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REVIEW

Lipotoxicity in type 2 diabetic cardiomyopathy

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As obesity and type 2 diabetes are becoming an epidemic in westernized countries, the incidence and prevalence of obesity- and diabetes-related co-morbidities are increasing. In type 2 diabetes ectopic lipid accumulation in the heart has been associated with cardiac dysfunction and apoptosis, a process termed lipotoxicity. Since cardiovascular diseases are the main cause of death in diabetic patients, diagnosis and treatment become increasingly important. Although ischaemic heart disease is a major problem in diabetes, non-ischaemic heart disease (better known as diabetic cardiomyopathy) becomes increasingly important with respect to the impairment of cardiac function and mortality in type 2 diabetes. The underlying aetiology of diabetic cardiomyopathy is incompletely understood but is beginning to be elucidated. Various mechanisms have been proposed that may lead to lipotoxicity. Therefore, this review will focus on the mechanisms of cardiac lipid accumulation and its relation to the development of cardiomyopathy.

Keywords Lipotoxicity • Diabetic cardiomyopathy • Diabetes • Lipid accumulation • Mitochondria

1. Introduction

Type 2 diabetic patients are at a significantly greater risk of developing both micro- and macrovascular disease.[1,2] Even after adjusting for concomitant risk, diabetic individuals remained at increased risk of heart failure. This phenomenon was first described by Rubler *et al.*[3,4] in 1972 in diabetic patients with heart failure but without hypertension or coronary artery disease. Since then this phenomenon has been confirmed by large epidemiological studies. This has led to the recognition of a new clinical entity now termed 'diabetic cardiomyopathy'. This disease is defined by structural changes in the heart, such as increased left ventricular (LV) mass, fibrosis and a dilation of the ventricles[5] in the absence of ischaemia or alterations in blood pressure.[3,6] Diabetic cardiomyopathy is mainly characterized by diastolic dysfunction, which may precede the development of systolic dysfunction.[7,8] Although the prevalence of this disease without any other cardiovascular co-morbidities is still quite rare in type 2 diabetes,[9,10] the co-incidence with micro- and macrovascular disease, might aggravate the existing pathology and lead to an increased mortality and morbidity in type 2 diabetes.[11,12] Therefore, a better understanding and treatment of this disease is needed.

It has been well established that indeed the greater incidence of physical inactivity, hypertension and hyperlipidaemia in obese patients are the main contributors to the development of cardiac disease in obesity and type 2 diabetes.[13,14] Furthermore, the reversal of these effects by reducing body mass and introducing a more physically active lifestyle appear to significantly reduce the risk of cardiovascular diseases.[15,16] Nonetheless, the underlying mechanisms are incompletely understood. There are many different theories about the mechanisms leading to structural changes of the diabetic heart.[17,18] As lipid accumulation and plasma free fatty acid (FFA) levels are increased in the obese state and are normalized after life-style intervention with a reduction in body mass, the negative effects of lipid accumulation have gained more attention in order to explain the aetiology of diabetic cardiomyopathy. Therefore, in this review we will focus on lipotoxic mechanisms possibly leading to diabetic cardiomyopathy in obesity and type 2 diabetes. Roughly these mechanisms can be divided into two main categories (i) lipotoxic mechanisms that impair cardiac energy metabolism and (ii) mechanisms that lead to lipoapoptosis.

Regarding cardiac energy metabolism, O_2 consumption of the heart has been shown to be increased and ATP/O ratios to be lower in type 2 diabetic hearts and in hearts under fatty acid (FA) infusion, indicating a decrease in cardiac efficiency in the prediabetic as well as in the diabetic state.[19–21] Possible underlying mechanisms will be discussed in more detail in Section 3.1.

At the basis of cardiac lipotoxicity is the excessive accumulation of fat or FA intermediates in cardiomyocytes. Therefore, understanding cardiac lipid uptake and mechanisms involved in the upregulation of cardiac lipid uptake is needed in order to understand the development of cardiac lipid accumulation and ultimately cardiac lipotoxicity.

2. Why does fat accumulate in cardiac muscle?

2.1 Role of FA uptake and transporters

Chronic overnutrition is associated with increased plasma concentrations of free fatty acids (FFA),[22,23] which are probably due to an

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increased adipose tissue insulin resistance.[24,25] A chronic nutrient overload leads to an increase in adipose tissue, and if the storage capacity and expandability of these adipocytes is low, the adipocytes will become hypertrophic. This may in turn create local hypoxia and lead to the release of pro-inflammatory factors. The latter has been shown to be able to suppress insulin signaling. In insulin-resistant adipocytes, lipolysis is incompletely suppressed, which causes an increased release of FFAs (for a more detailed overview see review[25]) leading to cardiac lipid accumulation.[26–28]

The increase in plasma FFA has been associated with an increased cardiac fatty acid (FA) uptake. For instance, in ob/ob mice (leptin-deficient mice), obesity increased plasma FA availability and resulted in an increased cardiac lipid content.[29] This mechanism is not pathological *per se*, as under physiological conditions such as fasting or exercise, myocardial lipid content can be elevated significantly.[30,31] This illustrates that elevated circulating FFA levels augment cellular uptake and stimulate storage of triglycerides. However, this raises the question of how FFA uptake is regulated in cardiac muscle and if this regulation is altered in obesity and type 2 diabetes.

Uptake of plasma FFAs can occur through passive diffusion via a so-called flip-flop mechanism.[32] Transbilayer flip-flop is the process by which lipids are moved between the two leaflets of the membrane bilayer spontaneously.[33] A protein-mediated process facilitates this FA transport. For cardiac tissue there are three recognized groups of FA transporting proteins: CD36 (human homologue of FA transporter protein FAT), FABP-pm (plasma membrane fraction of FA-binding protein) and FATP1,4 and 6 (FA translocase Protein 1, 4 and 6).[34]

In the heart FAT/CD36 and FABP-pm appear to be key transporters involved in FA uptake.[35] CD36 was shown to be responsible for up to 60% of the FA uptake in the heart.[36] Overexpression of CD36 in cardiac muscle increased the rate of FA uptake and increased FA metabolism, whereas knockdown of CD36/FAT reduced FA uptake and metabolism.[37,38] Similar to GLUT4, CD36 is present in cellular vesicles and can be translocated to the cell membrane rapidly upon acute stimulation by insulin, muscle contraction or AMP-kinase (AMPK) activation. This ability of CD36 to be able to translocate to the plasma membrane and thereby stimulate FA uptake under certain conditions is specific for CD36. Furthermore, CD36 can traffic between the endosomes and the nuclear membrane, whereas the other FA transport proteins do not have this capability.[39] Longer term regulation of CD36 involves ubiquitination. It was shown that insulin attenuates ubiquitination, thereby increasing the availability of CD36 for translocation and uptake of FFA. In contrast, FFA enhances ubiquination, thereby increasing CD36 degradation and creating a negative feedback.[40]

Although total concentration of CD36 was unchanged in obesity and diabetes, permanent translocation of CD36 to the plasma membrane seemed to occur.[41–43] It is suggested that the resulting increased uptake of FAs may lead to substrate competition with glucose, and therefore CD36 may also play an important role in the development of lipid-induced insulin resistance.[41,42]

Cardiac-specific FATP-1 overexpression has also been shown to increase FA uptake by 4-fold and cardiac lipid accumulation by 2-fold.[44] It has been speculated from studies in skeletal muscle that FATP-1-facilitated FA uptake mainly serves to fuel oxidation, though evidence concerning the heart is still lacking. Also, very little is known about the physiological stimuli that may regulate the expression of FATP-1.[45] FATP-4 is expressed in cardiac tissue, but the contribution to lipid uptake in cardiac tissue is still undetermined.[34,46]

FATP-6 is only expressed in the heart and has been shown to enhance FFA transport in cultured cardiomyocytes.[47] FATP-6 only promoted FA uptake slightly when expressed in yeast, and its role *in vivo* is still under debate.[48,49]

2.2 FA storage

Facilitated uptake of circulating FAs is an important regulatory step that affects overall cardiac fat content. However, next to FA uptake, triglyceride storage and lipolysis inside cardiomyocytes will ultimately determine the fate of the FAs.

One of the genes that has been intensively studied in the context of cardiolipotoxicity is Glycerol-3-phosphate acyltransferase (GPAT). GPAT is the rate-limiting step in TG synthesis, which can be inhibited by ACC-stimulated AMPK activation via malonyl-CoA. In some,[50,51] although not all[52] rodent models of diabetes, GPAT activity was found to be enhanced in cardiac tissue. Interestingly, deletion of GPAT1 protected mice from the effects of a high fat diet on cardiac dysfunction and fat accumulation.[51] So far, it remains unclear whether GPAT activity is increased in human diabetic cardiomyopathy. Another gene that has received considerable interest is diglyceride acyltransferase (DGAT). Thus, increased DGAT activity has been linked to lipid accumulation and cardiomyopathy. Glenn *et al.*[53] recently found increased lipid accumulation in a cardiomyocyte-selective DGAT1 transgenic mouse model. On the other hand, when DGAT1 overexpression was crossed with a model of lipotoxic cardiomyopathy, it seemed to protect against the development of cardiac dysfunction.[54] Therefore, it seems that DGAT might play a dual role in the development of lipotoxic cardiomyopathy.

Ueno *et al.*[55] investigated the role of HSL, which is involved in lipolysis of TG and DAG. In this study heart-specific HSL overexpressing transgenic mice (MHC-HSL) were used and diabetes was induced by administration of streptozotocin. Diabetic MHC-HSL mice had no lipid droplet formation in the heart upon a high fat diet, unlike the wild-type (WT) animals, thus demonstrating that the regulation of lipolysis is an important determinant of cardiac lipid content. Also, ATGL deficiency has been associated with a severe form of cardiac lipid accumulation and the development of cardiomyopathy both in mice[56,57] and in humans.[58] Therefore, a decent function of ATGL seems crucial for maintaining a normal cardiac function.

Taken together, a dysbalance between FA uptake, TG synthesis, and lipolysis may result in net fat storage in the heart, ultimately leading to cardiac fat accumulation. In Section 3.1.1 we will also discuss how alterations in FA and glucose oxidation may affect cardiac function and induce insulin resistance of the heart.

3. The association between cardiac steatosis and cardiomyopathy in type 2 diabetes

3.1 Impaired cardiac energetics

Low cardiac ATP production is associated with a decreased contractility in the absence of ischaemia.[19,59] For cardiomyocyte contraction, interactions between ATP and calcium are required in appropriate amounts.[60] Therefore, a decrease in ATP or impaired calcium handling, could drastically impair cardiac contractility (Figure 1).

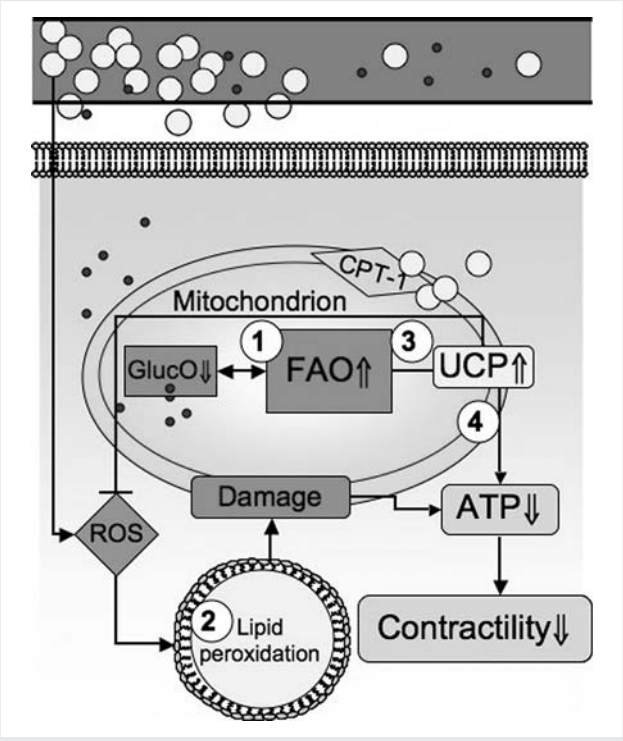

Figure 1 Mechanisms involved in decreasing cardiac energetics. (1) Change in substrate supply increases FA oxidation, decreases glucose oxidation and reduces ATP/O ratios. Furthermore, increased FA oxidation is associated with an increased ROS production. ROS oxidizes lipids stored in the cytoplasm into lipid peroxides (2), inducing cellular damage, as well as mitochondrial damage, further decreasing mitochondrial function. The presence of FAs also stimulates uncoupling (3), further decreasing energy production and contractility (4). On the other hand, uncoupling may also be cardioprotective by decreasing ROS production.

3.1.1 Altered substrate metabolism

The notion that type 2 diabetes leads to an altered regulation of cardiac metabolism is well established.[61,62] In the prediabetic and diabetic states, myocardial O_2 consumption and FA oxidation are increased and cardiac efficiency is decreased.[19,21] In type 2 diabetic patients a 2-fold increase in cardiac palmitate oxidation and a 30–40% decrease in glucose oxidation have been shown.[20,21] In *db/db* mice (mice with a defect in the leptin receptor) alterations in substrate metabolism were paralleled by a decreased contractility eventually leading to cardiomyopathy.[61,63] Other studies, performed with isolated working hearts of diabetic animals, confirmed that a high rate of FA oxidation is associated with ventricular dysfunction.[59,64] These results strongly suggest a causative role for altered substrate metabolism in the development of cardiomyopathy in db/db diabetic hearts.

For a long time, it was thought that the Randle cycle was the main contributor to the biochemical shift towards FA uptake and oxidation in type 2 diabetes. Randle proposed that a higher rate of release of FAs and ketone bodies for oxidation was responsible for the decrease in glucose oxidation and uptake[65]. Though substrate competition plays an important role in type 2 diabetes, increasing evidence suggests that there are also some cellular maladaptations that are important in the regulation of substrate oxidation. The gene regulatory pathway of peroxisome proliferator-activated receptor alpha (PPAR-α) was identified as an important determinant of the shift in substrate metabolism in type 2 diabetes. In mice with cardiac-restricted overexpression of PPARα (MHC-PPAR), it was found that PPAR-α is involved in the upregulation of CPT-1 in mitochondria, which increases the uptake of long-chain fatty acid into mitochondria and facilitates the FAs to undergo beta-oxidation.[66] In the same mouse model, the increase in FA oxidation was paralleled by decreased LV function on a high-fat diet. Paradoxically, chronic exposure to elevated FFAs seems to down-regulate PPAR-α in rodent cardiomyocytes. This down-regulation has been proposed to further decrease cardiac function by inhibition of FA oxidation and increased intracellular fat accumulation.[67,68] Human studies on PPAR expression in the heart are scarce. Marfella *et al.*[69] showed an increased expression of sterol regulatory element-binding protein 1c (SREBP1c) and PPAR-γ in patients with the metabolic syndrome, which was associated with an increased fat accumulation in the heart and poor cardiac function. Although PPAR-α was not changed in these subjects, the increased expression of SREBP1c and PPAR-γ might indicate that there are some aberrations in PPAR-regulated pathways, which lead to an increased fat uptake and storage in the metabolic syndrome. Conversely, other studies such as that of Anderson *et al.*,[21] did not detect a change in PPAR-α expression in the hearts of type 2 diabetes patients. Therefore, it remains unclear whether these mechanisms found in animal studies can be fully translated from animal studies to the human condition.

Although we focus on the negative effects of lipid accumulation in this review, it is important to note that the fate of glucose and FAs are intertwined. Besides inducing lipid accumulation and stimulating fat oxidation, PPAR-α activation can also decrease glucose utilization by increasing the expression of pyruvate dehydrogenase kinase 4 (PDK-4).[70] It is hypothesized that this mechanism protects against glucose overload in addition to the already present lipid overload and thereby making the heart insulin resistant.[65,71] This again contributes to a more rigorous shift in substrate metabolism towards FA oxidation. In a chronic state, this lipid-induced PDK-4 activity leads to increased intracellular glucose concentration, resulting in glucotoxicity. Chronically, a high glucose concentration increases the flux through the hexosamine biosynthethic pathway and increases *N*-acetyl-glucosamine production. This has been associated with insulin resistance, ROS production, hypertrophy and apoptosis in perfused hearts and cell studies, thus augmenting the lipotoxic effects.[72–74]

So it seems that the PPARα-induced increase in fat utilization in the diabetic heart may initially serve as a compensatory mechanism to adjust substrate oxidation to supply, though chronic derangements in cardiac metabolism may have maladaptive consequences, including glucotoxicity and functional cardiac abnormalities. However, the role of these metabolic changes in the development of cardiac dysfunction is poorly understood.

The shift in substrate oxidation itself is often hypothesized as one of the main factors contributing to the development of contractile dysfunction in diabetes.[75,76] It is suggested that reliance on FA oxidation will reduce cardiac energy efficiency, and thereby contributes to cardiomyopathy. Theoretically, more oxygen is required for the production of ATP from FAs compared with glucose.[77] Thus, when shifting from 100% palmitate oxidation to 100% glucose oxidation, a 12% decrease in the amount of oxygen required for ATP synthesis would occur.[77] However, a total shift from fat to glucose oxidation will never occur under physiological conditions. Therefore, while increased concentrations of exogenous FAs resulted in a marked

increase in basal myocardial oxygen requirements,[78,79] this increase in oxygen requirement and accompanying decrease in ATP production is too large to be explained solely by a shift from glucose to fat oxidation.[78]

3.1.2 Mitochondrial uncoupling, dysfunction and ROS production

Uncoupling describes the lowering of the proton gradient over the inner mitochondrial membrane without production of ATP. In this way, uncoupling lowers the production of mitochondrial ATP, thereby making the heart less efficient, which could contribute to the development of cardiac contractile dysfunction in type 2 diabetes.

In isolated hearts of ob/ob mice, perfusion with FFA compared with glucose led to increased oxygen consumption and a reduced ATP/O ratio. These changes in ATP/O ratio were too large to be accounted for by changes in substrate metabolism, which was interpreted as increased mitochondrial uncoupling.[75,80] Indeed, the increase in FA metabolism in these hearts was associated with an increased expression of mitochondrial uncoupling proteins.[81] Similar results were found for *db*/*db* mice.[63] It is known that FAs can induce uncoupling via PPAR-α-induced upregulation of UCP3.[67] Also in humans, the expression of UCP3 was related to circulating plasma FA levels.[82] In addition to an effect of FA on the induction of uncoupling proteins, FAs themselves may also activate the process of mitochondrial uncoupling themselves.[83,84] This uncoupling effect is due to a cyclic movement of undissociated FAs with the release of protons into the matrix.[85,86]

Though uncoupling might be detrimental for cardiac efficiency, a protective function has also been described. It is hypothesized that mitochondrial uncoupling serves to limit the production of reactive oxygen species by the mitochondria, as it has been shown that membrane potential displays a negative relationship with mitochondrial ROS production.[87,88] This suggests that a very mild lowering of the proton gradient may already markedly lower the production of ROS.[20,89] ROS can damage mitochondria and other cellular components by oxidizing proteins, converting lipids into reactive lipid peroxides, increase protein tyrosine nitration and damage DNA.[90,91] If extensive damage occurs, this can lead to mitochondrial dysfunction, altered cellular function or even apoptosis. Mitochondrial ROS production has been shown to impair contractility of cardiomyocytes *in vitro*.[92] Boudina *et al.*[93] showed an increased mitochondrial H_2O_2 production in cardiomyocytes of *ob*/*ob* mice. This was not only paralleled by increased cardiac lipid accumulation and mitochondrial dysfunction, but also by an increase in ROS scavengers in the heart.[63] Also in *db*/*db* mice an increased ROS production was found, which was associated with an increased apoptosis of cardiomyocytes.[68,94] The study of Anderson *et al.*[21] is the only study in humans indicating an increase in cardiac ROS production. They found that diabetic patients have an increased mitochondrial H_2O_2 emission during oxidation of lipid-based substrates compared with carbohydrate-based substrates and depleted glutathione, which are evidence of persistent oxidative stress in atrial tissue.[21]

As mentioned above, ROS can have direct negative effects on mitochondria, thereby impairing cardiac energetics. If ROS concentrations are indeed elevated in the diabetic heart, these patients are expected to be at increased risk for mitochondrial dysfunction. Indeed, it has been shown that the intrinsic function of cardiac mitochondria in type 2 diabetes is decreased. In mouse models of type 2 diabetes and obesity, myocardial mitochondrial function and ATP synthesis rates were impaired which was associated with cardiac dysfunction and lipid accumulation.[95–97] Boudina *et al.*[63] showed a decreased intrinsic mitochondrial function in db/db mice, measured in permeabilized cardiac fibres. This was paralleled by a decreased ATP production, lower ATPase expression, and a 2.2-fold increase in cardiac lipid accumulation. Palmitate infusion in these animals further deteriorated mitochondrial function, as uncoupled respiration increased and ATP/O ratios decreased, thus indicating induction of mitochondrial uncoupling in addition to the already existing deficit in mitochondrial respiration.[63] How *et al.*[97] showed that cardiac efficiency, expressed as the ratio of cardiac work [pressure–volume area (PVA)] over myocardial oxygen consumption (MVO_2), decreased as MVO_2 increased by around 86 and 57% in *db*/*db* and STZ-administered hearts, respectively. In another study[98] they showed that perfusing isolated working hearts with a higher concentration of FAs did not change the performance or contractile efficiency, though it did increase oxygen consumption, thus indicating an FA-induced increase in the unloaded MVO_2. This effect was more pronounced in the hearts of *db*/*db* mice. These data suggest that increase in FA availability makes the heart less efficient.

In human studies direct evidence of decreased cardiac mitochondrial function in type 2 diabetes comes from the study of Anderson *et al.*[21] They measured *ex vivo* mitochondrial respiratory capacity in permeabilized myofibres derived from atrial tissue of type 2 diabetic patients and found lower rates of respiration in diabetic patients. These findings were paralleled by increased cardiac lipid accumulation, indicating that lipid accumulation may be associated with impaired mitochondrial function. Furthermore, impaired intrinsic mitochondrial function of the diabetic heart is supported by studies using 31- P magnetic resonance spectroscopy (MRS) in humans. MRS can be used to determine energy status of the heart *in vivo* non-invasively by determining the phosphocreatine/ATP ratio (PCr/ATP ratio). MRS experiments demonstrated that type 2 diabetic patients had reduced PCr/ATP ratios, suggesting that mitochondrial high-energy phosphate metabolism may be impaired; however changes in the creatine pool and increased ATP utilization, as opposed to ATP production, may have also contributed to this observation.[99–101] Furthermore, it was shown that in diabetic patients with normal morphology of the heart, PCr/ATP correlates negatively with serum FFA[99], indicating that an increase in FFA availability might impair cardiac energy reserves. On the contrary, studies with well-controlled type 2 diabetic subjects do not show any changes in the PCr/ATP ratio.[20,102] Difference in these findings may be due to differences in co-morbidities of the study populations such as low glycemic control, high blood pressure and dyslipedemia.

Another lipotoxic mechanism linking mitochondrial dysfunction with cardiac structural changes is impaired calcium handling. Upon electrical stimulation, calcium is released from the sarcoplasmic reticulum in order for calcium to bind troponin C on the actin filaments. This leads to changes in the contractile regulatory proteins allowing interaction between actin and myosin cross-bridges. ATP is required for initiating a new contractile cycle and for the re-uptake of calcium in the sarcoplasmic reticulum via calcium-ATPase 2a. Besides the insulin-induced changes in calcium fluxes, a decrease in ATP production also leads to an impaired mitochondrial calcium handling, which contributes to a decreased cardiac contraction.[60,103]

In animal studies, calcium handling was altered in the type 2 diabetic (*db*/*db*) state.[59,104] Also, *ob*/*ob* mice had a slowed intracellular decay of

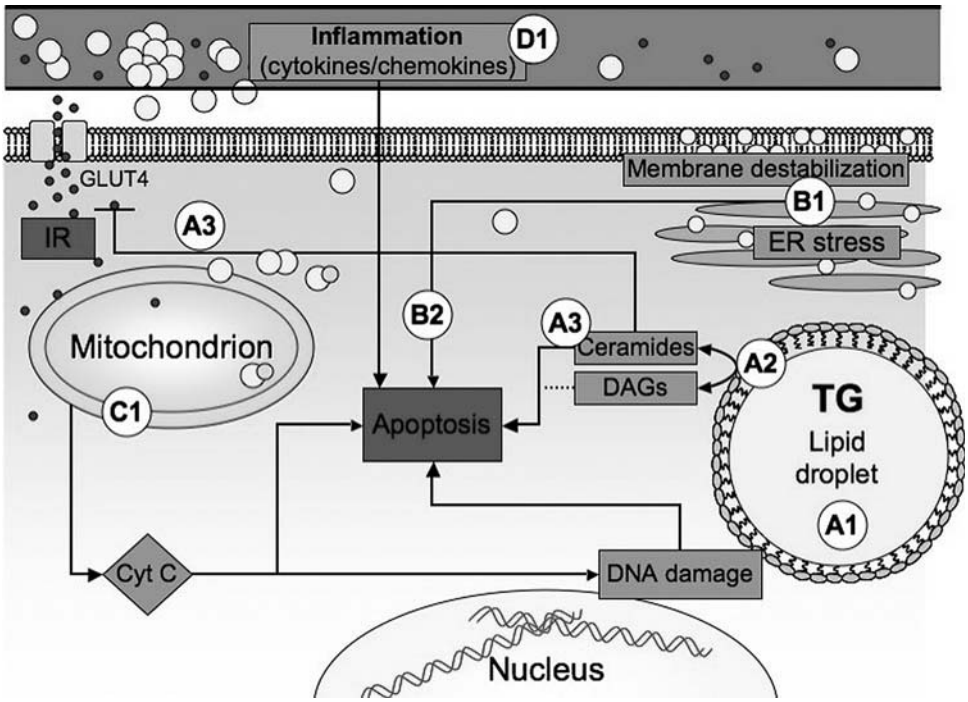

Figure 2 Possible mechanisms leading to lipoapoptosis in cardiomyocytes. (A1) Stored triglycerides in cardiomyocytes can be hydrolyzed, resulting in (A2) diacylglycerols and FAs that can be metabolized in non-oxidative pathways. In that way, saturated FAs can be converted into ceramides. Ceramides can initiate lipoapoptotic pathways (A3) via cytochrome C release of the mitochondria and can inhibit the insulin-signaling pathway via PKC resulting in IR. DAGs also induce IR via interference with the insulin-signaling pathways, but whether DAGs can also induce apoptosis in cardiac muscle still remains unknown. Lipids that cannot be stored can also be integrated as saturated phospholipids in the membranes and induce imbalances in the UFPR leading to ER stress (B1), which in turn (B2) can lead via mitochondrial pathways (C1) and cytochrome C release to lipoapoptosis. Also cytokines from systemic and locally inflamed adipose tissue (D1) might induce cellular signaling and attract immune cells thereby inducing local inflammation, leading to insulin resistance, cellular damage and apoptosis.

calcium and impaired mitochondrial calcium handling upon electrical stimulation, which was associated with increased intramyocellular lipid accumulation in these cells.[105,106] Upon high-fat feeding, it was shown that impaired calcium handling due to a lower cardiac ATP supply was the main cause of cardiac dysfunction in the absence of apoptosis.[107] Thus, while there is evidence that calcium handling is impaired in type 2 diabetes and mitochondria have been shown to play a part in impaired calcium handling in obesity models, it has not been directly investigated whether the impaired Ca^{2+} handling in diabetes is also caused by mitochondrial impairments.

Together, these data suggest that increased fat uptake in the heart could lead to reduced energy efficiency by inducing mitochondrial damage and uncoupling, increasing ROS production and via impairment of mitochondrial calcium handling. These studies highlight the importance of proper mitochondrial function in type 2 diabetes in order to maintain a normal cellular homeostasis.

3.2 Lipoapoptosis

Besides changes in cardiac efficiency, lipoapoptosis may also contribute to the decreased function of the fatty heart. Lipoapoptosis can be based on different mechanisms such as palmitate toxicity, ceramide and DAG formation, endoplasmic reticulum (ER) stress, membrane destabilization and inflammation (Figure 2).

3.2.1 Palmitate toxicity

Palmitate (a saturated FA) can induce apoptosis in isolated cardiomyocytes, independent of ceramide formation.[108,109] Although this toxicity of FA incubation *in vitro* has been implicated in the pathophysiology of cardiovascular disease, the effects on cardiac myocytes remain incompletely understood.

Several studies investigated the mechanisms through which palmitate may induce apoptosis. It was shown that palmitate-induced activation of the stress-associated protein kinases induced apoptosis in 20% of the cardiomyocytes.[110] Strikingly, it was found that the effects on apoptosis through palmitate exposure in cardiomyocytes were inhibited by titrating low concentrations of oleate.[110] Furthermore, increasing mitochondrial uptake of palmitate with L-carnitine decreased apoptosis, while decreasing uptake with the carnitine palmitoyl transferase-1 inhibitor perhexiline almost doubled palmitate-induced apoptosis.[110] This indicates that cytosolic accumulation of saturated FAs is detrimental for cellular function.

3.2.2 Ceramide formation

Ceramide-induced apoptosis is thought to be an important contributor to the development of lipotoxic cardiomyopathy. This concept was based on several *in vitro* and *in vivo* studies. When FA oxidation was reduced *in vitro*, intracellular triglycerides and ceramides were increased and induced caspase-3 activity and DNA laddering.[111,112]

In various rodent models of lipotoxic cardiomyopathy, diabetes, and obesity, increased myocardial ceramide content has been observed in association with cardiac dysfunction.[66,68] For instance, in the study of Park *et al.*[113] the lipotoxic dilated cardiomyopathy of the lipoprotein lipase (LPL)^{GPI} mice was rescued by pharmacological inhibition of ceramide synthesis via myriocin. This therapy restored ceramide levels to WT levels and normalized substrate utilization of the heart. Both changes were associated with the improvement of cardiac function.[113] Crossing the LPL^{GPI} mice with LCB1$^{+/-}$ mice that have a deletion in the serine palmitoyltransferase enzyme (the enzyme that initiates the conversion of fatty-acyl co-A into ceramides) gave similar results to that of the administration of myriocin alone.[68,113] Thus, these data suggest that ceramides play an important role in the development of lipotoxic cardiomyopathy.

There is only one human study that assessed the role of ceramides in lipoapoptosis in the human heart. Baranowski *et al.*[114] examined the relationship between myocardial apoptosis and ceramide levels in obese and type 2 diabetic patients. Compared with lean subjects, the markers for apoptosis were higher in the myocardium of obese patients and increased further in type 2 diabetic subjects. However, ceramide and sphingoid base content were similar in both groups. Furthermore, mRNA levels of enzymes involved in synthesis and degradation of ceramides were markedly increased in both obese and type 2 diabetic subjects. These results challenge the view that ceramides are the main determinant of apoptosis in the fatty heart of obese and diabetic humans.

Besides inducing apoptosis, ceramides can acutely inhibit insulin-stimulated glucose uptake, GLUT4 translocation and glycogen synthesis.[113] These effects appear to result from the ability of sphingolipid to block activation of either insulin receptor substrate 1 or AKT/protein kinase B. These effects could contribute to the vicious cycle of insulin resistance and thereby aggravate the existing cardiac impairments.

3.2.3 Diacylglycerol formation

It is known that in obesity and diabetes, due to an oversupply in lipids, DAGs can accumulate in different cellular compartments. It is hypothesized that DAGs interfere with the insulin-signaling cascade in the heart as they do in skeletal muscle,[115] leading to a decreased insulin-stimulated glucose uptake. Increased accumulation of DAG activates protein kinase epsilon and δ, which in turn interfere with the insulin-signaling cascade. A 10 week high fat diet in C57BL/6 mice, resulted in a decreased insulin-stimulated glucose oxidation in isolated working hearts. This was positively associated with increased accumulation of myocardial DAG, a concomitantly increase in GPAT and a decrease in DGAT, as well as an inhibition of insulin-signaling molecules.[116]

Whether DAG can also exert direct lipotoxic effects by inducing apoptosis in cardiac tissue remains unknown. Overexpression of DGAT in the heart reduced DAG levels and had a cardioprotective effect when crossed with a mouse model of cardiac lipotoxicity. However, in this model neutral lipid storage was increased. Besides the decrease in DAG levels, ceramide formation was decreased by 35%. Therefore, it remains unclear whether the cardioprotective effect was due to the lowering of DAG or ceramides levels.[54]

3.2.4 Membrane destabilization and ER stress

Another potential lipotoxic mechanism that may be involved in diabetic cardiomyopathy is membrane destabilization and ER stress.[117] The ER is a primary site for protein synthesis and folding. Most secreted and transmembrane proteins fold and mature in the lumen of the ER. Cells can adjust the protein-folding capacity of the ER, thereby ensuring that the necessary proteins can maintain cellular function in due time. The intracellular signalling pathway that mediates this regulation has been named the unfolded protein response (UFPR).[118]

In obesity, increased lipid delivery can stimulate an increased influx of unfolded proteins in the ER, which can lead to a mismatch between the UFPR and protein translation, inducing stress of the ER.[118] ER stress enhances calcium release and signaling to the mitochondria, ultimately resulting in increased apoptosis.[119]

In mice with streptozotocin-induced diabetes, cardiac ER stress was suggested by expression of ER chaperones and apoptosis was detected 5 months after diabetes onset. Also, in models of type 2 diabetes it was shown that cardiomyopathy was associated with ER stress. In *db*/*db* mice levels of the phosphorylated ER-stress makers were significantly elevated compared with lean controls.[120] It has been shown *in vitro* that ER stress is enhanced in lipotoxicity. Cells exposed to palmitate could rapidly convert palmitate into phospholipids, which were integrated into the microsomal membranes. This resulted in drastic membrane remodelling which was associated with dramatic dilatation of the ER and redistribution of protein-folding chaperones to the cytosol within 5 h, indicating compromised ER membrane integrity and further enhancing ER stress.[121] Although there is no data on ER stress in human cardiac tissue, data from animal studies strongly suggest that indeed ER stress is involved in the induction of apoptosis and that this mechanism is enhanced by lipid accumulation.

3.2.5 Systemic and local inflammation

It is well established that obesity and type 2 diabetes are characterized by a state of low-grade inflammation.[122,123] Systemic low-grade inflammation influences cellular processes in all tissues, including the heart and may lead to fibrosis and structural remodelling, which may contribute to the cardiac stiffness/diastolic impairment observed in diabetic cardiomyopathy.

Epicardial adipose tissue has been identified as an active source of adipokines and cytokines.[124] Due to the close relationship between this fat depot and the myocardial tissue, coupled with the lack of fascial boundaries, epicardial adipose tissue may locally interact and modulate the myocardium through secretion of pro- and anti-inflammatory cytokines.[125] Epicardial adipose tissue serves as a buffer for the storage of FFAs for the heart. However, the increased epicardial thickness as seen in obesity has been associated with pro-inflammatory cytokine production.[126] Thereby, epicardial adipose tissue derived cytokines may significantly contribute to the development of diabetic cardiomyopathy.

4. Implications and conclusions

In conclusion, accumulation of fat and a higher availability of FFAs are associated with an impaired cardiac efficiency and lipoapoptosis. Though many of the theories are confirmed in animal models, the evidence from human studies remains scarce. Nevertheless, the available data points toward the need for lipid lowering strategies to reduce ectopic fat accumulation and minimize lipotoxicity. To unravel the aetiology of diabetic cardiomyopathy in humans, novel non-invasive

imaging techniques will become increasingly important for *in vivo* investigations.

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