Body-weight lowering agents and their comparative metabolic and hepatic effects in obese mouse models of non-alcoholic fatty liver disease and steatohepatitis

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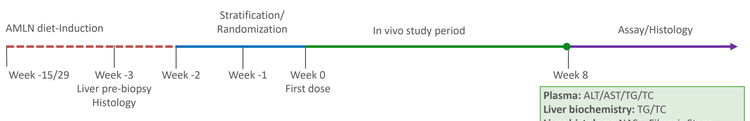
Introduction and Aim

The GLP-1 analogue liraglutide is an established treatment for obesity and type-2-diabetes. Here we aimed to compare the body weight lowering effects of liraglutide and the peroxisome proliferator activated receptor (PPAR) α/δ agonist, elafibranor, in diet-induced obese (DIO) and genetically obese mouse models of nonalcoholic fatty liver disease (NAFLD) and steatohepatitis (NASH).

Methods

Male wildtype C57BL/6J and leptin-deficient Lep$^{ob/ob}$ mice (5 weeks of age) were fed a diet high in trans-fat, fructose and cholesterol for a total of 26 weeks and 12 weeks, respectively, for induction of NASH. Only biopsy-confirmed steatotic and fibrotic animals were included and stratified into wildtype DIO-NASH and ob/ob-NASH treatment groups and treated for 8 weeks with vehicle (PO, QD), liraglutide (0.2 mg/kg, SC, BID) or elafibranor (30 mg/kg, PO, QD). At termination, blood samples were collected for plasma liver enzymes (alanine/aspartate aminotransferases; ALT/AST) and lipids (total cholesterol; TC, triglycerides; TG). Furthermore, liver post-biopsies and tissue samples were obtained for histological and biochemical analysis. Finally, a blinded histological evaluation of NAFLD Activity Score (NAS) (steatosis, inflammation, ballooning degeneration) including Fibrosis Stage was performed and compared to morphometric analyses of relative and total steatosis and fibrosis levels.

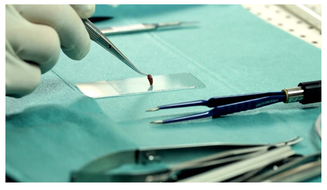
Study Design



Plasma: ALT/AST/TG/TC
Liver biochemistry: TG/TC
Liver histology: NAS + Fibrosis Stage + Steatosis + Col1a1 + Galectin-3
Liver RNAseq

Group #	Animal	Gender	Strain	Number of animals	Treatment	Administration form	Dosing volume	Daily Dosing concentration
5	LEAN-CHOW ob/ob-CHOW	Male	C57BL/6J/ Lep$^{ob/ob}$	10	**Vehicle**	QD, PO	5 ml/kg	-
1	DIO-NASH/ ob/ob-NASH	Male	C57BL/6J/ Lep$^{ob/ob}$	10-12	**Vehicle**	QD, PO	5 ml/kg	-
2	DIO-NASH/ ob/ob-NASH	Male	C57BL/6J/ Lep$^{ob/ob}$	10-12	**Liraglutide**	BID, SC	5 ml/kg	**0.4 mg/kg**
3	DIO-NASH/ ob/ob-NASH	Male	C57BL/6J/ Lep$^{ob/ob}$	10-12	**Elafibranor**	QD, PO	5 ml/kg	**30 mg/kg**

The liver biopsy procedure



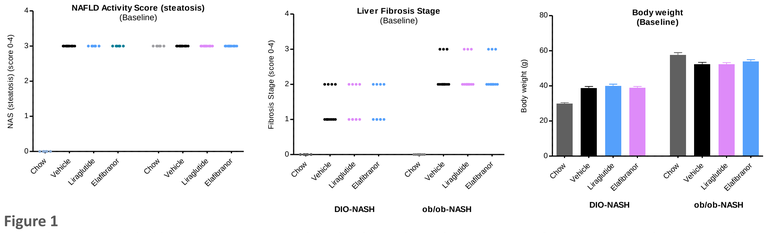


Figure 1
Animals were included based on a pre-study (week -3) histological assessment of steatosis stage (minimum 2) and fibrosis score (minimum 1)) and subsequently randomized into treatment groups based on steatosis score, fibrosis stage and body-weight.

Results

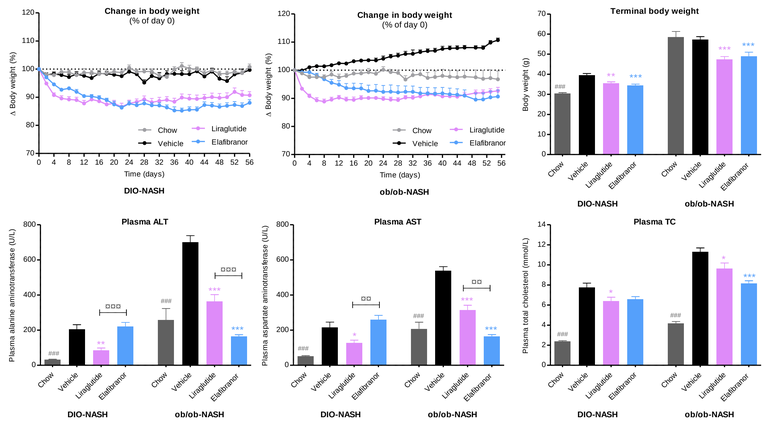


Figure 2
Metabolic parameters in DIO-NASH and ob/ob-NASH animals. Liraglutide and elafibranor induced a significant body weight reduction of approximately 10% in both NASH models (upper panel). Liraglutide reduced plasma ALT, AST and TC in both models, whereas elafibranor reduced ALT, AST and TC in ob/ob mice only (lower panel). Data presented as mean + SEM. ###p<0.001 vs. vehicle, Unpaired t-test; */***p<0.05-<0.001 vs. vehicle, ¤/¤¤¤ p<0.05-<0.001 vs. liraglutide, One-way ANOVA with Tukey Multiple Comparison Test.

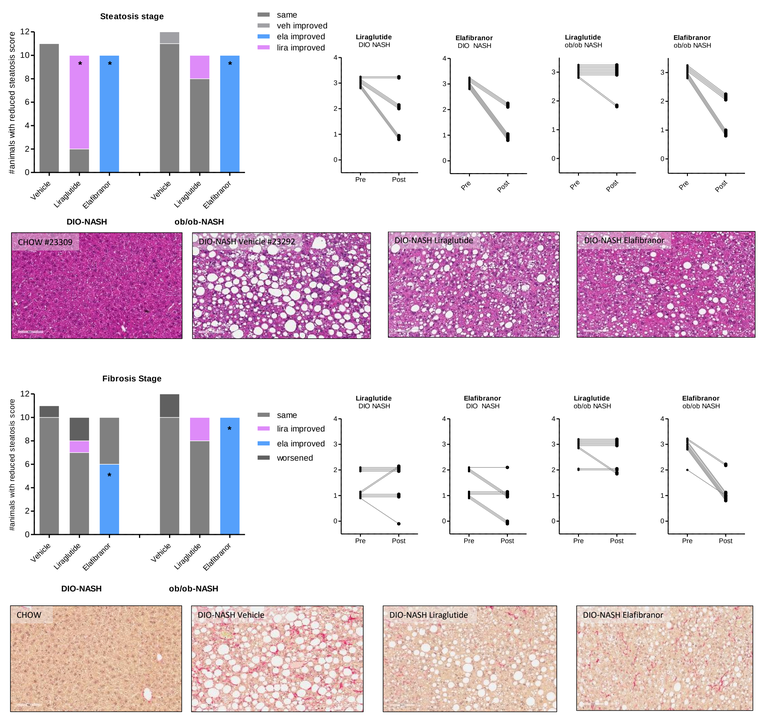


Figure 3
Summary of histopathological scoring of the pre- and post-study biopsies for steatosis (upper panel) and fibrosis (lower panel) in DIO-NASH and ob/ob-NASH mice . Whereas liraglutide and elafibranor reduced steatosis score in DIO-NASH mice, only elafibranor reduced steatosis in ob/ob NASH mice. Elafibranor also reduced fibrosis stage in both models. For each compound group, significance of number of animals with a lower score versus respective Vehicle was assessed using Fisher's exact test followed by adjustment for multiple correction using the Bonferroni method. *p<0.05. Ratios presented as mean ± SEM. ###p<0.001 vs. vehicle, Unpaired t-test; ***p<0.001 vs. vehicle, One-way ANOVA with Tukey Multiple Comparison Test.

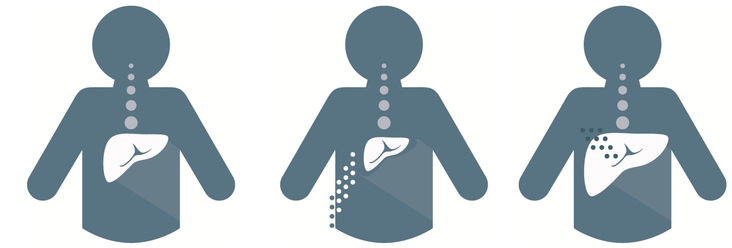


Figure 4
Schematic illustration demonstrating the inherent challenges in presenting relative data. A normal liver with relative fibrosis levels (left), being increased (relatively) due to fat depletion and subsequent shrinkage of total liver size (liraglutide, middle) or reduced (relatively) by liver hypertrophy due to peroxisome proliferation (elafibranor, right). The total amount of fibrosis is unchanged.

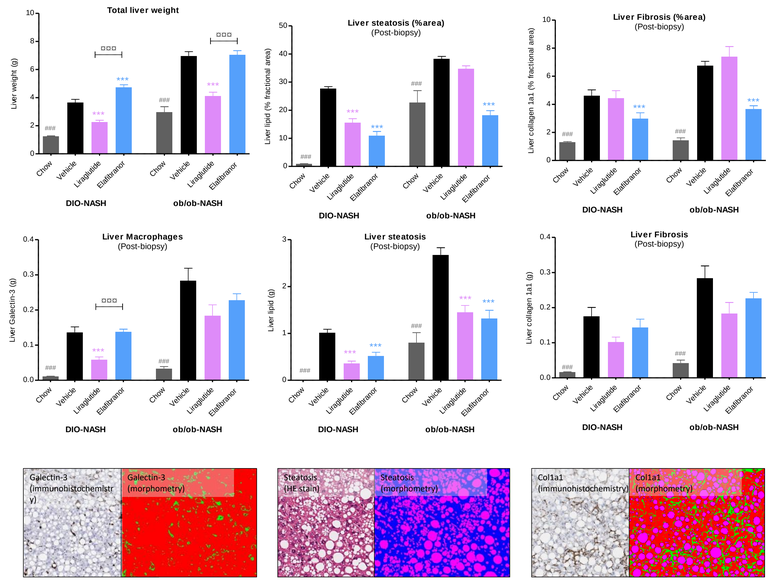


Figure 5
Liver weight and morphometry analyses of relative liver steatosis and fibrosis (upper level), with total levels of inflammation, steatosis and fibrosis (lower panel) in DIO-NASH and ob/ob-NASH animals. Liraglutide improves steatosis in both models when accounting for liver weight, decreases inflammation (Galectin-3) and tends to decrease fibrosis. The effect of elafibranor on liver fibrosis attains significance only when presented as relative values. Data presented as mean ± SEM. ###p<0.001 vs. vehicle, Unpaired t-test; */***p<0.05-<0.001 vs. vehicle, ¤/¤¤¤ p<0.01-<0.001 vs. liraglutide, One-way ANOVA with Tukey Multiple Comparison Test.

Conclusion

- **Pharmacological intervention with liraglutide and elafibranor induced distinct metabolic and liver histological effects, despite having similar efficacy on body weight loss in wildtype diet-induced and genetically obese mouse models of NASH.**
- **Elafibranor improved steatohepatitis and fibrosis in DIO-NASH and ob/ob-NASH mice when assessed by histopathology, as well as relative steatosis and fibrosis ratios.**
- **Liraglutide improved hallmarks of NASH in both models when incorporating effects on total liver size.**
- **The finding suggest that both weight-loss inducing agents have beneficial effects on hallmarks of NAFLD/NASH – but that different mechanism of action on liver size may impact conclusions drawn.**