HHS Public Access

Author manuscript

Hepatology. Author manuscript; available in PMC 2020 January 01.

Published in final edited form as:

Hepatology. 2019 January ; 69(1): 444–446. doi:10.1002/hep.30104.

Fasting Inhibits the Recruitment of Kinesin-1 to Lipid Droplets and Stalls Hepatic Triglyceride Secretion

Ryan J. Schulze[1,*] and **Mark A. McNiven**[1]

[1]Department of Biochemistry and Molecular Biology and the Center for Digestive Diseases, Mayo Clinic, 200 1st St SW, Rochester, MN, 55905, USA

In response to constantly changing levels of circulating free fatty acids (FFAs), the parenchymal cells of the liver, hepatocytes, possess a remarkable flexibility in the ability to alternate between the storage and secretion of lipids as appropriate to support organismal energy homeostasis. In the fed state, hepatocytes are generally observed to maintain only limited reservoirs of fat at any given time by virtue of an efficient utilization and repackaging of dietary FFAs into triacylglycerol-enriched very low-density lipoprotein (VLDL) particles for export. In the fasted state, however, the bolus of FFAs liberated from the adipose tissue results in a reduction of hepatocellular VLDL export relative to the significant intracellular accumulation of triglyceride sequestered within cytosolic lipid droplets (LDs). An unresolved question has therefore centered around the mechanisms whereby hepatocytes reign in VLDL secretion in the fasted state, thus preventing unwarranted releases of triglyceride into an already FFA-rich bloodstream. In a recent publication in the Proceedings of the National Academy of Sciences, Rai *et al.* (1) address this issue and uncover new insights at the cellular and molecular levels, demonstrating that a microtubule motor protein (kinesin-1) and a small regulatory GTPase (ARF1) function at the surface of cytosolic LDs in a fed/fasting-dependent manner to control hepatic triglyceride secretion. The authors demonstrate that ARF1 helps recruit kinesin-1 onto the surface of LDs in the fed condition, allowing transport of these neutral lipid-rich organelles to the smooth ER (sER) at the periphery of the cell, where VLDL packaging occurs. This is in contrast to what occurs during fasting, when kinesin-1 is no longer recruited to LDs and triglycerides instead rapidly accumulate within the hepatocyte. The ARF1-dependent recruitment of kinesin-1 appears to be regulated by insulin signaling, providing a potential link between the fed/fasted state and a novel mechanism for the regulatory control of hepatic lipid metabolism.

Using a combination of *in vivo* and *in vitro* approaches in rats and in rat-derived hepatocyte cell lines, Rai *et al.* demonstrate that hepatocellular LDs from fed animals are highly motile, with a tendency to congregate at the outer reaches of the cell. This localization is in accordance with the fact that the neutral lipids stored within LDs can be harvested for packaging into apoB-enriched VLDL particles at the sER. Before lipid redistribution can occur, however, LDs must first be actively transported out to the sER at the cell periphery. Might an understanding of organelle transport provide clues into the regulation of hepatic

*Corresponding Author: Schulze.Ryan@mayo.edu, Phone: +1 (507) 284-9081, Fax: +1 (507) 284-2053.

triglyceride secretion? Decades of work from the Mallik lab and others have provided seminal molecular and biophysical insights into the transport of organelles and vesicles along microtubule tracks within the cell (2). These long-range trafficking events are primarily mediated by the kinesins and dyneins, molecular motors catalyzing the movement of cargo toward the plus and minus ends of microtubules, respectively. One particular member of the kinesin superfamily (kinesin-1) was previously demonstrated to interact directly with LDs and mediate their intracellular movements in *Drosophila* embryos (3). In support of these findings, the authors of this study find hepatocellular LDs of fed rats to be highly motile and, upon isolation from cell homogenates, contain kinesin-1 on their surface.

Could changes in the activity of LD-localized kinesin-1 represent a novel mechanism for stemming the outward flow of LDs to the sER in preparation for VLDL packaging? Knockdown of kinesin-1 and live-cell microscopy show a significant decrease in microtubule plus-end directed motility of LDs to the cell periphery. Furthermore, the authors find that LDs purified from fasted animals exhibit reduced motility along purified microtubules in an *in vitro* setting. Ultimately, the physiological consequence of kinesin-1 perturbations amounted to a significant reduction in the levels of VLDL particles secreted into the cell culture medium.

Unexpectedly, this alteration in intracellular LD trafficking is not due to changes in the enzymatic activity of kinesin-1. Rather, LDs isolated from fasted rat livers appear to simply contain less kinesin-1 bound to their surface. Artificially synthesized LDs incubated together with liver lysates from fasted rat livers also contain less bound kinesin-1, suggesting the existence of a factor mediating the recruitment of this motor protein to the LD surface *in vivo*. Small GTPases frequently play such a role, orchestrating protein and organellar trafficking throughout the cell. Numerous small GTPases have been identified in proteomic analyses of purified LDs. Among these is a member of the ADP-ribosylation factor (ARF) gene family of small GTPases, ARF1, previously shown to be involved in LD protein targeting as well as mediating connections between the LD and the endoplasmic reticulum (4, 5). *In vitro* experiments with purified constitutively active (but membrane-binding defective) mutants of ARF1 showed that LD mobility was dramatically decreased, presumably due to the sequestration of kinesin-1 away from LDs.

These findings implicate two new players in the regulation of hepatic lipid metabolism. However, the signals governing the fed/fasted-dependent association of ARF1 and kinesin-1 with the LD remain unclear. Rai *et al.* propose that the key may lie with a phosphatidylcholine-specific phospholipase, PLD1. A known ARF1 interaction partner, PLD1 has also been shown to mediate the remodeling of the LD phospholipid monolayer, perhaps priming the surface for kinesin-1 binding and FFA transfer during VLDL production in the fed state. Importantly, ARF1 is additionally thought to mediate the insulin-dependent activation of PLD1. Indeed, levels of phospho-PLD1 were found to be dramatically reduced in the livers of fasted animals, where insulin levels would be expected to be reduced. Furthermore, the pharmacological inhibition of PLD1 prevented kinesin-1 recruitment to the LD surface. Therefore, the insulin-responsive activity of the ARF1-PLD1 complex may serve to regulate the amount of kinesin-1 recruited to hepatocellular LDs. In the fed liver, recruitment of this motor protein drives LD transport to the sER at the cell periphery, in

preparation for lipid re-packaging into VLDL particles. In the fasted liver, however, lower insulin levels ultimately result in the buildup of numerous kinesin-free LDs within the cytoplasm – LDs that remain 'stuck in neutral' until the kinesin-1 motor re-engages when VLDL export is once again desirable, e.g. during refeeding.

There are a number of implications for these new findings. This research adds another layer of regulatory complexity onto our understanding of the already intricate process of hepatic lipid metabolism. Current work has already begun to illuminate the important nature of LD-bound regulatory proteins in the reciprocal control of cytoplasmic lipolysis in adipose versus liver tissue (6). Now, novel insights into the roles played by the kinesin superfamily of proteins, involved in another process (LD trafficking), could further aid our understanding of the natural history of fatty liver disease. Indeed, recent studies have begun to examine relationships between these motor proteins and their roles in metabolic homeostasis. For example, an adipose tissue-specific knockout of KIF5B (kinesin heavy chain) was found to lead to enhanced hepatic triglyceride accumulation in mice fed a high-fat diet (7). Likewise, two additional kinesin superfamily members (KIF13B and KIF12) were also recently implicated in the pathogenesis of metabolic disease (8, 9). Future studies of the numerous other kinesin superfamily members and identification of potential connections to NAFLD development will therefore be informative. This study thus represents an important and exciting step forward by providing new insights and novel molecular machinery targets involved in the process of hepatic lipid metabolism and VLDL secretion.

References

1. Rai P, Kumar M, Sharma G, Barak P, Das S, Kamat SS, Mallik R. Kinesin-dependent mechanism for controlling triglyceride secretion from the liver. Proc Natl Acad Sci U S A. 2017; 114:12958–12963. [PubMed: 29158401]
2. Mallik R, Gross SP. Intracellular transport: how do motors work together? Curr Biol. 2009; 19:R416–418. [PubMed: 19467211]
3. Shubeita GT, Tran SL, Xu J, Vershinin M, Cermelli S, Cotton SL, Welte MA, et al. Consequences of motor copy number on the intracellular transport of kinesin-1-driven lipid droplets. Cell. 2008; 135:1098–1107. [PubMed: 19070579]
4. Guo Y, Walther TC, Rao M, Stuurman N, Goshima G, Terayama K, Wong JS, et al. Functional genomic screen reveals genes involved in lipid-droplet formation and utilization. Nature. 2008; 453:657–661. [PubMed: 18408709]
5. Wilfling F, Thiam AR, Olarte MJ, Wang J, Beck R, Gould TJ, Allgeyer ES, et al. Arf1/COPI machinery acts directly on lipid droplets and enables their connection to the ER for protein targeting. Elife. 2014; 3:e01607. [PubMed: 24497546]
6. Zhang X, Xie X, Heckmann BL, Saarinen AM, Czyzyk TA, Liu J. Targeted disruption of G0/G1 switch gene 2 enhances adipose lipolysis, alters hepatic energy balance, and alleviates high-fat diet-induced liver steatosis. Diabetes. 2014; 63:934–946. [PubMed: 24194501]
7. Cui J, Pang J, Lin YJ, Gong H, Wang ZH, Li YX, Li J, et al. Adipose-specific deletion of Kif5b exacerbates obesity and insulin resistance in a mouse model of diet-induced obesity. FASEB J. 2017; 31:2533–2547. [PubMed: 28242773]
8. Kanai Y, Wang D, Hirokawa N. KIF13B enhances the endocytosis of LRP1 by recruiting LRP1 to caveolae. J Cell Biol. 2014; 204:395–408. [PubMed: 24469637]
9. Yang W, Tanaka Y, Bundo M, Hirokawa N. Antioxidant signaling involving the microtubule motor KIF12 is an intracellular target of nutrition excess in beta cells. Dev Cell. 2014; 31:202–214. [PubMed: 25373778]