for 26 weeks from the induction of diabetes. As expected, given the results of our previous work, diabetic mice gained weight progressively over the duration of the study, primarily due to an increase in fat mass. Mice had a mild elevation in blood glucose and glycated haemoglobin, as well as a tendency for higher energy expenditure. A study in type 2 diabetes patients reported an elevation in 24 h energy expenditure (Bitz et al., 2004). Interestingly, studies have also shown that insulin signalling in the brain can influence whole body energy expenditure (Dodd and Tiganis, 2017; Loh et al., 2017). In relation to cardiac changes, the most prominent functional change in clinical diabetic cardiomyopathy is the presence of LV diastolic dysfunction (Loncarevic et al., 2016). In this study, diastolic dysfunction was evident in diabetic mice after 26 weeks of diabetes. This impairment developed in conjunction with myocardial fibrosis, a characteristic that has been reported in cardiac biopsies of patients with type 2 diabetes (Shimizu et al., 1993), as well as in several experimental models of diabetes (Wang et al., 2017; Tsai et al., 2018; Zhao et al., 2019).

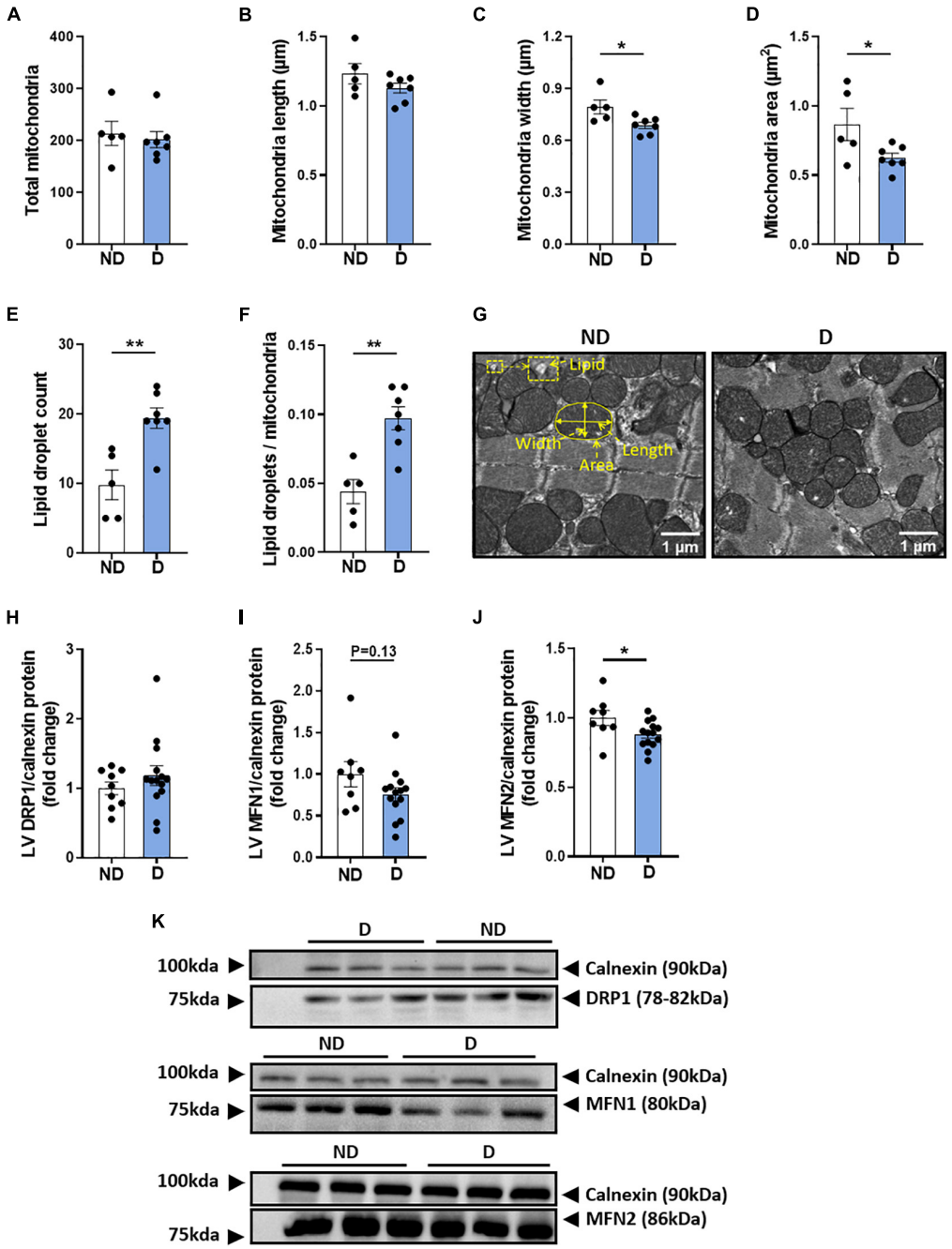

FIGURE 6 | LV mitochondria morphology in diabetic mice. Electron microscopy quantification of **(A)** total number of mitochondria, **(B)** mitochondria length, **(C)** mitochondria width, **(D)** mitochondria area, **(E)** total lipid droplet count, **(F)** lipid droplets/mitochondria ratio, **(G)** representative electron microscopy images of mitochondria structure (an enlarged image is available to view in **Supplementary Figure 2**). Regulators of mitochondrial morphology: **(H)** dynamin-related protein 1 (DRP1) and **(I)** Mitofusin 1 (MFN1), **(J)** Mitofusin 2 (MFN2), with **(K)** representative western blot images; Calnexin housekeeper are carried out separately for each protein of interest. Data represented as mean ± SEM. n = 5–14 per group (note individual data points). Data analysis used unpaired t-test. $^{*}P < 0.05$, $^{**}P < 0.01$. ND, non-diabetic; D, diabetes; LV, left ventricle.

An overproduction of ROS is a major driver of pathological remodelling in the diabetic heart (Kaludercic and Di Lisa, 2020). Clinical studies revealed elevated mitochondrial ROS production in cardiac biopsies of patients with type 2 diabetes (Anderson et al., 2009). Furthermore, elevated ROS has been reported in several experimental models of diabetes (Al-Rasheed et al., 2017; Li et al., 2019), including in the genetic models of diabetes, the *ob/ob* mouse and *db/db* mouse (Saraiva et al., 2007;

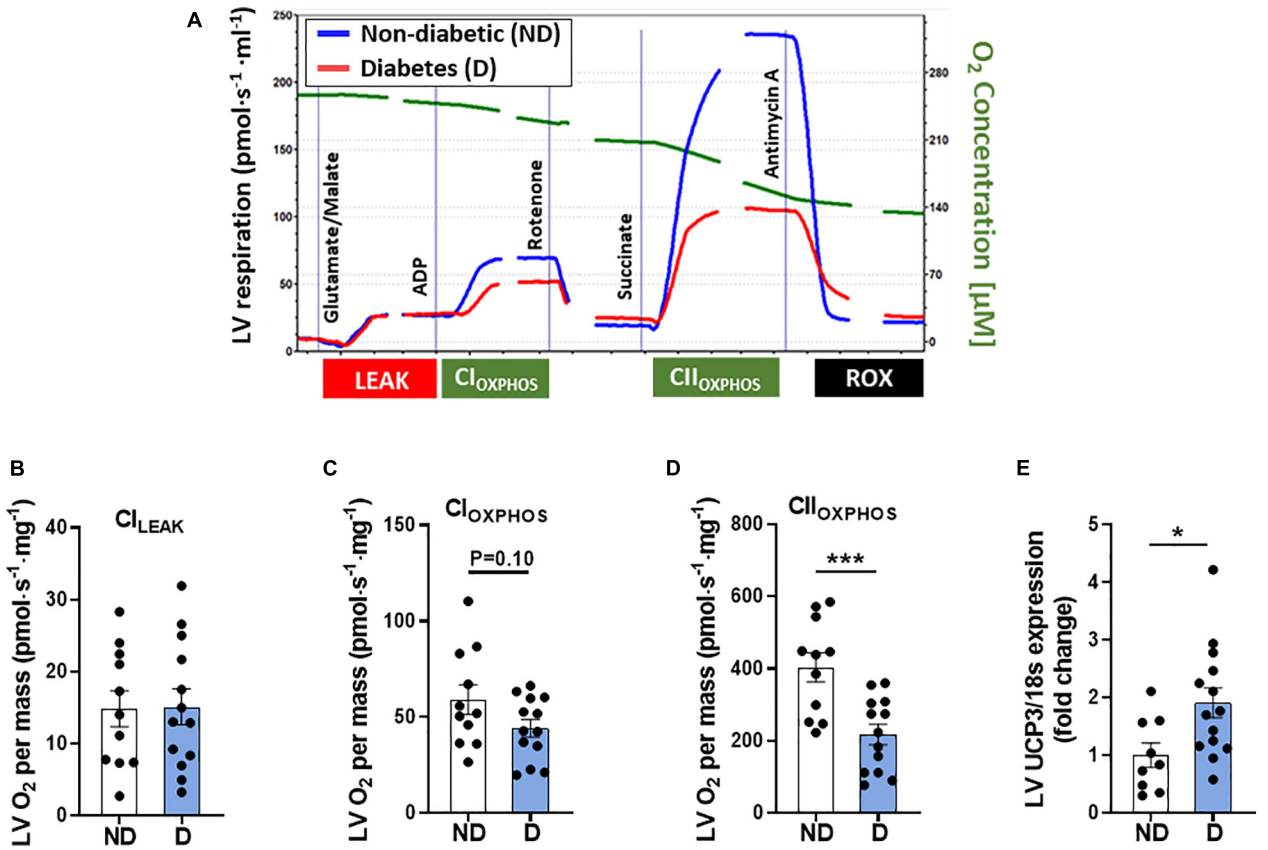

FIGURE 7 | LV mitochondria function in diabetic mice**. (A)** Representative mitochondrial oxygen consumption trace in diabetic mice (red line) and non-diabetic mice (blue line). Image has been cropped where lines are broken. Cytochrome C addition not shown. Oroboros high-resolution respirometry analysis of: **(B)** Complex-I^{LEAK} respiration, **(C)** Complex-I oxygen consumption, **(D)** Complex-II oxygen consumption. LV gene expression of **(E)** mitochondrial uncoupling protein-3 (UCP3). Data represented as mean ± SEM. n = 9–14 per group (note individual data points). Data analysis was carried out using unpaired t-test. $^{*}P < 0.05$, $^{***}P < 0.001$. ND, non-diabetic; D, diabetes; LV, left ventricle.

Huynh et al., 2012). In the current study, diabetic mice displayed a significant increase in LV superoxide production, coupled with a tendency toward a decrease in LV hydrogen peroxide levels. Reduced superoxide dismutase activity, an antioxidant enzyme that catalyses the dismutation of superoxide into hydrogen peroxide, has been implicated in the setting of diabetic cardiomyopathy (Tang et al., 2018). A preclinical study has demonstrated that the expression of cardiac superoxide dismutase-2 was reduced in *db/db* mice (Waldman et al., 2018). Similarly, diabetic mice in the present study also exhibited a reduction in the mRNA expression superoxide dismutase-2. Our findings suggest that there is an impairment in the processing of ROS in the hearts of diabetic mice, possibly due to a reduction in amount or activity in superoxide dismutase. Superoxide generating enzyme known as NADPH-oxidase is also thought to contribute to oxidative stress in diabetic cardiomyopathy (Liang et al., 2018). However, in this study there was no difference in the mRNA or protein levels of the NADPH-oxidase subunit NOX-4.

The mitochondria are of particular importance in the diabetic heart as they are the major site for ROS production (Kaludercic and Di Lisa, 2020). Previous studies have demonstrated that mitochondrial dysfunction contributes toward the progression of diabetic cardiomyopathy (Marciniak et al., 2014; Wang et al., 2015; Ni et al., 2020). Adverse cardiac mitochondrial remodelling has been reported in 12-week-old *db/db* mice exhibiting decreased diastolic function and fractional shortening (Hu et al., 2019). In a similar study using STZ-mice, cardiac contractile dysfunction was associated with mitochondrial cristae fusion and reduced levels of adenosine triphosphate (Li et al., 2018). In diabetic rabbits, depolarised cardiac mitochondrial membrane potential and mitochondrial swelling was associated with diastolic dysfunction (Zhang et al., 2018). Our primary objective was to assess mitochondria structure and function in the myocardium of this alternative mouse model of diabetic cardiomyopathy. No differences were seen in the total number of mitochondria in the myocardium, however, diabetic mice exhibited a significant reduction in mitochondria size and mitochondrial disorganisation. Other studies in STZ-induced diabetic rodents have reported a reduction in both total number of cardiac mitochondria and relative size (Zhou et al., 2018; Tao et al., 2019). In the hearts of

db/db mice, a decrease in mitochondria size and an increase in the number of mitochondria was observed; this was associated with decreased expression of mitochondria morphological regulator MFN but not DRP1 (Hu et al., 2019). Consistent with the *db/db* model, the protein expression of MFN2, but not DRP1, was also increased in diabetic mice (Hu et al., 2019). The downregulation of MFN2 may be partly the cause of smaller mitochondria size exhibited in diabetic mice in this model. These findings suggest that although cardiac mitochondrial structural changes are evident in the diabetic heart, morphological differences vary depending on the specific model and/or species. Increased availability of localised lipid droplets can impact mitochondrial dynamics in the diabetic heart by increasing the level of mitochondrial lipid uptake (Mazumder et al., 2004; Verma et al., 2017). In one study investigating cardiac tissue from diabetic mice, morphological changes in the mitochondria were parallelled with elevated mitochondrial fatty acid oxidation (Li et al., 2018). In our study, electron microscopy revealed an increased lipid-to-mitochondria ratio in diabetic mice.

Diabetes-induced changes in mitochondrial substrate utilisation can increase mitochondrial activity at the level of the respiratory complexes, leading to leakage of unpaired electrons and the formation of ROS (Alejandra Sánchez-Muñoz et al., 2018; Ding et al., 2019; Kaludercic and Di Lisa, 2020). A recent study demonstrated elevated protein expression of myocardial electron transport chain complexes (CI, CII, and CV) in diabetic mice (Wang et al., 2020). In this study there was an increase in complex-III and complex-V protein abundance, and a non-significant increase in complex-I and complex-IV. These findings indicate that a compensatory response to diabetes-induced excessive influx of reducing equivalents (NADH and FADH2) entering the mitochondrial complexes may be at play (Wu et al., 2017). This suggests a mismatch in energy expenditure (ATP consumption) and respiratory activity (reduced flow of electrons through the ETC) which may reflect the smaller mitochondria in the diabetic heart. Mitochondrial dysfunction is a known contributor to the pathological remodelling in diabetic cardiomyopathy (Ormazabal et al., 2018; Gollmer et al., 2020). Clinical studies have demonstrated a lower mitochondrial respiratory rate in the cardiac tissues of patients with diabetes (Croston et al., 2014; Montaigne et al., 2014). These findings also extend to experimental models of diabetic cardiomyopathy (Pham et al., 2014; Qaed et al., 2019). Consistent with previous studies, diabetic mice in this study exhibited similar reductions in LV mitochondrial oxygen consumption, particularly when measuring mitochondrial respiration by electron input through CII. Mitochondrial uncoupling proteins contribute to the protection of the myocardium by inducing mitochondrial respiration in response to a diabetes-induced reduction in the mitochondrial proton gradient (Dludla et al., 2018; Li et al., 2018). Studies have shown that an increase in mitochondrial uncoupling proteins is associated with decreased oxygen consumption (Hidaka et al., 1999; Pham et al., 2014; Fang et al., 2018). In this study, mRNA expression of mitochondrial UCP3 was significantly elevated with diabetes, suggesting that this may be a compensation mechanism in response to the diabetes-induced changes in substrate utilisation (Dludla et al., 2018; Li et al., 2018).

Limitations and Future Considerations

We acknowledge that there were some limitations with our study which we discuss here. While only male mice were utilised in this study, we acknowledge the importance of investigating the pathological mechanisms of diabetic cardiomyopathy in female mice. We have previously demonstrated that STZ-induced female mice are more susceptible to diastolic dysfunction than male mice despite exhibiting lower degree of hyperglycaemia (Chandramouli et al., 2018). Further, female *db/db* mice exhibit at least as severe an adverse cardiac remodelling phenotype as male *db/db* mice (and indeed it may occur earlier) (Bowden et al., 2015). Another limitation of the present study was a lack of data reporting the respiratory exchange ratio (RER) which would have informed us about substrate utilisation and respiration. We attempted to measure this in our study, unfortunately, there was a failure of the data acquisition programme at the time of the Promethion metabolic cage recordings. The only data that we could retrieve for every mouse studied was the data for energy expenditure (**Figure 3**). Mitochondrial DNA copy number, which is a measure of the number of mitochondrial genomes per cell, can inform on mitochondrial function and has been associated with several disease states. Previous preclinical studies have shown that mitochondria DNA copy number is increased in diabetic heart (Boudina et al., 2007; Fang et al., 2018). After performing analysis on the mitochondria structure and function, the remaining heart tissue in this study was insufficient to carry out this analysis. This analysis however, is worthy of attention in future studies of mitochondrial function in the setting of diabetes. Previous studies using models of diabetic cardiomyopathy such as *db/db* mice and STZ-C57BL/6 mice have also demonstrated a decrease in ATP production with associated reduction in reducing equivalent (NAD + /NADPH ratio) (Boudina et al., 2007; Guan et al., 2015; Sun et al., 2019; Wang et al., 2020; Yao et al., 2021). Although our data demonstrated an increase protein abundance in ATP-synthase subunit (ATP-synthase-C5-subunit v) in diabetic mice (**Figure 5**), ATP levels and reducing equivalents were not measured as there was insufficient sample to carry out these analyses. There may be a maladaptive compensatory mechanism at play whereby defective ATP synthase is upregulated in the diabetic heart. Investigating these parameters in future studies may further elucidate the functional changes of cardiac mitochondria in this model of diabetic cardiomyopathy.

Despite these limitations and considerations, overall, our findings demonstrate the presence of mitochondrial dysfunction and excess ROS production in a mouse model that replicates several clinical features of diabetic cardiomyopathy. It is anticipated that this model which combines the low-dose STZ approach with high-fat diet, resulting in a milder phenotype compared to existing genetic models, can be used alongside existing models to study oxidative stress and mitochondrial function in the diabetic heart.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by Alfred Medical Research and Education Precinct (AMREP) Animal Ethics Committee.

AUTHOR CONTRIBUTIONS

AP, MT, MJD, and RR performed conception and design of the research, drafted the manuscript, and edited and revised the manuscript. AP, MT, DP, MD, AW, DN, DD, SC, HK, CG, MC, MJD, and RR performed the experiments and approved final version of the manuscript. AP, MT, DP, MD, DN, DD, HK, MJD, and RR analysed the data. AP, MT, DP, DD, MJD, and RR interpreted results of the experiments. AP, MT, DD, HK, CG, MC, and RR prepared the figures. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.672252/full#supplementary-material

REFERENCES

Alejandra Sánchez-Muñoz, M., Valdez-Solana, M. A., Campos-Almazán, I., Flores-Herrera, Ó, Esparza-Perusquía, M., Olvera-Sánchez, S., et al. (2018). Streptozotocin-induced adaptive modification of mitochondrial supercomplexes in liver of wistar rats and the protective effect of *Moringa oleifera* lam. *Biochem. Res. Int.* 2018:5681081.

Al-Rasheed, N. M., Al-Rasheed, N. M., Hasan, I. H., Al-Amin, M. A., Al-Ajmi, H. N., Mohamad, R. A., et al. (2017). Simvastatin ameliorates diabetic cardiomyopathy by attenuating oxidative stress and inflammation in rats. *Oxid. Med. Cell Longev* 2017, 1–13. doi: 10.1155/2017/1092015

Amaral, N., and Okonko, D. O. (2015). Metabolic abnormalities of the heart in type II diabetes. *Diabetes Vasc. Dis. Res.* 12, 239–248. doi: 10.1177/1479164115580936

Anderson, E. J., Kypson, A. P., Rodriguez, E., Anderson, C. A., Lehr, E. J., and Neufer, P. D. (2009). Substrate-specific derangements in mitochondrial metabolism and redox balance in the atrium of the type 2 diabetic human heart. *J. Am. Coll. Cardiol.* 54, 1891–1898. doi: 10.1016/j.jacc.2009.07.031

Barouch, L. A., Berkowitz, D. E., Harrison, R. W., O'Donnell, C. P., and Hare, J. M. (2003). Disruption of leptin signaling contributes to cardiac hypertrophy independently of body weight in mice. *Circulation* 108, 754–759. doi: 10.1161/01.cir.0000083716.82622.fd

Bitz, C., Toubro, S., Larsen, T. M., Harder, H., Rennie, K. L., Jebb, S. A., et al. (2004). Increased 24-h energy expenditure in type 2 diabetes. *Diabetes Care* 27, 2416–2421. doi: 10.2337/diacare.27.10.2416

Boudina, S., Sena, S., Theobald, H., Sheng, X., Wright, J. J., Hu, X. X., et al. (2007). Mitochondrial energetics in the heart in obesity-related diabetes: direct evidence for increased uncoupled respiration and activation of uncoupling proteins. *Diabetes* 56, 2457–2466. doi: 10.2337/db07-0481

Bowden, M. A., Tesch, G. H., Julius, T. L., Rosli, S., Love, J. E., and Ritchie, R. H. (2015). Earlier onset of diabesity-Induced adverse cardiac remodeling in female compared to male mice. *Obesity* 23, 1166–1177. doi: 10.1002/oby.21072

Burke, S. J., Batdorf, H. M., Burk, D. H., Noland, R. C., Eder, A. E., Boulos, M. S., et al. (2017). db/db mice exhibit features of human type 2 diabetes that are not present in weight-matched C57BL/6J mice fed a western diet. *J. Diabetes Res.* 2017, 1–17. doi: 10.1155/2017/8503754

Chandramouli, C., Reichelt, M. E., Curl, C. L., Varma, U., Bienvenu, L. A., Koutsifeli, P., et al. (2018). Diastolic dysfunction is more apparent in STZ-induced diabetic female mice, despite less pronounced hyperglycemia. *Sci. Rep.* 8:2346.

Cho, N. H., Shaw, J. E., Karuranga, S., Huang, Y., Rocha Fernandes, J. D., da, et al. (2018). IDF diabetes Atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res. Clin. Pract.* 138, 271–281. doi: 10.1016/j.diabres.2018.02.023

Croston, T. L., Thapa, D., Holden, A. A., Tveter, K. J., Lewis, S. E., Shepherd, D. L., et al. (2014). Functional deficiencies of subsarcolemmal mitochondria in the type 2 diabetic human heart. *Am. J. Physiol. Hear. Circ. Physiol.* 307, 54–65.

Dabkowski, E. R., Baseler, W. A., Williamson, C. L., Powell, M., Razunguzwa, T. T., Frisbee, J. C., et al. (2010). Mitochondrial dysfunction in the type 2 diabetic heart is associated with alterations in spatially distinct mitochondrial proteomes. *Am. J. Physiol. - Hear. Circ. Physiol.* 299:H529.

Daghistani, H. M., Rajab, B. S., and Kitmitto, A. (2019). Three-dimensional electron microscopy techniques for unravelling mitochondrial dysfunction in heart failure and identification of new pharmacological targets. *Br. J. Pharmacol.* 176, 4340–4359. doi: 10.1111/bph.14499

Ding, W., Chang, W.-G., Guo, X.-C., Liu, Y., Xiao, D.-D., Ding, D., et al. (2019). Exenatide protects against cardiac dysfunction by attenuating oxidative stress in the diabetic mouse heart. *Front. Endocrinol.* 10:202. doi: 10.3389/fendo.2019.00202

Dludla, P. V., Nkambule, B. B., Tiano, L., Louw, J., Jastroch, M., and Mazibuko-Mbeje, S. E. (2018). Uncoupling proteins as a therapeutic target to protect the diabetic heart. *Pharmacol. Res.* 137, 11–24. doi: 10.1016/j.phrs.2018.09.013

Dodd, G. T., and Tiganis, T. (2017). Insulin action in the brain: roles in energy and glucose homeostasis. *J. Neuroendocrinol.* 29, e12513. doi: 10.1111/jne.12513

Donner, D. G., Kiriazis, H., Du, X.-J., Marwick, T. H., and McMullen, J. R. (2018). Improving the quality of preclinical research echocardiography: observations, training, and guidelines for measurement. *Am. J. Physiol. Heart Circ. Physiol.* 315, H58–H70.

Fang, W.-J., Wang, C.-J., He, Y., Zhou, Y.-L., Peng, X.-D., and Liu, S.-K. (2018). Resveratrol alleviates diabetic cardiomyopathy in rats by improving mitochondrial function through PGC-1α deacetylation. *Acta Pharmacol. Sin.* 39, 59–73. doi: 10.1038/aps.2017.50

Fillmore, N., Mori, J., and Lopaschuk, G. D. (2014). Mitochondrial fatty acid oxidation alterations in heart failure, ischaemic heart disease and diabetic cardiomyopathy. *Br. J. Pharmacol.* 171, 2080–2090. doi: 10.1111/bph.12475

Gollmer, J., Zirlik, A., and Bugger, H. (2020). Mitochondrial mechanisms in diabetic cardiomyopathy. *Diabetes Metab. J.* 44, 33–53. doi: 10.4093/dmj.2019.0185

Guan, S.-S., Sheu, M.-L., Wu, C.-T., Chiang, C.-K., and Liu, S.-H. (2015). ATP synthase subunit-β down-regulation aggravates diabetic nephropathy. *Sci. Rep.* 5:14561.

Hidaka, S., Kakuma, T., Yoshimatsu, H., Sakino, H., Fukuchi, S., and Sakata, T. (1999). Streptozotocin treatment upregulates uncoupling protein 3 expression in the rat heart. *Diabetes* 48, 430–435. doi: 10.2337/diabetes.48.2.430

Horscroft, J. A., Burgess, S. L., Hu, Y., and Murray, A. J. (2015). Altered oxygen utilisation in rat left ventricle and soleus after 14 days, but not 2 days, of environmental hypoxia. *PLoS One* 10:e0138564. doi: 10.1371/journal.pone.0138564

Hu, L., Ding, M., Tang, D., Gao, E., Li, C., Wang, K., et al. (2019). Targeting mitochondrial dynamics by regulating Mfn2 for therapeutic intervention in diabetic cardiomyopathy. *Theranostics* 9, 3687–3706. doi: 10.7150/thno.33684

Huynh, K., Kiriazis, H., Du, X.-J., Love, J. E., Jandeleit-Dahm, K. A., Forbes, J. M., et al. (2012). Coenzyme Q10 attenuates diastolic dysfunction, cardiomyocyte hypertrophy and cardiac fibrosis in the db/db mouse model of type 2 diabetes. *Diabetologia* 55, 1544–1553. doi: 10.1007/s00125-012-2495-3

Jia, G., Hill, M. A., and Sowers, J. R. (2018). Diabetic cardiomyopathy: an update of mechanisms contributing to this clinical entity. *Circ. Res.* 122, 624–638. doi: 10.1161/circresaha.117.311586

Kaludercic, N., Di Lisa, F. (2020). Mitochondrial ROS formation in the pathogenesis of diabetic cardiomyopathy. *Front. Cardiovasc. Med.* 7:12. doi: 10.3389/fcvm.2020.00012

Li, K., Zhai, M., Jiang, L., Song, F., Zhang, B., Li, J., et al. (2019). Tetrahydrocurcumin ameliorates diabetic cardiomyopathy by attenuating high glucose-induced oxidative stress and fibrosis via activating the SIRT1 pathway. *Oxid. Med. Cell. Longev* 2019, 1–15. doi: 10.1155/2019/6746907

Li, W., Yao, M., Wang, R., Shi, Y., Hou, L., Hou, Z., et al. (2018). Profile of cardiac lipid metabolism in STZ-induced diabetic mice. *Lipids Health Dis.* 17, 231.

Liang, E., Liu, X., Du, Z., Yang, R., and Zhao, Y. (2018). Andrographolide ameliorates diabetic cardiomyopathy in mice by blockage of oxidative damage and NF-κB-mediated inflammation. *Oxid. Med. Cell. Longev* 2018:9086747.

Liu, Q., Wang, S., and Cai, L. (2014). Diabetic cardiomyopathy and its mechanisms: Role of oxidative stress and damage. *J. Diabetes Invest.* 5, 623–634. doi: 10.1111/jdi.12250

Loh, K., Zhang, L., Brandon, A., Wang, Q., Begg, D., Qi, Y., et al. (2017). Insulin controls food intake and energy balance via NPY neurons. *Mol. Metab.* 6, 574–584. doi: 10.1016/j.molmet.2017.03.013

Loncarevic, B., Trifunovic, D., Soldatovic, I., and Vujisic-Tesic, B. (2016). Silent diabetic cardiomyopathy in everyday practice: a clinical and echocardiographic study. *BMC Cardiovasc. Disord.* 16:242. doi: 10.1186/s12872-016-0395-z

Marciniak, C., Marechal, X., Montaigne, D., Neviere, R., and Lancel, S. (2014). Cardiac contractile function and mitochondrial respiration in diabetes-related mouse models. *Cardiovasc. Diabetol.* 13:118.

Marwick, T. H., Ritchie, R., Shaw, J. E., and Kaye, D. (2018). Implications of underlying mechanisms for the recognition and management of diabetic cardiomyopathy. *J. Am. Coll. Cardiol.* 71, 339–351. doi: 10.1016/j.jacc.2017.11.019

Mazumder, P. K., O'Neill, B. T., Roberts, M. W., Buchanan, J., Yun, U. J., Cooksey, R. C., et al. (2004). Impaired cardiac efficiency and increased fatty acid oxidation in insulin-resistant ob/ob mouse hearts. *Diabetes* 53, 2366–2374. doi: 10.2337/diabetes.53.9.2366

Montaigne, D., Marechal, X., Coisne, A., Debry, N., Modine, T., Fayad, G., et al. (2014). Myocardial contractile dysfunction is associated with impaired mitochondrial function and dynamics in type 2 diabetic but not in obese patients. *Circulation* 130, 554–564. doi: 10.1161/circulationaha.113.008476

Ni, T., Lin, N., Huang, X., Lu, W., Sun, Z., Zhang, J., et al. (2020). Icariin ameliorates diabetic cardiomyopathy through apelin/sirt3 signalling to improve mitochondrial dysfunction. *Front. Pharmacol.* 11:256.

Nichols, G. A., Gullion, C. M., Koro, C. E., Ephross, S. A., and Brown, J. B. (2004). The incidence of congestive heart failure in type 2 diabetes: an update. *Diabetes Care* 27, 1879–1884. doi: 10.2337/diacare.27.8.1879

Ormazabal, V., Nair, S., Elfeky, O., Aguayo, C., Salomon, C., and Zuñiga, F. A. (2018). Association between insulin resistance and the development of cardiovascular disease. *Cardiovasc. Diabetol.* 17:122.

Pesta, D., and Gnaiger, E. (2012). High-resolution respirometry: OXPHOS protocols for human cells and permeabilized fibers from small biopsies of human muscle. *Methods Mol. Biol.* 810, 25–58. doi: 10.1007/978-1-61779-382-0_3

Pham, T., Loiselle, D., Power, A., and Hickey, A. J. R. (2014). Mitochondrial inefficiencies and anoxic ATP hydrolysis capacities in diabetic rat heart. *Am. J. Physiol. Cell Physiol.* 307, C499–C507.

Qaed, E., Wang, J., Almoiliqy, M., Song, Y., Liu, W., Chu, P., et al. (2019). Phosphocreatine improves cardiac dysfunction by normalizing mitochondrial respiratory function through JAK2/STAT3 signaling pathway in vivo and in vitro. *Oxid. Med. Cell. Longev.* 2019, 6521218.

Ritchie, R. H., and Abel, E. D. (2020). Basic mechanisms of diabetic heart disease. *Circ. Res.* 126, 1501–1525. doi: 10.1161/circresaha.120.315913

Rubler, S., Dlugash, J., Yuceoglu, Y. Z., Kumral, T., Branwood, A. W., and Grishman, A. (1972). New type of cardiomyopathy associated with diabetic glomerulosclerosis. *Am. J. Cardiol.* 30, 595–602. doi: 10.1016/0002-9149(72)90595-4

Saraiva, R. M., Minhas, K. M., Zheng, M., Pitz, E., Treuer, A., Gonzalez, D., et al. (2007). Reduced neuronal nitric oxide synthase expression contributes to cardiac oxidative stress and nitroso-redox imbalance in ob/ob mice. *Nitric Oxide* 16, 331–338. doi: 10.1016/j.niox.2006.12.001

Shimizu, M., Umeda, K., Sugihara, N., Yoshio, H., Ino, H., Takeda, R., et al. (1993). Collagen remodelling in myocardia of patients with diabetes. *J. Clin. Pathol.* 46, 32–36. doi: 10.1136/jcp.46.1.32

Soto, J. E., Burnett, C. M. L., Eyck, P., Ten, Abel, E. D., and Grobe, J. L. (2019). Comparison of the effects of high-fat diet on energy flux in mice using two multiplexed metabolic phenotyping systems. *Obesity* 27, 793–802.

Sun, Y., Teng, Z., Sun, X., Zhang, L., Chen, J., Wang, B., et al. (2019). Exogenous H2S reduces the acetylation levels of mitochondrial respiratory enzymes via regulating the NAD+-SIRT3 pathway in cardiac tissues of db/db mice. *Am. J. Physiol. Endocrinol. Metab.* 317, E284–E297.

Tang, Q., Len, Q., Liu, Z., and Wang, W. (2018). Overexpression of miR-22 attenuates oxidative stress injury in diabetic cardiomyopathy via Sirt 1. *Cardiovasc. Ther.* 36::e12318. doi: 10.1111/1755-5922.12318 [Epub ahead of print].

Tao, S., Chen, L., Song, J., Zhu, N., Song, X., Shi, R., et al. (2019). Tanshinone IIA ameliorates diabetic cardiomyopathy by inhibiting Grp78 and CHOP expression in STZ-induced diabetes rats. *Exp. Ther. Med.* 18, 729–734.

Tate, M., Prakoso, D., Willis, A. M., Peng, C., Deo, M., Qin, C. X., et al. (2019). Characterising an alternative murine model of diabetic cardiomyopathy. *Front. Physiol.* 10:1395. doi: 10.3389/fphys.2019.01395

Teshima, Y., Takahashi, N., Nishio, S., Saito, S., Kondo, H., Fukui, A., et al. (2014). Production of reactive oxygen species in the diabetic heart. *Circ. J.* 78, 300–306.

Tsai, T. H., Lin, C. J., Chua, S., Chung, S. Y., Chen, S. M., Lee, C. H., et al. (2018). Deletion of rasGRF1 attenuated interstitial fibrosis in streptozotocin-induced diabetic cardiomyopathy in mice through affecting inflammation and oxidative stress. *Int. J. Mol. Sci.* 19, 3094. doi: 10.3390/ijms19103094

Verma, S. K., Garikipati, V. N. S., and Kishore, R. (2017). Mitochondrial dysfunction and its impact on diabetic heart. *Biochim. Biophys. Acta Mol. Basis Dis.* 1863, 1098–1105. doi: 10.1016/j.bbadis.2016.08.021

Waldman, M., Cohen, K., Yadin, D., Nudelman, V., Gorfil, D., Laniado-Schwartzman, M., et al. (2018). Regulation of diabetic cardiomyopathy by caloric restriction is mediated by intracellular signaling pathways involving 'SIRT1 and PGC-1α'. *Cardiovasc. Diabetol.* 17:111.

Wang, H., Bei, Y., Lu, Y., Sun, W., Liu, Q., Wang, Y., et al. (2015). Exercise prevents cardiac injury and improves mitochondrial biogenesis in advanced diabetic cardiomyopathy with PGC-1α and Akt activation. *Cell. Physiol. Biochem.* 35, 2159–2168. doi: 10.1159/000374021

Wang, S., Wang, B., Wang, Y., Tong, Q., Liu, Q., Sun, J., et al. (2017). Zinc prevents the development of diabetic cardiomyopathy in db/db mice. *Int. J. Mol. Sci.* 18, 1–14.

Wang, S. Y., Zhu, S., Wu, J., Zhang, M., Xu, Y., Xu, W., et al. (2020). Exercise enhances cardiac function by improving mitochondrial dysfunction and maintaining energy homoeostasis in the development of diabetic cardiomyopathy. *J. Mol. Med.* 98, 245–261. doi: 10.1007/s00109-019-01861-2

Wu, J., Luo, X., Thangthaeng, N., Sumien, N., Chen, Z., Rutledge, M. A., et al. (2017). Pancreatic mitochondrial complex I exhibits aberrant hyperactivity in diabetes. *Biochem. Biophys. Rep*. 11, 119–129. doi: 10.1016/j.bbrep.2017.07.007

Xu, Z., Zhao, Y., Zhong, P., Wang, J., Weng, Q., Qian, Y., et al. (2017). EGFR inhibition attenuates diabetic nephropathy through decreasing ROS and endoplasmic reticulum stress. *Oncotarget* 8, 32655–32667. doi: 10.18632/oncotarget.15948

Yao, R., Cao, Y., Wang, C., Xu, L., Zhang, X., Deng, Y., et al. (2021). Taohuajing reduces oxidative stress and inflammation in diabetic cardiomyopathy through the sirtuin 1/nucleotide-binding oligomerization domain-like receptor protein 3 pathway. *BMC Complement. Med. Ther*. 21:78.

Zhang, X., Zhang, Z., Yang, Y., Suo, Y., Liu, R., Qiu, J., et al. (2018). Alogliptin prevents diastolic dysfunction and preserves left ventricular mitochondrial function in diabetic rabbits. *Cardiovasc. Diabetol.* 17:160.

Zhao, Y., Li, S., Quan, E., Zhang, H., Wu, Y., Luo, Y., et al. (2019). Trimetazidine inhibits cardiac fibrosis by reducing reactive oxygen species and downregulating connective tissue growth factor in streptozotocin-induced diabetic rats. *Exp. Ther. Med.* 18, 1477–1485.

Zhou, B., Lu, Y., Hajifathalian, K., Bentham, J., Cesare, M., Danaei, G, et al. (2016). Worldwide trends in diabetes since 1980: A pooled analysis of 751 population-based studies with 4.4 million participants. *Lancet* 387, 1513–1530.

Zhou, Y., Lian, S., Zhang, J., Lin, D., Huang, C., Liu, L., et al. (2018). Mitochondrial perturbation contributing to cognitive decline in streptozotocin-induced type 1 diabetic rats. *Cell. Physiol. Biochem*. 46, 1668–1682. doi: 10.1159/000489243

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MINI REVIEW
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Left Ventricular Hypertrophy in Diabetic Cardiomyopathy: A Target for Intervention

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Heart failure is an important manifestation of diabetic heart disease. Before the development of symptomatic heart failure, as much as 50% of patients with type 2 diabetes mellitus (T2DM) develop asymptomatic left ventricular dysfunction including left ventricular hypertrophy (LVH). Left ventricular hypertrophy (LVH) is highly prevalent in patients with T2DM and is a strong predictor of adverse cardiovascular outcomes including heart failure. Importantly regression of LVH with antihypertensive treatment especially renin angiotensin system blockers reduces cardiovascular morbidity and mortality. However, this approach is only partially effective since LVH persists in 20% of patients with hypertension who attain target blood pressure, implicating the role of other potential mechanisms in the development of LVH. Moreover, the pathophysiology of LVH in T2DM remains unclear and is not fully explained by the hyperglycemia-associated cellular alterations. There is a growing body of evidence that supports the role of inflammation, oxidative stress, AMP-activated kinase (AMPK) and insulin resistance in mediating the development of LVH. The recognition of asymptomatic LVH may offer an opportune target for intervention with cardio-protective therapy in these at-risk patients. In this article, we provide a review of some of the key clinical studies that evaluated the effects of allopurinol, SGLT2 inhibitor and metformin in regressing LVH in patients with and without T2DM.

Keywords: diabetic cardiomyopathy (DCM), heart failure, type 2 diabetes mellitus, metformin, allopurinol, SGLT2 inhibitors, left ventricular hypertrophy (LVH)

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INTRODUCTION

Diabetic cardiomyopathy (DCM) is defined as cardiac dysfunction, characterised by abnormal structural, functional and metabolic changes in the myocardium, that occurs in the absence of significant coronary, valvular or hypertensive diseases in individuals with diabetes (1). Diabetic cardiomyopathy was first described five decades ago (2), and the higher incidence of heart failure (HF) in patients with type 2 diabetes mellitus (T2DM) was further confirmed in several epidemiological studies, including the Framingham Heart Study (3–5). The aetiology of DCM in the diabetic heart is complex and is likely to multifactorial (**Figure 1**). In the early stages of its progression, DCM is usually asymptomatic and is characterised by subclinical structural and functional abnormalities including left ventricular hypertrophy (LVH), reduced LV compliance,

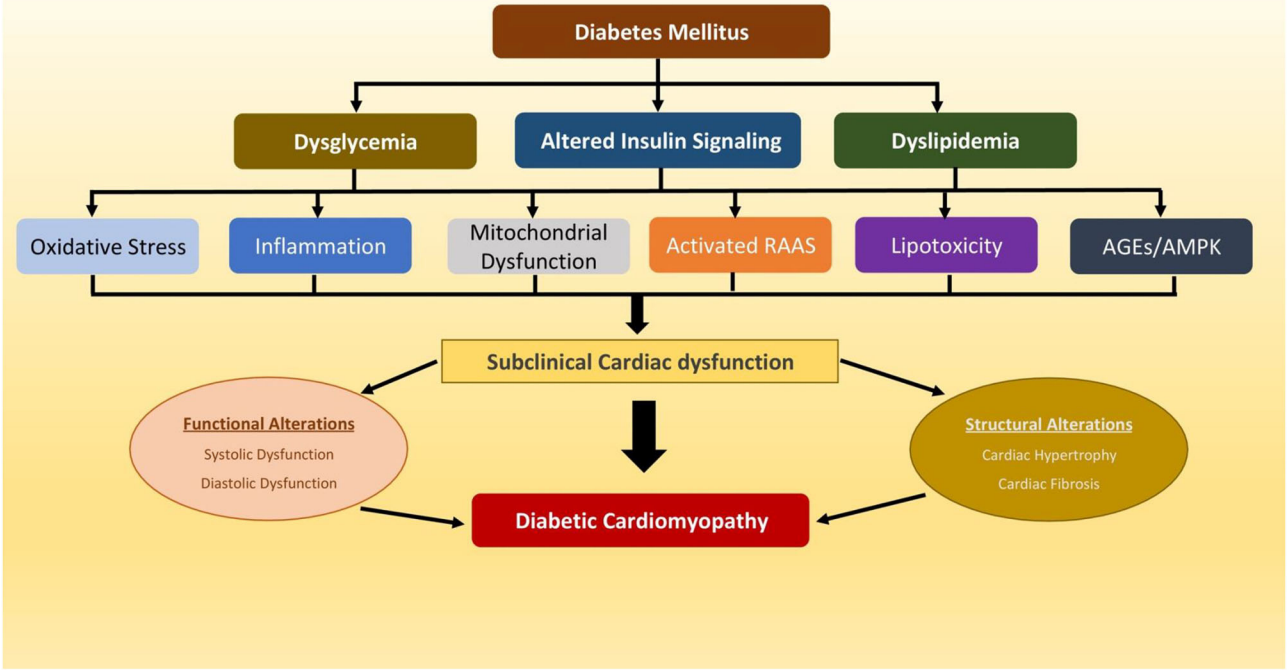

FIGURE 1 | A schematic diagram depicting multiple potential mechanisms that have been implicated in pathophysiology of diabetic cardiomyopathy. AGEs, advanced glycation end products; AMPK, AMP-activated protein kinase; RAAS, renin-angiotensin-aldosterone system.

myocardial fibrosis and stiffness (6). These pathophysiological changes associated with subclinical cardiac dysfunction can be progressive and lead to HF symptoms and to the clinical syndrome of HF (7). Despite the success of various antihyperglycemic agents in the intensive management of hyperglycemia in people with T2DM in reducing the risk of cardiovascular (CV) complications, the high prevalence of HF persists (8–10), that might suggest the role of other non-hyperglycemia associated pathophysiological mechanisms that might contribute to the development of DCM and consequent higher risk of HF in T2DM.

LVH is defined by an elevated left ventricular mass (LVM), either due to an increase in wall thickness or due to left ventricular cavity enlargement, or both. Typically, the LV wall thickening manifests in response to hemodynamic pressure overload, and chamber dilatation occurs in response to volumetric burden, with the caveat that other molecular and metabolic mechanisms may play a synergistic and potentially independent role. Importantly, LVH is strong predictor of CV events (11, 12), and is a common feature in the diabetic myocardium (7, 13). The reason why LVH is predictive of CV events is because it precedes many potentially fatal CV sequelae of events. For instance, the presence of LVH can lead to adverse cardiovascular (CV) outcome—LVH impedes left ventricular (LV) filling and can lead to diastolic heart failure; it reduces coronary perfusion reserve and can induce ischemia; it can lead to left atrial enlargement and subsequent atrial fibrillation and it is intrinsically arrhythmogenic and can cause sudden cardiac death (14). The development of LVH in T2DM is not fully explained by the hyperglycemia-associated cellular alterations alone (15). Specifically, there is extensive body of clinical and experimental underpinnings that supports the role of inflammation, oxidative stress, insulin resistance and AMP-activated kinase (AMPK) in mediating the development of LVH in T2DM (15–18). While the pathophysiology of LVH in diabetes is not yet fully elucidated, the increasing recognition that LVH is an exquisite orchestration of a wide array of pathophysiological process, that are not limited to hyperglycemia, hypertension and valvular disease, provided new opportunities to examine new pharmacological therapies in LVH regression.

As defined by the American College of Cardiology/American Heart Association (ACC/AHA) guidelines, HF progresses as a clinical continuum of four stages (19). Stage B HF represents patients with structural heart disease including LVH, but with no current or prior symptoms of HF (19). To prevent progression to symptomatic HF (Stage C HF), it is important to identify the presence of DCM at the early stages of its development. While the electrocardiogram (ECG) is a widely used method to diagnose LVH, its diagnostic accuracy is limited due to its poor sensitivity in detecting LVH (20, 21). Echocardiography has been conventionally considered a test of choice to evaluate the presence of cardiac dysfunction and early structural changes of the heart including LVH (22). Its sensitivity is significantly higher than ECG in detecting LVH (23), and can also diagnosis

other subclinical cardiac abnormalities and valvular heart disease. However, cardiac magnetic resonance imaging is considered the gold standard due to high precision and reproducibility in estimating LV mass (24) and other structural cardiac abnormalities, albeit its use is limited due to its high costs and limited availability.

In addition to multiple comorbidities and several molecular and metabolic mechanisms, several studies have reported that LVH is also influenced by various other factors such as age, gender, ethnicity and genetic factors. For instance, LVH is more prevalent in blacks compared with other race/ethnic groups (25, 26). The prevalence of LVH is not reported to be dissimilar between men and women, irrespective of diagnostic criteria applied (27). The prevalence of LVH also increased significantly with age, occurring in 33% of men and 49% of women over 70 (28). Furthermore, genome wide association studies (GWAS) have also reported substantial heritability for LVH in diverse populations including diabetic patients (29, 30).

In this mini-review, we summarise the epidemiology of LVH in T2DM and the key pathophysiological mechanisms and provide a review of some of the key studies that evaluated the effects of different classes drugs on LVH regression.

LEFT VENTRICULAR HYPERTROPHY IN DIABETIC CARDIOMYOPATHY

Prevalence and Economic Impact

It is becoming increasingly recognised that T2DM is an independent risk factor for LVH, even in the absence of overt cardiovascular diseases (CVD) (31, 32). T2DM is often associated with cardiomyopathy, manifested by LVH, and the reported prevalence of LVH in patients with T2DM ranges from 32 to 71% in different studies, depending on the criteria used for defining LVH (12, 33–36). Importantly, previous research has demonstrated that LVH regression *per se* independently reduces CV mortality and events (37, 38). Thus, routine screening of LVH, followed by targeted intervention could be a promising way of reducing CV events and mortality in people with T2DM. Furthermore, disability caused by CVD carries major health economic consequences at both individual levels, and the society as a whole, and this burden is expected to increase in the future. Therefore, routine screening of LVH and targeted treatment may have the potential to reduce the economic burden of CVD. While it is difficult to evaluate the economic value of saving life, the costs of screening for and treating LVH is anticipated to be low, primarily due to the high prevalence of the condition and the low unit cost of echocardiography (39).

Efficacy and Cost-Effectiveness of Screening

The efficacy and cost effectiveness of early screening for traditional CV risk factors has already been proven in the general population (40–42). However, an ongoing challenge is to develop adequate screening strategies that are cost-effective that can identify individuals at risk for future CV events, prior to developing overt CVD. Since people with T2DM are at a higher risk for future CV events than non-diabetic people, it may be more cost-effective to target subclinical cardiac abnormalities including LVH in diabetic population. Whilst this approach may initially increase healthcare costs, especially with the costs associated with CV imaging and treatment, such an approach may in the longer term reduce CV related mortality and morbidity in patients with T2DM. Witham et al. in a hypothetical analysis of the cost-effectiveness of screening LVH, suggested that this may indeed be a very cost-effective strategy to reduce CV events in high-risk people including those with T2DM (39). Biomarkers may prove to be an alternate tool that is even more cost-effective, when used in CVD risk prognostication (43). In this context, previous studies have utilised a biomarker-based approach for the identification of subclinical LV abnormalities in patients without CVD. For instance, in patients with T2DM and without HF, higher levels of B-type natriuretic peptide (BNP) associated with LVH and lower left ventricular ejection fraction (44). Another study of non-diabetic individuals without overt CVD, found that high sensitivity cardiac troponin T (hs-cTnT) and BNP may be suitable for (the c-statistic for screening with BNP ± hs-cTnT was 0·81) identifying asymptomatic cardiac diseases including LVH (45). If the results of these studies can be confirmed in future large studies, pre-screening of people with T2DM using the biomarker approach may offer a cost-effective modality in screening LVH. Further systematic health economics studies are warranted to assess the cost-effectiveness of screening T2DM patients for LVH, and then pre-emptive treatment.

Pathophysiology of LVH and Potential Targets

Inflammation

Inflammation is a prominent hallmark of LVH. Pro-inflammatory cytokines are found to modulate left ventricle LV structure and play a critical role in LV remodelling (46). Inflammatory mediators can result in changes in cardiac size, shapes, and composition, including myocyte hypertrophy, alterations in expression of foetal gene, and progressive myocyte loss through apoptosis (46). Pro-inflammatory cytokines such as IL-1, IL-6 and tumour necrosis factor-alpha (TNF-α) are normally not expressed in the heart, but they are activated and up regulated as response to myocardial injury or mechanical stress, contributing to cardiac remodelling (46). Soon after ischemic myocardial injury, TNF-α and IL-6 are elaborated and trigger additional cellular inflammatory responses (47). Chronically, to repair and remodelling, these cytokines activate matrix metalloproteinases and collagen formation, and regulate angiogenesis and progenitor cell mobilisation, resulting in development of myocardial hypertrophy, collagen deposition, and fibrosis (47). In macrophages, lipopolysaccharide (LPS), tumour necrosis factor-α, and interferon-γ, has been shown to be implicated in cardiac hypertrophy via their effects on the expression of microRNA-155 (miR-155) expression, a powerful mediator of cardiac hypertrophy (48). Notably, variable myocyte hypertrophy and inflammatory cell infiltration with activation of nuclear factor kappa B (NF-κB) have been identified in endomyocardial tissue of patients with Hypertrophic

cardiomyopathy (HCM) while the levels of in interleukins (IL-1β, IL-1RA, IL-6, IL-10) and high-sensitivity C-reactive protein (hsCRP) have been found to be significantly higher in patients with HCM than in control subjects (49). These suggest that a low-grade inflammatory response may play an important role in the development of cardiac hypertrophy in patients with HCM and support the causative significance of inflammatory signalling in hypertrophic heart disease, demonstrating the feasibility of therapeutic targeting of inflammation in heart failure.

Oxidative Stress

Growing evidence points to the potential involvement of oxidative stress in the pathophysiology of cardiac hypertrophy *via* different possible mechanisms. Dysregulated expression of nitrogen oxides (NOX) proteins, which are predominant isoforms expressed in cardiac tissue, has been proposed to contribute to the development of cardiac hypertrophy and is believed to driven by eroxisome proliferator-activated receptor α (PPARα) downregulation (50). Upregulation of NOX4 by angiotensin II, which is primary in mitochondria of cardiomyocytes, can also result in inducing nuclear export of histone deacetylase 4 (HDAC4), a crucial precursor of cardiac hypertrophy (51). NOX2 and NOX4 have also been found to be associated with cardiac hypertrophy and fibrosis in diabetic rats, and the elevation of NOX2 has been shown to be associated with an increase in cardiomyocytes size in mice subjected to high fat diet (50). Furthermore, In an experimental guinea pig model of pressure-overload induced cardiac hypertrophy, increased oxidative stress can result in an increase in cardiac ROS production, which induces cardiac hypertrophy through activation of redox-sensitive protein kinases such as mitogen activated protein kinase (MAPK) (52). In neonatal cardiomyocytes, however, ROS has been found to active a wide variety of hypertrophic signalling kinases and transcription factors, including the tyrosine kinase Src, GTP-binding protein Ras, protein kinase C, mitogen-activated protein kinases (extracellular response kinase and extracellular signal–regulated kinase), and Jun-nuclear kinase, NK-KB and Phosphoinositol 3-kinase, which is required for H2O2-induced hypertrophy (53). Finally, in diabetic patients, it is hypothesised that myocardial kinases β isoform of protein kinase C (PKCβ), which is preferentially overexpressed in diabetic myocardium accompanied with increased upregulation of pro-oxidant enzyme NAPH oxidase, that is a major upstream moderator of oxidative stress and that inhibition of PKCβ can attenuate myocardial hypertrophy (54). All these observations support the role of oxidative stress in the pathophysiology of cardiac hypertrophy.

ROLE OF INSULIN SIGNALLING, MTOR, AND AMPK IN CARDIAC HYPERTROPHY

Insulin resistance has long been recognised as a common factor in and contributing towards the intersection between diagnosis of heart disease and diabetes in the same patient (55). In brief, development of insulin resistance over time, initially compensated by hyperinsulinemia, but ultimately resulting in beta cell failure, likely results in successive periods where there is first overstimulation and then under stimulation of anabolic insulin-sensitive signalling pathways in cardiomyocytes and other tissues, any of which, as well as the metabolic flexibility inherent in insulin resistance, may contribute to pathology. Many of the proteins mediating insulin signalling are encoded by more than one gene, which has complicated mechanistic genetic analysis. Consistent with the likely complex role of insulin signalling in heart disease, deletion of IRS1 and IRS2 docking proteins in liver results in heart failure in mice, whereas deletion of the same genes in cardiac tissue resulted in smaller ventricular mass (56). These findings also illustrate that insulin signalling in cardiac tissue and in non-cardiac tissue, are both likely to have an important impact on progression of heart disease. More work is required to determine the critical metabolic regulatory pathways underlying these effects.

One of the metabolic signalling pathways most studied in cardiac dysfunction is mammalian target of rapamycin (mTOR) signalling. The mTOR signalling pathways are at the crossroads of anabolic and catabolic cellular process (57). mammalian target of rapamycin complex 1 (mTORC1) promotes anabolic metabolism in response to growth factor signalling, nutrients (particularly amino acids), and increased energy supply. mTORC1 also suppresses autophagy (58). mTORC1 phosphorylates Unc-51 like autophagy activating kinase (ULK1), a kinase that forms a complex with ATG13, FIP2000, and ATG101 and drives autophagosome formation, disrupting the interaction between Ulk1 and AMPK and prevent Ulk1 activation by AMPK (59). At transcriptional level, mTORC1 can also regulate autophagy. It prevents nuclear transport of the transcription factor EB (TFEB), which drives genes expression for lysosomes biogenesis and the autophagy machinery (60). In these ways mTORC1 is understood largely to determine the extent of cellular anabolic and catabolic processes, whilst in contrast mTORC2 seems to function mainly as an effector of insulin/IGF-1 signalling ref 8. mTORC2 activates and phosphorylates protein kinase B (PKB), a key effector of insulin/PI3K signalling. Phosphorylation of PKB results in cell promotion and proliferation via activation and suppression of many key substrates including the transcription factors of FoxO1/3a, the metabolic regulator (GSK3β), and the mTORC1 inhibitor (TSC2) (58). mTORC2 is also regulated by mTORC1 through a negative feedback loop between mTORC1 and insulin PI3K signalling. mTORC1 activates (Grb10), a negative regulator of insulin/IGF-1 receptor signalling upstream of Akt and mTORC2 (58).

In the cardiovascular system, mTOR pathways are understood to be important regulators of the hypertrophic response (61). Loss of function genetic studies indicate that mTORC1 activation is indispensable for development of cardiac hypertrophy in response to pressure overload. An animal study showed that the deletion of Rheb 1, which mediates mTORC1 activation, in cardiomyocytes confers cardioprotection against pathological remodelling in pressure overload (62). The mTORC1 regulates cardiac function and myocyte survival through 4E-BP1 inhibition in mice. A study showed a significant improvement in apoptosis and heart function when Mtor and the gene encoding 4E-BP1, an mTOR-containing multiprotein complex-1

(mTORC1) substrate that inhibits translation initiation, was deleted together (63).

Furthermore, in mice, ablation of cardiac raptor, a gene required for normal cardiac physiological function and for heart adaptation to increased workload, is shown to impair adaptive hypertrophy, change metabolic gene expression, and causing heart failure (64). In raptor-cKO mice, pressure overload causes a significant cardiac dilatation and immediate reduction in ejection fraction (64).

Consistent with this, pharmacological inhibition of mTORC1 also suppresses hypertrophy. Rapamycin, a specific inhibitor of mTOR, inhibits established cardiac hypertrophy via a complete suppression the phosphorylation of S6K1 and S6 phosphorylation in response to pressure overload (65). This can explain the attenuation of the increase in myocyte cell size induced by aortic constriction after administering Rapamycin to mice (66). Rapamycin is also shown to extend lifespan of mice (67). However, although it is shown that rapamycin can reverse age-dependent defects in cardiac function (68) and can protect against the changes of atherosclerosis (69), it is still unclear if an increase in lifespan owes to effects on cardiovascular system (67).

AMPK is another protein kinase that regulates many aspects of cellular energy balance and is seen as a promising target for anti-hypertrophic drugs. AMPK activation reverses increased protein O-GlcNAcylation, which is associated with cardiac hypertrophy, mainly through controlling the glutamine: fructose-6-phosphate aminotransferase (GFAT) phosphorylation, resulting in a decrease in O-GlcNAcylation of proteins such as troponin T (70). In line with this, an increase in cellular O-GlcNAc levels in response to O-linked N-acetylglucosamine (O-GlcNAc) inducers completely supresses the anti-hypertrophic effect of AMPK (70).

Metformin and AICAR have each been shown in preclinical studies to inhibit cardiac hypertrophy. Long-term metformin treatment significantly increases AMPK phosphorylation and attenuates cardiac hypertrophy induced by Transverse aortic constriction (TAC) (71). Interestingly, the antihypertrophic effects of metformin were not observed in AMPKα2−/− mice (71), suggesting that the chronic activation of AMPK during the development of cardiac hypertrophy is an important mechanism that mediates the beneficial effect of metformin. Moreover, long-term activation of AMPK by AICAR has been shown to block load-induced calcineurin–NFAT pathway as calcineurin is also regulated by MAPK pathway. Thus, the activation of AMPK may counteract MAPK pathway though blocking calcineurin–NFAT pathway (72).

However, counterbalancing these promising findings is recent evidence that a pan-AMPK activator improves glucose homeostasis, but at the cost of increased hypertrophy. Chronic activation of MK-8722, pan-AMPK activator, increases glucose uptake into skeletal muscle, but it results in cardiac hypertrophy associated with increased cardiac glycogen contents (73). To determine which of these beneficial and less beneficial aspects of the drugs really do owe to AMPK, further genetic studies are required.

Role of Drug Repurposing in LVH Regression

Due to the high attrition rates, significant economic burden and lengthy new drug discovery process, repurposing of 'old' drugs (also known as drug repositioning, reprofiling or re-tasking) to treat both common and rare diseases is increasingly becoming a promising field in drug discovery. Drug repurposing identifies new therapeutic uses for already approved drugs that are outside the scope of the original use (74). This is an attractive proposition because it involves the use of existing compounds that have undergone rigorous testing with potentially lower overall development costs and shorter development timelines.

Hypertension is the most common causes of LVH and therefore, controlling blood pressure (BP) especially with drugs that blocks the renin-angiotensin system (RAS) is the standard approach to the management of LVH. However, this approach is only partially effective since LVH persists in 20% of hypertensives that attain target BP (75). Thus "normotensive LVH" is very common (12). Indeed, BP only contributes 25% to the variability in LV mass seen in a population (76). Despite a "normal" BP, normotensive LVH is just as risky as is hypertensive LVH (77). Nevertheless, regressing LVH irrespective of BP changes is an effective way to reduce the incidence of all major CV events including specifically sudden deaths, heart failure hospitalisations, new onset AF and strokes (37, 38, 78–83). The LIFE study demonstrates that LVH regression *per se* reduces future cardiovascular events irrespective of BP (84). Since controlling BP and using a RAS blocker is only partially effective at regressing LVH, we now need additional ways of regressing LVH. In addition to BP, as we have discussed above, the pathophysiology of LVH may involve a complex cocktail of various non-hemodynamic disease processes including inflammation, oxidative stress, obesity, and insulin resistance (15, 29, 31, 53, 85–90).

Below, we provide a narrative review of some of the key clinical trials that evaluated the effects of interventions targeting LVH in patients with and without T2DM, in the context of normal BP.

Targeting LVH With Allopurinol

Allopurinol, a xanthine oxidase (XO) inhibitor, has been the mainstay of treatment for patients with gout associated with hyperuricemia for several decades. In addition, there is mounting evidence to suggest cardioprotective effects of allopurinol (91–93). Allopurinol has been shown to exert its cardioprotective effects through three key mechanisms: (i) reduction of uric acid concentrations which has pro-inflammatory effects; (ii) inhibiting xanthine oxidase mediated production of reactive oxygen species (ROS) which aggravate endothelial dysfunction and atherosclerosis plaque instability; and (iii) increasing local tissue availability of adenosine triphosphate and oxygen by inhibiting purine metabolism (94).

Allopurinol has been shown to regress LVM in different cohorts along the cardiovascular spectrum with significant pre-existing disease, oxidative stress and inflammation. In a RCT of people with T2DM, 9 months treatment of allopurinol

treatment significantly reduced absolute left ventricular mass (LVM) (allopurinol −2.65 ± 5.91 g vs. placebo group +1.21 ± 5.10 g; $p = 0.012$) and LVM indexed (LVMI) to body surface area (allopurinol −1.32 ± 2.84 g/m(2) vs. placebo group +0.65 ± 3.07 g/m(2); $p = 0.017$) (18). In another RCT of people with ischemic heart disease, allopurinol treatment significantly reduced LVM (allopurinol −5.2 ± 5.8 g vs. placebo −1.3 ± 4.48 g; $p = 0.007$) and LVMI (allopurinol −2.2 ± 2.78 g/m(2) vs. placebo −0.53 ± 2.5 g/m(2); $p = 0.023$) (17). Furthermore, Kao et al., in another RCT of patients with severe renal disease (CKD 3) reported significant reduction in LVM with 9 months allopurinol treatment (95). In contrast, a recent study by Gingles et al., reported that LVM regression was significantly reduced with allopurinol treatment than placebo, suggestive of a potential adverse effect (96). However, unlike other studies, in this RCT, the study population had well-controlled BP and were normouricemic with low oxidative stress. This may have negated any direct effect of allopurinol, who rely on urate for antioxidant defence, in reducing ROS generation by XO inhibition, and consequent null effect on LVH. Therefore, the regressive effect of allopurinol may not be observed universally in all study populations.

Targeting LVH With Metformin

Metformin is an oral antihyperglycemic agent that has been used widely for the treatment of T2DM for over many decades. Beyond its antihyperglycemic effects, there is now accumulating evidence to suggest that metformin is cardioprotective (97). While the exact mechanism of cardioprotective actions of metformin is not fully understood, several ancillary mechanisms have been proposed to explain the metformin induced LVH regression. First, as stated already, insulin resistance, inflammation, oxidative stress, endothelial dysfunction and obesity is understood to contribute to the development of LVH (15, 53, 85–89), and metformin has been shown to reduce insulin resistance (98), inflammation (99), oxidative stress (100–104), central obesity (105) and endothelial dysfunction (106), albeit the latter is not a consistent finding (107). Second, *in vivo* studies have reported activation of AMP-activated protein kinase (AMPK) as one of the putative mechanisms for the anti-hypertrophic effect of metformin (15), leading to the view that AMPK stimulation is a promising new strategy to prevent or reduce LVH (108–110).

For clinical trials, to date, only one study: The MET-REMODEL trial, has explicitly investigated the effect of metformin on LVH in non-diabetic CAD patients identified to have IR and/or diabetes. This study demonstrated that 12 months metformin treatment (2 g/day) significantly reduced LVMI (absolute mean difference −1.37 (95% CI: −2.63 to −0.12, $P = 0.033$) (111). In this study, metformin also significantly reduced LVM, systolic blood pressure, and oxidative stress. In line with these findings, few other clinical studies and a network analysis also reported anti-hypertrophic effects of metformin (112–114).

Targeting LVH With SGLT2 Inhibitors: Multipronged Approach

The sodium-glucose linked cotransporter type 2 (SGLT2) class of inhibitors was developed as a novel anti-diabetic agent that acts independent of the insulin-incretin pathway to lower blood sugar. Various classes of SGLT2 inhibitors such as empagliflozin, canagliflozin and dapagliflozin have been shown to reduce cardiovascular mortality in patients with diabetes mellitus (115–118), but its cardioprotective mechanism remains elusive. More recent evidence from RCTs, suggest the potential of dapagliflozin in reducing the risk of worsening heart failure or CV mortality, even in non-diabetic population (119, 120). However, it is not clear whether the cardioprotective effects of SGLT2 inhibition

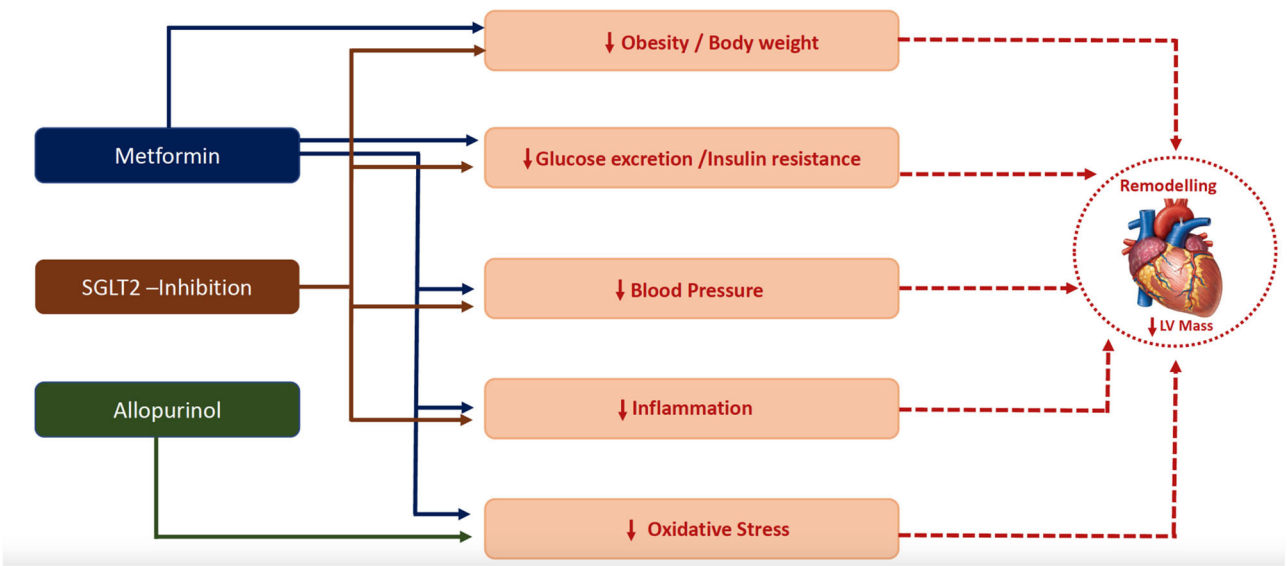

FIGURE 2 | Plausible mechanisms by which metformin (111), SGLT-2 inhibitor (128) and allopurinol (17, 18) regressed left ventricular hypertrophy.

in non-diabetic patients is class effect or drug-specific effect (121–124). Unlike other antidiabetic agents that are dependent on pancreatic beta-cell function and insulin sensitivity for the glucose lowering effect, the principal mechanism by which SGLT2 inhibitors lower blood glucose is by excreting excess glucose by enhancing urinary glucose excretion. Part of the off-target effects that have been observed with this class of drugs include weight loss, improved glycemia/lipid profile, arterial stiffness, reduce preload and afterload (blood pressure) and diuresis (125, 126)—all of which are key risk factors implicated in the development of LVH (15).

The DAPA-LVH study was the first placebo controlled RCT to investigate the efficacy of dapagliflozin in regressing LVH in normotensive patients with T2DM, without pre-existing CVD (127). At 12 months, dapagliflozin treatment significantly reduced LVM in people with T2DM, as assessed by cardiac magnetic resonance imaging (128). In this study, dapagliflozin was also shown to significantly reduce systolic BP, body weight, abdominal obesity (both visceral and subcutaneous), insulin resistance, and hsCRP. A similar finding was observed in the EMPA-HEART study that reported anti-hypertrophic effect of empagliflozin (129). In the DAPA-LVH trial, LVH regression was greater in those with higher baseline LVM (128). It is to be noted that a recent subgroup analysis of the EMPA-REG OUTCOME trial reported lower incidence of CVD in patients with LVH compared with those without LVH (130). Furthermore, *post-hoc* exploratory analysis from DAPA-LVH trial suggest dapagliflozin may improve subclinical dysfunction, as evidenced by improved myocardial longitudinal function (131). Taken together, there is compelling evidence to suggest that SGLT2 have the potential to promote reverse LV remodelling in patients with diabetes, which may, at least in part, explain the cardioprotective effects observed in large outcome trials of SGLT2.

CONCLUSIONS

In this mini-review, we have argued that LVH is a good surrogate marker of diabetic cardiomyopathy and discussed the trials targeting LVH as a manifestation of Stage B Cardiomyopathy, and potential mechanisms behind LVH regression (**Figure 2**) in patients with T2DM or in insulin-resistant individuals. We believe that cardiovascular outcome trials are still needed to provide definitive evidence for the cardio-protective role of the proposed repurposed drugs. With respect to metformin, the results of the on-going outcome trials such as the VA IMPACT trial (VA IMPACTNCT02915198) and Glucose Lowering in Non-diabetic hyperglycaemia Trial (GLINT; ISRCTN34875079), will be informative and might provide the needed evidence for recommending metformin in these at risk patients.

FUTURE RESEARCH

All the clinical studies discussed in this manuscript were "proof of concept" studies, conducted in small sample size, to evaluate the possible mechanisms behind the purported cardio-protective effects of each drugs. Unlike, the LVH regression observed in previous hypertension trials, the magnitude of the LVH regression observed in these trials were small, which may be, at least in part due to the shorter follow-up period and treatment duration. The results of these proof-of-concept trials are encouraging and help underpin.future large cardiovascular outcome trials, with longer follow-up period, incorporating better hard end points. One such trial is the VA-IMPACT trial (VA IMPACT NCT−02915198) of metformin. Such trials will be informative and help provide the medical evidence to support the use these drugs in diabetic cardiomyopathy.

AUTHOR CONTRIBUTIONS

CCL and MM conceived the idea. MM designed the figure. MM and AD wrote the first draft of the manuscript and was responsible for synthesizing the evidence, the search strategy, and conducted the literature search. IM, AMC, GR, and CCL critically reviewed and revised the manuscript. All authors read and approved the final manuscript.

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REFERENCES

1. Jia G, Hill MA, Sowers JR. Diabetic cardiomyopathy: an update of mechanisms contributing to this clinical entity. *Circ Res.* (2018) 122:624–38. doi: 10.1161/CIRCRESAHA.117.311586
2. Rubler S, Dlugash J, Yuceoglu YZ, Kumral T, Branwood AW, Grishman A. New type of cardiomyopathy associated with diabetic glomerulosclerosis. *Am J Cardiol.* (1972) 30:595–602. doi: 10.1016/0002-9149(72)90595-4
3. Cas D, Khan SS, Butler J, Mentz RJ, Bonow RO, Avogaro A, et al. Impact of diabetes on epidemiology, treatment, and outcomes of patients with heart failure. *JACC Heart Fail.* (2015) 3:136–45. doi: 10.1016/j.jchf.2014.08.004
4. Tousoulis D, Oikonomou E, Siasos G, Stefanadis C. Diabetes mellitus and heart failure. *Eur Cardiol.* (2014) 9:37–42. doi: 10.15420/ecr.2014.9.1.37
5. Aune D, Schlesinger S, Neuenschwander M, Feng T, Janszky I, Norat T, et al. Diabetes mellitus, blood glucose and the risk of heart failure: a systematic review and meta-analysis of prospective studies. *Nutr Metab Cardiovasc Dis.* (2018) 28:1081–91. doi: 10.1016/j.numecd.2018.07.005
6. Jia G, DeMarco VG, Sowers JR. Insulin resistance and hyperinsulinaemia in diabetic cardiomyopathy. *Nat Rev Endocrinol.* (2016) 12:144–53. doi: 10.1038/nrendo.2015.216
7. Lee M, Gardin JM, Lynch JC, Smith VE, Tracy RP, Savage PJ, et al. Diabetes mellitus and echocardiographic left ventricular function in free-living elderly men and women: the cardiovascular health study. *Am Heart J.* (1997) 133:36–43. doi: 10.1016/S0002-8703(97)70245-X
8. Moritz T, Duckworth W, Abraira C. Veterans Affairs diabetes trial—corrections. *N Engl J Med.* (2009) 361:1024–5. doi: 10.1056/NEJMc0 96250
9. Patel A, MacMahon S, Chalmers J, Neal B, Billot L, Woodward M, et al. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *N Engl J Med.* (2008) 358:2560–72. doi: 10.1056/NEJMoa0802987

10. Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ.* (2000) 321:405–12. doi: 10.1136/bmj.321.7258.405
11. Gosse PS. Left ventricular hypertrophy–the problem and possible solutions. *J Int Med Res.* (2005) 33 (Suppl 1):3A−11A. doi: 10.1177/14732300050330S102
12. Dawson A, Morris AD, Struthers AD. The epidemiology of left ventricular hypertrophy in type 2 diabetes mellitus. *Diabetologia.* (2005) 48:1971–9. doi: 10.1007/s00125-005-1896-y
13. Devereux RB, Roman MJ, Paranicas M, O'Grady MJ, Lee ET, Welty TK, et al. Impact of diabetes on cardiac structure and function: the strong heart study. *Circulation.* (2000) 101:2271–6. doi: 10.1161/01.CIR.101.19.2271
14. Artham SM, Lavie CJ, Milani RV, Patel DA, Verma A, Ventura HO. Clinical impact of left ventricular hypertrophy and implications for regression. *Prog Cardiovasc Dis.* (2009) 52:153–67. doi: 10.1016/j.pcad.2009.05.002
15. Mohan M, McSwiggan S, Baig F, Rutherford L, Lang CC. Metformin and its effects on myocardial dimension and left ventricular hypertrophy in normotensive patients with coronary heart disease (the MET-REMODEL study): rationale and design of the MET-REMODEL study. *Cardiovasc Ther.* (2015) 33:1–8. doi: 10.1111/1755-5922.12101
16. Öncel CR. Left ventricular hypertrophy, inflammation, insulin resistance. *Anatol J Cardiol.* (2016) 16:142. doi: 10.14744/AnatolJCardiol.2015.6857
17. Rekhraj S, Gandy SJ, Szwejkowski BR, Nadir MA, Noman A, Houston JG, et al. High-dose allopurinol reduces left ventricular mass in patients with ischemic heart disease. *J Am Coll Cardiol.* (2013) 61:92632. doi: 10.1016/j.jacc.2012.09.066
18. Szwejkowski BR, Gandy SJ, Rekhraj S, Houston JG, Lang CC, Morris AD, et al. Allopurinol reduces left ventricular mass in patients with type 2 diabetes and left ventricular hypertrophy. *J Am Coll Cardiol.* (2013) 62:2284–93. doi: 10.1016/j.jacc.2013.07.074
19. Goldberg LR, Jessup M. Stage B heart failure: management of asymptomatic left ventricular systolic dysfunction. *Circulation.* (2006) 113:2851–60. doi: 10.1161/CIRCULATIONAHA.105.600437
20. Pewsner D, Jüni P, Egger M, Battaglia M, Sundström J, Bachmann LM. Accuracy of electrocardiography in diagnosis of left ventricular hypertrophy in arterial hypertension: systematic review. *BMJ.* (2007) 335:711. doi: 10.1136/bmj.39276.636354.AE
21. Burgos PF, Luna Filho B, Costa FA, Bombig MT, Souza D, Bianco HT, et al. Electrocardiogram performance in the diagnosis of left ventricular hypertrophy in hypertensive patients with left bundle branch block. *Arq Bras Cardiol.* (2017) 108:47–52. doi: 10.5935/abc.20160187
22. Foppa M, Duncan BB, Rohde LE. Echocardiography-based left ventricular mass estimation. How should we define hypertrophy? *Cardiovasc Ultrasound.* (2005) 3:17. doi: 10.1186/1476-7120-3-17
23. Levy D, Labib SB, Anderson KM, Christiansen JC, Kannel WB, Castelli WP. Determinants of sensitivity and specificity of electrocardiographic criteria for left ventricular hypertrophy. *Circulation.* (1990) 81:815–20. doi: 10.1161/01.CIR.81.3.815
24. Pennell DJ, Sechtem UP, Higgins CB, Manning WJ, Pohost GM, Rademakers FE, et al. Cardiology. Clinical indications for cardiovascular magnetic resonance (CMR): Consensus Panel report. *Eur Heart J.* (2004) 25:1940–65. doi: 10.1016/j.ehj.2004.06.040
25. Drazner MH, Dries DL, Peshock RM, Cooper RS, Klassen C, Kazi F, et al. Left ventricular hypertrophy is more prevalent in blacks than whites in the general population: the Dallas Heart Study. *Hypertension.* (2005) 46:124–9. doi: 10.1161/01.HYP.0000169972.96201.8e
26. Lewis AA, Ayers CR, Selvin E, Neeland I, Ballantyne CM, Nambi V, et al. Racial differences in malignant left ventricular hypertrophy and incidence of heart failure: a multicohort study. *Circulation.* (2020) 141:957–67. doi: 10.1161/CIRCULATIONAHA.119.043628
27. Cuspidi C, Sala C, Negri F, Mancia G, Morganti A, Italian Society of Hypertension. Prevalence of left-ventricular hypertrophy in hypertension: an updated review of echocardiographic studies. *J Hum Hypertens.* (2012) 26:343–9. doi: 10.1038/jhh.2011.104
28. Levy D, Anderson KM, Savage DD, Kannel WB, Christiansen JC, Castelli WP. Echocardiographically detected left ventricular hypertrophy: prevalence and risk factors. The Framingham Heart Study. *Ann Intern Med.* (1988) 108:7–13. doi: 10.7326/0003-4819-108-1-7
29. Bella JN, Göring HH. Genetic epidemiology of left ventricular hypertrophy. *Am J Cardiovasc Dis.* (2012) 2:267–78.
30. Parry HM, Donnelly LA, Van Zuydam N, Doney AS, Elder DH, Morris AD, et al. Genetic variants predicting left ventricular hypertrophy in a diabetic population: a Go-DARTS study including meta-analysis. *Cardiovasc Diabetol.* (2013) 12:109. doi: 10.1186/1475-2840-12-109
31. Palmieri V, Tracy RP, Roman MJ, Liu JE, Best LG, Bella JN, et al. Relation of left ventricular hypertrophy to inflammation and albuminuria in adults with type 2 diabetes: the strong heart study. *Diabetes Care.* (2003) 26:2764–9. doi: 10.2337/diacare.26.10.2764
32. Fang ZY, Prins JB, Marwick TH. Diabetic cardiomyopathy: evidence, mechanisms, therapeutic implications. *Endocr Rev.* (2004) 25:543–67. doi: 10.1210/er.2003-0012
33. Muddu M, Mutebi E, Mondo C. Prevalence, types and factors associated with echocardiographic abnormalities among newly diagnosed diabetic patients at Mulago Hospital. *Afr Health Sci.* (2016) 16:183–93. doi: 10.4314/ahs.v16i1.25
34. Srivastava PM, Calafiore P, Macisaac RJ, Patel SK, Thomas MC, Jerums G, et al. Prevalence and predictors of cardiac hypertrophy and dysfunction in patients with Type 2 diabetes. *Clin Sci.* (2008) 114:313–20. doi: 10.1042/CS20070261
35. Somaratne JB, Whalley GA, Poppe KK, ter Bals MM, Wadams G, Pearl A, et al. Screening for left ventricular hypertrophy in patients with type 2 diabetes mellitus in the community. *Cardiovasc Diabetol.* (2011) 10:29. doi: 10.1186/1475-2840-10-29
36. Wu PY, Huang JC, Chen SC, Chen LI. Type 2 diabetes mellitus-related changes in left ventricular structure and function in patients with chronic kidney disease. *Oncotarget.* (2018) 9:14661–8. doi: 10.18632/oncotarget.24482
37. Okin PM, Devereux RB, Harris KE, Jern S, Kjeldsen SE, Julius S, et al. Regression of Electrocardiographic left ventricular hypertrophy is associated with less hospitalization for heart failure in hypertensive patients. *Annals of Internal Medicine.* (2007) 147:311–9. doi: 10.7326/0003-4819-147-5-200709040-00006
38. Wachtell K, Okin PM, Olsen MH, Dahlof B, Devereux RB, Ibsen H, et al. Regression of electrocardiographic left ventricular hypertrophy during antihypertensive therapy and reduction in sudden cardiac death: the LIFE Study. *Circulation.* (2007) 116:700–5. doi: 10.1161/CIRCULATIONAHA.106.666594
39. Witham MD, Davies JI, Dawson A, Davey PG, Struthers AD. Hypothetical economic analysis of screening for left ventricular hypertrophy in high-risk normotensive populations. *QJM.* (2004) 97:87–93. doi: 10.1093/qjmed/hch016
40. Selvarajah S, Haniff J, Kaur G, Guat Hiong T, Bujang A, Chee Cheong K, et al. Identification of effective screening strategies for cardiovascular disease prevention in a developing country: using cardiovascular risk-estimation and risk-reduction tools for policy recommendations. *BMC Cardiovasc Disord.* (2013) 13:10. doi: 10.1186/1471-2261-13-10
41. Barton P, Andronis L, Briggs A, McPherson K, Capewell S. Effectiveness and cost effectiveness of cardiovascular disease prevention in whole populations: modelling study. *BMJ.* (2011) 343:d4044. doi: 10.1136/bmj.d4044
42. Chamnan P, Simmons RK, Khaw KT, Wareham NJ, Griffin SJ. Estimating the population impact of screening strategies for identifying and treating people at high risk of cardiovascular disease: modelling study. *BMJ.* (2010) 340:c1693. doi: 10.1136/bmj.c1693
43. Vasan RS. Biomarkers of cardiovascular disease: molecular basis and practical considerations. *Circulation.* (2006) 113:2335–62. doi: 10.1161/CIRCULATIONAHA.104.482570
44. Dawson A, Davies JI, Morris AD, Struthers AD. B-type natriuretic Peptide is associated with both augmentation index and left ventricular mass in diabetic patients without heart failure. *Am J Hypertens.* (2005) 18(12 Pt 1):1586–91. doi: 10.1016/j.amjhyper.2005.06.016
45. Nadir MA, Rekhraj S, Wei L, Lim TK, Davidson J, MacDonald TM, et al. Improving the primary prevention of cardiovascular events by using biomarkers to identify individuals with silent heart disease. *J Am Coll Cardiol.* (2012) 60:960–8. doi: 10.1016/j.jacc.2012.04.049

46. Mann DL. Inflammatory mediators and the failing heart: past, present, and the foreseeable future. *Circ Res.* (2002) 91:988–98. doi: 10.1161/01.RES.0000043825.01705.1B
47. Nian M, Lee P, Khaper N, Liu P. Inflammatory cytokines and postmyocardial infarction remodeling. *Circ Res.* (2004) 94:1543–53. doi: 10.1161/01.RES.0000130526.20854.fa
48. Heymans S, Corsten MF, Verhesen W, Carai P, van Leeuwen RE, Custers K, et al. Macrophage microRNA-155 promotes cardiac hypertrophy and failure. *Circulation.* (2013) 128:1420–32. doi: 10.1161/CIRCULATIONAHA.112.001357
49. Kuusisto J, Karja V, Sipola P, Kholova I, Peuhkurinen K, Jaaskelainen P, et al. Low-grade inflammation and the phenotypic expression of myocardial fibrosis in hypertrophic cardiomyopathy. *Heart.* (2012) 98:1007–13. doi: 10.1136/heartjnl-2011-300960
50. Ramachandra CJA, Cong S, Chan X, Yap EP, Yu F, Hausenloy DJ. Oxidative stress in cardiac hypertrophy: From molecular mechanisms to novel therapeutic targets. *Free Radic Biol Med.* (2021) 166:297–312. doi: 10.1016/j.freeradbiomed.2021.02.040
51. Okabe K, Matsushima S, Ikeda S, Ikeda M, Ishikita A, Tadokoro T, et al. DPP (Dipeptidyl Peptidase)-4 Inhibitor Attenuates Ang II (Angiotensin II)-Induced Cardiac Hypertrophy via GLP (Glucagon-Like Peptide)-1-Dependent Suppression of Nox (Nicotinamide Adenine Dinucleotide Phosphate Oxidase) 4-HDAC (Histone Deacetylase) 4 Pathway. *Hypertension.* (2020) 75:991–1001. doi: 10.1161/HYPERTENSIONAHA.119.14400
52. Li JM, Gall NP, Grieve DJ, Chen M, Shah AM. Activation of NADPH oxidase during progression of cardiac hypertrophy to failure. *Hypertension.* (2002) 40:477–84. doi: 10.1161/01.HYP.0000032031.30374.32
53. Takimoto E, Kass DA. Role of oxidative stress in cardiac hypertrophy and remodeling. *Hypertension.* (2007) 49:241–8. doi: 10.1161/01.HYP.0000254415.31362.a7
54. Liu Y, Lei S, Gao X, Mao X, Wang T, Wong GT, et al. PKCbeta inhibition with ruboxistaurin reduces oxidative stress and attenuates left ventricular hypertrophy and dysfunction in rats with streptozotocin-induced diabetes. *Clin Sci.* (2012) 122:161–73. doi: 10.1042/CS20110176
55. MacDonald MR, Petrie MC, Hawkins NM, Petrie JR, Fisher M, McKelvie R, et al. Diabetes, left ventricular systolic dysfunction, and chronic heart failure. *Eur Heart J.* (2008) 29:1224–40. doi: 10.1093/eurheartj/ehn156
56. Qi Y, Xu Z, Zhu Q, Thomas C, Kumar R, Feng H, et al. Myocardial loss of IRS1 and IRS2 causes heart failure and is controlled by p38alpha MAPK during insulin resistance. *Diabetes.* (2013) 62:3887–900. doi: 10.2337/db13-0095
57. Eichner R, Fernandez-Saiz V, Targosz BS, Bassermann F. Cross talk networks of mammalian target of rapamycin signaling with the ubiquitin proteasome system and their clinical implications in multiple myeloma. *Int Rev Cell Mol Biol.* (2019) 343, 219–97. doi: 10.1016/bs.ircmb.2018.06.001
58. Saxton RA, Sabatini DM. mTOR signaling in growth, metabolism, and disease. *Cell.* (2017) 168:960–76. doi: 10.1016/j.cell.2017.02.004
59. Kim J, Kundu M, Viollet B, Guan KL. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol.* (2011) 13:132–41. doi: 10.1038/ncb2152
60. Martina JA, Chen Y, Gucek M, Puertollano R. MTORC1 functions as a transcriptional regulator of autophagy by preventing nuclear transport of TFE*Autophagy B.* (2012) 8:903–14. doi: 10.4161/auto.19653
61. Sciarretta S, Forte M, Frati G, Sadoshima J. New insights into the role of mTOR signaling in the cardiovascular system. *Circ Res.* (2018) 122:489–505. doi: 10.1161/CIRCRESAHA.117.311147
62. Wu X, Cao Y, Nie J, Liu H, Lu S, Hu X, et al. Genetic and pharmacological inhibition of Rheb1-mTORC1 signaling exerts cardioprotection against adverse cardiac remodeling in mice. *Am J Pathol.* (2013) 182:2005–14. doi: 10.1016/j.ajpath.2013.02.012
63. Zhang D, Contu R, Latronico MV, Zhang J, Rizzi R, Catalucci D, et al. MTORC1 regulates cardiac function and myocyte survival through 4E-BP1 inhibition in mice. *J Clin Invest.* (2010) 120:2805–16. doi: 10.1172/JCI43008
64. Shende P, Plaisance I, Morandi C, Pellieux C, Berthonneche C, Zorzato F, et al. Cardiac raptor ablation impairs adaptive hypertrophy, alters metabolic gene expression, and causes heart failure in mice. *Circulation.* (2011) 123:1073–82. doi: 10.1161/CIRCULATIONAHA.110.977066
65. McMullen JR, Sherwood MC, Tarnavski O, Zhang L, Dorfman AL, Shioi T, et al. Inhibition of mTOR signaling with rapamycin regresses established cardiac hypertrophy induced by pressure overload. *Circulation.* (2004) 109:3050–5. doi: 10.1161/01.CIR.0000130641.08705.45
66. Shioi T, McMullen JR, Tarnavski O, Converso K, Sherwood MC, Manning WJ, et al. Rapamycin attenuates load-induced cardiac hypertrophy in mice. *Circulation.* (2003) 107:1664–70. doi: 10.1161/01.CIR.0000057979.36322.88
67. Miller RA, Harrison DE, Astle CM, Fernandez E, Flurkey K, Han M, et al. Rapamycin-mediated lifespan increase in mice is dose and sex dependent and metabolically distinct from dietary restriction. *Aging Cell.* (2014) 13:468–77. doi: 10.1111/acel.12194
68. Flynn JM, O'Leary MN, Zambataro CA, Academia EC, Presley MP, Garrett BJ, et al. Late-life rapamycin treatment reverses age-related heart dysfunction. *Aging Cell.* (2013) 12:851–62. doi: 10.1111/acel.12109
69. Mueller MA, Beutner F, Teupser D, Ceglarek U, Thiery J. Prevention of atherosclerosis by the mTOR inhibitor everolimus in LDLR-/- mice despite severe hypercholesterolemia. *Atherosclerosis.* (2008) 198:39–48. doi: 10.1016/j.atherosclerosis.2007.09.019
70. Gelinas R, Mailleux F, Dontaine J, Bultot L, Demeulder B, Ginion A, et al. AMPK activation counteracts cardiac hypertrophy by reducing O-GlcNAcylation. *Nat Commun.* (2018) 9:374. doi: 10.1038/s41467-017-02795-4
71. Fu YN, Xiao H, Ma XW, Jiang SY, Xu M, Zhang YY. Metformin attenuates pressure overload-induced cardiac hypertrophy via AMPK activation. *Acta Pharmacol Sin.* (2011) 32:879–87. doi: 10.1038/aps.2010.229
72. Li HL, Yin R, Chen D, Liu D, Wang D, Yang Q, et al. Long-term activation of adenosine monophosphate-activated protein kinase attenuates pressure-overload-induced cardiac hypertrophy. *J Cell Biochem.* (2007) 100:1086–99. doi: 10.1002/jcb.21197
73. Myers RW, Guan HP, Ehrhart J, Petrov A, Prahalada S, Tozzo E, et al. Systemic pan-AMPK activator MK-8722 improves glucose homeostasis but induces cardiac hypertrophy. *Science.* (2017) 357:507–11. doi: 10.1126/science.aah5582
74. Ashburn TT, Thor KB. Drug repositioning: identifying and developing new uses for existing drugs. *Nat Rev Drug Discov.* (2004) 3:673–83. doi: 10.1038/nrd1468
75. Mancia G, Carugo S, Grassi G, Lanzarotti A, Schiavina R, Cesana G, et al. Prevalence of left ventricular hypertrophy in hypertensive patients without and with blood pressure control: data from the PAMELA population. Pressioni Arteriose Monitorate E Loro Associazioni. *Hypertension.* (2002) 39:744–9. doi: 10.1161/hy0302.104669
76. Fraser R. Studying genes and the development of cardiac hypertrophy: convenient intermediate phenotypes in man. *J Hypertens.* (2003) 21:873–4. doi: 10.1097/00004872-200305000-00010
77. Brown DW, Giles WH, Croft JB. Left ventricular hypertrophy as a predictor of coronary heart disease mortality and the effect of hypertension. *Am Heart J.* (2000) 140:848–56. doi: 10.1067/mhj.2000.111112
78. Verdecchia P, Schillaci G, Borgioni C, Ciucci A, Gattobigio R, Zampi I, et al. Prognostic significance of serial changes in left ventricular mass in essential hypertension. *Circulation.* (1998) 97:48–54. doi: 10.1161/01.CIR.97.1.48
79. Verdecchia P, Angeli F, Borgioni C, Gattobigio R, de Simone G, Devereux RB, et al. Changes in cardiovascular risk by reduction of left ventricular mass in hypertension: a meta-analysis. *Am J Hypertens.* (2003) 16(11 Pt 1):895–9. doi: 10.1016/S0895-7061(03)01018-5
80. Verdecchia P, Angeli F, Gattobigio R, Sardone M, Pede S, Reboldi GP. Regression of left ventricular hypertrophy and prevention of stroke in hypertensive subjects. *Am J Hypertens.* (2006) 19:493–9. doi: 10.1016/j.amjhyper.2005.10.018
81. Okin PM, Devereux RB, Jern S, Kjeldsen SE, Julius S, Nieminen MS, et al. Regression of electrocardiographic left ventricular hypertrophy during antihypertensive treatment and the prediction of major cardiovascular events. *JAMA.* (2004) 292:2343–9. doi: 10.1001/jama.292.19.2343
82. Okin PM, Wachtell K, Devereux RB, Harris KE, Jern S, Kjeldsen SE, et al. Regression of electrocardiographic left ventricular hypertrophy and decreased incidence of new-onset atrial fibrillation in patients with hypertension. *JAMA.* (2006) 296:1242–8. doi: 10.1001/jama.296.10.1242

83. Koren MJ, Ulin RJ, Koren AT, Laragh JH, Devereux RB. Left ventricular mass change during treatment and outcome in patients with essential hypertension. *Am J Hypertens.* (2002) 15:1021–8. doi: 10.1016/S0895-7061(02)03061-3
84. Dahlöf B, Devereux RB, Kjeldsen SE, Julius S, Beevers G, de Faire U, et al. Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. *Lancet.* (2002) 359:995–1003. doi: 10.1016/S0140-6736(02)08089-3
85. Rababa'h AM, Guillory AN, Mustafa R, Hijjawi T. Oxidative stress and cardiac remodeling: an updated edge. *Curr Cardiol Rev.* (2018) 14:53–9. doi: 10.2174/1573403X14666180111145207
86. de Simone G, Izzo R, De Luca N, Gerdts E. Left ventricular geometry in obesity: is it what we expect? *Nutr Metab Cardiovasc Dis.* (2013) 23:905–12. doi: 10.1016/j.numecd.2013.06.012
87. Cuspidi C, Rescaldani M, Sala C, Grassi G. Left-ventricular hypertrophy and obesity: a systematic review and meta-analysis of echocardiographic studies. *J Hypertens.* (2014) 32:16–25. doi: 10.1097/HJH.0b013e328364fb58
88. Masiha S, Sundström J, Lind L. Inflammatory markers are associated with left ventricular hypertrophy and diastolic dysfunction in a population-based sample of elderly men and women. *J Hum Hypertens.* (2013) 27:13–7. doi: 10.1038/jhh.2011.113
89. Gamrat A, Surdacki MA, Chyrchel B, Surdacki A. Endothelial dysfunction: a contributor to adverse cardiovascular remodeling and heart failure development in type 2 diabetes beyond accelerated atherogenesis. *J Clin Med.* (2020) 9:2090. doi: 10.3390/jcm9072090
90. Gottdiener JS, Reda DJ, Massie BM, Materson BJ, Williams DW, Anderson RJ. Effect of single-drug therapy on reduction of left ventricular mass in mild to moderate hypertension: comparison of six antihypertensive agents. The department of veterans affairs cooperative study group on antihypertensive agents. *Circulation.* (1997) 95:2007–14. doi: 10.1161/01.CIR.95.8.2007
91. Higgins P, Dawson J, Walters M. The potential for xanthine oxidase inhibition in the prevention and treatment of cardiovascular and cerebrovascular disease. *Cardiovasc Psychiatry Neurol.* (2009) 2009:282059. doi: 10.1155/2009/282059
92. Higgins P, Dawson J, Lees KR, McArthur K, Quinn TJ, Walters MR. Xanthine oxidase inhibition for the treatment of cardiovascular disease: a systematic review and meta-analysis. *Cardiovasc Ther.* (2012) 30:217–26. doi: 10.1111/j.1755-5922.2011.00277.x
93. Guedes M, Esperança A, Pereira AC, Rego C. What is the effect on cardiovascular events of reducing hyperuricemia with allopurinol? An evidence-based review. *Rev Port Cardiol.* (2014) 33:727–32. doi: 10.1016/j.repce.2014.06.003
94. Singh TP, Skalina T, Nour D, Murali A, Morrison S, Moxon JV, et al. A meta-analysis of the efficacy of allopurinol in reducing the incidence of myocardial infarction following coronary artery bypass grafting. *BMC Cardiovasc Disord.* (2018) 18:143. doi: 10.1186/s12872-018-0881-6
95. Kao MP, Ang DS, Gandy SJ, Nadir MA, Houston JG, Lang CC, et al. Allopurinol benefits left ventricular mass and endothelial dysfunction in chronic kidney disease. *J Am Soc Nephrol.* (2011) 22:1382–9. doi: 10.1681/ASN.2010111185
96. Gingles CR, Symon R, Gandy SJ, Struthers AD, Houston G, MacDonald TM, et al. Allopurinol treatment adversely impacts left ventricular mass regression in patients with well-controlled hypertension. *J Hypertens.* (2019) 37:2481–9. doi: 10.1097/HJH.0000000000002189
97. Rena G, Lang CC. Repurposing metformin for cardiovascular disease. *Circulation.* (2018) 137:422–4. doi: 10.1161/CIRCULATIONAHA.117.031735
98. Kirpichnikov D, McFarlane SI, Sowers JR. Metformin: an update. *Ann Intern Med.* (2002) 137:25–33. doi: 10.7326/0003-4819-137-1-200207020-00009
99. Cameron AR, Morrison VL, Levin D, Mohan M, Forteath C, Beall C, et al. Anti-inflammatory effects of metformin irrespective of diabetes status. *Circ Res.* (2016) 119:652–65. doi: 10.1161/CIRCRESAHA.116.308445
100. Esteghamati A, Eskandari D, Mirmiranpour H, Noshad S, Mousavizadeh M, Hedayati M, et al. Effects of metformin on markers of oxidative stress and antioxidant reserve in patients with newly diagnosed type 2 diabetes: a randomized clinical trial. *Clin Nutr.* (2013) 32:179–85. doi: 10.1016/j.clnu.2012.08.006
101. Chakraborty A, Chowdhury S, Bhattacharyya M. Effect of metformin on oxidative stress, nitrosative stress and inflammatory biomarkers in type 2 diabetes patients. *Diabetes Res Clin Pract.* (2011) 93:56–62. doi: 10.1016/j.diabres.2010.11.030
102. Formoso G, De Filippis EA, Michetti N, Di Fulvio P, Pandolfi A, Bucciarelli T, et al. Decreased in vivo oxidative stress and decreased platelet activation following metformin treatment in newly diagnosed type 2 diabetic subjects. *Diabetes Metab Res Rev.* (2008) 24:231–7. doi: 10.1002/dmrr.794
103. Skrha J, Prazny M, Hilgertova J, Kvasnicka J, Kalousova M, Zima T. Oxidative stress and endothelium influenced by metformin in type 2 diabetes mellitus. *Eur J Clin Pharmacol.* (2007) 63:1107–14. doi: 10.1007/s00228-007-0378-1
104. Pavlovic D, Kocic R, Kocic G, Jevtovic T, Radenkovic S, Mikic D, et al. Effect of four-week metformin treatment on plasma and erythrocyte antioxidative defense enzymes in newly diagnosed obese patients with type 2 diabetes. *Diabetes Obes Metab.* (2000) 2:251–6. doi: 10.1046/j.1463-1326.2000.00089.x
105. Srinivasan S, Ambler GR, Baur LA, Garnett SP, Tepsa M, Yap F, et al. Controlled trial of metformin for obesity and insulin resistance in children and adolescents: improvement in body composition and fasting insulin. *J Clin Endocrinol Metab.* (2006) 91:2074–80. doi: 10.1210/jc.2006-0241
106. Mather KJ, Verma S, Anderson TJ. Improved endothelial function with metformin in type 2 diabetes mellitus. *J Ame Coll Cardiol.* (2001) 37:1344–50. doi: 10.1016/S0735-1097(01)01129-9
107. Wong AK, Symon R, AlZadjali MA, Ang DS, Ogston S, Choy A, et al. The effect of metformin on insulin resistance and exercise parameters in patients with heart failure. *Eur J Heart Fail.* (2012) 14:1303–10. doi: 10.1093/eurjhf/hfs106
108. Chan AY, Dyck JR. Activation of AMP-activated protein kinase (AMPK) inhibits protein synthesis: a potential strategy to prevent the development of cardiac hypertrophy. *Can J Physiol Pharmacol.* (2005) 83:24–8. doi: 10.1139/y04-107
109. Dolinsky VW, Dyck JR. Role of AMP-activated protein kinase in healthy and diseased hearts. *Am J Physiol Heart Circ Physiol.* (2006) 291: H2557–69. doi: 10.1152/ajpheart.00329.2006
110. Wong AKF, Howie J, Petrie JR, Lang CC. AMP-activated protein kinase pathway: a potential therapeutic target in cardiometabolic disease. *Clin Sci.* (2009) 116:607–20. doi: 10.1042/CS20080066
111. Mohan M, Al-Talabany S, McKinnie A, Mordi IR, Singh JSS, Gandy SJ, et al. A randomized controlled trial of metformin on left ventricular hypertrophy in patients with coronary artery disease without diabetes: the MET-REMODEL trial. *Eur Heart J.* (2019) 40:3409–17. doi: 10.1136/heartjnl-2018-BCS.6
112. Hugo Velázquez, Meaney A, Galeana C, Zempoalteca JC, Gutiérrez-Salmeán G, Nájera N, et al. Metformin enhances left ventricular function in patients with metabolic syndrome. *Rev Colomb Cardiol.* (2016) 27:16–25.
113. Al Ali L, Hartman MT, Lexis CP, Hummel YM, Lipsic E, van Melle JP, et al. The effect of metformin on diastolic function in patients presenting with st-elevation myocardial infarction. *PLoS ONE.* (2016) 11:e0168340. doi: 10.1371/journal.pone.0168340
114. Ida S, Kaneko R, Murata K. Effects of oral antidiabetic drugs on left ventricular mass in patients with type 2 diabetes mellitus: a network meta-analysis. *Cardiovasc Diabetol.* (2018) 17:129. doi: 10.1186/s12933-018-0773-1
115. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med.* (2015) 373:2117–28. doi: 10.1056/NEJMoa1504720
116. Neal B, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erondu N, et al. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *N Engl J Med.* (2017) 377:644–57. doi: 10.1056/NEJMoa1611925
117. Wiviott SD, Raz I, Bonaca MP, Mosenzon O, Kato ET, Cahn A, et al. Dapagliflozin and cardiovascular outcomes in type 2 diabetes. *N Engl J Med.* (2019) 380:347–57. doi: 10.1056/NEJMoa1812389
118. Zelniker TA, Wiviott SD, Raz I, Im K, Goodrich EL, Bonaca MP, et al. SGLT2 inhibitors for primary and secondary prevention of cardiovascular and renal outcomes in type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. *Lancet.* (2019) 393:31–9. doi: 10.1016/S0140-6736(18)32590-X
119. McMurray JJV, Solomon SD, Inzucchi SE, Køber L, Kosiborod MN, Martinez FA. Dapagliflozin in patients with heart failure and reduced ejection fraction. *N Engl J Med.* (2019) 381:1995–2008. doi: 10.1056/NEJMoa1911303

120. Nassif ME, Windsor SL, Tang F, Khariton Y, Husain M, Inzucchi SE, et al. Dapagliflozin effects on biomarkers, symptoms, and functional status in patients with heart failure with reduced ejection fraction: the DEFINE-HF trial. *Circulation.* (2019) 140:1463–76. doi: 10.1161/CIRCULATIONAHA.119.042929
121. Packer M, Anker SD, Butler J, Filippatos G, Zannad F. Effects of sodium-glucose cotransporter 2 inhibitors for the treatment of patients with heart failure: proposal of a novel mechanism of action. *JAMA Cardiol.* (2017) 2:1025–9. doi: 10.1001/jamacardio.2017.2275
122. Maack C, Lehrke M, Backs J, Heinzel FR, Hulot JS, Marx N, et al. Heart failure and diabetes: metabolic alterations and therapeutic interventions: a state-of-the-art review from the Translational Research Committee of the Heart Failure Association-European Society of Cardiology. *Eur Heart J.* (2018) 39:4243–54. doi: 10.1093/eurheartj/ehy596
123. Santos-Gallego CG, Requena-Ibanez JA, San Antonio R, Ishikawa K, Watanabe S, Picatoste B, et al. Empagliflozin ameliorates adverse left ventricular remodeling in nondiabetic heart failure by enhancing myocardial energetics. *J Am Coll Cardiol.* (2019) 73:1931–44. doi: 10.1016/j.jacc.2019.01.056
124. Yurista SR, Silljé HHW, Oberdorf-Maass SU, Schouten EM, Pavez Giani MG, Hillebrands JL, et al. Sodium-glucose co-transporter 2 inhibition with empagliflozin improves cardiac function in non-diabetic rats with left ventricular dysfunction after myocardial infarction. *Eur J Heart Fail.* (2019) 21:862–73. doi: 10.1002/ejhf.1473
125. Packer M. Cardioprotective effects of sirtuin-1 and its downstream effectors: potential role in mediating the heart failure benefits of SGLT2 (Sodium-Glucose Cotransporter 2) Inhibitors. *Circ Heart Fail.* (2020) 13:e007197. doi: 10.1161/CIRCHEARTFAILURE.120.007197
126. Tentolouris A, Vlachakis P, Tzeravini E, Eleftheriadou I, Tentolouris N. SGLT2 inhibitors: a review of their antidiabetic and cardioprotective effects. *Int J Environ Res Public Health.* (2019) 16:2965. doi: 10.3390/ijerph16162965
127. Brown AJM, Lang C, McCrimmon R, Struthers A. Does dapagliflozin regress left ventricular hypertrophy in patients with type 2 diabetes? A prospective, double-blind, randomised, placebo-controlled study. *BMC Cardiovasc Disord.* (2017) 17:229. doi: 10.1186/s12872-017-0663-6
128. Brown AJM, Gandy S, McCrimmon R, Houston JG, Struthers AD, Lang CC. A randomized controlled trial of dapagliflozin on left ventricular hypertrophy in people with type two diabetes: the DAPA-LVH trial. *Eur Heart J.* (2020) 41:3421–32. doi: 10.1093/eurheartj/ehaa419
129. Verma S, Mazer CD, Yan AT, Mason T, Garg V, Teoh H, et al. Effect of empagliflozin on left ventricular mass in patients with type 2 diabetes mellitus and coronary artery disease: the EMPA-HEART cardiolink-6 randomized clinical trial. *Circulation.* (2019) 140:1693–702. doi: 10.1161/CIRCULATIONAHA.119.042375
130. Verma S, Mazer CD, Bhatt DL, Raj SR, Yan AT, Verma A, et al. Empagliflozin and cardiovascular outcomes in patients with type 2 diabetes and left ventricular hypertrophy: a subanalysis of the EMPA-REG OUTCOME trial. *Diabetes Care.* (2019) 42: e42–4. doi: 10.2337/dc18-1959
131. Brown A, Gandy S, Mordi IR, McCrimmon R, Ramkumar PG, Houston JG, et al. Dapagliflozin improves left ventricular myocardial longitudinal function in patients with type 2 diabetes. *JACC Cardiovasc Imaging.* (2021) 14:503–4. doi: 10.1016/j.jcmg.2020.07.025

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REVIEW
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Molecular Mechanisms and Epigenetic Regulation in Diabetic Cardiomyopathy

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Diabetes mellitus (DM) is an important lifestyle disease. Type 2 diabetes is one of the prime contributors to cardiovascular diseases (CVD) and diabetic cardiomyopathy (DbCM) and leads to increased morbidity and mortality in patients with DM. DbCM is a typical cardiac disease, characterized by cardiac remodeling in the presence of DM and in the absence of other comorbidities such as hypertension, valvular diseases, and coronary artery disease. DbCM is associated with defective cardiac metabolism, altered mitochondrial structure and function, and other physiological and pathophysiological signaling mechanisms such as oxidative stress, inflammation, myocardial apoptosis, and autophagy. Epigenetic modifiers are crucial players in the pathogenesis of DbCM. Thus, it is important to explore the role of epigenetic modifiers or modifications in regulating molecular pathways associated with DbCM. In this review, we have discussed the role of various epigenetic mechanisms such as histone modifications (acetylation and methylation), DNA methylation and non-coding RNAs in modulating molecular pathways involved in the pathophysiology of the DbCM.

Keywords: diabetes mellitus, diabetic cardiomyopathy, apoptosis, oxidative stress, mitochondrial function, cardiac remodeling, epigenetics

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INTRODUCTION

Diabetic cardiomyopathy (DbCM) is a cardiac disease characterized by functional and structural abnormalities in cardiac tissue in patients having diabetes mellitus (DM) but no other comorbidities such as hypertension, valvular diseases, and coronary artery disease (1). Framingham Heart Study observed that women and men with DM have 5- and 2.4-fold higher incidence of heart failure (HF), respectively (2). Patients with diabetes have a high prevalence of HF ranging from 19 to 26% (3–5). A case-control study found that the prevalence of HF was 1.3 times higher in diabetic subjects in comparison with the non-diabetic subjects (6). In both type I diabetes (T1D) and type II diabetes (T2D), patients showed a strong correlation between glycated hemoglobin A(1c) (HbA1c) and HF. With every 1% increase in HbA1c, there is a 30 and 8% higher incidence of HF in T1D and T2D, respectively, independent of other risk factors (7, 8). The initial phase of DbCM is characterized by extensive cardiac hypertrophy and mild to moderate fibrosis, leading to defects in the systolic and diastolic function of the heart (9).

Experimental and clinical studies have identified sustained hyperglycemia (HG), insulin resistance, aberrant insulin signaling, impaired glucose metabolism, abnormal free fatty acid (FFA) uptake, oxidative stress, increased renin–angiotensin–aldosterone (RAAS) activity, cardiac

inflammation, and aberrant mitochondrial function as the key determinants for biochemical alterations leading to a vicious cycle of disease. Cardiac fibrosis, left ventricular (LV) hypertrophy, and increased cardiomyocyte cell death are the most important mechanisms to explain the pathophysiology of the disease (10, 11). Multiple molecular mechanisms have been identified contributing to pathophysiological changes in DbCM (**Figure 1**), which include O-GlcNAcylation of cardiac proteins, decreased insulin and AMPK signaling, activated MAPK, peroxisome proliferator-activated receptors, and aberrant protein kinase C activity.

Recent studies suggest that epigenetic regulatory mechanisms such as DNA methylation, histone modifications (acetylation and methylation), deregulated microRNAs (miRNAs), circular RNA (circRNAs), and long non-coding RNA (lncRNAs) play an important role in the pathogenesis of DbCM (12, 13). In this review, we provide a comprehensive overview of the role of epigenetic modifications in various molecular pathways associated with DbCM.

MOLECULAR MECHANISMS AND THEIR EPIGENETIC REGULATIONS IN DbCM

Cardiac Remodeling in DbCM

Cardiomyocyte hypertrophy and fibrosis are the important features of DbCM. Cardiac fibrosis is a dominant mechanism contributing to the disease pathology of the diabetic human heart. There is a very pronounced deposition of collagen in interstitial and perivascular spaces in diabetic cardiac tissues (14). The major contributing pathways for the aggravated deposition of collagen types I and III are transforming growth factor-β1 (TGF-β1) and wingless-related integration site (WNT) signaling pathways (15). Additionally, there is remodeling of matrix metalloproteinases (MMPs) leading to dysregulated degradation of extracellular matrix in diabetic hearts (15–18). Activation of the TGF-β1 pathway and accelerated extracellular matrix degradation are mainly consequences of stimulation of RAAS resulting in heightened advanced glycation end products (AGEs)-mediated signaling, HG, and insulin resistance (19). Decreased availability of nitric oxide (NO), oxidative stress, activation of TGF-β1 signaling pathway, in association with deregulated insulin signaling leads to high cardiac collagen deposition and fibronectin content, leading to interstitial fibrosis (20). Several clinical and animal studies provide substantial evidence of cardiac fibrosis in diabetes-induced heart failure (HF) (18, 21–23).

An increase in LV hypertrophy represented by high LV mass and its association with DM is well-established (24–26). Thickened LV is a major hallmark of cardiac hypertrophy in humans (27). Cardiac fibrosis, hypertrophy, and myocardial cell apoptosis must be taken into account for the overall increase in LV mass (27, 28). In DbCM, there are other contributors in addition to cardiac hypertrophy such as insulin resistance, HG in the milieu, and oxidative stress-activating cardiac hypertrophic genes, such as β-myosin heavy chain (ß-MHC), atrial natriuretic factor (ANP), and brain natriuretic factor (BNP) (29). Heightened insulin levels induce cardiac hypertrophy. Insulin-like growth factor (IGF-1) induces cardiomyocyte hypertrophy through activation of the mitogen-activated protein kinase 1 (Erk1/2) and phosphoinositide 3-kinases (PI3K) signaling pathways (30). Several studies in animal models of DbCM have also shown the role of DM in the development of cardiac or cardiomyocyte hypertrophy (31–33).

Epigenetic Regulation of Cardiac Remodeling in DbCM

MicroRNAs are small, non-coding RNAs, which regulate cellular gene expression. Aberrant expression of ∼30% miRNAs (that is 300 out of 1,000 total miRNAs) has been observed in DM heart tissues (34). Several miRNAs have been found to regulate cardiac fibrosis and cardiac hypertrophy in DbCM. For example, miRNA-221 was shown to be highly upregulated in the cardiac tissue of diabetic mice (35). miRNA-212 was found to regulate the process of cardiac hypertrophy by directly regulating Forkhead box O3 (Foxo3) (35). Raut et al. reported that miRNA-30c mediates increased expression of hypertrophy genes, cell division control protein 42 homolog (Cdc42), and Rac1-activated kinase 1 (Pak1) in DbCM (36). Other miRNAs, such as 181a and 200c, were shown to play a pivotal role in cardiac remodeling (35, 37–39). The expression of miRNA-199a was elevated in cardiac hypertrophy (34). Recently, it was reported that silencing of miR-199a led to the reversal of cardiac hypertrophy by rescuing the mitochondrial fatty acid oxidation through targeting peroxisome proliferator-activated receptor-gamma coactivator (PGC-1α) (40). miRNA-30a, miRNA-1, and miRNA-29b levels were found to be downregulated in the diabetic heart (34). miRNA-144 and miRNA-133a are among the important key players, involved in the pathophysiology of diabetes-mediated HF (41, 42). Singh et al. showed that miRNA-200c promoted cardiac hypertrophy by modulating dual-specific phosphatase 1 (DUSP1) expression in DbCM (39).

Decreased levels of miRNA-133a were observed in the diabetic murine model (43). It was also seen that increase in miRNA-133a levels improved the systolic function and reduced fibrosis by decreasing the collagen (44). miRNA-21 has been established as a biomarker for cardiac fibrosis (45). Several groups including ours have shown upregulated miRNA-21 levels in rat cardiac fibroblasts in the hyperglycemic milieu and in diabetic hearts, which leads to advanced collagen synthesis and fibroblast proliferation (46, 47). miRNA-21 was also found to directly regulate dual-specific phosphatase 8 (DUSP8) by perturbing c-Jun N-terminal kinase (JNK) and p38 MAP kinase (MAPK) signaling pathways (46).

Long non-coding RNAs, a type of non-coding RNAs which are longer than miRNAs, have been implicated in various disease pathways (48). Nuclear lncRNAs act at the transcriptional level, and cytoplasmic lncRNAs often interact with miRNAs to regulate gene expression post-transcriptionally. Several lncRNAs have been recently shown to be involved in the pathophysiology of CVDs, including DbCM, contributing to cardiac hypertrophy and fibrosis. Myocardial infarction-associated transcript (MIAT) acts as prohypertrophic lncRNA as it has a sponging activity for antihypertrophic miRNAs,

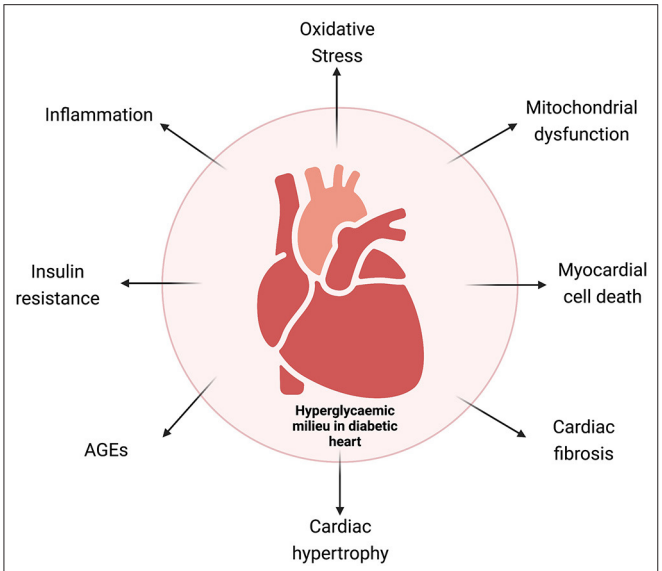

FIGURE 1 | Schematic representation of the various mechanisms involved in diabetic cardiomyopathy. AGEs, advanced glycation end products.

miRNA-150 (49), and miRNA-93 (50). Additionally, MIAT levels were higher in the myocardium and compete with miRNA-24 levels to regulate TGF-β1 expression and thus cardiac fibrosis (51). LncRNA Kcnq1ot1 ablation ameliorates TGF-β1 signaling and, thus, reduces fibrotic lesions in diabetic mice (52). The dysregulated ncRNAs, both miRNA and lncRNA, explain HG-related myocardial insult.

Histone modifications also have been found to play a crucial role in the cardiac remodeling in DbCM. Non-specific inhibitor-based silencing of histone deacetylases (HDACs) has been shown to attenuate cardiac hypertrophy and fibrosis, by increasing the glucose transporter 1 acetylation and MAPK-mediated phosphorylation in animal models of diabetic heart disease (53). The use of specific HDAC3 inhibitors such as RGFP966 also showed improved cardiac function and reversed DM-induced cardiac remodeling in diabetic mice. It was found that RGFP966 decreased cardiac hypertrophy by epigenetic modulation of the ERK1/2 pathway mediated by DUSP5 (54). In contrast, Sir2 is known to have a beneficial effect on DCM. It improves contractile dysfunction in leptin receptor-deficient db/db mice through a histone deacetylase Sir2-driven pathway (54), suggesting its potential as a therapeutic molecule in DbCM. (55).

Role of Epigenetics in Regulating Cell Death Mechanisms in DbCM

Diabetic cardiomyopathy has a strong association with high cardiomyocyte cell death. Apoptosis and autophagy are the important deregulated mechanisms responsible for this phenomenon (56). Several fold higher apoptosis rates have been reported in cardiomyocytes, fibroblasts, and endothelial cells in myocardial tissues of patients with DbCM. The death rate of cardiomyocytes was the highest, followed by that of endothelial cells and fibroblasts (57). Increased cardiomyocyte cell death results in cell loss in the heart, remodeling such as cardiac hypertrophy and fibrosis, leading to cardiomyopathy and cardiac failure (58).

Various mechanisms are proposed for increased cardiomyocyte cell death in diabetic hearts. HG, insulin resistance, lipid peroxidation, increased angiotensin II signaling, oxidative stress, and endoplasmic stress have been implicated as major triggers of cardiomyocyte apoptosis in the diabetic milieu. HG is the major causative factor for increased oxidative stress and endoplasmic stress mediating cardiomyocyte death in diabetic hearts (59). HG mediates these actions through localized increased angiotensin II (Ang II) (60). Kobayashi et al. have recently shown that HG may also induce cardiomyocyte cell death by inducing lysosomal membrane permeabilization and increased cathepsin D expression and lysosomal release in cardiomyocytes resulting in cell death (61).

Apoptosis and Its Epigenetic Regulation

The expression of several miRNAs was deregulated in HG-induced cardiomyocyte apoptosis and diabetic hearts (34). These include miRNA-30c, miRNA-181, miRNA-378, miRNA-34a, miRNA-1, miRNA-195, miRNA-144, and miRNA-483-3p. It was shown that miR-1 is upregulated in HG-treated H9c2 cardiomyocytes along with increased apoptosis. They reported that miR-1 promotes cardiomyocyte apoptosis by inhibiting IGF-1 expression; IGF-1 increased expression was shown to inhibit glucose-induced cytochrome c release and apoptosis, suggesting that miRNA-1 promotes apoptosis by regulating IGF-1 (62). miRNA-34a is highly expressed in cardiomyocytes and regulates the expression of several proteins including prosurvival protein, sirtuin 1 (SIRT1). miRNA-34a is upregulated in diabetic hearts and glucose-treated cardiomyocytes. Fomison-Nurse et al. reported that upregulation of miRNA-34a was associated with downregulation of SIRT1 and increased the activity of proapoptotic caspases in HG-treated cultured cardiomyocytes. Inhibition of miRNA-34a was found to reduce HG-induced cardiomyocyte apoptosis, indicating its potential therapeutic role (63). Qiao et al. showed that miRNA-483-3p was involved in HG-induced cardiomyocyte apoptosis by repressing the expression of its target gene, IGF-1. They reported elevated expression of this miRNA in diabetic mice and hyperglycemic cardiomyocytes (64). Downregulation of miRNA-30c and miRNA-181 was observed in diabetic hearts and hyperglycemic cardiomyocytes (38). These miRNAs promote cardiomyocyte apoptosis by deregulation of a p53-p21 axis (38). It was reported that miRNA-195 upregulation induces apoptosis in streptozotocin (STZ) and leptin receptor-deficient type 2 diabetic murine hearts *via* downregulation of SIRT1 and B cell leukemia 2 (Bcl2) (65). Altered expression of miRNA-144 was observed in hearts and also cardiomyocytes in hyperglycemic conditions. miRNA-144-3p was found to be upregulated in T2D (66). Karolina et al. reported that miRNA-144 controls the expression of IRS-1 in diabetes (66). Recently, Song et al. have reported increased miRNA-144 levels in HG-treated cardiomyocytes (67). They showed that miRNA-144-targeted C1q/TNF-related protein 3 (CTRP3)/JNK pathway and inhibition of miRNA-144 attenuated cardiomyocyte apoptosis. In another study, Tao et al. observed decreased miRNA-144 levels in HG-treated cardiomyocytes and diabetic hearts (68).

Cellular overexpression of miRNA-144 resulted in improved mitochondrial function and decreased myocyte apoptosis by regulating Rac family small GTPase 1 (Rac-1) levels, which in turn regulated apoptosis *via* 5' AMP-activated protein kinase (AMPK) phosphorylation and PGC-1α deacetylation (68). Thus, the precise role of this miRNA remains to be fully elucidated. Altered PI3K/Akt signaling stimulates apoptosis, fibrosis, and hypertrophy of cardiomyocytes and leads to DbCM progression (69). It was reported that miRNA-203 overexpression inhibited PIK3CA and activated of PI3K/Akt signaling, thus inhibiting myocardial hypertrophy, fibrosis, and apoptosis (69). Recently, miRNA-532 has been shown to exhibit a positive association with cardiomyocyte apoptosis in diabetic heart disease. miRNA-532 was shown to be upregulated in cardiac tissues of patients with type 2 DM, thus decreasing the expression of its main target, the antiapoptotic protein (ARC). It was shown that miRNA-532 upregulation leads to the activation of proapoptotic caspases activity and vice versa in HG-treated cardiomyocytes (70). Another study showed decrease in expression of antiapoptotic protein, Hsp60 in the diabetic heart. miRNA-1 and miRNA-206 modulated myocardial Hsp60 post-transcriptionally and its downregulation was an important proapoptotic signal in the diabetic myocardium (71).

Besides miRNAs, several lncRNAs have been identified mediating cardiac cell death in hyperglycemic or diabetic conditions (72). Decreased expression of lncRNA H19 in DCM and HG-treated cardiomyocytes was observed and improved ventricular function by inhibiting reduced apoptosis in diabetic rats (73). H19 functions by inhibiting miR-675-mediated expression of voltage-dependent anion channel 1 (VDAC1), a proapoptotic molecule, which promotes cell death (73). Yang et al. showed escalated expression of another lncRNA Kcnq1ot, in the hearts of diabetic mice. They further showed that inhibition of Kcnq1ot1 improved cardiac function and attenuated pyroptosis (52). Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is another long non-coding RNA that regulates HG-induced cardiomyocyte apoptosis (74). MALAT1 was also shown to downregulate miR-141 or miR-181a-5p levels by sponging and inducing NLR family pyrin domain containing 3 (NLRP3) inflammasome activity and TGF-β1/Smad signaling (75). In a very recent study, MALAT1 has been shown to influence cardiomyocyte apoptosis by EZH2, a histone methyltransferase, and upregulating ATP-binding cassette transporter A1 (ABCA1) (76). HOTAIR is another lncRNA that has been shown to protect cardiac cell death in hyperglycemic conditions and DbCM. HOTAIR was decreased in the hearts of the diabetic mice, and its cardiac-specific overexpression attenuated cardiomyocyte death in STZ diabetic mice (77). It was shown to regulate miR-34a levels by acting as competing endogenous RNA (ceRNA) and increasing its target protein SIRT1, which has antiapoptotic activity (77). MEG3 is a lncRNA that is upregulated in HG-treated cardiomyocytes and induces apoptosis *via* sponging miR-145 and increasing proapoptotic programmed cell death 4 (PDCD4) levels (78). Recently, expression of Lnc NKILA (nuclear factor-κ B interacting long non-coding RNA) was found to be highly increased in patients with DbCM and its *in vitro* silencing decreased HG-induced cardiac cell death (79). Similarly, increased lncRNA LUCAT1 (lung cancer-associated transcript 1) levels were found in HG-treated AC 16 cardiomyocytes and its inhibition reduced HG-induced cardiomyocyte apoptosis by downregulating aldosterone synthase (CYP11B2) (80).

The DNA and histone methylation and acetylation are important epigenetic mechanisms that regulate gene expression and associated cellular mechanisms. The role of these mechanisms in diabetic cardiomyocyte cell death has not been well-investigated but emerging research suggests that they might have an important role. Yu et al. reported that HDAC1 mediates repression of IGF-1R in HG-treated cardiomyocytes (81). They showed that the association of histone 4 with p53-HDAC1 is increased and the association of histone 4 with IGF-1R is decreased (81). HDAC inhibition was later shown to inhibit HG-induced cardiac apoptosis by increasing GLUT1 acetylation and decreasing caspase 3 activity in diabetic mice (53).

Endoplasmic reticulum (ER) stress, an important mediator of DbCM, has also been implicated in the induction of apoptosis of cardiac cells (82). The role of epigenetic regulation of ER stress in DM-induced cardiac apoptosis was further confirmed by Guo et al. They reported that activation of SIRT1, a deacetylase, attenuates ER stress and apoptosis in cardiomyocytes of diabetic rats (83). Nitrosative stress induced by increased nitric oxide production resulting in nitrosylation of proteins has been found to induce apoptosis in heart of diabetic rats (84). Puthanveetil et al. reported that HC-induced iNOS expression in cardiomyocytes leads to increased nitrosylation of caspase 3 that facilitates apoptosis. They showed that nitrosylation of the proteins was mediated by Foxo1. Foxo1-mediated nitrosylation of caspase 3 resulted in increased cell death under HG conditions (84).

Autophagy and Its Epigenetic Regulation

Autophagy is a physiological process that removes or recycles damaged cell components such as organelles, proteins, and metabolites from the cell. It is an important process to maintain cell homeostasis. Both repression and augmentation of autophagy have been reported in diabetic hearts and HG-exposed cardiomyocytes (85–87). Mellor et al. reported increased autophagy (LC3B-II: LC3B-I ratio) in hearts of fructose-fed diabetic mice, suggesting myocardial autophagy activation in DbCM (88). However, Xie et al. reported repressed cardiac AMPK activity and autophagy in OVE26 diabetic mice (89). To date, there is no unequivocal consensus on the role of myocardial autophagy in the pathophysiology of DbCM. A recent review on autophagy in diabetic heart showed that autophagy might act as a double-edged sword, with initial activation helping in the removal of damaged mitochondria, peroxisomes, and protein aggregates and improving antioxidant mechanisms through the activation of antioxidant transcription factors such as nuclear factor erythroid 2-related factor 2 (Nrf2). However, this increased autophagy in the cell may result in self-digestion and enhanced reactive oxygen species (ROS) generation, causing cardiac damage (87).

Few reports suggest that miRNAs may regulate diabetes-induced autophagy. Chen et al. reported that circulatory miRNA-30c levels were highly reduced in patients with DM. Similar

results have been found in an animal model of diabetes and cardiomyocytes. It was observed that miR-30c directly regulates Beclin-1 expression. Thus, downregulation of miR-30cenhanced autophagy by increasing proautophagic Beclin-1 expression in diabetic hearts. Further, miR-30c directly regulates Beclin-1, thus controlling autophagy in DM (90).

There are a few studies suggesting the involvement of LncRNAs in DbCM. Feng et al. showed a marked increase in expression of LncRNAs, DCM-related factor (DCRF) in DbCM in the diabetic mice model (91). They showed that DCRF increased cardiomyocyte autophagy by sponging miR-551b-5p, thereby increasing protocadherin 17 (PCDH17) expression (89). Similarly, Zhou et al. reported decreased expression of LncRNA H19 in DbCM. They observed that LncRNA H19 regulates GTP-binding protein Di-Ras3 (DIRAS3) expression and promotes mTOR phosphorylation, thus inhibiting autophagy in DbCM (92).

Pyroptosis and Its Epigenetic Regulation

Pyroptosis or inflammation-induced cell death has been shown to contribute to increased cardiomyocyte cell loss in DbCM (93). miRNA-30d promoted cardiomyocyte pyroptosis in hyperglycemic conditions by repressing Forkhead box O3 (Foxo3a) and its downstream effector activity regulated cytoskeleton-associated protein (ARC), an apoptotic repressor leading to caspase-1 activation and increasing proinflammatory molecules (94). Jeyabal et al. have also reported that miRNA-9 may have a role in HG-induced cardiomyocyte pyroptosis (95). They showed that expression of miRNA-9 was significantly decreased in HG-treated cardiomyocytes *in vitro* and in human diabetic hearts *in vivo*. The proinflammatory ELAV-like protein 1 (ELAVL1) was shown to be the target protein of miRNA-9. The authors reported that upregulation of miRNA-9 attenuated HG-induced cardiomyocyte pyroptosis by downregulating ELAVL1 expression, indicating that miRNA-9 has an antiapoptotic role in diabetic hearts (95).

Epigenetic Regulation of Mitochondrial Dysfunction in DbCM

Mitochondria play a vital role in the maintenance of cardiac function and metabolism. Loss of mitochondrial function is implicated in DbCM (96). In adult cardiomyocytes, oxidative phosphorylation is the major source of intracellular ATP production in mitochondria. During DM, there is a switch in the ATP production pathway from glucose to FFA oxidation in mitochondria (97). This impaired oxidative phosphorylation increases mitochondrial ROS generation (98). Further, faulty Ca^{2+} flux in mitochondria leads to apoptosis in cardiomyocytes (99). This dysregulated Ca^{2+} flux also induces permeability in mitochondrial membranes, resulting in increased cardiomyocyte autophagy (100).

MicroRNAs have a significant role in fatty acid metabolism in the diabetic heart tissues. miRNA-133a levels were decreased in the cardiac tissue of the diabetic murine model (43). Mechanistically, miRNA-133a controls the CD36 expression by directly regulating testicular protein 4 (101). This explains the increase in CD36 expression in diabetic rat hearts (102). Peroxisome proliferator-activated receptor alpha (PPAR-α) regulates the oxidation of fatty acids in cardiomyocytes (103). miRNA-29a directly regulates the coactivator of PPAR-α (104). It was also reported that miRNA-29a levels are decreased in diabetic rat hearts, and this explains the increased fatty acid oxidation mediated by PPAR-α (105). In another study, it was reported that miRNA-210 levels are 2.5 folds higher in human diabetic failing hearts compared with non-diabetic failing hearts (106). miRNA-210 is a direct regulator of ISCU1/2, iron sulfur complex protein, which drives the electron transport chain (ETC) by regulating the function of aconitase and complex I (107). Another group reported that there is an increase in miRNA-141 levels in type 1 diabetic hearts (108). miRNA-141 regulates inorganic phosphate transport in the mitochondria by regulating the solute carrier family 25 members 3 (SLC25A3), this, in turn, affects the ATP synthesis in mitochondria (108). Similarly, miRNA-378 that negatively regulates ATP synthase was found to be elevated in interfibrillar mitochondria in streptozotocin-induced diabetic hearts of mice (109). All studies suggest that miRNAs are important players in mitochondrial function and energy metabolism in the diabetic heart.

Histone modifiers such as deacetylases and acetyltransferases regulate global acetylation levels in various physiological states of the cell. They maintain homeostasis by acetylation or deacetylation of histone substrates (110). It was reported that increased HDAC activity leads to myocardial ischemia mediated by Foxo3a/Bim in the diabetic heart (111). Well-known cardiac protector resveratrol reduces mitochondrial dysfunction through regulation of SIRT1 activation in a murine model of DM and increased histone deacetylation of PGC-1α (112, 113). In the murine model of DM, HDAC inhibition resulted in elevated expression of cardiac PPAR-α and resulted in reduced expression of peroxisome proliferator-activated receptor gamma (PPAR-γ), suggesting the role of HDAC abrogation in regulating the fatty acid oxidation in DbCM (114).

Mitochondrial Oxidative Stress and Its Epigenetic Regulation in DbCM

Oxidative stress plays a crucial role in the pathogenesis and progression of DbCM by increasing insulin resistance in cardiomyocytes (**Figure 2**). During oxygen metabolism in mitochondria, ROS is produced as a by-product (97). Under abnormal conditions such as insulin resistance and HG, there is an increase in NADH in the mitochondrial respiratory chain leading to shunting of ETC at complex III and leading to tremendous ROS production (115). High NADPH oxidase activity is seen in cardiomyocytes of patients with obesity and cardiac insulin resistance (31). This increase in NADPH oxidase activity led to increased ROS generation. In DbCM, ROS levels also get elevated because of increased xanthine oxidase activity and NO synthase uncoupling (116). Mitochondrial dysfunction leads to increased ROS accumulation. Major ROS determinants are hydrogen peroxide, hydroxyl radical, superoxide molecules, and reduced oxygen in patients with DbCM (116–118).

Several miRNAs such as miRNA-1, miRNA-19b, and miRNA-144 have been associated with oxidative stress (34, 41).

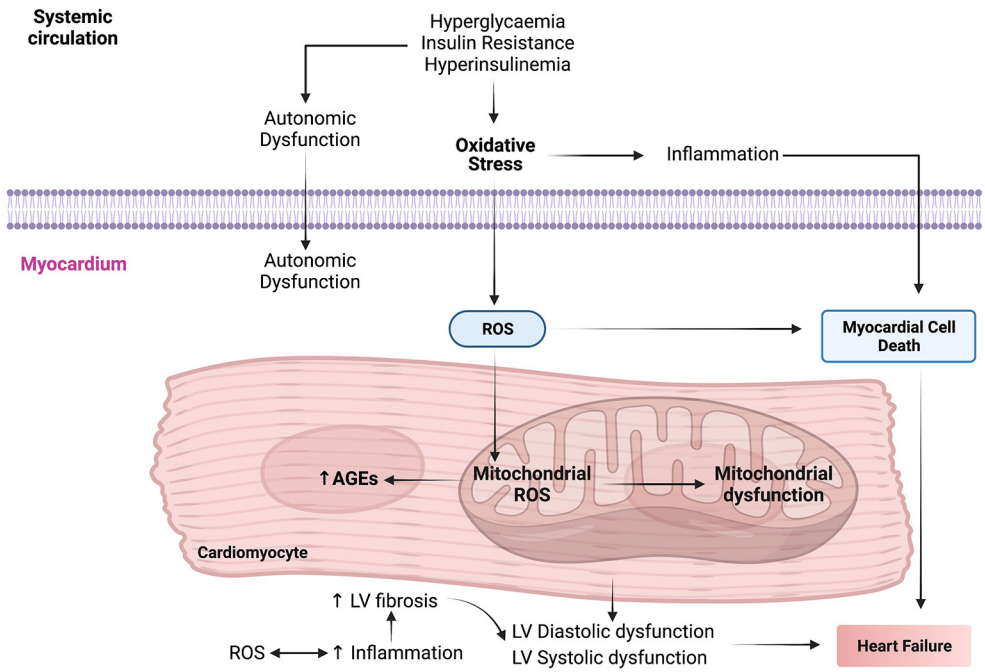

FIGURE 2 | The molecular mechanisms interactome in the pathophysiology of diabetic cardiomyopathy (DbCM). AGEs, advanced glycation end products; LV, left ventricle; ROS, reactive oxygen species.

It was shown that miR-1 levels decrease in cardiomyocytes treated with high glucose and treatment with N-acetylcysteine (NAC) leads to the rescue of cardiac phenotype proving the role of miRNA in oxidative stress-dependent DbCM (119). Similarly, miRNA-144 levels were found to be downregulated in hyperglycemic conditions. miRNA-144 is shown to regulate ROS levels directly through Nrf2 expression (41). Moreover, an increase in miRNA-141 in type I diabetic mice heart inhibited mitochondrial phosphate carrier (Slc25a) resulting in increased ROS and decreased mitochondrial ATP generation (108). Additionally, miRNA-210 has been reported to regulate mitochondrial metabolism by targeting the molecules involved in the ROS generation (120). Another report suggests that miRNA-373 levels were decreased in DbCM due to glucose-induced oxidative stress-mediated cardiac hypertrophy (121).

Few lncRNAs have been reported to regulate diabetes-induced oxidative stress. LncRNA H19 was shown to be downregulated in diabetic rat hearts, and enforced overexpression leads to attenuation of oxidative stress and thus, in turn, alleviates the LV dysfunction (73).

Epigenetic modulators such as histone DNA deacetylases have been also found to play an important role in oxidative stress-mediated pathophysiology of DbCM. Kumar et al. reported that dysregulated SIRT1 and methyltransferase 3b (Dnmt3b) activity resulted in increased histone H3 acetylation and CpG demethylation at the *p66Shc* (prooxidant adaptor protein) promoter in diabetes-induced vascular oxidative stress (122, 123). Similarly, Mortuza et al. looked into the mechanisms that decreased SIRT1 activity and suggested the role of SIRT1 and FOXO1 axis in ROS-mediated stress (124).

Electrical Remodeling and Its Epigenetic Regulation in the Progression of DbCM

Structural cues such as cardiac remodeling (fibrosis and hypertrophy) lead to functional abnormality (altered electrical activation) ultimately leading to electrical remodeling of the heart during HF. Electrical remodeling is characterized by compensatory or maladaptive prolonged disturbances in ion channels that might be reversible or irreversible, respectively. The remodeling of the electrical conduction system is considered to be the main reason for lethal arrhythmias (125). There are various etiologies of CVDs but delay in cardiac action potential repolarization is a common mechanism of electrical remodeling (125–127). Most electrophysiological studies suggest that a dip in the K+ currents plays a key role in electrical remodeling (126–129). There is experimental evidence suggesting that alterations in glucose metabolism in cardiomyocytes led to the remodeling of various channels in the ventricle. It will be interesting to learn that how the K+ channel gets altered in DM. Mechanisms behind the upregulation of K^+ channel activity in cardiomyocytes of patients with DbCM have derailed insulin signaling and glucose utilization. It was shown in the streptozotocin-induced DM murine model that insulin treatment is quite promising in achieving the normal transient outward current (129–131).

In the case of DbCM (induced by type I or type II diabetes), prolonged QT is seen (132–135), increasing the risk of ventricular arrhythmia (136, 137). At the molecular level, this lengthening of

the action potential is mainly driven by deregulated expression of various ion channel proteins and their properties (138–140).

Recently, it was found that epigenetic regulators such as miRNAs also participate in myocardial electrical remodeling (141). The expression of voltage-gated potassium channel Kv4.2 is regulated by miR-301a in diabetes (141). Overexpression of miRNA-29 in the diabetic murine model led to structural damage in the heart (142). In another study using a murine model of diabetes, it was seen that an increase in miRNA-141 levels affects ATP production by decreasing mitochondrial phosphate transport (108).

Various studies have underscored the role of HDACs in the regulation of ion channel expression but their exact function still needs to be elucidated. One such study elucidates the regulation of sodium–calcium exchanger (NCX)1 by HDAC5. The NCX1 is involved in Ca^{2+} efflux out of the cells and its expression is regulated by NK2 homeobox 5 (NKX2.5). It is involved in the recruitment of HDAC5 to the NCX gene promoter (143). Another study showed that acetylation of NKX2.5 increases its interaction with HDAC5, whereas deacetylation of NKX2.5 increases its affinity toward the p300 complex (144). Epigenetic regulation of HDACs affects Ca^{2+} flux in cardiomyocytes (145). In this study, the authors reported that the N-terminal of HDAC4 inhibits MEF2 activity, resulting in reduced expression of nuclear orphan receptor NR4A, suppressing the hexamine biosynthetic pathway (145). HDAC2 was downregulated in the porcine model of HF, affecting the potassium channel and prolonging the QT interval (146), leading to inhibition of HDAC2 and affecting the action potential. Inhibition of HDACs using class I inhibitor entinostat is a plausible therapeutic modality for HF that reduces the electrical and structural remodeling in HF (147).

Histone Deacetylases Inhibitors: A Prospective Therapeutic Modality for DbCM

Histone deacetylases are molecules with pleiotropic function and are involved in crucial homeostatic processes such as proliferation, cell death, and cell cycle. HDAC inhibitors (HDACIs) specifically block Zn^{2+}-dependent HDAC enzymes involved in histone acetylation. Recently, the US FDA has approved HDACIs for cancer treatment in clinics (148). Moreover, few reports suggest that regulation of histone acetylation is a promising strategy for the treatment of cardiovascular disease in the preclinical model (149). HDACIs are divided into five categories based on their structure:

A) Hydroxamic acid derivates: (e.g., panobinostat, trichostatin A)

B) Short-chain fatty (aliphatic) acids (e.g., valproic acid (VPA), sodium butyrate)

C) Cyclic peptides (e.g., romidepsin)

D) Benzamides (e.g., entinostat)

E) Sirtuin inhibitors

These HDACIs have been approved by US FDA (150–152). To date, HDACIs are not used in clinical trials for fibrotic diseases, but they have been used in cardiac and lung fibrosis (153–155). The major player in the fibrotic condition is the transition of fibroblast into myofibroblasts (156). There are studies suggesting that HDACIs have reversed myofibroblasts activation in animal models of HF. VPA combated fibrosis in the hypertension murine model by regulating the acetylation of corticoid receptors (157). In the pressure overload mice model, VPA abrogated cardiac remodeling (158). VPA also ameliorated cardiac fibrosis by regulating the ERK1/2 phosphorylation (159). A recent study demonstrated that VPA decreased the remodeling process, therefore leading to the onset of atrial fibrillation (160). Similarly, pan-HDACIs also showed antifibrotic activities, MPT0E014 decreased the expression of Ang II and TGF-β receptors in a murine model of cardiomyopathy (161). Mocetinostat downregulated the expression of HDACs in an HF model mechanistically by increasing apoptosis and reducing the myofibroblast phenotype (162). HDAC6 silencing or inhibition using tubacin reduced the TGF-β1 expression and, thus, decreased cardiac fibrosis (163). There is a need for more comprehensive studies looking into the potential of selective HDACIs for DbCM treatment.

CONCLUSION AND FUTURE PROSPECTIVE

Diabetic cardiomyopathy is a pleiotropic metabolic disease, with complex etiology and cumulative effects of crosstalk between genetic and epigenetic factors. The diabetic milieu has several inducers of cardiomyopathy such as ROS-mediated oxidative stress, hyperglycemic conditions, cytokines-mediated inflammation, cell death (apoptosis, autophagy, and pyroptosis), and epigenetic regulation of the dysregulated molecular pathways induced by these mediators. Epigenetic modifications range from deregulated ncRNAs (miRNAs and lncRNAs), histone modifications (acetylation and methylation), and DNA promoter methylation, which regulates the expression of important molecules of various pathways mediating DbCM. In summary, previous studies showed that interaction between environmental and genetic factors strongly determine the pathogenesis of DbCM through epigenetic changes in cellular signaling pathways (**Figure 3**).

The past decade has shown that miRNAs and lncRNAs are important regulators of major molecular pathways such as cell death, oxidative stress, mitochondrial dysfunction, and electrical remodeling (**Tables 1**, **2**). Cardiac fibrosis is an important phenomenon of the cardiac remodeling process in DbCM. There is substantial evidence that epigenetics plays a major role in diabetes-associated cell death. Epigenetic regulatory mechanisms such as histone changes, DNA methylation, miRNAs, and non-coding RNAs regulate cardiac cell death in the diabetic milieu. Similarly, other mechanisms such as mitochondrial dysfunction, oxidative stress, and electrical remodeling are also regulated by miRNAs and by HDACs. The elucidation of these epigenetic mechanisms can provide newer therapeutic strategies for the DbCM. miRNAs and lncRNAs have shown

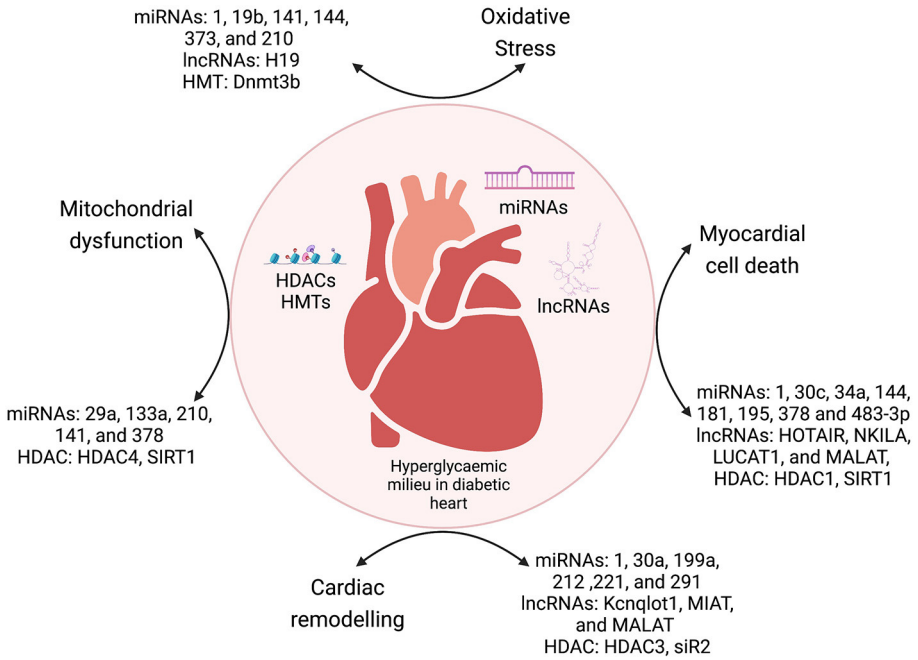

FIGURE 3 | The crosstalk between epigenetic modulators and various mechanisms of DbCM. miRNAs, microRNAs; lncRNAs, long non-coding RNAs; HDACs, histone deacetylases; HMTs, histone methyltransferases; DNMT, DNA methyltransferases.

TABLE 1 | Deregulated miRNAs in diabetic cardiomyopathy (DbCM).

miRNAs	Process associated	Target genes	References
miRNA-212	Cardiac hypertrophy	FOXO3	(35)
miRNA-30c	Cardiac hypertrophy	Cdc42 and Pak1	(36)
miRNA-30c & 181	Cardiac hypertrophy	p53	(38)
miRNA-199a	Cardiac hypertrophy	PGC-1α	(40)
miRNA-200c	Cardiac hypertrophy	DUSP-1	(39)
miRNA-133a	Cardiac fibrosis	TGF-ß1	(43)
miRNA-21	Cardiac fibrosis	DUSP-8	(46)
miRNA-1	Cardiac apoptosis	IGF-1	(62)
miRNA-34a	Cardiac apoptosis	SIRT-1	(77)
miRNA-483-3p	Cardiac apoptosis	IGF-1	(64)
miRNA-195	Cardiac apoptosis	SIRT-1	(65)
miRNA-144	Cardiac apoptosis	IRS	(66)
miRNA-203	Cardiac apoptosis	PIK3CA	(69)
miRNA-532	Cardiac apoptosis	ARC	(70)
miRNA-30c	Cardiac autophagy	Beclin1	(90)
miRNA-30d	Cardiac pyroptosis	Foxo3A	(94)
miRNA-9	Cardiac pyroptosis	ELAVL1	(95)
miRNA-29a	Mitochondrial dysfunction	PPARα	(103)
miRNA-210	Mitochondrial dysfunction	ISCU1/2	(107)
miRNA-141	Mitochondrial dysfunction	SLC25A3	(108)
miRNA-378	Oxidative stress	ATP synthase	(109)
miRNA-144	Oxidative stress	Nrf2	(41)
miRNA-301	Electrical remodeling	Kv4.2	(141)

TABLE 2 | Deregulated lncRNAs in DbCM.

lncRNAs	Process associated	Target genes	References
MIAT	Hypertrophy; fibrosis	TLR4; TGF-ß1	(49–51)
Kcnq1ot	Fibrosis; pyroptosis	TGF-ß1	(52)
H19	Apoptosis; autophagy	VDAC1; DIRAS3	(73, 92)
MALAT 1	Apoptosis	NLRP3; TGF-ß1; ABCA1	(74–76)
HOTAIR	Apoptosis	SIRT-1	(77)
MEG3	Apoptosis	PDCD 4	(78)
LUCAT1	Apoptosis	CYP11B2	(80)
DCRF	Autophagy	PCDH17	(91)

translational potential as diagnostic and prognostic biomarkers and therapeutic modalities for DbCM. It was also shown that HDACs are important regulators in the pathophysiology of DbCM. Inhibition of HDACs using inhibitors has shown promising data in the context of cardiac fibrosis. We have presented a detailed discussion on HDAC inhibitors as promising therapeutic targets for DbCM. However, there is a need to investigate regulatory mechanisms such as chromatin modifications and circular RNAs as contributors to DbCM.

With the advancement in genomics and molecular biology techniques, such as transposase-accessible chromatin (ATAC) sequencing, deep sequencing, and ChIP-sequencing, high-throughput data on DNA methylomes can be generated. This genome-wide data will provide a comprehensive picture of DbCM. The information, thus, acquired will help to understand the role of epigenetic modulators in DbCM in a pathway-specific manner. This review aims to help in understanding the role of various epigenetic factors in conjunction with specific pathways in DbCM.

AUTHOR CONTRIBUTIONS

AM performed the data collection, data approval, manuscript drafting, and manuscript editing. MK performed data collection, manuscript drafting, and manuscript editing. AB performed data manuscript editing. RG performed manuscript editing during revision. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Rubler S, Dlugash J, Yuceoglu YZ, Kumral T, Branwood AW, Grishman A. New type of cardiomyopathy associated with diabetic glomerulosclerosis. *Am J Cardiol.* (1972) 30:595–602. doi: 10.1016/0002-9149(72)90595-4
2. Kannel WB, Hjortland M, Castelli WP. Role of diabetes in congestive heart failure: the Framingham study. *Am J Cardiol.* (1974) 34:29–34. doi: 10.1016/0002-9149(74)90089-7
3. Rydén L, Armstrong PW, Cleland JGF, Horowitz JD, Massie BM, Packer M, et al. Efficacy and safety of high-dose lisinopril in chronic heart failure patients at high cardiovascular risk, including those with diabetes mellitus. Results from the ATLAS trial. *Eur Heart J.* (2000) 21:1967–78. doi: 10.1053/euhj.2000.2311
4. Shindler DM, Kostis JB, Yusuf S, Quinones MA, Pitt B, Stewart D, et al. Diabetes mellitus, a predictor of morbidity and mortality in the studies of left ventricular dysfunction (SOLVD) trials and registry. *Am J Cardiol.* (1996) 77:1017–20. doi: 10.1016/S0002-9149(97)89163-1
5. Thrainsdottir IS, Aspelund T, Thorgeirsson G, Gudnason V, Hardarson T, Malmberg K, et al. The association between glucose abnormalities and heart failure in the population-based Reykjavík study. *Diabetes Care.* (2005) 28:612–6. doi: 10.2337/diacare.28.3.612
6. Aronow WS, Ahn C. Incidence of heart failure in 2,737 older persons with and without diabetes mellitus. *Chest.* (1999) 115:867–8. doi: 10.1378/chest.115.3.867
7. Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ.* (2000) 321:405. doi: 10.1136/bmj.321.7258.405
8. Lind M, Bounias I, Olsson M, Gudbjörnsdottir S, Svensson A-M, Rosengren A. Glycaemic control and incidence of heart failure in 20,985 patients with type 1 diabetes: an observational study. *Lancet.* (2011) 378:140–6. doi: 10.1016/S0140-6736(11)60471-6
9. Westermeier F, Riquelme JA, Pavez M, Garrido V, Díaz A, Verdejo HE, et al. New molecular insights of insulin in diabetic cardiomyopathy. *Front Physiol.* (2016) 7:125. doi: 10.3389/fphys.2016.00125
10. Jia G, Hill MA, Sowers JR. Diabetic cardiomyopathy: an update of mechanisms contributing to this clinical entity. *Circ Res.* (2018) 122:624–38. doi: 10.1161/CIRCRESAHA.117.311586
11. Jia G, Whaley-Connell A, Sowers JR. Diabetic cardiomyopathy: a hyperglycaemia- and insulin-resistance-induced heart disease. *Diabetologia.* (2018) 61:21–8. doi: 10.1007/s00125-017-4390-4
12. Khullar M, Cheema BS, Raut SK. Emerging evidence of epigenetic modifications in vascular complication of diabetes. *Front Endocrinol.* (2017) 8:237. doi: 10.3389/fendo.2017.00237
13. Singh GB, Khanna S, Raut SK, Sharma S, Sharma R, Khullar M. *DUSP-1* gene expression is not regulated by promoter methylation in diabetes-associated cardiac hypertrophy. *Ther Adv Cardiovasc Dis.* (2017) 11:147–54. doi: 10.1177/1753944717704590
14. Shimizu M, Umeda K, Sugihara N, Yoshio H, Ino H, Takeda R, et al. Collagen remodelling in myocardia of patients with diabetes. *J Clin Pathol.* (1993) 46:32–6. doi: 10.1136/jcp.46.1.32
15. Chiu J, Farhangkhoee H, Xu BY, Chen S, George B, Chakrabarti S. PARP mediates structural alterations in diabetic cardiomyopathy. *J Mol Cell Cardiol.* (2008) 45:385–93. doi: 10.1016/j.yjmcc.2008.06.009
16. Westermann D, Rutschow S, Jäger S, Linderer A, Anker S, Riad A, et al. Contributions of inflammation and cardiac matrix metalloproteinase activity to cardiac failure in diabetic cardiomyopathy: the role of angiotensin type 1 receptor antagonism. *Diabetes.* (2007) 56:641–6. doi: 10.2337/db06-1163
17. Singh VP, Le B, Khode R, Baker KM, Kumar R. Intracellular angiotensin II production in diabetic rats is correlated with cardiomyocyte apoptosis, oxidative stress, and cardiac fibrosis. *Diabetes.* (2008) 57:3297–306. doi: 10.2337/db08-0805
18. Mizushige K, Yao L, Noma T, Kiyomoto H, Yu Y, Hosomi N, et al. Alteration in left ventricular diastolic filling and accumulation of myocardial collagen at insulin-resistant prediabetic stage of a type II diabetic rat model. *Circulation.* (2000) 101:899–907. doi: 10.1161/01.CIR.101.8.899
19. Bando YK, Murohara T. Diabetes-related heart failure. *Circ J.* (2014) 78:576–83. doi: 10.1253/circj.CJ-13-1564
20. Jia G, Habibi J, DeMarco VG, Martinez-Lemus LA, Ma L, Whaley-Connell AT, et al. Endothelial mineralocorticoid receptor deletion prevents diet-induced cardiac diastolic dysfunction in females. *Hypertension.* (2015) 66:1159–67. doi: 10.1161/HYPERTENSIONAHA.115.06015
21. Huynh K, McMullen JR, Julius TL, Tan JW, Love JE, Cemerlang N, et al. Cardiac-specific IGF-1 receptor transgenic expression protects against cardiac fibrosis and diastolic dysfunction in a mouse model of diabetic cardiomyopathy. *Diabetes.* (2010) 59:1512–20. doi: 10.2337/db09-1456
22. Van Linthout S, Seeland U, Riad A, Eckhardt O, Hohl M, Dhayat N, et al. Reduced MMP-2 activity contributes to cardiac fibrosis in experimental diabetic cardiomyopathy. *Basic Res Cardiol.* (2008) 103:319–27. doi: 10.1007/s00395-008-0715-2
23. Bowden MA, Tesch GH, Julius TL, Rosli S, Love JE, Ritchie RH. Earlier onset of diabesity-Induced adverse cardiac remodeling in female compared to male mice. *Obesity.* (2015) 23:1166–77. doi: 10.1002/oby.21072
24. Galderisi M, Anderson KM, Wilson PW, Levy D. Echocardiographic evidence for the existence of a distinct diabetic cardiomyopathy (the Framingham Heart Study). *Am J Cardiol.* (1991) 68:85–9. doi: 10.1016/0002-9149(91)90716-X
25. Devereux RB, Roman MJ, Paranicas M, O'Grady MJ, Lee ET, Welty TK, et al. Impact of diabetes on cardiac structure and function: the strong heart study. *Circulation.* (2000) 101:2271–6. doi: 10.1161/01.CIR.101.19.2271
26. Dawson A, Morris AD, Struthers AD. The epidemiology of left ventricular hypertrophy in type 2 diabetes mellitus. *Diabetologia.* (2005) 48:1971–9. doi: 10.1007/s00125-005-1896-y
27. Bugger H, Abel ED. Molecular mechanisms of diabetic cardiomyopathy. *Diabetologia.* (2014) 57:660–71. doi: 10.1007/s00125-014-3171-6
28. Huynh K, Bernardo BC, McMullen JR, Ritchie RH. Diabetic cardiomyopathy: mechanisms and new treatment strategies targeting antioxidant signaling pathways. *Pharmacol Ther.* (2014) 142:375–415. doi: 10.1016/j.pharmthera.2014.01.003
29. Rosenkranz AC, Hood SG, Woods RL, Dusting GJ, Ritchie RH. B-type natriuretic peptide prevents acute hypertrophic responses in the diabetic rat heart. *Diabetes.* (2003) 52:2389–95. doi: 10.2337/diabetes.52.9.2389
30. Sundgren NC, Giraud GD, Schultz JM, Lasarev MR, Stork PJS, Thornburg KL. Extracellular signal-regulated kinase and phosphoinositol-3 kinase mediate IGF-1 induced proliferation of fetal sheep cardiomyocytes. *Am J Physiol Regul Integr Comp Physiol.* (2003) 285:R1481–9. doi: 10.1152/ajpregu.00232.2003
31. Jia G, Habibi J, Aroor AR, Hill MA, DeMarco VG, Lee LE, et al. Enhanced endothelium epithelial sodium channel signaling prompts left ventricular diastolic dysfunction in obese female mice. *Metabolism.* (2018) 78:69–79. doi: 10.1016/j.metabol.2017.08.008

32. Feng B, Chen S, Chiu J, George B, Chakrabarti S. Regulation of cardiomyocyte hypertrophy in diabetes at the transcriptional level. *Am J Physiol Endocrinol Metab.* (2008) 294:E1119–26. doi: 10.1152/ajpendo.00029.2008
33. Hua Y, Zhang Y, Dolence J, Shi G-P, Ren J, Nair S. Cathepsin K knockout mitigates high-fat diet-induced cardiac hypertrophy and contractile dysfunction. *Diabetes.* (2013) 62:498–509. doi: 10.2337/db12-0350
34. Costantino S, Paneni F, Lüscher TF, Cosentino F. MicroRNA profiling unveils hyperglycaemic memory in the diabetic heart. *Eur Heart J.* (2016) 37:572–6. doi: 10.1093/eurheartj/ehv599
35. Ucar A, Gupta SK, Fiedler J, Erikci E, Kardasinski M, Batkai S, et al. The miRNA-212/132 family regulates both cardiac hypertrophy and cardiomyocyte autophagy. *Nat Commun.* (2012) 3:1078. doi: 10.1038/ncomms2090
36. Raut SK, Kumar A, Singh GB, Nahar U, Sharma V, Mittal A, et al. miR-30c mediates upregulation of Cdc42 and Pak1 in diabetic cardiomyopathy. *Cardiovasc Ther.* (2015) 33:89–97. doi: 10.1111/1755-5922.12113
37. Su M, Wang J, Wang C, Wang X, Dong W, Qiu W, et al. MicroRNA-221 inhibits autophagy and promotes heart failure by modulating the p27/CDK2/mTOR axis. *Cell Death Differ.* (2015) 22:986–99. doi: 10.1038/cdd.2014.187
38. Raut SK, Singh GB, Rastogi B, Saikia UN, Mittal A, Dogra N, et al. miR-30c and miR-181a synergistically modulate p53–p21 pathway in diabetes induced cardiac hypertrophy. *Mol Cell Biochem.* (2016) 417:191–203. doi: 10.1007/s11010-016-2729-7
39. Singh GB, Raut SK, Khanna S, Kumar A, Sharma S, Prasad R, et al. MicroRNA-200c modulates DUSP-1 expression in diabetes-induced cardiac hypertrophy. *Mol Cell Biochem.* (2017) 424:1–11. doi: 10.1007/s11010-016-2838-3
40. Yan H, Wang H, Zhu X, Huang J, Li Y, Zhou K, et al. Adeno-associated virus-mediated delivery of anti-miR-199a tough decoys attenuates cardiac hypertrophy by targeting PGC-1alpha. *Mol Ther Nucleic Acids.* (2021) 23:406–17. doi: 10.1016/j.omtn.2020.11.007
41. Yu M, Liu Y, Zhang B, Shi Y, Cui L, Zhao X. Inhibiting microRNA-144 abates oxidative stress and reduces apoptosis in hearts of streptozotocin-induced diabetic mice. *Cardiovasc Pathol.* (2015) 24:375–81. doi: 10.1016/j.carpath.2015.06.003
42. Chavali V, Tyagi SC, Mishra PK. MicroRNA-133a regulates DNA methylation in diabetic cardiomyocytes. *Biochem Biophys Res Commun.* (2012) 425:668–72. doi: 10.1016/j.bbrc.2012.07.105
43. Chen S, Puthanveetil P, Feng B, Matkovich SJ, Dorn GW, Chakrabarti S. Cardiac miR-133a overexpression prevents early cardiac fibrosis in diabetes. *J Cell Mol Med.* (2014) 18:415–21. doi: 10.1111/jcmm.12218
44. Kambis TN, Shahshahan HR, Kar S, Yadav SK, Mishra PK. Transgenic expression of miR-133a in the diabetic akita heart prevents cardiac remodeling and cardiomyopathy. *Front Cardiovasc Med.* (2019) 6:45. doi: 10.3389/fcvm.2019.00045
45. Reddy S, Hu D-Q, Zhao M, Blay E, Sandeep N, Ong S-G, et al. miR-21 is associated with fibrosis and right ventricular failure. *JCI Insight.* (2017) 2:e91625. doi: 10.1172/jci.insight.91625
46. Liu S, Li W, Xu M, Huang H, Wang J, Chen X. Micro-RNA 21Targets dual specific phosphatase 8 to promote collagen synthesis in high glucose-treated primary cardiac fibroblasts. *Can J Cardiol.* (2014) 30:1689–99. doi: 10.1016/j.cjca.2014.07.747
47. Kumar A, Raut SK, Saikia UN, Sharma R, Khullar M. Abstract 15227: microrna-21 contributes to diabetic cardiomyopathy associated cardiac fibrosis. *Circulation.* (2011) 124:A15227. doi: 10.1161/circ.124.suppl_21.A15227
48. Statello L, Guo C-J, Chen L-L, Huarte M. Gene regulation by long non-coding RNAs and its biological functions. *Nat Rev Mol Cell Biol.* (2021) 22:96–118. doi: 10.1038/s41580-020-00315-9
49. Zhu X-H, Yuan Y-X, Rao S-L, Wang P. LncRNA MIAT enhances cardiac hypertrophy partly through sponging miR-150. *Eur Rev Med Pharmacol Sci.* (2016) 20:3653–60.
50. Li Y, Wang J, Sun L, Zhu S. LncRNA myocardial infarction-associated transcript (MIAT) contributed to cardiac hypertrophy by regulating TLR4 via miR-93. *Eur J Pharmacol.* (2018) 818:508–17. doi: 10.1016/j.ejphar.2017.11.031
51. Qu X, Du Y, Shu Y, Gao M, Sun F, Luo S, et al. MIAT Is a Pro-fibrotic long non-coding RNA governing cardiac fibrosis in post-infarct myocardium. *Sci Rep.* (2017) 7:42657. doi: 10.1038/srep42657
52. Yang F, Qin Y, Lv J, Wang Y, Che H, Chen X, et al. Silencing long non-coding RNA Kcnq1ot1 alleviates pyroptosis and fibrosis in diabetic cardiomyopathy. *Cell Death Dis.* (2018) 9:1000. doi: 10.1038/s41419-018-1029-4
53. Chen Y, Du J, Zhao YT, Zhang L, Lv G, Zhuang S, et al. Histone deacetylase (HDAC) inhibition improves myocardial function and prevents cardiac remodeling in diabetic mice. *Cardiovasc Diabetol.* (2015) 14:99. doi: 10.1186/s12933-015-0262-8
54. Xu Z, Tong Q, Zhang Z, Wang S, Zheng Y, Liu Q, et al. Inhibition of HDAC3 prevents diabetic cardiomyopathy in OVE26 mice via epigenetic regulation of DUSP5-ERK1/2 pathway. *Clin Sci.* (2017) 131:1841–57. doi: 10.1042/CS20170064
55. Dong F, Ren J. Fidarestat improves cardiomyocyte contractile function in db/db diabetic obese mice through a histone deacetylase Sir2-dependent mechanism. *J Hypertens.* (2007) 25:2138–47. doi: 10.1097/HJH.0b013e32828626d1
56. Christidi E, Brunham LR. Regulated cell death pathways in doxorubicin-induced cardiotoxicity. *Cell Death Dis.* (2021) 12:339. doi: 10.1038/s41419-021-03614-x
57. Frustaci A, Kajstura J, Chimenti C, Jakoniuk I, Leri A, Maseri A, et al. Myocardial cell death in human diabetes. *Circ Res.* (2000) 87:1123–32. doi: 10.1161/01.RES.87.12.1123
58. Hu X, Bai T, Xu Z, Liu Q, Zheng Y, Cai L. pathophysiological fundamentals of diabetic cardiomyopathy. *Compr Physiol.* (2017) 7:693–711. doi: 10.1002/cphy.c160021
59. Boudina S, Abel ED. Diabetic cardiomyopathy, causes and effects. *Rev Endocr Metab Disord.* (2010) 11:31–9. doi: 10.1007/s11154-010-9131-7
60. Fiordaliso F, Li B, Latini R, Sonnenblick EH, Anversa P, Leri A, et al. Myocyte death in streptozotocin-induced diabetes in rats is angiotensin II- dependent. *Lab Invest.* (2000) 80:513–27. doi: 10.1038/labinvest.3780057
61. Kobayashi S, Zhao F, Kobayashi T, Hagiwara M, Kaminaris A, Li C, et al. Hyperglycemia-induced cardiomyocyte death is mediated by lysosomal membrane injury and aberrant expression of cathepsin D. *Biochem Biophys Res Commun.* (2020) 523:239–45. doi: 10.1016/j.bbrc.2019.12.051
62. Yu X-Y, Song Y-H, Geng Y-J, Lin Q-X, Shan Z-X, Lin S-G, et al. Glucose induces apoptosis of cardiomyocytes via microRNA-1 and IGF-1. *Biochem Biophys Res Commun.* (2008) 376:548–52. doi: 10.1016/j.bbrc.2008.09.025
63. Fomison-Nurse I, Saw EEL, Gandhi S, Munasinghe PE, Van Hout I, Williams MJA, et al. Diabetes induces the activation of pro-ageing miR-34a in the heart, but has differential effects on cardiomyocytes and cardiac progenitor cells. *Cell Death Different.* (2018) 25: 1336–1349. doi: 10.1038/s41418-017-0047-6
64. Qiao Y, Zhao Y, Liu Y, Ma N, Wang C, Zou J, et al. miR-483-3p regulates hyperglycaemia-induced cardiomyocyte apoptosis in transgenic mice. *Biochem Biophys Res Commun.* (2016) 477:541–7. doi: 10.1016/j.bbrc.2016.06.051
65. Zheng D, Ma J, Yu Y, Li M, Ni R, Wang G, et al. Silencing of miR-195 reduces diabetic cardiomyopathy in C57BL/6 mice. *Diabetologia.* (2015) 58:1949–58. doi: 10.1007/s00125-015-3622-8
66. Karolina DS, Armugam A, Tavintharan S, Wong MTK, Lim SC, Sum CF, et al. MicroRNA 144 impairs insulin signaling by inhibiting the expression of insulin receptor substrate 1 in type 2 diabetes mellitus. *PLoS ONE.* (2011) 6:e22839. doi: 10.1371/journal.pone.0022839
67. Song Y, Mai H, Lin Y, Wang Y, Wang X, Gu S. MiR-144 affects proliferation and apoptosis of high glucose-induced AC16 cardiomyocytes by regulating CTRP3/JNK signaling. *In J Clin Exp Pathol.* (2020) 13:142–52.
68. Tao L, Huang X, Xu M, Yang L, Hua F. MiR-144 protects the heart from hyperglycemia-induced injury by regulating mitochondrial biogenesis and cardiomyocyte apoptosis. *The FASEB Journal.* (2020) 34:2173–2197. doi: 10.1096/fj.201901838R
69. Yang X, Li X, Lin Q, Xu Q. Up-regulation of microRNA-203 inhibits myocardial fibrosis and oxidative stress in mice with diabetic cardiomyopathy through the inhibition of PI3K/Akt signaling pathway via PIK3CA. *Gene.* (2019) 715:143995. doi: 10.1016/j.gene.2019.143995
70. Chandrasekera DNK, Neale JPH, van Hout I, Rawal S, Coffey S, Jones GT, et al. Upregulation of microRNA-532 enhances

cardiomyocyte apoptosis in the diabetic heart. *Apoptosis.* (2020) 25:388–99. doi: 10.1007/s10495-020-01609-1
71. Shan Z-X, Lin Q-X, Deng C-Y, Zhu J-N, Mai L-P, Liu J-L, et al. miR-1/miR-206 regulate Hsp60 expression contributing to glucose-mediated apoptosis in cardiomyocytes. *FEBS Lett.* (2010) 584:3592–600. doi: 10.1016/j.febslet.2010.07.027
72. Guo Y, Feng X, Wang D, Kang X, Zhang L, Ren H, et al. Long non-coding RNA: a key regulator in the pathogenesis of diabetic cardiomyopathy. *Front Cardiovasc Med.* (2021) 8:655598. doi: 10.3389/fcvm.2021.655598
73. Li X, Wang H, Yao B, Xu W, Chen J, Zhou X. lncRNA H19/miR-675 axis regulates cardiomyocyte apoptosis by targeting VDAC1 in diabetic cardiomyopathy. *Sci Rep.* (2016) 6:36340. doi: 10.1038/srep36340
74. Zhang M, Gu H, Xu W, Zhou X. Down-regulation of lncRNA MALAT1 reduces cardiomyocyte apoptosis and improves left ventricular function in diabetic rats. *Int J Cardiol.* (2016) 203:214–6. doi: 10.1016/j.ijcard.2015.10.136
75. Che H, Wang Y, Li H, Li Y, Sahil A, Lv J, et al. Melatonin alleviates cardiac fibrosis via inhibiting lncRNA MALAT1/miR-141-mediated NLRP3 inflammasome and TGF-β1/Smads signaling in diabetic cardiomyopathy. *FASEB J.* (2020) 34:5282–98. doi: 10.1096/fj.201902692R
76. Wang C, Liu G, Yang H, Guo S, Wang H, Dong Z, et al. MALAT1-mediated recruitment of the histone methyltransferase EZH2 to the microRNA-22 promoter leads to cardiomyocyte apoptosis in diabetic cardiomyopathy. *Sci Total Environ.* (2021) 766:142191. doi: 10.1016/j.scitotenv.2020.142191
77. Gao L, Wang X, Guo S, Xiao L, Liang C, Wang Z, et al. LncRNA HOTAIR functions as a competing endogenous RNA to upregulate SIRT1 by sponging miR-34a in diabetic cardiomyopathy. *J Cell Physiol.* (2019) 234:4944–58. doi: 10.1002/jcp.27296
78. Chen Y, Zhang Z, Zhu D, Zhao W, Li F. Long non-coding RNA MEG3 serves as a ceRNA for microRNA-145 to induce apoptosis of AC16 cardiomyocytes under high glucose condition. *Biosci Rep.* (2019) 39:BSR20190444. doi: 10.1042/BSR20190444
79. Li Q, Li P, Su J, Liu S, Yang X, Yang Y, et al. LncRNA NKILA was upregulated in diabetic cardiomyopathy with early prediction values. *Exp Ther Med.* (2019) 18:1221–5. doi: 10.3892/etm.2019.7671
80. Yin Y, Yang Z-F, Li X-H, Zhou L-Q, Zhang Y-J, Yang B. Knockdown of long non-coding RNA LUCAT1 reverses high glucose-induced cardiomyocyte injury via targeting CYP11B2. *Eur Rev Med Pharmacol Sci.* (2019) 23:8560–5. doi: 10.26355/eurrev_201910_19171
81. Yu X-Y, Geng Y-J, Liang J-L, Lin Q-X, Lin S-G, Zhang S, et al. High levels of glucose induce apoptosis in cardiomyocyte via epigenetic regulation of the insulin-like growth factor receptor. *Exp Cell Res.* (2010) 316:2903–9. doi: 10.1016/j.yexcr.2010.07.004
82. Özcan U, Cao Q, Yilmaz E, Lee A-H, Iwakoshi NN, Özdelen E, et al. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science.* (2004) 306:457–61. doi: 10.1126/science.1103160
83. Guo R, Liu W, Liu B, Zhang B, Li W, Xu Y. SIRT1 suppresses cardiomyocyte apoptosis in diabetic cardiomyopathy: an insight into endoplasmic reticulum stress response mechanism. *Int J Cardiol.* (2015) 191:36–45. doi: 10.1016/j.ijcard.2015.04.245
84. Puthanveetil P, Zhang D, Wang Y, Wang F, Wan A, Abrahani A, et al. Diabetes triggers a PARP1 mediated death pathway in the heart through participation of FoxO1. *J Mol Cell Cardiol.* (2012) 53:677–86. doi: 10.1016/j.yjmcc.2012.08.013
85. Kanamori H, Takemura G, Goto K, Tsujimoto A, Mikami A, Ogino A, et al. Autophagic adaptations in diabetic cardiomyopathy differ between type 1 and type 2 diabetes. *Autophagy.* (2015) 11:1146–60. doi: 10.1080/15548627.2015.1051295
86. Bravo-San Pedro JM, Kroemer G, Galluzzi L. Autophagy and mitophagy in cardiovascular disease. *Circ Res.* (2017) 120:1812–24. doi: 10.1161/CIRCRESAHA.117.311082
87. Dewanjee S, Vallamkondu J, Kalra RS, John A, Reddy PH, Kandimalla R. Autophagy in the diabetic heart: a potential pharmacotherapeutic target in diabetic cardiomyopathy. *Ageing Res Rev.* (2021) 68:101338. doi: 10.1016/j.arr.2021.101338
88. Mellor KM, Bell JR, Young MJ, Ritchie RH, Delbridge LMD. Myocardial autophagy activation and suppressed survival signaling is associated with insulin resistance in fructose-fed mice. *J Mol Cell Cardiol.* (2011) 50:1035–43. doi: 10.1016/j.yjmcc.2011.03.002
89. Xie Z, Lau K, Eby B, Lozano P, He C, Pennington B, et al. Improvement of cardiac functions by chronic metformin treatment is associated with enhanced cardiac autophagy in diabetic OVE26 mice. *Diabetes.* (2011) 60:1770–8. doi: 10.2337/db10-0351
90. Chen C, Yang S, Li H, Yin Z, Fan J, Zhao Y, et al. Mir30c is involved in diabetic cardiomyopathy through regulation of cardiac autophagy via BECN1. *Mol Ther Nucleic Acids.* (2017) 7:127–39. doi: 10.1016/j.omtn.2017.03.005
91. Feng Y, Xu W, Zhang W, Wang W, Liu T, Zhou X. LncRNA DCRF regulates cardiomyocyte autophagy by targeting miR-551b-5p in diabetic cardiomyopathy. *Theranostics.* (2019) 9:4558–66. doi: 10.7150/thno.31052
92. Zhuo C, Jiang R, Lin X, Shao M. LncRNA H19 inhibits autophagy by epigenetically silencing of DIRAS3 in diabetic cardiomyopathy. *Oncotarget.* (2016) 8:1429–37. doi: 10.18632/oncotarget.13637
93. Zeng C, Wang R, Tan H. Role of pyroptosis in cardiovascular diseases and its therapeutic implications. *Int J Biol Sci.* (2019) 15:1345–57. doi: 10.7150/ijbs.33568
94. Li X, Du N, Zhang Q, Li J, Chen X, Liu X, et al. MicroRNA-30d regulates cardiomyocyte pyroptosis by directly targeting foxo3a in diabetic cardiomyopathy. *Cell Death Dis.* (2014) 5:e1479. doi: 10.1038/cddis.2014.430
95. Jeyabal P, Thandavarayan RA, Joladarashi D, Suresh Babu S, Krishnamurthy S, Bhimaraj A, et al. MicroRNA-9 inhibits hyperglycemia-induced pyroptosis in human ventricular cardiomyocytes by targeting ELAVL1. *Biochem Biophys Res Commun.* (2016) 471:423–9. doi: 10.1016/j.bbrc.2016.02.065
96. Kim J-A, Wei Y, Sowers JR. Role of mitochondrial dysfunction in insulin resistance. *Circ Res.* (2008) 102:401–14. doi: 10.1161/CIRCRESAHA.107.165472
97. Jia G, DeMarco VG, Sowers JR. Insulin resistance and hyperinsulinaemia in diabetic cardiomyopathy. *Nat Rev Endocrinol.* (2016) 12:144–153. doi: 10.1038/nrendo.2015.216
98. Anderson EJ, Kypson AP, Rodriguez E, Anderson CA, Lehr EJ, Neufer PD. Substrate-specific derangements in mitochondrial metabolism and redox balance in the atrium of the type 2 diabetic human heart. *J Am Coll Cardiol.* (2009) 54:1891–8. doi: 10.1016/j.jacc.2009.07.031
99. Anderson EJ, Rodriguez E, Anderson CA, Thayne K, Chitwood WR, Kypson AP. Increased propensity for cell death in diabetic human heart is mediated by mitochondrial-dependent pathways. *Am J Physiol Heart Circ Physiol.* (2011) 300:H118–24. doi: 10.1152/ajpheart.00932.2010
100. Shaikh S, Troncoso R, Criollo A, Bravo-Sagua R, García L, Morselli E, et al. Regulation of cardiomyocyte autophagy by calcium. *Am J Physiol Endocrinol Metab.* (2016) 310:E587–96. doi: 10.1152/ajpendo.00374.2015
101. Peng X-P, Huang L, Liu Z-H. miRNA-133a attenuates lipid accumulation via TR4-CD36 pathway in macrophages. *Biochimie.* (2016) 127:79–85. doi: 10.1016/j.biochi.2016.04.012
102. Pelsers MMAL, Lutgerink JT, van Nieuwenhoven FA, Tandon NN, van der Vusse GJ, Arends J-W, et al. A sensitive immunoassay for rat fatty acid translocase (CD36) using phage antibodies selected on cell transfectants: abundant presence of fatty acid translocase/CD36 in cardiac and red skeletal muscle and up-regulation in diabetes. *Biochem J.* (1999) 337:407–14. doi: 10.1042/bj3370407
103. Liang F, Wang F, Zhang S, Gardner DG. Peroxisome proliferator activated receptor (PPAR)α agonists inhibit hypertrophy of neonatal rat cardiac myocytes. *Endocrinology.* (2003) 144:4187–94. doi: 10.1210/en.2002-0217
104. Liang J, Liu C, Qiao A, Cui Y, Zhang H, Cui A, et al. MicroRNA-29a-c decrease fasting blood glucose levels by negatively regulating hepatic gluconeogenesis. *J Hepatol.* (2013) 58:535–42. doi: 10.1016/j.jhep.2012.10.024
105. Diao X, Shen E, Wang X, Hu B. Differentially expressed microRNAs and their target genes in the hearts of streptozotocin-induced diabetic mice. *Mol Med Rep.* (2011) 4:633–40. doi: 10.3892/mmr.2011.489
106. Greco S, Fasanaro P, Castelvecchio S, D'Alessandra Y, Arcelli D, Di Donato M, et al. MicroRNA dysregulation in diabetic ischemic heart failure patients. *Diabetes.* (2012) 61:1633–41. doi: 10.2337/db11-0952
107. Chan SY, Zhang Y-Y, Hemann C, Mahoney CE, Zweier JL, Loscalzo J. MicroRNA-210 controls mitochondrial metabolism during hypoxia by repressing the iron-sulfur cluster assembly proteins ISCU1/2. *Cell Metab.* (2009) 10:273–84. doi: 10.1016/j.cmet.2009.08.015

108. Baseler WA, Thapa D, Jagannathan R, Dabkowski ER, Croston TL, Hollander JM. miR-141 as a regulator of the mitochondrial phosphate carrier (Slc25a3) in the type 1 diabetic heart. *Am J Physiol Cell Physiol.* (2012) 303:C1244–51. doi: 10.1152/ajpcell.00137.2012
109. Jagannathan R, Thapa D, Nichols CE, Shepherd DL, Stricker JC, Croston TL, et al. Translational regulation of the mitochondrial genome following redistribution of mitochondrial microRNA in the diabetic heart. *Circ Cardiovasc Genet.* (2015) 8:785–802. doi: 10.1161/CIRCGENETICS.115.001067
110. Li B, Carey M, Workman JL. The role of chromatin during transcription. *Cell.* (2007) 128:707–19. doi: 10.1016/j.cell.2007.01.015
111. Wu Y, Leng Y, Meng Q, Xue R, Zhao B, Zhan L, Xia Z. Suppression of excessive histone deacetylases activity in diabetic hearts attenuates myocardial ischemia/reperfusion injury via mitochondria apoptosis pathway. *J Diabetes Res.* (2017) 2017:8208065. doi: 10.1155/2017/8208065
112. Fang W, Wang C, He Y, Zhou Y, Peng X, Liu S. Resveratrol alleviates diabetic cardiomyopathy in rats by improving mitochondrial function through PGC-1α deacetylation. *Acta Pharmacol Sin.* (2018) 39:59–73. doi: 10.1038/aps.2017.50
113. Ma S, Feng J, Zhang R, Chen J, Han D, Li X, et al. SIRT1 Activation by resveratrol alleviates cardiac dysfunction via mitochondrial regulation in diabetic cardiomyopathy mice. *Oxid Med Cell Long.* (2017) 2017:e4602715. doi: 10.1155/2017/4602715
114. Lee T-I, Kao Y-H, Tsai W-C, Chung C-C, Chen Y-C, Chen Y-J. HDAC inhibition modulates cardiac PPARs and fatty acid metabolism in diabetic cardiomyopathy. *PPAR Res.* (2016) 2016:5938740. doi: 10.1155/2016/5938740
115. Teshima Y, Takahashi N, Nishio S, Saito S, Kondo H, Fukui A, et al. Production of reactive oxygen species in the diabetic heart. Roles of mitochondria and NADPH oxidase. *Circ J.* (2014) 78:300–6. doi: 10.1253/circj.CJ-13-1187
116. Giacco F, Brownlee M. Oxidative stress and diabetic complications. *Circ Res.* (2010) 107:1058–70. doi: 10.1161/CIRCRESAHA.110.223545
117. Rolo AP, Palmeira CM. Diabetes and mitochondrial function: role of hyperglycemia and oxidative stress. *Toxicol Appl Pharmacol.* (2006) 212:167–78. doi: 10.1016/j.taap.2006.01.003
118. Shen X, Zheng S, Thongboonkerd V, Xu M, Pierce WM Jr, Klein JB, et al. Cardiac mitochondrial damage and biogenesis in a chronic model of type 1 diabetes. *Am J Physiol.* (2004) 287:E896–905. doi: 10.1152/ajpendo.00047.2004
119. Yildirim SS, Akman D, Catalucci D, Turan B. Relationship between downregulation of miRNAs and increase of oxidative stress in the development of diabetic cardiac dysfunction: junctin as a target protein of miR-1. *Cell Biochem Biophys.* (2013) 67:1397–408. doi: 10.1007/s12013-013-9672-y
120. Magenta A, Greco S, Gaetano C, Martelli F. Oxidative stress and microRNAs in vascular diseases. *Int J Mol Sci.* (2013) 14:17319–46. doi: 10.3390/ijms140917319
121. Shen E, Diao X, Wang X, Chen R, Hu B. micrornas involved in the mitogen-activated protein kinase cascades pathway during glucose-induced cardiomyocyte hypertrophy. *Am J Pathol.* (2011) 179:639–50. doi: 10.1016/j.ajpath.2011.04.034
122. Kumar S, Kim Y-R, Vikram A, Naqvi A, Li Q, Kassan M, et al. Sirtuin1-regulated lysine acetylation of p66Shc governs diabetes-induced vascular oxidative stress and endothelial dysfunction. *Proc Natl Acad Sci USA.* (2017) 114:1714–9. doi: 10.1073/pnas.1614112114
123. Wei D, Loeken MR. Increased DNA methyltransferase 3b (Dnmt3b)-mediated CpG island methylation stimulated by oxidative stress inhibits expression of a gene required for neural tube and neural crest development in diabetic pregnancy. *Diabetes.* (2014) 63:3512–22. doi: 10.2337/db14-0231
124. Mortuza R, Chen S, Feng B, Sen S, Chakrabarti S. High glucose induced alteration of SIRTs in endothelial cells causes rapid aging in a p300 and FOXO regulated pathway. *PLoS ONE.* (2013) 8:e54514. doi: 10.1371/journal.pone.0054514
125. Tomaselli GF, Marbán E. Electrophysiological remodeling in hypertrophy and heart failure. *Cardiovasc Res.* (1999) 42:270–83. doi: 10.1016/S0008-6363(99)00017-6
126. Kaprielian R, Wickenden AD, Kassiri Z, Parker TG, Liu PP, Backx PH. Relationship between K+ channel down-regulation and [Ca2+]i in rat ventricular myocytes following myocardial infarction. *J Physiol.* (1999) 517(Pt 1):229–45. doi: 10.1111/j.1469-7793.1999.0229z.x
127. Näbauer M, Kääb S. Potassium channel down-regulation in heart failure. *Cardiovasc Res.* (1998) 37:324–34. doi: 10.1016/S0008-6363(97)00274-5
128. Rozanski GJ, Xu Z, Zhang K, Patel KP. Altered K+ current of ventricular myocytes in rats with chronic myocardial infarction. *Am J Physiol Heart Circ Physiol.* (1998) 274:H259–65. doi: 10.1152/ajpheart.1998.274.1.H259
129. Xu L, Patel KP, Rozanski GJ. Metabolic basis of decreased transient outward K+ current in ventricular myocytes from diabetic rats. *Am J Physiol Heart Circ Physiol.* (1996) H2190–6. doi: 10.1152/ajpheart.1996.271.5.H2190
130. Shimoni Y, Ewart HS, Severson D. Insulin stimulation of rat ventricular K+ currents depends on the integrity of the cytoskeleton. *J Physiol.* (1999) 514 (Pt 3):735–745. doi: 10.1111/j.1469-7793.1999.735ad.x
131. Shimoni Y, Ewart HS, Severson D. Type I and II models of diabetes produce different modifications of K+ currents in rat heart: role of insulin. *J Physiol.* (1998) 507(Pt 2):485–96. doi: 10.1111/j.1469-7793.1998.485bt.x
132. Chambers JB, Sampson MJ, Sprigings DC, Jackson G. QT prolongation on the electrocardiogram in diabetic autonomic neuropathy. *Diabetic Med.* (1990) 7:105–10. doi: 10.1111/j.1464-5491.1990.tb01342.x
133. Ninkovic VM, Ninkovic SM, Miloradovic V, Stanojevic D, Babic M, Giga V, et al. Prevalence and risk factors for prolonged QT interval and QT dispersion in patients with type 2 diabetes. *Acta Diabetol.* (2016) 53:737–44. doi: 10.1007/s00592-016-0864-y
134. Bellavere F, Ferri M, Guarini L, Bax G, Piccoli A, Cardone C, et al. Prolonged QT period in diabetic autonomic neuropathy: a possible role in sudden cardiac death? *Heart.* (1988) 59:379–83. doi: 10.1136/hrt.59.3.379
135. Brown DW, Giles WH, Greenlund KJ, Valdez R, Croft JB. Impaired fasting glucose, diabetes mellitus, and cardiovascular disease risk factors are associated with prolonged QTc duration. results from the third national health and nutrition examination survey. *J Cardiovasc Risk.* (2001) 8:227–33. doi: 10.1177/174182670100800407
136. Tse G, Lai ETH, Tse V, Yeo JM. Molecular and electrophysiological mechanisms underlying cardiac arrhythmogenesis in diabetes mellitus. *J Diabetes Res.* (2016) 2016:e2848759. doi: 10.1155/2016/2848759
137. Hegyi B, Bers DM, Bossuyt J. CaMKII signaling in heart diseases: emerging role in diabetic cardiomyopathy. *J Mol Cell Cardiol.* (2019) 127:246–59. doi: 10.1016/j.yjmcc.2019.01.001
138. Lengyel C, Virág L, Kovács PP, Kristóf A, Pacher P, Kocsis E, et al. Role of slow delayed rectifier K+-current in QT prolongation in the alloxan-induced diabetic rabbit heart. *Acta Physiol.* (2008) 192:359–68. doi: 10.1111/j.1748-1716.2007.01753.x
139. Torres-Jacome J, Gallego M, Rodríguez-Robledo JM, Sanchez-Chapula JA, Casis O. Improvement of the metabolic status recovers cardiac potassium channel synthesis in experimental diabetes. *Acta Physiol.* (2013) 207:447–59. doi: 10.1111/apha.12043
140. Gallego M, Fernández D, Ahyayauch H, Casis E, Casis O. Reduced calmodulin expression accelerates transient outward potassium current inactivation in diabetic rat heart. *CPB.* (2008) 22:625–34. doi: 10.1159/000185546
141. Panguluri SK, Tur J, Chapalamadugu KC, Katnik C, Cuevas J, Tipparaju SM. MicroRNA-301a mediated regulation of Kv4.2 in diabetes: identification of key modulators. *PLoS ONE.* (2013) 8:e60545. doi: 10.1371/journal.pone.0060545
142. Arnold N, Koppula PR, Gul R, Luck C, Pulakat L. Regulation of cardiac expression of the diabetic marker microRNA miR-29. *PLoS ONE.* (2014) 9:e103284. doi: 10.1371/journal.pone.0103284
143. Harris LG, Wang SH, Mani SK, Kasiganesan H, Chou CJ, Menick DR. Evidence for a non-canonical role of HDAC5 in regulation of the cardiac Ncx1 and Bnp genes. *Nucleic Acids Res.* (2016) 44:3610–7. doi: 10.1093/nar/gkv1496
144. Chandrasekaran S, Peterson RE, Mani SK, Addy B, Buchholz AL, Xu L, et al. Histone deacetylases facilitate sodium/calcium exchanger up-regulation in adult cardiomyocytes. *FASEB J.* (2009) 23:3851–64. doi: 10.1096/fj.09-132415
145. Lehmann LH, Jebessa ZH, Kreusser MM, Horsch A, He T, Kronlage M, et al. A proteolytic fragment of histone deacetylase 4 protects the heart from

failure by regulating the hexosamine biosynthetic pathway. *Nat Med.* (2018) 24:62–72. doi: 10.1038/nm.4452
146. Syren P, Rahm A-K, Schweizer PA, Bruehl C, Katus HA, Frey N, et al. Histone deacetylase 2-dependent ventricular electrical remodeling in a porcine model of early heart failure. *Life Sci.* (2021) 281:119769. doi: 10.1016/j.lfs.2021.119769
147. Freundt JK, Frommeyer G, Spieker T, Wötzel F, Grotthoff JS, Stypmann J, et al. Histone deacetylase inhibition by Entinostat for the prevention of electrical and structural remodeling in heart failure. *BMC Pharmacol Toxicol.* (2019) 20:16. doi: 10.1186/s40360-019-0294-x
148. Qin H-T, Li H-Q, Liu F. Selective histone deacetylase small molecule inhibitors: recent progress and perspectives. *Expert Opin Ther Pat.* (2017) 27:621–36. doi: 10.1080/13543776.2017.1276565
149. Kim HJ, Rowe M, Ren M, Hong J-S, Chen P-S, Chuang D-M. Histone deacetylase inhibitors exhibit anti-inflammatory and neuroprotective effects in a rat permanent ischemic model of stroke: multiple mechanisms of action. *J Pharmacol Exp Ther.* (2007) 321:892–901. doi: 10.1124/jpet.107.120188
150. Marks PA. Histone deacetylase inhibitors: a chemical genetics approach to understanding cellular functions. *Biochim Biophys Acta.* (2010) 1799:717–25. doi: 10.1016/j.bbagrm.2010.05.008
151. Liu N, Zhuang S. Treatment of chronic kidney diseases with histone deacetylase inhibitors. *Front Physiol.* (2015) 6:121. doi: 10.3389/fphys.2015.00121
152. Eckschlager T, Plch J, Stiborova M, Hrabeta J. Histone deacetylase inhibitors as anticancer drugs. *Int J Mol Sci.* (2017) 18:E1414. doi: 10.3390/ijms18071414
153. Choi SY, Piao ZH, Jin L, Kim JH, Kim GR, Ryu Y, et al. Piceatannol attenuates renal fibrosis induced by unilateral ureteral obstruction via downregulation of histone deacetylase 4/5 or p38-MAPK signaling. *PLoS ONE.* (2016) 11:e0167340. doi: 10.1371/journal.pone.0167340
154. Li M, Zheng Y, Yuan H, Liu Y, Wen X. Effects of dynamic changes in histone acetylation and deacetylase activity on pulmonary fibrosis. *Int Immunopharmacol.* (2017) 52:272–80. doi: 10.1016/j.intimp.2017.09.020
155. Korfei M, Skwarna S, Henneke I, MacKenzie B, Klymenko O, Saito S, et al. Aberrant expression and activity of histone deacetylases in sporadic idiopathic pulmonary fibrosis. *Thorax.* (2015) 70:1022–32. doi: 10.1136/thoraxjnl-2014-206411
156. Kis K, Liu X, Hagood JS. Myofibroblast differentiation and survival in fibrotic disease. *Expert Rev Mol Med.* (2011) 13:e27. doi: 10.1017/S1462399411001967
157. Kang S-H, Seok YM, Song M, Lee H-A, Kurz T, Kim I. Histone deacetylase inhibition attenuates cardiac hypertrophy and fibrosis through acetylation of mineralocorticoid receptor in spontaneously hypertensive rats. *Mol Pharmacol.* (2015) 87:782–91. doi: 10.1124/mol.114.096974
158. Liu Y, Li S, Zhang Z, Lv Z, Jiang H, Tan X, Liu F. Effects of valproic acid on sympathetic activity and left ventricularmyocardial remodelling in rats during pressure overload. *Turk J Med Sci.* (2017) 47:1651–1660. doi: 10.3906/sag-1704-142
159. Zhang Y, Gao F, Tang Y, Xiao J, Li C, Ouyang Y, et al. Valproic acid regulates Ang II-induced pericyte-myofibroblast trans-differentiation via MAPK/ERK pathway. *Am J Transl Res.* (2018) 10:1976–89.
160. Scholz B, Schulte JS, Hamer S, Himmler K, Pluteanu F, Seidl MD, et al. HDAC (Histone Deacetylase) inhibitor valproic acid attenuates atrial remodeling and delays the onset of atrial fibrillation in mice. *Circ Arrhythm Electrophysiol.* (2019) 12:e007071. doi: 10.1161/CIRCEP.118.007071
161. Kao Y-H, Liou J-P, Chung C-C, Lien G-S, Kuo C-C, Chen S-A, et al. Histone deacetylase inhibition improved cardiac functions with direct antifibrotic activity in heart failure. *Int J Cardiol.* (2013) 168:4178–83. doi: 10.1016/j.ijcard.2013.07.111
162. Nural-Guvener HF, Zakharova L, Nimlos J, Popovic S, Mastroeni D, Gaballa MA. HDAC class I inhibitor, Mocetinostat, reverses cardiac fibrosis in heart failure and diminishes CD90+ cardiac myofibroblast activation. *Fibrogenesis Tissue Repair.* (2014) 7:10. doi: 10.1186/1755-1536-7-10
163. Tao H, Yang J-J, Hu W, Shi K-H, Li J. HDAC6 promotes cardiac fibrosis progression through suppressing RASSF1A expression. *Cardiology.* (2016) 133:18–26. doi: 10.1159/000438781

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REVIEW
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Novel Insights Into the Pathogenesis of Diabetic Cardiomyopathy and Pharmacological Strategies

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Diabetic cardiomyopathy (DCM) is a severe complication of diabetes developed mainly in poorly controlled patients. In DCM, several clinical manifestations as well as cellular and molecular mechanisms contribute to its phenotype. The production of reactive oxygen species (ROS), chronic low-grade inflammation, mitochondrial dysfunction, autophagic flux inhibition, altered metabolism, dysfunctional insulin signaling, cardiomyocyte hypertrophy, cardiac fibrosis, and increased myocardial cell death are described as the cardinal features involved in the genesis and development of DCM. However, many of these features can be associated with broader cellular processes such as inflammatory signaling, mitochondrial alterations, and autophagic flux inhibition. In this review, these mechanisms are critically discussed, highlighting the latest evidence and their contribution to the pathogenesis of DCM and their potential as pharmacological targets.

Keywords: diabetes, heart, inflammation, mitochondria, cardiomyopathy, pyroptosis, mitophagy

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INTRODUCTION

Diabetic cardiomyopathy (DCM) is one of the most severe complications of diabetes. DCM is phenotypically defined as the structural or functional changes of the heart occurring in a diabetic patient independent of other comorbidities such as hypertension, coronary disease, and valvular disease as well as independent of other conventional cardiovascular risk factors. Although the mentioned DCM definition is clear, the specific phenotyping of diagnosed patients is still a matter of discussion mainly due to the potential difference in clinical features among patients. An example of this could be the reported differences in the DCM clinical presentation for the South Asian population in respect to Europeans. Although diastolic impairment expressed as reduced E/A ratio (comparison between the early and late trans mitral flow) was similar, differences in hypertrophy expressed as left ventricular mass and myocardial lipid content were found (1, 2). Another fact pointing in the same direction is that poorly controlled DCM can progress to heart failure (HF). Furthermore, DCM can be featured by either the systolic or diastolic dysfunction, thus generating the so-called HF with reduced ejection fraction or HF with preserved ejection fraction, respectively, causing a substantial detriment to the patient's quality of life (3).

For the clinical presentation, the heart-related data obtained from preclinical animal models of diabetes are also a matter of discussion. One common strategy for generating a type 1 diabetes mellitus (T1DM) model is to induce pancreatic damage using streptozotocin (STZ). At the same time, the most usual approach to develop a T2DM model is to feed animals with a high-fat diet (HFD). HFD and STZ can be used together to mimic T2DM with a stronger hyperglycemic component because of a strong reduction in STZ-induced insulin secretion. Finally, several genetic models of obesity and T2DM, i.e., db/db mice, ob/ob mice, are also used (4).

Considering a variety of models of T1DM and T2DM, the difference in their etiologies generates different functional outcomes in DCM (5). Insulin may play a causal role in the difference. Myocardial insulin signaling in both types of diabetes is quite different as T1DM is characterized by insulin deficiency and T2DM is characterized by insulin resistance. However, in both types of diabetes, a key factor for preventing the progression of DCM to HF is an adequate glycemic control (6). Therefore, despite the different etiologies of both diabetes, several shared molecular alterations are taking place in the myocardium that will be presented as follows.

The aforementioned heterogeneity in the clinical presentation and in the preclinical models of DCM is also projected into the myocardial molecular and cellular physiopathology. Several molecular alterations, such as increased production of reactive oxygen species (ROS), chronic inflammation, fibrosis, mitochondrial dysfunction, autophagic flux inhibition, altered metabolic pathways, altered insulin signaling, cardiomyocyte hypertrophy, and increased myocardial cell death, among others, are described (7). Despite a variety of factors listed earlier, many of them could be subordinated as complementary mechanisms or consequences of broader cellular alterations. However, the approaches that intervene in some of these features, particularly inflammatory signaling, mitochondrial alterations, and autophagic flux, have been described, and hence modifies the DCM phenotype. Therefore, in this review, we focused on the last three mechanisms to outline an updated view of the key mediators in the pathophysiology of DCM. A particular focus is made to propose pharmacological targets to treat DCM. To this aim, we extensively reviewed the data mainly derived from preclinical models, identifying the novel molecular players in the DCM pathophysiology, which have been successfully intervened to achieve the goal of attenuating or improving the DCM phenotype in a structural or functional fashion. The mediators that met the mentioned criteria were considered as pharmacological targets, and the interventions on these pathways were considered as possible pharmacological strategies.

INFLAMMATION IN THE PATHOGENESIS OF DCM

Low-grade chronic inflammation is a main contributor to the pathogenesis of DCM. Different DCM-associated stimuli, such as hyperglycemia, hyperinsulinemia, and hyperlipidemia, induce inflammatory signaling pathways, including nuclear factor-kappa b (NF-κB), toll-like receptors (TLRs), inflammasome, and pyroptosis. Also, micro-RNAs (miRNA) and long noncoding RNAs (lncRNA) have been recognized as the regulators of inflammatory signaling in diabetic hearts. Moreover, searching for new targets to modulate the inflammatory response in DCM in cardiomyocytes, fibroblast, endothelium, and vascular smooth muscle cells (VSMCs) has become an interesting research area.

TLRs in the Pathogenesis of Cardiovascular Effects of Diabetes and Its Implications in DCM

Toll-like receptors have been associated with VSMC damage in cellular models of diabetes (8). Additionally, TLR2 and TLR4 are expressed in endothelial cells under diabetes-associated inflammation (9, 10). A T2DM model obtained by feeding LDL receptor-deficient ($LDLR^{-/-}$) mice with HFD generates large atherosclerotic lesions with increased intimal layer, macrophage, collagen accumulation, and expression of pro-inflammatory cytokines. These alterations are strongly reduced by using the TLR4 antagonist *Rhodobacter sphaeroides* LPS (Rs-LPS) (11). Another inhibitor of TLR4, TAK242, a cell-permeable cyclohexanecarboxylate that directly binds to the Cys747 residue of TLR4 reduces infarct size, edema, hemorrhagic transformation index, and excess hemoglobin. These effects were described in a STZ plus HFD diabetes model, which was subjected to an induced neurovascular injury (12). Also, the viral inhibitory peptide (VIPER), a specific TLR4 blocker, has cardioprotective effects improving diastolic function in a rat model of hypertension induced by angiotensin II (13). Furthermore, in rat VSMC, saturated free fatty acids (FFAs), such as palmitate, activates TLR4 signaling pathway, triggering an inflammatory response, assessed as an increase in monocyte chemoattractant protein 1 (MCP-1) expression (14). Moreover, in human VSMCs, palmitate induces IL-8 expression through the activation of TLR4-NF-κB signaling (15), and also induces apoptosis and oxidative stress through TLR4 pathway (8).

In primary cultured aortic endothelial cells, the depletion of TLR-2 using siRNA prevents the palmitate-induced attenuation of insulin signaling, evaluated as Y612 phosphorylation of insulin receptor substrate 1 (p-IRS1-Y^{612}), and blunted insulin-induced endothelial nitric oxide synthase (eNOS) phosphorylation (16). A similar phenomenon is described in wild-type mice fed with HFD for 10 weeks (16). Moreover, in TLR2-KO mice, the insulin-induced mesenteric artery vasorelaxation is unchanged even when fed with HFD (16).

On the other hand, high glucose also induces the TLR2 and TLR4 expression in human macrovascular aortic endothelial cells (HMAECs). This effect is prevented by ROS inhibitors such as N-acetyl cysteine or apocynin (17). Exposure of VSMC to high glucose also triggers TLR4 signaling by increasing the protein level and activity of TLR4, myeloid differentiation factor 88 (MyD88), and NF-κB, augmenting intracellular ROS production. The same findings are found in mesenteric arteries from STZ-induced diabetic rats (18).

In the heart of diabetic mice (STZ and db/db models), Wang et al. showed that advanced glycation end products (AGEs) induce the formation of a complex between myeloid differentiation 2 (MD2) and TLR4. Activated TLR4 triggers a signaling cascade that increases cytokine levels responsible for the development of DCM (19). In neonatal rat cardiomyocytes, oxidized low density lipoproteins (ox-LDL) also induce TLR4 and NF-κB-dependent apoptosis (20). Moreover, global silencing of TLR4, using a systemic administration of TLR4 siRNA, prevents a decline in the systolic function and the ventricular remodeling reported for STZ-treated mice (21). Therefore, TLRs can be considered as a molecule of interest in the pathophysiology of DCM. Although the specific cardiac effects of TLRs are still under investigation, more research is required to clarify the fundamental role of TLRs in the development of DCM. From a pharmacological point of view, it will be interesting to assess whether TLR inhibitors are safe and effective in preventing DCM in several animal models of diabetes.

Inflammasome Complexes in the Pathogenesis of DCM

Inflammasome complexes are composed of a receptor that is activated by pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs), an adaptor protein, and effectors that initiate the inflammatory signaling (22). Nucleotide-binding oligomerization domain, leucine-rich repeat, and pyrin domain-containing protein (NLRP) subfamily are the receptors characterized by a pyrin-containing domain involved in the inflammasome formation. After the binding of PAMPs or DAMPs to NLRPs, the pyrin domain interacts with the pyrin domain of the apoptosis-associated speck-like protein (ASC), which activates bound pro-caspase-1 to caspase-1, leading to the secretion of IL-1β and IL-18, or pyroptosis activation (22).

NLRP3 inflammasome is activated in HFD + STZ diabetic rats (23), where there is an increase in NLRP3, ASC, pro-caspase-1, caspase-1, pro-IL-1β, and IL-1β protein levels (23). The expression of sirtuin 3 (SIRT3), a protein member of class III histone deacetylases dependent on nicotinamide adenine dinucleotide (NAD^+), is decreased in the myocardial samples of STZ-induced diabetic mice, affecting cell energy metabolism and activating NLRP3 inflammasome. The SIRT3 KO animals display ventricular diastolic and systolic dysfunction, and necroptosis. Interestingly, cardiac dysfunction is prevented by inhibiting the NLRP3 inflammasome activation (24). The inhibition of miR-223, a miRNA expressed in diabetes, downregulates the markers of inflammation (NLRP3), fibrosis (collagens I and III), and apoptosis (caspase-3 and Bax), attenuating the cardiac effects as well in the systolic function and ventricular remodeling of STZ-induced diabetes (24). The same effect is obtained by metformin. In hearts from STZ-treated mice, metformin inhibits the increases in NLRP3, caspase-1, and IL-1β in an AMP-activated protein kinase/mammalian target of rapamycin (AMPK/mTOR-) dependent manner (25). Melatonin reduces cardiac fibrosis and improves cardiac systolic function in diabetic mice *via* inhibiting lncR-MALAT1/miR-141-mediated NLRP3 inflammasome activation (26). Another widely used treatment for T2DM is the combination of sodium-glucose cotransporter-2 inhibitors (SGLT2i) with dipeptidyl peptidase-4 (DPP-4) inhibitors (27). The treatment with dapagliflozin (an SGLT2i) and saxagliptin (a DPP-4 inhibitor) has an additive effect on the cardio protection of BTBR *ob/ob* diabetic mice by decreasing NLRP3 protein levels (28). In BTBR *ob/ob* mice, the combination of dapagliflozin with ticagrelor (P2Y12 receptor antagonist) also reduces cardiac hypertrophy, apoptosis, inflammation, fibrosis, and NLRP3 inflammasome markers associated with DCM (29).

Gypenosides (Gps), the main active compound in the herbaceous plant *Gynostemma pentaphylla Makino*, decrease the NLRP3 inflammasome activation in hearts from STZ + HFD-treated rats and exerts anti-inflammatory properties improving myocardial histological changes associated to DCM although there is a lack of cardiac functional data associated with the treatment (30). Visceral adipose tissue-derived serine protease inhibitor (vaspin) decreases NLRP3 levels in an autophagy-dependent mechanism in STZ-induced diabetes model, associated with the improvement of a systolic function (31). Finally, rosuvastatin, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, can alleviate the consequences of diabetes in rat hearts through the downregulation of NLRP3 (32). Other modulators of NLRP3 expression in STZ-treated diabetic rats are Spleen tyrosine kinase (SYK) and c-Jun N-terminal kinase (JNK). The treatment with the JNK inhibitor (SP) and the SYK inhibitor (BAY61-3606) inhibits NLRP3 activation in cellular models (33).

Another subfamily of the inflammasome complex is absent in melanoma 2 (AIM2), a protein that participates in inflammasome formation in the presence of cytoplasmic DNA (34). AIM2 is increased in the heart from STZ rats, and AIM2 knockdown attenuates cardiac hypertrophy accompanied by improvements in the diastolic and systolic function (35).

Only a few studies have addressed the activation of NLRP3 inflammasome in human heart samples. Fender et al. showed that right atrial appendages from patients with T2DM display higher levels of PAR4 than nondiabetic atrial tissue, along with an increased abundance of cleaved caspase-1, IL-1β and the plasma membrane pore-forming protein N-terminal gasdermin D, a protein required for IL-1β secretion (36). Immunohistochemistry evaluation of heart tissue samples obtained from the failing human hearts at the time of transplantation shows an increase in caspase-1 expression in human diabetic hearts as compared to nondiabetic heart tissue sections. The increase in caspase-1 expression is associated with a higher infiltration of inflammatory cells into the myocardium of diabetic heart tissues (37). These results suggest that in human hearts, as in animal models, NLRP3 inflammasome is activated in DCM.

Despite a great variety of molecules used in several diabetes animal models to reduce NLRP3/AIM2 inflammasome activation (**Figure 1**), and hence, to prevent cardiac injury, clinical trials are still required to study the possible benefit of NRLP3 targeted therapies in humans.

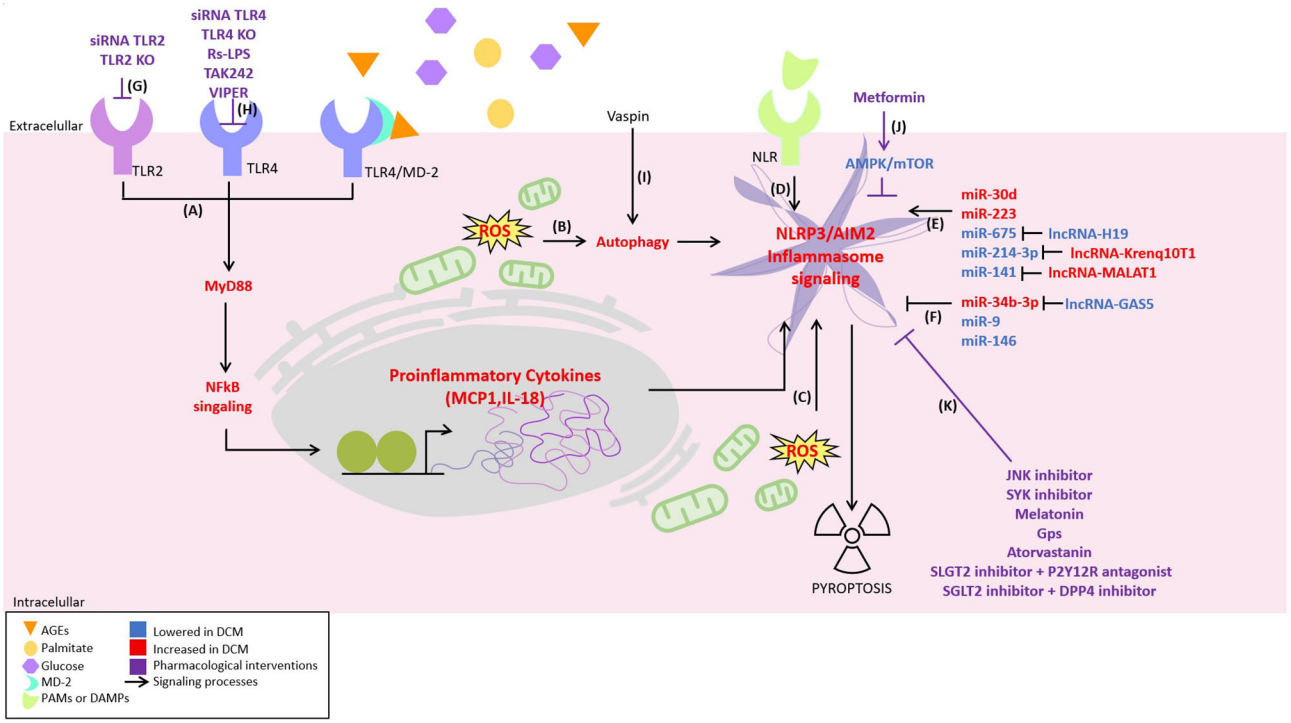

FIGURE 1 | Novel inflammatory signaling pathways implicated in diabetic cardiomyopathy (DCM) pathogenesis and its possible pharmacological interventions. Inflammatory signaling has been proposed to be responsible for the development of DCM. It is activated by different pathways, including: **(A)** nuclear factor-kappa b (NF-κB) signaling activated by toll-like receptor 2 (TLR2), TLR4, and myeloid differentiation 2 (MD2)/TLR4 complex, **(B)** oxidative stress-induced autophagy, **(C)** oxidative stress, **(D)** activation of the NLR by pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs). Moreover, some non-coding RNAs are described to **(E)** positively or **(F)** negatively regulate the inflammasome NLPR3. On the other hand, possible therapeutical strategies attenuating inflammatory signaling are illustrated as follows: **(G)** TLR2 depletion using siRNA TLR2 or TLR2-KO. **(H)** TLR4 depletion using siRNA TLR4 or TLR4-KO, TLR4 antagonist *Rhodobacter sphaeroides* LPS (Rs-LPS) and TLR4 blocker TAK242 and viral inhibitory peptide (VIPER), **(I)** decreasing NLRP3 by vaspin in an autophagy-dependent mechanism, **(J)** the inhibition of NLRP3 by metformin in an AMPK/mTOR-dependent manner, and **(K)** c-Jun N-terminal kinase (JNK) and spleen tyrosine kinase (SYK) inhibitor, melatonin, gypenosides (Gps), atorvastatin, SLGT2 inhibitor + P2Y12R antagonist, and SGLT2 inhibitor + DPP4 inhibitor.

Pyroptotic Cell Death in the Pathogenesis of DCM

Many of the studies have linked the different types of cell death to the development of DCM. Within the inflammatory context, the different types of programmed necrosis predominate, including pyroptosis (26, 38, 39). Several pharmacological interventions have been tested in DCM models (40). Exendin-4, an analog of the incretin GLP-1 used to treat diabetes, prevents the systolic dysfunction in HFD-fed mice, also reduces the markers of pyroptosis, such as caspase-1 activation and IL-1β and IL-18 release, in mice cardiomyocytes exposed to high glucose (33). Chemerin is an adipokine that is upregulated in obesity and diabetes and has been associated with cardiovascular disorders (41, 42). Chemerin and its receptor CMKLR1 are increased in the cardiac tissue of HFD + STZ rats, along with the markers of pyroptosis, such as NLRP3, cleaved caspase-1, and IL-1β (43). CMKLR1 knockdown reduces pyroptosis markers in the heart tissue of diabetic rats and reduces cardiac fibrosis and hypertrophy associated with an improvement in cardiac systolic and diastolic function (43).

In diabetic hearts, noncoding RNAs are described as the regulators of pyroptosis. Using neonatal rat cardiomyocytes treated with high glucose and hearts from STZ + HFD mice, Li et al. demonstrated the presence of pyroptosis, which was mediated by the upregulation of micro-RNA-30d (miR-30d) (44). miR-30d directly represses the expression of FoxO3, which in turn mediates the expression of an apoptosis repressor with caspase recruitment domain (ARC), an endogenous inhibitor of caspase activation. The consequent decrease in ARC levels in these DCM models activates caspase-1 and the pyroptotic pathway (44). miR-214-3p, which targets caspase-1, is also downregulated in diabetic mice and in human cardiomyocytes exposed to a high-glucose (HG) medium, leading to increased levels of caspase-1 and the activation of pyroptosis (45–47). A minimum of two noncoding RNAs endogenously regulate this microRNA, lncRNA Kcnq1ot1 (46, 47), and caspase-1-associated circular RNA (CACR) (45). These two noncoding RNAs are competitors for miRNA binding, generating a miRNA sponge effect. Kcnq1ot1 is upregulated in the serum of diabetic patients. Silencing this lncRNA reduces pyroptosis both *in vitro* and *in vivo* and ameliorates the systolic dysfunction in diabetic mice (46, 47). Similarly, CACR is also

upregulated in HG-treated cardiomyocytes and serum of diabetic patients. Knockdown of CACR in cardiomyocytes reduces both caspase-1 activation and pyroptosis (45). Another example is miR-34b-3p, which is markedly upregulated in a DCM mice model. miR-34b-3p targets the aryl hydrocarbon receptor (AHR), a negative regulator of NLRP3 inflammasome and pyroptosis (48). miR-34b-3p also has an endogenous regulator, the lncRNA GAS5, whose expression is strongly diminished in the hearts of diabetic mice (48). Furthermore, the overexpression of GAS5 reduces pyroptosis markers in the heart tissue and improves a systolic function (48). miR-9 also regulates pyroptosis in the diabetic heart (37). This miRNA is markedly downregulated in human diabetic hearts and HG exposed human cardiomyocytes, leading to the increase of its target, the ELAV-like protein 1 (ELAVL1) (37). ELAVL1 is an RNA-binding protein that stabilizes mRNAs of inflammatory genes, including NLRP3 and caspase-1, causing increased IL-1β production in the cardiac tissue (37). Accordingly, human cardiomyocytes transfected with miR-9 mimics reduced ELAVL1, caspase-1, and IL-1β expression (37).

In total, these studies show that caspase-1-induced pyroptosis in the heart may be an essential mediator of DCM pathogenesis (**Figure 1**) and could be regulated by noncoding RNAs, offering new therapeutic targets for treating this disease.

MiRNAs as Regulators of DCM and Its Inflammatory Features

In addition to the role of the abovementioned miRNAs in cardiac pyroptosis, other miRNAs have been described to regulate the different aspects related to inflammation in DCM (49). miR-675, along with its precursor lncRNA H19, is downregulated in STZ rats and neonatal rat cardiomyocytes exposed to high glucose (50). miR-675 targets the mitochondrial protein VDAC, which is increased in the diabetic model, leading to apoptosis of cardiomyocytes. The overexpression of H19 normalizes VDAC levels to reduce apoptosis, inflammation, and oxidative stress of cardiac tissues and improves cardiac systolic and diastolic function (50). Cardiac levels of miR-146a are also decreased in STZ-treated mice, specifically in endothelial cells, but not in cardiomyocytes (51). Endothelial-specific overexpression of miR-146a in diabetic mice reduces inflammatory markers such as IL-6, IL-1β, TNF-α, and p65-NF-κB, reducing cardiac fibrosis and diastolic dysfunction (51). Furthermore, a downregulation of miR-181a-5p, due to its regulator KCNQ1OT1, in both HG-cultured cardiomyocytes and STZ-treated mice is observed. However, its overexpression decreases inflammatory cytokine levels, myocardial apoptosis, and fibrosis *in vivo,* thus improving the DCM phenotype.

Cardiac fibrosis is also a characteristic of DCM, where cardiac fibroblasts show an activated phenotype with a greater production of extracellular matrix, a process regulated by the TGF-β1 and Smad2/3 signaling pathway. Neonatal cardiac fibroblasts exposed to a HG medium show increased TGF-β1 signaling and the production of collagens I and III with a parallel increase in miR-150-5p levels (52). miR-150-5p reduces Smad7 expression, a protein that binds to a TGF-β1 receptor and prevents the activation of a Smad2/3 pathway. Silencing miR-150-5p in cardiac fibroblast restored Smad7 levels and prevented the increase in pro-fibrotic and pro-inflammatory markers *in vitro* (52). miR-223 has also been associated with cardiac inflammation and fibrosis in STZ-induced diabetic rats (24). The silencing of miR-223 reduces fibrosis, NLRP3 expression, and apoptosis and ameliorates cardiac function in diabetic rats (24).

Menopause has been shown to aggravate DCM in women. To simulate postmenopausal estrogen deficiency in older diabetic women, Jia et al. treated ovariectomized (OVX) mice with STZ. A worsening of cardiac function compared to mice subjected to STZ alone is found (53). This cardiac dysfunction is associated with an imbalance between pro-inflammatory type 1 macrophages (M1) and anti-inflammatory type 2 macrophages (M2) infiltrated in the heart tissue. miR-155, which has been previously linked to pro-inflammatory imbalance during viral myocarditis (54), is markedly upregulated in OVX/STZ mice (53). Interestingly, the delivery of gold nanoparticles containing antagomiR-155, which are preferentially phagocytosed by macrophages, counteracts M1/M2 imbalance and restores cardiac function, reducing cardiac hypertrophy, fibrosis, and apoptosis of cardiac cells (53).

A 5-year longitudinal study on patients with T2DM, compared with nondiabetic age-matched controls, showed an increase in cardiac hypertrophy associated with a progressive impairment in cardiac strain that was paralleled by the upregulation of miR122-5p (55). The miRNA regulates macrophage polarization (56) and mediates inflammation in a model of acute lung injury induced by lipopolysaccharide (57). Interestingly, miR-208a, described originally to be increased in a mouse model of cardiac hypertrophy (58), also regulates the synthesis of IL-10 in human macrophages (59). The miRNA is increased in atrial samples derived from diabetic patients (60), suggesting a possible cross-regulation between cardiac hypertrophy and inflammation in DCM hearts. Moreover, the use of an anti-miR-208a in Dahl hypertensive rats prevents cardiac remodeling while improving cardiac function (61).

In total, these studies show that various miRNAs regulate different aspects of inflammation in DCM (**Figure 1**), through the regulation of numerous target genes. In the near future, understanding how these miRNAs act on the pathogenesis of DCM will offer new therapeutic targets for the treatment of this disease.

MITOCHONDRIAL ALTERATIONS IN THE PATHOGENESIS OF DCM

Mitochondria have been a top research topic for decades. Dysfunctional mitochondria are involved in the pathogenesis of multiple chronic diseases from cancer to neurodegenerative disorders, and DCM is not an exception (62). Alterations in mitochondrial substrate utilization and mitochondrial dynamics have been described as pathological features in the diabetic heart (63). Several mitochondrial approaches to revert, prevent, or attenuate the DCM phenotype have been described. In the following sections, updated evidence about interventions

targeting mitochondrial processes and their relevance in the DCM pathogenesis will be described.

Mitochondrial Substrate Utilization as a Pathological Feature in DCM

A healthy heart uses fatty acids as the main source of adenosine triphosphate (ATP) (70%). However, cardiomyocytes can also obtain acetyl-CoA from glucose, ketone bodies, lactate, and amino acids (64). Despite the increased availability of glucose due to the DCM-associated hyperglycemia, a decrease or loss in the capacity to oxidize glucose with a subsequent increase of fatty acid utilization is observed. Fatty acid oxidation uses 12% more oxygen than glucose oxidation (64). Left ventricular myocardium from patients with T2DM and nondiabetic patients undergoing coronary artery bypass graft surgery was obtained using subepicardial needle biopsy. The myocardium samples from patients with T2DM have a decreased mitochondrial respiration fueled by palmitoyl-carnitine. Diabetic myocardium also has a diminished activity of hydroxyacyl-CoA dehydrogenase and accumulates more lipid droplets (65). These results suggest that diabetic hearts exhibit a decreased mitochondrial capacity for β-oxidation with increased accumulation of intracellular lipids. This change in substrate utilization is believed to contribute to cardiac hypertrophy, along with ventricular dysfunction, both cardinal characteristics of DCM.

Recently, to promote glucose utilization in a model of diabetes induced by low-dose STZ, Wende et al. developed a mouse model with an inducible cardio-specific expression of GLUT4 (66). Surprisingly, despite the display of increased glucose uptake and utilization using the transgenic model, no improvement in the DCM phenotype is observed. Moreover, GLUT4 induction increases the diastolic dysfunction associated with the decrease of mitochondrial respiratory complex activity by O-GlcNAcylation (OGA) and reduces the transcript levels of electron transfer chain subunits (66). In STZ + HFD diabetes mice models, hyperglycemia is also associated with reduced cardiac expression of β-hydroxybutyrate-dehydrogenase (BDH1) and succinyl-CoA:3-oxoacid CoA transferase (OXCT1) due to OGA. This phenotype is recapitulated by a dominant-negative transgenic mouse for OGA enzyme responsible for OGA removal (67). Reliable and relevant data about OGA and its implications in the diabetic heart suggest that OGA could be targeted to design a protective intervention to prevent DCM. In this context, substantial attenuation of the structural and functional cardiac changes induced by diabetes is obtained by OGA overexpression (68) although its association with increased ketone utilization remains unclear. On the other hand, the overexpression of BDH1 increases ketone utilization by 1.7 folds and displays protective actions on cardiac structural and functional injury induced by transverse aortic constriction (TAC) surgery, suggesting that increasing ketone body uptake could be beneficial beyond DCM interventions (69). However, the link between ketone metabolism and DCM remains to be fully explored (68).

The CANVAS trial, which analyzed the efficacy and safety of canagliflozin (an SGLT2i) in 10,142 patients with T2DM and high cardiovascular risk, reported that SGLT2i significantly reduced cardiovascular risk and decreased hospitalization due to HF (70). Ferrannini et al. (71) analyzed the EMPA-REG OUTCOME clinical study (72) involving patients with T2DM and cardiovascular risk and found that empagliflozin modulated myocardial energy metabolism increasing the ketone body utilization. These ketone bodies are oxidized in preference to fatty acids, improving the efficiency of mitochondrial metabolism and oxygen consumption (71). These researchers propose SGLT2i as cardioprotective agents with hypoglycemic effects, which are beneficial for DCM (71). Furthermore, Li and Zhou summarize the beneficial impact of SGLT2i on DCM in preclinical studies, highlighting its metabolic, anti-fibrosis, and antioxidative functions (73).

Calpains are upregulated in the diabetic heart and directly affect the protein level and activity of ATP synthase (ATP5A1), decreasing mitochondrial ATP synthesis (74). Calpain inhibition increases the activity of ATP5A1, prevents the mitochondrial ROS, reduces cardiac hypertrophy markers, and improves the myocardial function determined by the increased fractional shortening and recovery of E/A ratio (74). Similarly, in STZ-induced diabetes model, the overexpression of calpastatin (endogenous calpain inhibitor) and the knockout *capn4,* a subunit required for calpain activity, prevents cardiomyocyte apoptosis and ventricular remodeling (75, 76).

In summary, more research is still required to perform pharmacological modulation of mitochondrial metabolism to slow down the progress and development of DCM. Furthermore, based on the recent evidence of ketone glucose utilization by cardiac cells, it remains to fully elucidate whether the metabolic changes are protective adaptations or whether those are the main problems in the pathogenesis of DCM.

Mitochondrial Oxidative Stress in DCM

Augmented ROS production in the diabetic heart is one of the leading factors in the development of DCM. Therefore, the strategies for reducing ROS or increasing antioxidant mechanisms to improve or maintain myocardial function in diabetes are of significant interest (77). There are several sources of cytosolic ROS, including NADPH oxidases (NOX), xanthine oxidases, and uncoupled nitric oxide synthases (NOS), most of which are involved in the development of DCM and have been reviewed previously (78–81). However, mitochondria are the primary sources of cellular ROS, specifically from complexes I and III of the electron transport chain, where the superoxide radical is generated by the transfer of electrons to molecular oxygen (62). Mitochondrial oxidative stress is triggered when the generation of superoxide increases and/or the defense capacity of the antioxidant system decreases (82, 83). Mitochondrial ROS increase in both STZ-induced T1DM and db/db T2DM models, indicating oxidative stress, which is associated with mitochondrial dysfunction, together with HF and DCM (84–86).

Several mechanisms that trigger mitochondrial oxidative stress associated with DCM have been described so far (82). TOM70 is an outer mitochondrial membrane (OMM) translocase that is downregulated in diabetic hearts. Knockdown of TOM70 generates an exacerbated DCM phenotype in db/db mice and increases cytosolic and mitochondrial ROS *in*

vitro in HG/high-fat-treated neonatal mouse cardiomyocytes. Conversely, lentiviral overexpression of TOM70 prevents hypertrophy, fibrosis, and ventricular dysfunction (87). TOM70 participates in the import of several mitochondrial proteins with potential antioxidant effects; however, the mechanism by which TOM70 reduces ROS remains to be fully elucidated.

As mentioned earlier, the diabetic heart exhibits an increase in the oxidation of fatty acids, which leads to mitochondria overload, with the consequent overproduction of ROS (82). Forkhead box protein O 1 (FOXO1) is a transcription factor that participates in the expression of lipid uptake and lipid metabolism-related genes, and it is increased in the heart of genetic (db/db) and HFD-induced diabetes models (88). In STZ-treated mice, FOXO1 indirectly increases NADPH oxidases 4 (NOX4) expression, increasing cytosolic ROS, and increases peroxisome proliferator-activated receptor alpha (PPARα), promoting fatty acid uptake and mitochondrial overload, with a consequent increase in mitochondrial ROS production (89). Accordingly, one study has shown that microRNA 30c (miR-30c) is an interesting new therapeutic target for DCM as its overexpression reduces the levels of PGC-1β, which is an important coactivator of PPARα, thus reducing lipid accumulation and ROS production in the heart of db/db mice (90).

Thus, the studies concerning mitochondrial oxidative stress and DCM seem to involve the antioxidant defense systems and the modulation of upstream proteins involved in superoxide formation by the electron transport chain (**Figure 2**). However, the underlying mechanisms are still being investigated, but they are a growing source for new therapeutic targets for DCM.

Mitochondrial Dynamics in the Pathogenesis of DCM

Mitochondria are highly dynamic organelles as they perform coordinated cycles of fission (mitochondrial fragmentation) and fusion (mitochondrial elongation) to regulate shape, size, subcellular distribution, and the number of mitochondria per cell (91). These processes, termed as mitochondrial dynamics, allow rapid and transitory morphological changes that regulate mitochondrial function and cellular processes related to apoptosis, metabolism, mitochondrial quality control, cell cycle, etc. (91). Mitochondrial fusion and fission are controlled by specific machinery. During mitochondrial fusion, mitofusins 1 and 2 (MFN1 and MFN2) and optic atrophic protein 1 (OPA1) maintain the fusion of the OMM and the IMM, respectively (91, 92). Mitochondrial fission depends on the recruitment of the Dynamin-related protein 1 (Drp1) GTPase from the cytosol of the GTPase Drp1 to the OMM by different surface receptor proteins. This binding allows the oligomerization of Drp1 and the constriction of mitochondria until the complete division into two-daughter mitochondria (92). The processes of mitochondrial fusion and fission need to be balanced to maintain a healthy mitochondrial population, which is essential for a normal heart function. Therefore, alterations in mitochondrial dynamics are considered to be one of the crucial mechanisms related to the development of DCM (93, 94).

Diabetic cardiomyopathy has been related to a largely fragmented mitochondrial phenotype. Westermeier et al. reviewed different diabetes models to address a direct relationship between mitochondrial fission/fusion and insulin resistance (63). Hyperglycemia induces mitochondrial fragmentation, increases ROS, and activates apoptotic pathways in neonatal rat ventricular myocytes and H9c2 cells. Hyperglycemia-dependent effects are prevented by a Drp1 dominant-negative, DRPK38A, demonstrating the involvement of mitochondrial fission (95, 96).

Recently, it was described in db/db mice models that the proportion of long and short isoforms of OPA1 (L-OPA1/S-OPA1) is decreased (97). The balance of long and short isoforms is necessary for the fusion of IMM. Therefore, an excess of S-OPA1 or a reduction in L-OPA1 prevents mitochondrial fusion. Moreover, Drp1 and Fis1, a Drp1 receptor protein, are also increased in hearts from db/db mice, which agrees with the observed mitochondrial fragmentation phenotype. Furthermore, as mitochondrial ATP production and oxygen consumption are decreased and ROS production is augmented, these mitochondria are also dysfunctional (97). These changes were prevented by feeding the mice with a ketogenic diet, accompanied by an increase in L-OPA1/S-OPA1 ratio, a drop in Fis-1, and no changes in Drp1 (97). Indeed, the restoration of OPA1 function also improves the systolic function in STZ diabetic rats (98). Therefore, the imbalance in mitochondrial dynamics triggered by an alteration in OPA1 contributes to the development of DCM.

Mitofusin 2 has also been shown to be a potential therapeutic strategy for DCM. In neonatal rat cardiomyocytes, PPARα positively regulates the expression of MFN2 by directly binding to its promoter. The expression of PPARα is decreased in cardiomyocytes cultured in high glucose/high lipid (OXO1) media producing a decrease in MFN2 levels and consequently inducing mitochondrial fission leading to mitochondrial dysfunction (99). *In vivo*, the restoration of MFN2 improved the systolic function and attenuated the diastolic changes associated with DCM (99).

In right atrial myocardium samples obtained from patients with T2DM, MFN1 levels are decreased and myofiber contractibility and oxygen consumption are reduced compared with obese individuals (100). However, this result must be taken cautiously because neither patients with obesity nor T2DM were affected by cardiomyopathy (100). Nevertheless, this result suggests that in the progression of obesity to diabetes, an alteration in mitochondria dynamics and function has taken place.

Therefore, regulating mitochondrial dynamics from the point of view of mitochondrial morphology seems to be an interesting therapeutic target in DCM (**Figure 2**). Restoring or preventing the downregulation of proteins involved in mitochondrial fusion could normalize the balance between mitochondrial fusion

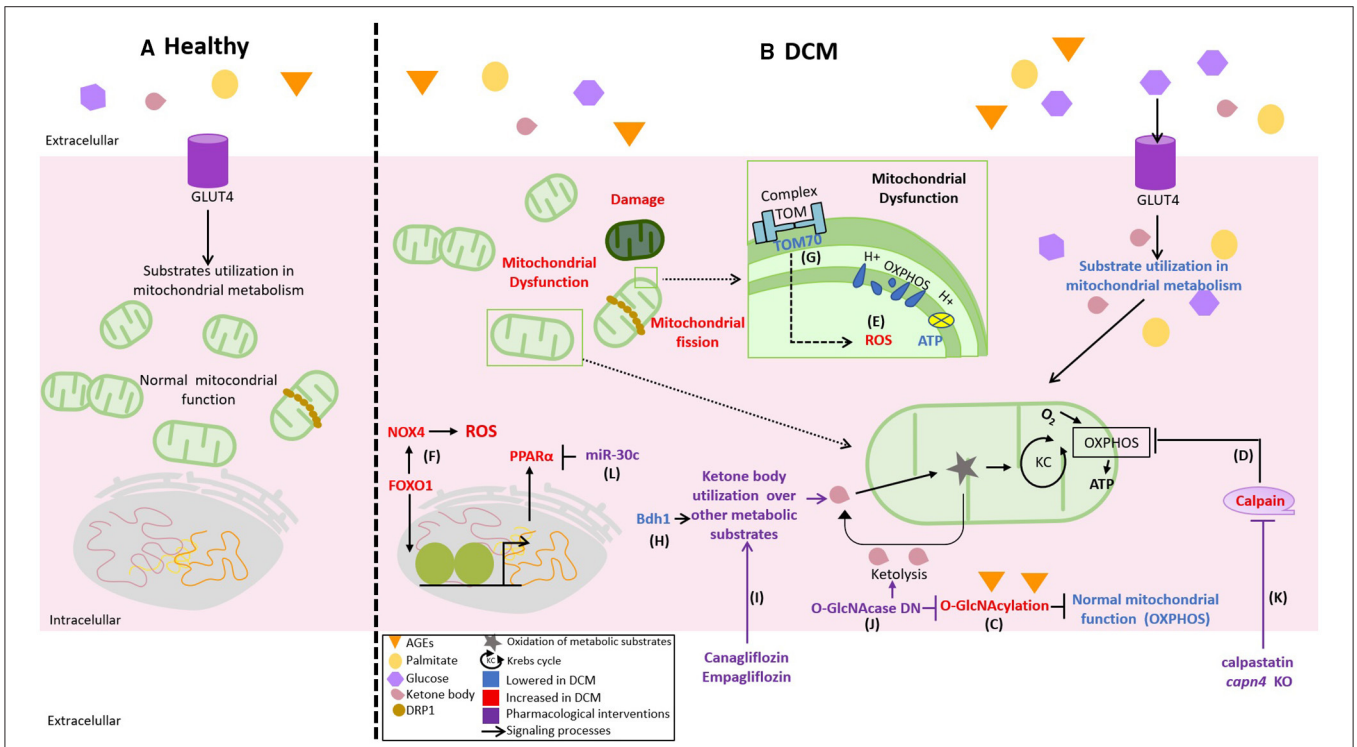

FIGURE 2 | Novel differences between healthy and DCM mitochondrial function and its pharmacological targets. **(A)** Normally, the mitochondria used glucose, lipids, and ketones as the fuel to obtain adenosine triphosphate (ATP) in the oxidative phosphorylation (OXPHOS) according to the necessities of the cell, **(B)** but in DCM, there is an excess of available fuels, increasing glucose, palmitate, and advanced glycation end products (AGEs) that affect the flexibility in substrate use by the mitochondria triggering mitochondrial dysfunction. This process is evidenced by a lower mitochondrial membrane potential, decreased production of ATP by OXPHOS, and increased reactive oxygen species (ROS). The mechanisms of mitochondrial deterioration in DCM are illustrated as follows: **(C)** the decrease in OXPHOS activity by O-GlcNAcylation (OGA), **(D)** the upregulation of calpains in the diabetic heart, **(E)** the abnormal production of mitochondrial ROS, **(F)** the production of cytosolic ROS by an increase in the expression of NADPH oxidases 4 (NOX4) indirectly regulated by FOXO1, and **(G)** the downregulation of TOM70 in the diabetic heart. Thus, therapeutic strategies aiming to improve a mitochondrial function are represented as follows: **(H,I)** the overexpression of β-hydroxybutyrate-dehydrogenase (BDH1) and Canagliflozin or Empagliflozin to increase the use of ketones, **(J)** dominant-negative for OGA (O-GlcNAcase DN), **(K)** inhibition of calpain activity by calpastatin (endogenous calpain inhibitor) or *capn4* knockout, and **(L)** micro-RNA 30c (miR-30c) reduces the proliferator-activated receptor alpha (PPARα) coactivator PGC-1β.

and fission, which is directly related to the improvement in mitochondrial activity and, consequently, in cardiac function.

AUTOPHAGY ALTERATIONS IN DCM

Autophagy is a cellular catabolic process responsible for recycling macromolecules and futile organelles as part of cellular homeostasis. This process is tightly regulated by autophagy-related genes (ATGs) (101). Three types of autophagy are described. Micro-autophagy occurs when proteins are degraded by direct entry into the lysosome. Chaperone-mediated autophagy, a process where proteins with the recognition sequence KFERQ binds to the chaperone 70 kDa heat shock protein (Hsp70) responsible for transporting these proteins to the lysosome in a lysosome-associated membrane protein 2- (LAMP2-) dependent fashion. Finally, macro-autophagy, commonly referred to as autophagy, implicates the formation of a phagophore, which is a nascent two-layered vesicle that encapsulates cytosolic components; the formed phagophore requires to be maturated and finally fused with the lysosome (101–103).

Several pieces of evidence suggest that autophagy dysregulation is involved in the pathogenesis of DCM (102). Most of the data show that autophagic flux is decreased or blunted in the heart of T2DM models and, conversely, increased in some models of T1DM (101, 104). Indeed, regarding T1DM, there is a recent report showing that FoxO1 mediates over-reactive autophagy, which has a causal role in the pathogenesis of DCM in STZ-treated mice. In these animals, the administration of angiotensin IV suppresses the increased autophagy and attenuates the pathological ventricular phenotype (105). It has been proposed that in T2DM, autophagy is a critical factor in the pathogenesis of DCM, while in T1DM, autophagy is a mechanism to limit the glucotoxicity damage in the heart (104). This dichotomy about the role of autophagy in diabetes is still a matter of discussion.

Decreased Autophagic Flux as a Feature of DCM

As described earlier, in STZ treatment-induced T1DM models, the autophagic flux in the heart is induced (104). However, chronic T1DM (6 months) also causes autophagic flux inhibition

(106). The same authors reported that cardiomyocyte-specific ATG5 knockout imitates the autophagic inhibition of the long-standing STZ approach and accelerates the progression of the heart dysfunction through a nuclear factor-erythroid factor 2-related factor 2- (Nrf2-) dependent mechanism. Nrf2 knockout rescues the phenotype induced by the deletion of ATG5 gene, concluding that Nrf2 could exert deleterious aberrant signaling once autophagy is inhibited (106). The final results are very interesting as Nrf2 has been proposed to be a therapeutic strategy for DCM due to a plethora of antioxidant and anti-inflammatory genes transactivated by this transcriptional factor (107). To summarize, both T1DM and T2DM are featured by autophagic inhibition but at chronic stages of the disease.

AMP-activated protein kinase is an important sensor of cell energy status (101). AMPK activation by an increase in the AMP/ATP ratio stimulates autophagic flux (101). AMPK activity is inhibited in STZ + HFD-induced T2DM mice. Furthermore, in HFD-fed mice, AMPK activity is slightly inhibited and the decrease in AMPK activity is related to a decrease in autophagic flux, measured by the autophagic marker LC3 II (108, 109). Treatment with sulforaphane, a naturally occurring isothiocyanate in plants, positively regulates the expression of Nrf2 and its downstream genes. This compound prevents diabetes-induced AMPK inhibition associated with an increase in LC3 II (108). On the other hand, in STZ-induced T1DM mice model, the inhibition of autophagic flux by AMPK 6 months after the onset of diabetes is described (106). This finding is also observed in H9C2 cells when autophagic flux is evaluated using a mCherry-Gfp-Lc3 construct (106). All these findings support the idea that autophagy is inhibited in the chronic stages of both T1DM and T2DM (101, 110, 111). Because there is altered energy metabolism and dysfunctional insulin signaling, the accumulation of metabolic substrates produced by hyperlipidemia and hyperglycemia in the chronic stages of TD1M and T2DM can lead to the inhibition of AMPK activity with the consequent inhibition of autophagic flow. However, the use of LC3 II as the only marker of autophagy should be done cautiously (104). In hyperlipidemia-dependent T2DM mice models, such as those generated using HFD, LC3 II is also located on the surface of lipid droplets (112). Therefore, AMPK and LC3 II changes could be related to the selective degradation of lipid droplets in the cytoplasm of cardiac cells rather than associated to autophagosome formation.

Several cardioprotective molecules regulating the autophagic flux in DCM have been described. Lin-28 homolog A (Lin28a) is decreased in high glucose conditions and inhibits the mammalian sterile 20-like kinase 1 (Mst1) (113). Lin28a overexpression activates autophagy in cardiomyocytes (113). Interestingly, the Lin28a-dependent protective effect against high glucose conditions was abolished when autophagy was inhibited by 3-metil-adenine. This data suggest that autophagy is involved in *in vitro* cardio protection (113). miR34a is upregulated in the myocardial tissue of diabetic patients and mice, leading to DCM (114). Flavanol (dihydromyricetin) and astragaloside-IV (triterpenoid saponin) downregulate miR34a and ameliorate DCM in STZ-treated rats in an autophagy-dependent mechanism (114, 115). Although, in these papers, the exact mechanism by which miR34a affected the autophagic flux was not disclosed, Pang et al. described in other models that miR34a targeted autophagy-related 9 A (ATG9A) (116). miR207 that targets LAMP2 is also upregulated and inhibits the autophagic flux in DCM (117). However, the inhibition of miR207 or the overexpression of LAMP2 for reverting the DCM phenotype is still not examined *in vivo* (117).

Cardiokines, which are defined as peptides released from the heart (118), are also an emerging topic in DCM. IL-33, a known cardiokine downregulated in the diabetic heart, prevents the onset of diastolic dysfunction in db/db mice through autophagy and endoplasmic reticulum stress-dependent mechanisms (119). Moreover, fibroblast growth factor 21 (FGF21), another cardiokine, prevents the systolic dysfunction associated to DCM in STZ-treated animals (120, 121).

Despite the defective autophagic flux in DCM and the success achieved for some interventions aiming for restoration (**Figure 3**), it is still a matter of discussion, which are the main molecular targets downregulated among the many proteins participating in the autophagic process. Knowledge about the precise molecular targets could be an invaluable tool for the future development of therapies.

Moreover, according to the preclinical data, an increase of autophagy in human right atrial samples derived from patients with T2DM is also described. Electron microscopy shows a larger number of autophagosomes associated with increased levels of LC3B-2 and Beclin-1 (122). Conversely, a recent report using atrial samples derived from patients with T2DM who underwent cardiopulmonary bypass showed that autophagy was suppressed when compared with the samples from nondiabetic patients, measured using Beclin-1 and ATG4 levels (123). On the other hand, the samples from diabetic HF patients submitted to the left ventricle-assisted device (LVAD) have shown that several autophagic markers such as LC3B, ATG3, and Beclin-1 were upregulated and associated with a decrease in the levels of miR-133a when compared with non-diabetic HF patients (124). Therefore, most of the preclinical and clinical data show that autophagy plays a role in the pathogenesis of cardiac complications of diabetes. However, the controversy of whether it is upregulated or downregulated still remains to be elucidated. Furthermore, the available data have led us to think that the role of autophagy is far more complex than merely a regulation of activation level. It seems to be related to the physio-pathological stage.

Impaired Mitophagy as a Feature of DCM

Mitophagy is a specialized catabolic process aiming to eliminate defective or damaged mitochondria through autophagy. This process is triggered by a decrease in the mitochondrial membrane potential, which stabilizes in the OMM the serine-threonine kinase PTEN-induced kinase 1 (PINK1), which in turn recruits the cytosolic protein E3-ligase Parkin (102). The PINK1/Parkin pathway functions as a molecular tag for the removal of damaged mitochondria. Mitophagy is essential in the maintenance of healthy cardiomyocytes, and defective mitophagy is a feature of several cardiac diseases, including DCM (94).

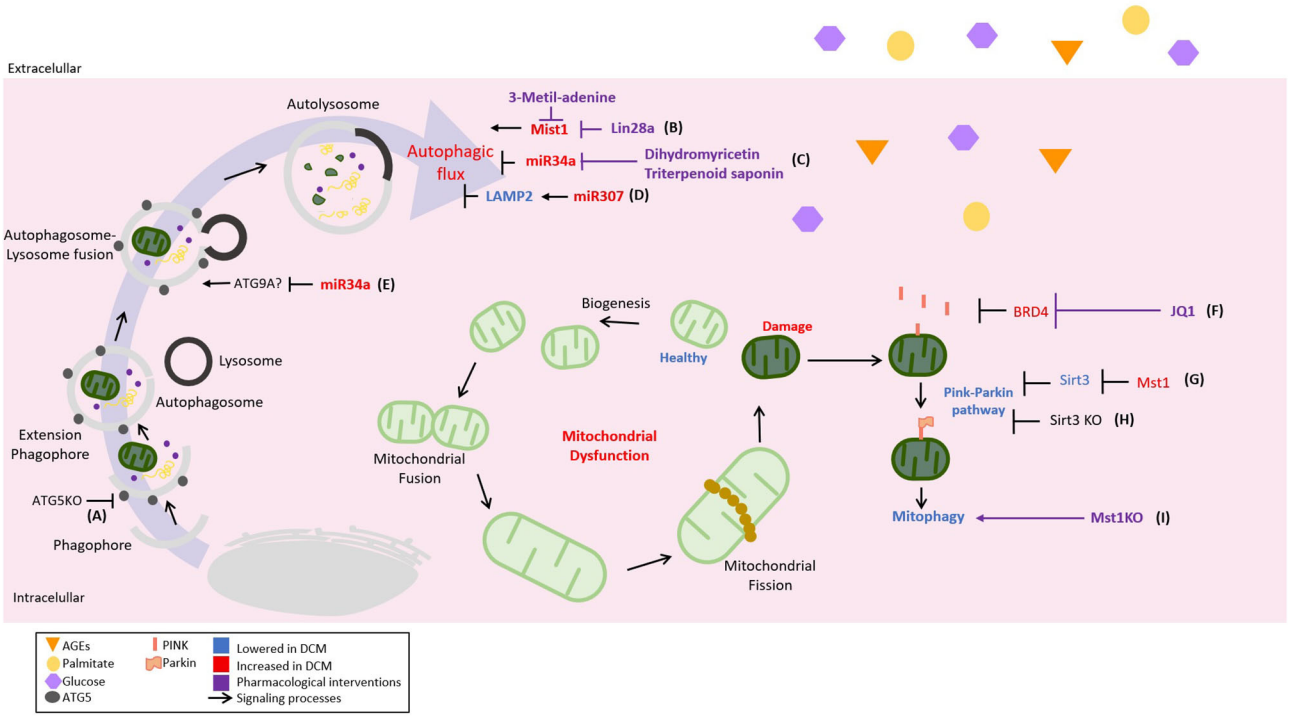

FIGURE 3 | Novel regulations of cellular catabolic pathways in DCM and its therapeutical strategies. The autophagic inhibition plays a critical role in the development and progression of DCM; therefore, different pathological mechanisms and their pharmacological strategies have been described. It can be illustrated as follows: **(A)** acceleration of cardiac dysfunction progression in type 1 diabetes mellitus (T1DM) with autophagy-related gene 5 (ATG5) KO, **(B)** the promotion of cardiac dysfunction by mammalian sterile 20-like kinase 1- (Mst1-) inhibited autophagic flow. Lin-28 homolog A (Lin28a) can inhibit Mst1 activating autophagy, **(C)** the inhibition of autophagic flow by the upregulation of miR-34a in the diabetic heart. As a pharmacological strategy, dihydromethicetin decreases the expression of miR-34a, restoring the impaired autophagy, **(D)** diabetic heart upregulates miR-207, which inhibits the autophagic flow in an LAPM2-dependent mechanism, and **(E)** miR-34a could inhibit the autophagic flux by targeting autophagy-related 9 A (ATG9A). Mitophagy, the mechanism by which damaged or defective mitochondria are eliminated, is strongly inhibited in DCM, being considered a pathological feature. For this reason, mechanisms and therapeutic strategies aiming at mitophagy recovery have also been included and represented as follows: **(F)** the inhibition of mitophagy by bromodomain-containing protein 4 (BRD4) that is restored by JQ1 in a PTEN-induced kinase 1- (PINK1-) dependent mechanism, **(G)** Mst1 decreases mitophagy using a PARKIN-dependent mechanism by inhibiting sirtuin 3 (SIRT3) expression; therefore, **(H)** SIRT3 KO reduces mitophagy also in a Parkin-dependent manner, and **(I)** Mst1 KO to induce mitophagy.

PTEN-induced kinase 1-Parkin-mediated mitophagy was suppressed in DCM induced by HFD (125, 126). In diabetic mouse hearts, the upregulation of bromodomain-containing protein 4 (BRD4), a member of the BET family of epigenetic regulators, inhibits PINK1/Parkin-mediated mitophagy, triggering the accumulation of damaged mitochondria with the subsequent impairment of cardiac structure and function (125). The inhibition of BRD4 with JQ1 restores mitochondrial function, cardiac structure, and the systolic and diastolic function through a PINK1-dependent mechanism (125). In HFD mice, the deletion of Parkin partially inhibits mitophagy and triggers a worsening of myocardial lipid accumulation and diastolic dysfunction (126). Moreover, the restoration of Sirt3-Foxo3A-Parkin signaling upregulates mitophagy and protects the development of DCM in STZ mice (127). On the other hand, Mst1 can induce DCM in a Parkin-dependent mechanism. Mst1 inhibits Sirt3 expression leading to the downregulation of Parkin, cardiomyocyte mitophagy inhibition, and DCM development (128). Melatonin administration inhibits Mst1 and activates Parkin pathway. This treatment rescues the impairment of cardiac mitophagy and alleviates systolic disfunction associated with DCM (129). The role of Mst1 in the development of DCM is further corroborated in the Mst1 knockout mice. The deletion of Mst1 gene reduces mitochondrial fission and alleviates left ventricular remodeling and systolic dysfunction in diabetic mice (130). Most of the previous studies are conducted using STZ mice models. However, in a db/db mice model, Mst1 knockdown also alleviates cardiac lipotoxicity and inhibits the development of DCM (131). These data suggest that Mst1/Parkin is a common and general mechanism for developing DCM in both T1DM and T2DM. Furthermore, despite the interesting data from animal models (**Figure 3**), there is a lack of knowledge about the clinical relevance of mitophagy stimulation as a therapy in DCM.

DISCUSSION AND FUTURE PERSPECTIVES

Diabetic cardiomyopathy is characterized by several cellular and molecular features. The low-grade inflammation appears to be

central as several experimental anti-inflammatory approaches, particularly involving the TLR4/MD2 pathway, prevent the development of DCM (19). Moreover, systemic interference of TLR4 expression using siRNA also prevents the development of DCM in STZ-treated mice (21). Despite these successful interventions, there are only a few articles linking TLR signaling with the pathogenesis of cardiac diseases. On the other hand, although most of the available data connect the NRLP3 inflammasome with the induction of DCM, probably other inflammasome complexes could also be involved in the DCM physiopathology, i.e., AIM2 inflammasome (35). Therefore, more research is required to clarify the role of all inflammasome complexes and their utility as a target for novel therapies for DCM. The increasing body of evidence, showing the strategies that successfully attenuated DCM by targeting inflammation (**Figure 1**), has converted this topic into a promising source for pharmacological interventions.

Despite the many functions described for miR presented earlier, other protective miR approaches have also been described in T1DM genetic models using the Akita mice. In these papers, the overexpression of miR-133a improves a systolic function and prevents structural remodeling, suggesting that miR-133a has protective actions in DCM (132, 133). However, it is also important to go deeper into the molecular mechanisms of the protective effects of miR-133a and is necessary to evaluate whether the intervention can successfully attenuate the DCM phenotype in other animal models.

The myocardial reprogramming on mitochondrial substrate utilization from glucose oxidation toward fatty acid oxidation has always been considered as a pathophysiological feature that could be responsible for the development of DCM. However, because of the latest information, this metabolic change is now seen as an adaptation to limit glucotoxicity in the heart (66). Currently, another change in myocardial substrate utilization, the ketone bodies, is becoming the main interest. Clinical trials using SGLT2i suggest that the increase of ketone body utilization in the diabetic heart could be protective. It remains to be elucidated if the modulation of OGA modifies the ketone body metabolism by preventing the OGA of key enzymes in the pathway. Although the pharmacological interest has clearly focused on the modulation of ketone metabolism, this evidence suggests that metabolic alterations have a causal role in the development of the DCM phenotype.

Regarding mitochondrial dynamics, OPA1 and Mfn2 are possible targets to prevent DCM development (97, 99). As several interventions modulating mitochondrial physiology achieved the goal of modifying the fate of the DCM phenotype, they could be considered as pharmacological strategies (**Figure 2**). However, pharmacological interventions to modulate mitochondrial dynamics suitable to perform clinical trials still need to be developed.

In DCM, the catabolic pathways such as autophagy or mitophagy are strongly suppressed (106). The mentioned suppression is probably propitiating the aberrant signaling of known cellular mediators like the cited example of Nrf2. That led us to think that there will be more examples of aberrant signaling implicated in DCM, once the inhibition of recycling pathways has taken place. For the mentioned reasons, more experimental interventions aiming to normalize autophagic flux are required in DCM. In the case of mitophagy, the evidence is strongly pointing to decreased PINK1/Parkin pathway (**Figure 3**), and future interventions aiming to recover its activity of improving mitochondrial quality control must be taken in consideration to look for a clinical application.

Cardiac delivery is a current challenge of DCM pharmacology. Several approaches are currently used to ensure the cardiac delivery of therapeutic agents, thus overcoming possible side effects produced by an untargeted distribution (134). However, the application of cardiac selective delivery to DCM is still not a widely used strategy. Conversely, accomplishing the criteria of attenuating or preventing the DCM phenotype is achieved in several examples. Nanotechnology has been used to selectively deliver the therapeutic molecules. For instance, liposomes loaded with a non-mitogenic acid fibroblast growth factor and associated to a microbubble are designed to be precisely destroyed with the targeted ultrasound. This approach led to an improved systolic function and milder structural remodeling in STZ-treated rats (135). Effective cardiac gene delivery using the natural tropism of adeno-associated virus serotypes 9 and 6 toward the DCM myocardium are described (89, 136, 137). The clinical safety of adeno-associated vectors has been tested in many clinical trials, and there are drugs using this technology on the market (138). For this reason, gene therapy using this technology is a promising tool to achieve the goal of cardiac specific delivery in DCM.

Several antidiabetic agents have been demonstrated to be cardioprotective in a clinical setup, including several ISGLT2 inhibitors, liraglutide, semaglutide, and metformin. However, the reported benefits of these molecules are in HF patients and there is scarce information available specifically for DCM. One of the available studies shows that dapagliflozin produces a regression in the increased left ventricular mass of patients with T2DM (139). A similar finding is reported for gliclazide when compared to other antidiabetic agents such as glyburide, voglibose, metformin, pioglitazone, rosiglitazone, and sitagliptin. A small clinical trial with liraglutide also shows the improvement of a diastolic function but is still not clear if this improvement delays the onset of symptomatic DCM (140, 141). Conversely, other drugs like exantenide and sitagliptin do not improve the systolic or diastolic dysfunction in patients with T2DM (142, 143). However, sitagliptin is used to prevent the exacerbation of DCM in T2DM when it is used together with other antidiabetic treatments (144). Finally, it is important to take a second look at the already approved therapies in the search for new DCM drugs because not only some of them can be useful but also the study of the mechanisms can lead to the discovery of new molecules with better therapeutical properties.

In total, the presented data led us to propose that for the case of DCM having a bunch of cellular and molecular alterations taking place at the same time in the myocardium, it is convenient to explore the health of broad cellular processes like the ones mentioned earlier because many of the cardinal features of the diabetic

heart may be consequences of alterations of broad cellular pathways. Thus, the approaches capable of alleviating these alterations give us a better chance to achieve the goal of a new treatment.

AUTHOR CONTRIBUTIONS

FM-C, CH-F, CL-C, MT, XC, and AG-M wrote the manuscript. LG, MC, PC, and SL designed, supervised, revised the manuscript, and approved the final version. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Paiman EHM, van Eyk HJ, Bizino MB, Dekkers IA, de Heer P, Smit JWA, et al. Phenotyping diabetic cardiomyopathy in Europeans and South Asians. *Cardiovasc Diabetol.* (2019) 18:133. doi: 10.1186/s12933-019-0940-z
2. Seferovic PM, Paulus WJ. Clinical diabetic cardiomyopathy: a two-faced disease with restrictive and dilated phenotypes. *Eur Heart J.* (2015) 36:1718–27, 27a—27c. doi: 10.1093/eurheartj/ehv134
3. Yap J, Tay WT, Teng TK, Anand I, Richards AM, Ling LH, et al. Association of diabetes mellitus on cardiac remodeling, quality of life, and clinical outcomes in heart failure with reduced and preserved ejection fraction. *J Am Heart Assoc.* (2019) 8:e013114. doi: 10.1161/JAHA.119.013114
4. Riehle C, Bauersachs J. Of mice and men: models and mechanisms of diabetic cardiomyopathy. *Basic Res Cardiol.* (2018) 114:2. doi: 10.1007/s00395-018-0711-0
5. Holscher ME, Bode C, Bugger H. Diabetic cardiomyopathy: does the type of diabetes matter? *Int J Mol Sci.* (2016) 17:2136. doi: 10.3390/ijms17122136
6. Salvatore T, Pafundi PC, Galiero R, Albanese G, Di Martino A, Caturano A, et al. The contributing pathophysiological mechanisms. *Front Med (Lausanne).* (2021) 8:695792. doi: 10.3389/fmed.2021.695792
7. Ritchie RH, Abel ED. Basic mechanisms of diabetic heart disease. *Circ Res.* (2020) 126:1501–25. doi: 10.1161/CIRCRESAHA.120.315913
8. Zhang Y, Xia G, Zhang Y, Liu J, Liu X, Li W, et al. Palmitate induces VSMC apoptosis via toll like receptor (TLR)4/ROS/p53 pathway. *Atherosclerosis.* (2017) 263:74–81. doi: 10.1016/j.atherosclerosis.2017.06.002
9. Jialal I, Rajamani U, Adams-Huet B, Kaur H. Circulating pathogen-associated molecular pattern - binding proteins and High Mobility Group Box protein 1 in nascent metabolic syndrome: implications for cellular Toll-like receptor activity. *Atherosclerosis.* (2014) 236:182–7. doi: 10.1016/j.atherosclerosis.2014.06.022
10. Salvador B, Arranz A, Francisco S, Cordoba L, Punzon C, Llamas MA. and Fresno M: Modulation of endothelial function by Toll like receptors. *Pharmacol Res.* (2016) 108:46–56. doi: 10.1016/j.phrs.2016.03.038
11. Lu Z, Zhang X, Li Y, Lopes-Virella MF, Huang Y. TLR4 antagonist attenuates atherogenesis in LDL receptor-deficient mice with diet-induced type 2 diabetes. *Immunobiology.* (2015) 220:1246–54. doi: 10.1016/j.imbio.2015.06.016
12. Abdul Y, Abdelsaid M, Li W, Webb RC, Sullivan JC, Dong G, et al. Inhibition of toll-like receptor-4 (TLR-4) improves neurobehavioral outcomes after acute ischemic stroke in diabetic rats: possible role of vascular endothelial TLR-4. *Mol Neurobiol.* (2019) 56:1607–17. doi: 10.1007/s12035-018-1184-8
13. Dange RB, Agarwal D, Masson GS, Vila J, Wilson B, Nair A, et al. Central blockade of TLR4 improves cardiac function and attenuates myocardial inflammation in angiotensin II-induced hypertension. *Cardiovasc Res.* (2014) 103:17–27. doi: 10.1093/cvr/cvu067
14. Ishikado A, Nishio Y, Yamane K, Mukose A, Morino K, Murakami Y, et al. Soy phosphatidylcholine inhibited TLR4-mediated MCP-1 expression in vascular cells. *Atherosclerosis.* (2009) 205:404–12. doi: 10.1016/j.atherosclerosis.2009.01.010
15. Quan J, Liu J, Gao X, Liu J, Yang H, Chen W, et al. Palmitate induces interleukin-8 expression in human aortic vascular smooth muscle cells via Toll-like receptor 4/nuclear factor-kappaB pathway (TLR4/NF-kappaB-8). *J Diabetes.* (2014) 6:33–41. doi: 10.1111/1753-0407.12073
16. Jang HJ, Kim HS, Hwang DH, Quon MJ, Kim JA. Toll-like receptor 2 mediates high-fat diet-induced impairment of vasodilator actions of insulin. *Am J Physiol Endocrinol Metab.* (2013) 304:E1077–88. doi: 10.1152/ajpendo.00578.2012
17. Pahwa R, Nallasamy P, Jialal I. Toll-like receptors 2 and 4 mediate hyperglycemia induced macrovascular aortic endothelial cell inflammation and perturbation of the endothelial glycocalyx. *J Diabetes Complications.* (2016) 30:563–72. doi: 10.1016/j.jdiacomp.2016.01.014
18. Carrillo-Sepulveda MA, Spitler K, Pandey D, Berkowitz DE, Matsumoto T. Inhibition of TLR4 attenuates vascular dysfunction and oxidative stress in diabetic rats. *J Mol Med (Berl).* (2015) 93:1341–54. doi: 10.1007/s00109-015-1318-7
19. Wang Y, Luo W, Han J, Khan ZA, Fang Q, Jin Y, et al. MD2 activation by direct AGE interaction drives inflammatory diabetic cardiomyopathy. *Nat Commun.* (2020) 11:2148. doi: 10.1038/s41467-020-15978-3
20. Wang X, Sun Y, Yang H, Lu Y, Li L. Oxidized Low-Density Lipoprotein Induces Apoptosis in Cultured Neonatal Rat Cardiomyocytes by Modulating the TLR4/NF-kappaB Pathway. *Sci Rep.* (2016) 6:27866. doi: 10.1038/srep27866
21. Zhang Y, Li Y, Huang X, Zhang F, Tang L, Xu S, et al. Systemic delivery of siRNA specific for silencing TLR4 gene expression reduces diabetic cardiomyopathy in a mouse model of streptozotocin-induced type 1 diabetes. *Diabetes Ther.* (2020) 11:1161–73. doi: 10.1007/s13300-020-00802-4
22. Zhou W, Chen C, Chen Z, Liu L, Jiang J, Wu Z, et al. A novel mediator in cardiovascular disease. *J Immunol Res.* (2018) 2018:5702103. doi: 10.1155/2018/5702103
23. Luo B, Li B, Wang W, Liu X, Xia Y, Zhang C, et al. NLRP3 gene silencing ameliorates diabetic cardiomyopathy in a type 2 diabetes rat model. *PLoS ONE.* (2014) 9:e104771. doi: 10.1371/journal.pone.0104771
24. Xu D, Zhang X, Chen X, Yang S, Chen H. Inhibition of miR-223 attenuates the NLRP3 inflammasome activation, fibrosis, and apoptosis in diabetic cardiomyopathy. *Life Sci.* (2020) 256:117980. doi: 10.1016/j.lfs.2020.117980
25. Yang F, Qin Y, Wang Y, Meng S, Xian H, Che H, et al. Metformin inhibits the NLRP3 inflammasome via AMPK/mTOR-dependent effects in diabetic cardiomyopathy. *Int J Biol Sci.* (2019) 15:1010–19. doi: 10.7150/ijbs.29680
26. Chen Y, Hua Y, Li X, Arslan IM, Zhang W, Meng G. Distinct types of cell death and the implication in diabetic cardiomyopathy. *Front Pharmacol.* (2020) 11:42. doi: 10.3389/fphar.2020.00042
27. Williams DM, Stephens JW. Combination therapy with saxagliptin and dapagliflozin for the treatment of type 2 diabetes. *Expert Opin Pharmacother.* (2015) 16:2373–9. doi: 10.1517/14656566.2015.1088830
28. Ye Y, Bajaj M, Yang HC, Perez-Polo JR, Birnbaum Y. SGLT-2 Inhibition with dapagliflozin reduces the activation of the Nlrp3/ASC inflammasome and attenuates the development of diabetic cardiomyopathy in mice with type 2 diabetes. Further augmentation of the effects with saxagliptin, a DPP4 inhibitor. *Cardiovasc Drugs Ther.* (2017) 31:119–32. doi: 10.1007/s10557-017-6725-2
29. Chen H, Tran D, Yang HC, Nylander S, Birnbaum Y, Ye Y. Dapagliflozin and ticagrelor have additive effects on the attenuation of the activation of the NLRP3 inflammasome and the progression of diabetic cardiomyopathy: an AMPK-mTOR interplay. *Cardiovasc Drugs Ther.* (2020) 34:443–61. doi: 10.1007/s10557-020-06978-y

30. Zhang H, Chen X, Zong B, Yuan H, Wang Z, Wei Y, et al. Gypenosides improve diabetic cardiomyopathy by inhibiting ROS-mediated NLRP3 inflammasome activation. *J Cell Mol Med.* (2018) 22:4437–48. doi: 10.1111/jcmm.13743
31. Li X, Ke X, Li Z, Li B. Vaspin prevents myocardial injury in rats model of diabetic cardiomyopathy by enhancing autophagy and inhibiting inflammation. *Biochem Biophys Res Commun.* (2019) 514:1–8. doi: 10.1016/j.bbrc.2019.04.110
32. Luo B, Li B, Wang W, Liu X, Liu X, Xia Y, et al. Rosuvastatin alleviates diabetic cardiomyopathy by inhibiting NLRP3 inflammasome and MAPK pathways in a type 2 diabetes rat model. *Cardiovasc Drugs Ther.* (2014) 28:33–43. doi: 10.1007/s10557-013-6498-1
33. Wei H, Bu R, Yang Q, Jia J, Li T, Wang Q, et al. Exendin-4 protects against hyperglycemia-induced cardiomyocyte pyroptosis via the AMPK-TXNIP pathway. *J Diabetes Res.* (2019) 2019:8905917. doi: 10.1155/2019/8905917
34. Zhao ZZ, Zheng XL, Jiang ZS. Emerging roles of absent in melanoma 2 in cardiovascular diseases. *Clin Chim Acta.* (2020) 511:14–23. doi: 10.1016/j.cca.2020.08.031
35. Wang X, Pan J, Liu H, Zhang M, Liu D, Lu L, et al. AIM2 gene silencing attenuates diabetic cardiomyopathy in type 2 diabetic rat model. *Life Sci.* (2019) 221:249–58. doi: 10.1016/j.lfs.2019.02.035
36. Fender AC, Kleeschulte S, Stolte S, Leineweber K, Kamler M, Bode J, et al. Thrombin receptor PAR4 drives canonical NLRP3 inflammasome signaling in the heart. *Basic Res Cardiol.* (2020) 115:10. doi: 10.1007/s00395-019-0771-9
37. Jeyabal P, Thandavarayan RA, Joladarashi D, Suresh Babu S, Krishnamurthy S, Bhimaraj A, et al. MicroRNA-9 inhibits hyperglycemia-induced pyroptosis in human ventricular cardiomyocytes by targeting ELAVL1. *Biochem Biophys Res Commun.* (2016) 471:423–9. doi: 10.1016/j.bbrc.2016.02.065
38. Zeng C, Wang R, Tan H. Role of pyroptosis in cardiovascular diseases and its therapeutic implications. *Int J Biol Sci.* (2019) 15:1345–57. doi: 10.7150/ijbs.33568
39. Jia C, Chen H, Zhang J, Zhou K, Zhuge Y, Niu C, et al. Role of pyroptosis in cardiovascular diseases. *Int Immunopharmacol.* (2019) 67:311–18. doi: 10.1016/j.intimp.2018.12.028
40. Nauck MA, Quast DR, Wefers J, Meier JJ. GLP-1 receptor agonists in the treatment of type 2 diabetes - state-of-the-art. *Mol Metab.* (2021) 46:101102. doi: 10.1016/j.molmet.2020.101102
41. Kaur J, Mattu HS, Chatha K, Randeva HS. Chemerin in human cardiovascular disease. *Vascul Pharmacol.* (2018) 110:1–6. doi: 10.1016/j.vph.2018.06.018
42. Helfer G, Wu QF. Chemerin: a multifaceted adipokine involved in metabolic disorders. *J Endocrinol.* (2018) 238:R79–94. doi: 10.1530/JOE-18-0174
43. Xie Y, Huang Y, Ling X, Qin H, Wang M, Luo B. Chemerin/CMKLR1 axis promotes inflammation and pyroptosis by activating nlrp3 inflammasome in diabetic cardiomyopathy rat. *Front Physiol.* (2020) 11:381. doi: 10.3389/fphys.2020.00381
44. Li X, Du N, Zhang Q, Li J, Chen X, Liu X, et al. MicroRNA-30d regulates cardiomyocyte pyroptosis by directly targeting foxo3a in diabetic cardiomyopathy. *Cell Death Dis.* (2014) 5:e1479. doi: 10.1038/cddis.2014.430
45. Yang F, Li A, Qin Y, Che H, Wang Y, Lv J, et al. A novel circular RNA mediates pyroptosis of diabetic cardiomyopathy by functioning as a competing endogenous RNA. *Mol Ther Nucleic Acids.* (2019) 17:636–43. doi: 10.1016/j.omtn.2019.06.026
46. Yang F, Qin Y, Lv J, Wang Y, Che H, Chen X, et al. Silencing long non-coding RNA Kcnq1ot1 alleviates pyroptosis and fibrosis in diabetic cardiomyopathy. *Cell Death Dis.* (2018) 9:1000. doi: 10.1038/s41419-018-1029-4
47. Yang F, Qin Y, Wang Y, Li A, Lv J, Sun X, et al. LncRNA KCNQ1OT1 mediates pyroptosis in diabetic cardiomyopathy. *Cell Physiol Biochem.* (2018) 50:1230–44. doi: 10.1159/000494576
48. Xu Y, Fang H, Xu Q, Xu C, Yang L, Huang C. LncRNA GAS5 inhibits NLRP3 inflammasome activation-mediated pyroptosis in diabetic cardiomyopathy by targeting miR-34b-3p/AHR. *Cell Cycle.* (2020) 19:3054–65. doi: 10.1080/15384101.2020.1831245
49. Zhang W, Xu W, Feng Y, Zhou X. Non-coding RNA involvement in the pathogenesis of diabetic cardiomyopathy. *J Cell Mol Med.* (2019) 23:5859–67. doi: 10.1111/jcmm.14510
50. Li X, Wang H, Yao B, Xu W, Chen J, Zhou X. lncRNA H19/miR-675 axis regulates cardiomyocyte apoptosis by targeting VDAC1 in diabetic cardiomyopathy. *Sci Rep.* (2016) 6:36340. doi: 10.1038/srep36340
51. Feng B, Chen S, Gordon AD, Chakrabarti S. miR-146a mediates inflammatory changes and fibrosis in the heart in diabetes. *J Mol Cell Cardiol.* (2017) 105:70–6. doi: 10.1016/j.yjmcc.2017.03.002
52. Che H, Wang Y, Li Y, Lv J, Li H, Liu Y, et al. Inhibition of microRNA-150-5p alleviates cardiac inflammation and fibrosis via targeting Smad7 in high glucose-treated cardiac fibroblasts. *J Cell Physiol.* (2020) 235:7769–79. doi: 10.1002/jcp.29386
53. Jia C, Chen H, Wei M, Chen X, Zhang Y, Cao L, et al. Gold nanoparticle-based miR155 antagonist macrophage delivery restores the cardiac function in ovariectomized diabetic mouse model. *Int J Nanomedicine.* (2017) 12:4963–79. doi: 10.2147/IJN.S138400
54. Corsten MF, Papageorgiou A, Verhesen W, Carai P, Lindow M, Obad S, et al. MicroRNA profiling identifies microRNA-155 as an adverse mediator of cardiac injury and dysfunction during acute viral myocarditis. *Circ Res.* (2012) 111:415–25. doi: 10.1161/CIRCRESAHA.112.267443
55. Derumeaux GA. d'Humieres T. MicroRNA, miR-122-5p, stiffens the diabetic heart. *JACC Cardiovasc Imaging.* (2021) 14:1143–45. doi: 10.1016/j.jcmg.2021.01.025
56. Zhao Z, Zhong L, Li P, He K, Qiu C, Zhao L, et al. Cholesterol impairs hepatocyte lysosomal function causing M1 polarization of macrophages via exosomal miR-122-5p. *Exp Cell Res.* (2020) 387:111738. doi: 10.1016/j.yexcr.2019.111738
57. Lu Z, Feng H, Shen X, He R, Meng H, Lin W, et al. MiR-122-5p protects against acute lung injury via regulation of DUSP4/ERK signaling in pulmonary microvascular endothelial cells. *Life Sci.* (2020) 256:117851. doi: 10.1016/j.lfs.2020.117851
58. Callis TE, Pandya K, Seok HY, Tang RH, Tatsuguchi M, Huang ZP, et al. MicroRNA-208a is a regulator of cardiac hypertrophy and conduction in mice. *J Clin Invest.* (2009) 119:2772–86. doi: 10.1172/JCI36154
59. Krishnamoorthy S, Recchiuti A, Chiang N, Fredman G, Serhan CN. Resolvin D1 receptor stereoselectivity and regulation of inflammation and proresolving microRNAs. *Am J Pathol.* (2012) 180:2018–27. doi: 10.1016/j.ajpath.2012.01.028
60. Rawal S, Nagesh PT, Coffey S, Van Hout I, Galvin IF, Bunton RW, et al. Early dysregulation of cardiac-specific microRNA-208a is linked to maladaptive cardiac remodelling in diabetic myocardium. *Cardiovasc Diabetol.* (2019) 18:13. doi: 10.1186/s12933-019-0814-4
61. Montgomery RL, Hullinger TG, Semus HM, Dickinson BA, Seto AG, Lynch JM, et al. Therapeutic inhibition of miR-208a improves cardiac function and survival during heart failure. *Circulation.* (2011) 124:1537–47. doi: 10.1161/CIRCULATIONAHA.111.030932
62. Lopez-Crisosto C, Pennanen C, Vasquez-Trincado C, Morales PE, Bravo-Sagua R, Quest AFG, et al. Sarcoplasmic reticulum-mitochondria communication in cardiovascular pathophysiology. *Nat Rev Cardiol.* (2017) 14:342–60. doi: 10.1038/nrcardio.2017.23
63. Westermeier F, Navarro-Marquez M, Lopez-Crisosto C, Bravo-Sagua R, Quiroga C, Bustamante M, et al. Defective insulin signaling and mitochondrial dynamics in diabetic cardiomyopathy. *Biochim Biophys Acta.* (2015) 1853:1113–8. doi: 10.1016/j.bbamcr.2015.02.005
64. Bai J, Liu C, Zhu P, Li Y. Novel insights into molecular mechanism of mitochondria in diabetic cardiomyopathy. *Front Physiol.* (2020) 11:609157. doi: 10.3389/fphys.2020.609157
65. Ljubkovic M, Gressette M, Bulat C, Cavar M, Bakovic D, Fabijanic D, et al. Disturbed fatty acid oxidation, endoplasmic reticulum stress, and apoptosis in left ventricle of patients with type 2 diabetes. *Diabetes.* (2019) 68:1924–33. doi: 10.2337/db19-0423
66. Wende AR, Schell JC, Ha CM, Pepin ME, Khalimonchuk O, Schwertz H, et al. Maintaining myocardial glucose utilization in diabetic cardiomyopathy accelerates mitochondrial dysfunction. *Diabetes.* (2020) 69:2094–111. doi: 10.2337/db19-1057
67. Brahma MK, Ha CM, Pepin ME, Mia S, Sun Z, Chatham JC, et al. Increased glucose availability attenuates myocardial ketone body utilization. *J Am Heart Assoc.* (2020) 9:e013039. doi: 10.1161/JAHA.119.013039
68. Prakoso D, Lim SY, Erickson JR, Wallace RS, Lees JG, Tate M, et al. Fine-tuning the cardiac O-GlcNAcylation regulatory enzymes governs the

functional and structural phenotype of the diabetic heart. *Cardiovasc Res.* (2021). doi: 10.1093/cvr/cvab043
69. Uchihashi M, Hoshino A, Okawa Y, Ariyoshi M, Kaimoto S, Tateishi S, et al. Cardiac-specific Bdh1 overexpression ameliorates oxidative stress and cardiac remodeling in pressure overload-induced heart failure. *Circ Heart Fail.* (2017) 10:e004417. doi: 10.1161/CIRCHEARTFAILURE.117.004417
70. Neal B, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erondu N, et al. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *N Engl J Med.* (2017) 377:644–57. doi: 10.1056/NEJMoa1611925
71. Ferrannini E, Mark M, Mayoux E, CV. Protection in the EMPA-REG OUTCOME Trial: A "Thrifty Substrate" hypothesis. *Diabetes Care.* (2016) 39:1108–14. doi: 10.2337/dc16-0330
72. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med.* (2015) 373:2117–28. doi: 10.1056/NEJMoa1504720
73. Li N, Zhou H. SGLT2 inhibitors: a novel player in the treatment and prevention of diabetic cardiomyopathy. *Drug Des Devel Ther.* (2020) 14:4775–88. doi: 10.2147/DDDT.S269514
74. Ni R, Zheng D, Xiong S, Hill DJ, Sun T, Gardiner RB, et al. Mitochondrial calpain-1 disrupts ATP synthase and induces superoxide generation in type 1 diabetic hearts: a novel mechanism contributing to diabetic cardiomyopathy. *Diabetes.* (2016) 65:255–68. doi: 10.2337/db15-0963
75. Li Y, Li Y, Feng Q, Arnold M, Peng T. Calpain activation contributes to hyperglycaemia-induced apoptosis in cardiomyocytes. *Cardiovasc Res.* (2009) 84:100–10. doi: 10.1093/cvr/cvp189
76. Li Y, Ma J, Zhu H, Singh M, Hill D, Greer PA, et al. Targeted inhibition of calpain reduces myocardial hypertrophy and fibrosis in mouse models of type 1 diabetes. *Diabetes.* (2011) 60:2985–94. doi: 10.2337/db10-1333
77. Wang SY, Zhu S, Wu J, Zhang M, Xu Y, Xu W, et al. Exercise enhances cardiac function by improving mitochondrial dysfunction and maintaining energy homoeostasis in the development of diabetic cardiomyopathy. *J Mol Med (Berl).* (2020) 98:245–61. doi: 10.1007/s00109-019-01861-2
78. Arad M, Waldman M, Abraham NG, Hochhauser E. Therapeutic approaches to diabetic cardiomyopathy: Targeting the antioxidant pathway. *Prostaglandins Other Lipid Mediat.* (2020) 150:106454. doi: 10.1016/j.prostaglandins.2020.106454
79. Byrne NJ, Rajasekaran NS, Abel ED, Bugger H. Therapeutic potential of targeting oxidative stress in diabetic cardiomyopathy. *Free Radic Biol Med.* (2021) 169:317–42. doi: 10.1016/j.freeradbiomed.2021.03.046
80. Hansen SS, Aasum E, Hafstad AD. The role of NADPH oxidases in diabetic cardiomyopathy. *Biochim Biophys Acta Mol Basis Dis.* (2018) 1864:1908–13. doi: 10.1016/j.bbadis.2017.07.025
81. Wilson AJ, Gill EK, Abudalo RA, Edgar KS, Watson CJ, Grieve DJ. Reactive oxygen species signalling in the diabetic heart: emerging prospect for therapeutic targeting. *Heart.* (2018) 104:293–99. doi: 10.1136/heartjnl-2017-311448
82. Kaludercic N, Di Lisa F. Mitochondrial ROS Formation in the Pathogenesis of Diabetic Cardiomyopathy. *Front Cardiovasc Med.* (2020) 7:12. doi: 10.3389/fcvm.2020.00012
83. Murphy MP. How mitochondria produce reactive oxygen species. *Biochem J.* (2009) 417:1–13. doi: 10.1042/BJ20081386
84. Ni R, Cao T, Xiong S, Ma J, Fan GC, Lacefield JC, et al. Therapeutic inhibition of mitochondrial reactive oxygen species with mito-TEMPO reduces diabetic cardiomyopathy. *Free Radic Biol Med.* (2016) 90:12–23. doi: 10.1016/j.freeradbiomed.2015.11.013
85. Wang D, Yin Y, Wang S, Zhao T, Gong F, Zhao Y, et al. FGF1(DeltaHBS) prevents diabetic cardiomyopathy by maintaining mitochondrial homeostasis and reducing oxidative stress via AMPK/Nur77 suppression. *Signal Transduct Target Ther.* (2021) 6:133. doi: 10.1038/s41392-021-00542-2
86. Qi B, He L, Zhao Y, Zhang L, He Y, Li J, et al. Akap1 deficiency exacerbates diabetic cardiomyopathy in mice by NDUFS1-mediated mitochondrial dysfunction and apoptosis. *Diabetologia.* (2020) 63:1072–87. doi: 10.1007/s00125-020-05103-w
87. Wang P, Wang D, Yang Y, Hou J, Wan J, Ran F, et al. Tom70 protects against diabetic cardiomyopathy through its antioxidant and antiapoptotic properties. *Hypertens Res.* (2020) 43:1047–56. doi: 10.1038/s41440-020-0518-x
88. Battiprolu PK, Hojayev B, Jiang N, Wang ZV, Luo X, Iglewski M, et al. Metabolic stress-induced activation of FoxO1 triggers diabetic cardiomyopathy in mice. *J Clin Invest.* (2012) 122:1109–18. doi: 10.1172/JCI60329
89. Kyriazis ID, Hoffman M, Gaignebet L, Lucchese AM, Markopoulou E, Palioura D, et al. KLF5 Is induced by FOXO1 and causes oxidative stress and diabetic cardiomyopathy. *Circ Res.* (2021) 128:335–57. doi: 10.1161/CIRCRESAHA.120.316738
90. Yin Z, Zhao Y, He M, Li H, Fan J, Nie X, et al. MiR-30c/PGC-1beta protects against diabetic cardiomyopathy via PPARalpha. *Cardiovasc Diabetol.* (2019) 18:7. doi: 10.1186/s12933-019-0811-7
91. Tilokani L, Nagashima S, Paupe V, Prudent J. Mitochondrial dynamics: overview of molecular mechanisms. *Essays Biochem.* (2018) 62:341–60. doi: 10.1042/EBC20170104
92. Bravo-Sagua R, Parra V, Lopez-Crisosto C, Diaz P, Quest AF, et al. Calcium Transport and Signaling in Mitochondria. *Compr Physiol.* (2017) 7:623–34. doi: 10.1002/cphy.c160013
93. Chang X, Zhang W, Zhao Z, Ma C, Zhang T, Meng Q, et al. Regulation of mitochondrial quality control by natural drugs in the treatment of cardiovascular diseases: potential and advantages. *Front Cell Dev Biol.* (2020) 8:616139. doi: 10.3389/fcell.2020.616139
94. Morales PE, Arias-Duran C, Avalos-Guajardo Y, Aedo G, Verdejo HE, Parra V, et al. Emerging role of mitophagy in cardiovascular physiology and pathology. *Mol Aspects Med.* (2020) 71:100822. doi: 10.1016/j.mam.2019.09.006
95. Yu T, Robotham JL, Yoon Y. Increased production of reactive oxygen species in hyperglycemic conditions requires dynamic change of mitochondrial morphology. *Proc Natl Acad Sci U S A.* (2006) 103:2653–8. doi: 10.1073/pnas.0511154103
96. Yu T, Sheu SS, Robotham JL, Yoon Y. Mitochondrial fission mediates high glucose-induced cell death through elevated production of reactive oxygen species. *Cardiovasc Res.* (2008) 79:341–51. doi: 10.1093/cvr/cvn104
97. Guo Y, Zhang C, Shang FF, Luo M, You Y, Zhai Q, et al. Ketogenic diet ameliorates cardiac dysfunction via balancing mitochondrial dynamics and inhibiting apoptosis in type 2 diabetic mice. *Aging Dis.* (2020) 11:229–40. doi: 10.14336/AD.2019.0510
98. Ding M, Liu C, Shi R, Yu M, Zeng K, Kang J, et al. Mitochondrial fusion promoter restores mitochondrial dynamics balance and ameliorates diabetic cardiomyopathy in an optic atrophy 1-dependent way. *Acta Physiol (Oxf).* (2020) 229:e13428. doi: 10.1111/apha.13428
99. Hu L, Ding M, Tang D, Gao E, Li C, Wang K, et al. Targeting mitochondrial dynamics by regulating Mfn2 for therapeutic intervention in diabetic cardiomyopathy. *Theranostics.* (2019) 9:3687–706. doi: 10.7150/thno.33684
100. Montaigne D, Marechal X, Coisne A, Debry N, Modine T, Fayad G, et al. Myocardial contractile dysfunction is associated with impaired mitochondrial function and dynamics in type 2 diabetic but not in obese patients. *Circulation.* (2014) 130:554–64. doi: 10.1161/CIRCULATIONAHA.113.008476
101. Gatica D, Chiong M, Lavandero S, Klionsky DJ. Molecular mechanisms of autophagy in the cardiovascular system. *Circ Res.* (2015) 116:456–67. doi: 10.1161/CIRCRESAHA.114.303788
102. Lavandero S, Chiong M, Rothermel BA, Hill JA. Autophagy in cardiovascular biology. *J Clin Invest.* (2015) 125:55–64. doi: 10.1172/JCI73943
103. Nemchenko A, Chiong M, Turer A, Lavandero S, Hill JA. Autophagy as a therapeutic target in cardiovascular disease. *J Mol Cell Cardiol.* (2011) 51:584–93. doi: 10.1016/j.yjmcc.2011.06.010
104. Kanamori H, Takemura G, Goto K, Tsujimoto A, Mikami A, Ogino A, et al. Autophagic adaptations in diabetic cardiomyopathy differ between type 1 and type 2 diabetes. *Autophagy.* (2015) 11:1146–60. doi: 10.1080/15548627.2015.1051295
105. Zhang M, Sui W, Xing Y, Cheng J, Cheng C, Xue F, et al. Angiotensin IV attenuates diabetic cardiomyopathy via suppressing FoxO1-induced excessive autophagy, apoptosis and fibrosis. *Theranostics.* (2021) 11:8624–39. doi: 10.7150/thno.48561
106. Zang H, Wu W, Qi L, Tan W, Nagarkatti P, Nagarkatti M, et al. Autophagy inhibition enables Nrf2 to exaggerate the progression of diabetic cardiomyopathy in mice. *Diabetes.* (2020) 69:2720–34. doi: 10.2337/db19-1176

107. Ge ZD, Lian Q, Mao X, Xia Z. Current status and challenges of NRF2 as a potential therapeutic target for diabetic cardiomyopathy. *Int Heart J.* (2019) 60:512–20. doi: 10.1536/ihj.18-476
108. Zhang Z, Wang S, Zhou S, Yan X, Wang Y, Chen J, et al. Sulforaphane prevents the development of cardiomyopathy in type 2 diabetic mice probably by reversing oxidative stress-induced inhibition of LKB1/AMPK pathway. *J Mol Cell Cardiol.* (2014) 77:42–52. doi: 10.1016/j.yjmcc.2014.09.022
109. Borghetti G, von Lewinski D, Eaton DM, Sourij H, Houser SR, Wallner M. Diabetic cardiomyopathy: current and future therapies. *Beyond Glycemic Control Front Physiol.* (2018) 9:1514. doi: 10.3389/fphys.2018.01514
110. Desjardins EM, Steinberg GR. Emerging role of AMPK in brown and beige adipose tissue (BAT): implications for obesity, insulin resistance, and type 2 diabetes. *Curr Diab Rep.* (2018) 18:80. doi: 10.1007/s11892-018-1049-6
111. Carling D, AMPK. signalling in health and disease. *Curr Opin Cell Biol.* (2017) 45:31–7. doi: 10.1016/j.ceb.2017.01.005
112. Shibata M, Yoshimura K, Furuya N, Koike M, Ueno T, Komatsu M, et al. The MAP1-LC3 conjugation system is involved in lipid droplet formation. *Biochem Biophys Res Commun.* (2009) 382:419–23. doi: 10.1016/j.bbrc.2009.03.039
113. You P, Cheng Z, He X, Deng J, Diao J, Chen H, et al. Lin28a protects against diabetic cardiomyopathy through Mst1 inhibition. *J Cell Physiol.* (2020) 235:4455–65. doi: 10.1002/jcp.29321
114. Ni T, Lin N, Lu W, Sun Z, Lin H, Chi J, et al. Dihydromyricetin prevents diabetic cardiomyopathy via mir-34a suppression by activating autophagy. *Cardiovasc Drugs Ther.* (2020) 34:291–301. doi: 10.1007/s10557-020-06968-0
115. Zhu Y, Qian X, Li J, Lin X, Luo J, Huang J, et al. Astragaloside-IV protects H9C2(2-1) cardiomyocytes from high glucose-induced injury via miR-34a-mediated autophagy pathway. *Artif Cells Nanomed Biotechnol.* (2019) 47:4172–81. doi: 10.1080/21691401.2019.1687492
116. Pang J, Xiong H, Lin P, Lai L, Yang H, Liu Y, et al. Activation of miR-34a impairs autophagic flux and promotes cochlear cell death via repressing ATG9A: implications for age-related hearing loss. *Cell Death Dis.* (2017) 8:e3079. doi: 10.1038/cddis.2017.462
117. Xing R, Liu D, Cheng X, Tian X, Yan C, Han Y. MiR-207 inhibits autophagy and promotes apoptosis of cardiomyocytes by directly targeting LAMP2 in type 2 diabetic cardiomyopathy. *Biochem Biophys Res Commun.* (2019) 520:27–34. doi: 10.1016/j.bbrc.2019.09.092
118. Wu YS, Zhu B, Luo AL, Yang L, Yang C. The role of cardiokines in heart diseases: beneficial or detrimental? *Biomed Res Int.* (2018) 2018:8207058. doi: 10.1155/2018/8207058
119. Wu MX, Wang SH, Xie Y, Chen ZT, Guo Q, Yuan WL, et al. Interleukin-33 alleviates diabetic cardiomyopathy through regulation of endoplasmic reticulum stress and autophagy via insulin-like growth factor-binding protein 3. *J Cell Physiol.* (2021) 236:4403–19. doi: 10.1002/jcp.30158
120. Gao J, Liu J, Meng Z, Li Y, Hong Y, Wang L, et al. Ultrasound-assisted C3F8-filled PLGA nanobubbles for enhanced FGF21 delivery and improved prophylactic treatment of diabetic cardiomyopathy. *Acta Biomater.* (2021) 130:395–408. doi: 10.1016/j.actbio.2021.06.015
121. Wu F, Wang B, Zhang S, Shi L, Wang Y, Xiong R, et al. FGF21 ameliorates diabetic cardiomyopathy by activating the AMPK-paraoxonase 1 signaling axis in mice. *Clin Sci (Lond).* (2017) 131:1877–93. doi: 10.1042/CS20170271
122. Munasinghe PE, Riu F, Dixit P, Edamatsu M, Saxena P, Hamer NS, et al. Type-2 diabetes increases autophagy in the human heart through promotion of Beclin-1 mediated pathway. *Int J Cardiol.* (2016) 202:13–20. doi: 10.1016/j.ijcard.2015.08.111
123. Zhang Q, Feng R, Chaudhary O, Mahmood E, Baribeau Y, Rashid R, et al. Cardiopulmonary bypass suppresses forkhead box o3 and downstream autophagy in the diabetic human heart. *Ann Thorac Surg.* (2021) 111:937–44. doi: 10.1016/j.athoracsur.2020.05.142
124. Nandi SS, Duryee MJ, Shahshahan HR, Thiele GM, Anderson DR, Mishra PK. Induction of autophagy markers is associated with attenuation of miR-133a in diabetic heart failure patients undergoing mechanical unloading. *Am J Transl Res.* (2015) 7:683–96.
125. Mu J, Zhang D, Tian Y, Xie Z, Zou MH. BRD4 inhibition by JQ1 prevents high-fat diet-induced diabetic cardiomyopathy by activating PINK1/Parkin-mediated mitophagy *in vivo*. *J Mol Cell Cardiol.* (2020) 149:1–14. doi: 10.1016/j.yjmcc.2020.09.003
126. Tong M, Saito T, Zhai P, Oka SI, Mizushima W, Nakamura M, et al. Mitophagy is essential for maintaining cardiac function during high fat diet-induced diabetic cardiomyopathy. *Circ Res.* (2019) 124:1360–71. doi: 10.1161/CIRCRESAHA.118.314607
127. Yu W, Gao B, Li N, Wang J, Qiu C, Zhang G, et al. Sirt3 deficiency exacerbates diabetic cardiac dysfunction: Role of Foxo3A-Parkin-mediated mitophagy. *Biochim Biophys Acta Mol Basis Dis.* (2017) 1863:1973–83. doi: 10.1016/j.bbadis.2016.10.021
128. Wang S, Zhao Z, Fan Y, Zhang M, Feng X, Lin J, et al. Mst1 inhibits Sirt3 expression and contributes to diabetic cardiomyopathy through inhibiting Parkin-dependent mitophagy. *Biochim Biophys Acta Mol Basis Dis.* (2019) 1865:1905–14. doi: 10.1016/j.bbadis.2018.04.009
129. Wang S, Zhao Z, Feng X, Cheng Z, Xiong Z, Wang T, et al. Melatonin activates Parkin translocation and rescues the impaired mitophagy activity of diabetic cardiomyopathy through Mst1 inhibition. *J Cell Mol Med.* (2018) 22:5132–44. doi: 10.1111/jcmm.13802
130. Feng X, Wang S, Yang X, Lin J, Man W, Dong Y, et al. Mst1 Knockout Alleviates Mitochondrial Fission and Mitigates Left Ventricular Remodeling in the Development of Diabetic Cardiomyopathy. *Front Cell Dev Biol.* (2020) 8:628842. doi: 10.3389/fcell.2020.628842
131. Xiong Z, Li Y, Zhao Z, Zhang Y, Man W, Lin J, et al. Mst1 knockdown alleviates cardiac lipotoxicity and inhibits the development of diabetic cardiomyopathy in db/db mice. *Biochim Biophys Acta Mol Basis Dis.* (2020) 1866:165806. doi: 10.1016/j.bbadis.2020.165806
132. Kambis TN, Shahshahan HR, Kar S, Yadav SK, Mishra PK. Transgenic expression of miR-133a in the diabetic akita heart prevents cardiac remodeling and cardiomyopathy. *Front Cardiovasc Med.* (2019) 6:45. doi: 10.3389/fcvm.2019.00045
133. Nandi SS, Shahshahan HR, Shang Q, Kutty S, Boska M, Mishra PK. MiR-133a mimic alleviates T1DM-induced systolic dysfunction in akita: an mri-based study. *Front Physiol.* (2018) 9:1275. doi: 10.3389/fphys.2018.01275
134. Pala R, Anju VT, Dyavaiah M, Busi S, Nauli SM. Nanoparticle-mediated drug delivery for the treatment of cardiovascular diseases. *Int J Nanomedicine.* (2020) 15:3741–69. doi: 10.2147/IJN.S250872
135. Zhang M, Zhu NW, Ma WC, Chen MJ, Zheng L. Combined treatment with ultrasound-targeted microbubble destruction technique and NM-aFGF-loaded PEG-nanoliposomes protects against diabetic cardiomyopathy-induced oxidative stress by activating the AKT/GSK-3beta1/Nrf-2 pathway. *Drug Deliv.* (2020) 27:938–52. doi: 10.1080/10717544.2020.1785052
136. Dang Z, Avolio E, Thomas AC, Faulkner A, Beltrami AP, Cervellin C, et al. Transfer of a human gene variant associated with exceptional longevity improves cardiac function in obese type 2 diabetic mice through induction of the SDF-1/CXCR4 signalling pathway. *Eur J Heart Fail.* (2020) 22:1568–81. doi: 10.1002/ejhf.1840
137. Prakoso D, De Blasio MJ, Tate M, Kiriazis H, Donner DG, Qian H, et al. Gene therapy targeting cardiac phosphoinositide 3-kinase (p110alpha) attenuates cardiac remodeling in type 2 diabetes. *Am J Physiol Heart Circ Physiol.* (2020) 318:H840–H52. doi: 10.1152/ajpheart.00632.2019
138. Kuzmin DA, Shutova MV, Johnston NR, Smith OP, Fedorin VV, Kukushkin YS, et al. The clinical landscape for AAV gene therapies. *Nat Rev Drug Discov.* (2021) 20:173–74. doi: 10.1038/d41573-021-00017-7
139. Brown AJM, Gandy S, McCrimmon R, Houston JG, Struthers AD, Lang CC, et al. randomized controlled trial of dapagliflozin on left ventricular hypertrophy in people with type two diabetes: the DAPA-LVH trial. *Eur Heart J.* (2020) 41:3421–32. doi: 10.1093/eurheartj/ehaa419
140. Ida S, Kaneko R, Murata K. Effects of oral antidiabetic drugs on left ventricular mass in patients with type 2 diabetes mellitus: a network meta-analysis. *Cardiovasc Diabetol.* (2018) 17:129. doi: 10.1186/s12933-018-0773-1
141. Lambadiari V, Pavlidis G, Kousathana F, Varoudi M, Vlastos D, Maratou E, et al. Effects of 6-month treatment with the glucagon like peptide-1 analogue liraglutide on arterial stiffness, left ventricular myocardial deformation and oxidative stress in subjects with newly diagnosed type 2 diabetes. *Cardiovasc Diabetol.* (2018) 17:8. doi: 10.1186/s12933-017-0646-z
142. Chen WJY, Diamant M, de Boer K, Harms HJ, Robbers L, van Rossum AC, et al. Effects of exenatide on cardiac function, perfusion, and energetics in type 2 diabetic patients with cardiomyopathy: a randomized controlled trial against insulin glargine. *Cardiovasc Diabetol.* (2017) 16:67. doi: 10.1186/s12933-017-0549-z

143. Oe H, Nakamura K, Kihara H, Shimada K, Fukuda S, Takagi T, et al. Comparison of effects of sitagliptin and voglibose on left ventricular diastolic dysfunction in patients with type 2 diabetes: results of the 3D trial. *Cardiovasc Diabetol.* (2015) 14:83. doi: 10.1186/s12933-015-0242-z
144. Yamada H, Tanaka A, Kusunose K, Amano R, Matsuhisa M, Daida H, et al. Effect of sitagliptin on the echocardiographic parameters of left ventricular diastolic function in patients with type 2 diabetes: a subgroup analysis of the PROLOGUE study. *Cardiovasc Diabetol.* (2017) 16:63. doi: 10.1186/s12933-017-0546-2

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Perinatal Combinational Exposure to Bisphenol A and a High-Fat Diet Contributes to Transgenerational Dysregulation of Cardiovascular and Metabolic Systems in Mice

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Both bisphenol A (BPA) and high-fat diet (HFD) exert unfavorable effects on animals and humans; moreover, they could affect the health of their offspring. BPA and HFD often coexist in modern lifestyles; however, the long-term effects of simultaneous exposure of mothers to BPA and HFD during the perinatal period on the cardiovascular and metabolic systems of the offspring remain unclear. This study aimed to examine the effect of simultaneous exposure of mothers to BPA and HFD on the risk of metabolic and cardiovascular abnormalities in offspring. Institute of Cancer Research female mice (F0) were exposed to BPA and fed with HFD before and during gestation until the end of lactation. F0 mice were mated with untreated males to produce the first generation (F1); subsequently, adult F1 males/females were mated with normal females/males to produce the second generation (F2). Combined maternal exposure to BPA and HFD caused myocardial hypertrophy and aortic tunica media thickening as well as increased the cross-sectional area of cardiomyocytes and blood pressure in the matrilineal F2 generation. These cardiovascular changes might be associated with reduced endothelial nitric oxide synthase (eNOS) levels. The patrilineal female F2 was more likely to be obese than the patrilineal male F2. Re-feeding with a HFD showed a more significant weight gain and reduced energy expenditure. However, the aforementioned effects were not observed with exposure to HFD or BPA alone during the perinatal period. Our findings suggest that perinatal combinational exposure to BPA and HFD could cause metabolic and cardiovascular disorders in the offspring, Further, our findings demonstrate that the synergistic effects of HFD and BPA could be transmitted to future generations in a sex-dependent manner.

Keywords: bisphenol A, cardiovascular system, obesity, offspring, transgenerational inheritance

INTRODUCTION

There has been a worldwide increase in the prevalence of cardiovascular and metabolic diseases, which have made them a grave public health concern (NCD Risk Factor Collaboration (NCD-RisC), 2017; Devos and Menard, 2020). In addition to genetic susceptibility, other risk factors for hypertension and metabolic diseases include lifestyle and environmental factors, especially nutrition (Riccardi et al., 2004; Bergman et al., 2012; Kataria et al., 2017; Dai et al., 2020; Hochsmann et al., 2021). Furthermore, exposure of rodents and humans (F0) to some adverse environmental factors can cause phenotype changes and increased susceptibility to diseases (Kolb and Martin, 2017; Di Ciaula and Portincasa, 2019), with these adverse outcomes being possibly passed down to future generations (F1, F2, etc.) even without direct exposure (Barua and Junaid, 2015). This is considered the transgenerational inheritance of the adverse effects of environmental factors (Krishnan et al., 2018; Schellong et al., 2020). Bisphenol A (BPA) is a typical endocrine-disrupting chemical (EDC) with estrogenic activity. It is the precursor of polycarbonate and epoxy resin and is widely used in food packaging, medical devices, and other daily products (Krishnan et al., 2018; Geens et al., 2021). BPA exerts intergenerational effects on neurodevelopmental abnormalities, hypertension, and metabolic disorders (Wolstenholme et al., 2013; Gawlinski et al., 2021). Exposure to BPA or a high-fat diet (HFD) during pregnancy has similar effects, which increases the risk of hypertension and obesity in offspring (Provvisiero et al., 2016; Bae et al., 2017; Lomas-Soria et al., 2018; Figueiredo et al., 2020). Several large epidemiological studies have demonstrated an association of high exposure levels to BPA or HFD with an increased risk of metabolic and cardiovascular diseases (Han and Hong, 2016; Bao et al., 2020; Choi et al., 2020).

People are often exposed to various adverse factors during their lifetime (Jeon et al., 2015); further, it is more realistic to examine the effects of exposure to multiple, rather than single, factors on health and the offspring (Di Ciaula and Portincasa, 2019). Since high energy diets are very common in modern society, there is a need to determine the effect of combined exposure to a high energy diet and EDCs on health. However, it remains unclear whether the symptoms caused by maternal perinatal period exposure to BPA or HFD in F0, including abnormal blood pressure regulation and metabolic abnormalities, could be passed down to F1, F2, or further generations even without direct exposure to BPA or HFD. Moreover, the effects of perinatal combined exposure to BPA and HFD on the offspring's health remain unclear.

MATERIALS AND METHODS

Animals

We purchased 6-week-old Institute of Cancer Research female mice from the Experimental Animal Center of Chongqing Medical University. They adapted to the facility for 1 week before being transferred to the Specific Pathogen Free animal room. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Chongqing Medical University. Based on our previous study, we selected a dose of 500 μg/kg/day (Lv et al., 2017). Mice randomly received four treatments: a control low-fat diet (LFD, with 10% of calories from fat, D12450B, Research Diet, New Brunswick, NJ, United States); BPA (500 μg/kg/day, > 99% purity, Sigma-Aldrich, St Louis, MO, United States); HFD (60% of calories from fat, D12492, Research Diet, New Brunswick, NJ, United States); HFD + BPA (500 μg/kg/day BPA combined with HFD). BPA was dissolved in ethanol, and serial dilutions by phosphate-buffered saline were then performed which allowed for BPA working solution in a final ethanol concentration of 0.01% (BPA final concentration: 50 μg/ml). The solution was freshly prepared for daily gavage. After treatment for 10 weeks, the female mice were mated with age-matched healthy males to produce F1 offspring. The same treatment was maintained during 3-week gestation and lactation periods. All F1 mice received an LFD. The F1 mice were mated with untreated 12-week-old males/females to produce F2 offspring. A vaginal plug indicated successful conception; subsequently, the pregnant mice were housed individually throughout the gestation period until postnatal day 21. The F2 mice received a HFD again at the age of 13 weeks to determine among-group differences in metabolic and cardiovascular phenotypes. **Figure 1A** shows the experimental design. F2 mice received an LFD for 12 weeks. Subsequently, some mice received a HFD from the 13th week to the 21st week, while the remaining mice received an LFD until the 21st week before being sacrificed. Blood and tissues were collected and stored at −80°C.

Determination of Serum Hormone Levels

Blood was collected from the retro-orbital sinus puncture before sacrifice. We obtained serum through centrifugation (3,000 g, 15 min, 4°C) for insulin measurement (CSB-E05071m, CUSABIO, Wuhan, China). Analyses were performed in duplicate following the manufacturer's instructions. The detection range was 15.6–1,000 nIU/ml. In case the measurement was lower than the minimum detection limit, we used the minimum detection limit.

Measurement of Blood Pressure

We measured the blood pressure rate using a noninvasive computerized tail-cuff blood pressure system (Visitech Systems, Apex, NC, United States). Briefly, we allowed the adapted mice to calm down in a tunnel maintained at 37°C; subsequently, blood pressure was measured in triplicate. The final blood pressure and heart rate were determined by the mean values.

Echocardiography

Cardiac function was assessed using echocardiography (IE33; Philips, Amsterdam, Netherlands). Mice were anesthetized using 2% isoflurane in 100% oxygen; moreover, images were acquired in the short-axis view to evaluate cardiac function.

Glucose Tolerance Test

Following fasting (12 h deprivation of diet: 8:00 p.m. to 8:00 a.m.), these mice were intraperitoneally injected with a solution of D-glucose (2 g/kg body weight). We obtained

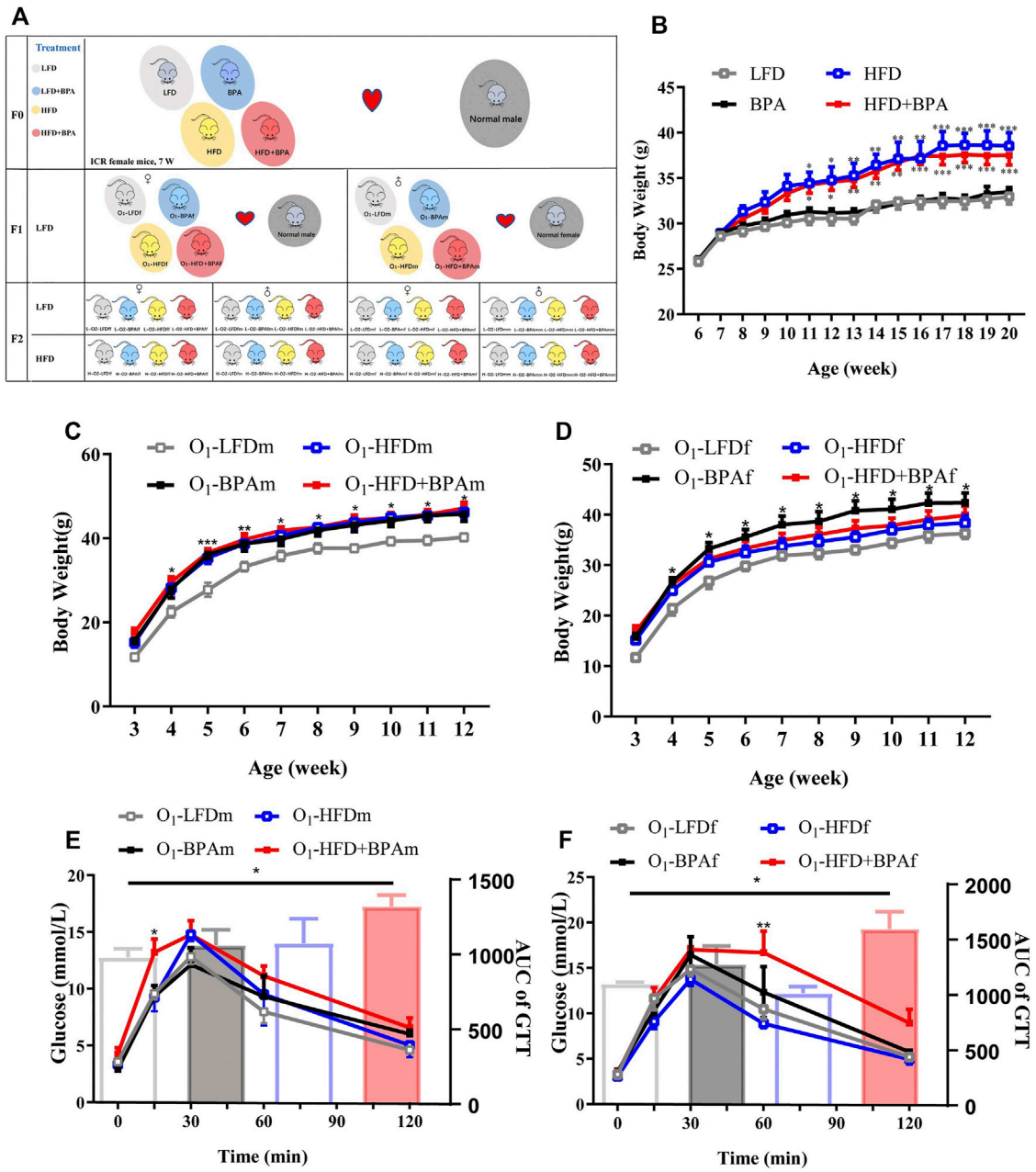

FIGURE 1 | HFD, BPA and their combination affect weight gain, metabolic profile of F0 and F1. **(A)** Schematic diagram of experimental design and animal groups. **(B)** The body weight difference of F0 female exposed to LFD, BPA, HFD, and HFD plus BPA. **(C,E)** Body weight and GTT of F1 male offspring. **(D,F)** Body weight and GTT of F1 female offspring. Data were presented as mean ± SEM and were analyzed by two-way ANOVA with a post hoc test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, BPA, HFD, BPA + HFD vs. LFD.

blood samples from the tail vein of the mice at 0 min (just before glucose load), as well as 15, 30, 60, 90, and 120 min after glucose administration. Blood glucose levels were measured using the glucometer, followed by calculation of the area under the curve (AUC).

Histological Examination

F1 and F2 animals were euthanized at the age of 24 weeks, followed by collection of the aorta and heart after heart perfusion. The aorta and heart were embedded in paraffin and serially sectioned at 4-μm thickness for hematoxylin-eosin

staining, followed by observation using the SLIDEVIEW VS200 (Olympus, Tokyo, Japan) under 20× conditions. For morphometric analysis of the aorta, the intima-media thickness was detected using OlyVIA V3.3 software (Olympus, Tokyo, Japan).

Wheat Germ Agglutinin Staining

The heart was harvested and fixed in 4% paraformaldehyde overnight at 4°C. Tissue sections (5-μm thickness) were stained with wheat germ agglutinin (WGA, L4895, Sigma-Aldrich, St Louis, MO, United States) to assess the cardiomyocyte cross-sectional area in myocardial sections.

Energy Expenditure

We determined O_2 consumption, CO_2 production, and energy expenditure using the PhenoMaster/LabMaster Caging System (TSE System, Bad Homburg, Germany). We individually monitored 22-week-old mice for 48 h; additionally, data were collected at 27-min intervals after being allowed to adapt for 1 day.

Western Blotting

Aortic rings were used for Western blot analysis. Briefly, we isolated intact aortic rings from the different treated offspring. Subsequently, the rings were homogenized in RIPA buffer containing protease and phosphatase inhibitors. The homogenates were ultrasonicated for 15 s, followed by centrifugation at 4°C for 10 min at 10,000 g. The supernatants were collected to determine the protein levels. Samples with equal protein were loaded and separated on 10% SDS-PAGE. The membranes were incubated with primary antibodies, followed by a secondary horseradish peroxidase-conjugated goat antirabbit antibody. We used the rabbit antibodies against eNOS (32027s, 1: 1000, Cell Signaling Technology, Beverly, MA, United States) and anti-β-actin (BM5422, 1:10000, Boster, Wuhan, China). Signals were detected using ECL Western Blotting Detection reagent (Advansta, Menlo Park, CA, United States) and FUSION FX (Vilber Lourmat, Marne La Vallee, France). The band intensity was quantified using FusionCapt Advance FX5 software (Vilber Lourmat, Marne La Vallee, France).

Statistical Analysis

Results were expressed as the mean ± standard error of the mean. Statistical analyses were performed using GraphPad Prism software version 8 (GraphPad Prism Software Inc., La Jolla, CA, United States). Among-group differences were analyzed using the two-way analysis of variance (ANOVA). Statistical significance was set at $p < 0.05$.

RESULTS

HFD, BPA, and Their Combination Affect Weight Gain and Metabolic Profile of F0 and F1 Mice

Figure 1A shows the experimental design and animal groups. There was a significant increase in the bodyweight of female mice in the HFD and HFD + BPA group than of those in the LFD group; moreover, BPA had no direct effect on body weight in F0 mice (**Figure 1B**). In the F1 generations, males in the HFD, BPA, and HFD + BPA groups were heavier than those in the LFD group (**Figure 1C**). However, for F1 female mice, only the BPA group weighed heavier than the LFD group (**Figure 1D**). In the GTT, F1 male mice in the HFD + BPA group showed a significant increase in their glucose levels at 30 min after glucose loading when compared with those in the LFD group (**Figure 1E**). Contrastingly, F1 female mice in the HFD + BPA group showed a significant increase in glucose levels at 60 min after glucose loading compared with those in the LFD group (**Figure 1F**).

Effects of Combined Exposure to BPA and HFD on Body Weight, Glucose Tolerance, Blood Pressure, and Cardiac Structure of Matrilineal Male F2 Mice

There was no significant change in the body weight and glucose tolerance of matrilineal male F2 mice from the 4th week to the 21st week (**Figures 2A,B**). However, there was a significantly higher systolic blood pressure (SBP) in the L-O_2-HFD + BPAfm group than in matrilineal male F2 mice in the LFD group (**Figure 2C**). Echocardiographical analysis revealed significantly higher interventricular septum thickness and left ventricular mass (**Figures 2D,F**) in the L-O_2-HFD + BPAfm group than in matrilineal male F2 mice in the LFD group. WGA staining revealed a significant increase in the cardiomyocyte area in the L-O_2-HFD + BPAfm group than in matrilineal male F2 mice in the LFD group (**Figures 2E,G**). Additionally, the arterial media thickness (**Figures 2H,I**) was significantly higher in the L-O_2-HFD + BPAfm group than in matrilineal male F2 mice in the LFD group. Western blot analysis revealed significantly lower aortic eNOS expression in the L-O_2-HFD + BPAfm group than in matrilineal male F2 mice in the LFD group (**Figures 2J,K**).

Effects of Combined Exposure to BPA and HFD on Body Weight, Glucose Tolerance, Blood Pressure, and Heart Structure of Matrilineal Female F2 Mice

There was no significant difference in body weight and glucose tolerance of matrilineal male F2 from weaning to 21 weeks (**Figures 3A,B**). SBP was significantly higher in the L-O_2-HFD + BPAfm group than in matrilineal female F2 mice in the LFD group (**Figure 3C**). Echocardiography revealed significantly higher interventricular septum thickness and left ventricular posterior wall thickness (**Figures 3D,F**) were significantly thicker in the L-O_2-HFD + BPAfm group than in matrilineal female F2 mice in the LFD group. WGA staining revealed a significantly higher cardiomyocyte area in the L-O_2-HFD + BPAff group than in matrilineal female F2 mice in the LFD group (**Figures 3E,G**). The L-O_2-HFD + BPAff group had a significantly higher arterial media thickness

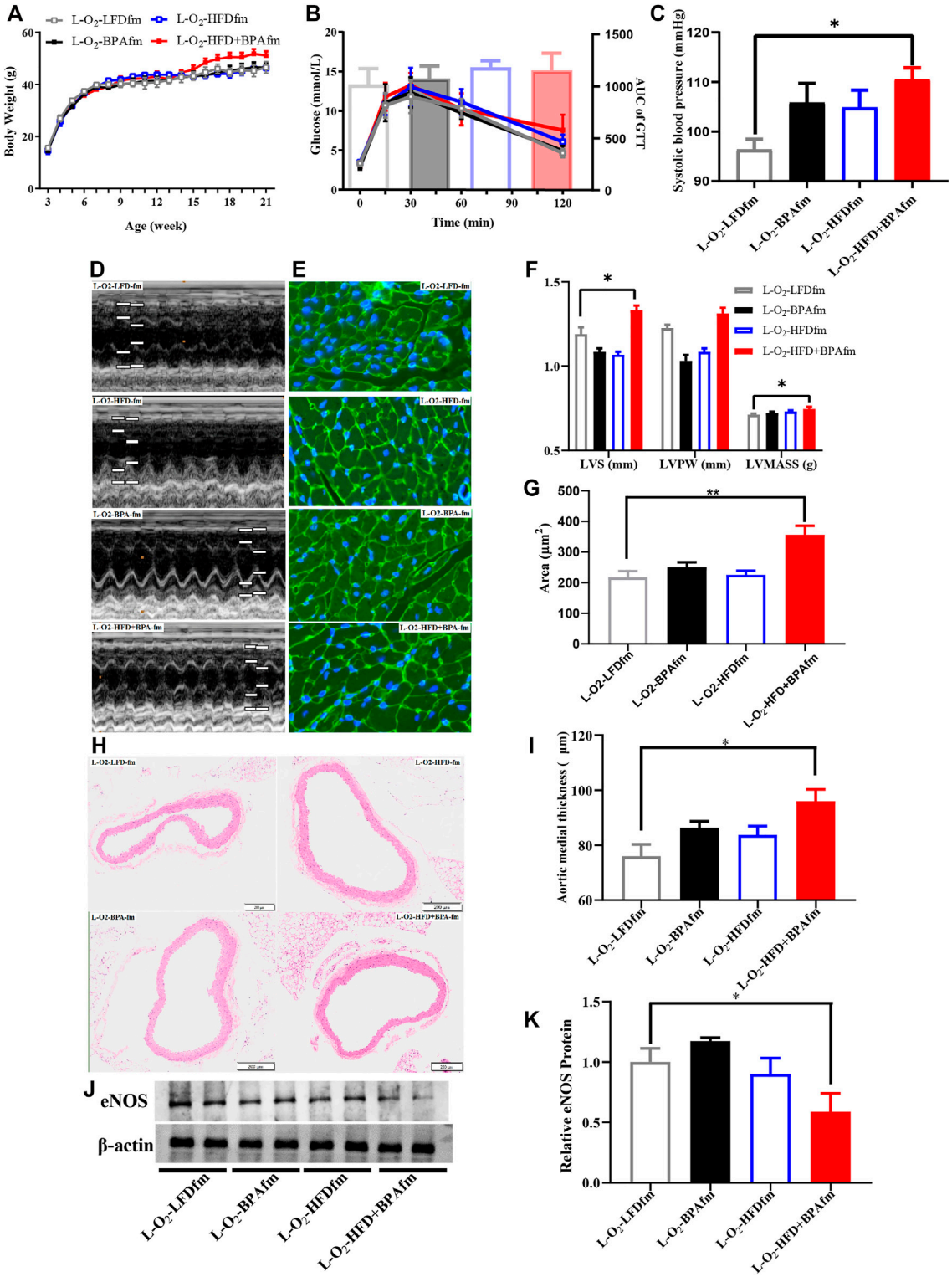

FIGURE 2 | HFD, BPA and their combination affect weight gain, metabolic profile of matrilineal male F2. **(A,B)** The weight and glucose tolerance difference among the four groups of matrilineal male F2 from weaning to 21 weeks. **(C)** The SBP among the four groups of matrilineal male F2. **(D,F)** Echocardiograms among the four groups of matrilineal male F2. **(E,G)** Representative photomicrographs depicting WGA staining of cardiomyocytes and average cardiomyocyte area of matrilineal male F2. **(H,I)** Representative photomicrographs depicting hematoxylin eosin staining of aorta and arterial media thickness. **(J,K)** Western blotting results and quantitative analysis for aorta of matrilineal male F2. Data are mean ± SEM and were analyzed by two-way ANOVA with a posthoc test. *$p < 0.05$, **$p < 0.01$, BPA, HFD, BPA + HFD vs. LFD.

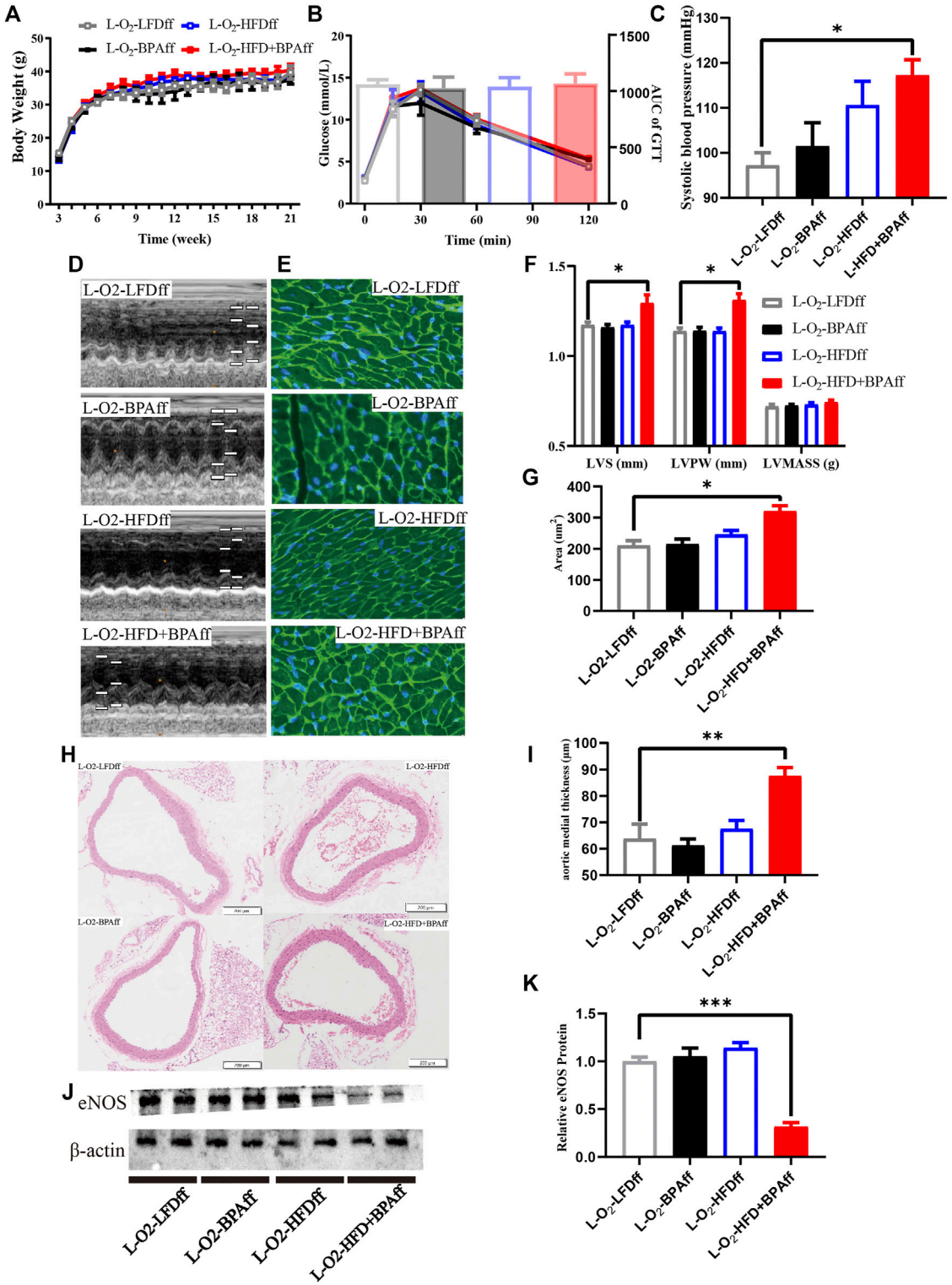

FIGURE 3 | HFD, BPA and their combination affect weight gain, metabolic profile of maternal female F2. **(A,B)** The weight and glucose tolerance difference among the four groups of matrilineal female F2 from weaning to 21 weeks. **(C)** The SBP among the four groups of matrilineal female F2. **(D,F)** Echocardiograms among the four groups of matrilineal female F2. **(E,G)** Representative photomicrographs depicting WGA staining of cardiomyocytes and Average cardiomyocyte area of matrilineal female F2. **(H,I)** Representative photomicrographs depicting hematoxylin eosin staining of aorta and arterial media thickness. **(J,K)** Western blotting results and quantitative analysis for aorta of matrilineal female F2. Data are mean ± SEM and were analyzed by two-way ANOVA with a posthoc test. $^{*}p < 0.05$. $^{**}p < 0.01$. $^{***}p < 0.001$, BPA, HFD, BPA + HFD vs LFD.

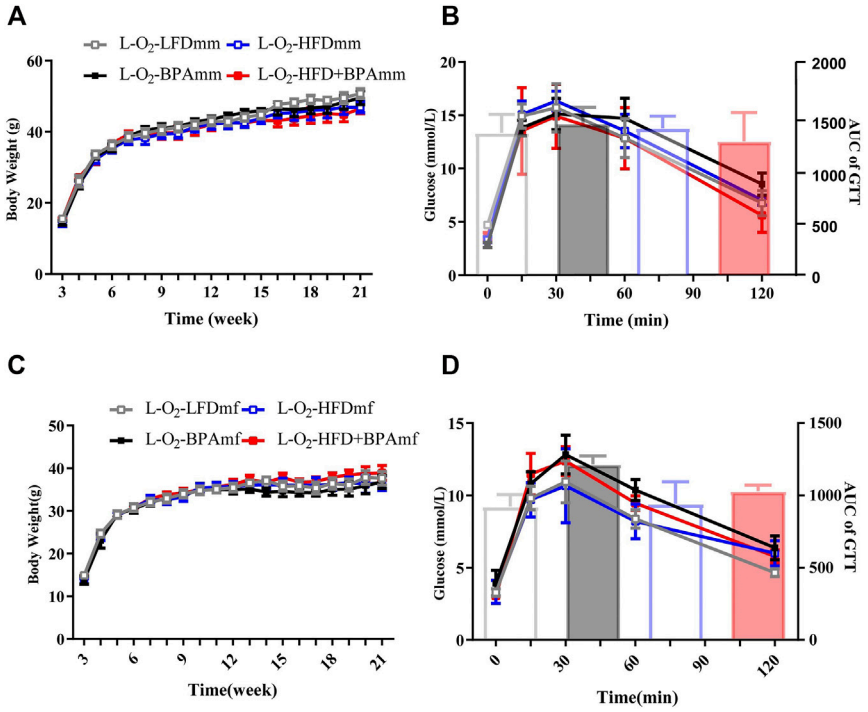

FIGURE 4 | HFD, BPA and their combination affect weight gain, glucose of patrilineal F2 with low fat diet. **(A,B)** Body weight and glucose tolerance of patrilineal male F2. **(C,D)** Body weight and glucose tolerance of patrilineal female F2. Data are mean ± SEM and were analyzed by two-way ANOVA with a posthoc test.

(**Figures 3H,I**) than matrilineal female F2 mice in the LFD group. Western blot analysis revealed significantly lower aortic eNOS expression in the L-O_2-HFD + BPAff group than in matrilineal female F2 mice in the other three groups (**Figures 3J,K**).

HFD, BPA, and Their Combination Affect Metabolic Profile and Blood Pressure of Patrilineal F2 Mice

There were no significant sex differences in body weight and glucose tolerance in adult patrilineal F2 mice (**Figures 4A–D**). Adult patrilineal F2 mice were fed a HFD from the 12th to 21st week to examine changes in metabolic or cardiovascular phenotypes after re-administration of a HFD. However, there were no significant changes in body weight and glucose tolerance in patrilineal male F2 mice (**Figures 5A–C**). However, there was a significant increase in the body weight and impaired glucose tolerance in HFD-fed adult patrilineal female F2 mice from ancestors exposed to both HFD and BPA compared with their counterparts from ancestors exposed to an LFD diet during the perinatal period (H-O2-HFD + BPAmf vs. H-O2-LFDmf, **Figures 5D,E**). There was no significant change in blood pressure among HFD-fed patrilineal female F2 mice (**Figures 5C,F**). Female patrilineal F2 mice showed significantly higher serum insulin levels and homeostatic model assessment–insulin resistance (HOMA-IR) in the H-O2-HFD + BPAmf group than in the LFD group (**Figures 5G,H**). Metabolic cage testing revealed significantly decreased energy expenditure, oxygen intake, and carbon dioxide production in the H-O2-HFD + BPAfm group than in the H-O2-LFDmf group (**Figures 5I–K**). These findings suggested that decreased energy expenditure may contribute to increased body weight, impaired glucose tolerance, and insulin resistance in the H-O2-HFD + BPAmf group.

Effects of Combined Exposure to BPA and HFD on Insulin of Matrilineal F2 and Their Metabolic Profile for Exposing HFD Again

The L-O2-HFD + BPAff group showed significantly higher serum insulin levels and HOMA-IR in the LFD group (**Figures 6A–D**). Adult matrilineal F2 mice were fed a HFD from the 12th to 21st week to examine the metabolic profile, which revealed significantly increased body weight and impaired glucose tolerance. However, these effects were mainly attributed to BPA or HFD rather than their synergistic effect (**Figures 6E,F**). There was a significant increase in the bodyweight of HFD-fed adult matrilineal female F2 mice from ancestors exposed to both HFD and BPA compared with counterparts from ancestors exposed to an LFD diet during the perinatal period (**Figure 6G**). However, the AUC did not reveal a significant change in glucose tolerance; however, the H-O2-HFD + BPAff group showed a significant increase in glucose levels at 15 min after glucose loading (**Figure 6H**).

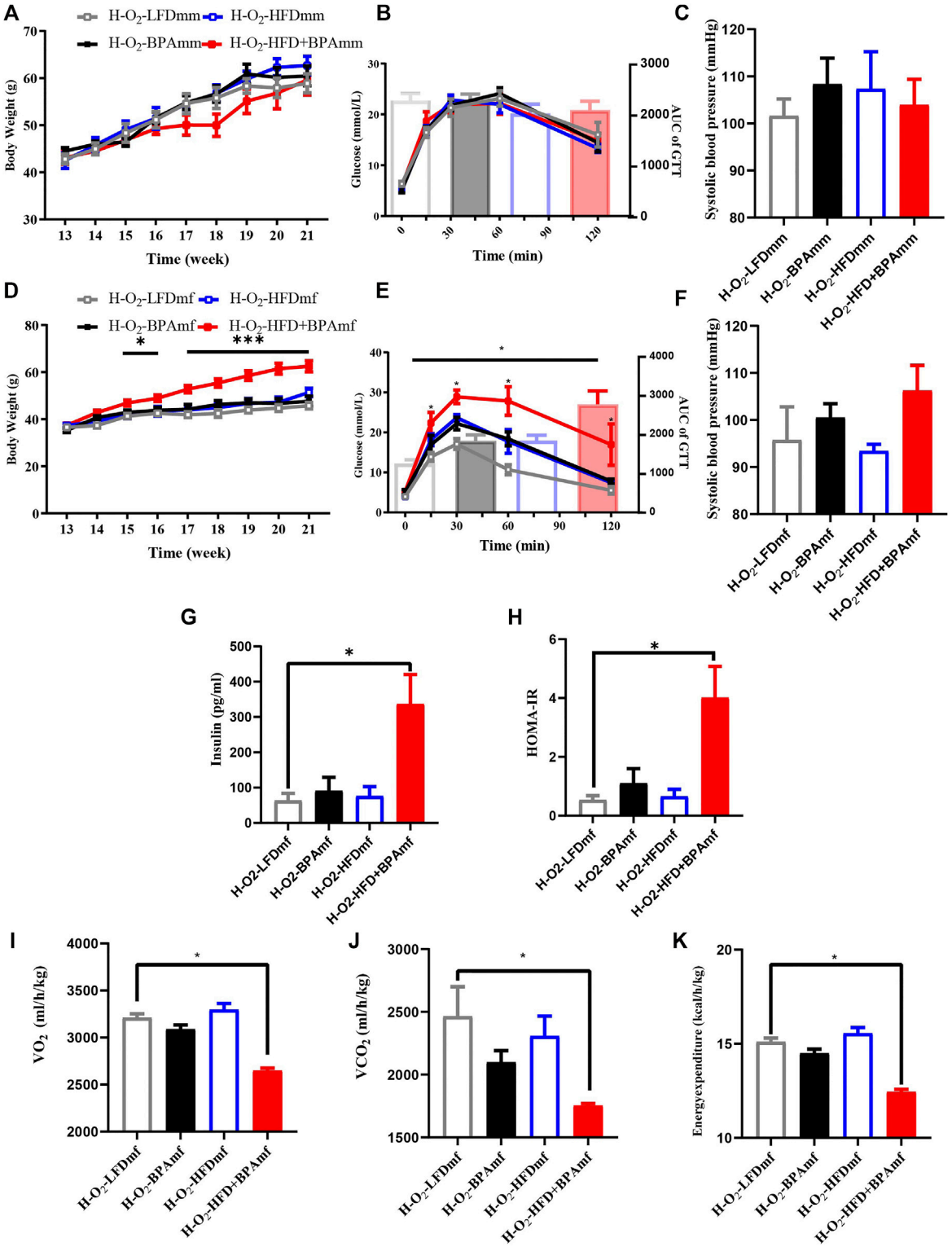

FIGURE 5 | HFD, BPA and their combination affect weight gain, metabolic profile and blood pressure of patrilineal F2 with HFD. **(A–C)** Body weight, glucose tolerance and systolic blood pressure of patrilineal male F2 with HFD. **(D–F)** Body weight, glucose tolerance and systolic blood pressure of females and males of patrilineal female F2 with HFD. **(G,H)** Serum insulin concentration and HOMA-IR of patrilineal female F2 with HFD. **(I–K)** metabolism cage of patrilineal female F2 with HFD. Data are means ± SEMs and were analyzed by two-way ANOVA with a posthoc test. $^{*}p < 0.05$, $^{***}p < 0.001$, BPA, HFD, BPA + HFD vs. LFD.

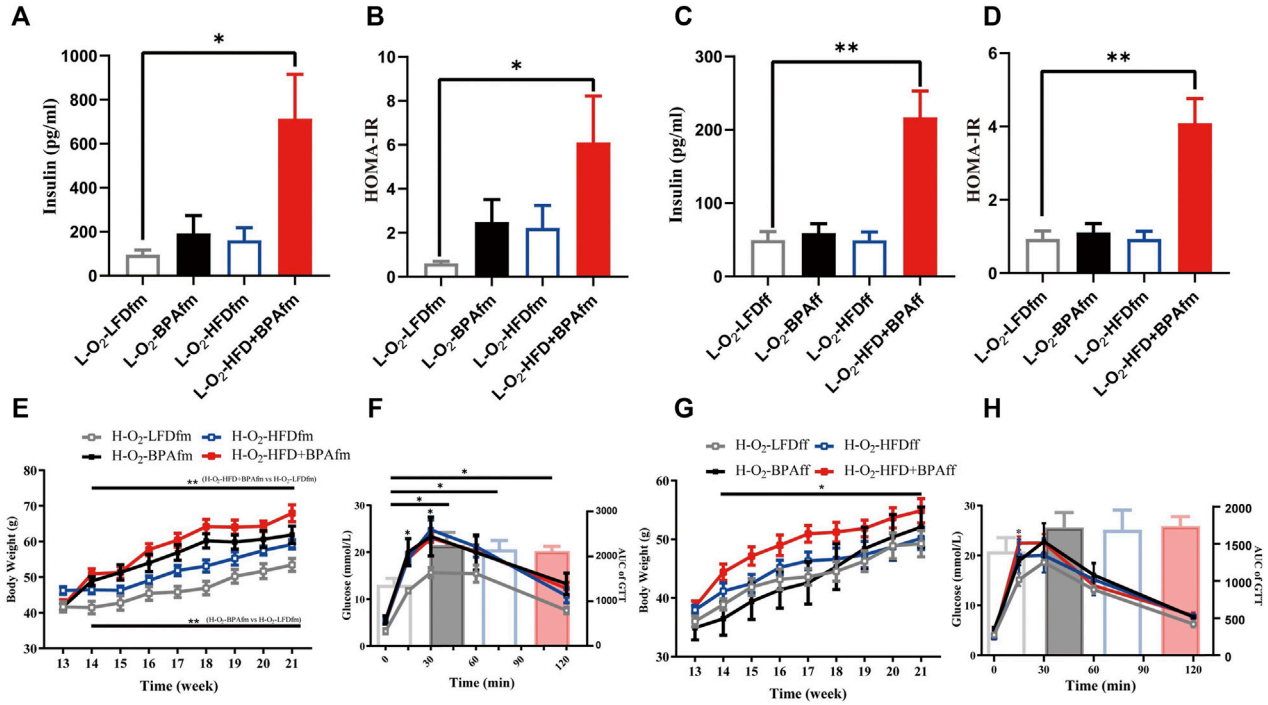

FIGURE 6 | Effects of combined exposure to BPA and HFD on insulin of matrilineal F2 and their metabolic profile for exposing HFD again. **(A–D)** Serum insulin concentration and insulin resistance index. **(E–H)** Body weight and glucose tolerance of maternal F2 when exposed to HFD again. Data are means ± SEMs and were analyzed by two-way ANOVA with a posthoc test. $^{*}p < 0.05$, $^{**}p < 0.01$, BPA, HFD, BPA + HFD vs LFD.

DISCUSSION

This study examined the transgenerational inheritance of adverse outcomes after combinational exposure of female mice (F0) to BPA and HFD during the perinatal period, with a particular focus on metabolic and cardiovascular changes in F2. In contrast to studies on the direct toxicity of exposure to BPA or HFD (Lecoutre et al., 2016; Bansal et al., 2017; Tain et al., 2017; Lomas-Soria et al., 2018; Choi et al., 2020; Filardi et al., 2020), we examined the long-term and synergistic effects of BPA and HFD. Notably, we observed that patrilineal female F2 mice mainly showed metabolic dysfunction signs, including increased weight gain, impaired glucose tolerance, and insulin resistance. Contrastingly, matrilineal F2 mice primarily showed cardiovascular changes, including increased blood pressure, cardiac remodeling, and aorta intimal thickening. This suggests that the adverse effects of combined exposure to BPA and HFD during the perinatal period could be sex-specific. Other studies have reported these sex differences, which indicates that the outcomes of perinatal exposure to adverse factors could have sex-dependent effects on offspring (Tain et al., 2017; Lomas-Soria et al., 2018; Sarker et al., 2018). Additionally, compared with exposure to the LFD, HFD, or BPA, exposure to both BPA and HFD decreased aortic eNOS expression in F2 mice, which indicates a significant synergistic effect of combined exposure to HFD and BPA. Further, this demonstrates that exposure to multiple adverse factors during the perinatal period has a more profound impact than exposure to a single adverse factor (Hsu et al., 2019). Moreover, patrilineal female mice, as well as matrilineal female and male mice, showed increased serum insulin levels and insulin resistance, which is consistent with previous reports that BPA or HFD exposure can cause insulin resistance (Naville et al., 2019; Farrugia et al., 2021; Gao et al., 2021; Huang et al., 2021; Yang et al., 2021). This may in turn contribute to metabolic- and cardiovascular-related phenotypic changes. Finally, we found that exposure to HFD or BPA alone did not significantly affect F2 mice; however, combined exposure to BPA and HFD yielded a synergistic effect, which caused significant and long-term changes in metabolic and cardiovascular phenotypes. Our findings demonstrated that early-life exposure to multiple factors may synergistically lead to more severe adverse outcomes, with these changes being passed on to the future generation. In summary, the toxicity of BPA could be significantly aggravated by the co-presence of HFD, which suggests environmental pollutants with the high energy diets may exert more severe and longer adverse impacts on human health and therefore should be further addressed in future work.

The mechanisms underlying the induction of metabolic and cardiovascular changes after early-life exposure to BPA and HFD remain unclear. Maternal exposure to adverse factors during the perinatal period could alter the intrauterine environment, which increases the risk of chronic disease in offspring (Nivoit et al., 2009; Vickers, 2014; Argyraki et al., 2019). For example, *in utero* malnutrition can cause changes in the fetal conditions, which promote dysfunction of certain organs in later life. These effects

could be multigenerational in some cases (Bale, 2014; Picton and Balen, 2019). Transgenerational transmission of metabolic dysfunction is potentially related to epigenetics. For example, maternal exposure to BPA causes pancreatic islet dysfunction in the next generation, which may be associated with methylation of pancreatic islet genes Igf2 and Esr1 (Bansal et al., 2017). Maternal exposure to a HFD causes metabolic disorders in the offspring through epigenetics (Fleming et al., 2018). Future studies should clarify the involvement of epigenetics in our model. Moreover, our findings demonstrated sexual dimorphism. Human and mouse studies have showed sexual dimorphism in susceptibility to metabolic disease (Mauvais-Jarvis, 2015; Kautzky-Willer et al., 2016; Monrroy et al., 2019; Lefebvre and Staels, 2021). These sex differences could be attributed to differences in energy metabolism and the actions of sex hormones (Xiao et al., 2008; Tramunt et al., 2020). Furthermore, prenatal effects may be affected by sex-specific differences. There are sex differences in epigenetic regulation of the fetus and placenta, which could contribute to the differential sensitiveness of males and females in metabolic and cardiovascular disease (Kautzky-Willer et al., 2016).

In our study, when only the F0 generation was directly exposed to unfavorable factors, compared with the LFD group, maternal F2 mice in the HFD + BPA group showed significant cardiovascular changes. Contrastingly, for patrilineal F2 female mice who were re-exposed to a HFD, the HFD + BPA group showed more obvious metabolic disorders. However, for matrilineal F2 mice, re-exposure to a HFD did not show a synergistic effect of exposure to HFD and BPA on the metabolic phenotype. Therefore, the weight gain, insulin resistance, hypertension, and cardiac hypertrophy in F2 may result from the direct transgenerational inheriting effects caused by the synergistic effects of BPA and HFD exposure in F0 generation. These results demonstrate that perinatal exposure of the F0 generation to BPA and HFD can cause harmful changes in metabolic and cardiovascular systems in their F2 offspring.

This study has several limitations. First, we mainly focused on the phenotype, and the mechanism should be further explored. Thus, our future studies will examine changes in mitochondrial function, reactive oxygen species (ROS) levels and global gene expression profile and clarify whether epigenetic mechanism, especially DNA methylation will influence the effect of maternal adverse factors exposure on metabolism and cardiovascular system of the offspring. Second, we did not explore the effects of different doses of BPA exposure on offspring to determine whether they would lead to different phenotypes in adult offspring.

Our findings demonstrated that combinational exposure of female mice to HFD and BPA during the perinatal period can cause susceptibility to insulin resistance, obesity, impaired glucose tolerance, increased blood pressure, cardiac hypertrophy, and impaired endothelial function in their F2 offspring. In addition, these inherited transgenerational abnormalities showed a sex-specific pattern. Our findings demonstrate the requirement of adjusting lifestyle and alleviating exposure to environmental EDSs during pregnancy to reduce the risk of metabolic and cardiovascular diseases in the offspring.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by the Biomedical ethics committee of Chongqing Medical University.

AUTHOR CONTRIBUTIONS

JL: Methodology, Conceptualization, Investigation, Formal analysis, Data curation, Visualization, Writing—original draft. ML: Methodology, Resources, Formal analysis. RH: Investigation, Validation, Formal analysis. YY: Validation. XJL: Investigation, Validation. HY: Resources. LF: Methodology. YZ: Validation. XYL: Methodology, Writing—review and editing. JL: Data curation, Methodology, Writing—review and editing. XX: Conceptualization, Methodology, Project administration, Supervision, Writing—review and editing.

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REFERENCES

Argyraki, M., Damdimopoulou, P., Chatzimeletiou, K., Grimbizis, G. F., Tarlatzis, B. C., Syrrou, M., et al. (2019). In-utero Stress and Mode of conception: Impact on Regulation of Imprinted Genes, Fetal Development and Future Health. *Hum. Reprod. Update* 25, 777–801. doi:10.1093/humupd/dmz025

Bae, S., Lim, Y.-H., Lee, Y. A., Shin, C. H., Oh, S.-Y., and Hong, Y.-C. (2017). Maternal Urinary Bisphenol A Concentration during Midterm Pregnancy and

Children's Blood Pressure at Age 4. *Hypertension* 69, 367–374. doi:10.1161/hypertensionaha.116.08281

Bale, T. L. (2014). Lifetime Stress Experience: Transgenerational Epigenetics and Germ Cell Programming. *Dialogues Clin. Neurosci.* 16, 297–305. doi:10.31887/DCNS.2014.16.3/tbale

Bansal, A., Rashid, C., Xin, F., Li, C., Polyak, E., Duemler, A., et al. (2017). Sex- and Dose-specific Effects of Maternal Bisphenol A Exposure on Pancreatic Islets of First- and Second-Generation Adult Mice Offspring. *Environ. Health Perspect.* 125, 097022. doi:10.1289/ehp1674

Bao, W., Liu, B., Rong, S., Dai, S. Y., Trasande, L., and Lehmler, H.-J. (2020). Association between Bisphenol a Exposure and Risk of All-Cause and Cause-specific Mortality in Us Adults. *JAMA Netw. Open* 3, e2011620. doi:10.1001/jamanetworkopen.2020.11620

Barua, S., and Junaid, M. A. (2015). Lifestyle, Pregnancy and Epigenetic Effects. *Epigenomics* 7, 85–102. doi:10.2217/epi.14.71

Bergman, A., Heindel, J., Jobling, S., Kidd, K., and Zoeller, R. T. (2012). State-of-the-science of Endocrine Disrupting Chemicals, 2012. *Toxicol. Lett.* 211, S3. doi:10.1016/j.toxlet.2012.03.020

Choi, Y.-J., Lee, Y. A., Hong, Y.-C., Cho, J., Lee, K.-S., Shin, C. H., et al. (2020). Effect of Prenatal Bisphenol A Exposure on Early Childhood Body Mass index through Epigenetic Influence on the Insulin-like Growth Factor 2 Receptor (IGF2R) Gene. *Environ. Int.* 143, 105929. doi:10.1016/j.envint.2020.105929

Dai, H., Alsalhe, T. A., Chalghaf, N., Riccò, M., Bragazzi, N. L., and Wu, J. (2020). The Global burden of Disease Attributable to High Body Mass index in 195 Countries and Territories, 1990-2017: An Analysis of the Global Burden of Disease Study. *Plos Med.* 17, e1003198. doi:10.1371/journal.pmed.1003198

Devos, P., and Ménard, J. (2020). Trends in Worldwide Research in Hypertension over the Period 1999-2018. *Hypertension* 76, 1649–1655. doi:10.1161/hypertensionaha.120.15711

Di Ciaula, A., and Portincasa, P. (2019). Diet and Contaminants: Driving the Rise to Obesity Epidemics? *Cmc* 26, 3471–3482. doi:10.2174/0929867324666170518095736

Farrugia, F., Aquilina, A., Vassallo, J., and Pace, N. P. (2021). Bisphenol A and Type 2 Diabetes Mellitus: A Review of Epidemiologic, Functional, and Early Life Factors. *Int. J. Environ. Res. Public Health* 18, 716. doi:10.3390/ijerph18020716

Figueiredo, L. S., Oliveira, K. M., Freitas, I. N., Silva, J. A., Jr., Silva, J. N., Favero-Santos, B. C., et al. (2020). Bisphenol-A Exposure Worsens Hepatic Steatosis in Ovariectomized Mice Fed on a High-Fat Diet: Role of Endoplasmic Reticulum Stress and Fibrogenic Pathways. *Life Sci.* 256, 118012. doi:10.1016/j.lfs.2020.118012

Filardi, T., Panimolle, F., Lenzi, A., and Morano, S. (2020). Bisphenol A and Phthalates in Diet: An Emerging Link with Pregnancy Complications. *Nutrients* 12, 525. doi:10.3390/nu12020525

Fleming, T. P., Watkins, A. J., Velazquez, M. A., Mathers, J. C., Prentice, A. M., Stephenson, J., et al. (2018). Origins of Lifetime Health Around the Time of conception: Causes and Consequences. *The Lancet* 391, 1842–1852. doi:10.1016/s0140-6736(18)30312-x

Gao, X., Liu, S., Ding, C., Miao, Y., Gao, Z., Li, M., et al. (2021). Comparative Effects of Genistein and Bisphenol A on Non-alcoholic Fatty Liver Disease in Laying Hens. *Environ. Pollut.* 288, 117795. doi:10.1016/j.envpol.2021.117795

Gawlinski, D., Gawlińska, K., and Smaga, I. (2021). Maternal High-Fat Diet Modulates Cnr1 Gene Expression in Male Rat Offspring. *Nutrients* 13 (8), 2885. doi:10.3390/nu13082885

Geens, T., Aerts, D., Berthot, C., Bourguignon, J. P., Goeyens, L., Lecomte, P., et al. (2021). A Review of Dietary and Non-dietary Exposure to Bisphenol-A. *Food Chem. Toxicol.* 50, 3725–3740. doi:10.1016/j.fct.2012.07.059

Han, C., and Hong, Y.-C. (2016). Bisphenol A, Hypertension, and Cardiovascular Diseases: Epidemiological, Laboratory, and Clinical Trial Evidence. *Curr. Hypertens. Rep.* 18, 11. doi:10.1007/s11906-015-0617-2

Höchsmann, C., Dorling, J. L., Martin, C. K., Newton, R. L., Jr., Apolzan, J. W., Myers, C. A., et al. (2021). Effects of a 2-Year Primary Care Lifestyle Intervention on Cardiometabolic Risk Factors. *Circulation* 143, 1202–1214. doi:10.1161/circulationaha.120.051328

Hsu, C. N., Lin, Y. J., and Tain, Y. L. (2019). Maternal Exposure to Bisphenol A Combined with High-Fat Diet-Induced Programmed Hypertension in Adult Male Rat Offspring: Effects of Resveratrol. *Int. J. Mol. Sci.* 20, 4382. doi:10.3390/ijms20184382

Huang, W. C., Liao, K. Y., Hsieh, S. K., Pan, P. H., Kuan, Y. H., Liao, S. L., et al. (2021). Magnesium Lithospermate B Supplementation Improved Prenatal Bisphenol A Exposure-induced Metabolic Abnormalities in Male Offspring. *Environ. Toxicol.* 36, 1932–1943. doi:10.1002/tox.23313

Jeon, J. Y., Ha, K. H., and Kim, D. J. (2015). New Risk Factors for Obesity and Diabetes: Environmental Chemicals. *J. Diabetes Invest.* 6, 109–111. doi:10.1111/jdi.12318

Kataria, A., Levine, D., Wertenteil, S., Vento, S., Xue, J., Rajendiran, K., et al. (2017). Exposure to Bisphenols and Phthalates and Association with Oxidant Stress, Insulin Resistance, and Endothelial Dysfunction in Children. *Pediatr. Res.* 81, 857–864. doi:10.1038/pr.2017.16

Kautzky-Willer, A., Harreiter, J., and Pacini, G. (2016). Sex and Gender Differences in Risk, Pathophysiology and Complications of Type 2 Diabetes Mellitus. *Endocr. Rev.* 37, 278–316. doi:10.1210/er.2015-1137

Kolb, H., and Martin, S. (2017). Environmental/lifestyle Factors in the Pathogenesis and Prevention of Type 2 Diabetes. *BMC Med.* 15, 131. doi:10.1186/s12916-017-0901-x

Krishnan, K., Mittal, N., Thompson, L. M., Rodriguez-Santiago, M., Duvauchelle, C. L., Crews, D., et al. (2018). Effects of the Endocrine-Disrupting Chemicals, Vinclozolin and Polychlorinated Biphenyls, on Physiological and Sociosexual Phenotypes in F2 Generation sprague-dawley Rats. *Environ. Health Perspect.* 126, 97005. doi:10.1289/EHP3550

Lecoutre, S., Deracinois, B., Laborie, C., Eberlé, D., Guinez, C., Panchenko, P. E., et al. (2016). Depot- and Sex-specific Effects of Maternal Obesity in Offspring's Adipose Tissue. *J. Endocrinol.* 230, 39–53. doi:10.1530/joe-16-0037

Lefebvre, P., and Staels, B. (2021). Hepatic Sexual Dimorphism - Implications for Non-alcoholic Fatty Liver Disease. *Nat. Rev. Endocrinol.* 17 (11), 662–670. doi:10.1038/s41574-021-00538-6

Lomas-Soria, C., Reyes-Castro, L. A., Rodríguez-González, G. L., Ibáñez, C. A., Bautista, C. J., Cox, L. A., et al. (2018). Maternal Obesity Has Sex-dependent Effects on Insulin, Glucose and Lipid Metabolism and the Liver Transcriptome in Young Adult Rat Offspring. *J. Physiol.* 596, 4611–4628. doi:10.1113/jp276372

Lv, Q., Gao, R., Peng, C., Yi, J., Liu, L., Yang, S., et al. (2017). Bisphenol A Promotes Hepatic Lipid Deposition Involving Kupffer Cells M1 Polarization in Male Mice. *J. Endocrinol.* 234, 143–154. doi:10.1530/joe-17-0028

Mauvais-Jarvis, F. (2015). Sex Differences in Metabolic Homeostasis, Diabetes, and Obesity. *Biol. Sex. Differ.* 6, 14. doi:10.1186/s13293-015-0033-y

Monrroy, H., Pribic, T., Galan, C., Nieto, A., Amigo, N., Accarino, A., et al. (2019). Meal Enjoyment and Tolerance in Women and Men. *Nutrients* 11, 119. doi:10.3390/nu11010119

Naville, D., Gaillard, G., Julien, B., Vega, N., Pinteur, C., Chanon, S., et al. (2019). Chronic Exposure to a Pollutant Mixture at Low Doses Led to Tissue-specific Metabolic Alterations in Male Mice Fed Standard and High-Fat High-Sucrose Diet. *Chemosphere* 220, 1187–1199. doi:10.1016/j.chemosphere.2018.12.177

NCD Risk Factor Collaboration (NCD-RisC) (2017). Worldwide Trends in Body-Mass index, Underweight, Overweight, and Obesity From 1975 to 2016: A Pooled Analysis of 2416 Population-Based Measurement Studies in 128.9 Million Children, Adolescents, and Adults. *Lancet* 390, 2627–2642. doi:10.1016/S0140-6736(17)32129-3

Nivoit, P., Morens, C., Van Assche, F. A., Jansen, E., Poston, L., Remacle, C., et al. (2009). Established Diet-Induced Obesity in Female Rats Leads to Offspring Hyperphagia, Adiposity and Insulin Resistance. *Diabetologia* 52, 1133–1142. doi:10.1007/s00125-009-1316-9

Picton, H. M., and Balen, A. H. (2019). Transgenerational PCOS Transmission. *Nat. Med.* 25, 1818–1820. doi:10.1038/s41591-019-0678-x

Provvisiero, D. P., Pivonello, C., Muscogiuri, G., Negri, M., de Angelis, C., Simeoli, C., et al. (2016). Influence of Bisphenol A on Type 2 Diabetes Mellitus. *Int. J. Environ. Res. Public Health* 13, 989. doi:10.3390/ijerph13100989

Riccardi, G., Giacco, R., and Rivellese, A. A. (2004). Dietary Fat, Insulin Sensitivity and the Metabolic Syndrome. *Clin. Nutr.* 23, 447–456. doi:10.1016/j.clnu.2004.02.006

Sarker, G., Berrens, R., von Arx, J., Pelczar, P., Reik, W., Wolfrum, C., et al. (2018). Transgenerational Transmission of Hedonic Behaviors and Metabolic

Phenotypes Induced by Maternal Overnutrition. *Transl. Psychiatry* 8, 195. doi:10.1038/s41398-018-0243-2

Schellong, K., Melchior, K., Ziska, T., Rancourt, R. C., Henrich, W., and Plagemann, A. (2020). Maternal but Not Paternal High-Fat Diet (HFD) Exposure at conception Predisposes for 'diabesity' in Offspring Generations. *Int. J. Environ. Res. Public Health* 17, 4229. doi:10.3390/ijerph17124229

Tain, Y. L., Lin, Y. J., Sheen, J. M., Yu, H. R., Tiao, M. M., Chen, C. C., et al. (2017). High Fat Diets Sex-Specifically Affect the Renal Transcriptome and Program Obesity, Kidney Injury, and Hypertension in the Offspring. *Nutrients* 9, 357. doi:10.3390/nu9040357

Tramunt, B., Smati, S., Grandgeorge, N., Lenfant, F., Arnal, J.-F., Montagner, A., et al. (2020). Sex Differences in Metabolic Regulation and Diabetes Susceptibility. *Diabetologia* 63, 453–461. doi:10.1007/s00125-019-05040-3

Vickers, M. (2014). Early Life Nutrition, Epigenetics and Programming of Later Life Disease. *Nutrients* 6, 2165–2178. doi:10.3390/nu6062165

Wolstenholme, J. T., Goldsby, J. A., and Rissman, E. F. (2013). Transgenerational Effects of Prenatal Bisphenol A on Social Recognition. *Horm. Behav.* 64, 833–839. doi:10.1016/j.yhbeh.2013.09.007

Xiao, D., Xu, Z., Huang, X., Longo, L. D., Yang, S., and Zhang, L. (2008). Prenatal Gender-Related Nicotine Exposure Increases Blood Pressure Response to Angiotensin II in Adult Offspring. *Hypertension* 51, 1239–1247. doi:10.1161/hypertensionaha.107.106203

Yang, J., Wang, H., Du, H., Xu, L., Liu, S., Yi, J., et al. (2021). Serum Bisphenol A, Glucose Homeostasis, and Gestational Diabetes Mellitus in Chinese Pregnant Women: a Prospective Study. *Environ. Sci. Pollut. Res.* 28, 12546–12554. doi:10.1007/s11356-020-11263-4

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