

# Therapeutic Strategies for Diabetes and Complications: A Role for Sphingolipids?

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### Abstract

**D**iabetes is a debilitating chronic disease that has no cure and can only be managed by pharmaceutical or nutritional interventions. Worldwide, the incidence of diabetes and diabetic complications is dramatically increasing. This may reflect the incomplete knowledge base underlying the role of inflammatory or nutritional stresses to exacerbate diabetic complications. Despite the knowledge that hyperlipidemia is a cardinal feature of both Type 1 and 2 diabetes, the actual lipid species that contribute to complications such as diabetic nephropathy, retinopathy, neuropathy and cardiovascular disease have not been well defined, or have not elucidated new treatment strategies. Sphingolipids comprise only a fraction of total lipids but a body of evidence has now identified dysfunctional sphingolipid metabolism and/or generation of specific sphingolipid metabolites as contributors to diabetic complications. This review suggests that pharmacological therapies that target dysfunctional sphingolipid metabolism and/or signaling may prove beneficial in decreasing the chronic pathology of hyperglycemia and hyperlipidemia. Moreover, the review suggests that these treatment options may also prove beneficial to ameliorate or delay pancreatic beta cell failure.

### Diabetes and Insulin Resistance

Diabetes affects over 246 million people worldwide and is increasing at an alarming rate in both developed and developing countries.<sup>1</sup> Worldwide, diabetes is one of the top five leading causes of disease-related death.<sup>1</sup> Despite insulin treatment, good nutritional support as well as new treatment modalities to increase the effectiveness of insulin; diabetic complications continue to plague diabetic patients. Moreover, a worldwide obesity epidemic contributes to the increase in metabolic syndrome and diabetic patients.

Simply stated, diabetes is the failure of the body to correctly utilize glucose due to diminished insulin signaling. Insulin is an essential anabolic hormone and growth factor that promotes cell function and survival. Diminished insulin levels and/or insulin signaling lead to the catabolic features of diabetes; hyperglycemia, hyperlipidemia and accelerated protein degradation. This failure in insulin signaling can reflect either impaired insulin production (Type 1 diabetes) or impaired insulin responsiveness (Type 2 diabetes). Both of these pathologies lead to hyperglycemia, which contributes to serious pathological complications, including nephropathy, neuropathy, retinopathy or cardiovascular disease. The underlying etiology of both diseases are still unknown, but may reflect an autoimmune disease targeting pancreatic beta islet cells (Type 1) and an inflammatory

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condition exacerbated by nutritional stress or obesity (Type 2) that impairs insulin signaling in insulin responsive cell types.<sup>2</sup> Even though the initial insult may be distinctly different for Type 1 and 2 patients, the resultant insulin resistant phenotype contributes to diabetic complications in both diseases.

Type 1 patients develop secondary insulin resistance in insulin responsive tissues like Type 2 patients.<sup>3</sup> Type 2 patients develop secondary pancreatic symptoms, similar to Type 1 patients, which further worsens clinical outcomes. Several clinical studies are now reporting that global insulin resistance and resultant diabetic complications is more than just an increase in glucose levels and may reflect nutritional stresses. In fact, the EURODIAB Prospective Complications Study showed that insulin resistance is a major predictive factor for the development of complications in Type 1 diabetes.<sup>4</sup> These findings have been confirmed in other studies showing dramatic increased risk of complications with body mass index  $>25 \text{ kg/m}^2$ .<sup>5</sup> In addition, persons with Type 1 diabetes and the metabolic syndrome have a 3.7 fold increased risk of severe retinopathy, a 22.8 fold increased risk of stroke and a 7.3 fold increased risk of peripheral vascular disease.<sup>6</sup> A recent study shows that the estimated glucose disposal rate, but not metabolic syndrome or insulin dose, is highly related with the development of retinopathy, nephropathy and macrovascular disease in Diabetes Control and Complications Trial (DCCT) subjects.<sup>7</sup> This finding is in line with the observation that insulin doses parallel plasma triglyceride concentrations in children with Type 1 diabetes.<sup>8</sup> Together, these findings indicate that complications of diabetes depend on factors other than hyperglycemia and include insulin resistance and overall nutritional status.

The contribution of hyperlipidemia to diabetes is being defined. While sphingolipids typically constitute a relatively minor component of the lipid environment compared to glycerolipids and sterols, altered sphingolipid metabolism has been implicated in diabetes pathogenesis. In vitro studies are beginning to define the biochemical and biophysical signaling mechanisms by which sphingolipid metabolites contribute to diabetic complications. Particular areas in which sphingolipids are being investigated include the detrimental effects of the generation of reactive oxygen species,<sup>9</sup> induction of the hexosamine biosynthetic pathway,<sup>10,11</sup> inflammation<sup>12</sup> and increases in advanced glycated end products,<sup>13-15</sup> all of which may contribute to diabetic complications. As these mechanisms, including signaling, have been reviewed elsewhere,<sup>16-19</sup> we have focused the present review on the in vivo animal and human studies that have implicated dysfunctional sphingolipid metabolism and/or accumulation of sphingolipid metabolites in insulin resistance and/or diabetic complications.

## Insulin Resistance and Altered Sphingolipid Metabolism

Classically, insulin resistance is typically associated with the liver, skeletal muscle and adipose tissue. Consequences of insulin resistance in these tissues include glucose intolerance and hyperlipidemia. Thus, it is not surprising that altered sphingolipid metabolism has been observed in multiple obese rodent models of Type 2 diabetes. What is intriguing is the restoration of insulin sensitivity by pharmacological strategies that target dysfunctional sphingolipid metabolism.

Recent studies have begun to define the role of hyperlipidemia to alter sphingolipid metabolites and regulate insulin resistance.<sup>20</sup> Ceramide accumulates in skeletal muscle and liver after infusion of lard oil emulsions into the bloodstream of Sprague-Dawley rats. Treating these animals with inhibitors of serine palmitoyltransferase (SPT) prevented the lard oil-induced increases in ceramide accumulation and maintained insulin-stimulated glucose utilization. Using an ex vivo approach, administration of saturated fats, including palmitate, a precursor necessary for serine palmitoyltransferase-induced generation of de novo sphingolipids, to isolated rodent muscles induced ceramide-dependent insulin resistance and impaired 2-deoxyglucose uptake. Knock-out animal models have also been quite useful to define a role for sphingolipid metabolism in insulin resistance. Palmitate-induced insulin resistance was abrogated in isolated soleus muscles from mice lacking one allele of dihydroceramide desaturase 1.<sup>20</sup> Deficiency of the acid sphingomyelinase gene product protected against saturated fat diet-induced hyperglycemia,

insulin resistance and impaired hepatic triacylglyceride accumulation.<sup>21</sup> Similarly, hyperlipidemia and altered sphingolipid metabolites was noted in the liver of *ob/ob* mice where C<sub>16:0</sub> and C<sub>18:0</sub> ceramides positively correlated with liver triglycerides.<sup>22</sup> Yet, despite evidence that dysfunctional sphingolipid metabolism contributes to insulin resistance, the role of specific glucosylated or phosphorylated sphingolipid metabolites to contribute to insulin resistance are only recently being identified.

It should not be surprising that glycosphingolipids accumulate in obese animal models of diabetes. As examples, Zucker diabetic fatty *fa/fa* rats (ZDF) and *ob/ob* mice display increased levels of glucosylceramide in liver,<sup>23</sup> consistent with an increase in GM3 synthase expression in adipose tissue.<sup>24</sup> Zucker diabetic fatty rats also have elevated skeletal muscle GM3 ganglioside levels.<sup>25</sup> However, the latter finding is in contrast to the first mentioned group,<sup>23</sup> which found that neither glucosylceramide nor GM3 gangliosides were elevated in muscle or liver of ZDF rats.<sup>23</sup> These apparent discrepancies are usually dismissed as consequences of different tissues or animal models. Yet, these contradictions may also reflect different analytical strategies to characterize and quantify sphingolipid metabolites in addition to age and diets of the animals. Alternatively, they may reflect a “global” increase in sphingolipid metabolism, manifested by transcriptional, translational and posttranslational modifications of sphingolipid and glycosphingolipid metabolic enzymes. As an example, TNF $\alpha$ -induced insulin resistance can be reversed by inhibiting ceramide production<sup>26</sup> or via inhibition of glucosylceramide synthase.<sup>24</sup> Alluding to the contribution of glycosphingolipids to diabetes complications, the ganglioside, GM3, can impair insulin signaling<sup>24,27</sup> and GM3 knockout mice have increased insulin sensitivity.<sup>28</sup> Similarly, GD3 has been implicated in TNF $\alpha$  and CD95 (Fas)-induced apoptosis<sup>29</sup> and GT1b has been demonstrated to inhibit pro-survival Akt activation in diabetic organs.<sup>30,31</sup> Of clinical interest, two recent publications have demonstrated increased glycemic control and insulin sensitivity upon pharmacological inhibition of glucosylceramide synthase.<sup>23,25</sup> In addition to Type 2 obese models, elevated concentrations of GM3 have also been observed in the Type 1 streptozotocin-induced diabetic rat model.<sup>31,32</sup> Finally, accumulating sphingolipid metabolites are not limited to glucosylated species, as elevated levels of sphingosine, at the expense of sphingomyelin or ceramide, has been observed in adipose tissue of diabetic *ob/ob* mice.<sup>33</sup>

Studies of human populations have not been as conclusive as the above animal experiments. Obese insulin-resistant human subjects displayed higher concentrations of ceramide, but not other sphingolipids, in skeletal muscle compared with lean subjects.<sup>34</sup> Similarly, a relationship was observed between decreased insulin sensitivity and increased ceramides in human skeletal muscle.<sup>34,35</sup> Consistent with these human studies, are additional studies demonstrating increased ceramide content in the skeletal muscle of insulin-resistant rats.<sup>36,37</sup> In contrast, a recent study observed no significant alterations in ceramide levels in skeletal muscle of Type 2 patients.<sup>38</sup> These disparate results for ceramide mass in diabetic populations may, in part, reflect overall dysfunctional sphingolipid metabolism and altered levels of phosphorylated or glucosylated sphingolipid metabolites. For example, it has also been demonstrated that plasma sphinganine and sphingosine were elevated in Type 2 diabetics compared with healthy control subjects, which may be consistent with elevated ceramide levels.<sup>39</sup> The presence of sulfated lactosylceramide in blood samples of Type 2 patients and low blood levels of sulfatides may also be linked to insulin resistance.<sup>40</sup> Thus far, these studies have involved analysis of relatively small numbers of patients or volunteers and have not revealed whether ceramide accumulation and/or altered sphingolipid metabolism predicts insulin resistance in lean or obese individuals. Again, biopsied tissues subjected to lipidomic analyses will be essential in defining the “sphingolipidsome” in controlled and poorly controlled diabetic patients, and establishing the contributions of specific sphingolipid metabolites to diabetic complications. Analysis of multiple sphingolipid species has the potential to define diabetic complications as a consequence of global dysfunctional sphingolipid metabolism. Moreover, the integrated use of lipidomics and metabolomics offers the potential to identify novel surrogate biomarkers of diabetes that can be monitored during pharmacological or nutritional treatment regimens.

## Diabetic Pancreatic Dysfunction and Sphingolipids

The primary defect in Type 1 patients is autoimmune-mediated destruction of pancreatic beta cells. Deterioration of beta cell function in Type 2 diabetes, commonly referred to as beta cell exhaustion, occurs as a result of a compensatory mechanism by which the pancreas responds to global insulin resistance. Similar to insulin responsive tissues, altered sphingolipid metabolism or metabolites have been observed in pancreas tissue from diabetic animal models. Again, more importantly, pancreatic beta cell destruction can be reduced by pharmacological strategies that reduce sphingolipid metabolism. Serine palmitoyltransferase is upregulated in pancreatic islet cells of ZDF rats and inhibition of serine palmitoyltransferase reduces beta cell death.<sup>41,42</sup>

Consistent with elevated sphingolipid metabolism, ceramide is elevated in prediabetic and diabetic islets of ZDF rats. Mechanistically, palmitate (a de novo ceramide synthesis precursor) blocks insulin gene expression in primary rat islets<sup>43</sup> and induces beta-cell death,<sup>44</sup> possibly via de novo ceramide production. An alternative mechanism implicates reactive oxygen species in ceramide-induced pancreatic dysfunction. Treatment of ZDF rats with the antioxidant, NAC (N-acetylcysteine) between 6 and 12 weeks of age prevented beta-cell failure and ameliorated the hyperglycemic state.<sup>45</sup> Sphingolipid metabolites contribute to the regulation of cellular redox homeostasis, and reactive oxygen species scavengers have been demonstrated to prevent ceramide-induced cell death.<sup>9</sup> It is of note that while ceramide may be destructive to the pancreas that sphingosine-1-phosphate (S1P) and dihydroS1P may actually promote beta-cell survival and insulin secretion.<sup>46,47</sup> Yet, these S1P data must be interpreted with caution, as discussed later S1P may also exacerbate diabetic complications.

Altered levels of specific glycosylated metabolites of ceramide may ultimately be shown to modulate pancreatic islet cell destruction. In the pancreas of STZ-diabetic rats, c-series gangliosides (GT3, GT2, GQ1c, GP1c) are drastically reduced.<sup>32</sup> Similarly, in *ob/ob* and *db/db* mice, models of Type 2 diabetes, C<sub>16:0</sub> sulfatides are drastically diminished in the pancreas.<sup>48</sup> More importantly, treatment of ZDF rats with C<sub>16:0</sub> sulfatides increased the amount of insulin in the blood and improved the first-phase insulin response.<sup>49</sup> Such an effect may be mediated through activation of potassium channels to increase insulin secretion<sup>50</sup> and/or serving as a molecular chaperone for insulin.<sup>51</sup> In addition to decreases or increases in specific sphingolipid metabolites, pancreatic dysfunction may also reflect an immunological component that targets these altered glycosphingolipid metabolites. Autoantibodies to sulfatides, GT3, GD3 and GM2-1 have been identified in patients with Type 1 diabetes and may target immune cells to the pancreas and contribute to their destruction.<sup>52</sup> Similarly, ganglioside-specific autoantibodies to GM3 have also been found in patients with Type 2 diabetes.<sup>53</sup> Autoimmunity may not be restricted to the pancreas. GM1 autoantibodies have been found in both Type 1 and Type 2 patients with peripheral neuropathy.<sup>54</sup> In fact, increased titers of autoantibodies to GM1, GD1b, GD1a and sulfatides correlated with more pronounced neuropathic changes.<sup>55</sup>

## Diabetic Cardiovascular Dysfunction and Sphingolipids

Cardiovascular disease is the major cause of death of patients with diabetes. Both Type 1 and 2 patients have an increased incidence and severity of atherosclerosis, myocardial infarctions and strokes that is exacerbated by poor glycemic control or obesity. Importantly, a recent clinical trial has shown that the sphingolipid metabolite, S1P, is more predictive of obstructive coronary artery disease than other well-established risk factors, including age, sex, family history, diabetes, lipid profile and hypertension.<sup>56</sup> The heart and aorta from the Type 1 STZ-induced diabetic rat demonstrated increased sphingosine kinase activity, which was diminished in animals on an insulin pump.<sup>57</sup> Mechanistically, hyperglycemia induces sphingosine kinase activity, corresponding S1P levels and high glucose induced-leukocyte adhesion to endothelial cells was blocked by overexpression of a kinase-dead sphingosine kinase 1 mutant.<sup>57</sup> In contrast to these reports, in the Type 1 nonobese diabetic (NOD) mouse model, S1P prevented monocyte/endothelial interactions through S1P1 receptor activation.<sup>58</sup> Also, in Type 2 *KK/Ay* diabetic mice, sphingosine kinase 1 gene delivery by adenoviruses via intravenous injection, reduced blood glucose and

improved plasma lipid profiles (reduced cholesterol, triglycerides, LDL, nonesterified fatty acids and increased HDL), and prevented cardiac injury.<sup>59</sup> Moreover, subcutaneous injection of sphingosine-1-phosphate also has been shown to improve wound healing through improved vascularization of the wounded tissue.<sup>60</sup> The protective or detrimental actions of S1P have not as yet been completely defined in diabetes and will need to be interpreted in light of future studies that will utilize well defined selective S1P receptor modulators in clinical models.

The role of ceramide to contribute to cardiovascular disease in diabetic patients is also not well documented. Acid sphingomyelinase activity levels are elevated in the blood of Type 2 diabetic patients.<sup>61</sup> While the link to elevated circulating sphingomyelinase in diabetes is unknown, sphingomyelinase (and presumably ceramide) has been linked to the development of atherosclerosis.<sup>62</sup> In addition, sphingomyelinase inhibition as well as diminishing ceramide levels through preconditioning can diminish apoptosis and infarct size after cardiac ischemia.<sup>63</sup> Ceramide and other sphingolipid metabolites has also been shown to be a major component of lipoprotein vesicles.<sup>64</sup> Yet, intriguing data have suggested that ceramide nanofilms coated on balloon embolectomy catheters actually prevent coronary and carotid neointimal hyperplasia after vascular trauma in porcine and rabbit models.<sup>65,66</sup> These disparate findings might suggest that ceramide itself decreases vascular smooth muscle cell growth, while ceramide metabolites, including ceramide-1-phosphate or sphingosine-1-phosphate exacerbate restenotic or atherosclerotic injury. In fact, ceramide coated catheters promote wound healing responses, possibly as a consequence of metabolism of ceramide to other sphingoid metabolites within the endothelium.<sup>66</sup> Consistent with these interpretations, recent studies have suggested a novel anti-inflammatory antileukocytic effect for nanoliposomal ceramide, but not ceramide-1-phosphate or sphingosine-1-phosphate) in models of corneal keratitis.<sup>67</sup>

## Diabetic Nephropathy and Sphingolipids

Diabetic nephropathy is one of the most frequent causes of renal dysfunction. Diabetic nephropathy results from angiopathy of the capillaries in the glomeruli. Diabetes also causes an initial mesangial cell proliferation followed by growth arrest and hypertrophy. This contributes to an overproduction of extracellular matrix proteins, resulting sequentially in impaired blood filtration, increased proteinuria and renal failure.

Again, evidences implicate altered sphingolipids contributing to diabetic dysfunction of peripheral organs. The paradigm continues that pharmacological strategies to restore sphingolipid metabolism to basal levels will dissipate diabetic complications in the kidney. Accumulation of glucosylceramides at the expense of ceramide in streptozotocin (STZ)-induced diabetes was shown to contribute to renal hypertrophy.<sup>68</sup> Elevation of the ganglioside GM3 was also observed. More importantly, an inhibitor of glucosylceramide synthase, PMP, limited diabetes-induced mesangial cell hypertrophy and decreased glomeruli volume.<sup>68</sup> Such increases in glycosphingolipids have also been implicated in renal hypertrophy/diabetic nephropathy, possibly mediated by advanced glycation end products (AGEs).<sup>15,68</sup> Yet, controversy again exists in the literature, as ganglioside content, particularly GM3 and sialic acid content, is decreased from glomeruli of STZ-induced diabetic rats,<sup>69</sup> in contrast to *Zador et al.*<sup>68</sup> The answers to these disparities may again need to include a more comprehensive, integrated lipidomic approach, reflecting simultaneous measurements of multiple sphingolipid metabolites. Similar to glucosylceramide metabolites, phosphorylated sphingosine metabolites have also been implicated in glomerular mesangial cell proliferation, concomitant with increased neutral ceramidase and sphingosine kinase activities.<sup>70</sup>

## Diabetic Retinopathy and Sphingolipids

Diabetic retinopathy is the leading cause of blindness among working age adults. A misconception about diabetic retinopathy is that it is solely a microvascular disease of the retina based upon visual clinical manifestations such as hemorrhages, microaneurysms, exudates, edema and neovascularization. A large body of work has demonstrated that diabetic retinopathy is a complex complication that, in addition to the microvasculature (endothelial cells and pericytes), also affects macroglial cells (Müller and astrocytes), microglia cells and neurons.<sup>71</sup> The vascular changes actually occur

later on in the pathogenesis of this disease and thus understanding mechanisms that contribute to earlier pathology may reveal new therapeutic targets.

As already discussed, altered sphingolipids may be involved in insulin resistance in peripheral organs. In fact, the EURODIAB Prospective Complications Study showed that insulin resistance is a major predictive factor for the development of retinopathy in Type 1 diabetes.<sup>4</sup> It should be noted that the retina is an insulin responsive tissue and exhibits high basal insulin receptor activity, reminiscent of postprandial livers.<sup>72</sup> Furthermore, retinal insulin receptor and kinases that can be downstream (PI<sub>3</sub>K, Akt and p70 S6 kinase) are impaired in models of Type 1 diabetes<sup>73,74</sup> and can only be partially resored with insulin therapy.<sup>75</sup> In addition, retina-specific insulin receptor deletion impairs the ability of the retina to withstand light induced stress.<sup>76</sup> The roles of sphingolipids in this insulin-signaling impairment are unknown, but in vitro evidence in a retinal neuronal cell line demonstrates that glucosylceramide synthase inhibitors can augment insulin signaling.<sup>10</sup> In neuronal tissues, glycosphingolipids have been implicated in increasing sensitivity to neurotoxic agents such as the excitatory amino acid neurotransmitter, glutamate,<sup>77</sup> a potential contributor to diabetic retinopathy.<sup>75,78</sup> Thus, it is not surprising that lipidomic analysis has recently revealed dysregulated sphingolipid metabolism in retinal tissue from Type 1 diabetic models. Specifically, in the streptozotocin-induced model of diabetes<sup>10</sup> as well as the Ins2<sup>Akita</sup> mouse model (unpublished observations), a decrease in ceramides with a corresponding increase in glucosylceramides<sup>10</sup> has been observed. The in vivo consequence of this is still unknown; however, as discussed above, mounting in vitro evidence has implicated elevated glucosylceramide metabolites in the inflammatory or immunomodulatory regulation of retinal and pancreatic cell death, cardiovascular abnormalities and dysfunctional insulin signaling.

Similar to glycosphingolipid metabolites, phosphorylated metabolites (S1P) may also contribute to diabetic retinopathy. For example, a sphingosine kinase inhibitor has been demonstrated to inhibit retinal vascular permeability in streptozotocin-induced diabetic rats.<sup>79</sup> Knockout of the S1P2 receptor, but not S1P1 or S1P3 receptors, suppressed some inflammatory mediators (COX-2 and eNOS) and shifted the phenotype of vascular changes from pathogenic to normal in a model of retinopathy of prematurity.<sup>80</sup> Furthermore, a monoclonal antibody that binds to S1P (sonopczumab), suppressed laser-induced choroidal revascularization in mice and did not demonstrate any overt toxicology to the retina in nonhuman primates.<sup>81</sup> Often, these models of induced vascularization serve as surrogate models to study the mechanisms by which dysfunctional vasculature contribute to diabetic retinopathy. Our studies have not found significant differences in retinal S1P, but we (unpublished observations from the streptozotocin-induced diabetic rats and Ins2<sup>Akita</sup> diabetic mice) have observed elevated S1P from the plasma of these Type 1 diabetes models. Thus, it may be that retinal vascular alterations may be influenced by S1P in the blood. In fact, elevated sphingosine kinase activity within the retina may not be sufficient to induce retinopathy, as intraocular administration of adenoviruses carrying the sphingosine kinase gene was unable to induce neovascularization.<sup>82</sup>

## Therapeutics That Target Sphingolipid Metabolism or Sphingolipid Signaling in Diabetes

Given the large body of in vitro evidence that glycosphingolipids exacerbate diabetic complications, it is not surprising that treatment strategies that inhibit glucosylceramide synthase (GCS) have proven effective in diabetic animal models. Treatment of *ob/ob* mice with the selective GCS inhibitor, AMP-DNM, lowered blood glucose levels, improved oral glucose tolerance, reduced hemoglobin A1C and improved insulin sensitivity in muscle and liver.<sup>23</sup> Similar beneficial metabolic effects were observed in high fat—fed mice and ZDF rats.<sup>23</sup> Furthermore, AMP-DNM in normal mice lowered triglyceride and cholesterol levels in plasma.<sup>83</sup> Analogous results were also observed with a structurally distinct glucosylceramide synthase inhibitor Genz-123346 in ZDF rats and diet-induced obese mice, including diminished blood glucose, improved glucose tolerance and reduced A1C levels. Furthermore, this inhibitor

limited the loss of pancreatic beta-cell function.<sup>25</sup> Yet, interpretation of these provocative *in vivo* studies must be cautioned, as administration of specific exogenous glycosphingolipids could have beneficial effects. For example, GM1 administration to NOD mice reduced the incidence of diabetes onset and the degree of pancreatic islet injury.<sup>84</sup> Treatment with other glycosphingolipids, including sulfatide and galactosylceramide, were also able to decrease the incidence of diabetes in NOD mice.<sup>85</sup> Likewise, synthetic homologs of nonphysiological alpha-galactosylceramides, such as OCH and KRN7000, have also proven beneficial in NOD mice, possibly through several mechanisms which include increasing T<sub>H</sub>2 responses and recruitment of tolerogenic dendritic cells.<sup>86-90</sup>

Likewise, treatment regimens to regulate S1P mass, sphingosine kinase activity or S1P receptor activity must be coached in light of the conflicting evidence suggesting pancreatic islet survival despite some evidence supporting exacerbation of cardiovascular and microvascular complications by S1P. FTY720 (Fingolimod) is a myriocin analog with structural similarity to sphingosine, that when phosphorylated may serve as a S1P receptor modulator.<sup>91</sup> FTY720 has shown significant immunomodulatory actions, limiting lymphocyte egress from lymphoid tissues.<sup>91</sup> FTY720 has been shown to limit autoimmune diabetes in rats<sup>92</sup> and mice.<sup>93-95</sup> Furthermore, survival of both islet allografts and xenografts in rodents and nonhuman primates were augmented when treated with FTY720.<sup>96-99</sup> Further studies will prove if the immunological or antiinflammatory actions of FTY720 in diabetic models are manifested by alterations in S1P receptor signaling or through inhibition of cytosolic phospholipase A<sub>2</sub><sup>100</sup> or ceramide synthase<sup>101</sup> and/or possibly other mechanisms.

Several studies are now correlating the actions of patient approved therapies with altered sphingolipid metabolism. In addition to tight insulin control and proper diet, Type 2 patients are further managed by strategies to increase pancreatic insulin secretion (sulfonylureas or meglitinides), decrease hepatic gluconeogenesis (biguanides/metformin), improve insulin sensitivity in peripheral tissues (thiazolidinediones (Peroxisome proliferator-activated receptor-gamma (PPAR-gamma)) and to prevent breakdown of complex carbohydrates into simple sugars (alpha-glucosidase inhibitors). It is not surprising that thiazolidinediones, which regulate a transcriptional complex for insulin-responsive genes that control glucose and lipid metabolism, could affect sphingolipid metabolism. Yet, the literature reveal inconsistent findings. Various thiazolidinediones, (pioglitazone, troglitazone and rosiglitazone) can reduce ceramide levels in rat and mouse skeletal muscle, likely through *de novo* inhibition.<sup>102-104</sup> Similarly, troglitazone reduced ceramide level in the heart of ZDF rats.<sup>105</sup> In contrast, pioglitazone has been shown to increase *de novo* ceramide synthesis in rat hearts.<sup>106</sup> Also, transcriptome analyses of rosiglitazone-treated cardiac tissue of *db/db* mice revealed increases in *ASAH2* (ceramidase), *ST3GAL5* (GM3 biosynthesis) and *B4GALNT1* (GM2 and GD2 biosynthesis).<sup>107</sup> Thus, it is possible that specific thiazolidinediones could increase sphingolipid metabolism and possibly explain recent observations that thiazolidinediones have been linked to increased cardiovascular complications in diabetic patients.<sup>108</sup> In a similar manner, Metformin also alters sphingolipid metabolism, reducing ceramide content in skeletal muscle of high fat-fed rats, which was further augmented with exercise.<sup>109</sup> Again, a comprehensive “omic” approach, integrating LC/MS and NMR-based lipidomics and metabolomics, has the power to define sphingolipid and other metabolites as novel surrogate biomarkers that can be monitored during therapy.

## Conclusion

The review identifies dysfunctional sphingolipid metabolism as contributors to diabetes and diabetic complications. Novel therapeutic strategies that target dysfunctional sphingolipid metabolism are identified that could decrease diabetic complications. Additional targets and/or biomarkers can be identified in animal models by integrating lipidomic approaches with functional genomics, proteomics and metabolomics, coupled with detailed organ-specific natural histories. In additional, integrated “omic” approaches coupled with therapeutic interventions can be used to firmly determine mechanisms underlying diabetic complications.

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