VIEWS AND COMMENTARIES

Recycling the danger via lipid droplet biogenesis after autophagy

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ABSTRACT

Fatty acids are an important cellular energy source under starvation conditions. However, excessive free fatty acids (FFAs) in the cytoplasm cause lipotoxicity. Therefore, it is important to understand the mechanisms by which cells mobilize lipids and maintain a homeostatic level of fatty acids. Recent evidence suggests that cells can break down lipid droplets (LDs), the intracellular organelles that store neutral lipids, via PNPLA2/adipose triglyceride lipase and a selective type of macroautophagy/autophagy termed lipophagy, to release FFAs under starvation conditions. FFAs generated from LD catabolism are either transported to mitochondria for β -oxidation or converted back to LDs. The biogenesis of LDs under starvation conditions is mediated by autophagic degradation of membranous organelles and requires diacylglycerol O-acyltransferase 1, which serves as an adaptive cellular protective mechanism against lipotoxicity.

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Lipid droplets (LDs) are intracellular organelles that primarily store triacylglycerol (TAG) and sterol esters as a bioenergy source. Under starvation conditions, cells shift their metabolism from reliance on glycolysis to fatty acid oxidation through the breakdown of LDs and then transport free fatty acids (FFAs) to mitochondria for β -oxidation as an adaptive response. In addition to being a nutrient source, excessive FFAs in the cytoplasm may be detrimental to cells via the generation of reactive oxygen species (ROS) and the subsequent damage to mitochondria and other cellular components. It is essential for cells to develop mechanisms to control the cytoplasmic levels of FFAs. Cells can use two major pathways for LD catabolism: lipolysis via cytosolic neutral lipases such as patatin-like phospholipase domain containing 2 (PNPLA2)/adipose triglyceride lipase (ATGL) and the autophagic/lysosomal pathway. Upon starvation, it was reported that lipophagy is induced for the removal of LDs.^{1,2} This would predict that nutrient starvation should cause a decrease of LDs due to enhanced autophagic activity. However, evidence suggests a different view of the role of autophagy in the biogenesis of LDs. It was reported that in mouse embryonic fibroblasts (MEFs), the number of intracellular LDs increases during starvation for amino acids, glucose, and serum, despite enhanced autophagy or possible lipophagy (a form of autophagy selective for LDs).³ In a recent study, Nguyen et al. further investigated this phenomenon that is seemingly paradoxical to the concept of starvation-induced “lipophagy”.⁴ They found that upon autophagic degradation of intracellular membranes, which generates FFAs, cells increase LD formation to channel the FFAs as an adaptive protective mechanism against FFA accumulation and lipotoxicity. They further demonstrate that this process is dependent on

diacylglycerol O-acyltransferase 1 (DGAT1), an enzyme that mediates the final step in TAG synthesis.

The authors first determined the dynamics of LDs during starvation by culturing MEFs in either complete medium or in Hank's balanced salt solution (HBSS), which lacks amino acids, glucose, glutamine, and serum. They found that indeed HBSS rapidly induces the number of LDs that reach the plateau at around 16 h. By selectively depleting each nutrient component, the authors found that deprivation of amino acids or glutamine but not glucose or serum can induce autophagic flux, and importantly, increase the number of intracellular LDs. These results suggest that glucose or serum starvation is not an ideal condition to trigger autophagy. Inhibition of autophagy either by treatment with bafilomycin A₁ (BafA1) that inhibits the lysosomal vacuolar-type H⁺-translocating ATPase or 3-methyladenine (3-MA) that inhibits the class III phosphatidylinositol 3-kinase PIK3C3/VPS34 blocks starvation-induced LD formation, supporting the notion that increased autophagic flux correlates with the accumulation of LDs during starvation. Starvation-induced LD formation is independent of de novo fatty acid (FA) synthesis because fatty acid synthase inhibition by TVB-3166 cannot block LD formation. Notably, LD formation requires the autophagic degradation of membrane lipids but not LC3-II formation, because BafA1 blocks starvation-induced LD formation despite increased levels of LC3-II. This is different from a previous report that LC3-II localizes on the LD surface and is involved in LD formation in mouse hepatocytes under starvation conditions.⁵

Because starvation inhibits the activity of mechanistic target of rapamycin [serine/threonine kinase] complex 1 (MTORC1), the authors next examined whether the formation of LDs is

mediated by MTORC1 signaling during starvation. Inhibition of MTORC1 by Torin1 or ablation of LAMTOR1/p18, a subunit of the Ragulator complex that is required for MTORC1 lysosomal translocation and activation,⁶ increases the number of LDs even in complete medium. There is no further increase of LDs in the presence of Torin1 or in LAMTOR1-deficient MEFs during HBSS starvation. Furthermore, MEFs lacking nitrogen permease regulator-like 2, a subunit of the Gator1 complex that negatively regulates RAG GTPases and inhibits MTORC1 activity,⁷ fail to undergo starvation-induced LD accumulation. Therefore, inhibition of MTORC1 is necessary and sufficient for autophagy-dependent biogenesis of LDs.

Next, the authors determined the role of DGAT1 and DGAT2, the two enzymes that mediate the final step in TAG synthesis,⁸ in LD biogenesis during starvation or FA overload. They found that selective inhibition of DGAT1 is sufficient to block starvation-induced LD biogenesis, whereas inhibition of both DGAT1 and DGAT2 is required to abolish oleic acid overload-induced LD formation. It remains unclear why starvation-induced LD biogenesis only relies on DGAT1 but not DGAT2. Inhibition of DGAT1 does not affect autophagic flux, which thus ruled out the possibility that decreased LD formation upon DGAT1 inhibition is due to decreased autophagy. Having observed the increased LD biogenesis during starvation, the authors next determined the mechanisms by which the pre-existing and starvation-induced LDs are degraded. They found that degradation of pre-existing and starvation-induced LDs is blocked by ATGListatin, an inhibitor of LD-associated PNPLA2/ATGL. These results suggest that PNPLA2-mediated LD lipolysis plays an important role in LD catabolism under starvation conditions. It was reported recently that PNPLA2 can also affect autophagy and it is unclear whether the inhibition of LD breakdown by ATGListatin also leads to decreased autophagy.⁹ Thus, the contribution of lipophagy vs PNPLA2-mediated LD breakdown under starvation conditions remains elusive.

Interestingly, using Bodipy staining for LDs and MitoTracker for mitochondria, the authors found that LDs clustered in close proximity to mitochondria, raising the possibility that FAs are transferred via the LD-mitochondria contact sites into mitochondria for β -oxidation. However, DGAT1 inhibition during starvation markedly increases the levels of C16:0 and C18:0 acylcarnitines, which are generated from FAs by mitochondrial carnitine palmitoyltransferase 1 (CPT1). Because DGAT1 inhibition decreases LD biogenesis during starvation, these results suggest that FA delivery to mitochondria does not require LD formation and may even increase when DGAT1 activity is inhibited. It is likely that the LD-mitochondrial contact site may only affect the existing LDs and is less efficient compared with direct transfer of FFAs to mitochondria for β -oxidation. This notion is supported by the increased level of acylcarnitine in the presence of DGAT1 inhibitor (which results in fewer LDs).

Finally, the authors determined the physiological significance of autophagy and PNPLA2-dependent LD biogenesis during starvation. It has been generally thought that FFAs are toxic to cells, hence converting FFAs to LDs may protect cells against lipotoxicity.¹⁰ Indeed, the authors found that inhibition of DGAT1 during starvation markedly decreases cell viability.

To further determine the molecular mechanism of increased cell death under the DGAT1 inhibition and starvation conditions, the authors investigated the generation of ROS, markers of ER stress and mitochondrial functions. Inhibition of DGAT1 in HBSS-starved cells increases neither ROS production nor ER stress, whereas mitochondrial oxygen consumption and mitochondrial membrane potential are markedly decreased. Because increased level of acylcarnitines is detected in response to DGAT1 inhibition during starvation, the authors further investigated the role of acylcarnitines in mitochondrial damage using etomoxir, a pharmacological inhibitor of CPT1. Etomoxir rescues DGAT1 inhibition-induced loss of mitochondrial membrane potential during starvation. Moreover, direct addition of exogenous palmitoylcarnitine is sufficient to depolarize isolated mitochondria. These results suggest that DGAT1-mediated LD formation can serve as a protective mechanism against mitochondrial dysfunction by preventing the accumulation of acylcarnitines during nutrient deprivation.

Overall, this study provides a novel mechanism as to how cells cope with lipotoxicity, which may be caused by FFAs generated by autophagic degradation of intracellular membranes upon starvation. Unlike the conventional notion that autophagy may decrease LD numbers via lipophagy, starvation-induced autophagy promotes LD biogenesis in a DGAT1-dependent manner. Whereas starvation-induced LDs are in close proximity to mitochondria, LDs are not required for FA trafficking to mitochondria but serve as buffering storage to avoid accumulation of acylcarnitines and subsequent mitochondrial dysfunction and lipotoxicity. These novel findings are quite significant because lipotoxicity contributes to a variety of metabolic tissue injuries including pancreatic β cells, vascular endothelial cells, adipocytes, cardiomyocytes and hepatocytes.

While this study has largely extended our understanding of how cells adapt to FA accumulation after autophagy, several important questions remain to be answered. For example, the study was mainly performed in cultured MEFs, which is not the most physiologically relevant system. In response to starvation, mammals including humans increase the mobilization of FFAs from adipose tissues to other metabolic tissues such as liver and muscles. Increased LDs in these tissues are largely due to increased uptake of FFAs. While autophagy is also activated in the liver and muscles under starvation conditions, it is unclear how much autophagy-dependent degradation of membranes would contribute to LD biogenesis compared with the uptake of FFAs from adipose tissue lipolysis. Furthermore, for the pre-existing LDs such as the accumulated LDs in non-alcoholic fatty liver disease (NAFLD), it is also unclear how much pre-existing LDs in NAFLD are degraded via PNPLA2-mediated lipolysis or autophagy-dependent lipophagy. Future work to differentiate the contributions of different pathways for LD catabolism may help refine better targets for treating NAFLD.

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