

## CLINICAL IMPLICATIONS OF BASIC RESEARCH

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## Nonalcoholic Fatty Liver Disease, Insulin Resistance, and Ceramides

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Insulin resistance is present in most, but not all, obese and elderly patients and even in some young and lean persons. It is a precursor to and accelerant of coexisting conditions such as type 2 diabetes, atherosclerosis, nonalcoholic fatty liver disease, and probably obesity-associated cancers. There is broad consensus that insulin resistance is associated with ectopic lipid deposition in skeletal muscle and liver. However, multiple lipid species are implicated, and the molecular mechanisms impairing the action of insulin are debated.<sup>1</sup> In one model, an increase in *sn*-1,2-diacylglycerol impairs insulin signaling. In this model,

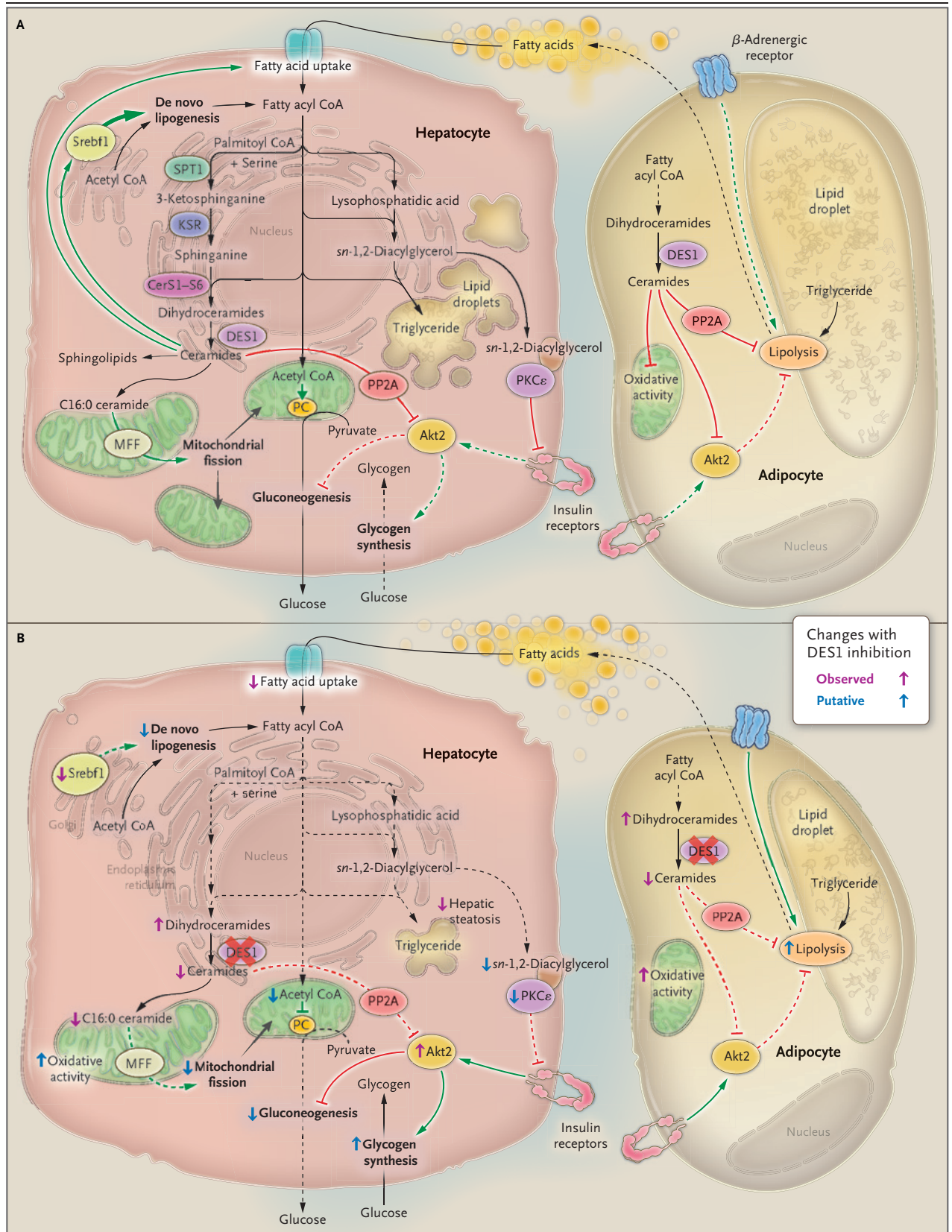
diacylglycerol activates an intracellular enzyme (such as protein kinase C [PKC] isoform  $\epsilon$  [PKC $\epsilon$ ], one in a class of diacylglycerol-dependent and calcium-independent [or “novel”] PKC isoforms), which in turn inhibits the ability of insulin to directly regulate cellular processes, such as the synthesis of hepatic glycogen or inhibition of lipolysis in white adipocyte tissue (Fig. 1). Dysregulated lipolysis then promotes hepatic  $\beta$ -oxidation and increases hepatic acetyl-CoA content, which, together with increased glycerol flux, augments hepatic gluconeogenesis.<sup>1</sup>

In addition, a substantial body of work sup-

**Figure 1 (facing page). Lipids and Hepatic Cellular Insulin Resistance.**

Panel A shows the effects of lipid excess. Ceramide synthesis begins with the condensation of serine and palmitoyl CoA by serine palmitoyl transferase 1 (SPT1), subsequent reduction by 3-ketosphinganine reductase (KSR), the addition of a second fatty acyl CoA through specific isoforms of ceramide synthase (CerS1–S6), and finally the addition of a double bond by dihydroceramide desaturase 1 (DES1). Ceramides serve as precursors for other membrane sphingolipids. Ceramides (and perhaps specifically C16:0 ceramides synthesized by CerS6) are implicated in numerous cellular pathways, including alteration of mitochondrial function. C16:0 ceramides interact with mitochondrial fission factor (MFF), promoting mitochondrial fission. Ceramides have also been shown to promote fatty acid uptake in hepatocytes and to activate genes involved in *de novo* lipogenesis controlled by sterol responsive binding factor 1 (Srebf1). Ceramides are implicated in impaired activation of Akt2 either through protein phosphatase 2A (PP2A) or protein kinase c (PKC) isoform  $\zeta$  (not shown here). In the steatotic liver, increases in other ectopic lipids can impair metabolism. Increases in *sn*-1,2-diacylglycerol at the plasma membrane activate PKC isoform  $\epsilon$  (PKC $\epsilon$ ), which then impairs insulin receptor signaling. Increases in hepatic acetyl CoA will allosterically activate pyruvate carboxylase and, together with substrate push from glycerol (not depicted here) promote gluconeogenesis. In adipose tissue, ceramides may also affect mitochondrial oxidative function and, through PP2A, may impair insulin signaling and adrenergic mediated lipolysis.

Panel B shows the effects of DES1 inhibition. Chaurasia et al.<sup>2</sup> recently reported the effects of knocking out *Degs1*, the mouse orthologue of *DES1*. The changes they observed are indicated with purple arrows, and putative other changes are shown with blue arrows. DES1 inhibition increases the level of dihydroceramides and reduces that of the ceramides, including C16:0 ceramides, possibly decreasing mitochondrial fission. Targeting DES1 also decreases fatty acid uptake, expression of Srebf1 and its key targets, and probably *de novo* lipogenesis. The reduction in ceramide-mediated mitochondrial fission may preserve the capacity of the mitochondrion to oxidize fatty acids — which is almost certainly key to decreasing hepatic steatosis. The concordant decrease in hepatic acetyl CoA will decrease activation of pyruvate carboxylase, while the reduction in *sn*-1,2-diacylglycerol will decrease PKC $\epsilon$ -mediated impairments of the insulin receptor. Together, these changes would improve hepatic insulin action (a decrease in gluconeogenesis and increase in glycogen synthesis). In adipocytes, targeting DES1 improves mitochondrial oxidative activity and may also lead to increased lipolysis, which would account for the decreased size of adipocytes in *Degs1*-deficient mice.



ports a role for ceramides in promoting insulin resistance.<sup>3</sup> Ceramides, like the diacylglycerols, are bioactive lipid intermediates. But whereas diacylglycerols give rise to energy-storing triglycerides, ceramides and their derivatives (e.g., sphingomyelin) are structural membrane lipids. Specific enzymes, called desaturases, convert dihydroceramides into ceramides, which alter membrane biophysical properties.<sup>3</sup> Explanations for ceramide-associated insulin resistance include alterations in insulin signaling, mitochondrial function, and inflammatory pathways.<sup>4</sup> Both ceramides and dihydroceramides have been associated with insulin resistance.

A recent study by Chaurasia et al. established the importance of ceramides through the genetic manipulation of dihydroceramide desaturase 1 (Des1, which is encoded by the gene *Degs1*) in mice.<sup>2</sup> An inducible whole-body deletion of *Degs1* in both lean and obese (leptin-deficient) mice altered energy balance, reduced body weight and adiposity, and brought about the attendant metabolic benefits: decreased hepatic steatosis and increased insulin sensitivity and glucose tolerance. Tissue-specific deletion of *Degs1* in liver and adipose tissue conferred modest metabolic improvements without detectable changes in body weight or whole-body energy balance. Finally, an adeno-associated vector containing a small hairpin RNA, targeting *Degs1* messenger RNA for degradation, improved metabolic measures in mice that either were already obese or were of normal weight but subsequently placed on a high-fat diet, with the added benefit of preventing obesity in the latter. In sum, deletion of *Degs1* improved insulin signaling and mitochondrial function and decreased lipogenesis and lipid uptake.

However, these findings do not establish the necessity of ceramides for the development of insulin resistance. Multiple studies in rodents and humans have disassociated hepatic ceramide content from changes in hepatic insulin sensitivity.<sup>1</sup> In contrast, knockdown of PKC $\epsilon$ , or mutation of its target residue in the insulin receptor, prevents hepatic insulin resistance without changing energy balance or affecting intracellular lipids (including ceramides), which suggests that activation of this axis is necessary for the development of hepatic insulin resistance.<sup>1</sup> Chaurasia et al. did not find differences in the concentration of hepatic diacylglycerol in the *Degs1*-deficient mice, although changes in the relatively small pool of

bioactive diacylglycerols in the plasma membrane may be difficult to detect when total diacylglycerol content is measured in whole cells. It would be interesting to assay specific species of diacylglycerol, such as *sn*-1,2-diacylglycerol (the type of diacylglycerol that activates diacylglycerol-dependent, calcium-independent PKC isoforms), in distinct cellular compartments purified by cell fractionation.

Models of ceramide- and diacylglycerol-mediated insulin resistance can be reconciled by considering how ceramides affect mitochondrial function. For example, myriocin, an inhibitor of ceramide synthesis, improves insulin sensitivity in mice but also enhances mitochondrial activity and  $\beta$ -oxidation of fatty acids.<sup>3</sup> Conversely, overexpression of ceramide synthase 6 increases levels of a specific ceramide species, known as C16:0 ceramides, in mitochondrial membranes, where they interact with mitochondrial fission factor and thereby promote mitochondrial fragmentation.<sup>5</sup> Genetic ablation of ceramide synthase 6 prevents weight gain and glucose intolerance in mice, similar to the effect of targeting DES1. However, silencing of mitochondrial fission factor in mice with simultaneous overexpression of ceramide synthase 6 prevented mitochondrial fragmentation and glucose intolerance, despite increases in levels of C16:0 ceramides.<sup>5</sup> Thus, ceramide-mediated mitochondrial dysfunction may account for the observed association with metabolic diseases, which include a sensory neuropathy and macular telangiectasia type 2, as recently reported in the *Journal* by Gantner and colleagues.<sup>6</sup>

Increased mitochondrial fatty acid oxidation decreases ectopic lipid content in the liver. Decreases in plasma membrane *sn*-1,2-diacylglycerol and hepatic acetyl CoA can improve insulin signaling and decrease gluconeogenesis, respectively. Liver-targeted mitochondrial uncouplers increase lipid oxidation and reverse hepatic insulin resistance, hyperlipidemia, hepatic steatosis, and hyperglycemia independently of changes in the level of hepatic ceramides,<sup>1</sup> which probably exert more subtle effects on cellular energy balance. Chaurasia et al. found that mice in which *Degs1* was systemically knocked out had a lower body weight than control mice, as well as higher mitochondrial activity in isolated adipocytes. However, differences in body-weight gain or energy balance were not apparent in mice in which *Degs1* was knocked out specifically in liver, adipose

tissue, or both. Moreover, knockdown of *Degs1* (by small hairpin RNA) could prevent but not reverse obesity in mice. Perhaps augmented mitochondrial function, brought about through suppression of DES1, improves whole-body glucose metabolism by reducing ectopic lipid and intracellular lipid metabolites (such as plasma membrane *sn*-1,2-diacylglycerol, acetyl-CoA) independent of weight loss.<sup>1</sup>

Insulin resistance probably encompasses heterogeneous subtypes with discrete mechanisms, but in most clinical situations it ultimately reflects ectopic lipid accumulation in insulin-responsive organs. In some patients, concurrent increases in intracellular ceramides may impair mitochondrial function and exacerbate lipid accumulation and insulin resistance. Modest weight loss can be effective in many scenarios, but is difficult to achieve and sustain without using meal replacements, medication, or bariatric surgery. The findings of Chaurasia et al. are consistent with those of previous studies that support the development of experimental medications that enhance hepatic mitochondrial oxidation, possibly by reducing ceramide biosynthesis (e.g., by inhibiting DES1 or

ceramide synthase 6) or by promoting hepatic mitochondrial uncoupling to treat persons with nonalcoholic fatty liver disease and type 2 diabetes who are unable to achieve therapeutic weight loss.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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DOI: 10.1056/NEJMcibr1910023

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