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SPOTLIGHT REVIEW

Metabolic remodelling in diabetic cardiomyopathy

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Abstract Diabetes is a risk factor for heart failure and cardiovascular mortality with specific changes to myocardial metabolism, energetics, structure, and function. The gradual impairment of insulin production and signalling in diabetes is associated with elevated plasma fatty acids and increased myocardial free fatty acid uptake and activation of the transcription factor PPARα. The increased free fatty acid uptake results in accumulation of toxic metabolites, such as ceramide and diacylglycerol, activation of protein kinase C, and elevation of uncoupling protein-3. Insulin signalling and glucose uptake/oxidation become further impaired, and mitochondrial function and ATP production become compromised. Increased oxidative stress also impairs mitochondrial function and disrupts metabolic pathways. The diabetic heart relies on free fatty acids (FFA) as the major substrate for oxidative phosphorylation and is unable to increase glucose oxidation during ischaemia or hypoxia, thereby increasing myocardial injury, especially in ageing female diabetic animals. Pharmacological activation of PPARγ in adipose tissue may lower plasma FFA and improve recovery from myocardial ischaemic injury in diabetes. Not only is the diabetic heart energetically-impaired, it also has early diastolic dysfunction and concentric remodelling. The contractile function of the diabetic myocardium negatively correlates with epicardial adipose tissue, which secretes proinflammatory cytokines, resulting in interstitial fibrosis. Novel pharmacological strategies targeting oxidative stress seem promising in preventing progression of diabetic cardiomyopathy, although clinical evidence is lacking. Metabolic agents that lower plasma FFA or glucose, including PPARγ agonism and SGLT2 inhibition, may therefore be promising options.

Keywords Diabetes • Diabetic cardiomyopathy • Diabetic heart • Metabolism • Metabolic remodelling

This article is part of the Spotlight Issue on Metabolic Cardiomyopathies.

1. Diabetic cardiomyopathy: an individual entity

Although heart failure and diabetes were thought to co-exist as a single entity as early as in 1881,[1] it was only in 1972 that Rubler and colleagues provided the first evidence in four diabetic patients with overt heart failure: they described ventricular hypertrophy and diffuse myocardial fibrosis, independent of alcohol consumption, structural, vascular, and coronary disease.[2] The term 'diabetic cardiomyopathy' (DCM) was coined from then and is commonly used to describe myocardial structural and functional changes that occur in patients with diabetes. Although it is known that diabetes increases the risk of developing heart failure by two- to three-fold after adjustment for other cardiovascular (CV) risk factors,[3] a clear diagnostic algorithm for DCM is lacking. Clinical distinction between DCM and cardiomyopathies of other aetiologies is limited, making it diagnostically impractical. Nevertheless, there are pathophysiological differences (discussed further in this *Spotlight issue*) and metabolic remodelling (discussed in this review) characteristic of DCM, suggesting that it is indeed a unique entity requiring further investigation and early intervention to halt disease progression.

2. Effects of diabetes on myocardial metabolism

2.1 The healthy heart

The heart, predominantly an aerobic organ, relies heavily on the oxidation of substrates, such as free fatty acids (FFA), glucose, lactate, ketone bodies, and some amino acids, to generate adenosine triphosphate (ATP), the major source of energy. The process of substrate selection is

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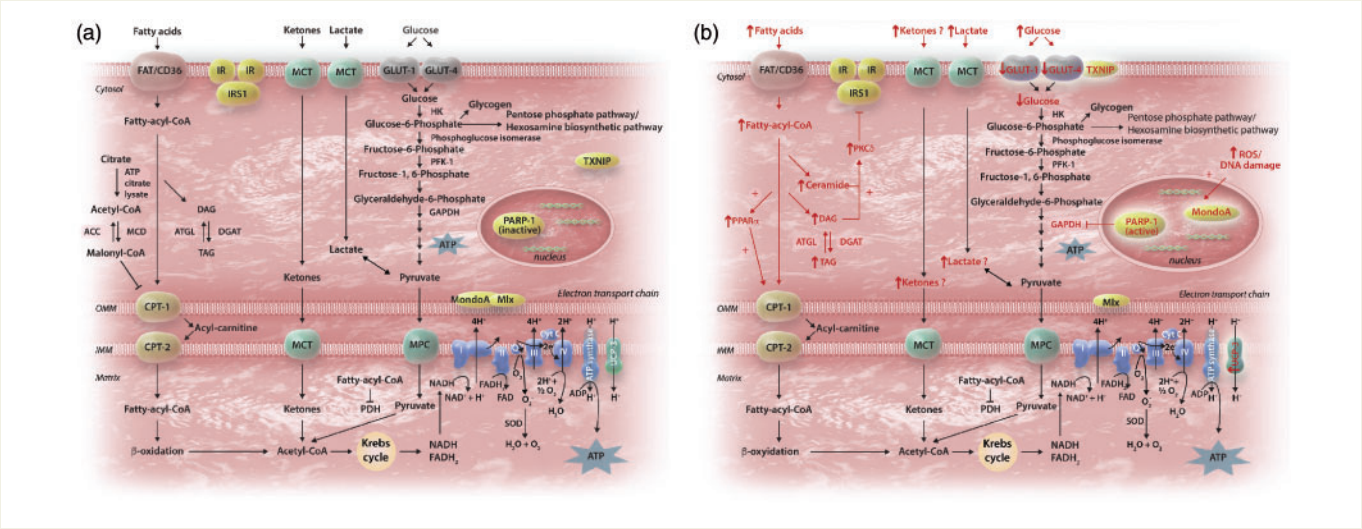

Figure 1 (*A*) The healthy heart. The reciprocal relationship between myocardial substrate oxidation is governed by Randle cycle for mitochondrial generation of ATP: oxidation of fatty acid leads to increased fatty acyl-CoA which inhibits pyruvate dehydrogenase; whereas glucose oxidation increases cytosolic citrate, a precursor of malonyl-CoA which inhibits CPT-1. In healthy heart, the predominant substrate used is fatty acid and glucose, and occasionally lactate, pyruvate or ketone bodies. (*B*) The diabetic heart. Hyperglycaemia increases ROS and activates PARP-1, which then inhibits GAPDH and increases glycolytic intermediates. Under the transcription factor MondoA, TXNIP also shuttles from cytosol to plasma membrane and inhibits GLUT-1, reducing further uptake of glucose. Furthermore, as diabetes progresses, fatty acids (and potentially ketone bodies and other substrates) become increasingly relied on as the substrate for oxidation; parallels the upregulation of UCP-3. However, the uncoupling between uptake and oxidation of fatty acids leads to accumulation of toxic metabolites, which activates protein kinase C and further impairs insulin signalling. ACC, acyl-coA carboxylase; ATGL, adipose triglyceride lipase; CPT-1/2, carnitine palmitoyl transferase-1/2; DAG, diacyglycerol; DGAT, diacylglycerol transferase; IR, insulin receptor; IRS1, insulin receptor substrate-1; FAT, fatty acid transporter; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GLUT, glucose transporter; HK, hexokinase; MCD, malonyl-CoA decarboxylase; MCT, monocarboxylase transporter; MPC, mitochondrial pyruvate carrier; O/IMM, outer/inner mitochondrial membrane; PDH, pyruvate dehydrogenase; PFK-1, phosphofructose kinase-1; PKC, protein kinase C; PPAR, peroxisome proliferator activated receptor; ROS, reactive oxygen species; SOD, superoxide dismutase; UCP, uncoupling protein; TAG, triacylglycerol; TXNIP, Thioredoxin interacting protein.

dynamic and depends largely on substrate availability, oxygen concentration, and myocardial workload. Such dynamic processes are integrated to ensure that myocardial contractile performance and housekeeping functions are maintained. Under normal physiological conditions, more than 90% of ATP is generated in mitochondria, and 60–70% from the oxidation of FFA.[4] Interaction between substrate utilization is governed by the 'Randle cycle', the reciprocal and dependent metabolic relationship between FFA and glucose oxidation (see *Figure 1*).

Free fatty acids enter the cytosolic compartment via transporters, such as FA translocase/CD36 (FAT/CD36), plasma membrane FA binding protein (FABPpm), and FA transport proteins (FATP1 and 6).[5] In response to certain stimuli, such as increased insulin or activation of AMP-activated protein kinase (AMPK), FAT/CD36 'shuttles' from intracellular vesicles to the sarcolemma to increase the uptake of FA.[6] Upon entry into the cytosol, the non-esterified FA are esterified to fatty acyl-CoA. Depending on myocardial demand, fatty acyl-CoA is either stored in the myocardial lipid pool or enters the mitochondria for β-oxidation via the carnitine shuttle: carnitine palmitoyl transferase-1 (CPT-1) being the rate-limiting enzyme for mitochondrial uptake of FA. Oxidation of FFA triggers an increase in mitochondrial ratios of [acetyl-CoA]/[CoA] and [NADH]/[NAD^+], both of which inhibit the activity of PDH complex. Ketone bodies, produced from FFA in the liver, can also be metabolised to acetyl-CoA for entry into Krebs cycle.[5]

Glucose uptake is facilitated by transporters, most notably the insulin-independent GLUT1 and insulin-dependent GLUT4 in the heart. Similar to FAT/CD36, glucose transporters also 'shuttle' between intracellular vesicles and the sarcolemma in response to stimuli. After entering the cytosol, glucose is phosphorylated by hexokinase to glucose-6-phosphate, which enters glycolysis, glycogenesis, the pentose phosphate pathway, or the hexosamine biosynthetic pathway. Glycolysis generates a small amount of ATP independent of oxygen availability, and is regulated mainly by phosphofructokinase, which is inhibited by cytosolic citrate from the Krebs cycle. Cytosolic citrate is also the major precursor of malonyl-CoA, which inhibits CPT-1. The end product of glycolysis is pyruvate, which enters mitochondria for oxidation in normoxia or is reduced to lactate in the cytosol under hypoxia. Mitochondrial pyruvate dehydrogenase (PDH) is the key enzyme governing the oxidative decarboxylation of pyruvate to acetyl-CoA. Lactate, readily extracted from the bloodstream, can be converted to pyruvate in the cytosol and further metabolized to acetyl-CoA for ATP generation.[5]

Arising from the oxidation of a variety of substrates, acetyl-CoA enters the Krebs cycle to produce NADH and $FADH_2$, which donate electrons to the electron transport chain thereby creating the proton electrochemical gradient needed to generate ATP. Oxidation of FFAs generates more ATP compared to glucose, but at the expense of greater oxygen consumption. Therefore, when oxygen availability is low, glucose oxidation is more 'metabolically efficient'.

2.2 The diabetic heart

Normally, with post-prandial elevated blood glucose, pancreatic β-cells take up glucose leading to increased generation of mitochondrial ATP, which closes the ATP-sensitive K_{ATP} channel, with accumulation of

K^+ ions that depolarises the plasma membrane.[7] Depolarisation activates calcium channels, causing an influx of Ca^{2+} and the eventual exocytosis of insulin.[7] However, this process is defective in diabetes for various reasons, which may include decreased glucokinase activity[8,9] and reduction in mitochondrial mass or in the ability to generate ATP.[7] Metabolically, it is characterised by rapid defective (type 1 diabetes, T1D) or gradual diminution (type 2 diabetes, T2D) of insulin secretion, leading to increased extracellular glucose and greater reliance on fatty acid oxidation. Early in T2D, the lack of response to insulin in peripheral organs is over-compensated by increased insulin secretion, resulting in hyperinsulinaemia. Hyperinsulinaemia may be sustained for a long time, or may cause a gradual loss of pancreatic function, resulting in hypoinsulinaemia and hyperglycaemia.[5] In both T1D and T2D, failure of insulin to suppress hormone sensitive lipase in adipose tissue and very low-density lipoprotein secretion in the liver increases circulating FFAs. This, in turn activates peroxisome proliferator activated receptor-α (PPARα), a transcription factor that upregulates myocardial FFA uptake and metabolism while decreasing GLUT4,[10,11] leading to systemic hyperglycaemia. Consequently, therapeutic strategies should perhaps focus on minimizing the delivery or type of substrates supplied.[12]

In the T2D male GK rat hearts, despite normal basal glucose uptake, insulin-stimulated glucose uptake was 50% lower in diabetic rat hearts than controls.[13] Initial decreases occurred in the GLUT-4, phosphorylated insulin receptor β-subunit, insulin receptor substrate-1, and PI3-kinase,[13] suggesting that these pre-receptor changes are responsible for early insulin resistance.

2.2.1 Impact of substrate overload on myocardial mitochondria

The overabundance of food, as occurs in certain western cultures, is thought to cause metabolic dysfunction, obesity, and diabetes, and is a risk factor for developing DCM. Used in many sweeteners, high dietary fructose induced cardiac dysfunction in diabetes via post-translational modifications, including the formation of advanced glycation end products and *O*-GlcNAcylation (for a comprehensive review, see ref.[14]). On the other hand, 3 weeks of high fat diet in male Wistar rat led to decreased myocardial efficiency (hydraulic power divided by oxygen consumption) despite normal glucose oxidation. This was driven by elevated FFA oxidation and mitochondrial uncoupling with increased expression of mitochondrial uncoupling protein-3 (discussed later).[15] Nine-week-old wild-type mice (C57BL/6NJ) provided with 4 months of high fat diet exhibited diastolic dysfunction associated with impaired mitochondrial respiration and ATP production.[16] However, in different animal models of heart failure a high fat diet did not further impair cardiac and mitochondrial function,[17–19] suggesting that the originating stimulus may be important.

2.2.2 Myocardial steatosis

Diabetic myocardium has an increased triacylglycerol (TAG) content, largely owing to greater FA availability than oxidation. In several clinical studies, proton (^{1}H)-MRS has revealed that diabetic patients have between 1.5- and 2.3-fold higher myocardial TAG levels compared to non-diabetic controls,[20–22] the levels predicting concentric left ventricular (LV) remodelling and subclinical contractile dysfunction.[20] However, substrate oxidation and metabolic flexibility were not assessed in humans, making it difficult to determine whether it was the overabundance of substrate, or excessive substrate oxidation, leading to oxidative stress that leads to the cardiac dysfunction.[23–25]

Increased plasma FFA concentrations increase the flux through myocardial FFA oxidation via activation of the PPARα transcription factor,[15,26] leading to the upregulation of enzymes involved in FFA oxidation, such as the acyl-CoA dehydrogenases. However, because cardiomyocytes are not specialised to store lipids, increased long-chain fatty acyl-CoA is diverted towards the production of diacylglycerol and ceramide (*Figure 1*). Such intermediates are thought to be toxic, compromising ATP production and overall cell viability, via the activation of several stress kinases, including protein kinase C (PKC; for review, see ref.[27]). PKC inhibits the metabolic action of insulin by phosphorylating the serine/threonine residues on the insulin receptor and/or its substrates,[28] disrupting insulin signalling, inhibiting insulin-stimulated translocation of GLUT4, inducing apoptosis, and leading to lower basal expression of hypoxia inducible factor-1α and vascular endothelial growth factor.[27] Importantly, pharmacological inhibition of PKC ameliorates FFA-mediated inhibition of basal and insulin-stimulated glucose oxidation and normalizes diastolic function in the STZ-treated T1D heart without altering the circulating metabolites.[29]

2.2.3 Ketosis: friend or foe for the diabetic heart?

The ketone bodies, acetoacetate and β-hydroxybutyrate (β-OHB), are generated by the liver from non-esterified FAs in response to hypoinsulinaemia and hypoglycaemia, and are oxidized by most body tissues to form acetyl-CoA. Ketosis has always been feared in patients with diabetes, being associated with life-threatening acidosis. However, a recent study showed that, amongst patients with type 2 diabetes who presented with hyperglycaemic crisis, those with ketosis (but not acidosis) had lower all-cause mortality than those without,[30] suggesting that ketosis may potentially be protective in diabetes. Two recent independent studies also showed that ketone body metabolism is elevated in the failing (albeit non-diabetic) myocardium.[31,32] Given that exogenous D-β-hydroxybutyrate, consumed as a ketone ester drink, was metabolised by exercising skeletal muscle to increase endurance performance in athletes and healthy rats,[33,34] it may be that increased ketone metabolism in the diabetic heart is compensating for defects in mitochondrial energy transduction associated with acute insulin deficiency.[35]

2.2.4 Uncoupling proteins

Uncoupling proteins (UCPs) are mitochondrial anion carriers that dissipate the proton electrochemical gradient by transferring protons, generated during oxidative phosphorylation, back into the mitochondrial matrix without the concomitant synthesis of ATP (*Figure 1*). In patients undergoing coronary bypass surgery, upregulation of cardiac UCP-3 correlated positively with plasma concentrations of FFA.[36] In mice, elevation of UCP-3 expression is mediated via increased FFA stimulation of nuclear transcription factor, PPARα.[26] In chronically infarcted or high-fat diet induced rat hearts, increased UCP-3 concentrations are associated with mitochondrial uncoupling and decreased cardiac efficiency.[37,15] The *db*/*db* mice has increased myocardial UCP3 that increased mitochondrial inefficiency following ischaemia.[38] Activation of UCPs may be controlled by reactive oxygen species (ROS), potentially via glutathionylation.[39]

3. Oxidative stress and metabolic dysfunction in diabetic cardiomyopathy

Diabetes is often linked to inflammation and is associated with increased levels of C-reactive protein and interleukin-6.[40] Although there is a long-standing idea that insulin resistance and ectopic adiposity confer an increased risk of CV events, a new school of thought is that myocardial

insulin resistance maybe a defence against glucotoxicity and oxidative stress.[12] This is based on pre-clinical evidence that impaired mitochondrial oxidative capacity is not an early event in the development of insulin resistance, but follows increased ROS production with inhibition of mitochondrial ROS production reversing insulin resistance.[41]

Mitochondrial respiration is the major source of ROS, central to a number of biological processes, including cell proliferation, differentiation, adaptation to hypoxia, autophagy, immune function, hormone signalling, and cell survival. ROS production is usually counterbalanced by clearance via cellular antioxidant defence systems, such as superoxide dismutase, glutathione peroxidase, catalase, the thioredoxin system, and antioxidant molecules, such as vitamin E. However, in diabetes, ROS accumulates and causes non-specific oxidative damage to DNA, proteins, lipids, or other macromolecules.[42]

Hyperglycaemia also induces cellular damage via four major pathways: activation of the PKC pathway via diacylglycerol, increased hexosamine pathway flux, increased advanced glycation end products, and increased polyol pathway flux.[43,44] All pathways increase ROS production and activated nuclear poly-(ADP-ribose)-polymerase (PARP), which cleaves NAD^+ into nicotinamide and ADP-ribose.[44] Overactivation of PARP in hyperglycaemia forces the cell to synthesize NAD^+ via the salvage pathway which consumes ATP.[45] The process also leads to the ribosylation and inactivation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which in turn increases glycolytic intermediates and activates the proinflammatory transcription factor NF-κB.[44] Although pharmacological inhibition of PARP abolishes hyperglycaemia-induced cardiac structural dysfunction in T1D models of female NOD mice and STZ-induced male Wistar rats,[46] to date there has been no evidence that PARP inhibition improves the systemic metabolic profile in diabetes.

Catalase plays an important role in catabolizing hydrogen peroxide, and cardiac catalase activity is elevated in diabetes potentially as an early defence against reactive oxidants produced during aerobic metabolism.[47–49] Inhibition of cardiac catalase (by 3-amino-1,2,4-triazole) reduced the antioxidant transcription factor, nuclear factor erythroid-factor-2 (Nrf2), elevating PARP-1 and lipid peroxidation in STZ-induced T1D animals.[50] Importantly, both direct and indirect activation of catalase in STZ-induced T1D and KK T2D rats prevented protein nitration, inflammation, and cardiomyopathy.[48,50,51] However, clinical evidence in this area is lacking and it remains unknown if targeting inflammation or oxidative stress in DCM confers benefit.

In 2002, thioredoxin interacting protein (TXNIP) was reportedly the gene most upregulated by high glucose concentrations in a human islet oligonucleotide gene expression microarray;[52] and one of the most responsive genes to blood glucose levels and insulin signalling in T2D patients.[53] Ubiquitously expressed and pro-apoptotic, TXNIP exerts its effect via inhibition of the antioxidant thioredoxin, but also has some thioredoxin-independent effects,[54] including direct inhibition of glucose uptake by GLUT1[55,56] through the transcriptional complex, MondoA:Mlx.[57] In both high dose STZ-induced T1D and *ob/ob* T2D mice, administration of a calcium channel blocker reduced the cardiac expression of TXNIP and cleaved caspases *in vivo*,[58] but it is not known if cardiac function was preserved. Although TXNIP may reduce hypertrophy, making its reduction undesirable in DCM, the reported effects of TXNIP on cardiac hypertrophy are conflicting and inconclusive.[59,60] Stimuli of TXNIP, such as shear stress, commonly cause hypertrophy and agents that reduced TXNIP are anti-hypertrophic.[58,61] Interestingly, patients with diabetes who use calcium channel blockers, in particular verapamil, have lower serum glucose levels than non-users,[62] suggesting a potential protective role in pancreatic islet cells and DCM.

4. How does the diabetic heart cope with hypoxia or ischaemia?

Even in the normal heart, hypoxia or ischaemia cause profound changes in metabolic substrate utilization and oxidation. In particular, myocardial FFA oxidation, PPARα expression (together with its downstream targets, such as UCP3), and mitochondrial oxygen consumption are decreased in chronic hypoxia, whereas glycolysis is enhanced.[63–65] In mice with activated PPARα, myocardial FFA oxidation is increased and associated with a reduction in cardiac efficiency and decreased recovery of contractile function post low-flow ischaemia,[66,67] suggesting that mechanical dysfunction occurs as a result of the inability to increase glycolysis during a decrease in oxygen availability.

It is increasingly recognised that it is the lack of metabolic flexibility, rather than specific substrate preference that predisposes the diabetic heart to injury. Abnormal myocardial substrate metabolism was attenuated when high-fat/low dose STZ-induced T2D rats were subjected to chronic hypoxia; suggesting that the diabetic heart retained sufficient metabolic plasticity to adapt to hypoxia.[68] Additionally, during low-flow ischaemia, the isolated T1D rat heart used FFA oxidation for oxidative phosphorylation and production of ATP, suggesting a protective non-deleterious role of FFAs when glucose metabolism was down-regulated.[69] Myocardial TAG may be a dynamic, instead of inert, reservoir for FFAs[23–25] (for review, see ref.[70]). Diabetic hearts contain a high TAG content, which contributes significantly to overall oxidative metabolism.[71] While some studies suggested that accumulation of TAG is cardioprotective by virtue of channeling FFA oxidation away from toxic metabolites[72] and improves cardiac function from ischaemia,[73] others argue that lower TAG protects against DCM (in Akita and STZ-T1D mice and T2D patients).[74,75] Overall, TAG contribution to ischaemic recovery has not been explored in diabetes.

Activation of AMPK by metformin, a metabolic-sensing 'master switch' that promotes both cellular uptake of glucose and β-oxidation of FFAs, not only reduces ischaemic-reperfusion injury and limits myocardial infarct size, but also attenuates remodelling and heart failure in diabetes. However, animal experiments involving pharmacological activation of PPAR in diabetic hearts are inconclusive; potentially due to the specificity of the agent for the various PPAR isoforms. Whilst all, except for tetradecythioacetic acid, TTA, a PPARα agonist that also has potent antioxidant properties[76] demonstrated reduction in circulating FFA and increased glucose oxidation, overall cardiac effects were inconsistent: those employing a PPARγ agonist rosiglitazone and TTA demonstrated improved ischaemic tolerance;[76–78] whereas others using BM17.0744 or 2-(2-(4-phenoxy-2-propylphenoxy)ethyl)indole-5-acetic acid (PPARα and PPARγ agonists, respectively) showed no difference.[79,80] It has been suggested that there may be an interaction between substrate availability, PPARα activation and ceramide formation,[70] in which rats treated with a PPARα agonist and fed with high fat diet (34% fat) have increased myocardial ceramide, when the effect was attenuated in rats fed with normal chow diet (3% fat).[81] Although ceramide formation was not assessed, rats fed a high fat diet had increased PPARα expression, elevated FA oxidation, increased UCP3 expression, reduced glycolysis and consequent contractile dysfunction when subjected to hypoxia.[65]

4.1 Age and gender

Age-dependent studies in animals (*db/db* mice, Zucker *fa/fa*, and Goto-Kakizaki rats) reveal that diabetic hearts rely increasingly on FFA oxidation and less on glucose oxidation for the formation of acetyl-CoA with

increasing age, potentially due to substrate availability.[78,82] Age was associated with increased FFA oxidation, reduced glucose oxidation, worsened contractility and decreased recovery from ischaemic insult.[78,82,83] Compared to age-matched, non-diabetic counterparts, both the young and ageing diabetic rats had increased FFA oxidation.[78] However, glucose uptake and lactate production were unchanged regardless of diabetes in the younger rats during ischaemia. On the other hand, the ageing *fa/fa* rats had lower glucose uptake and lactate production than the age-matched controls, suggesting an overreliance of ageing diabetic hearts on FFA oxidation.[78]

With respect to gender, female diabetic animals typically display greater myocardial abnormalities than those of the male, including increased cardiac hypertrophy and lower insulin-stimulated glucose uptake,[82,84] mimicking clinical observations in diabetic patients.[85] Female STZ-induced T1D animals developed diastolic and systolic dysfunction much earlier than their male counterparts, with earlier ventricular remodelling, including increased LV dilation and reduced ejection fraction.[86] These changes were associated with down regulation of pro-survival Pim-1, and upregulation of proapoptotic signalling caspases, microRNA-1, and microRNA-208a[86] (see ref.[87] for comprehensive review).

5. Energetic changes in diabetic heart: evidence from magnetic resonance imaging studies

The clinical assessment of myocardial energetic status can be determined using the ratios of PCr to ATP (PCr/ATP) non-invasively via phosphorus-31 cardiac magnetic resonance spectroscopy (^{31}P-MRS). ^{31}P-MRS yields peaks for PCr and the three phosphorus atoms of ATP, all of which are proportional to the cellular concentration of the metabolites. The myocardial PCr/ATP ratio also correlates well with the New York Heart Association functional status, indices of systolic or diastolic function and survival rate in heart failure patients.[4] Despite 'normal' cardiac function measured using echocardiography and the lack of known coronary artery disease or ECG detectable ischaemic changes, diabetic patients have a lower myocardial PCr/ATP than the matched healthy controls, suggesting that diabetic patients are 'cardiac energy-deficient'.[88] The PCr/ATP ratios also correlated negatively with fasting plasma FFA concentrations.[88] Additionally, the pre-existing energetic deficit in DCM was exacerbated by exercise (*Figure 2*),[89] supporting the notion that the cardiac metabolic reserve is impaired in T2D.

In 1999, Cline and colleagues used ^{13}C- and ^{31}P-MRS to measure intracellular concentrations of glucose, glucose-6-phosphate and glycogen in gastrocnemius muscle of T2D patients, to demonstrate that insulin-stimulated glycogen synthesis is impaired.[90] Additionally, studies using ^{18}F-fluorodeoxyglucose positron emission tomography showed that T2D patients had lower insulin-stimulated glucose uptake in the skeletal muscle,[91] with either normal[91] or lower[92] glucose uptake in the myocardium. The disparities in the glucose findings may be due to difference in the severity of diabetes.

Multidetector-computed tomography, MRI, ultrasonography, and ^{1}H-MRS have been used to quantify lipid content within an organ, and to examine the association of fat depots with both systemic and local manifestations of disease as the distribution of excess fat may be an important determinant of CV risk.[93] As compared to subcutaneous adiposity, ectopic, and visceral adiposity or 'acquired lipodystrophy' is linked to insulin resistance and diabetes.[94] Epicardial adipose tissue (EAT), a form of visceral fat, has no anatomical barriers with the myocardium and by secreting proinflammatory adipokines and cytokines may play a significant role in diabetic heart. Supporting this, there is a negative correlation between EAT volumes and cardiac contractile function in obese T2D patients.[95]

6. Myocardial structural and functional changes in diabetes

Although an increased LV mass is independently associated with diabetes,[96] often the increase in patients with diabetes is modest. Frequently reported in patients with T2D, LV concentric remodelling represents the main structural characteristic and is more predictive of CV mortality than eccentric remodelling.[20,97,98] Importantly, a stepwise multivariable regression study revealed myocardial steatosis to be the only independent predictor of concentric remodelling in patients with T2D.[20] Although it is tempting to suggest that myocardial steatosis represents a major link between T2D and the development of LV concentric remodelling, a cause-effect relationship has yet to be established.

Interstitial fibrosis has also been implicated in the pathogenesis of LV hypertrophy and occurs in the more advanced stages of DCM.[2] In stable/early DCM the role of interstitial fibrosis is much less clear, as abnormal myocyte hypertrophy rather than fibrosis appears to predominate.[99] Cardiac magnetic resonance (CMR) T1 mapping for extracellular volume (ECV) quantification allows the non-invasive measurement of fibrosis[100] that correlates closely with collagen area in histology.[101] Using this technique, two studies have demonstrated no significant increase in ECV and native (pre-contrast) T1 mapping in young patients with well-controlled T2D, suggesting the absence of significant fibrosis in the presence of LV concentric remodelling and diastolic dysfunction.[20]

Diastolic abnormalities are an early functional defect in the diabetic heart, with the prevalence rates in asymptomatic, normotensive diabetic patients ranging from 15 to 75%.[95,102,103] Yet, there is mild to little systolic dysfunction,[95] which may depend on the severity or duration of disease. Detection of subclinical dysfunction is made available via the use of echocardiographic strain imaging or CMR, with reduced longitudinal contractility and impaired systolic circumferential strain.

7. Rescuing diabetic cardiomyopathy: clinical perspectives

Over the past 10–20 years, the therapeutic approach to the prevention and/or treatment of DCM has largely been aimed at both reducing the incidence of CV events associated with diabetes and halting the progression of diabetic heart towards heart failure. After concerns of cardiac adverse events related to the use of the PPARγ activator, Rosiglitazone, were raised, it has become mandatory for national drug regulatory bodies to enforce the evaluation of CV safety of new anti-diabetic medications. One such medication is Empagliflozin, a renal sodium-glucose cotransporter-2 (SGLT2) inhibitor. Initially designed to evaluate CV safety, the EMPA-REG OUTCOME trial[104] showed significant reduction of CV or all-cause mortality and the incidence of new heart failure, even though the impact on glucose concentrations was modest (about 0.4% reduction of HbA1c over 94 weeks in the empaglifozin arm compared

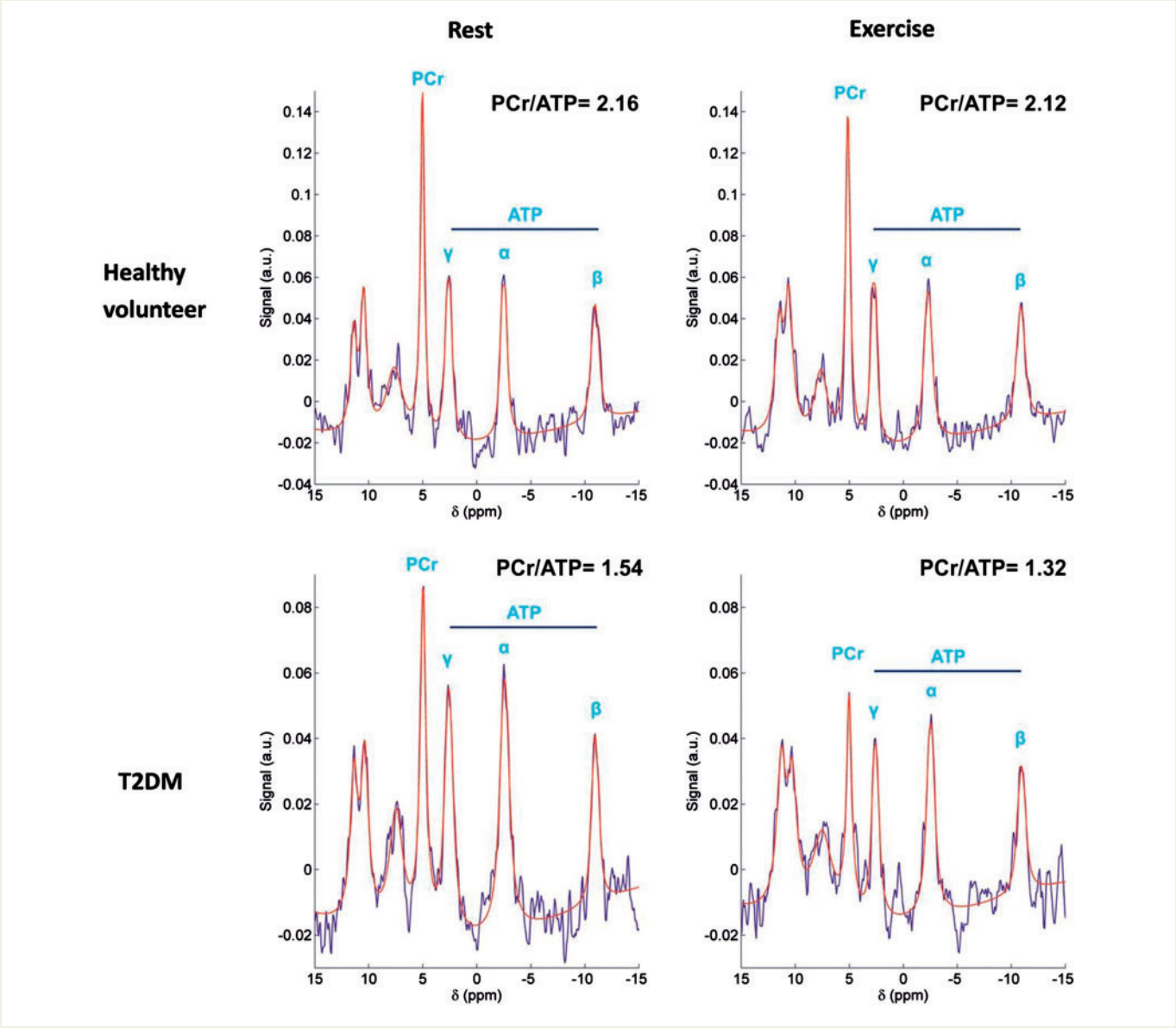

Figure 2 Rest and exercise myocardial ^{31}P-MR spectra in a healthy volunteer (*top row*) and a T2DM patient (*bottom row*). T2DM was associated with significantly lower myocardial PCr/ATP than control at rest, and the decrease was exacerbated during exercise, suggesting a pre-existing myocardial energy deficit in type 2 diabetes mellitus. (Reprinted with permission).

to control).[104] The mortality benefits were remarkable, as the beneficial effect was almost immediate (< 3 months), suggesting that the benefits may well extend beyond SGLT2 inhibition. Explanations for the effects include the inhibition of renin-angiotensin-aldosterone system beyond ACE-inhibition,[105] activation of AMPK,[106] and improved energetics via induction of mild ketosis.[107,108] Plasma concentrations of β-OHB rose from 0.25 to 0.56 mM after 4 weeks of treatment;[107] ketone body oxidation yields more ATP per oxygen consumption than palmitate, so being more more 'energy-efficient'.[109]

A number of other metabolic agents have also been proposed to benefit the diabetic heart. Theoretically PPARγ agonism may be beneficial, but the clinical utility is limited by the associated sodium/water retention properties. Agents that lower substrate availability by means of slowing gastric emptying (such as a glucagon-like-peptide[110]), inhibiting hepatic gluconeogenesis (such as glucagon antagonist and metformin[111,112]), or inhibiting renal reabsorption of glucose (such as SGLT2 inhibitors) have yielded relatively positive CV outcomes.

Newer therapeutic approaches towards DCM have focused on reducing oxidative stress associated with diabetes (for comprehensive review, see ref.[113]). Epidemiological studies show that inhibition of the renin-angiotensin-aldosterone-system reduces CV adverse events in T2D. The mechanism(s) of benefit are not completely understood, although prevention of mitochondrial dysfunction and oxidative stress are commonly postulated. By virtue of reducing cellular oxidative activity, several other agents, including calcium channel antagonists and statins may also limit DCM. Pre-clinical therapies of DCM targeting oxidative stress include upregulation of enzymes such as catalase, superoxide dismutase, glutathione peroxidase or thioredoxin, and antioxidants such as

vitamin C, vitamin E, zinc, resveratrol or coenzyme Q10. Depending on the disease severity, the timing of an intervention, especially on antioxidant enzymes, may be critical, as it is likely that excessive production of free radicals in chronic disease may render such interventions impractical.[47] With the exception of vitamin C and E (which showed no evidence for prevention or reversal of CV events in patients with diabetes),[114,115] other approaches have not yet been examined clinically. Similarly, ruboxistaurin, a protein kinase C-β inhibitor, limits DCM,[116] nephropathy,[117] and retinopathy[118] in animal studies. Although effects on limiting other complications of diabetes have been promising in phase 2-3 clinical trials,[119,120] an effect of ruboxistaurin on DCM has not yet been demonstrated in humans.

8. Conclusions

Metabolic changes in the diabetic heart are complicated. In general, it is characterised by failure of insulin to promote glucose uptake, substrate overload and increased reliance on fatty acid oxidation. The initial adaptation and subsequent maladaptation of the diabetic heart reflects not only a loss of metabolic flexibility, but also abnormal molecular signalling cascades including accumulation of toxic metabolites, upregulation of UCPs, and activation of stress kinases. Significant advances have been made in characterising the myocardial metabolic changes during the development and progression of DCM, including the myocardial metabolic response to ischaemia or hypoxia, and the impact of ageing and gender on the challenges. Although clinical evidence is still lacking, novel therapies targeting oxidative stress and downstream signalling seem promising. Therefore anti-diabetic therapies with pleiotropic actions may remain a mainstream strategy to treat DCM.

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