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

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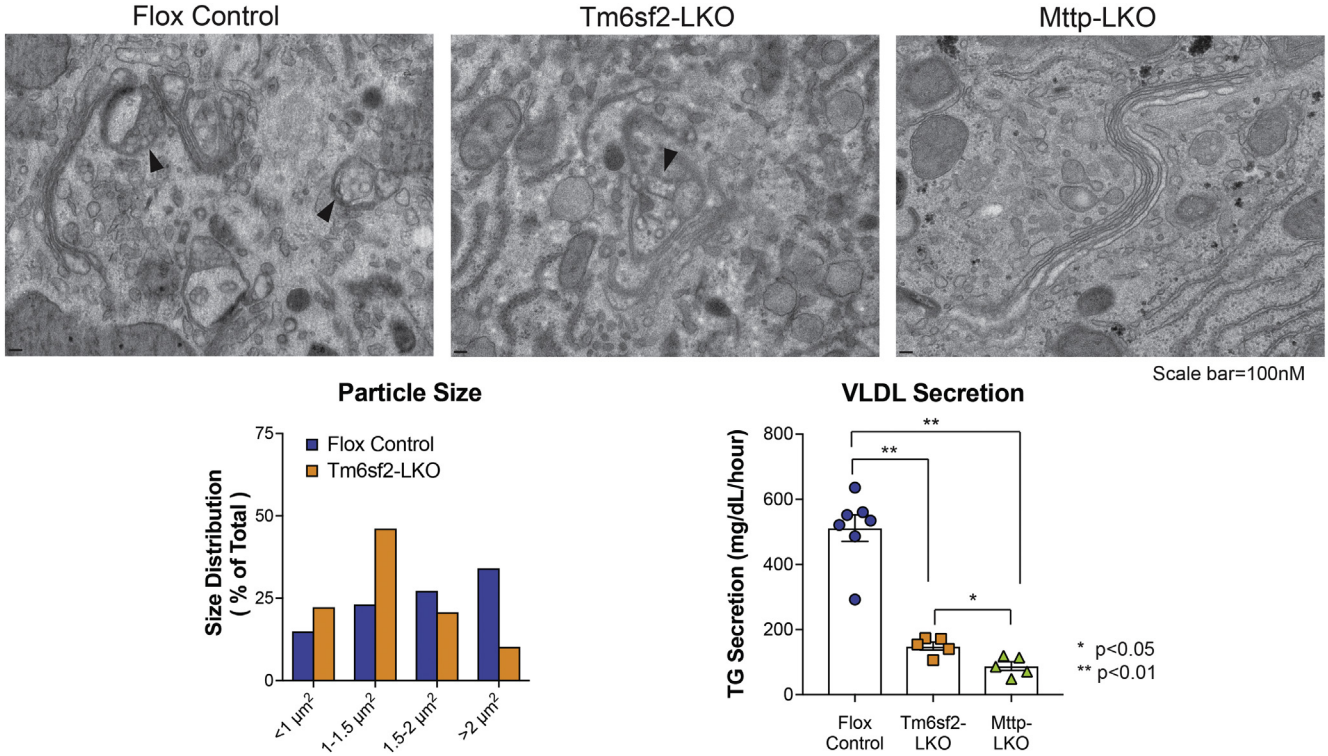

Hepatic VLDL assembly is proposed as a two-step process in which a small primordial lipoprotein particle is formed in the smooth ER by cotranslational lipidation of apolipoprotein B (APOB) by the lipid transfer chaperone microsomal triglyceride transfer protein (MTTP) (1). The nascent VLDL particles 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* deletor 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 $CaCl_2$ 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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