**"Mechanistic Insight into Ceramide Synthase 6 (CerS6)-induced Insulin Resistance in Obesity"**

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# Abstract

Obesity contributes to changes in the cellular lipid content, also in non-adipose tissues, and to alterations in mitochondrial integrity in the pathophysiology of insulin resistance. Identification of the specific lipid metabolites and the mechanisms by which they induce insulin resistance will help to better understand the causes underlying the adverse health outcomes of obesity in rodent models and human patients. In obesity, members of the heterogeneous group of sphingolipids, known as ceramides, accumulate ectopically, and $C_{16:0}$ ceramides promote weight gain and glucose intolerance in mice by interfering with mitochondrial fatty acid oxidation in liver and brown adipose tissue.

This dissertation reports on a distinction between the two $C_{16:0}$ ceramide-producing enzymes, ceramide synthase (CerS)5 and CerS6, while only abrogation of the latter in mice prevents obesity-induced hepatic mitochondrial fragmentation, liver steatosis, and insulin resistance. These differential effects are likely due to the selective ability of CerS6 to regulate the $C_{16:0}$ ceramide content of mitochondria and mitochondria-associated membranes (MAM) in the liver. Chemo-proteomic screening for sphingolipid-binding proteins in cultured cells revealed candidates potentially interacting with either CerS5- or CerS6-derived sphingolipids. In this context, the protein mitochondrial fission factor (MFF) was captured only in the presence of CerS6, indicative of specific binding of MFF to CerS6-derived sphingolipids. In the liver of mice, MFF and CerS6 cooperatively promote obesity-related hepatic mitochondrial fragmentation and insulin re-sistance. In addition, the timely controlled disruption of CerS6 or MFF in obesity improves glucose metabolism, highlighting the therapeutic potential of inhibiting CerS6 or the $C_{16:0}$ sphingo-lipid/MFF interaction to treat metabolic disorders in obesity.

In sum, the present study demonstrates the complexity of ceramide deregulation in obesity, while the intracellular site of $C_{16:0}$ ceramide accumulation was identified as critical in the regulation of mitochondrial fragmentation and development of insulin resistance. Further evidence is provided for a mechanism that links hepatic lipid accumulation to morphological and functional alterations of mitochondria, namely via CerS6-dependent regulation of MFF, thereby promoting the develop-ment of metabolic diseases in obesity.