Shedding light on the enigma of myocardial lipotoxicity: the involvement of known and putative regulators of fatty acid storage and mobilization

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Review

THERE IS A GROWING BODY OF EVIDENCE demonstrating that increased fatty acid (FA) availability contributes to certain deleterious cardiac effects associated with type 2 diabetes (38, 51, 130). Indeed, hearts from diabetic and/or obese animals show an overreliance on FA for energy production and have accelerated rates of FA oxidation (FAO) (17, 21, 70). Associated with this increase in FAO is decreased myocardial efficiency (14, 17, 24, 98, 100) and increased production of mitochondrial reactive oxygen species (14, 17, 47, 171) that eventually damage mitochondria (14, 141), further compromising ATP production and cardiac function. Interestingly, although FAO is increased in hearts from obese and diabetic animals, many of these animal models are also characterized by increased lipid accumulation in cardiomyocytes (1, 12). These data suggest that FA uptake can often exceed even accelerated FAO with the resulting effect of increased FA storage as triacylglycerol (TG). This increase in TG is correlated with contractile dysfunction in several genetically altered and mutant murine models, (1, 12, 28, 174), and it has been termed lipotoxic cardiomyopathy. Interestingly, the role that increased TG plays in lipotoxic cardiomyopathy is yet to be fully elucidated. One possibility is that myocardial TG accumulation protects the heart by “storing away” detrimental lipid intermediates such as diacylglycerol (DG), long-chain fatty acyl-CoA esters, and/or ceramides. A second possibility is that excessive TG storage contributes to the presence of detrimental lipid intermediates.

These, in turn, induce cardiac dysfunction (27, 29, 55, 137, 152) by multiple mechanisms. These include cardiomyocyte apoptosis (29, 178) and/or impaired insulin signaling followed by decreased glucose use, an energetically compromised heart, and contractile dysfunction (3, 28, 29, 44, 115, 142, 178).

The phenomenon of cardiomyocyte lipoproteinosis and the resulting consequences have been reviewed extensively (88, 138, 149, 160, 168). Briefly, saturated, but not unsaturated, FA trigger cardiomyocyte apoptosis acutely through mechanisms such as ceramide accumulation (71, 144), decreased cardiolipin synthesis (118), and release of cytochrome c from mitochondria and cytosolic enzymes from cardiomyocytes (36, 72, 111, 118, 144). Palmitate-induced apoptosis of cardiomyocytes is reported to increase oxidative stress, and this effect was decreased by carnitine, which increases FAO, or low concentrations of olate (111). However, it was concluded from other studies that oxidative stress was not involved in palmitate-induced apoptosis in neonatal cardiomyocytes (72).

Pancreatic β-cells, hepatocytes, and other cell types are also susceptible to saturated FA-induced apoptosis. A contribution of ceramide accumulation and nitric oxide-mediated lipotoxicity has been reported (143, 157), although different apoptotic pathways, including the endoplasmic reticulum (ER) stress pathway, might be involved depending on the cell type (48, 80, 94, 167). Furthermore, studies in skeletal muscle cells showed that saturated FA can decrease insulin-stimulated signaling pathways through the activation of PKCδ and NF-κB by DG accumulation, leading to IRS-1 phosphorylation and inhibition of Akt signaling (26, 31, 112). Ceramides also decrease insulin-stimulated glucose uptake through preventing the translo-
cation of GLUT4 transporters to the cell surface (163). Additionally, ceramide production from saturated FA leads to increased PKCζ activation and decreased insulin-stimulated Akt phosphorylation (26, 126). Finally, the detrimental effects of saturated FA are abrogated by the presence of unsaturated FA. The prevailing model is that treatment with unsaturated FA increases the incorporation of the saturated FA into TG, which is thought to prevent the short-term accumulation of DG, ceramide, and highly saturated phosphatidylglycerol (36, 93, 118).

However, the long-term effect of chronic FA overload, regardless of chain saturation, has been determined in obese and/or diabetic animals as well as in transgenic mouse models with cardiac-specific TG accumulation. Under these conditions, the increased reliance of the heart on FA results in decreased myocardial efficiency, production of reactive oxygen species, and mitochondrial damage (13, 14, 109, 141). Furthermore, the development of cardiac steatosis is associated with cardiac hypertrophy, myocardial systolic and diastolic dysfunction, increased ceramide levels, cardiomyocyte apoptosis, and premature death (29, 133, 170). The question of whether TG accumulation is a protective response to elevated FA or a detrimental consequence of chronic fuel imbalance will be addressed below.

This review focuses on what is known about the pathways that control intramyocardial lipid accumulation and how this process is likely to be regulated based on work from other tissues. We also discuss further how lipid accumulation in cardiomyocytes is associated with lipotoxicity.

Myocardial Energy Metabolism

In the healthy heart, contractile function depends on the production of intracellular ATP derived primarily from a balance between FA and carbohydrate oxidation. Although the ratio between FAO and glucose oxidation varies throughout the day, the healthy adult heart obtains 50–75% of acetyl-CoA-derived ATP from long-chain FA (101). This dynamic use of FA and glucose for energy production is largely a result of fluctuations in plasma FA availability and the resulting changes in FA entry into the cardiac myocyte (22) (discussed later). Immediately following uptake, FA are converted into long-chain acyl-CoA esters that can be used to form acylcarnitines for transport into mitochondria. Once inside the mitochondria, the re-formed acyl-CoA esters undergo β-oxidation to produce acetyl-CoA that, upon entering into the tricarboxylic acid (TCA) cycle, undergoes further metabolism to provide the energy for ATP production (Fig. 1). Cytoplasmic long-chain acyl-CoA esters that are not immediately diverted to acylcarnitines are incorporated into TG and phospholipids (159). Although there can be significant detrimental consequences associated with accelerated rates of FAO in the heart, these have been discussed elsewhere (99, 146). As such, this review focuses on the regulation of FA uptake, TG storage, and TG mobilization in cardiomyocytes.

FA and TG in Insulin Resistance and Diabetes

Excessive lipolysis in expanded visceral adipose tissue in android obesity predisposes to insulin resistance (15) because of large FA loads delivered to the liver, skeletal muscle, and heart (9, 60, 91, 110). Additionally, a deficiency in FA trapping by adipose tissue could contribute significantly to the increase in FA concentrations, particularly in the postprandial state (30, 49). Increased FA and glucocorticoid actions stimulate hepatic gluconeogenesis, TG synthesis, and very low density lipoprotein (VLDL) secretion (15). Glucocorticoids increase the capacity for hepatic TG synthesis through increased phosphatidate phosphatase (PAP) activity (131). This is associated with a fatty liver and hypertriglyceridemia in diabetes. Glucocorticoids also stimulate apolipoprotein B (apoB) production and VLDL secretion, and insulin antagonizes these effects (107, 162). In diabetes, hepatic TG production relies mainly on FA liberated from adipose tissue in excess of the capacity for FAO. In addition, hyperinsulinemia increases the expression and activity of the transcription factor sterol-regulatory element-binding protein-1c, which upregulates the expression of lipogenic genes in the liver and thus the secretion of newly synthesized FA in VLDL (42).

Thus, in diabetes, the heart has a rich supply of nonesterified FA released by adipose tissue and FA derived from VLDL or chylomicrons. The uptake of FA from these lipoproteins by the heart is regulated by lipoprotein lipase activity in blood capillaries, which is increased in insulin resistance and diabetes (121, 128). If FA uptake exceeds the heart’s capacity for FAO, then excess FA are incorporated into TG, resulting in cardiac steatosis (101). These TG can provide a FA reserve for increased FAO (43, 86) while also possibly providing protection against lipotoxicity. It is also clear that other tissues, such as the liver and skeletal muscle, exhibit similar responses to elevated FA (11, 129) and that TG synthesis often increases as a companion pathway to increased FAO (131). The accumulation of TG in nonadipose tissues appears to be a concerted disruption of normal TG homeostasis, which could temporarily avoid the build-up of toxic lipid metabolites in the face of saturated rates of FAO (93, 156, 158). Moreover, there is considerable evidence showing that FAO in the tissues of obese type 2 diabetic subjects decreases due to mitochondrial damage and dysfunction, possibly resulting from the chronically elevated FA concentrations (43, 76, 92). Eventually, the chronic imbalance of lipid storage vs. lipid uptake, oxidation, and secretion in these nonadipose tissues could result in mechanical dysfunction or organ failure (138, 156).

TG accumulation in myocytes and epicardial adipose tissue is highly correlated with the development and severity of insulin resistance and cardiovascular disease (38, 91). Epicardial adipose tissue could modulate the function of the heart and vasculature through the paracrine secretion of proinflammatory cytokines (e.g., tumor necrosis factor-α, interleukin-1 and -6) and FA (77). This could play a possible role in adiposity-related inflammation and atherosclerosis. On the other hand, epicardial fat could exert a protective effect through adiponectin and adrenomedullin secretion (77). Chronic and excessive accumulation of intramyocardial TG has detrimental consequences. Indeed, TG accumulation in the heart could be a maladaptive response associated with lipotoxicity (155). To begin to address these possible adaptive and maladaptive outcomes, we will first discuss the mechanisms that regulate FA uptake, FAO, esterification, and TG turnover.
Proteins That Facilitate FA Uptake and Export by the Heart

Although diffusion contributes to FA transport into cardiomyocytes, the high-energy demand of the heart makes protein-mediated transport especially important (Fig. 1). Sarcolemmal proteins that are central to the uptake mechanism in cardiac myocytes are CD36 (78), plasma membrane FA-binding protein (FABPpm), and FA transport proteins (FATP), specifically FATP-1 and -6 (59). Although all of these proteins appear to be involved, inhibition of CD36 decreases total FA taken up by the heart by more than 50%. This result indicates that CD36 is a major contributor to the FA transport process (61, 78).

Interestingly, cardiac CD36 expression is markedly increased in diabetes, insulin resistance, and other situations of cardiac steatosis (33, 34, 85, 119), CD36 may, therefore, play a central role in regulating intramyocardial TG accumulation during these conditions (103).

In addition to regulation by the protein transporters, FA uptake is also governed by the rates of FAO as well as other intracellular fates of FA. As a consequence, many proteins involved in these pathways also contribute to the uptake process. For example, intracellular proteins such as cytoplasmic FABP (FABPc) and long-chain fatty acyl-CoA synthetases (ACS1–6) can facilitate FA uptake by either binding to or decreasing the intracellular FA pool, thereby creating an intracellular "sink" for FA and establishing an inward FA gradient (108). Moreover, proteins that regulate FA storage can also drive FA uptake into cardiomyocytes.

The cardiac TG pool turns over rapidly, and this turnover may provide a mechanism to balance intracellular FA levels and metabolic flexibility in energy production (135, 145). In addition, TG accumulation in the heart is decreased by the expression of microsomal triglyceride transfer protein (MTP), which facilitates TG secretion in apoB-containing particles (7). Cardiac MTP is increased by fasting and fat feeding. The physiological significance of MTP activation and lipoprotein secretion from the heart in response to increased systemic FA supply was demonstrated in mice with cardiac-specific deletion of the MTP-A isoform (7). Cardiac MTP-A deficiency resulted in significantly increased myocardial TG levels upon fasting and fat feeding. Conversely, cardiac apoB overexpression pre-

Fig. 1. Schematic representation of the interactions among pathways of fatty acid (FA) uptake, oxidation, and esterification and triacylglycerol (TG) turnover in cardiomyocytes. Albumin-bound FA and FA liberated from chylomicrons and VLDL by lipoprotein lipase enter the cardiomyocyte through FA transporters such as CD36 and FATP1/6. Acyl-CoA synthase (ACS) isoforms 1–6 convert FA to acyl-CoA. These acyl-CoAs are used for β-oxidation or are incorporated into complex lipids. Acyl-CoAs (particularly when saturated, e.g., palmitoyl-CoA) are incorporated into ceramide (Cer) by Cer synthase, which is present on the endoplasmic reticulum (ER). Ceramides provide signals that lead to apoptosis and insulin resistance. The glycerolipid biosynthetic pathway consists of two sequential esterifications catalyzed by glycerol 3-phosphate (G-3-P) and acylglycerolphosphate acyltransferases (GPAT and AGPAT) located in mitochondria and ER. The PA that is formed serves as the precursor for the synthesis of phosphatidylglycerol and cardiolipin. Phosphatidate (PA) is also dephosphorylated to form DG by lipin-1 (phosphatidate phosphatase) when it translocates to the surface of the ER. Diacylglycerol (DG), formed by lipin-1, is converted to TG by two diacylglycerolacyltransferases (DGATs) or is used for production of phosphatidylcholine (PC) or phosphatidylethanolamine (PE). DG also activates downstream signals such as through PKCs (PKCe and -δ), which can cause insulin resistance. TG stored in lipid droplets are hydrolyzed to DG, monoacylglycerol (MG), and glycerol sequentially by adipose tissue TG lipase (ATGL), hormone-sensitive lipase (HSL), and MG lipase. Liberated FA are converted by ACSs to acyl-CoA, which can undergo a reesterification-lipolysis cycle. Alternatively, the acyl-CoA formed after release of FA from TG stores are transported into mitochondria through the carnitine carrier system involving the carnitine palmitoyltransferases (CPT I and II) and carnitine acylcarnitine translocase (CACT) so that they can undergo β-oxidation to provide energy. In the nucleus, PPARγ coactivator-1α (PGC-1α) and PPARα, in conjunction with other transcriptional coactivators, e.g., lipin-1, control the transcriptional regulation of genes involved in FA uptake and oxidation. Additional abbreviations: ETC, electron transport chain; G-3-P, glyceral 3-phosphate; IMM, inner mitochondrial membrane; LPA, lysophosphatidate; PM, plasma membrane; Sph, sphingosine; TCA, tricarboxylic cycle.
vented induction of lipid metabolizing genes and TG accumulation in the hearts of mice fed a high-fat diet (7). Importantly, fat-fed apoB transgenic mice also displayed improved heart function compared with the control mice. These findings support the hypothesis that cardiac lipid export through lipoprotein secretion plays a significant physiological role in preventing excessive TG accumulation in the heart in obesity or diabetes. Thus, stimulation of cardiac lipoprotein secretion could limit the development of obesity and diabetes-related cardiac steatosis and lipotoxicity (7).

Proteins Involved in Myocardial FA Esterification to Phosphatidate

Cardiac TG are synthesized de novo through the Kennedy pathway (Fig. 1), in which the initial step is FA esterification with glycerol 3-phosphate (G-3-P) to lysophosphatidate through glycerol-3-phosphate acyltransferases (GPAT). In addition, TG turnover probably also involves reesterification of DG and possibly monoaoylglycerol (MG) (148). There are four GPAT enzymes in the heart: two mitochondrial (GPAT1 and -2) and two in the ER, GPAT3 and -4 (20, 90, 151). The most studied enzyme is GPAT1, which is located at the outer mitochondrial membrane and accounts for up to 30% of total GPAT activity in the heart (90). Mice deficient in GPAT1 are protected from diet-induced increases in myocardial TG accumulation despite the fact that the levels of TG and FA in the plasma of control and GPAT1-deficient mice were similar. This suggests that decreased TG deposition in the hearts of GPAT1-KO mice, which were fed high-fat or high-sucrose diets, was not caused by the diminished availability and uptake of plasma lipids (90). The results point to an important role for cardiac GPAT1 in TG accumulation following the consumption of lipogenic or high-fat diets. Microsomal GPATs contribute most of the GPAT activity in the majority of tissues, including the heart, but their individual contribution to cardiac TG synthesis remains to be determined. Interestingly, GPAT activity in the sarcoplasmic reticulum of rat ventricles exhibited a fourfold greater $K_m$ for G-3-P than the microsomal GPAT of adipose tissue (148). The fact that G-3-P concentrations in cardiomyocytes are normally lower than the $K_m$ of GPAT suggests that cardiac TG synthesis is regulated by G-3-P levels, provided FA concentrations are not limiting (148). This may underlie the augmented TG synthesis under conditions that raise cardiomyocyte G-3-P levels, e.g., ischemia or increased lactate supply (148).

The next step of TG synthesis is catalyzed by acylglycerol-3-phosphate acyltransferases (AGPATs), which acylate lysophosphatidate to form phosphatidate. At least four AGPAT isoforms are expressed in the murine heart (102). Total cardiac AGPAT activity and mRNA expression of at least two cardiac AGPAT enzymes appear to be regulated by peroxisome proliferator-activated receptor-α (PPARα) (102), but their specific roles in myocardial TG synthesis and energy metabolism require further elucidation.

Role of PAP in Lipid Synthesis and FAO

The phosphatidate (PA) that is synthesized de novo is converted by PAP to DG, a necessary precursor for the synthesis of TG, phosphatidylcholine, and phosphatidylethanolamine. The identity of PAP was discovered only recently from work with yeast (67). PAP activity in mammals is catalyzed by four mammalian lipins consisting of lipin-1A and -1B (splice variants) and lipin-2 and lipin-3 (41, 131). Adult hearts express mainly lipin-1A/1B as demonstrated by the apparent lack of cardiac PAP activity in adult fld (lipin-1-deficient) mice (69). The Mg2+-dependent PAP activities are catalyzed by the haloacid dehalogenase motif, DxDxT, of the lipins (41). Mammalian lipins also contain an LxxIL motif, which is responsible for transcription factor binding (131). For example, lipin-1 expression is essential for PPARγ action, the expression of adipogenic genes, and thus the production of mature adipocytes (131). This LxxIL site also enables lipin-1 to interact with PPARα and the PPARγ coactivator (PGC-1α) to promote the expression of enzymes involved in FAO in the liver (53). This regulation by the PGC-1α/PPARα system could be especially important in the heart since activation of PPARα and PPARβ/δ, but not PPARγ (57), stimulates the transcription of genes that encode for proteins involved in FA uptake (e.g., CD36, FABP), mitochondrial biogenesis, and FAO [e.g., m-acyl-CoA decarboxylase, carnitine palmitoyltransferase 1 (CPT I) and enzymes of β-oxidation] (6, 19, 52) while decreasing glucose uptake through lowered GLUT4 expression (55).

Although little is known about the role of lipins in the heart, this dual function of the lipins in coordinating TG synthesis and FAO could be especially significant for controlling the balance of FA vs. glucose oxidation that is used for cardiac energy production. The cardiac PGC-1α/PPARα system is activated in type 1 and type 2 diabetes (52), but some studies showed diminished expression of PPARα target genes (37, 178). Chronic activation of PPARα is detrimental to cardiac recovery during reperfusion after ischemia (137). Overexpression of PPARα in the heart mimics the diabetic phenotype (55) by increasing FA uptake through CD36 and decreasing glucose transport by GLUT4 (55). However, those authors found no evidence that PPARα or DG acyltransferases (DGAT) were PPARα targets, although there was increased steatosis after fasting even in the presence of increased FAO (55).

Increased TG synthesis could depend on increased lipin-1 expression, but there is relatively little information about the regulation of cardiac lipin-1 expression (139, 148). Our unpublished work establishes that lipin-1 expression in cardiomyocytes is increased by glucocorticoids, an effect that is synergized by cAMP and decreased by insulin, similarly to the regulation in liver (105, 131). These findings explain why cardiac (139) and hepatic (131) PAP activities are increased in insulin-deficient diabetes to provide a reservoir of cytosolic lipid-1 activity. This reservoir is activated by the increased accumulation of FA in cells resulting from increased lipolysis in adipose tissue followed by the consequent increase in FA uptake and accumulation in heart and liver (23, 132). By contrast, the specific activities of PAP were lower in the hearts of Zucker diabetic fatty (ZDF) rats and in atrial tissue from human patients with type 2 diabetes mellitus (18).

PAP activity is mainly cytosolic, whereas other enzymes of TG synthesis are in the ER or mitochondria (Fig. 1). Unsaturated FA, rather than saturated FA, promote the physiological expression of PAP activity in liver (62), heart (139, 148), and...
adipose tissue (69, 136) through translocation from the cytosol to the ER, where TG are synthesized (131). This explains why unsaturated FA stimulate the incorporation of palmitate to TG by accelerating the PAP reaction (75). Cells are unable to synthesize significant quantities of tripalmitoylglycerol in response to exposure to palmitate alone, and they undergo apoptosis (93). Oleate alleviates palmitate-induced apoptosis by facilitating TG synthesis (93, 111). Lipins, therefore, act as physiological sensors for unsaturated FA, and translocation to the ER provides the reserve capacity that matches TG synthesis to excess FA supply (131). The conversion of acyl-CoA to TG also regenerates the CoA required for further FA uptake and metabolism (Fig. 1).

PAP translocation to the ER in hepatocytes depends on its phosphorylation state, since okadaic acid (inhibitor of protein phosphatases 1 and 2) displaces PAP from membranes in the absence, or presence, of oleate and decreases DG concentrations (63). Lipin-1 is phosphorylated by different kinases on more than 15 different residues, and this controls its subcellular distribution (69). Insulin displaces lipin-1 from the membrane compartment of adipocytes, a finding that was unexpected since insulin stimulates TG synthesis in adipose tissue (69). This insulin effect on the subcellular distribution of lipin-1 depends on the phosphorylation of lipin-1 and its interaction with 14-3-3 proteins (123). By contrast, PAP and lipin-1 expression is increased in diabetes (139, 169, 177). There is also increased association of PAP activity with ER membranes in the presence of glucagon or epinephrine (through cAMP) in the presence of FA (69, 125). This appears to facilitate limited FA reesterification during enhanced lipolysis in adipose tissue and enables the liver to sequester excess FA as TG in diabetes. Interestingly, perfusion of the heart with glucagon increases membrane-associated PAP activity (139), which could contribute to the increased capacity for TG synthesis in diabetes.

Lipin-1 is a regulator of fuel homeostasis, since increased lipin-1 expression in skeletal muscle causes obesity and insulin resistance linked to decreased energy expenditure (131). Conversely, enhanced lipin-1 expression in adipose tissue improves insulin sensitivity despite increased adiposity (124). It was suggested previously that preferential FA storage in adipose tissue prevents ectopic lipid deposition and thus improves insulin sensitivity (83, 156). Thus, enhanced sequestration of FA by lipin-1 overexpression in adipose tissue may underlie the improved insulin action seen in these transgenic mice (124, 131). By contrast, increasing lipin-1 expression in skeletal muscle is associated with insulin resistance (124).

Role of Diacylglycerol Acyltransferases

Two DG acyltransferases (DGAT1 and DGAT2) are responsible for converting DG to TG in the heart (173, 175). DGAT1 belongs to a family that includes acyl-CoA:cholesterol acyltransferase, and it can also use other alcohols, such as retinol, as a substrate. DGAT2 appears to have a more restricted substrate preference, and it belongs to a family that includes acyl-CoA:MG acyltransferases (173, 175). It was proposed that DGAT2 preferentially incorporates endogenously synthesized oleate into TG, but FA synthesis de novo is not an active pathway in cardiomyocytes. DGAT1 appears to be involved in the esterification of FA that are taken up by cells or those that are undergoing recycling from TG stores (173).

Information on the regulation of DGAT1 in the heart is limited, although total cardiac DGAT was reported to be decreased by glucagon (139). In addition, exercise-induced cardiac hypertrophy was associated with stimulation of DGAT1 expression and increased TG levels in the heart (95). Cardiac-specific overexpression of DGAT1 correspondingly resulted in augmented myocardial TG stores. In parallel, cardiac levels of ceramide, DG, and free FA were lowered, suggesting that efficient sequestration of FA in the TG pool reduces potentially lipotoxic FA metabolites in this mouse model (95). Combined overexpression of DGAT1 with long-chain ACS1 in the hearts of transgenic mice improved heart function; fractional shortening increased by 74%, and diastolic function improved compared with ACS1 transgenic mice. Survival was also improved compared with mice with only long-chain ACS1 overexpression, which develop lipotoxic cardiomyopathy. These beneficial effects of DGAT1 overexpression on the long-chain ACS1 transgenic background were associated with reduced cardiac ceramide, FA, and DG content as well as diminished cardiomyocyte apoptosis and enhanced FA oxidation (95).

In line with the findings in the heart, overexpression studies in skeletal muscle suggest that DGAT1 may protect against lipotoxicity by increasing TG synthesis and reducing DG and ceramide levels (97). This was accompanied by decreased activation of DG-responsive PKCs and decreased serine phosphorylation of IRS-1. Myotubes of muscle-specific DGAT1 transgenic mice also exhibited increased FA oxidation (96). These findings in both cardiac and skeletal muscle indicate that DGAT1 influences not only TG synthesis but also mitochondrial FAO capacity due to mechanisms that are yet to be explored. However, this work (96) also demonstrates that FAO and TG synthesis can be considered as companion rather than antagonistic pathways.

Similar to muscle-specific DGAT1 overexpression, increased expression of DGAT2 in glycolytic muscle of mice increased the levels of TG and decreased DG content (89). However, this was paralleled by augmented concentrations of ceramides and unsaturated long-chain acyl-CoA, which was associated with muscle insulin resistance and whole body glucose intolerance. The divergent phenotypes of DGAT1- and DGAT2-KO mice suggest that the two DGAT isoforms play different roles in lipid and energy metabolism (173), but little is known about the mechanisms for this differential effect in the heart.

Enzymes in Cardiac Triacylglycerol Degradation

Myocardial lipolysis involves adipose tissue triglyceride lipase (ATGL) (64, 176), carboxylesterase 3/TG hydrolase (Ces3/TGH) (40), hormone-sensitive lipase (HSL) (74, 172), and MG lipase (81) (Fig. 1). An essential role was demonstrated for ATGL, which specifically catalyzes the first and rate-limiting step in cardiac lipolysis (64). Mice with global ATGL deficiency display decreased lipolysis in adipose and nonadipose tissues, leading to chronically decreased plasma ceramide and unsaturated long-chain acyl-CoA, which was associated with muscle insulin resistance and whole body glucose intolerance. The divergent phenotypes of DGAT1- and DGAT2-KO mice suggest that the two DGAT isoforms play different roles in lipid and energy metabolism (173), but little is known about the mechanisms for this differential effect in the heart.
examined, indicating that ATGL is critical for efficient TG catabolism even in tissues where its expression level is relatively low. Interestingly, hearts of ATGL-KO mice exhibited over 20-fold increases in TG content (64). This ultimately precipitated congestive heart failure leading to the premature death of ATGL-KO mice. Echocardiographic analysis showed severely impaired cardiac function with a 50% decrease in the ejection fraction of the left ventricle, suggestive of systolic dysfunction (64). Decreased plasma FA, in combination with diminished mobilization of endogenous FA from TG stores and increased cardiac glucose uptake (64), means that hearts of ATGL-KO mice exhibit reduced FAO and rely more on glucose oxidation.

Consistent with this, case reports of patients with ATGL/PNPLA2 mutations indicate that the lipase plays a similar role in the human heart. Hirano et al. (73) documented marked TG accumulation in cardiomyocytes as well as in the atherosclerotic coronary arteries in one patient with a mutation in the ATGL gene (PNPLA2). This patient underwent cardiac transplantation due to congestive heart failure. Although less detailed information is available on other patients with ATGL mutations, cardiomyopathy is a common feature (140). Taken together, the studies that have been published identify ATGL activity as being essential for maintaining cardiac TG and energy homeostasis and show that alternative TG hydrolases appear not to compensate for its loss.

Overexpression of ATGL in cultured myotubes increases endogenous FAO (165) possibly due to increased provision of ligands (FA) for PPAR activation. The rate of FAO might thus depend on the balance of the lipolysis/reesterification cycle. Enhanced expression of PPARγ target genes suggests increased total FAO capacity that is forced by ATGL expression and TG hydrolysis in these muscle cells (164). It is likely that overexpression of ATGL in skeletal and cardiac muscle will have a similar effect on FAO in these tissues in vivo. Interestingly, ATGL overexpression in rat skeletal muscle also results in increased concentrations of DG and ceramides, two potentially lipotoxic intermediates (165).

ATGL generates the DG, which is further catabolized by HSL (Fig. 1). Although HSL was originally considered to be rate limiting for TG hydrolysis, it was subsequently shown to be essential for efficient DG catabolism since this is its preferred substrate (172, 176). Mice deficient in HSL accumulate DG in many tissues including the heart, and TG hydrolysis in heart homogenates is unchanged (65). Moreover, myocardial TG content was unexpectedly decreased (66) or unchanged (120) in HSL-deficient mice that were fed normal chow. Hyperinsulinemic-euglycemic clamp studies showed that insulin-stimulated glucose uptake by the myocardium is decreased by 40% in HSL-KO mice fed a chow diet (120). However, the hearts of HSL-deficient mice were protected from high-fat diet-induced insulin resistance, which was associated with an attenuated deposition of myocardial TG in response to high-fat feeding and reduced plasma FA levels in HSL-KO mice (120).

A role of HSL in lipid handling and FAO in the myocardium was established in mice with cardiac-specific overexpression of HSL (147, 153). A 16-h fast increased myocardial TG content twofold in control mice; however, there was no increase in HSL transgenic mice (147). Serum FA concentrations were not different between genotypes. These results indicate that cardiac HSL overexpression diverts FA away from storage and toward FAO, consistent with the increased expression of genes involved in FAO in fasted HSL transgenic mice (147). In addition, mice with cardiac-specific HSL overexpression showed less myocardial lipid accumulation following induction of diabetes with streptozotocin (153). Besides protection from cardiac steatosis, HSL overexpression also decreased the effect of diabetes on cardiac interstitial fibrosis and mortality. Diabetes-induced increases of cardiac PPARα, PPARγ, and lipoprotein lipase mRNA expression were blunted in HSL transgenic mice. Furthermore, compared with the diabetic control mice, HSL transgenic mice displayed reduced mRNA expression for proteins involved in FA transport and TG synthesis (153). Possibly, reduced FA uptake combined with decreased TG synthesis and increased endogenous TG and DG hydrolysis contribute to the beneficial effect of HSL overexpression in the diabetic heart.

MG resulting from HSL-mediated hydrolysis can be further converted to FA and glycerol, possibly through the activity of MG lipase that is expressed at significant levels in the heart (81). However, the contribution of MG lipase to FA production for either reesterification into lipids or for FAO is currently unknown.

Similarly, the role of Ces3/TGH in cardiac lipid metabolism is unclear. Ces3/TGH functions in the lipolysis/reesterification cycle to promote the provision of TG for VLDL assembly in hepatocytes (58, 166), and it might serve a similar function in the heart (7).

Consequences of Lipid Accumulation for Lipotoxicity

Although the healthy heart relies normally on FAO for its predominant energy supply, an overreliance on FAO compared with glucose for energy production has been associated with ventricular dysfunction (14, 17, 24, 98, 100). Increased FA uptake in diabetes favors the oxidation of FA rather than glucose, especially when combined with insulin resistance. If FA supply exceeds the capacity for FAO, then the excess FA are incorporated into TG, resulting in cardiac steatosis. Saturated FA, such as palmitate, are also incorporated into ceramides (Fig. 1), which can cause apoptosis of cardiomyocytes (117) and insulin resistance in cells (25, 163). Significantly, ceramides accumulate in the heart after high-fat feeding, and this depends on PPARα activity (5, 54). The diversion of FA into TG rather than ceramide synthesis should alleviate these ceramide-induced effects. This could explain why unsaturated FA protect cardiomyocytes against palmitate-induced cell death (16, 36, 93). Significantly, oleate promotes the translocation of PAP (lipin-1) activity to the ER to increase TG synthesis, whereas saturated FA, like palmitate, have little effect on PAP activation (62). The glucocorticoid-induced expression of lipin-1 in the heart that occurs in diabetes probably provides extra capacity for sequestering excess FA as TG.

It is difficult to assess the physiological significance of studies on palmitate-induced apoptosis, although they do demonstrate the effects of compromised TG synthesis and the diversion of palmitate into ceramide synthesis. Cells in vivo are never exposed to palmitate alone, since circulating FA would always consist of approximately equivalent proportions of unsaturated FA (mainly oleate) to saturated FA (mainly palmitate) (114), which would enable palmitate to be incorpo-
rated efficiently into TG. However, ceramide concentrations often increase with TG concentrations when there is excessive diversion of FA to non adipose tissues, including the heart (155). This could contribute to insulin resistance and apoptosis of cardiomyocytes. By contrast, fibroblasts are relatively resistant to ceramide-induced apoptosis because they respond to ceramides by stimulating survival pathways, including activation of tyrosine kinases, Ras, and phosphatidylinositol 3-kinase (68). This result is significant since cardiac steatosis is associated with interstitial fibrosis (64, 153).

A further consequence of steatosis is that DG concentrations often increase with increased TG levels. DG accumulation activates novel PKCs, which act as DG sensors and cause the serine phosphorylation of target proteins including the insulin receptor and IRS-1 (39, 113). This decreases the tyrosine phosphorylation of these proteins and thereby insulin signaling. At present, it is unclear how the balance of DG formation through ATGL and the PAP activity of lipin-1 vs. DG hydrolysis by HSL influences insulin responses in the heart. Increased accumulation of DG activates PKCε or PKCδ, which appear to contribute to the effects of FA in causing insulin resistance (39). This conclusion is supported with observations that the overexpression of lipin-1 in muscle tissue results in whole body insulin resistance (131) and with decreased insulin-stimulated glucose uptake by the myocardium in HSL-KO mice (120).

Increased lipin-1 could augment the action of PGC-1α/PPARα in increasing the expression of enzymes involved in promoting FA uptake and oxidation. This would increase the reliance of the heart on FA rather than glucose as a fuel, resulting in adverse effects on cardiac metabolism and decreasing the ability to recover from ischemia. In this sense, TG synthesis should not be considered an antagonistic pathway to FAO, but a companion pathway that sequesters excess FA until they can be oxidized. Another explanation for coordinating the regulation of TG synthesis and FAO is that some FA must be converted to TG after their uptake by cardiomyocytes and then turned over before they can be efficiently oxidized. Therefore, there appears to be tight coordination among FA transport, TG synthesis, and FAO. At present, we know very little about how the regulation of these pathways is coordinated in the heart.

Pharmacological Approaches to Controlling Cardiac Lipid Metabolism and Normalizing Cardiac Function

The development of therapeutics that modify cardiac metabolism, and consequently cardiac mechanical function, has also progressed with the advance in knowledge concerning the regulation of cardiac metabolism (99). As mentioned above, the diabetic heart uses predominantly FA as an energy source, which requires more oxygen and is less energetically efficient. There are a significant number of studies that demonstrate the beneficial effects of shifting fuel usage from FA to glucose oxidation in several pathological conditions (104, 116, 150, 161). Drugs that promote the oxidation of glucose relative to FA include etomoxir and oxefinicine, which inhibit CPT I (116, 150), as well as trimetazidine and ranolazine, which are postulated to inhibit enzymes involved in the terminal steps of β-oxidation (56, 100, 134, 161). Glucose utilization can also be promoted by using malonyl-CoA decarboxylase inhibitors, which through increased malonyl-CoA concentrations inhibit β-oxidation (45). Drugs that block β-oxidation may prove beneficial in the treatment of diabetic cardiomyopathy. However, the benefits of favoring glucose over FA oxidation may have to be weighed against the possible contribution of decreased FAO to the development of cardiac hypertrophy, and impaired recovery from ischemia (35, 46, 76, 154).

Statins and fibrates are two classes of drugs that have positive cardiovascular effects (4, 8, 50). Although fibrates are classified as PPARα agonists, their mode of action depends on the tissue type. Their beneficial effects on cardiovascular function appear to depend mainly on an indirect mechanism through the liver and an improvement in plasma lipid and high-density lipoprotein levels (1, 2, 32, 106, 127).

Thiazolidinediones, which are PPARγ ligands, have also been proposed to have beneficial effects on cardiac function through pleiotropic effects on inflammation and lowering plasma glucose levels. However, there is an increased risk of heart failure, especially with rosiglitazone (82, 179).

Concluding Remarks

Our conclusion as to the enigma of whether increased TG synthesis is protective against lipotoxicity and insulin resistance or whether it is a maladaptive response is “yes” to both possibilities. Sequestering excess FA as TG in insulin resistance and diabetes provides an initial buffer against the accumulation of FA and acyl-CoA esters or the diversion of palmitate into ceramide synthesis. It also serves a physiological role by providing an internal store of FA that can be turned over to fuel FAO. On the negative side, an overreliance of the heart on FAO compared with glucose oxidation is associated with cardiac dysfunction and impaired recovery from ischemia. Steatosis is characterized by the excessive accumulation of TG in tissues, but it is often associated with increased concentrations of DG and ceramides that aggravate insulin resistance. Ceramides can also cause apoptosis in cardiomyocytes (10, 84, 87, 122). Furthermore, prolonged steatosis is associated with fibrosis, deficiencies in mechanical contraction, and congestive heart failure.

It is clear that the elucidation of the mechanisms regulating cardiac FA metabolism will continue to provide vital information regarding the progression of cardiac dysfunction in the diabetic heart. With increasing knowledge of the regulation of cardiac lipid metabolism, we can start to delineate which proteins involved in FA and lipid metabolism we should target as well as the ones that are unsuitable for pharmacological intervention.

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DISCLOSURES

No conflicts of interest are reported by the author(s).

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Cardiac Lipid Metabolism


