Chapter 6

Current Views of the Fat Cell as an Endocrine Cell: Lipotoxicity

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1. INTRODUCTION

Lipotoxicity can be defined as lipid-induced metabolic damage [1]. It occurs when lipid uptake exceeds capacity to store lipids and lipid oxidative capacity [2]. The principal function of adipose tissue is to store energy, and lipids are a particularly efficient form in which to store energy because of their high caloric density. However, lipids can be cytotoxic and nonadipose tissues have limited capacity to store lipids [3]. Fat tissue is protected against lipotoxicity, but if fat tissue function becomes dysregulated, lipotoxicity in other tissues can ensue. Fatty acids (FAs), the essential role of which is to serve as fuels and to form phospholipid bilayers and phospholipid messengers, are particularly damaging to nonadipose tissues when present in excess [4, 5]. The causes, mechanisms, and consequences of lipotoxicity are considered, with particular regard to the role of adipose tissue in lipotoxicity in other tissues and to possible reasons why adipose tissue is resistant to lipotoxicity.

2. FUNCTIONS OF FAT TISSUE

In addition to storing energy, fat tissue has important immune, endocrine and homeostatic, regenerative, mechanical, and thermal functions. Fat tissue defends against bacterial and fungal infection, as well as tissue injury. To do so, it produces a number of cytokines, chemokines, and hemostatic factors. Indeed, preadipocytes, which account for 15% to 50% of the cells in fat tissue, have gene expression profiles closer to those of macrophages than fat cells [6].

FAs may play a larger than generally recognized role in the defensive function of fat tissue. While there is a lack of information about local FA concentrations in fat tissue, concentrations are likely very high near fat cells, particularly during lipolysis. Direct measurements of FA concentrations adjacent to fat cells are not available. However, the decrease in intracellular pH that accompanies FA transfer across fat cell membranes following induction of

lipolysis with isoproterenol or forskolin is as high as that which occurs when cells are exposed to 65 μM oleic acid without albumin (see Figures 1 and 2 in [7]). This suggests that FA concentrations in the immediate vicinity of fat cells could reach levels equivalent to the mid-millimolar range in the presence of physiological albumin concentrations. These levels are lethal to most types of cells. Much lower concentrations are effective in killing *Helicobacter pylori* [8], pneumococcus [9], *Mycobacterium avium* [10], and tuberculosis [11]. Somehow, preadipocytes and fat cells are resistant to these high local FA concentrations. Thus, fat tissue, which is located under the skin and around viscera at points susceptible to invasion by microorganisms, produces both FAs and inflammatory mediators that protect against infection. Indeed, bacterial or fungal infections of fat tissue are rare. Thus, lipotoxicity appears to have been adapted by fat tissue as a defense mechanism. Further, fat cells can use the lipotoxic effects of FAs to regulate function of other cells. For example, human fat cells can release sufficient polyunsaturated FAs in bone marrow to inhibit osteoblastic proliferation without inducing apoptosis [12].

The homeostatic, paracrine, and endocrine functions of adipose tissue are, in part, related to its immune function. Indeed, many of the endocrine and paracrine factors released by adipose tissue with metabolic effects are cytokines (e.g., leptin), while others are lipids. Fat tissue can exert endocrine control over other tissues in a number of ways. It has a traditional endocrine function through releasing protein hormones and processing steroids that act at a distance from fat tissue. Fat cells can also regulate function of other tissues in a nontraditional endocrine manner by taking up residence in nonadipose tissues and exerting effects by producing paracrine factors and lipids. Fat cells can release or fail to remove metabolites, including lipids, that impact function of other tissues. When fat cell numbers increase or their function is dysregulated, they could conceivably contribute to dysfunction of other tissues through lipotoxicity.

3. CONDITIONS ASSOCIATED WITH LIPOTOXICITY

Several conditions, including obesity, diabetes, the metabolic syndrome, aging, lipodystrophies, and certain drugs have been associated with lipotoxicity in pancreatic β-cells, skeletal muscle, cardiac muscle, hepatocytes, and osteoblasts. Other tissues are likely affected analogously.

Fat tissue is the repository of surplus lipid. In otherwise normal rats, a 60% fat diet for 8 weeks causes a 150% increase in body fat, but only a small increase in pancreatic, liver, heart, and skeletal muscle fat [5]. However, some individuals with obesity, particularly massive obesity, develop lipid accumulation in nonadipose tissues (a sign that lipotoxicity may be occurring). Hepatic,

cardiac, skeletal muscle, and pancreatic steatosis have been found in *ob/ob* and *db/db* mice and *fa/fa* rats, which have obesity together with increased appetite, hyperlipidemia, and increased blood free FAs (FFAs) [2, 5, 13]. Obese human subjects can have increased intramyocellular lipid in skeletal muscle [14], increased myocardial lipid by positron emission tomography (PET) scanning [15, 16], and hepatic steatosis [1] associated with dysfunction in each of these tissues. Indeed, cardiac triglyceride (TG) accumulation appears to be an early metabolic marker of cardiac dysfunction in obese subjects [15]. Intramyocardial TG overload occurs in approximately 30% of patients with nonischemic heart failure [13]. Why some, but not all, obese subjects develop lipotoxicity in nonadipose tissues is a potentially illuminating issue that remains to be explained. Among the factors that could account for this are dyslipidemia, genetic traits, altered regional fat distribution, fat tissue dysfunction, aging, extent of adipokine and inflammatory response, hormonal status, coexisting diseases, and activity.

As with obesity, diabetes and insulin resistance are associated with lipid accumulation, cytotoxicity, and dysfunction in a number of tissues. For example, proton magnetic resonance studies suggest that increased intramyocellular lipid content is associated with reduced insulin sensitivity in healthy humans [17]. Type 2 diabetes is associated with increased FA uptake into cardiac myocytes and mitochondria, altered mitochondrial function, and decreased cardiac contractility [2]. Lipotoxicity may be an early event in type 2 diabetes, because inhibiting lipolysis, which results in reduced fasting plasma FFA (but no change in adipokines) improves insulin sensitivity in subjects predisposed to develop diabetes [18]. Of course, obesity and insulin resistance are linked and are components of the metabolic syndrome. The failure of antilipotoxic protection associated with obesity and insulin resistance may even be a cause of the metabolic syndrome [1].

Defective adipose tissue may promote lipotoxicity in peripheral tissues and be a key link among obesity, insulin resistance, and type 2 diabetes [19]. This is highlighted by the observations that aging and congenital lipodystrophies, conditions associated with altered fat tissue function, are themselves associated with the metabolic syndrome and accumulation of lipid associated with dysfunction of nonadipose tissues [5, 20–22]. Congenital lipodystrophies are the most severe of lipotoxic diseases, with little adipose tissue in which to store lipid, low adiponectin and leptin, hyperlipidemia, cardiomyopathy, diabetes, and liver steatosis [5, 23]. Certain drugs associated with fat tissue redistribution and dysfunction are also associated with lipotoxicity. Glucocorticoids cause lipotoxicity with diabetes, steatosis, and hyperlipidemia in rodents [24]. HIV protease inhibitors impede adipogenesis [25] and result in fat redistribution, cardiomyopathy, and diabetes in some patients [26].

4. MECHANISMS OF LIPOTOXICITY

Several mechanisms probably contribute to the cytotoxicity associated with lipid accumulation in nonadipose tissues. These include increased lipid synthesis, detergent effects on membranes, increased lipolysis or reduced ability to suppress lipolysis in adjacent lipid-containing cells, β-oxidation of FAs, reactive oxygen species (ROS) generation, lipid peroxides, effects on protein kinase B (PKB) and PKC activity, ceramide, stimulation of apoptotic or inhibition of antiapoptotic pathways, necrosis, and promotion of inflammatory cytokine release.

Under most conditions, extensive lipid storage and synthesis, particularly of TGs, is restricted to adipose cells, with smaller amounts being made by liver, muscle, myelin-forming, and steroidogenic cells. Under certain conditions, lipotoxicity can occur in nonadipose cells when lipid synthesis is increased. For example, overexpressing acyl coenzyme A (CoA) synthase (ACS) in cardiomyocytes can induce lipotoxic cardiomyopathy [27]. ACS increases FA import (Figure 1), leading to lipid accumulation with apoptosis, myofiber disorganization, interstitial fibrosis, left ventricular dysfunction, and dilated cardiomyopathy [27]. Decreased ability to suppress lipolysis may contribute to increased local FA concentrations and lipotoxicity. Diabetes and obesity with insulin resistance lead to decreased ability to suppress lipolysis [28] and are associated with lipotoxicity. Thus, increased production or release of FA by cells can contribute to lipotoxicity.

Decreased FA β-oxidation may contribute to lipotoxicity by decreasing removal of cytotoxic FA, while increased β-oxidation may raise production of cytotoxic ROS. Impaired β-oxidation may contribute to increased intramyocellular lipid in obesity and diabetes [29, 30]. Impeding β-oxidation (e.g., by inhibiting ACC activity; Figure 1) can increase levels of potentially lipotoxic nonoxidative metabolites of FAs [5]. Fatty acyl CoA accumulation might be the main factor that leads to cardiac lipotoxicity [31]. Leptin, which increases FA oxidation [32], protects against lipotoxicity in lipodystrophy [33–35]. Thus, reduced β-oxidation may contribute to lipotoxicity. On the other hand, increased β-oxidation can result in ROS generation and lipotoxicity, with the impaired β-oxidation in diabetes and obesity being a compensatory response to protect against excess ROS production [2, 36]. Increasing FA abundance can itself result in increased β-oxidation, possibly through FA binding to peroxisome proliferator activated receptors (PPARs), leading to increased CPT-1 activity and FA oxidation that exceeds energy needs [2, 5, 37] (Figure 1). Indeed, FA oxidation is increased in hearts of obese *db/db* and *ob/ob* animals [38, 39] and cardiac PPARα [13] and PPARγ coactivator-1α (PGC-1α) [40] are increased in diabetes. ROS generation may contribute to palmitate-induced cell death [41]. Fluorescence of an oxidant-sensitive probe is increased

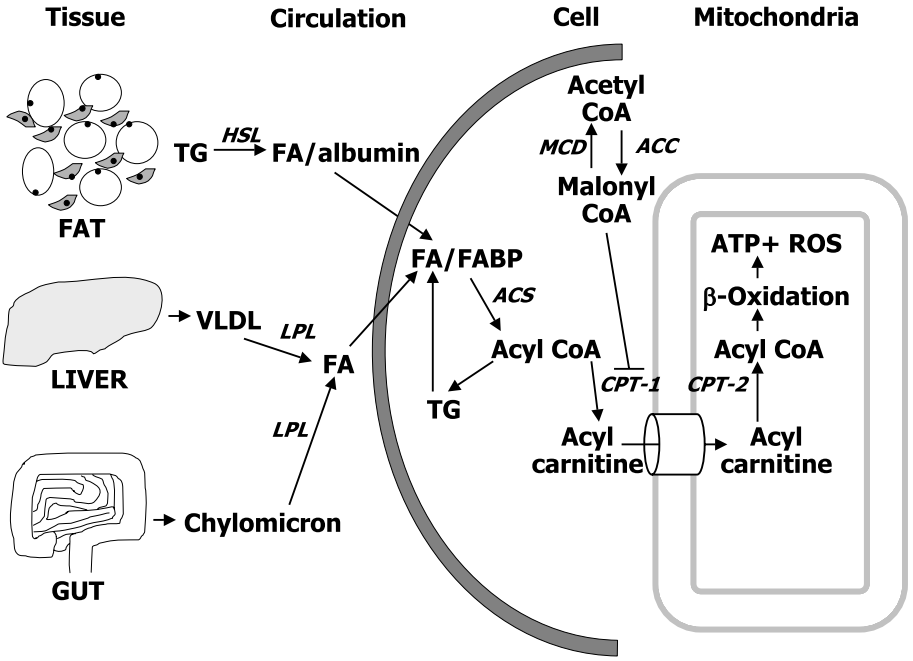

Figure 1. Fatty acid utilization pathways. Triglycerides (TGs) absorbed by the gut circulate as chylomicrons and TGs exported by the liver as lipoproteins. Fatty acids (FAs) released by hormone-sensitive lipase-catalyzed hydrolysis of TGs, circulate as complexes with albumin. TG is hydrolyzed by lipoprotein lipase (LPL) to FA near cell surfaces. FAs diffuse across the cell membrane and are complexed to FA binding proteins (FABP) in the cytosol. Acyl-CoA synthetases (ACS) convert FA to fatty acyl-CoA (acyl CoA). Acyl CoA, in turn, can be incorporated into intracellular TGs or converted into acyl carnitine by carnitine palmitoyl transferase-1 (CPT-1) located in the outer mitochondrial membrane. CPT-1 can be inhibited by malonyl CoA, the concentration of which is determined by a balance between synthesis from acetyl-CoA by acetyl-CoA carboxylase (ACC) and degradation by malonyl CoA decarboxylase (MCD). Once generated by CPT-1, acyl carnitine is transferred into mitochondria by a translocase. After conversion back into acyl CoA by CPT-2 (located in the inner mitochondrial membrane), acyl groups undergo β-oxidation and energy production that entails generation of reactive oxygen species (ROS). PPARα increases ACS (resulting in increased acetyl CoA), MCD (resulting in decreased malonyl CoA), and CPT-1 (enhancing β-oxidation).

by palmitate exposure. Palmitate-induced apoptosis can be blocked by compounds that scavenge reactive intermediates. Thus, increases or decreases in FA β-oxidation can set off events that culminate in cell death.

These findings suggest that lipotoxicity arises from a constellation of cytotoxic mechanisms and is not a single, unified process. This contention is underscored by the observations that such diverse processes as accumulation of peroxidized FA (as a result of increased ROS due to β-oxidation or increased presence of lipid susceptible to peroxidation [2, 42–44]), inhibition of protein

kinase B45 or induction or inhibition of certain protein kinase C isoforms by FAs [46–48], and ceramide accumulation (in palmitate- but not oleate-induced lipotoxicity [1, 27, 41, 49, 50] can be involved in cytotoxic effects of FAs.

Exogenous FAs can cause apoptosis within hours in cultured cells [51]. Palmitic acid is a particularly potent apoptosis inducer [51]. Indeed, saturated FAs are generally more lipotoxic than unsaturated FA: excess palmitic acid is more lipotoxic than oleic acid in a number of cell types [3, 41, 50, 52–55]. This has been attributed to generation of specific proapoptotic lipid species or signaling molecules that may vary across cell types: ROS [41], ceramide [56], and nitric oxide [57], decreases in phosphatidylinositol-3-kinase [54] as well as primary effects on mitochondrial structure or function [58]. Exogenous or endogenously generated unsaturated FAs, such as oleate, can rescue palmitate-induced apoptosis by promoting palmitate incorporation into TGs in Chinese hamster ovary (CHO) cells [3]. In cells in which activity of stearoyl-CoA desaturase 1 (SCD1), which catalyzes desaturation of palmitate, is increased, TG accumulation after exposure to palmitate also increases. This suggests that endogenously produced unsaturated FAs can promote TG accumulation. Further, by increasing SCD1 activity, less apoptosis occurs following palmitate exposure. Thus, enhancing ability to synthesize TGs can protect against development of lipotoxicity. Unsaturated FAs reduce lipotoxicity by increasing incorporation of saturated FAs into TGs.

Long-chain FA can suppress Bcl2, an antiapoptotic factor, leading to increased susceptibility to apoptosis in pancreatic cells [59]. Activity of serine/threonine protein phosphatase type 2C is stimulated by certain unsaturated FAs, including oleic acid, and this enzyme dephosphorylates Bad, resulting in increased apoptosis in human umbilical vein endothelial, rat cortical and hippocampal, and human neuroblastoma SH-SY5Y cells [60, 61]. Palmitate and, to a lesser extent, oleate can induce apoptosis in pancreatic β-cells [62]. Both FAs induce endoplasmic reticulum stress response elements (C/EBP homologous protein, activating transcription factor-4 and -6, and immunoglobulin heavy chain binding protein mRNAs and alternative splicing of X-box binding protein-1), but not NFκB. Thus, FFAs can cause apoptosis by activating ER stress responses through an NFκB- and nitric oxide-independent mechanism. In endothelial cells, palmitate is also more effective than oleate in inducing apoptosis, but NO synthase is increased by FA in these cells [63]. Also, elevated FFAs can cause apoptosis of β-cells partly as a result of ceramide generation [50, 64]. Again, cytotoxicity of palmitate is higher than oleate under these conditions. In CHO cells, palmitate, but not oleate, can induce apoptosis through the generation of ROS independently of ceramide synthesis [41]. Thus, FA can cause apoptosis in multiple cell types through diverse mechanisms.

TGs can also cause cell death, in some cases by necrosis rather than apoptosis. In macrophages, exposure to TGs under conditions in which no FFAs were detectable caused cell death in a dose-dependent fashion without an increase in caspase-3 activity [51]. Indeed, caspase-3 activity was reduced in the presence of TGs. Cell death was associated with increased ROS generation by mitochondrial complex 1. Thus, although TGs induce less lipotoxicity than FFAs, they are not completely neutral. The processes through which TGs mediate changes in cell function and death appear to be distinct from those of FAs.

5. INHERENT PROPERTIES OF CELLS CONTRIBUTE TO SUSCEPTIBILITY TO LIPOTOXICITY

Different cell types vary in susceptibility to lipotoxicity (e.g., pancreatic β-cells compared to other pancreatic cell types, or fat cells compared to hepatocytes). This is compatible with the contention that susceptibility to lipotoxicity is partly determined by inherent properties of cells. Studies of effects of aging also support this contention. Dysfunctional cells containing lipid can accumulate with aging in various tissues such as muscle, liver, and bone marrow [20]. Even preadipocytes isolated from animals of different ages maintained for several cell generations under identical culture conditions become increasingly susceptible to FA-induced apoptosis with increasing age [65], pointing to a predisposition to lipotoxicity caused by inherent changes in cell function. With aging, progenitors of a variety of mesenchymal cell types (e.g., muscle satellite cells, osteoblasts) accumulate lipid, express some markers associated with fat cells such as PPARγ2 or FA binding protein 4 (aP2), and continue to express some transcription factors and markers characteristic of their own cells type, but do not develop into functional differentiated cells. This occurs even when these progenitors are maintained under identical culture conditions without exposure to any of the changes in circulating lipids, hormones, or paracrine factors that may occur with aging. Although these adipocyte-like cells contain lipid and are dysfunctional, it is not clear if they really result from lipotoxicity or changes in transcription factor expression related to cell autonomous aging events.

6. ASSOCIATION BETWEEN LIPIDS AND INFLAMMATORY RESPONSES

In addition to causing cytotoxicity directly, lipids can induce immune responses that amplify extent of tissue damage. FAs regulate macrophage gene expression and can induce expression of inflammatory cytokines in

macrophages [11, 66]. Given the close relationship between preadipocytes and macrophages, and because inflammatory cytokine expression increases in obesity, it would not be surprising if FAs, particularly saturated FAs, elicited increased inflammatory cytokine expression in adipose tissue with an impact on other organs.

7. MECHANISMS OF DEFENSE AGAINST LIPOTOXICITY

Tissues employ a variety of strategies for protection from the lipotoxic effects of lipids. Lipid depletion is effective in protecting cells from lipotoxicity. For example, lipid depletion protects pancreatic β-cells from apoptotic effects of cytokines [67]. Depletion of intramyocellular lipid is associated with improved insulin sensitivity, reduced ACC mRNA, and increased GLUT4 expression [68]. Overexpression of apolipoprotein B leads to a reduction in cardiac TG stores and increased TG secretion [69, 70], but it is important to acknowledge that lipoprotein secretion has not been demonstrated in cardiac tissue of wild type mice [2]. Insulin can induce lipid accumulation acutely and through up regulating SREBP-1c, which induces lipogenic enzyme expression [71]. Paradoxically, insulin resistance may protect against lipid accumulation, because excluding glucose from cells reduces glucose-derived lipogenesis. Thus, mechanisms that can potentially defend against lipotoxicity include lipoprotein secretion (in cells containing microsomal TG transfer protein), FA export, and insulin resistance.

Control of circulating lipids is another defense against lipotoxicity. While diabetes and obesity can result in increased plasma FAs [72, 73], fasting FFAs are not consistently increased in obese subjects [74], although marked variations in plasma FFAs occur in response to feeding and fasting. FFAs might be elevated at night or integrated basal FFA levels may be higher in obese subjects with the metabolic syndrome than in lean subjects, an area warranting further study. Also, increased circulating lipoproteins and *de novo* lipogenesis from glucose may predispose to lipotoxicity. However, the fact that TG content in cell types other than adipocytes remains within a very narrow range, despite excess caloric intake sufficient to increase fat cell TGs, is consistent with a system of FA homeostasis to protect against lipotoxicity [75]. Normally rats can tolerate a 60% fat diet because 96% of surplus fat is deposited in adipocytes [42].

Although TGs can induce cell necrosis, TGs are less cytotoxic than FAs [3, 50]. While TG accumulation is an indicator of ectopic lipid deposition, storage as TG is probably the least toxic means for sequestering surplus lipids. However, intracellular TG can become part of the problem. Intracellular TG is a potential source of FAs in excess of oxidative needs and can contribute to an

increase in the pool of FA CoA, a substrate and regulator of many pathways of nonoxidative FA metabolism. Of nonadipocytes, liver and muscle have highest tolerance to surplus TG: liver can export surplus TG as very low density lipoprotein (VLDL), while muscle can β-oxidize lipid. Also, fat cells present in nonadipose tissues may actually protect those tissues from lipotoxicity by storing or processing excess FAs locally.

Exercise is associated with protection against potentially adverse effects of intramyocellular lipid [76]. Endurance training results in increased intramyocellular lipid despite increased β-oxidation. In obesity and diabetes, increased intramyocellular TG correlates with insulin resistance and is associated with increased lipid peroxidation, but not in endurance-trained subjects [43]. This suggests that endurance training increases intramyocellular antioxidant enzyme activity. Further, increased intramyocellular TG may be a constantly utilized source of energy for ATP production in endurance-trained subjects, while in obese subjects, intramyocellular TG may be stored but not mobilized. Thus, intramyocellular TG accumulation does not necessarily indicate lipotoxicity.

Adiponectin and leptin can defend against cytotoxic effects of lipids. Adiponectin protects against metabolic syndrome [77–80]. It increases AMP-activated protein kinase (AMPK) activity and enhances FA oxidation [81]. Leptin also increases AMPK activity [82] and FA oxidation [32]. In obese, leptin-deficient Zucker rats, adenoviral overexpression of leptin in the liver protects from hepatic fat accumulation and hypertriglyceridemia [32]. Thus, leptin and other factors produced by subcutaneous fat may protect against lipotoxicity [1]. Indeed, increased leptin or transplantation of normal fat ameliorates the lipotoxicity caused by lipodystrophy: leptin reduces the steatosis and diabetes of lipodystrophy in mice and humans [33–35]. Infection with an adenovirus that increases circulating leptin improves lipotoxic cardiomyopathy and decreases blood FA and TG, elevates cardiac expression of anti-apoptotic Bcl2, and decreases expression of proapoptotic Bax [83]. In addition to increasing AMPK, high levels of leptin reduce lipogenic transcription factor expression (SREBP-1C in liver and PPARγ [and ACC and FAS] in fat), increase PGC-1α (increasing numbers of mitochondria) [42]), and prevent the FA-mediated decline in Bcl2 [59]. Thus, the increase in leptin or other adipokines in diet-induced obesity may protect against lipotoxicity in nonadipose tissues, although resistance to effects of these adipokines may eventually develop, as occurs with leptin.

AMPK activation decreases ACC activity, reducing malonyl CoA, resulting in increased CPT1 activity and β-oxidation (Figure 1). AMP kinase activating agents (leptin [82, 84, 85], adiponectin [84], thiazolidinediones [86], metformin [87], and 5-aminoimidazole 4-carboxamide 1-β-D-ribofuranoside AICAR [88, 89]) decrease lipotoxicity. AMP kinase activation reduces the diabetes and ectopic lipid accumulation that occur in Zucker rats [89]. Thus,

AMP kinase appears to have an important role in the lipotoxicity associated with obesity and fat tissue dysfunction.

Despite the importance of adipokines in the genesis of some forms of lipotoxicity, lipotoxicity can occur independently of altered adipokine levels. Transgenic mice with muscle- or liver-specific overexpression of lipoprotein lipase have increased muscle and liver TG content and insulin resistance because of altered insulin signaling [90]. These defects in insulin action are associated with increases in diacylglycerol, fatty acyl CoA, and ceramides. Thus, increased TG synthesis can cause accumulation of intracellular FA-derived metabolites and insulin resistance through alterations in insulin signaling independently of circulating adipokines.

Exogenous or endogenously generated unsaturated FA can rescue palmitate-induced apoptosis in CHO cells [3]. Oleate promotes palmitate incorporation into TG and prevents increased ROS and ceramide generation resulting from palmitate. In cells with increased stearoyl-CoA desaturase 1 (SCD1), TG accumulation is increased in the presence of palmitate, suggesting that endogenously produced unsaturated FAs can promote TG accumulation. These cells are resistant to palmitate-induced apoptosis. Thus, generation of unsaturated FAs can protect against lipotoxicity by increasing incorporation of saturated FAs into TGs.

Other mechanisms may also provide protection from lipotoxicity. For example, removal of ceramide can reduce lipotoxicity. Ceramide is formed by the condensation of palmitoyl CoA and serine, catalyzed by serine palmitoyl transferase (SPT [91]). Reducing palmitoyl CoA and SPT decreases apoptosis in pancreatic islets. Caloric restriction and thiazolidinediones reduce SPT activity and lead to protection from apoptosis [5]. Sirtuins, which promote fat mobilization [92] and are activated by dietary flavinoids, may turn out to be involved in protection from lipotoxicity. In pancreatic β-cells, PKB activation can prevent apoptosis through inhibition of the proapoptotic proteins glycogen synthase kinase-3α/β, FoxO1, and p53 [45].

8. PREADIPOCYTES AND FAT CELLS ARE MORE RESISTANT THAN OTHER CELL TYPES TO FA

Defenses against lipotoxicity are best developed in adipose tissue. Nonadipose tissues have very limited capacity to store lipids [3]. Lipotoxicity does not seem to occur in fat tissue itself [93], at least under most conditions. Preadipocytes, which account for 15% to 50% of the cells in fat tissue, are resistant to levels of FAs that would destroy other cell types. 3T3-L1 cells are resistant to 1.5 mM palmitic acid [93]. Fat cells themselves are resistant to FA. Treatment of collagenase-isolated rat epididymal adipocytes for up to 24 hours with 1.5 mM oleate or palmitate at an FFA: albumin ratio of 2.5:1 results in

no significant effects on IRS-1, PI3 kinase, PKB, phosphorylated PKB, GLUT4, insulin-stimulated glucose uptake, or basal or cAMP-stimulated lipolysis or inhibition of lipolysis by insulin [93].

How do preadipocytes and fat cells protect themselves against the consequences of exposure to very high concentrations of FAs? Very few data are available about this. Possible mechanisms include the following. Fat cells express abundant aP2 and other FA-binding proteins, which may provide protection against high intracellular levels of FAs and their metabolites. Long-chain FAs induce preadipocyte aP2 expression [94]. aP2 and other intracellular lipid-binding proteins may also function as lipid chaperones, facilitating the movement of FA out of fat cells [95]. Fat cells likely have well developed antiapoptotic mechanisms, because there are high local concentrations of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) in adipose tissue that they must defend themselves against. Fat tissue turns over at a greater rate than generally appreciated—with fat cell numbers increasing throughout life in some fat depots [96], permitting replacement of damaged cells. There is a large pool of fat cell progenitors that can replace damaged adipocytes. Fat cells have highly developed machinery to esterify potentially lipotoxic FAs into TGs. Also, β-oxidation occurs in fat cells, providing another means to dispose of acyl-CoA. Further, fat cells are resistant to potentially high levels of ROS resulting from FAs. Interestingly, the dicarboxylate carrier is expressed at higher levels in adipocytes than in any other cell type [97]. Overexpression of the mitochondrial dicarboxylate carrier leads to hyperpolarization of the mitochondrial membrane, resulting in increased ROS formation [98]. Exposure of primary rat adipocytes to hyperglycemic conditions *in vitro* reduces insulin sensitivity and increases ROS levels [99]. Adipocytes isolated from mice fed high-fat have significantly elevated ROS [100]. ROS are increased in primary adipocytes isolated from mice exposed to nutrient excess *in vivo* [98]. Further, differentiation of murine 3T3-L1 preadipocytes into adipocytes is associated with the acquisition of apoptotic resistance accompanied by upregulation of cell survival genes even under conditions in which ROS production is increased [101]. Thus, ROS in fat cells may be high and these cells appear to have well developed mechanisms to resist ROS damage.

There may be situations in which even cells in fat tissue become paradoxically susceptible to lipotoxicity. An example of this is the increasing susceptibility of preadipocytes to apoptosis induced by FA with aging [65]. Perhaps other disease states, such as fat redistribution and the metabolic syndrome associated with HIV protease inhibitors that interfere with adipogenesis, may also prove to involve this hypothetical mechanism. Such processes could set up a cycle of lipotoxicity in fat tissue (Figure 2), with FA contributing to preadipocyte dysfunction, impeding adipogenesis with failure to store FAs as

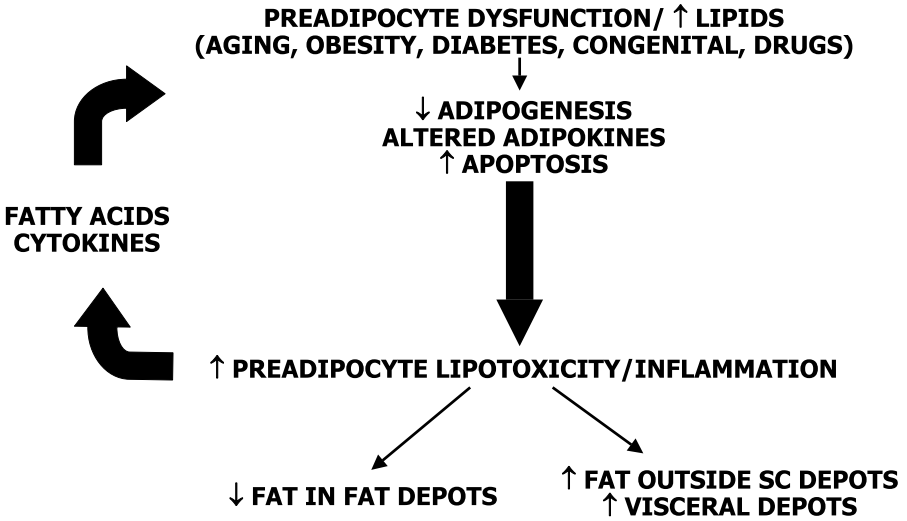

Figure 2. Hypothetical lipotoxicity cycle in fat tissue. Fat cells and preadipocytes presumably have stronger defenses against lipotoxic effects of potentially high local FA concentrations and flux than cells in other tissues. Should these defenses (including capacity to undergo adipogenesis, FA binding proteins, β-oxidation, mechanisms to remove reactive oxygen species, resistance to apoptosis) fail, a cycle of reduced FA removal leading to more damage, resulting in further reduction in capacity to remove FA could ensue. This could contribute to increased fat tissue inflammatory cytokine generation and reduced capacity to store FA as TG, with spillover into nonadipose tissues and other fat depots.

TGs, leading to further increases in FAs, compounding fat tissue dysfunction and causing reduced adiponectin and leptin production and increased inflammatory cytokine generation.

9. SUMMARY

Lipotoxicity, defined as lipid-induced metabolic damage, occurs when net capacity to store and utilize lipids is exceeded. In diabetes, obesity, the metabolic syndrome, lipodystrophies, aging, and other conditions, lipotoxicity can result in systemic dysfunction. However, lipotoxicity can be adaptive, possibly providing defense against infection and accumulation of dysfunctional cells. Fatty acids are more lipotoxic than triglycerides, and different fatty acids vary in extent and mechanisms of lipotoxicity. Lipotoxicity is predisposed to by multiple factors, occurs through diverse mechanisms, and can cause cell removal through apoptosis or necrosis. Fat cells and preadipocytes are particularly resistant. Thus, lipotoxicity is not a single process and can have adaptive as well as detrimental consequences.

10. CONCLUSIONS

Although it is tempting to consider lipotoxicity to be a single process, this is probably simplistic. A diversity of triggers and pathways can lead to the lipid accumulation and cell death that are features of lipotoxicity. With respect to triggers, increased external lipid concentrations, decreased adiponectin or leptin, increased glucocorticoids, and intracellular processes, such as mitochondrial dysfunction with aging, may all predispose to lipotoxic cell death. None of these processes appears to be uniformly required for intracellular lipid accumulation and then cell death to occur. With respect to pathways involved, increases as well as decreases in FA β-oxidation, depending on cellular context, have been associated with mechanisms culminating in cell death. Accumulation of ceramide, which is likely important in the lipotoxicity resulting specifically from palmitic acid exposure, is much less likely to be a key factor in the lipotoxicity resulting from oleic acid. Deficiency of adiponectin or leptin may predispose to lipotoxicity, but lipotoxicity can occur without this, for example, in the setting of increased lipoprotein lipase activity. Even the mechanisms of cell death resulting from exposure to increased concentrations of various types of lipids differ: FAs are associated with apoptosis while TGs induce necrotic cell death. Thus, lipotoxicity is a group of processes predisposing to cell death through diverse triggers and pathways. A search for a unifying mechanism leading to cell death from lipids in all tissues is unlikely to be revealing. Although description of the diverse mechanisms resulting in cell death due to lipids is important, it is even more important to understand the tissue- and situation-specific processes that defend against cell death in order to devise specific therapies.

Lipotoxicity is not uniformly detrimental. It can be an adaptive process that removes dysfunctional cells or invading organisms, provides a means for regulating tissue development (e.g., osteoprogenitor function), and defends against overshoot effects of chronically high insulin levels by contributing to insulin resistance. The high FA levels likely present in fat tissue have been incorporated into its metabolic storage, regulatory, and immune roles. Obesity, a state rarely found in nature, and other types of fat tissue dysfunction may subvert these normal responses, resulting in lipotoxicity in fat and other tissues. The metabolic syndrome might be a particularly extreme example of this.

Thus, lipotoxicity is not a single process with uniformly destructive effects. The extent of lipotoxicity is predisposed to by multiple factors (including type of lipid, cellular context, inflammatory cytokines, hormonal status, drugs), proceeds through diverse mechanisms, and can have beneficial as well as destructive consequences.

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