**Hepatic stellate cells retain the capacity to synthesize retinyl esters and to store neutral lipids in small lipid droplets in the absence of LRAT**

ABSTRACT

Hepatic stellate cells (HSCs) play an important role in liver physiology and under healthy conditions they have a quiescent and lipid-storing phenotype. Upon liver injury, HSCs are activated and rapidly lose their retinyl ester-containing lipid droplets. To investigate the role of lecithin:retinol acyltransferase (LRAT) and acyl-CoA:diacylglycerol acyltransferase 1 (DGAT1) in retinyl ester synthesis and lipid droplet dynamics, we modified LC–MS/MS procedures by including multiple reaction monitoring allowing unambiguous identification and quantification of all major retinyl ester species. Quiescent primary HSCs contain predominantly retinyl palmitate. Exogenous fatty acids are a major determinant in the retinyl ester species synthesized by activated HSCs and LX-2 cells, indicating that HSCs shift their retinyl ester synthesizing capacity from LRAT to DGAT1 during activation. Quiescent LRAT−/− HSCs retain the capacity to synthesize retinyl esters and to store neutral lipids in lipid droplets ex vivo. The median lipid droplet size in LRAT−/− HSCs (1080 nm) is significantly smaller than in wild type HSCs (1618 nm). This is a consequence of an altered lipid droplet size distribution with 50.5 ± 9.0% small (≤ 700 nm) lipid droplets in LRAT−/− HSCs and 25.6 ± 1.4% large (1400–2100 nm) lipid droplets in wild type HSC cells. Upon prolonged (24 h) incubation, the amounts of small (≤ 700 nm) lipid droplets strongly increased both in wild type and in LRAT−/− HSCs, indicating a dynamic behavior in both cell types. The absence of retinyl esters and reduced number of lipid droplets in LRAT-deficient HSCs in vivo will be discussed.

**Keyword:** Hepatic stellate cells; LRAT; DGAT1; Retinyl esters; Lipid droplets; Lipidomics