Targeting Ceramide Synthesis to Reverse Insulin Resistance

Carsten Schmitz-Peiffer

Insulin resistance is a key feature of type 2 diabetes, and the strong association between fat oversupply and defective insulin action in target tissues, especially skeletal muscle and liver, has motivated the search for intracellular lipid mediators that can interfere with insulin signaling and glucose homeostasis. Two of the best-studied candidates are diacylglycerol (DAG) and ceramide (1–3), to the extent that the tissue levels of these intermediate species are now routinely measured in models of insulin resistance and in human studies. Correlative evidence for their roles has accumulated over the last 10–15 years, but unfortunately, there are now several conflicting reports concerning the relative importance of these molecules (4–7). To resolve this situation, it is necessary to go beyond descriptive studies in order to address causation and elucidate the mechanisms involved.

In the case of ceramide, the inhibitor myriocin is proving to be a useful tool. This fungal metabolite is a potent and specific blocker of serine palmitoyltransferase (SPT), the first enzyme in the pathway of de novo ceramide synthesis (8). By reducing the condensation of serine with palmitoyl-CoA to form 3-ketosphinganine (Fig. 1), the inhibitor is able to decrease ceramide synthesis without elevating intermediates such as sphinganine, which are upstream of ceramide formation and also have biological effects (9). Myriocin treatment of rats infused with different lipid cocktails for 6 h to induce insulin resistance in an acute fashion demonstrated that a reduction in ceramide accumulation in skeletal muscle could prevent defects in glucose disposal (10). Longer-term administration of the inhibitor was also able to improve glucose tolerance in genetically obese Zucker diabetic fatty (ZDF) rats and fat-fed mice. Finally, this study also supported the conclusion, drawn from in vitro work (11), that saturated fatty acids contribute to ceramide accumulation, whereas unsaturated fatty acids induce insulin resistance by ceramide-independent mechanisms involving, for example, DAG-activated protein kinase C (PKC) isoforms or phosphatidic acid (1,12).

Mechanistic investigations carried out in cell models have elucidated direct effects of ceramide on insulin signaling components, especially the inhibition of Akt through activation of phosphatases or atypical PKCζ, which could account for the induction of insulin resistance (13,14). Recent reports of the use of myriocin to reverse preexisting insulin resistance in vivo, especially in models of diet-induced obesity, *1*) indicate a broader role for ceramide accumulation in the development of insulin resistance and the metabolic syndrome, and *2*) suggest that this may be a clinically useful approach (15–17). In this issue of *Diabetes*, Ussher et al. (16) report the effects of myriocin treatment on glucose homeostasis in mice fed a high-fat diet for 12 weeks. In addition to substantiating the role of ceramide in muscle insulin resistance, they provide some new insights into the actions of the lipid intermediate on glucose and lipid metabolism in vivo. Myriocin, administered for 2 weeks, improved glucose and insulin tolerance and also restored insulin signal transduction in skeletal muscle at the level of Akt, as might be predicted from its effects in vitro and upon acute lipid infusion.

More unexpectedly, the inhibitor also reversed fat diet–induced defects in whole-body oxygen consumption and mitochondrial function. Furthermore, the authors were able to link ceramide accumulation with the contention that incomplete fatty acid oxidation contributes to insulin resistance (18). Myriocin reduced the accumulation of short-chain acylcarnitine esters and increased muscle triglyceride accumulation, suggesting that fatty acid oxidation was inhibited, whereas glucose metabolism was improved. The mechanistic link between ceramide accumulation and mitochondrial dysfunction was not explored but could involve processes such as the generation of reactive oxygen species or an increase in membrane permeability (rev. in [19]). The in vivo situation is likely to be complex, involving interplay between different metabolic tissues. Yang et al. (15) have also recently examined the effects of myriocin treatment in dietary and genetic models of obesity, reporting beneficial changes in the regulation of energy expenditure in liver and adipose, linked with the expression of suppressor of cytokine signaling-3 and uncoupling protein-3. It is possible that the improvements in insulin signaling reported in each study were at least in part secondary to the action of myriocin on fuel metabolism (Fig. 1).

The effects of myriocin may not, however, be limited to direct actions on ceramide accumulation in insulin target tissues. Ceramide-dependent pathways regulate several aspects of the immune response (20), and the inhibitor possesses potent immunosuppressant activity in vivo (21). Chronic inflammation is associated with insulin resistance, especially under conditions of long-term fat oversupply (22), and it is therefore likely that myriocin has indirect effects on muscle insulin action by reducing the activation of macrophages either locally or in adipose tissue, to reduce the release of inhibitory cytokines (G.I. Lancaster and M.A. Febbraio, personal communication). Ussher et al. (16) did not observe any increase in the

From the Garvan Institute of Medical Research, Sydney, Australia; and the Faculty of Medicine, St. Vincent's Clinical School, University of New South Wales, Sydney, Australia.
Corresponding author: Carsten Schmitz-Peiffer, c.schmitz-peiffer@garvan.org.au.
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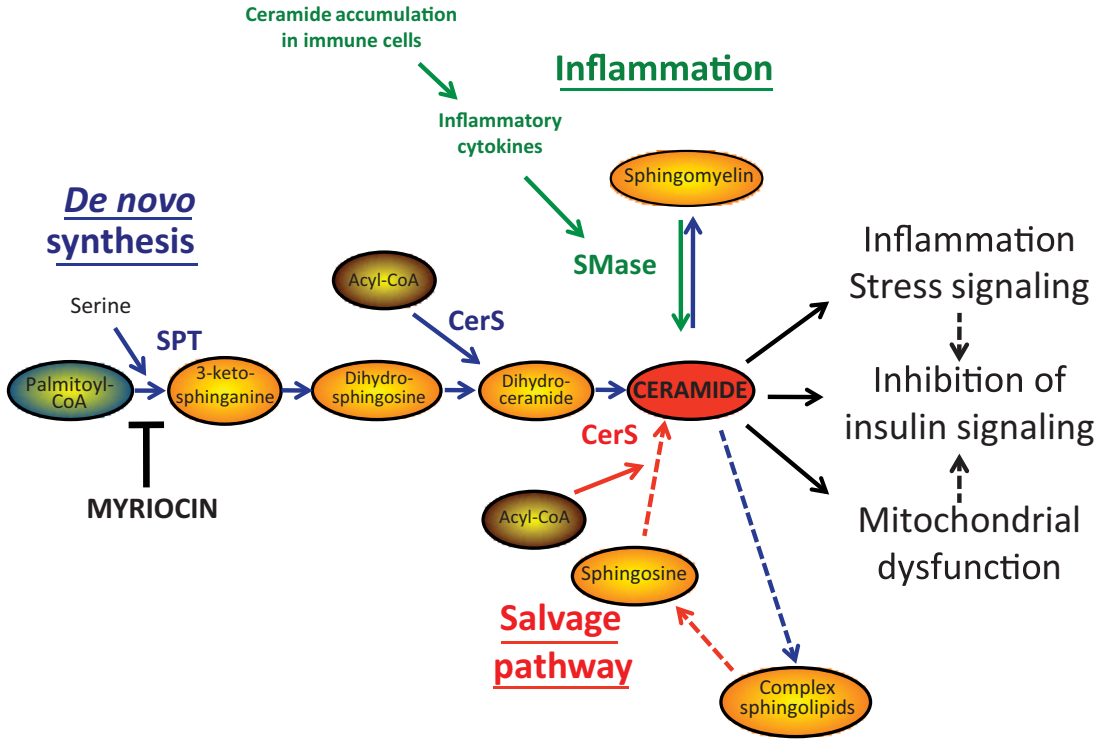

FIG. 1. Roles of SPT, CerS, and sphingomyelinase (SMase) in the metabolism of ceramides. De novo synthesis of ceramide is initiated by the condensation of serine with palmitoyl-CoA by SPT to generate the sphingoid backbone. A range of saturated and unsaturated fatty acids (acyl-CoA) can be subsequently incorporated by CerS (LASS) isoforms to form the ceramide sidechain. The salvage pathway, responsible for the cycling between ceramide and complex sphingolipids, also involves CerS activity and can also promote ceramide accumulation upon fatty acid oversupply. See references (9) and (24) for further details of sphingolipid metabolism. Inflammation can promote ceramide release from sphingomyelin through the activation of SMase by cytokines. Studies in intact cells and in vivo suggest that ceramide (and possibly also complex sphingolipids) can affect insulin action at least in part by inducing further stress signaling and mitochondrial dysfunction as well as by directly inhibiting insulin signal transduction.

activation of inflammatory signaling through p38 mitogen-activated protein kinase or c-Jun NH_2-terminal kinase in muscle in their dietary model, although the possibility remains that myriocin inhibited inflammatory mechanisms elsewhere that impinged on muscle insulin action. Indeed, Yang et al. (15) reported that the inhibitor reduced the release of plasminogen activator inhibitor-1 and monocyte chemoattractant protein-1 from adipose tissue. Furthermore, the anti-inflammatory agent lisofylline also reduces muscle ceramide accumulation and improves glucose tolerance in fat-fed mice (17), indicating that disruption of the cycle between inflammation and ceramide production, by means other than direct inhibition of ceramide synthesis, can be beneficial for glucose homeostasis (Fig. 1).

Ussher et al. further examined ceramide accumulation and the effects of myriocin treatment in muscle from *db/db* mice. No increase in ceramide was observed in untreated *db/db* mice compared with *db/+* controls, whereas ceramide levels were reduced and glucose homeostasis was improved by administration of myriocin. This indicates that the relationship between ceramide accumulation and insulin action can be less direct, and the authors suggest that their data are consistent with the view that ceramide metabolites such as glucosylceramides can also play a role in the generation of insulin resistance (3). It has become apparent that the cycling between ceramide and complex sphingolipids through a salvage pathway, which involves hydrolysis to sphingosine and reacylation (Fig. 1), is at least as important in ceramide action as the de novo synthetic route (23). In each case, both saturated and unsaturated fatty acids of varying chain lengths are incorporated by ceramide synthases (CerS1-6, encoded by the longevity assurance homologue [LASS] genes) to (re)generate the ceramide sidechain (23) (Fig. 1). An important corollary is that the use of the saturated fatty acyl-CoA derived from palmitate by SPT to form the sphingoid backbone is rate-limiting only for de novo synthesis of ceramide (Fig. 1). Thus, in contrast to the findings from acute lipid infusions (10), ceramides can accumulate upon the oversupply of either saturated or unsaturated fatty acids in the longer term (17), most likely as a consequence of salvage pathway activity. Through their specificity and level of expression, LASS isoforms determine the array of ceramide species with differing acyl sidechains that can be detected (15,17). Changes in the expression of CerS1, the most abundant isoform in skeletal muscle, are associated with alterations in ceramide levels and glucose tolerance in muscle from fat-fed mice (17). An important next step in the understanding of ceramide action is therefore to determine whether there are specific roles for particular species of ceramide and the corresponding complex sphingolipids. Targeting CerS isoforms rather than SPT may in fact represent a better therapeutic strategy to reduce ceramide accumulation given the potential for specificity and the ability to inhibit the salvage pathway as well as de novo synthesis.

Finally, the continued controversy regarding the relative importance of ceramide and DAG in the generation of lipid-induced insulin resistance remains to be resolved. Although it appears that lipid composition is a key factor in vitro and in acute in vivo models, it is likely that further explanations are required to reconcile the discrepancies

between findings made using longer term dietary and genetic models and in human studies. As well as the analysis of individual ceramide and DAG species, investigation of the cellular locations of these inhibitory mediators by subcellular fractionation may shed more light on this issue. For example, total DAG levels, comprising both DAG released at the plasma membrane and also that synthesized at the endoplasmic reticulum, mitochondria, and lipid droplets (19), may not always reflect the accumulation of the pool(s) of this intermediate which can promote PKC activation. Similarly, differential intracellular distribution of CerS isoforms is likely to give rise to pools of ceramide with distinct roles (23).

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