Lipotoxicity: when tissues overeat

Jean E. Schaffer

Purpose of review

This review will provide the reader with an update on our understanding of the adverse effects of fatty acid accumulation in non-adipose tissues, a phenomenon known as lipotoxicity. Recent studies will be reviewed. Cellular mechanisms involved in the lipotoxic response will be discussed. Physiologic responses to lipid overload and therapeutic approaches to decreasing lipid accumulation will be discussed, as they add to our understanding of important pathophysiologic mechanisms.

Recent findings

Excess lipid accumulation in non-adipose tissues may arise in the setting of high plasma free fatty acids or triglycerides. Alternatively, lipid overload results from mismatch between free fatty acid import and utilization. Evidence from human studies and animal models suggests that lipid accumulation in the heart, skeletal muscle, pancreas, liver, and kidney play an important role in the pathogenesis of heart failure, obesity and diabetes. Excess free fatty acids may impair normal cell signaling, causing cellular dysfunction. In some circumstances, excess free fatty acids induce apoptotic cell death.

Summary

Recent studies provide clues regarding the cellular mechanisms that determine whether excess lipid accumulation is well tolerated or cytotoxic. Critical in this process are physiologic mechanisms for directing excess free fatty acids to specific tissues as well as cellular mechanisms for channeling excess fatty acid to particular metabolic fates. Insight into these mechanisms may contribute to the development of more effective therapies for common human disorders in which lipotoxicity contributes to pathogenesis.

Keywords

lipotoxicity, free fatty acids, heart failure, diabetes, obesity

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Center for Cardiovascular Research, Departments of Internal Medicine and Molecular Biology and Pharmacology, Washington University School of Medicine, St Louis, Missouri, USA

Correspondence to Jean E. Schaffer, Center for Cardiovascular Research, Washington University School of Medicine, 660 South Euclid Avenue, Box 8086, St Louis, MO 63110-1010, USA
Tel: +1 314 362 8717; fax: +1 314 362 0186; e-mail: jschaff@im.wustl.edu

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Abbreviations

CoA	coenzyme A
FFA	free fatty acid
IκB	inhibitor of nuclear factor-κB
NASH	non-alcoholic steatohepatitis
NFκB	nuclear factor-κB
PKC	protein kinase C
PPAR	peroxisome proliferator-activated receptor
ZDF	obese *fa/fa* Zucker diabetic fatty

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Introduction

Accumulation of excess lipids in non-adipose tissues leads to cell dysfunction or cell death. This phenomenon, known as lipotoxicity, may play an important role in the pathogenesis of diabetes and heart failure in humans. In this review, recent findings on the consequences of fatty acid overload on organ and cell functions will be discussed. The relationship of experimental animal models and cell culture studies to human pathophysiology will be highlighted.

Lipid homeostasis

Normal cellular fatty acid homeostasis reflects a balance between processes that generate or deliver fatty acids and processes that utilize these molecules. In mammalian cells, free fatty acids (FFAs) are generated through the de-novo synthetic pathway and liberated when triglycerides and phospholipids are hydrolyzed by cellular lipases. FFAs can also be imported into mammalian cells by both protein- and non-protein-mediated mechanisms, either when cellular demand is high or when extracellular FFA concentrations are high [1]. FFAs derived from each of these processes can be utilized for membrane biosynthesis, energy production through β-oxidation, generation of lipid signaling molecules, post-translational protein modification, and transcriptional regulation.

When cells accumulate more FFAs than are required for anabolic or catabolic processes, excess lipid is esterified and stored as triglyceride in lipid droplets. These single-membrane bound compartments are dynamic and fatty acids stored within may be mobilized through the actions of cellular lipases, in a process regulated by hormones and by droplet-associated proteins. Adipocytes have a unique capacity to store large amounts of excess FFAs in cytosolic lipid droplets. However, cells of non-adipose tissues have a limited capacity for storage of lipids. When this capacity is exceeded, resultant cellular dysfunction or cell death is termed lipotoxicity.

Disease states associated with lipid overload

High plasma FFA and triglyceride levels lead to increased import of FFAs into non-adipose tissues, contributing to intracellular lipid accumulation. In addition to primary hyperlipidemias, serum triglycerides [2,3] and FFAs [4,5] are elevated in type 1 and type 2 diabetics and plasma FFA levels are elevated in obese individuals [6]. More rare are congenital or acquired lipodystrophies in which absence of functioning adipose tissues likely leads to the observed high serum

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triglyceride and FFA levels that promote excess lipid accumulation in the liver and skeletal muscle [7]. In each of these disorders, excess FFAs may be taken up directly by cardiac myocytes, hepatocytes, jejunal enterocytes and adipocytes, since increased substrate concentration leads to increased transport into these cells [8]. Triglycerides and triglyceride-rich lipoproteins also increase delivery of FFA to the myocardium, either directly through the uptake of remnant particles [9] or through uptake of FFAs liberated by lipoprotein lipase-mediated hydrolysis of triglycerides within the vascular space.

Another mechanism for lipid accumulation is observed in tissues with high turnover/metabolism of FFAs, such as the heart, when utilization of FFAs is impaired in the face of continued FFA import or production. While long-chain FFAs are the major source of energy in the normal adult mammalian heart, acquired and inherited cardiac disorders are associated with a switch in energy substrate utilization from FFAs to glucose. In idiopathic dilated cardiomyopathy and other forms of heart failure, decreased FFA oxidation [10] is associated with triglyceride accumulation [11]. Moreover, in inherited fatty acid oxidation disorders, failure to utilize long-chain FFAs is associated with up to 100-fold increase in myocardial triglyceride content [12]. Similarly, pharmacological inhibition of FFA β-oxidation in a rat model leads to intramyocellular lipid accumulation, which is exacerbated in the setting of a high fat diet [13].

Pathophysiology of lipid overload

Adverse consequences of lipid overload have been observed in many organ systems. This review will focus on recent data on lipid overload in the heart, skeletal muscle, pancreas, liver, and kidney.

Heart

Systemic metabolic alterations that result in cardiac lipid accumulation are associated with cardiomyopathy. In humans, the cardiac lipid overload that occurs with inherited defects in the mitochondrial fatty acid oxidation pathway is associated with heart failure and sudden death [14]. Lipid accumulation in cardiomyocytes of mice that are null for long-chain acyl-coenzyme A (CoA) dehydrogenase leads to replacement fibrosis pathologically, which may provide foci for initiation of arrhythmias [15]. Cardiac dysfunction is also observed in obese *fa/fa* Zucker diabetic fatty (ZDF) rats in which a loss-of-function mutation in the leptin receptor leads to progressive elevation of serum FFA and triglyceride levels and diabetes [16•]. In this model, deposition of fat in cardiac myocytes is followed by evidence of apoptotic cardiomyocyte death and a modest decrease in systolic function (fractional shortening 56% transgenic versus 65% wild type at 20 weeks of age).

In both fatty acid oxidation disorders and in the ZDF rat model of diabetes, lipid accumulation in the heart is associated with death of cardiac myocytes that likely contributes to impairment of systolic cardiac performance. However, energy starvation in the case of fatty acid oxidation disorders and excessive fatty acid oxidation in the ZDF rat may also contribute to cardiomyopathy. Thus, several recent studies examined the effects of cardiac lipid accumulation in transgenic mice in isolation from systemic metabolic disturbances. First, cardiac restricted overexpression of acyl-CoA synthetase leads to increased cardiac myocyte FFA uptake and intramyocyte accumulation of triglyceride and phospholipid [17•]. Lipid accumulation in this model is associated with early evidence of cardiomyocyte apoptosis, initial cardiac hypertrophy, dramatic decrease in systolic function (fractional shortening 28% transgenic versus 65% wild type at 6 weeks), and premature death. In a second model, overexpression of lipoprotein lipase at the surface of cardiomyocytes of transgenic mice [18•] leads to increased cardiac uptake of radiolabeled VLDL triglyceride, cardiac hypertrophy, increased mortality, cardiomyocyte accumulation of FFAs and cholesteryl esters, and poor contractile function (fractional shortening 33% transgenic versus 58% wild type). Induction of genes involved in FFA metabolism and the absence of triglyceride accumulation in cardiac myocytes of these mice suggest that β-oxidation is upregulated in this model. In a third model of increased lipid utilization by the heart, cardiac restricted overexpression of peroxisome proliferator activated receptor (PPAR)α leads to increased expression of genes of myocardial lipid uptake and β-oxidation [19]. When these mice are fasted or fed a high fat diet, triglyceride accumulation in the heart results from an imbalance between FFA uptake and utilization and is associated with ventricular hypertrophy and systolic ventricular dysfunction (fractional shortening decreases from 58.5 to 43.2%). Together, these models show that lipid accumulation that results from mismatch between lipid import and utilization in the heart can lead to systolic ventricular dysfunction.

Skeletal muscle

Increased plasma FFAs lead to intramyocellular lipid accumulation in humans that has been proposed to play a critical role in the genesis of insulin resistance and type 2 diabetes [20]. Intracellular FFAs or their metabolites activate a serine/threonine kinase cascade that ultimately results in reduced insulin receptor substrate-1 tyrosine phosphorylation, reduced insulin receptor substrate-1-associated phosphatidylinositol 3-kinase activity and failure to promote translocation of the GLUT4 glucose transporter to the plasma membrane in response to insulin stimulation [21]. Intramyocellular lipid accumulation is associated with activation of protein kinase C (PKC)-θ [21], PKC-ε [22], and activation/translocation of

PKC-β and -δ isoforms from the cytosol to the cell membrane [23]. As well, decreased activation of atypical PKC isoforms ($\zeta/\lambda/\iota$) has been observed [24]. Alterations in PKC activation may not only interfere with normal insulin signaling, but also may contribute to activation of the nuclear factor-κB (NFκB) pathway [23,25•]. These changes in signaling pathways downstream of the insulin receptor are most closely associated with accumulation of intracellular fatty acyl-CoA and diacylglycerol, rather than triglyceride and ceramide [26•].

Pancreas

Lipid overload in pancreatic β-cells leads to dysregulated insulin secretion with short-term increases and chronic decreases [27–29]. While these effects may occur through acute alterations in signaling pathways that lead to insulin secretion, there is also evidence that FFAs have effects on expression of PPARα, glucokinase, the GLUT2 glucose transporter, prepro-insulin, and pancreatic/duodenal homeobox-1 (PDX-1) [30]. Additionally, FFAs serve as ligands for PPARα, which may modulate insulin secretion [31,32].

In addition to FFA-induced β-cell dysfunction, accumulation of excess FFAs also causes β-cell apoptosis. In ZDF rats, triglyceride accumulation in islets is associated with decreased β-cell mass and decline in insulin production with evidence for DNA laddering [33]. *In vitro*, excess FFAs lead to apoptosis in primary rat pancreatic β-cells and β-cell lines [34,35] and in isolated human islets [36]. The finding that the extent of FFA-induced cell death and the magnitude of caspase activation increases with increasing glucose concentrations suggests that the toxic effects of glucose and FFAs are synergistic [35].

Liver

Evidence for the toxic effects of excess lipid in the liver is found in animal models and in human disease. In a mouse model of impaired β-oxidation due to lack of mitochondrial trifunctional protein, moderate to severe lipid accumulation in the liver may lead to cell dysfunction, manifest as failure to appropriately carry out gluconeogenesis [37]. In these mice, neonatal hypoglycemia contributes to excess early mortality. In humans, triglyceride and FFA accumulation in the liver is associated with non-alcoholic steatohepatitis (NASH), characterized by an inflammatory response with evidence of hepatocyte damage and fibrosis that can progress to cirrhosis [38]. NASH has been described in obese individuals, in diabetics, and in patients with lipodystrophy.

Kidney

In the progression of renal failure, FFAs carried on albumin in the proximal tubule are thought to have toxic effects on proximal tubular epithelial cells and contribute to pathological changes of the tubulointerstitium [39]. Evidence for this hypothesis comes in part from studies using a model of protein-overload proteinuria. Rats injected with FFA-carrying bovine serum albumin had significantly greater macrophage infiltration in the outer cortex, tubular cell apoptosis, and cortical cell proliferation than rats injected with FFA-depleted bovine serum albumin or saline. This study suggests that FFAs carried by filtered albumin in the setting of proteinuria play a role in the genesis of tubulointerstitial injury.

Mechanisms of lipid-induced cell death

A number of studies have focused on characterization of specific lipid species that initiate signals for apoptosis. Observations that a non-metabolized fatty acid (2-bromopalmitate) induces apoptosis in normal rat β-cells [40•] and that acyl-CoA synthetase activity in 293 and HT29 cells is inversely correlated with levels of arachidonic acid-induced apoptosis [41] suggest that unmetabolized FFAs may be the culprits. In addition to FFA effects on signal transduction pathways, FFAs may act directly on mitochondria to induce mitochondrial membrane permeability transition and cytochrome C release [42,43]. Alternatively, several FFA metabolites may promote, if not initiate, apoptosis. The observation that long-chain saturated but not unsaturated FFAs induce apoptosis suggests that metabolites made from saturated species may be central [40,44,45,46•,47,48]. For example, saturated, but not unsaturated FFAs, are precursors for de-novo synthesis of ceramide, a lipid signaling molecule that is known to induce apoptosis. Although incubation of cultured cells with palmitate leads to increased ceramide biosynthesis, only in some cells does this mediator play a critical role in palmitate-induced apoptosis [36,44,49,50]. Another difference between saturated and unsaturated FFAs is that saturated species are poor substrates for cardiolipin biosynthesis and lead to a decrease in mitochondrial cardiolipin content that promotes cytochrome c release [51].

Signaling cascades stimulated by excess FFA may also promote apoptosis. FFA-induced apoptosis in a variety of cell types correlates with reduced protein kinase B phosphorylation, whereas constitutive overexpression of active protein kinase B may inhibit pro-apoptotic signals and prevent FFA-induced apoptosis [52,53]. Decreases in phosphatidylinositol 3-kinase activation [47] and activation of NFκB pathways [25•,54] have also been implicated.

Excess FFAs also may increase cellular oxidative stress to initiate apoptosis. Increased nitric oxide production is observed when islets are cultured in media containing excess FFAs, and FFA-induced apoptosis in cultured

islets is inhibited by nicotinamide, aminoguanidine, and N-acetyl cysteine [55,56]. Reactive intermediate generation in Chinese hamster ovary cells occurs prior to caspase 3 activation and DNA laddering, and FFA-induced apoptosis is blocked by agents that scavenge reactive intermediates [44]. In addition to upregulation of inducible nitric oxide synthase, FFAs may lead to reactive intermediates as byproducts of oxidative phosphorylation [57].

Approaches to limiting or preventing lipotoxicity

Three general approaches to the prevention or treatment of lipotoxicity have been considered (Fig. 1). First, measures that decrease overall the lipid content of non-adipose tissues decrease lipotoxicity. Second, lipotoxicity can be decreased by diversion of excess lipid away from non-adipose tissues and by diversion of excess lipid from cellular pathways that lead to cell dysfunction and cell death. A third approach has been to target signaling or metabolic pathways that are critical for FFA-induced cell dysfunction or cell death.

Lipid content of non-adipose tissues may be diminished by decreasing serum FFA and triglyceride levels or by increasing tissue lipid export. Significant weight loss in morbidly obese humans is associated with decreases in skeletal muscle long-chain acyl-CoA content (presumably due to lower serum FFAs and triglycerides) that is associated with decreased fasting insulin levels and increased total body insulin sensitivity [58•]. Non-adipose tissue lipid content may be reduced not only by decreasing plasma lipid levels, but also by endogenous mechanisms for lipid export such as secretion of lipoprotein particles. Secretion of excess lipid in the form of lipoproteins lowers cardiomyocyte triglyceride stores in fasted mice, in mice null for long-chain acyl-CoA dehydrogenase [59•] and in mice with strepto-zotocin-induced diabetes [60•]. In the latter model, decreased cardiac triglyceride levels are associated with improved cardiac function.

Lipid content may also be decreased by increasing metabolism of excess lipid within non-adipose tissues. In the A-ZIP/F-1 mouse model of severe lipoatrophic diabetes, treatment with a specific PPARα agonist reduces serum triglyceride and FFA, decreases muscle and liver triglyceride content, and is associated with improved glucose and insulin levels [61•]. In this case, the PPARα agonist likely decreases lipid accumulation through stimulation of β-oxidation. Leptin treatment improves hyperlipidemia, hepatic steatosis, insulin sensitivity, and diabetes in the ZDF rat [62•] and in the A-Zip/F-1 mouse model of lipotoxicity [63]. Moreover, treatment of lipodystrophic human patients with recombinant leptin decreases serum and tissue triglyceride levels, improves glycemic control, and improves insulin-stimulated hepatic and peripheral glucose metabolism [64,65•]. Leptin may be acting, in part, through upregulation of peripheral FFA metabolism.

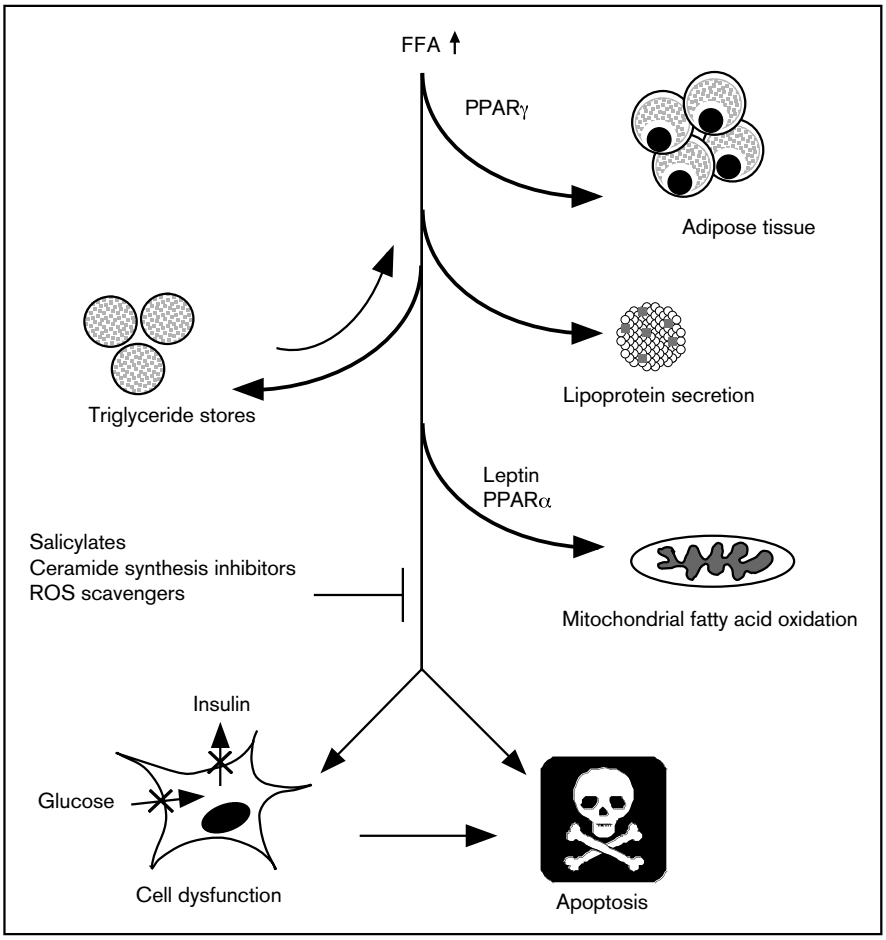
Strategies that divert excess lipid away from non-adipose tissues have been shown to decrease lipotoxicity. In ZDF rats daily administration of troglitazone, a PPARγ agonist, prevents diabetes and cardiomyopathy by decreasing ectopic deposition of fat and presumably increasing adipose tissue accumulation [16•,66]. In human patients with type 2 diabetes, 3-month treatment with troglitazone decreased serum FFA concentrations and improved insulin-simulated muscle glucose metabolism [67]. The failure of troglitazone to rescue FFA cytotoxicity in cultured pancreatic β-cells may reflect the absence of appropriate fat depots (e.g. adipose tissue) in a cultured cell system [68].

Within cells, it may also be possible to channel excess FFA away from pathways that induce apoptosis. In cultured rat islets [46•], rat β-cells [40•], and Chinese hamster ovary cells [69•], saturated long-chain FFAs are not well incorporated into triglyceride pools and are associated with apoptosis. On the other hand, monounsaturated long-chain FFAs are readily incorporated into triglyceride pools and are associated with cell proliferation. Moreover, co-incubation of cells with oleic and palmitic acid rescues the palmitic acid-induced apoptosis. Measures that increase cellular triglyceride accumulation in response to palmitate supplementation decrease palmitate-induced apoptosis, whereas measures that decrease triglyceride accumulation in response to oleic acid render this FFA species cytotoxic as well [69•]. Thus, while triglyceride accumulation is the *sine qua non* of lipid overload states, cellular triglyceride accumulation may initially serve a protective role. Accumulation of excess FFAs in triglyceride pools likely diverts these molecules from pathways that lead to cytotoxicity and may thus serve as a buffer against lipotoxicity. In pathologic states, lipotoxicity may occur over time, despite triglyceride accumulation, when either the cellular capacity for triglyceride storage is exceeded or when triglyceride pools are hydrolyzed, resulting in increased cellular FFA levels.

Studies in which inhibition of specific metabolic or signaling pathways decreases lipotoxicity provide evidence for the importance of these mechanisms in lipotoxic disease and suggest potential therapeutic targets. When pre-diabetic ZDF rats are treated with nicotinamide or aminoguanidine to prevent production of nitric oxide, or L-cycloserine to block ceramide production, β-cell apoptosis is partially prevented and hyperglycemia ameliorated [49,55]. These results are consistent with important roles for nitric oxide and

Figure 1. Determinants of lipotoxicity

Excess accumulation of free fatty acid (FFA) in non-adipose tissues leads to cell dysfunction (e.g. impaired insulin-stimulated glucose transport or diminished insulin secretion). Excess FFA accumulation may also lead to apoptotic cell death. The effects of FFA accumulation depend on the cell type, the nature of the lipid, and the degree and duration of lipid overload. Lipotoxicity may be ameliorated or prevented if lipid can be redirected to adipose tissues (e.g. through stimulation of peroxisome proliferator-activated receptor (PPAR)γ pathways) or secreted in the form of lipoproteins. Alternatively, within the lipid-overloaded cell, channeling of FFAs to triglyceride stores or to increased β oxidation (e.g. through stimulation of PPARα pathways) may protect against lipotoxicity. Agents such as salicylates, ceramide synthesis inhibitors and scavengers of reactive oxygen species (ROS) may block specific signaling pathways and fatty acid metabolic pathways that are critical for lipotoxicity.



ceramide in FFA-induced lipotoxicity in this model. Loss of function or inhibition of IκB kinase β by high-dose salicylate decreases insulin resistance in rodent models [70•,71•] and improves glucose metabolism in human patients [25•]. These studies suggest that IκB kinase β, an inhibitor of NFκB, may be proximal to the defects in insulin receptor signaling in lipotoxicity or that activation of NFκB plays an important role in the lipotoxic response.

Conclusion

In humans and in animal models of human disease, excess lipid accumulation in non-adipose tissues is associated with cell dysfunction and in some cases cell death. Ultimately, the duration and extent of lipid overload as well as the nature of the accumulated lipid species may determine whether a cell is protected or damaged. Recent studies in cultured cells and in animal models provide plausible mechanisms through which lipotoxicity occurs and suggest therapeutic approaches to human diseases associated with lipotoxicity.

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