The heart uses large amounts of fatty acids (FAs) as energy-providing substrates. More than 70% of all substrates used for ATP generation are derived from FAs, with the remaining sources being glucose, lactate, ketone bodies, and amino acids. The relatively tight coupling between lipid uptake and oxidation prevents accumulation of excess lipids. Several processes that affect cardiac function, including ischemia, obesity, diabetes mellitus, sepsis, and heart failure, lead to altered fatty acid oxidation and often also to the accumulation of lipids. There is now mounting evidence associating certain species of these lipids with cardiac lipotoxicity and subsequent myocardial dysfunction. Experimental and clinical data are discussed and pathways to reduction of toxic lipids as a means to improve cardiac function are suggested.

**Abstract:** The heart utilizes large amounts of fatty acids as energy-providing substrates. The physiological balance of lipid uptake and oxidation prevents accumulation of excess lipids. Several processes that affect cardiac function, including ischemia, obesity, diabetes mellitus, sepsis, and heart failure, are associated with a reduction in FA oxidation (FAO) with a relative increase in anaerobic glycolysis and, in some cases, accumulation of nonoxidized FA derivatives in the form of lipids. In addition, excess circulating FA levels in type 2 diabetes mellitus and its precursor, the metabolic syndrome, also cause cardiac lipid accumulation. There is increasing evidence that many of these lipids worsen heart function and lead to structural myocardial damage, including cardiac fibrosis, myocyte apoptosis, and reduced contractility, which is often thought to be caused by mitochondrial dysfunction. However, the reversibility of this dysfunction along with several key steps of oxidative and glycolytic metabolism after the correction of cardiac stress and failure suggest that mitochondrial dysfunction is a reversible adaptation and is secondary to the altered metabolic pathways. Several altered metabolic pathways lead to lipid accumulation. Experimental and clinical data provide evidence that lipid accumulation causes or exacerbates heart dysfunction, a process termed cardiac lipotoxicity. In support of this hypothesis, several recent studies have shown that reduction of toxic lipids is associated with improved metabolism and function of the heart.

**Key Words:** ceramide ■ diabetes mellitus ■ heart failure ■ lipids and lipoprotein metabolism ■ lipotoxicity ■ obesity ■ triglyceride
Evidence of Lipotoxicity in the Human Heart

The role of intracellular lipid accumulation for the development of myocardial dysfunction is supported by studies of patients with inborn errors of FAO who develop cardiac abnormalities, including cardiac and skeletal myopathies, insulin resistance, arrhythmias, and sudden cardiac death. Patients with heart failure show a profound switch from FAO to preferential use of glucose as a substrate for ATP generation through glycolysis and utilization of alternative sources of energy, including lactate, ketone bodies, and amino acids. Further, flux through anaerobic glycolysis is increased. Overall, these changes in metabolic pathways and usage of substrates for energy provision leads to a chronically altered state with decreased ATP production and energy depletion of the failing myocardium.

Cardiac lipid accumulation has also been reported in humans with metabolic cardiovascular complications, such as diabetes mellitus and metabolic syndrome. More than 50 years ago, accumulation of lipids around the heart epicardial fat was reported for obese patients and correlated with cardiac dysfunction. Increased cardiac lipid content has been linked to impaired systolic function and increased left ventricular mass. Recent studies with more advanced imaging methods showed increased intramyocardial lipid content in patients with nonischemic heart failure, which is further exacerbated with obesity, diabetes mellitus, and metabolic syndrome.

A key study by Sharma et al described a group of heart failure patients with severe metabolic dysregulation characterized by intramyocardial triacylglycerol (TAG) accumulation and a transcriptional profile similar to that of an animal model of lipotoxicity and contractile dysfunction. Along with a proinflammatory phenotype of the failing myocardium, these data suggested dysregulation of FA metabolism contributing to cardiac dysfunction. Noninvasive imaging studies have demonstrated impaired lipid uptake and decreased FA utilization in the failing myocardium along with increased glucose oxidation. Further evidence of dysregulated FAO and accumulation of toxic lipid intermediates was found in samples from patients undergoing left ventricular assist device placement and heart transplantation. These studies demonstrated increased myocardial ceramide and decreased neutral TAG levels in the failing human myocardium. Increased ceramides were linked to protein kinase C (PKC) activation and insulin resistance through inhibition of Akt signaling, as well as abnormal 5’ adenosine monophosphate-activated protein kinase (AMPK) activity. These findings indicate that cardiac lipotoxicity is primarily driven by ceramides and diacylglycerol (DAG) and not TAG. Accordingly, mechanical unloading of these hearts with implantation of left ventricular assisting devices increased cardiac TAG and reduced ceramides and DAG. Additional studies revealed abnormal transcriptional regulation with a central role of KLF15 (kruppel-like factor 15), impaired amino acid metabolism, and mitochondrial dysfunction.

Excess cardiac lipid accumulation has also been associated with 2 forms of cardiomyopathy in patients with abnormal glucose homeostasis and metabolic syndrome: obesity-related cardiomyopathy, a cardiomyopathy associated with normal coronary arteries and sudden death, and diabetic cardiomyopathy with decreased cardiac function. Studies of pathological specimens, cardiac lipid uptake and oxidation, and magnetic resonance cardiomy TAG analysis showed that cardiac dysfunction is associated with deranged cardiac lipid metabolism and lack of intracellular TAG-derived FA mobilization that leads to TAG accumulation. Of note, early studies in the field of cardiac metabolism have highlighted the role of FA overload in the development of mitochondrial dysfunction and the uncoupling of oxidative phosphorylation in cardiomyocytes and other tissues by long-chain FAs. It remains unclear whether cardiomyopathy is as a result of abnormal FAO or accumulation of toxic lipids or both.

Diabetes mellitus and the metabolic syndrome are associated with a distinct form of cardiomyopathy that is characterized by early diastolic changes with increased interstitial fibrosis and myocardial lipid accumulation. Accumulation of intramyocardial lipids occurs in diabetes mellitus and is associated with increased myocardial infarction events and heart failure compared with individuals with less cardiac lipid droplet formation. Increased incidence and prevalence of heart failure in diabetic and obese patients with the metabolic syndrome is independent from the development of accelerated atherosclerosis. A link seems to exist between obesity and diabetes mellitus and left ventricular hypertrophy in correlation with TAG accumulation and cardiac steatosis. Although the exact pathophysiological mechanism for the development of this metabolic cardiomyopathy is not yet fully elucidated, increased supply of FAs along with insulin resistance and myocardial inflammation as well as reduced FAO because of mitochondrial dysfunction all might contribute to the development of a lipotoxic phenotype. Increased TAG and ceramides have both been described in the heart of diabetic and obese patients. The increased cardiac lipid content in diabetes mellitus may reflect increased uptake of FA destined for cardiac ATP production because insulin resistance may diminish cardiac glucose uptake and eventually glucose catabolism. A functional genomics study, which aimed to identify cardiac genes that are differentially regulated in obese individuals, discovered that apolipoprotein O is overexpressed in hearts from diabetic patients. A follow-up study that included both animal and human cardiac samples showed that apolipoprotein O localizes to mitochondria and compromises their function by promoting uncoupling of oxidative metabolism from phosphorylation of ADP. Mitochondrial uncoupling has been associated with cardiac lipotoxicity and has a major role in the development of cardiomyopathy in obesity and diabetes mellitus.
The role of the metabolic syndrome was highlighted in a study of a large cohort of patients undergoing aortic valve replacement. Increased lipid deposits associated with higher sterol regulatory element–binding protein-1c and peroxisomal proliferator–activated receptor (PPAR)-γ and lower levels of SERCA2a (sarcoplasmic reticulum Ca2+-ATPase) were found in the myocardium of patients with left ventricular hypertrophy and the metabolic syndrome compared with patients without the metabolic syndrome. These changes correlated with left ventricular dysfunction, suggesting a link between molecular markers of TAG synthesis and abnormal calcium handling with impaired ventricular function. Not surprisingly, patients with the metabolic syndrome had more insulin resistance, which, however, did not correlate with lipid accumulation in the failing myocardium.

It is currently unclear whether lipid accumulation in failing myocardium results from increased uptake of FAs, increased TAG synthesis, or impaired degradation of lipids. Although the phenotype of increased lipid accumulation in the failing myocardium, in particular in the setting of diabetes mellitus and the metabolic syndrome, has been established, it is not clear what specific type of lipid intermediates accumulate in the failing myocardium. The role of insulin resistance and impaired insulin signaling on myocardial lipid accumulation, as well as the specific lipid composition in human heart failure, is also currently unclear. Therefore, a systematic approach to characterize lipid content and type as well as intracellular compartmentalization of lipids is needed.

### Cardiac Lipid Accumulation and Storage

Under a variety of circumstances, the heart can accumulate nonpolar stored lipids and polar lipids that can activate subcellular structures that sequester nonpolar lipids, such as TAG, cholesteryl esters, and retinyl esters. A protein and phospholipid coat, analogous to those encapsulating circulating lipoproteins, encloses and contains the lipids. This coat protects the lipids but also contains proteins that modulate the release of the lipid esters, which occurs via the exposure of the lipids to the surface of the lipid droplet followed by activation of esterases. Although the number and variety of lipid droplet proteins keeps expanding, within the heart, the major proteins are members of the perilipin (Plin) family: Plin 2, previously deleted adipocyte differentiation–related protein; Plin 3, tail interacting protein of 47 kDa (Tip 47); Plin 4, S3-12; and Plin 5, also described as myocardial lipid droplet protein or (oxidative tissue) perilipin, adipophilin, and TIP47 (OXPHAT/PAT-1) protein. Knockout models have suggested that these proteins protect the droplet from cytosolic lipases as deletion of Plin5 leads to a marked reduction in lipid droplets and in some cases increased FAO. Transgenic overexpression of Plin 5 reduces cardiac FA hydrolysis, a result interpreted as evidence that this protein specifically protects the stored lipids. However, analogous to circulating lipoproteins where overexpression of many apolipoproteins, including apoC-II that activates lipoprotein lipase (LpL), results in hypertriglyceridemia, excess protein coating of the lipid droplet by amphipathic proteins prevents lipolysis by nonphysiological processes.

TAG lipolysis occurs via the actions of a series of enzymes that remove each of the 3 glycerol-attached FAs. Within the heart, the l-major lipase is adipose triglyceride lipase (ATGL, also known as PNPLA2). Deletion of this enzyme induces marked accumulation of TAG within the heart, heart failure, and premature death. In this case, lipid accumulation is, at least partially, because of defective FAO. Nevertheless, activation of oxidation pathways via treatment of ATGL knockout mice with a PPARα agonist reduced TAG accumulation, ameliorated heart failure, and reduced premature death. Two other enzymes hormone-sensitive lipase and monoaoyl–glycerol lipase are expressed in the heart and release the remaining FAs on TAG. In addition, TAG release from the droplet can occur via lipophagy in which the droplet is engulfed and exposed to lysosomal lipases. Mechanisms of TAG turnover are important for the regulation of cardiac lipid metabolism and seem to be affected by cardiac hypertrophy and failure. Of note, the overexpression of the FA transport protein cluster of differentiation 36 (CD36) changes TAG turnover dynamics in the heart, suggesting a regulatory role of cardiac FA uptake for TAG turnover.

Unlike the liver, the heart is not a site of active de novo synthesis of FAs from glucose or amino acids. Its high energy requirements, primarily fueled by lipids, require uptake of FAs from the circulation. Both nonesterified FAs released from adipose tissue as well as TAG contained in lipoproteins are used by the heart (Figure 2). LpL, the primary enzyme required for hydrolysis of TAG within circulating lipoproteins, is expressed at highest levels in the heart. At least in the rodent, LpL expression by the heart alone is sufficient to prevent hypertriglyceridemia, and cardiomyocyte-specific deletion of LpL leads to systemic hypertriglyceridemia.

Cholesterol is required by all mammalian cells because it is an essential component of the cell membrane. The heart seems to obtain cholesterol in an low-density lipoprotein receptor–independent manner. Hearts have low expression of the low-density lipoprotein receptor and also are not a site of robust cholesterol synthesis. How the cholesterol from the very low–density lipoprotein and chylomicrons is acquired by the heart after lipolysis has not been completely explored. Because lipolysis is required, the uptake could be via remnant lipoproteins interacting with lipoprotein receptors, although the lack of obvious cardiac defects in low-density lipoprotein receptor and apolipoprotein E knockout mice does not support this. Another option is that the surface lipids that are liberated from these particles are internalized by some as yet unknown process. Lipids obtained via hydrolysis of TAG-rich lipoproteins (very low–density lipoprotein and chylomicrons) are sufficient for the cholesterol needs of the heart (Figure 3).

### Animal Models of Lipid Toxicity

Although it has been argued that greater FAO will increase reactive oxygen formation and lead to toxicity, several experimental situations that increase FAO do not adversely affect heart function unless there is inappropriate lipid accumulation. Oxidation of long chain FAs such as palmitate requires the transfer of FA-CoA into the mitochondria by carnitine...
Intracellular triglyceride storage and release. Triglycerides are stored within cardiomyocytes in lipid droplets (shown in yellow) that are surrounded by phospholipids and several proteins; the most abundant are the perilipins (PLINs such as PLIN 2, 3, and 5). These proteins modulate the actions of the major triglyceride hydrolytic enzyme adipose triglyceride lipase (ATGL), which removes the first fatty acid (FFA) from triglyceride. The second fatty acid is removed by hormone-sensitive lipase (HDL) and the final by monoglycerol lipase (MGL). The released fatty acid complex with CoA via long chain acyl coA synthetases (ACSL).

Figure 1.

Cellular fatty acid uptake. Fatty acids generated by lipoprotein lipase (LpL) or as nonesterified fatty acids associated with albumin enter cells via a cell surface receptor such as cluster of differentiation 36 (CD36) or at high levels are acquired via nonspecific movement across the cell membrane. Once inside the cells, fatty acids are complexed to CoA and then either used for ATP generation or stored within lipid droplets. ATGL indicates adipose triglyceride lipase; DGAT, DAG acyl transferase; FFA, first fatty acid; PPAR, peroxisomal proliferator–activated receptor; and VLDL, very low-density lipoprotein.

Figure 2.
overexpression is beneficial for mice with pressure overload.108 However, this benefit cannot be attributed to increased FAO, which is surprisingly reduced, possibly as a consequence of the increased glucose utilization. In a mouse with total knock-out of the other important cardiac lipase, hormone-sensitive lipase, cardiac TAG lipase activity was decreased, but cardiac TAG was not dramatically changed, and there was no overt cardiac phenotype.108 Thus, massive cardiac lipid overload associated with decreased FAO causes heart dysfunction, whereas increased utilization of stored lipids seems to be beneficial.

The most informative of these models is perhaps the creation and then treatment of mice with cardiomyocyte-specific overexpression of members of the PPAR transcription factors. PPARs are central regulators of proteins that are involved in FAO. The PPAR family consists of 3 members, PPARα, PPARβ/δ, and PPARγ. PPARα regulates FAO in heart89 and skeletal muscle.109 PPARα activates FAO in the heart,91 whereas PPARγ is a major regulator of lipogenesis,92,93 and it also contributes to regulation of FAO in cardiac94 and skeletal95 muscle. PPARα-mediated FAO in the heart relies on the activation of peroxisomal and mitochondrial enzymes, such as acyl-CoA oxidase and CPT-1, malonyl-CoA carboxylase and UCP3. The transcriptional function of PPARα requires interaction with the co-activator, PPARγ-coactivator-1.96 Heart failure,97 as well as myocardial infarction,98 hypoxia,99,100 inflammatory mediators, such as IL-1β,101 IL-6,101 NF-κB,102 and reactive oxygen species,102 all downregulate PPARα expression.

Both PPARα and PPARγ-coactivator-1 gene expression levels are increased by AMPK,103–105 which also enhances cardiac transporter–mediated FA uptake106 and oxidation.107 AMPK is activated by a decline in the ATP/AMP ratio. Mice that express a dominant negative form of AMPK cannot increase mitochondrial biogenesis in response to energy starvation.108 Similarly, mice that express inactive AMPK show impaired fasting-induced expression of lipid oxidation-related genes.109 Mice expressing constitutively active AMPK also show increased transcript levels of FAO genes.109–111 Cardiomyocyte-specific PPARα transgenic mice have increased expression of FAO-related genes, greater FAO, and decreased glucose oxidation and GLUT4 expression.90 The α-myosin heavy chain (MHC)-PPARα mice fed with a long chain fatty acid–containing diet develop severe lipotoxic cardiomyopathy. Lipotoxicity was ameliorated when diet was switched to a medium-chain TAG-enriched diet.112 Reduction of lipid uptake in the PPARγ transgenic mice via either global deletion of CD36112 or a cardiac-specific deletion of LpL114 corrected the features of cardiomyopathy.

PPARγ transgenic mice show a similar increase in FA metabolic genes, but no decrease in GLUT4.49 Despite increased FAO, both αMHC-PPARα and αMHC-PPARγ mice develop lipotoxicity rather than reduced cardiac lipid stores, most likely because of increased expression of lipid uptake–related proteins, such as CD36 and LpL. PPARγ overexpression in cardiomyocytes results in lipid accumulation, heart failure, and sudden cardiac death with ventricular fibrillation.91,115 Interestingly, cardiomyocyte-specific overexpression of PPARγ in PPARα−/− background improved FAO, cardiac function, and survival rates, despite similar cardiac TAG and toxic lipids, DAG, and ceramide accumulation, as compared with PPARγ−/−.91 Acylcarbinitine content was decreased and so were apoptosis, reactive oxygen species (ROS) levels, and endoplasmic reticulum (ER) stress markers. Although these models suggest that lipid accumulation accounts for toxicity, at least partially, the toxic effects of excess lipid oxidation in perfused heart models of ischemia116 do not rule out toxicity emanating from excess lipid oxidation.

When FAO was reduced by a tissue-specific knockout of PPARδ, lipid accumulation and cardiomyopathy occurred.117 Conversely, constitutive cardiomyocyte-specific expression of PPARδ induced the expression of FAO–associated genes and did not lead to lipid accumulation and cardiac dysfunction.118 Besides elevated FAO, the prevention of cardiac lipid accumulation and organ dysfunction in the αMHC-PPARδ mice may be attributed to increased expression of angiopeptin-like 4,119 which is an inhibitor of LpL and therefore may minimize cardiac lipid uptake.

**Efforts to Elucidate the Toxic Lipids**

TAG accumulation is often correlated with toxicity but its direct role in myocardial dysfunction is not certain. Increased TAG levels correlate with insulin resistance, but several studies suggest that TAG accumulation is not toxic per se but just coincides with elevation of other lipid species that actually account for cellular toxicity.

Figure 3. Metabolism of circulating triglyceride–rich lipoproteins. Triglycerides (TG) within the circulation are predominantly carried by chylomicrons and very low-density lipoprotein (VLDL). Chylomicrons carry dietary lipids. Along with the lipids, it contains apolipoproteins including apoB-48 and C-II, the activator of lipoprotein lipase (LPL). VLDL contains apoB-100 and carries triglycerides secreted from the liver. Lipolysis converts triglycerides to fatty acids (FA) and also leads to the shedding of surface components that contain cholesterol (Chol). Defective lipolysis leads to reduced acquisition of fatty acids, cholesterol, and vitamin A by the heart. CE indicates cholesteryl ester; LDL, low-density lipoprotein; and LDL-R, low-density lipoprotein receptor.
In contrast to TAG, a series of relatively nonpolar lipids also accumulate in tissues, such as the heart, but in nonsequestered forms. These partially charged lipids are free in the cytosol, intercalated in membranes, and associated with carrier proteins. Nonesterified FAs (NEFAs) are rapidly complexed with CoA, which traps them intracellularly and neutralizes their charge. Although there may be many charged and potentially toxic intracellular lipids, DAGs and ceramides are the most thoroughly investigated. Saturated FAs, such as palmitic acid (16:0), are considered a more potent cause of lipotoxicity than unsaturated FAs, such as oleic acid (18:1). Incubation of isolated cardiomyocytes with palmitic acid results in higher levels of ceramide and DAG compared with incubation with oleic acid. Moreover, the greater propensity of oleic acid for sequestration in TAG has been associated with its protective role.

DAG is another lipid that could mediate FA-induced toxicity. DAG has been strongly associated with insulin resistance in skeletal muscle and liver. DAG acyl transferase (DGAT) incorporates fatty acyl–CoA and converts the toxic DAG to TAG. There are 2 isoforms of DGAT, DGAT1 and DGAT2. Overexpression of DGAT1 in hearts of lipotoxic models, such as the αMHC-acyl CoA synthetase and the αMHC–PPARγ mice, reversed cardiac dysfunction, despite increased lipid accumulation. Moreover, cardiomyocyte-specific expression of DGAT1 improved cardiac function after ischemia. Thus, DGAT1-mediated TAG synthesis seems to be protective for cardiac function in several pathological contexts. On the contrary, DGAT1-deficient mice are resistant to diet-induced obesity, which seems to be because of increased total energy expenditure because these mice have increased physical activity. The mechanisms that underlie ceramide and DAG toxicity in cardiomyocytes are not described thoroughly but several hypotheses have been proposed.

Both ceramides and DAGs bind to and activate isoforms of PKCs, which then translocate to the sarcolemma and cytosolic membrane. The PKC family includes 12 serine/threonine protein kinases. Several PKCs are highly expressed in adult myocardium and regulate contractility, gene expression, and cell growth. Overexpression of PKCβ specifically in the myocardium of transgenic mice, leads to cardiomyopathy because of combined myocardial necrosis and thickened left and right ventricular walls, resulting from the increase in the number of cardiomyocytes and the size of the interstitial extracellular matrix. High-fat diet increases PKCβ2 activation and causes cardiac hypertrophy in male Sprague–Dawley rats. Several PKC isoforms are activated during heart failure. PKCα and PKCε confer negative inotropic effects in cardiomyocytes. PKCβ impairs Ca+2 handling, increases cardiomyocyte necrosis, and promotes ventricular wall thickening. Genetic and pharmacologic inhibition of PKCs improves cardiac responsiveness to catecholamines and heart function in mice with cardiomyopathy. Cardiac tissue from heart failure patients, mouse models of cardiac lipotoxicity, and a palmitate-treated human cardiomyocyte cell line have increased PKCα and PKCδ activation. Moreover, several lipotoxic heart models exhibit abnormal activation of PKC and defective adrenergic signaling pathways. Thus, PKC signaling is activated by toxic lipids and is associated with heart failure and also changes in heart rhythm.

PKC activation blocks insulin signaling pathways, inactivates adrenergic receptors, and increases cellular apoptosis. Transgenic overexpression of several PKCs leads to heart failure. DAGs are thought to accumulate when cardiomyocytes take up more FAs than can be converted to TAGs, that is, when the TAG esterification pathway is saturated. As noted earlier, overexpression of DGAT1 reduces cardiac DAG, increases TAG stores, and ameliorates some forms of cardiac lipid–induced toxicity. Surprisingly, DGAT1 expression is reduced in cardiac tissue from patients with severe heart failure, who also have DAG accumulation. Selective cardiomyocyte DGAT1 knockout mice reproduces this lipid abnormality and causes heart failure. Thus, DGAT1 seems to control the intracellular concentration of cardiotoxic lipids. Of note, many of the interventions that affect heart levels of DAGs also affect other lipids, such as ceramides, and maybe others because lipid metabolic pathways are interconnected. Thus, changes in DGAT1 that alter DAGs also change total ceramides in the same direction, perhaps because it shunts palmitate from ceramide to TAG synthesis.

Ceramide is synthesized via 3 major pathways. De novo synthesis includes conversion of palmitate to palmitoyl-CoA, which is then converted to 3-keto-sphinganine with the contribution of serine palmitoyltransferase. Subsequent reactions lead to the synthesis of sphinganine, dihydroceramide, and ceramide. Sphinganine is an inhibitor of post-lysosomal cholesterol transport. Ceramide is also produced from sphingomyelin that is hydrolyzed by sphingomyelinase. Ceramides are also produced through the salvage pathway from sphingosine-1-phosphate and the sphingomyelinase pathway.

Mice expressing glycosylphosphatidylinositol (GPI)-anchored human LpL specifically in cardiomyocytes (αMHC-LpL) have increased cardiac uptake and accumulation of lipids that are derived from circulating lipoproteins. These mice develop a dilated cardiomyopathy, which is accounted for, at least partially, by ceramide. Treatment of these mice with myriocin, a de novo ceramide synthesis inhibitor, normalizes intramyocardial ceramide levels and alleviates cardiac hypertrophy. However, this treatment improves survival only slightly, indicating that other nonceramide mechanisms may also mediate cardiac lipotoxicity.

Further evidence of the toxic role of ceramides has been derived from animal models of ceramidase modulation. This enzyme controls the degradation and therefore detoxification of ceramides. Ceramidase activity is regulated by adiponectin, and adiponectin gene deletion mice develop a phenotype of increased apoptosis mediated through a sphingolipid-mediated pathway. Overexpression of adiponectin decreases caspase-8-mediated cell death. These data suggest a role for adiponectin-mediated sphingolipid metabolism through the regulation of ceramidase activity and ceramide homeostasis. Ceramides and their regulation through ceramidase have been suggested to play a crucial role in the development and progression of insulin resistance both in diabetes mellitus and heart failure.
Phospholipids constitute another lipid class that accounts for cardiac lipotoxicity. The toxic effect of phospholipids has been attributed to either indirect crosstalk of metabolic pathways of phospholipids with those of TAG or other lipids or direct signaling events triggered by changes in phospholipid content. Increased NEFA content promotes degradation of phospholipids in rat cardiomyocytes and increases cell death. On the contrary, increased phospholipid synthesis is cardioprotective in rats. Phospholipid synthesis of cardiac function associated with elevated concentrations of cardiac TAG levels. Phosphatidylethanolamine depletion activated the sterol regulatory element–binding protein pathway that promotes FA synthesis and lipogenesis. Suppression of the sterol regulatory element–binding protein pathway in these flies alleviated cardiac lipid accumulation and improved heart function. A study in mice with defective phosphatidylethanolamine synthesis because of global CTP:phosphoethanolamine cytidylyltransferase (Pcyt2) haploinsufficiency showed that mice have lower expression of cardiac FA metabolism genes, as well as hypertriglyceridemia and increased ROS, as well as inhibition of cardiac insulin signaling. These findings indicate reduced cardiac metabolic rates that may lead to increased toxic lipid accumulation. Thus, alterations in phospholipid metabolism affect cellular lipid homeostasis and signaling with potential consequences in cardiac function.

**In Vivo Data Supporting a Role for Lipids in Cardiac Toxicity**

The importance of lipid toxicity as a cause or an accessory to human heart failure is gaining acceptance. Lipids might alone lead to heart failure, and they may aggravate disease that is primarily caused by ischemia or other forms of cardiomyopathy. Aside from the increased concentration of DAG and ceramides in hearts of humans with severe heart failure, some experimental studies have found lipid accumulation in hearts after acute ischemia. This is presumably because of an imbalance as the ischemic hearts continue to acquire FAs while hypoxia switches cardiac metabolism toward glucose and reduced FAO.

By modifying lipid metabolic pathway, investigative studies in mice have confirmed that lipid accumulation alone can lead to heart failure (reviewed in Goldberg et al) and in some cases sudden death. These studies are also consistent with the hypothesis that TAG storage within lipid droplets is unlikely to be the culprit, despite its use as a marker for overall lipid accumulation. Thus, increasing lipid uptake pathways or increasing the trapping of NEFAs lead to cardiac lipid overload. Overexpression of both PPARα and PPARγ increase FAO but cause an imbalance in cardiac lipid metabolism because uptake exceeds oxidation and leads to lipid accumulation. Finally, marked reduction of FAO can also lead to lipid accumulation and heart failure.

As mentioned earlier, Atgl−/− mice exhibit reduced FAO and massive cardiac lipid accumulation and severe cardiac dysfunction that is corrected when PPARα is activated pharmacologically. This indicates that FA-mediated PPARα activation relies on intracellular TAG lipolysis, and ATGL overexpression is beneficial for mice with pressure overload. However, this benefit cannot be attributed to increased FAO, which is surprisingly reduced, possibly as a consequence of the increased glucose utilization. In hormone-sensitive lipase gene deletion mice, cardiac TAG lipase activity was decreased, but cardiac TAG was not dramatically changed and there was no overt cardiac phenotype.

Animal models have also been used to evaluate methods to treat lipotoxicity. Not unexpectedly, reductions in cardiomyocyte lipid uptake, increased secretion of lipids, and greater conversion of toxic lipids to nonpolar stored forms all improve heart function. Several hormones seem to directly affect cardiac lipid content and have been used to improve heart function in these models. Adiponectin stimulates ceramidase and improves heart function in mice. Glucagon-like peptide 1 improved heart function in mice with genetic deletion of DGAT1.

**Lipid-Driven Signaling Pathways Associated With Cardiac Dysfunction**

An incompletely answered question is how the excess lipids cause cardiac dysfunction and heart failure. Cardiac lipotoxicity is associated with apoptotic mechanisms in obesity, diabetes mellitus, and aging. Apoptosis is one of the major cofactors of cardiac dysfunction. Saturated FAs induce apoptosis in a cellular environment of increased lipid content and excess lipid oxidation. Treatment of isolated neonatal rat ventricular myocytes with palmitic acid compromises mitochondrial physiology and leads to apoptosis associated with cardiopin loss, cytochrome c release, mitochondrial swelling, and DNA laddering. Generation of ROS has also been implicated in palmitate-induced programmed cell death in one study, but this could not be confirmed by another study. The former study, which was performed in Chinese hamster ovary cells, showed that 2 ROS scavengers prevented palmitic acid–mediated apoptosis. In contrast, palmitate-induced apoptosis was neither associated with increased ROS nor rescued by antioxidants in neonatal rat cardiomyocytes. In cultured aortic endothelial cells, NEFAs increased ROS production, especially in the setting of hyperglycemia. Defective insulin signaling is one of the earliest observed cardiac defects in mice fed with high-fat diet and frequently is ignited by cardiac lipid accumulation. Predominant utilization of FA for cardiac ATP production, decreased glucose uptake, defective contractile response to insulin, and decreased...
cardiac efficiency because of oxygen waste for noncontractile purposes are some of the major events that occur with cardiac insulin resistance. Conversely, mice with a cardiacspecific deletion of insulin receptors demonstrate increased glucose uptake and oxidation and develop smaller hearts.

Both ceramide and DAG have been implicated in defective insulin signaling and reduced glucose uptake in muscle. Saturated fat feeding causes insulin resistance, most likely via alterations in ceramide metabolism. Although the mechanism is not fully elucidated, it has been shown that ceramide blocks insulin-mediated activation of Akt/PKB via direct inhibition of Akt/ PKB phosphorylation or by stimulating protein phosphatase 2A that dephosphorylates Akt/ PKB. In accordance with this observation, overexpression of acid ceramidase, which reduces intracellular ceramides by catalyzing their conversion to sphingosine attenuated the inhibitory effects of saturated NEFAs on insulin signaling of C2C12 myotubes. DAG blocks insulin signaling by promoting IRS-1 phosphorylation, resulting in its deactivation. This process, at least in skeletal muscle, may be mediated by activation of PKC or other PKCs. Systemic insulin resistance in patients with heart failure is accompanied by increased toxic lipid intermediates, DAG, and ceramide. Mechanical unloading with left ventricular assist device implantation decreased DAG and ceramide levels and activated insulin/phosphatidylinositol-3 kinase/Akt pathway.

Mitogen-activated protein kinases have been implicated in cardiac development and disease, as well as in cardiomyocyte apoptosis. In addition, there are findings that implicate mitogen-activated protein kinases in FA-induced toxicity. Treatment of primary neonatal rat ventricular myocytes with palmitic acid activates extracellular signal–regulated kinase (Erk)1/2, p38 mitogen-activated protein kinase (p38 MAPK), and c-Jun N-terminal kinase (JNK). However, a mitogen-activated protein kinase (MAP)1/2 inhibitor or a p38 MAPK inhibitor had no effect on baseline or palmitate-induced apoptosis. Activation of JNK1 is involved in the induction of apoptosis in rat cardiomyocytes that undergo ischemia/reperfusion stress. The apoptotic effect of ceramide in rat cardiomyocytes can be mediated by activation of JNK and attenuated by antisense JNK1 or JNK2. JNK interacts with proapoptotic Bax on the mitochondrial membrane. Treatment of the same cells with a low concentration of oleate along with palmitate inhibited both palmitate-induced JNK activation and apoptotic events. Inhibition of JNK is also associated with increased FAO in the hearts of septic mice. These data suggest that mitogen-activated protein kinases may be involved in lipid-mediated apoptosis or suppression of FAO and may therefore account for impaired cardiac function.

Accumulation of FAs also causes ER stress. Specifically, palmitate induces oxidative stress and generation of ROS that eventually lead to ER stress and cell death. Moreover, the incorporation of palmitate in phospholipid and TAG compromises the integrity of ER membrane and releases protein-folding chaperones to the cytosol. Another study has reported that the esterification of palmitate can directly cause ER ligation. Myocardial ER stress markers were elevated in a rat heart failure model (left anterior descending coronary artery ligation), and their expression was alleviated by treatment with atorvastatin, which improved left ventricular function and reduced cardiac fibrosis. Atorvastatin blocks cholesterol, not FA, synthesis, and so how this intervention altered intracellular lipids is unclear. In cardiomyocytes, palmitate-induced ER stress is prevented by combined treatment of cardiomyocytes with oleate that promotes TAG formation. The same study also showed that the protective effect of oleate is abolished on overexpression of ATGL that increases the release of NEFAs from the intracellular TAG pool. Thus, cardiac lipid accumulation may contribute in ER stress and the development of heart failure primarily because of elevation of palmitate.

**Septic Cardiac Dysfunction**

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. Unless treated promptly, sepsis can lead to septic shock, which is a lethal condition because of combined hypotension, ischemia, and multiple organ failure. Cardiac dysfunction is a major event of sepsis. Nevertheless, septic patients in advanced stages show impaired cardiac contractility, diastolic dysfunction, reduced cardiac index, and a low ejection fraction. The clinical importance of cardiac dysfunction in the pathophysiology of sepsis is signified by the higher mortality of septic patients with systolic or diastolic dysfunction compared with those diagnosed with sepsis but without diastolic or systolic dysfunction. The mechanisms that underlie myocardial depression during septic shock may be driven either by elevated inflammation or impaired metabolism and reduced ATP production in the heart.

Reduced ATP production in cardiomyocytes is primarily caused by impaired FA and glucose metabolism. The impairment of FA utilization in sepsis causes intracellular lipid accumulation that occurs despite reduced cardiac lipid uptake. Although cardiac lipid accumulation has been reported in septic animal models, the lipids that mediate cardiac toxicity have not been identified. A study that analyzed ceramide species did not indicate any association of cardiac dysfunction or improvement in cardiac function with alteration of ceramide levels in the heart.

Inhibition of intracellular FA mobilization and oxidation is accounted for by reduced expression of FA-binding protein, acyl-CoA synthetase, and Cpt1. Furthermore, cardiac expression of transcriptional factors that regulate FAO, such as PPARs, retinoid-X receptors, and thyroid receptors, which drive the expression of various FA metabolism–related proteins, is reduced. The coactivator of these nuclear receptors, PPARγ-coactivator-1, has also decreased cardiac expression during sepsis. Accordingly, cardiomyocyte-specific constitutive expression of PPARγ-coactivator-1 or PPARγ pharmacological activation of PPARγ, prevention of PPARα downregulation, and cecal ligation and puncture. This suggests that improved ATP production (and...
likely lower lipid accumulation) in organs of septic mice is crucial for the function of organs besides the heart. As PPARγ activation is also associated with reduced inflammation, it is tempting to speculate that the improvement in survival during sepsis is because of alleviation of inflammation and not altered lipid accumulation and use. However, when PPARγ activation was applied to mice that did not express adiponectin, an adipocyte-derived protein that promotes FAO in peripheral organs, including the heart, the survival benefit was abolished. In accordance with animal studies that associated improved FAO with reduced sepsis-related mortality, a clinicometabolic study that analyzed plasma from septic patients showed that markers of reduced FAO utilization in tissues, such as higher concentration of carnitine esters and FAs, were associated with increased lethality. Thus, improvement in FA mobilization that eventually leads to increased energy production seems to confer survival benefit at least for the early stages of the disease.

Metabolic Modulation as a Therapeutic Intervention in Lipotoxic Cardiomyopathies

The role of weight loss for the correction of cardiac lipid accumulation in obesity and diabetes mellitus is controversial. Limited data suggest that weight loss indeed reduces cardiac TAG levels and reduces FA uptake, leading to improved diastolic function. Of note, short-term fasting (eg, overnight) increases cardiac lipid droplets and TAG content likely because of impaired FAO in the absence of nutritional glucose supply. However, fasting does not lead to reduced heart function; this is further evidence that not all causes of TAG accumulation are detrimental.

In animal models, FAO has also been inhibited using pharmacological inhibitors of Cpt-1, such as etomoxir, ethyl-2-tetradecyl glycine, and oxefinicine. It has been proposed that this change from FAO to greater glucose oxidation with less oxygen requirements should be beneficial in the response to ischemia. In patients with heart failure, reduction of FAO by reducing plasma NEFA levels was not beneficial, and in some acute studies, reducing NEFA levels was harmful. Pharmacological compounds such as perhexiline which blocks mitochondrial FA via inhibition of CPT-1 and CPT-2 have been used in both ischemic and nonischemic heart failure patients. Treatment with perhexiline was associated with improved cardiac function and symptoms. Trimetazidine reduces FAO and slightly improves cardiac function and insulin sensitivity in patients with idiopathic dilated ischemic ischemia. Depletion of circulating FAs through a hyperlipopidemic agent (acipimox) that aimed to reduce FA uptake by the heart and storage into triglycerides did not improve cardiac function in patients with heart failure. Therefore, the overall benefit of reducing FAO in heart failure is still unclear. One possible reason is the poorly defined nature of heart failure, suggesting that a better characterization of cardiomyopathies is necessary before inclusion into subsequent trials. It is unclear whether patients might also require reduced myocardial substrate uptake. Further, it remains to be seen whether normalizing substrate uptake and reducing lipotoxicity can be the next major advance or a complementary treatment in heart failure therapy.

Open Questions

Both ischemia and heart failure are associated with a switch to greater glucose utilization through glycolysis and reduced FAO. It remains to be clarified whether this is a short- or long-term adaptive response of the failing heart or whether this constitutes a maladaptive change that leads to energy depletion and further deleterious cardiac effects. It remains unclear what lipid species are involved in mediating cardiac lipotoxicity that has been associated with lower ATP production and mitochondrial dysfunction. Finally, although some studies have demonstrated sex differences in cardiac metabolism and the myocardial response to stressors, it remains unclear whether this also affects mechanisms of cardiac lipotoxicity.

In regard to lipid intermediates, in particular, ceramides, the specific nature of its toxicity and the direct impact of different chain length are the topic of ongoing investigations. The same direct evidence is missing for DAG and TAG species, as well as other lipids. This is directly linked to the question whether it is possible to adjust the delicate balance of synthesis and consumption or use and storage of different lipids and, therefore, affect and prevent the toxicity associated with increased levels of these intermediates. In this regard, the recently reported marked reduction in heart failure because of the use of a sodium glucose co-transporter 2 inhibitor has been interpreted as evidence that reducing heart glucose uptake and increasing FAO and lipid accumulation might improve cardiac function. However, whether changing the rate of FAO and its relationship to lipid accumulation alters lipotoxicity remains to be addressed. One possible option is to reduce lipid uptake for the treatment of lipid toxicity, but this has to be balanced by the possible effects of energy depletion because FAs are the main source for ATP production in the normal, nonfailing heart. Despite the need for greater oxygen use, another option would be to stimulate FAO to improve heart function during failure, which would indicate that the long-term switch to greater glucose use is a maladaptive response.

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Disclosures

None.

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