Effect of OCA treatment on metabolic parameters, hepatic pathology and NAFLD Activity Score including Fibrosis Stage in female and male biopsy-confirmed DIO-NASH mice

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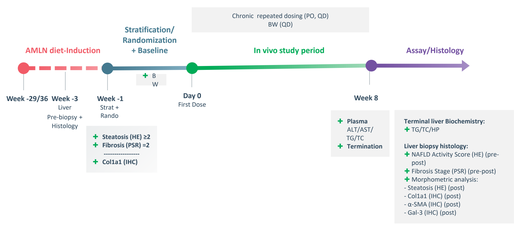
INTRODUCTION AND AIM

Nonalcoholic fatty liver disease and its more severe form, nonalcoholic steatohepatitis (NASH) are considered to be the hepatic manifestation of the metabolic syndrome and the prevalence of NASH is increasing worldwide. In humans, NASH is twice as common in men than in women until age 60, where the prevalence shifts to become higher in women. In rodent studies, it is believed that males are more sensitive to NASH development compared to females due to estrogen and its protective effects. The aim of this study was to explore the development of metabolic, biochemical and histopathological endpoints related to NASH as well as the response to treatment of a late-stage clinically relevant drug candidate, the FXR agonist obeticholic acid (OCA), for both male and female mice in a diet-induced obese (DIO) mouse model of biopsy-confirmed NASH with fibrosis.

METHODS

Male and female C57BL/6J mice were fed Amylin Liver NASH (AMLN, D09100301, Research Diets, USA) diet high in fat, fructose and cholesterol or chow (Altromin 1324, Brogården, Denmark) for 36 and 29 weeks, respectively prior to liver pre-biopsy collection. Only DIO-NASH animals with biopsy-confirmed steatosis (score =3) and fibrosis (stage =F2) were included. DIO-NASH mice were stratified and randomized into treatment groups based on liver collagen 1a1 (% fractional area). The Chow mice received vehicle (PO, QD) and DIO-NASH mice received either vehicle (PO, QD) or OCA (30 mg/kg, PO, QD) for 8 weeks. Pre-post liver biopsy histopathology was performed for within-subject evaluation of changes in composite NAFLD Activity Score (NAS) and Fibrosis Stage. Also, terminal quantitative liver histology, blood and liver biochemistry was assessed.

STUDY DESIGN



GROUPS

No. #	Group name	Number of animals	Animal model	Dose [mg/kg]	Dosing Frequency	Route of administration
1	Male Chow Vehicle	10	LEAN-CHOW	NA	QD	PO
2	Male NASH Vehicle	11	DIO-NASH	NA	QD	PO
3	Male OCA 30mg/kg	10	DIO-NASH	30	QD	PO
4	Female Chow Vehicle	9	LEAN-CHOW	NA	QD	PO
5	Female NASH Vehicle	11	DIO-NASH	NA	QD	PO
6	Female OCA 30 mg/kg	11	DIO-NASH	30	QD	PO

RESULTS

Baseline characteristics in female and male DIO-NASH mice

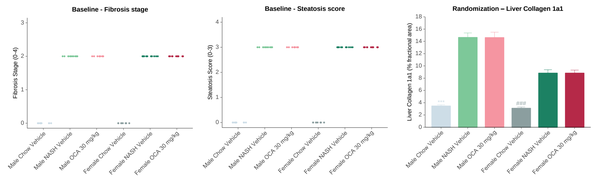


Figure 1 | Liver pre-biopsy stratification and randomization into study groups after 26/33 weeks on AMLN diet. Inclusion criteria for DIO-NASH mice were based on steatosis score (=3), fibrosis stage (=2) and randomization based on collagen 1a1 (% fractional area) by histomorphometric image analysis.

Effect on OCA treatment on metabolic and biochemical parameters in female and male DIO-NASH mice

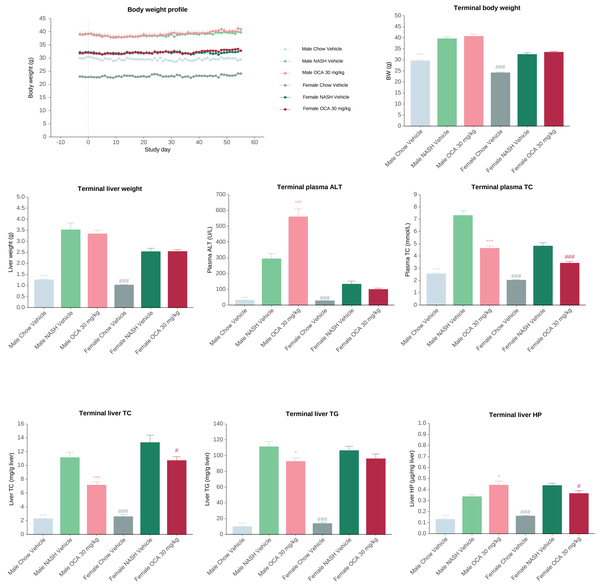


Figure 2 | Body weight profile, terminal body weight, liver weight and biochemical metabolic parameters. ALT: alanine transaminase, TG: total triglycerides, TC: total cholesterol, HP: hydroxyproline. *: P < 0.05 **: P < 0.01 ***: P < 0.001 compared to Male NASH Vehicle. #: P < 0.05 ##: P < 0.01 ###: P < 0.001 compared to Female NASH Vehicle

Effect of OCA treatment on histopathological NAFLD Activity Score and Fibrosis Stage in female and male DIO-NASH mice

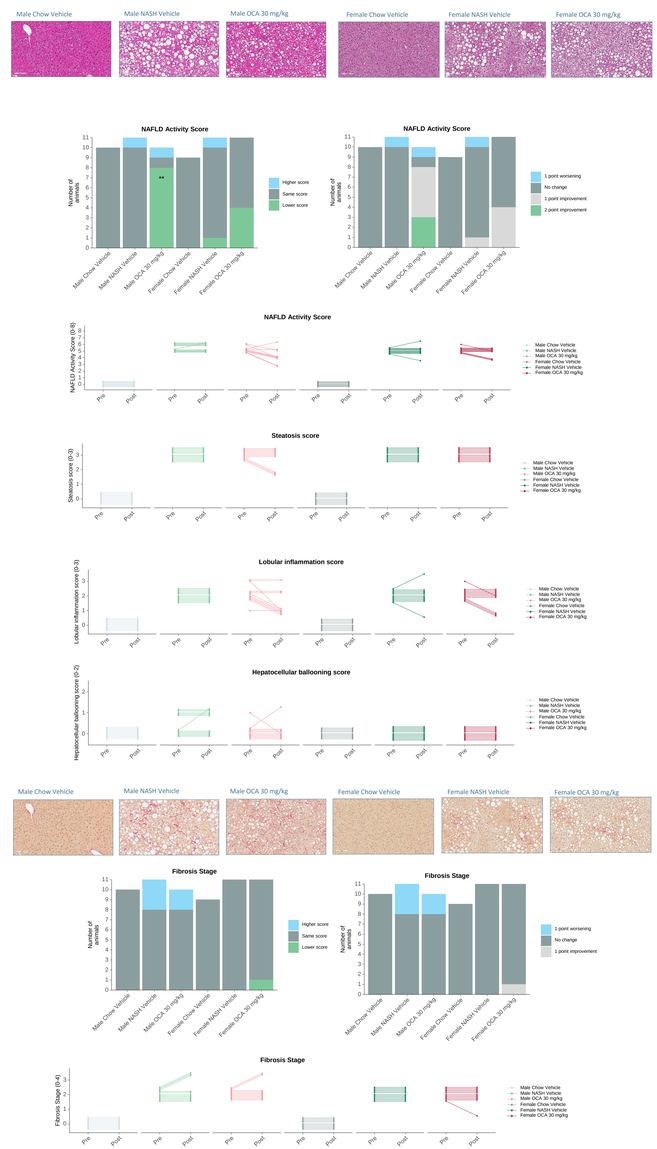


Figure 3 Representative images of Hematoxylin-Eosin and PSR staining. Histopathological scoring (pre-to-post) for liver biopsies for all animals separated by groups. **: P < 0.001 compared to Male NASH Vehicle.

Effect of OCA treatment on histomorphometric steatosis, inflammation and fibrosis in female and male DIO-NASH mice

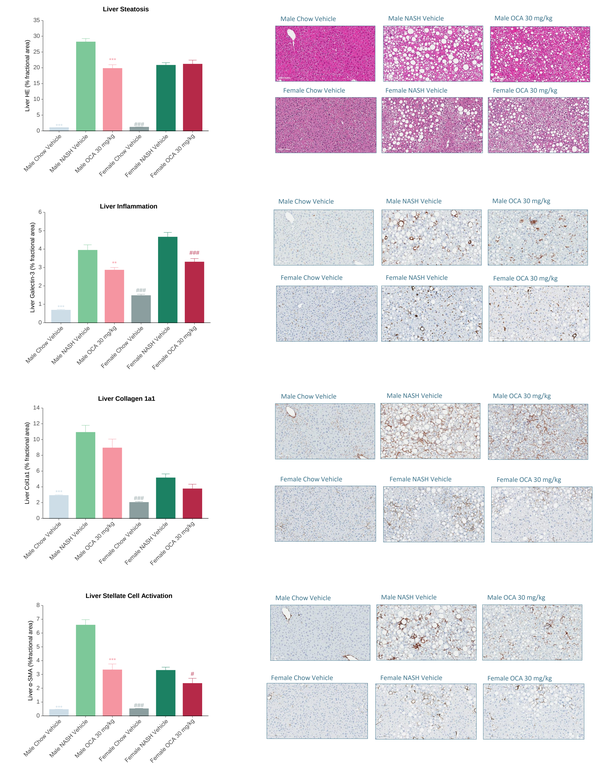


Figure 4. Representative images of Liver HE, Galectin-3, Collagen 1a1 and α-SMA staining. Terminal liver % fractional area (FA) for steatosis (HE), inflammation (Galectin-3), collagen (Collagen 1a1) and stellate cell activation (α-SMA) determined by histomorphometry . ***: P < 0.001 compared to Male NASH Vehicle. #: P < 0.05, ###: P < 0.001 compared to Female NASH Vehicle.

CONCLUSION

- Male and female DIO-NASH mice both exhibits key features of metabolic (obesity, elevated liver plasma enzymes and hypercholesterolemia) and hepatic (hepatomegaly, steatosis, inflammation and fibrosis) parameters of NASH in comparison to LEAN-CHOW control groups.
- OCA treatment reduced plasma and liver TC levels and decreased liver inflammation and fibrogenesis in both male and female DIO-NASH mice.
- OCA treatment increased plasma ALT and liver HP levels only in male DIO-NASH mice.
- OCA treatment reduced liver HP levels only in female DIO-NASH mice.
- OCA treatment reduced liver steatosis and improved NAFLD Activity Score (pre-to-post) only in male DIO-NASH mice.
- Differential gender drug efficacy can be explored in male and female DIO-NASH mouse models.