The ART of Lowering Ceramides

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Ceramides are lipid metabolites implicated in the metabolic dysregulation that accompanies dyslipidaemia and obesity. Using a genetic mouse model to acutely degrade ceramides in adipose tissue or the liver (i.e., by conditionally expressing acid ceramidase), in this issue of *Cell Metabolism* Xia et al. (2015) identify roles for these molecules in insulin resistance, steatohepatitis, and interorgan communication.

The accumulation of lipid metabolites in tissues not suited for nutrient storage gives rise to the panoply of defects that underlie diabetes and cardiovascular disease. Of the lipids that accrue, sphingolipids such as ceramides are particularly deleterious. Interventional studies unequivocally demonstrate that inhibition of sphingolipid biosynthesis ameliorates insulin resistance, steatohepatitis, and other metabolic disorders (Chavez and Summers, 2012; Holland et al., 2007; Turpin et al., 2014). In this issue of *Cell Metabolism*, Xia et al. (Xia et al., 2015) identify a new therapeutic strategy for lowering ceramides and improving metabolic health—driving ceramide degradation.

The study focuses on ceramidase, an enzyme that removes an acyl-chain from ceramide, converting it to sphingosine, which does not oppose insulin signaling or induce apoptosis (Chavez et al., 2005; Lang et al., 2011). The impetus for this study was the observation that the broad spectrum of activities of the antidiabetic and cardioprotective adiponectin could be explained by its activation of a ceramidase, likely one intrinsic to its receptors (Holland et al., 2011). Adiponectin receptors bear sequence homology with ceramidase enzymes, and substitution of residues predicted to be important for ceramidase activity renders them ineffectual. Infusion of recombinant adiponectin or transgenic overexpression of adiponectin in mice increases ceramidase activity and depletes ceramides in various tissues. Moreover, genetic ablation of adiponectin receptors exacerbates sphingolipid-dependent toxicity. These findings suggest that ceramide depletion could be a unifying mechanism to explain the pleiotropic actions of the adipokine.

Xia and colleagues investigated whether acutely increasing ceramidase activity was sufficient to recapitulate adiponectin action in liver and adipose tissue. They generated transgenic mice that express acid ceramidase (AC) driven by tissue-specific, tetracycline response elements, allowing for the selective and acute deacylation of ceramides in either the liver or adipose tissue. The authors observed profound improvements in hepatic steatosis, adipose morphology, and glucose metabolism following ceramidase expression in either locale (Figure 1).

Doxycycline-inducible, liver-specific ceramidase overexpression was achieved by generating mice expressing an albumin promoter-driven Cre recombinase, a Cre-dependent tetracycline-controlled transactivator, and a tetracycline-responsive acid ceramidase (Alb-AC). In Alb-AC mice fed a high-fat diet, doxycycline increased insulin sensitivity owing to improved insulin signaling and action in both the liver (i.e., increased repression of hepatic glucose output) and adipose tissue (i.e., increased glucose uptake). The regimen did not affect glucose disposal in muscle. Transgene expression also reduced liver triglycerides owing to increased hepatic triglyceride export and decreased fatty acid uptake.

Doxycycline-inducible, adipose-specific ceramidase expression was achieved by crossing the Tet-responsive acid ceramidase mice with ones harboring an *a*diponectin-*r*everse *t*etracycline (Art) transgene. Overexpression of ceramidase in adipose tissue protected these Art-AC mice from diet-induced insulin resistance, again due to increased insulin action in both adipose tissue and the liver. Adipose tissue also displayed reduced adipose inflammation and fibrosis.

These findings revealed the existence of a novel crosstalk mechanism between the liver and adipose tissue. The beneficial metabolic effects were independent of changes in body weight, and ceramidase expression was confined to the intended tissue. The intervention selectively targeted sphingolipids, as other lipids implicated in insulin resistance (e.g., diacylglycerol) were unaffected. Thus, changes in the sphingolipidome in one tissue affected the metabolic health of the other.

Xia and colleagues also demonstrated that acute ceramidase activation reversed pre-existing steatosis and insulin resistance. The rapidity with which adipose ceramidase overexpression reversed metabolic impairment was impressive, occurring within 3 days. Expression of ceramidase in the liver also resolved insulin resistance and steatosis, but the effect took considerably longer.

Several mechanisms have been described to explain ceramide effects on insulin sensitivity and liver steatosis. Ceramides inhibit insulin signaling to Akt through parallel pathways involving protein phosphatase 2A and protein kinase C-ζ (PKCζ) (Chavez and Summers, 2012). Certain ceramides (i.e., C16-ceramides) also have been reported to induce liver steatosis through the inhibition of oxidative phosphorylation (Raichur et al., 2014; Turpin et al., 2014). Xia and colleagues described a new role, with ceramide inducing lipid uptake into the liver, but not adipose tissue. Both Alb-AC and Art-AC mice displayed doxycycline-dependent increases in expression of CD36, a protein required for fatty acid uptake. PKCζ again emerged as the critical intermediary. Exogenous ceramides stimulated CD36 translocation to the membrane and lipid uptake via a PKCζ-dependent mechanism. The pathway appeared relevant in vivo, as injection

with a PKCζ inhibitor rapidly improved insulin sensitivity and reduced hepatic lipid accumulation.

The role of ceramides in hepatic insulin resistance and steatosis has been contentious. Though numerous studies reveal that interventions which deplete ceramides improve metabolic homeostasis in mice (Chavez and Summers, 2012; Holland et al., 2007; Turpin et al., 2014), correlational studies investigating relationships between ceramides, hepatic steatosis, and insulin resistance have been discordant (Kumashiro et al., 2011; Yetukuri et al., 2007). A major concern with the correlational studies is the challenge of profiling sphingolipids that do not exist at steady-state concentrations or which reside in distinct intracellular pools. More complete temporal-spatial profiling of the sphingolipidome (e.g., in relation to feeding) could inform this debate. Other important questions also remain. The precise sensing mechanisms through which very small changes in ceramides modulate PKCζ or other nutrient sensors are unresolved. Moreover, the nature of the signal between adipose tissue and the liver is unclear. One possibility is that circulating sphingolipids themselves are the signaling entities. Determination of the tissue of origin of the impactful lipids has significant therapeutic implications. Is adipose a source for ceramides or a sink where they are removed from the circulation?

In summary, the development of an acute, genetic means to modulate ceramide levels in adult mouse tissues reveals new insight into our understanding of the regulatory mechanisms linking nutrient sensing to metabolic regulation. Enzymes controlling ceramide synthesis and metabolism hold enormous promise in the treatment of a broad spectrum of metabolic disorders.

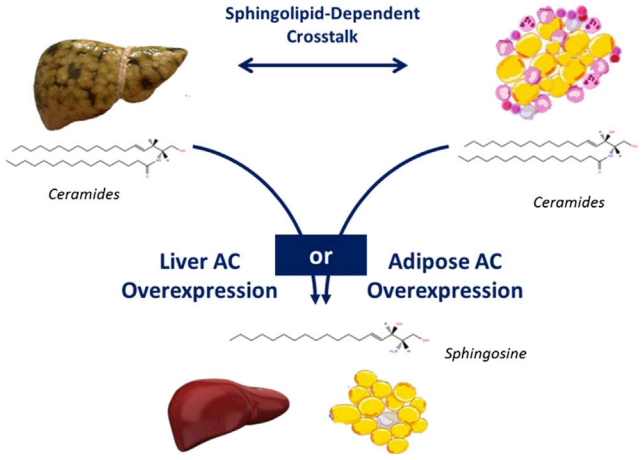

Figure 1. Schematic Depicting the Effect of Adipose and Liver Ceramidase Activation on Metabolic Homeostasis

Xia and colleagues (Xia et al., 2015) studied new mouse models allowing for the acute induction of acid ceramidase in liver and adipose tissue. Increasing rates of ceramide deacylation in either location markedly improved the metabolic health of both tissues, revealing new roles for sphingolipids in liver-fat crosstalk.

REFERENCES

Chavez, J.A., and Summers, S.A. (2012). Cell Metab. *15*, 585–594.

Chavez, J.A., Holland, W.L., Bär, J., Sandhoff, K., and Summers, S.A. (2005). J. Biol. Chem. *280*, 20148–20153.

Holland, W.L., Brozinick, J.T., Wang, L.P., Hawkins, E.D., Sargent, K.M., Liu, Y., Narra, K., Hoehn, K.L., Knotts, T.A., Siesky, A., et al. (2007). Cell Metab. *5*, 167–179.

Holland, W.L., Miller, R.A., Wang, Z.V., Sun, K., Barth, B.M., Bui, H.H., Davis, K.E., Bikman, B.T., Halberg, N., Rutkowski, J.M., et al. (2011). Nat. Med. *17*, 55–63.

Kumashiro, N., Erion, D.M., Zhang, D., Kahn, M., Beddow, S.A., Chu, X., Still, C.D., Gerhard, G.S., Han, X., Dziura, J., et al. (2011). Proc. Natl. Acad. Sci. USA *108*, 16381–16385.

Lang, F., Ullrich, S., and Gulbins, E. (2011). Expert Opin. Ther. Targets *15*, 1061–1071.

Raichur, S., Wang, S.T., Chan, P.W., Li, Y., Ching, J., Chaurasia, B., Dogra, S., Öhman, M.K., Takeda, K., Sugii, S., et al. (2014). Cell Metab. *20*, 687–695.

Turpin, S.M., Nicholls, H.T., Willmes, D.M., Mourier, A., Brodesser, S., Wunderlich, C.M., Mauer, J., Xu, E., Hammerschmidt, P., Brönneke, H.S., et al. (2014). Cell Metab. *20*, 678–686.

Xia, J.Y., Holland, W.L., Kusminski, C.M., Sun, K., Sharma, A.X., Pearson, M.J., Sifuentes, A.J., McDonald, J.G., Gordillo, R., and Scherer, P.E. (2015). Cell Metab. *22*, this issue, 266–278.

Yetukuri, L., Katajamaa, M., Medina-Gomez, G., Seppänen-Laakso, T., Vidal-Puig, A., and Oresic, M. (2007). BMC Syst. Biol. *1*, 12.