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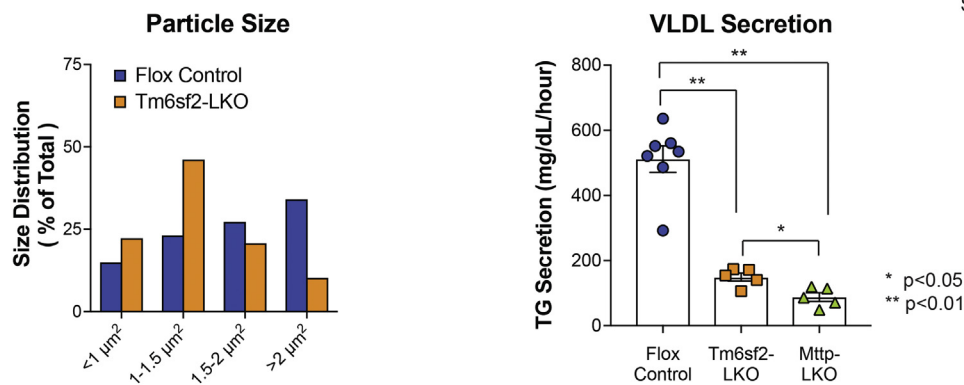
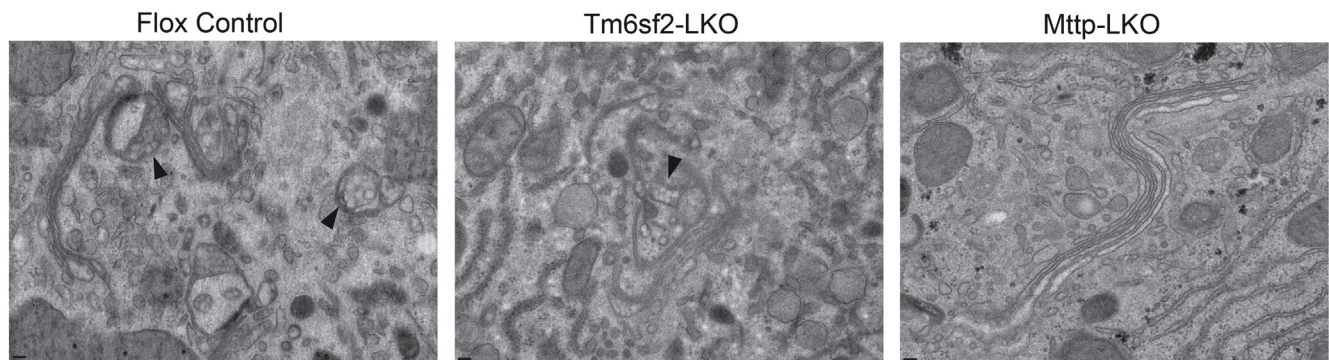
Liver-specific deletion of *Mttp* versus *Tm6sf2* reveals distinct defects in stepwise VLDL assembly

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Hepatic VLDL assembly is proposed as a two-step process in which a small primordial lipoprotein particle is formed in the smooth ER by cotranslational lipidation of apolipoprotein B (APOB) by the lipid transfer chaperone microsomal triglyceride transfer protein (MTTP) (1). The nascent VLDL particles are progressively lipidated (step 2) through fusion with ER lipid droplets in a process that requires MTTP and other players, including TM6SF2 (1). Because MTTP is required for both step 1 and step 2, mice with liver-specific deletion of *Mttp* (*MtTp-LKO*) develop hepatic steatosis and complete abrogation of VLDL and APOB secretion (2). Both germline (3) and liver-specific *Tm6sf2* deleter mice (*Tm6sf2-LKO*, *Tm6sf2*^{f/f} Albumin Cre^{Tg}) (4) exhibit steatosis and reduced VLDL triglyceride, but no change in APOB secretion (even in an APOB100-only background (4)), consistent with secretion of small and underlipidated VLDL particles in *Tm6-LKO* mice (3, 4). We used transmission electron microscopy to visualize intracellular and nascent lipoprotein particles in the ER and Golgi of *MtTp-LKO*, *Tm6sf2-LKO*, and Flox control mice. Lipoprotein particles, which appear translucent in the absence of imidazole-buffered osmium staining (2), were observed in Golgi of control and *Tm6sf2-LKO* mice (arrowheads, left and middle panels), but none were detected in the ER or Golgi of *MtTp-LKO* mice, with Golgi stacks appearing either flat or dilated, with no distinct structures inside (right). Nascent VLDL particles in *Tm6sf2-LKO* Golgi were smaller compared with controls (lower left, $n = 68-74$ particles/genotype; ImageJ software; NIH). Relative VLDL secretion rate is shown for each genotype (lower right).

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EQUIPMENT: Leica EM UC7 ultramicrotome (Leica-Microsystems, Vienna, Austria) and JEM-1400 transmission electron microscopy (JEOL, Peabody, MD) with an AMT XR111 8 Megapixel scintillated CCD camera.

METHODS: Livers were perfused in situ (2.5% glutaraldehyde, 2% paraformaldehyde, 2 mM CaCl₂ in 0.15 M cacodylate buffer, 5 min), excised, and fixed overnight in fresh fixative. The tissue was postfixed (1% osmium tetroxide and 0.3% potassium ferrocyanide in 0.15 M cacodylate), washed, and stained en bloc with 2% aqueous uranyl acetate (overnight, 4°C). Tissues were dehydrated in 30%, 50%, 70%, and 100% (3×) ethanol, infiltrated in a Spurr's resin/ethanol-graded series, embedded in 100% Spurr's resin, and polymerized at 60°C for 48 h. About 70 nm ultrathin sections were picked up on copper formvar/carbon support film grids and poststained with 1% tannic acid (2 min), 1% osmium tetroxide (2 min), 2% uranyl acetate (15 min), and Sato's lead for 2 min. Serum triglyceride was measured at 0 and 4 h after injection with Pluronic F-127 (10 µl/g body weight) to examine VLDL secretion.

Author contributions

E. P. N. conceptualization, investigation, and validation; G. W. S. methodology and validation; J. A. J. F. methodology, validation, supervision, and funding acquisition; and N. O. D. conceptualization, writing, review and editing, and funding acquisition.

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REFERENCES

1. Ginsberg, H. N. (2021) ApoB SURFs a Ride from the ER to the Golgi. *Cell Metab.* **33**, 231–233
2. Raabe, M., Veniant, M. M., Sullivan, M. A., Zlot, C. H., Bjorkegren, J., Nielsen, L. B., Wong, J. S., Hamilton, R. L., and Young, S. G. (1999) Analysis of the role of microsomal triglyceride transfer protein in the liver of tissue-specific knockout mice. *J. Clin. Invest.* **103**, 1287–1298
3. Smagris, E., Gilyard, S., BasuRay, S., Cohen, J. C., and Hobbs, H. H. (2016) Inactivation of Tm6sf2, a gene defective in fatty liver disease, impairs lipidation but not secretion of very low density lipoproteins. *J. Biol. Chem.* **291**, 10659–10676
4. Newberry, E. P., Hall, Z., Xie, Y., Molitor, E. A., Bayguinov, P. O., Strout, G. W., Fitzpatrick, J. A. J., Brunt, E. M., Griffin, J. L., and Davidson, N. O. (2021) Liver specific deletion of mouse Tm6sf2 promotes steatosis, fibrosis and hepatocellular cancer. *Hepatology*. <https://doi.org/10.1002/hep.31771>