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Lipid oversupply, selective insulin resistance, and lipotoxicity: Molecular mechanisms

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Abstract

The accumulation of fat in tissues not suited for lipid storage has deleterious consequences on organ function, leading to cellular damage that underlies diabetes, heart disease, and hypertension. To combat these lipotoxic events, several therapeutics improve insulin sensitivity and/or ameliorate features of metabolic disease by limiting the inappropriate deposition of fat in peripheral tissues (i.e. thiazolidinediones, metformin, and statins). Recent advances in genomics and lipidomics have accelerated progress towards understanding the pathogenic events associated with the excessive production, underutilization, or inefficient storage of fat. Herein we review studies applying pharmacological or genetic strategies to manipulate the expression or activity of enzymes controlling lipid deposition, in order to gain a clearer understanding of the molecular mechanisms by which fatty acids contribute to metabolic disease.

Keywords

Lipotoxicity; Diabetes; Lipid; Sphingolipids; Insulin resistance; Genetic manipulation; Metabolism; Metabolic syndrome; Lipid metabolism

1. Introduction

“Gluttony is the source of all our infirmities, and the fountain of all our diseases. As a lamp is choked by a superabundance of oil, a fire extinguished by excess of fuel, so is the natural health of the body destroyed by intemperate diet.” Robert Burton, English writer, philosopher and humorist, 1621

In a 1992 paper entitled “What if Minkowski had been ageusic—an alternative angle on diabetes,” Dennis McGarry suggested that the glucose-centric view of diabetes had led researchers astray, and that the disease would be “more readily understood if viewed in the context of underlying abnormalities of lipid metabolism” [1]. This seminal paper challenged existing dogma about the aetiology of the disease, and introduced a new and controversial model suggesting that increased action of insulin, specifically its ability to drive lipid synthesis in the liver, rather than insulin resistance, was the key feature in the progression towards diabetes. Some 17 years later, we know that Dr. McGarry was not only correct in many of his predictions, but that disorders in lipid metabolism and the excess delivery of fat to peripheral

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tissues underlie most of the more prevalent diseases affecting mankind. In the article below, we discuss progress elucidating the molecular mechanisms by which abnormalities in lipid metabolism contribute to diabetes and other metabolic diseases.

The psychological and economic toll of diabetes is well documented, and the increasing rate of occurrence of the condition is disconcerting and unequivocal [2]. The astounding proliferation of the disease is undoubtedly related to population changes in body composition, as the prevalence of obesity in the USA has risen from percentages in the mid-teens at the time of Dr. McGarry's article to nearly 33% today [3]. With obesity rates increasing in developed and developing countries across the globe [4], the World Health Organization estimates that a staggering number of individuals are currently dealing with complications of disrupted lipid homeostasis, 1.6 billion adults are overweight, 400 million are obese, and 180 million are diabetic [5]. Cardiovascular disease, which is the major complication of diabetes, is the cause of death of 17 million [6].

Based on our review of the literature, we surmise that the metabolic diseases associated with obesity, including diabetes and cardiovascular disease, derive from a common molecular pathogenesis characterized of two independent stages: (a) *selective* insulin resistance; and (b) lipotoxicity (Fig. 1).

Stage One: Selective Insulin Resistance. When circulating glucose reaches a critical threshold level, pancreatic β -cells secrete insulin, the major anabolic hormone in the body. Insulin has two major actions: to lower circulating glucose levels by facilitating its uptake into skeletal muscle while inhibiting its production by the liver; and, to promote the storage of available nutrients, predominantly in the form of glycogen and fat. Obese individuals display a decreased capacity for insulin-stimulated glucose disposal, as muscle fibres adjust to utilize the calorically dense fatty acids, rather than glucose, as a primary energy source. However, insulin-stimulated lipogenesis, particularly in the liver and adipose tissue, is unaltered or enhanced. This selective insulin resistance is one of several factors which predispose individuals to cardiovascular disease and diabetes [7]

The relative importance of insulin resistance in the pathogenesis of metabolic disease is subject to debate. At best, insulin resistance is a benign predictor of metabolic disease—an independent marker of a condition where the supply of nutrients exceeds demand, and muscle is adapting to oxidize lipids, while sparing glucose. At worst, this selective insulin resistance exacerbates and accelerates the path towards metabolic disease, as it promotes hyperinsulinemia, leading to an increase in triglyceride synthesis and enhanced delivery of lipoprotein-bound fats to peripheral tissues.

Stage Two: Lipotoxicity in Peripheral Tissues. Diseases related to obesity probably result from the delivery of lipids to peripheral tissues in excess of their oxidative or storage capacities. Indeed, experimental manipulations aimed to enhance lipid delivery to peripheral tissues (e.g. pancreatic β -cells or the heart) or prevent their storage in white adipose tissue is sufficient to recapitulate many of the features of these diseases [8–19]. Moreover, pharmacological therapies for warding off the condition almost invariably reduce the accumulation of ectopic fat in non-adipose tissues (e.g. thiazolidinediones, metformin, or statins).

Ultimately, both of these stages likely result from the excess delivery of fat to peripheral tissues, and the body appears capable of sensing small changes in cellular lipids. During selective insulin resistance, elevation in intracellular lipid metabolites leads to an inhibition of GLUT4 glucose transporters, thus decreasing net rates of glucose influx into the cell. During lipotoxicity, this increased lipid deposition leads to cellular events that compromise tissue function, such as ER and oxidative stress, apoptosis, etc. However, an understanding of the mechanisms underlying these varied responses has been elusive.

In virtually all tissues, fatty acids face one of three major metabolic fates: (a) they can be converted to glycerolipids, including triglycerides, diglycerides, and major membrane phospholipids (Fig. 2); (b) they can be converted to sphingolipids, including sphingomyelin and ceramide (Fig. 3); and, (c) they can be oxidized for energy (Fig. 4). As the genes and enzymes that control these anabolic and catabolic pathways have been sequenced and characterized, accompanying experiments manipulating their expression and/or activity has revealed that modulating rates of lipid synthesis and degradation has profound effects on insulin-sensitivity, diabetes, and cardiovascular disease.

2. Manipulation of glycerolipid synthesis: impact on selective insulin resistance and lipotoxicity

Energy-dense triglycerides (TAGs) have two major physiological roles: first, they are energy stores, and their accumulation, preferentially in adipocytes, allows organisms to prepare for periods when food supplies are scarce; second, they are the primary form in which lipids are transported between tissues, as they are the major constituents of lipoproteins. In most tissues, *de novo* TAG synthesis involves a series of enzymatic reactions localized in the endoplasmic reticulum, where fatty acyl-CoA intermediates are coupled onto a glycerol backbone (e.g. glycerolipids) (Fig. 1) [20,21]. Most of the biochemical reactions involved in this pathway were elucidated in Eugene Kennedy's laboratory in the 1950's [22].

2.1. Manipulation of GPAT1

Glycerol-phosphate acyltransferase (GPAT) isoforms 1–4 catalyze the first, rate-limiting step in glycerolipid synthesis, which involves the synthesis of lysophosphatidic acid from glycerol-3-phosphate and long-chain acyl-CoA (Fig. 2, Reaction A) [23,24]. In most tissues, the majority of GPAT activity (GPAT3 and 4) resides in the endoplasmic reticulum, but mitochondrial isoforms (GPATs 1 and 2) play a more prominent role in the liver. Knockout mice lacking the mitochondrial GPAT1 display a partial reduction in liver triglycerides and resistance to obesity [25,26]. In particular, the animals have a smaller fat pad, decreased body weight, a lower rate of VLDL secretion, and increased insulin sensitivity [25,26]. This profound metabolic improvement occurs despite the fact that the GPAT1^{-/-} mice demonstrate markedly elevated acyl-CoA levels. Interestingly, the effects of global GPAT1-deficiency on glucose tolerance are highly dependent upon gender and the carbohydrate content of the diet [27], reflecting the increased role of triglyceride synthesis which accompanies carbohydrate consumption. Selective knockdown of GPAT1 in the liver produces similar findings [28], while hepatic overexpression of the enzyme induces hepatic and peripheral insulin resistance [29]. These data strongly suggest that glycerolipid intermediates modulate hepatic glucose output in addition to controlling the rate of delivery of lipids to other peripheral tissues.

2.2. Manipulation of AGPATs

Acyl-CoA:1-acylglycerol-sn-3-phosphate acyltransferases (AGPATs, also referred to as lysophosphatidate acyltransferases, or LPAATs) catalyze the second step in the Kennedy pathway, acylating lysophosphatidic acid to produce phosphatidic acid (Fig. 2, Reaction B). A number of enzymes have been identified which have sequence homology and the capacity to serve as acyltransferases, but most have only been characterized at the biochemical or cellular level [30]. The one exception is AGPAT2, which is present in high levels in adipose, liver, heart, and pancreas, and more modestly in other tissues [30]. Humans with mutations in the AGPAT2 gene are lipodystrophic, and suffer from severe insulin resistance, hepatic steatosis, hypertriglyceridemia, and diabetes [31]. These patients have virtually no adipose tissue and have extremely low serum leptin and adiponectin levels [32–35]. AGPAT2 knockout

mice have a comparable phenotype, including nearly complete loss of adipose tissue, insulin resistance, hepatic steatosis, and hyperglycemia [36].

2.3. Manipulation of lipins

Phosphatidate phosphatases (i.e. lipins) catalyze the third step in the Kennedy pathway: the dephosphorylation of phosphatidic acid to form diacylglycerol (Fig. 2, Reaction C). Of the three isoforms, lipin-1 has been characterized most extensively, and the enzyme accounts for all of the phosphatidic acid phosphatase (PAP) activity in adipose tissue and skeletal muscle. In addition, lipin-1 may additionally have coactivator functions which drive the expression of pro-adipogenic genes in adipocytes [37] and lipid oxidation genes in the liver [38]. Lipin-1-deficient *fld* mice are lipodystrophic, displaying profound hepatic steatosis, hypertriglyceridemia, and insulin resistance [39–41].

Transgenic approaches have been used to assess the role of lipin-1 in adipose tissue and skeletal muscle, and its overexpression in these tissues induces obesity, albeit through separable mechanisms. Animals overexpressing the enzyme in adipose tissue become obese, due to the upregulation in TAG synthesis, but retain insulin sensitivity [42]. Animals overexpressing lipin1 in skeletal muscle also become obese but display marked decreases in whole-body energy expenditure and fat utilization, as well as insulin resistance [42]. In fact, replacing lipin in skeletal muscle of the *fld* mouse is sufficient to reduce energy metabolism, without increasing adiposity.

2.4. Manipulation of DAGK

The reversal of the step 3 reaction listed above involves the phosphorylation of DAG to produce phosphatidic acid, and at least 10-different DAG-kinases (DAGK) are capable of catalyzing this reaction (Fig. 2, Reaction D). Mice lacking the DAGK δ isoform, which localizes in the ER [43], display a particularly severe phenotype, dying within 24 h after birth [44]. Newborn DGK δ -knockout mice have open eyelids, which is characteristic of reduced epidermal growth factor signalling [44]. Moreover, studies in cell culture reveal that DGK δ deficiency leads to downregulation of EGF-receptors via a PKC-related mechanism [44]. Studies described below suggest that DGK δ may similarly modulate insulin receptor activity through a comparable mechanism.

Noting that DAGK δ was decreased in skeletal muscle from poorly controlled type 2 diabetic patients, the Zierath group investigated whether mice haploinsufficient for the enzyme displayed alterations in their metabolic characteristics [45]. Enzyme assays revealed that such heterozygosity reduced total DAGK activity in muscle and fat, but not liver. At 9 weeks of age, the DAGK haploinsufficiency did not affect body weight, but at 36 weeks both the body weight and epididymal fat pad weight were increased. The older animals also displayed glucose intolerance and insulin resistance. Under these conditions, DAG accumulated in muscle but not liver, suggesting this tissue was a major site of action. The mice also demonstrated metabolic inflexibility as they had diminished lipid oxidation and were unable to shift between lipid and glucose oxidation during the transition between rested and active states. These changes preceded obesity. Moreover, studies in isolated issues suggested that defects in glucose transport were also evident prior to the animals showing an increase in adipose mass. Moreover, studies in cell culture revealed that the mechanism for the inhibition of insulin action likely involved PKC and the subsequent inhibition of insulin receptor substrate-1 (IRS-1).

2.5. Manipulation of DGATs

In rodents, two acylCoA:diacylglycerol acyltransferase (DGAT) isoforms catalyze incorporation of a fatty acid onto a diacylglycerol backbone, which is the final step in the biosynthetic pathway leading to triglyceride synthesis (Fig. 2E, Reaction E). DGAT2 appears

to play a dominant role in most tissues, and mice lacking this isoform die within a few hours after birth due to a severe reduction in triglycerides needed for oxidative metabolism and in essential fatty acids needed for epidermal barrier function [46]. However, studies on DGAT1^{-/-} mice provide insight into the role of glycerolipids in insulin resistance, hepatic steatosis, and lipotoxicity.

Knockout mice lacking DGAT1 are resistant to obesity and remain insulin sensitive when placed on a high fat diet [47]. The reduced adiposity is due to decreased lipid absorption in the intestine [48] and an increase in energy expenditure [47], and results in diminished accumulation of lipids in peripheral tissues, including skeletal muscle and the liver. Surprisingly, DGAT ablation is not associated with increases in other lipid intermediates, such as diacylglycerol (DAG). Many of the effects of whole body DGAT knockouts can be recapitulated by transplanting adipocytes from DGAT1-deficient mice, suggesting that the anti-obesity phenotype is due to functions within this tissue [49]. One predicts that the small adipocytes from the DGAT1 knockout mice retain a favourable endocrine profile. In particular, DGAT deficiency increases leptin sensitivity, and the anti-obesity effects of DGAT-deficiency are lost in mice lacking leptin [50]. In contrast, adiponectin appears dispensable for the improved insulin sensitivity associated with DGAT deficiency [51]. Transgenic overexpression of DGAT1 in adipose tissue of C57Bl/6 mice exacerbates obesity without impacting peripheral insulin sensitivity or glucose homeostasis [52], supporting the hypothesis that lipotoxic accumulation of fat in peripheral tissues, rather than obesity itself, is a key component of glucose intolerance associated with an elevated BMI.

Inhibition of DGAT activities within the liver has been investigated as a therapeutic strategy for limiting lipid delivery to peripheral tissues, but the studies are mixed, depending on the duration of knockdown. Short-term reduction of hepatic DGAT2 using antisense oligonucleotides reduced hepatic steatosis in obese mice and rats, improved hepatic insulin sensitivity, and promoted lipid oxidation [53,54]. Paradoxically, this experimental approach led to reductions in various glycerolipid intermediates, such as DAG and lysophosphatidic acid. Long-term knockdown of DGAT2 using antisense approaches also protected from hepatic steatosis in mice fed a methionine/choline-deficient diet, but increased levels of hepatic fatty acids, oxidative stress, fibrosis and necrosis [55]. DGAT1 knockdown did not produce comparable effects.

Overexpression of DGAT1 or 2 in the liver enhances steatosis in the absence of hepatic insulin resistance [56]. Surprisingly, these transgenic mice have increased levels of other putative lipotoxic intermediates, including DAG, ceramides, and unsaturated FFAs, without evidence of altered hepatic glucose output or peripheral insulin resistance.

Intramyocellular triglyceride levels correlate well with the severity of peripheral insulin resistance, except in endurance-trained athletes, who demonstrate markedly elevated triglyceride levels in type I (oxidative) fibers but remarkably enhanced insulin sensitivity [57–59]. These studies suggest that TAGs are inert entities in muscle, and studies with DGAT reinforce this idea. Overexpression of DGAT1 in skeletal muscle using electroporation or the creation of transgenic mice increases intramyocellular triglyceride levels and improves insulin sensitivity and glucose homeostasis [60,61]. These manipulations are associated with decreased levels of DAG and ceramide, and suggest that TG production in muscle protects from lipotoxicity caused by the accumulation of glycerolipid and sphingolipid intermediates. Of note, manipulating rates of glycerolipid synthesis here seemed to impact sphingolipid synthesis, suggesting that the rate of flux of FFAs through the two arms are interrelated. Interestingly, transgenic overexpression of DGAT2 in type II (glycolytic) fibers leads to the accumulation of TAG and reduced DAG levels, but has a paradoxical decrease in insulin sensitivity and glucose tolerance [62]. However, in this circumstance, the tissues displayed

elevated FFA and ceramide content, suggesting that DGAT overexpression somehow leads to a secondary enhancement of sphingolipid synthesis.

2.6. Manipulation of SCD1

Though not technically part of the glycerolipid biosynthetic pathway, stearoyl-CoA desaturase, which catalyzes the synthesis of Δ^9 -monounsaturated fatty acids, markedly impacts rates of glycerolipid synthesis. As noted previously [63], SCD1-deficient animals display a phenotype remarkably similar to the DGAT1 knockout, including resistance to obesity and enhanced insulin sensitivity [64,65]. The enzyme has been shown to co-localize with DGAT2 [66], and clearly impacts rates of glycerolipid synthesis. Liver-specific depletion of SCD1 using antisense nucleotides protects from hepatic steatosis and insulin resistance [67,68].

Like DGAT, SCD1 expression in peripheral tissues appears to protect cells from lipotoxic events associated with fat exposure, while its inhibition renders them susceptible to lipotoxic insults. In human myotubes, overexpression of SCD1 enhances triglyceride synthesis and prevents inflammatory and ER-stress responses to palmitate [69], suggesting that it prevents lipotoxicity by sequestering lipids into inert triglyceride pools. In the liver, SCD1 deficiency sensitizes cells to injury, despite the concomitant decrease in triglycerides [70]. SCD1 inhibition in various other mouse models has been shown to improve insulin sensitivity and prevent steatosis but to exacerbate molecular events associated with atherosclerotic plaque formation [71] and pancreatic β -cell failure [72,73].

SCD1 clearly promotes triglyceride synthesis, though the mechanism is for this effect remains elusive. Thus, its deficiency in liver leads to diminished steatosis and enhanced lipid oxidation [74], and consequently decreasing adiposity and reducing the delivery of triglycerides to peripheral tissues. By contrast, the enzyme's deficiency in other cell-types, including β -cells, likely leads to the formation of saturated lipid intermediates that impair tissue function. A more detailed discussion of putative metabolites and their role in lipotoxic events is included below.

2.7. Manipulation of TAG hydrolysis

Most triglycerides in the body reside in lipid droplets, which are dynamic organelles comprised of TG and cholesterol esters surrounded by a phospholipid monolayer containing regulatory proteins (e.g. perilipin, adipophilin, TIP47, etc.). The impact of these proteins on lipid dynamics is fascinating and complex, and is only starting to be fully realized. Ultimately, triglyceride hydrolysis involves the sequential action of three lipases: (a) adipose triglyceride lipase (ATGL) (Fig. 2, Reaction F), which shows higher affinity for TAG; hormone sensitive lipase (HSL) (Fig. 2, Reaction G), which is rat-limiting in the hydrolysis of DAG; and monoglyceride lipase [75,76]. HSL was cloned and characterized first, and knockout mice lacking the enzyme have a paradoxical reduction in WAT mass and resistance to obesity, owing to a downregulation of triglyceride synthesis [77,78]. In this circumstance, HSL depletion reduces β -adrenergic-stimulated lipolysis by a modest 30–40% and promotes DAG accumulation [79,80]. Other mechanisms accounting for the decrease in adiposity in the HSL knockouts include an increase in necrotic adipocyte death and an upregulation of uncoupling protein-1 [81,82].

Reports on glucose homeostasis in HSL^{-/-} mice have shown mixed and contradictory results. In some studies, HSL knockout mice have signs of enhanced glucose uptake, increased insulin sensitivity, protection from diet-induced insulin resistance, and decreased muscle triglycerides [83,84]. Conversely, other groups have reported that HSL^{-/-} mice have decreased insulin sensitivity [85,86]. In addition to affecting insulin-responsive tissues, HSL depletion impacts β -cells, and islets isolated from the whole-body knockouts show impaired insulin secretion [86], owing evidently to the enzyme's ability to liberate free cholesterol needed for the

exocytosis to insulin granules [87]. Indeed, mice lacking HSL selectively in β -cells display hyperglycaemia and disrupted insulin release [88].

Unlike mice lacking HSL, those devoid of ATGL exhibit a predicted phenotype including enlarged fat pads, disrupted lipolysis, and cold sensitivity [89]. All of these findings are consistent with the animals having an impaired lipolytic response, which is decreased by more than 75%. ATGL-deficiency has severe metabolic consequences associated with the accumulation of TAG in peripheral tissues including the heart, skeletal muscle, testis, kidney, and pancreas. The lipid accumulation in cardiac myocytes causes cardiac failure and premature death, evidenced at only 12 weeks of age. The reduced availability of FFAs is accompanied by increased utilization of glucose, improved glucose tolerance, and insulin sensitivity [89]. Thus, in the ATGL knockouts, insulin resistance can be dissociated from lipotoxicity. In a small number of patients, mutations in ATGL leads to abnormal lipid deposition and myopathy [76].

Less is known about the role of ATGL in non-adipose tissues. In the liver, overexpression of ATGL (or HSL) reduces hepatic lipid stores and promotes lipid oxidation [90]. In muscle, whole-animal ATGL deficiency is associated with increased lipid accumulation, glucose uptake, and glucose utilization [89,91]. Overexpression of the enzyme in muscles using electrotransfer increased the oxidation of fatty acids and elevated DAG and ceramide levels [92]. Moreover, its forced overexpression in obese, insulin resistant rats reduced TAG content but did not restore insulin sensitivity [92]. In islets, ATGL may additionally impact insulin secretion. Peyot and colleagues [93] noted that ATGL^{-/-} mice demonstrated increased insulin sensitivity and decreased GSIS. Moreover, islets from ATGL^{-/-} mice or cultured islets in which ATGL was knocked down with short hairpins showed impaired insulin secretion in response to a number of different secretagogues.

2.8. Summary—molecular mechanisms through which glycerolipids contribute to selective insulin resistance and lipotoxicity

Teasing out molecular mechanisms underlying the pathogenesis of metabolic disease from the complex phenotypes of knockout mice is challenging, but the data give us clues regarding the potential role of some distinct lipid metabolites. Moreover, the findings identify several enzymes involved in glycerolipid synthesis or metabolism as potential therapeutic targets. In general, partial inhibition of triglyceride synthesis in WAT (e.g. DGAT1 knockouts) or liver e.g. (GPAT1 or SCD1 knockouts) appears to have beneficial effects owing to a reversal of selective insulin resistance in the liver, an overall lack of delivery of lipids to peripheral tissues, and an improved endocrine profile of adipocytes. In each of these cases, adipose and/or liver triglyceride levels are greatly decreased. Surprisingly, the animals are able to adequately deal with the excess lipid that is not stored as triglycerides, and the condition appears to be associated with either decreased intestinal absorption or increased oxidation of the excess fat. In contrast, complete ablation of enzymatic intermediates in the biosynthetic pathway leading to triglyceride synthesis, particularly in WAT (e.g. AGPAT2 and Lipin1), promotes a lipodystrophic syndrome characterized by increased lipid deposition and severe malfunction of peripheral tissues, resembling genetically engineered “fatless” mice where transgenic manipulations lead to complete adipocyte apoptosis [94,95].

The anti-obesity and/or anti-steatosis properties of the whole-animal knockouts make it difficult to glean whether glycerolipids play direct roles in skeletal muscle or mediate other lipotoxic events associated with obesity (e.g. β -cell or cardiomyocyte apoptosis, etc.). In these whole-body knockouts, the changes in muscle insulin sensitivity are likely to be secondary to changes in the adipocyte (e.g. effects on adipokines) or the liver (e.g. synthesis of VLDLs, and thus delivery to peripheral tissues). Nonetheless, our interpretation of the studies with GPAT1^{-/-} and DAGK^{-/+} mice is that glycerolipid intermediates likely contribute to the

desensitization of insulin action in liver and muscle, respectively. But which glycerolipid metabolite is the culprit? As described above, overexpression of DGAT or SCD1 in peripheral tissues appears to protect cells from lipotoxic events associated with fat exposure by diverting lipids into inert TAG stores [60,61,69]. Conversely, inhibition of these enzymes renders peripheral tissues susceptible to lipotoxic insults [55,70–73]. These observations are consistent with studies in cultured cells suggesting that one can ward off the lipotoxic effects of saturated fats by supplementing with oleate, which promotes incorporation of the excess lipid into the triglyceride pool [96–98]. Thus, TAG is unlikely to be anything other than an inert bystander.

An idea which has received considerable attention is that intermediates in the *de novo* synthesis pathway are antagonists of glucose transport and modulators of cell function. Indeed, both DAG and PA are well established as cell signalling intermediates, though their role in signalling is normally attributed to plasma membrane functions, rather than those in the ER. For example, DAG involved in cell signalling is generally produced at the cell membrane via the hydrolysis of phosphatidylcholine. However, in the *lipin1* deficient animals, Nadra et al. [99] found that the accumulation of phosphatidic acid, through its ability to activate the MEK/ERK signalling pathway, accounted for peripheral neuropathy. Thus, intermediates in the *de novo* glycerolipid pathway are capable of serving as signalling intermediates, as well as lipotoxic modulators of cell function.

But which glycerolipids serve as modulators of insulin sensitivity in muscle or liver? Because of the data involving the *DAGK δ ^{-/+}* mice [98], DAG emerges as a strong candidate. Indeed, DAG has received considerable attention as an inducer of insulin resistance, and the prevailing hypothesis has been that its ability to activate PKC isoforms leads to the phosphorylation of insulin receptor substrates and the inhibition of phosphatidylinositol 3-kinase [100,101], with the latter being an obligate intermediate in insulin-stimulated glucose uptake and anabolic metabolism. The aforementioned studies by Chibalin et al. [98], which revealed that PKC inhibitors restored insulin sensitivity in cultured myotubes, support this hypothesis. However, there have been two lines of evidence that introduce doubts about the importance of DAG as a modulator of glucose homeostasis. First, though DAG often correlates with insulin sensitivity, in some experimental models large increases in liver or muscle DAG are not sufficient to induce insulin resistance [56,102]. Second, the James lab has recently suggested that insulin resistance (i.e. the inhibition of glucose transport in muscle) caused by lipids occurs independently of signalling to IRS-1, the supposed target of DAG/PKC-induced insulin resistance [103]. Indeed, effects on IRS-1 are unlikely to account for the *selective* insulin resistance associated with obesity, as inhibition of PI3K would be predicted to prevent hepatic lipogenesis [104]. Curiously, in cultured myotubes, overexpression of another DAGK isoform, DAGK ϵ , prevented lipid (i.e. linoleate) activation of PKCs, but exacerbated insulin resistance [105]. In this study, the authors identified phosphatidic acid as the primary antagonist of insulin action [105].

A unique feature of the glycerolipid pathway is its dependence upon mono-unsaturated fats, as evidenced by the impact of SCD expression or oleate supplementation to drive lipids into the triglyceride pool [96–98]. As described below, saturated and unsaturated fats induce insulin resistance by separable mechanisms, with the former being reliant on sphingolipid signalling [102]. An intriguing possibility is that the fatty acid composition of glycerolipid intermediates influences their signalling capabilities. Indeed, prior studies suggest that DAG comprised of unsaturated acyl-chains is a far more potent activator of PKC [106], raising the possibility that distinct, unsaturated DAG subspecies will emerge as important contributors to insulin resistance and metabolic disease.

3. Manipulation of sphingolipid synthesis: impact on selective insulin resistance and lipotoxic diseases

Many of the circulating factors associated with obesity (e.g. inflammatory cytokines, saturated fatty acids, glucocorticoids, etc.) induce sphingolipid synthesis. As in *de novo* glycerolipid synthesis, most of the action occurs in the ER, where the substrates palmitoyl-CoA, serine, and another fatty acyl-CoA are converted to ceramide [107]. Vesicular transport mechanisms or ceramide transport proteins move ceramide to the golgi [108], where additional biosynthetic reactions convert it to sphingomyelin, ceramide-phosphate, glucosylceramides, and other complex sphingolipids. Manipulation of enzymes controlling sphingolipid synthesis or degradation *in vitro* or *in vivo* has a potent effect on insulin resistance and ameliorates lipotoxic responses associated with obesity [109].

3.1. Manipulation of serine palmitoyltransferase

Serine palmitoyltransferase (SPT), which condenses palmitoyl-CoA and serine to produce 3-ketosphinganine, is the initial, rate-limiting enzyme in the biosynthetic pathway driving ceramide production (Fig. 3, Reaction A) [110]. In seminal studies manipulating this enzyme, the Unger laboratory found that inhibiting SPT with the broad-spectrum antibiotic cycloserine prevented lipotoxic effects of palmitate in Zucker fa/fa beta cells *in vitro* and *in vivo* [111, 112], leading the authors to conclude that “the ceramide pathway seems to be the most important of the destructive routes” involved in lipoapoptosis [113]. Subsequent studies involving the manipulation of this enzyme with higher specificity inhibitor myriocin and genetic manipulations strongly support this hypothesis.

In lipid-infused, high fat fed, and ob/ob mice as well as dexamethasone-treated, Zucker fa/fa, and ZDF rats, treatment with the SPT inhibitor myriocin markedly improves insulin sensitivity and glucose tolerance [102,114]. Hyperinsulinemic–euglycemic clamp studies confirm that myriocin improves insulin sensitivity in both muscle and the liver [102]. In the ZDF rats, SPT inhibition additionally preserved pancreatic insulin (Holland and Summers, unpublished observation) and prevented the onset of frank diabetes [102], confirming the Unger hypothesis that depleting ceramide preserves pancreatic β -cells. Studies in cultured cells and/or isolated tissues strongly suggest that these effects can result from tissue-autonomous mechanisms, as SPT inhibition negates lipid-induced insulin resistance and apoptosis in isolated muscles (and cultured myotubes) or pancreatic islets, respectively [96,102,111,112,115–117].

Though these findings universally support the enzyme’s role in insulin sensitivity in both skeletal muscle and the liver, some important differences emerge that warrant elucidation. The relative effects of myriocin on body weight are unclear, due to some discrepancies in the literature. In a prior study, the insulin sensitizing effects of myriocin were independent of changes in body weight or a reduction in obesity [102]. In apolipoprotein-E deficient mice (ApoE^{-/-}) fed a high fat diet (discussed below), myriocin also had only a minor impact on body weight [118]. However, in a subsequent study in high fat fed and ob/ob mice, myriocin reduced body weight and increased energy expenditure [114]. Moreover, in this latter study myriocin reduced hepatic steatosis [114], though it has been shown by others to have no acute effect on glycerolipid synthesis (e.g. during lipid infusion [102] or lipid-incubation of hepatocytes [119]). These are important differences that have profound implications on our understanding of the mechanism of sphingolipid action.

Studies using myriocin additionally suggest that ceramide is related to cardiovascular (e.g. atherosclerosis and cardiomyopathy) complications of lipid excess, even in the absence of obesity. Several groups have found that treating ApoE^{-/-} mice with myriocin decreases the formation of atherosclerotic lesions [118,120–122]. Moreover, the Goldberg laboratory found

that myriocin ameliorated symptoms of cardiomyopathy, including cardiac hypertrophy, enlarged and abnormal myocytes, left ventricular dilatation, and reduced fractional shortening, in animals overexpressing lipoprotein lipase in the heart [123]. In these studies, myriocin reduced sphingolipid levels without impacting glycerolipid synthesis. Importantly, the Goldberg laboratory was the first to demonstrate that haploinsufficiency for a key subunit of SPT also prevented features of metabolic disease, as the manipulation rendered mice resistant to this form of lipotoxic cardiomyopathy [124].

3.2. Manipulation of dihydroceramide desaturase

Dihydroceramide desaturase (DES) inserts a critical double bond into dihydroceramide, converting it into the bioactive molecule ceramide (Fig. 3, Reaction B). Of the two DES isoforms, DES1 is essential for ceramide synthesis in most tissues, with DES2 playing more specialized roles in making phytosphingolipids which are in the gut [102]. Mice that are haploinsufficient for DES1 are refractory to dexamethasone-induced insulin resistance. Moreover, muscles isolated from these mice remain insulin sensitive, even in a profoundly hyperlipidemic environment [102]. Interestingly, the small molecule fenretinide has recently been identified as a potent inhibitor of DES1 [125], and the compound improves insulin sensitivity in mice fed a high fat diet [126].

3.3. Manipulation of glucosylceramide synthase and gm3 synthase

Ceramides can be glucosylated by glucosylceramide synthases (Fig. 3, Reaction C), and the product can be further modified to give rise to a large family gangliosides (Fig. 3, Reaction D). Studies with two unrelated glucosylceramide synthase inhibitors suggest that these intermediates may contribute to obesity-induced insulin resistance. Specifically, the GCS inhibitors N-(5'-adamantane-1'-yl-methoxy)-pentyl-1-deoxynojirimycin (AMP-DNM) and Genz-123346 improve glucose tolerance and increase insulin sensitivity in muscle and liver of ob/ob and DIO mice, as well as ZDF rats [127,128]. When given to ob/ob mice, inhibitors of GCS prevent hepatic steatosis by modulating the expression of various lipogenic genes [129]. However, these drugs do not impact susceptibility to atherosclerosis [130].

Through the addition of various additional carbohydrate residues, glucosylceramide gets converted into distinct gangliosides. One of these, GM3 ganglioside, is widely distributed, and serves as a precursor for most of the more complex species. Mice lacking the GM3-synthase gene display lower fasting glucose levels and improved glucose tolerance [131]. When challenged with high fat diets, the GM3-synthase null mice maintained superior glucose tolerance, improved insulin stimulated glucose uptake, and enhanced suppression of hepatic glucose output.

3.4. Manipulation of lysosomal ceramide degradation (e.g. acid ceramidase and acid sphingomyelinase)

Sphingomyelinases convert sphingomyelin back into ceramide and phosphocholine (Fig. 3, Reaction E). Nikolova-Karakashian and colleagues [119] crossed mice lacking the acidic, lysosomal form of the enzyme (ASMase^{-/-}) with mice lacking the low density lipoprotein receptor (ldlr^{-/-}), with the latter prone to metabolic disease when placed on western diets enriched in saturated fat and cholesterol. The ablation of ASMase produced a striking phenotype including a marked reduction in hepatic triglycerides, increased insulin sensitivity, and improvements in glucose tolerance. The animals demonstrated a substantial increase in hepatic ceramide. Desipramine, an inhibitor of ASMase, but not myriocin, reduced rates of triglyceride synthesis in isolated hepatocytes. These results suggest a highly interesting link between acid sphingomyelinase activity and rates of triglyceride synthesis.

Once ceramide accumulates within the lysosome, acid ceramidases further deacylate it to produce sphingosine (Fig. 3, Reaction F). Mice that are haploinsufficient for acid ceramidase (*Asah1^{-/+}*) show a phenotype remarkably different than that of the *ASMase* mice mentioned above, including the development of a progressive lipid storage disorder, with particularly abundant fat accumulation within the liver [132].

3.5. Summary—molecular mechanisms through which sphingolipids contribute to selective insulin resistance and lipotoxicity

The studies reviewed herein strongly suggest that one or more sphingolipid entities are important contributors to selective insulin resistance and lipotoxicity, as the inhibition of early steps in the sphingolipid pathway has large beneficial effects in rodent models of obesity, diabetes, atherosclerosis, and cardiomyopathy. But which metabolite is the critical factor, and what is the mechanism by which small increases in sphingolipid intermediates affect cell function?

Of the sphingolipids, ceramide, including that generated by *de novo* synthesis as well as by acute sphingomyelin hydrolysis, has emerged as a particularly important player in cellular responses to stress. With regards to insulin signalling, ceramide or a ceramide-metabolite inhibits activation of Akt/PKB, a serine/threonine kinase important for glucose uptake (muscle) and repression of hepatic glucose output (liver), via a two independent pathways involving protein phosphatase 2A and protein kinase C α/ζ [133]. In cultured myotubes, we found that blocking ceramide glucosylation or deacylation antagonized insulin signalling, leading us to conclude that ceramide was the likely signalling intermediate [134].

Taniguchi and colleagues [104] have suggested that Akt/PKB and PKC λ play differential roles on glucose output and lipogenesis, respectively. Thus, the differential effect of ceramide on Akt/PKB and atypical PKCs is intriguing, as it provides a potential explanation for selective insulin resistance (Fig. 1). However, Akt/PKB has also been implicated in events underlying lipid accumulation in liver [135], and it is still unclear whether ceramide is capable of modulating lipid synthesis in this tissue. This issue is important to resolve.

In addition to its modulation of Akt/PKB, ceramide may contribute to insulin resistance and lipoapoptosis through a number of additional mechanisms. In skeletal muscle, ceramide or a related lipid has also been linked to distal events controlling glucose transporter 4 translocation [103,136], which is a key step in postprandial glucose disposal. Moreover, ceramide has been strongly linked to the induction of mitochondrial damage and apoptosis, which can contribute to the demise of pancreatic β -cells, cardiomyocytes, and others. Specific mechanisms include the following: (a) recruitment of pro-apoptotic Bax to mitochondrial membranes [137–140]; (b) generation of reactive oxygen species through the inhibition of the mitochondrial ubiquinone pool of complex III [141,142] or regulation of NADPH oxidase [143,144]; (c) clustering of death receptors [145–147]; and (d) directly increasing mitochondrial membrane permeability [148–154].

Though the pharmacological and knockout data listed above strongly implicate sphingolipids in metabolic disease, several pieces of data argue that ceramide is not the key bioactive intermediate. First, though ceramide levels sometimes correlate with various indices of metabolic disease, sometimes disease symptoms are manifest under conditions where no ceramide elevation is detected [155]. Second, under certain experimental conditions, large inductions in liver ceramide are not associated with hepatic insulin resistance [56,156]. Third, the increase in ceramide during obesity, when observed, is typically rather small, and it is difficult to understand how subtle differences in ceramide levels could account for such profound metabolic defects. These data suggest that a ceramide-metabolite, rather than ceramide itself, may be the key bioactive molecule. Alternatively, ceramide may not be

working through tissue autonomous mechanisms, and the primary site of action has not been evaluated.

Other target which have received attention include the glucosyl-ceramides, due to the therapeutic benefit of the glucosylceramide synthase inhibitors. One mechanism that has been put forth is that glucosylceramides interfere with insulin binding to its receptor, and studies in cultured cells reinforce the hypothesis that glucosylceramide interfere with early steps in insulin signalling [157,158]. However, as with most signalling cascades, low levels of receptor occupancy are typically sufficient to promote a full insulin response. Moreover, the aforementioned studies by Hoehn et al. argue against such a proximal defect in insulin resistance [103]. Lastly, this mechanism would not explain how the compounds reduce hepatic lipid levels [129]. A more likely explanation is that the protective effects of this class of compound are secondary to their lowering of liver triglycerides, and the mechanism underlying this effect warrants further study.

4. Manipulation of lipid oxidation: impact on selective insulin resistance and lipotoxic diseases

In the 1960s, Randle and colleagues [159] introduced the hypothesis that mitochondria served as the primary sensors of excess lipid, and that fatty acids and glucose could serve as competitive substrates for oxidation. The mechanism put forth (i.e. the Randle Hypothesis) was that increased acetyl-CoA, produced during rounds of lipid oxidation, would trigger a chain of events leading to the accumulation of glucose 6-phosphate, which in turn inhibits hexokinase. The resulting decrease in glucose phosphorylation would reduce rates of glucose entry into the cell. While it is now clear that insulin resistance can occur in the absence of increased G6P (e.g. during lipid infusion [160]), the relative role of the Randle hypothesis, mitochondrial lipid sensing, and/or mitochondrial dysfunction in insulin resistance continues to be a hot topic of interest and debate [161,162].

4.1. Manipulation of CPT-1

Lipid entry into mitochondria involves the carnitine palmitoyl-transferase system, which exchanges carnitine for coenzyme-A to create a fatty acid-carnitine conjugate capable of being transported across the inner mitochondrial membrane (Fig. 4, Reaction A). Inhibition of CPT-1 with a pharmacological reagent (i.e. etomoxir) increases lipid deposition and exacerbates insulin resistance in rats fed a high-fat diet [163]. Moreover, modest overexpression of CPT1 in distal hindlimb muscles of rats protects against lipid-induced insulin resistance [164]. These data suggest that mitochondria do not serve as primary sensors of lipid excess.

4.2. Manipulation of ACC1 and ACC2

Malonyl-CoA is a potent inhibitor of CPT1, and a key determinant of rates of lipid oxidation. Acetyl-CoA carboxylase (ACC) generates malonyl-CoA from acetyl-CoA (Fig. 4, Reaction B), and thus is an important regulator lipid oxidation. Of the two distinct isoforms, the cytosolic ACC1 generally produces cytosolic malonyl-CoA for lipogenesis, while mitochondrial ACC2 produces the malonyl-CoA which regulates CPT1. However, there is evidence of compensation between the two isoforms (see below).

Genetic ablation of ACC1 in mice is embryonically lethal [165]. However, elimination of ACC2 in mice reduces fat mass, increases energy expenditure, improves insulin sensitivity, and prevents obesity-induced diabetes [166,167]. Moreover, soraphen, an inhibitor of the ACC system, prevents weight gain and improves peripheral insulin sensitivity in mice fed a high-fat diet [168]. As a result, ACC has emerged as an attractive therapeutic target [169].

Tissue-specific manipulations of ACC isoforms reveals some surprising findings. Liver-specific ACC knockouts demonstrate marked reductions in hepatic malonyl-CoA and decreases in hepatic triglyceride [170]. However, despite these changes, these mice were not resistant to obesity or insulin resistance [170]. Harada et al. also made liver-specific ACC1 knockout mice [171]. These animals demonstrated no changes in lipogenesis or in hepatic malonyl-CoA, suggesting that ACC2 could compensate in this experimental model. Intraperitoneal injection of antisense oligonucleotide inhibitors against ACC1 or ACC2 has also been shown to effectively reduce each isoform. Knockdown of ACC1, but not ACC2, was associated with decreased lipogenesis [172]. After high fat feeding, inhibiting both isoforms simultaneously, but not either one independently, reduced hepatic malonyl-CoA, lowered hepatic lipids, and improved peripheral insulin sensitivity [172].

4.3. Manipulation of MCD

Malonyl-CoA-decarboxylase (MCD) promotes β -oxidation by converting malonyl-CoA into acetyl-CoA (Fig. 4, Reaction C), thus relieving the inhibition of CPT1. Koves and colleagues [173] revisited the hypothesis that glucose and lipids served as competitive substrates for oxidation by testing MCD^{-/-} mice fed a high fat diet. These mice demonstrated improved glucose tolerance and insulin sensitivity, as assessed by glucose and insulin tolerance tests. These data are consistent with observations that mitochondrial fatty acid β -oxidation is sometimes enhanced in muscles from insulin resistant rodents or diabetic patients [162,174–177]. Noting that high fat feeding leads to an increase in acyl-carnitines, Muoio and colleagues hypothesized that increased flux of acyl-CoAs into mitochondria induces stress, as the substrates exceed the oxidative capacity of this organelle. They hypothesized this “incomplete β -oxidation” leads to the formation of intermediates capable of inhibit correct mitochondrial function [173,178]. This finding is consistent with studies in isolated human myocytes, where knockdown of MCD increased glucose oxidation [179,180].

Of particular note, the MCD effect appears to be specific to skeletal muscle, as MCD1 overexpression in the liver has opposing effects, preventing hepatic steatosis and reversing insulin resistance [181]. Surprisingly, the whole-animal MCD^{-/-} mice described above did not display noticeable changes in lipid oxidation in the liver [173]. Of note, the liver isoform of CPT1 is much less sensitive to malonyl-CoA, potentially explaining the muscle-specific findings in the MCD^{-/-} mice.

4.4. Manipulation of FAS

Fatty acid synthase (FAS) reduces malonyl-CoA by converting it to palmitoyl-CoA during *de novo* lipogenesis (Fig. 4, Reaction D). Deletion of the gene producing cytoplasmic FAS is embryonically lethal [182]. Manipulation of this reaction with pharmacological inhibitors has potent effects on body weight, due to hypothalamic effects on food intake [183]. Conditional ablation of FAS in the hypothalamus and pancreatic recapitulates the resistance to weight gain and decreased appetite, and reveals a role in physical activity and increased energy expenditure [184,185].

In the periphery, liver-specific FAS knockouts (FASKOL) have a paradoxical predisposition to hypoglycemia and hepatic steatosis, particularly when starved or when placed on a no fat/high carbohydrate diet that promotes lipogenesis [186]. The phenotype is attributable to a reduction in β -oxidation, owing to a three-fold increase in malonyl-CoA levels [186]. Moreover, most of the effects were reversed by treating with agonists of the PPAR α transcription factor [186], suggesting that “new fat” produced by the FAS reaction served as endogenous ligands for this important transcriptional regulator of genes involved in lipid metabolism.

4.5. Summary—molecular mechanisms through which mitochondrial lipid oxidation influences insulin resistance and lipotoxicity

The data herein demonstrate that increasing lipid oxidation in the liver (e.g. MCD overexpression, ACC knockdowns) prevents hepatic steatosis and insulin resistance. Thus, these enzymes emerge as putative therapeutic targets, particularly for siRNA or antisense strategies which can effect a conditional effect in the liver.

In contrast, studies in skeletal muscle gave mixed findings, and are more difficult to interpret. Whole animal CPT inhibition induces insulin resistance, and CPT overexpression in muscle improves insulin sensitivity. In contrast, ablation of MCD, which blocked lipid oxidation in muscle, had the opposite effect. How does one reconcile these paradoxical findings? It is worth noting that metformin, which is widely prescribed as insulin sensitizing therapeutic, acts essentially as a mitochondrial poison which alters intracellular ATP/AMP ratios, leading to an activation of AMP-protein kinase [187,188]. An apparently important action of AMPK is to promote lipid oxidation, which it does by reducing ACC and increasing MCD activities, respectively. Muoio and Newgard [178] propose that metformin is working primarily working in the liver, and this idea warrants further scrutiny. Alternatively, one wonders whether metformin is associated with compensatory changes which improve the capacity of the TCA cycle to metabolize incoming fat.

Considering the role of incomplete lipid oxidation in the induction of insulin resistance in muscle, one is tempted to speculate on potential mechanisms. Muoio and colleagues noted through metabolic profiling that acylcarnitines were elevated in insulin resistant rodents, but it is unclear whether acylcarnitines are the key signal, or are merely a marker of the incomplete oxidative state. Indeed, acylcarnitines are generally considered to be part of a detoxification method for removing excess acyl group from mitochondria, and carnitine supplementation improves insulin sensitivity [189]. Another possibility is that reactive oxygen species (ROS) are the culprits, and many investigators have proposed that excessive lipid supply forces the mitochondria to produce ever-increase amounts of ROS, impairing mitochondrial function and inducing a concomitant development of insulin resistance. Houstis et.al. [190] found that chronic treatment with the antioxidant agent manganese (iii) tetrakis (4-benzoic acid) porphyrin (MnTBAP) improved insulin sensitivity and glucose homeostasis in insulin-resistant ob/ob mice. More recently, Hoehn and colleagues [103] found that ROS scavengers prevented insulin resistance in L6-myotubes exposed to a number of factors which induce insulin resistance, including various exogenous fatty acids. In this study, sphingolipid synthesis inhibitors also prevented the insulin resistance, suggesting that sphingolipids and ROS may function by interrelated mechanisms.

5. Future directions

Experimental manipulation of enzymes controlling lipid synthesis and metabolism has profound effects on selective insulin resistance and lipotoxicity, and many of the enzymes discussed herein have emerged as putative therapeutic drug targets. What is clear, however, is that no single lipid entity fully accounts for the lipotoxic events underlying metabolic disease, and a wide array of metabolites likely control tissue function by additive and synergistic mechanisms. One might anticipate that these different lipid species may have differing levels of importance in individual patients or patient populations.

A problem with each of these experimental approaches, particularly when it comes to trying to elucidate precise molecular mechanisms involved in lipid sensing and action, is that the inhibition of a single enzyme in a cascade of interrelated events is likely to have a large impact on the entire lipidome, as fatty acyl-CoAs get diverted from one pathway to the next. For example, inhibiting early steps in glycerolipid or sphingolipid synthesis impacts all of the

downstream metabolites. Moreover, inhibition of one pathway will increase the flux of nutrients into the other. Thus, the implementation of blunt, sledgehammer approaches to tackle delicate and intricate biological questions has not yet produced a particularly strong understanding of mechanism. Advances in lipidomics, when combined in full force with conditional genetic knockouts, knockdown approaches, and more specific and efficacious small molecule inhibitors, will accelerate progress towards understanding how specific lipid metabolites perturb metabolic homeostasis.

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Fig. 1.

Schematic diagram depicting the flux of nutrients during the proposed stages of metabolic disease. (A) In healthy individuals, insulin facilitates postprandial nutrient deposition by (a) inhibiting hepatic glucose production, (b) stimulating glucose uptake into muscle, and promoting triglyceride synthesis in (c) liver and (d) adipose. (B) The obese become selectively resistant to insulin effects on glucose metabolism, such that muscle glucose transport is blocked and hepatic glucose output is enhanced (denoted by red X's). However, other effects of insulin remain intact, and the enhanced lipogenesis in the liver leads to increased circulating triglyceride levels (i.e. VLDLs). As circulating insulin levels rise, due to the residual glucose in the blood, the lipogenic effects of the hormone become dominant, and a vicious cycle ensues. Increased production of triglycerides leads to an increasing rate of their delivery to peripheral tissues, greater insulin resistance, more profound hyperinsulinemia, and exacerbation of the lipid synthesis effects of the hormone. (C) The final stages of metabolic disease involve additional defects in the adipocyte. Impairments in adipocyte plasticity or storage capacity

limit its utility as a reservoir for excess fat. Moreover, impairment of insulin action in the tissue leads to enhanced lipolysis, further increasing delivery of fatty acids to the liver. Ultimately, lipid delivery to peripheral tissues exceeds their storage and oxidative capacity, and tissue damage ensues.

Fig. 2.

Schematic diagram illustrating key reactions in glycerolipid synthesis. Most glycerolipid synthesis occurs in the ER, though some enzymes have been found in mitochondria and/or mitochondrial associated membranes. Enzymes discussed in the text are noted with the black circles. A major substrate is glycerol-3-phosphate derived either from the reduction of the glycolytic intermediate dihydroxyacetone phosphate or from ATP-dependent phosphorylation of glycerol by glycerol kinase. Glycerol-3-phosphate undergoes successive esterifications with acyl-CoAs to produce lysophosphatidic acid (LPA) (catalyzed by GPATs, Reaction A) and phosphatidic acid (PA) (catalyzed by AGPATs, Reaction B). Phosphatidic acid is a precursor for both to membrane glycerophospholipids and triglycerides. Lipins dephosphorylate PA to produce DAG (Reaction C), and diacylglycerol kinases (Reaction D) catalyze the reverse reaction. DGATs add the final acyl moiety, to produce triglycerides. Hydrolysis of TAG includes the sequential actions of ATGL (Reaction F) and HSL (Reaction G).

Fig. 3.

Schematic diagram illustrating key reactions in sphingolipid synthesis. *De novo* synthesis of ceramide occurs in the ER, though some of the biosynthetic enzymes have additionally been found in ER and mitochondria. Enzymes discussed in the text are noted with the black circles. Serine palmitoyltransferase (Reaction A) catalyzes the initial step, which involves the condensation of serine and palmitoyl-CoA to form 3-ketosphinganine. 3-ketosphinganine reductase reduces 3-ketosphinganine to form the sphingoid base sphinganine. A family of (dihydro)Ceramide synthase catalyzes sphinganine acylation, producing dihydroceramide. Dihydroceramide desaturase (Reaction B) oxidizes inactive dihydroceramide into active ceramide. Once generated, ceramide is the common precursor of complex sphingolipids. In the golgi, glucosylceramide synthase (Reaction D) and sphingomyelin synthases convert the precursor ceramide into glucosylceramides and sphingomyelin, respectively. Glucosylceramides are the substrate for further reactions leading to the production of complex gangliosides. One of these, a GM3 synthase (reaction D), is discussed in the text. Within the lysosome (Reaction E) and plasma membrane, sphingomyelinases convert sphingomyelin back into ceramide, which can be further deacylated to produce sphingosine (Reaction F).

Fig. 4.

Schematic representation of fatty acid oxidation pathway. Fatty acyl-CoAs are transported into the mitochondrial matrix by the carnitine transport system, with CPT1 being a key and rate-limiting component (Reaction A). Once inside the mitochondrial matrix, fatty acyl-CoAs are oxidized in a series of repeated steps that each release acetyl-CoA. The basic outlines of this pathway includes the following: transfer to carnitine to acyl-CoAs by CPT-1; transport of acyl-carnitines through inner mitochondrial membranes; regeneration of acyl-CoA by carnitine acyltransferase II (CPT-2); successive rounds of β -oxidation of fatty acids. Acetyl-CoA then enters the citric acid cycle, where it is oxidized in the same fashion as the acetyl-CoA derived from glycolysis. As acetyl-CoA levels rise, acetyl-CoA carboxylases (ACCs, Reaction B) convert it into malonyl-CoA, first committed intermediated in fatty acid biosynthesis and a potent inhibitor of CPT-1. Key regulators of malonyl-CoA levels include malonyl-CoA decarboxylase (MCD, Reaction C) and fatty acid synthase (FAS, Reaction D).