Lipid metabolism and its implications for type 1 diabetes-associated cardiomyopathy

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Abstract

Diabetic cardiomyopathy was first defined over four decades ago. It was observed in small post-mortem studies of diabetic patients who suffered from concomitant heart failure despite the absence of hypertension, coronary disease or other likely causal factors, as well as in large population studies such as the Framingham Heart Study. Subsequent studies continue to demonstrate an increased incidence of heart failure in the setting of diabetes independent of established risk factors, suggesting direct effects of diabetes on the myocardium. Impairments in glucose metabolism and handling receive the majority of the blame. The role of concomitant impairments in lipid handling, particularly at the level of the myocardium, has however received much less attention. Cardiac lipid accumulation commonly occurs in the setting of type 2 diabetes and has been suggested to play a direct causal role in the development of cardiomyopathy and heart failure in a process termed as cardiac lipotoxicity. Excess lipids promote numerous pathological processes linked to the development of cardiomyopathy, including mitochondrial dysfunction and inflammation. Although somewhat underappreciated, cardiac lipotoxicity also occurs in the setting of type 1 diabetes. This phenomenon is, however, largely understudied in comparison to hyperglycaemia, which has been widely studied in this context. The current review addresses the changes in lipid metabolism occurring in the type 1 diabetic heart and how they are implicated in disease progression. Furthermore, the pathological pathways linked to cardiac lipotoxicity are discussed. Finally, we consider novel approaches for modulating lipid metabolism as a cardioprotective mechanism against cardiomyopathy and heart failure.

Key Words
- diabetes
- lipid metabolism
- cardiomyopathy
- heart
- mouse models

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Introduction

Diabetic cardiomyopathy is associated with changes in the structure and function of the heart, and thus, is typically characterised by fibrosis and hypertrophy, ultimately resulting in cardiac dysfunction. This phenomenon was first observed in the 1970s after post-mortem studies of four individuals exhibiting concomitant diabetes and heart failure, in the absence of hypertension, coronary artery disease (CAD) or other likely causal factors (Rubler *et al.* 1972, Regan *et al.* 1977). Furthermore, the Framingham Heart Study demonstrated that individuals

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with diabetes exhibited a 2.5- to 5-fold increase in the incidence of heart failure, independent of CAD and hypertension (Kannel *et al*. 1974). Although the concept of diabetic cardiomyopathy is often considered in individuals specifically affected by type 2 diabetes (T2D), a metabolically induced cardiomyopathy, independent of hypertension, nephropathy or ischaemic heart disease is also evident in individuals with type 1 diabetes (T1D) (Gotzsche *et al*. 1996). Thus, direct pathological effects of diabetes on the myocardium, regardless of aetiology, likely play a causal role in the development of diabetes-associated cardiomyopathy.

Elevated glucose levels, known as hyperglycaemia, are widely considered as a key contributor to the development of metabolic, structural and functional abnormalities observed in the heart in the setting of diabetes (Huynh *et al*. 2014). Hyperglycaemia results from the inability of hyperinsulinaemia to compensate for insulin resistance, as commonly seen in the setting of T2D. It can also result from insufficient release of insulin from pancreatic beta-cells (due to their autoimmune destruction), as seen in individuals with T1D. Hyperglycaemia has been shown to promote a variety of pathological pathways linked to the development of cardiac abnormalities (Fang *et al*. 2004, Boudina & Abel 2007, Bugger & Abel 2014, Huynh *et al*. 2014). These include the accumulation of advanced glycation end products, altered calcium handling, increased reactive oxygen species (ROS) and activation of the renin-angiotensin system (Fiordaliso *et al*. 2004, Ligeti *et al*. 2006, Goh & Cooper 2008, Yao & Brownlee 2010, Thomas *et al*. 2013). Indeed, a study of almost 50,000 individuals with diabetes demonstrated that, for each 1% increase in glycated haemoglobin, there was an 8% increase in the risk of heart failure (Iribarren *et al*. 2001). Interestingly, this effect was observed independent of hypertension status, consistent with the finding that diabetic cardiomyopathy can occur in the absence of hypertension as mentioned previously (Rubler *et al*. 1972, Regan *et al*. 1977).

A further, but often overlooked, consequence of hyperglycaemia is the modulation of lipid metabolism. Diabetes is associated with impaired myocardial glucose uptake, and thus, there is an increased reliance on fatty acids (FA) as a source of adenosine triphosphate (ATP) in the heart. This effect is potentiated by the presence of dyslipidaemia, which is frequently observed in the setting of diabetes and results in an excess availability of lipids. However, the importance of lipids in the development of diabetic cardiomyopathy has been somewhat underappreciated, particularly in comparison to hyperglycaemia, and forms the basis of this review, as discussed below.

Lipid metabolism and diabetic cardiomyopathy

As far back as the 1970s, Regan and coworkers demonstrated increased deposition of cholesterol and triglycerides in ventricular autopsy specimens of individuals with diabetes compared to those without (Regan *et al*. 1977). Intramyocardial lipid overload in failing human hearts has also been shown to be greater in those with diabetes than those free of diabetes (Sharma *et al*. 2004). Increased myocardial triglyceride accumulation has been associated with increased left ventricular (LV) mass as well as systolic and diastolic dysfunction (Szczepaniak *et al*. 2003, Rijzewijk *et al*. 2008). Interestingly, intramyocardial lipid accumulation has even been observed in diabetic individuals prior to the onset of cardiac dysfunction, further implicating a causal role for lipids in the development of cardiomyopathy (McGavock *et al*. 2007). Cardiac dysfunction due to excess accumulation of lipids, termed as lipotoxic cardiomyopathy or fatty heart, has been an underappreciated clinical entity (Szczepaniak *et al*. 2007); however, increasing evidence has demonstrated the importance of lipids in the development of diabetic cardiomyopathy and heart failure, as will be discussed. It must be noted that dyslipidaemia, or more specifically, elevated levels of low-density lipoprotein cholesterol and triglycerides and low levels of high-density lipoprotein, are commonly observed in individuals with diabetes (Mahaney *et al*. 1995, UKPDS33 1998). Indeed, each of these factors has been associated with an increased risk of CAD (Turner *et al*. 1998). For example, it is well established that oxidised LDL has been shown to drive many pathogenic pathways that contribute to atherosclerotic lesion development (Glass & Witztum 2001). Therefore, it is likely that the cardiac dysfunction observed in individuals with diabetes is a net result of both CAD-driven cardiac dysfunction due to effects of lipids on the vasculature as well as a direct pathological effect of lipids on the myocardium promoting cardiomyopathy (Rubler *et al*. 1972).

Substrate utilisation

The heart is flexible in its ability to use different substrates depending upon their availability, as a source of energy to generate ATP, although in the absence of disease, up to 70% of energy is obtained from the oxidation of

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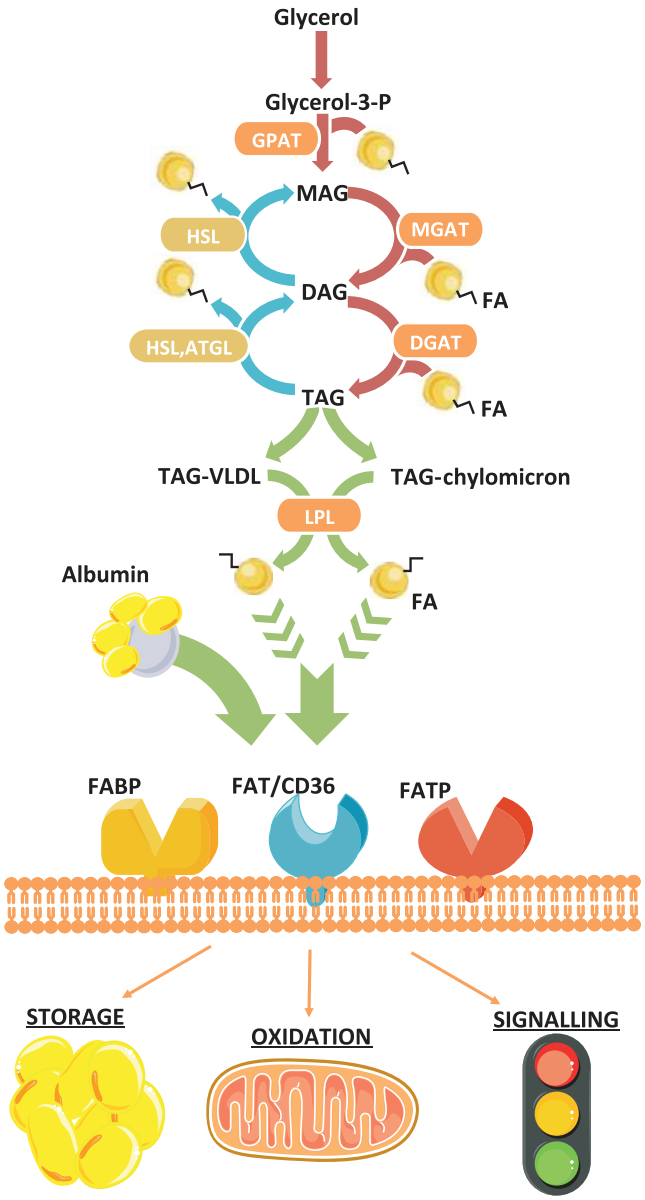

Figure 1
Simplified schema of fatty acid metabolism. ATGL, adipose triglyceride lipase; CD36, cluster of differentiation 36; CGI-58, comparative gene identification 58; DAG, diacylglycerol; DGAT, diacylglycerol acyltransferase; FA, fatty acid; FABP, fatty acid binding protein; FAT, fatty acid translocase; FATP, fatty acid transport protein; GPAT, glycerol-3-phosphate acyltransferase; HSL, hormone sensitive lipase; MAG, monoacylglycerol; MGAT, monoacylglycerol acyltransferase; TAG, triacylglycerol; VLDL, very low density lipoprotein.

FAs (Bing *et al.* 1954, Wisneski *et al.* 1990). In contrast to the liver, the heart has a limited capacity to carry out *de novo* synthesis of FAs (Bayeva *et al.* 2013). Therefore, cardiomyocytes acquire FAs from the circulation or from hydrolysis of stored lipids (Fig. 1). Circulating free FAs bound to albumin can be taken up into cells by transporters such as fatty acid transport protein (FATP) or fatty acid-binding protein. Quantitative studies have, however, determined that facilitated diffusion via cluster of differentiation (CD)36/fatty acid translocase is the major mechanism for FA uptake by cardiomyocytes, contributing up to 70% of total uptake of FAs (Habets *et al.* 2007). Interestingly, Glatz and coworkers have demonstrated the feasibility of targeting CD36 to modulate cardiac substrate utilisation *in vitro* (Glatz *et al.* 2013), and CD36 has been shown to be necessary for the development of lipotoxic cardiomyopathy in mice (Yang *et al.* 2007). In humans, CD36 deficiency is associated with reduced myocardial FA uptake (Kusaka *et al.* 2008). A non-receptor-mediated phenomenon, known as 'flip-flop', in which lipids solubilise within the plasma membrane is also thought to contribute to fatty acid uptake (Kamp *et al.* 1995). In addition to being transported in the circulation bound to albumin, free FAs can be esterified to form triacylglycerols (TAGs), which are transported in the circulation within lipoproteins as chylomicrons or very-low-density lipoproteins. FAs are subsequently released from these lipoproteins at the surface of capillaries via lipoprotein lipase (LPL). Interestingly, mice overexpressing LPL in a cardiac-specific manner exhibit LV systolic dysfunction accompanied by structural features of cardiomyopathy (Yagyu *et al.* 2003). Subsequent to their release, FAs are then transported to the mitochondria where they undergo oxidation or are esterified for storage as TAGs for subsequent hydrolysis dependent upon the energy demands of the cell. Beta-oxidation yields acetyl-CoA, which proceeds into the tricarboxylic acid cycle to generate ATP (Bayeva *et al.* 2013). There is a concomitant activation of pyruvate dehydrogenase kinase (PDK) and inhibition of pyruvate dehydrogenase, which together result in a decrease in glucose oxidation. This allows the heart to 'switch' between energy sources dependent upon availability, a phenomenon described by the Randle principle (Randle *et al.* 1963). Peroxisome proliferator-activated receptor (PPAR)-alpha is a master regulator of many of these processes, modulating the expression of genes involved in FA uptake and modification, as well as lipid and glucose oxidation (Burkart *et al.* 2007). Indeed, overexpression of *Ppar*-alpha in a cardiac-specific manner mimics much of the changes observed in the setting of diabetes including increased FA oxidation and decreased glucose oxidation, with concomitant cardiac hypertrophy and dysfunction (Finck *et al.* 2002).

In settings of both T1D and T2D, the heart becomes almost completely reliant upon FAs as a source of energy. Although the mechanisms by which this occurs are

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distinct for the two major types of diabetes as will be discussed below, there is ultimately an increased uptake and utilisation of FAs in both settings. The pathological consequences of this have been extensively reviewed in detail elsewhere (Poornima *et al*. 2006, Goldberg *et al*. 2012, Bayeva *et al*. 2013, Schulze *et al*. 2016). Thus, we will just briefly address the key pathways by which pathological lipid accumulation is thought to contribute toward the development of cardiomyopathy in the setting of diabetes (Fig. 2).

Pathological effects of lipids in the setting of diabetes

Lipids and their intermediates are in a constant state of flux, and it is their downstream products, including fatty acyl-CoA, diacylglycerol (DAG) and ceramides, that likely mediate much of the lipotoxic effects. Indeed, these pathological lipid species have been shown to interfere with signalling via modulation of serine/threonine kinases such as protein kinase C, jun N-terminal kinase (JNK), IκB kinase and mechanistic target of rapamycin, resulting in lipid-induced insulin resistance, as well as promoting inflammation, apoptosis and hypertrophy (Goldberg *et al*. 2012). The promotion of insulin resistance drives a further increase in insulin levels, which have pathological effects on the heart *per se*. FAs can also influence contractility as elevated levels of fatty acyl-CoA levels within cardiomyocytes can promote the opening of the K_{ATP} channel, which leads to reduced calcium flux (Liu *et al*. 2001). Intramyocardial lipid accumulation can also promote mitochondrial dysfunction, and in turn, impaired mitochondrial function may further lead to an accumulation of lipotoxic medium-chain acyl carnitines, creating a futile cycle of dysfunction and also promoting ROS production (Koves *et al*. 2008). These finding are supported by observations in humans, demonstrating that myofibres from right atrial appendages of diabetic individuals exhibited mitochondria that were impaired in their maximal capacity to oxidise FAs and glutamate, with elevated mitochondrial hydrogen peroxide release (Anderson *et al*. 2009). Finally, FAs can act as ligands for PPAR-alpha, promoting the upregulation of genes involved in FA uptake, transport and oxidation (Burkart *et al*. 2007). Indeed, *Ppar*-alpha overexpression in a cardiac-specific manner promotes a phenotype mimicking diabetic cardiomyopathy, thus further promoting the accumulation of lipids and promoting this futile cycle. The lipid-induced upregulation of these key pathological pathways, namely inflammation, increased ROS production, mitochondrial dysfunction, dysregulated insulin signalling, apoptosis and altered calcium handling, have consequent effects to promote contractile dysfunction, impaired cardiac relaxation and fibrosis, ultimately leading to cardiomyopathy. In support of this, individuals with diabetes exhibit cardiac lipid accumulation in association with cardiac hypertrophy and LV systolic and diastolic dysfunction (Szczepaniak *et al*. 2003, Rijzewijk *et al*. 2008).

The implications of cardiac lipotoxicity are not limited to cardiomyopathy and can also be detrimental in the setting of myocardial infarction. During ischaemia, the heart is reliant on anaerobic glycolysis to generate ATP. Thus, there is a rapid switch from fat to glucose utilisation. However, the consequence of excess lipid availability is a reciprocal reduction in the utilisation of glucose as a substrate (Randle *et al*. 1963). Furthermore, impaired insulin signalling, as a result of lipid-induced insulin resistance, also attenuates glucose utilisation. Indeed, individuals with diabetes not only have an increased risk of myocardial infarction, but also have a poorer prognosis (Aronson *et al*. 1997, Kannel & McGee 1979, Lehto *et al*. 1994). Thus, cardiac lipotoxicity has implications for both cardiomyopathy and myocardial infarction. However, further discussion of ischaemia is beyond the scope of this review.

Much of the discussed studies have been performed in the setting of T2D with regard to both rodent studies and studies in humans. In contrast, there is a paucity of information regarding the mechanisms underlying cardiac lipid accumulation in the setting of T1D, and thus, the remainder of this review will focus on cardiomyopathy in the setting of insulin-dependent diabetes.

Type 1 diabetes: changes to lipid metabolism in the human heart

There are a number of metabolic disturbances in the setting of T1D that are thought to contribute to the cardiomyopathy often seen in these individuals. T1D differs from T2D in that hyperglycaemia occurs much earlier in life and this occurs in the relative absence of hyperinsulinaemia (American Diabetes Association 2009). Due to insulin insufficiency as a result of autoimmune beta-cell destruction, reduced myocardial glucose uptake is observed, and this results in decreased glucose oxidation. As a consequence, the heart becomes heavily reliant on FAs as a source of energy (Herrero *et al*. 2006). This in itself is thought to promote ROS production, mitochondrial dysfunction and altered calcium handling, contributing to the development of cardiomyopathy often observed

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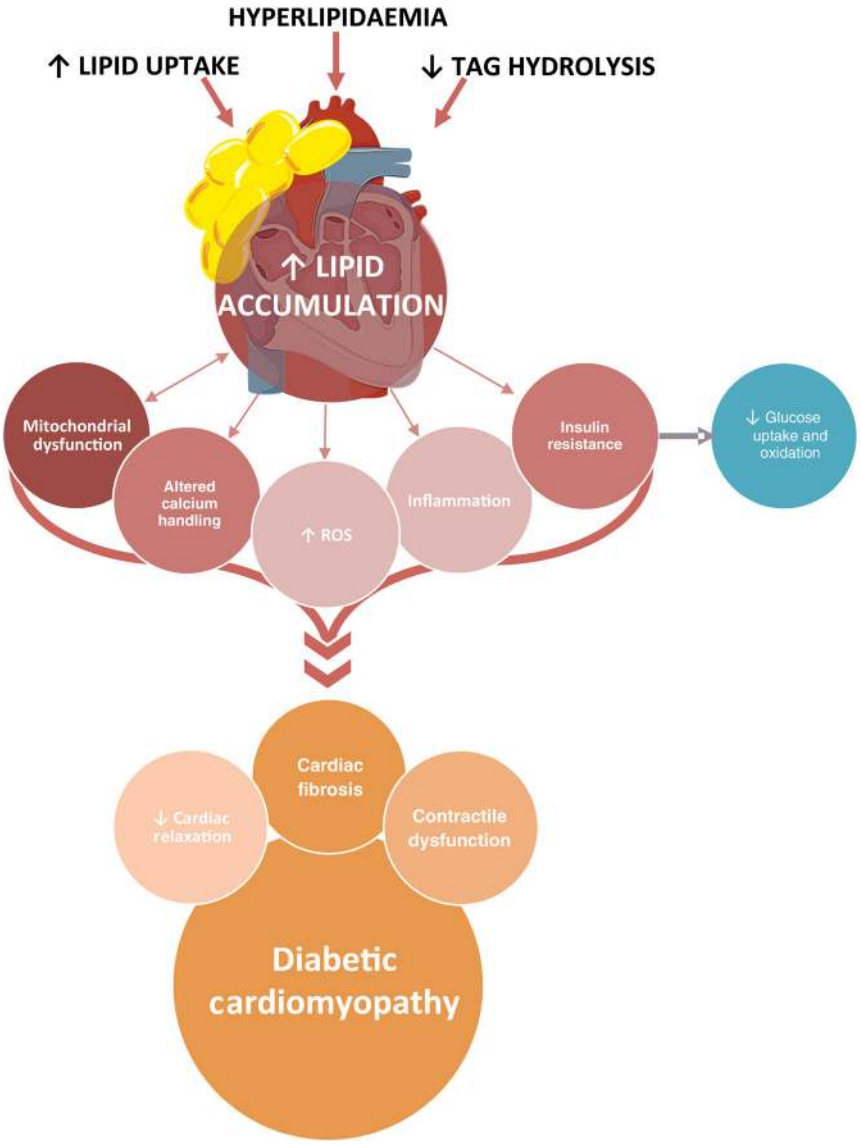

Figure 2
Disturbances in lipid metabolism in the setting of diabetes and their consequential effects on cardiac pathology, structure and function leading to the development of cardiomyopathy. ROS, reactive oxygen species; TAG, triacylglycerols.

in these individuals and as discussed previously (Liu *et al*. 2001, Koves *et al*. 2008). Furthermore, hyperlipidaemia is often present in individuals with T1D, resulting in increased lipid delivery to the myocardium. This further promotes FA utilisation, oxidation and esterification as well as reduced hydrolysis of triglyceride stores.

Herrero and coworkers specifically sought to assess whether higher dependence on FAs occurred in individuals with T1D by quantitating myocardial glucose and FA metabolism. They demonstrated in young females with T1D that myocardial FA utilisation and oxidation were increased, and myocardial glucose uptake was lower, than non-diabetic individuals, despite hyperglycaemia and elevated plasma FA levels (Herrero *et al*. 2006). Doria and coworkers assessed myocardial metabolism in individuals with T1D absent of CAD, and also demonstrated that, despite hyperglycaemia, diabetic individuals exhibited reduced myocardial glucose uptake and significantly increased uptake of ketones and FAs (Doria *et al*. 1991). Furthermore, they demonstrated that net balance of glucose, pyruvate and lactate across the myocardium was inversely related to simultaneous levels of ketone bodies and FAs. These findings on cardiac substrate handling are consistent with the principle of the Randle cycle described previously (Randle *et al*. 1963). Avogaro and coworkers reported similar findings, and because the observed metabolic abnormalities were normalised by the administration of insulin, which restored glycaemia, they postulated that the metabolic disturbances were a consequence of hypoinsulinaemia and hyperglycaemia

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rather than being the primary defect (Avogaro *et al.* 1990). Interestingly, using positron emission tomography, Peterson and coworkers demonstrated that fasted T1D subjects with similar glucose and plasma FFA levels to non-diabetic individuals had similar rates of myocardial glucose use and FA oxidation to non-diabetic fasting control subjects, despite higher insulin levels, also indicating the presence of some degree of insulin resistance (Peterson *et al.* 2008). They also demonstrated that myocardial glucose and FA uptake can be manipulated by altering the plasma environment with regard to hormonal and substrate availability. This emphasises the need to reduce excess FA availability to the heart, highlighting the importance of lipid-lowering therapies in these individuals. This is particularly pertinent in the setting of ischaemia in which there is a predominant requirement for glucose utilisation. Finally, a study by Hammer and coworkers assessed the effects of hyperglycaemia due to 24 h of partial insulin deprivation in individuals with T1D (Hammer *et al.* 2008). Plasma FAs increased by ~50%; however, no effect on myocardial triglyceride content or LV diastolic function (as measured by E/A ratio) was observed. The 24-h time frame is likely too short to see changes in TAG content and a longer period of elevated FAs may be required to elicit the true impact on LV function and preclinical models offer utility in this regard.

Whilst studies have identified specific lipid species that accumulate in the hearts of individuals with T2D, no such studies have been performed in humans with T1D, highlighting a clear knowledge gap, at least in humans. However, much information has been gained from mouse models of T1D, and genetic manipulation of key pathways involved in the regulation of lipid metabolism in these models, as discussed below.

Preclinical models of type 1 diabetes-associated cardiomyopathy

There are a number of mouse models that have been utilised to study diabetic cardiomyopathy in the context of T1D that are generated via either genetic manipulation or chemical induction. The majority of studies focus on three key models, mice treated with the beta-cell toxin, streptozotocin (STZ), the OVE26 mouse and the Akita mouse. The features of each of these models with regard to cardiac pathology are outlined in Table 1, including their advantages and disadvantages. The changes in lipid metabolism observed in each of these models are described below.

Streptozotocin-diabetic mouse

STZ is a beta-cell toxin that promotes insulinitis via induction of type C viruses within beta-cells (Rossini *et al.* 1977). Consequent effects include raised blood glucose levels 5–6 days after administration. Over time, this can develop into overt diabetes dependent upon the susceptibility of the model used. Lipid phenotypes in STZ-diabetic mice vary significantly, and this is likely due to differences in the protocol of STZ administration or the background strain of mice. Indeed, studies vary in the age at induction of diabetes, from 6 weeks to 16 weeks of age, as well as the regimen, from a single dose of 185 mg/kg to 7 doses of 40 mg/kg (Finck *et al.* 2002, 2003, Nielsen *et al.* 2002, Ueno *et al.* 2008, Pulinilkunnil *et al.* 2013). Findings in the different models are described below, in descending order from the highest dose of STZ administered.

Pulinilkunnil and coworkers demonstrated that STZ-induced diabetes via a single dose of 165–185 mg/kg was associated with ~4-fold increase in plasma triglyceride levels (Pulinilkunnil *et al.* 2013). Significant elevations in cardiac TAG, ceramide and palmitoyl CoA accumulation were observed in these mice, although no change in DAG levels was observed. Concomitant elevations in protein expression of the regulator of TAG hydrolysis, adipose triglyceride lipase (ATGL), as well as PPAR-alpha, perilipin (PLIN)-5 and uncoupling protein (UCP)-3 were observed in the hearts of diabetic mice. Finck and coworkers demonstrated a 2- to 3-fold upregulation in the expression of key regulators of lipid handling, *Ppar*-alpha, acyl-CoA oxidase, carnitine palmitoyl transferase and PPAR gamma co-activator 1 alpha in the hearts of mice rendered diabetic via a single dose of STZ at 180 mg/kg (Finck *et al.* 2002). Furthermore, TAG-containing long-chain FAs were elevated in the hearts of diabetic mice compared to their non-diabetic counterparts (Finck *et al.* 2003). Han and coworkers performed lipidomic analysis of cardiac lipids after the induction of diabetes with STZ (165 mg/kg) to 4-month-old C57BL/6 mice (Han *et al.* 2007). They particularly focused on cardiolipins, a subclass of phospholipids that are primarily located on the inner mitochondrial membrane and play a role in mitochondrial bioenergetics and apoptosis. A dramatic remodelling of cardiolipin species with STZ diabetes was demonstrated, with some species being depleted within days of the induction of diabetes and others increasing over the duration of diabetes. Thus, these changes appeared to precede changes in mitochondrial morphology or function or TAG accumulation. The authors postulated that these changes in cardiolipins could precipitate mitochondrial

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dysfunction, resulting in the inability of mitochondria to deal with the increased lipid burden seen in the setting of diabetes, namely TAG accumulation (Han *et al*. 2007). Ueno and coworkers demonstrated that administration of STZ (150 mg/kg) to 12- to 16-week-old C57BL/6 mice until blood glucose readings of 350 mg/dL were achieved resulted in the presence of lipid droplets in hearts after just 3 weeks of diabetes (Ueno *et al*. 2008). Plasma TAG levels were increased by ~70% and cardiac TAG and DAG levels were both significantly elevated in diabetic mice. Interestingly, key genes involved in the regulation of lipid uptake, oxidation, FA release, lipolysis and uptake, namely *Ppar-alpha, Lpl, Atgl*, hormone-sensitive lipase (*Hsl*) and *CD36* were all significantly upregulated in the hearts of diabetic mice. LV interstitial fibrosis and increased collagen deposition were also observed in diabetic mice (Ueno *et al*. 2008). A similar protocol to render mice diabetic via STZ (150 mg/kg) in slightly older C57BL/6 mice (10–14 weeks of age) administered every 3–4 days until blood glucose exceeded 350 mg/dL, resulted in a significant elevation in cardiac TAG, DAG, FA and ceramide accumulation, despite no changes in plasma lipid levels (Kuramoto *et al*. 2014). Consistent with these findings, diabetes was also associated with elevated levels of *Cd36, Fatp* as well as *Plin5*, shown to protect lipid droplets from lipolytic attack (Kuramoto *et al*. 2014). Xu and coworkers demonstrated that a single dose of STZ (150 mg/kg) to C57BL/6 mice was associated with a 2-fold increase in plasma triglycerides and a 1.5- to 2-fold increase in plasma-free FAs from 3 weeks after STZ administration but did not increase further by 9 weeks of diabetes (Xu *et al*. 2013). Yan and coworkers rendered C57BL/6 mice diabetic via 6 injections of STZ (60 mg/kg), resulting in a ~2-fold increase in plasma triglyceride levels as well as increased cardiac TAG levels and *Cd36* mRNA expression by 4-month diabetes duration (Yan *et al*. 2015). Interestingly, plasma levels of fibroblast growth factor (FGF)-21, known for its role in regulating energy homeostasis, were markedly reduced with the induction of diabetes. Given their role in the modulation of mitochondrial function, which has been shown to be an important mediator of diabetic cardiomyopathy, Novgorodov specifically investigated the effect of cardiac sphingolipid metabolism in mice administered with 50 mg/kg STZ for 5 days (Novgorodov *et al*. 2016). Diabetic mice exhibited increased expression of desaturase 1, dihydroceramide synthase 2, serine palmitoyl transferase and the rate of ceramide formation by mitochondrial ceramide synthase, indicative of an upregulation of ceramide biosynthesis; however, no changes in ceramide levels were observed, suggesting a compensatory upregulation of ceramide metabolising pathways (Novgorodov *et al*. 2016). Concomitant mitochondrial defects were observed in these mice. Finally, and in contrast to other studies, Nielsen and coworkers demonstrated no changes in plasma FAs, triglycerides or cholesterol associated with STZ diabetes in C57BL/6 mice; however, they did observe ~50% increase in cardiac TAG levels (Nielsen *et al*. 2002). No changes in other lipids species such as cholesterol, sphingomyelin, phosphatidylcholine or phosphatidylethanolamine were observed in the hearts of these diabetic mice. However, it is possible that changes may have been observed in subspecies that can have functional consequences; however, only total levels were reported for each class. Nielsen and coworkers induced diabetes with a lower dose of STZ (40 mg/kg) more frequently (7 daily injections), a less toxic and more reproducible approach, which may explain the lack of effect on plasma lipids.

OVE26 RIP-calmodulin mouse

Epstein and coworkers first described the OVE26 mouse model (Epstein *et al*. 1989). These mice carry the calmodulin minigene driven by the rat insulin promoter, such that mice exhibit a 5-fold increase in the content of calmodulin in beta-cells. Calmodulin is a calcium-binding messenger protein, which has been implicated in the control of insulin release from the beta-cell. Subsequent studies in OVE26 mice revealed compromised granule formation and apoptosis of beta-cells that preceded the onset of hyperglycaemia, which occurred as early as 2–3 weeks of age as well as decreased glucose-mediated insulin secretion (Epstein *et al*. 1992, Yu *et al*. 2002). Cardiac dysfunction was observed in these mice by 3 months of age. There is limited information regarding the lipid alterations seen in these mice. Studies have reported that OVE26 mice exhibit ~2-fold increases in plasma FAs and ~3-fold increase in plasma triglycerides by 5 months of age on an FVB background, which is supported by the findings of others (Liang *et al*. 2002, Ye *et al*. 2003, Xu *et al*. 2013). However, no studies have assessed cardiac lipid levels in this model.

AKITA Ins2$^{+/-}$ mouse

A more recent mouse model for the study of T1D is the Akita mouse (Yoshioka *et al*. 1997, Wang *et al*. 1999). These mice have a single-nucleotide polymorphism in the insulin 2 gene, *Ins2*, resulting in an amino acid substitution, C96Y. This mutation is associated with

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Table 1 Cardiac features of mouse models of type 1 diabetes.

Model name/parameter	C57Bl/6J+STZ	FVB/N+STZ	OVE26	Akita
Function				
Diastolic function	↓ Decreased (Nielsen *et al.* 2002, Westermann *et al.* 2009, Pulinilkunnil *et al.* 2013, Xu *et al.* 2013, Yan *et al.* 2015, Novgorodov *et al.* 2016)	↓ Decreased (Huynh *et al.* 2010, Wold *et al.* 2006)	↓ Decreased (Zhang *et al.* 2003, Shen *et al.* 2004, Ye *et al.* 2004, Kralik *et al.* 2005, Xie *et al.* 2011)	↓ Decreased (Basu *et al.* 2009, Mishra *et al.* 2010, LaRocca *et al.* 2012, Prathipati *et al.* 2016)
			Normal/minor change (Vadvalkar *et al.* 2013)	Unchanged (Park *et al.* 2009)
Systolic function	↓ Decreased (Nielsen *et al.* 2002, Westermann *et al.* 2009, Pulinilkunnil *et al.* 2013, Xu *et al.* 2013, Kuramoto *et al.* 2014, Novgorodov *et al.* 2016)	↓ Decreased (Wold *et al.* 2006, Xie *et al.* 2011)	↓ Decreased (Zhang *et al.* 2003, Shen *et al.* 2004, Ye *et al.* 2004, Kralik *et al.* 2005, Xie *et al.* 2011, Xu *et al.* 2013)	↓ Decreased (Lu *et al.* 2007)
		Unchanged (Huynh *et al.* 2010)	Unchanged/minor change (Vadvalkar *et al.* 2013)	Unchanged (Bugger *et al.* 2008, Basu *et al.* 2009, Park *et al.* 2009, Mishra *et al.* 2010, LaRocca *et al.* 2012, Prathipati *et al.* 2016)
Structure				
Hypertrophy	↑ Increased (Pulinilkunnil *et al.* 2013) Unchanged (Westermann *et al.* 2009)	↑ Increased (Huynh *et al.* 2010)	↑ Increased (Li *et al.* 2011, Wang *et al.* 2013)	↑ Increased (Chavali *et al.* 2014) Unchanged (Bugger *et al.* 2008, Basu *et al.* 2009)
Fibrosis	↑ Increased (Ueno *et al.* 2008, Westermann *et al.* 2009, Xu *et al.* 2013, Kuramoto *et al.* 2014, Yan *et al.* 2015)	↑ Increased (Huynh *et al.* 2010)	↑ Increased (Li *et al.* 2011, Wang *et al.* 2013)	Unchanged (Basu *et al.* 2009)
Substrate metabolism				
Plasma lipids	↑ Increased (Ueno *et al.* 2008, Pulinilkunnil *et al.* 2013, Xu *et al.* 2013, Yan *et al.* 2015) Unchanged (Nielsen *et al.* 2002, Kuramoto *et al.* 2014)		↑ Increased (Liang *et al.* 2002, Xu *et al.* 2013)	↑ Increased (Bugger *et al.* 2008)
Cardiac lipids	↑ Increased (Nielsen *et al.* 2002, Han *et al.* 2007, Ueno *et al.* 2008, Pulinilkunnil *et al.* 2013, Kuramoto *et al.* 2014, Yan *et al.* 2015, Novgorodov *et al.* 2016)			↑ Increased (Bugger *et al.* 2008, Basu *et al.* 2009, Pulinilkunnil *et al.* 2013)
Glucose oxidation	↓ Decreased (Pulinilkunnil *et al.* 2013)			↓ Decreased (Bugger *et al.* 2008, Basu *et al.* 2009)
Fatty acid oxidation	↑ Increased (Pulinilkunnil *et al.* 2013)			↑ Increased (Bugger *et al.* 2008, Basu *et al.* 2009)
Pathways modulated				
Inflammation	↑ Increased (Westermann *et al.* 2009)		↑ Increased (Wang *et al.* 2013)	↑ Increased (Chavali *et al.* 2014)
Mitochondrial function	Dysfunction/abnormal (Pulinilkunnil *et al.* 2013, Xu *et al.* 2013, Novgorodov *et al.* 2016)		Dysfunction/abnormal (Shen *et al.* 2004, 2006, Ye *et al.* 2004, Xie *et al.* 2011, Vadvalkar *et al.* 2013, Xu *et al.* 2013)	Dysfunction/abnormal (Bugger *et al.* 2008, 2009)

(Continued)

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Table 1 Continued.

Model name/parameter	C57Bl/6J + STZ	FVB/N + STZ	OVE26	Akita
Apoptosis	↑ Increased (Xu *et al.* 2013)	↑ Increased (Wold *et al.* 2006)	↑ Increased (Xie *et al.* 2011, Xu *et al.* 2013)	
Oxidative stress	↑ Increased (Kuramoto *et al.* 2014, Westermann *et al.* 2009, Xu *et al.* 2013, Yan *et al.* 2015)	↑ Increased (Wold *et al.* 2006)	↑ Increased (Liang *et al.* 2002, Ye *et al.* 2004, Song *et al.* 2007, Wang *et al.* 2013, Xu *et al.* 2013)	Unchanged (Bugger *et al.* 2008)
Calcium mobilisation		↓ Decreased (Wold *et al.* 2006)	↓ Decreased (Ye *et al.* 2004, Kralik *et al.* 2005)	↓ Decreased (Lu *et al.* 2007, Bugger *et al.* 2009, Mishra *et al.* 2010, LaRocca *et al.* 2012, Prathipati *et al.* 2016)
Advantages and disadvantages				
Advantages	• Wide range of STZ dose allows for easy manipulation of disease severity • High percentage of successful induction of diabetes with low mortality of experimental animals • Exhibits other complications of diabetes		• Survive >1 year, allowing for long-term effects of diabetes to be investigated in the heart	• Onset of T1D at 3–6 weeks similar to humans (15–25 years) • No confounding effects such as those seen in STZ treatment • Exhibits other complications of diabetes including retinopathy, neuropathy and nephropathy
Disadvantages	• Potential extra-pancreatic toxic effects, particularly with high dose STZ • Severity of diabetes can vary considerably	• Potential extra-pancreatic toxic effects, particularly with high dose STZ	• Develop diabetes in the first weeks postpartum, which may influence cardiac development	• Relatively few studies available to assess cardiac phenotype due to recent generation of model

impaired folding of proinsulin leading to endoplasmic reticulum (ER) stress in pancreatic islets such that Akita mice exhibit progressive loss of beta-cells, which correlates with diabetes development. Hyperglycaemia is evident from 3 to 4 weeks of age. There are a limited number of studies that have described the lipid phenotype observed in these mice, discussed below.

Basu and coworkers reported elevated levels of palmitoyl CoA, oleoyl CoA and steroyl CoA, ceramides, DAG and TAG in hearts of 3-month-old Akita mice compared to their wild-type counterparts (Basu *et al.* 2009). Concomitant elevations in PDK4, CD36 and FATP expression were observed as well as an upregulation of palmitate oxidation. No change in glucose oxidation was observed. Lipid droplets were also observed in Akita mice at 3 months of age, and this was more pronounced at 6 months of age at which time an elevation of long-chain acyl-CoA dehydrogenase was also observed. These changes were observed in the context of diastolic dysfunction; however, myocardial hypertrophy, fibrosis and systolic dysfunction were absent, highlighting the early stage at which perturbation of lipid metabolism within the heart is evident in this model. Pulinilkunnil and coworkers have also demonstrated a marked elevation in myocardial TAG content in Akita mice (Pulinilkunnil *et al.* 2013). A concomitant increase in ATGL expression was observed, although this was insufficient to handle the elevated TAG accumulation observed. Elevated levels of serum FAs and triglycerides have also been observed in this model at 24 weeks of age (Bugger *et al.* 2008). Consistent with the previously mentioned findings, isolated working hearts from Akita mice demonstrated increased palmitate oxidation with a concomitant reduction in glucose oxidation. Despite this, these mice exhibited no change in insulin sensitivity or cardiac efficiency (Bugger *et al.* 2008). Furthermore, no changes in mitochondrial uncoupling were observed, likely due to the absence of elevated oxidative stress. Further studies by Bugger demonstrated elevated cardiac levels of mitochondrial FA oxidation proteins, consistent with their previous findings of increased cardiac FA oxidation in Akita mice (Bugger *et al.* 2009). They also demonstrated compromised cardiac mitochondrial function in these mice and suggested that altered mitochondrial membrane lipid content may have contributed to such a phenotype.

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Mechanistic insights gained from mouse of models type 1 diabetes

There are limited studies examining the underlying mechanisms behind the altered lipid metabolism seen in the setting of T1D. However, the use of knockout and transgenic mice to manipulate key regulators of lipid metabolism in the heart in the setting of T1D has generated some understanding of the metabolic defects that contribute to lipotoxic cardiomyopathy.

Cardiac lipid uptake and hydrolysis

FGF21 is well known for its role in regulating energy homeostasis. Yan and coworkers demonstrated that diabetic *Fgf21* KO mice exhibit no difference in plasma lipid levels compared to their wild-type diabetic counterparts (Yan *et al.* 2015); however, these mice exhibited a significant elevation in cardiac lipid accumulation beyond their wild-type diabetic counterparts. They suggested this enhanced lipid accumulation was likely due to nuclear respiratory factor-2-mediated upregulation of CD36 driving the enhanced oxidative stress, cardiac remodelling and ultimately cardiac dysfunction observed in these mice as assessed by increased 3-nitrotyrosine, 4-hydroxynonenal and the marker of fibrosis, connective tissue growth factor. Reduced ejection fraction and fractional shortening were also observed in these mice.

Pulinilkunnil and coworkers studied the effect of modulating *Ppar* through the use of heterozygous *Atgl* deletion and cardiac-specific *Atgl* overexpression. Deletion of just one allele of *Atgl* was associated with no difference in plasma and cardiac TAG levels, systolic cardiac function, cardiac dilatation in non-diabetic mice; however, diabetic *Atgl* heterozygous mice exhibited features of diastolic dysfunction, including increased mitral valve deceleration and isovolumetric relaxation time. Partial *Atgl* deficiency did not have an impact on the diabetes-induced upregulation of *Ppar*-alpha and its targets, *Ucp-3* or *Plin5* or accumulation of palmitoyl CoA or ceramides; however, lipid droplets were larger and mitochondrial abnormalities were observed in the hearts of these mice. Conversely, mice overexpressing *Atgl* in a heart-specific manner via the alpha-myosin heavy-chain (alpha-*Mhc*) promoter, displayed no difference in plasma TAG levels but were resistant to diabetes-associated myocardial TAG accumulation, lipotoxicity and cardiac dysfunction. *Ex vivo* studies demonstrated enhanced utilisation of glucose and reduced palmitate oxidation. This shift away from lipid utilisation resulted in reduced accumulation of lipotoxic intermediates such as palmitoyl CoA and ceramides compared to that seen in wild-type diabetic mice. Concomitant protection from the diabetes-associated decline in LV systolic and diastolic function was observed in these mice, as indicated by measures including mitral valve E-wave velocity, LV volume and isovolumetric relaxation time. Finally, reductions in the diabetes-associated increase in PPAR-alpha, diglyceride acyltransferase-2, CD36, UCP3 and PLIN5 as well as reduced markers of ER stress and mitochondrial complex protein remodelling were observed in alpha-*Mhc-Atgl* mice compared to their diabetic counterparts (Pulinilkunnil *et al.* 2013). These findings provide the rationale for the protection from diabetes-associated cardiac dysfunction seen in these mice.

HSL also plays a role in hydrolysis acting downstream of ATGL to promote the formation of monoacylglycerols from DAGs. Cardiac-specific overexpression of *Hsl* (alpha-*Mhc-Hsl*) rendered mice resistant to diabetes-associated cardiac steatosis and fibrosis (Ueno *et al.* 2008). Specifically, alpha-*Mhc-Hsl* mice exhibited no difference in plasma lipid levels; however, they were protected from the diabetes-associated increase in cardiac TAG accumulation, lipid droplet formation and mRNA expression of *Ppar-alpha, Gpat* and *Lpl* compared to their wild-type diabetic counterparts. Concomitant reductions in markers of inflammation (nuclear factor kappa-light-chain-enhancer of activated B cells and inducible nitric oxide synthase) and fibrosis (transforming growth factor-beta, matrix metalloproteinase-2, collagen types I, III, IV) and increased antioxidant markers were also observed in alpha-MHC-HSL, highlighting the importance of TAG accumulation in the development of cardiomyopathy.

Altered lipid handling

Overexpression of human apolipoprotein B in a heart-specific manner in STZ-diabetic mice was not associated with any significant changes in plasma triglycerides, FAs or blood glucose (Nielsen *et al.* 2002). Despite this, these mice exhibited protection from the ~50% increase in cardiac TAG accumulation seen with diabetes. Furthermore, cardiac brain natriuretic peptide levels and heart function as assessed by echocardiography were normalised to that seen in non-diabetic mice (Nielsen *et al.* 2002). These findings highlight the effect of cardiac TAG accumulation on the development of cardiac pathology. Moreover, they emphasise the importance of lipoprotein formation to modulate cardiac lipid metabolism.

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Lipid droplets are a means of storing TAGs and other neutral lipids and perilipins reside at the surface of lipid droplets to prevent hydrolysis from lipases such as ATGL. Remarkably, *Plin5*-knockout mice lacked detectable lipid droplets in their hearts and exhibited an attenuation of cardiac accumulation of TAG below the level of non-diabetic mice (Kuramoto *et al*. 2014). Marked reductions in cardiac levels of FAs, DAGs and ceramides were also observed. Diabetic *Plin5*-deficient mice exhibited a concomitant attenuation of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunits, $p47^{phox}$, $p67^{phox}$ and the oxidative stress marker, malondialdehyde, as well as the fibrotic marker, collagen 1. *Plin5* deletion also normalised the diabetes-associated attenuation in fractional shortening.

PPAR-alpha drives the transcriptional regulation of lipid uptake and oxidation, and its expression is upregulated in the myocardium of STZ-diabetic mice. Studies led by Kelly and coworkers investigated the importance of PPAR-alpha in the regulation of cardiac lipid regulation through the use of mouse models with cardiac-restricted *Ppar*-alpha overexpression (alpha-*Mhc*-*Ppar*-alpha) or global PPAR-alpha deletion (Finck *et al*. 2002, 2003). STZ-diabetic *Ppar*-alpha-null mice did not exhibit the diabetes-associated increase in biventricular/body weight ratio seen in diabetic wild-type mice. Conversely, alpha-*Mhc*-*Ppar*-alpha mice exhibited a potentiation of this parameter as well as reduced LV shortening. A concomitant increase in neutral lipid deposition was observed in the hearts of these mice, and mass spectrometry analysis revealed that the most marked increase was in TAG-containing long-chain FAs. The derangements in lipid metabolism, in this case attributed to PPAR-alpha, can have a significant impact on cardiac structure and the development of cardiomyopathy.

Cardiac lipid accumulation and mitochondrial function

Vadvalkar and coworkers observed metabolic inflexibility in the hearts of OVE26 mice (Vadvalkar *et al*. 2013). Specifically, they observed large deficits in mitochondrial respiration in response to non-FA substrates. Indeed, the excess availability of FAs seen in the setting of diabetes likely results in incomplete oxidation of FAs which in turn, results in mitochondrial dysfunction and insulin resistance and may promote increased production of ROS (St-Pierre *et al*. 2002, Koves *et al*. 2008, Zhang *et al*. 2010). The interaction between these pathways is highlighted in Fig. 2. Others have demonstrated cardiac mitochondrial dysfunction in the setting of diabetes secondary to modulations of lipid metabolism as described in the abovementioned models (Han *et al*. 2007, Ueno *et al*. 2008, Pulinilkunnil *et al*. 2013). Moreover, modulation of lipid metabolism can restore the mitochondrial dysfunction seen in T1D. This is exemplified by Novgorodov and colleagues who increased lactosylceramide via knockdown of neutral ceramidase (Novgorodov *et al*. 2016). As mentioned previously, Han and coworkers postulated that early changes in cardiolipin species play a causal role in the mitochondrial dysfunction seen in T1D, which results in an inability of mitochondria to deal with the excess lipid burden in this setting (Han *et al*. 2007). The role of mitochondrial dysfunction and ROS accumulation in the setting of diabetes warrants further discussion but is beyond the scope of the current review. Nevertheless, reversal of mitochondrial dysfunction or increasing antioxidant levels have both been shown to improve cardiac function in models of T1D (Ye *et al*. 2003, Shen *et al*. 2005, 2006, Baseler *et al*. 2013).

Cardiac lipid accumulation and inflammation

Dong and coworkers studied the role of toll-like receptor (*Tlr*)-4 on cardiac lipid accumulation and function utilising non-obese diabetic (NOD) $Tlr4^{+/+}$ and NOD $Tlr4^{-/-}$ mice (Dong *et al*. 2012). NOD mice are a model of autoimmune disease that spontaneously develop diabetes due to autoreactive T lymphocyte infiltration and destruction of pancreatic beta-cells (Makino *et al*. 1980). They observed no significant differences in plasma triglyceride and FA levels between diabetic NOD $Tlr4^{+/+}$ and NOD $Tlr4^{-/-}$ mice. In contrast, cardiac lipid accumulation, as assessed by oil red O-staining, was significantly reduced in *Tlr*4-null NOD mice up to 3 months after diabetes development, after which no difference was seen between groups. This suggested a role for TLR4 in cardiac lipid accumulation in early diabetes. Concomitant reductions in myeloid differentiation primary response gene 88, p38 mitogen-activated protein kinase (MAPK) and LPL were observed at 1 and 2 months of diabetes. Furthermore, an increase in phosphorylated adenosine monophosphate-activated protein kinase and acetyl-CoA carboxylase as well as an attenuation in phosphorylated JNK were observed in the absence of *Tlr*4. Diabetes-associated cardiac dysfunction was somewhat ameliorated by the deletion of *Tlr*4, providing a key link between inflammation, lipid accumulation and their effects on cardiac dysfunction.

Together, these studies demonstrate that cardiac lipid accumulation can occur in the absence of elevated

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plasma lipid levels in the setting of diabetes, highlighting the importance of disturbances in cardiac metabolism to promote such a phenotype. Moreover, these studies highlight the significance of altered lipid metabolism to the development of diabetic cardiomyopathy, with inflammation, ROS and mitochondrial function all playing roles in this process.

Targeting lipid metabolism as a therapeutic approach to attenuate diabetic cardiomyopathy

The abovementioned studies emphasise the causal role and impact of disturbances in lipid metabolism on the development of diabetic cardiomyopathy. Moreover, they provide rationale for modulation of lipid pathways to correct the metabolic disturbances seen in the heart in the setting of diabetes and ultimately improve or prevent the cardiac dysfunction seen in this setting.

Considerable research attention has been paid to targeting individual pathological pathways such as ROS accumulation or inflammation in the treatment of cardiomyopathy. As highlighted previously, changes in lipid metabolism are suggested to be upstream of these various pathways. Therefore, targeting lipotoxicity is a particularly attractive therapeutic strategy as it has the potential to mediate beneficial effects on numerous pathways. Many of the abovementioned key regulators of lipid metabolism provide therapeutic potential for the treatment of cardiomyopathy in the setting of diabetes. However, signalling pathways that provide consequential benefit via changes in lipid metabolism also offer promise. For example, previous studies have shown that deletion of MAPK-activated protein kinase-2 (MK2), a downstream mediator of p38 MAPK, has benefits on ischaemia-reperfusion and pressure overload-induced hypertrophy (Shiroto *et al*. 2005, Streicher *et al*. 2010). In recent findings, Ruiz and coworkers further demonstrated that deletion of *Mk2* was associated with protection against STZ diabetes-induced cardiomyopathy (Ruiz *et al*. 2016). Indeed, diabetic *Mk2*$^{-/-}$ mice did not exhibit the elevated plasma-free FA levels, diastolic dysfunction, increased beta-*Mhc*/alpha-*Mhc* ratio, decreased SERCA2a levels or attenuated p38MAPK expression seen in Mk2$^{+/+}$ diabetic mice. However, a reduction in plasma glucose levels and glycated haemoglobin was observed in these mice. Thus, it is difficult to ascertain how much the observed improvements in cardiac function can be attributed to direct effects on the heart or are secondary to systemic alterations due to the global nature of Mk2 deletion. Furthermore, the relative contribution of lipids to this phenotype is unclear.

Given the heterogeneous nature of lipid metabolism biology throughout the body, it would be anticipated that the most appropriate strategy would be a tissue-specific approach. Gene therapy provides such an approach and is being tested in clinical trials worldwide with over 2000 trials having been completed, underway or approved (http://www.abedia.com/wiley/index.html). This includes viral approaches such as adenovirus making up 21.7% of studies, retrovirus (18.3%) and adeno-associated virus (AAV; 6.7%) as well as naked/plasmid deoxyribonucleic acid (DNA) (17.4%). 177 of these studies have been or are targeted at cardiovascular disease.

AAVs provide a particularly attractive strategy, and the first gene therapy approved in the western world was an AAV vector expressing *Lpl*, for the treatment of autosomal recessive *Lpl* deficiency (Kaeppel *et al*. 2013). AAV is a small, human non-pathogenic virus of the parvovirus family and is the only DNA virus that mediates site-specific integration into the genome (Kotin *et al*. 1990, Samulski *et al*. 1991). All viral genes are usually absent in AAV vectors and thus they are unable to integrate into the host genome in either a host genome or random way; yet, they retain their infectious properties (Zacchigna *et al*. 2014). Their advantages have been reviewed elsewhere (Zacchigna *et al*. 2014) but include their ability to deliver DNA to post-mitotic tissues for long periods of time, advantageous for targeting the heart. There are currently 10 open trials in phases 1–3 using AAVs for the treatment of cardiovascular disease, with a majority targeting *Serca2* for the treatment of heart failure (http://www.abedia.com/wiley/index.html). Thus, AAVs provide a promising approach for the treatment of lipid derangements in the heart. Moreover, studies defining the importance of disturbances in lipid metabolism to cardiac dysfunction continue to provide a rationale for the use of such therapies as interventions for the treatment of diabetic cardiomyopathy.

Conclusion

Cardiac lipid accumulation plays an underappreciated but central role in the promotion of key pathological pathways linked to the development of diabetic cardiomyopathy. Studies in rodent models have given insight into the changes in lipid metabolism that lead to the pathological accumulation of lipids in the heart in

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the setting of T1D; however, limited studies in humans have provided validation of these studies. Indeed, future studies in humans are required to identify key lipid species that accumulate in the T1D heart as well as to validate key pathways involved in this process and the subsequent metabolic disturbances. Nevertheless, studies in preclinical models are critical to the identification, understanding and validation of novel candidates, which have the potential to be targeted for therapeutic intervention in the treatment of diabetic cardiomyopathy.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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