

Obeticholic acid attenuates fibrosis development in a high fat diet induced NASH model (LDLr^{-/-}.Leiden mice)

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Introduction

The LDLr^{-/-}.Leiden mouse is a translational, diet-inducible model for non-alcoholic steatohepatitis (NASH) with associated fibrosis, displaying many clinically relevant features of NASH. Here, we aimed to study whether the progression of fibrosis in this model can be retarded or reverted by switch to a healthy diet or by therapeutically intervention using obeticholic acid.

Methods

We fed LDLr^{-/-}.Leiden mice a high-fat diet (HFD) for 24w, after which mice were randomized into 4 groups: one group was sacrificed, one continued on HFD, one was switched to chow (dietary intervention) and one received HFD + 10 mg/kg obeticholic acid (OCA) for the remainder of the study (up to 34w; see Figure 1). Development of NASH and hepatic fibrosis was assessed blindly by a pathologist, as well as by direct measuring collagen synthesis rates (assessed as the incorporation of deuterium from heavy water into the stable C-H bonds of hydroxyproline (OHP) in the newly synthesized protein).

Results – NASH

Liver weights did not differ between groups. Continuation on HFD did not increase the liver steatosis (Fig 3B and 3C). Dietary switch to chow resulted in significantly less steatosis, in particular macrovesicular steatosis. OCA intervention did show less total hepatic triglycerides, although macro vesicular steatosis was not significantly lower. 34 weeks on HFD markedly increased inflammatory aggregates, which tended to be lower by dietary switch to chow and OCA intervention (Fig 3D).

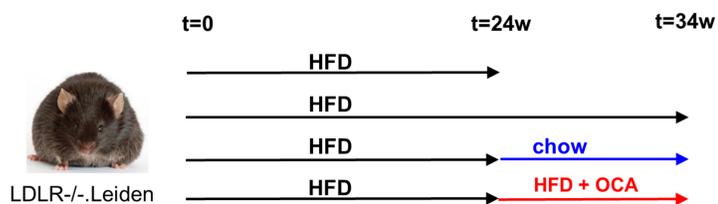


Figure 1: Experimental set-up

Results

OCA did not significantly influence body weight, while switch to chow resulted in moderate body weight loss. Circulating ALT levels and plasma lipids were significantly reduced by both switch to chow and OCA interventions (Figure 2).

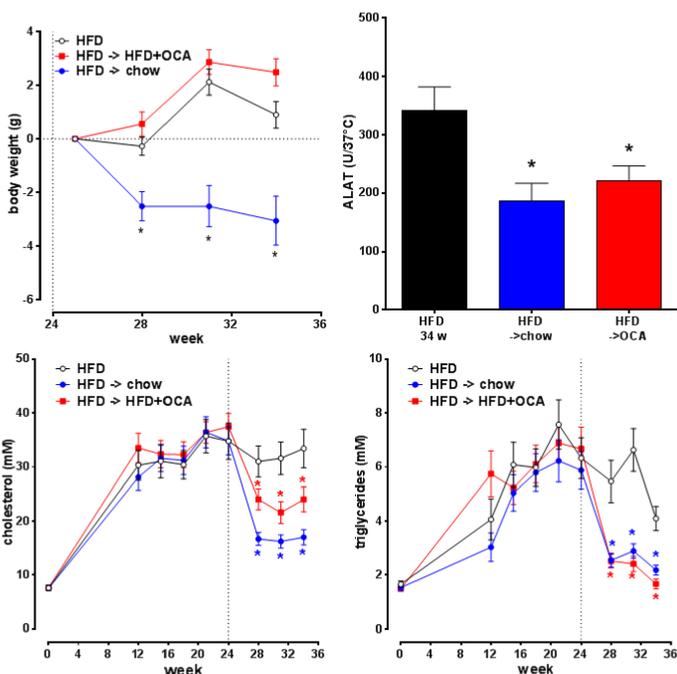


Figure 2: Body weight, plasma ALT at 34 weeks and plasma cholesterol and triglycerides over time. *P<0.05 vs HFD 34 weeks.

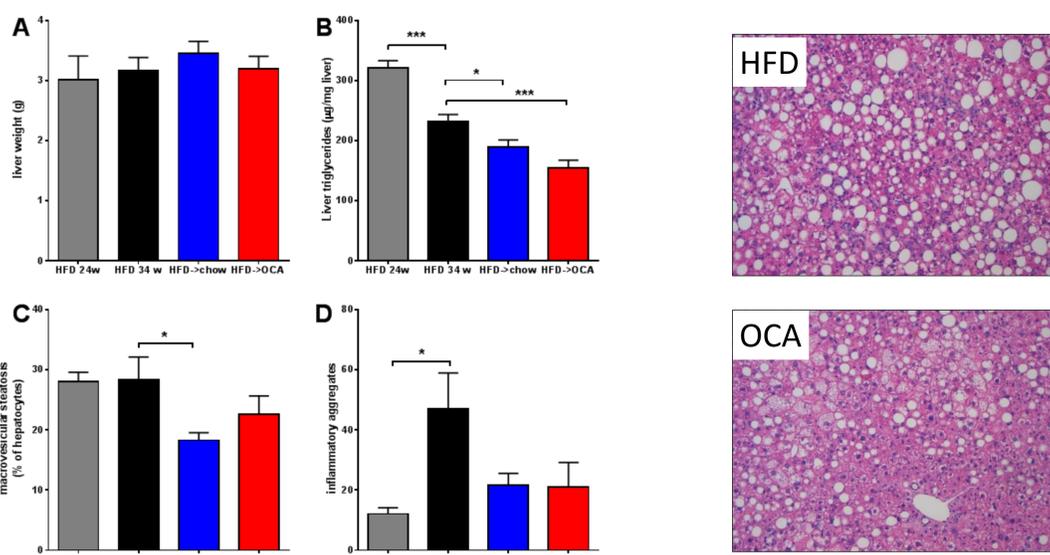


Figure 3: Liver weight (A), total liver triglycerides (B), macrovesicular steatosis (C) and inflammatory aggregates (D). Additionally, representative photomicrographs of HE stained liver cross section for HFD 34w and HFD+OCA are shown.

Results – liver fibrosis

Both switch to chow and OCA interventions reduced the progression of fibrosis, analyzed by pathologist (Fig4A) and confirmed by image analysis (Fig 4B). Newly synthesized guanidine soluble as well as guanidine insoluble collagen Ia1 was reduced by dietary and OCA intervention compared to HFD (Fig 4C and 4D).

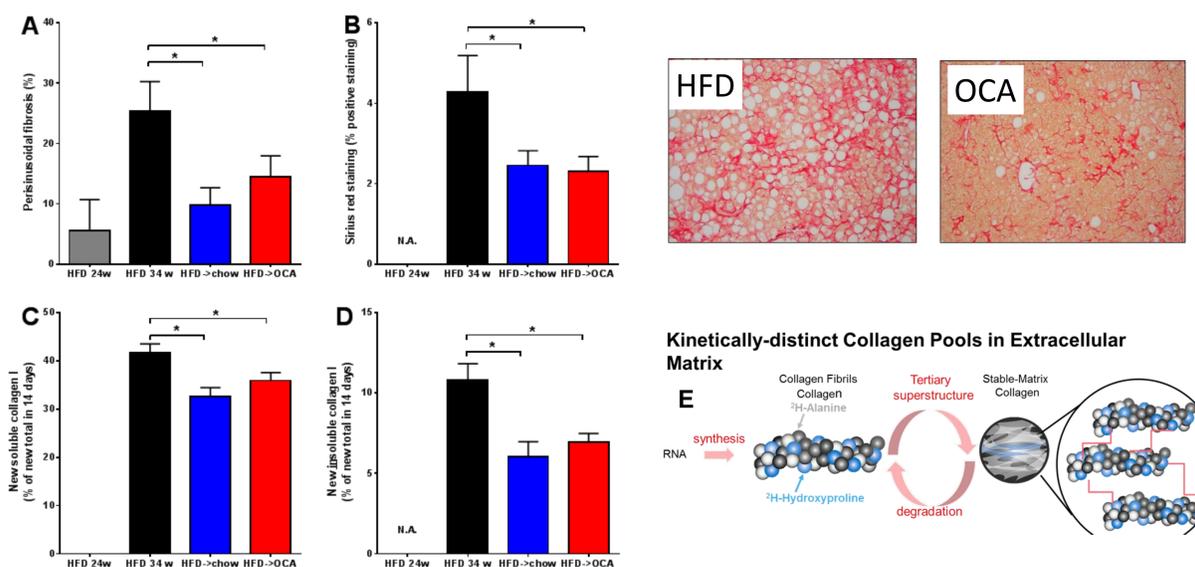


Figure 4: Liver fibrosis analyzed by pathologist (A) and by image analysis (B). Sirius red stained cross sections are shown. De novo collagen synthesis was analyzed both for guanidine soluble (C) as well as guanidine insoluble (D) fractions, illustrated in the cartoon (E).

Conclusions

- LDLr^{-/-}.Leiden mice develop NASH (steatosis and inflammation) with progressive fibrosis upon HFD feeding.
- The fibrosis progression can be reduced by dietary (switch to chow) and pharmaceutical (OCA) interventions.
- D₂O technology allows to analyse newly synthesized collagen and to dissociate between guanidine soluble and guanidine insoluble.