

Ceramide and Sphingosine 1-Phosphate in adipose dysfunction

Zijian Fang, Susan Pyne and Nigel J Pyne*

Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, 161
Cathedral St, Glasgow, G4 0RE, Scotland, UK

Abstract –The increased adipose tissue mass of obese individuals enhances the risk of metabolic syndrome, type 2 diabetes and cardiovascular diseases. During pathological expansion of adipose tissue, multiple molecular controls of lipid storage, adipocyte turnover and endocrine secretion are perturbed and abnormal lipid metabolism results in a distinct lipid profile. There is a role for ceramides and sphingosine 1-phosphate (S1P) in inducing adipose dysfunction. For instance, the alteration of ceramide biosynthesis, through the de-regulation of key enzymes, results in aberrant formation of ceramides (e.g. C_{16:0} and C_{18:0}) which block insulin signaling and promote adipose inflammation. Furthermore, S1P can induce defective adipose tissue phenotypes by promoting chronic inflammation and inhibiting adipogenesis. These abnormal changes are discussed in the context of possible therapeutic approaches to re-establish normal adipose function and to, thereby, increase insulin sensitivity in type 2 diabetes. Such novel approaches include blockade of ceramide biosynthesis using inhibitors of sphingomyelinase or dihydroceramide desaturase and by antagonism of S1P receptors, such as S1P₂.

* To whom correspondence should be addressed (n.j.pyne@strath.ac.uk)

Introduction

Type 2 diabetes (T2D) is a multifactorial metabolic disorder that results in hyperglycaemia due to the resistance of peripheral tissues to insulin and the failure of pancreatic β cells to secrete insulin; the latter is termed β cell decompensation [1,2]. 392 million people were diagnosed with T2D in 2015 and the prevalence of the disease is estimated to rise globally to approx. 600 million by 2035 [3,4]. Insulin resistance is a pathological condition that develops early in the disease and precedes the onset of hyperglycaemia and hyperinsulinemia, thereby affecting the endocrine control of peripheral tissue metabolism [2,5]. Meta-analyses of patient populations indicate that the relative risk of developing the disease is much higher in obese and overweight individuals compared with those with a normal BMI [6,7]. However, not all obese patients develop insulin resistance or diabetic complications (termed healthy obesity) [8,9]. The pathogenesis of T2D involves a dysfunctional adiposity phenotype, which includes excess visceral fat, chronic inflammation and insulin resistance [10–12]. The risk of pre-diabetes and T2D is increased by visceral obesity rather than general adiposity [13]. The defective function of adipose tissue constitutes a mechanistic link between obesity and T2D. For instance, hyperglycaemia and hyperlipidaemia arising from excess adiposity can interfere with insulin signaling and cellular metabolism in non-adipose tissue (termed glucolipotoxicity), which eventually results in chronic inflammation, insulin resistance and abnormal islet function [14–19]. In addition, the abnormal secretion of inflammatory cytokines and adipokines from dysregulated adipose tissue accelerates systemic inflammation and promotes peripheral insulin resistance [20]. Therefore, adipose dysfunction in obesity appears to have a significant role in the development of systemic insulin resistance in T2D and metabolic syndrome.

Preventive therapy that slows the onset and reverses the progression of T2D requires an improved understanding of the basic molecular biology of adipose tissue in response to the

imbalance between food intake and energy expenditure. Sphingolipids are a class of cellular lipids containing a sphingoid base and various head groups, many members of which (e.g. ceramide and sphingosine 1-phosphate (S1P)) are emerging players in metabolic syndrome, obesity and T2D [21,22]. In this regard, ceramide and S1P regulate a myriad of cellular events, such as insulin signaling, inflammatory responses and intracellular metabolism in an organ-specific manner [23–25]. Therefore, this review focuses on the role of ceramide/S1P metabolism and signaling in dysregulated adipose tissue function under conditions of surplus dietary energy intake.

Physiological role of adipose tissue in whole-body metabolism

Adipose tissue is composed of various cell types, including adipocytes, pre-adipocytes, mesenchymal stem cells and immune cells (e.g. adipose tissue macrophages, ATM), along with different cellular compositions of fat depots. These fat depots are found in distinct anatomical locations which determine their special physiological properties [26,27]. The primary cell type within white adipose tissue is the white adipocyte, which contains a large high energy lipid droplet. In contrast, adipocytes of brown adipose tissue contain large mitochondria with a high level of uncoupling protein-1 (UCL-1) that results in more extensive lipid oxidation to generate heat, thereby enabling thermogenic regulation [1]. Interestingly, white adipocytes can be transdifferentiated into brown-like adipocytes (known as beige adipocyte) with thermogenic capacity in response to cold and β -adrenergic receptor stimulation [28,29]. Adipocyte progenitor cells from the adipose tissue stromal fraction are responsible for maintaining the normal ‘turn-over’ of adipocytes [28,30], while there is also a spectrum of immune cells (including M1 and M2 macrophages), which are involved in adipose remodeling and in regulating insulin sensitivity [31,32].

White adipose tissue maintains normal metabolism by absorbing and exporting fat and glucose in a non-oxidative pathway during feeding and fasting respectively, thereby meeting the physiological demand between meals. The esterification of glycerol with non-esterified fatty acids (NEFA) produces triacylglycerol (TAG), which is then packaged into the lipid droplet inside the white adipocyte. The lipolytic pathway in adipocytes is tightly controlled by the neuroendocrine system and circulating nutrient molecules. These modulate the expression and activity of lipid droplet-associated proteins and lipid hydrolytic enzymes (*e.g.* hormone-sensitive lipase (HSL), adipose triglyceride lipase (ATGL) and monoglyceride lipase (MAGL)) and which are regulated by cAMP- and cGMP-dependent signaling pathways [33]. For example, the interaction of perilipin1, the predominant protein associated with the lipid droplet, and HSL can be enhanced by the protein kinase A (PKA) catalysed phosphorylation of perilipin1, which increases the enzymatic activity of HSL [34,35]. In addition, PKA can directly phosphorylate HSL and induce its association with the lipid droplet [36]. Another important function of adipose tissue is the endocrine control of whole-body metabolism and appetite and this is achieved by regulating production of adipokines, pro-inflammatory cytokines (*e.g.* MCP-1, TNF α and interleukin-1 β) and adipocyte-specific hormones (*e.g.* leptin, adiponectin and vaspin). Circulating adiponectin enhances energy consumption and lipid/glucose metabolism in the liver and skeletal muscle whereas leptin, acting in the hypothalamus, reduces appetite [20].

The lipolytic/lipogenic pathways in adipose tissue are fine-tuned to regulate circulating glucose and lipid levels and this is controlled by the endocrine and neuronal systems [33,37]. High fat diet (HFD) challenge in the short term does not cause adipose dysfunction or systemic metabolic defects because the expansion and redistribution of fat depots compensate for the lipid burden in metabolically active tissues [38]. Indeed, pre-adipocyte differentiation and

proliferation (hyperplasia) and existing adipocyte enlargement (hypertrophy) in fat depots enables functional plasticity of the adipose tissue that is in positive caloric balance [39]. The upregulation of the transcription factors such as peroxisome proliferator-activated receptor gamma (PPAR γ), CCAAT/enhancer-binding proteins (C/EBPs) and sterol regulatory element-binding transcription factor 1 (SREBF1) and inhibition of Wnt/ β -catenin signaling promotes differentiation of pre-adipocytes into lipid-storing adipocytes. This results in the expression of adipocyte-specific genes including the glucose transporter type 4 (GLUT-4) and fatty acid-binding protein 4 (FABP4) that maintain glucose and lipid homeostasis [28,40,41]. Considering the beneficial role of PPAR γ and other transcription factors involved in TAG biosynthesis, *de novo* adipogenesis ensures normal glucose/NEFA metabolism in adipose tissue and maintains overall insulin sensitivity during subcutaneous expansion [40]. Indeed, the disruption of adipogenesis in adipocyte-specific *Stat3*^{-/-} mice leads to increased general adiposity, reduced energy expenditure and high liver TAG levels [42].

Visceral adipose expansion through increased lipid filling is dependent on adipocyte enlargement, which results from a high expression of anti-adipogenic genes such as *Gata2* and *Tgfb2* allied with reduced expression of pro-adipogenic genes such as *Pparg*, *Bmp2* and *Bmp4*. [43,44]. Indeed, the enlargement of fat cells leads to increased hormone-induced lipolysis and suppressed insulin-stimulated lipid and glucose uptake [45–48]. Furthermore, up-regulation of pro-inflammatory cytokines (e.g. TNF α and MCP-1) and altered secretion of adipokines (e.g. adiponectin and leptin) are evident in hypertrophic adipocytes [20,49–51]. Therefore, while adipogenesis is the predominant form of adaptation to accommodate over-nutrition, hypertrophy is likely to be involved in promoting insulin resistance and hyperlipidaemia thereby leading to diabetic complications [39,52–54]. This is supported by the finding that hypertrophic obesity is associated with suppressed adipogenic rates in pre-adipocyte [55]. Of

note, there is different sensitivity of pre-adipocytes to adipogenic stimuli in different fat pads. For example, adipogenesis during the development of obesity is much higher in subcutaneous adipose tissue compared with visceral compartments, and this results in the distinct changes in adipocyte size, number and metabolism [54]. Intriguingly, the hypertrophic adipocyte, with exhausted storage capacity, in visceral regions is associated with derangement of lipid metabolism and the development of dyslipidemia [56]. Furthermore, subcutaneous adipose hypertrophy correlates with impaired insulin signaling and glucose handling, suggesting the pathological effects of adipose hypertrophy are also depot-specific [56].

The lipid overload and excess TAG deposition in adipose tissue leads to impaired adipogenesis and increased adipocyte hypertrophy. This is characterized by a hyper-lipolytic and pro-inflammatory phenotype that is resistant to insulin [1,2]. The increased secretion of MCP-1 from hypertrophic adipocytes initiates the recruitment and proliferation of ATMs, which subsequently release chemokines and cytokines that promote chronic inflammation [20,57,58]. In addition, the release of TNF α and IFN γ from macrophages, T cells and NK cells inhibits the differentiation of pre-adipocytes and reduces insulin signaling in mature adipocytes [59,60]. Therefore, the lipolytic pathway in hypertrophic adipocytes is further amplified by chronic inflammation and insulin resistance. As a result, the increased NEFA and lipid metabolites delivery from dysfunctional adipose tissue overwhelms the oxidation rate to cause ectopic fat deposition in the heart, liver and skeletal muscle and this leads to the onset of systemic metabolic disease [10]. A good example of this is the increase in NEFA influx in skeletal muscle and liver that results in higher intracellular diacylglycerol (DAG) levels, which activate protein kinase C (PKC) isoforms, PKC θ and PKC ϵ , that can functionally abrogate insulin signaling [61].

Dyslipidaemia involves a large number of different lipids, which arise from dysregulated lipid metabolism pathways in adipose tissue. TAG and NEFA become elevated in plasma and high levels of circulating ceramide in T2D patients appear to contribute to the development of peripheral insulin resistance [62]. It is not surprising then, that the reduction in whole body and abdominal adiposity after physical exercise is associated with decreased plasma ceramide levels and improved systemic insulin sensitivity in T2D patients [63]. The elevation of circulating S1P levels in obese animal models and T2D patients is positively associated with central abdominal obesity and metabolic syndrome [64–66], thereby suggesting a relationship between ceramide/S1P metabolism, adipose dysfunction and T2D. However, whether this represents a causal mechanism is currently unknown.

Insulin resistance of adipose tissue

Insulin regulates glucose and fat metabolism in metabolically active tissues through canonical insulin-dependent signaling to reduce plasma concentrations of NEFA and glucose. At the molecular level, insulin activates the insulin receptor (IR) tyrosine kinase, which catalyses the tyrosine phosphorylation of itself and IR substrate (IRS). The latter acts as cytosolic adaptor protein, enabling recruitment and activation of phosphoinositide 3-kinase (PI3K) and thereby elevation of phosphatidylinositol (3,4,5)-trisphosphate (PIP₃), an activator of phosphoinositide-dependent kinases (PDK) which, together with the mTOR complex 2 (mTORC2), catalyses the phosphorylation of protein kinase B (PKB/AKT) on Thr308 and Ser473. The downstream effectors of AKT modulate insulin-induced metabolism; these include effects on the glucose transporter, GLUT-4, lipid synthesis (e.g. SREBP1c) and gluconeogenic and lipogenic enzyme expression (e.g. FOXO) [67]. In addition, the insulin-induced activation of the Ras/mitogen-activated protein kinase (MAPK) pathway via IRS regulates cell growth and differentiation. A complex and integrated network is formed by the extensive cross-talk

between insulin-, leptin-, insulin growth factor-1- and inflammatory cytokine-mediated signaling pathways to modulate reversible phosphorylation cascades and transcriptional/translational programmes [67,68].

Insulin regulates glucose transportation, glycolysis and glycogen synthesis in adipose tissue [69]. At a molecular level, insulin activates cAMP phosphodiesterase 3B (PDE3B), downstream of AKT to reduce intracellular cAMP levels thereby, blocking PKA-induced lipolysis. Other proteins that limit lipolysis include protein phosphatase-1 (PP-1) which, when stimulated by insulin, deactivates HSL via a cAMP-independent mechanism [35,70,71]. Insulin also transcriptionally suppresses ATGL and thereby accelerates TAG accumulation *via* inhibition of the nuclear translocation of Forkhead box protein O1 (FOXO1) [70,72]. PPAR γ -mediated adipogenic programmes via activation of the AKT/mTORC1 pathway are also stimulated by insulin [38,73]. Finally, the insulin-dependent activation of MAPK pathways promotes the stimulation of transcriptional factors, such as Med23 and Elk1; key regulators of adipogenesis [74]. Indeed, the inducible deletion of phosphatase and tensin homolog (PTEN), which catalyses the dephosphorylation of PIP₃ in mature adipocytes results in enhanced insulin signaling to potentiate adipogenesis and to reduce adipose inflammation during the long-term HFD insult [75]. Therefore, the maintenance of normal adipose tissue function provides many whole-body metabolic advantages, such as low liver fat accumulation and improved glucose tolerance in adult mice [75].

The ectopic lipid deposition in obesity and resulting from excess NEFA influx establishes a large lipid metabolite pool in adipose tissue [18]. Consequently high DAG and ceramide levels activate PKC ϵ and PKC ζ respectively, which catalyse inhibitory serine/threonine phosphorylation of IR and IRS to block insulin signaling [17,18]. Concurrent with lipid-

mediated insulin resistance, inflammatory mediators, such as TNF α , IL-1 β and IL-6, derived from adipocytes and ATM, stimulate c-Jun N-terminal kinases (JNKs), inhibitor of nuclear factor kappa-B kinase subunit β (IKK β), ribosomal protein S6 kinase (S6K) and the mammalian target of rapamycin (mTOR). This results in the serine phosphorylation of IRS-1, which reduces insulin signaling [19,67,76]. It is also significant in the context of insulin resistance that TNF α and IFN γ increase expression of the suppressor of cytokine signaling 3 (SOCS3) which is a specific inhibitor of IRS [77–79] (Figure 1).

Lipid oversupply and activation of PKC can also induce IKK- and JNK-dependent phosphorylation of IRS and IR, thereby stimulating pro-inflammatory STAT-1 and NF κ B-dependent signaling and insulin resistance [80]. Moreover, saturated NEFAs are ligands of Toll-like receptor 4 (TLR4) and are able to directly promote IKK β and JNK activation in adipocytes [31,81]. The oversupply of glucose and lipid interferes with the oxidative system in mitochondria and protein synthesis in the endoplasmic reticulum (ER), thereby resulting in oxidative and ER stress respectively. This is important because these stress pathways can induce activation of JNK-AP1 and IKK β -NF κ B, the transcription of pro-inflammatory genes [18,82–85] and the increase cytokine secretion in an autocrine and paracrine manner [5]. The net effect is that extracellular pro-inflammatory mediator secretion induced by lipid overload accelerates the impairment of insulin sensitivity in a positive regulatory loop. For example, TNF α increases the hydrolysis of TAG through transcriptional, translational and post-translational down-regulation of PPAR γ [17] and the subsequent over-activation of the lipolytic programme exacerbates lipid burden and PKC-mediated insulin resistance in adipocytes. In most cases, lipid-mediated signaling and metabolic inflammatory pathways are therefore, interconnected and function in synergy to oppose insulin signaling (Figure 1).

Deregulation of ceramide biosynthesis in adipose tissue

While the majority of NEFA is either oxidized in the mitochondria or stored as glycolipids, a small proportion is used for the biosynthesis of phospholipids and sphingolipids. Ceramide is a major hub of sphingolipid metabolism and is composed of sphingosine, a long-chain amino alcohol, with an N-linked fatty acyl group of various carbon chain lengths. Ceramide also constitutes the backbone of complex sphingolipids and can be converted into sphingosine, catalyzed by the enzyme, ceramidase. There is particular interest in ceramide because it can promote insulin resistance [86]. This is exemplified by studies showing that the treatment of 3T3-L1 adipocytes with the cell permeant short chain C₆-ceramide reduces GLUT4 transcription and GLUT4 mRNA stability leading to impaired glucose uptake [87]. The underlying mechanism by which ceramide impairs glucose transport is linked with the dephosphorylation of AKT by protein phosphatase 2A (PP2A). This is supported by the fact that PP2A is activated by ceramide [88–90]. PKC ζ is also activated by ceramide and inhibits AKT recruitment and activation by catalyzing the phosphorylation of Thr34 in the PH-domain of AKT, thereby reducing binding of PIP₃ to AKT [91,92]. Other mechanisms also operate as caveolar ceramide induces the association of PKC ζ with AKT, the recruitment of PTEN and the retention of AKT within caveolin-enriched micro-domains (CEM) [93]. The PKC ζ -AKT interaction represses AKT in CEM, and the localization of PTEN in these membrane domains enables dephosphorylation of PIP₃ to prevent AKT activation [93]. Ceramide also stimulates the NLRP3 inflammasome to increase IL-1 β levels [94] and enhances TNF α , MCP-1 and IL-6 expression in adipocytes [95], which might contribute to chronic inflammation and the development of insulin resistance. Finally, the blockade of ceramide synthesis results in the downregulation of MCP-1 and plasminogen activator inhibitor-1 (PAI-1) in adipose tissue of diet-induced obese (DIO) rodent models [96]. Therefore, ceramide-mediated insulin resistance

is the result of an integrated inflammation and lipid signaling responses that are linked with the hallmarks of adipose dysfunction (Figure 2).

Ceramide is formed by cellular compartment-specific mechanisms (Figure 3). Therefore, alteration of these specific pathways may influence the intracellular distribution of ceramide. One key mechanism of ceramide production is *via* the ‘so-called’ *de novo* synthesis pathway on the ER. The condensation of serine and palmitoyl Co-A by serine palmitoyltransferase (SPT), to produce 3-keto-sphinganine, is usually the rate limiting step in this biosynthetic pathway [97]. Therefore, the modulation of SPT activity can affect the profile of sphingolipid metabolites in cells [98]. This is exemplified by studies showing that SPT deficiency in adipose tissue results in reduced ceramide, sphingomyelin, S1P and sphinganine 1-phosphate levels and these changes are associated with impaired adipocyte development and adipocyte death [99]. The rapid conversion of 3-keto-sphinganine into sphinganine is catalyzed by 3-keto-sphinganine reductase. A second fatty acid CoA is then incorporated into sphinganine to form dihydroceramide, catalysed by ceramide synthase (CerS). Dihydroceramide is then desaturated by dihydroceramide desaturase (Dggs1) to produce ceramide through the introduction of a 4, 5 *trans* double bond. Six distinct CerS isoforms use different fatty acid CoAs to catalyse acylation of sphinganine to produce ceramides with different acyl chain lengths. Specificity is determined by 11 amino acids in an N-terminal luminal loop of the enzyme [100]. The fatty acid CoAs range from C_{14:0} to C_{26:0} [21,101,102]. For example, the concerted action of Dggs1 and CerS2 results in the synthesis of ceramides with the very long acyl chains varying from C_{20:0} to C_{26:0}, whereas CerS6 and Dggs1 catalyse the formation of ceramides containing C_{14:0} and C_{16:0} fatty acids [88,103]. In contrast, Dggs1 and CerS4 catalyse the synthesis of C_{18:0} to C_{20:0} acyl chain ceramides [88,103]. The formation of C_{16:0} and C_{18:0} ceramides can lead to abnormalities in glucose hemostasis and lipid metabolism. Indeed, the loss of CerS5 and CerS6

protects mice from HFD-induced obesity and insulin resistance [104–106]. Furthermore, ceramide, glucosylceramide and dihydroceramide containing stearate (C_{18:0}) are associated with insulin resistance and inflammation in human skeletal muscle [107]. In contrast, the low expression of CerS2 in the liver of CerS2 heterozygous (CerS2^{-/+}) mice leads to decreased levels of long-chain ceramides (i.e. C_{22:0}, C_{24:0} and C_{24:1} ceramides) but there is also a compensatory increase in C_{16:0}-containing sphingolipids in hepatocytes, which are associated with hepatic insulin resistance and mitochondrial respiratory chain disorders [108]. Dggs1 is the last step in *de novo* ceramide synthesis and might play a key role in obesity. In this regard, GWAS studies indicate that *Dggs1* is a pre-disposing gene for fat mass accumulation [109]. This is supported by evidence showing that genetic deletion of the Dggs homologue in *Drosophila* results in high levels of dihydroceramides and increased fat storage [110].

The acid and neutral sphingomyelinases (aSMase and nSMase) hydrolyse membrane sphingomyelin into ceramide and phosphocholine [86]. Deficiency in aSMase underlies the lysosomal storage Niemann-Pick disease where there is accumulation of sphingomyelin in lysosomes. Intriguingly, the low body weight of Niemann-Pick patients might indicate that aSMase has a role in regulating fat depots. This is supported by results demonstrating that mice with deletion of aSMase (*Smpd1*^{-/-}) cannot accumulate fat in liver and adipose tissue [111]. The lysosomal recycling of complex sphingolipids, such as sphingomyelin or glucosylceramide produces ceramide, which is rapidly deacylated to sphingosine and NEFA. This sphingosine can be resynthesized into ceramide by CerS or phosphorylated to S1P by sphingosine kinases (SphK) [23,112]. The intracellular levels of ceramide do not remain constant but undergo temporal and spatial fluctuations due to different rates of metabolism, which is likely to determine impact on insulin sensitivity and adipose function [88,90].

The infusion of circulating saturated lipid into adipose tissue provides substrates to produce ceramide and other sphingolipids because the metabolic flux through SPT is dependent on the availability of palmitate [97,113]. For example, NEFA is used for ceramide biosynthesis in human skeletal muscle [114]. However, the ability of lipid oversupply, through *de novo* ceramide synthesis, to affect adipose tissue dysfunction is confounded by the finding that the exposure of 3T3-L1 adipocytes to palmitate does not enhance ceramide synthesis and does not affect insulin sensitivity [89]. Nevertheless, there is substantial evidence that ceramide synthesis is, indeed, upregulated in adipose tissue during inflammation [115,116]. This was demonstrated by the intraperitoneal administration of the inflammatory mediator TNF α , which stimulates ceramide synthesis in adipose tissue by increasing the expression of aSMase, nSMase, and SPT in C57/BL6 mice [95]. In addition, pro-inflammatory lipids, such as palmitate, and lipopolysaccharides (LPS) activate TLR4/IKK β signaling and increase the expression of SPT2, CerS1, CerS2 and CerS6 and Degr1 linked with ceramide-induced insulin resistance in C2C12-derived myotubes [117]. Wild-type mice also have a higher C_{16:0} ceramide level in subcutaneous fat compared with mice carrying adipocyte-specific TLR4 deletion [118]. It has also been shown that the expression of sphingomyelinases is increased in inflamed adipose tissue of obese female patients [119]. However, there are no detectable changes in *de novo* ceramide biosynthesis in adipocytes and macrophages, thereby suggesting a more prominent role for the sphingomyelinase pathway [119]. Taken together, this evidence suggests that, in obesity, there might be a switch from the *de novo* ceramide pathway to the sphingomyelinase pathway in dysregulated adipose tissue. Indeed, it has been proposed that *de novo* ceramide synthesis is actually reduced as the expression of Degr1 mRNA is decreased in white adipose tissue of HFD-fed and ob/ob mice [120].

The expression level of CerS6 in visceral and subcutaneous fat depots is positively correlated with BMI in humans. This is significant as CerS6 regulates the formation of C_{16:0} ceramide in adipocytes of DIO mice [106] and this ceramide species is linked with adipose dysfunction and T2D. Importantly, this might suggest that it is the formation of specific molecular species of ceramide in adipose tissue that promotes adipose dysfunction, even though the net production of ceramide by *de novo* synthesis is reduced.

Recently, Siddique et al. [121] reported that mouse embryonic fibroblasts lacking *Degs1* have increased dihydroceramide levels and enhanced AKT/protein kinase B (PKB) signaling, which is a pro-survival pathway that blocks apoptosis. In addition, *Degs1*^{-/-} cells exhibited high levels of autophagy as a result of impaired ATP synthesis and activation of AMP-activated protein kinase (AMPK). Therefore, *Degs1* deletion is associated with the induction of both anabolic and catabolic signaling pathways. We propose that these findings suggest that there might be two populations of Degs1, which are functionally opposed. The first population is native Degs1 which catalyses formation of ceramides which, we propose, act to block AKT/PKB signaling. Thus, removal of Degs1 will increase anabolic AKT signaling and this might contribute to sensitisation of cells to insulin. In addition, we have shown that Degs1 is subject to polyubiquitination in HEK293T cells and PANC1 cancer cells [122]. Therefore, the second population of Degs1 might be represented by these polyubiquitinated forms, which we have shown exhibit a 'gain of function' and appear to have a different substrate specificity compared with the native form [122]. Alternatively, these polyubiquitinated forms might be relocated to specialized lipid micro-domains with access to specific dihydroceramides. The polyubiquitinated forms of Degs1 are linked to positive regulation of p38 MAPK [122], which is an inhibitor of autophagy [123]. Therefore, loss of polyubiquitinated forms of Degs1 might increase autophagy because of the reduction in p38 MAPK signaling. We have also shown that

the native Degr1 is pro-apoptotic while the polyubiquitinated forms promote cell survival [122]. Genetic deletion of *Degr1* will eliminate both native and polyubiquitinated forms, and it is possible that deficiency of the native form has a predominant effect in promoting cell survival [121] (Figure 4).

In contrast with the findings described above, the shRNA mediated decrease in Degr1 expression in 3T3-L1 cells increases the dihydroceramide/ceramide ratio and reduces adipogenic and lipogenic programmes [120]. The down-regulation of Degr1 reduces PPAR γ activity and cyclins, such as D1, D3, E, as well as decreasing cdk2, which regulate PPAR γ activity during adipogenesis. These findings are correlated with adipose dysfunction in diabetic patients as the levels of dihydroceramide are higher compared with non-diabetic controls [124].

Targeting ceramide biosynthesis in adipose tissue

High content of DAG and ceramide, together with increased macrophage infiltration are found in subcutaneous adipose tissue of obese women with increased liver fat [125], suggesting that ceramide might also amplify chronic inflammation and insulin resistance in adipose tissue [125]. In addition, the levels of ceramides including C_{14:0}, C_{16:0}, C_{16:1} and C_{18:1} species are elevated in the white adipose tissue of obese and diabetic patients [106,124,126,127] and C_{16:0} and C_{18:0} ceramides levels are increased in adipose tissue of HFD-fed mice and are correlated with the development of adipocyte-specific insulin resistance [128]. The adipose tissue of C57BL mice on long-term HFD (16 or 18 weeks) also have increased C_{16:0} and C_{18:0} ceramide levels but decreased C_{24:0} ceramide [106,129]. Indeed, in a large multi-ethnic Dallas Heart Study, plasma short-chain saturated ceramides, such as C_{16:0} and C_{18:0} were positively linked with insulin resistance, dyslipidemia and visceral adiposity. In contrast, plasma

polyunsaturated ceramides (C_{24:2}, C_{30:10} and C_{32:11}) were inversely associated with these unfavorable phenotypes [130]. These findings suggest that C_{16:0} and C_{18:0} ceramides are linked with de-regulated adiposity. Nevertheless, there is still some controversy regarding the role of ceramides in adipose dysfunction. Thus, while SPT, aSMase, and nSMase mRNA levels are increased in obese human subcutaneous and abdominal adipose tissue, ceramide accumulation does not occur [131]. However, this might be explained by increased expression of acid and alkaline ceramidase, which might rapidly convert these ceramides into sphingosine [95]. It is also worth noting that, despite no overt increase in ceramide levels, subtle changes in certain ceramide species in subcellular compartments might be sufficient to induce abnormalities in metabolism and insulin signaling [132,133].

Targeting the biosynthesis of ceramide shows promising results in restoring normal adipose function. For instance, DIO mice treated with myriocin, a potent inhibitor of SPT, results in a decrease in intracellular ceramide levels and an increase in the expression of thermogenic and browning/beiging genes in white adipose tissue [124]. The consequence of this is to direct adipocytes into a metabolically active state with increased glucose uptake and lipid oxidation, potentially arising from normalization of insulin sensitivity [124]. The loss of adipocyte-specific SPT2 also recapitulates this phenotype and improves mitochondrial activity [124]. Furthermore, pharmacological blockade or genetic loss of SPT suppresses chemokines and cytokines expression and promotes an anti-inflammatory M2 macrophages phenotype in fat depots [96,124]. In addition, mice deficient in CerS6 and challenged with HFD exhibit reduced C_{16:0} ceramide levels in white and brown adipose tissue. This results in suppressed adipose inflammation and increased β -oxidative capacity [106]. Additional support for this concept, is that the elevation of C_{16:0} ceramide levels induced by HFD in epididymal white adipose tissue is reduced in *Cers5*^{-/-} mice compared with wild type littermates. This results in upregulation of

C/EBP α and PPAR γ that promote adipogenesis concomitant with down-regulation of pro-inflammatory genes [134]. The role of *Degs1* has been investigated using the *Degs1* inhibitors, fenretinide and GT-11. Fenretinide blocks ceramide accumulation in liver and skeletal muscle and prevents peripheral insulin resistance and hepatic steatosis in DIO mice [135]. Fenretinide also improves adipokine profiles, with increased levels of adiponectin and resistin and decreased levels of leptin and retinol binding protein 4 (RBP4) [136]. Paradoxically, the *Degs1* inhibitor, GT-11 blocks rosiglitazone (PPAR γ agonist)-induced adipocyte differentiation [120] and exogenous dihydroceramides interfere with the early phase of adipocyte differentiation. The implication of these findings is that *Degs1* inhibitors might worsen the adipocyte phenotype in obesity [120]. However, the action of GT-11 is somewhat different from genetic loss of *Degs1* as AKT or AMPK signaling in response to insulin is unaltered. Furthermore, the action of GT-11 is distinct from fenretinide, which does not block rosiglitazone-induced adipocyte differentiation. Fenretinide also normalises mitochondrial metabolism as indicated by the low level of TCA cycle intermediates and oxidative stress markers, in white adipocyte of HFD-fed mice. In common with the *Degs1*^{-/-}, fenretinide induces autophagy in mature adipocytes, indicating that its mechanism of action is recapitulated [121]. This is further supported by studies using a metabolite of fenretinide, 4-oxo-N-(4-hydroxyphenyl)retinamide (termed 4-OXO), which is also a potent inhibitor of *Degs1* [137] and has poor effects on retinoic acid receptor. Significantly, 4-OXO does not inhibit adipogenesis of 3T3-L1 adipocytes [138], but instead increases the expression level of adipogenic markers. In addition, 4-OXO promotes AKT phosphorylation and autophagy induction independent of retinol gene modulation [138]. These findings raise the question as to why GT-11 and fenretinide have distinct mechanisms of action yet both target *Degs1*. We propose that this difference might be due to the ability of fenretinide, but not GT-11 to induce the polyubiquitination of *Degs1*, which inhibits autophagy by promoting p38 MAPK signaling [122]. However, polyubiquitinated

Degs1 will eventually be degraded by the proteasome, which, if fast in adipocytes, might result in the loss of Degs1 in response to fenretinide. This would then recapitulate the effects of genetic loss of *Degs1* on AKT signaling and autophagy [121]. These studies highlight the need to more fully understand the role of Degs1 in adipose dysfunction. It is therefore, important to know whether different classes of Degs1 inhibitors are anti-adipogenic or pro-adipogenic as highlighted by issues raised from use of GT-11 *versus* fenretinide. However, the balance of the data, in our opinion favours the use of Degs1 inhibitors that induce proteasomal degradation of Degs1 to treat T2D. This is supported by evidence showing that Degs1^{+/-} mice exhibit normal glucose levels and enhanced insulin sensitivity and are refractory to dexamethasone-induced insulin resistance compared with wild type littermates [139].

Additional evidence to support a role for ceramide in adipose dysfunction comes from studies in which the deletion of *Smpd1*, encoding aSMase, has been reported to be protective against HFD-induced hyperglycemia and insulin resistance in mice lacking LDL receptor [111]. *Ldlr*^{-/-} mice with *Smpd1* deletion fed on a HFD exhibit reduced adipose and liver fat accumulation compared with *Smpd1*^{+/+}/*Ldlr*^{-/-} littermates. These effects are recapitulated by the aSMase inhibitor, amitriptyline, which reduces plasma ceramide and attenuates adiposity, insulin resistance and glomerular injury in HFD C57BL/6J mice [140]. Finally, the genetic loss of *Smpd1* prevents adipocyte hypertrophy and promotes brown adipose tissue differentiation via a mechanism involving alterations in gene expression of adipocytes in Western diet-fed mice [141]. The improvement in adipose tissue function contributes to diminished liver steatosis [141].

S1P metabolism and signaling

Sphingosine-1-phosphate (S1P) is a bioactive lipid that appears to have an important role in obesity and T2D. S1P levels are governed by the availability of sphingosine, and the catalytic activity of S1P metabolising enzymes e.g. sphingosine kinases (SphK), S1P phosphatase and S1P lyase. Ceramidases catalyse the deacylation of ceramide to produce sphingosine which can be phosphorylated, using ATP, by two isoforms of sphingosine kinase, SphK1 and SphK2, to produce S1P. These isoforms are encoded by two different genes and regulate overlapping and non-overlapping signaling pathways [142]. Their differing protein sizes, tissue and subcellular localisations and biochemical properties determine their differing biological roles in physiological and pathophysiological states [25,143].

In response to extracellular stimuli, such as growth factors or inflammatory mediators, the catalytic activity of SphK1 can be increased by an ERK-1/2-catalysed phosphorylation of Ser225 in the R-loop [144]. ERK-1/2 is downstream of growth factor receptor tyrosine kinases (e.g. EGFR and VEGFR), TNF receptors and PKC [145–147]. Ser225 phosphorylation stimulates the translocation of cytosolic SphK1 to the plasma membrane where the enzyme can access sphingosine [144,148]. Intracellular S1P confers E3 ligase activity to TRAF2 to activate the NF κ B pathway [149,150] and can bind to PPAR γ to induce transcriptional activity in endothelial cells [151]. Similarly, epidermal growth factor (EGF) and the PKC activator, phorbol 12-myristate 13-acetate (PMA) promote activation of SphK2 via ERK-1-catalysed phosphorylation of Ser351 and Thr578 [152,153]. SphK2 is also regulated by a PKD catalysed phosphorylation of its nuclear export motif, which causes its export from the nucleus to the cytoplasm [154,155], thereby altering its subcellular localisation. In the nucleus SphK2 is associated with histone H3 and histone deacetylase 1 and 2 (HDAC-1/2) and the subsequently formed S1P inhibits HDAC1/2-catalysed deacetylation of histone H3 to up-regulate the

expression of cyclin dependent kinase inhibitor p21 and the transcriptional regulator c-fos [156]. In addition, S1P produced by mitochondrial SphK2 binds to prohibitin 2 to facilitate the assembly of respiratory complex IV and to thereby regulate oxidative phosphorylation in the inner mitochondrial membrane [157].

However, S1P is also released from cells through cell-specific transporters (e.g. ATP-binding cassette transporters and the spinster 2 transporter) and is an agonist of S1P-specific G protein-coupled receptors (a family of five, termed S1P₁-S1P₅). S1P₁ couples exclusively with G_i and inhibits adenylyl cyclase, while S1P₄ and S1P₅ couple to G_i and G_{12/13} [158,159]. S1P₂ and S1P₃ have a broader G protein coupling specificity and interact with G_i, G_q and G_{12/13} [158,160]. S1P receptors coupled to G_i/G_q activate phospholipase C (PLC) and promote the elevation of intracellular Ca²⁺ [161]. The activation of S1P₁ decreases intracellular cAMP levels and stimulates the Ras/ERK-1/2 and PI3K/AKT pathways to promote cell survival [158]. S1P binding to S1P₁ also induces PI3K/Rac pathway to stimulate cytoskeletal rearrangement and cell migration [162]. In contrast, signaling through G_{12/13} results in the activation of Rho to inhibit cell motility [163]. Therefore, S1P₁ and S1P₂ are functionally opposed in regulating cell migration [164,165].

Intracellular S1P is either irreversibly cleaved into phosphoethanolamine and hexadecenal by S1P lyase, which represents the only exit point of sphingolipid metabolism [166], or is dephosphorylated to sphingosine by S1P-specific phosphatases [167]. *Sgpl*^{-/-} mice exhibit increased levels of several sphingolipids (S1P, ceramide and sphingomyelin) and other lipid metabolites (cholesterol and phospholipid) in serum. Moreover, the absence of S1P lyase is associated with altered expression of key genes involved in lipid metabolism in the liver, such as *Sptlc1/2* and *PPARγ* [168]. Lipid phosphate phosphatases (LPPs) can also catalyse

dephosphorylation of S1P, thereby regulating the levels of this bioactive lipid in intracellular and extracellular compartments [169,170]. The overexpression of LPP1 also downregulates typical PKC isoform expression to suppress GPCR and receptor tyrosine kinase signaling [171].

S1P and insulin signaling in adipocytes

S1P levels in adipocytes are significantly elevated in obese patients and SphK1/S1P signaling in adipocytes is increased in obese and T2D rodent models [126,172]. The effect of S1P on cellular response is regulated by the ‘so-called’ sphingolipid-rheostat, which involves inter-conversion of ceramide, sphingosine and S1P. Ceramide generally induces cell apoptosis/senescence and S1P promotes proliferation/survival pathways [173,174]. Therefore, the balance of this rheostat might determine cell fate. This is exemplified by studies showing that the stimulation of SphK reduces ceramide levels to exert dramatic effects on cell survival, insulin signaling and gene expression [24]. Indeed, the overexpression of SphK1 in HFD-treated transgenic mice improves insulin sensitivity in muscle [175]. In addition, glucose-induced activation of SphK2 increases intracellular S1P enhances insulin secretion [176] and improves mitochondria homeostasis by modulating prohibitin expression in the pancreatic β cell [177]. S1P also promotes an anti-apoptotic effect in pancreatic β cells [178,179]. Two S1P receptor-like motifs in CerS2 are bound by S1P to inhibit its activity [180], which might prevent accumulation of ceramide within the sphingolipid rheostat, indicating that forward and backward reactions are likely reciprocally regulated. Furthermore, the over-expression of SphK1 enhances S1P formation in INS-1 β cells and prevents palmitate-induced apoptosis, accompanied by down-regulation of CerS4 to limit the synthesis of specific ceramide species *via* an S1P receptor-independent mechanism [181]. S1P also directly attenuates the pro-apoptotic effect of ceramide in pancreatic β cells [181,182] and affects glucose homeostasis by increasing the rate of glucose uptake in 3T3-L1 adipocytes [183]. The bioactive lipid might

also have a role in leptin-deficient and DIO rodent models, where organ-specific over-expression of adiponectin receptor in adipose and liver reduces C_{16:0} and C_{18:0} ceramides levels via an activation of ceramidase [184,185]. Increased ceramidase activity likely also contributes to a metabolically active state in adipose tissue [186] and removal of S1P by LPP3 is a key regulatory step in adipose function. Thus, deletion of LPP3 ameliorates insulin resistance without altering TAG synthesis or adipocyte differentiation in HFD-feeding mice [187]. Interestingly, the elimination of LPP3 leads to down-regulation of SPT and improves sphingolipid profiles with a reduction in ceramide and sphingomyelin and an increase in S1P levels in adipocytes. These findings reveal that the increase in the S1P/ceramide ratio in adipocytes might be potentially beneficial in maintaining or restoring insulin sensitivity.

S1P also activates the PI3K/AKT pathway in cells; this therefore, might represent a convergent point with insulin signaling [188,189]. This is exemplified by the use of FTY720 phosphate (an analogue of S1P), which is an agonist of all S1P receptor types with the exception of S1P₂. In the adipose tissue of DIO mice, FTY720 phosphate prevents the reduction in AKT-dependent signaling caused by a HFD [190]. However, it should be noted that crosstalk between S1P receptors and canonical insulin signaling pathways results in different outcomes dependent on the cell type. For instance, in C2C12 skeletal muscle myoblasts, S1P₂ activation induces the formation of reactive oxygen species which promotes the oxidation and enzymatic inhibition of protein-tyrosine phosphatase 1B (PTP1B), a major negative regulator of IR-mediated signaling [191]. S1P also stimulates IR phosphorylation and increases glucose uptake in myoblasts [191] and expression of SphK2 and increased S1P levels are induced by ER stress leading to AKT phosphorylation in mouse primary hepatocytes [192]. This results in improved lipid metabolism as a consequence of increased expression of genes involved in fatty acid

oxidation [192]. In contrast, an opposing role for S1P involves S1P₂, suppression of AKT phosphorylation and inhibition of glycogen synthesis in hepatocytes [193].

S1P and adipose tissue remodeling

S1P functions as an important lipid mediator in regulating adipose distribution and whole-body fat mass; achieved by modulating lipolysis/lipogenesis and the pre-adipocyte differentiation/proliferation state (Table 1). SphK1 and SphK2 are upregulated during the hormone-stimulated differentiation of 3T3-L1 pre-adipocytes whereas pharmacological inhibition or gene silencing of SphK1 reduces lipid storage and adipogenesis [201,202]. Both SphK1 and SphK2 appear to regulate adipogenesis, while SphK1 also regulates the differentiation of pre-adipocytes. Even though the expression of SphK1 and SphK2 are increased with differentiation, their total enzymatic activity declines in the late phase of terminal differentiation [202]. In addition, mRNA expression levels of S1P₁₋₃ are reduced during the late phase of adipogenesis and the addition of exogenous S1P cannot re-establish the adipogenic programme. These findings suggest that S1P receptors might not be sufficient to influence the pre-adipocyte differentiation state under physiological conditions [202].

Even though SphK1/S1P promotes pre-adipocyte differentiation in *in vitro* models, there are reports of restricted adipose tissue expansion upon activation of specific S1P receptors. In addition, S1P lyase deficiency reduces adiposity in mice fed on a normal diet [168]. Relevant to these issues is the finding that high concentrations of S1P can induce lipolysis through S1P receptor-linked cAMP-PKA signaling in cultured rat white adipocytes [194]. It is notable that cAMP formation in coronary artery smooth muscle cells is promoted by activation of S1P₂, which induces arachidonic acid release. In this case, S1P appears to promote an ERK-1/2–dependent phosphorylation and activation of PLA₂ which produces prostacyclin that can, in

turn, act on the PGI₂ receptor (which is coupled via G_s) to stimulate cAMP formation [203]. S1P also induces the down-regulation of adipocyte-specific transcriptional factors, such as PPAR γ , C/EBP α and adiponectin, which abrogates adipogenesis to suppress lipid deposition in 3T3-L1 pre-adipocytes [195]. This might be mediated by S1P₂ receptor and JNK signaling [195], based on the finding that the adenoviral-mediated overexpression of S1P₂ in 3T3-L1 pre-adipocytes inhibits the JNK pathway and induces down-regulation of PPAR γ expression [200]. S1P also increases the expression of S1P₂ during the differentiation of 3T3-L1 pre-adipocytes and this is associated with the inhibition of adipogenesis and lipid accumulation [198]. In this regard, antagonism of S1P₂, with JTE-013, abolishes the S1P-dependent downregulation of adipogenic genes [198] and increases the proliferation of 3T3-L1 pre-adipocytes through the ERK-1/2 pathway [194]. The inhibitory role of S1P₂ in regulating adipogenesis is further evident by the finding that epididymal adipocytes isolated from HFD-fed *S1pr2*^{-/-} mice proliferate and exhibit improved insulin sensitivity and glucose tolerance [199]. Furthermore, oral administration of the S1P₂ antagonist, JTE-013 to ob/ob mice increases insulin sensitivity, accompanied by reduced adipocyte hypertrophy [199].

Despite the evidence described above which identifies S1P₂ as a potential therapeutic target, there are examples where the anti-adipogenic effects of S1P involve other S1P receptor types. This is based on the finding that FTY720 phosphate, which does not bind to S1P₂, inhibits adipogenesis and promotes lipolysis in DIO mice, resulting in weight and fat mass loss [190]. Indeed, the major effect of FTY720 phosphate might be through internalization and persistent signaling of S1P₁, which is subsequently degraded [204,205]. This functional antagonistic effect of FTY720 phosphate on S1P₁ signaling leads to the sequestration of circulating T-lymphocytes in lymph nodes and suppresses autoreactive T lymphocyte recirculation in, for instance, multiple sclerosis [206,207]. However, it is notable that the administration of low

dose FTY720 (0.04 mg/kg twice per week), that does not induce lymphopenia, promotes AKT/GSK3 β and AMPK signaling and reduces adipogenesis in DIO mice. S1P also inhibits early phase differentiation of C3H10T1/2 multipotent stem cells into adipocytes [196]. This involves an S1P₁- and G_i-dependent decrease in cAMP levels, thereby inhibiting C/EBP β , PPAR γ and FABP4 expression [196]. The involvement of S1P₁ is also evident from studies showing that the S1P_{1/3} antagonist, VPC-23019 induces the differentiation of 3T3-F442A pre-adipocytes [199]. These findings are supported by the siRNA-mediated loss of S1P₁ which promotes differentiation of 3T3-F442A pre-adipocytes [199]. The pro-lipolytic effect of FTY720 phosphate involves increased transcriptional regulation of HSL, ATGL and perilipin allied with increased Ser563 phosphorylation of HSL. These findings have been confirmed by *in vitro* experiments in 3T3-L1 adipocytes [190]. In addition, FTY720 promotes insulin resistance and reduces glucose uptake in mature adipocytes [208–210]. Although FTY720 is an activator of PP2A, this is likely excluded as a mechanism of action, because PP2A catalyses the dephosphorylation of HSL and inhibits hormone-stimulated lipolysis in an obese rodent model [211]

In summary, S1P-mediated lipolysis in mature adipocytes reduces the lipid burden and limits adipose tissue expansion. In contrast, the inhibition of pre-adipocyte differentiation, mediated by S1P₁ or S1P₂, reduces adaptive expansion of adipose tissue in response to positive caloric balance. Of note, high concentrations of S1P might activate cAMP/PKA via PGI₂ to promote lipolysis and lipid release, whereas the inhibition of adipogenesis might involve S1P_{1/2}. These responses could potentially account for adipose dysfunction and peripheral insulin resistance. However, the biological functions of each S1P receptor subtype needs to be validated *in vivo* as cell models do not recapitulate the micro-environmental interaction of adipose tissue with the stromal vascular fraction under pathological conditions. Nevertheless, we propose that the

pro-inflammatory and hypertrophic phenotype of adipocytes under excess NEFA influx renders S1P more likely to inhibit adipogenesis and increase lipolysis.

S1P and adipose inflammation

SphK1/S1P regulate cytokines secretion and promote inflammatory responses in *in vitro* and *in vivo* models and these responses might also contribute to impaired adipogenesis leading to insulin resistance. For instance, S1P promotes increased expression of pro-thrombotic proteins (*e.g.* PAI-1) and pro-inflammatory cytokines (*e.g.* TNF, IL-6, MCP-1 and keratinocyte-derived chemokine) in 3T3-L1 adipocytes [95] (Table 2). Importantly, LPS also stimulates the up-regulation of SphK1 in primary rat adipocytes and SphK1 activity this is positively associated with chemokine (C-C motif) ligand 5 (CCL5) levels in subcutaneous white adipose tissue in rats [212].

The activation of β_3 adrenoceptor (ADRB3)/HSL signaling also upregulates SphK1 via JNK- and activator protein-1 (AP-1)-dependent pathways to promote IL-6 secretion in white adipose tissue [213]. These findings suggest the involvement of S1P in regulating lipolysis-dependent IL-6 synthesis, release and local inflammation [213]. Consistent with this, the expression of anti-inflammatory mediators, such as IL-10 and adiponectin are elevated in epididymal adipose tissue of *Sphk1*^{-/-} mice, which exhibit HFD-induced obesity [172] and decreased expression of pro-inflammatory mediators, such as TNF, IL-6, and MCP-1 [172]. In addition, deletion of *Sphk1* in adipose tissue decreases expression of SOCS3 [172] leading to improved insulin sensitivity and glucose tolerance that is partly attributed to impairment of the inflammatory response [172]. Similarly, the use of SphK1/2 inhibitor, SPHK I (also known as SKI-II, 2-(*p*-Hydroxyanilino)-4-(*p*-chlorophenyl) thiazole) on lean Zucker rats prevents LPS-induced up-regulation of CCL5, IL-6, pentraxin 3 (Ptx3) and TNF α in subcutaneous white adipose tissue

[212]. The up-regulation of cytokine production can also be abolished by silencing SphK1 in 3T3-L1 cells [212]. These findings suggest an interesting parallel with colitis-associated cancer cell biology, where the up-regulation of SphK1 expression increases the production of S1P, which persistently activates the canonical NF κ B activation pathway, leading to enhanced IL-6 formation and STAT3 activation [214]. Therefore, a SphK1/S1P₁/STAT3 axis may amplify the inflammatory response and promote insulin resistance in adipose tissue through a regulatory loop.

S1P also regulates immune cell trafficking and re-circulation [215,216]. The administration of FTY720 (acting as FTY720 phosphate after its phosphorylation by SphK2) down-regulates S1P₁ receptor expression in C57BL/6 DIO mice and this prevents the recruitment of circulating monocytes into adipose tissue, thereby reducing the presence of pro-inflammatory M1-macrophages [204]. FTY720 also decreases adipose tissue lymphocytes and increases the presence of CD11c-negative, anti-inflammatory macrophages, which is associated with increased insulin sensitivity [204]. S1P might also contribute to M1 macrophage-mediated inflammatory responses in the adipose tissue microenvironment. This is supported by histological analysis of epididymal fat tissues isolated from HFD-fed *S1pr2*^{-/-} mice, which exhibit a low level of M1 macrophage infiltration. However, wide-type littermates on HFD exhibit many crown-like structures, which are formed by M1 macrophages that surround dying or dead adipocytes [199].

SphK1 is also up-regulated in M1 macrophages from DIO and ob/ob rodent models and palmitate-induced lipotoxicity in mouse macrophage-like RAW264.7 cells is associated with increased SphK1 activity and enhanced survival of a macrophage population with a pro-inflammatory phenotype [217]. These findings demonstrate that S1P derived from adipose not

only acts as a chemoattractant for macrophages and monocytes but also facilitates the polarization of adipose-derived anti-inflammatory M2 macrophages into a pro-inflammatory M1 phenotype.

Taken together, modulation of the sphingolipid rheostat in adipose tissue might suggest that SphK1/2 can remove harmful ceramide. However, the S1P formed has the potential to promote inflammation and to inhibit adipogenesis, thereby representing a key mechanism for adipose dysfunction in T2D. It is possible that increased SphK activity, accompanied by S1P signaling, initially reduces accumulation of TAG and ceramide in adipose tissue, thereby reducing the fat burden on adipocytes. However, the inhibition of adipogenesis via S1P receptor-dependent mechanism(s) appears to direct adipocytes into a dangerous hypertrophic phenotype. The S1P/SphK1 pathway also initiates an inflammation cascade by promoting the secretion of pro-inflammatory cytokines, which ablate adipogenesis [20]. Notably, up-regulation of pro-inflammatory genes and the participation of immune cells is enhanced during the transition from acute to chronic inflammation in adipose tissue [218]. As macrophage-mediated adipose inflammation is a key component in HFD-induced insulin resistance, M1 macrophage recruitment and polarization in response to S1P might have a very significant role in inducing adipose malfunction [57,218].

Conclusion

It has been proposed that depleting intracellular ceramide levels might represent an attractive therapeutic strategy for treatment of T2D and obesity [219]. Considering the involvement of glucosylceramide and sphingomyelin in the acquisition of glucose intolerance and insulin resistance [21], S1P appears to be a less harmful product of ceramide metabolism in skeletal muscle, liver and pancreatic β cells (Figure 5). However, S1P/S1P₂ signaling impairs insulin

action, glycogen synthesis and hepatic cell regeneration in the liver. This suggests that therapeutic antagonism of the S1P₂ receptor might be a more attractive therapeutic strategy [193,220]. S1P is also pro-inflammatory and has a role in IL-6-induced insulin resistance [221]. The activation of S1P receptors, especially S1P₁ and S1P₂, impairs adipogenesis and promotes the secretion of cytokines that indirectly block adipocyte differentiation and insulin signaling. S1P also has a role in macrophage recruitment and polarization that promotes adipose dysfunction during chronic inflammation.

It must be kept in mind that the interactions between adipose tissue and the circulatory system are important in whole-body homeostatic control of metabolism. On the one hand, the lipid spill over from adipose tissue may contribute to the high circulating ceramide levels in obese individuals. In addition, NEFA from de-regulated adipose tissue can be utilized for sphingolipid biosynthesis in metabolically active tissues, which results in ceramide formation in the liver and skeletal muscle [218]. In addition, the adipose tissue itself is a potential source of circulating ceramides [115] and S1P derived from adipose tissue also promotes systemic inflammation in obesity. The majority of plasma S1P is associated with apoM rich high-density lipoproteins (HDL) with small quantities binding to low-density lipoproteins (LDL) or very-low-density lipoproteins (VLDL) [222,223]. ApoM deficiency in mice results in defective S1P₁ and S1P₃ signaling in the endothelium and brown adipose tissue respectively and this leads to enhanced TAG metabolism and brown adipose development [224]. These findings suggest that the binding of circulating HDL-S1P to S1P receptors in brown adipose tissue might be responsible for restricting metabolic activity [224,225]. As there are high levels of circulating S1P and ceramide levels in obese and diabetic individuals, the pathological actions of blood-borne bioactive lipids on adipose metabolism and inflammation should not be neglected [62,64].

The pharmacological intervention strategies targeting adipose function are limited and it is possible that tissue specific effects might be required. Since ceramide has a significant role in metabolic syndrome and cardiovascular diseases [226], it is reasonable to speculate that targeting adipose ceramide in T2D and obesity is a worthwhile therapeutic approach. The inhibition/loss of key enzymes (e.g. Dggs1 inhibitors) that directly contribute to ceramide synthesis might effectively attenuate negative effects on insulin sensitivity. In addition, the upregulation of the sphingomyelinase pathway in dysfunctional adipose tissue provides a rationale for the use of sphingomyelinase inhibitors. Functional inhibitors of acid sphingomyelinase (FIASMA), a large group of pharmacological compounds with diverse chemical structures, can insert into the inner membrane of lysosomes, thereby removing the membrane-bound aSMase, which is then degraded within lysosomes [227,228]. However, pharmacological manipulation of sphingolipid metabolism by converting ceramide into sphingosine and, subsequently, S1P does not seem to hold promise. A greater understanding of the precise actions of each S1P receptor subtype in adipose tissue is required to validate their full therapeutic potential and provide impetus for novel treatment strategies in T2D. Nevertheless, we conclude that the blockade of the S1P receptor signaling system, such as S1P₁ and S1P₂, might provide therapeutic utility by restoring adipogenesis and inhibiting inflammation to re-establish normal adipose tissue function and insulin sensitivity in T2D

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References

- [1] Guilherme A, Virbasius J V., Puri V, Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nat Rev Mol Cell Biol* 2008;9:367–77. doi:10.1038/nrm2391.
- [2] Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* 2006;444:840–6. doi:10.1038/nature05482.
- [3] Guariguata L, Whiting DR, Hambleton I, Beagley J. Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Res Clin Pract* 2014;103:137–49. doi:10.1016/j.diabres.2013.11.002.
- [4] Vos T, Allen C, Arora M, Barber RM, Bhutta ZA, Brown A, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 2016;388:1545–602. doi:10.1016/S0140-6736(16)31678-6.
- [5] Ye J. Mechanisms of insulin resistance in obesity. *Front Med* 2013;7:14–24. doi:10.1007/s11684-013-0262-6.
- [6] Hartemink N, Boshuizen HC, Nagelkerke NJD, Jacobs MAM, Van Houwelingen HC. Combining risk estimates from observational studies with different exposure cutpoints: A meta-analysis on body mass index and diabetes type 2. *Am J Epidemiol* 2006;163:1042–52. doi:10.1093/aje/kwj141.
- [7] Abdullah A, Peeters A, de Courten M, Stoelwinder J. The magnitude of association between overweight and obesity and the risk of diabetes: A meta-analysis of prospective cohort studies. *Diabetes Res Clin Pract* 2010;89:309–19. doi:10.1016/j.diabres.2010.04.012.

- [8] Eckel RH, Kahn SE, Ferrannini E, Goldfine AB, Nathan DM, Schwartz MW, et al. Obesity and type 2 diabetes: What Can be unified and what needs to be individualized? *Diabetes Care* 2011;34:1424–30. doi:10.2337/dc11-0447.
- [9] Blüher M. The distinction of metabolically “healthy” from “unhealthy” obese individuals. *Curr Opin Lipidol* 2010;21:38–43. doi:10.1097/MOL.0b013e3283346ccc.
- [10] Després J-P, Lemieux I. Abdominal obesity and metabolic syndrome. *Nature* 2006;444:881–7. doi:10.1038/nature05488.
- [11] McLaughlin T, Lamendola C, Liu A, Abbasi F. Preferential fat deposition in subcutaneous versus visceral depots is associated with insulin sensitivity. *J Clin Endocrinol Metab* 2011;96:1756–60. doi:10.1210/jc.2011-0615.
- [12] Preis SR, Massaro JM, Robins SJ, Hoffmann U, Vasani RS, Irlbeck T, et al. Abdominal subcutaneous and visceral adipose tissue and insulin resistance in the framingham heart study. *Obesity* 2010;18:2191–8. doi:10.1038/oby.2010.59.
- [13] Neeland IJ, Turer AT, Ayers CR, Powell-Wiley TM, Vega GL, Farzaneh-Far R, et al. Dysfunctional Adiposity and the Risk of Prediabetes and Type 2 Diabetes in Obese Adults. *JAMA* 2012;308:1150. doi:10.1001/2012.jama.11132.
- [14] Kashyap S, Belfort R, Gastaldelli A, Pratipanawatr T, Berria R, Pratipanawatr W, et al. A sustained increase in plasma free fatty acids impairs insulin secretion in nondiabetic subjects genetically predisposed to develop type 2 diabetes. *Diabetes* 2003;52:2461–74. doi:10.2337/diabetes.52.10.2461.
- [15] Kashyap SR, Belfort R, Berria R, Suraamornkul S, Pratipranawatr T, Finlayson J, et al. Discordant effects of a chronic physiological increase in plasma FFA on insulin signaling in healthy subjects with or without a family history of type 2 diabetes. *Am J Physiol Metab* 2004;287:E537–46. doi:10.1152/ajpendo.00541.2003.

- [16] Kusminski CM, Shetty S, Orci L, Unger RH, Scherer PE. Diabetes and apoptosis: Lipotoxicity. *Apoptosis* 2009;14:1484–95. doi:10.1007/s10495-009-0352-8.
- [17] Samuel VT and SGI. Integrating Mechanisms for Insulin Resistance: Common Threads and Missing Links. *Cell* 2013;148:852–71. doi:10.1016/j.cell.2012.02.017.Integrating.
- [18] Boden G. Obesity, insulin resistance and free fatty acids. *Curr Opin Endocrinol Diabetes Obes* 2011;18:139–43. doi:10.1097/MED.0b013e3283444b09.45Obesity.
- [19] Sears B, Perry M. The role of fatty acids in insulin resistance. *Lipids Health Dis* 2015;14:1–9. doi:10.1186/s12944-015-0123-1.
- [20] Maury E, Brichard SM. Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. *Mol Cell Endocrinol* 2010;314:1–16. doi:10.1016/j.mce.2009.07.031.
- [21] Meikle PJ, Summers SA. Sphingolipids and phospholipids in insulin resistance and related metabolic disorders. *Nat Rev Endocrinol* 2017;13:79–91. doi:10.1038/nrendo.2016.169.
- [22] Iqbal J, Walsh MT, Hammad SM, Hussain MM. Sphingolipids and Lipoproteins in Health and Metabolic Disorders. *Trends Endocrinol Metab* 2017;28:506–18. doi:10.1016/j.tem.2017.03.005.
- [23] Bikman BT, Summers SA. Ceramides as modulators of cellular and whole-body metabolism. *J Clin Invest* 2011;121:4222–30. doi:10.1172/JCI57144.
- [24] Fayyaz S, Japtok L, Kleuser B. Divergent role of sphingosine 1-phosphate on insulin resistance. *Cell Physiol Biochem* 2014;34:134–47. doi:10.1159/000362990.
- [25] Pyne S, Adams DR, Pyne NJ. Sphingosine 1-phosphate and sphingosine kinases in health and disease: Recent advances. *Prog Lipid Res* 2016;62:93–106. doi:10.1016/j.plipres.2016.03.001.

- [26] Rosen ED, MacDougald OA. Adipocyte differentiation from the inside out. *Nat Rev Mol Cell Biol* 2006;7:885–96. doi:10.1038/nrm2066.
- [27] Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, et al. Dynamics of fat cell turnover in humans. *Nature* 2008;453:783–7. doi:10.1038/nature06902.
- [28] Cristancho AG, Lazar MA. Forming functional fat: A growing understanding of adipocyte differentiation. *Nat Rev Mol Cell Biol* 2011;12:722–34. doi:10.1038/nrm3198.
- [29] Harms M, Seale P. Brown and beige fat: Development, function and therapeutic potential. *Nat Med* 2013;19:1252–63. doi:10.1038/nm.3361.
- [30] Rodeheffer MS, Birsoy K, Friedman JM. Identification of White Adipocyte Progenitor Cells In Vivo. *Cell* 2008;135:240–9. doi:10.1016/j.cell.2008.09.036.
- [31] Wernstedt Asterholm I, Tao C, Morley TS, Wang QA, Delgado-Lopez F, Wang Z V., et al. Adipocyte inflammation is essential for healthy adipose tissue expansion and remodeling. *Cell Metab* 2014;20:103–18. doi:10.1016/j.cmet.2014.05.005.
- [32] Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J Clin Invest* 2007;117:175–84. doi:10.1172/JCI29881.
- [33] Nielsen TS, Jessen N, Jorgensen JOL, Moller N, Lund S. Dissecting adipose tissue lipolysis: molecular regulation and implications for metabolic disease. *J Mol Endocrinol* 2014;52:R199–222. doi:10.1530/JME-13-0277.
- [34] Miyoshi H, Souza SC, Zhang HH, Strissel KJ, Christoffolete MA, Kovsan J, et al. Perilipin promotes hormone-sensitive lipase-mediated adipocyte lipolysis via phosphorylation-dependent and -independent mechanisms. *J Biol Chem* 2006;281:15837–44. doi:10.1074/jbc.M601097200.

- [35] Duncan RE, Ahmadian M, Jaworski K, Sarkadi-Nagy E, Sul HS. Regulation of Lipolysis in Adipocytes. *Annu Rev Nutr* 2007;27:79–101. doi:10.1146/annurev.nutr.27.061406.093734.
- [36] Granneman JG, Moore HPH. Location, location: protein trafficking and lipolysis in adipocytes. *Trends Endocrinol Metab* 2008;19:3–9. doi:10.1016/j.tem.2007.10.006.
- [37] Paar M, Jüngst C, Steiner NA, Magnes C, Sinner F, Kolb D, et al. Remodeling of Lipid Droplets during Lipolysis and Growth in Adipocytes. *J Biol Chem* 2012;287:11164–73. doi:10.1074/jbc.M111.316794.
- [38] Jeffery E, Church CD, Holtrup B, Colman L, Rodeheffer MS. Rapid depot-specific activation of adipocyte precursor cells at the onset of obesity. *Nat Cell Biol* 2015;17:376–85. doi:10.1038/ncb3122.
- [39] Wang QA, Tao C, Gupta RK, Scherer PE. Tracking adipogenesis during white adipose tissue development , expansion and regeneration. *Nat Med* 2013:1–8. doi:10.1038/nm.3324.
- [40] Ahmadian M, Suh JM, Hah N, Liddle C, Atkins AR, Downes M, et al. PPAR γ signaling and metabolism: the good, the bad and the future. *Nat Med* 2013;99:557–66. doi:10.1038/nm.3159.
- [41] Sohn EJ, Jung D-B, Lee J, Yoon SW, Won G-H, Ko HS, et al. CCR4-NOT2 Promotes the Differentiation and Lipogenesis of 3T3-L1 Adipocytes via Upregulation of PPAR γ , CEBP α and Inhibition of P-GSK3 α/β and β -Catenin. *Cell Physiol Biochem* 2015;37:1881–9. doi:10.1159/000438549.
- [42] Cernkovich ER, Deng J, Bond MC, Combs TP, Harp JB. Adipose-specific disruption of signal transducer and activator of transcription 3 increases body weight and adiposity. *Endocrinology* 2008;149:1581–90. doi:10.1210/en.2007-1148.

- [43] Tchoukalova YD, Koutsari C, Votruba SB, Tchkonina T, Giorgadze N, Thomou T, et al. Sex- and Depot-Dependent Differences in Adipogenesis in Normal-Weight Humans. *Obesity* 2009;18:1875–80. doi:10.1038/oby.2010.56.
- [44] Macotela Y, Emanuelli B, Mori MA, Gesta S, Schulz TJ, Tseng Y-H, et al. Intrinsic Differences in Adipocyte Precursor Cells From Different White Fat Depots. *Diabetes* 2012;61:1691–9. doi:10.2337/db11-1753.
- [45] Farnier C, Krief S, Blache M, Diot-Dupuy F, Mory G, Ferre P, et al. Adipocyte functions are modulated by cell size change: Potential involvement of an integrin/ERK signalling pathway. *Int J Obes* 2003;27:1178–86. doi:10.1038/sj.ijo.0802399.
- [46] Laurencikiene J, Skurk T, Kulyté A, Hedén P, Åström G, Sjölin E, et al. Regulation of lipolysis in small and large fat cells of the same subject. *J Clin Endocrinol Metab* 2011;96:2045–9. doi:10.1210/jc.2011-1702.
- [47] Jernås M, Palming J, Sjöholm K, Jennische E, Svensson PA, Gabrielsson BG, et al. Separation of human adipocytes by size: hypertrophic fat cells display distinct gene expression. *FASEB J* 2006;20:1540–2. doi:10.1096/fj.05-5678fje.
- [48] Franck N, Stenkula KG, Öst A, Lindström T, Strålfors P, Nystrom FH. Insulin-induced GLUT4 translocation to the plasma membrane is blunted in large compared with small primary fat cells isolated from the same individual. *Diabetologia* 2007;50:1716–22. doi:10.1007/s00125-007-0713-1.
- [49] Maury E, Noël L, Detry R, Brichard SM. In Vitro hyperresponsiveness to tumor necrosis factor- α contributes to adipokine dysregulation in omental adipocytes of obese subjects. *J Clin Endocrinol Metab* 2009;94:1393–400. doi:10.1210/jc.2008-2196.
- [50] Hube F, Birgel M, Lee YM, Hauner H. Expression pattern of tumour necrosis factor receptors in subcutaneous and omental human adipose tissue: Role of obesity and non-

- insulin-dependent diabetes mellitus. *Eur J Clin Invest* 1999;29:672–8.
doi:10.1046/j.1365-2362.1999.00520.x.
- [51] Sartipy P, Loskutoff DJ. Monocyte chemoattractant protein 1 in obesity and insulin resistance. *Proc Natl Acad Sci* 2003;100:7265–70. doi:10.1073/pnas.1133870100.
- [52] Klötting N, Fasshauer M, Dietrich A, Kovacs P, Schön MR, Kern M, et al. Insulin-sensitive obesity. *Am J Physiol - Endocrinol Metab* 2010;299:E506-515.
doi:10.1152/ajpendo.00586.2009.
- [53] Gustafson B, Hedjazifar S, Gogg S, Hammarstedt A, Smith U. Insulin resistance and impaired adipogenesis. *Trends Endocrinol Metab* 2015:1–8.
doi:10.1016/j.tem.2015.01.006.
- [54] Laforest S, Labrecque J, Michaud A, Cianflone K, Tchernof A. Adipocyte size as a determinant of metabolic disease and adipose tissue dysfunction. *Crit Rev Clin Lab Sci* 2015;52:301–13. doi:10.3109/10408363.2015.1041582.
- [55] Lessard J, Laforest S, Pelletier M, Leboeuf M, Blackburn L, Tchernof A. Low abdominal subcutaneous preadipocyte adipogenesis is associated with visceral obesity, visceral adipocyte hypertrophy, and a dysmetabolic state. *Adipocyte* 2014;3:197–205.
doi:10.4161/adip.29385.
- [56] Hoffstedt J, Arner E, Wahrenberg H, Andersson DP, Qvisth V, Löfgren P, et al. Regional impact of adipose tissue morphology on the metabolic profile in morbid obesity. *Diabetologia* 2010;53:2496–503. doi:10.1007/s00125-010-1889-3.
- [57] McNelis JC, Olefsky JM. Macrophages, Immunity, and Metabolic Disease. *Immunity* 2014;41:36–48. doi:10.1016/j.immuni.2014.05.010.
- [58] Amano SU, Cohen JL, Vangala P, Tencerova M, Nicoloso SM, Yawe JC, et al. Local proliferation of macrophages contributes to obesity-associated adipose tissue inflammation. *Cell Metab* 2014;19:162–71. doi:10.1016/j.cmet.2013.11.017.

- [59] Yang H, Youm Y-H, Vandanmagsar B, Ravussin A, Gimble JM, Greenway F, et al. Obesity Increases the Production of Proinflammatory Mediators from Adipose Tissue T Cells and Compromises TCR Repertoire Diversity: Implications for Systemic Inflammation and Insulin Resistance. *J Immunol* 2010;185:1836–45. doi:10.4049/jimmunol.1000021.
- [60] O'Rourke RW, Metcalf MD, White AE, Madala A, Winters BR, Maizlin II, et al. Depot-specific differences in inflammatory mediators and a role for NK cells and IFN- γ in inflammation in human adipose tissue. *Int J Obes* 2009;33:978–90. doi:10.1038/ijo.2009.133.
- [61] Erion DM, Shulman GI. Diacylglycerol-mediated insulin resistance. *Nat Med* 2010;16:400–2. doi:10.1038/nm0410-400.
- [62] Haus JM, Kashyap SR, Kasumov T, Zhang R, Kelly KR, Defronzo RA, et al. Plasma ceramides are elevated in obese subjects with type 2 diabetes and correlate with the severity of insulin resistance. *Diabetes* 2009;58:337–43. doi:10.2337/db08-1228.
- [63] Kasumov T, Solomon TPJ, Hwang C, Huang H, Haus JM, Zhang R, et al. Improved insulin sensitivity after exercise training is linked to reduced plasma C14:0 ceramide in obesity and type 2 diabetes. *Obesity* 2015;23:1414–21. doi:10.1002/oby.21117.
- [64] Kowalski GM, Carey AL, Selathurai A, Kingwell BA, Bruce CR. Plasma Sphingosine-1-Phosphate Is Elevated in Obesity. *PLoS One* 2013;8:1–7. doi:10.1371/journal.pone.0072449.
- [65] Ito S, Iwaki S, Koike K, Yuda Y, Nagasaki A, Ohkawa R, et al. Increased plasma sphingosine-1-phosphate in obese individuals and its capacity to increase the expression of plasminogen activator inhibitor-1 in adipocytes. *Coron Artery Dis* 2013;24:1. doi:10.1097/MCA.0000000000000033.

- [66] Tanaka S, Kanazawa I, Sugimoto T. Visceral fat accumulation is associated with increased plasma sphingosine-1-phosphate levels in type 2 diabetes mellitus. *Diabetes Res Clin Pract* 2018;143:146–50. doi:10.1016/j.diabres.2018.07.003.
- [67] Haeusler RA, McGraw TE, Accili D. Biochemical and cellular properties of insulin receptor signalling. *Nat Rev Mol Cell Biol* 2017. doi:10.1038/nrm.2017.89.
- [68] Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: Insights into insulin action. *Nat Rev Mol Cell Biol* 2006;7:85–96. doi:10.1038/nrm1837.
- [69] Dimitriadis G, Mitrou P, Lambadiari V, Maratou E, Raptis SA. Insulin effects in muscle and adipose tissue. *Diabetes Res Clin Pract* 2011;93:S52–9. doi:10.1016/S0168-8227(11)70014-6.
- [70] Bolsoni-Lopes A, Alonso-Vale MIC. Lipolysis and lipases in white adipose tissue – An update. *Arch Endocrinol Metab* 2015;59:335–42. doi:10.1590/2359-3997000000067.
- [71] Degerman E, Ahmad F, Chung YW, Guirguis E, Omar B, Stenson L, et al. From PDE3B to the regulation of energy homeostasis. *Curr Opin Pharmacol* 2011;11:676–82. doi:10.1016/j.coph.2011.09.015.
- [72] Chakrabarti P, Kandror K V. FoxO1 controls insulin-dependent adipose triglyceride lipase (ATGL) expression and lipolysis in adipocytes. *J Biol Chem* 2009;284:13296–300. doi:10.1074/jbc.C800241200.
- [73] Zhang HH, Huang J, Düvel K, Boback B, Wu S, Squillance RM, et al. Insulin stimulates adipogenesis through the Akt-TSC2-mTORC1 pathway. *PLoS One* 2009;4. doi:10.1371/journal.pone.0006189.
- [74] Wang W, Huang L, Huang Y, Yin J wen, Berk AJ, Friedman JM, et al. Mediator MED23 Links Insulin Signaling to the Adipogenesis Transcription Cascade. *Dev Cell* 2009;16:764–71. doi:10.1016/j.devcel.2009.04.006.

- [75] Morley TS, Xia JY, Scherer PE. Selective enhancement of insulin sensitivity in the mature adipocyte is sufficient for systemic metabolic improvements. *Nat Commun* 2015;6:7906. doi:10.1038/ncomms8906.
- [76] Osborn O, Olefsky JM. The cellular and signaling networks linking the immune system and metabolism in disease. *Nat Med* 2012;18:363–74. doi:10.1038/nm.2627.
- [77] Jorgensen SB, O’Neill HM, Sylow L, Honeyman J, Hewitt KA, Palanivel R, et al. Deletion of skeletal muscle SOCS3 prevents insulin resistance in obesity. *Diabetes* 2013;62:56–64. doi:10.2337/db12-0443.
- [78] Shi H, Cave B, Inouye K, Bjørbaek C, Flier JS. Overexpression of suppressor of cytokine signaling 3 in adipose tissue causes local but not systemic insulin resistance. *Diabetes* 2006;55:699–707.
- [79] McGillicuddy FC, Chiquoine EH, Hinkle CC, Kim RJ, Shah R, Roche HM, et al. Interferon γ Attenuates Insulin Signaling, Lipid Storage, and Differentiation in Human Adipocytes via Activation of the JAK/STAT Pathway. *J Biol Chem* 2009;284:31936–44. doi:10.1074/jbc.M109.061655.
- [80] Nandipati KC, Subramanian S, Agrawal DK. Protein kinases: mechanisms and downstream targets in inflammation-mediated obesity and insulin resistance. *Mol Cell Biochem* 2017;426:27–45. doi:10.1007/s11010-016-2878-8.
- [81] Tanti JF, Ceppo F, Jager J, Berthou F. Implication of inflammatory signaling pathways in obesity-induced insulin resistance. *Front Endocrinol (Lausanne)* 2013;3:1–15. doi:10.3389/fendo.2012.00181.
- [82] Lin Y, Berg AH, Iyengar P, Lam TKT, Giacca A, Combs TP, et al. The Hyperglycemia-induced Inflammatory Response in Adipocytes. *J Biol Chem* 2005;280:4617–26. doi:10.1074/jbc.M411863200.

- [83] Guo W, Wong S, Xie W, Lei T, Luo Z. Palmitate modulates intracellular signaling, induces endoplasmic reticulum stress, and causes apoptosis in mouse 3T3-L1 and rat primary preadipocytes. *Am J Physiol Metab* 2007;293:E576–86. doi:10.1152/ajpendo.00523.2006.
- [84] Pagliassotti MJ, Kim PY, Estrada AL, Stewart CM, Gentile CL. Endoplasmic reticulum stress in obesity and obesity-related disorders: An expanded view. *Metabolism* 2016;65:1238–46. doi:10.1016/j.metabol.2016.05.002.
- [85] Jiao P, Ma J, Feng B, Zhang H, Alan Diehl J, Eugene Chin Y, et al. FFA-induced adipocyte inflammation and insulin resistance: Involvement of ER stress and IKK β pathways. *Obesity* 2011;19:483–91. doi:10.1038/oby.2010.200.
- [86] Aburasayn H, Al Batran R, Ussher JR. Targeting ceramide metabolism in obesity. *Am J Physiol - Endocrinol Metab* 2016;311:E423–35. doi:10.1152/ajpendo.00133.2016.
- [87] Long SD, Pekala PH. Lipid mediators of insulin resistance: ceramide signalling down-regulates GLUT4 gene transcription in 3T3-L1 adipocytes. *Biochem J* 1996;319 (Pt 1:179–84.
- [88] Chaurasia B, Summers SA. Ceramides - Lipotoxic Inducers of Metabolic Disorders. *Trends Endocrinol Metab* 2015;26:538–50. doi:10.1016/j.tem.2015.07.006.
- [89] Chavez JA, Summers SA. Characterizing the effects of saturated fatty acids on insulin signaling and ceramide and diacylglycerol accumulation in 3T3-L1 adipocytes and C2C12 myotubes. *Arch Biochem Biophys* 2003;419:101–9. doi:10.1016/j.abb.2003.08.020.
- [90] Blouin CM, Prado C, Takane KK, Lasnier F, Garcia-Ocana A, Ferre P, et al. Plasma Membrane Subdomain Compartmentalization Contributes to Distinct Mechanisms of Ceramide Action on Insulin Signaling. *Diabetes* 2010;59:600–10. doi:10.2337/db09-0897.

- [91] POWELL DJ, TURBAN S, GRAY A, HAJDUCH E, HUNDAL HS. Intracellular ceramide synthesis and protein kinase C ζ activation play an essential role in palmitate-induced insulin resistance in rat L6 skeletal muscle cells. *Biochem J* 2004;382:619–29. doi:10.1042/BJ20040139.
- [92] Powell DJ, Hajduch E, Kular G, Hundal HS. Ceramide Disables 3-Phosphoinositide Binding to the Pleckstrin Homology Domain of Protein Kinase B (PKB)/Akt by a PKC ζ -Dependent Mechanism. *Mol Cell Biol* 2003;23:7794–808. doi:10.1128/MCB.23.21.7794.
- [93] Hajduch E, Turban S, Le Liepvre X, Le Lay S, Lipina C, Dimopoulos N, et al. Targeting of PKC ζ and PKB to caveolin-enriched microdomains represents a crucial step underpinning the disruption in PKB-directed signalling by ceramide. *Biochem J* 2008;410:369–79. doi:10.1042/BJ20070936.
- [94] Vandanmagsar B, Youm YH, Ravussin A, Galgani JE, Stadler K, Mynatt RL, et al. The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. *Nat Med* 2011;17:179–89. doi:10.1038/nm.2279.
- [95] Samad F, Hester KD, Yang G, Hannun YA, Bielawski J. Altered adipose and plasma sphingolipid metabolism in obesity: A potential mechanism for cardiovascular and metabolic risk. *Diabetes* 2006;55:2579–87. doi:10.2337/db06-0330.
- [96] Yang G, Badeanlou L, Bielawski J, Roberts AJ, Hannun YA, Samad F. Central role of ceramide biosynthesis in body weight regulation, energy metabolism, and the metabolic syndrome. *Am J Physiol Metab* 2009;297:E211–24. doi:10.1152/ajpendo.91014.2008.
- [97] Holland WL, Summers SA. Sphingolipids, insulin resistance, and metabolic disease: New insights from in vivo manipulation of sphingolipid metabolism. *Endocr Rev* 2008;29:381–402. doi:10.1210/er.2007-0025.

- [98] Hanada K. Serine palmitoyltransferase, a key enzyme of sphingolipid metabolism. *Biochim Biophys Acta - Mol Cell Biol Lipids* 2003;1632:16–30. doi:10.1016/S1388-1981(03)00059-3.
- [99] Alexaki A, Clarke BA, Gavrilova O, Ma Y, Zhu H, Ma X, et al. De Novo Sphingolipid Biosynthesis Is Required for Adipocyte Survival and Metabolic Homeostasis. *J Biol Chem* 2017;292:3929–39. doi:10.1074/jbc.M116.756460.
- [100] Tidhar R, Zelnik ID, Volpert G, Ben-Dor S, Kelly S, Merrill AH, et al. Eleven residues determine the acyl chain specificity of ceramide synthases. *J Biol Chem* 2018;293:9912–21. doi:10.1074/jbc.RA118.001936.
- [101] Aguilera-Romero A, Gehin C, Riezman H. Sphingolipid homeostasis in the web of metabolic routes. *Biochim Biophys Acta - Mol Cell Biol Lipids* 2014;1841:647–56. doi:10.1016/j.bbalip.2013.10.014.
- [102] Mullen TD, Hannun YA, Obeid LM. Ceramide synthases at the centre of sphingolipid metabolism and biology. *Biochem J* 2012;441:789–802. doi:10.1042/BJ20111626.
- [103] Levy M, Futerman AH. Mammalian ceramide synthases. *IUBMB Life* 2010;62:NA-NA. doi:10.1002/iub.319.
- [104] Gosejacob D, Jäger PS, vom Dorp K, Frejno M, Carstensen AC, Köhnke M, et al. Ceramide Synthase 5 Is Essential to Maintain C 16:0 -Ceramide Pools and Contributes to the Development of Diet-induced Obesity. *J Biol Chem* 2016;291:6989–7003. doi:10.1074/jbc.M115.691212.
- [105] Hla T, Kolesnick R. C16 : 0-Ceramide Signals Insulin Resistance. *Cell Metab* 2014;20:703–5. doi:10.1016/j.cmet.2014.10.017.
- [106] Turpin SM, Nicholls HT, Willmes DM, Mourier A, Brodesser S, Wunderlich CM, et al. Obesity-Induced CerS6-Dependent C16:0 Ceramide Production Promotes Weight

- Gain and Glucose Intolerance. *Cell Metab* 2014;20:678–86.
doi:10.1016/j.cmet.2014.08.002.
- [107] Bergman BC, Brozinick JT, Strauss A, Bacon S, Kerege A, Bui HH, et al. Muscle sphingolipids during rest and exercise: a C18:0 signature for insulin resistance in humans. *Diabetologia* 2016;59:785–98. doi:10.1007/s00125-015-3850-y.
- [108] Raichur S, Wang ST, Chan PW, Li Y, Ching J, Chaurasia B, et al. CerS2 Haploinsufficiency Inhibits β -Oxidation and Confers Susceptibility to Diet-Induced Steatohepatitis and Insulin Resistance. *Cell Metab* 2014;20:687–95.
doi:10.1016/j.cmet.2014.09.015.
- [109] Parks BW, Nam E, Org E, Kostem E, Norheim F, Hui ST, et al. Resource Genetic Control of Obesity and Gut Microbiota Composition in Response to High-Fat , High-Sucrose Diet in Mice. *CMET* 2013;17:141–52. doi:10.1016/j.cmet.2012.12.007.
- [110] Walls SM, Attle SJ, Brulte GB, Walls ML, Finley KD, Chatfield DA, et al. Identification of Sphingolipid Metabolites That Induce Obesity via Misregulation of Appetite, Caloric Intake and Fat Storage in *Drosophila*. *PLoS Genet* 2013;9.
doi:10.1371/journal.pgen.1003970.
- [111] Deevska GM, Rozenova KA, Giltiay N V., Chambers MA, White J, Boyanovsky BB, et al. Acid sphingomyelinase deficiency prevents diet-induced hepatic triacylglycerol accumulation and hyperglycemia in mice. *J Biol Chem* 2009;284:8359–68.
doi:10.1074/jbc.M807800200.
- [112] Van Brocklyn JR, Williams JB. The control of the balance between ceramide and sphingosine-1-phosphate by sphingosine kinase: Oxidative stress and the seesaw of cell survival and death. *Comp Biochem Physiol Part B Biochem Mol Biol* 2012;163:26–36. doi:10.1016/j.cbpb.2012.05.006.

- [113] Merrill AH. De novo sphingolipid biosynthesis: A necessary, but dangerous, pathway. *J Biol Chem* 2002;277:25843–6. doi:10.1074/jbc.R200009200.
- [114] Adams JM, Pratipanawatr T, Berria R, Wang E, DeFronzo RA, Sullards MC, et al. Ceramide Content Is Increased in Skeletal Muscle From Obese Insulin-Resistant Humans. *Diabetes* 2004;53:25–31. doi:10.2337/diabetes.53.1.25.
- [115] Kang SC, Kim BR, Lee SY, Park TS. Sphingolipid metabolism and obesity-induced inflammation. *Front Endocrinol (Lausanne)* 2013;4:1–11. doi:10.3389/fendo.2013.00067.
- [116] Glass CK, Olefsky JM. Inflammation and Lipid Signaling in the Etiology of Insulin Resistance. *Cell Metab* 2012;15:635–45. doi:10.1016/j.cmet.2012.04.001.
- [117] Holland WL, Bikman BT, Wang L, Yuguang G, Sargent KM, Bulchand S, et al. Lipid-induced insulin resistance mediated by the proinflammatory receptor TLR4 requires saturated fatty acid-induced ceramide biosynthesis in mice. *J Clin Invest* 2011;121:1858–70. doi:10.1172/JCI43378.
- [118] Tao C, Holland WL, Wang QA, Shao M, Jia L, Sun K, et al. Short-term versus long-term effects of adipocyte toll-like receptor 4 activation on insulin resistance in male mice. *Endocrinology* 2017;158:1260–70. doi:10.1210/en.2017-00024.
- [119] Kolak M, Gertow J, Westerbacka J, Summers SA, Liska J, Franco-Cereceda A, et al. Expression of ceramide-metabolising enzymes in subcutaneous and intra-abdominal human adipose tissue. *Lipids Health Dis* 2012;11:115. doi:10.1186/1476-511X-11-115.
- [120] Barbarroja N, Rodriguez-Cuenca S, Nygren H, Camargo A, Pirraco A, Relat J, et al. Increased Dihydroceramide/Ceramide Ratio Mediated by Defective Expression of *degs1* Impairs Adipocyte Differentiation and Function. *Diabetes* 2015;64:1180–92. doi:10.2337/db14-0359.

- [121] Siddique MM, Li Y, Wang L, Ching J, Mal M, Ilkayeva O, et al. Ablation of Dihydroceramide Desaturase 1, a Therapeutic Target for the Treatment of Metabolic Diseases, Simultaneously Stimulates Anabolic and Catabolic Signaling. *Mol Cell Biol* 2013;33:2353–69. doi:10.1128/MCB.00226-13.
- [122] Alsanafi M, Kelly SL, Jubair K, McNaughton M, Tate RJ, Merrill AH, et al. Native and polyubiquitinated forms of dihydroceramide desaturase are differentially linked to human embryonic kidney cell survival. *Mol Cell Biol* 2018. doi:10.1128/MCB.00222-18.
- [123] He Y, She H, Zhang T, Xu H, Cheng L, Yepes M, et al. p38 MAPK inhibits autophagy and promotes microglial inflammatory responses by phosphorylating ULK1. *J Cell Biol* 2018;217:315–28. doi:10.1083/jcb.201701049.
- [124] Chaurasia B, Kaddai VA, Lancaster GI, Henstridge DC, Sriram S, Galam DLA, et al. Adipocyte Ceramides Regulate Subcutaneous Adipose Browning, Inflammation, and Metabolism. *Cell Metab* 2016;24:820–34. doi:10.1016/j.cmet.2016.10.002.
- [125] Kolak M, Westerbacka J, Velagapudi VR, Wågsäter D, Yetukuri L, Makkonen J, et al. Adipose Tissue Inflammation and Increased Ceramide Content Characterize Subjects With High Liver Fat Content Independent of Obesity. *Diabetes* 2007;56:1960–8. doi:10.2337/db07-0111.
- [126] Blachnio-Zabielska AU, Koutsari C, Tchkonja T, Jensen MD. Sphingolipid Content of Human Adipose Tissue: Relationship to Adiponectin and Insulin Resistance. *Obesity* 2012;20:2341–7. doi:10.2337/db14-0359.
- [127] Candi E, Tesauro M, Cardillo C, Lena AM, Schinzari F, Rodia G, et al. Metabolic profiling of visceral adipose tissue from obese subjects with or without metabolic syndrome. *Biochem J* 2018;475:1019–35. doi:10.1042/BCJ20170604.

- [128] Turner N, Kowalski GM, Leslie SJ, Risis S, Yang C, Lee-Young RS, et al. Distinct patterns of tissue-specific lipid accumulation during the induction of insulin resistance in mice by high-fat feeding. *Diabetologia* 2013;56:1638–48. doi:10.1007/s00125-013-2913-1.
- [129] Shah C, Yang G, Lee I, Bielawski J, Hannun YA, Samad F. Protection from High Fat Diet-induced Increase in Ceramide in Mice Lacking Plasminogen Activator Inhibitor 1. *J Biol Chem* 2008;283:13538–48. doi:10.1074/jbc.M709950200.
- [130] Neeland IJ, Singh S, McGuire DK, Vega GL, Roddy T, Reilly DF, et al. Relation of plasma ceramides to visceral adiposity, insulin resistance and the development of type 2 diabetes mellitus: the Dallas Heart Study. *Diabetologia* 2018. doi:10.1007/s00125-018-4720-1.
- [131] Błachnio-Zabielska AU, Pułka M, Baranowski M, Nikołąjuk A, Zabielski P, Górka M, et al. Ceramide metabolism is affected by obesity and diabetes in human adipose tissue. *J Cell Physiol* 2012;227:550–7. doi:10.1002/jcp.22745.
- [132] Perreault L, Newsom SA, Strauss A, Kerege A, Kahn DE, Harrison KA, et al. Intracellular localization of diacylglycerols and sphingolipids influences insulin sensitivity and mitochondrial function in human skeletal muscle. *JCI Insight* 2018;3:1–21. doi:10.1172/jci.insight.96805.
- [133] Chung JO, Koutsari C, Blachnio-Zabielska AU, Hames KC, Jensen MD. Intramyocellular Ceramides: Subcellular Concentrations and Fractional De Novo Synthesis in Postabsorptive Humans. *Diabetes* 2017;66:2082–91. doi:10.2337/db17-0082.
- [134] Gosejacob D, Jäger PS, Dorp K Vom, Frejno M, Carstensen AC, Köhnke M, et al. Ceramide synthase 5 is essential to maintain C16:0-Ceramide pools and contributes to

- the development of diet-induced obesity. *J Biol Chem* 2016;291:6989–7003.
doi:10.1074/jbc.M115.691212.
- [135] Bikman BT, Guan Y, Shui G, Siddique MM, Holland WL, Kim JY, et al. Fenretinide Prevents Lipid-induced Insulin Resistance by Blocking Ceramide Biosynthesis. *J Biol Chem* 2012;287:17426–37. doi:10.1074/jbc.M112.359950.
- [136] Mcilroy GD, Delibegovic M, Owen C, Stoney PN, Shearer KD, McCaffery PJ, et al. Fenretinide treatment prevents diet-induced obesity in association with major alterations in retinoid homeostatic gene expression in adipose, Liver, and hypothalamus. *Diabetes* 2013;62:825–36. doi:10.2337/db12-0458.
- [137] Rahmaniyan M, Curley RW, Obeid LM, Hannun YA, Kravka JM. Identification of dihydroceramide desaturase as a direct in vitro target for fenretinide. *J Biol Chem* 2011;286:24754–64. doi:10.1074/jbc.M111.250779.
- [138] Mcilroy GD, Tammireddy SR, Maskrey BH, Grant L, Doherty MK, Watson DG, et al. Fenretinide mediated retinoic acid receptor signalling and inhibition of ceramide biosynthesis regulates adipogenesis, lipid accumulation, mitochondrial function and nutrient stress signalling in adipocytes and adipose tissue. *Biochem Pharmacol* 2016;100:86–97. doi:10.1016/j.bcp.2015.11.017.
- [139] Holland WL, Brozinick JT, Wang L-P, Hawkins ED, Sargent KM, Liu Y, et al. Inhibition of Ceramide Synthesis Ameliorates Glucocorticoid-, Saturated-Fat-, and Obesity-Induced Insulin Resistance. *Cell Metab* 2007;5:167–79.
doi:10.1016/j.cmet.2007.01.002.
- [140] Boini KM, Zhang C, Xia M, Poklis JL, Li P-L. Role of Sphingolipid Mediator Ceramide in Obesity and Renal Injury in Mice Fed a High-Fat Diet. *J Pharmacol Exp Ther* 2010;334:839–46. doi:10.1124/jpet.110.168815.

- [141] Sydor S, Sowa JP, Megger DA, Schlattjan M, Jafoui S, Wingerter L, et al. Acid sphingomyelinase deficiency in Western diet-fed mice protects against adipocyte hypertrophy and diet-induced liver steatosis. *Mol Metab* 2017;6:416–27. doi:10.1016/j.molmet.2017.03.002.
- [142] Gao P, Peterson YK, Smith RA, Smith CD. Characterization of isoenzyme-selective inhibitors of human sphingosine kinases. *PLoS One* 2012;7. doi:10.1371/journal.pone.0044543.
- [143] Chan H, Pitson SM. Post-translational regulation of sphingosine kinases. *Biochim Biophys Acta - Mol Cell Biol Lipids* 2013;1831:147–56. doi:10.1016/j.bbalip.2012.07.005.
- [144] Pitson SM, Moretti PAB, Zebol JR, Lynn HE, Xia P, Vadas MA, et al. Activation of sphingosine kinase 1 by ERK1/2-mediated phosphorylation. *EMBO J* 2003;22:5491–500. doi:10.1093/emboj/cdg540.
- [145] Johnson KR, Becker KP, Facchinetti MM, Hannun YA, Obeid LM. PKC-dependent Activation of Sphingosine Kinase 1 and Translocation to the Plasma Membrane. *J Biol Chem* 2002;277:35257–62. doi:10.1074/jbc.M203033200.
- [146] Shu X, Wu W, Mosteller RD, Broek D. Sphingosine Kinase Mediates Vascular Endothelial Growth Factor-Induced Activation of Ras and Mitogen-Activated Protein Kinases. *Mol Cell Biol* 2002;22:7758–68. doi:10.1128/MCB.22.22.7758-7768.2002.
- [147] Foust KD, Kaspar BK. The role of sphingosine kinase-1 in EGFRvIII-regulated growth and survival of glioblastoma cells. *NIH Public Access* 2010;8:4017–8. doi:10.1007/s11060-010-0345-z.
- [148] Stahelin R V., Hwang JH, Kim JH, Park ZY, Johnson KR, Obeid LM, et al. The mechanism of membrane targeting of human sphingosine kinase 1. *J Biol Chem* 2005;280:43030–8. doi:10.1074/jbc.M507574200.

- [149] Alvarez SE, Harikumar KB, Hait NC, Allegood J, Strub GM, Kim EY, et al. Sphingosine-1-phosphate is a missing cofactor for the E3 ubiquitin ligase TRAF2. *Nature* 2010;465:1084–8. doi:10.1038/nature09128.
- [150] Park ES, Choi S, Shin B, Yu J, Yu J, Hwang JM, et al. Tumor necrosis factor (TNF) receptor-associated factor (TRAF)-interacting protein (TRIP) negatively regulates the TRAF2 ubiquitin-dependent pathway by suppressing the TRAF2-sphingosine 1-phosphate (S1P) interaction. *J Biol Chem* 2015;290:9660–73. doi:10.1074/jbc.M114.609685.
- [151] Parham KA, Zebol JR, Tooley KL, Sun WY, Moldenhauer LM, Cockshell MP, et al. Sphingosine 1-phosphate is a ligand for peroxisome proliferator-activated receptor- that regulates neoangiogenesis. *FASEB J* 2015;29:3638–53. doi:10.1096/fj.14-261289.
- [152] Hait NC, Sarkar S, Le Stunff H, Mikami A, Maceyka M, Milstien S, et al. Role of sphingosine kinase 2 in cell migration toward epidermal growth factor. *J Biol Chem* 2005;280:29462–9. doi:10.1074/jbc.M502922200.
- [153] Hait NC, Bellamy A, Milstien S, Kordula T, Spiegel S. Sphingosine kinase type 2 activation by ERK-mediated phosphorylation. *J Biol Chem* 2007;282:12058–65. doi:10.1074/jbc.M609559200.
- [154] Siow D, Wattenberg B. The compartmentalization and translocation of the sphingosine kinases: Mechanisms and functions in cell signaling and sphingolipid metabolism. *Crit Rev Biochem Mol Biol* 2011;46:365–75. doi:10.3109/10409238.2011.580097.
- [155] Igarashi N, Okada T, Hayashi S, Fujita T, Jahangeer S, Nakamura SI. Sphingosine Kinase 2 Is a Nuclear Protein and Inhibits DNA Synthesis. *J Biol Chem* 2003;278:46832–9. doi:10.1074/jbc.M306577200.

- [156] Hait NC, Allegood J, Maceyka M, Strub GM, Harikumar KB, Singh SK, et al. Regulation of histone acetylation in the nucleus by sphingosine-1-phosphate. *Science* (80-) 2009;325:1254–7. doi:10.1126/science.1176709.
- [157] Strub GM, Paillard M, Liang J, Gomez L, Allegood JC, Hait NC, et al. Sphingosine-1-phosphate produced by sphingosine kinase 2 in mitochondria interacts with prohibitin 2 to regulate complex IV assembly and respiration. *FASEB J* 2011;25:600–12. doi:10.1096/fj.10-167502.
- [158] O’Sullivan C, Dev KK. The structure and function of the S1P1 receptor. *Trends Pharmacol Sci* 2013;34:401–12. doi:10.1016/j.tips.2013.05.002.
- [159] Li W, Xu H, Testai FD. Mechanism of action and clinical potential of fingolimod for the treatment of stroke. *Front Neurol* 2016;7:1–11. doi:10.3389/fneur.2016.00139.
- [160] Sharman JL, Spedding M, Peters JA, Harmar AJ. the Concise Guide To Pharmacology 2013 / 14 : GPCRs. *Br Journal Pharmacol* 2013:1706–96. doi:10.1111/bph.12444/full.
- [161] Lucki NC, Li D, Sewer MB. Sphingosine-1-phosphate rapidly increases cortisol biosynthesis and the expression of genes involved in cholesterol uptake and transport in H295R adrenocortical cells. *Mol Cell Endocrinol* 2012;348:165–75. doi:10.1016/j.mce.2011.08.003.
- [162] Singleton PA, Dudek SM, Chiang ET, Garcia JGN. Regulation of sphingosine 1-phosphate-induced endothelial cytoskeletal rearrangement and barrier enhancement by S1P1 receptor, PI3 kinase, Tiam1/Rac1, and alpha-actinin. *FASEB J* 2005;19:1646. doi:10.1096/fj.05-3928com.
- [163] Suzuki N, Hajicek N, Kozasa T. Regulation and physiological functions of G12/13-mediated signaling pathways. *NeuroSignals* 2009;17:55–70. doi:10.1159/000186690.

- [164] Lepley D, Paik J-H, Hla T, Ferrer F. The G Protein–Coupled Receptor S1P₂ Regulates Rho/Rho Kinase Pathway to Inhibit Tumor Cell Migration. *Cancer Res* 2005;65:3788–95. doi:10.1158/0008-5472.CAN-04-2311.
- [165] Malchinkhuu E, Sato K, Maehama T, Mogi C, Tomura H, Ishiuchi S, et al. S1P₂ receptors mediate inhibition of glioma cell migration through Rho signaling pathways independent of PTEN. *Biochem Biophys Res Commun* 2008;366:963–8. doi:10.1016/j.bbrc.2007.12.054.
- [166] Serra M, Saba JD. Sphingosine 1-phosphate lyase, a key regulator of sphingosine 1-phosphate signaling and function. *Adv Enzyme Regul* 2010;50:349–62. doi:10.1016/j.advenzreg.2009.10.024.
- [167] Mandala SM. Sphingosine-1-phosphate phosphatases. *Prostaglandins Other Lipid Mediat* 2001;64:143–56. doi:10.1016/S0090-6980(01)00111-3.
- [168] Bektas M, Laura Allende M, Lee BG, Chen WP, Amar MJ, Remaley AT, et al. Sphingosine 1-phosphate lyase deficiency disrupts lipid homeostasis in liver. *J Biol Chem* 2010;285:10880–9. doi:10.1074/jbc.M109.081489.
- [169] Brindley DN, Pilquil C. Lipid phosphate phosphatases and signaling. *J Lipid Res* 2009;50:S225–30. doi:10.1194/jlr.R800055-JLR200.
- [170] Brindley DN. Lipid phosphate phosphatases and related proteins: Signaling functions in development, cell division, and cancer. *J Cell Biochem* 2004;92:900–12. doi:10.1002/jcb.20126.
- [171] Long JS, Yokoyama K, Tigyi G, Pyne NJ, Pyne S. Lipid phosphate phosphatase-1 regulates lysophosphatidic acid- and platelet-derived-growth-factor-induced cell migration. *Biochem J* 2006;394:495–500. doi:10.1042/BJ20051674.

- [172] Wang J, Badeanlou L, Bielawski J, Ciaraldi TP, Samad F. Sphingosine kinase 1 regulates adipose proinflammatory responses and insulin resistance. *AJP Endocrinol Metab* 2014;306:E756–68. doi:10.1152/ajpendo.00549.2013.
- [173] Pyne S, Chapman J, Steele L, Pyne NJ. Sphingomyelin-derived lipids differentially regulate the extracellular signal-regulated kinase 2 (ERK-2) and c-Jun N-terminal kinase (JNK) signal cascades in airway smooth muscle. *Eur J Biochem* 1996;237:819–26. doi:10.1111/j.1432-1033.1996.0819p.x.
- [174] Cuvillier O, Pirianov G, Kleuser B, Vanek PG, Coso OA, Gutkind JS, et al. Suppression of ceramide-mediated programmed cell death by sphingosine-1-phosphate. *Nature* 1996;381:800–3. doi:10.1038/381800a0.
- [175] Bruce CR, Risis S, Babb JR, Yang C, Kowalski GM, Selathurai A, et al. Overexpression of Sphingosine Kinase 1 Prevents Ceramide Accumulation and Ameliorates Muscle Insulin Resistance in High-Fat Diet-Fed Mice. *Diabetes* 2012;61:3148–55. doi:10.2337/db12-0029.
- [176] Stanford JC, Morris AJ, Sunkara M, Popa GJ, Larson KL, Özcan S. Sphingosine 1-phosphate (S1P) regulates glucose-stimulated insulin secretion in pancreatic beta cells. *J Biol Chem* 2012;287:13457–64. doi:10.1074/jbc.M111.268185.
- [177] Hong S-W, Lee J, Kwon H, Park SE, Rhee E-J, Park C-Y, et al. Deficiency of Sphingosine-1-Phosphate Reduces the Expression of Prohibitin and Causes β -Cell Impairment via Mitochondrial Dysregulation. *Endocrinol Metab* 2018;33:403. doi:10.3803/EnM.2018.33.3.403.
- [178] Laychock SG, Sessanna SM, Lin MH, Mastrandrea LD. Sphingosine 1-phosphate affects cytokine-induced apoptosis in rat pancreatic islet β -cells. *Endocrinology* 2006;147:4705–12. doi:10.1210/en.2006-0456.

- [179] Moon Hosik, Chon Jinyoung, Joo Jindeok KD, Janghyeok I, Haejin L, Jaesik P, Choi Jinwoo. FTY720 preserved islet β -cell mass by inhibiting apoptosis and increasing survival of β -cells in db/db mice. *Diabetes Metab Res Rev* 2012;32:13–23. doi:10.1002/dmrr.
- [180] Laviad EL, Albee L, Pankova-Kholmyansky I, Epstein S, Park H, Merrill AH, et al. Characterization of ceramide synthase 2: Tissue distribution, substrate specificity, and inhibition by sphingosine 1-phosphate. *J Biol Chem* 2008;283:5677–84. doi:10.1074/jbc.M707386200.
- [181] Véret J, Coant N, Gorshkova IA, Giussani P, Fradet M, Riccitelli E, et al. Role of palmitate-induced sphingoid base-1-phosphate biosynthesis in INS-1 β -cell survival. *Biochim Biophys Acta - Mol Cell Biol Lipids* 2013;1831:251–62. doi:10.1016/j.bbalip.2012.10.003.
- [182] Véret J, Coant N, Berdyshev E V., Skobeleva A, Therville N, Bailbé D, et al. Ceramide synthase 4 and de novo production of ceramides with specific N-acyl chain lengths are involved in glucolipotoxicity-induced apoptosis of INS-1 β -cells. *Biochem J* 2011;438:177–89. doi:10.1042/BJ20101386.
- [183] Yea K, Kim J, Lim S, Park HS, Park KS, Suh PG, et al. Lysophosphatidic acid regulates blood glucose by stimulating myotube and adipocyte glucose uptake. *J Mol Med* 2008;86:211–20. doi:10.1007/s00109-007-0269-z.
- [184] Holland WL, Miller RA, Wang Z V., Sun K, Barth BM, Bui HH, et al. Receptor-mediated activation of ceramidase activity initiates the pleiotropic actions of adiponectin. *Nat Med* 2011;17:55–63. doi:10.1038/nm.2277.
- [185] Holland WL, Xia JY, Johnson JA, Sun K, Pearson MJ, Sharma AX, et al. Inducible overexpression of adiponectin receptors highlight the roles of adiponectin-induced

- ceramidase signaling in lipid and glucose homeostasis. *Mol Metab* 2017;6:267–75.
doi:10.1016/j.molmet.2017.01.002.
- [186] Reibe-Pal S, Febbraio MA. Adiponectin serenades ceramidase to improve metabolism. *Mol Metab* 2017;6:233–5. doi:10.1016/j.molmet.2017.01.011.
- [187] Federico L, Yang L, Brandon J, Panchatcharam M, Ren H, Mueller P, et al. Lipid phosphate phosphatase 3 regulates adipocyte sphingolipid synthesis, but not developmental adipogenesis or diet-induced obesity in mice. *PLoS One* 2018;13:e0198063. doi:10.1371/journal.pone.0198063.
- [188] Roztocil E, Nicholl SM, Davies MG. Mechanisms of sphingosine-1-phosphate-induced akt-dependent smooth muscle cell migration. *Surgery* 2009;145:34–41. doi:10.1016/j.surg.2008.08.001.
- [189] Matsuzaki E, Hiratsuka S, Hamachi T, Takahashi-Yanaga F, Hashimoto Y, Higashi K, et al. Sphingosine-1-phosphate promotes the nuclear translocation of β -catenin and thereby induces osteoprotegerin gene expression in osteoblast-like cell lines. *Bone* 2013;55:315–24. doi:10.1016/j.bone.2013.04.008.
- [190] Park SY, Moon MH, Jeong JK, Lee JH, Park YG, Lee YJ, et al. Antiobesity activity of a sphingosine 1-phosphate analogue FTY720 observed in adipocytes and obese mouse model. *Exp Mol Med* 2012;44:603–14. doi:10.3858/emmm.2012.44.10.069.
- [191] Rapizzi E, Taddei ML, Fiaschi T, Donati C, Bruni P, Chiarugi P. Sphingosine 1-phosphate increases glucose uptake through trans-activation of insulin receptor. *Cell Mol Life Sci* 2009;66:3207–18. doi:10.1007/s00018-009-0106-3.
- [192] Lee S-Y, Hong I-K, Kim B-R, Shim S-M, Sung Lee J, Lee H-Y, et al. Activation of sphingosine kinase 2 by endoplasmic reticulum stress ameliorates hepatic steatosis and insulin resistance in mice. *Hepatology* 2015;62:135–46. doi:10.1002/hep.27804.

- [193] Fayyaz S, Henkel J, Japtok L, Krämer S, Damm G, Seehofer D, et al. Involvement of sphingosine 1-phosphate in palmitate-induced insulin resistance of hepatocytes via the S1P2 receptor subtype. *Diabetologia* 2014;57:373–82. doi:10.1007/s00125-013-3123-6.
- [194] Jun DJ, Lee JH, Choi BH, Koh TK, Ha DC, Jeong MW, et al. Sphingosine-1-phosphate modulates both lipolysis and leptin production in differentiated rat white adipocytes. *Endocrinology* 2006;147:5835–44. doi:10.1210/en.2006-0579.
- [195] Moon M-H, Jeong J-K, Lee Y-J, Seol J-W, Park S-Y. Sphingosine-1-phosphate inhibits the adipogenic differentiation of 3T3-L1 preadipocytes. *Int J Mol Med* 2014;34:1153–8. doi:10.3892/ijmm.2014.1856.
- [196] Hashimoto Y, Matsuzaki E, Higashi K, Takahashi-Yanaga F, Takano A, Hirata M, et al. Sphingosine-1-phosphate inhibits differentiation of C3H10T1/2 cells into adipocyte. *Mol Cell Biochem* 2014;401:39–47. doi:10.1007/s11010-014-2290-1.
- [197] Nincheri P, Luciani P, Squecco R, Donati C, Bernacchioni C, Borgognoni L, et al. Sphingosine 1-phosphate induces differentiation of adipose tissue-derived mesenchymal stem cells towards smooth muscle cells. *Cell Mol Life Sci* 2009;66:1741–54. doi:10.1007/s00018-009-9181-8.
- [198] Moon MH, Jeong JK, Park SY. Activation of S1P2 receptor, a possible mechanism of inhibition of adipogenic differentiation by sphingosine 1phosphate. *Mol Med Rep* 2015;11:1031–6. doi:10.3892/mmr.2014.2810.
- [199] Kitada Y, Kajita K, Taguchi K, Mori I, Yamauchi M, Ikeda T, et al. Blockade of Sphingosine 1-Phosphate Receptor 2 Signaling Attenuates High-Fat Diet-Induced Adipocyte Hypertrophy and Systemic Glucose Intolerance in Mice. *Endocrinology* 2016;157:1839–51. doi:10.1210/en.2015-1768.

- [200] Jeong JK, Moon MH, Park SY. Modulation of the expression of sphingosine 1-phosphate 2 receptors regulates the differentiation of pre-adipocytes. *Mol Med Rep* 2015;12:7496–502. doi:10.3892/mmr.2015.4388.
- [201] Hashimoto T, Igarashi J, Kosaka H. Sphingosine kinase is induced in mouse 3T3-L1 cells and promotes adipogenesis. *J Lipid Res* 2009;50:602–10. doi:10.1194/jlr.M800206-JLR200.
- [202] Mastrandrea LD. Role of sphingosine kinases and sphingosine 1-phosphate in mediating adipogenesis. *J Diabetes Mellit* 2013;03:52–61. doi:10.4236/jdm.2013.32009.
- [203] Damirin A. Sphingosine 1-Phosphate Receptors Mediate the Lipid-Induced cAMP Accumulation through Cyclooxygenase-2/Prostaglandin I2 Pathway in Human Coronary Artery Smooth Muscle Cells. *Mol Pharmacol* 2005;67:1177–85. doi:10.1124/mol.104.004317.
- [204] Kendall MR, Hupfeld CJ. FTY720, a sphingosine-1-phosphate receptor modulator, reverses high-fat diet-induced weight gain, insulin resistance and adipose tissue inflammation in C57BL/6 mice. *Diabetes, Obes Metab* 2008;10:802–5. doi:10.1111/j.1463-1326.2008.00910.x.
- [205] Gräler MH, Goetzl EJ. The immunosuppressant FTY720 down-regulates sphingosine 1-phosphate G-protein-coupled receptors. *FASEB J* 2004;18:551–3. doi:10.1096/fj.03-0910fje.
- [206] Chiba K, Yanagawa Y, Masubuchi Y, Kataoka H, Kawaguchi T, Ohtsuki M, et al. FTY720, a novel immunosuppressant, induces sequestration of circulating mature lymphocytes by acceleration of lymphocyte homing in rats. I. FTY720 selectively decreases the number of circulating mature lymphocytes by acceleration of lymphocyte homing. *J Immunol* 1998;160:5037–44. doi:10.1161/01.

- [207] Choi JW, Gardell SE, Herr DR, Rivera R, Lee C-W, Noguchi K, et al. FTY720 (fingolimod) efficacy in an animal model of multiple sclerosis requires astrocyte sphingosine 1-phosphate receptor 1 (S1P1) modulation. *Proc Natl Acad Sci* 2011;108:751–6. doi:10.1073/pnas.1014154108.
- [208] Beg M, Srivastava A, Shankar K, Varshney S, Rajan S, Gupta A, et al. PPP2R5B, a regulatory subunit of PP2A, contributes to adipocyte insulin resistance. *Mol Cell Endocrinol* 2016;437:97–107. doi:10.1016/j.mce.2016.08.016.
- [209] Rahman MM, Prünte L, Lebender LF, Patel BS, Gelissen I, Hansbro PM, et al. The phosphorylated form of FTY720 activates PP2A, represses inflammation and is devoid of S1P agonism in A549 lung epithelial cells. *Sci Rep* 2016;6:6–13. doi:10.1038/srep37297.
- [210] Tay KH, Jin L, Tseng HY, Jiang CC, Ye Y, Thorne RF, et al. Suppression of PP2A is critical for protection of melanoma cells upon endoplasmic reticulum stress. *Cell Death Dis* 2012;3. doi:10.1038/cddis.2012.79.
- [211] Kinney BP, Qiao L, LeVaugh JM, Shao J. B56 α /protein phosphatase 2A inhibits adipose lipolysis in high-fat diet-induced obese mice. *Endocrinology* 2010;151:3624–32. doi:10.1210/en.2010-0245.
- [212] Tous M, Ferrer-Lorente R, Badimon L. Selective inhibition of sphingosine kinase-1 protects adipose tissue against LPS-induced inflammatory response in Zucker diabetic fatty rats. *Am J Physiol Endocrinol Metab* 2014;307:E437–46. doi:10.1152/ajpendo.00059.2014.
- [213] Zhang W, Mottillo EP, Zhao J, Gartung A, VanHecke GC, Lee JF, et al. Adipocyte lipolysis-stimulated interleukin-6 production requires sphingosine kinase 1 activity. *J Biol Chem* 2014;289:32178–85. doi:10.1074/jbc.M114.601096.

- [214] Liang J, Nagahashi M, Kim EY, Harikumar KB, Yamada A, Huang WC, et al. Sphingosine-1-Phosphate Links Persistent STAT3 Activation, Chronic Intestinal Inflammation, and Development of Colitis-Associated Cancer. *Cancer Cell* 2013;23:107–20. doi:10.1016/j.ccr.2012.11.013.
- [215] Schwab SR, Cyster JG. Finding a way out: Lymphocyte egress from lymphoid organs. *Nat Immunol* 2007;8:1295–301. doi:10.1038/ni1545.
- [216] Spiegel S, Milstien S. The outs and the ins of sphingosine-1-phosphate in immunity. *Nat Rev Immunol* 2011;11:403–15. doi:10.1038/nri2974.
- [217] Gabriel TL, Mirzaian M, Hooibrink B, Ottenhoff R, van Roomen C, Aerts JMFG, et al. Induction of Sphk1 activity in obese adipose tissue macrophages promotes survival. *PLoS One* 2017;12:e0182075. doi:10.1371/journal.pone.0182075.
- [218] Lee YS, Li P, Huh JY, Hwang IJ, Lu M, Kim JI, et al. Inflammation Is Necessary for Long-Term but Not Short-Term High-Fat Diet-Induced Insulin Resistance. *Diabetes* 2011;60:2474–83. doi:10.2337/db11-0194.
- [219] Bellini L, Campana M, Mahfouz R, Carlier A, Véret J, Magnan C, et al. Targeting sphingolipid metabolism in the treatment of obesity/type 2 diabetes. *Expert Opin Ther Targets* 2015;19:1037–50. doi:10.1517/14728222.2015.1028359.
- [220] Ikeda H, Watanabe N, Ishii I, Shimosawa T, Kume Y, Tomiya T, et al. Sphingosine 1-phosphate regulates regeneration and fibrosis after liver injury via sphingosine 1-phosphate receptor 2. *J Lipid Res* 2009;50:556–64. doi:10.1194/jlr.M800496-JLR200.
- [221] Ross JS, Hu W, Rosen B, Snider AJ, Obeid LM, Cowart LA. Sphingosine kinase 1 is regulated by peroxisome proliferator-activated receptor α in response to free fatty acids and is essential for skeletal muscle interleukin-6 production and signaling in diet-induced obesity. *J Biol Chem* 2013;288:22193–206. doi:10.1074/jbc.M113.477786.

- [222] Okajima F. Plasma lipoproteins behave as carriers of extracellular sphingosine 1-phosphate: is this an atherogenic mediator or an anti-atherogenic mediator? *Biochim Biophys Acta - Mol Cell Biol Lipids* 2002;1582:132–7. doi:10.1016/S1388-1981(02)00147-6.
- [223] Thuy A V, Reimann C-M, Hemdan NYA, Gräler MH. Sphingosine 1-Phosphate in Blood: Function, Metabolism, and Fate. *Cell Physiol Biochem* 2014;34:158–71. doi:10.1159/000362992.
- [224] Christoffersen C, Federspiel CK, Borup A, Christensen PM, Madsen AN, Heine M, et al. The Apolipoprotein M/S1P Axis Controls Triglyceride Metabolism and Brown Fat Activity. *Cell Rep* 2018;22:175–88. doi:10.1016/j.celrep.2017.12.029.
- [225] Dahlbäck B. Lean ApoM $-/-$ Mice with Hyperactive Brown Adipose Tissue. *Trends Endocrinol Metab* 2018;29:283–4. doi:10.1016/j.tem.2018.02.011.
- [226] Summers SA. Could Ceramides Become the New Cholesterol? *Cell Metab* 2018;27:276–80. doi:10.1016/j.cmet.2017.12.003.
- [227] Kölzer M, Werth N, Sandhoff K. Interactions of acid sphingomyelinase and lipid bilayers in the presence of the tricyclic antidepressant desipramine. *FEBS Lett* 2004;559:96–8. doi:10.1016/S0014-5793(04)00033-X.
- [228] Kornhuber J, Tripal P, Reichel M, Mühle C, Rhein C, Muehlbacher M, et al. Functional Inhibitors of Acid Sphingomyelinase (FIASMA): A Novel Pharmacological Group of Drugs with Broad Clinical Applications. *Cell Physiol Biochem* 2010;26:9–20. doi:10.1159/000315101.

Abbreviations:

ADRB3, β_3 -adrenergic receptor; AKT, protein kinase B; AMSC, adipose tissue-derived mesenchymal stem cell; ApoM, apolipoprotein M; AP1, activator protein 1; aSMase, acid sphingomyelinase; α SMA, α -smooth muscle actin; ATGL, adipose triglyceride lipase; ATM, adipose tissue macrophages; BMI, body mass index; BMP, bone morphogenetic protein; CCL5, chemokine ligand 5; C/EBPs, CCAAT/enhancer-binding proteins; CEM, caveolin-enriched micro-domains; CerS, ceramide synthase; CL, CL-316243 (β_3 -adrenergic agonist); DAG, diacylglycerol; Degs1, dihydroceramide desaturase; DIO, diet-induced obesity; DMS, dimethylsphingosine; DHS, dihydrosphingosine; EGF, epidermal growth factor; ER, endoplasmic reticulum; ERK1/2, extracellular signal-regulated kinase 1 and 2; FABP4, fatty acid-binding protein 4; FOXO, forkhead box protein O; FTY-720, fingolimod (2-Amino-2-[2-(4-octyl-phenyl)-ethyl]-propane-1,3-diol); GLUT4, glucose transporter 4; GSK3 β , glycogen synthase kinase 3 beta; GWAS, genome-wide association study; HDAC1/2, histone deacetylase 1 and 2; HDL, high density lipoprotein; HFD, high fat diet; HSL, hormone-sensitive lipase; IFN γ , interferon gamma; IL-1 β , interleukin-1 β ; IKK- β , inhibitor of nuclear factor kappa-B kinase subunit beta; IR, insulin receptor; IRS, insulin receptor substrate; JAK, Janus kinases; c-JNK, c-Jun N-terminal kinase; JTE-013, S1P₂ antagonist; KC, keratinocyte-derived chemokine; LDL, low density lipoprotein; LPP, lipid phosphate phosphatase; LPS, lipopolysaccharides; MAGL, monoglyceride lipase; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemoattractant protein 1; mTOR, mammalian target of rapamycin; NEFA, Non esterified fatty acid; NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B cells; NLRP3, NACHT, LRR and PYD domains-containing protein 3; nSMase, neutral sphingomyelinase; PCNA, proliferating cell nuclear antigen; PDE3B, phosphodiesterase 3B; PDK, phosphoinositide-dependent kinases; PHB2, prohibitin 2; PMA, phorbol 12-myristate 13-acetate; PI3K, phosphoinositide 3-kinase; PIP₃, phosphatidylinositol (3,4,5)-trisphosphate; PKA, protein kinase A; PKC, protein kinase C; PKD, protein kinase D; PLC, phospholipase C; PPAR γ , peroxisome proliferator-activated receptor gamma; PP2A, protein phosphatase-2A; PTEN, phosphatase and tensin homologue; PTP1B, protein-tyrosine phosphatase 1B; S1P, sphingosine 1-phosphate; S1P₁₋₅, S1P receptor 1-5; S6K, ribosomal protein S6 kinase; SPHK I or SKI-II (2-(p-Hydroxyanilino)-4-(p-chlorophenyl) thiazole); *Smpd1*, acid sphingomyelinase gene; SphK, sphingosine kinase; SOCS3, Suppressor of cytokine signaling 3; SPT, serine palmitoyl transferase; SREBF1, sterol regulatory element-binding transcription factor 1; STAT3, signal transducer and activator of transcription 3; T2D, type 2 diabetes; TGF β , transforming growth factor beta; TLR, Toll-like receptors; TNF α , tumor necrosis factor alpha; TRAF2, TNF receptor-associated factor 2; UCL-1, uncoupling protein-1; VEGF, vascular endothelial growth factor; VLDL, very low density lipoprotein; VPC23019, S1P_{1/3} antagonist; ZLC, Zucker lean normoglycemic control; 5c, 2,2-dimethyl-4S-(1-oxo-2-hexadecyn-1-yl)-1,1-dimethylethylester-3-oxazolidine-carboxylic acid.

Figure Legends

Figure 1. Pro-inflammatory and lipid signaling act synergistically in insulin resistance.

The increased NEFA influx causes the elevation of diacylglycerol and ceramide levels in adipocytes and which activate PKC ϵ and PKC ζ respectively. These PKC isoforms not only

catalyse inhibitory serine/threonine phosphorylation of IR and IRS but also activate other protein kinase pathways, such as JNK, which are additionally regulated by inflammatory mediators, ER stress and ROS. The activation of JNK, IKK β , S6K and mTOR induce up-regulation of SOCS3 that antagonizes IRS to block insulin responsiveness. Pro-inflammatory mediators, such as TNF α , IFN γ and saturated fatty acid also promote upregulation of IL-6 and TNF α , which suppress PPAR γ to inhibit lipogenesis and interfere intracellular metabolism through transcriptional and post-transcriptional regulation.

Figure 2. Ceramide metabolism modulation and action in adipocyte. Ceramide is a key lipid metabolite that reduces AKT-mediated signaling and responsiveness to insulin. Glucose transport is impaired by intracellular ceramide, which activates PP2A and PKC ζ . PP2A dephosphorylates Ser473-phosphorylated AKT and blocks insulin receptor signaling. PKC ζ interrupts the recruitment of AKT and PTEN, retaining AKT in caveolin-enriched microdomains of the plasma membrane by phosphorylating Thr34 in AKT. Ceramide increases NLRP3 inflammasome activity; which stimulates conversion of pro-IL1 β into IL-1 β , to promote insulin resistance. The accumulation of ceramides is promoted by the activation of TLR4 and TNFR. In the contrast, the binding of adiponectin to adiponectin receptor1/2 induces ceramidase activity and accelerates ceramides metabolism.

Figure 3. Ceramide biosynthesis. The *de novo* ceramide synthesis pathway occurs in the endoplasmic reticulum and is initiated by serine palmitoyl transferase, the activity of which is affected by the availability of substrates, palmitate and serine. After a series of reactions, ceramide is generated in ER and is transported the Golgi apparatus for further biosynthesis of complex sphingolipids. Sphingomyelinase catalyses the hydrolysis of complex sphingolipids to produce ceramide in different sub-cellular compartments. The key metabolic enzymes that may play a role in adipose dysfunction in obesity are highlighted in red. The genetic deletion and pharmacological inhibition of these enzymes improve adipose metabolism and insulin sensitivity.

Figure 4. Role of native and polyubiquitinated forms of Degs1 in regulating anabolic/catabolic signaling. Native Degs1 which is pro-apoptotic can be polyubiquitinated

to a form which has pro-survival function. We propose here that these forms might account for the anabolic/catabolic phenotype of fibroblasts in which *Degs1* expression has been eliminated. In this case, the native form of *Degs1* might be responsible for the synthesis of ceramides which can block Akt signaling and thereby induce insulin resistance. In contrast, the polyubiquitinated forms are linked with p38 MAPK signaling that functions to reduce autophagy by phosphorylation of ULK1. We propose that certain *Degs1* modulators such as fenretinide and 4-oxo-N-(4-hydroxyphenyl)retinamide but not GT-11 induce the ubiquitin-proteasomal degradation and loss of *Degs1*, which recapitulates the phenotype in fibroblasts where *Degs1* has been genetically eliminated.

Figure 5. Ceramide and S1P function in concert to promote adipose dysregulation.

Excessive fatty acid influx and inflammation promotes ceramide accumulation through the upregulation of enzymes involved in ceramide synthesis in adipose tissue to disrupt insulin signaling. The upregulation of pro-inflammatory genes can be induced by ceramide and can amplify the inflammatory responses in adipose tissue. The increased production of ceramide and upregulation of SphK1 contribute to the enhanced S1P formation. The binding of S1P to S1P_{1/2} also reduces adipogenesis to hinder adaptation of adipose tissue to excessive fat deposition. The SphK1/S1P/S1PR axis is also involved in pro-inflammatory cytokines secretion and macrophages recruitment and differentiation. The direct consequence is the chronic inflammation that leads to adipose dysfunction and insulin resistance.

Table 1. S1P and downstream signaling in adipocyte metabolism and differentiation				
Cell type or animal	Intervention	Downstream signal	Biological outcome	Ref
(rat) white adipocyte	S1P (1–30 μ M, 20 m [*])	\uparrow cytosolic Ca ²⁺ and cAMP	\uparrow lipolysis \downarrow insulin-induced leptin production	[194]
3T3-L1 adipocytes	S1P (5 μ M, 10 m)	Not detected	\uparrow glucose uptake	[183]
3T3-L1 adipocytes	S1P (0.5-50 μ M, during adipogenesis)	\downarrow PPAR γ , C/EBP α and adiponectin \downarrow P-JNK1/2, P-p38 MAPK	\downarrow adipogenesis	[195]
C3H10T1/2 cells	S1P (0.1-1 μ M, 1-8 d)	\downarrow cAMP, C/EBP β , FABP4; \downarrow PPAR γ -driven transcription	\downarrow adipogenic differentiation	[196]
AMSC	S1P (1 μ M, 1-10 d)	\uparrow α SMA/transgelin; \uparrow L-type and T-type Ca ²⁺ currents	\uparrow differentiation to smooth muscle phenotype	[197]
ASMC	JTE-013 (1 μ M)	\downarrow S1P-induced α SMA/transgelin up-regulation; \downarrow S1P-activated L-type Ca ²⁺ currents	\downarrow S1P-induced differentiation to smooth muscle phenotype	[197]
3T3-L1 adipocytes	JTE-013 (0.02-2 μ M, during adipogenesis)	\downarrow S1P-induced down-regulation of PPAR γ , C/EBP α and adiponectin	\uparrow adipogenesis \uparrow lipid accumulation	[198]
3T3-L1 adipocyte	JTE-013 (10 μ M, 1 h P-ERK; 24 h proliferation)	\uparrow P-ERK	\uparrow adipocyte proliferation	[199]
3T3-F442A adipocyte	VPC-23019 (10 μ M, 5 d)	\uparrow adipocyte-specific gene expression (<i>Fabp4</i> , <i>Lpl</i> , <i>Adipoq</i>)	\uparrow adipogenic differentiation	[199]
3T3-L1 adipocytes	siRNA S1P ₂	\downarrow S1P-induced downregulation of PPAR γ and P-JNK	\downarrow S1P-mediated inhibition of lipid accumulation	[200]
3T3-L1 adipocytes	DMS or DHS (5 μ M, 8 d of differentiation)	\downarrow AP2 (mRNA)	\downarrow adipogenesis and lipid accumulation	[201]
3T3-L1 adipocytes	SKI-II (10 μ M, during adipogenesis)	Not detected	\downarrow lipogenesis and differentiation	[202]
3T3-L1 adipocytes	siRNA SphK1	\downarrow (mRNA) AP2, PPAR γ , C/EBP α	\downarrow adipogenesis and lipid accumulation	[201]
S1P ₂ ^{-/-} mice	HFD for 4 weeks	\uparrow PCNA expression	\uparrow adipocyte hyperplasia	[199]
Male C57B/6J DIO mice	FTY720 (0.04 mg/kg, i.p. 2x per w; 6 w)	\downarrow HFD-induced AKT and AMPK dephosphorylation; \uparrow HSL, perilipin, ATGL, and HSL phosphorylation	\downarrow fat accumulation \uparrow lipolysis	[190]

*AMSC, adipose tissue-derived mesenchymal stem cells; **d**, day; **h**, hour; **m**, minute; **w**, week

Table 2. S1P and downstream signaling in adipose inflammation				
Cell type or animal	Intervention	Downstream signal	Biological outcome	Reference
3T3-L1 adipocytes	S1P (50, 100 nM, 3 h)	↑ TNF- α and KC ↑ IL-6	↑ proinflammatory cytokine secretion	[95]
C57BL/6 male DIO mice	FTY720 (1 or 3 mg/kg, i.p., 8 w)	↓ circulating monocytes	↓ adipose inflammation phenotype	[204]
Zucker lean normo-glycemic control (ZLC) rats	SPHK I (10 mg/kg, i.p., 60 m before LPS (10 mg/kg))	↓ LPS-induced CCL5 ↓ up-regulation of inflammation markers (Cd68, Cd163, IL-6, CCL2, and TNF α) in white adipose tissue	↓ LPS-induced adipose inflammation ↓ serum CCL5	[212]
C57BL/6 male DIO mice	SKI-II (20 mg/kg, i.p.)	↓ isoproterenol- and CL-316243-mediated IL-6 upregulation	↓ ADRB3/HSL-mediated adipose inflammation	[213]
<i>sphk1</i> ^{-/-} mice	HFD (16 w)	↑ PPAR γ , AP2, GLUT4 ↓ PPAR γ Ser112 phosphorylation ↓ TNF α , IL-6, and MCP-1, SOCS3 ↑ IL-10, adiponectin	↑ adipogenesis, ↓ adipose inflammation ↑ insulin sensitivity	[172]
DIO mice	5c (2mg/kg, i.p., 1x per d, 3d)	↑ insulin-mediated P-AKT ↓ (mRNA) TNF α , IL-6, MCP-1, SOCS3 ↑ (mRNA) IL-10, adiponectin	↑ glucose hemostasis ↓ adipose inflammation	[172]
S1P ₂ ^{-/-} mice	HFD for 4 weeks	abolish HFD-induced upregulation of M1 macrophage markers (Cd11c and Nos2)	↓ M1 macrophage recruitment	[199]

Fig. 1

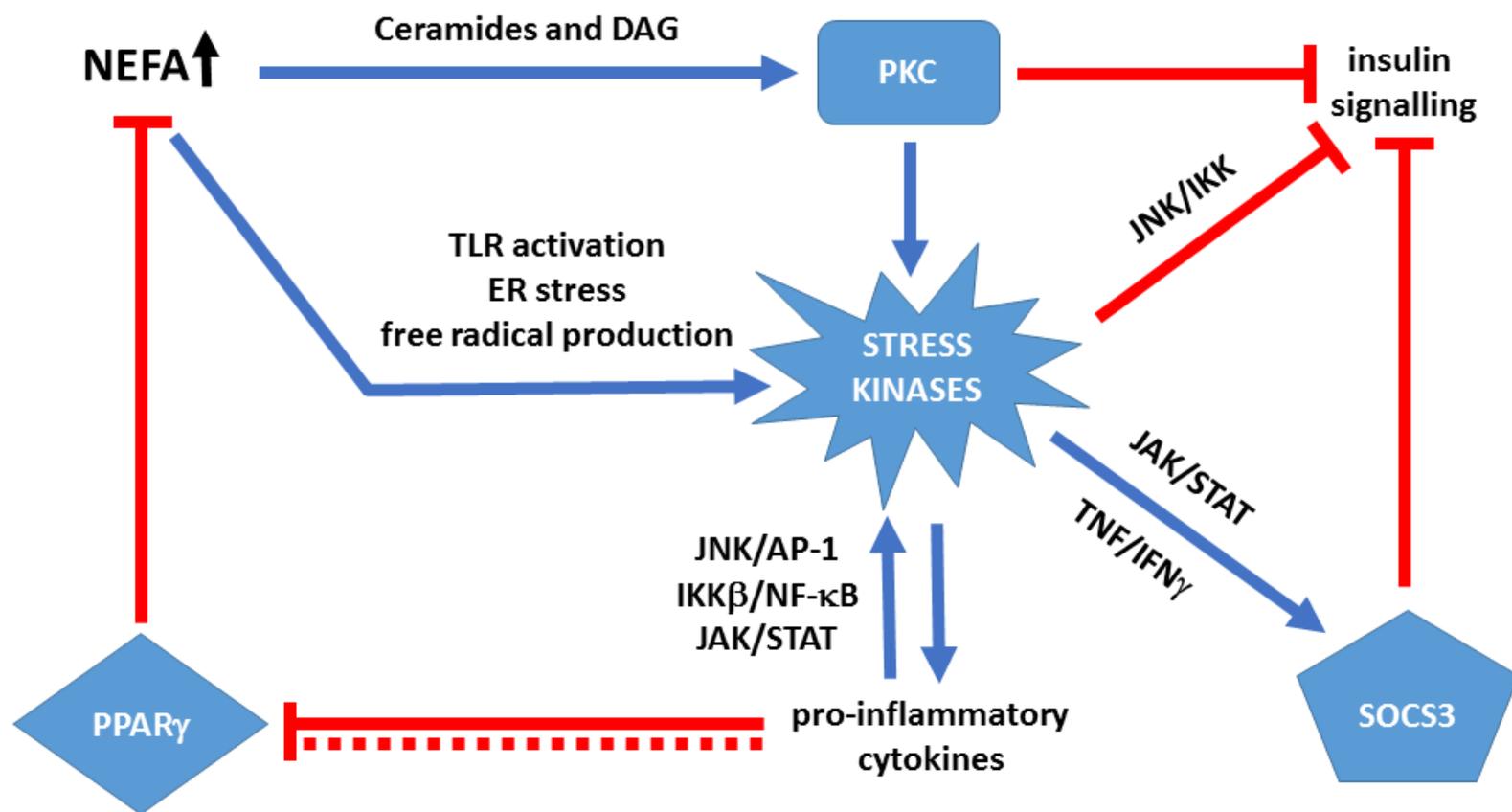


Fig. 2

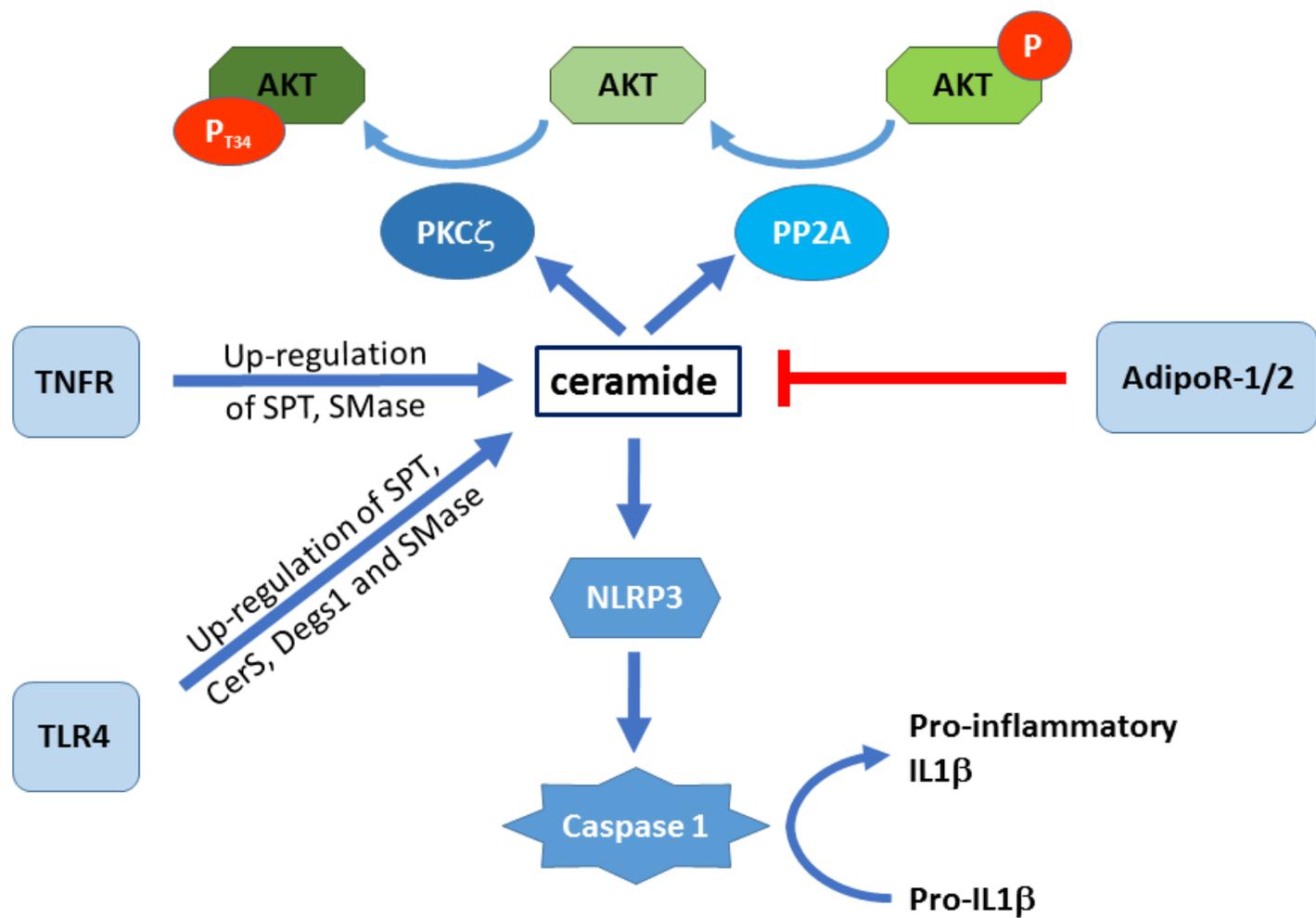


Fig. 3

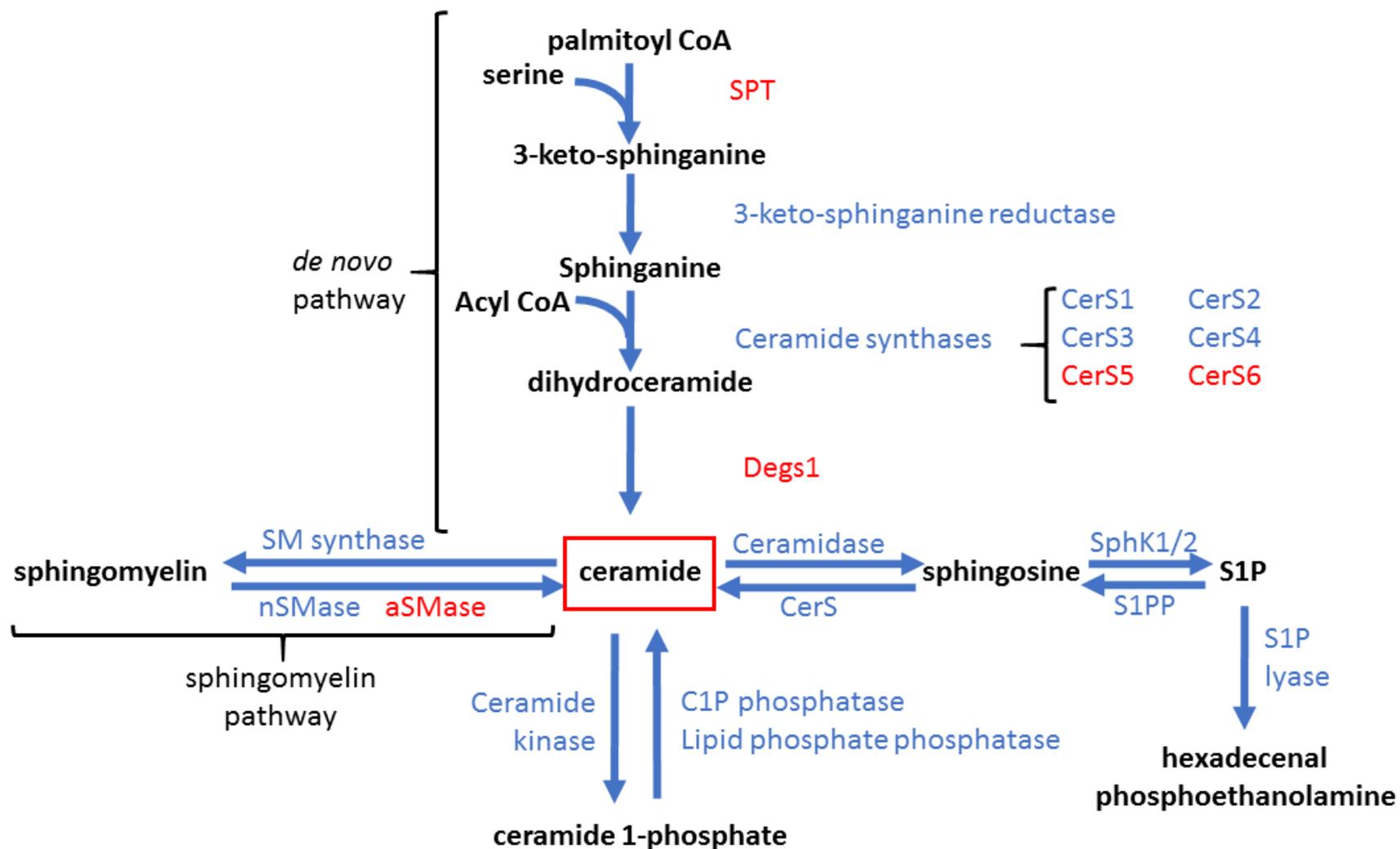


Fig. 4

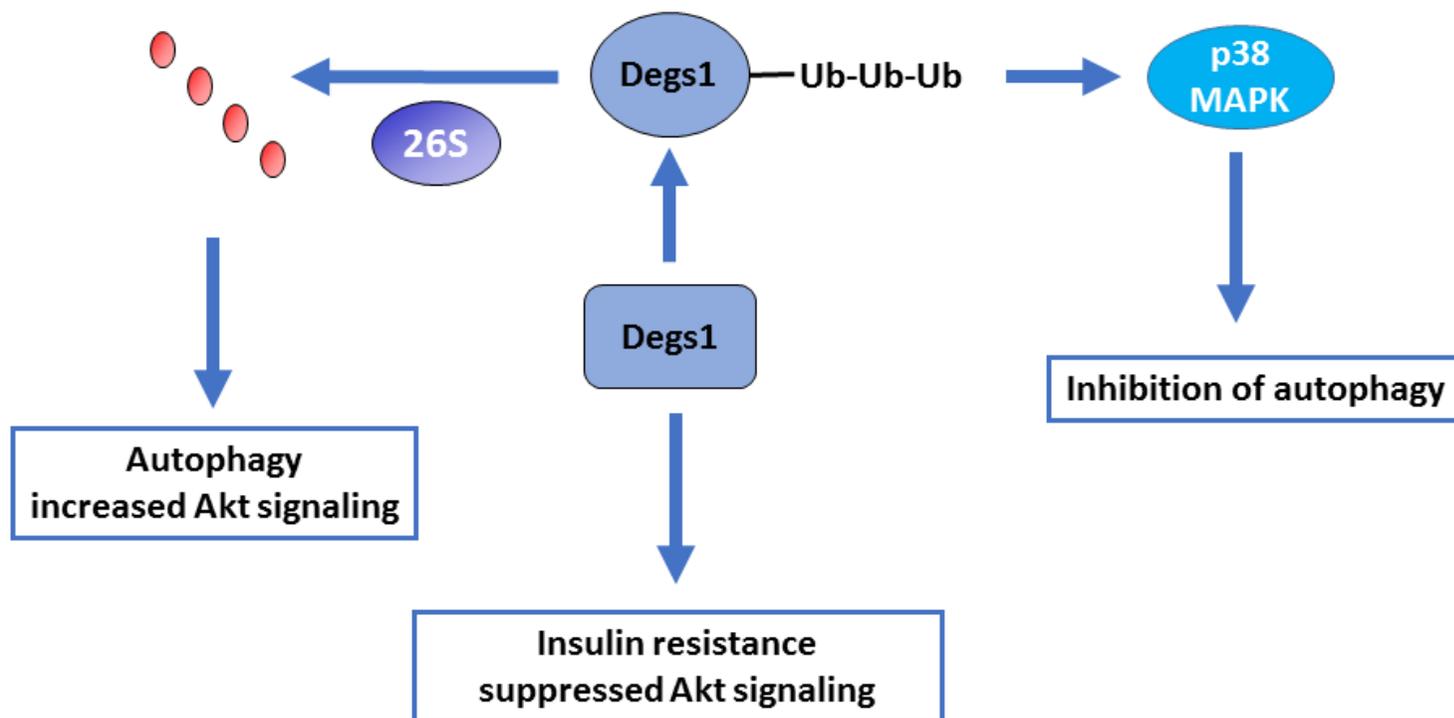


Fig. 5

