Roles of ceramide and sphingolipids in pancreatic β-cell function and dysfunction

Ebru Boslem,1,2 Peter J. Meikle3 and Trevor J. Biden1,2,*

1Diabetes and Obesity Program; Garvan Institute of Medical Research; Darlinghurst, NSW Australia; 2St. Vincent’s Clinical School; Faculty of Medicine; University of New South Wales; Sydney, NSW Australia; 3Baker IDI Heart and Diabetes Institute; Melbourne, VIC Australia

Keywords: pancreatic β-cell, islet, ceramide, endoplasmic reticulum stress, trafficking, lipidomics, lipotoxicity, palmitate, apoptosis, type 1 diabetes, type 2 diabetes

Abbreviations: CerS, ceramide synthase; DRM, detergent resistant membrane; ER, endoplasmic reticulum; FA, fatty acid; GalCer, galactosylceramide; GluCer, glucosylceramide; IAPP, islet amyloid polypeptide; IL-1β, interleukin-1β; LPS, lipopolysaccharide; NOD, non-obese diabetic; SMS, sphingomyelin synthase; SIP, sphingosine-1-phosphate; SM, sphingomyelin; SPT, serine palmitoyltransferase; T1D, type 1 diabetes; T2D, type 2 diabetes; TNFα, tumor necrosis factor α; ZDF, Zucker diabetic fatty

Recent technical advances have re-invigorated the study of sphingolipid metabolism in general, and helped to highlight the varied and important roles that sphingolipids play in pancreatic β-cells. Sphingolipid metabolites such as ceramide, glycosphingolipids, sphingosine-1-phosphate and gangliosides modulate many β-cell signaling pathways and processes implicated in β-cell diabetic disease such as apoptosis, β-cell cytokine secretion, ER-to-golgi vesicular trafficking, islet autoimmunity and insulin gene expression. They are particularly relevant to lipotoxicity. Moreover, the de novo synthesis of sphingolipids occurs on many subcellular membranes, in parallel to secretory vesicle formation, traffic and granule maturation events. Indeed, the composition of the plasma membrane, determined by the activity of neutral sphingomyelinases, affects β-cell excitability and potentially insulin exocytosis while another glycosphingolipid, sulfatide, determines the stability of insulin crystals in granules. Most importantly, sphingolipid metabolism on internal membranes is also strongly implicated in regulating β-cell apoptosis.

Together with glycerophospholipids and neutral lipids, sphingolipids constitute one of the major classes within the mammalian lipidome. Sphingolipids are structurally diverse, synthesized and modified in a wide variety of cellular localizations, and play multiple roles acting as both signaling molecules and structural components of membranes.1–3 Perhaps to an even greater extent than for the other lipid classes, recent methodological advances have provided great insights into biological roles of particular sphingolipid metabolites, helped resolve older controversies in this field, and generated unexpected avenues for future investigation.1–3 As with other biological systems, there is a disparate and long-standing literature that implicates sphingolipids in β-cell dysfunction (predominately). Until recently, however, a coherent picture has been difficult to establish, primarily due to methodological limitations. A handfull of newer studies, using the latest genetic and lipidomic approaches, have provided a more comprehensive analyses of sphingolipid metabolism in β-cell biology, thereby generating new insights that both aid in the re-interpretation of the earlier work, and raise new experimental questions to be addressed. Our aim here is to review the current understanding of sphingolipid biology in β-cells and highlight some areas where further knowledge is needed.

Sphingolipid Metabolism

The central sphingolipid metabolite is ceramide, comprising a sphingoid base as a backbone to which is attached a single fatty acid (FA) side-chain of varying length and degree of saturation1–3 (see Fig. 1). The sphingoid backbone can either be synthesized de novo (sphinganine) or salvaged (as sphingosine) via the breakdown of more complex sphingolipids.4 Although the latter pathway is thought to pre-dominate in most differentiated cells under normal conditions,4 de novo synthesis is the ultimate precursor of cellular ceramide and may be especially important under certain pathophysiological conditions such as lipotoxicity (see below).5 The first and rate-limiting step of de novo synthesis is catalyzed by serine palmitoyltransferase (SPT) and condenses two abundant nutrient metabolites, serine and palmitoyl-CoA.5 Sphinganine is generated after several further modifications and serves (as does sphingosine from the salvage pathway) as a substrate for ceramide synthase (CerS formerly known as Lass). This is now known to comprise a family of six enzymes, which display differing selectivity for the type of FA chain they attach to the sphingoid base.6 As such, this reaction accounts for the diversity of sphingolipid isoforms in different tissues and under different conditions.6,7 For example, C18 ceramide (containing a stearic acid side-chain) is abundant in many tissues such as brain and muscle, but much less

*Recent work demonstrates that ceramide is present in the circulation, so its uptake might potentially contribute significantly to total ceramide in a small well-vascularized tissue bed such as pancreatic islets. To our knowledge, however, this possibility has not been addressed specifically.
Ceramide-1-phosphate. All of these compounds can in turn be catabolized, thereby regenerating ceramide which can be further catabolized to sphingosine, via the action of a variety of ceramide synthases (CerS). This occurs in a number of cellular compartments as part of the salvage pathway. Sphingosine can be reconverted to ceramide, as discussed above, or phosphorylated by sphingosine kinases to form sphingosine-1-phosphate (S1P).

A major determinant of the fate of ceramide is the cellular compartmentalization of many of the metabolic pathways described above in references 10 and 11. All of the steps of de novo synthesis, and the regeneration of ceramide from sphingosine, take place on the external leaflet of the endoplasmic reticulum (ER). For generation of GluCer and SM this ceramide must be transported to the Golgi compartment. Unlike SM, which is produced on the surface of the golgi, GluCer is found in the lumen, implying a requirement for transmembrane transport. Whether this occurs at the golgi itself via a flippase, or whether so in (at least murine) β-cells (see below). The product of CerS in the de novo pathway, dihydroceramide, must be desaturated to generate ceramide, but this enzyme produces ceramide directly when the salvaged base sphingosine is used as a substrate.

Ceramide serves as the precursor for a series of more complex sphingolipids (Fig. 2) of which the most abundant in mass terms is sphingomyelin (SM). This is generated (along with diacylglycerol) from the condensation of ceramide with the phospholipid phosphatidylcholine, or in some cases phosphatidylethanolamine. This reaction is catalyzed by one of two SM synthase enzymes (SMS1 or 2). The other major route of ceramide metabolism is via glycosylation, which gives rise to a range of products (glycosphingolipids) that vary depending on the type and number of sugar residues attached. The most abundant mono-hexosyl derivatives are glucosylceramide (GluCer) and galactosylceramide (GalCer), which are formed by the actions of GluCer synthase and ceramide galactosyltransferase respectively. GluCer serves as the precursor for a plethora of compounds of the ganglioside family, whereas GalCer can be sulfated to produce sulfatide or sialosylated to GM4 ganglioside. Finally, ceramide can also be phosphorylated by the enzyme ceramide kinase to generate ceramide-1-phosphate. All of these compounds can in turn be catabolized, thereby regenerating ceramide which can be further catabolized to sphingosine, via the action of a variety of ceramide synthases (CerS). This occurs in a number of cellular compartments as part of the salvage pathway. Sphingosine can be reconverted to ceramide, as discussed above, or phosphorylated by sphingosine kinases to form sphingosine-1-phosphate (S1P).

A major determinant of the fate of ceramide is the cellular compartmentalization of many of the metabolic pathways described above in references 10 and 11. All of the steps of de novo synthesis, and the regeneration of ceramide from sphingosine, take place on the external leaflet of the endoplasmic reticulum (ER). For generation of GluCer and SM this ceramide must be transported to the Golgi compartment. Unlike SM, which is produced on the surface of the golgi, GluCer is found in the lumen, implying a requirement for transmembrane transport. Whether this occurs at the golgi itself via a flippase, or whether
that serve as platforms for specialized signaling functions, and to this end SM and cholesterol concentrations are tightly and coordinately regulated at multiple levels. Activation of (especially neutral) SMases also occurs in specific signaling cascades, especially those downstream of receptors for cytokines such as interleukin-1β (IL-1β) and tumor necrosis factorα (TNFα).

**Sphingolipids and Type 1 Diabetes**

β-cell cytokine signaling. Early investigations of ceramide generation in β-cells focused in the area of Type 1 diabetes (T1D), prompted by studies of inflammatory signaling cascades in other cell systems. It was first shown that exogenous ceramide provided...
either directly or generated following addition of SMase was sufficient to reproduce some of the effects of IL-1β in repressing insulin production and promoting β-cell death in fetal islets and β-cell lines. These treatments did not activate the NFκB pathway that mediates induction of iNOS and generation of nitric oxide. Direct evidence for activation of SMase by cytokines, however, has been harder to demonstrate using β-cells than in other systems. IL-1β reportedly promoted a transient (5 min) decrease in SM in clonal RINm5F cells, but this was not consistent with other studies using rat islets or β-TC3 cells possibly explained by technical limitations of the tracer methodologies that had been employed in all three reports. Moreover, increases in ceramide mass under these conditions were only observed in RINm5F cells, but not β-TC3 cells. These modest or non-significant effects are perhaps consistent with observations that cytokines can activate both neutral and acidic ceramidases in β-cells, which would tend to counteract any ceramide accumulation. Pharmacological inhibitors of ceramide synthesis or SMase activity also failed to alter IL-1β-stimulated responses in a rat β-cell line, although the potentiation of IL-1β effects on cell death by TNFα might involve an enhanced sensitivity to basal acid SMase. Finally, IL-1β and TNFα augment SIP in β-cells, which might represent a protective response against cell death via SIP receptor mediated pathways. The overall evidence would thus tend to argue against alterations in sphingolipid metabolism playing major roles in cytokine-mediated β-cell destruction in the context of T1D. However, this conclusion is perhaps not definitive and this topic might benefit from further investigation focusing on longer-term effects of cytokines, employing more sensitive assay methods, and extending measurements to metabolites in addition to ceramide and SM.

**Immune cell infiltration in type 1 diabetes.** The in vitro studies discussed above focused on the downstream responses of β-cells to the extracellular milieu of cytotoxic cytokines encountered during autoimmune cellular destruction of the islets in T1D. These cytokines come from the many different immune cell types that migrate to the islet in response to islet self-antigens. Fewer studies have investigated the effect of altering sphingolipid metabolism from the reverse perspective; whether alterations in the sphingolipid content of β-cells, immune cells or serum might modulate immune cell infiltration, or otherwise act upstream of cytokine release. This seems feasible given known effects of sphingolipid modulators in other inflammatory diseases, and some recent indications that downregulation of certain SM species in the circulation is associated with decreased progression to T1D in both humans and non-obese diabetic (NOD) mice. However, SM was only one of several lipids that were altered and this observation was not pursued mechanistically. Other studies have focused on glycosphingolipids that can serve as ligands for cell surface receptors. One such is the macrophage scavenger receptor, CD14, which is primarily expressed on monocytes, macrophages and other immune cell types with lipopolysaccharide (LPS) as its natural ligand. CD14 is also expressed on a subset of β-cells from human islets. In addition to LPS, GalCer, acting via the CD14 receptor and presumably in an auto/paracrine manner, was also found to also stimulate β-cells to secrete TNFα, IL-1β and IL-8. Conversely sulfatide, another endogenous glycolipid found in insulin secretory granules, inhibited LPS effects mediated via the CD14 receptor. However, the real physiological role of CD14 on the β-cell surface and whether it contributes to β-cell death seen in diabetes is as yet unclear.

There is some evidence that S1P may be critical in the protection of β-cell destruction by cytokines. Recent studies of islet allografts in diabetic mice demonstrated that the S1P receptor agonist, FTY720, enabled long-term survival of these grafts. This S1P analog was found to prevent lymphoangiogenesis that was key to subsequent T cell infiltration, inflammation and β-cell destruction of these allografts. Long-term FTY720 treatment also prevented the onset of diabetes in rodent models of T1D by halting islet immune cell infiltration and pro-inflammatory cytokine-mediated destruction. Modulation of S1P signaling may therefore be an attractive candidate for future studies focused on the preservation of islet architecture for the prevention of T1D.

There is a longer-standing literature suggesting that various sphingolipids in β-cells act as auto-antigens. Dotta and colleagues identified GM2-1 as a pancreatic islet specific species of ganglioside that was a target of IgG islet cell autoantibodies. The presence of these antibodies not only correlated strongly with progression to diabetes in relatives of subjects with T1D, but the content of this glycolipid was hyperexpressed in the NOD mouse. Furthermore, this ganglioside is present in β-cell secretory granules, similar to other autoantigens in Type 1 diabetes such as insulin and carboxypeptidase H. More recently, cell surface patches of SM were shown to be the epitope for IC2 a monoclonal antibody that specifically recognizes the surface of β-cells. Since this antibody was developed from the diabetic BB rat, which possesses circulating auto-antibodies to β-cells, these findings at least raise the possibility that cell surface SM might be involved in β-cell autoimmunity.

**Sphingolipids and Type 2 Diabetes**

Ceramide and β-cell apoptosis. Although ceramide had long been implicated in apoptosis, its involvement in lipotoxicity was first suggested by studies in hemopoietic cells showing that saturated but not unsaturated FAs induced cell death, which was associated with enhanced de novo synthesis of ceramide. This concept was extended to β-cells in pioneering work from the Unger laboratory using the Zucker diabetic fatty (ZDF) rat model of T2D. Key evidence included observations that ceramide content was augmented in islets of the diabetic animals and that the accompanying enhancement of apoptosis was reduced by fumonisin B1, an inhibitor of SPT. Tracer analyses implicated an enhancement of de novo ceramide synthesis, which was consistent with observed increases in SPT expression in islets of ZDF rats, and islets of control rats treated with elevated FAs. In vivo treatment of diabetic animals with an SPT inhibitor also reduced apoptosis of islets assayed ex vivo. This work proved seminal for the future development of the lipotoxicity field, not only in β-cells, but also in the broader context of the whole metabolic syndrome. In retrospect, however, it could be argued that the ZDF model
represented an extreme case in that these islets accumulate massive amounts of lipid. Indeed, the partitioning of palmitate tracer into ceramide in islets of ZDF rats greatly outweighs the amount that is oxidized. More recent work using another animal model of the metabolic syndrome has shown only modest increases in ceramide late in the progression of β-cell failure, and in fact β-cells appear relatively resistant to lipid overload as compared with other tissues in this model. A milder and much more commonly employed model of lipotoxicity comprises the chronic exposure of β-cells to elevated concentrations of FAs in vitro. A caveat of this approach is that the actual concentration of free FA depends on the ratio of total FA to albumin in the medium, and so can vary markedly between protocols and in a manner that is not always easy to evaluate. The discussion below is focused on reports that are less likely to be confounded by the use of very high levels of free FAs. The general conclusion from these studies is that whereas both saturated and unsaturated FAs promote defects in glucose-stimulated insulin secretion, β-cell apoptosis is much more restricted to saturated FAs, and indeed unsaturated FAs appear to be protective in this case. Islets from ZDF rats appear to be an exception as these were sensitive to mixtures (2:1 unsaturated to saturated) of FA but this might reflect the high overall concentrations employed or be a special feature of the ZDF model as discussed above. Although a variety of mechanisms have been proposed to account for β-cell lipo-apoptosis one of the best substantiated relates to ER stress. Evidence in support of this hypothesis includes the observation that ER stress is preferentially triggered by saturated FAs, and that (at least mild) lipo-apoptosis is reduced by inhibition of ER stress signaling. A separate and longer standing body of work has pointed to involvement of ceramide synthesis in β-cell apoptosis due to saturated FAs, consistent with the substrate preference of SPT for palmitate. This has been supported by recent observations showing that 1H-serine incorporation into ceramide (also catalyzed by SPT) was augmented by pre-exposure to palmitate. Enzymatic measurements of ceramide mass, however, have generally reported only modest increases with FA pretreatment unless glucose is concomitantly elevated. And yet inhibitors of de novo ceramide synthesis, acting at the level of either SPT (cycloserine or myriocin) or CerS (fumonisin B1), generally reduce lipo-apoptosis in β-cells although this is not universally observed, particularly with higher concentrations of FAs or when combined with elevated glucose. A possible explanation for these somewhat disparate observations might be that apoptosis is triggered by increases in ceramide that are restricted to particular species or cellular locations, and which are less apparent when the total cellular content is assayed. Alternatively, ceramide generated via different pathways (de novo synthesis vs. salvage pathway or SMase activity) might differ in its apoptotic potential. Finally, the toxic intermediate might not be ceramide itself, but a metabolite of it.

To some extent these possibilities have been resolved by two recent studies using β-cell lines that combine genetic modulation of sphingolipid metabolism with accurate quantification of ceramide mass using mass spectroscopy. Our work, aimed at identifying the lipid metabolite responsible for lipotoxic ER stress, comprised a comprehensive and unbiased lipidomic screen and largely excluded involvement of phospholipids or neutral lipids. Following pretreatment of the MIN6 β-cells with palmitate for 48 h we were unable to detect increases in major ceramide species at the whole cell level, but hexosylceramide (predominately GluCer) was augmented, as was flux through the de novo synthetic pathway as determined by incorporation of 1H-serine. However, overexpression of GluCer synthase to enhance conversion of ceramide to GluCer did not exacerbate lipotoxicity, but partially protected against apoptosis and induction of ER stress markers. This intervention also overcame the defects in ER-togolgi protein trafficking due to palmitate pretreatment that we had previously postulated contributes to lipotoxic ER stress through protein overload. These results suggested that chronic palmitate induces ER stress via increases in a subpool of ceramide, without altering total ceramide mass. Broadly consistent with our findings Veret et al. were able to show increases in ceramide using INS-1 cells treated with higher concentrations of palmitate, but that this effect was maximal at 12 h and was no longer apparent at 48 h, except in the presence of elevated glucose concentrations. The increased ceramide was observed with most side-chain species, not just C16, and appeared to arise from the de novo synthetic pathway because there were accompanying increases in di-hydroceramide and sphinganine, but not sphingosine. In this model, however, there was minimal lipo-apoptosis unless glucose was also augmented and this correlated best with accumulation of C18:0, C22:0 and C24:1 ceramide species, also consistent with increased expression of CerS4 under these conditions. Finally, up or downregulation of CerS4 expression enhanced or reduced glucolipotoxicity respectively. The conclusion that C18:0 and C22:0 Cer are the toxic metabolites would be consistent with unpublished data that these species are also selectively increased in our model of lipotoxicity. One caveat, however, is that C18:0 and C22:0 ceramide are very minor metabolites in MIN6 cells and mouse islets (Boslem E, Meikle PJ, Biden TJ, unpublished). Indeed there may be pronounced species differences since C16:0 greatly abounds over C24:0 ceramide in mouse β-cells (Boslem E, Meikle PJ, Biden TJ, unpublished), but the opposite is true in rat INS-1 cells. It will thus be important to investigate key aspects of sphingolipid metabolism in rat, and ultimately human islets, to confirm findings obtained in the cell lines, and to determine which of the rodent species is the better model for human islets.

Although de novo synthesis appears to be the major mechanism for lipotoxic ceramide generation there are other possibilities. Ramanadham and co-authors have demonstrated that strong ER stress, triggered either pharmacologically in vitro or using a genetic model of pro-insulin misfolding itself promotes ceramide accumulation secondary to the activation of
neutral SMase 2. In this instance, ceramide accumulation in the mitochondria would be one mechanism whereby ER stress might trigger apoptosis. Whether ceramide generated via de novo synthesis during lipotoxicity could also act in this manner (perhaps independently of ER stress) is yet to be determined. In contrast, using our milder lipotoxic model, ER stress was neither necessary nor sufficient for any of the observed alterations in lipid metabolites, including ceramide, SM and GluCer. Nevertheless it cannot be excluded that small, perhaps localized, increases in ceramide and decreases in SM might contribute to lipo-apoptosis, either up or downstream of ER stress. This will require detailed analyses of sphingolipid metabolites in different subcellular compartments, and testing the functional effects of manipulating those metabolites in selected compartments.

**Mechanisms of ceramide-induced apoptosis in β-cells.** Our studies based on the protection afforded by GluCer synthase overexpression clearly implicate ceramide as acting upstream of defective protein trafficking and the activation of lipotoxic ER stress. This mechanism does not appear to apply in liver, defective protein trafficking and the activation of lipotoxic ER overexpression clearly implicate ceramide as acting upstream of ER stress. This has not been assessed in liver. Nevertheless it cannot be excluded that small, perhaps localized, increases in ceramide and decreases in SM might contribute to lipo-apoptosis, either up or downstream of ER stress. This will require detailed analyses of sphingolipid metabolites in different subcellular compartments, and testing the functional effects of manipulating those metabolites in selected compartments.

As intimated above, ceramide could promote apoptosis by mechanisms other than ER stress, especially during glucolipotoxicity or very strong lipotoxicity. One such possibility would be disruption of mitochondrial function as mentioned previously in reference 62, and which has been implicated as causative in some aspects of defective β-cell function using SMS1 knockout mice. This is discussed further below. Although exogenous ceramide is sufficient to disrupt mitochondrial function there is currently no evidence linking ceramide accumulation in mitochondria to lipo-apoptosis in β-cells. Ceramide also has the ability to form large stable channels in membranes which directly affects membrane conductance and hence may have biological relevance at the mitochondria or plasma membrane, particularly in regard to cytochrome c release and apoptotic signaling. Another potential apoptotic mechanism would be alterations in plasma membrane DRMs. Indirect evidence for this comes from studies of β-cell apoptosis due to islet amyloid peptide (IAPP) accumulation of which is linked to the pathogenesis of T2D. In this instance IAPP appeared to augment ceramide, and apoptosis was not observed in islets of mice deficient in acid SMase. The latter enzyme was also required for IAPP-mediated inhibition of a K⁺ current that had previously been implicated in apoptosis and whose function might depend on clustering in DRMs. If confirmed such a mechanism would potentially apply to many signaling pathways in addition to the gating of K⁺ channels. A further means whereby ceramide might mediate β-cell lipotoxicity is via inhibition of the anti-apoptotic kinase, Akt, as best described in muscle. In β-cells, however, Akt is inhibited by both olate and palmitate, which might argue against involvement of ceramide because of the substrate specificity of SPT. Moreover, several labs have shown that chronic exposure to moderately elevated palmitate activates rather than inhibits Akt and high FA concentrations stimulate Akt phosphorylation at early time points, and only inhibit at 24 h or more. In muscle activation of the protein phosphatase PP2A is implicated in mediating the palmitate/ceramide effects on Akt. It is therefore conceivable that PP2A might contribute to β-cell lipotoxicity via substrates other than Akt, although this remains to be investigated.

**Ceramide and insulin gene expression.** Co-treatment with palmitate inhibits the augmentation of pro-insulin mRNA levels due to chronically elevated glucose. The fact that this did not seem to occur with olate and that ceramide was sufficient to repress insulin content potentially implicated de novo ceramide synthesis in this context. This was directly confirmed by Kelpe et al. who demonstrated that inhibitors of the de novo synthetic pathway overcome both the increases in ceramide due to glucolipotoxicity, as well as the accompanying reduction in pro-insulin gene expression. Mechanistically there appears to be a key role for the transcription factor C/EBPβ, whose expression is enhanced by palmitate but not olate and which competes with Pdx1 and MafA on the proinsulin gene promoter. Administration of exogenous ceramide or blockade of further ceramide metabolism in the presence of palmitate is sufficient to activate ERK or modulate proinsulin and C/EBPβ gene expression respectively. Whether ERK activation occurs upstream or independently of C/EBPβ remains to be resolved. To date, however, there is no direct evidence that ceramide accumulation is actually necessary for the enhancement of C/EBPβ function due to palmitate, although this would seem to be likely and could easily be tested by genetic or pharmacological intervention. With the use of lipidomic tools such as mass spectrometry, the contribution of endogenous ceramides to transcriptional regulation will be easier to elucidate, obviating the reliance on short-chain length exogenous ceramides (such as C2-ceramide), whose physiological relevance within the cell has often been questioned.

**Ceramide and insulin secretory defects.** In contrast to the strong evidence favoring ceramide as a mediator for lipo-apoptosis and reduced insulin gene expression, a corresponding role in defective insulin secretion appears less probable. Ceramide analogs have only marginal effects on glucose-stimulated insulin secretion and inhibitors of de novo ceramide synthesis do not overcome the secretory defects due to lipotoxicity. This is probably not surprising since unsaturated FAs (as well as saturated FAs) inhibit glucose-stimulated insulin secretion and these are not substrates for SPT. It is possible that chronic exposure to olate might augment ceramide through other mechanisms, perhaps via side-chain incorporation. This seems unlikely, however, since we observed no evidence for accumulation of sphingolipid metabolites in a palmitate-resistant subline of MIN6 cells, which display an augmented ratio of unsaturated FAs to saturated FAs due to enhanced expression of stearoyl CoA desaturase. Even so, it is conceivable that localized, or more subtle, alterations in sphingolipid metabolism might still impact on stimulus-secretion coupling. Indeed a whole body knockout of SMS1 resulted in the expected decreases in SM and increases in ceramide in β-cells and was accompanied by defects...
in insulin secretion and mitochondrial function and increased ROS production.\(^9\) However, because these mice also display lipodystrophy it is possible that there is disruptive lipid accumulation in β-cells secondary to increases in circulating FAs. Confirming whether this interesting secretory phenotype is truly intrinsic to the β-cell would require further investigation using tissue-specific knockout mice. However, knockdown or pharmacological inhibition both SMS1 and SMS2 in INS-1 has also been recently shown to inhibit insulin secretion in vitro.\(^8\) The mechanism in this instance appears to involve defective export of secretory proteins from the golgi to the plasma membrane. Whether this is explained by increased ceramide, or decreases in either SM or DAG, which are both products of SMS activity, was not addressed. Although taken together these studies suggest that SMS function is essential for the maintenance insulin secretion, it remains to be determined whether loss of SMS activity contributes to the secretory defects observed in the setting of diabetes.

The sequestration of SM and/or ceramide within DRMs post-stimulus, such as in the case of IAPP accumulation described above,\(^7\) promotes clustering of receptors and other plasma membrane proteins into these microdomains to augment downstream signaling. Recent work characterizing the expression, gating and association of potassium and calcium channels at the plasma membrane of β-cells has revealed a regulated compartmentalization of these ion channels into DRMs during the time-frame of insulin release.\(^9\) Voltage-gated Ca\(^2+\) channels (Ca\(_{v}1.2\)) and β\(_v\) channels (K\(_{v}2.1\)), two critical determinants of β-cell electrical excitability, are directly associated within these DRMs with exocytotic proteins such syntaxin 1A, SNAP-25 and VAMP2. Dissolution or disordering of those lipid microdomains with β-cyclodextrin (which removes cholesterol from the membrane), inhibited K\(_{v}2.1\) activity, but not Ca\(_{v}1.2\) channel activity and this actually enhanced single-cell exocytotic events and insulin secretion.\(^8\) It is therefore possible that alterations in sphingolipid metabolism that impact on DRMs could influence insulin secretion, but that this has yet to be tested directly.

Although it is unclear whether ceramide itself mediates defective insulin secretion, the minor yet highly physiologically active ceramide metabolite, S1P, has recently been shown to accumulate in response to glucose in both MIN6 and pancreatic islets.\(^7\) Furthermore, the activity of SK2 was responsible for this S1P accrual and its inhibition reduced GSIS in vitro and impaired insulin availability and glucose tolerance in vivo. The downstream signaling of S1P from its five G-protein coupled receptors may therefore play an essential and as yet to be defined role in GSIS.

**Glycosphingolipids in Type 2 Diabetes**

Glycosphingolipids such as GM3 have been implicated in T2D, most specifically in the development of insulin resistance.\(^9\) GM3 ganglioside synthase levels were found to be upregulated in Zucker fa/ta rat and ob/ob mouse models of T2D.\(^9\) Recently, studies in ZDF rats\(^9\) have observed cytoprotective effects within the pancreatic islets of these animals following dietary supplementation with small molecule glycosphingolipid synthase inhibitors Genz-123346\(^9\) and AMP-DNM.\(^9\) Both these studies reported a partial protection of β-cell content and islet architecture following chronic (10 week\(^9\) or 60 d\(^9\)) glycosphingolipid synthase inhibition. Basal\(^9\) and glucose-stimulated insulin serum content\(^9\) was diminished, corresponding to a lower serum glucose levels in both the fasted and non-fasted rats,\(^9\) indicating an increase in whole body insulin sensitivity. The possibility of gangliosides being directly involved in lipo-apoptotic signaling pathways was entertained as a mechanism behind this protection from β-cell destruction,\(^9\) but this was not assessed directly. In contrast, subcellular remodelling of β-cell glycosphingolipid species in response to saturated lipid oversupply was seen to be cytoprotective in our recent publication,\(^5\) in which enhanced synthesis of GluCer prevented β-cell lipopapotosis, ER stress and a previously reported protein trafficking defect.\(^5\) This discrepancy between the in vitro\(^9\) and in vivo\(^9\) effects of GluCer synthase inhibitors on β-cell function possibly reflects differences between responses intrinsic to the β-cell vs. those mediated indirectly via whole body effects.

As discussed above, there is a limited literature addressing the role of glycosphingolipids in the inflammation of T1D. However, the growing realization that inflammation also contributes to β-cell dysfunction in T2D\(^9\) raises the possibility of a broader role. There is only very limited data addressing this using the Cohen diabetes sensitive rat, which under environmental pressure (high sucrose diet), develops diabetes characterized by blunted glucose stimulated insulin secretion, glucose intolerance and various pancreatic lesions including exocrine steatosis and IL-1β positive macrophage infiltration.\(^6\) In a study where these animals were co-treated for one month with daily IP injections of β-glucosyl and β-lactosylceramides, which are known stimulators of natural killer T and CD8 lymphocytes (via dendritic cells), pancreatic steatosis was markedly decreased and glucose stimulated insulin secretion was restored.\(^7\) The beneficial effects of these glycosphingolipids upon the islet were therefore deemed to be mediated by immunomodulation of T cells.

**Sulfatide.** A glycosphingolipid derivative of GalCer, sulfatide, appears to be particularly important to secretory cells such as the pancreatic β-cell and neuronal cells. This lipid, GalCer-3-O-sulfate, is sulfated by sulfate transferase and present in β-cells but not exocrine tissue of the islets in humans and other species including rat, mouse, pig and monkey.\(^8\) Sulfatide is synthesized in the golgi and is packaged into insulin secretory granules with insulin in the trans-golgi network.\(^9\) It binds to insulin crystals to preserve the crystal structure at pH 5.5 as well as aiding the conversion of insulin hexamers to monomers at pH 7.4 at the cell surface.\(^1\) It also promotes proinsulin folding and oxidation within the secretory pathway.\(^1\) Patch clamp studies show that sulfatide negatively regulates glucose stimulated insulin secretion potentially via its action on K\(_{ATP}\) channels.\(^1\) Furthermore, loss of the C16:0 isoform of sulfatide in β-cells has been implicated in the pathogenesis of T2D. This specific isoform is lacking in islets from ob/ob and db/db mouse models of T2D as compared with normal human pancreatic tissue, BALB/c mice and the non-diabetic Lewis rat.\(^9\) Moreover, C16:0 sulfatide significantly improves insulin crystal preservation.\(^9\) As discussed
earlier, the co-secretion of sulfatide with insulin also appears to negatively regulate CD14 signaling to prevent excessive secretion of pro-inflammatory cytokines from the β-cell that may precipitate β-cell destruction.28 Collectively these studies make a strong case for sulfatide playing an important role in β-cell biology, and this is another topic for reinvestigation using newer genetic and analytical tools.

**Concluding Remarks**

It is becoming increasingly apparent that sphingolipids have varied roles in many critical aspects of β-cell biology; these are summarized in Table 1. The composition of DRMs determined by the activity of neutral SMases at the plasma membrane facilitate the proper complexing and activation of the ion channels and cell surface receptors that can impact on insulin or cytokine secretion as well as responses to other extracellular stimuli. The glycosphingolipids within the plasma membrane also function as receptors that signal to β-cell cytokine secretion, which might in turn modulate recruitment and activation of immune cells. The pancreatic specific ganglioside GM2-1 also elicits autoantibody production, although the relevance of these glycosphingolipids within both T1D and T2D are as yet unknown. This and other glycosphingolipids such as sulfatide are found within insulin secretory granules, where they might regulate other β-cell processes including the stabilization of mature insulin crystals.

Ceramide is postulated to be the lipid ‘second messenger’ responsible for β-cell death due to saturated FA exposure yet how ceramide accumulation mediates this end remains elusive. It can impact on known protein signaling intermediates such as Akt and C/EBPβ, which are thought to contribute to apoptosis induction and blunted insulin gene expression. The accumulation of certain species of ceramide in membranes of organelles such as the ER and mitochondria might in theory modulate shape and function of these membranes. This itself may impact on vesicle formation, protein trafficking and processing of secretory cargo, as well as mitochondrial membrane potential. These responses could exert diverse effects on ER stress, apoptosis and

### Table 1. Sphingolipid metabolites in the β-cell and their biological functions

<table>
<thead>
<tr>
<th>Sphingolipid metabolite</th>
<th>References</th>
<th>Biological process (enzymes involved)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceramide</td>
<td>35–38</td>
<td>Proapoptotic; accumulates in the ZDF rat model of lipotoxicity</td>
</tr>
<tr>
<td></td>
<td>56, 58</td>
<td>Proapoptotic; induces ER stress in mild β-cell models of lipotoxicity, longer chain ceramide species implicated in toxicity</td>
</tr>
<tr>
<td></td>
<td>62–64</td>
<td>Proapoptotic; accumulates at the ER and mitochondria in response to strong ER stressors (nSMase 2, iPLA2β)</td>
</tr>
<tr>
<td></td>
<td>56, 69</td>
<td>Disrupts ER-to-golgi traffic</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>Proapoptotic; intraislet IAPP deposits induce ceramide accumulation (aSMase)</td>
</tr>
<tr>
<td></td>
<td>55, 84</td>
<td>Represses transcription of proinsulin mRNA</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>27</td>
<td>SM serum content in NOD mice and humans with T1D associated with T1D progression</td>
</tr>
<tr>
<td></td>
<td>34</td>
<td>Pancreatic β-cell specific surface SM possesses the epitope for IC2 monoclonal antibody found in the diabetic BB rat</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>Regulates GSIS and ROS homeostasis in mouse pancreatic islets (SMS1)</td>
</tr>
<tr>
<td></td>
<td>89</td>
<td>Regulates GSIS from β-cell line, INS-1 (SMS1)</td>
</tr>
<tr>
<td>Sphingosine 1-phosphate</td>
<td>91</td>
<td>Regulates GSIS in β-cell line MIN6 and pancreatic islets (SK)</td>
</tr>
<tr>
<td></td>
<td>24, 25</td>
<td>Antiapoptotic; stimulates cytokine-induced S1P signaling (SK)</td>
</tr>
<tr>
<td></td>
<td>29, 30</td>
<td>Anti-inflammatory; inhibits lymphangiogenesis and immune cell infiltration of islet allografts (SK)</td>
</tr>
<tr>
<td></td>
<td>31, 32</td>
<td>Anti-inflammatory; prevents autoimmune mediated islet destruction in a rat model of T1D (SK)</td>
</tr>
<tr>
<td>Galactosylceramide</td>
<td>28</td>
<td>Activates β-cell CD14 receptor to secrete TNFα, IL-1β and IL-8</td>
</tr>
<tr>
<td>Glucosylceramide κβ</td>
<td>93, 94</td>
<td>Contributes to insulin sensitivity in ZDF rats</td>
</tr>
<tr>
<td>β-Glucosylceramide, β-lactosylceramide</td>
<td>97</td>
<td>Exerts immunomodulatory effects on NKT and CD8 T cells to prevent pancreatic steatosis in the Cohen diabetic rat</td>
</tr>
<tr>
<td>Sulfatide</td>
<td>28</td>
<td>Modulates β-cell CD14 receptor signaling</td>
</tr>
<tr>
<td></td>
<td>101, 102</td>
<td>Binds to and stabilizes insulin crystals within granules, modulates β-cell K⁺-channel activity and exocytosis</td>
</tr>
<tr>
<td>GM2-1</td>
<td>33</td>
<td>Pancreatic islet specific ganglioside that is the target of IgG autoantibodies correlating strongly with T1D progression in patients with diabetic relatives</td>
</tr>
</tbody>
</table>

Abbreviations: aSMase, acid sphingomyelinase; BB, biobreeding; ER, endoplasmic reticulum; GSIS, glucose stimulated insulin secretion; iPLA2β, calcium-independent phospholipase A2 β; IL-1β, interleukin 1-β; NOD, non-obese diabetic; nSMase, neutral sphingomyelinase; IL-8, interleukin-8; NKT, natural killer T; ROS, reactive oxygen species; S1P, sphingosine 1-phosphate; SK, sphingosine kinase; SM, sphingomyelin; SMS1, sphingomyelin synthase 1; T1D, type 1 diabetes; TNF-α, tumor necrosis factor α; ZDF, Zucker diabetic fatty.
even autophagy. In our opinion, future studies should focus on modulating sphingolipids in different cellular compartments, using genetic or pharmacological intervention, as a means of addressing the actions of ceramide, SM or glycosphingolipid on β-cell secretion, insulin expression, apoptosis and ER function.

Despite the biochemical and biological challenges that such studies will entail, we believe that this approach will not only expand our understanding of the roles of sphingolipids in β-cell biology, and but also shed light on the fundamental mechanisms underlying β-cell failure in both forms of diabetes.

References


16. Weldh N. Interleukin-1β-induced ceramide and diacylglycerol generation may lead to activation of the c-Jun NH2-terminal kinase and the transcription factor ATF2 in the insulin-producing cell line RIN5F5E. J Biol Chem 1996; 271:8307-12; PMID:8626526.


55. Vanhoutte PM, Volchuk A. Chronic palmitate but not oleate triglyceride formation is higher in insulin-producing MIN6 cells exposed to oleate compared to palmitate. J Cell Biochem 2010; 111:497-507; PMID:20524206; http://dx.doi.org/10.1002/jcb.22734.


55. Vanhoutte PM, Volchuk A. Chronic palmitate but not oleate triglyceride formation is higher in insulin-producing MIN6 cells exposed to oleate compared to palmitate. J Cell Biochem 2010; 111:497-507; PMID:20524206; http://dx.doi.org/10.1002/jcb.22734.


Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:
Boslem, E;Meikle, PJ;Biden, TJ

Title:
Roles of ceramide and sphingolipids in pancreatic beta-cell function and dysfunction

Date:
2012-05-01

Citation:

Persistent Link:
http://hdl.handle.net/11343/259110

License:
CC BY-NC