# Uncovering the dual role of hepatocyte-derived Apolipoprotein E in lipoprotein metabolism

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**Background and Aims:** Apolipoprotein E (ApoE) is a key regulator of lipid metabolism, controlling transport and clearance of triglyceriderich lipoproteins. Although ApoE is produced in several tissues, the liver, especially hepatocytes, is the main source of circulating ApoE. Wholebody ApoE knockout mice develop severe dyslipidemia, whereas mice lacking ApoE only in hepatocytes (ApoEΔHep) display only mild alterations on a standard diet, suggesting compensation from extrahepatic sources and raising questions about the specific contribution of hepatic ApoE. This work investigated the distinct role of hepatocytederived ApoE in hepatic lipid handling.
**Methods:** ApoEΔHep mice were generated by crossing ApoE flox/flox mice with AlbuminCre mice. Animals were kept on a standard chow diet for 12 weeks. Lipid metabolism was assessed by FPLC, Western blotting, hepatic geneexpression analysis and functional assays of lipoprotein production and clearance.
**Results:** Plasma WB confirmed that hepatocyte-derived ApoE is the main circulating source of the protein, which was absent in ApoEΔHep mice. Total plasma cholesterol was similar to controls under both standard diet (76,19±9,99 vs. 85,64±15,20 mg/dL) and highcholesterol diet (154,7±26,45 vs. 151,4±14,35 mg/dL). However, lipoprotein profiling revealed a shift toward LDLenriched cholesterol in ApoEΔHep mice, in contrast to the VLDLdominant pattern of global ApoEKO mice. ApoEΔHep animals showed delayed clearance of triglycerides, consistent with impaired removal of triglyceriderich lipoproteins, and a 46% reduction ($p < 0.0001$) in VLDL production, indicating a role for hepatocyte ApoE in the synthesis and secretion of these particles. Hepatic transcript analysis pointed to deregulated lipidmetabolic pathways.
**Conclusions:** This work highlights a dual role of hepatocyte-derived ApoE in both the production and clearance of TG-rich lipoproteins, pointing to distinct function of ApoE at systemic versus hepatocellular level. Ongoing molecular analyses aim to further dissect the intracellular mechanisms of lipoprotein synthesis and clarify the hepatocyte-specific contributions of ApoE to lipid metabolism and dyslipidemia.

# Evaluation of siRNA therapeutic approaches for the regulation of ceramide synthesis

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**Background and Aim:** Since increased plasma ceramide levels have recently emerged as a pro-atherogenic factor, selective modulation of hepatic ceramide biosynthesis might represent a novel therapeutic approach to reduce cardiovascular risk.
**Methods:** The efficacy of siRNA-based treatments (siPOOLs®) on ceramide level modulation was tested by targeting single enzymes or couples of enzymes playing key roles in ceramide metabolism. *In vitro*, the effects of siRNAs on target gene expression and lipidome changes were evaluated in Hepa1c1c7 murine hepatocytes (96 hours after treatment, 6 nM). For *in vivo* studies, siPOOLs® were encapsulated in lipid nanoparticles (LNPs) and first tested for hepatic tropism by confocal microscopy (single intraperitoneal injection of Cy5-labelled LNPs, 2 mg/kg, in C57Bl/6 male mice). *In vivo* gene silencing efficacy, effects on plasma and liver lipidome, and histological analyses were assessed after four intraperitoneal injections (2 mg/kg every 72 hours) in 8-week-old C57Bl/6 male mice.
**Results:** *In vitro* validation identified siRNAs effective in reducing target gene expression and lowering ceramide levels in hepatocytes. Biodistribution studies showed that siRNA-LNPs accumulated primarily in the liver and in the spleen. *In vivo* testing showed variable silencing efficacy of siRNA-LNPs, ranging from –17% (Sptlc1) to –80% (Sptssa), depending on the target and the combination/dose administered. Despite a promising silencing effect, lipidomic analyses of plasma and liver did not show effective modulation of ceramide levels. Treated mice displayed extramedullary hematopoiesis in the spleen and, in a few cases, hepatic necrosis.
**Conclusions:** The siRNA-LNP approach resulted in efficient silencing but did not translate into plasma ceramide modulation. A second strategy, consisting of N-acetyl galactosamine-conjugated siRNAs, will be tested *in vitro* and *in vivo*, with the aim of achieving higher efficacy and reduced toxicity.