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commentary

Regulation of lipophagy in NAFLD by cellular metabolism and CD36[1]

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Nonalcoholic fatty liver disease (NAFLD) progresses in a subset of patients to nonalcoholic steatohepatitis (NASH) with inflammation, fibrosis, and increased risk of hepatocellular carcinoma (1). A better understanding of the factors involved in the development, progression, or resolution of hepatic steatosis and NAFLD will expand the repertoire of tools available for treatment of NASH and its associated comorbidities.

The hallmark of NAFLD is an excessive accumulation of liver triglycerides in the form of intracellular lipid droplets (LDs). Lipid homeostasis is impaired, because lipid uptake or synthesis exceeds lipid oxidation or from impaired lipid secretion via VLDLs. As a result, LD biogenesis surpasses LD catabolism (2). Lipid mobilization from LDs in response to nutritional or hormonal cues occurs through two main pathways; by lipolysis through lipases acting at the LD surface and by a form of autophagy termed lipophagy. Autophagy is the orderly degradation and recycling of cellular components by lysosomes in response to stresses, such as nutrient deprivation or the accumulation of damaged proteins or organelles (3-5). The type of cargo delivered to lysosomes is used to classify autophagy. For example, mitophagy and pexophagy refer to mitochondrial and peroxisomal cargos, respectively, and likewise, lipophagy refers to lipid cargo. Lipophagy involves incorporation of small LDs into autophagosomes for delivery to lysosomes, where triglycerides are degraded by lysosomal acid lipase, releasing FAs for mitochondrial oxidation and ATP generation (2, 3).

Autophagy and lipophagy are dynamic processes that are tightly linked to the cellular metabolic state. Nutrient deprivation is a potent inducer of autophagy (6), while chronic high fat feeding (7) or overnutrition, as in the ob/ob mouse (8), are associated with autophagy suppression. Autophagy plays an important role in hepatocyte LD turnover, as supported by the findings that mice lacking the autophagy gene Atg7 in the liver display increased hepatic triglyceride content and reduced rates of both mitochondrial FA oxidation and VLDL secretion (7). In ob/ob mice, overexpression of ATG7 in the liver, by contrast, effectively prevents hepatic steatosis (8).

In this issue of the *Journal of Lipid Research*, Li et al. (9) identify the FA transporter CD36 as a negative regulator of lipophagy in hepatocytes. The authors observe that livers of mice fed a high-fat diet have reduced autophagy and increased CD36 protein. Although these two observations have been previously reported (7, 8, 10), the potential relationship between CD36 and autophagy had not been explored. The authors show that CD36 overexpression in hepatoma-derived cell lines (HepG2 and Huh7) decreases autophagy and increases LD content, while CD36 knockdown has the opposite effect. *Cd36* deletion in mice resulted in increased autophagy, which was abolished by CD36 rescue. The mechanism proposed for the negative effect of CD36 on autophagy is its action to suppress adenosine monophosphate-activated protein kinase (AMPK) (11), which is a major enhancer of autophagy (12). Li et al. show that CD36 deficiency increases autophagy via activation of AMPK, which enhances activity of uncoordinated 51-like kinase 1 (ULK1) and Beclin1, two key protein complexes important for autophagosome biogenesis. Both ULK1 and Beclin1 are activated through AMPK phosphorylation (13).

CD36 is a membrane receptor that recognizes multiple lipid (FAs, lipoproteins) and nonlipid (thrombospondins, collagen) ligands. CD36 interaction with its ligands triggers intracellular signaling that connects to a range of pathways involving metabolism, immunity, and matrix remodeling (14–16). An established physiological function of CD36 is its facilitation of the cellular uptake of FAs, a process implicated in cellular LD accumulation. Less well appreciated is the regulatory role of CD36 signaling in cellular metabolism. CD36 associates with various metabolic proteins and influences protein associations and signal transduction by controlling localization and activity of a number of partner kinases (14). Among the CD36-associated metabolic proteins are AMPK, LKB1, the insulin receptor (IR), PI3, and src kinases, notably Fyn. CD36 expression has been shown to suppress AMPK activation (11) while it enhances insulin signaling (17). These effects are mediated by Fyn phosphorylation of LKB1 and IR, resulting in opposite effects on the signaling activity of these proteins. FA addition reverses the effects of CD36 on AMPK and IR, reducing

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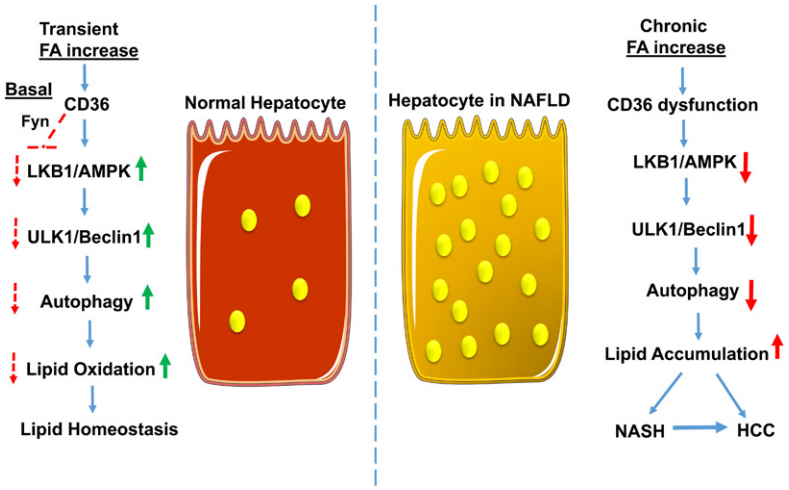

Fig. 1. Lipid regulation of autophagy in the healthy and fatty liver. Autophagy is suppressed by CD36 through Fyn recruitment to phosphorylate LKB1, which prevents LKB1 access to AMPK (left panel, red arrows). Under normal conditions (left panel), acute FA addition disrupts the CD36/FYN association and activates AMPK to increase FA oxidation (11) (green arrows). AMPK activation enhances autophagy by activating ULK1 and Beclin1, two key protein complexes important for autophagosome biogenesis. The enhanced lipid consumption induced by FA maintains hepatic lipid homeostasis. The right panel illustrates the condition where the liver is chronically exposed to excess FA. CD36 becomes dysfunctional and the added FA does not elicit AMPK activation. This might reflect augmented palmitoylation of hepatic CD36, which was reported to associate with NAFLD (10). Fatty acids induce CD36 internalization into vesicles where it colocalizes with LKB1 (11). Possibly the augmented palmitoylation prevents CD36 internalization by FA and AMPK activation. Under such conditions, deletion of CD36 alleviates steatosis as shown by Li et al. (9). HCC, hepato-cellular carcinoma.

insulin-stimulated glucose metabolism and activating AMPK. Thus, the influence of CD36 on cellular preference between glucose and FAs provides a basis for its relationship to autophagy, which is coupled to the nutritional state of the cell.

Previous studies suggest that lipid regulation of autophagy is likely to differ in the healthy, as compared with the fatty, liver. In contrast to chronic lipid exposure, which suppresses autophagy, acute lipid addition activates autophagy, which helps reestablish homeostasis by enhancing lipid consumption (7). The enhanced respiratory metabolism correlates positively with the extent of autophagy induction (6). Oleic acid treatment of hepatocytes activates lipophagy (7) and both palmitic and oleic acids acutely stimulate autophagy in vitro and in vivo (18). Interestingly, AMPK depletion only inhibited palmitic acid induction of autophagy, suggesting that distinctive mechanisms mediate effects of saturated versus unsaturated FAs (18). Li et al. mention that addition of palmitic acid to cells depleted of CD36 did not alter AMPK activation (Fig. 6G). However, this would be expected because palmitate activation of AMPK requires CD36 (11). Thus, a limitation of the study is that it only addresses autophagy in mice chronically fed a high-fat diet and does not shed light on autophagy regulation under normal conditions.

In summary, the available data suggest that lipids activate autophagy to increase oxidative metabolism, but this adaptive response fails with chronic lipid exposure (**Fig. 1**). CD36 expression is normally low in hepatocytes, but both expression and membrane localization are increased in NAFLD (10). A recent report showed that this might be caused by augmented CD36 palmitoylation, which impairs the ability of CD36 to enhance FA oxidation, a process important for autophagy induction. The report further showed that inhibition of CD36 palmitoylation prevents development of steatohepatitis (10). CD36 is subject to a number of posttranslational modifications, and it is possible that targeting these modifications could afford a more specific approach for controlling the function and metabolic effects of the protein.

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